Industrial Polymers Handbook Volume 4

Industrial Polymers Handbook

Products, Processes, Applications

Edward S. Wilks (Editor)

Volume 4

Biopolymers and their Derivatives (continued)
Indexes

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6. Gelatin

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6.1. Introduction

Gelatin is a mixture of high molecular mass polypeptides produced from collagenous animal tissues such as hide splits, pigskin, and bones. Collagen is the most commonly occurring protein in the human and animal body, accounting for about 30 % of the total protein content. Since animal horns and hoofs are not composed of collagen they cannot serve as raw materials, contrary to popular belief. The name gelatin has been used since about 1700 and is derived from the Latin *gelatus* which means frozen [1]. There is evidence that gelatin has been used for at least 4000 years [2]. The great variety of applications of gelatin depend on its solubility in hot water, its availability in a wide range of qualities, and its ability to form thermally reversible gels, which is unique for a

protein and is due to the fact that the primary structure of collagen still exists in collagen-derived polypeptides.

6.2. Structure and Properties

6.2.1. Structure

Collagen is not a uniform substance but an entire protein family. To date, some 19 polymorphs of collagen have been identified. All types of collagen have a triple-helix structure in which three protein chains are intertwined to form a rigid strand (Fig. 1). The length of the triple helix and the type and position of the nonhelical regions vary from one collagen type to an other.

The "classical" type I collagen, the most important type in the manufacture of gelatin, occurs in skin and bone. Type II collagen occurs almost exclusively in cartilage tissue, and type III collagen occurs besides type I in skin. The concentration of type III collagen is strongly dependent on age: embryonic skin can contain up to 50 %, but older skin only 5 – 10 %. The other collagen types occur only in low concentrations and fulfil mostly organ-specific functions.

Collagen consists of three helical polypeptide chains wound around each other and connected by intermolecular cross-links. The triple helix is formed intercellularly from three individual chains (α-chains). Chain selection occurs via intra- and intermolecular disulfide bridges in the region of the N- and C-terminal peptides, additionally supported by glycopeptide structural elements. While collagen types II and III contains three identical chains (homotrimer $[\alpha_1(II)]_3$), type I collagen consists of two identical and one somewhat differing chain (heterotrimer $[\alpha_1(I)]_2\alpha_2(I)$). The triple helix molecule is 300 nm long and 1.4 nm in cross section. The amino acid sequences of the α_1 and α_2 chains are highly homologous (ca. 90 % correspondence).

Collagen contains large amounts of two unusual amino acids: 4-hydroxyproline and ε-hydroxylysine, which are the result of post-translational modification: special enzyme systems facilitate the intracellular hydroxylation of proline and lysine on the growing collagen chain. Other enzymes are responsible for the glycosylation of individual hydroxylysine residues. Type I collagen, for example, contains 0.5 wt % carbohydrate, and type III about 10 %. The carbohydrate portion comprises galactose and a 1,2-linked galactose – glucose disaccharide. The ether-like bond between collagen and the saccharide occurs between the C1 atom of galactose and the ε-hydroxyl group of hydroxylysine.

The enzyme-catalyzed modification steps (hydroxylation and glycolyzation) are restricted to growing individual chains; as soon as triple-helix formation is initiated, modification ceases. As stability of the helix is dependent on the hydroxyproline content, helix formation and proline hydroxylation are interdependent and self-regulating.

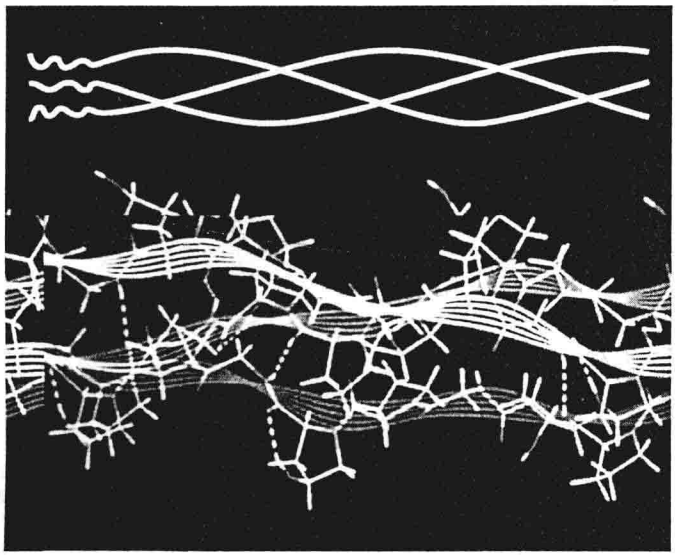

Figure 1. Schematic representation of the triple helix

The helical part of all three collagen types contains 1014 amino acids per chain, and the N- and C-terminal nonhelical extension peptides (telopeptides) are between 9 and 26 amino acids long. The triple helix molecule has a molecular weight of about 290 000, including the short N- and C-terminal globular extension peptides.

Collagen consists of one-third glycine and 22 % of the imino compounds proline and hydroxyproline; the remaining 45 % comprises some 17 other amino acids (Table 1). The content of acidic and basic amino acids is relatively high. About one-third of the acidic amino acids glutamic acid and aspartic acid is present in the amidated form as glutamine and asparagine. Cysteine is completely absent, and of the sulfur-containing amino acids only methionine is present at low concentration (exception: type III which has two cysteine residues per 1000 amino acids).

The "classical collagen" types are characterized by repetitive tripeptide units — $(\text{glycine-X-Y-})_n$ — where proline occurs in the X and Y positions and 4-hydroxyproline exclusively in the Y position. Several other amino acids also have preferred positions, presumably for steric reasons. For example, glutamic acid, phenylalanine, and leucine occur frequently in the X position, and arginine mostly in the Y position. Generally, collagen types from different species are to a high degree homologous (usually about 90 %); and, within the same species, type I collagen from skin, bones, and tendons are identical.

Triple-helix, water-soluble collagen (types I to III) is converted extracellularly by cross-linking and fibril formation into highly associated, water-insoluble collagen. These intra- and inter-molecular cross-links are responsible for a dramatic increase in stability of the collagen molecule relative to thermal, mechanical, and enzymatic influence. The type and extent of the covalent cross-links are age-dependent, an aspect that is of considerable importance for the gelatin industry. Process parameters and extraction

Table 1. Comparison of the amino acid composition of collagen and gelatin: number of amino acids per 1000 (rounded off)

Amino acid	Type I collagen (bovine)	Type A gelatin *	Type B gelatin **
Alanine	114	112	117
Arginine	51	49	48
Aspargine	16	16	-
Aspartic acid	29	29	46
Glutamine	48	48	-
Glutamic acid	25	25	72
Glycine	332	330	335
Histidine	4	4	4
4-Hydroxyproline	104	91	93
ε-Hydroxylysine	5	6	4
Isoleucine	11	10	11
Leucine	24	24	24
Lysine	28	27	28
Methionine	6	4	4
Phenylalanine	13	14	14
Proline	115	132	124
Serine	35	35	33
Threonine	17	18	18
Tyrosine	4	3	1
Valine	22	26	22

* Type A gelatin: acid-pretreated pigskin gelatin. ** Type B gelatin: alkali-pretreated bone gelatin [1]

conditions must hence be adapted to the age of the raw materials (e.g., young pig or calf skin / old cattle hide).

6.2.2. Physical Properties

Commercial gelatin is a vitreous solid with a faint color; it is almost tasteless and odorless and usually contains 9 – 13 % moisture. It gives typical protein reactions and is hydrolyzed by most proteolytic enzymes to give its peptide or amino acid components.

Depending on the degree of hydrolysis, two different types of gelatin are obtained:

1) Gelatin, gelling type [*9000-70-8*]: When the granules are immersed in cold water they are hydrated to discrete swollen particles. On heating, these swollen particles dissolve to form a solution.
2) Gelatin hydrolysate (also known as collagen hydrolysate), nongelling type [*68410-45-7*]: In this product range the molecular mass is reduced to such an extent (< 15000 D) that no gelation is observed. This type, mostly produced as a fine powder is soluble in cold water. Gelatin and gelatin hydrolysate are insoluble in less polar organic solvents such as benzene, petroleum ether, ethanol, acetone and tetrachloromethane.

   Gelatin and gelatin hydrolysate are insoluble in less polar organic solvents such as benzene, petroleum ether, ethanol, acetone and tetrachloromethane. Gelatin is

amphoteric: in acidic solution gelatin is positively charged and migrates as a cation in an electric field; in alkaline solution it is negatively charged and migrates as an anion. The intermediate point, where the net charge is zero and no migration occurs, is known as the isoionic or isoelectric point. Type A gelatin, produced by acidic processing of collagenous raw materials, has a broad isoelectric region between pH 6.0 and 9.5. Type B gelatin, produced by the alkaline processing of collagenous raw materials, has an isoelectric point between pH 4.7 and 5.6. The difference in the isoelectric points of type A and type B gelatins is the result of partial desamidation of glutamine and asparagine to the corresponding glutamic acid and aspartic acid. Mixtures of type A and B, as well as gelatins produced by modifications of the above-mentioned processes, may exhibit isoelectric points outside these ranges. Gelatin in solutions containing no ions other than H^+ and OH^- is known as isoionic gelatin [3]. These solutions can be readily prepared by the use of ion-exchange resins.

One of the most important properties of gelatin (gelling type) is the formation of heat-reversible gels in water. Gelatin readily forms gels over a wide range of pH with a variety of solutes. The formation and stability of a gelatin gel is influenced by a number of factors. When an aqueous solution of gelatin with a concentration greater than ca. 1 % is cooled to about 35 – 40 °C it first increases in viscosity and on further cooling it forms a gel. The strength of the gel depends on concentration and the intrinsic strength of gelatin used, which is a function of structure and molecular mass. Gel formation is not fully understood, but is believed to result from hydrogen bonding; the gelatin molecules are arranged in micelles that form a semisolid gel and bind water. Quantitative measurement of this property is important, both for control and to determine the amount of gelatin required for a given purpose because gelatin is used in many products for its gel-forming properties. Modern theory proposes that the first step in gelation is the formation of locally ordered regions caused by the partial random return (renaturation) of gelatin to collagen-like helices (collagen fold). Next, a continuous fibrillar three-dimensional network of fringed micelles forms throughout the system, probably due to nonspecific bond formation between the more ordered segments of the chains. Hydrophobic, hydrogen, and electrostatic bonds may be involved in cross-linking. Since these bonds are disrupted on heating, the gel is thermoreversible. Formation of cross-links is the slowest part of the process, so that under ideal conditions the strength of the gel increases with time as more cross-links are formed. The total effect is a time-dependent increase in average molecular mass and in order. Renaturation involves association between components (peptide chains) that differ in degree of cross-linking, chain length, and chemical composition [4], [5].

Gelatin is stable for long periods of time when suitably stored in sealed containers, at normal ambient temperatures to prevent ingress or loss of moisture. When gelatin is heated above 45 °C in air at relatively high humidity it may lose its ability to swell and dissolve [6].

Gel Strength is determined with a Bloom gelometer [7], [8], [10]–[13]. A 6.67 % solution of the gelatin sample is prepared in a special wide-mouthed test bottle, which is then cooled to 10.0 ± 0.1 °C and kept for 17 ± 1 h for maturation at this temperature. The firmness of the resulting gel is then measured with a gelometer. This instrument impresses a standard plunger (12.7 mm diameter, plane surface, sharp edges) into the surface of the gel. The force required to depress the plunger 4 mm into the gel is the gel strength or Bloom value of the gelatin. Commercial gelatins vary from ca. 50 to 300 g Bloom.

Viscosity is usually determined by using a calibrated viscosity pipette (Bloom pipette), which measures the efflux time of 100 mL of a 6.67 % solution at 60 °C.

For gelatin hydrolysate, the viscosity of a 10 % or 20 % solution at 25 °C is determined, but other concentrations or temperatures are possible, according to the different uses. For measurement of higher viscosity values, rotation viscometers are also used.

Molecular mass fractions (≥ 200 000D) have some impact on viscosity, medium molecular mass fractions (55 000 – 300 000 D) have an influence on gel strength. The viscosity of gelatin solutions increases with increasing gelatin concentration and with decreasing temperature; viscosity is at a minimum at the isoionic point. Gelatin solutions should not be exposed to temperatures above 60 °C for prolonged periods of time because the gel strength and viscosity decrease [14].

Color and clarity of gelatin and gelatin hydrolysate can be important for certain applications. These parameters can be measured with a spectrophotometer, but visual assessment against standard gelatins is also common practice. The color of gelatin depends on the raw material and the production process. Pigskin gelatins usually are less strongly colored than those made from bone or hide. Turbidity may be due to insoluble or foreign matter in the form of emulsions or dispersions that are stabilized by the protective-colloid action of gelatin, or can be caused by an isoelectric haze. This haze is at a maximum at the isoelectric point in ca. 2 % solution [15].

Gelatin typically contains ca. 9 – 13 % *moisture*, which can be determined by drying a sample at 105 °C for 17 h.

Technical gelatins are normally specified by their gelling power and viscosity. The test methods employed are generally similar to those used for edible or pharmaceutical gelatins, but different gelatin concentrations may be employed. For lower grades of technical gelatins (and animal glues) 12.5 % solutions (15 g in 105 mL water) are used.

Methods for sampling and testing of animal glues are described in an international standard [16]. In addition to gel strength and viscosity the measurement of moisture content, melting and setting point, foam characteristics, pH and fat content are also described.

In some countries the viscosity of technical gelatin is measured with a cup viscometer, normally the Engler viscometer [17], [18], [19], and using a 17.75 % solution at 40 °C. The viscosity (in degrees Engler, °E) is the flow time of the gelatin solution compared with that of an equal volume of water at 20 °C. Relationships between viscosity measured by capillary and by the Engler method are available [19], [20].

Gelatin also undergoes a phenomenon called coacervation when colloidal particles separate from the liquid phase. Coacervates are formed when two hydrophilic sols carrying opposite charges are mixed in suitable amounts [21]. If gelatin from an acid-treated precursor is mixed with gelatin from an alkali-treated precursor and the resulting gel is prepared at pH 5 – 7, different degrees of turbidity result. This effect depends on the ratio of the two gelatins in the mixture and the pH. This phenomenon arises from the formation of a complex coacervate between the gelatin micelles with opposite charge. Adjustment of the pH above or below the isoelectric range of both gelatins gives a clear gel; both gelatins are now either positively or negatively charged and hence are mutually compatible. A common application of complex coacervation is the use of gelatin and gum arabic to produce oil-containing microcapsules for carbonless paper manufacture [22], [23].

By addition of plasticizers such as glycerin, sorbitol and lubricants like stearate it is possible to produce a blend with thermoplastic properties that can be used in extrusion and injection molding processes [24].

6.2.3. Chemical Properties

Gelatin is formed by the particle hydrolysis of collagen protein resulting in a mixture of protein fragments of varying molecular mass (gelatin 15 000 – 400 000 Dalton, gelatin hydrolysate < 15 000 D). The amino acid composition (see Table 1) corresponds to that of the collagen from which it is derived. Hydroxyproline and hydroxylysine are two unusual amino acids found in gelatin. The determination of the hydroxyproline content can be used for identification purposes [25].

The quantitative analysis of the elements gives about 50.5 % carbon, 25.2 % oxygen, 17.0 % nitrogen and 6.8 % hydrogen. The ash content of commercial gelatin varies with the origin of the raw material and the method of processing. Gelatins derived from pigskin contain small amounts of chlorides and sulfates resulting from acid treatment before extraction; gelatin from bone and hide contains calcium and sodium salts from the lime used in pretreatment.

Dry gelatin stored in airtight containers at room temperature remains unchanged for long periods. Degradation in solution may be caused by extremes of pH, temperature and by proteolytic enzymes such as papain or trypsin.

Gelatin can be chemically modified to change its properties. It undergoes typical protein reactions, including acylation and carbamylation [26]. Such products include succinylated, phthalated, and carbamylated gelatin. These products are used for special pharmaceutical and photographic applications. A new type is methacrylated gelatin, which is suitable for technical applications. Permanent cross-linking of gelatin can be achieved by reaction with aldehydes such as formaldehyde, glyoxal, or glutaraldehyde [26].

6.2.4. Microbiological Analysis

Microbiological testing is in most cases carried out according to methods described in the European Pharmacopeia (Ph. Eur.) and the United States Pharmacopeia (U.S.P.). Important test parameters are total aerobic count, including molds and yeasts, after an incubation period of up to 5 d at incubation temperatures of 30 – 35 °C and 20 – 25 °C and counts of colony-forming units on tryptic soy agar and Sabouraud agar. Tests for *Escherichia coli* and Salmonella species include at least one enrichment step and detection of typical growth on selective nutrition media.

6.2.5. Quality Specifications

Gelatin, as a protein, is subject to contamination by microorganisms; good manufacturing practice must be followed to ensure a clean product. In addition to rendering gelatin unacceptable for human consumption, bacteria can degrade gelatin to a point at which it loses its gel-forming property. Conductivity and pH have a major impact on gelatin quality due to their importance regarding optimal growth conditions for bacteria [27].

Most gelatin manufacturing plants meet the strict requirements of ISO 9000 [28] and ensure consistent high quality by the implementation of HACCP (hazard analysis and critical control points) techniques [29]. The result is one of the purest proteins available to the food, pharmaceutical, and photographic industries. As a pure, natural protein, gelatin is a food, not an additive and, does not carry an E number.

The majority of commercial gelatins contain less than 3000 nonpathogenic bacteria per gram. For pharmaceutical-grade gelatin the pharmacopeia (Ph. Eur., U.S.P.) set a limit of 1000 bacteria per gram, and Salmonella species and *Escherichia coli* must be absent in 10 g and 1 g, respectively [9], [10]. Physical and chemical characteristics of gelatin are listed in Table 2, together with microbiological specifications for pharmaceutical-grade gelatin.

Up to now there are no official limits for microbiological, physical or chemical parameters for food-grade gelatin. Therefore the Gelatin Manufacturers of Europe (GME) cooperate with official authorities to set microbiological, physical, and chemical limits for food-grade gelatin. It is planned to release a monograph for food-grade gelatin which contains not only the quality limits but describes also the test methods to ensure compliance with those limits.

Since the raw materials are exclusively obtained from animals fit for human consumption, and the production process includes numerous purification steps like washing, filtration, ion exchange and a final UHT sterilization step, gelatin is a safe product with regard to bacteriological, viral, and TSE (transmissible spongiform encephalopathies, e.g., Scrapie and BSE) safety [30], [31] and therefore does not carry any risk for the health of consumers.

Table 2. Gelatin specifications

Parameter	Ph. Eur. limits	U.S. P. limits
pH	3.8–7.6	
Total ash, %	2	2
Loss on drying, %	15	
Sulfur dioxide, mg/kg	200	40 *
Peroxides, mg/kg	100	
Arsenic, mg/kg	1	0.8
Heavy metals, mg/kg	50	50
Phenolic preservatives	0	
Total viable count CFU **	1000/g	1000/g
Escherichia coli	0 in 1 g	0 in 10 g
Salmonella	0 in 10 g	0 in 10 g

* Gelatin used for capsule manufacture can contain up to 1500 mg/kg. ** CFU = colony-forming units.

6.3. Raw Materials and Production

6.3.1. Raw Materials

Gelatin is commercially derived from animal collagen of skins and bones. The principle raw materials worldwide are cattle hides, bones and pigskins. In principle all raw materials are fit for human consumption. Sources from other mammalian species and fish sometimes are also used. Each raw material requires special treatment to remove noncollagenous extraneous substances such as fat and minerals. The resulting pure collagen is then hydrolyzed to gelatin, which is soluble in hot water.

Bones. The so-called green bones are supplied by slaughterhouses and meat packers. The fresh raw material is transported directly to the degreasing plants of the gelatin producer. After preselection the bones are crushed into small particles, degreased, dried and sorted according to size. The mineral components of bones are hydroxyapatite and calcium carbonate. To remove the inorganic components the bones are treated for about a week or more with cold diluted acid. The resulting collagen is called ossein, the starting material for subsequent gelatin extraction.

Cattle Hides. Skins from calves and beef are obtained from animals slaughtered for human use. The subcutaneous, fat-containing tissue is mechanically separated and the skins are split horizontally into two parts: the part previously covered with hair is tanned and subsequently used as a high-quality leather. The parts previously in contact with the flesh are the so called hide splits. These residual thinner sections remain untanned and are dried or preserved with salt or calcium hydroxide to become the raw material used in the production of gelatin.

Pigskin. Pigskin is the most important raw material for production of edible gelatin in Europe and United States. Pigskins are supplied by slaughterhouses and meat processing plants and are frequently cooled or even frozen to prevent deterioration.

6.3.2. Production

Each raw material requires a special pretreatment of the collagen containing material to render it soluble in hot water. During pretreatment procedures the collagen swells and softens, peptides and cross-links are hydrolyzed, and various substances, regarded as impurities, are extracted. Two main types of gelatin are distinguished: type A from acid pretreated raw material and type B from alkaline processing. The production process is shown in Figure 2.

Acid Process. The acid process is used for pigskin and sometimes for special types of ossein. Washed pigskins are treated with diluted mineral acids for approximately 24 h at low temperature. This pretreatment is sufficient to break acid-labile peptide bonds in pigskin collagen. After partial neutralization the gelatin is then extracted with hot water. Usually, the gelatin is extracted stepwise with successive increase of temperature and time. Gelatins from successive extractions show different physical and chemical properties. The first extract has the highest gel strength and molecular mass and a very low color. Later extracts, obtained at elevated temperatures, contain a higher amount of low molecular weight peptides and therefore have lower gel strength and are more intensive in color and clarity.

Alkali Process. For the production of type B gelatin, cattle hides and ossein are pretreated with alkali (lime or sodium hydroxide) at ambient temperature. The liming time varies from several weeks to several months. The treatment removes impurities and splits cross links and peptides. The liming time is a critical parameter for the further processing.

The dilute solutions obtained by hot-water extraction contain 3 – 10 % gelatin. The liquors are filtered and deionized to remove suspended matter and undesirable amounts of inorganic ions. The clarified, dilute gelatin solution is concentrated to 25 – 35 wt % by vacuum evaporation, filtered, and sterilized. The concentrated liquor is rapidly chilled to a gel, extruded as noodles, which are deposited on a stainless steel net for drying with hot, sterile air. The net passes slowly through a drying chamber, which is divided into several zones with controlled temperature and humidity. Typical temperature range is from about 30 °C at the beginning up to about 70 °C in the final zone. The dried gelatin, having a moisture content of ca. 10 %, is broken and milled. The physical, chemical and microbiological properties of each extract are tested.

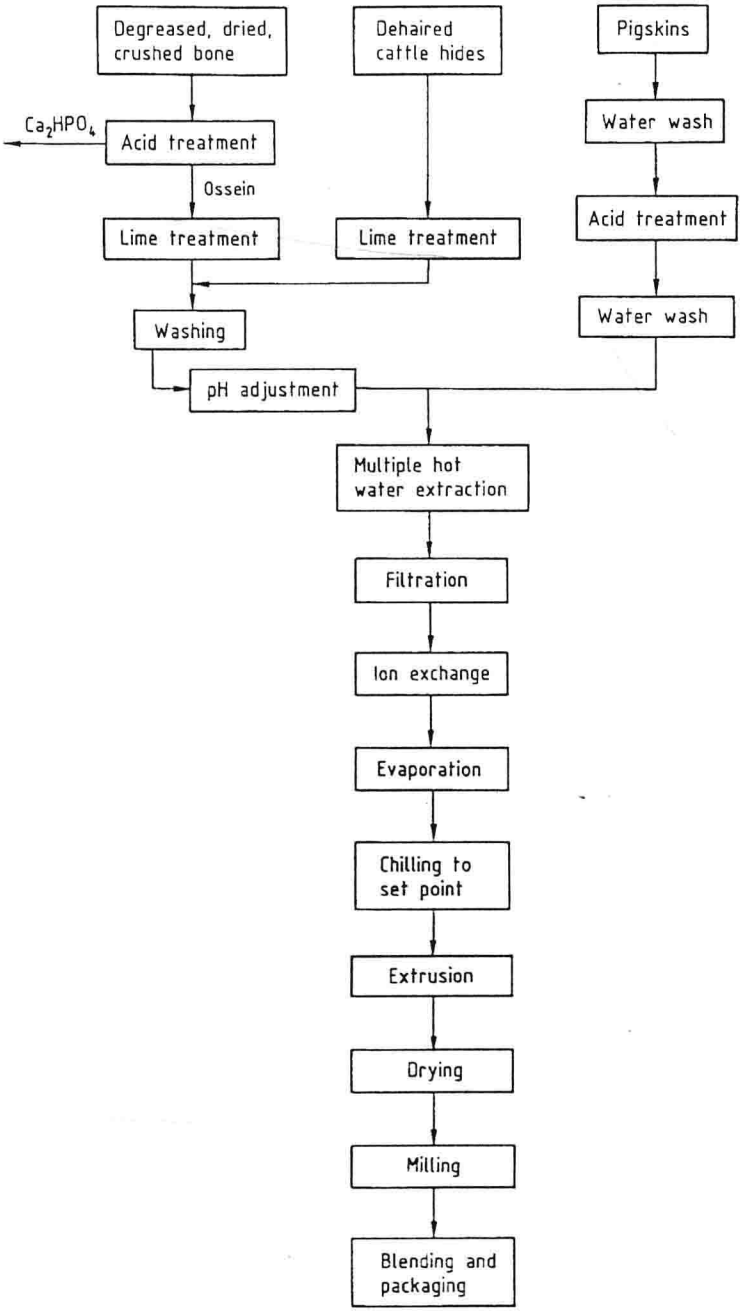

Figure 2. Gelatin production process