Training Toolkit: Lessons and Exercises.
Unit 1: Quality Control and Standardization

WP 2.1.

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INNOLEA: Innovation for the Leather Industry in Jordan and Egypt
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(*) Action: C = Creation, I = Insert, U = Update, R = Replace, D = Delete

Disclaimer

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1. Introduction

INNOLEA project aims to fill an apparent gap in the area of specialized services for the leather sector with the establishment of four leather centres in local Universities, two in Jordan and two in Egypt, utilizing the experience and expertise of EU partners in the area of services for the leather sector.

Through the creation of these centres and the further tasks that will be implemented in this project, the leather sectors in Jordan and Egypt will be offered access to business development services, such as quality testing, product certification, training, fashion trends, production organization, BtoB and funding opportunities, and subsequently the Jordanian and Egyptian leathers sector will have a valuable ally for its further development.

The project also aims to create and maintain a link between Universities and businesses of the leather sector that will foster innovation and the manufacturing of high value quality products, as well as further cooperation between EU and Jordan and Egypt Universities and leather businesses.

The project also aims to help encourage the Egyptian and Jordanian governments to favour the establishment of leather centres within universities and to promote research and projects between EU and Egypt and Jordan universities in the leather sector, by creating a research innovation and training network, which will continue to operate after the end of the current project.

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2. UNIT 1: Quality Control and Standardization

2.1. Objectives

1. To acquire technical competences and skills (on standardization and quality control);
2. To acquire key skills and competences;
3. To acquire more knowledge about quality characteristics of raw hides and skins, semi-processed, finished leather
4. To know evaluation technics for assessing quality of materials
5. To have a deep understanding about quality and certification issues.

2.2. Lesson 1: Quality management in a laboratory accredited according to ISO/IEC 17025

Author: Viorica ROSCULET - INCDTP-ICPI

- Quality management following the ISO/IEC 17025 standard
- Products certification
- Enterprise certification
- Quality labels

2.2.1. Definitions

- **Product:** result of a process
- **Procedure:** specified way to carry aut an activity or a process
- **Specified requirement:** need or expectation that is stated
- **Testing:** determination of one or more characteristics of an object of conformity assessment, according to a *procedure*
- **Sampling:** provision of a sample of the object of conformity assessment, according to a *procedure*
- **Review:** verification of the suitability, adequacy and effectiveness of selection and determination activities, and the results of these activities, with regard to fulfilment of *specified requirements* by an object of conformity assessment
- **Attestation:** issue of a statement, based on o decision following *review*, that fulfilment of a *specified requirements* has been demonstrated
- **Scope of attestation:** range or characteristics of objects of conformity assessments covered by *attestation*
- **Inspection:** examination of a product design, *product*, process or installation and determination of its conformity with specific requirements or, on the basis of professional judgement, with general requirements

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- **Audit:** systematic, independent, documented process for obtaining records, statements of fact or other relevant information and assessing them objectively to determine the extent to which *specified requirements* are fulfilled
- **Conformity assessment:** demonstration that *specified requirements* relating to a *product*, process, system, person or body are fulfilled
- **First-party conformity assessment:** conformity assessment activity that is performed by a person or organization that provides the object
- **Second-party conformity assessment:** conformity assessment activity that is performed by a person or organization that has a user interest in the object
- **Third-party conformity assessment:** conformity assessment activity that is performed by a person or organization that is independent of the person or organization that provides the object, and of user interests in that object
- **Conformity assessment body:** body that performs conformity assessments services
- **Accreditation body:** authoritative body that performs *accreditation*
- **Conformity assessment system:** rules, *procedures* and management for carrying out *conformity assessment*
- **Conformity assessment scheme/ programme:** *conformity assessment system* related to specified objects of conformity assessment, to which the same *specified requirements*, specific rules and *procedures* apply
- **Declaration:** first-party *attestation*
- **Certification:** third-party *attestation* related to products, processes, systems or persons
- **Accreditation:** third-party *attestation* related to a *conformity assessment body* conveying formal demonstration of its competence to carry out specific conformity assessments tasks
- **Surveillance:** systematic iteration of conformity assessments activities as a basis for maintaining the validity of the statement of conformity
- **Suspension:** temporary invalidation of the statement of conformity for all or part of the specified *scope of attestation*
- **Withdrawal:** revocation cancellation of the statement of conformity

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- **Appeal:** request by the provider of the object of conformity assessment to the *conformity assessment body* or *accreditation body for* reconsideration by the body of a decision it has made relating to that object
- **Complaint:** expression of dissatisfaction, other than *appeal*, by any person or organization to a *conformity assessment body* or *accreditation body*, relating to the activities of that body, where a response is expected
- **Approval:** permission for a *product* or process to be marketed or used for stated purposes or under stated conditions
- **Designation:** governmental authorization of a *conformity assessment body* to perform specified conformity assessment activities
- **Equivalence of conformity assessment results:** sufficiency of different conformity assessment results to provide the same level of assurance of conformity with regard to the same *specified requirements*
- **Recognition of conformity assessment results:** acknowledgement of the validity of a conformity assessment result provided by another person or body
- **Acceptance:** use of a conformity assessment result provided by another person or body.

2.2.2. Normative references

- ISO/IEC Guide 99, International vocabulary of metrology-Basic and general concepts and associated terms (VIM- also known as JCGM 200)
- ISO/IEC 17000- Conformity assessment - Vocabulary and general principles

RELATED NORMATIVE REFERENCES

- ISO 5725-1 ISO/IEC 12207 ISO/IEC 17065
- ISO 5725-2 ISO 15189 ISO 17511
- ISO 5725-3 ISO 15194 ISO 19011
- ISO 5725-4 ISO/IEC 17011 ISO 21748
- ISO 5725-6 ISO/IEC 17020 ISO 31000
- ISO 9000 ISO/IEC 17021-1
- ISO 9001 ISO 17034
- ISO 10012 ISO/IEC 17043

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- ISO Guide 30
- ISO Guide 31
- ISO Guide 33
- ISO Guide 35
- ISO Guide 80
- ISO/IEC Guide 98-3
- ISO/IEC Guide 98-4
- ISO/IEC Guide 115

2.2.3. ISO/IEC 17025:2017 Requirements

General requirements for the competence of testing and calibration laboratories

Scope: This International Standard specifies general requirements for competence, impartiality and laboratory consistent activity.

This document applies to all organizations which perform laboratory activities regardless of the number of staff.

Normative references: Specify the documents, in whole or in part, which are normatively referenced in this document and are indispensable for its application.

Terms and definitions: Specify the terms and definitions which apply for the purposes of the document.

General requirements

Impartiality: Means the presence of objectivity. The laboratory activities shall perform and manage to assure the impartiality and the laboratory management shall provide evidence of its commitment for ensuring the impartiality.

Confidentiality: Laboratory shall be responsible through legal commitments for managing all information obtained or created during the activities of the laboratory.

Structural requirements

- Laboratory shall be a legally constituted entity or part of a legal entity that is legally responsible for its laboratory activities.
- Laboratory shall identify who has overall responsibility for management of the laboratory.
- Laboratory shall define and document the range of the laboratory activities that complies with this document.

Resources

General:

- Laboratory shall have staff, facilities, equipment, systems and support services needed to manage and perform its laboratory activities.

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People

- All laboratory personnel, both internally and externally, who could influence laboratory operations, shall act impartially, shall be responsible and shall work in accordance with the management system of the laboratory.
- Laboratory shall document competency requirements for each function that influence the results of laboratory activities, including requirements relating to education, qualification-ability, training, technical knowledge, skills and experience.

Infrastructure and environment: The facilities and environmental conditions shall be suitable for laboratory activities and should not affect the validity of the results.

Equipment: Laboratory shall have access to the equipment (including but not limited to measuring instruments, software, measurement standards, reference materials, reference data, consumables and auxiliary apparatus) necessary for correct laboratory activities and which can influence the results.

Metrological traceability: Laboratory shall establish and maintain metrological traceability of its measurement results by means of a calibration and documented unbroken chain, each contributing to the uncertainty of measurement, related the results to an adequate reference.

Products and services supplied from external providers: Laboratory shall ensure that the products and services that affect the laboratory activities, if externally provided, are used only if appropriate.

2.2.4. Requirements for the process

Analysis requests, tenders and contracts: Laboratory shall have a procedure for the analysis of requests, offers and contracts.

Selection, verification and validation of the methods: Laboratory shall use methods and procedures for all laboratory activities and, where appropriate, for the evaluation of measurement uncertainty as well as statistical techniques for data analysis.

Sampling: Laboratory shall have a sampling plan and method, when carrying out sampling of substances, materials or products for subsequent testing or calibration.

Handling of test and calibration objects: Laboratory shall have a procedure for the transport, reception, handling, protection, storage and disposal or return of objects for test or calibration, including all the necessary measures to protect the integrity of the object to test or calibration, and to protect the interests of the laboratory and the customer.

Technical records: Laboratory shall ensure that technical records for each laboratory activity contain results, reports and sufficient information to facilitate, if possible, identification of factors affecting the measurement result and associated measurement uncertainty and to permit repetition of laboratory activity in conditions as close as possible to the original.

Evaluation of measurement uncertainty: Laboratories shall identify contributions to measurement uncertainty.

Ensuring the validity of results: Laboratory shall have a procedure for monitoring the validity of its results.

Reporting results: The results shall be analyzed and approved prior to release.

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Complaints: Laboratory shall have a documented process for receiving, evaluating and deciding on complaints.

Nonconforming activity: Laboratory shall have a procedure to be implemented when any aspect of laboratory activities or results of laboratory activities do not conform to its own procedures or to the agreed requirements of the client.

Data control and information management: Laboratory shall have access to data and information necessary to perform laboratory activities.

Quality management system requirements: Laboratory shall establish, document, implement and maintain a management system (according to option A or option B) capable of supporting and demonstrating consistent fulfillment of the requirements of this document and ensure the quality of laboratory results.

Options: Option A - no other QMS implemented; Option B - QMS according to ISO 9001.

Quality management system documentation (option A)

Control of quality management system documents (option A)

Control of the records (option A)

Actions to address risks and opportunities (option A)

Improvement (option A)

Corrective actions (option A)

Internal audit (option A)

Management review (option A)

Annex A (informative) - Metrological traceability;

Annex B (informative) - Quality management system options.

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2.3. Lesson 2: Chemical tests for leather

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- Physical and mechanical quality controls available
- Evaluation techniques for assessing the quality of materials
- Quality tools

2.3.1. Introduction

Official methods of analysis and testing, internationally accepted, are imperative for delineating standards and specifications. International official methods are needed to protect tanner and manufacturer, to avoid trade disputes and misinterpretation and to allow them to sell their leather and products on export markets imposing performance standards.

Two international organizations are dealing with leather testing, the *International Organization for Standardization*, **ISO**, and the *International Union of Leather Technologists and Chemists Societies*, **IULTCS**.

The *Comite Europeen de Normalisation*, **CEN**, covering the countries member of the European Union - previously the European Economic Community (EEC) and the countries of the European Free Trade Association (EFTA), includes standardized leather testing methods and also mandatory specifications for performance standards.

Many countries have their national standards bureau, their standardized leather and leather products testing methods and sometimes specifications for performance.

The *International Organization for Standardization*, **ISO**, is a worldwide federation of national bodies, the world's largest non-governmental system for voluntary industrial and technical collaboration at the international level. ISO work is decentralized, being carried out by technical committees and sub-committees. The Central Secretariat in Geneva assists in coordinating ISO operations, administers voting and approval procedures, and publishes the International Standards. The ISO TC/120 is the committee for hides and skins and for leather. The ISO Committee TC/45 and TC/137 covers leather and non-leather footwear and TC/176 the quality assurance standards.

Most of the **ISO** Technical Committees are bureaucratic and ISO TC/120 *Leather* has not been particularly active. Recently, the ISO Board of Directors recognized the IULTCS as the legal organism specialized in the preparation, definition and drafting of standard testing methods for leather. The Secretariat has also agreed to existing IULTCS methods as official ISO methods after translation into ISO format. Only the standards on raw and semi-processed hides and skins are remaining in the activities of the Technical Committee TC/120.

Since 1990 many of the IU methods have become joint IULTCS and ISO Standards. From 2005 it was agreed that ISO will be responsible for publishing all new joint IULTCS and ISO Standards. Member countries of ISO very often use the ISO Standards to establish their own National Standards.

The *International Union of Leather Technologists and Chemists Societies*, **IULTCS**, grouping some 40 countries over the world, has three International Commissions working on leather testing:

- the IUC International Commission for chemical analysis
- the IUP International Commission for physical testing
- the IUF International Commission for fastness testing.

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The Commissions are working with the Leather and Footwear Institutes and with members of the chemical and tanning industries. They select and study methods, compare and evaluate the results of testing in different laboratories, propose the draft methods for adoption by the Council of Delegates at the biennial Congresses of the Union.

In order to avoid possible technical obstacles to a wider competitivity in the markets, the Commission of the European Community has created the CEN standardization committee dealing inter alia with leather and leather products, for which the CEN/TC 289 Committee has been created with three working groups:

WG 1 Terminology

WG 2 Sampling and analysis, subdivided in 3 task groups:

TG 1 Chemical tests

TG 2 Physical/mechanical tests

TG 3 Fastness tests

WG 3 Guidelines for leather performances

Set out below is a complete list of the IULTCS official methods together with the reference numbers for the equivalent ISO Standards and European Norm (EN) methods. Standards with numbers that include DIS (Draft International Standard) or FDIS (Final Draft International Standard) are still in preparation. They can be obtained from Standards Associations but are not yet officially approved Standards.

The IULTCS official methods of analysis for leather, including the equivalent ISO and EN Standards

- update version: September 2018

Table 2.1. IULTCS - CHEMICAL TEST METHODS

IUC Test method	Method name	ISO Standard	EN Standard
IUC 1 (1965)	General comments	-	-
IUC 2 (2002)	Sampling location (same as IUP 2)	**ISO 2418:2002**	**EN ISO 2418**
IUC 3 (2017)	Preparation of chemical test samples	**ISO 4044:2017**	**EN ISO 4044**
IUC 4 (2018)	Determination of matter soluble in dichloromethane and free fatty acid content	**ISO 4048:2018**	**EN ISO 4048**
IUC 5 (2005)	Determination of volatile matter	**ISO 4684:2005**	**EN ISO 4684**
IUC 6 (2018)	Determination of water-soluble matter, water soluble inorganic matter and water-soluble organic matter	**ISO 4098:2018**	**EN ISO 4098**
IUC 7 (1977)	Determination of sulphated total ash and sulphated water insoluble ash	**ISO 4047:1977**	**EN ISO 4047**
IUC 8-1 (2018)	Determination of chromic oxide content Part 1: Quantification by titration	**ISO 5398-1:2018**	**EN ISO 5398-1**
IUC 8-2 (2009)	Determination of chromic oxide content Part 2: Quantification by colorimetric determination	**ISO 5398-2:2009**	**EN ISO 5398-2**
IUC 8-3 (2018)	Determination of chromic oxide content Part 3: Quantification by atomic absorption spectrometry	**ISO 5398-3:2018**	**EN ISO 5398-3**
****IUC 8-4** (2018)	Determination of chromic oxide content Part 4: Quantification by inductively coupled plasma (ICP-OES)	****ISO 5398-4:2018**	****EN ISO 5398-4**
IUC 9 (1984)	Determination of water-soluble magnesium salts	**ISO 5399:1984**	**EN ISO 5399**
IUC 10 (1984)	Determination of nitrogen and hide substance	**ISO 5397:1984**	-
IUC 11 (2018)	Determination of pH and difference figure	**ISO 4045:2018**	**EN ISO 4045**
IUC 13 (1975)	Determination of zirconium	-	-
IUC 15 (1973)	Determination of phosphorus	-	-

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IUC Test method	Method name	ISO Standard	EN Standard
IUC 16 (1969)	Determination of aluminium	-	-
IUC 17 (1980)	Determination of hydroxyproline in materials containing collagen	-	-
IUC 18-1 (2017)	Determination of hexavalent chromium content – Part 1; Colorimetric method	**ISO 17075-1:2017**	**EN ISO 17075-1**
IUC 18-2 (2017)	Determination of hexavalent chromium content – Part 2; Ion chromatographic method	**ISO 17075-2:2017**	**EN ISO 17075-2**
****IUC 19-1** (2008)	Determination of formaldehyde content in leather. Part 1: Quantification by HPLC	****ISO 17226-1:2008**	****EN ISO 17226-1**
****IUC 19-2** (2008)	Determination of formaldehyde content in leather. Part 2: Quantification by colorimetric analysis	****ISO 17226-2:2008**	****EN ISO 17226-2**
IUC 19-3 (2011)	Determination of formaldehyde content in leather - Part 3: Formaldehyde emissions from leather	**ISO 17226-3:2011**	**EN ISO 17226-3**
****IUC 20-1** (2015)	Chemical tests for the determination of certain azo colorants in dyed leathers - Part 1: Determination of certain aromatic amines derived from azo colorants	****ISO 17234-1:2015**	****EN ISO 17234-1**
IUC 20-2 (2011)	Chemical tests for the determination of certain azo colorants in dyed leathers, Part 2: Determination of 4-aminoazobenzene derived from azo colorants	**ISO 17234-2:2011**	**EN ISO 17234-2**
IUC 21 (2003)	Method for the detection of certain azo colourants in dyestuff mixtures.	-	-
IUC 22 (2003)	Determination of aluminium oxide content of aluminium tanning agents	-	-
IUC 24 (2003)	Determination of basicity of aluminium tanning agents.	-	-
IUC 25 (2015)	Determination of tetrachlorophenol-, trichlorophenol-, dichlorophenol-, monochlorophenol-isomers and pentchlorophenol content	**ISO 17070:2015**	**EN ISO FDIS 17070**
****IUC 26** (2009)	Determination of free-formaldehyde content in leather processing chemicals	****ISO 27587:2009**	****EN ISO 27587**
****IUC 27-1** (2011)	Chemical determination of metal content. Part 1: Extractable metals	****ISO 17072-1:2011**	****EN ISO 17072-1**
****IUC 27-2** (2011)	Chemical determination of metal content. Part 2: Total metal content	****ISO 17072-2:2011**	****EN ISO 17072-2**
IUC 28-1 (2015)	Determination of ethoxylated alkylphenols in leather. Part 1: Direct method	**ISO 18218-1:2015**	**EN ISO 18218-1**
IUC 28-2 (2018)	Determination of ethoxylated alkylphenols in leather. Part 2: Indirect method	**ISO 18218-2:2018**	**EN ISO 18218-2**
****IUC 29** (2011)	Determination of preservative content (TCMTB-OPP- CMK-OIT) in leather	****ISO 13365:2011**	****EN ISO 13365**
IUC 30 (2015)	Determination of chlorinated hydrocarbons in leather - method for short-chain chlorinated paraffins (SCCP)	**ISO 18219:2015**	**EN ISO 18219**
IUC 30-1 (2018) **(replacement for ISO 18219, draft is the same)**	Leather - Chemical determination of chlorinated hydrocarbons in leather. Part 1: Chromatographic method for short chain chlorinated paraffins (SCCP)	**ISO/DIS 18219-1:2018**	**prEN ISO/DIS 18219-1**
IUC 30-2 (2018) **(draft at DIS enquiry stage)**	Leather - Chemical determination of chlorinated hydrocarbons in leather – Part 2: Chromatographic method for middle chain chlorinated paraffins (MCCP)	**ISO/DIS 18219-2:2018**	**prEN ISO/DIS 18219-2**
-	Determination of organo-tin compounds in leather by GC/MS method (**Project transferred to ISO/TC 216 Footwear**)	**ISO/TS 16179:2012** (Footwear method)	**CEN ISO/TS 16179**
****IUC 32** (2012)	Quantitative analysis of tanning agents by filter method	****ISO 14088:2012**	****EN ISO 14088**

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IUC Test method	Method name	ISO Standard	EN Standard
IUC 33 (2013)	Leather - Determination of tan content of synthetic tanning agents	**ISO 17489:2013**	**EN ISO 17489**
IUC 34 (2016)	Leather - Determination of N-methylpyrrolidone in leather	**ISO 19070:2016**	**EN ISO 19070**
IUC 35 (2016)	Leather - Determination of Cr(VI) and its reductive potential in leather chemicals	**ISO 19071:2016**	**EN ISO 19071**
IUC 36 (2017)	Leather - Guidelines for testing critical chemicals in leather	**ISO 20137:2017**	**EN ISO 20137**
****IUC 37** (2017)	Leather - Determination of degradability by micro- organisms	****ISO 20136:2017**	****EN ISO 20136**
IUC 38 (2017) **(draft at FDIS formal vote stage)**	Leather - Determination of pesticide residues content in leather	**ISO/FDIS 22517:2017**	-
IUC 39-1 (2018)	Leather - Organic fluorine - Part 1: Determination of non-volatile compounds by extraction method using liquid chromatography	**ISO 23702-1:2018**	**EN ISO 23702-1**
IUC 40	Free – original document changed to IUC 30-2		
IUC 41(2018)	Determination of hexavalent chromium content – Pre- ageing for chemical determination of hexavalent chromium	**ISO 10195:2018**	-

** Standard is undergoing revision and an updated version is in preparation.

Table 2.2. IULTCS – PHYSICAL TEST METHODS

IUP Test method	Method name	ISO Standard	EN Standard
IUP 1 & IUP 3 (2012)	Sample preparation and conditioning	**ISO 2419:2012**	**EN ISO 2419**
IUP 2 (2017)	Sampling location (same as IUC 2)	**ISO 2418:2017**	**EN ISO 2418**
IUP 4 (2016)	Measurement of thickness	**ISO 2589:2016**	**EN ISO 2589**
IUP 5 (2017)	Measurement of apparent density	**ISO 2420:2017**	**EN ISO 2420**
IUP 6 (2011)	Measurement of tensile strength and percentage elongation	**ISO 3376:2011**	**EN ISO 3376**
IUP 7 (2016)	Measurement of static absorption of water	**ISO 2417:2016**	**EN ISO 2417**
IUP 8 (2016)	Measurement of tear load – Double edge tear	**ISO 3377-2:2016**	**EN ISO 3377-2**
IUP 9 (2015)	Measurement of distension and strength of grain by the ball burst test	**ISO 3379:2015**	**EN ISO 3379**
IUP 10-1 (2011)	Water resistance of flexible leather. Part 1: Linear compression method (Penetrometer)	**ISO 5403-1:2011**	**EN ISO 5403-1**
IUP 10-2 (2011)	Water resistance of flexible leather. Part 2: Angular compression method (Maeser)	**ISO 5403-2:2011**	**EN ISO 5403-2**
IUP 11 (2011)	Measurement of water resistance of heavy leather	**ISO 5404:2011**	**EN ISO 5404**
IUP 12 (2002)	Measurement of resistance to grain cracking and the grain crack index	**ISO 3378:2002**	**EN ISO 3378**
IUP 13 (1961)	Measurement of two dimensional extension	-	-
IUP 14 (1960)	Measurement of waterproofness of gloving leathers	-	-
IUP 15 (2012)	Measurement of water vapour permeability	**ISO 14268:2012**	**EN ISO 14268**
IUP 16 (2015)	Measurement of shrinkage temperature up to 100ºC	**ISO 3380:2015**	**EN ISO 3380**
IUP 17 (1966)	Assessment of the resistance of air dry insole leathers to heat	-	-
IUP 18 (1969)	Resistance of air dry lining leathers to heat	-	-
IUP 19 (1969)	Resistance of air dry upper leather to heat	-	-
****IUP 20-1** (2017)	Determination of flex resistance. Part 1: Flexometer method	****ISO 5402-1:2017**	****EN ISO 5402-1**
IUP 21 (1963)	Measurement of set in lasting	-	-
IUP 22 (1963)	Assessment of scuff damage by use of the viewing box	-	-
IUP 23 (1963)	Measurement of scuff damage	-	-
IUP 24 (1964)	Measurement of surface shrinkage by immersion in boiling water	-	-

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IUP Test method	Method name	ISO Standard	EN Standard
IUP 26 (1993)	Measurement of resistance to abrasion of heavy leather	-	-
IUP 28 (1969)	Measurement of the resistance to bending of heavy leather	-	-
IUP 29 (2017)	Measurement of cold crack temperature of surface coatings	**ISO 17233:2017**	**EN ISO 17233**
IUP 30 (1983)	Measurement of water vapour absorption and desorption (See IUP 42)	-	-
IUP 32 (2014)	Measurement of area	**ISO 11646:2014**	**EN ISO 11646**
IUP 35 (2002)	Determination of the dimensional stability of leather (Old title: Measurement of dry heat resistance of leather)	**ISO 17227:2002**	**EN ISO 17227**
IUP 36 (2015)	Measurement of leather softness	**ISO 17235:2015**	**EN ISO 17235**
IUP 37 (2017)	Measurement of water repellency of garment leather	**ISO 17231:2017**	**EN ISO 17231**
IUP 38 (2017)	Measurement of heat resistance of patent leather	**ISO 17232:2017**	**EN ISO 17232**
IUP 39 (2015)	Determination of flex resistance. Part 2: Vamp flex method	**ISO 5402-2:2015**	**EN ISO 5402-2**
IUP 40 (2011)	Measurement of tear load – Single edge tear	**ISO 3377-1:2011**	**EN ISO 3377-1**
IUP 41 (2011)	Measurement of surface coating thickness	**ISO 17186:2011**	**EN ISO 17186**
IUP 42 (2016)	Measurement of water vapour absorption	**ISO 17229:2016**	**EN ISO 17229**
IUP 43 (2016)	Measurement of extension set	**ISO 17236:2016**	**EN ISO 17236**
****IUP 44** (2017)	Measurement of stitch tear resistance	****ISO 23910:2017**	****EN ISO 23910**
IUP 45 (2006)	Measurement of water penetration pressure	**ISO 17230:2006**	**EN ISO 17230**
IUP 46 (2006)	Measurement of fogging characteristics	**ISO 17071:2006**	**EN ISO 17071**
IUP 47 (2006)	Measurement of resistance to horizontal spread of flame	**ISO 17074:2006**	**EN ISO 17074**
****IUP 48-1** (2011)	Measurement of abrasion resistance. Part 1: Taber method	****ISO 17076-1:2011**	****EN ISO 17076-1**
IUP 48-2 (2011)	Measurement of abrasion resistance. Part 2: Martindale ball plate method	**ISO 17076-2:2011**	**EN ISO 17076-2**
IUP 49 (Draft: 2002)	Measurement of bagginess	-	**CEN/TS 14689:2006**
IUP 50	*Free (original document changed to IUP 53-2)*	-	-
IUP 51 (Draft: 2002)	Measurement of Surface Friction	-	-
IUP 52 (Draft: 2002)	Measurement of Compressibility	-	-
****IUP 53-1** (2012)	Determination of soiling. Part 1: Martindale method	****ISO 26082-1:2012**	****EN ISO 26082-1**
IUP 53-2 (2012)	Determination of soiling. Part 2: Tumbling method	**ISO 26082-2:2012**	**EN ISO 26082-2**
IUP 54 (2011)	Determination of flexural properties	**ISO 14087:2011**	**EN ISO 14087**
IUP 55 (2013)	Determination of dimensional change	**ISO 17130:2013**	**EN ISO 17130**
IUP 56 (2012)	Identification of leather with microscopy	**ISO 17131:2012**	**EN ISO 17131**
IUP 57 (2015)	Determination of water absorption by capillary action (wicking)	**ISO 19074:2015**	**EN ISO 19074**
IUP 58 (2016)	Measurement of leather surface – Using electronic techniques	**ISO 19076:2016**	**EN ISO 19076**

** Standard is undergoing revision and an updated version is in preparation

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2.3.2. Chemical tests

Before any leather products are placed on the market, there are legal obligations to be met to ensure they are „fit for purpose". Chemical testing of leather can form part of a good production control system to ensure that performance requirements are met. Testing can also be used to indicate possible causes of problems and/or faults in the finished article.

Due to the heterogeneity of leather, care has to be taken in the sampling, in the number of samples to draw from a lot or a consignment to form a gross sample, in the location of test pieces in each item (hide or skin), in the number of test pieces. The gross sample should be representative of the lot. The location of sampling, important for physical testing and to some extent for chemical analysis, should be representative of the mean structure configuration of the leather. The number of test pieces is related to the accuracy of the test results.

Sampling should be a compromise between accuracy and reproducibility through taking enough pieces in valuable location in the skin and, at the same time, avoiding to spoil too much leather. In the future, non-destructive methods for testing mechanical properties will probably be applicable to leather, for example methods based on acoustic emission.

The analytical results of a full analysis of leather consist of an analytically determined fraction and a calculated fraction (IUC 1 General comments).

For physical testing, the sampling method is given by standard IUP 2 and for chemical analysis by standard IUC 2 (ISO 4044). Both methods may be used on finished leather or on leather during processing (lime, pickled, wet blue, crust, etc.) The sample pieces should be orientated with a mark in such a way that the position related to the backbone can be assessed.

Sampling according to IUC/2 and IUP/2 and ISO 2418, Leather - Chemical, physical and mechanical and fastness tests - Sampling location

1. Sampling location for skins, whole hides and sides

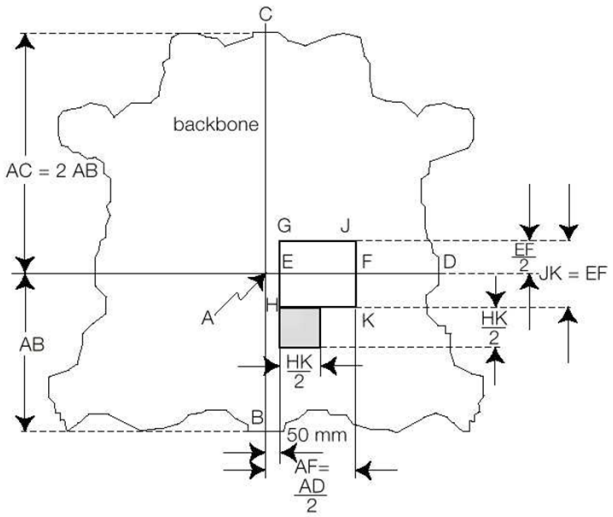

Figure 2.1. Sampling location for skins - whole hides

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2. Sampling location for shoulders

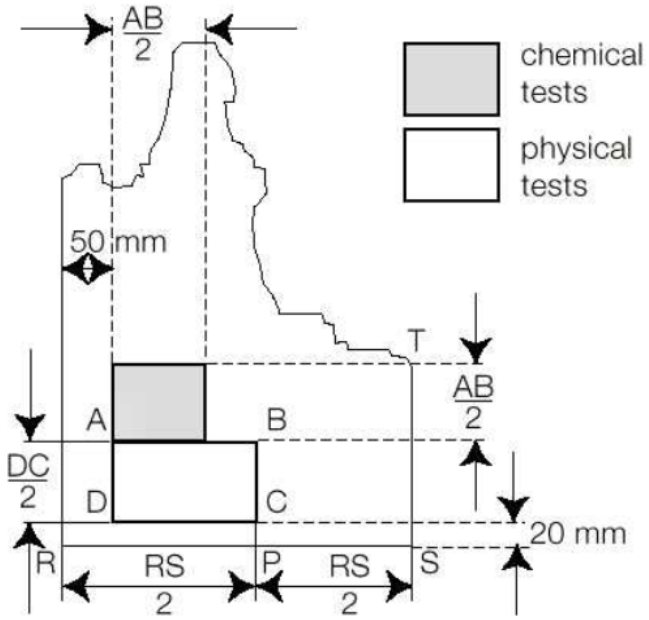

Figure 2.2. Sampling location for skins - shoulders

3. Sampling location for bends

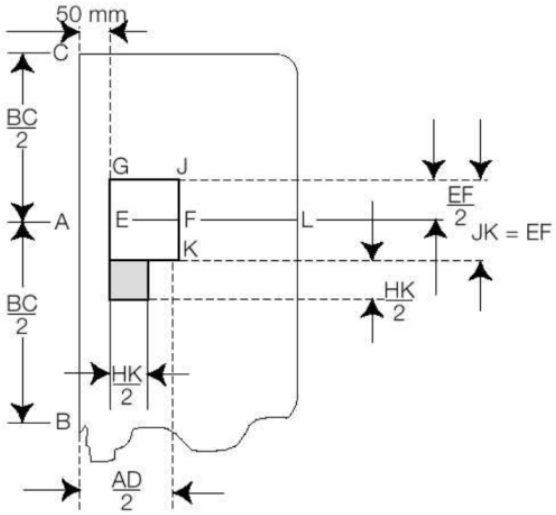

Figure 2.3. Sampling location for skins - locations for bends

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4. Sampling location for bellies (flanks)

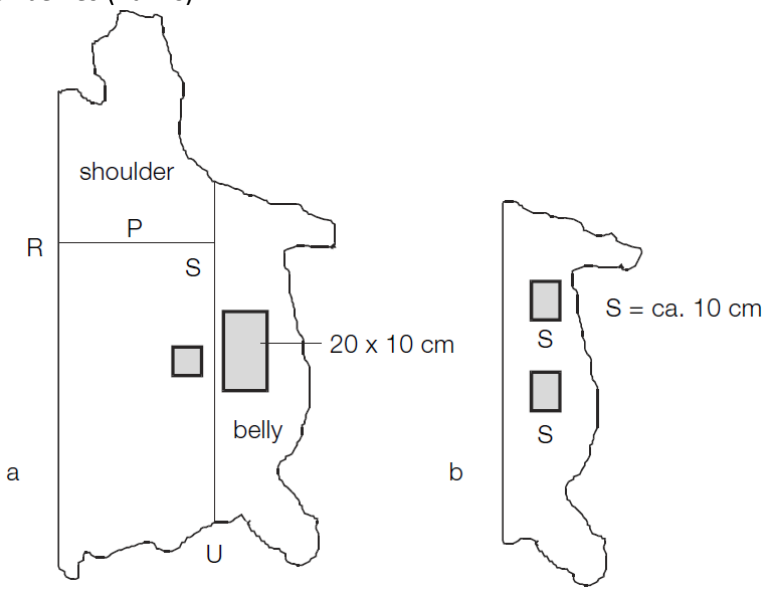

Figure 2.4.
a. sampling location, if no physical tests are required
b. sampling location, if physical tests are required

For chemical analysis, leather of all kinds must be ground in a cutter mill. Leather samples (location and identification IUC 2 / ISO 2418) for analysis need to be grounded (IUC 3 / ISO 4044, *Leather - Preparation of chemical test samples*) in order to have a more homogeneous sample and a better contact between leather and the reagents. It gives also the conditions for drying and conditioning of wet or damp samples of leather.

Determination of water content (IUC 5/ ISO 4684:2005, Leather – Chemical tests - Determination of volatile matter)

Moisture is an important characteristic of leather and will change depending on the temperature and relative humidity of its surrounding environment. Moisture and small amounts of volatile oils or solvents are included in a determination of volatile matter. Changes in moisture can affect the physical proprietes of leather – one important factor being a gain or reduction in the area of a hide or skin. Ideal moisture content is ofen considered as being 12 to 14 per cent. A sample of ground leather is dried to a constant mass at a temperature above 100^0C and the mass loss is calculated.

$$\text{Water content in \%} = \frac{G_1 - G_2}{G1} \times 100$$

G_1 = weight of sample before drying
G_2 = weight of sample after drying

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Determination of substances extractable with dichloromethane (IUC 4 /ISO 4048:2018 - Leather - Chemical tests - Determination of matter soluble in dichloromethane and free fatty acid content)

Oil and fats give suppleness and softness to leather. The level of fatlliquor (often referred to as grease content) present in leather is controlled during fatliquoring process. The final characteristics and performance of leather will be determinated to some degree by the amount and type of fatliquors used. Poor control during this part of the production process may result in leather that is harder or softer than desired, or a leather that feels greasy. Another problem that can be attributed to the fatliquors is the possible formation of a fatty acids spue on the surface of the leather. This is caused by migration of fatty acids(predominately steric and palmitic acids) to the surface where they solidify, resulting in a while waxy or grasy substance on the surface. Not all fatty and similar substances can be extracted from leather with organic solvents; they may be in part soluble and partly bound to the leather. On the other hand, the solvent can dissolve non-fatty substances, for example sulfur and impregnants, both of which cause difficulty in the determination of the acid value and saponification value of the fat.

The amount of grease is determined by extracting with dichloromethane from leather semple, using the Soxhlet apparatus. The solvent is evaporated from the extract, which is then dried at 102 °C.This method is applicable to all types of leather.

The matter extractable in dichloromethane is given, as a percentage by mass on dry matter:

$$\text{Extractable substances in \%} = \frac{\text{g extract x 100}}{\text{g weight of sample}}$$

As the extraction is frequently done in conjunction with determination of the free fatty acid content of the leather, a suitable procedure for determination of the free fatty acids extracted by this method is included. The fatty acid content of the extractable substances as determined by this method and expressed as oleic acid, $C_{18}H_{34}O_2$.

Determination of water soluble matter, water soluble inorganic matter and water soluble organic matter (IUC 6/ ISO 4098:2018 - Leather — Chemical tests — Determination of water-soluble matter, water-soluble inorganic matter and water-soluble organic matter)

The level of water soluble matter present in leather can indicate the degree of unbound tans and non-tans, salts and other substances present. The tanning process and chemicals/substances used will result in a proportion of the tannins being bound to the skin collagen structure by hydrogen bonding. However, there will always be a proportion will remain unbound and these, along with water soluble salts and unfixed dyes, can be quantified. The level of water solubles present is determined by shaking the degreased leather in water. This resulting solution is filtered and a portion is evaporated, thus allowing the solubles to be quantified. The ratio of organic to inorganic substances can be also calculated by adding sulphuric acid to the dried extract (there by converting any inorganic salts to the stable sulphate form) and then ashing to remove the organic portion.

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a. Total loss by washing in % = $\frac{\text{g solids}}{\text{g weight of sample}}$ x 100

b. Sulfate ash removable by washing in % = $\frac{\text{g sulfated residue on ignition}}{\text{g weight of sample}}$ x 100

c. Organic substances removable by washing in % = difference between total loss by washing and sulfated ash removable by washing.

Determination of water-soluble matter, water-soluble inorganic matter and water-soluble organic matter is applicable to all leather types. The result obtained by this analysis depends on factors such as:

- the degree to which the leather is ground;
- the extraction temperature;
- the extraction period;
- the ratio of leather to water.

To obtain comparable results, it is consequently imperative that test conditions be accurately reproduced.

Determination of ash (IUC 7/ ISO 4047:1977 Leather - Determination of sulphated total ash and sulphated water-insoluble ash)

The residue left from burning leather at a given temperature is defined as total ash. The residues obtained after extracting the leather with water, ashing and sulphating, are defined as water insoluble ash. With the introduction of cleaner technologies and the reduction of water consumption in the tanneries, the salt content in leather tends to be high because of replacing of rinsing by washing and working in short floats. Hence the importance of salts determination in the water extract or in the ashes of leather. The principle consists of the carbonization of the leather followed by treatment with sulphuric acid and ashing in an open crucible. The method is applicable to all types of leather.

a. Total ash in % = $\frac{\text{g total sulfated ash}}{\text{g weight of sample}}$ x 100

b. water-insoluble ash in % (determined by calculation) = % total sulfated ash minus % sulfated ash removable by washing.

Determination of chrome (IUC 8 / ISO 5398, Leather — Chemical tests - Determination of Chromic Oxide content)

The tanning process, which is the conversion of hides and skins into leather, commonly uses chromium salts. When determining the level of chromium present, the result is expressed as chromic oxide (Cr_2O_3). Knowledge of the chromic oxide content is a useful quality control technique, and a reduction in thermal stability (poor heat resistance) could be attributed to a lower than required chromic oxide content. There are a number of techniques avaible to determine the amount of chromium present in leather.

ISO 5398 consists of the following parts, under the general title Leather — Chemical determination of chromic oxide content:

- – Part 1: Quantification by titration - ISO 5398-1:2018
- – Part 2: Quantification by colorimetric determination - ISO 5398-2:2009
- – Part 3: Quantification by atomic absorption spectrometry - ISO 5398-3:2018
- – Part 4: Quantification by inductively coupled plasma — optical emission spectrometer (ICP-OES) - ISO 5398-4:2018.

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One such technique involves ashing the leather, followed by conversing of the chromium into the hexavalent state and finally iodometric titration conform ISO 5398-1: 2018 and is intended to be applicable to chromium tanned leathers which are expected to have chromic oxide contents in excess of 0,25%.
Amount of chromium in leather, determined by this method and reported as chromic oxide in percentage by mass, based on dry matter.

$$\%Cr_2O_3 = \frac{V_1 \times 0.00253 \times 100 \times F}{m_0}$$

where:

V_1 is the volume, in millilitres (ml), of 0,1 mol/l thiosulfate solution used for the titration;

m_0 is the mass of the original leather sample, in grams (g);

NOTE 1ml of exactly 0,1 mol/l thiosulfate solution is equivalent to 0,00253g Cr_2O_3

F is the factor to correct to 0 % volatile matter; it is calculated as follows:

$$F = \frac{100}{100 - W}$$

where:

W = water content, based on ISO 4684, in percent by mass

NOTE. It is also permissible if required to quote the results based on the dry, degreased weight of the sample.

ISO 5398-2: 2009 describes the determination of chrome by colorimetric means. It is applicable to leathers which are expected to have chromic oxide contents in excess of 0,05 %. Ash obtained from leather is fused by an alkaline mixture. The chromium present in the leather is solubilized in the hexavalent state followed by analysis of the solution after reaction with 1,5-diphenyl carbazide to diphenyl carbazone by photometric means at a wavelength of 540 nm. The different techniques have been described to reflect the variations in industrial practice compared with the more sensitive analytical equipment available for test laboratories. Variations also exist in the range of chromic oxide that the methods are deemed suitable to quantify. This document describes a traditional technique applied in industry that does not require the use of advanced analytical equipment.

Determination of pH value (IUC 11 / ISO 4045:2018 - Leather -- Chemical tests - Determination of pH and difference figure)

The acidity or alkalinity of the aqueous leather extract is determined by the hydrogen or hydroxyl ions activity with the conventional pH scale. The strength of acids or bases is given by the difference figure which is the difference between the pH value of a solution and its tenfold dilution. If the difference figure amounts to 0.7 to 1.0 a solution contains a free strong acid or a free strong base. The majority of tanned leathers is in the acidic range. Presence of acid in leather is not so harmful for the leather itself but is dangerous for the textile materials used in combination with leather in the manufacture of leather articles. A low pH value can promote the deterioration of footwear components and result in allergic reactions.

The difference value is the difference between the pH of a solution and that of its 1: 10 dilution.

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Figure 2.5. Equipment for pH determination

Determination of Nitrogen and Hide Substance. (IUC 10 / ISO 5397:1984 - Leather - Determination of nitrogen content and hide substance - Titrimetric method)

This test method covers the determination of the nitrogen content of all types of leather. The nitrogen content is used to calculate the hide substance (protein fiber) content of leather. The method is applicable to all types of leather in all types of tannage.
The Kjeldahl procedure involves three major steps: Digestion, Distillation, Titration

A. Digestion:
The ground leather specimen prepared according to an accepted procedure is digested with acid in the presence of a catalyst to convert the nitrogen to ammonium ion. The ammonium ion formed is nonvolatile under these highly acid conditions.

$$Protein\ (-N) + H_2SO_4 \xrightarrow[Catalyst]{Sample} (NH_4)_2SO_4 + CO_2 + H_2O$$

B. Distillation:
During the distillation step the ammonium ions (NH_4^+) are converted into ammonia (NH_3) by adding alkali (NaOH).

$$(NH_4)_2SO_4 + 2NaOH \Leftrightarrow 2NH_3\ (gas) + Na_2SO_4 + 2H_2O$$

The ammonia liberated is distilled into a boric acid solution which absorbs the ammonia. The ammonia is quantitatively captured by the boric acid solution forming solvated ammonium ions.

$$B(OH)_3 + NH_3 + H_2O \Leftrightarrow NH_4^+ + B(OH)_4^-$$

C. Titration:
The amount of ammonia in the boric acid is then determined by back titration with standardized acid using phenolphthalein as indicator (sharp color change green to purple) to determine the end point.

$$H_2SO_4\ (total) + 2NH_3 \rightarrow SO_4^{2-} + 2NH_4^+$$

The calculations for % nitrogen must take into account which type of receiving solution was used and any dilution factors used during the distillation process. In the equations below, “N” represents normality. “ml blank” refers to the milliliters of base needed to back titrate a reagent blank if standard acid is the receiving solution, or refers to milliliters of standard acid needed to titrate a reagent blank if boric acid is the receiving solution.

$$\text{Nitrogen, \%} = [((A \pm B) \times N \times 0.014)/W] \times 100$$

where:

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A = milliliters of H_2SO_4 required for titration of the sample,

B = milliliters of H_2SO_4 required for titration of the blank (or equivalent millilitres of H_2SO_4 in terms of 0.1 N NaOH solution calculated in 9.1) (use plus B in the formula above if the blank was acidic and required titration by alkali. Use minus B if the blank was alkaline and required titration by acid),

N = normality of the H_2SO_4,

W = grams of sample.

$$\text{Nitrogen, \% (moisture-free basis)} = [C/(100 - M)] \times 100$$

where:

C = percent nitrogen (as-received basis)

M = percent volatile matter or moisture determined according to ISO 4684.

Figure 2.6. Equipment Velp Scientifica for Distillation

The nitrogen content as determined by this test method is normally considered to be related to the amount of hide substance (protein fiber) present in the leather sample. A factor of 5.62 is normally used to calculate the hide substance from the nitrogen content. The 5.62 factor represents the average result of many analyses of animal hides, but it cannot be considered to be accurate since it varies somewhat from hide to hide of the same type, from type of hide to type of hide, and also with the thickness of hide retained in the final leather (split thickness as compared to original hide thickness). As a result of these variations, the true factor for any given leather may be expected to vary from 5.44 to 5.80 or about 63 %.

The determination of "hide substance" from nitrogen content is based on the fact that, according to Schroder and Passler, the grease- and ash-free dry substance of the pelts of various animals has a somewhat different, but practically constant, nitrogen content for certain types of animal.

Other nitrogenous substances (e.g. certain fixatives, synthetic tannins, cationic fats and dyestuffs) falsify the value for the "hide substance". If these materials are present, it is not possible to obtain an accurate result for the "hide substance".

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Chemical tests – instrumental analysis

ISO 17226:2008 Leather - Chemical determination of formaldehyde content – Part 1: Method using high performance liquid chromatography

Scope

This part of ISO 17226 specifies a method for the determination of free and released formaldehyde in leathers. This method is based on high performance liquid chromatography (HPLC). It is selective and not sensitive to colored extracts.
The formaldehyde content is taken to be the quantity of free-formaldehyde and formaldehyde extracted through hydrolysis contained in a water extract from the leather under standard conditions.

Principle

The process is selective. Formaldehyde is separated and quantified as a derivative from other aldehydes and ketones by liquid chromatography. Detected is the free-formaldehyde and formaldehyde which is hydrolyzed during extraction to yield free-formaldehyde.
The sample is eluted with detergent solution at 40 °C. The eluate is mixed with 2,4-dinitrophenylhydrazine, whereby aldehydes and ketones react to give the respective hydrazones. These are separated by means of a reversed-phase HPLC method, detected at 360 nm and quantified.

Apparatus

HPLC system with UV detection, 360 nm.

Calculation of the formaldehyde content in leather samples

$$w_F = \frac{\rho_S \times F}{m}$$

where

***w*F** is the concentration of formaldehyde in the sample in milligrams per kilogram (mg/kg)

S is the concentration of formaldehyde obtained from the calibration graph in micrograms per 10 ml (µg/10 ml);

F is the dilution factor in milliliters (ml);

m is the mass of leather weighed in grams (g).

Expression of results

Express the formaldehyde concentration to the nearest 0,1 mg/kg based on the mass of the leather sample tested.
Result based on dry matter:

$$w_{Cr(VI)} - dry = w_{Cr(VI)} \times D$$

where

D is the factor for conversion to dry matter:

$$D = \frac{100}{100 - w}$$

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w is the mass fraction of the volatile matter determined using ISO 4684, expressed as a percentage.

ISO 17226-2:2008 Leather - Chemical determination of formaldehyde content – Part 2: Method using colorimetric analysis

Scope

This part of ISO 17226 specifies a method for the determination of free and released formaldehyde in leathers. This method is based on colorimetric analysis.
The formaldehyde content is taken to be the quantity of free-formaldehyde and formaldehyde extracted through hydrolysis contained in a water extract from the leather under standard conditions.
This process is not absolutely selective for formaldehyde. Other compounds such as extracted dyes could interfere at 412 nm.

Principle

The leather sample is eluted with detergent solution at 40 °C. The elute is treated with acetyl acetone, whereby formaldehyde reacts to give a yellow compound (3,5-diacetyl-1,4-dihydrolutidine). The absorbance of this compound is measured at 412 nm. The amount of formaldehyde corresponding to the absorbance value for the test specimen is obtained from a calibration curve prepared under identical conditions.

Apparatus

Spectrophotometer, with suitable cells capable of measuring absorbance at 412 nm.

Calculation of the content of formaldehyde of the leather sample

$$w_p = \frac{(E_p + E_e) \times V_o \times V_f}{F \times m \times V_a}$$

where

w_p is the concentration of formaldehyde in the sample in milligrams per kilogram (mg/kg), rounded off to 0,1 mg/kg;

E_p is the absorbance of the filtrate after reaction with acetyl acetone;

E_e is the absorbance of the filtrate (initial color);

V_o is the volume of elution in milliliters (ml) (*standard conditions: 50 ml*);

V_a is the aliquot taken from the filtrate in milliliters (ml) (*standard conditions: 5 ml*);

V_f is the volume of solution obtained in 7.2.3 after reaction, in milliliters (ml) (*standard conditions:10 ml*);

F is the gradient of calibration curve (y/x), in milliliters per microgram (ml/µg);

m is the mass of leather, in grams (g).

Expression of results

Express the formaldehyde concentration to the nearest 0,1 mg/kg based on the mass of the leather sample tested.
Result based on dry matter:

$$w_{Cr(VI)} - dry = w_{Cr(VI)} \times D$$

where

D is the factor for conversion to dry matter:

$$D = \frac{100}{100 - w}$$

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w is the mass fraction of the volatile matter determined using ISO 4684, expressed as a percentage.

ISO 17075-1:2017 Leather — Chemical determination of chromium (VI) content in leather — Part 1: Colorimetric method

Scope

This International Standard specifies a method for determining chromium (VI) in solutions leached from leather under defined conditions. The method described is suitable to quantify the chromium (VI) content in leathers down to 3 mg/kg.
The results obtained from this method are strictly dependent on the extraction conditions. Results obtained by using other extraction procedures (extraction solution, pH, extraction time, etc.) are not comparable with the results produced by the procedure described in this standard.

Principle

Soluble chromium (VI) is leached from the sample in phosphate buffer at pH 7,0 to 8,0 and co-extracted coloured substances which influence the detection are removed by solid phase extraction if necessary. The chromium (VI) in solution oxidizes 1,5-diphenylcarbazide to 1,5-diphenylcarbazone to give a red/violet complex with chromium which can be quantified photometrically at 540 nm

Apparatus and materials

Spectrophotometer or **filter photometer**, wavelength 540 nm.

Calculation of chromium (VI) content

$$W_{Cr(VI)} = \frac{(A_1 - A_2) x\ V_0\ x\ V_1\ x\ V_2}{V_1\ x\ V_3\ x\ m\ x\ F}$$

where

$w_{Cr(VI)}$ is the mass fraction, expressed in milligrams per kilogram (mg/kg), of soluble Cr(VI) in leather;
A_1 is the absorbance of sample solution with DPC;
A_2 is the absorbance of sample solution without DPC;
F is the gradient of calibration curve (y/x), expressed in milliliters per microgram (ml/µg);
m is the mass of the leather sample taken, expressed in grams (g);
V_0 is the extract volume of the initial sample, expressed in milliliters (ml);
V_1 is the aliquot taken from the extract volume of the initial sample, expressed in milliliters (ml);
V_2 is the total elute ($S1$) volume, after passage through the SPE column, to which the aliquot V_1 was made up, expressed in milliliters (ml);
V_3 is the aliquot taken from solution $S1$, expressed in milliliters (ml);
V_4 is the final make-up volume of the aliquot from $S1$, expressed in milliliters (ml).

Result based on dry matter:

$$w_{Cr(VI)} - dry = w_{Cr(VI)} \times D$$

where

D is the factor for conversion to dry matter:

$$D = \frac{100}{100 - w}$$

w is the mass fraction of the volatile matter determined using ISO 4684, expressed as a percentage.

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ISO 17075-2:2017 Leather — Chemical determination of chromium (VI) content in leather — Part 2: Chromatographic method

Scope

This International Standard specifies a method for determining Chromium (VI) in solutions leached from leather under defined conditions. The method described is suitable to quantify the chromium VI content in leathers down to 3 mg/kg.
This document is applicable to all leather types. The results obtained from this method are strictly dependent on the extraction conditions. Results obtained by using other extraction procedures (extraction solution, pH, extraction time, etc.) are not comparable with the results produced by the procedure described in this standard.

Principle

Soluble chromium (VI) is leached from the sample in phosphate buffer at pH 7,0 to 8,0. An aliquot of the filtered extract is analyzed for Cr (VI) using ion-exchange chromatography with UV-VIS detection.

Apparatus and materials

Ion-exchange chromatograph with UV detector or high performance liquid chromatograph (HPLC) with anion-exchange column and UV detector. It is recommended a photo Diode Array Detector (DAD).

Calculation of chromium (VI) content

$$W_{Cr(VI)} = \frac{(A-b)x\ V_0\ x\ V_c}{V_M\ x\ m\ x\ F}$$

where

$w_{Cr(VI)}$ is the mass fraction, expressed in milligrams per kilogram (mg/kg), of soluble Cr(VI) in leather;
A is the area of the peak of chromate in the chromatogram of the extract of the sample;
F is the gradient of calibration curve (y/x), expressed in milliliters per microgram (ml/µg);
b is the intercept of calibration curve (y/x);
m is the mass of the leather sample taken, expressed in grams (g);
V_0 is the extract volume of the sample, expressed in milliliters (ml);
V_C is the injection volume in the calibration, expressed in microliters (µl);
V_M is the injection volume in the sample analysis, expressed in microliters (µl);

Result based on dry matter:

$$w_{Cr(VI)} - dry = w_{Cr(VI)} \times D$$

where

D is the factor for conversion to dry matter:

$$D = \frac{100}{100 - w}$$

w is the mass fraction of the volatile matter determined using ISO 4684, expressed as a percentage.

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ISO 17234-1:2015 Leather — Chemical tests for the determination of certain azo colorants in dyed leathers — Part 1: Determination of certain aromatic amines derived from azo colorants

Scope

This International Standard specifies a method for determining the use of certain azo colorants which may release certain aromatic amines.
According to the current state of scientific knowledge, the use of banned azo colorants in the manufacture or treatment of leathers is considered as proved, if the coloured leather yields upon cleavage under the conditions of this procedure (9.2) one or more of the above indicated amines and the determined amount of any of these amines exceeds 30 mg/kg – table 1.

Table 2.3. Aromatic amines listed in Appendix 8 of EU regulation 1907/2006 and/or GB 20400

No.	Substances	CAS number
1	biphenyl-4-ylamine	92-67-1
2	benzidine	92-87-5
3	4-chloro-o-toluidine	95-69-2
4	2-naphthylamine	91-59-8
5[a]	o-aminoazotoluene	97-56-3
6[a]	5-nitro-o-toluidine	99-55-8
7	4-chloroaniline	106-47-8
8	4-methoxy-m-phenylenediamine	615-05-4
9	4,4'-methylenedianiline 4,4'-diaminodiphenylmethane	101-77-9
10	3,3'-dichlorobenzidine	91-94-1
11	3,3'-dimethoxybenzidine o-dianisidine	119-90-4
12	3,3'-dimethylbenzidine 4,4'-bi-o-toluidine	119-93-7
13	4,4'-methylenedi-o-toluidine	838-88-0
14	6-methoxy-m-toluidine p-cresidine	120-71-8
15	4,4'-methylene-bis-(2-chloro-aniline)	101-14-4
16	4,4'-oxydianiline	101-80-4
17	4,4'-thiodianiline	139-65-1
18	o-toluidine 2-aminotoluene	95-53-4
19	4-methyl-m-phenylenediamine	95-80-7
20	2,4,5-trimethylaniline	137-17-7
21	o-anisidine 2-methoxyaniline	90-04-0
22[b]	4-aminoazobenzene	60-09-3
23[c]	2,4-xylidine 2,4-dimethylbenzene-1-amine	95-68-1
24[c]	2,6-xylidine 2,6-dimethylbenzene-1-amine	87-62-7

[a]The CAS-numbers 97-56-3 (No. 5) and 99-55-8 (No. 6) are further reduced to CAS-numbers 95-53-4 (No. 18) and 95-80-7 (No. 19).
[b]Azo colorants that are able to form 4-aminoazobenzene, generate under the condition of this method aniline and/or 1,4-phenylenediamine. The presence of these colorants shall be tested using

Principle

After degreasing, the leather sample is treated with sodium dithionite in an aqueous buffer solution (pH 6) at 70 °C in a closed vessel. The amines released in the process of reductive cleavage are transferred to a t-butyl methyl ether phase by means of liquid-liquid extraction using Kieselgur columns. The t-butyl methyl ether

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extract is then concentrated under mild conditions in a rotary vacuum evaporator and the residue is dissolved in a suitable solvent, depending on the method used to determine the amines.

Determination of the amines is performed by means of high pressure liquid chromatography using a diode array detector (HPLC/DAD), thin layer chromatography (TLC, HPTLC) and densitometric quantification, capillary gas chromatography with a flame ionization detector and/or a mass specific detector (GC/FID and/or MSD), or by capillary electrophoresis with a diode array detector (CE/DAD).

The amines shall be identified by means of at least two different chromatographic separation methods in order to avoid any possible misinterpretations caused by interfering substances (such as position isomers of the amines to be identified) and hence any incorrect statements. Amine quantification shall be performed by HPLC/DAD.

Apparatus

HPLC with gradient controller, preferably with DAD, or HPLC-MS.

Calculation of azo colorants content

Calculate the amine concentration based on the peak areas of the individual amine components with reference to the 30 µg/ml calibration group of amines. Calculate the content of the amine as mass portions, *w*, in milligrams of the individual component per kilogram of leather material according to the following equation:

$$w = \frac{A_P \; x \; \rho_K x \; V}{A_K \; x \; m}$$

where

A_p is the peak area of the amine, in area units, in the sample;
A_K is the peak area of the amine, in area units, in the calibration solution;
ρ_K is the concentration of the amine in the calibration solution, in micrograms per millilitre;
V is the volume to which the sample is made up in 9.3 (final sample volume), in milliliters;
m is the mass portion accounted for by the leather sample in the final sample volume, in grams.

Result based on dry matter:

$$w_{Cr(VI)} - dry = w_{Cr(VI)} \times D$$

where

D is the factor for conversion to dry matter:

$$D = \frac{100}{100 - w}$$

w is the mass fraction of the volatile matter determined using ISO 4684, expressed as a percentage.

ISO 17070:2015 Leather — Chemical tests — Determination of tetrachlorophenol-, trichlorophenol-, dichlorophenol-, monochlorophenol-isomers and pentachlorophenol content

Scope

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This International Standard specifies a method for determining the content of tetrachlorophenol- (TeCP), trichlorophenol- (TrCP), dichlorophenol- (DiCP), monochlorophenol- (MoCP) isomeres and pentachlorophenol (PCP), its salts and esters in leather.

Principle

First of all, the leather sample is submitted to steam-distillation.

After extraction into *n*-hexane the chlorinated phenols (CP) are acetylated by acetic anhydride and the chlorinated acetates are analysed by gas-chromatography with an electron capture detector (ECD) or mass selective detector (MSD). Quantification is performed by an external standard and correction made with an internal standard.

Apparatus

- Gas chromatography with ECD or MSD

Reagents

Unless otherwise specified, analytical grade chemicals should be used

Table 2.4. Chlorinated phenol mix, which contains the following isomers, 100 µg/ml in acetone

No.	Substances	CAS-Number
1	2-Chlorophenol	95-57-8
2	3-Chlorophenol	108-43-0
3	4-Chlorophenol	106-48-9
4	2,3-Dichlorophenol	576-24-9
5	2,4-Dichlorophenol	120-83-2
6	2,5-Dichlorophenol	583-78-8
7	2,6-Dichlorophenol	87-65-0
8	3,4-Dichlorophenol	95-77-2
9	3,5-Dichlorophenol	591-35-5
10	2,3,4-Trichlorophenol	15950-66-0
11	2,3,5-Trichlorophenol	933-78-8
12	2,3,6-Trichlorophenol	933-75-5
13	2,4,5-Trichlorophenol	95-95-4
14	2,4,6-Trichlorophenol	88-06-2
15	3,4,5-Trichlorophenol	609-19-8
16	2,3,4,5-Tetrachlorophenol	4901-51-3
17	2,3,4,6-Tetrachlorophenol	58-90-2
18	2,3,5,6-Tetrachlorophenol	935-95-5
19	Pentachlorophenol	87-86-5

Calculation of chlorinated phenol mix content

Compare the areas of the single peaks with the areas of the standard which are analysed simultaneously and calculated.

Calculate the CP concentration as a mass fraction, w_{CP} , in milligrams per kilogram (mg/kg) leather sample, according to the following equation:

$$w_{CP} = \frac{A_{CP-S}\ x\ c_{CP-St}\ x\ A_{TCG-St}\ x\ V\ x\ \beta}{A_{CP-St}\ x\ c_{TCG-S}\ x\ m}$$

where

A is the peak area;

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c is the concentration of the chlorinated Phenol in the calibration standard in micrograms per millitre, µg/ml

m is the mass of the sample in grams, g;

V is the final sample volume in millilitres, ml;

β is the dilution factor;

Indices:

CP-St is the concentration of the standard

TCG-S is the Area of the internal standard in the sample

TCG-St is the Area of the internal standard in the standard

Result based on dry matter:

$$w_{Cr(VI)} - dry = w_{Cr(VI)} \times D$$

where

D is the factor for conversion to dry matter:

$$D = \frac{100}{100 - w}$$

w is the mass fraction of the volatile matter determined using ISO 4684, expressed as a percentage.

The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time).

2.3.3. Chromatography

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase,* which carries it through a structure holding another material called the *stationary phase.* The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation – figure 2.7.

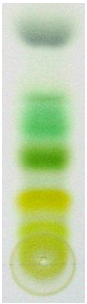

Figure 2.7. Thin layer chromatography is used toseparate components of a plant extract, illustrating the experiment with plant pigments that gave chromatography its name

Chromatography, pronounced/ˌkroʊməˈtɒgrəfi, is derived from Greek χρῶμα *chroma*, which means "color", and γράφειν *graphein*, which means "to write".[2]

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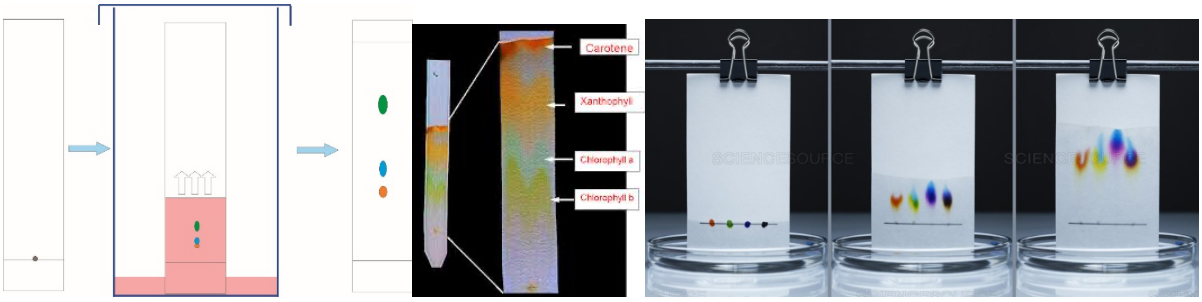
Chromatography technique developed substantially as a result of the work of Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s, for which they won the 1952 Nobel Prize in Chemistry.[3] They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of several chromatographic methods: paper chromatography, gas chromatography, and what would become known as high-performance liquid chromatography.

Paper chromatography

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of *chromatography paper*. The paper is placed in a container with a shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture, which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.



a – principle b - exemples

Figure 2.8. Paper chromatography

Gas chromatography

Gas chromatography (GC) is a separation technique in which the mobile phase is a gas. Gas chromatographic separation is always carried out in a column, which is typically "packed" or "capillary". Capillary columns generally give far superior resolution and although more expensive are becoming widely used, especially for complex mixtures. Both types of column are made from non-adsorbent and chemically inert materials. Stainless steel and glass are the usual materials for packed columns and quartz or fused silica for capillary columns.

a **b**

Figure 2.9. Packed columns (a), quartz or fused silica (b)

Liquid chromatography

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. It can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as high-performance liquid chromatography (HPLC).

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In HPLC the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane.

Figure 2.10. HPLC columns

Gas chromatography–mass spectrometry (GC-MS)

Gas chromatography–mass spectrometry (**GC-MS**) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample.
The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer.

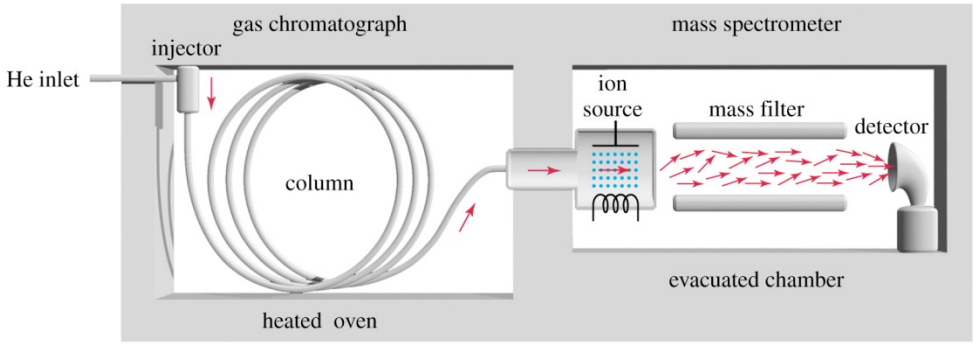

Figure 2.11. GC-MS schematic display

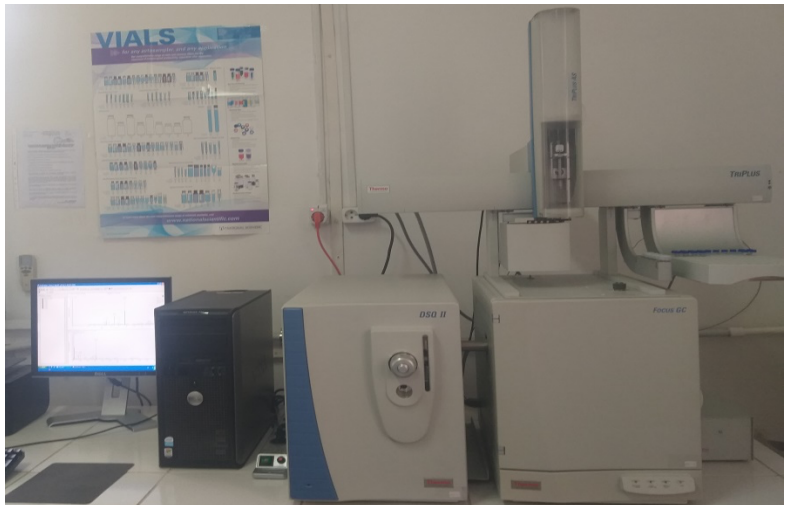

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Figure 2.12. GC-MS device

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone.

The gas chromatograph utilizes a capillary column which depends on the column's dimensions as well as the phase properties. The mass spectrometer breaks each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer.

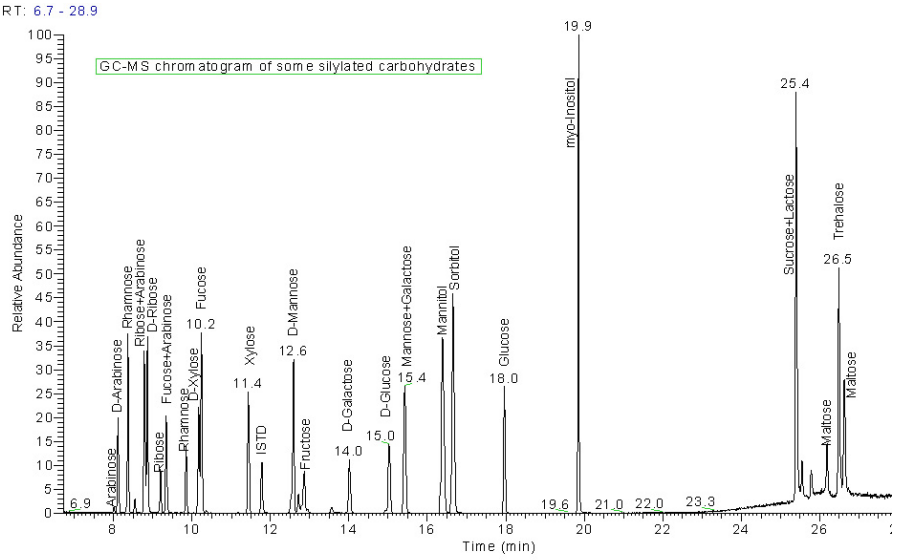

Figure 2.13. GC-MS chromatogram

Types of Mass Spectrometer Detectors

The most common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupole mass spectrometer, trade name "Mass Selective Detector" (MSD). Another relatively common detector is the ion trap mass spectrometer. Additionally, one may find a magnetic sector mass spectrometer, however these particular instruments are expensive and bulky and not typically found in high-throughput service laboratories. Other detectors may be encountered such as time of flight (TOF), tandem quadrupoles (MS-MS).

Ionization

After the molecules travel the length of the column, pass through the transfer line and enter into the mass spectrometer they are ionized by various methods with typically only one method being used at any given

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time. Once the sample is fragmented it will then be detected, usually by an electron multiplier, which essentially turns the ionized mass fragment into an electrical signal that is then detected.

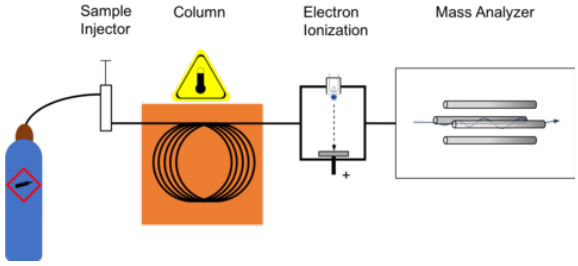

Figure 2.14. Block diagram for gas chromatography using electron ionization for collecting mass spectrum

Electron ionization

Standard form of ionization is electron ionization (EI). The molecules enter into the MS where they are bombarded with free electrons emitted from a filament. The electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way. This "hard ionization" technique results in the creation of more fragments of low mass-to-charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit. The molecular fragmentation pattern is dependent upon the electron energy applied to the system, typically 70 eV (electron Volts). The use of 70 eV facilitates comparison of generated spectra with library spectra using manufacturer-supplied software or software developed by the National Institute of Standards (NIST-USA). Spectral library searches employ matching algorithms such as Probability Based Matching[4] and dot-product[5] matching that are used with methods of analysis written by many method standardization agencies. Sources of libraries include NIST,[6] Wiley,[7] the AAFS,[8] and instrument manufacturers.

Cold electron ionization

The "hard ionization" process of electron ionization can be softened by the cooling of the molecules before their ionization, resulting in mass spectra that are richer in information.[9][10] In this method named cold electron ionization (cold-EI) the molecules exit the GC column, mixed with added helium make up gas and expand into vacuum through a specially designed supersonic nozzle, forming a supersonic molecular beam (SMB). Collisions with the make up gas at the expanding supersonic jet reduce the internal vibrational (and rotational) energy of the analyte molecules, hence reducing the degree of fragmentation caused by the electrons during the ionization process.[9][10] Cold-EI mass spectra are characterized by an abundant molecular ion while the usual fragmentation pattern is retained, thus making cold-EI mass spectra compatible with library search identification techniques. The enhanced molecular ions increase the identification probabilities of both known and unknown compounds, amplify isomer mass spectral effects and enable the use of isotope abundance analysis for the elucidation of elemental formulae.[11]

Chemical ionization

In chemical ionization a reagent is a gas, typically methane or ammonia is introduced into the mass spectrometer. Depending on the technique (positive CI or negative CI) chosen, this reagent gas will interact with the electrons and analyte and cause a 'soft' ionization of the molecule of interest. A softer ionization fragments the molecule to a lower degree than the hard ionization of EI. One of the main benefits of using

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chemical ionization is that a mass fragment closely corresponding to the molecular weight of the analyte of interest is produced.
In positive chemical ionization (PCI) the reagent gas interacts with the target molecule, most often with a proton exchange. This produces the species in relatively high amounts.
In negative chemical ionization (NCI) the reagent gas decreases the impact of the free electrons on the target analyte.

Analysis

A mass spectrometer is typically utilized in one of two ways: full scan or selective ion monitoring (SIM). The typical GC-MS instrument is capable of performing both functions either individually or concomitantly, depending on the setup of the particular instrument.
The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original.
When collecting data in the full scan mode, a target range of mass fragments is determined and put into the instrument's method. An example of a typical broad range of mass fragments to monitor would be *m/z* 50 to *m/z* 400. The determination of what range to use is largely dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences. A MS should not be set to look for mass fragments too low or else one may detect air (found as *m/z* 28 due to nitrogen), carbon dioxide (*m/z* 44) or other possible interference.
A “full spectrum” analysis considers all the “peaks” within a spectrum. Conversely, selective ion monitoring (SIM) only monitors selected ions associated with a specific substance.

Full scan is useful in determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method.

Applications

Environmental monitoring and cleanup
GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies.

Criminal forensics
GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing and Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate.

Law enforcement
GC-MS is increasingly used for detection of illegal narcotics, and may eventually supplant drug-sniffing dogs. A simple and selective GC-MS method for detecting marijuana usage was recently developed by the Robert Koch-Institute in Germany. It involves identifying an acid metabolite of tetrahyhydrocannabinol (THC), the

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active ingredient in marijuana, in urine samples by employing derivatization in the sample preparation.[12] GC-MS is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased. In drug screening, GC-MS methods frequently utilize liquid-liquid extraction as a part of sample preparation, in which target compounds are extracted from blood plasma.[13]

Sports anti-doping analysis

GC-MS is the main tool used in sports anti-doping laboratories to test athletes' urine samples for prohibited performance-enhancing drugs, for example anabolic steroids.[14]

Security

A post–September 11 development, explosive detection systems have become a part of all US airports. These systems run on a host of technologies, many of them based on GC-MS. There are only three manufacturers certified by the FAA to provide these systems one of which is Thermo Detection (formerly Thermedics), which produces the EGIS, a GC-MS-based line of explosives detectors. The other two manufacturers are Barringer Technologies, now owned by Smith's Detection Systems, and Ion Track Instruments, part of General Electric Infrastructure Security Systems.

Chemical warfare agent detection

As part of the post-September 11 drive towards increased capability in homeland security and public health preparedness, traditional GC-MS units with transmission quadrupole mass spectrometers, as well as those with cylindrical ion trap (CIT-MS) and toroidal ion trap (T-ITMS) mass spectrometers have been modified for field portability and near real-time detection of chemical warfare agents (CWA) such as sarin, soman, and VX.[15] These complex and large GC-MS systems have been modified and configured with resistively heated low thermal mass (LTM) gas chromatographs that reduce analysis time to less than ten percent of the time required in traditional laboratory systems.[16] Additionally, the systems are smaller, and more mobile, including units that are mounted in mobile analytical laboratories (MAL), such as those used by the United States Marine Corps Chemical and Biological Incident Response Force MAL and other similar laboratories, and systems that are hand-carried by two-person teams or individuals, much ado to the smaller mass detectors.[17] Depending on the system, the analytes can be introduced via liquid injection, desorbed from sorbent tubes through a thermal desorption process, or with solid-phase micro extraction (SPME).

Chemical engineering

GC-MS is used for the analysis of unknown organic compound mixtures. One critical use of this technology is the use of GC-MS to determine the composition of bio-oils processed from raw biomass.[18]

Food, beverage and perfume analysis

Foods and beverages contain numerous aromatic compounds, some naturally present in the raw materials and some forming during processing. GC-MS is extensively used for the analysis of these compounds which include esters, fatty acids, alcohols, aldehydes, terpenes etc. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful and which are often controlled by governmental agencies, for example pesticides.

Astrochemistry

Several GC-MS have left earth. Two were brought to Mars by the Viking program.[19] Venera 11 and 12 and Pioneer Venus analysed the atmosphere of Venus with GC-MS.[20] The Huygens probe of the Cassini–Huygens

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mission landed one GC-MS on Saturn's largest moon, Titan.[28] The material in the comet 67P/Churyumov–Gerasimenko will be analysed by the Rosetta mission with a chiral GC-MS in 2014.[22]

Medicine

Dozens of congenital metabolic diseases also known as inborn errors of metabolism (IEM) are now detectable by newborn screening tests, especially the testing using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering with metabolic disorders. This is increasingly becoming a common way to diagnose IEM for earlier diagnosis and institution of treatment eventually leading to a better outcome. It is now possible to test a newborn for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS.

In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of ^{13}C as the labeling and the measurement of ^{13}C-^{12}C ratios with an isotope ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few select ions and return values as ratios.

2.3.4. High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (**HPLC)** is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. The schematic of a HPLC instrument typically includes a degasser, sampler, pumps, and a detector.

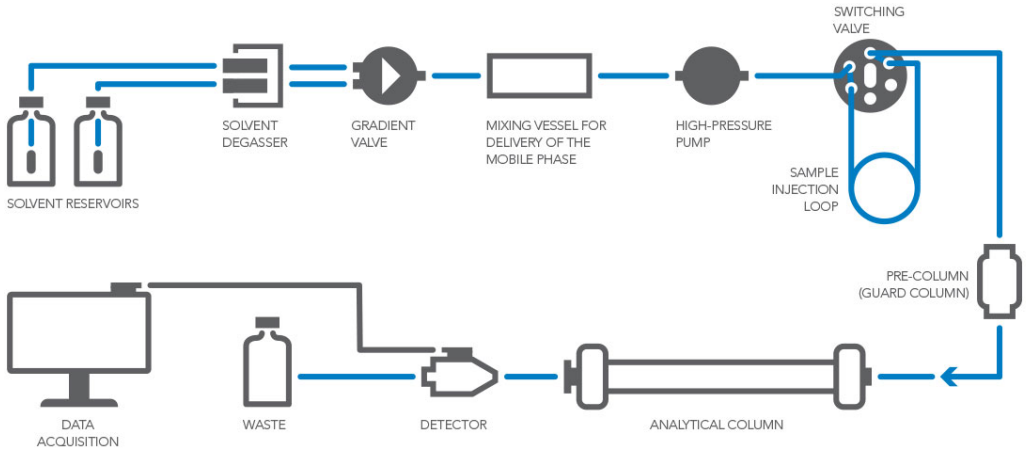

Figure 2.14. Schematic form of HPLC

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Figure 2.15. HPLC system

Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

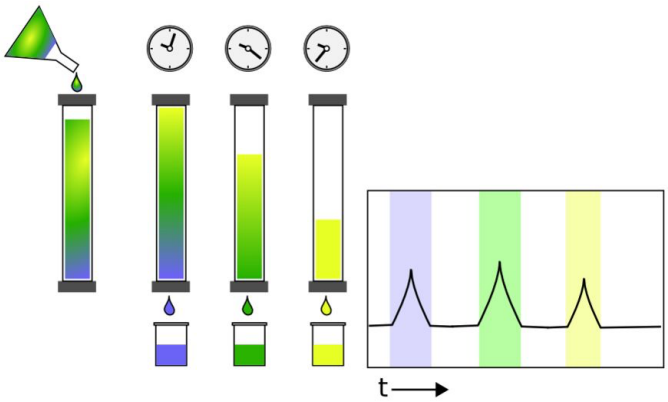

Figure 2.16. HPLC separation model

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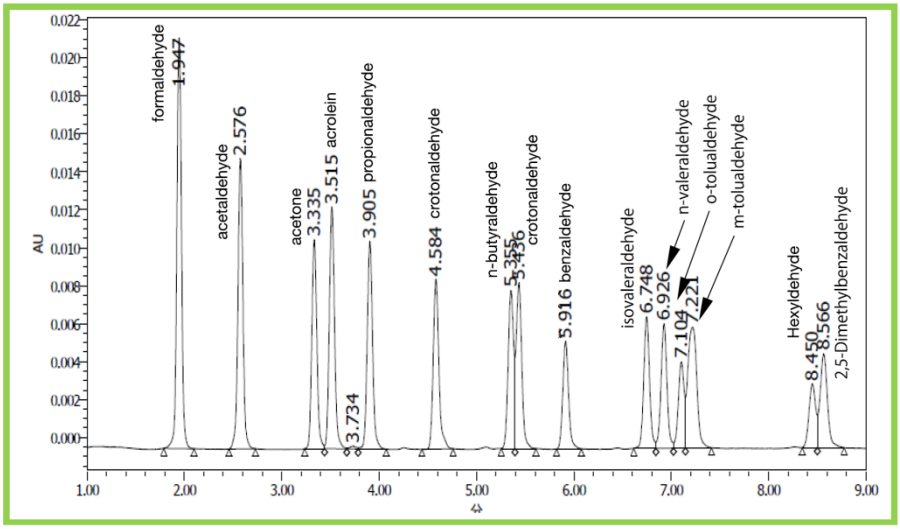

Figure 2.17. HPLC chromatogram

HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with adsorbent, leading to the separation of the sample components. The active component of the column, the adsorbent, is typically a granular material made of solid particles (silica, polymers, etc.), 2–50 µm in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the adsorbent particles. The pressurized liquid is typically a mixture of solvents (water, acetonitrile and/or methanol) and is referred to as a "mobile phase".

Pumps in a HPLC instrument can mix multiple solvents together in ratios changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/Vis, photodiode array (PDA) or based on mass spectrometry. Most HPLC instruments also have a column oven that allows for adjusting the temperature at which the separation is performed.

The composition of the mobile phase may be kept constant ("isocratic elution mode") or varied ("gradient elution mode") during the chromatographic analysis. Isocratic elution is typically effective in the separation of sample components that are very different in their affinity for the stationary phase. In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength. The eluting strength of the mobile phase is reflected by analyte retention times with high eluting strength producing fast elution (=short retention times).

Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and adsorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination.

HPLC is distinguished from traditional ("low pressure") liquid chromatography because operational pressures are significantly higher (50–350 bar), while ordinary liquid chromatography typically relies on the force of gravity to pass the mobile phase through the column. Due to the small sample amount separated in analytical HPLC, typical column dimensions are 2.1–4.6 mm diameter, and 30–250 mm length.

The internal diameter of an HPLC column is an important parameter that influences the detection sensitivity and separation selectivity in gradient elution. Larger columns are usually seen in industrial applications, such as the purification of a drug product for later use.

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Analytical scale columns (4.6 mm) have been the most common type of columns. They are used in traditional quantitative analysis of samples and often use a UV-Vis absorbance detector.

Narrow-bore columns (1–2 mm) are used for applications when more sensitivity is desired either with special UV-vis detectors, fluorescence detection or with other detection methods like liquid chromatography-mass spectrometry.

Capillary columns (under 0.3 mm) are used almost exclusively with alternative detection means such as mass spectrometry.

HPLC detectors use two main categories: universal or selective. Universal detectors typically measure a bulk property (refractive index) by measuring a difference of a physical property between the mobile phase and mobile phase with solute while selective detectors measure a solute property (UV-Vis absorbance) by simply responding to the physical or chemical property of the solute.[24] HPLC most commonly uses a UV-Vis absorbance detector, however, a wide range of other chromatography detectors can be used. A universal detector that complements UV-Vis absorbance detection is the Charged aerosol detector (CAD). A kind of commonly utilized detector includes refractive index detectors, which provide readings by measuring the changes in the refractive index of the effluent as it moves through the flow cell. In certain cases, it is possible to use multiple detectors, for example LC-MS normally combines UV-Vis with a mass spectrometer.

Applications

Manufacturing

HPLC has many applications in both laboratory and clinical science. It is a common technique used in pharmaceutical development, as it is a dependable way to obtain and ensure product purity.[18] While HPLC can produce extremely high quality (pure) products, it is not always the primary method used in the production of bulk drug materials.[19]

Legal

This technique is also used for detection of illicit drugs in urine. The most common method of drug detection is an immunoassay.[20] This method is much more convenient. As HPLC is a method of determining purity, using HPLC alone in evaluating concentrations of drugs is somewhat insufficient. Liquid chromatography in conjunction with MS is used to detect a variety of agents like doping agents, drug metabolites, glucuronide conjugates, amphetamines, opioids, cocaine, BZDs, ketamine, LSD, cannabis, and pesticides.[23][24]

Research

Similar assays can be performed for research purposes, detecting concentrations of potential clinical candidates like anti-fungal and asthma drugs.[25] This technique is obviously useful in observing multiple species in collected samples, as well, but requires the use of standard solutions when information about species identity is sought out. It is used as a method to confirm results of synthesis reactions, as purity is essential in this type of research.

Medical

Medical use of HPLC can include drug analysis, but falls more closely under the category of nutrient analysis. While urine is the most common medium for analyzing drug concentrations, blood serum is the sample collected for most medical analyses with HPLC.[26] Other methods of detection of molecules that are useful for clinical studies have been tested against HPLC, namely immunoassays. In one example of this, competitive protein binding assays (CPBA) and HPLC were compared for sensitivity in detection of vitamin D. Useful for diagnosing vitamin D deficiencies in children, it was found that sensitivity and specificity of this CPBA reached

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only 40% and 60%, respectively, of the capacity of HPLC.[27] While an expensive tool, the accuracy of HPLC is nearly unparalleled.

2.3.5. Spectroscopy

Spectroscopy and spectrography are terms used to refer to the measurement of radiation intensity as a function of wavelength and are often used to describe experimental spectroscopic methods. Spectral measurement devices are referred to as spectrometers, spectrophotometers, spectrographs or spectral analyzers.
One of the central concepts in spectroscopy is a resonance and its corresponding resonant frequency. Spectra of atoms and molecules often consist of a series of spectral lines, each one representing a resonance between two different quantum states.

Ultraviolet–visible spectroscopy

The instrument used in ultraviolet–visible spectroscopy is called a UV/Vis **spectrophotometer**. It measures the intensity of light passing through a sample (***I***), and compares it to the intensity of light before it passes through the sample (I_0). The ratio is called the *transmittance*, and is usually expressed as a percentage (%T).

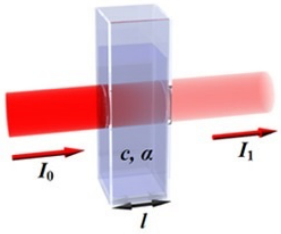

Figure 2.18. Light before and after passes through the sample

The Beer–Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length.[28] It is necessary to know how quickly the absorbance changes with concentration.
The method is most often used in a quantitative way to determine concentrations of an absorbing species in solution, using the Beer–Lambert law:

$$A = log_{10}\left(\frac{I_0}{I}\right) = \epsilon cL$$

where

A is the measured absorbance (in Absorbance Units (AU)),
I_0 is the intensity of the incident light at a given wavelength
I is the transmitted intensity,
L the path length through the sample
c the concentration of the absorbing species.

A spectrophotometer can be either *single beam* or *double beam*. In a single beam instrument all of the light passes through the sample cell – figure 2.19.

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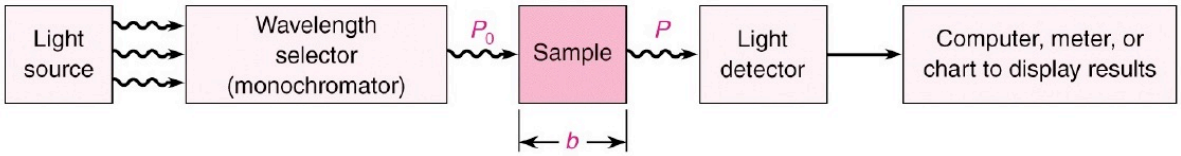

Figure 2.19. Simplified schematic of a mono beam UV–visible spectrophotometer

In a single-beam instrument, the cuvette containing only a solvent has to be measured first.

In a double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as the reference; the other beam passes through the sample. The reference beam intensity is taken as 100% Transmission (or 0 Absorbance), and the measurement displayed is the ratio of the two beam intensities. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beam are measured at the same time.

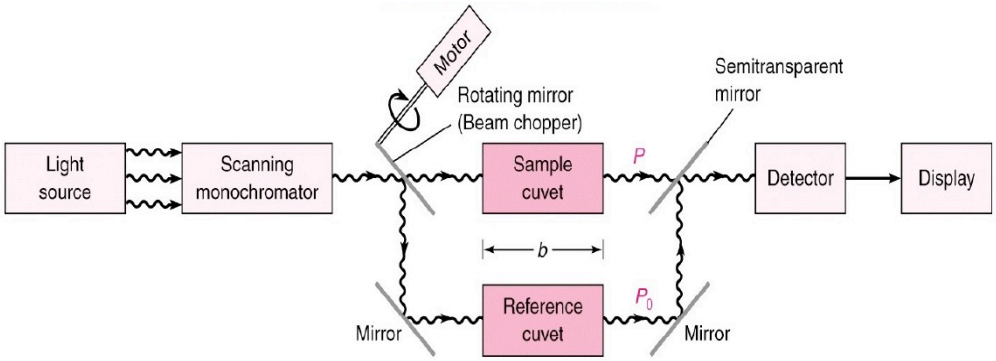

Figure 2.20. Simplified schematic of a double beam UV–visible spectrophotometer

The light source consists of a Xenon flash lamp for the ultraviolet (UV) as well as for the visible (VIS) and near-infrared wavelength regions covering a spectral range from 190 up to 1100 nm.

Samples for UV/Vis spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. The most widely applicable cuvettes are made of high quality fused silica or quartz glass because these are transparent throughout the UV, visible and near infrared regions. Glass and plastic cuvettes are also common, although glass and most plastics absorb in the UV, which limits their usefulness to visible wavelengths.[29]

Ultraviolet–visible spectroscopy or ultraviolet–visible spectrophotometry (UV–Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

Principle of Ultraviolet–Visible Absorption

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals.[30] The more easily excited the electrons, the longer the wavelength of light it can absorb. There are four possible types of transitions (π–π*, n–π*, σ–σ*, and n–σ*), and they can be ordered as follows: σ–σ* > n–σ* > π–π* > n–π*.

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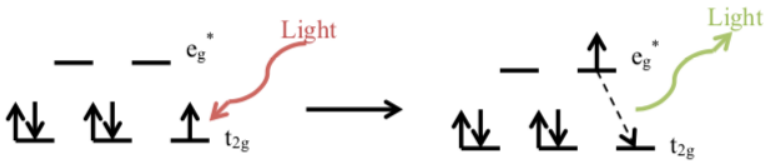


Figure 2.21. Schematic display of UV-Vis light emision

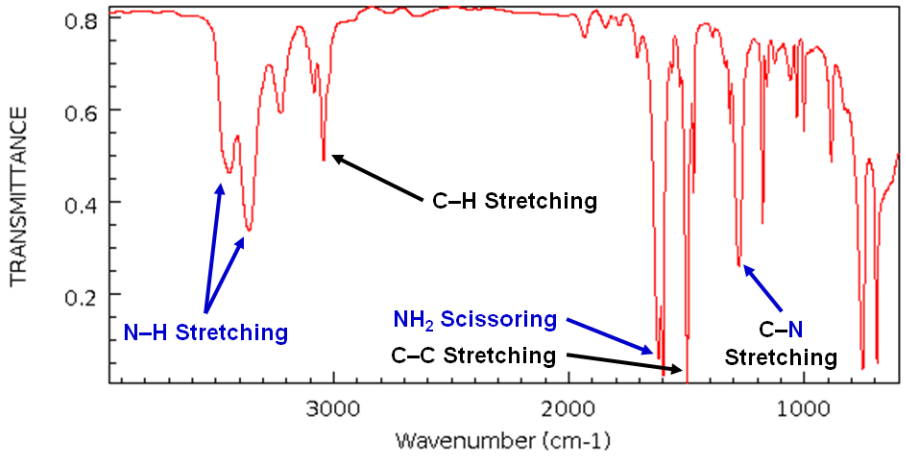
Applications

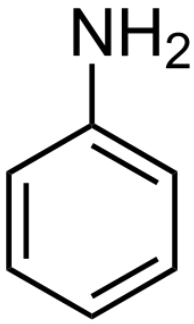
UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

Organic compounds absorb light in the UV or visible regions of the electromagnetic spectrum. Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths. Solvent polarity and pH can affect the absorption spectrum of an organic compound. A UV/Vis spectrophotometer may be used as a detector for **HPLC**. The presence of an analyte gives a response assumed to be proportional to the concentration. The wavelengths of absorption peaks can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups within a molecule. The spectrum alone is not, however, a specific test for any given sample. The nature of the solvent, the pH of the solution, temperature, high electrolyte concentrations, and the presence of interfering substances can influence the absorption spectrum.

Infrared spectroscopy

The method or technique of infrared spectroscopy is conducted with an instrument called an **infrared spectrometer** (or spectrophotometer) to produce an **infrared spectrum**.





a) Infrared spectrum **b) Chemical formula**

Figure 2.22. Aniline

The infrared spectrum of a sample is recorded by passing a beam of infrared light through the sample. When the frequency of the IR is the same as the vibrational frequency of a bond or collection of bonds, absorption occurs. Examination of the transmitted light reveals how much energy was absorbed at each frequency (or wavelength).

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This technique is commonly used for analyzing samples with covalent bonds. Simple spectra are obtained from samples with few IR active bonds and high levels of purity. More complex molecular structures lead to more absorption bands and more complex spectra.

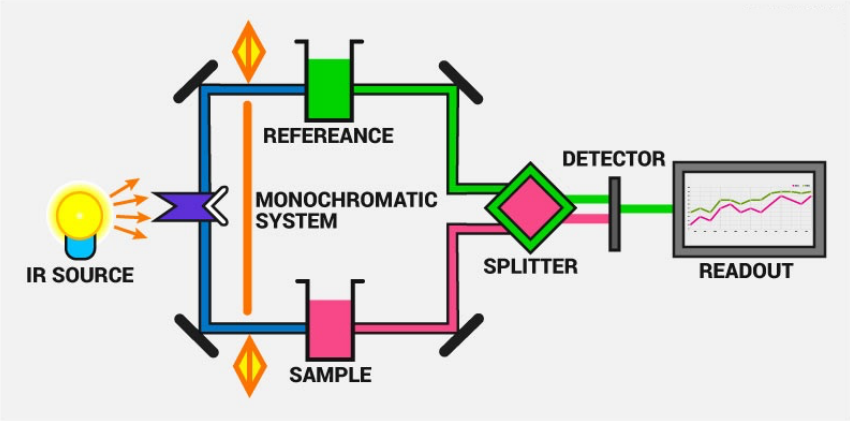

Figure 2.23. Infrared device schematic display

The resonant frequencies are also related to the strength of the bond and the mass of the atoms at either end of it. Thus, the frequency of the vibrations is associated with a particular normal mode of motion and a particular bond type.

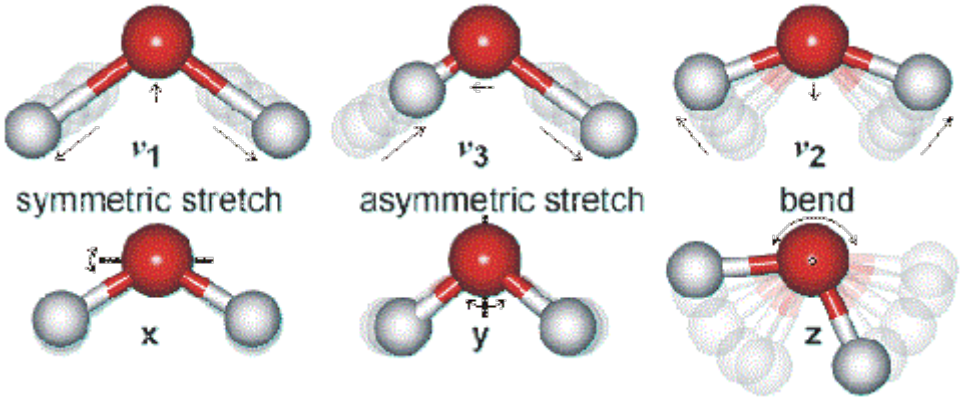

Figure 2.24. Molecule vibrations types

Infrared spectroscopy (IR spectroscopy or vibrational spectroscopy) involves the interaction of infrared radiation with matter. As with all spectroscopic techniques, it can be used to identify and study chemicals. Samples may be solid, liquid, or gas.

An IR spectrum can be visualized in a graph of infrared light absorbance (or transmittance) on the vertical axis vs. frequency or wavelength on the horizontal axis. Typical units of frequency used in IR spectra are reciprocal centimeters (sometimes called wave numbers), with the symbol cm^{-1}. Units of IR wavelength are commonly

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given in micrometers (formerly called "microns"), symbol µm, which are related to wave numbers in a reciprocal way.

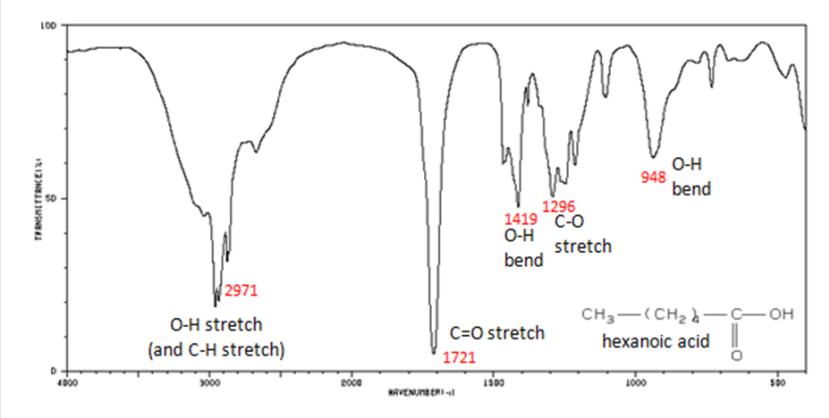

Figure 2.25. Infrared spectra

The infrared portion of the electromagnetic spectrum is usually divided into three regions:

- the near-infrared - 14000–4000 cm^{-1} can excite overtone or harmonic vibrations.
- mid-infrared - 4000–400 cm^{-1} may be used to study the fundamental vibrations and associated rotational-vibrational structure
- far- infrared - approximately 400–10 cm^{-1}.

Absorption Bands

IR spectroscopy is often used to identify structures because functional groups give rise to characteristic bands both in terms of intensity and position (frequency).

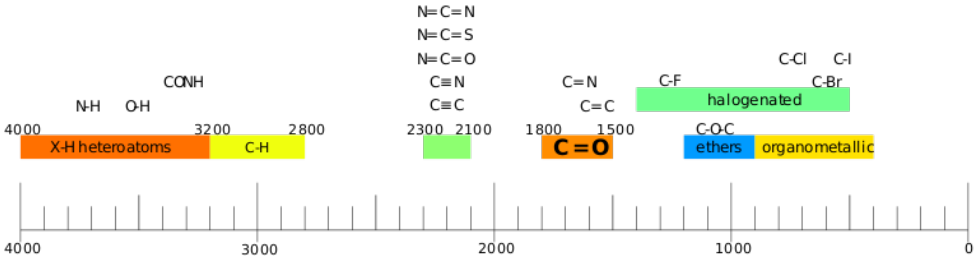

Figure 2.25. List of main IR spectroscopy bands. For example, the carboxyl group will contain a C = O band at 1700 cm^{-1} and an OH band at 3500 cm^{-1}(total group -COOH)

Regions

A spectrograph is often interpreted as having two regions.[31]

- **functional group region**
  - In the functional region there are one to a few troughs per functional group.[31]
- **fingerprint region**
  - In the fingerprint region there are many troughs which form an intricate pattern which can be used like a fingerprint to determine the compound.[31]

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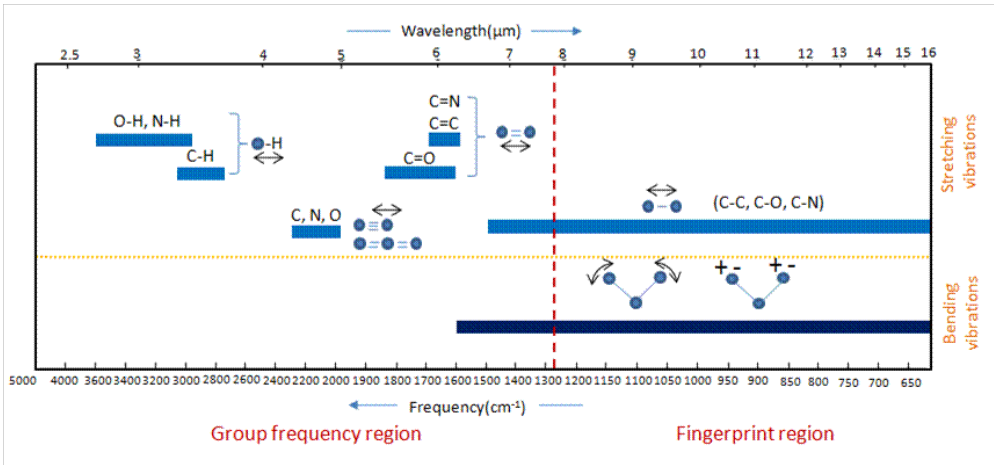

Figure 2.26. Infrared regions separation

Applications

Infrared spectroscopy is a simple and reliable technique widely used in both organic and inorganic chemistry, in research and industry. It is used in quality control, dynamic measurement, and monitoring applications such as the long-term unattended measurement of CO_2 concentrations in greenhouses and growth chambers by infrared gas analyzers.
It is also used in forensic analysis in both criminal and civil cases, for example in identifying polymer degradation. It can be used in determining the blood alcohol content of a suspected drunk driver.

IR-spectroscopy has been successfully used in analysis and identification of pigments in paintings[32] and other art objects such as illuminated manuscripts.[34]

Some instruments also automatically identify the substance being measured from a store of thousands of reference spectra held in storage. Infrared spectroscopy is also useful in measuring the degree of polymerization in polymer manufacture. Changes in the character or quantity of a particular bond are assessed by measuring at a specific frequency over time. This can be done whilst simultaneous measurements are made using other techniques. This makes the observations of chemical reactions and processes quicker and more accurate.

Infrared spectroscopy has also been successfully utilized in the field of semiconductor microelectronics:[35] infrared spectroscopy can be applied to semiconductors like silicon, gallium arsenide, gallium nitride, zinc selenide, amorphous silicon, silicon nitride, etc.

Another important application of Infrared Spectroscopy is in the food industry to measure the concentration of various compounds in different food products[36][37]

In February 2014, NASA announced a greatly upgraded database, based on IR spectroscopy, for tracking polycyclic aromatic hydrocarbons (PAHs) in the Universe. According to scientists, more than 20% of the carbon in the Universe may be associated with PAHs, possible starting materials for the formation of life. PAHs seem to have been formed shortly after the Big Bang, are widespread throughout the universe, and are associated with new stars and exoplanets.[38]

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2.4. Lesson 3: Physical – mecanical tests for leather

Authors: Gabriela MACOVESCU and Ciprian CHELARU - INCDTP-ICPI

2.4.1. Climatic conditioning (IUP 3 / ISO 2419 Leather — Physical and mechanical tests — Sample preparation and conditioning)

To ascertain leather's over-all performance or to establish its suitability for particular end-uses, certain tests must be performed, which will differ according to the end-use. Not all tests need to be performed on all types of leather. It is necessary to determine which tests to use for which leathers.

A distinction has to be drawn between tests on the leather to determine, on one hand, the behavior of the leather's external appearance - all fastness tests like ageing, light, rub and other tests like flex resistance, finish adhesion - and, on the other hand, to determine the strength of the fibre structure (grain crack, tear, stitch tear, tensile). Tests will also be selected in relation with the techniques in the leather transforming manufactures, e.g. solvent resistance, water absorption, steam and heat resistance, migration, fat content, etc. The end-uses can determine the selection of specific tests like perspiration resistance for linings and some garments, flame resistance for industrial and furniture leathers, fogging for car upholstery leathers.

Many physical properties of leather depend on the water vapour present in the fibre structure. To obtain reproducible results in physical testing, the humidity content of the leather shall be maintained at constant level by storage in a conditioned atmosphere.

The climatic conditioning is specified in method IUP 3 (ISO 2419) and is for a temperature of 23 ± 2°C and a relative humidity of 50% ± 5%. Leather samples and test pieces shall be stored during at least 24 hours in the standard climatic conditions. Most of the tests, certainly those taking a long time, should be performed in the standard atmosphere. In some countries it is difficult to obtain the above conditions, alternative atmospheres are defined is 20 ± 2° C and 65 + 5% RH. However, it should be noted that the numerical values of test results will not necessarily be the same if atmospheres other than the standard reference atmosphere of 23°C and 50% are used. For conditioning of samples and test pieces, the required relative humidity (RH) can be maintained in a closed space (desiccator) either by use of certain salts in water (in which the solid phase is also present in excess) or by the use of a solution, of given concentration, of sulphuric acid in water. Any deviation from these conditions shall be mentioned in the test reports.

2.4.2. Measurement of thickness (IUP 4/ISO 2589:2016 - Leather -- Physical and mechanical tests - Determination of thickness)

The hide or the skin does not have the same thickness over all its cross-section. On heavy leathers thickness differences can reach 25% and on light leathers 20%. Thickness of leather can be modified by stretching or compressing, by splitting, shaving, buffing or skiving. As many properties will depend on the thickness, this will be measured in order to express the test results in relation to the thickness. Standard IUP 4/ISO 2589 gives the method of measurement of thickness and specifies the characteristics of the measuring gauge. The results of the thickness measurement are expressed in millimeters to the nearest 0.01 mm. The method is applicable to all types of leather of any tannage. The measurement is valid for both the whole leather and a test sample.

To evaluate the mechanical properties of leathers in relation to the end-uses, mainly footwear manufacture, the most important tests are the measurement of resistance of grain in the lastometer, of the tear resistance and the flexing endurance.

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2.4.3. Measurement of shrinkage temperature - IUP 16 / ISO 3380:2015 - Leather - Physical and mechanical tests - Determination of shrinkage temperature up to 100 °C

The process of tanning a hide or skin confers a number of important properties on the leather produced – the primary one being the resistance to decay. Another property is that the leather can be much more resistant to shrinkage when subjected to moist heat, compared to raw or untanned hide or skin. There are a variety of different processes which can be used to tan leather such as chrome tanning, vegetable tanning and aldehyde tanning. Different types of tanning (both primary tanning and re-tanning) produce different physical properties, including levels of resistance to moist heat in the resulting leather.

The way to determine that the tanning process has been carried correctly is to measure the 'hydrothermal stability' – its resistance to wet heat – more commonly referred to as the 'shrinkage temperature'. A characteristic of hides, skins and leathers is that if they are gradually heated in water, they reach a temperature at which they are subject to sudden, irreversible shrinkage. Raw hides or skins shrink very easily at temperatures of about 65°C, whereas chrome tanning, for example, increases the point at which shrinkage occurs to temperatures up to a maximum of around 120ºC. As the shrinkage temperature of tanned leathers (for instance, chrome-tanned leathers) will often be above 100ºC, it is necessary to conduct the test under pressure. This raises the temperature at which the water will boil, hence allowing tests to be conducted above 100°C.This increased resistance to wet heat is an important requirement for leather when making a wide range of types of footwear in which the leather is subjected to moisture and high temperatures as part of the manufacturing process.

Table 2.5. Shrinkage temperature for leather in different technological stages

Condition/tanning method	Shrinkage temperature
Rawhide	60°C - 65°C (140 - 149 °F)
After pickling before tanning	40°C - 60°C (104 - 140 °F)
Vegetable-tanned leather	70°C - 80°C (158 - 176 °F)
Chrome-tanned	85°C - 100°C (185 - 212 °F)
Synthetic tanned	75°C - 85°C (167 - 185 °F)
Chamois leather	60°C - 70°C (104 - 158 °F)

Figure 2.27. Apparatus for Measurement of Shrinkage Temperature

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The apparatus is filled with water and the wetted 'along' direction specimen is suspended in the sight chamber. The apparatus is closed, creating a pressure-tight system. After an initial dwell period, the water is heated (at approximately 4°C/min) by applying the external heat source to the boiler compartment. The apparatus includes a thermometer which allows the temperature of the water surrounding the test specimen to be measured. One end of the leather is suspending from a hook in the water within the sight chamber and a small weight is attached to the lower end. The position of the lower end is indicated by an adjustable marker outside the tube to help judge when shrinkage occurs. The test continues with the specimen under observation until the length of the test specimen rapidly decreases by at least several millimetres.

The temperature at which this occurs, to the nearest degree Celsius, is recorded as the 'shrinkage temperature' for the along direction. The water in the apparatus is allowed to cool down before opening and the specimen replaced with the across direction specimen. The test is repeated and the shrinkage temperature recorded for the across direction. The shrinkage temperature for the leather under assessment is the average of the two shrinkage temperatures obtained.

Thermal microscopy, Micro Hot Table method – MHT, (in house method) allows hydrothermal stability determination (the evaluation of the shrinkage activity) of the collagen-based material fibres. The MHT measurements were made by using equipment composed of a micro hot plate, equipped with an automatic system for temperature control, a digital microscope and a computer for programming and data acquisition. The sample fibres of parchment or leather (0,1 mg), taken from the corium (flesh side) of the material, were thoroughly wetted in demineralised water on a microscope slide and separated using a pair of fine needles. The sample was covered with a cover glass, placed on the micro hot plate, under the microscope and heated at 2 °C min-1, in the temperature range 25–100^{o}C.

Figure 2.28. Thermal microscopy, Micro Hot Table device

2.4.4. Resistance to tear

IUP 40-1/ ISO 3377-1:2011 - Leather — Physical and mechanical tests - Determination of tear load — Part 1: Single edge tear

The resistance to tear is very important for all kinds of upper leathers. The official method IUP 7/ISO 3377:2011 measures the tear load on a specimen in which a slot has been cut and which is slipped over the turned-up ends of a pair of holders attached to the jaws of a tensile strength machine. A rectangular test specimen

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partially slit from one short edge is pulled so that a tear is propagated from the end of the slit. The mean force exerted during separation of the test piece is recorded.
From the sample, cut 6 test pieces in accordance with ISO 2419, 3 test pieces with the longer sides parallel to the backbone and 3 test pieces with the longer sides perpendicular to the backbone. Condition the test pieces in accordance with ISO 2419.Measure the thickness of the test pieces in accordance with ISO 2589.

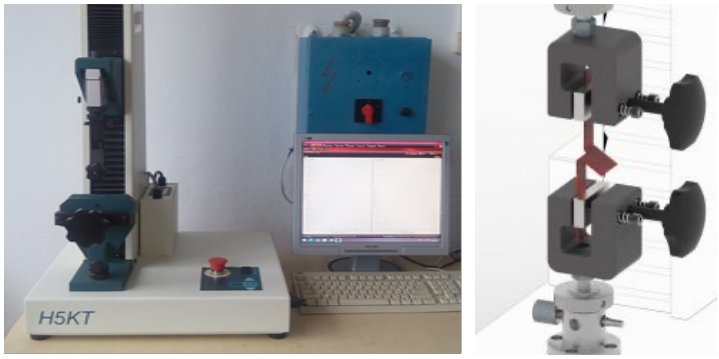

Figure 2.29. Echipment Tinus Olsen for Determination of tear load

The method is sometimes described as a trouser tear. It is applicable to all types of leather. The forces exerted during separation of the holders are recorded and the highest force is taken as the tearing load and expressed in Newtons. The initial load and the mean load can also be taken from the recorded diagram. Tearing load can be expressed as the quotient of the load by the thickness of the sample. Values obtained from the various tear resistance tests give reliable information to the leather products manufacturers on the need for reinforcing during manufacture of leather articles.

IUP 8/ISO 3377-2:2016 Leather - Physical and mechanical tests - Determination of tear load - Part 2: Double edge tear

This part of ISO 3377 specifies a method for determining the tear strength of leather using a double edged tear. The method is sometimes described as the Baumann tear. It is applicable to all types of leather. A rectangular test piece with a hole of specified shape is placed over the turned up ends of a pair of holders attached to the jaws of a tensile testing machine. The highest force exerted during tearing of the test piece is recorded.

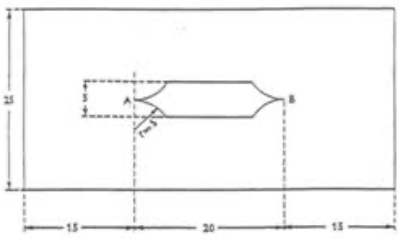

Figure 2.30. Shape of test piece for Double edge tear

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Figure 2.31. Determination of tear load, Double edge tear

Measurement of tearing load (split tear strength) conform IUC/8,
Split tear force in N = mean value of tearing load;
Tearing load in N/mm = force applied to tear specimen
Stitch tear strength = force in N

IUP 6/ ISO 3376:2011 - Leather — Physical and mechanical tests — Determination of tensile strength and percentage extension

ISO 3376:2011 specifies a method for determining the tensile strength, elongation at a specified load and elongation at break of leather. It is applicable to all types of leather. In the measurement of the tensile strength - IUP 6/ISO 3376 - the "medium" specimen is normally used for testing light leathers. To avoid that the specimen should slip out of the jaws during the test, it is useful to increase the area of the leather fixed within the jaws from 20 to 30 mm length and 20 to 25 mm width and to change the cutting knives accordingly. Constant clamp loads are generated automatically with pneumatic jaws. To avoid breaking near the jaws, a proposal was made to change the shape of the test piece and to give it curved sides.

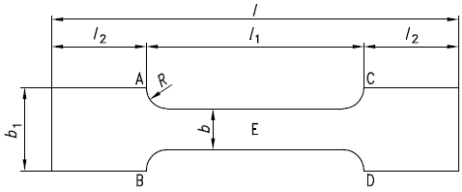

Figure 2.32. Shape of test piece

Designation	l	l_1	l_2	b	b_1	R
Standard	110	50	30	10	20	5
Large	190	100	45	20	40	10

Figure 2.33. Dimension of test pieces

From the sample, cut six test pieces in accordance with ISO 2419 by applying a press knife to the grain surface, three test pieces with the longer sides parallel to the backbone and three test pieces with the longer sides perpendicular to the backbone. Measure the thickness of each test piece in accordance with ISO 2589. If previous testing has shown that there is slippage of the test piece in the jaws, use the large press knife. If

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there is a requirement for more than two hides or skins to be tested in one batch, then only one test piece in each direction need be taken from each hide or skin, provided that the overall total is not less than three test pieces in each direction.

$$\text{Tensile strength in N/mm}^2 = \frac{\text{breaking load N}}{\text{thickness in mm x width in mm}}$$

Breaking load N = highest load reached at break

$$\text{Elongation at break in \%} = \frac{\text{mm length at break} - \text{mm initial length}}{\text{mm initial length}} \times 100$$

Figure 2.34. Determination of tensile strength of leather

2.4.5. Flexing endurance

Flexing endurance of light leathers and their surface finishes (IUP 20) is partly a physical test but as it is applied mainly for testing the finishes it is also a fastness test. The apparatus used for this test is known as Bally Flexometer. The test may be performed on conditioned leather samples or on wet samples, in standard climatic conditions or in a cold atmosphere. Some criticism rise recently because the test is not representative of how leather is flexed during wearing.

ISO 5402 consists of the following parts, under the general title Leather — Determination of flex resistance

- **— Part 1: Flexometer method**
- **— Part 2: Vamp flex method**

ISO/5402-1:2017/ IUP 20 Leather — Determination of flex resistance — Part 1: Flexometer method

This part of ISO 5402 specifies a method for determining the wet or dry flex resistance of leather and finishes applied to leather. It is applicable to all types of flexible leather below 3,0 mm in thickness. A test piece is folded with the surface to be tested inwards and clamped in an upper moveable clamp and with the surface to be tested outwards in a lower fixed clamp. Movement of the upper clamp causes a fold in the test piece to run along it. The test piece is examined periodically for damage.

From the sample, in accordance with ISO 2418, cut a minimum of four pieces for dry test and/or four pieces for wet test by applying the press knife to the surface to be tested. Cut a minimum of two test pieces with the long edge parallel to the backbone and two with the long edge perpendicular to the backbone. If there is a

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requirement for more than two hides or skins to be tested in one batch, then only one test piece in each direction need be taken from each hide or skin, provided that the overall total is not less than three pieces in each direction.

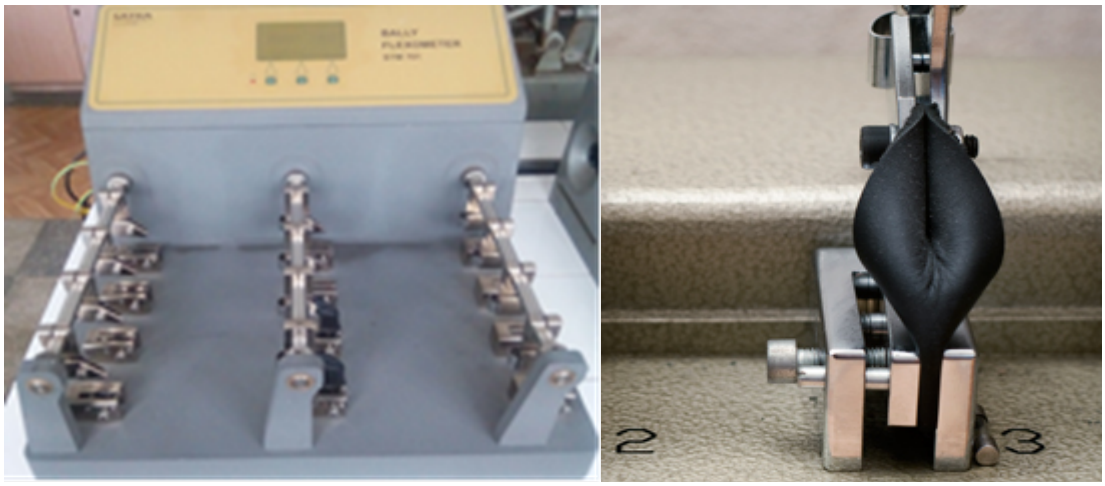

Figure 2.35. Bally Leathers Flexing Tester

Run the machine for the required number of flex cycles selected from the following list:

Dry flex: 500; 1 000; 5 000; 10 000; 20 000; 25 000; 50 000; 100 000; 150 000; 200 000; 250 000 cycles;

Wet flex: 500; 1 000; 2 500; 10 000; 20 000; 25 000; 50 000 cycles.

In addition to the above inspection points remove the wet test pieces from the machine every 25 000 cycles and examine them for spue before re-wetting and replacing in the test machine. The test pieces should flex without excessive bulging at the sides. If they do not flex in this mode include this in the test report. Stop the test machine and remove the test piece. Fold it along the longitudinal axis and examine visually in good light using the naked eye and with the magnifier. Record any damage in the flexed area, ignoring damage in the clamped area. Use the following wording to describe the cracks:

- ✓ cracks: visible with naked eye
- ✓ fine cracks: visible with magnifier
- ✓ micro cracks: visible with microscope 25 times

IUP 39 / ISO 5402 – 2:2015 - Leather — Determination of flex resistance — Part 2: Vamp flex method

This International Standard specifies a method for determining the wet or dry flex resistance of leather and finishes applied to leather. It is applicable to all types of leather below 3,0 mm in thickness. A test piece is folded with the surface to be tested outwards over two inverted “V” shaped clamps. Relative movement of the clamps flexes the sample producing one downward crease surrounded by four upward creases. The test piece is examined periodically for damage.

ISO 5403 consists of the following parts, under the general title - Leather — Determination of water resistance of flexible leather:

- ✓ *Part 1: Repeated linear compression (penetrometer)*
- ✓ *Part 2: Repeated angular compression (Maeser)*

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IUP 10-1 / ISO 5403-1:2011 Leather - Determination of water resistance of flexible leather - Part 1: Repeated linear compression (penetrometer)

ISO 5403-1:2011 specifies a method for determining the dynamic water resistance of leather by means of repeated linear compression. It is applicable to all flexible leathers but is particularly suitable for leathers intended for footwear applications. This property is related to the penetration of water in footwear and the associated materials.

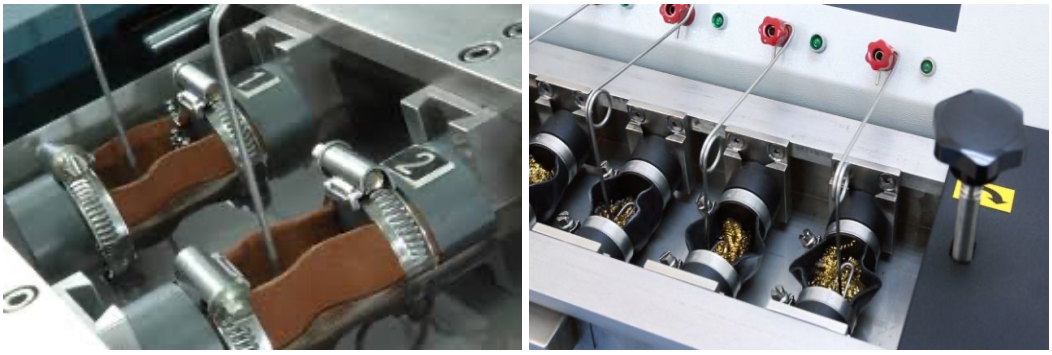

Figure 2.36. Bally penetrometer

The most suitable flexing amplitude depends on the thickness of the leather. Penetration time is recorded in minutes.

$$\text{Water absorption in \%} = \frac{\text{weight of sample after flexing} - \text{weight before flexing}}{\text{weight of sample before flexing}} \times 100$$

Water transmitted in g = gain in weight of absorbent cloth.

IUP 10-2 / ISO 5403-2:2011 Leather - Determination of water resistance of flexible leather - Part 2: Repeated angular compression (Maeser)

ISO 5403-2:2011 specifies a method for determining the dynamic water resistance of leather by means of repeated angular compression. The number of flexes endured by the leather at the point at which the water penetrates are counted. The dynamic water absorption is often tested after 15000 to 20000 flexes or at the point of penetration. It is applicable to all flexible leathers but is particularly suitable for leathers intended for footwear applications. It uses a Maeser-type machine and includes an option for electronic detection.

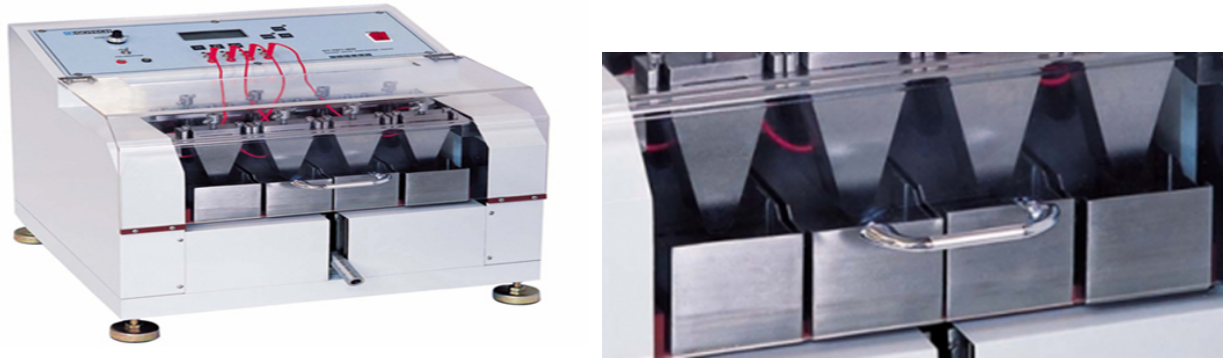

Figure 2.37. Maeser Water Penetration Tester

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2.4.6. Comfort and hygienic properties

For evaluation of **comfort and hygienic properties**, water vapour absorption WVA and water vapour permeability WVP are primordial. Water absorption and desorption is related with material capacity has to absorb and desorbs water. This property is important to remove humidity from foot skin surface and promote a higher comfort. Water vapor permeability is related with materials breathability, in other words, its capacity in the passage and absorption of water vapor. It's necessary to assure a good breathability of materials in footwear to promote the feet comfort.

IUP 42 / ISO 17229:2016 - Leather -- Physical and mechanical tests -- Determination of water vapour absorption

This standard specifies a method for determining the water vapour absorption of leather. The method is applicable for all leathers but is particularly relevant for leathers intended for footwear uppers and linings.
The test piece and an impermeable material are clamped over the opening of metal container containing 50 ml of water for a specified time. The water vapor absorption of the test piece is determined by increase in mass.

IUP 15 / ISO 14268:2012 (R 2018) Leather -- Physical and mechanical tests -- Determination of water vapour permeability

This standard describes a method for determining the water vapour permeability of leather and provides alternative methods of sample preparation. The test piece is clamped over the opening of container which contains a solid desiccant and is placed in a strong current of air in a standard atmosphere. The air inside the container is constantly agitated by the desiccant which is kept in motion by rotation of the container. The container is weighed at start and the end of the test and the mass of moisture which has been absorbed by the desiccant is determined from difference. The mass increase of the desiccant corresponds to the amount of moisture transmitted by the specimen and is thus used to calculate its water vapour permeability.

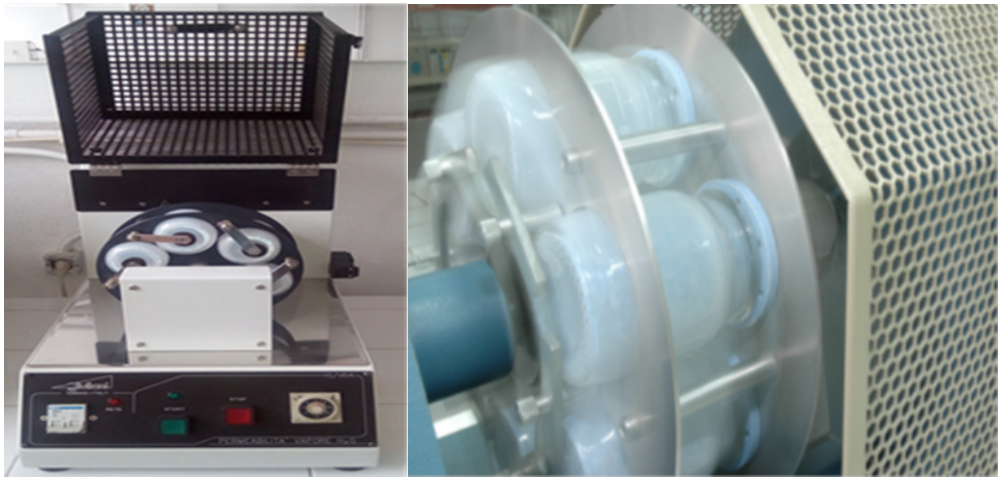

Figure 2.38. Apparatus for determining the water vapour permeability of leather

$$\text{Water vapour permeability in mg/cm}^2 \cdot \text{h} = \frac{7640 \text{ x m}}{d^2 \text{ x t}}$$

m = gain in weight in mg between two weighings
d = inner diameter in mm in bottle neck

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t = time in minutes between two weightings

IUP 7/ ISO 2417:2016 - Leather -- Physical and mechanical tests -- Determination of static absorption of water

ISO 2417:2016 specifies a method for determining the water absorption of leather, after Kubelka, under static conditions. The method is applicable to all leather, particularly heavy leather. A test piece of known mass or volume is immersed in water for a known period of time and the volume of water absorbed measured. The graduated scale shall be readable to 0,1 ml with an accuracy of ± 0,1 ml. The total volume of the bulb (A) and the graduated tube shall be 75 ml ± 2 ml. Rubber stopper (C), fitted with a glass rod or a nickel or stainless steel wire of diameter about 1 mm and of sufficient length to keep the test piece at the end of the cylinder (B) distant from the stopper (C).

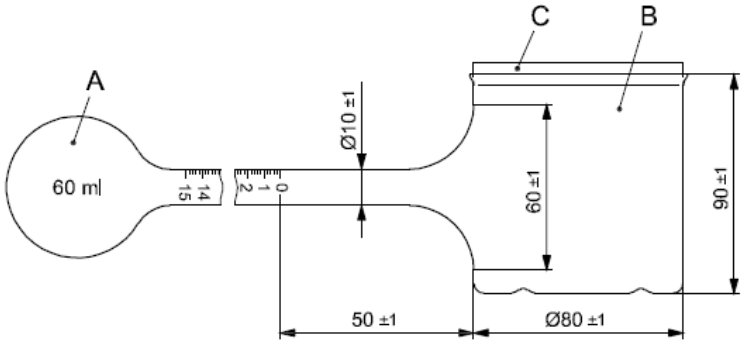

Figure 2.39. Kubelka apparatus and stopper

The bulb and tube unto the zero mark are then filed up with distilled water and the leather sample of known weight is put into the cup with flesh side up. If there are no projected two glass bars at the bottom surface of the cup, the leather surface is put on two glass rods, placed horizontally on the floor of the cup otherwise the bottom grain surface of the leather sample may not sufficiently come in contact with water. The grain side of the sample is kept downwards because in shoes soles are fitted to insoles in such a way that their grain sides touch the ground surface. The entire quantity of water is then transferred to the cup by tilting the apparatus. The leather sample should be immersed in water in the cup for a specified period of time with the open to surface of the cup covered with a glass plate (to avoid evaporation of water during the experiment).

After specified period of time the entire water is carefully transferred into the bulb by tilting the apparatus. The water level will go down in the tube from zero reading showing directly the volume of water absorbed by the leather sample. Water absorption readings are generally taken after ½ hour, 2 hour and 24 hours of soaking in the cup.

$$\text{Water absorption in \% by vol.} = \frac{\text{volume of water absorbed in ml}}{\text{volume of sample in cm}^3}$$

$$\text{Water absorption in \% by wt.} = \frac{\text{weight of water absorbed in g}}{\text{mass weight of sample in g}}$$

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2.4.7. Testing of leather dyeing

IUF 420 / ISO 15700:1998 Leather - Tests for colour fastness – Colour fastness to water spotting

This International Standard specifies a method for assessing the effect, on leather of all kinds, caused by spotting with water. The method is suitable for assessing the change in physical appearance and the colour change of the leather. Two drops of distilled water are placed at separate spots on the leather. After 30 min., any surplus water is removed with filter paper from one of the drops and any physical effects are observed. The other drop is allowed to evaporate overnight and the change in colour of the leather is assessed with the standard grey scale. Since the finish of a patent leather and other plastic coated leathers is impervious to water, an alternative procedure is required in which the water spots are placed on the inside surface of the leather.

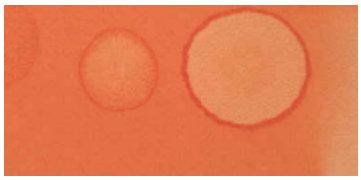

Figure 2.40. Water spotting leather test

IUF 442 / ISO 15701:2015 Leather - Tests for colour fastness – Colour fastness to migration into plasticized poly vinyl chloride (PVC)

This International Standard specifies a method for assessing the propensity of dyes and pigments to migrate from leather to a synthetic substrate by determining the transfer of colour from the leather to white plasticized poly vinyl chloride in contact with it.

This method is suitable for leather of all kinds at any stage of processing. The side under test of the leather specimen is placed on a white pigmented sheet of plasticized poly vinyl chloride and the resultant composite specimen is exposed to heat under pressure in a suitable apparatus. The transfer of colour from the leather to the white sheet is assessed with the standard grey scale for assessing staining and, if applicable, any change in hue of the staining is also assessed.

The use of standards sheets of plasticized poly vinyl chloride makes it possible to determine the tendency of colour to migrate from the leather to synthetic materials used in conjunction with the leather. If the leather has a finish, the test may be carried out with the finish intact or broken.

Figure 2.41. Apparatus for Colour fastness to migration into PVC

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IUF 450 / ISO 11640: 2018 - Leather - Tests for colour fastness - Colour fastness to cycles of to–and-fro rubbing

This International Standard specifies a method for determining the behaviour of the surface of leather on rubbing with wool felt. It is applicable to leathers of all kinds. One side of the leather to be tested is rubbed with pieces of reference wool felt under a given pressure for a given number of forward and backward motions.

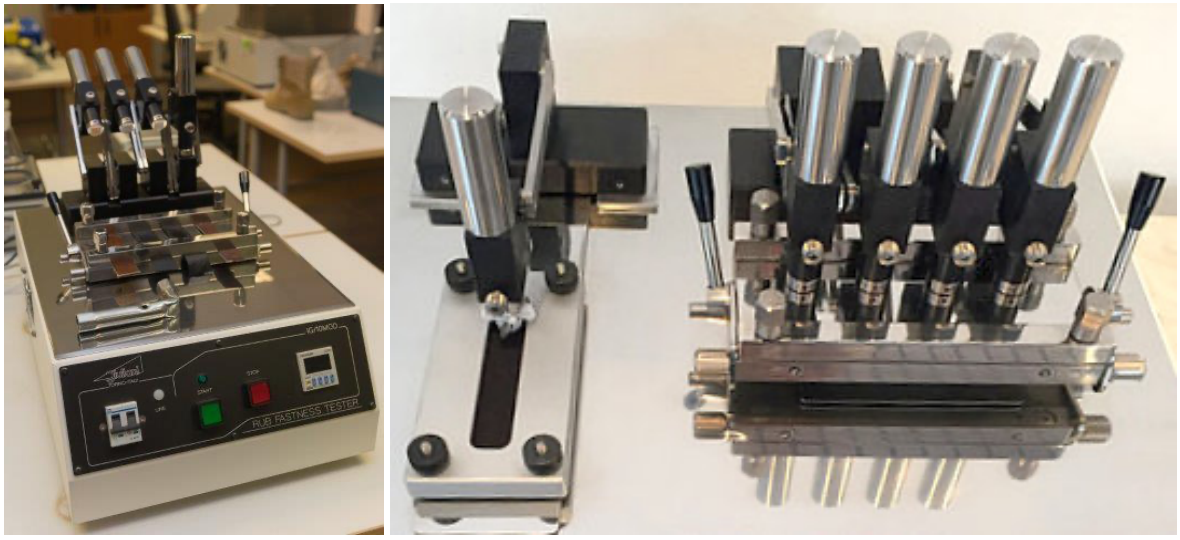

Figure 2.42. Apparatus for Colour fastness to cycles of to–and-fro rubbing

The change in colour of the pieces of felt and of the leather is assessed with the grey scales. Any other visible change in or damage to the surface of the leather is also reported. The general colour fastness testing principles shall be in accordance with those described in **ISO 105-A01** *Textiles — Tests for colour fastness — Part A01: General principles of testing*, taking into account that the substrate is leather.

The test can be performed on dry leather with dry felt, on dry leather with wet felt and on wet leather with dry felt. Leather and felt may be wetted with demineralized water or with an artificial perspiration solution.

IUF 426 / ISO 11641:2012 (R 2018) Leather - Tests for colour fastness - Colour fastness to perspiration

In many leather articles, the leather comes in direct contact with the human skin. Human perspiration can also migrate through garment and be absorbed by the leather, causing staining or changes of the leather appearance.

This International Standard specifies a method for determining the colour fastness to perspiration of leather of all kinds at all stages of processing, but it applies particularly to gloving, clothing and lining leathers, various belt, strap or orthopaedic leathers, as well as leather for the uppers of unlined shoes. The method uses an artificial perspiration solution to simulate the action of human perspiration. Since perspiration varies widely from one individual to the next, it is not possible to design a method with universal validity, but the alkaline artificial perspiration solution specified below will give results corresponding to those with natural perspiration in most cases.

A leather specimen is soaked in artificial perspiration solution and a piece of adjacent fabric, also soaked in artificial perspiration solution, lay against each side to be tested. The composite test specimen is placed under a load of 4.5 kg (123 N/cm2 = 125 p/cm2) at 37 ± 2 °C for one hour in a suitable apparatus. The leather

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specimen and adjacent fabric are then dried, the change in colour of the specimen and the staining of the adjacent fabric assessed with the grey scales. Leathers with a finish may be tested intact or with the finish broken. The general colour fastness testing principles shall be in accordance with those described in **ISO 105-A01**, taking into account that the substrate is leather.

When the composite specimen is dry, using D65 illumination according to ISO 105-A01 illumination visually assess the staining of each kind of fibre in the adjacent fabric(s), using the appropriate grey scale in accordance with **ISO 105-A03** *Textiles — Tests for colour fastness — Part A03: Grey scale for assessing staining*. Also assess the change in colour of the leather specimen in accordance with **ISO 105-A02** *Textiles — Tests for colour fastness — Part A02: Grey scale for assessing change in colour*.

IUF 421/ISO 11642:2012 Leather — Tests for colour fastness — Colour fastness to water

This International Standard specifies a method for determining the colour fastness to water of leather of all kinds at all stages of processing. A leather specimen is soaked in demineralised water and a piece of adjacent fabric, also soaked in demineralised water, lay against each side to be tested. The composite specimen is left under pressure for a specified time in a suitable apparatus. The leather specimen and adjacent fabric are then dried, and the change in colour of the specimen and the staining of the adjacent fabric assessed with the grey scales. Leathers with a finish may be tested intact or with the finish broken.The general colour fastness testing principles shall be in accordance with those described in **ISO 105-A01**, taking into account that the substrate is leather.

IUF 434 / ISO 11643:2009 (R 2014) - Leather - Test for colour fastness - Colour fastness of small samples to dry–cleaning solutions

This International Standard specifies a method for determining the resistance to dry-cleaning solutions of the colour and the finish of unused, and not yet dry-cleaned, leather. It does not cover composite materials or complete leather garments. It is not intended to be used to give the dry-cleaner any guidance as to the process to be employed for cleaning.

During the test, the adjacent fabric used may become stained, the finish of the leather may be damaged and the colour of the leather may change. The presence of absorbed water in the leather, adjacent fabric or solvent has not been found to be a critical factor in assessing the colour fastness.

A composite specimen of the leather and an adjacent fabric is agitated, together with PTFE rods, in a solvent which may contain triolein (and possibly a detergent), then squeezed and dried at ambient temperature. The change in colour of the specimen and staining of the adjacent fabric are assessed with the grey scales and any changes in the finish are noted.

IUF 412 / ISO 17228:2015 - Leather — Tests for colour fastness — Change in colour with accelerated ageing

Over time, the surface colour of leather and the leather itself change due to ageing and to the action of the surroundings on the leather. The purpose of the various ageing procedures described in this International Standard is to obtain an indication of the changes that could occur when leather is exposed to a certain environment for a prolonged time. The test conditions to be used depend on the type of leather and its intended use. This procedure can also be used to age specimens for the test of dimensional change according to ISO/FDIS 17130. Leather – Physical and mechanical tests - Determination of dimensional change

Prior to cutting out specimens, condition the piece of leather in accordance with ISO 2419.

Cut out two representative test specimens measuring not less than 100 mm x 100 mm.

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If the piece of leather available for testing is a whole hide or skin, then the specimens shall be taken in accordance with standard procedures given in ISO 2418.

A test specimen of leather is exposed to at least one of the three following conditions:

- **Ageing by heat alone** (Clause 6); - The purpose of this procedure is to simulate prolonged ageing by the application of heat. Two different types of change can be involved:

  a) the change in colour of the substances in the leather;

  b) the evaporation of volatile substances or the migration of substances, leading to a change in the colour and/or other properties of the leather or finish.

  As both the exposure time and the temperature can be varied, this procedure can be used for a variety of purposes, including preparing specimens for other tests.

Table 2.6. Ageing by heat

Method	Conditions for ageing by heat	Recommended use
6A	24 h ± 1 h at 60 °C ± 2 °C	General-purpose ageing conditions
6B	24 h ± 1 h at 100 °C ± 2 °C	Specifically for yellowing of individual products in undyed leather
6C	72 h ± 2 h at 60 °C ± 2 °C	Extended general-purpose ageing
6D	72 h ± 2 h at 100 °C ± 2 °C	Ageing of automotive leather
6E	168 h ± 2 h at 90 °C ± 2 °C	Extended ageing of automotive leather
6F	168 h ± 2 h at 60 °C ± 2 °C	Extended ageing at moderate temperature
6G	168 h ± 2 h at 100 °C ± 2 °C	Extended ageing at elevated temperature
6H	4 h ± 0,2 h at 100 °C± 2 °C	Short ageing of automotive leather
6J	144 h ± 2 h at 100 °C± 2 °C	Ageing of automotive leather
6K	168 h ± 2 h at 120 °C± 2 °C	Extended ageing of automotive leather at high temperature

- **Ageing by heat and elevated humidity** (Clause 7); This procedure is similar to the application of heat, but the moisture present acts as a mild hydrolysing agent, thus simulating prolonged ageing at ambient conditions with some degree of humidity. At high humidities, some substances can migrate to the surface. As different levels of humidity can be used, and the time and temperature can be varied, this procedure can be used for a variety of purposes, including preparing specimens for other tests.

Table 2.7. Ageing by heat and elevated humidity

Method	Conditions for ageing by heat and elevated relative humidity (r.h.)	Recommended use
7A	24 h ± 1 h at 50 °C ± 2 °C and 90 % r.h. ± 5 % r.h.	General-purpose ageing conditions
7B	96 h ± 2 h at 50 °C ± 2 °C and 90 % r.h. ± 5 % r.h.	Extended general-purpose ageing
7C	12 h ± 1 h at 70 °C ± 2 °C and 90 % r.h. ± 5 % r.h.	Migration test for finished leather
7D	48 h ± 1 h at 55 °C ± 2 °C and 80 % r.h. ± 5 % r.h.	Climate test for automotive leather
7E	168 h ± 2 h at 40 °C ± 2 °C and 95 % r.h. ± 5 % r.h.	Extended climate test for automotive leather
7F	16 h ± 1 h at 40 °C ± 2 °C and 95 % r.h. ± 5 % r.h.	General-purpose ageing conditions
7G	72 h ± 1 h at 60 °C ± 2 °C and 85 % r.h. ± 5 % r.h.	Simulate transport in closed container
7H	168 h ± 2 h at 70 °C± 2 °C and 75 % r.h.± 5 % r.h.	Climate test for automotive leather
7J	168 h ± 2 h at 70 °C± 2°C and 95 % r.h.± 5 % r.h.	Climate test for automotive leather

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- **Cyclic variation in heat and elevated humidity** (Clause 8). This procedure is similar to those in Clauses 6 and 7, but the temperature and humidity are varied cyclically to simulate the changes which could be experienced during a typical day. This procedure is especially used for automotive leather. As different levels of humidity can be used, and the time and temperature can be varied, the procedure can be used for a variety of other purposes, including preparing specimens for other tests.

Table 2.8. **Ageing under cyclic temperature/humidity conditions**

Method	Conditions for cycling at various temperatures and relative humidities (r.h.)	Recommended use
8A	1 cycle consists of: 24 h ± 1 h at 38 °C ± 2 °C and 95 % r.h. ± 5 % r.h.; 24 h ± 1 h at 100 °C ± 2 °C. Run 3 cycles under these conditions.	General-purpose climate test for automotive leather
8B	1 cycle consists of: 4,0 h ± 0,2 h at 70 °C ± 2 °C and 20 % r.h. ± 5 % r.h.; 16 h ± 1 h at 38 °C ± 2 °C and 95 % r.h. ± 5 % r.h.; 4,0 h ± 0,2 h at –30 °C ± 2 °C. Run 10 cycles under these conditions.	Extended climate test for automotive leather (including temperatures below freezing)
8C	1 cycle consists of: 4,0 h ± 0,2 h at 40 °C ± 2 °C and 90 % r.h. ± 5 % r.h.; 2,0 h ± 0,2 h heating to 120 °C (above 90 °C, switch humidity control off); 4,0 h ± 0,2 h at 120 °C ± 2 °C; 2,0 h ± 0,2 h cooling to 40 °C ± 2 °C and 90 % r.h. ± 5 % r.h., (switch humidity control back on only after reaching 40 °C,). Run 20 cycles under these conditions.	Simulation of warm, humid climate for automotive leather
8D	1 cycle consists of: 4,0 h ± 0,2 h at 10°C ± 2 °C and 92 % r.h. ± 3 % r.h.; 2,0 h ± 0,2 h at 90°C ± 2 °C and max. 20 % rates of heating: approx. 1,5 °C/min (temperature should not exceed 50 °C at 40 % r.h. during heating and cooling)	Simulation of warm, humid climate for automotive leather
	Run 10 (or if required 30) cycles under these conditions.	
8E	Pre-conditioning: 900 min ± 10 min at 23 °C and 50 % r.h.; 1 cycle consists of: 29 min heating to 70 °C and 50 % r.h.; 31 min heating to 120 °C and < 10 % r.h. 120 min at 120 °C 20 min cooling to 70 °C and 50 % r.h.; 40 min cooling to 15 °C and 95 % r.h. 120 min at 15 °C and 95 % r.h. Run 9 cycles under these conditions. Post-run: 29 min heating to 70 °C and 50 % r.h.; 31 min heating to 120 °C and < 10 % r.h. 180 min at 120 °C Unless given, the maximum deviations are: for time: ± 2 min for temperature: ± 2 °C for relative humidity: ± 5 % r.h.	Simulation of warm, humid climate for automotive leather

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The sets of conditions given have been selected to provide a range of conditions for different applications. If other conditions are used, then they shall be clearly noted in the test report. After the period of time has elapsed, remove the test specimen from the climate chamber. Allow the specimen to cool. Then recondition both the test and the reference specimen under standard conditions according to ISO 2419 for 24 h. Either visually assess the colour difference between the aged specimen and the reference specimen using the appropriate grey scale in accordance with ISO 105-A02 (4.4) or ISO 105-A03 (4.5) or alternatively, assess the grey scale colour difference instrumentally in accordance with ISO 105-A05 or ISO 105-A04. Note any change in appearance, hue or flexibility and any shrinkage of the aged specimen in comparison with the reference specimen.

Grey Scale for Assessing Change in Colour ISO 105-A02

This Grey Scale is for assessing changes in colour of leather in colour fastness tests, tests, for example, wash fastness, perspiration fastness, etc. The scale consists of nine pairs of grey colour chips each representing a visual difference and contrast.
The fastness rating goes step-wise from:

Note 5 = no visual change (best rating)
To Note 1 = a large visual change (worst rating).
The grey scale has the 9 possible values: 5, 4-5, 4, 3-4, 3, 2-3, 2, 1-2, 1.

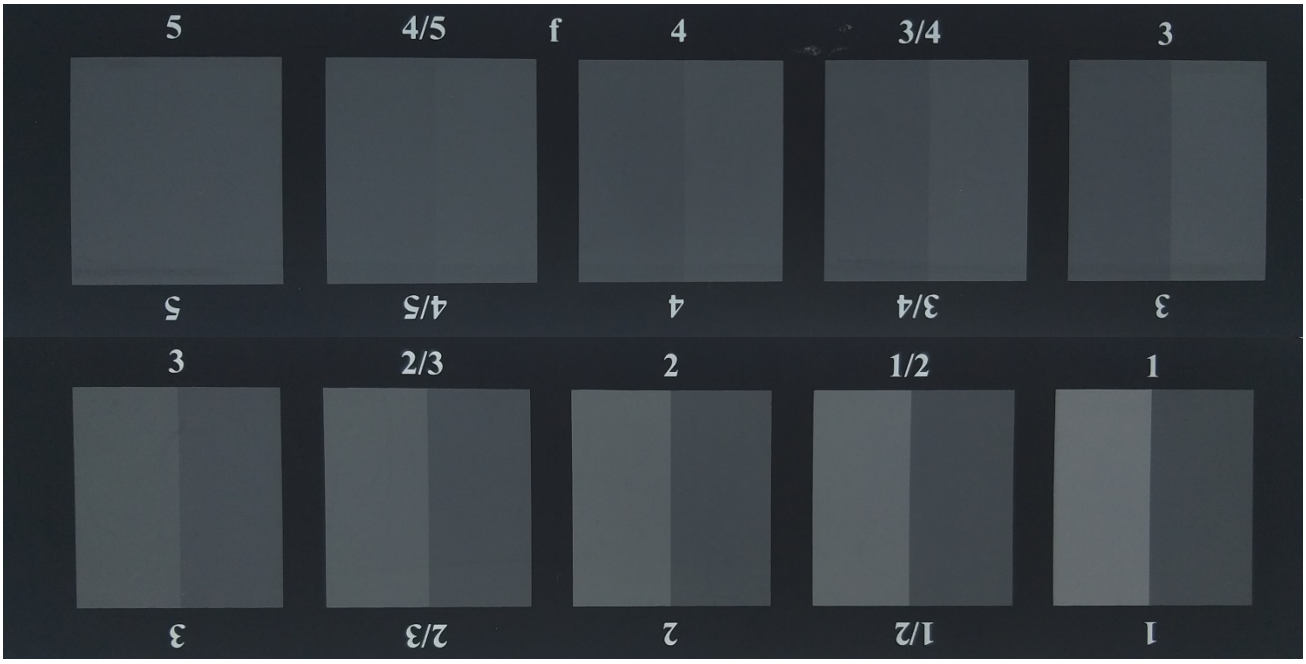

Figure 2.43. Grey Scale for Assessing Change in Colour

It is now quite common to measure the Grey Scale change in colour instrumentally. This is made using a suitable reflectance spectrophotometer according to the test method procedure, **ISO 105-A05.**

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Grey Scale for Assessing Staining ISO 105-A03

This Grey Scale is for assessing the degree of staining caused by a dyed leather in colour fastness tests. For example, the staining of wool and cotton fabrics in the wash fastness, perspiration fastness, etc. The scale consists of nine pairs of grey colour chips each representing a visual difference and contrast.

The fastness rating goes step-wise from:
Note 5 = no visual change (best rating)
To Note 1 = a large visual change (worst rating).
The grey scale has the 9 possible values: 5, 4-5, 4, 3-4, 3, 2-3, 2, 1-2, 1.

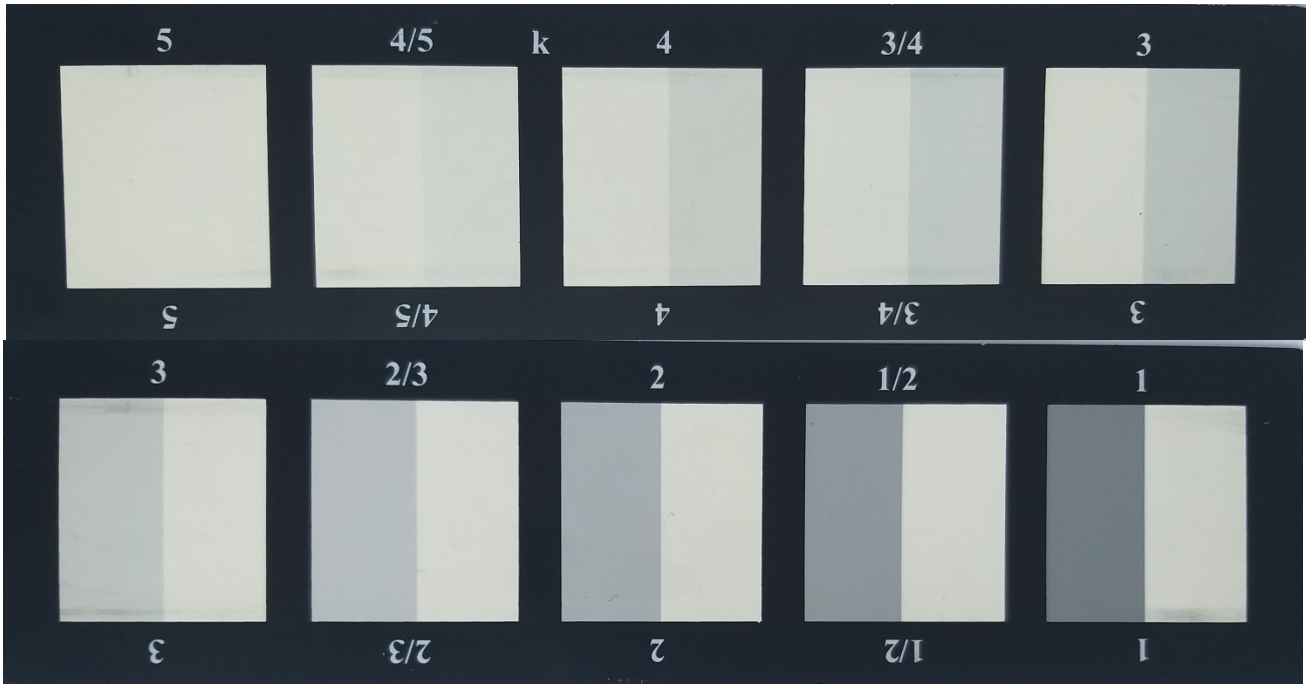

Figure 2.44. Grey Scale for Assessing Staining

It is now quite common to measure the Grey Scale for assessing the degree of staining instrumentially. This is made using a suitable reflectance spectrophotometer according to the test method procedure, **ISO 105-A04**.

Colour Fastness of Leather to Light: Xenon Lamp - ISO 105-B02:2014 Textiles - Tests for colour fastness part B02 Colour Fastness to artificial light: Xenon arc fading lamp test

This method is intended for determining the resistance of the colour of leather to the action of a standard artificial light source. The Xenon lamp has an emission wavelength profile close to daylight. In most (but not all) dye supplier's pattern cards, the values reported are for dyeing on full grain chrome leather. It should be clearly understood that the light fastness rating obtained for dyed leather will change significantly depending on the type of leather and amount of dye applied. The side to be tested of the leather sample is exposed to light from a Xenon Lamp, under controlled conditions, along with eight blue dyed wool standards (blue scale). The light fastness is assessed by comparing the fading of the leather with the fading of the blue standards. The fading is typically made in 2 exposure times to better assist the evaluation. The exposure time can be varied depending on the lamp intensity and the blue scale level required. To measure up to a maximum blue scale level 3 an exposure of 20h and 80h (as below) is often sufficient for a quick checking of the light fastness

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rating. Important: for measuring the light fastness in the blue scale 3 – blue scale 6 range, exposure times of 144h and 280h are necessary.

The Blue Scale is a card with 8 sticked blue scale gradations in *pure wool*, which allows to determine the *resistance of the color of fabrics or leathers* to the action of an **artificial light source** (Xenon lamp, Xenotest or Sunlight Test). The value of the light fastness depending on the type of fabric / leather and the quantity of the pigment used. To verify the light fastness needs to compare the fabric / leather with the tonality of the blue standards. The values present in the blue scale start from the lowest (1 = low solidity) to the highest (8 = maximum solidity).

Figure 2.45. Blue Scale for Assessing Staining

2.4.8. Testing of leather finishes

IUF 470 / ISO 11644: 2009 (R 2015) - Leather - Test for adhesion of finish

Depending on the way the leather has been finished, the adhesion of the finish to the leather can be so weak over the whole area, or part of it, that the finish separates from the leather during use. With finishes consisting of several layers, the separation may occur between the layers, for example between the pigmented top layer and the base coat. This International Standard specifies a method for measuring the adhesion of the finish to the leather or the adhesion between two adjacent layers of the finish.

The method is valid for all finished leathers with a smooth surface which can be bonded to an adherent plate without the adhesive penetrating into the finish. Preliminary experiments might be necessary to determine whether these conditions are met. This test method is valid for finished leathers with a finish-coat thickness of at least 15 µm.

The finished side of part of a strip of leather is bonded to a carrier plate by means of heat-reactivated adhesive film. Force is applied to the free end of the strip to peel the leather from the finish over a given distance, the finish layer remaining on the carrier plate together with the film of adhesive. The force required is measured and reported as the adhesion of the finish to the leather.

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2.5. Lesson 3: Chemical tests for leather

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- Chemical quality controls available
- Evaluation techniques for assessing the quality of materials

2.5.1. Introduction

Whether surfactants, tanning agents, fatliquoring agents or special auxiliaries – the leather sector includes all the chemical tools needed to turn the raw skin/hide into a leather according to your specifications.

The following is a list of chemicals commonly used in leather making:

Beamhouse and Tanyard

- Biocides prevent the growth of bacteria which can damage the hides or skins during the soaking process;
- Surfactants are used to help with the wetting back of the hides or skins;
- Degreasers help with the removal of natural fats and greases from the hides or skins;
- Swell regulating agents help prevent uneven swelling of the hides or skins during liming;
- Lime is used to swell the hides or skins;
- Sodium sulphide chemically destroys the hair on hides or skins;
- Sodium hydrosulphide chemically destroys the hair on hides or skins. It does not create as much swelling as sodium sulphide;
- Low sulphide unhairing agents help to reduce the amount of sulphides used in a tannery thus reducing the environmental impact of tanneries;
- Caustic soda is used during the liming process to help swell the hides or skins;
- Soda ash is used during the soaking or liming processes to help raise the pH of the hides or skins;
- Ammonium sulphate is used during the deliming process and helps remove lime from the hides or skins;
- Enzyme preparations are used after the deliming process and helps bate the hides or skins;
- Ammonium chloride is used during the deliming process and helps remove lime from the hides or skins;
- Sodium metabisulphite is used during the deliming process and helps prevent the formation of toxic hydrogen sulphide gas during deliming. It also acts as a bleaching agent;
- Formic/acetic acid is used during the pickling process to change the pH of the hides or skins;
- Sulphuric acid is used during the pickling process to lower the pH of the hides or skins;
- Sodium chloride is used during the pickling process to prevent acid swelling of the hides or skins;
- Sodium formate is used during the tanning process to assist with the penetration of chromium tanning salts into the hides or skins;
- Chromium tanning material is the tanning agent used to make wet blue;
- Aldehyde tanning agents are tanning agents used to make wet white;
- Magnesium oxide is used during basification and raises the pH of the hide or skin to allow the chromium or aldehyde to chemically bind to the skin protein;
- Fungicide are chemicals that are used to prevent the growth of moulds or fungi on tanned hides or skins;

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Dyehouse

- Surfactants/ Wetting agents help in the wetting back of the wet blue in the dyehouse;
- Degreasers help remove grease or fats that may be present on the wet blue as a result of the wet blue coming into contact with machinery;
- Sodium formate helps raise the pH during the neutralization process;
- Sodium bicarbonate helps raise the pH during the neutralization process;
- Formic acid reduces the pH for the rechroming process or helps with chemically fixing dyehouse chemicals to the leather at the end of the dyehouse processes;
- Chrome syntans are used during rechroming to improve the softness of the final leather;
- Syntans are used to give properties such as softness, fullness, roundness to the leather;
- Resins are used to give fullness and a tight grain to the leather;
- Polymers are used to give fullness and a tight grain to the leather;
- Dyes are used to give the leather a colour desired by the customer;
- Dyeing auxiliaries help disperse the dyes evenly;
- Fatliquors are oils that are added to leather to give softness to the final leather;

Finishing

- Acrylic resins give specific properties to the leather finish such as adhesion, water resistance;
- Butadiene resins give specific properties to the leather finish such as good coverage;
- Polyurethane resins give specific properties to the leather finish such as good toughness and good lightfastness;
- Fillers help fill small blemishes on the leather surface;
- Dullers help reduce the gloss of the finish;
- Crosslinkers are used to toughen the leather finish and improve the water resistance properties of polyurethanes;
- Handle modifiers are used to give the leather surface a waxy or slippery feel;
- Nitrocellulose lacquers are used in the top coat of a leather finish;
- Acrylic lacquers are used in the top coat of a leather finish;
- Polyurethane lacquers are used in the top coat of a leather finish;
- Viscosity modifiers are used to increase the viscosity of a finish mixture;
- Pigments are colouring agents that help hide defects on the leather surface;
- Dyes are colouring agents that are used to slightly change the colour of the leather finish or to give the leather finish a more natural look;
- Defoamers are used to prevent bubbles from forming in the finish mixture.

All materials and auxiliaries used in leather processing can be divided into three groups in conformity with their complexity:

1. Simple organic or inorganic materials (sodium chloride, sodium sulphide, acetic acid, formic acid etc.);

2. Complex materials (dyes, enzymes, detergents, vegetable tannins etc.);

3. Mixtures of materials purchased as technical products (enzyme preparations, fatliquors, binders, materials for finish etc.).

The testing of the complex materials or the technical products mostly are very complicated and usually requires specific and expensive equipment. Accordingly, the analysis of such materials are carried out in

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specialised chemical laboratories. Additionally, the companies, which supply the materials for leather processing, usually do not reveal the compositions of the supplied materials (enzyme preparations, fatliquors, fillers, materials for finish etc.). Therefore, the main aim of the chemical laboratory in leather center is to be able to execute chemical tests for simple chemicals used for leather processing to establish their quality or concentrations in working solutions.

The lesson "Chemical Tests for Auxiliaries in Leather Processing" presents the test methods of materials used in leather industry. The test methods are based on corresponding ISO standards and on literature specialised in leather technology.

Reference – https://sites.google.com/site/isttschool/useful-information/chemicals-used-in-leather-processing.

2.5.2. Testing methods

Determination of total soluble alkalinity of sodium carbonate

Principle of the method is based on a preparation of solution of a test portion, filtration of the solution and titration of the total soluble alkalinity with a standard volumetric solution of hydrochloric acid, using methyl orange as indicator.

Reagents

During the analysis, use only reagents of recognized analytical reagent grade and only distilled water or water of equivalent purity.

(1) Hydrochloric acid, 1 N standard volumetric solution.

(2) Methyl orange, 0.5 g/l solution (the methyl orange may be replaced by any other indicator giving the same end-point).

Equipment - ordinary laboratory apparatus.

Procedure

Weigh test portion of sodium carbonate, to the nearest 0.01 g, a mass of the test sample of 50±0.1 g, 59±0.1 g, 110±0.1 g or 135±0.1 g, depending on whether the product is anhydrous or mono-, hepta- or decahydrate.

Dissolve the test portion by pouring it in small quantities at a time, while stirring, into a beaker of suitable capacity (for example 600 ml) containing 200 ml of water at about 50 ^{o}C.

Filter the decanted solution through a medium-speed filter, collecting the filtrate in a 500 ml one-mark volumetric flask.

Wash the insoluble matter onto the filter with water at about 50 ^{o}C. Complete the washing, collecting all the washings in the volumetric flask. Allow to cool, dilute to the mark and mix.

Transfer 25.0 ml of the test solution to a 500 ml conical flask. Add approximately 75 ml of water, 5 drops of the methyl orange solution (2) and titrate with the standard volumetric hydrochloric acid solution (1) until the indicator turns from yellow to orange-pink.

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NOTE - If required, this determination may also be carried out by means of back-titration, using 1 N standard volumetric hydrochloric acid solution and 1 N or 0.1 N standard volumetric sodium hydroxide solution. If this method is adopted, it shall be taken into account in the calculation of results.

Expression of results

The total soluble alkalinity (*TSA*), expressed as a percentage by mass of sodium carbonate (Na_2C0_3), is given by the formula:

$$TSA = V \times \frac{500}{25} \times \frac{100}{m} \times 0.0530 = 106\frac{V}{m}$$

where

V is the volume, in millilitres, of the standard volumetric hydrochloric acid solution (1) used for the titration;

m is the mass, in grams, of the test portion;

0.0530 is the mass, in grams, of sodium carbonate equivalent to 1 ml of exactly 1 N standard volumetric hydrochloric acid solution.

Notes

1 If the concentration of the standard volumetric solution used is not exactly as specified in the list of reagents, an appropriate correction should be made.

2 If it is desired to express the result on the basis of non-volatile matter at 250 °C, multiply the result obtained on the product as received by the ratio

$$\frac{100}{100 - \text{loss of mass at } 250\ ^\circ\text{C in } \%\ (\text{m/m})}$$

Reference – IS0 740-1976: Sodium carbonate for industrial use - Determination of total soluble alkalinity - Titrimetric method

Determination of sodium sulphide

Principle of the method is based on a preparation of solution of a test portion and titration of the sulphides with a standard volumetric solution of potassium hexacyanoferrate(III), using ***sodium nitroprusside*** as indicator.

Reagents

During the analysis, use only reagents of recognized analytical grade, and only distilled water or water of equivalent purity, neutral to methyl red.

(1) Sodium hydroxide, 0.1 N volumetric solution;

(2) Potassium hexacyanoferrate(III) ($K_3Fe(CN)_6$), 0.1 N standard volumetric solution;

(3) ***Sodium nitroprusside (***$Na_2[Fe(CN)_5NO]$), 4 g/l solution in distilled water.

Apparatus

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Ordinary laboratory apparatus and

Burette, graduated in 0.05 ml;

1000 ml one-mark volumetric flask;

25 ml pipette.

Procedure

Weigh test portion of sodium sulphide in a weighing dish, previously tared to the nearest 0.0001 g, weigh, to the nearest 0.0001 g, approximately 10 g of the test sample.

Quantitatively transfer the test sample into a 1000 ml one-mark volumetric flask. Pour about half of flask 0.1 N sodium hydroxide (1) and dissolve the test portion. Dilute to the mark by 0.1 N sodium hydroxide solution (1) and mix getting a sample solution.

Take with pipette 25 ml sample solution. Transfer quantitatively to 500 ml flask; add 10 ml 0.1 N sodium hydroxide solution (1), 50 ml distilled water and about 1 ml ***sodium nitroprusside*** 4 g/l solution (3) and promptly titrate with potassium hexacyanoferrate(III) ($K_3Fe(CN)_6$), 0.1 N standard volumetric solution (2) until the colour changes from violet to light yellow.

Expression of results

The total amount (TA) of sodium sulphide, expressed as a percentage by mass of, is given by the formula:

$$\mathrm{TA} = \frac{V \times 0.0039 \times 1000}{25} \times \frac{100}{m} = \frac{V \times 15.6}{m}$$

where

V is the volume, in millilitres, of the potassium hexacyanoferrate solution (2) used for the titration;

m is the mass, in grams, of the test portion;

0.0039 is the mass, in grams, of sodium sulphide corresponding to 1 ml of exactly 0.1 N potassium hexacyanoferrate solution (2).

Reference – Golovtejeva A.A., Kucidi L.B., Sankin L.B. Laboratory compendium of chemistry and technology of leather and fur. Moscow: Legkaiya promyslennost, 1982.

Determination of calcium hydroxide

Principle of the method is based on a preparation of solution of a test portion and titration of the calcium hydroxide with a standard volumetric solution of hydrochloric acid, using ***phenolphthalein*** as indicator.

Reagents

During the analysis, use only reagents of recognized analytical grade, and only distilled water or water of equivalent purity, neutral to methyl red.

(1) Hydrochloric acid, 0.1 N volumetric solution;

(2) ***Phenolphthalein*** 1 g dissolve in 70 ml 95 % (V/V) ethanol and ad 30 ml of distilled water.

Apparatus

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Ordinary laboratory apparatus and

Burette, graduated in 0.05 ml;

1000 ml one-mark volumetric flask;

50 ml pipette.

Procedure

Weigh test portion of calcium hydroxide in a weighing dish, previously tared to the nearest 0.0001 g, weigh, to the nearest 0.0001 g, approximately 1 g of the test sample.

Quantitatively transfer the test sample into a 1000 ml one-mark volumetric flask. Pour about 800-900 ml of distilled water and dissolve the test portion. Dilute to the mark with distilled water and mix getting a sample solution.

Take with pipette 50 ml sample solution. Transfer quantitatively to 500 ml flask; add 3-4 drops of ***phenolphthalein*** solution (2) and titrate with hydrochloric acid 0.1 N standard volumetric solution (1) until the violet-red colour disappears.

Expression of results

The total amount (TA) of calcium hydroxide, expressed as a percentage by mass of, is given by the formula:

$$\mathrm{TA} = \frac{V \times 0.0037 \times 1000}{50} \times \frac{100}{m} = \frac{V \times 7.4}{m}$$

where

V is the volume, in millilitres, of the hydrochloric acid solution (1) used for the titration;

m is the mass, in grams, of the test portion;

0.0037 is the mass, in grams, of calcium hydroxyde corresponding to 1 ml of exactly 0.1 N hydrochloric acid solution (1).

Reference – Golovtejeva A.A., Kucidi L.B., Sankin L.B. Laboratory compendium of chemistry and technology of leather and fur. Moscow: Legkaiya promyslennost, 1982.

Testing of ammonia sulphate: determination of ammoniacal nitrogen content

Principle – the method is based on a distillation of the ammonia after displacement by an alkali solution; absorption in an excess of standard volumetric sulphuric acid solution and back-titration with standard volumetric sodium hydroxide solution in the presence of an indicator.

Reagents

During the analysis, use only reagents of recognized analytical reagent grade and only distilled water or water of equivalent purity.

(1) Sodium hydroxide, 450 g/l solution;

(2) Sulphuric acid, 0.5 N standard volumetric solution.

(3) Sodium hydroxide, 0.5 N standard volumetric solution.

(4) Mixed indicator, ethanol solution. Dissolve 0.1 g of methyl red in about 50 ml of 95 % (V/V) ethanol, add 0.05 g of methylene blue and, after dissolution, dilute to 100 ml with the same ethanol.

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Apparatus

Ordinary laboratory apparatus and:

Distillation apparatus, with, preferably, spherical ground glass joints, or any apparatus that will ensure quantitative distillation and absorption. The apparatus may, for example, be made up from the following items (see figure):

distillation flask (A), capacity 1000 ml, with a female joint;

splash head (B), with male joints and parallel inlet and outlet into which is fused a cylindrical *dropping funnel* (C), capacity 50 ml;

Liebig condenser (D), effective length about 400 mm, fitted with a female joint at the inlet and a male joint at the outlet;

conical flask (E), capacity 500 ml, with a female joint, fitted with two side bulbs;

spring clamps (F).

Procedure

Prepare the test portion (*m*) by weighting, to the nearest 0.001 g, about 10 g of the test sample.

Carry out a blank test at the same time as the determination, following the same procedure and using the same quantities of all the reagents as used for the determination.

Prepare the sample solution by placing the test portion in a 500 ml one-mark volumetric flask. Dissolve the test portion in water, dilute to the mark and mix.

Place 50 ml of the sample solution in the distillation flask (A). Add about 350 ml of water and a few anti-bumping granules.

Coat the joints of the apparatus with a Silicone grease. Mount the splash head (B) on the flask (A), and connect it to the condenser (D).

Place 40 ml of the standard volumetric sulphuric acid solution (2), about 80 ml of water and a few drops of the mixed indicator solution (4) into the flask (E). Connect the flask (E) to the condenser (D), ensuring that all the joints of the apparatus are firm by means of the spring clamps (F).

Introduce 20 mi of the sodium hydroxide solution (1) into the flask (A) through the dropping funnel (C), taking care to leave at least a few millimetres of liquid above the tap.

Distil until the volume of liquid in the flask (E) has reached about 250 to 300 ml. Stop the heating, open the tap of the dropping funnel (C), disconnect the splash head (B) and wash the condenser (D) carefully, collecting the wash water in the flask (E). Finally disconnect the flask (E).

Carefully mix the solution contained in the flask (E) and in the two side bulbs, and back-titrate the excess of the standard volumetric sulphuric acid solution (2) with the standard volumetric sodium hydroxide solution (3). During the titration, stir carefully to ensure that the solution is completely mixed.

Note. The procedure is described in terms of the apparatus specified in Fig. and will require modification if other apparatus is used.

Expression of results

The ammoniacal nitrogen content, expressed as a percentage by mass of nitrogen (N), is given by the formula:

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$$\mathrm{N} = (V_1 - V_2) \times 0.007004 \times \frac{500}{50} \times \frac{100}{m} = \frac{7.004 \times (V_1 - V_2)}{m}$$

where

V_1 is the volume, in millilitres, of the standard volumetric sodium hydroxide solution (3) used for the back-titration of the excess of the Standard volumetric sulphuric acid Solution (2) placed in the flask (E) for the blank test;

V_2 is the volume, in millilitres, of the standard volumetric sodium hydroxide solution (3) used for the back-titration of the excess of the standard volumetric sulphuric acid solution (2) placed in the flask (E) for the determination;

m is the mass, in grams, of the test portion;

0.007004 is the mass, in grams, of nitrogen corresponding to 1 ml of 0.5 N standard volumetric sulphuric acid solution.

Note. If the concentrations of the standard volumetric solutions are not exactly as specified in the list of reagents, appropriate corrections should be made.

Reference – ISO 3332-1975: Ammonium sulphate for industrial use - Determination of ammoniacal nitrogen content – Titrimetric method after distillation.

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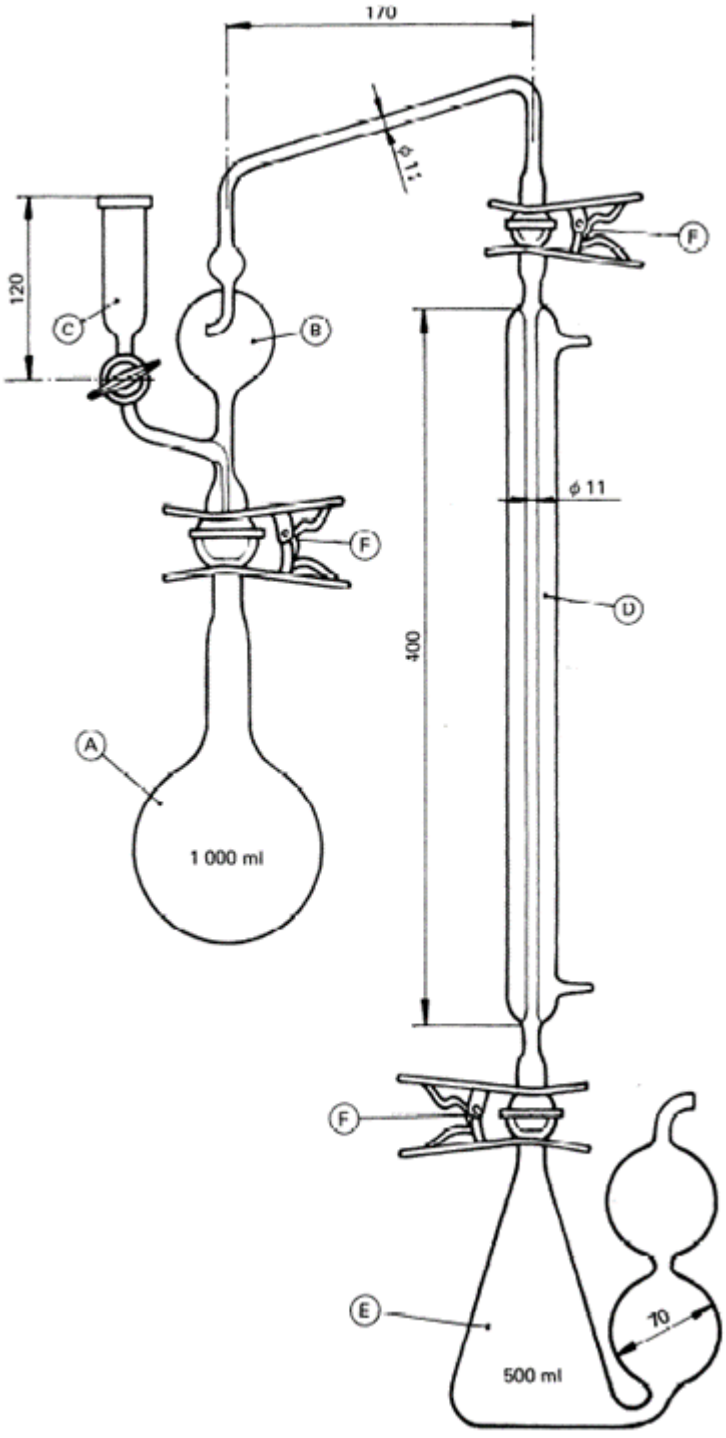

Figure 2.46. Typical distillation apparatus (dimensions in mm)

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Proteolytic activity: testing of enzyme or enzyme preparation by TEGEWA method

Principle. The method is based on ability break peptide bonds in substrate by proteolytic enzymes. In this assay, casein acts as a substrate. When the protease we are testing digests casein, the amino acid tyrosine is liberated along with other amino acids and peptide fragments. Folin and Ciocalteus Phenol (F-C), or Folin's reagent primarily reacts with free tyrosine to produce a blue colored chromophore, which is quantifiable and measured as an absorbance value on the spectrophotometer. The more tyrosine that is released from casein, the more the chromophores are generated and the stronger the activity of the protease. Absorbance values generated by the activity of the protease are compared to a standard curve, which is generated by reacting known quantities of tyrosine with the F-C reagent to correlate changes in absorbance with the amount of tyrosine in micromoles. From the standard curve the activity of protease samples can be determined in terms of Units, which is the amount in micromoles of tyrosine equivalents released from casein per minute.

Reagents

During the analysis, use only reagents of recognized analytical reagent grade and only distilled water or water of equivalent purity.

(1) 50 mM potassium phosphate buffer, pH 7.5. Prepare using 11.4 mg/ml of potassium phospate dibasic, trihydrate in purified water and adjusting pH with 1M HCl. This solution is placed at 37°C prior to use.

(2) 0.65% weight/volume casein solution, prepared by mixing 6.5 mg/ml of the 50 mM potassium phosphate buffer. The solution temperature is gradually increased with gentle stirring to 80-85 °C for about 10 minutes until a homogenous dispersion is achieved. It is very important not to boil the solution. The pH is then adjusted if necessary with NaOH and HCl.

(3) 110 mM trichloroacetic acid solution (TCA), prepared by diluting a 6.1N stock 1:55 with purified water. Trichloroacetic acid is a strong acid and should be handled with care.

(4) 0.5 M F-C, or Folin's Phenol Reagent, which is the solution that will react with tyrosine to generate a measurable color change that will be directly related to the activity of proteases. Folin's Phenol Reagent is an acid and should be handled with care.

(5) 500 mM sodium carbonate solution, prepared using 53 mg/ml of anhydrous sodium carbonate in purified water.

(6) An enzyme diluent solution, which consists of 10 mM sodium acetate buffer with 5mM calcium, pH 7.5, at 37°C. This solution is used to dissolve solid protease samples or dilute liquid enzyme solutions. If necessary, a solid protease sample of predetermined activity can be used, which is dissolved using enzyme diluent to 0.1-0.2 units/ml. This solution serves as a positive control for the quality control assay and as validation for the calculations which will be performed to determine enzyme activity.

(7) 1mM L-tyrosine Standard stock solution. Prepared using 0.2 mg/ml L-tyrosine in purified water and heated gently until the tyrosine dissolves. As with the casein, do not boil this solution. Allow the L-tyrosine standard to cool to room temperature. This solution will be diluted further to make our standard curve.

NOTE:

Apparatus

Ordinary laboratory apparatus and:

0.45 µm polyethersulfone syringe filter and syringe;

Dram vials or polypropylene tubes capable of holding 15 ml of solution;

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Spectrophotometer;

Cuvettes;

Pipettes;

Stir/Hot plate;

Stir bar;

Scale;

pH Meter

Procedure

To begin this assay, find suitable vials that will hold about 15 ml. For each enzyme that you will test, you will need 4 vials. One vial will be used as a blank, and three others will be used to assay activity of three dilutions of the protease. Three dilutions are useful when checking our final calculations against each other. To each set of four vials add 5 ml of our 0.65% casein solution (2), and let them equilibrate in a water bath at 37°C for about 5 minutes. Then, add varying volumes of enzyme solution you want to test to three of the test sample vials, but not the blank. Mix them by swirling and incubate for 37 °C for exactly ten minutes. The protease activity and consequential liberation of tyrosine during this incubation time is what will be measured and compared between test samples.

NOTE. If solid sample is used, prepare the test portion by weighing, to the nearest 1 mg g, about 100-1000 mg (*m*) of the solid sample. Put the sample into a 50-100 ml glass beaker, pour about 10 ml of diluent solution (6) and thoroughly grind with glass stick the sample to obtain homogenous solution (suspension). Quantitatively transfer the test portion into a 100 ml one-mark volumetric flask (when solid sample is used transfer the ground test portion into volumetric flask by washing the diluent solution (6)). Dilute to the mark with diluent solution (6) and mix getting a sample solution. Use the sample solution in the same way as liquid enzyme.

After this 10 minute incubation, add the 5 ml of the TCA reagent (3) to each tube to stop the reaction. Then an appropriate volume of enzyme solution is added to each tube, even the blank, so that the final volume of enzyme solution in each tube is 1 ml. This is done to account for the absorbance value of the enzyme itself and ensure that the final volume in each tube is equal. Now incubate the solutions at 37°C for 30 minutes.

During this 30 minute incubation, set up your tyrosine standard dilutions, which is done using 6 dram vials (dram vials can be substituted with polypropylene tubes) that can easily hold 8 ml. To the six vials the 1.1 mM tyrosine standard stock solutions (7) is added with the following volumes in ml: 0.05, 0.10, 0.20, 0.40, 0.50. Don't add any tyrosine standard to the blank. Lower standards may be needed for impure test samples with that will yield little color change. Once the tyrosine standard solution has been added, add an appropriate volume of purified water to each of the standards to bring the volume to 2 ml.

After the 30 minute incubation, filter each of the test solutions and the blank using a 0.45 um polyethersulfone syringe filter. Filtration is required to remove any insolubles from the samples. The filtration 2 ml of the test samples and blank filtrate is then added to 4 dram vials that can hold at least 8 ml. Use the same type of vial in which the standards are prepared. To all of the vials containing the standards and standard blank, 5 ml of sodium carbonate (5) is added, and for best results, 1 ml of F-C or Folin's reagent (4) is added immediately afterwards. sodium carbonate (5) is added to regulate any pH drop created by the addition of the Folin's reagent. Sodium carbonate (5) is then added to test samples and test blank. Notice that these solutions become cloudy after the addition of sodium carbonate. Then, the F-C or Folin's reagent (4) is added, which

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will react primarily with free tyrosine. The dram vials are then mixed by swirling and incubated at 37°C for 30 minutes.

After this incubation, notice that the standards have a gradation of colour correlating with the amount of tyrosine added; the highest concentrations of tyrosine appearing darkest. You can also notice appreciable colour change in our test samples. 2 ml of these solutions are filtered using a 0.45 um polyethersulfone syringe filter into suitable cuvettes. Measure the absorbance of the solutions and record the absorbance values.

The absorbance of the samples is measured by a spectrophotometer using a wavelength of 660 nm. The light path is set to 1 cm. Record the absorbance values for the standards (see Table), standard blank, the different test samples, and test blank. Once all of the data has been collected, create standard curve. In order to generate the curve, difference in absorbance between the standard and standard blank must be calculated. This is the absorbance value attributable to the amount of tyrosine in the standard solutions (7). After this simple calculation, draw the standard curve by plotting the change in absorbance of standards on the Y axis, versus the amount in micromoles for each of 5 standards on the X axis and generate a line of best fit and corresponding slope equation.

Table 2.9. Volume of tyrosine standard

Volume of tyrosine standard (7), ml	µMoles tyrosine
0.05	0.055
0.10	0.111
0.20	0.221
0.40	0.442
0.50	0.553

Calculation and expression of results

Find the change in absorbance in test samples by calculating the difference between test sample absorbance and the absorbance of test blank. Inserting the absorbance value for one of the test samples into the slope equation and solving will result in the micromoles of tyrosine liberated during this particular proteolytic reaction. To get the activity of enzyme in units per/ml of liquid enzyme, perform the following calculation:

$$\text{Units / ml enzyme} = \frac{(\mu\text{mole tyrosine equivalents released}) \times 11}{1 \times 10 \times 2}$$

where

11 is total volume of assay, ml;

10 is time of assay as per Unit definitiom, minutes;

1 is volume of liquid enzyme used, ml;

2 is volume used in colorimetric determination, ml.

To get the activity of enzyme in units per/mg of solid enzyme, use the formula:

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$$\text{Units / mg enzyme} = \frac{(\text{units / ml enzyme}) \times 100}{m}$$

100 is the total volume of solid sample solution, ml;

m is the mass of solid enzyme sample, taken for the preparation of the sample solution, mg.

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[1] https://www.sigmaaldrich.com/video/life-science/universal-protease-activity-assay.html
[2] GOST 20264.2-88. Enzyme preparations. Methods for determination of proteolytic activity. http://docs.cntd.ru/document/1200023426.

Determination of total acidity of sulphur acid which contents equal to or lower than 98 % (m/m)

Principle of the method is based on a titration of the total acidity with a standard volumetric sodium hydroxide solution, in the presence of methyl red as indicator.

Reagents

During the analysis, use only reagents of recognized analytical grade, and only distilled water or water of equivalent purity, neutral to methyl red.

(1) Hydrogen peroxide, 60 g/l solution, neutral to methyl red;

(2) Sodium hydroxide, 1 N standard volumetric solution;

(3) Methyl red, 1 g/l solution in 95 % (V/V) ethanol.

Apparatus

Ordinary laboratory apparatus and

Flask, capacity approximately 500 ml, with neck of diameter about 30 mm, with ground glass stopper.

Burette, graduated in 0.05 ml;.

Conical flask, capacity 500 ml, with ground glass stopper.

Procedure

Weigh test portion of sulphur acid in a weighing bottle, previously tared to the nearest 0.0001 g, weigh, to the nearest 0.0001 g, approximately 2 g of the test sample.

Transfer the test portion quantitatively to a 500 ml conical flask containing approximately 300 ml of water.

Add 5 ml of the hydrogen peroxide solution (1), heat to boiling and boil gently for 10 min.

Allow to cool, add 2 drops of the methyl red solution (3) and titrate with the sodium hydroxide solution (2) until the colour changes from red to yellow.

Expression of results

The total acidity (TA), expressed as a percentage by mass of sulphuric acid (H_2SO_4), is given by the formula:

$$\text{TA} = \frac{V \times 0.04904 \times 100}{m} = \frac{V \times 4.904}{m}$$

where

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V is the volume, in millilitres, of the sodium hydroxide solution (2) used for the titration;

m is the mass, in grams, of the test portion;

0.04904 is the mass, in grams, of sulphuric acid corresponding to 1 ml of exactly 1 N sodium hydroxide solution.

Reference – IS0 910-1977: Sulphuric acid and oleum for industrial use – Determination of total acidity, and calculation of free sulphur trioxide content of oleum - Titrimetric method.

Testing of acetic acid

Principle of the method is based on a preparation of solution of a test portion and titration of the acetic acid with a standard volumetric solution of sodium hydroxide, using ***phenolphthalein*** as indicator.

Reagents

During the analysis, use only reagents of recognized analytical grade, and only distilled water or water of equivalent purity, neutral to methyl red.

(1) Sodium hydroxide, 0.1 N volumetric solution;

(2) ***Phenolphthalein*** 1 g dissolve in 70 ml 95 % (V/V) ethanol and ad 30 ml of distilled water.

Apparatus

Ordinary laboratory apparatus and

Burette, graduated in 0.05 ml;

1000 ml one-mark volumetric flask;

50 ml pipette.

Procedure

Weigh test portion of calcium hydroxide in a weighing beaker, previously tared to the nearest 0.001 g, weigh, to the nearest 0.001 g, approximately 1 g of the test sample.

Quantitatively transfer the test sample into a 1000 ml one-mark volumetric flask by washing with distilled water. Dilute to the mark with distilled water and mix getting a sample solution.

Take with pipette 50 ml sample solution. Transfer quantitatively to 250-500 ml flask; add 3-4 drops of ***phenolphthalein*** solution (2) and titrate with sodium hydroxide 0.1 N standard volumetric solution (1) until the light pink colour appears, which does not disappear during 20 minutes.

Expression of results

The total amount (TA) of acetic acid, expressed as a percentage by mass of, is given by the formula:

$$\mathrm{TA} = \frac{V \times 0.006 \times 1000}{50} \times \frac{100}{m} = \frac{V \times 12}{m}$$

where

V is the volume, in millilitres, of the sodium hydroxide solution (1) used for the titration;

m is the mass, in grams, of the test portion;

0.0037 is the mass, in grams, of acetic acid corresponding to 1 ml of exactly 0.1 N sodium hydroxide solution (1).

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Reference – Golovtejeva A.A., Kucidi L.B., Sankin L.B. Laboratory compendium of chemistry and technology of leather and fur. Moscow: Legkaiya promyslennost, 1982.

Sodium chloride analysis

Determination of matter insoluble in water or in acid and preparation of principal solutions for other determinations

Principle – the determination is based on a preparation of a solution of a test portion in water. Filtration, drying and weighing of the insoluble residue. Dilution of the filtrate to form the principal solution (solution A) for carrying out other determinations.

Reagents

Distilled water, or water of equivalent purity, shall be used in the test.

(1) Silver nitrate, 5 g/l nitric solution. Dissolve 0.5 g of silver nitrate in a little water, add 10 ml of nitric acid solution d = 1.4 g/ml approximately, and dilute to 100 ml.

Equipment

Ordinary laboratory apparatus and

Filter crucible or funnel, glass or porcelain, approximately 30 mm diameter and of a porosity grade P 10 or P 16 (pore size index 4-16 µm).

Electric oven, ventilated by convection and capable of being controlled at 110±2 °C.

Desiccator, containing silica gel, phosphorus pentoxide or a molecular sieve.

Procedure

Weigh, to the nearest 0.01 g, approximately 100 g of the test sample.

Place the test portion in a 600 ml beaker and add 350 ml of water. Heat at just below boiling for 10 min, with stirring, and then transfer the beaker, covered with a watch glass, to a boiling water bath for 30 min. Cool to approximately 20°C.

Filter by vacuum on the filter crucible, previously dried at 110°C, cooled in the desiccator, and weighed to the nearest 0.1 mg. Then wash the insoluble matter, in groups of five successive washings, using 20 ml of water each time, disconnecting the vacuum after each washing in order to bring the insoluble matter into suspension for approximately 1 min before filtering, and checking for absence of chloride from the filtrate after the fifth, tenth or fifteenth washing. 10 ml of the washing water shall remain clear 5 min after adding 10 ml of the nitric silver nitrate solution (1). Cease washing as soon as the check indicates absence of chlorides.

Dry the crucible and its contents in the oven (2) controlled at 110±2°C for 1 h, cool in the desiccator and weigh to the nearest 0.1 mg. Repeat this operation until two weightings differ by not more than 0.2 mg.

NOTE - If the insoluble matter is so finely divided as to clog the filter, repeat the determination and add, after the 350 ml of water, 1.5 g, weighed to the nearest 0.1 mg, of a filter aid (kieselguhr) of analytical quality. The minimum particle size of the filter aid should be µm and it should be dried, at about 110°C, to constant mass before use.

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Preparation of the principal solution for other determinations **(solution A)**

Quantitatively transfer the filtrate obtained, after filtering and washing of the insoluble matter, to a 1000 ml one-mark volumetric flask. Dilute to the mark and mix. Keep this solution for the other determinations.

Expression of results

The matter insoluble in water (M) is given, as a percentage by mass, by the formula:

$$\mathrm{M} = (m_1 - m_2)\frac{100}{m_0}$$

where

m_0 is the mass, in grams, of the test portion;

m_1 is the mass, in grams, of the filter crucible and insoluble matter;

m_2 is the mass, in grams, of the filter crucible alone.

NOTE - If a filter aid has been used, deduct its mass from ml (see NOTE in ***Procedure***).

Reference – ISO 2479-1972: Sodium chloride for industrial use – Determination of matter insoluble in water or in acid and preparation of principal solutions for other determination

Determination of halogens, expressed as chlorine, by mercurimetric method

Principle – the method is based on a titration of the halogen ions with mercury (II) nitrate in the presence of diphenylcarbazone as indicator.

Reagents

Distilled water, or water of equivalent purity, shall be used in the test.

(1) Nitric acid, *d* = 1.40 g/ml, approximately 68 % (m/m) or approximately 14 N solution;

(2) Nitric acid, approximately 2 N solution.

(3) Sodium chloride, 0.1 N standard reference solution.

Weigh, to the nearest 0.1 mg, 5.8443 g of sodium chloride, previously dried for 1 h at 500°C and cooled in a desiccator. Dissolve it in water in a 1000 ml one-mark volumetric flask, dilute to the mark and mix.

(4) Standard end-point matching solution.

Prepare this standard solution immediately before use. Pour 200 ml of water into a 500 ml conical flask, followed by 3 drops of bromophenol blue solution (6) and the nitric acid solution (2), added drop by drop until the colour changes from blue to yellow. Add an excess of 3 drops of this acid, between 0.5 and 1.0 ml of the diphenylcarbazone solution (7) and the volume of mercury(II) nitrate solution (5) (from a burette) necessary to change the colour of the solution from yellow to mauve (about 1 drop).

(5) Mercury(II) nitrate, 0.1 N standard volumetric solution.

Preparation of the solution. Weigh 10.85 ± 0.01 g of mercury(ll) oxide (HgO) and dissolve it in 10 ml of the nitric acid solution (1), in a 1000 ml one-mark volumetric flask, dilute to the mark and mix.

Standardize this solution, following the procedure described in *Standardization of the solution,* adjusting it to the exact concentration if necessary.

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Standardization of the solution

Transfer 40.0 ml of the sodium chloride standard reference solution (3) to a 500 ml conical flask, followed by 160 ml of water and 3 drops of the bromophenol blue solution (6). Add the nitric acid solution (2) drop by drop until the colour of the indicator changes from blue to yellow.

Add an excess of 3 drops of this acid and same volume of the diphenylcarbazone solution (7) as in the standard end-point matching solution (4). Titrate the chloride with the mercury(II) nitrate solution to be standardized until the colour matches the mauve of the standard end-point matching solution (4) and deduct the volume of mercury(ll) nitrate solution (5) added during the preparation of this standard end-point matching solution (about 1 drop).

The correct amount is 40.00ml.

(6) Bromophenol blue, 1 g/l solution in 95 % (V/V) ethanol.

(7) Diphenylcarbazone, 5 g/l solution in 95 % (V/V) ethanol. Store this solution in a refrigerator and replace it when it no longer gives a sharp colour change.

Apparatus

Ordinary laboratory apparatus.

Procedure

Preparation of test portion. Take 50 ml of the principal solution A (see subchapter 2.8.1 Determination of matter insoluble in water or in acid and preparation of principal solutions for other determinations: *Preparation of the principal solution for other determinations* (solution A)), containing 100 g of the test sample per 1000 ml.

Determination. Get a sample solution by placing the test portion in a 500 ml one-mark volumetric flask. Dilute the test portion to the mark and mix.

Place 25 ml of the sample solution in a 500 ml conical flask. Dilute to 200 ml, then add 3 drops of the bromophenol blue solution (6) and the nitric acid solution (2) drop by drop, until the colour changes from blue to yellow. Add a further 3 drops of this acid and the same volume of the diphenylcarbazone solution (7) as in the standard end-point matching solution (4).

Titrate with the mercury(II) nitrate solution (5) until the colour matches the mauve of the standard end-point matching solution (4).

Expression of results

The halogens content, expressed as chlorine (Cl), is given, as a percentage by mass, by the formula:

$$\mathrm{Cl} = (V - V_1) \times \frac{500}{25} \times \frac{1000}{50} \times \frac{100}{m} \times 0.003545 = \frac{141.8(V - V_1)}{m}$$

where

m is the mass, in grams, of the test portion used for preparing the **principal solution A**.

V is the volume, in millilitres, of volumetric mercury(II) nitrate solution (5) used for titration;

V_1 is the volume, in millilitres, of the standard volumetric mercury(II) nitrate solution (5) used in the preparation of the standard end-point matching solution;

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0.003545 is the mass, in grams, of chlorine corresponding to 1 ml of the standard volumetric mercury(II) nitrate solution (5).

Reference – ISO 2481-1973: Sodium chloride for industrial use – Determination of halogens, expressed as chlorine – Mercurimetric method.

Testing of tanning materials

Analysis of vegetable and synthetic tanning materials

Principle – the method is based on an indirect gravimetric analysis of vegetable and synthetic tanning agents through fixing of the absorbent compounds on low-chromed hide powder.

Reagents

During the analysis, use only reagents of recognized analytical reagent grade and only distilled water or water of equivalent purity.

(1) Freshly prepared distilled water. The pH value of the water shall be between 5 and 6. When using methyl red, the water should not turn red. The evaporation residue of 100 ml should be less than 1 mg;

(2) Hide powder containing not more than 0.5 % chromium oxide and with a humidity not more than 13 %.

(3) Gelatine solution, of 1 g gelatine and 10 g sodium chloride, filled up to 100 ml with distilled water, adjusted to pH = 4.7.

Apparatus

The glass equipment shall be resistant to the action of distilled water. The flasks and tubes shall be Class A. Use normal laboratory equipment and, in particular, the following.

Desiccator, with an airtight cover and containing silica orange gel;

Evaporation dishes, suitable for slowly evaporating water. These shall be short with flat bases and measure 7 cm to 8.5 cm in diameter. Use silver dishes. If this is not possible, preferably use dishes made of stainless steel or, if necessary, ceramic or glass;

Water bath;

Drying oven, whose temperature shall be kept at the operating range of (102±2)°C;

Analytical balance, with a precision of 0.2 mg at a load of 200 g;

Technical balance, with a precision of 0.1 g at a load of 1000 g;

Procter bell (see Figure 1), composed of a cylindrical glass bell (length of the cylindrical part 90mm±1mm, internal diameter of the cylindrical pan: 28mm±1mm). A perforated rubber cork is inserted into the narrow part of the bell. A capillary glass tube (internal diameter 1.5mm) with two right-angled bends is inserted into the hole in the cork. The end of the shortest part can fit right down to the base of the cork;

Polyethylene tube, the tube shall be the right size to fit onto the bell's capillary glass tube;

Hoffman clamp;

1000 ml volumetric flasks;

50 ml pipette;

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Vacuum filter system (e.g. Figure 2);

Cellulose acetate membrane filters, with pores of 0.45µm and 3µm;

50 ml and 100 ml measuring cylinders.

Procedure

Sample preparation. There should be a generous, representative sample of the tanning agents for analysis; this should be thoroughly mixed. If the particles are heterogeneous, resort to manual or mechanical milling to homogenize the size of the particles. The particle size should not be smaller than 300 µm to avoid blocking the Procter bell.

Preparation of the analytical solution.

a) *Vegetable tanning agents in powder/solid form*:

Weigh the appropriate quantity (see Table) of vegetable tanning agents on an analytical balance. Add this to 800 ml of hot (60 °C to 80 °C) distilled water in a 1000 ml volumetric flask. Shake the flask to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

The aim is to obtain an analytical solution containing between 3.75 g and 4.25 g of substances absorbed by the hide powder. If the tanning content in the solution goes beyond these limits, repeat the analysis with a sample of suitable quantity.

Table 2.10. Tanning agents

Tanning agents, %	Approximate amount to weigh, g
50	8. 0
55	7.3
60	6.6
65	6.1
70	5.7
75	5.3
80	5.0
90	4.2

b) *Vegetable tanning agents in liquid form:*

Weigh the tanning agents on an analytical balance, taking into account the percentage of content in dry form. Add this to in a 1000 ml volumetric flask containing 800 ml of hot (60 °C to 80 °C) distilled water Shake the flask to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

The aim is to obtain an analytical solution containing between 3.75 g and 4.25 g of substances absorbed in the hide powder. If the tanning content in the solution goes beyond these limits, repeat the analysis with a sample of suitable quantity.

c) *Synthetic tanning agents in powder form:*

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Weigh about (4±0.1) g of tanning agents on an analytical balance. Add this to a 1000 ml volumetric flask containing 800 ml of hot (40 °C to 50 °C) distilled water. Shake the flash to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

In the case of breakthrough of the tanning agent, repeat the analysis using a lower mass.

d) *Synthetic tanning agents in liquid form:*

Weigh about (8±0.1) g of tanning agents on an analytical balance. Add it to a 1000 ml volumetric flask containing 800 ml of hot (40 °C to 50 °C) distilled water. Shake the flask to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

In the case of breakthrough of the tanning agent, repeat the analysis using a lower mass.

e) *Vegetable tanning agents organic solvent extracted in powder form:*

Weigh about (4±0.1) g of tanning agents on an analytical balance. Add this to a 1000 ml volumetric flask containing 800 ml of hot (40 °C to 50 °C) distilled water. Shake the flask to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

Select the amount in function of the quality of the extract desired. The final concentration of the analytical solution should contain about 4 g of tanning compound per litre.

Preparation of the bell

Place a layer of cotton wool at the top of the bell to prevent the hide powder from entering the capillary tube. Put the rubber cork containing the glass capillary tube in the bell. Weigh 7.0 g of hide powder on a technical balance and introduce it uniformly in the bell, pressing it down, up to the top of the rim. Check that the hide powder is fully pressed down to ensure that it will be completely tanned. Put the polyethylene tube in the glass capillary tube and use the Hoffman clamp.

De-tanning the analytical solution (determination of the non-tanning agents)

Place the bell containing hide powder in a beaker of suitable capacity. Fill the beaker with the unfiltered analytical solution up to the neck of the bell. When the hide powder is completely soaked, suck on the longer end of the capillary tube to create a slight depression and start siphoning the solution.

Use the Hoffman clamp to adjust the flow of the solution so that about 8 to 10 drops of the de-tanned solution drip through per minute. The resulting solution shall be clear.

Collect a total of 90 ml in (120±10) min.

The first 30 ml of the filtrate should be collected in a 50 ml glass measuring cylinder and disposed of.

The next 60 ml should be collected in a perfectly dry 100 ml glass measuring cylinder to determine the non-tanning agents. To control the breakthrough of tanning agents use 5 ml of the collected solution and add 0.5 ml gelatine solution. The pH of the total solution should be lower than 5. If necessary, use a few drops of formic acid to reduce the pH. A white precipitate is an indication of a breakthrough. In this case, repeat the analysis with a lower sample mass.

The solution should be at a temperature no less than 18 °C and no more than 20 °C. Use the pipette to transfer 50 ml of the filtered solution into a previously dried and weighed silver dish.

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Place the dish on the water bath and wait for complete evaporation. Put the dish in the oven at (102±2) °C to attain constant mass (about 18 h±2 h). Put the dish in the silica gel dryer and weigh it after 15 min on analytical balance.

Determination of soluble substances

To filter the analytical solutions, use the filter system indicated in Figure 2. Use the cellulose acetate membranes with 0.45 µm pores. If filtration proves awkward, use membranes with 3.0 µm pores and then pass the pre-filtered solution through the 0.45 µm membranes

If the filtration is not possible, the solution should be centrifuged. Collect about 100 ml of filtrate. Use the pipette to transfer 50 ml of the filtered solution into a previously dried and weighed silver dish.

Place the dish on the water bath and wait for complete evaporation. Put the dish in the oven at (102±2) °C to attain constant mass (about 18 h±2 h). Put the dish in the silica gel dryer and weigh it after 15 min on analytical balance.

Determination of total solids

Calibrate a silver dish in the oven at (102±2)°C and then cool it off in the dryer, for about 15 min, down to ambient temperature.

Weigh the dish with the analytical balance. Add about 3 g to 5 g of the sample to the dish. Record the mass as m_{P0}.

Put the dish in the oven at (102±2) °C to attain constant mass (about 18 h±2 h). The heating should be done without air circulation.

Put the dish in the silica gel dryer and weigh it after 15 min on analytical balance. Record the mass of dry residue as m_1.

Determination of hide powder blank value.

Place the bell containing hide powder in a beaker of suitable capacity. Fill the beaker with distilled water up to the neck of the bell. When the hide powder is completely soaked, suck on the longer end of the capillary tube to create a slight depression and start siphoning the solution.

Use the Hoffman clamp to adjust the flow of the solution so that about 8 to 10 drops of the solution drip through per minute. Collect a total of 90 ml in (120±10) min.

The first 30 ml of the filtrate should be collected in a 50 ml glass measuring cylinder and disposed of. The next 60 ml should be collected in a perfectly dry 100 ml glass measuring cylinder to determine the blank value.

Distilled water should be at a temperature no less than 18 °C and no more than 20 °C.

Use the pipette to transfer 50 ml of the filtered solution into a previously dried and weighed silver dish. Place the dish on the water bath and wait for complete evaporation. Put it in the oven at (102±2) °C to attain constant mass (about 18 h±2 h).

To determine the blank value, the procedure needs to be repeated at least 5 times for each batch purchased. Calculate the average of the values obtained. The result is the “blank value".

Calculation and expression of the results

Calculate the percentage content of dry substances (S_{t0}) (% total solid) using Equation (1).

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$$S_{t0} = \frac{m_1 \times 100}{m_{P0}} \qquad (1)$$

where

S_{t0} is the percentage content of dry substances (% total solid), in percent (%);

m_1 is the dry residue, in grams (g);

m_{P0} is the product's mass, in grams (g).

Calculate the percentage of soluble solids (S_{S0}) using Equation (2).

$$S_{S0} = \frac{m_2 \times 20 \times 100}{m_{P1}} \qquad (2)$$

where

S_{S0} is the percentage content of soluble solids, in percent (%);

m_2 is the dry residue of 50 ml of the filtered analytical solution, in grams (g);

m_{P1} the initial mass of the product (analytical solution), in grams (g).

Calculate the percentage of non-tanning solid (S_{nt}) using Equation (3).

$$S_{nt} = \frac{m_3 \times 20 \times 100}{m_{P1}} \qquad (3)$$

where

S_{nt} is the percentage of non-tanning solid, in percent (%);

m_3 is the dry residue of the non-tanning agent solution, deducted from the blank value, in grams (g);

m_{P1} the initial mass of the product (analytical solution), in grams (g).

Calculate the percentage of tanning agents (T) using Equation (4).

$$T = S_{S0} - S_{nt} \qquad (4)$$

Calculate the percentage of insoluble matter (I_M) using equation (5).

$$I_M = S_{t0} - S_{nt} \qquad (5)$$

Calculate the percentage of water (W) using Equation (6).

$$W = 100 - S_{t0} \qquad (6)$$

Calculate the ratio of tanning agent to non-tanning agent (R) using Equation (7)

$$\mathrm{R} = \frac{T}{S_{nt}} \qquad (7)$$

References:

[1] ISO 14088:2012: Leather–Chemical tests–Quantitative analysis of tanning agents by filter method.
[2] https://emeraldscientific.com/filtration-all-glass-vacuum-filter-holder-set-47mm/

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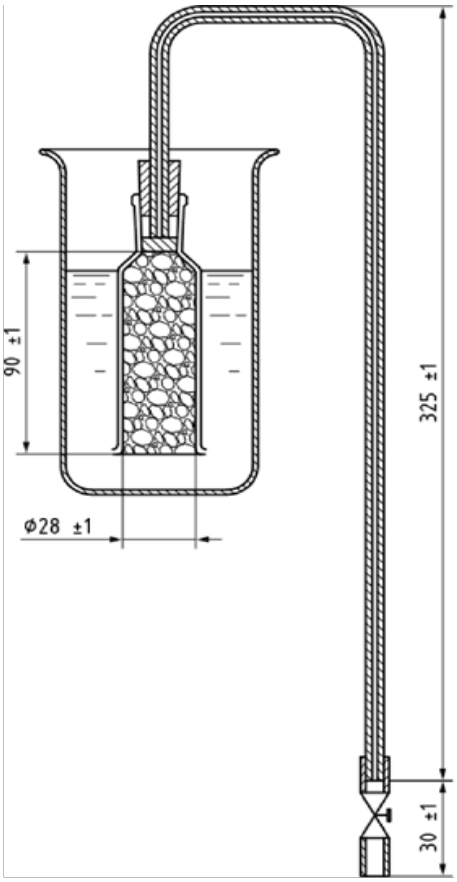

Figure 2.47. Procter bell (dimensions in mm)

Figure 2.48. Vacuum filter system

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Exploration of synthetic tanning materials

Principle – the method is based adsorption of polyphenols from the tanning agent by an insoluble copolymer absorbs. The dry content of the solution before and after mixing with the absorbing copolymer are measured. The difference is the adsorbable fraction, called the tan content.

Reagents

(1) Croslinked, insoluble vinylimidazole/vinylpyrrolidone copolymer (a suitable version of the insoluble vinylimidazole/vinylpyrrolidone copolymer powder, Divergan HM, is used widely in the wine industry; any other insoluble vinylimidazole/vinylpyrrolidone copolymer powders that give the same results can be used);

(2) Formic acid solution, a mass fraction of 50 %;

(3) Gelatine, pure, AR grade;

(4) Sodium chloride, AR grade;

(5) Deionised or distilled water.

Apparatus

Normal laboratory equipment and the following items:

Analytical balance, accurate to ±0.1 mg;

Drying oven, ventilated, capable of maintaining a temperature of 105 °C ±2 °C;

Magnetic stirrer;

PH meter with suitable combination electrode;

Stainless steel or aluminium dishes for evaporating aqueous solutions;

Desiccator, with drying agent;

Membrane filter, 50 mm diameter, 0.45 µm pore size or a suitable analytical grade filter paper;

Glass beakers, 1000 ml and 600 ml;

Volumetric flasks, 500 ml;

Pipette, 50 ml, analytical grade.

Procedure

Preparation of the synthetic tanning agent solution for analysis

In a 1 000 ml beaker, add the following.

For powder synthetic tanning agents:

– accurately weigh 1.3000 g to 1.7000 g of the powder and record the mass (m):

– add approximately 400 ml of warm deionised water (5).

For liquid synthetic tanning agents:

– accurately weigh 2.7000 g to 3.3000 g of the liquid and record the mass (*m*);

– add approximately 400 ml of deionised water (5) at room temperature. Stir so that the synthetic tanning agent is dissolved; cool the solution to 23 °C ± 1 °C. While stirring, adjust the pH to 2.0 to 2.3 by adding dropwise

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50% formic acid solution (2). Transfer the solution quantitatively to a 500 ml volumetric flask and fill to the mark with deionised or distilled water (5). This is the tanning solution to be used for the following test steps.

Prepare duplicate tanning solutions for each synthetic tanning agent to be tested.

Determination of the total residual dry content (duplicate determination)

Weigh (*T1*) the clean, dry stainless steel dish. With an analytical 50 ml pipette, add 50 ml of the tanning solution into the dish and evaporate most of the water carefully on a suitable hotplate or water bath. Place the dish in an oven at 105 °C ± 2 °C until constant mass is reached. Allow the dish to cool for approximately 2 h in a desiccator with a drying agent, for example, either calcium chloride ($CaCl_2$) or silica gel. Weigh the dish (*P1*).

The % dry content is calculated as follows:

$$\%\ \text{dry content} = \frac{(P_1 - T_1) \times 10 \times 100}{m}$$

Determination of the non-tanning agent content (i.e. non-adsorbable fraction)

In a 600 ml beaker, add the following:

— 40.0 g ± 0.1 g insoluble vinylimidazole/vinylpyrrolidone copolymer powder (5);

— 300 g ± 1 g of the tanning solution.

Stir the suspension for approximately 30 min and leave it to settle for approximately 90 min. If the upper aqueous phase is not clear, it can be centrifuged for 15 min at approximately 3000 rpm. Decant the upper aqueous phase through a 0.45 µm membrane filter or a suitable analytical grade filter paper if no membrane filter is available. Keep the filtrate to make a double determination approximately 130 ml of filtrate is needed.

To check the absence of tanning agent in the filtrate, prepare a gelatine solution by dissolving approximately 10 g gelatine (3) and approximately 100 g NaCl (4) in 1000 ml deionised or distilled water (5). To an approximately 5 ml sample of the filtrate, add approximately 2 ml of the gelatine solution. The test solution must remain clear. If precipitation or cloudiness occurs, then there is still residual tanning agent in the filtrate. Repeat the procedure in using a larger amount of the insoluble vinylimidazole/ vinylpyrrolidone copolymer powder (1) until the test solution is clear.

Carry out this section in duplicate. Weigh the clean, dry stainless steel dish (*T2*). With an analytical 50 ml pipette, add 50 ml of the tanning solution into the dish and evaporate most of the water carefully on a suitable hotplate or water bath. Place the dish in an oven at 105 °C ± 2 °C until constant mass is reached. Allow the dish to cool for approximately 2 h in a desiccator with a drying agent. Weigh the dish (*P2*).

The non-tanning agent content is calculated as follows:

$$\%\ \text{Non} - \text{tanning agent content} = \frac{(P_2 - T_2) \times 10 \times 100}{m}$$

Calculation and expression of results

Calculate the tan content (i.e. the adsorbable fraction) as follows:

% tan content = (% dry content) — (% non-tanning agent content)

Reference – ISO 17489:2013: Leather - Chemical tests - Determination of tan content in synthetic tanning agents.

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2.6. Lesson 4: Chemical Tests for Auxiliaries used in Leather Processing

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- Standardized chemical quality controls available for auxiliary substances
- Common practice chemical tests for auxiliaries

2.5.1. Introduction

Whether surfactants, tanning agents, fatliquoring agents or special auxiliaries – the leather sector includes all the chemical tools needed to turn the raw skin/hide into leather according to your specifications.

The following is a list of chemicals commonly used in leather making:

Beamhouse and Tanyard

- Biocides prevent the growth of bacteria which can damage the hides or skins during the soaking process;
- Surfactants are used to help with the wetting back of the hides or skins;
- Degreasers help with the removal of natural fats and greases from the hides or skins;
- Swell regulating agents help prevent uneven swelling of the hides or skins during liming;
- Lime is used to swell the hides or skins;
- Sodium sulphide chemically destroys the hair on hides or skins;
- Sodium hydrosulphide chemically destroys the hair on hides or skins. It does not create as much swelling as sodium sulphide;
- Low sulphide unhairing agents help to reduce the amount of sulphides used in a tannery thus reducing the environmental impact of tanneries;
- Caustic soda is used during the liming process to help swell the hides or skins;
- Soda ash is used during the soaking or liming processes to help raise the pH of the hides or skins;
- Ammonium sulphate is used during the deliming process and helps remove lime from the hides or skins;
- Enzyme preparations are used after the deliming process and helps bate the hides or skins;
- Ammonium chloride is used during the deliming process and helps remove lime from the hides or skins;
- Sodium metabisulphite is used during the deliming process and helps prevent the formation of toxic hydrogen sulphide gas during deliming. It also acts as a bleaching agent;
- Formic/acetic acid is used during the pickling process to change the pH of the hides or skins;
- Sulphuric acid is used during the pickling process to lower the pH of the hides or skins;
- Sodium chloride is used during the pickling process to prevent acid swelling of the hides or skins;
- Sodium formate is used during the tanning process to assist with the penetration of chromium tanning salts into the hides or skins;
- Chromium tanning material is the tanning agent used to make wet blue;
- Aldehyde tanning agents are tanning agents used to make wet white;
- Magnesium oxide is used during basification and raises the pH of the hide or skin to allow the chromium or aldehyde to chemically bind to the skin protein;
- Fungicide are chemicals that are used to prevent the growth of moulds or fungi on tanned hides or skins;

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Dyehouse

- Surfactants/ Wetting agents help in the wetting back of the wet blue in the dyehouse;
- Degreasers help remove grease or fats that may be present on the wet blue as a result of the wet blue coming into contact with machinery;
- Sodium formate helps raise the pH during the neutralization process;
- Sodium bicarbonate helps raise the pH during the neutralization process;
- Formic acid reduces the pH for the rechroming process or helps with chemically fixing dyehouse chemicals to the leather at the end of the dyehouse processes;
- Chrome syntans are used during rechroming to improve the softness of the final leather;
- Syntans are used to give properties such as softness, fullness, roundness to the leather;
- Resins are used to give fullness and a tight grain to the leather;
- Polymers are used to give fullness and a tight grain to the leather;
- Dyes are used to give the leather a colour desired by the customer;
- Dyeing auxiliaries help disperse the dyes evenly;
- Fatliquors are oils that are added to leather to give softness to the final leather;

Finishing

- Acrylic resins give specific properties to the leather finish such as adhesion, water resistance;
- Butadiene resins give specific properties to the leather finish such as good coverage;
- Polyurethane resins give specific properties to the leather finish such as good toughness and good lightfastness;
- Fillers help fill small blemishes on the leather surface;
- Dullers help reduce the gloss of the finish;
- Crosslinkers are used to toughen the leather finish and improve the water resistance properties of polyurethanes;
- Handle modifiers are used to give the leather surface a waxy or slippery feel;
- Nitrocellulose lacquers are used in the top coat of a leather finish;
- Acrylic lacquers are used in the top coat of a leather finish;
- Polyurethane lacquers are used in the top coat of a leather finish;
- Viscosity modifiers are used to increase the viscosity of a finish mixture;
- Pigments are colouring agents that help hide defects on the leather surface;
- Dyes are colouring agents that are used to slightly change the colour of the leather finish or to give the leather finish a more natural look;
- Defoamers are used to prevent bubbles from forming in the finish mixture.

All materials and auxiliaries used in leather processing can be divided into three groups in conformity with their complexity:

1. Simple organic or inorganic materials (sodium chloride, sodium sulphide, acetic acid, formic acid etc.);

2. Complex materials (dyes, enzymes, detergents, vegetable tannins etc.);

3. Mixtures of materials purchased as technical products (enzyme preparations, fatliquors, binders, materials for finish etc.).

The testing of the complex materials or the technical products mostly are very complicated and usually requires specific and expensive equipment. Accordingly, the analysis of such materials is carried out in

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specialised chemical laboratories. Additionally, the companies, which supply the materials for leather processing, usually do not reveal the compositions of the supplied materials (enzyme preparations, fatliquors, fillers, materials for finish etc.). Therefore, the main aim of the chemical laboratory in leather center is to be able to execute chemical tests for simple chemicals used for leather processing to establish their quality or concentrations in working solutions.

The lesson "Chemical Tests for Auxiliaries in Leather Processing" presents the test methods of materials used in leather industry. The test methods are based on corresponding ISO standards and on literature specialised in leather technology.

Reference – https://sites.google.com/site/isttschool/useful-information/chemicals-used-in-leather-processing.

2.6.1. Testing methods

Determination of total soluble alkalinity of sodium carbonate

Principle of the method is based on a preparation of solution of a test portion, filtration of the solution and titration of the total soluble alkalinity with a standard volumetric solution of hydrochloric acid, using methyl orange as indicator.

Reagents

During the analysis, use only reagents of recognized analytical reagent grade and only distilled water or water of equivalent purity.

(1) Hydrochloric acid, 1 N standard volumetric solution.

(2) Methyl orange, 0.5 g/l solution (the methyl orange may be replaced by any other indicator giving the same end-point).

Equipment - ordinary laboratory apparatus.

Procedure

Weigh test portion of sodium carbonate, to the nearest 0.01 g, a mass of the test sample of 50±0.1 g, 59±0.1 g, 110±0.1 g or 135±0.1 g, depending on whether the product is anhydrous or mono-, hepta- or decahydrate.

Dissolve the test portion by pouring it in small quantities at a time, while stirring, into a beaker of suitable capacity (for example 600 ml) containing 200 ml of water at about 50 °C.

Filter the decanted solution through a medium-speed filter, collecting the filtrate in a 500 ml one-mark volumetric flask.

Wash the insoluble matter onto the filter with water at about 50 °C. Complete the washing, collecting all the washings in the volumetric flask. Allow to cool, dilute to the mark and mix.

Transfer 25.0 ml of the test solution to a 500 ml conical flask. Add approximately 75 ml of water, 5 drops of the methyl orange solution (2) and titrate with the standard volumetric hydrochloric acid solution (1) until the indicator turns from yellow to orange-pink.

NOTE - If required, this determination may also be carried out by means of back-titration, using 1 N standard volumetric hydrochloric acid solution and 1 N or 0.1 N standard volumetric sodium hydroxide solution. If this method is adopted, it shall be taken into account in the calculation of results.

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Expression of results

The total soluble alkalinity (*TSA*), expressed as a percentage by mass of sodium carbonate (Na_2C0_3), is given by the formula:

$$TSA = V \times \frac{500}{25} \times \frac{100}{m} \times 0.0530 = 106\frac{V}{m}$$

where

V is the volume, in millilitres, of the standard volumetric hydrochloric acid solution (1) used for the titration;

m is the mass, in grams, of the test portion;

0.0530 is the mass, in grams, of sodium carbonate equivalent to 1 ml of exactly 1 N standard volumetric hydrochloric acid solution.

Notes

1 If the concentration of the standard volumetric solution used is not exactly as specified in the list of reagents, an appropriate correction should be made.

2 If it is desired to express the result on the basis of non-volatile matter at 250 °C, multiply the result obtained on the product as received by the ratio

$$\frac{100}{100 - \text{loss of mass at } 250\ ^\circ\text{C in \% (m/m)}}$$

Reference – IS0 740-1976: Sodium carbonate for industrial use - Determination of total soluble alkalinity - Titrimetric method

Determination of sodium sulphide

Principle of the method is based on a preparation of solution of a test portion and titration of the sulphides with a standard volumetric solution of potassium hexacyanoferrate(III), using ***sodium nitroprusside*** as indicator.

Reagents

During the analysis, use only reagents of recognized analytical grade, and only distilled water or water of equivalent purity, neutral to methyl red.

(1) Sodium hydroxide, 0.1 N volumetric solution;

(2) Potassium hexacyanoferrate(III) ($K_3Fe(CN)_6$), 0.1 N standard volumetric solution;

(3) ***Sodium nitroprusside (***$Na_2[Fe(CN)_5NO]$), 4 g/l solution in distilled water.

Apparatus

Ordinary laboratory apparatus and

Burette, graduated in 0.05 ml;

1000 ml one-mark volumetric flask;

25 ml pipette.

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Procedure

Weigh test portion of sodium sulphide in a weighing dish, previously tared to the nearest 0.0001 g, weigh, to the nearest 0.0001 g, approximately 10 g of the test sample.

Quantitatively transfer the test sample into a 1000 ml one-mark volumetric flask. Pour about half of flask 0.1 N sodium hydroxide (1) and dissolve the test portion. Dilute to the mark by 0.1 N sodium hydroxide solution (1) and mix getting a sample solution.

Take with pipette 25 ml sample solution. Transfer quantitatively to 500 ml flask; add 10 ml 0.1 N sodium hydroxide solution (1), 50 ml distilled water and about 1 ml ***sodium nitroprusside*** 4 g/l solution (3) and promptly titrate with potassium hexacyanoferrate(III) ($K_3Fe(CN)_6$), 0.1 N standard volumetric solution (2) until the colour changes from violet to light yellow.

Expression of results

The total amount (TA) of sodium sulphide, expressed as a percentage by mass of, is given by the formula:

$$\mathrm{TA} = \frac{V \times 0.0039 \times 1000}{25} \times \frac{100}{m} = \frac{V \times 15.6}{m}$$

where

V is the volume, in millilitres, of the potassium hexacyanoferrate solution (2) used for the titration;

m is the mass, in grams, of the test portion;

0.0039 is the mass, in grams, of sodium sulphide corresponding to 1 ml of exactly 0.1 N potassium hexacyanoferrate solution (2).

Reference – Golovtejeva A.A., Kucidi L.B., Sankin L.B. Laboratory compendium of chemistry and technology of leather and fur. Moscow: Legkaiya promyslennost, 1982.

Determination of calcium hydroxide

Principle of the method is based on a preparation of solution of a test portion and titration of the calcium hydroxide with a standard volumetric solution of hydrochloric acid, using ***phenolphthalein*** as indicator.

Reagents

During the analysis, use only reagents of recognized analytical grade, and only distilled water or water of equivalent purity, neutral to methyl red.

(1) Hydrochloric acid, 0.1 N volumetric solution;

(2) ***Phenolphthalein*** 1 g dissolve in 70 ml 95 % (V/V) ethanol and ad 30 ml of distilled water.

Apparatus

Ordinary laboratory apparatus and

Burette, graduated in 0.05 ml;

1000 ml one-mark volumetric flask;

50 ml pipette.

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Procedure

Weigh test portion of calcium hydroxide in a weighing dish, previously tared to the nearest 0.0001 g, weigh, to the nearest 0.0001 g, approximately 1 g of the test sample.

Quantitatively transfer the test sample into a 1000 ml one-mark volumetric flask. Pour about 800-900 ml of distilled water and dissolve the test portion. Dilute to the mark with distilled water and mix getting a sample solution.

Take with pipette 50 ml sample solution. Transfer quantitatively to 500 ml flask; add 3-4 drops of ***phenolphthalein*** solution (2) and titrate with hydrochloric acid 0.1 N standard volumetric solution (1) until the violet-red colour disappears.

Expression of results

The total amount (TA) of calcium hydroxide, expressed as a percentage by mass of, is given by the formula:

$$\mathrm{TA} = \frac{V \times 0.0037 \times 1000}{50} \times \frac{100}{m} = \frac{V \times 7.4}{m}$$

where

V is the volume, in millilitres, of the hydrochloric acid solution (1) used for the titration;

m is the mass, in grams, of the test portion;

0.0037 is the mass, in grams, of calcium hydroxyde corresponding to 1 ml of exactly 0.1 N hydrochloric acid solution (1).

Reference – Golovtejeva A.A., Kucidi L.B., Sankin L.B. Laboratory compendium of chemistry and technology of leather and fur. Moscow: Legkaiya promyslennost, 1982.

Testing of ammonia sulphate: determination of ammoniacal nitrogen content

Principle – the method is based on a distillation of the ammonia after displacement by an alkali solution; absorption in an excess of standard volumetric sulphuric acid solution and back-titration with standard volumetric sodium hydroxide solution in the presence of an indicator.

Reagents

During the analysis, use only reagents of recognized analytical reagent grade and only distilled water or water of equivalent purity.

(1) Sodium hydroxide, 450 g/l solution;

(2) Sulphuric acid, 0.5 N standard volumetric solution.

(3) Sodium hydroxide, 0.5 N standard volumetric solution.

(4) Mixed indicator, ethanol solution. Dissolve 0.1 g of methyl red in about 50 ml of 95 % (V/V) ethanol, add 0.05 g of methylene blue and, after dissolution, dilute to 100 ml with the same ethanol.

Apparatus

Ordinary laboratory apparatus and:

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Distillation apparatus, with, preferably, spherical ground glass joints, or any apparatus that will ensure quantitative distillation and absorption. The apparatus may, for example, be made up from the following items (see figure):

distillation flask (A), capacity 1000 ml, with a female joint;

splash head (B), with male joints and parallel inlet and outlet into which is fused a cylindrical *dropping funnel* (C), capacity 50 ml;

Liebig condenser (D), effective length about 400 mm, fitted with a female joint at the inlet and a male joint at the outlet;

conical flask (E), capacity 500 ml, with a female joint, fitted with two side bulbs;

spring clamps (F).

Procedure

Prepare the test portion (*m*) by weighting, to the nearest 0.001 g, about 10 g of the test sample.

Carry out a blank test at the same time as the determination, following the same procedure and using the same quantities of all the reagents as used for the determination.

Prepare the sample solution by placing the test portion in a 500 ml one-mark volumetric flask. Dissolve the test portion in water, dilute to the mark and mix.

Place 50 ml of the sample solution in the distillation flask (A). Add about 350 ml of water and a few anti-bumping granules.

Coat the joints of the apparatus with a Silicone grease. Mount the splash head (B) on the flask (A), and connect it to the condenser (D).

Place 40 ml of the standard volumetric sulphuric acid solution (2), about 80 ml of water and a few drops of the mixed indicator solution (4) into the flask (E). Connect the flask (E) to the condenser (D), ensuring that all the joints of the apparatus are firm by means of the spring clamps (F).

Introduce 20 mi of the sodium hydroxide solution (1) into the flask (A) through the dropping funnel (C), taking care to leave at least a few millimetres of liquid above the tap.

Distil until the volume of liquid in the flask (E) has reached about 250 to 300 ml. Stop the heating, open the tap of the dropping funnel (C), disconnect the splash head (B) and wash the condenser (D) carefully, collecting the wash water in the flask (E). Finally disconnect the flask (E).

Carefully mix the solution contained in the flask (E) and in the two side bulbs, and back-titrate the excess of the standard volumetric sulphuric acid solution (2) with the standard volumetric sodium hydroxide solution (3). During the titration, stir carefully to ensure that the solution is completely mixed.

Note. The procedure is described in terms of the apparatus specified in Fig. and will require modification if other apparatus is used.

Expression of results

The ammoniacal nitrogen content, expressed as a percentage by mass of nitrogen (N), is given by the formula:

$$\mathrm{N} = (V_1 - V_2) \times 0.007004 \times \frac{500}{50} \times \frac{100}{m} = \frac{7.004 \times (V_1 - V_2)}{m}$$

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where

V_1 is the volume, in millilitres, of the standard volumetric sodium hydroxide solution (3) used for the back-titration of the excess of the Standard volumetric sulphuric acid Solution (2) placed in the flask (E) for the blank test;

V_2 is the volume, in millilitres, of the standard volumetric sodium hydroxide solution (3) used for the back-titration of the excess of the standard volumetric sulphuric acid solution (2) placed in the flask (E) for the determination;

m is the mass, in grams, of the test portion;

0.007004 is the mass, in grams, of nitrogen corresponding to 1 ml of 0.5 N standard volumetric sulphuric acid solution.

Note. If the concentrations of the standard volumetric solutions are not exactly as specified in the list of reagents, appropriate corrections should be made.

Reference – ISO 3332-1975: Ammonium sulphate for industrial use - Determination of ammoniacal nitrogen content – Titrimetric method after distillation.

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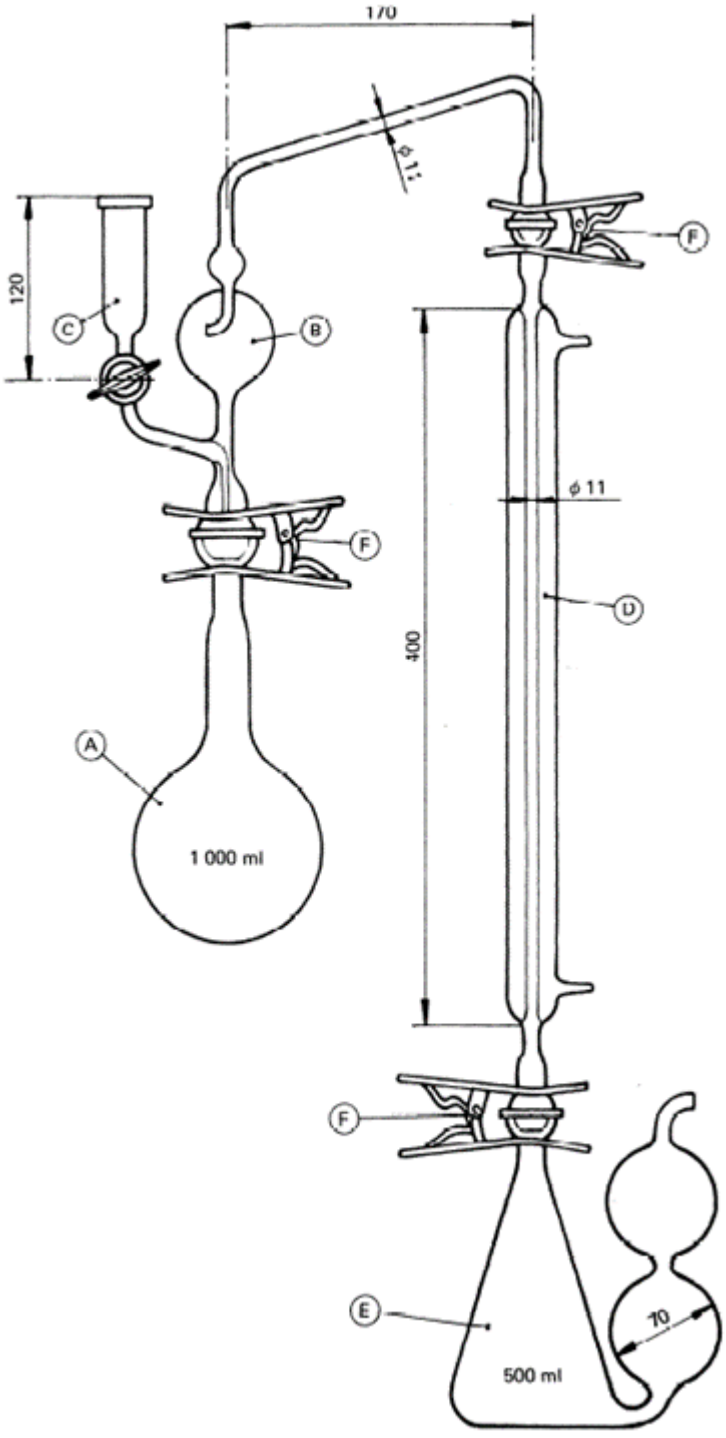

Figure 2.49. Typical distillation apparatus (dimensions in mm)

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Proteolytic activity: testing of enzyme or enzyme preparation by TEGEWA method

Principle. The method is based on ability break peptide bonds in substrate by proteolytic enzymes. In this assay, casein acts as a substrate. When the protease we are testing digests casein, the amino acid tyrosine is liberated along with other amino acids and peptide fragments. Folin and Ciocalteus Phenol (F-C), or Folin's reagent primarily reacts with free tyrosine to produce a blue colored chromophore, which is quantifiable and measured as an absorbance value on the spectrophotometer. The more tyrosine that is released from casein, the more the chromophores are generated and the stronger the activity of the protease. Absorbance values generated by the activity of the protease are compared to a standard curve, which is generated by reacting known quantities of tyrosine with the F-C reagent to correlate changes in absorbance with the amount of tyrosine in micromoles. From the standard curve the activity of protease samples can be determined in terms of Units, which is the amount in micromoles of tyrosine equivalents released from casein per minute.

Reagents

During the analysis, use only reagents of recognized analytical reagent grade and only distilled water or water of equivalent purity.

(1) 50 mM potassium phosphate buffer, pH 7.5. Prepare using 11.4 mg/ml of potassium phospate dibasic, trihydrate in purified water and adjusting pH with 1M HCl. This solution is placed at 37°C prior to use.

(2) 0.65% weight/volume casein solution, prepared by mixing 6.5 mg/ml of the 50 mM potassium phosphate buffer. The solution temperature is gradually increased with gentle stirring to 80-85 °C for about 10 minutes until a homogenous dispersion is achieved. It is very important not to boil the solution. The pH is then adjusted if necessary with NaOH and HCl.

(3) 110 mM trichloroacetic acid solution (TCA), prepared by diluting a 6.1N stock 1:55 with purified water. Trichloroacetic acid is a strong acid and should be handled with care.

(4) 0.5 M F-C, or Folin's Phenol Reagent, which is the solution that will react with tyrosine to generate a measurable color change that will be directly related to the activity of proteases. Folin's Phenol Reagent is an acid and should be handled with care.

(5) 500 mM sodium carbonate solution, prepared using 53 mg/ml of anhydrous sodium carbonate in purified water.

(6) An enzyme diluent solution, which consists of 10 mM sodium acetate buffer with 5mM calcium, pH 7.5, at 37°C. This solution is used to dissolve solid protease samples or dilute liquid enzyme solutions. If necessary, a solid protease sample of predetermined activity can be used, which is dissolved using enzyme diluent to 0.1-0.2 units/ml. This solution serves as a positive control for the quality control assay and as validation for the calculations which will be performed to determine enzyme activity.

(7) 1mM L-tyrosine Standard stock solution. Prepared using 0.2 mg/ml L-tyrosine in purified water and heated gently until the tyrosine dissolves. As with the casein, do not boil this solution. Allow the L-tyrosine standard to cool to room temperature. This solution will be diluted further to make our standard curve.

NOTE

Apparatus

Ordinary laboratory apparatus and:

0.45 µm polyethersulfone syringe filter and syringe;

Dram vials or polypropylene tubes capable of holding 15 ml of solution;

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Spectrophotometer;

Cuvettes;

Pipettes;

Stir/Hot plate;

Stir bar;

Scale;

pH Meter

Procedure

To begin this assay, find suitable vials that will hold about 15 ml. For each enzyme that you will test, you will need 4 vials. One vial will be used as a blank, and three others will be used to assay activity of three dilutions of the protease. Three dilutions are useful when checking our final calculations against each other. To each set of four vials add 5 ml of our 0.65% casein solution (2), and let them equilibrate in a water bath at 37°C for about 5 minutes. Then, add varying volumes of enzyme solution you want to test to three of the test sample vials, but not the blank. Mix them by swirling and incubate for 37 °C for exactly ten minutes. The protease activity and consequential liberation of tyrosine during this incubation time is what will be measured and compared between test samples.

NOTE. If solid sample is used, prepare the test portion by weighing, to the nearest 1 mg g, about 100-1000 mg (*m*) of the solid sample. Put the sample into a 50-100 ml glass beaker, pour about 10 ml of diluent solution (6) and thoroughly grind with glass stick the sample to obtain homogenous solution (suspension). Quantitatively transfer the test portion into a 100 ml one-mark volumetric flask (when solid sample is used transfer the ground test portion into volumetric flask by washing the diluent solution (6)). Dilute to the mark with diluent solution (6) and mix getting a sample solution. Use the sample solution in the same way as liquid enzyme.

After this 10 minute incubation, add the 5 ml of the TCA reagent (3) to each tube to stop the reaction. Then an appropriate volume of enzyme solution is added to each tube, even the blank, so that the final volume of enzyme solution in each tube is 1 ml. This is done to account for the absorbance value of the enzyme itself and ensure that the final volume in each tube is equal. Now incubate the solutions at 37°C for 30 minutes.

During this 30 minute incubation, set up your tyrosine standard dilutions, which is done using 6 dram vials (dram vials can be substituted with polypropylene tubes) that can easily hold 8 ml. To the six vials the 1.1 mM tyrosine standard stock solutions (7) is added with the following volumes in ml: 0.05, 0.10, 0.20, 0.40, 0.50. Don't add any tyrosine standard to the blank. Lower standards may be needed for impure test samples with that will yield little color change. Once the tyrosine standard solution has been added, add an appropriate volume of purified water to each of the standards to bring the volume to 2 ml.

After the 30 minute incubation, filter each of the test solutions and the blank using a 0.45 um polyethersulfone syringe filter. Filtration is required to remove any insolubles from the samples. The filtration 2 ml of the test samples and blank filtrate is then added to 4 dram vials that can hold at least 8 ml. Use the same type of vial in which the standards are prepared. To all of the vials containing the standards and standard blank, 5 ml of sodium carbonate (5) is added, and for best results, 1 ml of F-C or Folin's reagent (4) is added immediately afterwards. sodium carbonate (5) is added to regulate any pH drop created by the addition of the Folin's reagent. Sodium carbonate (5) is then added to test samples and test blank. Notice that these solutions become cloudy after the addition of sodium carbonate. Then, the F-C or Folin's reagent (4) is added, which

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will react primarily with free tyrosine. The dram vials are then mixed by swirling and incubated at 37°C for 30 minutes.

After this incubation, notice that the standards have a gradation of colour correlating with the amount of tyrosine added; the highest concentrations of tyrosine appearing darkest. You can also notice appreciable colour change in our test samples. 2 ml of these solutions are filtered using a 0.45 um polyethersulfone syringe filter into suitable cuvettes. Measure the absorbance of the solutions and record the absorbance values.

The absorbance of the samples is measured by a spectrophotometer using a wavelength of 660 nm. The light path is set to 1 cm. Record the absorbance values for the standards (see Table), standard blank, the different test samples, and test blank. Once all of the data has been collected, create standard curve. In order to generate the curve, difference in absorbance between the standard and standard blank must be calculated. This is the absorbance value attributable to the amount of tyrosine in the standard solutions (7). After this simple calculation, draw the standard curve by plotting the change in absorbance of standards on the Y axis, versus the amount in micromoles for each of 5 standards on the X axis and generate a line of best fit and corresponding slope equation.

Table 2.10. Volume of tyrosine standard

Volume of tyrosine standard (7), ml	µMoles tyrosine
0.05	0.055
0.10	0.111
0.20	0.221
0.40	0.442
0.50	0.553

Calculation and expression of results

Find the change in absorbance in test samples by calculating the difference between test sample absorbance and the absorbance of test blank. Inserting the absorbance value for one of the test samples into the slope equation and solving will result in the micromoles of tyrosine liberated during this particular proteolytic reaction. To get the activity of enzyme in units per/ml of liquid enzyme, perform the following calculation:

$$\text{Units / ml enzyme} = \frac{(\mu\text{mole tyrosine equivalents released}) \times 11}{1 \times 10 \times 2}$$

where

11 is total volume of assay, ml;

10 is time of assay as per Unit definitiom, minutes;

1 is volume of liquid enzyme used, ml;

2 is volume used in colorimetric determination, ml.

To get the activity of enzyme in units per/mg of solid enzyme, use the formula:

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$$\text{Units / mg enzyme} = \frac{(\text{units / ml enzyme}) \times 100}{m}$$

100 is the total volume of solid sample solution, ml;

m is the mass of solid enzyme sample, taken for the preparation of the sample solution, mg.

References:

[1] https://www.sigmaaldrich.com/video/life-science/universal-protease-activity-assay.html
[2] GOST 20264.2-88. Enzyme preparations. Methods for determination of proteolytic activity. http://docs.cntd.ru/document/1200023426.

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Determination of total acidity of sulphur acid which contents equal to or lower than 98 % (m/m)

Principle of the method is based on a titration of the total acidity with a standard volumetric sodium hydroxide solution, in the presence of methyl red as indicator.

Reagents

During the analysis, use only reagents of recognized analytical grade, and only distilled water or water of equivalent purity, neutral to methyl red.

(1) Hydrogen peroxide, 60 g/l solution, neutral to methyl red;

(2) Sodium hydroxide, 1 N standard volumetric solution;

(3) Methyl red, 1 g/l solution in 95 % (V/V) ethanol.

Apparatus

Ordinary laboratory apparatus and

Flask, capacity approximately 500 ml, with neck of diameter about 30 mm, with ground glass stopper.

Burette, graduated in 0.05 ml;.

Conical flask, capacity 500 ml, with ground glass stopper.

Procedure

Weigh test portion of sulphur acid in a weighing bottle, previously tared to the nearest 0.0001 g, weigh, to the nearest 0.0001 g, approximately 2 g of the test sample.

Transfer the test portion quantitatively to a 500 ml conical flask containing approximately 300 ml of water.

Add 5 ml of the hydrogen peroxide solution (1), heat to boiling and boil gently for 10 min.

Allow to cool, add 2 drops of the methyl red solution (3) and titrate with the sodium hydroxide solution (2) until the colour changes from red to yellow.

Expression of results

The total acidity (TA), expressed as a percentage by mass of sulphuric acid (H_2SO_4), is given by the formula:

$$\mathrm{TA} = \frac{V \times 0.04904 \times 100}{m} = \frac{V \times 4.904}{m}$$

where

V is the volume, in millilitres, of the sodium hydroxide solution (2) used for the titration;

m is the mass, in grams, of the test portion;

0.04904 is the mass, in grams, of sulphuric acid corresponding to 1 ml of exactly 1 N sodium hydroxide solution.

Reference – IS0 910-1977: Sulphuric acid and oleum for industrial use – Determination of total acidity, and calculation of free sulphur trioxide content of oleum - Titrimetric method.

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Testing of acetic acid

Principle of the method is based on a preparation of solution of a test portion and titration of the acetic acid with a standard volumetric solution of sodium hydroxide, using ***phenolphthalein*** as indicator.

Reagents

During the analysis, use only reagents of recognized analytical grade, and only distilled water or water of equivalent purity, neutral to methyl red.

(1) Sodium hydroxide, 0.1 N volumetric solution;

(2) ***Phenolphthalein*** 1 g dissolve in 70 ml 95 % (V/V) ethanol and ad 30 ml of distilled water.

Apparatus

Ordinary laboratory apparatus and

Burette, graduated in 0.05 ml;

1000 ml one-mark volumetric flask;

50 ml pipette.

Procedure

Weigh test portion of calcium hydroxide in a weighing beaker, previously tared to the nearest 0.001 g, weigh, to the nearest 0.001 g, approximately 1 g of the test sample.

Quantitatively transfer the test sample into a 1000 ml one-mark volumetric flask by washing with distilled water. Dilute to the mark with distilled water and mix getting a sample solution.

Take with pipette 50 ml sample solution. Transfer quantitatively to 250-500 ml flask; add 3-4 drops of ***phenolphthalein*** solution (2) and titrate with sodium hydroxide 0.1 N standard volumetric solution (1) until the light pink colour appears, which does not disappear during 20 minutes.

Expression of results

The total amount (TA) of acetic acid, expressed as a percentage by mass of, is given by the formula:

$$\mathrm{TA} = \frac{V \times 0.006 \times 1000}{50} \times \frac{100}{m} = \frac{V \times 12}{m}$$

where

V is the volume, in millilitres, of the sodium hydroxide solution (1) used for the titration;

m is the mass, in grams, of the test portion;

0.0037 is the mass, in grams, of acetic acid corresponding to 1 ml of exactly 0.1 N sodium hydroxide solution (1).

Reference – Golovtejeva A.A., Kucidi L.B., Sankin L.B. Laboratory compendium of chemistry and technology of leather and fur. Moscow: Legkaiya promyslennost, 1982.

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Sodium chloride analysis

Determination of matter insoluble in water or in acid and preparation of principal solutions for other determinations

Principle – the determination is based on a preparation of a solution of a test portion in water. Filtration, drying and weighing of the insoluble residue. Dilution of the filtrate to form the principal solution (solution A) for carrying out other determinations.

Reagents

Distilled water, or water of equivalent purity, shall be used in the test.

(1) Silver nitrate, 5 g/l nitric solution. Dissolve 0.5 g of silver nitrate in a little water, add 10 ml of nitric acid solution d = 1.4 g/ml approximately, and dilute to 100 ml.

Equipment

Ordinary laboratory apparatus and

Filter crucible or funnel, glass or porcelain, approximately 30 mm diameter and of a porosity grade P 10 or P 16 (pore size index 4-16 µm).

Electric oven, ventilated by convection and capable of being controlled at 110±2 °C.

Desiccator, containing silica gel, phosphorus pentoxide or a molecular sieve.

Procedure

Weigh, to the nearest 0.01 g, approximately 100 g of the test sample.

Place the test portion in a 600 ml beaker and add 350 ml of water. Heat at just below boiling for 10 min, with stirring, and then transfer the beaker, covered with a watch glass, to a boiling water bath for 30 min. Cool to approximately 20°C.

Filter by vacuum on the filter crucible, previously dried at 110°C, cooled in the desiccator, and weighed to the nearest 0.1 mg. Then wash the insoluble matter, in groups of five successive washings, using 20 ml of water each time, disconnecting the vacuum after each washing in order to bring the insoluble matter into suspension for approximately 1 min before filtering, and checking for absence of chloride from the filtrate after the fifth, tenth or fifteenth washing. 10 ml of the washing water shall remain clear 5 min after adding 10 ml of the nitric silver nitrate solution (1). Cease washing as soon as the check indicates absence of chlorides.

Dry the crucible and its contents in the oven (2) controlled at 110±2°C for 1 h, cool in the desiccator and weigh to the nearest 0.1 mg. Repeat this operation until two weightings differ by not more than 0.2 mg.

NOTE - If the insoluble matter is so finely divided as to clog the filter, repeat the determination and add, after the 350 ml of water, 1.5 g, weighed to the nearest 0.1 mg, of a filter aid (kieselguhr) of analytical quality. The minimum particle size of the filter aid should be µm and it should be dried, at about 110°C, to constant mass before use.

Preparation of the principal solution for other determinations **(solution A)**

Quantitatively transfer the filtrate obtained, after filtering and washing of the insoluble matter, to a 1000 ml one-mark volumetric flask. Dilute to the mark and mix. Keep this solution for the other determinations.

Expression of results

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The matter insoluble in water (M) is given, as a percentage by mass, by the formula:

$$M = (m_1 - m_2)\frac{100}{m_0}$$

where

m_0 is the mass, in grams, of the test portion;

m_1 is the mass, in grams, of the filter crucible and insoluble matter;

m_2 is the mass, in grams, of the filter crucible alone.

NOTE - If a filter aid has been used, deduct its mass from ml (see NOTE in ***Procedure***).

Reference – ISO 2479-1972: Sodium chloride for industrial use – Determination of matter insoluble in water or in acid and preparation of principal solutions for other determination

Determination of halogens, expressed as chlorine, by mercurimetric method

Principle – the method is based on a titration of the halogen ions with mercury (II) nitrate in the presence of diphenylcarbazone as indicator.

Reagents

Distilled water, or water of equivalent purity, shall be used in the test.

(1) Nitric acid, *d* = 1.40 g/ml, approximately 68 % (m/m) or approximately 14 N solution;

(2) Nitric acid, approximately 2 N solution.

(3) Sodium chloride, 0.1 N standard reference solution.

Weigh, to the nearest 0.1 mg, 5.8443 g of sodium chloride, previously dried for 1 h at 500°C and cooled in a desiccator. Dissolve it in water in a 1000 ml one-mark volumetric flask, dilute to the mark and mix.

(4) Standard end-point matching solution.

Prepare this standard solution immediately before use. Pour 200 ml of water into a 500 ml conical flask, followed by 3 drops of bromophenol blue solution (6) and the nitric acid solution (2), added drop by drop until the colour changes from blue to yellow. Add an excess of 3 drops of this acid, between 0.5 and 1.0 ml of the diphenylcarbazone solution (7) and the volume of mercury(II) nitrate solution (5) (from a burette) necessary to change the colour of the solution from yellow to mauve (about 1 drop).

(5) Mercury(II) nitrate, 0.1 N standard volumetric solution.

Preparation of the solution. Weigh 10.85 ± 0.01 g of mercury(ll) oxide (HgO) and dissolve it in 10 ml of the nitric acid solution (1), in a 1000 ml one-mark volumetric flask, dilute to the mark and mix.

Standardize this solution, following the procedure described in *Standardization of the solution,* adjusting it to the exact concentration if necessary.

Standardization of the solution

Transfer 40.0 ml of the sodium chloride standard reference solution (3) to a 500 ml conical flask, followed by 160 ml of water and 3 drops of the bromophenol blue solution (6). Add the nitric acid solution (2) drop by drop until the colour of the indicator changes from blue to yellow.

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Add an excess of 3 drops of this acid and same volume of the diphenylcarbazone solution (7) as in the standard end-point matching solution (4). Titrate the chloride with the mercury(II) nitrate solution to be standardized until the colour matches the mauve of the standard end-point matching solution (4) and deduct the volume of mercury(ll) nitrate solution (5) added during the preparation of this standard end-point matching solution (about 1 drop).

The correct amount is 40.00ml.

(6) Bromophenol blue, 1 g/l solution in 95 % (V/V) ethanol.

(7) Diphenylcarbazone, 5 g/l solution in 95 % (V/V) ethanol. Store this solution in a refrigerator and replace it when it no longer gives a sharp colour change.

Apparatus

Ordinary laboratory apparatus.

Procedure

Preparation of test portion. Take 50 ml of the principal solution A (see subchapter 2.8.1 Determination of matter insoluble in water or in acid and preparation of principal solutions for other determinations: *Preparation of the principal solution for other determinations* (solution A)), containing 100 g of the test sample per 1000 ml.

Determination. Get a sample solution by placing the test portion in a 500 ml one-mark volumetric flask. Dilute the test portion to the mark and mix.

Place 25 ml of the sample solution in a 500 ml conical flask. Dilute to 200 ml, then add 3 drops of the bromophenol blue solution (6) and the nitric acid solution (2) drop by drop, until the colour changes from blue to yellow. Add a further 3 drops of this acid and the same volume of the diphenylcarbazone solution (7) as in the standard end-point matching solution (4).

Titrate with the mercury(II) nitrate solution (5) until the colour matches the mauve of the standard end-point matching solution (4).

Expression of results

The halogens content, expressed as chlorine (Cl), is given, as a percentage by mass, by the formula:

$$\mathrm{Cl} = (V - V_1) \times \frac{500}{25} \times \frac{1000}{50} \times \frac{100}{m} \times 0.003545 = \frac{141.8(V - V_1)}{m}$$

where

m is the mass, in grams, of the test portion used for preparing the **principal solution A**.

V is the volume, in millilitres, of volumetric mercury(II) nitrate solution (5) used for titration;

V_1 is the volume, in millilitres, of the standard volumetric mercury(II) nitrate solution (5) used in the preparation of the standard end-point matching solution;

0.003545 is the mass, in grams, of chlorine corresponding to 1 ml of the standard volumetric mercury(II) nitrate solution (5).

Reference – ISO 2481-1973: Sodium chloride for industrial use – Determination of halogens, expressed as chlorine – Mercurimetric method.

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2.6.2. Testing of tanning materials

Analysis of vegetable and synthetic tanning materials

Principle – the method is based on an indirect gravimetric analysis of vegetable and synthetic tanning agents through fixing of the absorbent compounds on low-chromed hide powder.

Reagents

During the analysis, use only reagents of recognized analytical reagent grade and only distilled water or water of equivalent purity.

(1) Freshly prepared distilled water. The pH value of the water shall be between 5 and 6. When using methyl red, the water should not turn red. The evaporation residue of 100 ml should be less than 1 mg;

(2) Hide powder containing not more than 0.5 % chromium oxide and with a humidity not more than 13 %.

(3) Gelatine solution, of 1 g gelatine and 10 g sodium chloride, filled up to 100 ml with distilled water, adjusted to pH = 4.7.

Apparatus

The glass equipment shall be resistant to the action of distilled water. The flasks and tubes shall be Class A. Use normal laboratory equipment and, in particular, the following.

Desiccator, with an airtight cover and containing silica orange gel;

Evaporation dishes, suitable for slowly evaporating water. These shall be short with flat bases and measure 7 cm to 8.5 cm in diameter. Use silver dishes. If this is not possible, preferably use dishes made of stainless steel or, if necessary, ceramic or glass;

Water bath;

Drying oven, whose temperature shall be kept at the operating range of (102±2)°C;

Analytical balance, with a precision of 0.2 mg at a load of 200 g;

Technical balance, with a precision of 0.1 g at a load of 1000 g;

Procter bell (see Figure 1), composed of a cylindrical glass bell (length of the cylindrical part 90mm±1mm, internal diameter of the cylindrical pan: 28mm±1mm). A perforated rubber cork is inserted into the narrow part of the bell. A capillary glass tube (internal diameter 1.5mm) with two right-angled bends is inserted into the hole in the cork. The end of the shortest part can fit right down to the base of the cork;

Polyethylene tube, the tube shall be the right size to fit onto the bell's capillary glass tube;

Hoffman clamp;

1000 ml volumetric flasks;

50 ml pipette;

Vacuum filter system (e.g. Figure 2);

Cellulose acetate membrane filters, with pores of 0.45µm and 3µm;

50 ml and 100 ml measuring cylinders.

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Procedure

Sample preparation. There should be a generous, representative sample of the tanning agents for analysis; this should be thoroughly mixed. If the particles are heterogeneous, resort to manual or mechanical milling to homogenize the size of the particles. The particle size should not be smaller than 300 µm to avoid blocking the Procter bell.

Preparation of the analytical solution.

a) *Vegetable tanning agents in powder/solid form*:

Weigh the appropriate quantity (see Table) of vegetable tanning agents on an analytical balance. Add this to 800 ml of hot (60 °C to 80 °C) distilled water in a 1000 ml volumetric flask. Shake the flask to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

The aim is to obtain an analytical solution containing between 3.75 g and 4.25 g of substances absorbed by the hide powder. If the tanning content in the solution goes beyond these limits, repeat the analysis with a sample of suitable quantity.

Table 2.11. Tanning agents

Tanning agents, %	Approximate amount to weigh, g
50	8. 0
55	7.3
60	6.6
65	6.1
70	5.7
75	5.3
80	5.0
90	4.2

b) *Vegetable tanning agents in liquid form:*

Weigh the tanning agents on an analytical balance, taking into account the percentage of content in dry form. Add this to in a 1000 ml volumetric flask containing 800 ml of hot (60 °C to 80 °C) distilled water Shake the flask to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

The aim is to obtain an analytical solution containing between 3.75 g and 4.25 g of substances absorbed in the hide powder. If the tanning content in the solution goes beyond these limits, repeat the analysis with a sample of suitable quantity.

c) *Synthetic tanning agents in powder form:*

Weigh about (4±0.1) g of tanning agents on an analytical balance. Add this to a 1000 ml volumetric flask containing 800 ml of hot (40 °C to 50 °C) distilled water. Shake the flash to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

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In the case of breakthrough of the tanning agent, repeat the analysis using a lower mass.

d) *Synthetic tanning agents in liquid form:*

Weigh about (8±0.1) g of tanning agents on an analytical balance. Add it to a 1000 ml volumetric flask containing 800 ml of hot (40 °C to 50 °C) distilled water. Shake the flask to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

In the case of breakthrough of the tanning agent, repeat the analysis using a lower mass.

e) *Vegetable tanning agents organic solvent extracted in powder form:*

Weigh about (4±0.1) g of tanning agents on an analytical balance. Add this to a 1000 ml volumetric flask containing 800 ml of hot (40 °C to 50 °C) distilled water. Shake the flask to fully dissolve the tanning agents. There may be some residue if there is any insoluble matter in the sample. Leave it to cool down in a water bath at (20±2) °C and add distilled water up to the mark.

Select the amount in function of the quality of the extract desired. The final concentration of the analytical solution should contain about 4 g of tanning compound per litre.

Preparation of the bell

Place a layer of cotton wool at the top of the bell to prevent the hide powder from entering the capillary tube. Put the rubber cork containing the glass capillary tube in the bell. Weigh 7.0 g of hide powder on a technical balance and introduce it uniformly in the bell, pressing it down, up to the top of the rim. Check that the hide powder is fully pressed down to ensure that it will be completely tanned. Put the polyethylene tube in the glass capillary tube and use the Hoffman clamp.

De-tanning the analytical solution (determination of the non-tanning agents)

Place the bell containing hide powder in a beaker of suitable capacity. Fill the beaker with the unfiltered analytical solution up to the neck of the bell. When the hide powder is completely soaked, suck on the longer end of the capillary tube to create a slight depression and start siphoning the solution.

Use the Hoffman clamp to adjust the flow of the solution so that about 8 to 10 drops of the de-tanned solution drip through per minute. The resulting solution shall be clear.

Collect a total of 90 ml in (120±10) min.

The first 30 ml of the filtrate should be collected in a 50 ml glass measuring cylinder and disposed of.

The next 60 ml should be collected in a perfectly dry 100 ml glass measuring cylinder to determine the non-tanning agents. To control the breakthrough of tanning agents use 5 ml of the collected solution and add 0.5 ml gelatine solution. The pH of the total solution should be lower than 5. If necessary, use a few drops of formic acid to reduce the pH. A white precipitate is an indication of a breakthrough. In this case, repeat the analysis with a lower sample mass.

The solution should be at a temperature no less than 18 °C and no more than 20 °C. Use the pipette to transfer 50 ml of the filtered solution into a previously dried and weighed silver dish.

Place the dish on the water bath and wait for complete evaporation. Put the dish in the oven at (102±2) °C to attain constant mass (about 18 h±2 h). Put the dish in the silica gel dryer and weigh it after 15 min on analytical balance.

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Determination of soluble substances

To filter the analytical solutions, use the filter system indicated in Figure 2. Use the cellulose acetate membranes with 0.45 µm pores. If filtration proves awkward, use membranes with 3.0 µm pores and then pass the pre-filtered solution through the 0.45 µm membranes

If the filtration is not possible, the solution should be centrifuged. Collect about 100 ml of filtrate. Use the pipette to transfer 50 ml of the filtered solution into a previously dried and weighed silver dish.

Place the dish on the water bath and wait for complete evaporation. Put the dish in the oven at (102±2) °C to attain constant mass (about 18 h±2 h). Put the dish in the silica gel dryer and weigh it after 15 min on analytical balance.

Determination of total solids

Calibrate a silver dish in the oven at (102±2)°C and then cool it off in the dryer, for about 15 min, down to ambient temperature.

Weigh the dish with the analytical balance. Add about 3 g to 5 g of the sample to the dish. Record the mass as m_{P0}.

Put the dish in the oven at (102±2) °C to attain constant mass (about 18 h±2 h). The heating should be done without air circulation.

Put the dish in the silica gel dryer and weigh it after 15 min on analytical balance. Record the mass of dry residue as m_1.

Determination of hide powder blank value.

Place the bell containing hide powder in a beaker of suitable capacity. Fill the beaker with distilled water up to the neck of the bell. When the hide powder is completely soaked, suck on the longer end of the capillary tube to create a slight depression and start siphoning the solution.

Use the Hoffman clamp to adjust the flow of the solution so that about 8 to 10 drops of the solution drip through per minute. Collect a total of 90 ml in (120±10) min.

The first 30 ml of the filtrate should be collected in a 50 ml glass measuring cylinder and disposed of. The next 60 ml should be collected in a perfectly dry 100 ml glass measuring cylinder to determine the blank value.

Distilled water should be at a temperature no less than 18 °C and no more than 20 °C.

Use the pipette to transfer 50 ml of the filtered solution into a previously dried and weighed silver dish. Place the dish on the water bath and wait for complete evaporation. Put it in the oven at (102±2) °C to attain constant mass (about 18 h±2 h).

To determine the blank value, the procedure needs to be repeated at least 5 times for each batch purchased. Calculate the average of the values obtained. The result is the “blank value".

Calculation and expression of the results

Calculate the percentage content of dry substances (S_{t0}) (% total solid) using Equation (1).

$$S_{t0} = \frac{m_1 \times 100}{m_{P0}} \qquad (1)$$

where

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S_{t0} is the percentage content of dry substances (% total solid), in percent (%);

m_1 is the dry residue, in grams (g);

m_{P0} is the product's mass, in grams (g).

Calculate the percentage of soluble solids (S_{S0}) using Equation (2).

$$S_{S0} = \frac{m_2 \times 20 \times 100}{m_{P1}} \qquad (2)$$

where

S_{S0} is the percentage content of soluble solids, in percent (%);

m_2 is the dry residue of 50 ml of the filtered analytical solution, in grams (g);

m_{P1} the initial mass of the product (analytical solution), in grams (g).

Calculate the percentage of non-tanning solid (S_{nt}) using Equation (3).

$$S_{nt} = \frac{m_3 \times 20 \times 100}{m_{P1}} \qquad (3)$$

where

S_{nt} is the percentage of non-tanning solid, in percent (%);

m_3 is the dry residue of the non-tanning agent solution, deducted from the blank value, in grams (g);

m_{P1} the initial mass of the product (analytical solution), in grams (g).

Calculate the percentage of tanning agents (T) using Equation (4).

$$T = S_{S0} - S_{nt} \qquad (4)$$

Calculate the percentage of insoluble matter (I_M) using equation (5).

$$I_M = S_{t0} - S_{nt} \qquad (5)$$

Calculate the percentage of water (W) using Equation (6).

$$W = 100 - S_{t0} \qquad (6)$$

Calculate the ratio of tanning agent to non-tanning agent (R) using Equation (7)

$$\mathrm{R} = \frac{T}{S_{nt}} \qquad (7)$$

References:

1. ISO 14088:2012: Leather–Chemical tests–Quantitative analysis of tanning agents by filter method.

2. https://emeraldscientific.com/filtration-all-glass-vacuum-filter-holder-set-47mm/

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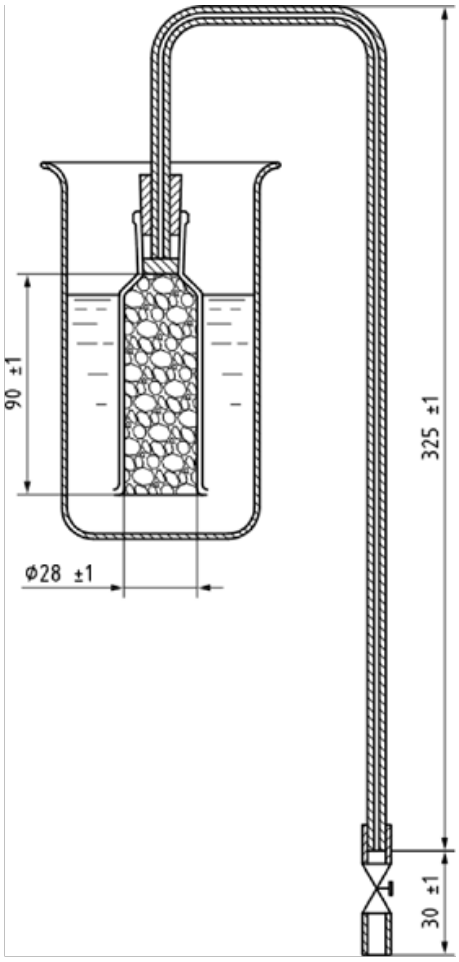

Figure 2.50. Procter bell (dimensions in mm)

Figure 2.51. Vacuum filter system

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Exploration of synthetic tanning materials

Principle – the method is based adsorption of polyphenols from the tanning agent by an insoluble copolymer absorbs. The dry content of the solution before and after mixing with the absorbing copolymer are measured. The difference is the adsorbable fraction, called the tan content.

Reagents

(1) Croslinked, insoluble vinylimidazole/vinylpyrrolidone copolymer (a suitable version of the insoluble vinylimidazole/vinylpyrrolidone copolymer powder, Divergan HM, is used widely in the wine industry; any other insoluble vinylimidazole/vinylpyrrolidone copolymer powders that give the same results can be used);

(2) Formic acid solution, a mass fraction of 50 %;

(3) Gelatine, pure, AR grade;

(4) Sodium chloride, AR grade;

(5) Deionised or distilled water.

Apparatus

Normal laboratory equipment and the following items:

Analytical balance, accurate to ±0.1 mg;

Drying oven, ventilated, capable of maintaining a temperature of 105 °C ±2 °C;

Magnetic stirrer;

PH meter with suitable combination electrode;

Stainless steel or aluminium dishes for evaporating aqueous solutions;

Desiccator, with drying agent;

Membrane filter, 50 mm diameter, 0.45 µm pore size or a suitable analytical grade filter paper;

Glass beakers, 1000 ml and 600 ml;

Volumetric flasks, 500 ml;

Pipette, 50 ml, analytical grade.

Procedure

Preparation of the synthetic tanning agent solution for analysis

In a 1 000 ml beaker, add the following.

For powder synthetic tanning agents:

– accurately weigh 1.3000 g to 1.7000 g of the powder and record the mass (m):

– add approximately 400 ml of warm deionised water (5).

For liquid synthetic tanning agents:

– accurately weigh 2.7000 g to 3.3000 g of the liquid and record the mass (*m*);

– add approximately 400 ml of deionised water (5) at room temperature. Stir so that the synthetic tanning agent is dissolved, cool the solution to 23 °C ± 1 °C. While stirring, adjust the pH to 2.0 to 2.3 by adding dropwise

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50% formic acid solution (2). Transfer the solution quantitatively to a 500 ml volumetric flask and fill to the mark with deionised or distilled water (5). This is the tanning solution to be used for the following test steps.

Prepare duplicate tanning solutions for each synthetic tanning agent to be tested.

Determination of the total residual dry content (duplicate determination)

Weigh (*T1*) the clean, dry stainless steel dish. With an analytical 50 ml pipette, add 50 ml of the tanning solution into the dish and evaporate most of the water carefully on a suitable hotplate or water bath. Place the dish in an oven at 105 °C ± 2 °C until constant mass is reached. Allow the dish to cool for approximately 2 h in a desiccator with a drying agent, for example, either calcium chloride ($CaCl_2$) or silica gel. Weigh the dish (*P1*).

The % dry content is calculated as follows:

$$\%\ \text{dry content} = \frac{(P_1 - T_1) \times 10 \times 100}{m}$$

Determination of the non-tanning agent content (i.e. non-adsorbable fraction)

In a 600 ml beaker, add the following:

— 40.0 g ± 0.1 g insoluble vinylimidazole/vinylpyrrolidone copolymer powder (5);

— 300 g ± 1 g of the tanning solution.

Stir the suspension for approximately 30 min and leave it to settle for approximately 90 min. If the upper aqueous phase is not clear, it can be centrifuged for 15 min at approximately 3000 rpm. Decant the upper aqueous phase through a 0.45 µm membrane filter or a suitable analytical grade filter paper if no membrane filter is available. Keep the filtrate, to make a double determination approximately 130 ml of filtrate is needed.

To check the absence of tanning agent in the filtrate, prepare a gelatine solution by dissolving approximately 10 g gelatine (3) and approximately 100 g NaCl (4) in 1000 ml deionised or distilled water (5). To an approximately 5 ml sample of the filtrate, add approximately 2 ml of the gelatine solution. The test solution must remain clear. If precipitation or cloudiness occurs, then there is still residual tanning agent in the filtrate. Repeat the procedure in using a larger amount of the insoluble vinylimidazole/vinylpyrrolidone copolymer powder (1) until the test solution is clear.

Carry out this section in duplicate. Weigh the clean, dry stainless steel dish (*T2*). With an analytical 50 ml pipette, add 50 ml of the tanning solution into the dish and evaporate most of the water carefully on a suitable hotplate or water bath. Place the dish in an oven at 105 °C ± 2 °C until constant mass is reached. Allow the dish to cool for approximately 2 h in a desiccator with a drying agent. Weigh the dish (*P2*).

The non-tanning agent content is calculated as follows:

$$\%\ \text{Non} - \text{tanning agent content} = \frac{(P_2 - T_2) \times 10 \times 100}{m}$$

Calculation and expression of results

Calculate the tan content (i.e. the adsorbable fraction) as follows:

% tan content = (% dry content) — (% non-tanning agent content)

Reference – ISO 17489:2013: Leather - Chemical tests - Determination of tan content in synthetic tanning agents.

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2.7. Lesson 5: Physical – chemical characteristics of the main leather assortments

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- Quality characteristics of leather types
- Quality characteristics of raw hides and skins
- Quality characteristics of semi-processed (wet blue, crust, pickled…)
- Quality characteristics of the finished leather

2.7.1. Quality characteristics of raw hides and skins

Raw material quality is a prime concern of tanners the world over. The tanning industry and the downstream industries which it supplies - footwear, furniture, automotive, clothing, leathergoods, saddlery - are entirely dependent for their raw material on supplies of cattle hides and sheep skins, plus a small number of goat and other skins. While supplies and quality of hides and skins are vital to the tanning industry, they are just by-products for the meat, dairy or wool industries. For the tanner, the raw hides and skins represent 50-60% of the cost of producing a piece of leather [1].

The quality of leather that the tanner can produce is determined primarily by the quality of the raw hides that he buys. But the quality of the hides cannot be fully assessed until after the hair or wool has been removed, and after the completion of the tanning process when the hide has been turned into leather. The value of the hide depends on the end use to which the leather goes. This eventually has to be reflected in what the tanner pays for his raw material.

Factors influencing leather quality

The best quality leather will depend on these important factors, most notably:

- Type of animal and breed;
- Physical location and climate where the animal lived;
- Portion of the hide the leather was cut from;
- Layer of the hide that is used (full grain, split);
- Quality and skill of the processing and tanning.

The quality of the hide or skin is to a large extent related to the amount of damage to the grain (or "outside") surface. The damage may be due to skin parasites that affect the live animal, related scratch, husbandry practices on the farm or in transport of the live animal (scratches, bruising, or dirt contamination); it may be due to damage during slaughter or removal of the hide; or it may be caused by inappropriate handling or inadequate preservation techniques. Most types of damage can be reduced or avoided altogether by better management of the animal or the hide.

Commercial classification of hides and skins

A. Cattle hides, calf skins, sheep skins, goat skins;
B. Pig skins;
C. Fish skins;
D. Reptiles;
E. Other types of hides and skins: deer, doe, antelope, dog, kangaroo and rabbit skins

Curing and disinfection of raw hides and skins

Purpose of curing is to protect freshly flayed hides and skins from attack by micro-organisms and render them storable for a prolonged period [2].

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Methods of curing:

a) Curing by drying;
b) Salting (most frequently) used at present;
c) Curing by pickling;
d) Short-time curing without using salt

Desinfection: bactericides and fungicides are used to inhibit bacteria and mould growth in soaking liquors and vegetable tan liquors, and on pickled pelts and wet leathers.

2.7.2. Quality characteristics of semi-processed leather

Wet-blue

Wet-blue is leather in a wet condition after chrome tanning. Wet Blue refers to moist chrome-tanned leather. In this phase, the leather is tanned, but neither dried, dyed nor finished. The bluish colouring is produced by the chrome tanning agent (Chromium (III) oxide), which is blue and is contained in the leather after tanning. Wet blue catle hides shall comply with the requirements given in Table 2.11 [3].

Figure 2.52. Wet- blue

Wet-white

Wet-white is leather in a wet condition after tanning with substances e.g. zirconium salts, aluminium salts modified aldehydes, glutaraldehydes and syntans, that confer a whitish colour.
Chemical characteristics of wet-blue and wetwhite leather are shown in Table 2.11.

Figure 2.53. Wet- white

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Table 2.11. Wet-blue and wet-white leather characteristics

No.	Characteristics	Wet-blue	Wet-white Ti-Al tanned	Wet-white organic tanned
1	Chrome oxide, %	3.5	0	0
2	Ti/Al oxides, %	0	3.12	0
3	Shrinkage temperature, °C	Over 100	75-78	70-74
4	Grease, %	1.2	2.1	4.5
5	pH of water extract	3.5	3.6	4.3
6	Moisture, %	59.0	66.33	55.0
7	Ash, %	16.6	12.59	10.5
8	Total nitroge n, %	12.7	13.84	12.9
9	Digestibility, %	51.9	61.3	65.5

Crust

Crust is leather which is tanned, fatliquored and dried, before finishing.

While most testing is often carried out on finished products, many brands and retailers often carry out testing on materials during manufacture. By conducting semi-processed leather testing, the hide is tested at a much earlier stage in the production process. Therefore, any potential contaminants or additives can be identified earlier and a solution created before the finished hide reaches the product manufacturing stage. The testing of semi-processed leather identifies fluctuations in: Grease content, Chrome content, pH, Shrinkage temperature.

Figure 2.54. Crust leather

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2.7.3. Main types of leather

Definition of leather

Hides and skins are a by-product - the main sources of raw material for the leather industry world-wide are cattle, sheep and goats, which are reared specifically for the production of meat, wool and dairy products. Typically, the value of cattle hides, sheep and goat skins represents in the region of 5-15% of the market value of an animal.

Leather is a noble, natural, durable and warm material, which is mainly used to make shoes, furniture, car interiors, clothing and leather bags. About 500,000 tons of leather is produced each year, equivalent to about 1.5 billion square metres. Leather can be manufactured and processed in a wide spectrum of varieties.

European Standard EN 15987:2015 [4] specify the following definition for leather: hide or skin with its original fibrous structure more or less intact, tanned to be imputrescible, where the hair or wool may or may not have been removed, whether or not the hide or skin has been split into layers or segmented either before or after tanning and where any surface coating or surface layer, however applied, is not thicker than 0.15 mm.

The British Standard BS: 2780 [5] leather definition is:

„Hide or skin with its original fibrous structure more or less intact, tanned to be imputrescible. The hair or wool may, or may not, have been removed. It is also made from a hide or skin that has been split into layers or segmented either before or after tanning". The amount of surface coating applied to the leather influences whether or not the item can be described as genuine leather. If the leather has a surface coating, the mean thickness of this surface layer, however applied, has to be 0.15mm or less, and does not exceed 30% of the overall thickness".

Eureka – Leather dictionary [6] leather definition is:

Leather is an imputrescible product obtained by treating the whole, or a main part, of a dehaired or dewooled appropriately prepared animal skin; the original fibrous structure being essentially retained. In ***Germany Standard RAL 060 A2*** defines the term "leather" and the differentiation from other materials.

'Genuine leather' article.

■ The description "Leather" or "Genuine Leather" conforms with local standards and laws, which differ from country to country. Sometimes there are even different rules for different uses of leather within a country.

■ In particular, with online businesses or discounted offers "leather" is frequently declared incorrectly. It is difficult for the customers to find out if it's really "Genuine Leather".

■ Different countries have different norms, standards, regulations and terms to define leather and leather types. These are not laws. But norms and regulations help in case of disagreements and are cited by experts in legal disputes.

Figure 2.55. Symbol for genuine leather

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The EC Directive 94/11 on Footwear Labelling [7] states that a genuine leather shoe must contain the following proportions of leather:

- Upper - 80% of surface area
- Lining/Sock - 80% of surface area
- Sole - 80% of volume

"If no one material accounts for at least 80%, information should be given on the two main materials used in the composition of the footwear".

The best quality leather will depend on these important factors, most notably:

- Type of animal and breed
- Physical location and climate where the animal lived
- Portion of the hide the leather was cut from
- Layer of the hide that is used (full grain, split)
- Quality and skill of the processing and tanning

The hide is made of two main integrated layers – the corium and the grain. Collagen fibres in the corium are thinner and more flexible, and become tighter and thicker as they move up toward the grain, where the fibres are tightly packed and very sturdy. The corium becomes thicker with age, which is why calfskins are thinner, smoother and softer than the hides of older animals.

Grain is outer side of leather once the hair or wool and epidermis has been removed, which is characterized by one of the following patterns, specific to each animal species:

- Pores from hair and wool;
- Feather follicles;
- Scales.

In general, there are three types of leather. These include: Full Grain Leather, Corrected Grain Leather, and Split Leather.

Full Grain Leather

Full Grain Leather is leather having kept its entire grain, with none of the surface removed by any corrective mechanical treatment [3].

High quality leather furniture and footwear are often made from full-grain leather. Full-grain leathers are typically available in two types:

- Aniline leather – leather whose natural grain is clearly and completely visible and where any surface coating with a non-pigmented finish is less than or equal to 0.01 mm.
- Semi-aniline leather – leather or split leather whose coated with a finish containing a small amount of pigment, so that the natural grain is clearly visible.

Corrected Grain Leather

Corrected Grain Leather - grain which has been partially removed by buffing or any similar mechanical treatment, and enhanced by a finishing treatment [3].
Most corrected-grain leather is used to make pigmented leather as the solid pigment helps hide the corrections or imperfections. Corrected grain leathers can mainly be bought as:

- Pigmented leather – leather whose natural grain is completely concealed with a finish containing pigments.
- Coated leather - leather where surface coating, applied to the outer side, does not exceed one third of the total thickness of the product but is in excess of 0.15 mm.

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- Patent leather - leather with generally a mirror-like effect, obtained by application of a layer of pigmented or non-pigmented varnishes, or synthetic resins, whose thickness does not exceed one third of the total thickness of product.

Split Leather

Split Leather - layer from hide or skin made from a flesh split or middle split, without any grain structure, tanned to be imputrescible [3].

A split is a layer of hide or skin obtained by dividing it horizontally (splitting) to obtain at least two separate layers; the top layer is called grain split, and the bottom layer is called flesh split; for heavy hides also a middle split can be obtained.

Split Leather can mainly be bought as:

- Pigmented split leather – whose surface is completely concealed with a finish containing pigments.
- Coated split leather – split leather where surface coating, applied to the outer side, does not exceed one third of the total thickness of the product but is in excess of 0.15 mm.
- Patent split leather – split leather with generally a mirror-like effect, obtained by application of a layer of pigmented or non-pigmented varnishes, or synthetic resins, whose thickness does not exceed one third of the total thickness of product.

Bonded Leather board / bonded leather fibre / recycled leather fibre

Bonded Leather board / bonded leather fibre / recycled leather fibre [3] – material having a minimum amount of 50% in weight of dry leather fibres, where tanned hides or skins are disintegrated mechanically and/or chemically into fibrous particles; small pieces or powders and then, with or without the combination of chemical binding agent, are made into sheets.

2.7.4. Physical-chemical characteristics of the main leather assortments

People use leather to make various goods—including clothing (e.g., shoes, hats, jackets, skirts, trousers, and belts), bookbinding, leather wallpaper, and as a furniture covering. It is produced in a wide variety of types and styles, decorated by a wide range of techniques. The main type of leather include: Shoe upper leather, Sole leather, Lining leather, Upholstery and light leather, Clothing leather, Technical leather.

Quality requirements for Shoe upper leather

The upper is leather on the visible side of the shoe. It makes up the bulk of the processed leather for shoes. The variety of processed leathers is unlimited, including skins and hides from many species.
It is common for uppers to be made from cowhide, calfskin, goatskin, sheepskin and even horsehide at a premium price. Skins of exotic animals are also used to make shoe uppers, including Crocodile leather, snake leather and many other exotic leathers. Depending on the desired look and finish, leather can be coarse or fine-grained, matt or glossy, metallic or luminous. Most upper leather is chrome-tanned.

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Table 2.12. Quality requirements for Shoe upper leather [8]

	Shoe upper leather								Standard method
	Box calf	Box side	Corrected grain side	Glazed kid	Water proof (comb. leather)	Water proof (chrome. leather)	Vegetable tanned leather	Suede (cattle, goat, sheep)	
Sulfate ash %	Max. 2 % above the content of tanning oxide								EN ISO 4047
Chromium oxide content %	>2.5	>2.5	>2.5	>2.5	>1.2	>2.5	-	>2.5	EN ISO 5398-1
Fatty substances %	3-8	5-16	5-16	4-8	<16	8-15	18-26	2-6	EN ISO 4048
Loss by washing %	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 6.0	-	EN ISO 4098
Degree of tannage	-	-	-	-	>30	-	>50	-	IUP 24 (1964)
pH (1:20)	aqueous extract not below pH 3.5								EN ISO 4045
Tensile strength, N/mm2	>20	>20	>20	>20	>25	>25	>25	>20	EN ISO 3376
Elongation at break %	>40	>40	>40	>40	>40	>40	>40	>40	EN ISO 3376
Elongation in % at 2 N/mm2	<14	<14	<14	<14	<16	<14	-	-	EN ISO 3376
Split tear force, N/mm	>40	>40	>25	>25	>50	>50	>40	-	EN ISO 3377-2
Stitch tear strength, N	>80	>100	>80	>80	>100	>120	>100	>80	EN ISO 23910
Water penetration in min (penetrometer)	>60	>20	>20	>20	>180	>120	>20	-	EN ISO 5301-1
Water absorption after 60 min	<20	<30	<30	<30	<20	<25	<30	-	EN ISO 2417
Water absorption - Kubelka after 2 h after 24 h	<60 <85	<60 <85	<60 <85	<60 <85	<30 <40	<30 <40	<35 <45	<100 <125	EN ISO 2417
Grain distension (Lastometer), mm	>7.0	>7.0	>7.0	>7.0	>7.0	>7.0	>7.0	>7.0	EN ISO 3379

Source: Pocket book for leather technologist, 4th edition, BASF, 2007

Quality requirements for Sole leather

When the sole of a shoe is made of leather, it's called a sole leather. Sole leather is thick (2.5 - 6 mm) not very pliable and solid, vegetable-tanned leather [7]. Definition of vegetable-tanned leather: hide or skin converted to leather by vegetable tanning agents, where the total content of tanning metals (Cr, Al, Ti, Zr,Fe) is less than or equal to 0.3% (mass of all metals/total dry weight of leather) [1].

Table 2.13. Quality requirements for sole leather [8]

	Sole leather			Standard method
	Sole leather modern tannage	Sole leather old pit tannage	Insole leather	
Fatty substances %	<3.5	<2.0	<4.0	EN ISO 4048
Loss by washing %	<14.0	<6.0	<10.0	EN ISO 4098
Degree of tannage	60-95	60-95	60-95	IUP 24 (1964)
pH (1:20)	at pH values below 4.0, difference value not above 0.7			EN ISO 4045
Tensile strength, N/mm2	>25	>25	>20	EN ISO 3376
Elongation at break %	<30	<35	<35	EN ISO 3376
Stitch tear strength, N/mm	>130	>130	>125	EN ISO 23910
Water absorption - Kubelka after 2 h after 24 h	 <40 <50	 <40 <50	 >50 -	EN ISO 2417

Source: Pocket book for leather technologist, 4th edition, BASF, 2007

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Quality requirements for lining leather

Shoes can have a leather lining inside. It is not the leather where the foot rests but the part which is in contact with the upper part of the foot in the shoe. A leather lining therefore needs to feel good and be permeable to water vapour, so you do not sweat.

Leather from cowhide, calfskin, goatskin, sheepskin and pigskin is used to make shoe linings.

Table 2.14. Quality requirements for lining leather [8]

	Lining leather				Standard method
	Insole, sock lining (sheep), combination tanned	Vegetable tanned	Combination tanned	Chrome tanned	
Chromium oxide content %	0.8	-	<0.5	<2.5	EN ISO 5398-1
Fatty substances %	<4.0	4-8	5-11	5-11	EN ISO 4048
Loss by washing %	<10.0	<6.0	<3.0	<3.0	EN ISO 4098
Degree of tannage	>50	>50	>40	-	IUP 24 (1964)
pH (1:20)	at pH values below 4.0, difference value not above 0.7				EN ISO 4045
Tensile strength, N/mm2	>10	>15	>15	>20	EN ISO 3376
Elongation at break %	<40	<70	<100	<100	EN ISO 3376
Split tear force, N/mm	-	>15	>15	>40	EN ISO 3377-2
Stitch tear strength N	-	>40	>40	>40	EN ISO 23910
Water absorption - Kubelka after 2 h after 24 h	 >50 -	 >75 >100	 >75 >100	 >75 >100	EN ISO 2417

Source: Pocket book for leather technologist, 4th edition, BASF, 2007

Quality requirements for Upholstery and light leather

The term "upholstery leather" is mainly used in connection with furniture leather. However, it includes all areas where leather is processed on upholstery. Upholstery leather also includes car leather, boat leather or leather in buses, aircraft and trains.

Large skins (due to the large areas) of robust and exotic animal species (due to load) are used as upholstery leather. This is mainly cow leather, because, for thousands of years, it has been available in sufficient quantities and has the necessary characteristics.

The upholstery leather can be vegetable tanned, combination tanned or chrome tanned. Vegetable tanning refers to leather that is tanned with oak and spruce bark. Also quebracho, tara pods, olive leaves, rhubarb roots or mimosa are common. These substances are placed in a pit along with the skins and hides. As these tannins are derived from plants, the leather is called vegetable-tanned leather.

Table 2.15. Quality requirements for Upholstery and light leather [8]

	Upholstery and light leather				Standard method
	Upholstery leather, veget. tanned	Upholstery leather, comb. tanned	Upholstery leather, chrome tanned	Light leather, veget. tanned	
Sulfate ash %	Max. 2 % above the content of tanning oxide				EN ISO 4047
Chromium oxide content %	-	>0.8	>2.5	-	EN ISO 5398-1
Fatty substances %	5-11	5-11	5-11	3-8	EN ISO 4048
Loss by washing %	<7.0	<7.0	-	<6.0	EN ISO 4098
Degree of tannage	>50	>30	-	>50	IUP 24 (1964)
pH (1:20)	aqueos extract not below pH 3.5				EN ISO 4045
Tensile strength, N/mm2	>20	>25	>27.5	>10	EN ISO 3376
Elongation at break %	<50	<50	<75	<50	EN ISO 3376

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Split tear force, N/mm	>40	>40	>50	>10	EN ISO 3377-2
Stitch tear strength N	>100	>100	>110	-	EN ISO 23910

Source: Pocket book for leather technologist, 4th edition, BASF, 2007

Quality requirements for Clothing leather

Jackets, trousers and gloves made of leather come under the heading of "leather clothing". The most common animal species used for clothing are cows, sheep, goats and pigs. This is because leather is almost exclusively processed from animals' hides/skins that are bred for their meat.

Cow leather is ideal for making clothing that is solid and stable. This is because cowhide is heavier, thicker and also more robust than other animal hides. Motorcycle leather suits and other protective clothing or water buffalo leather pants are typical examples of leather clothing. Thin and lightweight leisure jackets made mainly from cowhide are also very common and highly fashionable.

Lambskin is usually processed as smooth leather for lightweight casual jackets, skirts, dresses or leather trousers. In particular, expensive leather clothing is often made of lambskin.

Goatskin is usually used as suede for jackets, skirts or pants.

Pigskin is used to make low-cost leather clothing. It is commonly offered as suede and smooth leather. Pigskin is usually not as stable and soft as goatskin or Lamb leather.

Leather made of deer or reindeer skins (traditional costumes, conservative casual jackets), horses (oiled, heavy jackets), kangaroos (motorcycle racing suits) or animal fur (fur coats, jackets) are less common.

Leather is also processed for workwear. Heavy duty protective clothing, gloves or jackets

and trousers are commonly made using leather.

Hat sweat band leather is the only part of the completed *hat* that touches the head, so, it is imperative that the *leather* be soft, well-fitted, and absorbent. Most *hat* leathers are made from sheepskin and lambskin.

Table 2.16. Quality requirements for clothing leather [8]

	Clothing leather				Standard method
	Clothing leather, chrome tanned	**Glove leather, chrome tanned**	**Glove leather, Water-repellent**	**Hat sweat band leather, veget. tanned**	
Sulfate ash, %	Max. 2 % above the content of tanning oxide				EN ISO 4047
Chromium oxide content, %	>2.5	>2.5	>2.5	-	EN ISO 5398-1
Fatty substances, %	<16-18	10-18	<23	4-12	EN ISO 4048
Loss by washing, %	<2-3	<2.0	<2.0	<6.0	EN ISO 4098
Degree of tannage	-	-	-	>50	IUP 24 (1964)
pH (1:20)	aqueous extract not below pH 3.5				EN ISO 4045
Tensile strength, N/mm2	>25	>25	>20	>12	EN ISO 3376
Elongation at break, %	<60	<50	>50	-	EN ISO 3376
Elongation in % at 2 N/mm2	<20	<20	>20	-	EN ISO 3376
Split tear force, N/mm	>35	>35	>35	>15	EN ISO 3377-2
Stitch tear strength, N	>100	>100	>100	>30	EN ISO 23910
Water penetration in min (penetrometer)	>40	-	>180	-	EN ISO 5301-1
Water absorption after 60 min	<25(1)	-	<25 1)	-	EN ISO 2417
1)after 180 minutes					

Source: Pocket book for leather technologist, 4th edition, BASF, 2007

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Quality requirements for Technical leather

Oil-tanning is a process of tanning leather using natural oils after the initial vegetable tan. Typically fish oil, cod oil to be more specific. Oil tanned leather is water, stain and perspiration resistant, and has a more natural look and feel because less finishing materials have been applied, allowing its natural beauty to shine through. Oil-tanned leather is exceptionally durable.

Harness Leather - These sides feature superb strength and workability for riding tack, outdoor gear, linemen's belts and weightlifter's belts. Specially tanned.

Leather balls have existed for hundreds of years. The leather is shaped and cut so that it can be sewn into a ball. There are many sports across the world that use leather balls; the most well-known sports are football in the US (or American football), soccer in the US (or football elsewhere), baseball, cricket and many other ball games. Also medicine balls.

Ass leather is the term for a garment used by miners in past times as part of their gear. It was a belt-fastened leather apron, which was worn over the trousers and was supposed to protect the bottom, especially when the miner slipped through sliding shafts. Even today the ass leather can be seen as a part of the uniform in traditional miners' events.

Leather can be transparent. Parchment or seal intestines have these properties.

"***Chamois***" is leather made from the flesh split of sheepskin or lambskin or from sheepskin or lambskin from which the grain has been removed by frizzing and tanned by processes involving the oxidation of marine oils in the skin, using solely such oils (full-oil chamois) or first an aldehyde and then such oils(combination chamois) [4].

Table 2.17. Quality requirements for technical leather [8]

	Technical leather							Standard method
	Oil tanned leather	**Harness leather, veget. tanned**	**Harness leather, chrome tanned**	**Football leather, chrome tanned**	**ASS leather, chrome tanned**	**Raw hide a transp. leather**	**Chamois leather**	
Sulfate ash %	Max. 2 % above the content of tanning oxide							EN ISO 4047
Chromium oxide content %	-	-	>2.5	>2.5	>4.0	-	-	EN ISO 5398-1
Fatty substances %	<35	<25	<25	4-10	5-13	-	<10	EN ISO 4048
Loss by washing %		<7.0						EN ISO 4098
Degree of tannage		>30						IUP 24 (1964)
pH (1:20)	at pH values below 4.0, difference value not above 0.7*							EN ISO 4045
Tensile strength N/mm2	>35	>20	>27.5	>30	>15	>60	>10	EN ISO 3376
Elongation at break %	<90	<50	<75	<70	<70	<35	<50	EN ISO 3376
Elongation in % at 2 N/mm2	-	-	-	-	>8	-	-	EN ISO 3376
Split tear force N/mm	-	>40	>50	>40	>30	-	>15	EN ISO 3377-2
Stitch tear strength, N	-	>100	>110	>120	>75	-	>35	EN ISO 23910
Water absorption - Kubelka after 2 h after 24 h				<35 <70			>300[1] >400[2]	EN ISO 2417
*for chamois leather pH 4.0-10.0; for raw skin and transparent leather pH 4.0-8.0 [1] After 2 minutes [2] After 60 minutes								

Source: Pocket book for leather technologist, 4th edition, BASF, 2007

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Conclusions

- ✓ Hides and skins are mostly a by-*product* that is dependent on the meat and dairy industry.
- ✓ *Leather* is a unique *natural product* with many highly desirable characteristics and therefore differs from hide to hide. Variations in the texture of the *leather* should not be considered a fault but inherent of the natural beauty and uniqueness of the *leather*.
- ✓ According to the Global Leather Goods Market 2017-2021 Report [9], conducted by Technavio analysts, the global leather market is expected to grow at a compound annual growth rate (CAGR) of almost 5% from 2017-2021.
  - In 2017, the total market value was $217.49 billion. It is expected to reach $271.21 billion by 2021.
  - This segment is expected to represent an incremental growth of more than $53.72 billion during the forecast period.
- ✓ Leather made with state-of-the-art technology and chemicals is a very safe product. It is possible to manufacture leathers that comply with the most stringent requirements as to restricted substances and no health risks can be associated with such leather, which is formed from a natural product.
- ✓ Leather is a natural material which is permeable to air and moisture; it is supple and soft, warm, flexible and durable – its characteristic comfort properties have been appreciated since time immemorial. Uniquely developed and individual in the pattern of its pores, scars, surface structure and natural creasing, every leather is a unique piece in its own right. These features are evidence of the genuineness of this natural material. With its fine, slightly tinted lacquer finish, genuine leather is flameresistant, lightfast, oil-resistant and waterrepellent. It complies with today's most stringent standards. Easy to clean and care for, leather retains its original beauty for a very long time.
- ✓ Because of its durability and versatility, leather has always been a part of the world's cultural heritage, be it in the form of vellum or parchment for writing, for footwear, or for clothing.
- ✓ Specifications should form an integral part of a brand and quality management system.
- ✓ Specific laboratories have compiled a database of specifications, that target performance requirements across a range of products and material types.

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