Solubility of Structurally Complicated Materials: II. Bone

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Bone is a structurally complex material, formed of both organic and inorganic chemicals. The organic compounds constitute mostly collagen and other proteins. The inorganic or bone mineral components constitute predominantly calcium, phosphate, carbonate, and a host of minor ingredients. The mineralized bone is composed of crystals which are closely associated with a protein of which collagen is an acidic polysaccharide material. This association is very close and the protein integrates into the crystalline structure. The mineralization involves the deposition of relatively insoluble crystals on an organic framework. The solubility process takes place when the outermost ions in the crystal lattice breakaway from the surface and become separated from the crystal. This is characteristic for ions dissolving in water or aqueous solutions at the specified temperature. The magnitude of solubility is temperature and pH dependent. Bone is sparingly soluble in most solvents. Enamel is less soluble than bone and fluoroapatite is the least soluble of all apatites in acid buffers. Collagen is less soluble in neutral salt solution than in dilute acid solutions at ambient temperatures. The solubility of collagens in solvents gradually decreases with increasing age of the bone samples. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2360606]

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1. Introduction

In the preceding paper[1] the relevant structure features and physical and chemical properties of wood were drawn together with an emphasis on the solubility of wood in solvents. As the second part of this series on Structurally Complicated Materials, the objective of this paper is to review some of the important aspects of the solubility of bone in solvents and liquids.

From actual handbooks and literature sources, it is clear that there is a very practical need to raise the level of awareness of the scarcity of experimental data and to try to stimulate interest in the collection and dissemination of well-defined data that are as accurate as possible ⟨http://www.knovel.com⟩.

The bibliographic literature, searched using Chemical Abstract Service Online, provided very little information. The search results rarely indicated that the bibliographic abstracts contained information that pointed to needed data ⟨http://www.cas/websearch/html⟩.

In current literature citations, from journal publications to widely used and cited handbooks, data have not undergone sufficient updating and evaluation to assure their reliability and accuracy,[2] Despite the great demand for solubility data by scientists and engineers, experimental values published in

the open literature are very limited. Users of the solubility data have two choices: to determine or to estimate the required solubility.

This paper is not an attempt to make an exhaustive review, but rather to give the reader some insight into the structure and solubility relation between bone and solvents. It does not mean that this compilation replaces the critical data analysis, but only aids solubility data evaluators in making some powerful interpretations of already published experimental data.

There are different forces which hold the atoms and molecules together in solid materials. The interatomic and intermolecular forces include metallic bonds, electrical attraction, dipolar, hydrogen bonds, molecular association, covalent bond, and cohesive or van der Waals forces. The various forces in solid materials are comprehensively reviewed by Hirschfelder.[3]

The molecular forces in hydrides of oxygen, nitrogen, and fluorine are recognized as being capable of exhibiting hydrogen bonding. The bond established between a strong electronegative atom and a hydrogen atom is abnormally strong, as in H_2O, NH_3, HF, and CH_3NH_2. The hydrogen bond is a dipole like attraction but much smaller than ordinary dipole forces. Hydrogen bonds between molecules are approximately 1/10 as strong as covalent bonds between atoms within a molecule.

The hydrogen bond is usually considered to be a bond in which a hydrogen atom lies between two closely spaced electronegative atoms. In addition to O, N, and F atoms, occasionally Cl is identified in hydrogen bonding systems.

The compounds which form hydrogen bonds are classified as acidic or basic. A combination of a compound from the acid group with a compound from the basic group will be affected by hydrogen bond formation. However, hydrogen containing compounds do not necessarily have hydrogen bonds. According to the definition, paraffin and olefin series of hydrocarbons do not form hydrogen bonds. The following organic compounds form hydrogen bonds: acetylenes, aldehydes, and halogenated alkanes.[4–8]

The melting point of a solid material depends both upon the type of structure and upon the bonding strength. In principle the melting point of crystalline substance is determined by the symmetry of the molecules and the intermolecular forces between molecules. These two factors govern the crystal lattice strength.

In covalently bonded organic compounds, the dipolar and hydrogen bonding forces are dominating. The forces holding solid materials together depend on the temperature. By increasing the temperature the interparticle forces are diminishing and the bond must be broken down to form a liquid. In large molecules, although the covalent bonding may not be very strong, yet because many of the bonds have to be broken before melting can occur, a high temperature is required.

2. Theory

2.1. Structure and Properties of Bone

A fully developed human bone is wired with over a hundred trillion synaptic contacts that provide information on the complex shapes and sizes of the human skeleton and how that code is translated by cells during development. Cells differentiate, communicate, and interact morphologically and functionally. Together, they construct multicellular structures such as bone.

Shaping of bones from simple cells is explained by demonstrating that dioxyribonucleic acid (DNA) acted as a genetic code. The genome contains information about how to make distinct proteins, rRNA and fRNA, and how to replace itself.

There are about 100,000 genes in a human which contain the program that controls the order and pattern of gene expression. The various combinations of genes influence the different cells, organs, and body regions. The interaction among cells generated cells which move, migrate, and die according to genetically determined schedules.

Bone develops originally from embryonic mesenchymol cells that have a very broad range of developmental potentialities, giving rise to fat, muscle, and other cells.[9]

DNA molecules contain the complete genetic code for every enzyme in the cell. It is present as a major component of the genes which are located on the chromosomes in the cell nucleus. The nucleotide units in DNA are arranged in a double helix containing phosphoric acid, 2-deoxyribose and the nitrogenous bases—adenine, guanine, thymine, and cytosine. Most cells in the body contain a complete copy of their DNA which are essentially identical.

Each part of an organism in the body has the DNA and the necessary information to assemble a complete organism. However, when the organism dies, the DNA molecules degrade rapidly. After 100,000 years, no DNA is likely to be preserved in skeletal remains.

In order to analyze DNA, it is necessary to have a sufficient quantity of it in the sample.[10] This can be achieved by polymerase chain reaction (PCR). PCR can amplify a section of DNA from a few original molecules. The principle of DNA analysis of bone or tooth consists of three parts:

(1) Preparation of the bone or tooth powder (approximately 1 g); reaction with a chemical which removes the proteins and other compounds; after this treatment, the powder is more concentrated in DNA;
(2) The amplification of the DNA is carried out with PCR; and
(3) Analysis of the concentrated DNA by determining the nucleotide sequence.

In general, DNA is extracted from tissues, mostly bone and teeth surfaces, which has been cleaned with 10% bleach and ultraviolet (UV) irradiated to eliminate DNA contaminants.

In a typical example, between 0.124 and 0.358 g of bone powder is utilized in each extraction. The bone powder is

decalcified over the course of 3 days using 0.5 M (ethylenediaminetetraacetic acd) (EDTA), pH=8.0. The powdered bone (and negative controls) is digested using proteinase K overnight at 55 °C. In the case of fully decalcified bone powder, the digestion by proteinase K was complete. The extraction of the digested bone powder and negative controls was performed with a phenol–chloroform mixture and then filtered and concentrated.

The PCR products were separated on agarose gels through electrophoresis, stained with ethidium bromide, and visualized under UV light. Digital images of the electrophoretic gels were taken.

The PCR cocktail contains the following reagents:

0.6 mM primers
1× manufacturers's buffer with 1.5 mM $MgCl_2$
dd H_2O
1 mg/ml BSA
200 nM dNTPs

and the DNA template was UV cross linked for 10 min to eliminate any exogenous DNA contamination.

During DNA analysis, calcified tissue is destroyed in order to remove any DNA trapped in the mineral matrix. In practice, the removal of DNA from skeletal remains involves dissolving the bone in a calcium-chelating agent. Many analysts request samples of bone on the order of 15–30 g. This gentle decalcifying agent will leave any collagen that does exist in the bone in a form that can be used for radiocarbon dating.[11]

Since soft tissues rarely survive in archeological deposits, calcified tissues (bone and teeth) have been the usual sample material for paleodietary studies. Bone and tooth minerals usually survive much longer than collagen due to hydrolysis and dissolution of older specimens.[12] In warm and wet environments, no usable collagen remains after 10 000 years. Radiocarbon studies demonstrated that different kinds of diet produce characteristic differences between bone collagen and apatite ^{13}C content.

Man and all vertebrates have a skeleton which is a rigid supporting framework of the body. This is entirely within the body and grows with age. The human skeleton is made up of over 200 bones. It protects and supports the soft tissue of the body.

Most mammal bones have a central cavity filled with fatty yellow marrow. This fat does not get used for the metabolism. However, the red marrow forms blood cells which are intensely useful to the animals. The long bones of birds are filled with air. About 2/3 of the weight of bone is mineral, mostly calcium, phosphate, and carbonate. The rest is organic materials, consisting largely of the fibrous protein collagen, responsible for the strength of the bones. Molecular and cellular compositions of hard compact and trabecular bone tissue are identical. It is only the difference in porosity that separates these gross anatomical bone types. Cancellous bone has a spongy structure.[13]

Biological materials, such as bone, may be regarded as derived from the paraffin hydrocarbons either by the substitution of one or more inorganic atoms for hydrogen or by the insertion of one or more inorganic atoms between the carbon atoms constituting the carbon chain. Both the chemical and physical properties of the paraffin hydrocarbons are fundamentally changed by the introduction of these inorganic constituents.

It is usually possible to separate the components of a mixture of organic compounds into members of different homologous series as a result of their differing chemical reactions or more obvious physical differences. The solubility of a substance in a limited number of solvent will also provide valuable information as to the presence or absence of certain classes of organic compounds. Teeth are coated by an outer layer of enamel, a much harder substance that contains more calcium phosphate and less collagen.

Calcium and phosphorous form the greater part of the inorganic matter of bones, teeth, and many pathological deposits in the body. These elements have claimed much attention and study in biological fields. Calcium is capable of forming difficultly soluble salts with the common anions present, such as PO_4^{3-}, HPO_4^{2-}, CO_3^{2-}, and OH^-. Its effect on nerve and muscle permeability and irritability warrant consideration of the nature of its metabolism.

How does the body absorb calcium and phosphorous? There is evidence that solid $Ca_3(PO_4)_2$ cannot be absorbed by the body, only free Ca^{2+} ions or the various types of phosphate ions can pass through the wall of the intestine. The solubility product of calcium compounds determines the limits the concentrations of ions available for absorption.[14]

The intestinal contents are usually on the acidic side and PO_4^{3-} is small and other ions, such as Ca^{2+}, HPO_4^{2-}, and $H_2PO_4^-$, can exist without exceeding the solubility product of $Ca_3(PO_4)_2$. Increasing acidity increases the solubility of the calcium phosphate system. However, in alkaline environments, the absorption ceases. The search for this solubility product has led many investigators to study the solubility behavior of bone and basic calcium phosphates.

The calcification process in one formation involves an orderly precipitation of bone mineral within a highly organized organic matrix. Although it is, at present, difficult to see any specific properties in the components of bone matrix which can be specially correlated with the mechanism of calcification, it is evident that they are capable of providing a carefully balanced system for the control of mineralization.

Major bone types are: fiber bone, lamellar bone, primary non-Haversian, pseudo-Haversian, and true Haversian tissue. As there are different kinds of wood with different material properties,[1] similarly, different kinds of bones have different properties. Bones serve different functions. Therefore, they are made of varying compositions with suitable different properties.

The analysis of different kinds of bone showed that the calcium phosphate and other minerals in the bone were made up of various proportions. The mineral content of bones increases with age; that is, children's bones are three times stronger than old people's bones.

TABLE 1. Composition of human bone, dentin, and enamel (wt %)—LeGeros [16]

Contents	Bone	Dentin	Enamel
Calcium, Ca	37.44	36.80	37.70
Phosphorus, P	16.70	17.62	17.80
Ca/P ratio	1.74	1.62	1.64
Magnesium, Mg	0.48	1.11	0.35
Sodium, Na	1.01	0.88	0.65
Potassiu, K	0.03	0.05	0.08
Carbonate, CO_3	6.60	5.80	3.60
Chlorine, Cl	0.13	0.05	0.30
Total inorganic	65.0	70.0	97.0
Total organic	25.0	20.0	1.5
Absorbed H_2O	10.0	10.0	1.5

Bones and teeth are composed of a carbonated hydroxyapatite:

$$\{Ca_9[(PO_4)_{4.5}(CO_3)_{1.5}](OH)_{1.5}\}.$$

Bone is a relatively porous material composed of tiny hydroxyapatite crystals intermixed with ~30% (dry weight) organic matter. Tooth enamel is essentially nonporous and composed of relatively large crystals that include only minor amounts (<2%) of organic matter.[11]

The composition of adult bovine cortical bone has been reported in several sources. The average values obtained were 77% inorganic matter and 23% organic matter. The organic salts and collagen contents were extracted from the bone powder with hot water. The total mineral content of the collagen fraction was approximately 1.25%.

On chemical analysis, the chief components of bone are collagen, calcium, phosphate, and water, but there are also significant amounts of mucopolysaccharides, glycoproteins, lipids, carbonate, citrate, sodium, magnesium, fluoride, and a host of minor and possibly adventitious ingredients as well. One concludes that the collagen is mostly in the fibers, the calcium and phosphate in the crystals, and the rest in the cement.[15]

The solubility of bone and related biomaterials such as dentin and enamel can be described based on the studies of crystallinity (crystal size, lattice parameters, and strain), composition, chemical and thermal stabilities, effects of carbonate, fluoride, magnesium, and other elements (notably strontium), and factors influencing the formation and transformation of synthetic apatites, (see Table 1).[16]

Bone is less dense than dentin and enamel, reflecting the considerable differences in the ratios of their inorganic or mineral/organic phase and water content.[17]

The dissolution of dental minerals in organic acid buffer is in the order: bone > dentin > enamel.[18,19] Carbonate in minerals causes an increase in its solubility.[20,21]

Dentin is composed of materials similar to those found in bone, but the fibers are even more densely packed, and the concentration of crystals is even greater than in bone. Since the specific surface area of dentin is considerable higher that that of enamel, it is possible that a significant part of the carbonate content (6.6%) in dentin be present as adsorbed on the crystallite surfaces.[22] There is a canal system of dentinal tubules containing the cellular processes of cells called odontoblasts, but the tubules run approximately parallel to one another, and the cell bodies remain outside the matrix, whereas, in bone, the canaliculi radiate and branch freely, and the cell bodies are buried in the matrix.

The human skeleton is made up of 206 bones. Bone is a composite material, formed of protein (collagen) and mineral (hydroxyapatite). Despite the variety of external forms, the makeup of bones at the gross and microscopic level is remarkably constant. No matter what shape a bone takes at the molecule level, its tissue is basically the same in all mammals.[23,11]

About 1/3 of bone mass is in the form of mineral crystals, which are embedded in an extracellular matrix composed largely of a complex interwoven network of a tough fibrous protein, collagen. Bone cells, attached to one another by protoplasmic processes, small blood vessels, and variable amounts of extracellular and intracellular fluid, make up the rest of the organic matrix.[13]

The organic content of bone normally ranges from 24% to 26%. Corresponding values for dentin from deciduous and permanent teeth are both 19% and 21%. However, there is a significant difference between the values for the enamels, that is, 0.4%–0.9%.[24]

Bone calcification increased with age throughout life, and the degree of calcification appears related to the proportion of collagen (90%–96%) in the total matrix. Apart from collagen and also noncollagenous protein the only other organic constituents of importance in lipid-free cleaned bone are mucopolysaccharide and citric acid. Collagen is a large protein molecule, which constitutes about 90% of bone's organic content.

Hydroxyapatite is a dense inorganic filling which stiffen the bone. Crystals of calcium phosphate impregnate the collagen matrix which gives bone its hardness and rigidity.

When the mineral component dissolves in acid, then the bone becomes a rubber-like, flexible structure. If the collagen—organic content—is combusted or leached out with solvents, the bone becomes brittle and crumbles.

Soil acidity (pH) and permittivity, moisture, temperature, and micro-organisms can all affect the rate of skeletal deterioration, or biological composition of bone subsequent to death.

Collagen constitutes the major protein component of bone, tendon, skin, and other forms of connective tissue. It is a high molecular weight, relatively insoluble, fibrous protein.[25–27] The basic molecular unit of collagen is a triple-stranded coil, rod-like structure of about 14 Å in diameter, 2800 Å in length, and a weight average molecular weight of 300,000 Daltons.

The chemical and physical properties of collagen are unique, consequently it receives great interest from both the medical and industrial researchers. The new technology is based on the principle that collagen is disintegrated from its original fiber assembly and repolymerized into a new fibrous

TABLE 2. Contribution (atom %) of amino acids to the carbon of bone collagen—Howland *et al.*[29]

Number	Nonessential	Atom (%)	Essential	Atom (%)
1	Alanine	9.3	Histidine	0.7
2	Arginine	7.9	Isoleucine	1.7
3	Aspartate	4.7	Leucine	3.6
4	Glutamate	9.7	Lysine	4.5
5	Glycine	16.9	Methionine	1.1
6	Hydroxylysine	1.0	Phenylslanine	3.3
7	3-hydroxyproline	0.2	Threonine	2.1
8	4-hydroxyproline	13.0	Valine	2.4
9	Praline	14.7		
10	Serine	2.2		
11	Tyrosine	1.1		

state for industrial application. One of the several utilizations of repolymerized/regenerated collagen is the processing of sausage casings in the food industry.

The term collagen covers a large group of proteins which differ slightly in their composition and purity. The analytical results, obtained using calf skin collagen, are very different from those with tendon collagen.[28] Protein collagen obtained from various sites (calf skin, tendon, etc.) can be purified by dissolving it in 0.45 mol/L sodium chloride solution. A considerable portion of the protein of finely ground bone is readily soluble in water. More could be extracted by buffer.[24]

Bones contain fatty marrow in both their medofullary cavities and their travbecular regions. Fat can be obtained if the shafts are cracked and pulled apart. Fat in the spongy bone can be extracted by boiling the crushed trabecular potions to render the fat in cooking vessels.

Bone is found only in vertebrate animals, fishes, amphibians, reptiles, birds, and mammals. Like fiberglass, bone is stiff and reasonably strong both in tension and compression.

Fiberglass has two components (glass and resin), but bone has three. The three components are tiny crystals of calcium phosphate (a ceramic), collagen fibers (a fibrous polymer), and a jelly-like matrix containing protein molecules with sugars bonded to them The crystals adhere to the collagen fibers, which in turn are embedded in the matrix.[13]

Howland *et al.* [29] investigated the percentage contribution of each amino acid to the carbon of bon collagen (pig bone). The values are presented in Table 2.

Glycine constitutes about 1/3 of the amino acid residues. Proline and hydroproline together accounted for slightly more than 1/5, indicating that the dentin collagen, as in most collagens studied so far, are by far the most abundant. In addition to collagen, the organic matrix of dentin contains other proteins. One of these, a peptide rich in organically bound phosphorous, contains abundant serine and aspartic acid residues.

The six most abundant fatty acids extracted from bone and identified by gas chromatography (GC) and GC/mass spectrometry (MS) were myristic acid ($C_{14.0}$), palmitic acid ($C_{16.1}$), palmitoleic acid ($C_{16.1}$), stearic acid ($C_{18.0}$), oleic acid ($C_{18.1}$), and the essential fatty acid linoleic acid ($C_{18.2}$).

Collagen, the protein of tendon, cartilage, bone, and skin, is formed by the mutual bonding of three polypeptide chains as a triple helix and the interaction of numerous triple helices to form a tough fiber. Leather is almost pure collagen. Collagenous connective tissue consists of fibers. These, in turn, are made up of collagen fibrils, which have a cross-striated appearance. It has a molecular weight of approximately 100,000.

Polypeptide is a polymer of amino acids, forming chains that may consist of several thousand amino acid residues. The acid units are chemically bound together with amino linkages (–CO–NH–). Collagen can be transformed into a stringy, insoluble, and indigestible form in boiling water. This conversion involves hydrolysis of some of the covalent bonds of collagen.

If a lone bone is soaked in acid, the mineral matter dissolves and the organic part becomes soft. The mineral and collagen together are called the bone matrix.

Collagen fibrils consist of recurring polypeptide subunits called tropocollagen. From x-ray analysis it has been deduced that the tropocollagen subunits consist of three polypeptide chains tightly coiled into a three-strand rope. Tropocollagen has a molecular weight of about 300,000. Each of the three helically intertwined polypeptides have about 1000 amino acid resides. The amino acid sequence of the collagen chains are the longest found in any protein.

The three helical polypeptide chains are tightly twisted about each other. They are also cross linked to each other by hydrogen bonds and by covalent cross linkages. As the number of covalent cross linkages between tropocollagen units increase, the collagen fibrils become more rigid and brittle on ageing.

In a large number of plants and animals, calcium deposits mainly as carbonates or phosphates; however, oxalates and citrates are not uncommon. The most familiar calcified structures are bones, teeth, fish scales, eggshells, and corals.

Calcium ions have a strong attraction for electrons in oxygen-containing anions such as carbonates, phosphates, and sulfates. These anions are large and their outer electrons can easily break away and become associated with the calcium ion. This reduces the attraction of water for the ions, so that these minerals tend to be insoluble in water. Consequently, calcium has three important properties for a skeleton; it is available in large quantities (about 4% of the earth's crust), it is not poisonous, and it forms insoluble compounds.

The insolubility of skeletons and shells in water is an important property which provides the strength, stability, and protection and is associated with mineral salts in crystalline forms.

Bone minerals are rather variable but they have the general formula as hydroxyapatites $Ca_{10}(PO_4)_6(OH)_2$. The mineralized skeletons are composed of crystals which are closely associated with a protein of which collagen is an acidic polysaccharide material. This association is very close and the protein integrates into the crystalline structure. The mineralization involves the deposition of relatively insoluble

TABLE 3. Decalcification of dentin and enamel in acetic acid buffer at 25 °C—Leach [18]

Time of decalcification	5 min		24 h		48 h	
Concentration (mg)	Ca	P	Ca	P	Ca	P
Dentin	83.4	39.8	90.8	41.3	91.4	40.6
Enamel	56.7	26.1	62.2	28.1	61.8	28.8
Dentin with 0.43% F	45.6	19.3	50.4	21.2	51.1	23.8
Enamel with 0.53% F	34.3	15.2	39.4	16.5	39.8	16.8

TABLE 4. Solubility of enamel in 17.0 mM H_3PO_4 at 25 °C—Patel and Brown[32]

pH	Ca (mM)	P (mM)
5.14	4.83	12.2
4.69	9.62	20.3
4.58	11.6	23.7
4.53	12.5	25.2
4.50	12.9	25.9

crystals on an organic framework. The skeleton system is the ultimate achievement of the mineralization process.

The calcified skeletons are characterized by their insolubility in water and the crystallization which occurs on an organic base or matrix and becomes incorporated into the skeleton. Pure crystals dissolve in water when the outermost ions in the crystal lattice break away from the surface and become separated from the crystal. This is the solubility process in water. If a sufficient number of ions dissolve in water, then the concentration will increase and the solution becomes saturated at the specific temperature. The temperature effects the movements of the ions in the solution and thus their solubility product. The mineralization of biological tissues will only occur if the fluid at the site of calcification contains so many of the constituent ions that they exceed the solubility product constant.[18]

Biological apatites are rather complex, caused by the presence of several minor elements. Therefore, to study the effect of the individual elements on the properties of the apatite, it is necessary to prepare synthetic apatites for characterizing the individual effects of each element. The results are then extrapolated to bone, dentin, and enamel which have differences in the ratios of their inorganic or mineral/organic phases and water content.[16]

The effect of fluoride content on the rate of reaction of dentin and enamel in 0.2 M acetic acid buffer at room temperature has been reported by Leach.[18] The results show that the reaction was very rapid, but the decalcification was not complete after 48 h (Table 3).

The fluorinated samples were less soluble than the fluorine free dentin and enamel. Furthermore, the solubility of solid/solution ration decreased as their fluoride content increased. The rate of reaction depends on the fraction of the tooth material present to the acid and the diffusion rates of ions of the mineral to and from the crystal surfaces. The greater solubility of dentin than enamel is the result of the smaller crystal size and higher specific surface of dentin.

The role of fluoride in enamel caries has been the subject of a large number of studies.[30,31] Experiments with synthetic apatites showed that the fluoride incorporation will lower, while magnesium incorporation will increase the solubility of the apatite. The extent of dissolution was directly proportional to the CO_3 content of the apatite, but the incorporation of fluoride significantly minimized the adverse effect of CO_3.

Caries is initiated by the production of acid by plaque bacteria, causing the release of calcium and phosphate ions from the partial dissolution of the dental apatite crystals. Depending on the local pH and on the type of ions present in the immediate vicinity, different types of calcium phosphates and other calcium compounds can form.[16,20]

Enamel is the hardest calcified tissue. The organic matter of enamel seems to have little effect on its dissolution rate. To investigate the probable cause of caries, numerous studies have been reported that have attempted to relate solubility properties of enamel to caries experience.[32] Most of the reported solubility studies differ. There is no single value for the solubility of enamel mineral but rather a spectrum of solubility. Variable solubility of enamel is one of the properties that must be considered in elucidating the mechanism of caries formation. The solubility of enamel in H_3PO_4 was reported at various pH ranges at 25 °C (Table 4).

The solubility of human enamel has been extensively studied in numerous papers; for example, refer to the Journal of Dental Research, Special Issue, Volume 69 (1990). One of the more interesting studies was the comparison of the solubility of powdered human enamel with the shark enameloid in acidic buffer at 37 °C. The amount of calcium ions released in the 0.1 mol/L NaAc at pH=5 was 50% higher with human enamel relative to the shark enameloid. The difference in solubility was due mainly to the difference in the CO_3 and F contents. Greater content of CO_3 increases while greater content of F lower the dissolution.[31] The high F content of the shark enameloid was assumed to be the cause for suppressing the initial dissolution and for enhancing remineralization by providing fluorine ions.

Suspension of a human tooth in 0.1 mol/L NaH_2PO_4 solution at 37 °C resulted in the formation of large $CaHPO_4$ crystals on both the enamel and dentin surfaces at pH=3. The crystal sizes decreased at higher pH values. The same experiments with shark enameloid produced sparse formation of small $CaHPO_4$, suggesting lower solubility of the shark enameloid compared with that of the human enamel. Similar results were also observed when powdered enamel was suspended in dilute phosphoric acid solution or in acetate buffer.

2.2. Solvents and Solubility

The solubility mechanism of structurally complicated materials is rather complex particularly because the molecular structure is made up of both inorganic and organic compounds with various polarities.

The dissolution of structurally complicated materials can be divided in two major classes: reversible and irreversible solubility. In the first case, after the dissolution of a smaller or larger fraction of the materials, the dissolved components can be recovered from the solution by unit operations. In the latter case, the dissolved compounds change their original chemical structure, often through chemical reaction and become unrecoverable.

Inorganic compounds are recoverable from solutions. However, larger organic molecules like collagen and keratin lose their molecular structure in solutions and reduce into smaller molecules through decomposition or reaction.

The solubility of a material in a solvent is characterized by the reduction/diminishing of the weight of the solute. The solubility data represent the final state of equilibrium between the solute and solvent at the specified conditions. Whether the solute is a pure compounds or a mixture of diverse elements or compounds, this statement stands. The thermodynamic equilibrium condition is imperative for the definition of solubility.

For the true equilibrium of an aqueous solution of solids with various sizes of particles, the crystal should be uniform after equilibration. Such a requirement can be approached by the condition when neither net mineral formation nor net mineral dissolution can be detected over time. In several experiments, equilibrium conditions were assumed when the composition did not change significantly for a period of 3–4 days after an equilibration period of 15–21 days.[22]

Numerous studies have been devoted to the solubility of enamel and dentin of various sizes, impurities and degrees of substitution, for example, with CO_3, F, and Mg. The established solubility products of synthetic hydroxyl apatites have been reported as 3.7×10^{-58} and 5.5×10^{-55}. The discrepancy may be ascribed to the impurities or to the nonstoichiometric mineral.[33] The parameters describing the condition and environment of the solubility measurement are important contributors of the experimental results. The reproduction of the measurement is possible if all essential details are available and clearly stated.

While in a single compound material, the impurities play an important role. In structurally complicated materials, like wood, bone, and body parts, the number of components or impurities might be considerable and constitute the integral part of the whole structure, whether they are inorganic and/or organic substances.

The solubility concept is merely the interaction between like and unlike molecules. Whether the solute and solvent are chemically and physically similar, substances will influence the solubility data. If the molecule–molecule forces in the solute and the molecule–solvent forces in the solution are favorable, solubility will take place. The combination of hydrogen bonds between solute and solvent molecules is shown by the good miscibility between water and alcohols, which are also amphoteric in nature.

A classification of various liquid systems has been suggested by Ewell *et al.*[34] According to the proposal, the liquids are divided into five groups, based on their hydrogen-bond–formation tendencies. Whether a hydrogen bond is strong or weak depends on the nature of the atoms and the coordinating ability of the hydrogen atom between them.

Since the early years of study of mixture properties, the aim was to relate the behavior of the pure components to the mixture. In other words, the aim was to predict the nonideal properties of the solution from the physical properties of the pure components. Apart from difficulties in many cases, good approximations have been achieved by considering some phenomenon (for example, hydrogen bonding and polarity) that enables us to draw some sort of conclusion regarding the deviation from ideality. In general, the mixtures of homologous series (for example, ethanol–methanol, benzene–toluene, n-butane–n-heptane, 1,2-dichloroethane–benzene) approach ideal behavior. Similarly, the mixtures of isomeric compounds form ideal or almost ideal systems.

Whether a rule for the estimation or prediction techniques is acceptable or recommended for solubility, it is relevant to distinguish between the different sorts of molecules present in the system. In connection with solubility principles, the following types of molecules of solute and solvent have to be considered: polar, nonpolar, associated, nonassociated, and hydrogen bonding (both the formation of, and the lack of formation). For example, the regular solution theory developed for nonpolar molecules,[35] is not valid for aqueous solutions.

The more the solubility parameter of the solvent differs from that of the solute, the lower the solubility. If the solubility parameter of two solvents is similar then they will be miscible. However, if the difference between two solvents is about 2.5 at ambient temperature, then total miscibility is unlikely.[36] It is established that high temperature favors miscibility.

The molecular liquids belong either to the polar or to the nonpolar groups of fluids. Whether a compound is polar or nonpolar depends upon its dipole moment. The polarity of a solvent molecule represent is ability to interact with the solute molecules. Four factors are responsible for the dipole moment of the molecule:

(A) difference in the dimensions of the atomic orbital;
(B) displacement of the center of gravity of the charge of the electrons toward the more electronegative atom;
(C) hybridization, causing the asymmetry of electrons; and
(D) hybridization, causing asymmetric atomic orbital.

There are permanent and induced dipole moments. The shape or unsymmetric structure gives the permanent dipole moment of molecules. The induced dipole moment originates from an electric field which displaces the electrons and the molecules will show induced dipoles.

Dipole moments are calculated values often from the dielectric constant. The dielectric constant of a solvent is measured between two electrostatically charged plates of a condenser. Depending on whether the molecules tend strongly or weakly toward the charged plates, the solvent has a high or low dipole moment. The order of the dielectric constant in-

TABLE 5. Properties of common solvents at 25 °C—Griffiths and Pugh[81] and Marsh[82]

Solvent	Formula	Dielectric constant Permittivity, ε	Dipole moment μ (D)	Solubility parameter δ, (cal/cc)$^{1/2}$
Hydrogen fluoride	HF	84.0 at 0 °C	1.92	7.71
Water	H_2O	78.36	1.84	23.53
Ethylene glycol	$C_2H_6O_2$	37.7	2.28	13.55
Nitrobenzene	$C_6H_5NO_2$	34.78	4.03	10.00
Methyl alcohol	CH_3OH	32.66	1.60	14.51
Ethyl alcohol	C_2H_5OH	24.55	1.69	12.92
Propyl alcohol	C_3H_7OH	20.33	1.68	12.05
Ammonia	NH_3	16.90	1.50	12.41
Dichloroethane	$C_2H_4Cl_2$	10.37	1.75	9.56
Chlorobenzene	C_6H_5Cl	5.62	1.54	9.50
Dichlorobenzene	$C_6H_4Cl_2$	2.41	0.0	9.22
Benzene	C_6H_6	2.27	0.0	9.16
Carbon tetrachloride	CCl_4	2.23	0.0	9.34
Cyclohexane	C_6H_{12}	2.02	0.0	8.20

dicates how the solvent molecule orients its dipoles and electric charges. The polar molecules of the solute are attracted by the polar molecules of the solvent.

Regarding solute–solvent interaction from the hydrogen bonding point of view, Pimentel and McClellan[6] classified the solvents as follows:

(a) proton donors (acidic: e.g., $CHCl_3$ and C_2HCl_5;
(b) proton acceptors (basic): e.g., ketones, ethers, aldehydes, esters, olefins, aromatic hydrocarbons;
(a-b) both proton donors and acceptors (acid–base): e.g., water, alcohols carboxylic acids, primary and secondary amines; and
(n) nonhydrogen bonding: e.g., CS_2, CCl_4, paraffins.

The available solvents and their physical and chemical properties have been compiled in several comprehensive works by: Archer,[37] Ash and Ash,[38,39] Chermisinoff,[40] Flick,[41] Lide,[42,43] Marcus,[44,45] Mellan and Flick,[46] Reichardt,[47] Sedivec and Flek,[48] Smallwood,[49] Whim and Johnson,[50] and Wypych.[51–53]

A large number of organic compounds are nonpolar solvents. These chemicals have low dielectric constants owing to their small permanent dipole moments and ion-pairing effects (refer to Table 5). Common nonpolar solvents are benzene, carbon tetrachloride, and *p*-dichlorobenzene. The cohesive forces are very weak in these nonpolar solvents and therefore they cannot dissolve polar or ionic compounds with strong forces between the molecules. Consequently, the dissolving power of nonpolar solvents is limited to solutes having low polarity, such as propanol.

The magnitude of the static dielectric constant provides a good approximation for the dissolving power of a solvent. A solvent with a higher dielectric constant more easily dissolves polar and ionic compounds. Solvents of similar dielectric constant usually have similar dipole moments. The line that is usually drawn between polar and nonpolar solvents is at dielectric constants having a value of $\varepsilon=30$. In this division, CH_3OH ($\varepsilon=32$) lies below the polar group of compounds, whereas C_2H_5OH ($\varepsilon=24.55$) is a nonpolar solvent. Furthermore, both polar and nonpolar classes can be subdivided into protic and aprotic subclasses (i.e., hydrogen bonded and nonhydrogen bonded solvents). Aprotic molecules are more polar than that of protic solvents. Therefore, the ion dipole and molecule dipole solute–solvent interactions are stronger in dipolar aprotic solvents. These molecules are more polarizable than the molecules of protic solvents, and the London dispersion forces are also more pronounced in dipolar aprotic solvents.

The ionizing solvents consist of polar molecules. They are dissolving ionic substances, such as inorganic salts, and the solution contains dissolved anions and cations. The dissolving strength of the ionic solvent toward ionic compounds is measured by the magnitude of its dielectric constant. That is, a high dielectric constant indicates that the solvent is strongly polar and capable of dissolving ionic substance that have attractive forces between the oppositely charge ions. These solutions show electrical conductivity, contrary to the solutions of polar solvents, where the anions and cations have no independent mobility.

Liquids such as water and hydrogen fluoride have very high dielectric constants because of their strong permanent dipole moment, coupled with hydrogen bonding, so they are strongly ionizing solvents. Because of its hydrogen bonds, ammonia is also a good ionizing solvent, despite its low dielectric constant value. Liquid water is not only polar but is also an ionizing solvent. Consequently it dissolves ionic and covalent compounds of high polarity such as methanol and glycol. However, water does not dissolve nonpolar molecules that have small dipole moments, e.g., hydrocarbons.

Whether a substance is soluble or insoluble in water is determined by its structure. The binding forces between the solute molecules and those between the solvent molecules play a considerable role. The associated water molecules are bonded together by hydrogen bonds between the hydroxyl groups in the liquid state, and one expects that those substances will be soluble in water that can fit into the water structure and play the same or similar role as water molecules. Therefore, those organic substance that possess hydroxyl or/and carboxylic groups tend to be water soluble. However, hydrocarbons, halogenated hydrocarbons, and heterogeneous hydrocarbons which do not possess hydroxyl or carboxylic groups, cannot therefore form associative bonds with associated molecules.

The study of the biological fluids and tissues is of fundamental importance in toxicology and physiology. The solubility of all sort of pharmarcological active nonelectrolytes, such as gases, liquids, and solids has been comprehensively reviewed by Kamlet and co-workers (Kamlet *et al.*,[54–60] Abraham *et al.*,[61] and Leahy *et al.*[62]). They have come up with a universal equation that allows them to predict the solubility for any pure compound in any solvent as long as

they can estimate a few fundamental parameters of the compounds involved. The solubility between the solute and solvent depends on three terms:

(1) a cavity term describes the energy required to make a hole in the solvent that will be occupied by a solute molecules;
(2) a term which measures dipole interaction; and
(3) a term which gives the hydrogen bonding effects.

The hydrogen bonding effects account for the acid–base properties of solvents and solutes. Acidity is based on the ability of a molecule to act as a donor for hydrogen bonding. Meanwhile, basicity is measured by a molecule's ability to act as a hydrogen bond acceptor. The three terms coupled together account for essentially all the forces involved in dissolving a solute in a solvent.

Estimation techniques for checking the reported solubility data or for checking the consistency of the solubility with a series of similar substances have not yet been developed for the group of structurally complicated materials, such as wood, bone, and body parts. The few suggested estimation techniques are too crude and too limited a scope of classes of chemicals to be of use for multicomponent mixtures. In addition, there are generally too few data for one to adequately test the validity of an estimation technique.[63]

Predictive models are needed because time and cost make it impossible to run actual experiments in the field to determine the impact of chemicals, for example on groundwater quality. Accurate and reliable predictions will be a considerable benefit over the coming years, but these are only possible with accurate physical and chemical data.

The main reason for the difficulty of estimating and correlating is the fact that materials of practical interest are usually composed of molecules which are too complex to meet the basic requirements of the theoretical treatment. Only the properties of two component mixtures of crystalline solids can be estimated from the component properties and concentrations. The extreme specificity of molecular packing in crystals is the primary reason for the difficulties.

The first criterion for selecting a solvent or a solvent blend is its capacity to dissolve the bone. Whether a solvent dissolves a solute or not, the selection depends on the dissolving power of the solvent. The definition for the dissolving power of solvents, \$ (a dimensionless quantity), is given by the following equation of the form:

$$\$ = (\mu^\circ - \mu_{soln})/RT$$

where μ° is the chemical potential of the solute in its own phase and μ_{soln} is the chemical potential of the solute in the solution.

The dissolving power is a calculated quantity and there are several proposed methods in the literature.[64–66]

The selection of proper solvents for bone powder is a usual problem. The choice of the solvents is facilitated by use of numerical criterion of a solvent power. There are several approaches to estimate solvent power, most of which have been developed for polymer dissolution:[67]

Kauri-butanol value (KB),
Dilution ratio (DR),
Aniline point (AP),
Promote or decrease solubility (PDS), and
Solubility parameter (δ).

However, none of the approximate empirical scheme listed about has been applied to the solubility of bone in solvents.

One of the main objectives of the classification of solvents is to allocate the best candidates for a particular application. The choice is based on the consideration of the various parameters which influence the solute–solvent interaction. There are several reports and reviews on the choice and analysis of the basic variables.[68,69]

Despite the practical and useful application of the methods for solvent selection, the techniques do not go far enough for structurally complicated materials, such as bone, wood, and body parts. Consequently, with the present state of the art calculation, it is not possible to derive acceptable solubility values for the solubility of bone in organic solvents.

2.3. Solubility of Bone

The solubility of biological molecules is an important consideration in the development of pharmacological agents and in many biological processes. Researches at the University of Illinois, Chicago have used stem cells to form new bone and cartilage to create the ball structure of the joint in the human jaw. It means that both cartilage and bone-like tissues were grown from a single population of stem. Patients suffering from a variety of bone and joint injuries and diseases, including arthritic hips, damaged knees and ankles, have hope of growing back their own joints.

At the University of Pittsburgh, School of Medicine, a team of scientists studied the stem cell transplantation of patients with heart disease. The research patients were injected with stem cells taken from their own bone marrow. The cells grow into almost any type of human tissue and quickly evolve into healthy heart cells that augment and replace the diseased predecessors. Stem cell transplant could replace surgery as a way of treating heart failure.

The mineral content of calcified deposits in the vascular system, including arteries and heart valves, has been determined as carbonated hydroxyapatite. The same mineral has been found in bone.[70] The dissolution mechanism of this mineral phase has been extensively studied, including the dynamic biological processes of bone formation, remodeling, and resorption.

The dissolution rate of carbonated hydroxyapatite in hydrochloric acid solution at varying pH, osmolality (ionic strength), temperature, flow rate, mechanical agitation, and surface area of the powdered mineral has been studied by Hankermeyer *et al.*[71]

$$Ca_{8.8}(HPO_4)_{4.5}(CO_3)_{0.7}(OH)_{1.3} + 17.6H^+ \rightarrow 8.8Ca^{2+} + 5.2H_3PO_4 + 0.7H_2CO_3 + 1.3H_2O.$$

The linear relationship between 0.1 and 1.0 N$[H^+]$ indicates

that increasing the number of available protons will lead to a steady rise in the dissolution rate. The hydrogen ions act as the protonation agents of the phosphate and carbonate groups of carbonate hydroxyapatite. The overall dissolution rate was expressed as weight loss (mg) divided by time (min). In conclusion, the decalcification of arterial value plaques in vivo can be affected by use of acidic solutions.

A substance which binds ions might function equally well as an inhibitor or a nucleator, depending on how reversible the binding is, or how available the ions are, to exchange or to further interactions; or it might depend on the concentration ratio of binder to ions.

The solubility of bone and tooth in various solvents is complex. The solute–solvent interaction involves different molecules of both inorganic and organic compounds. In addition to the molecular forces, several other physical properties have to be considered during the solubility process.[72]

The structure of bone and tooth consist of molecules of various sizes which require accommodation in the solvent if the solubility is expected. To form holes in the solvent, energy is required, which is regained when the holes are filled with the solute molecules. If the solvent is associated, extra work will be required to make the holes and this will not be recovered when noninteracting molecules are used to fill the holes: for example, in such associated liquids as water, salt solutions and molten salts.

The solubility of bone can be expected to be generally low because the energy required for the breaking of a large number of solvent bonds during the dissolution is not adequately compensated for by the interaction between the solute and the solvent.

The higher solubility of bone in aromatic solvents as compared to aliphatic solvents can be explained on the basis of a better interaction between the solute and the solvent molecules in the former case.

The solubility of bone is important in understanding the resorption and remodeling course of actions. Resorption is taking place when the bone minerals are dissolved in weak acids and the system undergoes remolding. Furthermore, interest arises from the effect of aging on the solubility and crystallinity of bone minerals.[20] The investigation of the solubility and crystallinity of rat bone minerals has been found to correlate well with the age. Younger bone mineral being more soluble and less crystalline than older, more mature bond mineral. This is explained by the physicochemical differences in mineral from older rats when compared with younger rats, as shown in Table 6.[73]

In a study of the solubility of powdered bone, Nordin[74] obtained evidence suggesting that the agitation of the bone powder in water and inorganic solutions (temperature and pH were not specified) resulted in the extraction of organic matter from the bone.

The solubility of a material is dependent mainly on temperature and equilibration time, although it is slightly dependent on the size of the material (for example, crystal size)

TABLE 6. Solubility of rat bone mineral as a function of rat age—Barry *et al.*[73] [a]

Rat age (months)	Solubility in 0.10 M acetate buffer (pH=5) pK_{HAP}
1	109.3
5	110.0
8	111.0
14	111.7
18	111.8
25	111.9

[a]HAP=hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$; temperature of the solubility determinations was not reported.

and the pH of the solution. The solubility curve always refers to the stable equilibrium condition, except in metastable conditions.

Nordin[74] showed that when calf bone powder (about 100 mesh) was shaken with a synthetic ultrafiltrate at a strength of 0.15 M for a period of 24 h at a pH of about 6.7 and at 37 °C, the relative solubility obtained can be expressed in terms of the pK of the solubility product of the secondary

$$pK[CaHPO_4] = 6.1 - 6.7$$

and tertiary salts

$$pK[Ca_3(PO_4)_2] = 26 - 27.$$

The synthetic ultrafiltrate was made up of tris(hydrozxymethyl)aminomethane, cacodylate buffer or acetate. Further detail is given in Tables 7 (A–D).

Which are the suitable solvents for bringing bone in solution at ambient temperature? Although different studies have used different strengths of acetic acid to pretreat fossils, evidence suggest that exposure to strong (>1.0 N) acetic acid can cause bones to recrystallize.

The bone powder is usually prepared by a cut from bone cortex and after milling and passed through a 100 mesh sieve, the sample is washed with water, alcohol, and ether, and dried at 100 °C.

After pretreatment all samples were dissolved in 2.5 N HCl and Sr was extracted by standard ion exchange chromatography. The results confirm, that pretreatment of bones and teeth with sequential rinses in weak acetic acid, selectively removes some diagenetic Sr.[75]

From each specimen, 1–2 mg of bone or enamel was collected and ground to a fine powder using either a drill or agate mortar and pestle. Samples were soaked in a NaOCl solution overnight to remove organic material and then rinsed five times with de-ionized water. After rinsing, samples were transferred to acid-washed Teflon vials and dissolved in 1.5 mL of 2.5 N HCl at 120 °C overnight to ensure complete dissolution.[76]

To perform the analysis of the samples, 2 μL of HNO_3 was added to each vial to dissolve the sample, then 1 μL of this solution was collected and pipetted onto a rhenium filement and dried for ~2 min at 800 °C.

TABLE 7. Solubility of calf bone powder (100 mesh) at 37 °C—MacGregor and Nordin[83]

pH	$Ca^{2+} \times$ total P (10^6 mole/L)
A. in 0.15 mole/L tris buffer	
7.0	0.56
7.2	0.42
7.4	0.36
7.6	0.25
7.8	0.19
B. in 0.15 mole/L cocodyl buffer	
6.2	3.9
6.4	3.0
6.6	1.6
6.8	1.3
7.0	0.7
C. in 50 mL of 0.15 mole/L tris buffer	
Weight of bone powder (mg)	
100	0.205
500	0.286
1000	0.387
3000	0.376
5000	0.352

D. Solubility of human bone powder (22 mesh) at 37 °C

pH	$[Ca^{2+}] \times 10^3$	[P total] $\times 10^3$	$Ca^{2+} \times$ total P $\times 10^6$
In 0.15 mole/L tris buffer solution			
7.0	3.2	0.3	0.96
7.4	1.4	0.2	0.28
7.8	0.5	0.1	0.05
In 0.15 mole/L cocodylic acid solution			
6.2	8.0	0.42	3.36
6.6	5.3	0.40	2.12
7.0	3.2	0.30	0.96

A certain quantity of bone poser was added to each solvent to obtain an indication to ensure that extra solid would be present. A stirring bar was used to agitate the solutions in the dark for periods of not less than 25 h to ensure equilibration. The laboratory temperature varied between 23 and 25 °C during this period.

To test the time required for equilibration, the solubility of bone in three regular solvents was measured after 2, 3, and 5 days. The solubility in each solvent at three time periods was found to be the same within experimental error. Additional sonication, followed with stirring periods of the mixtures did not influence or change the measured solubility. Hence, the initial 1 day stirring was considered sufficient for equilibration. Care was taken to ensure that solid residues were left in the bottle after the experiment to serve as proof that the solution was not deficient in bone prior to filtering.

TABLE 8. Solubility (wt %) of collagen in solvents—Miller *et al.*[84]

Age of collagen	Solubility in 0.5% acetic acid	Solubility in 0.6% NaCl tris buffer
Fetal	7.60	9.18
3–6 weeks	5.77	7.01
18 months	3.42	2.72
40 months	2.70	2.37

During equilibration, part of the organic portion of the bone goes into colloidal solution and may materially influence the results. That is, the organic material of the bone may cause interference with the determination. Therefore, in the early studies, the organic matter from bone powder had been removed by heating with glycerol and KOH.[85] Since the bone mass is composed of approximately 23% organic components, it had been deproteinated by hydrazine or less frequently with NaOCl solution, followed by washing the residue with ethanol and finally with de-ionized water. After the treatment, the bone mineral samples were free from organic compounds, such as collagen.

The dissolution rates of bone, dentin, and enamel ion 0.1 M acetic acid–sodium acetate buffer over the acid pH range 3.5–5.1 have been compared by Apostolopoulos and Buonocore,[19] as shown in Table 8. It has been found that the presence of the organic matter of bone and, to a lesser extent that of dentin, retards the acid dissolution rates of calcified tissues. However, the organic matter of enamel has little effect on its dissolution rate in acetate buffers.

The explanation may be found in the complex structure of the organic matrix. In the case of bone and dentin the dominant component is collagen, while in the enamel a keratin-like structure is the principal constituent. Whether the samples are intact or inorganic enamels, the dissolution rates showed similar results, indicating that the interference of the organic matter is apparently small.

The retarding effect of the organic matter on dissolution rates of bone and dentin is related to the influence of the swelling of collagen but little or none of keratin. The swelling of the organic matter may in effect be considered as an increase in the thickness of the stationary layer which surrounds the dissolving particles, through which the diffusion of reactants and reaction products occurs.

As the pH is changing in the decalcifying medium, the net electric charge in a protein molecule varies. This exerts a retarding influence on the diffusion of hydronium, calcium, and phosphate ions to depress the dissolution rates.

Hydrazine treatment of bone powder showed no significant effect on the solubility and did not cause any change in the crystal structure of apatites. The result also showed that there was no appreciable change in the amounts of carbonate, calcium, and phosphate after hydrazine extractions of bone powder. Several measurements were used and the solubility values were within 10% of each other. The average values computed and reported. Bone is sparingly soluble in most solvents. Replicate measurements were conducted to confirm the relatively high solubility data.

The solubility behavior of bone was studied by bringing the sample into contact with a solvent or solution and to wait until the concentration of the solutes, as well as pH, do not change any more with time.[86] The samples of bone were obtained from well defined sources and locations. The precise description is essential for tests of reproduction.

After removing the adherent soft tissues, bisecting the calvicles longitudinally and removing the spongiosa, the cortical part was crushed into small pieces and pulverized using a steel mortar and pestle. The bone powder was defatted with 1:1 alcohol–ether for 24 h and stored in a desiccator. The particle size of bone, selected for solubility determination, was 80–120 mesh.

Triplicate bone samples were mechanically shaken continuously in buffer solutions at various pH levels for times ranging from one to several hours at 25 °C.

At the end of the equilibration, the solution was filtered through 5 μm pore diameter crucibles and the filtrates analyzed. The analytical values obtained included all metals. In most cases, a stoichiometric relationship of calcium to phosphorous was found.

Preparation of samples consisted of washing and cleaning in an ultrasonic bath. The demineralization of collagen was performed in 0.2 M hydrochloric acid. The lipids (fats and fat derived, relatively insoluble in water but soluble in organic solvents, such as benzene, chloroform, acetone, ether, etc.) were separated with a mixture of methanol:chloroform: water (2:1:0.8 by volume). Humic acid (a mixture of polymers containing aromatic and heterocyclic structures, carbonyl groups and nitrogen) was removed from bone samples with 0.1 M sodium hydroxide solution[29]

For the preparation of pure apatite samples, a 1.5% sodium hypochlorite solution was used to remove organic material, followed by 1 M acetic acid to separate calcite and carbonates.

Approximately 50 mg of each bone, enamel, and mineral fluoroapatite were prepared by milling in a freezer mill. A buffer mixture of 0.1 M acetic acid/sodium acetate at pH =4.5 was added to the powder and sonicated for 1 min. After centrifugation, the buffer and the residual powder separated. The solid floating on the surface was collected for analysis. The extracts were used for measuring the concentration of calcium and strontium. The results were reported as rations.

The solubility was found to vary by 7% (821 ppm Ca), the Ca/P ratio by 4%, the Sr by 7%, and the Sr/Ca ratio by 8%.[87] The composition of the solubility of bone, enamel, and mineral fluorapatite shows that the enamel is less soluble than bone and fluorapatite is the least soluble of all apatites in acid buffers. Most bone specimens are more soluble in acid buffers than enamel.

The solubility increases as collagen is associated with effect of increased pH and ionic strength. The ionic concentration is greater when 0.44% sodium tripolyphosphate (STPP) solution was combined with a NaCl solution (3%). The addition of STPP, and the subsequent pH and ionic strength effects, destabilize the hydrogen bonds and allows more complete releases of the soluble component after heating at 50 °C for 15 min. The increased pH produced by STPP changed the negative charge and thus affected the solubility. The solubility of collagen gradually increased from 50 to 70 °C in the presence of 3% NaCl and 0.44% STPP solutions.

The most basic functional properties of corium collagen are pH, ionic strength, and temperature. The solubility of these collagen products is dependent upon the physicochemical state of their molecules which are either favorably or adversely affected by hearting, drying, and other processing treatments during their manufacture and storage.

The heat denaturation of corium collagen results in changes to their solubility depending on the severity of the heat treatment. The quality of soluble nitrogen was significantly increased when the hydrothermal conditions were studied for 100% collagen substitution. At 70 °C under the same solvent conditions (3% NaCl solution), there was a marked increase in solubility attributable to the thermal denaturation of collagen after heating for 15 min.[88]

The initial increase in temperature may represent more extensive rupture of collagen crosslinks of mature collagen in addition to the soluble material released at 50 °C. For the remaining heating time, any increase in collagen solubility may be due strictly to the denaturation of more mature collagen fibrils.

Denaturing agents such as LiCl, KSCN, and guanidine salts at neutral pH increase the solubilizing up to 80% of the total amount of collagen. Using 5 M guanidine hydrochloride as denaturing agent resulted in a 17% collagen extraction from the total collagen. In another experiment 20% of the total collagen was extracted with 0.5 M acetic acid, followed by a further 64% extraction with 5 M guanidine hydrochloride.[89]

At 70 °C, sufficient heat may be present to break the continuity of the interchain waterbridges, hydroxyproline related stability, and the aldol type cross linkages associated with hydroxylysine.

The decreasing levels of solubility of collagen samples in acetic acid with increasing age has been reported by Miller *et al.*[84] Cross linking of the collagen structure increases with age. Collagenous tissue with more cross linkages is more resistant to swelling and has a lower water holding capacity.

In collagen, weak hydrogen bonds are the predominant form of intermolecular cross-linking. There bonds are susceptible to disruption by heating and by certain chemical reagents such as weak acids. With increasing age, more stable intermolecular and intramolecular cross links develop which are resistant to destruction by heat or acids.[90]

Acid-soluble collagen appears as the early collagen form in fetal skin and becomes increasingly stable as intermolecular and intramolecular cross-link form. Similarly, mature collagen fiber is more resistant to the action of pepsin than young collagen fiber which is more readily solubilized.

The physicochemical properties of bovine collagen are directly related to age-induced cross links and the selection of collagen material for use in the regenerating process.

The solubility of collagen in 0.5% acetic acid, 0.6%NaCl

TABLE 9. Dissolution rates of bone, dentin, and enamel in acetate buffer at 25 °C—Apostolopoulos and Buonocore[19]

Shaking time (min)	Dissolved calcium, mg/50 mL acetate buffer								
	pH=3.5			pH=5.0			pH=5.0		
	Bone	Dentin	Enamel	Bone	Dentin	Enamel	Bone	Dentin	Enamel
0	0	0	0	0	0	0	0	0	0
10	6.9	7.2	7.8	6.4	6.2	6.0	4.5	3.4	2.0
20	8.4	9.2	9.7	8.1	7.1	8.5	5.7	4.5	2.7
30	9.5	11.1	11.3	9.2	10.1	9.7	6.4	5.3	3.1
40	10.4	12.4	12.7	10.1	10.7	10.5	7.0	6.1	3.4
50	11.1	13.7	14.0	10.8	11.8	11.1	7.4	6.7	3.7
60	11.8	14.8	15.0	11.4	12.0	11.7	7.7	7.3	3.9

tris buffer at pH=7, and 0.5% pepsin-treated at pH=2.5 HCl adjusted was studied by Miller *et al.*[84] The data reported show that the solubility of collagen in the tested solvents gradually decreases with increasing age of the samples (refer to Table 9).

The insolubility of mature collagen is the result of intermolecular cross linking between adjacent tropocollagen molecules (macromolecules). Less than 0.5% collagen is soluble in neutral salt solution and less than 1% in dilute acid solution at ambient temperature.[89] However, if chicken bone collagen was frozen at −70 °C and thawed at 2 °C in the presence of 3% acetic acid solution, then 30% of the collagen solubilized.

The effective demineralization or extraction of bone mineral with acid also dissolves a small amount of soluble collagen.

Powder of calf bone was decalcified with a saturated solution of sodium EDTA (pH=8.5) at 22 °C for 48 h. The EDTA extract contained 35% of the total protein of the bone, including 20% of the collagen and acid mucopolysaccharide and glycoprotein fraction. Although small amounts of bone powder are solubilized with EDTA solution, the great majority of the collagen remains insoluble even after complete decalcification.

After the removal of dentine minerals by decalcification in acid or EDTA, the remaining organic matrix of human dentine contains about 90% collagen.[89]

The solubility behavior of biological apatites in surrounding tissue fluids has been intensively studied since 1950. Despite the numerous investigations of the possible physicochemical relationships between carbonated apatites in buffer (weak acid) solutions, the dissolution mechanism remains controversial.

In general, the main interest is focused on the solubility of bone powder mineral in buffer solutions at 37 °C. This type of investigation has been performed at the University of Utah and reported in a series of papers, mainly in the Journal of Colloid and Interface Science and Calcified Tissue International.

According to a group of investigators in the laboratory of the Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, rat bone mineral has been shown to have a unique solubility behavior, termed metastable equilibrium solubility (MES). Under the experimental conditions for solubility determination, the initial dissolution of bone mineral in weakly acidic media has occurred, however, followed by no further dissolution and no mineral nucleation or crystal growth. There was neither an appreciable rate of dissolution nor precipitation. This metastable equilibrium solubility is far removed from the true thermodynamic equilibrium state.

The dissolution mechanism is explained through the formation of a surface complex of hydroxyapatite. The surface complex has an interfacial structure at the surface of the mineral by rapid ion exchange between the solid and the solution (see Berg *et al.*,[91,92] Barry *et al.*,[73] Chhettry *et al.*,[93] and Hsu *et al.*[94]).

The majority of the papers in the literature dealing with carbonate incorporation into apatite are concerned with the metastable equilibrium solubility behavior of bone mineral. To avoid any interference of the organic components in the interpretation of the solubility data, the soft tissues were removed and the marrow was flushed from the remaining, mostly cortical bone, using normal saline. The proteins were removed by extraction with hydrazine. After 24 h incubation, the excess hydrazine was removed and the mineral was washed first with 100% ethanol, followed by rinsing with double-de-ionized warer and air dried. The bone mineral samples were then used to equilibrate in the buffer solutions. The buffer solution contained 0.10 M acetate with varying amounts of calcium and phosphate.

The solubility of the bone mineral was determined by placing the sample into 0.1 M acetate buffer for 48 h. After drying the metastable equilibrium solubility was established.

The solubility properties of dental enamel and hydroxyapatite in organic acids, which were produced from dietary carbohydrate by oral bacteria, have been investigaeted by Shellis and Wilson [95] The apparent solubility distributions at different pH values were different from that of previous reports. The average solubility was smaller than that found by previous measurements using conventional equilibration. The 24 h equilibration period might be insufficient for syn-

TABLE 10. Complete dissolution of bone powder

Type of bone	Solvent	t (°C)	Dissolution time (h)	Reference
Marine mammals	2.5 M HCl	120	12	Clementz *et al.* [76]
Seal				Hoppe *et al.* [75]
Pigs	6.0 M HCl	100	24	Howland *et al.* [29]
Deer	Concentrated HNO_3	110	1	Burton *et al.* [96]

thetic preparations of carbonate apatites , but enamel consists of densely packed crystals which need longer time to reach equilibrium with the solution.

3. Comments

The solubility of bone powder in a series of organic solvents has not been reported as yet.

All solubility data in this paper have been classified as tentative often only because comparable data have been lacking. We may conclude that there is a need for more, as well as more accurate, solubility data.

The solubility determination of solids, which are very sparingly soluble, presents several problems such as slow dissolution, the effect of impurities, and the heterogeneity in the energy content of the solid. Consequently, a discrepancy among the measured and reported values is quite common.[77]

The rate of dissolution is dependent upon the degree of saturation and the sum of the activities of the acidic species in solution, i.e., phosphoric and organic acids.[78]

Solubility is an equilibrium property. The equilibrium depends on the size of the solid, the amount of solute added, and the rate of stirring. The rate of equilibration in the solution will depend on the surface area of the solid available per cubic centimeter of solvent used.

The solubility of bone and tooth in solvents constitutes a rather mixed mechanism. As a matter of fact, the dissolution mechanism in weak acid solutions remains controversial. The bone and tooth masses are composed of mineral crystals which are embedded in an interwoven network of protein collagen.

During the equilibration in acid solutions, the bone minerals dissolve and part of the protein collagen goes into colloidal solution. The complete dissolution of bone powder has been reported (refer to Table 10).

In general terms, the dissolution of bone minerals increase rapidly as pH decreases.[79,80] As a rough estimate, in the pH range 4–6, the solubility can be calculated to increase with a factor of 7–8 each time the pH decreases by one unit. However, several studies have shown that when fluoride is present both in the solid apatite and in the aqueous environment, the solubility of apatite is significantly reduced.[18]

Most of the experimental investigations focused on the solubility of bone mineral in buffer solutions. The study investigated the effect of age on MES and crystallinity of rat bone mineral. Little attention has been addressed to the combined solubility of mineral and collagen of the same sample. The principle of the experimental measurement involves the equilibrium of the bone powder with a pure solvent, followed by the determination of the reduction of the weight of the original solute sample. The weight loss of the bone in the solvent represents the solubility, usually expressed in weight percent. Because of the multicomponent composition of bone, including inorganic and organic compounds, the capabilities of solvents are not very effective in dissolving both types of chemicals. The acidic solvents (e.g., acetic acid) are good for the inorganic compounds, while hydrazine dissolves protein collagen. As the solubility is pH and temperature dependent, the magnitude of the result will be affected.

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