

**EXTRACTION AND CHARACTERIZATION OF GELATIN  
FROM ANIMAL BONE FOR THE PRODUCTION OF  
PHOGRAPHIC FILM**

**BY**

***ANYANWU SUNDAY ANAYO***

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**DEPARTMENT OF CHEMICAL ENGINEERING,  
SCHOOL OF ENGINEERING AND ENGINEERING TECHNOLOGY  
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA  
NIGER STATE, NIGERIA.**

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## DEDICATION

This research work is dedicated to the Almighty God the Father, God the Son and God the Holy Spirit for the gift of Life, Ability and Inspiration to carry out this research work.

## CERTIFICATION

This is to certify that this research work '*Extraction and Characterization of Gelatin from Animal Bone for the Production of Photographic Film*' was carried out by Anyanwu Sunday Anayo, (2003/14960EH) under my supervision and approved for submission to the department of Chemical Engineering, Federal University of Technology, Minna. And that the research work is in partial fulfillment of the requirement for the award of Bachelor of Engineering Degree (B. ENG) in Chemical Engineering.



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Project Supervisor  
Engr Manasseh Auta

11-11-2008

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Date.

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Head of Department  
Dr. M O Edoga

---

Date

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External Supervisor

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Date

## ABSTRACT

The research aimed at the extraction and characterization of gelatin from animal bone (cow) for the production of photographic film. The bones were grounded to get various particle sizes of 1.40mm, 2.00mm, 2.80mm, 3.35mm and 5.00mm which gave yields of 22.104g, 21.004g, 18.573g, 14.804g and 12.756g of gelatin respectively. This shows that smaller bone sizes give higher yield of gelatin than the larger ones.

The type of gelatin produced is type A gelatin since acid was used to treat the bone sample.

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## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Historical Background Of Gelatin

Gelatin is a translucent, Colourless, brittle and nearly tasteless solid substance, extracted from the collagen inside animals' connective tissue. It has been commonly used as a gelling agent in food, pharmaceutical, photography and cosmetic manufacturing industries.

The exact time of the discovery of gelatin is not known, but considering it is derived after the boiling of animal skin and bone, it is quite likely to have been quite some time ago. Whenever it was, it is certain that chefs utilized its wonderful setting properties to create gastronomic works of art, which were both pleasing to the eye and tempting on the palate. (Hill, T.T, 1965)

In England during the Victorian era (late 1800's to early 1900s) gelatin was widely used in both savoury and sweet dishes. Cooks were renowned for using fancy moulds in all shapes and sizes, from tall and fluted to long and low and even some in the shape of fish and other animals

(Hill, T.T, 1965)

Commercially produced gelatin appears to have started in Holland around 1685, shortly followed by England in about 1700. The first commercial production of gelatin in the United States was in Massachusetts in 1808.

Gelatin does not occur "free" in nature. It has to be made or manufactured.

However, it is a natural ingredient, in that it is derived from natural ingredients and has no additives. Nowadays, it is made mainly from beef bones and hides and pork skin, which contain the protein collagen, the essential ingredient required for the production of gelatin.

Gelatin is a protein produced by partial hydrolysis of collagen extracted from the bones, connective tissues, organs, and some intestines of animals such as the domesticated cattle, and horses. The natural molecular bonds between individual collagen strands are broken down into a form that rearranges more easily. Gelatin melts when heated and solidifies when cooled again. Together with water, it forms a semi-solid colloid gel. Gelatin forms a solution of high viscosity in water, which sets to a gel on cooling, and its chemical composition is, in many respects, closely

## 2.2.1 The Process Of Producing Gelatin

The Process of Producing Gelatin Consist of Three Main Stages

1. Pretreatments to make the raw materials ready for the main extraction step and to remove impurities which may have negative effects on physicochemical properties of the final gelatin product,
2. The main extraction step, which is usually done with hot water or dilute acid solutions as a multistage extraction to hydrolyze collagen into gelatin, and finally,
3. The refining and recovering treatments including filtration, clarification, evaporation, sterilization, drying, rutting, grinding, and sifting to remove the water from the gelatin solution, to blend the gelatin extracted, and to obtain dried, blended and ground final gelatin.

### 1 Pretreatments

If the physical material which will be used in production is bones, dilute acid solutions should be used to remove calcium and similar salts. Hot water or several solvents may be used for degreasing. Maximum fat content of the material should not exceed 1% before the main extraction step. If the raw material is hides and skin, size reduction, washing, removing hair from the hides, and degreasing are the most important pretreatments used to make the hides and skins ready for the main extraction step.

Raw material preparation for extraction is done by three different methods:

- Acid treatments
- Alkali treatments
- Enzymatic treatments.

#### i Acid treatment

Acid treatment is especially suitable for less fully cross-linked materials such as pig skin collagen. Pig skin collagen is less complex than the collagen found in bovine hides. Acid treatment is faster than alkali treatment and requires normally 10 to 48 hours.

#### ii Alkali treatment

Alkali treatment is suitable for more complex collagen as being in bovine hides. This process requires longer time, normally several weeks. The purpose of the alkali treatment is to destroy certain chemical cross-linkages still present in collagen.

### **iii Enzymatic treatments**

Enzymatic treatments used for preparing raw material for the main extraction step are relatively new. Enzymatic treatments have some advantages in contrast to alkali treatment. Time required for enzymatic treatment is short, the yield is almost 100% in enzymatic treatment, the purity is also higher, and the physical properties of the final gelatin product are better.

The gelatin obtained from acid treated raw material has been called type-A gelatin, and the gelatin obtained from alkali treated raw material is referred to as type-B gelatin.

## **2 Extraction**

After preparation of the raw material, i.e., reducing cross-linkages between collagen components and removing some of the impurities such as fat and salts, partially purified collagen is converted into gelatin by extraction with either water or acid solutions at appropriate temperatures. This extraction is one of the most important steps in gelatin production. All industrially used processes are based on neutral or acid pH values because alkali treatments speed up conversion, but, at the same time, degradation processes are promoted.

Acid extract conditions are extensively used in the industry but the degree of acid varies with different processes. This extraction step is a multi stage process, and extraction temperature is usually increased in later extraction steps. This procedure ensures the minimum thermal degradation of the extracted gelatin.

## **3 Recovery**

This process includes several steps such as filtration, evaporation, sterilization, drying, grinding, and sifting. These operations are concentration-dependent and also dependent on the particular gelatin used. Degradation must be avoided or minimized. For this purpose, limiting the temperature as much as possible would be helpful. Rapid processing is required for most of them. All of these processing steps should be done in several stages to avoid extensive deterioration of peptide structure. Otherwise, low gelling strength would be obtained that is not generally desired.

### **2.3 Types of Gelatin**

There are two types of gelatin dependent on whether the preparation involves an alkaline pretreatment or acid treatment. Acid pretreatment (Type A gelatin) uses cow bones whereas alkaline treatment (Type B gelatin) makes use of cattle hides and skin.

## 2.4 Uses Of Gelatin

- **Photography**

Photographic film is a sheet of plastic (polyester, nitrocellulose or cellulose acetate) coated with an emulsion containing light-sensitive silver halide salts (bonded by gelatin)

This means that gelatin is used to hold silver halide crystals in an emulsion in virtually all photographic films and photographic papers. As a surface sizing, photographic grade gelatin smoothens glossy printing papers and maintains the wrinkles in crêpe paper.

Despite some efforts, no suitable substitutes with the stability and low cost of gelatin have been found. Industrial gelatin is light yellow or brown grain, which can pass the 4mm aperture standard sieve. It is mainly used in photographic paper, fixture and galvanoplastics liquid, match head and wooden furniture (Carroll, Burt H 1931, Pouradier, J. 1974)

- **Food and Nutrition**

Gelatin for food grade

Household gelatin comes in the form of sheets, granules, or powder. Instant types can be added to the food as they are; others need to be soaked in water beforehand.

Special kinds of gelatin indicate the specific animal origin that was used for its production. For example, Muslim halal or Jewish kosher customs may require gelatin from fish.

Probably best known as a gelling agent in cooking, different types and grades of gelatin are used in a wide range of food and non-food products.

Common examples of foods that contain gelatin are gelatin desserts, jelly, trifles, aspic, marshmallows, and confectioneries such as Peeps and gummy bears.

Gelatin may be used as a stabilizer, thickener, or texturizer in foods such as ice cream, jams, yogurt, cream cheese, and margarine; it is used, as well, in fat-reduced foods to simulate the mouth feel of fat and to create volume without adding calories( Islamic Services of America, 2006)

Gelatin is used for the clarification of juices, such as apple juice, and of vinegar. Isinglass, from the swim bladders of fish, is still in use as a fining agent for wine and beer. Beside hartshorn jelly, from deer antlers (hence the name "hartshorn"), isinglass was one of the oldest sources of gelatin. Edible Gelatin is light yellow to yellow fine grain. It should be kept dry, clean and even place without impurities. It can pass 4mm aperture sieve, that is 5 mesh (Chien, J.C.W 1975).

Gelatin is one of the few foods that cause a net loss of protein if eaten exclusively. In the 1970s, several people died of malnutrition while on popular liquid protein diets( FDA Consumer magazine, April 1990)

Several Russian researchers offer the following opinion regarding certain peptides found in gelatin: "gelatin peptides reinforce resistance of the stomach mucous tunic to ethanol and stress action, decreasing the ulcer area by twice.

- **Medicine and Pharmacy**

Gelatin typically constitutes the shells of pharmaceutical capsules in order to make them easier to swallow. Hypromellose is a vegan-acceptable alternative to gelatin, but is more expensive to produce

For decades, gelatin has been touted as a good source of protein. It has also been said to strengthen nails and hair. However, there is little scientific evidence to support such an assertion, one which may be traced back to Knox's revolutionary marketing techniques of the 1890s, when it was advertised that gelatin contains protein and that lack of protein causes dry, deformed nails. In fact, the human body itself produces abundant amounts of the proteins found in gelatin.

Medicine Gelatin is a kind of high level gelatin product made from animal skins and bones, which contains high protein and non fat, has good properties of in viscosity and freezing. Gelatin has also been claimed to promote general joint health. A study at Ball State University, sponsored by Nabisco (the former parent company of Knox gelatin), found that gelatin supplementation relieved knee joint pain and stiffness in athletes. These results have not yet been replicated by other researcher.( Pearson, David2007).

- **Technical Uses**

Bone Glue is amber, semi transparent pearl eye grain with good performances in freezing and low viscosity. It is widely used in emery cloth, sand paper and textile sizing, electroplated finishes and wooden furniture.

Certain professional lighting equipment uses color gels to change the beam color. These used to be made with gelatin, hence the name color gel.

Animal glues such as hide glue are essentially unrefined gelatin.

Used as a carrier, coating or separating agent for other substances, it, for example, makes beta-carotene water-soluble, thus imparting a yellow Colour to any soft drinks containing beta-carotene.

Gelatin is closely related to bone glue and is used as a binder in match heads and sandpaper.

Cosmetics may contain a non-gelling variant of gelatin under the name hydrolyzed collagen.

When added to boiling water and cooled, unflavored gelatin can make a home-made hair styling gel that is cheaper than many commercial hair styling products, but by comparison has a shorter shelf life (about a week) when stored in this form (usually in a refrigerator). After being applied to scalp hair, it can be removed with rinsing and some shampoo.

## **2.5 Properties and Characteristics of Gelatin**

It is well known that gelatin is a product of the structural and chemical degradation of collagen. Because of this, reviews and monographs dealing with the structure and properties of collagen also present data on gelatin thus providing additional information on collagen. Moreover, the few available reviews and monographs devoted specifically to gelatin deal, to a large extent, with the structure and properties of collagen. This naturally stems from the similarities in amino acid composition and, to some extent, in structure between collagen and gelatin and hence the similarity in properties between the two proteins.

At the same time, gelatin exhibits its own properties which are either absent in collagen or are developed only to a slight extent. Gelatin has some individual importance as a polymeric product widely utilized in the manufacture of various articles and materials. In most cases, except for the food industry, gelatin is used in the solid state. However, whereas the properties of gelatin gels and solutions have been thoroughly surveyed, only a few reviews have dealt with the solid state of gelatin and they provided only a brief outline, ignoring many aspects of the behaviour and properties of gelatin.

Alongside a large number of quite useful features inherent in gelatin, it also exhibits some significant shortcomings which are aggravated under unfavorable temperature and humidity conditions. Among these shortcomings there is, first of all, a high brittleness exhibited by solid gelatin at reduced humidity and elevated temperatures, the brittleness resulting in premature failure of gelatin articles. Because of this, the problem of modification of the properties of solid gelatin and, first of all, the problem of its plasticization has been the subject of much investigation for over a century and cannot yet be considered as completely resolved. This impelled us to discuss the methods for modifying the properties of solid gelatin and to compare these methods and outline their

capabilities in reducing brittleness of gelatin materials under severe environmental conditions.

### 2.5.1 The Structure of Gelatin

Gelatin contains many glycine (almost 1 in 3 residues, arranged every third residue), proline and 4-hydroxyproline residues. A typical structure is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-.

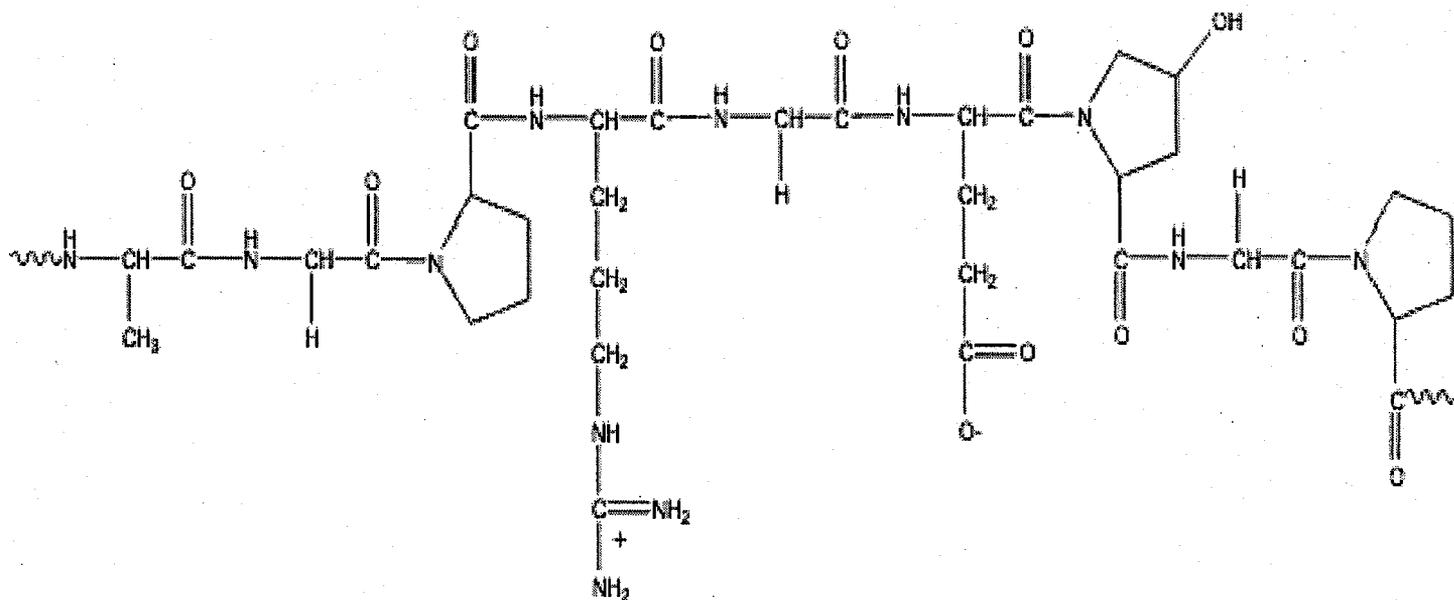


Fig. 2.1 Molecular structure of gelatin

Gelatin is a heterogeneous mixture of single or multi-stranded polypeptides, each with extended left-handed proline helix conformations and containing between 300 - 4000 amino acids. Gelatin consists of mixtures of these strands together with their oligomers and breakdown polypeptides. Solutions undergo coil-helix transition followed by aggregation of the helices by the formation of collagen-like right-handed triple-helical proline/hydroxyproline rich junction zones. Higher levels of these pyrrolidines result in stronger gels gelatin films containing greater triple-helix content swell less in water and are consequentially much stronger. Chemical cross-links can be introduced, to alter the gel properties, using transglutaminase to link lysine to glutamine residues or by use of glutaraldehyde to link lysine to lysine.

## 2.5.2 Structure, Chemical and Polymeric Characterization Of Gelatin

The characterization of gelatin as a product of the denaturation and structural degradation of collagen has been discussed not only in numerous experimental works but also in some review papers and monographs paper. (Kargin et al, 1993).

The properties of gelatin as a typical rigid-chain high molecular weight compound are in many respects similar to those of rigid-chain synthetic polymers. Gelatin exhibits essentially the same common properties typical of polymeric substances, which is not the case with native collagen. Thus, in a similar way to linear-chain synthetic polymers, in aqueous solutions gelatin macromolecules assume, at elevated temperatures, the conformation of a statistical coil. Under specific conditions (temperature, solvent, pH) gelatin macromolecules can display flexibility sufficient to realize a wide variety of conformations. This makes it possible to vary all the gelatin characteristics dependent on its molecular structure.

Besides, gelatin, similar to synthetic high polymers, shows a rather wide molecular weight distribution. Quite as interesting is the capacity of gelatin to form a large variety of super-molecular structures, from the simplest globular structure, typical of amorphous polymers, to a well developed fibrillar structure with various intermediate states. Clearly the differences in super-molecular structures should be reflected in the physico-mechanical properties of the gelatin materials.

Structural diversity of gelatin chain units determines the specific features of gelatin properties. Most synthetic polymers show no such features that are typical of most biopolymers.

The first peculiarity of gelatin common to all biopolymers arises from the presence of both acidic and basic functional groups in the gelatin macromolecules.

The second peculiarity of gelatin is its capacity to form a specific triple-stranded helical structure not observed in synthetic polymers (this structure is formed in solutions at low temperatures). The rate of the formation of a helical structure depends on many factors such as the presence of covalent cross-bonds, gelatin molecular weight, the presence of iminoacids and the gelatin concentration in the solution.

The third peculiarity of gelatin as a biopolymer is its specific interaction with water which is different to that observed with synthetic hydrophilic polymers. This peculiarity governs the structural and physico-mechanical properties of gelatin in the solid state.

### 2.5.3 The Effect of Water on the Structural and Mechanical Properties of Gelatin

In the past twenty years the state of water in biopolymers has been discussed extensively in the literature. As an inherent component of living cells, water plays an important role in all biological processes. Because collagen and gelatin are important commercial products, the elucidation of the effect of water on their structural and mechanical properties is of great practical significance. It is well known that the isotherms of water sorption by gelatin are S-shaped which is typical of most natural and synthetic hydrophilic polymers and is evidence for the varied degrees of interaction of the polymers with sorbed water. This S-shape has allowed many authors to apply the Brunauer, Emmett and Teller (BET) polymolecular adsorption theory to the gelatin-water system. A thorough study of the sorption parameters of hydrophilic polymers toward water has shown the linear region to correspond to the strongest interaction between the components of the system. The amount of water bound directly to gelatin is 12-14% and corresponds to the so-called 'monomolecular layer'. Further water sorption ('polymolecular layers') is accompanied by a far lower thermal effect though this water is also bound.

Water sorption by gelatin depends on a number of factors, both common to all substances and specific for gelatin and related polymers. Thus the sorption capacity of gelatin depends substantially on pH and increases with ionization of dissociating groups. At the same time, the sorption capacity of gelatin is independent of its molecular weight except probably in the case of a highly hydrolyzed product where the number of carboxyl and amino terminal groups is greatly increased, and also the sorption capacity of gelatin decreases with increasing temperature, which is of some practical importance. The specific behaviour of the gelatin sorption capacity toward water vapours, considered as a function of the conformational state of gelatin macromolecules, is also of interest. Comparison of the sorption capacities of films made of collagen with those of helical and coiled gelatin has shown that the higher the degree of molecular ordering in the protein the higher its sorption capacity toward water vapour at almost all air humidity. The analysis of the literature data on hydration of gelatin and collagen makes it possible to subdivide protein-bound water according to its state, function and influence on the properties of these materials. Hence the critical amount of water corresponding to the optimum physico-mechanical properties of gelatin materials may be determined. Contains the data reported by various authors on the amount of water bound in helical gelatin and in collagen.

A number of authors have thus considered water a necessary element for the formation of the collagen and gelatin helical structures. A definite amount of water is believed to be necessary to maintain the native conformation of collagen molecules. The specific role of

water in the formation of the helical structure has also been demonstrated for synthetic polypeptides isomorphous to collagen. The effect of water on the mechanical properties of collagen and gelatin also provides some information, though indirectly, on the role of water in the formation of the collagen and gelatin helical structures. Gelatin dehydrated below a 2% moisture level becomes insoluble in water because of cross-linking between the gelatins macromolecules. Dehydrated gelatin is thus a cross-linked polymer which makes it extremely brittle and hence unusable in the solid state. At  $25\pm 3\%$  moisture content, gelatin undergoes transformation from the glassy to the rubbery state at room temperature.

#### **2.5.4 Physico-Chemical Properties of Gelatin**

When heated, gelatin undergoes not only structural and mechanical but also, physico-chemical transformations such as partial or complete loss of solubility in water caused by cross-linking and lowering of molecular weight caused by thermal and thermo-oxidative destruction. These changes are observed in heating gelatin between  $140^{\circ}\text{C}$  and  $170^{\circ}\text{C}$ , gelatin is insoluble in hot water. Thermally treated gelatin occurs in water as an elastic gel-like film. The formation of chemical bonds between gelatin macromolecules in heating above  $140^{\circ}\text{C}$  is confirmed by its insolubility in hot saturated urea solutions. As soon as gelatin undergoes transformation to the rubbery state (at  $\sim 210^{\circ}\text{C}$ ) and its viscosity decreases, its liability to thermal destruction and, in the presence of air, thermo-oxidative destruction increases.

It therefore follows that the thermal stability of gelatin, determined by the temperature at which it begins to decompose, is close to its heat resistance determined by the super contraction temperature.

#### **2.5.5 Physico-Chemical Modification of Gelatin**

Physico-chemical modification of polymeric substances implies the preparation of two- or multi-component systems modifying the chemical composition and structure of the material, but involving no chemical reaction between the components. Physico-chemical modification includes the use of various methods for plasticization of polymers and for construction of heterogeneous systems.

#### **2.5.6 Chemical Modification of Gelatin**

The methods of chemically modifying gelatin take advantage of the presence of various functions in gelatin that are reactive towards various low- and high-molecular weight

compounds. These methods will be subdivided into three groups: (1) polymer-analogous conversions; (2) cross-linking; (3) graft copolymerization.

**Polymer-analogous conversions.** These methods were employed both for purely scientific purposes and to improve some technological processes for manufacturing new materials with the desired properties. The scientific utility of these methods comes mainly from the possibility they offer for elucidating the mechanism and nature of gel formation in gelatin. As for technological applications, gelatin thus modified is widely used in the manufacture of photographic emulsions by a solid phase deposition method, in micro-capsulation of hydrophobic substances, in medicine and various industries.

**Cross linking.** Chemical modification by cross-linking should directly affect the physico-mechanical properties of gelatin. These methods are widely used in the manufacture of various gelatin and collagen products and seem to offer limitless possibilities for modifying the properties of gelatin because the number of bi- and polyfunctional organic and inorganic compounds that can interact with the particular gelatin functions is very large indeed. Cross-linking of gelatin macromolecules is known to increase the viscosity of the gelatin solutions, strength and melting points of gelatin gels, and to affect all the physicochemical and mechanical properties of solid gelatin. A linear relationship between the swelling and rigidity modulus of hardened gelatin layers independent of the nature of the hardener, the temperature and the conformational state of the gelatin macromolecules has been observed. It is noteworthy that cross-linking of gelatin has no effect on the helix formation in solutions. As for solid gelatin, the cross linking has practically no effect on its heat resistance ( $T_s$ ) but substantially reduces its super contraction. It is also of interest that the presence of hardener does not hinder the decrease of  $T_s$  under the action of plasticizers<sup>1</sup>.

**Graft copolymerization.** The third group of methods employed for chemically modifying gelatin is the preparation of graft copolymers based on gelatin and synthetic polymers. The synthesis, structure, thermophysical and physico-mechanical properties of graft gelatin copolymers have been studied in detail. It is important that graft gelatin copolymers retain the valuable properties of the parent gelatin: the ability to form gels and helices and the high heat resistance. At the same time, gelatin acquires new properties depending on the nature of the grafted polymer. Thus grafting of rubber-like polybutylacrylate (PBAC) and rigid-chain polyacrylonitrile (PAN) onto gelatin yields products differing greatly in their properties. The dependence of the physico-mechanical properties of graft gelatin copolymers on the conformational state of gelatin macromolecules has attracted much

interest of researchers. The grafting of PBAC, a flexible-chain polymer, onto coiled gelatin produced a substantial increase in film elasticity.

## **2.6 Photographic Grade Gelatin**

### **2.6.1 Moisture Properties of Photographic Gelatin**

This article presents additional data on the temperature effect on the moisture equilibrium curve. However, unlike the work of Iwano (1994) and McCormick-Good Hart (1994, 1995, 1996), who studied the gelatin binder, this study presents information on the complete photographic film on cellulose triacetate base. Iwano interpreted his data as it pertains to the humidity inside sealed bags when used in accelerated incubation tests. A consideration for cold storage of photographic film is the rate of moisture conditioning at low temperatures (Sheppard, 1923).

A very practical aspect of moisture effects in storage is the moisture protection provided by various types of enclosure materials such as bags, boxes, and cans. Comparisons were made at both room temperature and at  $-16^{\circ}\text{C}$ . (Ward, 1977)

## **2.7 Animal Bone**

Bones are rigid organs that form part of the endoskeleton of vertebrates. They function to move, support, and protect the various organs of the body, produce red and white blood cells and store minerals. Because bones come in a variety of shapes and have a complex internal and external structure, they are lightweight, yet strong and hard, in addition to fulfilling their many other functions. One of the types of tissues that makes up bone is the mineralized osseous tissue, also called bone tissue, that gives it rigidity and honeycomb-like three-dimensional internal structure. Other types of tissue found in bones include marrow, endosteum and periosteum, nerves, blood vessels and cartilage. (Logan and Taylor, 1937)

Data on the protein, fat, moisture and ash content of whole bones from domestic animals and fowl of market weight and age are needed for at least three reasons: first, information on composition of bone may be useful in improving subjective estimates of bone maturity as they relate to U.S.D.A. grades; second, some components of bone from domestic animals and poultry may be a potential source of human food; and third, information on bone composition is needed so that a reliable method for accurately detecting the amount of bone in mechanically de-boned meat can be developed. Proximate analyses and calcium content of physically separated bone from cattle, pigs, sheep, turkeys and chickens of

different ages are presented in this report. In addition, hydroxyprolines was determined to give an indication of protein quality in whole bone.

### 2.7.1 General View of Animal Bone

#### Compact Bone & Spongy (Cancellous Bone)

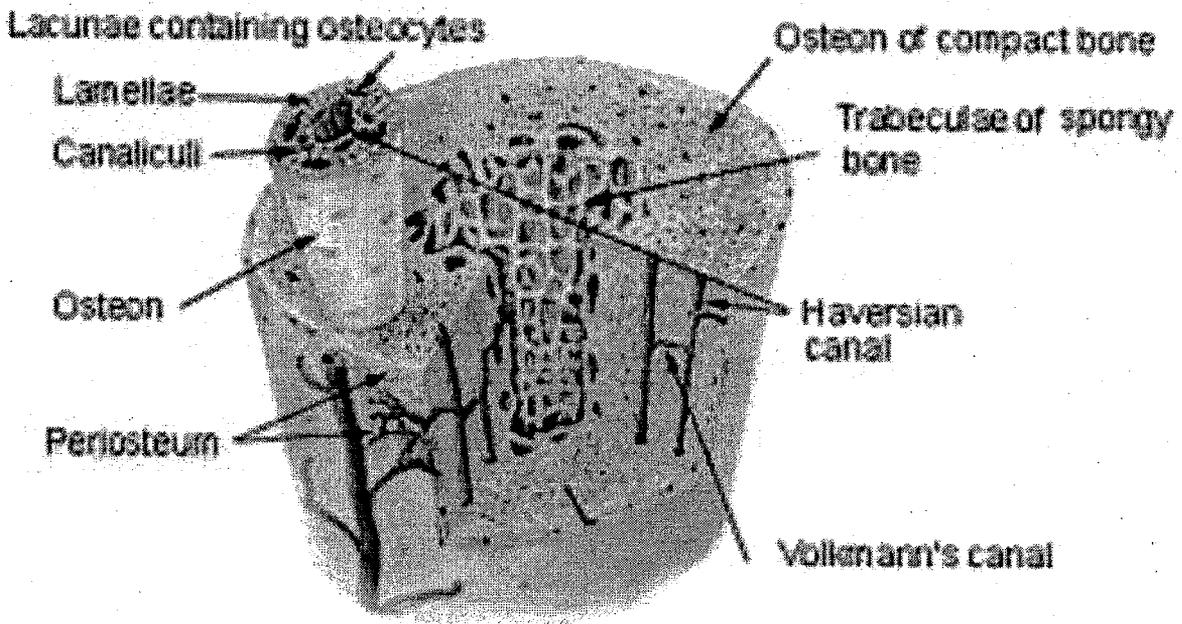
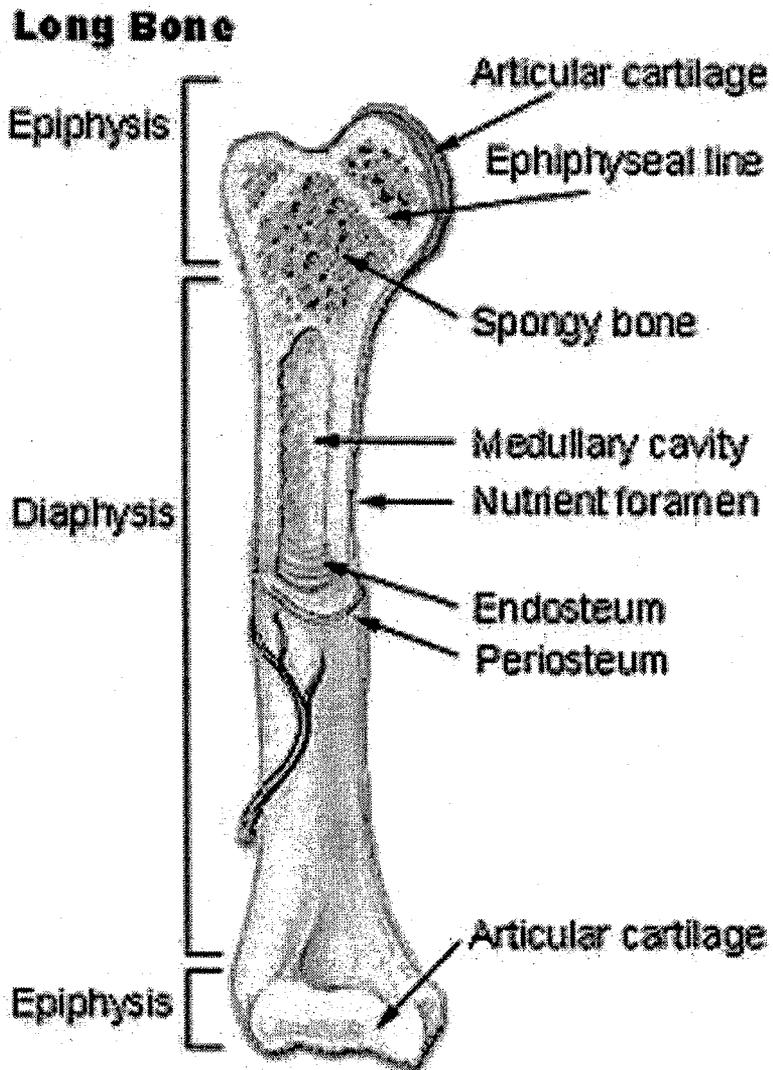


Fig 2.1 Transverse section of animal bone



## Fig 2.2 Femur of a Bovine

The hard outer layer of bones is composed of compact bone tissue, so-called due to its minimal gaps and spaces. This tissue gives bones their smooth, white, and solid appearance, and accounts for 80% of the total bone mass of an adult skeleton. Compact bone may also be referred to as dense bone

### 2.7.2 Composition of Animal Bone

#### 1. Composition

The composition of bones is somewhat variable according to age, state of nutrition & species. Bones also contain small amounts of Na, K, Cl & F, and traces of other elements. The ratio of calcium & phosphorus in the bone is about 2:1 (Maynard et al., 1979)

Table 2.1 Composition of Animal Bone

ITEM	%
Approximate Composition	
Water	45
Protein	25
Fat	10
Ash	
Composition of Ash (Moisture and Fat free Basis)	
Calcium	36
Phosphorus	17
Magnesium	0.8

Table 2.1 Composition of Animal Bone

### 2.7.3 Bone Structure of Animal Bone

Major constituents of animal bone are:

- Bone cells
- Organic matrix
- Minerals.

## **1. Bone cells:**

Bone cell is simply referred to as the cell that is part of the bone. Thus the major constituent of the bone cell are;

### **Osteoblasts**

This is referred to as the cell from which bone develops. It is found in active areas of bone formation. Osteoblast is responsible for the synthesis of bone matrix.

Most cells eventually rise to osteocytes, while others remain as osteoblasts for a long period of time, and some return to the state of "osteoprogenitor cell" (i.e., slightly differentiated mesenchymal cell).

### **Osteocytes**

This is the mature bone cells found in the bone matrix. It is less active as osteoblasts, and involved in maintenance of bony matrix. During destruction of the matrix (e.g., in the process of remodeling), some osteocytes die, whereas others return to the state of osteoprogenitor cell (Lee I. Chiba, 2007)

### **Osteoclasts**

This is the cell that is involved in the breakdown and resorption of bone tissues. It is the multinuclear, giant cell found in sites of bone resorption.

A precise role of osteoclasts in bone resorption is not clear, but those cells are responsible for secretion of collagenase, acid & proteolytic enzymes

## **2. Organic matrix**

The organic matrix is the body substance in which tissue cells are embedded. This body substance consist of 95% collagen, which is responsible for hardness & resistance of the bone. Others substances make-up 5% Amorphous (non-crystalline) ground substances.

Ground substances - Fill spaces, act as a lubricant & also serve as a barrier.

The organic matrix also contain glycosaminoglycans which is associated with proteins, (mucopolysaccharides) - e.g., Chondroitin 4-sulfate, chondroitin 6-sulfate and keratin sulfate.

## **3. Minerals**

The Inorganic matter constituent are mostly calcium & phosphorus which are deposited as tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ) - Amorphous and are predominant in immature bones.

$\text{Ca}_3(\text{PO}_4)_2$  undergoes changes to form hydroxyapatite  $\{3[\text{Ca}_3(\text{PO}_4)_2] \cdot \text{Ca}(\text{OH})_2 \text{ or } \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\}$  – Crystalline and predominant in mature bones( Crenshaw et al., 1981)

#### 2.7.4 Properties and Characteristics of Animal Bone

The primary tissue of bone, osseous tissue, is a relatively hard and lightweight composite material, formed mostly of calcium phosphate in the chemical arrangement termed calcium hydroxylapatite (this is the osseous tissue that gives bones their rigidity). It has relatively high compressive strength but poor tensile strength, meaning it resists pushing forces well, but not pulling forces. While bone is essentially brittle, it does have a significant degree of elasticity, contributed chiefly by collagen. All bones consist of living cells embedded in the mineralized organic *matrix* that makes up the osseous tissue.

#### 2.7.5 Functions and Uses of Animal Bone

Bone is essentially brittle and has a degree of elasticity contributed by its organic components mainly collagen. It has an internal mesh-like structure, the density of which may vary at different joints. Long bones can be connected to muscles via tendons and connect at joints by ligament(Legkaya Insustriya, Moscow, 1971)

Bone functions as a support and protector to the body structures Bones can serve to protect internal organs, such as the skull protecting the brain or the ribs protecting the heart and lung.s.

Other essential functions of animal bone include

- Shape — Bones provide a frame to keep the body supported.
- Blood production — The marrow, located within the medullary cavity of long bones and interstices of cancellous bone, produces blood cells in a process called haematopoiesis.
- Mineral storage — Bones act as reserves of minerals important for the body, most notably calcium and phosphorus.
- Movement — Bones, skeletal muscles, tendons, ligaments and joints function together to generate and transfer forces so that individual body parts or the whole body can be manipulated in three-dimensional space. The interaction between bone and muscle is studied in biomechanics.
- Acid-base balance — Bone buffers the blood against excessive pH changes by absorbing or releasing alkaline salts.

**EXTRACTION AND CHARACTERIZATION OF GELATIN  
FROM ANIMAL BONE FOR THE PRODUCTION OF  
PHOGRAPHIC FILM**

**BY**

***ANYANWU SUNDAY ANAYO***

**(2003/14960EH)**

**DEPARTMENT OF CHEMICAL ENGINEERING,  
SCHOOL OF ENGINEERING AND ENGINEERING TECHNOLOGY  
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA  
NIGER STATE, NIGERIA.**

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similar to that of its parent collagen (Ward, A.G; Courts, A. 1977). Gelatin solutions show visco-elastic flow and streaming birefringence.

The solubility of the gelatin is determined by the method of manufacture. Typically, gelatin can be dispersed in a relatively concentrated acid. Such dispersions are stable for 10-15 days with little or no chemical changes and are suitable for coating purposes or for extrusion into a precipitating bath. Gelatin is also soluble in most polar solvents. Gelatin gels exist over only a small temperature range.

The mechanical properties are very sensitive to temperature variations, previous thermal history of the gel, and time. The viscosity of the gelatin/water mixture increases with concentration and when kept cool ( Tucker, H. A., 1965)

## **2.2 Production Of Gelatin**

The worldwide production amount of gelatin is about 300,000 tons per year, roughly 600 million lbs ([www.gelatine.org/marketdata2005](http://www.gelatine.org/marketdata2005)). On a commercial scale, gelatin is made from by-products of the meat and leather industry, mainly pork skins, pork and cattle bones, or split cattle hides.

Recently, by-products of the fishery industry began to be considered as raw material for gelatin production because they eliminate most of the religious obstacles surrounding gelatin consumption (Islamic Services of America, 2006). Contrary to popular belief, horns and hooves are not commonly used. The raw materials are prepared by different curing, acid, and alkali processes which are employed to extract the dried collagen hydrolysate. These processes may take up to several weeks, and differences in such processes have great effects on the properties of the final gelatin products (GELITA Group, 2006).

Gelatin can also be prepared at home. Boiling certain cartilaginous cuts of meat or bones will result in gelatin being dissolved into the water. Depending on the concentration, the resulting broth, when cooled, will naturally form a jelly or gel.

While there are many processes whereby collagen can be converted to gelatin, they all have several factors in common. The intermolecular and intramolecular bonds which stabilize insoluble collagen rendering it insoluble must be broken, and the hydrogen bonds which stabilize the collagen helix must also be broken. (Ward, 1977).

## CHAPTER ONE

### INTRODUCTION

In recent years, the development of environmental friendly and biodegradable materials based on natural polymers have received increasing attention. This is due to attempt to substitute petroleum-based plastics which presents concerns in terms of pollution and sustainability. Among other biopolymers, gelatin has been extensively studied due to its low cost, biodegradability, biocompatibility and non immunogenic.

Gelatin, a protein substance obtained by boiling animal bones and connective tissue containing collagen in water or dilute acid or base is colorless, transparent, odorless brittle and tasteless in a purified form. Gelatin dissolves in hot water and forms a gel or jelly upon cooling. It is insoluble in organic solvents, such as ether, chloroform and benzene. When placed in cold water, gelatin takes up five to ten times its own weight and swells to an elastic transparent mass. (Budavari, 1996).

Isinglass is an impure form of gelatin. A purified form of gelatin obtained from air bladders of fish, including sturgeon, cod, catfish and carps is called isinglass. Gelatin is a heterogeneous mixture of water soluble proteins of high molecular weight (Budavari, 1996). On dry weight basis gelatin consist of 98-99% protein. The molecular weight of this large protein structures typically ranges between 20,000 and 250,000, with some aggregate weighing in the millions (Kennase, 1994).

Gelatin is obtained by thermal denaturation of physical and chemical degradation of collagen from animal skins and bones. Collagen, in which gelatin is obtained from is a fibrous protein that makes up a connecting tissue, that is, it is a proteinous chemical substance that is the main support of skin, tendons, bones cartilages, and connecting tissues.

Gelatin is amphoteric. Meaning that it is neither acidic nor alkaline but possesses both properties depending on the nature of the solution. (Budavari, 1996). The pH at which gelatin is charged in solution is neutral is known as isoelectric point. The isoelectric point of gelatin ranges between 4.8 and 9.4 with acid processed gelatin having higher isoelectric point than alkaline processed gelatin (Poppe, 1997).

Gelatin is not found in nature but is derived from collagen, a naturally occurring protein (Budavari, 1996). One possible exception may be undenatured isinglass (Ackerman, 1991) or isinglass that has been denatured by thermal treatment. (Hickman et al, 2000)

ur processes are used: acid, alkaline, enzymatic and heat/processes. Among all of these  
cesses, heat/pressure processes is done at a short time and no chemicals, but the  
racted gelatin has low bloom index. Gelatin is widely used in food, pharmaceutical  
ustries as well as in the biomedical field : hard and soft capsule, micro spheres,  
lants for vascular prostheses, wound dressing, adsorbent pad, for surgical use and  
plantable device. In spite of gelatin advantages, gelatin films has poor mechanical  
properties and are dissolvable in aqueous solutions very fast, which limit their possible  
plication as a biomaterial especially for long-term applications (Ward,1985).

latin in its purest form is used a constituents of food, being highly nutritious and easily  
gestible and absorbed. It cannot, however, completely replace other proteins because it  
lacks some essential amino acids. Gelatin is used in making jams and jellies, ice creams,  
and marshmallows and as a setting for other foods in spices.

is employed in photographic industries in the preparation of films plates and paper; in  
acteriology as a culture medium; and in medicine as a coating for capsules, pills, and  
some surgical dressings. It is also used dyeing and in photomechanical printing processing  
(Schmidt, 2004)

## **1 Aims/Objective**

### **Aim**

The aim of this research work to gelatin from the animal bone (cattle) for the production of  
photographic film.

### **Objective**

The objective of this research work is to produce and characterize the gelatin obtained  
from animal bone with a view to ensuring that it is suitable for the production of  
photographic film and other accessories.

## **2 Scope And Limitation**

This research work covers the production and the characterization of gelatin from animal  
bone that will be suitable for use in the photographic industry.

- Detoxification — Bone tissues can also store heavy metals and other foreign elements, removing them from the blood and reducing their effects on other tissues. These can later be gradually released for excretion
- Sound transduction — Bones are important in the mechanical aspect of hearing and protect the internal organs and facilitate movement in conjunction with muscles.

Some cut and polished bones from variety of animals are used as materials for jewelry and other crafts. Ground cattle bone is sometime used as fertilizer.

In the stone age, bones were used to manufacture art weapons needles etc.

The hydrolysis of collagen that is extracted from bone gives gelatin which has a wide range of industrial, pharmaceutical and technical applications.

### **2.7.6 Animal Bone as a Source of Gelatin**

The basic steps in the production of gelatin from animal bone are; Washing of the bone to remove dirt, blood and other soluble impurities. Drying and crushing of the bone to relatively small size. Curing of the bone sample. This involves the treatment of the bone sample with dilute HCl for about 5-days. The step is to kill bacterial and demineralize the bone.

Washing and liming of the cured sample. The liming process is whereby the washed sample is soaked in  $\text{Ca}(\text{OH})_2$  solution for about 2-days. This is to neutralize the acid to a pH of 7 which is suitable for gelatin extraction.

The extraction process involves boiling the treated bone (ossein) in distilled water for 2 hours and constantly stirred. During this process gelatin gradually dissolves in the boiling water.

The next stage is the filtration, concentration and evaporation of the filtrate. And finally the gelatin formed is dried at a temperature of about  $60^\circ\text{C}$

## **2.8 Photographic Film**

### **2.8.1 Background Study**

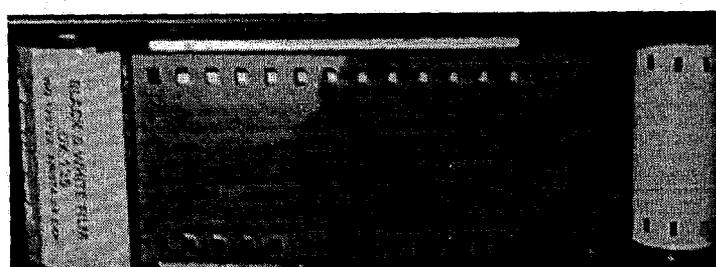
Photographic film is a sheet of plastic (polyester, nitrocellulose or cellulose acetate) coated with an emulsion containing light-sensitive silver halide salts (bonded by gelatin) with variable crystal sizes that determine the sensitivity, contrast and resolution of the film.

When the emulsion is sufficiently exposed to light (or other forms of electromagnetic radiation such as X-rays), it forms a latent (invisible) image. Chemical processes can then be applied to the film to create a visible image, in a process called film developing.

In black-and-white photographic film there is usually one layer of silver salts. When the exposed grains are developed, the silver salts are converted to metallic silver, which block light and appear as the black part of the film negative.

Color film uses at least three layers. Dyes, which adsorb to the surface of the silver salts, make the crystals sensitive to different colors. Typically the blue-sensitive layer is on top, followed by the green and red layers. During development, the exposed silver salts are converted to metallic silver, just as with black and white film. But in a color film, the by-products of the development reaction simultaneously combine with chemicals known as color couplers that are included either in the film itself or in the developer solution to form colored dyes. Because the by-products are created in direct proportion to the amount of exposure and development, the dye clouds formed are also in proportion to the exposure and development. Following development, the silver is converted back to silver salts in the bleach step. It is removed from the film in the fix step. This leaves behind only the formed color dyes, which combine to make up the colored visible image.

Photographic film is a chemically reactive material that records a fixed or still image when the film is exposed to light. Typically, film is placed in a camera, and light from the image being photographed is allowed to enter and is focused and sometimes made larger or smaller by the camera lens. The film is exposed to the image by opening a shutter in the camera body, and the combination of the speed of the shutter and the film speed (which is the chemical reactivity of the film) controls the amount of light that strikes the film. The image is recorded on the film, but it is a latent or invisible image. When the film is removed from the camera, it is developed by chemical processes into a visible image. This visible image is negative or the reverse in brightness of the way our eyes see light; the brightest parts of the photographed object appear the darkest on the negative where the film received the most exposure to light. The negative image is made positive, or as our eyes see it, by another type of processing whereby the negative is printed on sensitive paper. Color-reversal films are positives and are used for making slides. All of the elements of the process—the parts of the camera, the type and parts of the lens, the type of film, including its chemistry, the developing process, the printing process, and the type of paper—contribute to the sharpness or trueness of the finished photograph.



### 2.8.2 History of Photographic Film

Film was "discovered" in a chemistry laboratory. In 1727, Johann Henrich Schulze, a German doctor, mixed chalk, silver, and nitric acid in a flask to make silver nitrate. When the solution was exposed to sunlight, it changed color from white to purple. When Schulze pasted cutouts of letters and numbers on the outside of a flask of freshly made solution and exposed it to the light, the cutouts appeared to have been printed on the solution. Although the discovery marked the birth of photography, it was not used for over 100 years. In 1839, Louis Daguerre, a French painter, created a photographic process in which liquid iodine was placed on a silvered copper plate, and the plate was exposed to light. The liquid iodine was the emulsion, or light-reactive chemical, and the copper plate was the base for these photographs called "daguerreotypes." The American inventor Samuel F.B. Morse learned the art of daguerreotype and taught it to Matthew Brady, who made images of the Civil War that are treasured both as historical records and artistic landmarks in photography. Daguerreotype was cumbersome to use; the "wet plate" process was awkward, the box-type cameras had to hold the large plates, and the finished photographs were the size of the plates. While Daguerre was developing his process, William Henry Fox Talbot, an English archaeologist, created his own process called "calotype," meaning "beautiful picture" in 1841. Talbot coated a paper base with an emulsion of silver iodide and produced a negative by a developing process. The calotype is more like today's film and photographic process, and the intermediate step resulting in a negative permitted more than one print to be made. The flexibility of photography was improved further in 1871 when R.L. Maddox invented the "dry plate" process. Gelatin made from animal bones and hides was used to coat glass plates, and silver iodide was precipitated inside the gelatin layer. The plates and their dried jelly could be exposed, and then the photograph could be developed later by rewetting the gelatin. The complicated procedure of manufacturing the plate, exposing it, and processing it into the finished photograph was broken into parts that made the photographer's work easier and made photography and photo processing a manufacturing industry. George Eastman combined the paper base of Talbot's calotype with the gelatinous silver nitrate emulsion from Maddox's process to invent flexible roll film in 1884. Eastman quickly made the transition to an emulsion-bearing plastic, transparent film by 1889, which was a year after his company introduced the first Kodak camera. These developments

made photography a simple, compact, portable practice that is now the most popular hobby in the United States.

### **2.8.3 The Raw Materials**

A roll of film consists of the emulsion and base that compose the film itself, the cassette or cartridge, and outer protective packaging. The materials used to make the emulsion are silver, nitric acid, and gelatin. The base consists of cellulose and solvents that are mixed to form a thick fluid called dope. Film that is packed in a cassette (35-millimeter film is typically packed this way) requires a metal spool, the protective metal canister, and plastic strips at the canister opening where the film emerges. Other sizes of film including Polaroid film are protected from light and air by plastic cartridges or packs. Outer packaging, which varies among film products, is made from foil-lined paper, plastic, and thin cardboard cartons. The outer packaging is also insulating and protects the film from exposure to light, heat, and air.

### **2.8.4 The Manufacturing Process**

- **Base**

For most films, the base to which the light-sensitive emulsion is fixed consists of cellulose acetate, which is wood pulp or cotton linters (short cottonseed fibers) mixed with acetate to form a syrup. Solid pellets of cellulose acetate precipitate or separate out of the syrup and are washed and dried. The pellets are dissolved in solvents to form the transparent, honey-like dope. The dope is spread in a thin, even sheet on a wheel that is two stories in diameter. The wheel is plated with chromium for a smooth finish, and it turns slowly. The solvents in the dope volatilize or evaporate as the wheel turns. The process is much like the applying and drying of nail polish. The remaining base is a thin sheet of plastic that is of a uniform thickness measured in ten-thousandths of an inch. When it is dry, the base is removed from the wheel and wound on 54-inch (137 cm) diameter reels.

- **Emulsion**

Silver is the main ingredient of the emulsion. Pure silver bullion is received at the manufacturing plant in bars that are checked by weight and serial number. The bars are dissolved in a strong solution of nitric acid, and the process releases heat. After the acid has completely dissolved the silver, the solution is stirred constantly and cooled. Cooling causes crystals of silver nitrate to grow, much like salt crystals in water. The crystals are wet with water that also separates out. The crystals are removed from the solution and whirled in centrifuges with sieve-like openings to remove the water and keep the crystals

pure. At this point in the process, the chemical solutions are light-sensitive, so further manufacturing processes are completed in darkness.

Meanwhile, gelatin has been made using distilled water and treated with chemicals including potassium iodide and potassium bromide. The gelatin serves as a binding agent to hold the silver nitrate crystals, and also to fix them to the base. The gelatin and chemicals are mixed in cookers that are lined with silver so the emulsion remains pure. As the mixture cools, silver halide salts (chemical combinations of the silver, iodide, and bromide) form as fine crystals that remain suspended in the gelatin to make the emulsion.

- **Coating process**

The emulsion is pumped through a piping system to "coating alley," a huge work area that may be 200 feet (61 m) wide and five stories high. The area must be immaculately clean and dust-free, and the operations of the roll-coating machines are controlled by arrays of control panels in the fully automated process. Machines coat precise amounts of emulsion in micro-thin layers on the wide strips of plastic base; a single, dried layer of emulsion may be six one-hundred-thousandths of an inch thick. Successive layers of three emulsions are applied to the base to make color film, and each emulsion layer has its own color-forming chemicals called linked dyes. The three emulsion layers in color film respond to blue, green, and red light, so each photograph is a triple latent image with the sandwiched color range reproduced by processing. The strips of emulsion-coated base (now film) are cut into progressively narrower widths, perforated so the film can be advanced in the camera, and spooled, except for instant film and sheet film that are packed flat.

- **Packaging**

Film is packed in cartridges, cassettes, rolls, instant packs, or sheets. Cartridges are used in certain types of cameras and include a take-up spool that is built in so the exposed film and cartridge are removed as a unit. Cassettes are made for cameras that use film in the 35-millimeter format. They consist of a spool enclosed in a metal jacket. The tongue of the film is drawn over the pressure plate at the back of the camera to a take-up spool that is built into the camera. When the film is finished, it is rewound onto the spool in the cassette, and the unit is removed. Roll films consist of paper-backed film that is packed on a spool like the one in the camera. The film is wound onto the spool in the camera, and that spool and film are removed. The spool on which the film was packed originally can then be moved to the receiving side of the camera, and a new roll inserted. The packs for instant cameras contain 8 to 12 sheets that are ejected individually after each shot. Sheet film is used for specialized applications like x-ray film.

## 2.8.5 Properties of Photographic Film Raw Materials

Possible approaches to assess the effect of processing solutions or environmental factors such as temperature, and relative humidity on the stability of coated gelatin can be grouped into three categories:

1. Accelerated aging tests of photographic specimens, followed by quantitative tests such as tensile strength, elongation and dimensional changes.
2. Chemical analysis before and after a treatment, for example in an amino acid analyzer.
3. Monitoring of physical properties. Measurable properties of gelatin include:
  - o Bloom strength;
  - o Gel strength; and
  - o Viscosity;

And of coated gelatin layers:

- o Melting point;
- o Scratch resistance;
- o Resistance to abrasion during processing ("mushiness"); and
- o Swelling.

The results show that the stability of the gelatin is influenced by the type of support onto which it is 'coated. The results of experiments to examine the Physical properties of unsupported gelatin layers have also been reported. Generally speaking, gelatin layers have longevity comparable to that of the cellulose triacetate onto which they are coated, and are quite stable as long as they are kept dry. Photographic gelatin layers are usually acidic in nature. Gelatin is an anisotropic substance: its properties are independent of geometry. If, for example, a stripped layer of gelatin is placed in water to observe lateral swelling, no preferential expansion is noted: a square piece been reviewed by Current. Chemical analyses, even when performed in fully automated analytical equipment, are too complex and time-consuming to be carried out on large numbers of samples. One can monitor the chemical changes in the dyes of a faded colour photograph by measuring physical changes, such as density values; similarly, by determining changes in physical properties of gelatin, such as a decrease in the mushiness value, it is possible to infer chemical changes in the gelatin. For purposes of determining the influence of gelatin layers on the stability of processed photographic films, samples were reported to have been incubated at 1000 C in a dry oven, as well as at a temperature of 71° C, and a relative humidity of 50%.

(Hill, Thomas, 1977).

### **2.8.6 Emulsion Characteristics**

The emulsion itself is made up from light sensitive silver halide crystals suspended in gelatin.

Photographic papers are distinguished by the characteristics inherent in their different photographic emulsions. Black-and-white photographic paper is available in different grades which are usually numbered 0 to 5, with 0 being the softest, or least contrastful paper grade and 5 being the hardest, or most contrastful paper grade.

Photographic emulsions are also produced in a variable contrast type which permits the selection of any grade between 00 and 5. Variable contrast photographic paper is actually coated with a mixture of two types of emulsion, one of which is very low in contrast (0 or 00) and one of which is very high in contrast (5). The low contrast layer is activated by green light, the high contrast layer by blue light. The use of filters activates each layer in different proportions, thereby creating all contrast grades from 0 (or 00) to 5.

The trade names of variable contrast photographic papers typically include a reference to contrast variability, such as various contrasts, Multigrade, Vary con or similar. Some companies simply designate variable contrast type papers with the words variable or polycontrast.

The contrast of photographic papers can also be controlled during processing or by the use of bleaches or toners.

### **2.8.7 Quality Control in Photographic Film Industry**

In all phases of manufacture, photographic film is extremely sensitive to light, heat, dust, and impurities. Air flow into the film-manufacturing rooms is washed and filtered.

Temperature and humidity are carefully regulated. Production rooms are scrubbed clean daily, and plant workers wear protective clothing and enter sensitive work areas through air showers that clean personnel of dust and contaminants. Each step of manufacture is carefully inspected and controlled. For example, the chromium-plated wheel on which the base is formed is inspected to maintain a mirror-like finish because tiny imperfections will affect the quality of the film. Finally, samples of film are removed from completed batches and subjected to many tests, including the taking of photographs with the samples.

## CHAPTER THREE

### 3.0 MATERIALS AND METHODOLOGY

#### 3.1 Materials and Equipment

In an attempt to carry out this research many instruments and equipments were used. But only the main materials and equipments are presented in this chapter.

**Table 3.1 List of equipments**

Name	Model/Manufacturer
Beakers	Techmel/USA
Electronic Weighing Balance	CT1200/OHAUS
Sieve	Tower Brand
Hot Plate	Pyrex
Measuring Cylinder	Techmel/USA
pH Meter	3071/Jenway
Stirrer	Emmex
Thermometer	Sedim
Oven	Pyrex
Flow cup Viscometer	Cannon Fenske
Filter	—

**Table 3.2 List of Materials and reagents**

Material/Reagent	Formula	Comment
Animal Bone (Cow Bone)	—	Got from Minna Abattoir
Hydrochloric Acid	HCl	Laboratory reagent
Calcium Hydroxide	Ca(OH) <sub>2</sub>	Laboratory reagent
Distilled Water	H <sub>2</sub> O	Analytical reagent

#### 3.2.0 Procedure

##### 3.2.1 Raw Materials Preparation

The cow bone was washed to remove dirt, blood and other soluble impurities. It was then dried in an oven at a temperature of 98°C for 3hrs so as to remove fat and to enhance size

reduction of the bone. The ground bone was sieved to obtain different uniform particle size.

### **3.2.2 Curing of the Bones**

After sieving, 40g of the bone from the various particle sizes of 1.40mm, 2.00mm, 2.80mm, 3.35mm and 5.00mm was weighed and placed into different beakers. Thereafter, 6% concentrated hydrochloric acid solution was added to the bone sample in each beaker and was left to soak to 5 days. This treatment removes bacterial and demineralizes the bone. The samples were stirred thoroughly using stirrer on daily basis.

### **3.2.3 Washing of the Samples**

The cured bone samples were washed thoroughly with distilled water after 5 days of soaking in the acid until the water becomes clear.

### **3.2.4 Liming of the Samples**

The washed samples were limed by soaking them in 6% concentrated calcium hydroxide for 2 days so as to neutralize the acid and to provide the samples with a pH of 7.0 suitable for extraction.

### **3.2.5 Extraction of Gelatin**

The treated samples were placed separately according to particle sizes in beakers containing distilled water. Each of the filled beakers was placed one after the other and the content was heated at a temperature of 96°C for 2hrs 30minutes with constant stirring. During the process heating, gelatin dissolves in hot water.

### **3.2.6 Filtration**

The solution of the samples and the dissolved gelatin were filtered with the aid of a muslin cloth. The residue (bone) was removed after filtration. The filtrate (light liquor) containing gelatin was collected.

### **3.2.7 Concentration of Gelatin**

The collected liquor (filtrate) was heated until it becomes thickened and heavy liquor formed was formed as concentrated gelatins after cooling

### **3.2.8 Drying**

The set gelatin produced was dried using a tray at a temperature of 70°C and the dried product gelatin was obtained.

### **3.2.0 Characterization Procedure**

The following are the characterization procedure

#### **3.2.1 Viscosity**

The cannon Fenske viscometer was used to determine viscosity of gelatin.

Some quantity of the gelatin was put in the viscometer so that it just coincided with the level of the upper mark of the viscometer. The time taken for the meniscus of the sample (gelatin) to fall from the upper meniscus of the bulb was noted and the viscosity was calculated.

#### **3.2.2 pH Measurements**

The pH of the gelatin produced was measured by means of a pH meter. Sample of the gelatin produced was collected and put into a beaker and the pH value was determined by inserting the pH meter electrode into the beaker. The value was then read from the instrument.

#### **3.2.3 Moisture Content Analysis**

The moisture content of the gelatin was determined using the process outlined below

A 5g sample of the gelatin was put into a known mass of petri-dish. The sample and the petri-dish were put in an oven for 15minutes. Thereafter, the sample and the petri-dish were weighed. This was repeated severally until there is a constant weight.

The percentage moisture content was then calculated

## CHAPTER FOUR

### 4.0 RESULTS AND ANALYSIS

#### 4.1 Results

The results obtained from the experiment are shown below

Table 4.1 Weight Percentage Extract of Gelatin using 6% HCl acid

Bone Size Sample (mm)	Mass of Gelatin Extracted (g)	Percentage Extract %
1.40	22.104	55.26
2.00	21.004	52.51
2.80	18.573	46.43
3.35	14.804	37.01
5.00	12.756	31.89

Table 4.2 Characterization Results

Property	Samples					Standard Value
	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>4</sub>	M <sub>5</sub>	
pH (30°C)	6.1	6.3	6.2	5.8	5.9	5.5-7.0
Viscosity (32°C) (cp)	1.568	1.574	1.588	1.592	3.559	1.2-3.5
Moisture Content %	7.2	7.3	6.9	7.2	7.4	3.5-10.1

## 4.2 Discussion of Results

Table 4.1 shows that the yield of gelatin is inversely proportional to the bone sizes that are the smaller the bone size the higher the yield of gelatin. The bone sizes are 1.40mm, 2.00mm, 2.80mm, 3.35mm and 5.00mm gave yield of 22.104g, 21.004g, 18.573g, 14.804g and 12.756g of gelatin respectively. It is therefore advisable to have smaller bone sizes for higher yields of gelatin.

Table 4.2 shows the values of various properties of the samples (gelatin), from the table, the pH values of samples M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub> and M<sub>5</sub> are 6.1, 6.3, 6.2, 5.8 and 5.9 respectively. These values are considered reasonable as compare to the pH of standard values obtain from literature (5.5-7.0) for the standard photographic gelatin.

The viscosities of samples M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub> and M<sub>5</sub> are 1.568cp, 1.574cp, 1.588cp, 1.592cp and 3.559cp respectively. The result shows that M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> are closer compared to M<sub>5</sub>. The outrageous value of sample M<sub>5</sub> can be attributed to the fact that its value was not determined at the same time with the remaining samples. This is because the knowledge of polymerization shows that reaction often continue at room temperature and this leads to increase in molecular mass which has direct effect on the viscosity of the sample. This difference is considered to be as a result of the delay in the determination of the viscosity of sample M<sub>5</sub>.

Moisture content of M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub> and M<sub>5</sub> are 7.2%, 7.3%, 6.9%, 7.2% and 7.4% respectively. These values are considered reasonable as compared to the standard values obtained from literature. The results are close due to the fact that the same quantities of the samples were used for the test.

## **CHAPTER FIVE**

### **5.0 CONCLUSIONS AND RECOMMENDATIONS**

#### **5.1 Conclusions**

From the results and analysis, it can be concluded that higher yield of photographic gelatin is inversely proportional to the bone sizes. That is higher gelatin yield is obtained from the smaller particle sizes of bone than the larger ones. In addition animal is more economical in the production of photographic gelatin than other raw materials like animal skin, connective tissues etc., this is because animal bone is cheap and relatively available.

#### **5.2 Recommendations**

From the results obtained, I hereby recommend that, photographic grade gelatin should be Produced from animal bones. And that the particle size of the bone sample should be relatively small so as to enhance higher yield of gelatin

In order to have a generalized and established fact with respect to this research work, more information is required. Thus, it will be good if this research work can be made a continuous research process until all necessary facts and information are determined.

Similarly, industries should be setup to embark on the

## APPENDIX

### Concentration of Reagents Used

$$\% \text{ Concentration By Volume} = \frac{\text{Volume of Liquid}}{\text{Total Volume of Solution}} \times 100$$

#### For HCl acid

500ml of HCl was prepared with 6% concentration

$$6\% = \frac{\text{Volume of acid}}{500}$$

$$\text{Volume of acid} = \frac{500 \times 6}{100}$$

$$= 30\text{ml}$$

#### For Ca(OH)<sub>2</sub>

500ml of HCl was prepared with 6% concentration

$$6\% = \frac{\text{Volume of base}}{500}$$

$$\text{Volume of base} = \frac{500 \times 6}{100}$$

$$= 30\text{ml}$$

**% Extract of Gelatin using 6% concentration of HCl with 40g of bone**

$$\% \text{ extract} = \frac{\text{Mass Extracted}}{\text{Total Mass}} \times 100$$

**For 1.40mm of Bone size**

Mass of gelatin = 22.104g

$$\% \text{ extract} = \frac{22.104}{40} \times 100$$

$$= 55.26\%$$

**For 2.00mm of Bone size**

Mass of gelatin = 21.004g

$$\% \text{ extract} = \frac{21.004}{40} \times 100$$

$$= 52.51\%$$

**For 2.80mm of Bone size**

Mass of gelatin = 18.573g

$$\% \text{ extract} = \frac{18.573}{40} \times 100$$

$$= 46.43\%$$

**For 3.350mm of Bone size**

Mass of gelatin = 14.804g

$$\% \text{ extract} = \frac{14.804}{40} \times 100$$

$$= 37.01\%$$

**For 5.00mm of Bone size**

Mass of gelatin = 12.756g

$$\begin{aligned} \% \text{ extract} &= \frac{12.756}{40} \times 100 \\ &= 31.89\% \end{aligned}$$

**Viscosity Calculation**

The viscosity value determined using viscometer from the experiment was calculated thus:

Time taken for the gelatin to flow through flow cup viscometer, t

Stake's constant for the viscometer,  $S_c = 0.08$

$$\text{Viscosity} = S_c \times t$$

**For 1.40mm of Bone size**

t = 19.6s

$$\begin{aligned} \text{Viscosity} &= 0.08 \times 19.6 \\ &= 1.568\text{cp} \end{aligned}$$

**For 2.00mm of Bone size**

t = 19.68s

$$\begin{aligned} \text{Viscosity} &= 0.08 \times 19.68 \\ &= 1.574\text{cp} \end{aligned}$$

**For 2.80mm of Bone size**

t = 19.85s

$$\begin{aligned} \text{Viscosity} &= 0.08 \times 19.85 \\ &= 1.588\text{cp} \end{aligned}$$

**For 3.350mm of Bone size**

t = 19.9s

$$\begin{aligned} \text{Viscosity} &= 0.08 \times 19.9 \\ &= 1.592\text{cp} \end{aligned}$$

**For 5.00mm of Bone size**

$$t = 44.5\text{s}$$

$$\begin{aligned} \text{Viscosity} &= 0.08 \times 44.5 \\ &= 3.559\text{cp} \end{aligned}$$

**Moisture Content Calculation**

The moisture content of Gelatin is calculated thus:

$$\% \text{ moisture content} = \frac{\text{Moisture evaporated}}{\text{Initial mass}} \times 100$$

**For 1.40mm of Bone size**

$$\text{Moisture evaporated} = 1.5915$$

$$\begin{aligned} \% \text{ moisture content} &= \frac{1.5915}{22.104} \times 100 \\ &= 7.2\% \end{aligned}$$

**For 2.00mm of Bone size**

$$\text{Moisture evaporated} = 1.5333$$

$$\begin{aligned} \% \text{ moisture content} &= \frac{1.5333}{21.004} \times 100 \\ &= 7.3\% \end{aligned}$$

**For 2.80mm of Bone size**

$$\text{Moisture evaporated} = 1.2815$$

$$\begin{aligned} \% \text{ moisture content} &= \frac{1.2815}{18.573} \times 100 \end{aligned}$$

$$= 6.9\%$$

**For 3.350mm of Bone size**

$$\text{Moisture evaporated} = 1.0659$$

$$\begin{aligned} \text{\% moisture content} &= \frac{1.0659}{14.804} \times 100 \\ &= 7.2\% \end{aligned}$$

**For 5.00mm of Bone size**

$$\text{Moisture evaporated} = 0.9439$$

$$\begin{aligned} \text{\% moisture content} &= \frac{0.9439}{12.756} \times 100 \\ &= 7.4\% \end{aligned}$$

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