SECTION III

TECHNOLOGICAL MODIFICATIONS OF PROTEIN MATERIALS AND THE EFFECT ON STABILITY

PROTEIN ADHESIVES -- D. W. Von Endt

INTRODUCTION

The process of adhesion was actually designed by nature millions of years ago as evidenced, for instance, by barnacles and mussels which attach themselves to rocks. Perhaps the observation that dried blood would mat together hair and debris was one of the observations which later led humans to search for natural products that could serve as adhesives.

Thousands of years ago humans also discovered that the boiling of hide or bone could produce liquid substances which would thicken and bond things together. This process has lasted through the ages, and animal glues are still one of the common types of adhesives in use today. In a more purified form they also are used widely in the photographic industry as emulsions for securing light sensitive chemicals to film and printing paper. The ability of animal glues to perform effectively as adhesives is derived from the strength and polarity of the collagen molecule. Any reasonably complete explanation of why this is so would require a thorough knowledge of physical and polymer chemistry concepts and terminology used in the study of adhesives, so this description will be general and brief.

Adhesion has been defined as that state in which two surfaces are held together by forces consisting of molecular forces or interlocking action or both. An adhesive is then a substance which is capable of holding materials by surface attachment. In fact, excellent adhesion can occur when two materials contact each other sufficiently well to interact. In this case, van der Waals forces come into play. These represent molecular interactions other than covalent bonding, such as dipole-dipole interactions, hydrogen bonding, and London dispersion forces. The types of forces operating within glues and between adhesive and the material surface are listed below in Table IV along with the relative strength of these forces.

Table IV Bonding Forces

Type of Force	Energy in calories per mole
Chemical	
Ionic Bond	140-250,000
Covalent Bond	15-170,000
Metallic Bond	27-83,000
Intermolecular	
Hydrogen Bond	$\leq$ 12,000
Dispersion	$\leq$ 10,000
Dipole-Dipole	$\leq$ 5,000

The covalent bonds mentioned in Table IV are the strongest commonly encountered and are those involved in the primary structure of compounds such as proteins. In these bonds the atoms share their electrons equally, unlike ionic bonds in which one atom contains the fully developed positive (or negative) charge and will strongly attract an oppositely charged particle.

Metal bonds are quite different in that metals consist of a regularly spaced array of atoms with their valence electrons free to move through the whole mass as a sort of "electron gas". These electrons account for much of the interaction between a metal substrate and those adhesives having ionic, dipolar and/or nonpolar character.

Among the intermolecular bonding mentioned in the Table, dipole forces are those which arise from the partially ionic character of some covalent bonds. For instance, the amide bond of a protein contains a carboxyl group (an oxygen atom doubly bonded to carbon) where the electrons in the double bond are always in motion and spend slightly more of their time around the oxygen atom than around the carbon, giving the oxygen a slightly negative character. As a result, the relative dearth of electrons around the carbon lends to it a slightly positive character.

This separation of charges results in the formation of a dipole. The resultant attractions between oppositely charged dipoles on different molecules can result in a strong cummulative attracive force. Dipoles may also polarize neighboring neutral molecules; once this condition is induced, the molecules attract each other.

The hydrogen bonding mentioned in Table IV is somewhat similar in that a hydrogen atom attached to, say, an oxygen to form an alcohol, retains a slight positive charge because of the electron gathering capability of the oxygen. Therefore, the hydrogen can form an attraction to another slightly negatively charged atom.

Dispersion forces are for the most part individually quite weak even though the Table gives the impression that they are relatively strong. Only a few are on the order of hydrogen bonding forces; the rest are much smaller in magnitude--some as small as 20 calories per mole. Dispersion forces are due to the movement of electrons around the atoms of neutral molecules. This movement produces a small dipole in the molecule for an instant, even though there is no permanent separation of charges. The sum of these weak forces can provide a powerful intermolecular attraction between neutral molecules if the molecules are close enough together to interact in this manner. Good adhesion, then, is the result of most, or all of these forces coming into play between adhesive and substrate. Good adhesion is promoted when there is a great degree of molecular contact at the interface, allowing the various interactions to take place.

GELATIN (ANIMAL GLUE)

Animal glues are all collagen based, derived in varying degrees of purity by aqueous boiling of bone and skin waste products from slaughterhouses and tanneries. When the normally insoluble protein collagen is treated with hot water or dilute acids and bases, it slowly becomes soluble. If mild processes are used for the conversion and the starting material is quite clean, the resulting high molecular weight product (commonly termed gelatin) can be very pure. In fact, the collagen used for photograhic emulsions is the purest available, even more pure than that used for food. It is the ability of collagen to form clear, even, flexible suspensions in water which allows it to be used even today for photographic purposes. If the extraction process is more vigorous because of the source of the collagen, a more impure and degraded product (an impure and degraded form of gelatin) results. This product has been used as an adhesive.

Animal glues, being a solution of degraded collagen in water, require a surface which can be wetted by water in order to work. Thus, if two surfaces are to be joined, one of them must be permeable to water.

Although protein adhesives may be derived from a number of animal sources (skin and bone being the main ones), the chemical composition of the collagen derived from these sources varies little. In the making of glue from bones, the starting material must first be cleaned of adhering tissue and degreased to remove the considerable amount of fat which accompanies it. This is usually accomplished by using ultrasonic techniques and cold water to avoid degrading the collagen. The bones are then demineralized with acid and treated several times with steam then hot water to extract the collagen, associated proteins and protein polysaccharides comprising the protein portion of bone. The highest quality glues are produced in the early extractions, since these have been exposed to the least heat.

Hide glues are extracted from tannery waste after first treating the pieces of hide with a saturated water solution of lime. This pretreatment swells the skin and allows the collagen to be extracted more easily. The lime is neutralized with acid and removed with water. The collagen then is extracted with hot water. In both hide and bone glues the initial dilute solutions are finally concentrated by evaporation until the protein concentration reaches about 16-45%. Typically, these solutions then are allowed to set to a gel. The remaining water then is removed and the dried protein is ground into particles for use. When finished, the glue contains about 15% water, 1-4% of inorganic salts, a small amount of fat, and protein polysaccharides (bone glue may contain as much as 6% of this material) as impurities.

From the above, it is evident that even glue from a single source may vary in composition, depending on the processing used and the degree to which degredation has occurred in obtaining it from the native collagen. For instance, the average molecular weight of collagen-derived gelatin may vary by a factor of 10 (from 20,000 to above 200,000). So unless steps are taken to fractionate it, gelatin will contain molecules with a wide range of molecular weights.

Since collagen contains a variety of amino acids with ionizable groups, the net charge on the molecule varies with pH. Acidic conditions contribute protons, increasing the positive molecular charge; and bases remove protons, increasing the negativity. The point at which these charges balance (the iso-electric point) varies with different gelatins and generally lies between pH 4.8 and 9.2. This variation in molecular charge affects both the shape of the molecules and their ability to interact, and accounts to some degree for physical property changes of gelatin (as well as animal glues) with pH.

The presence of acids or bases in the solution leads to an uncoiling of the normal helical form of the collagen molecule, increasing the solution viscosity. The presence of salts, on the other hand, neutralizes the net molecular charge and the viscosity decreases. The rigidity of these glue gels are a function of the average molecular weight, the concentration, pH, and the amount of time intermolecular interactions are allowed to take place. Below a certain molecular weight, each molecule possesses insufficient ionizable groups to allow sufficient intermolecular attraction so that a permanent gelling takes place. This gel "cut-off" is thought to occur at a molecular weight of about 20,000, usually the result of vigorous extraction (and consequently degradation) conditions.

Dried gelatin films bond well and are tough and cohesive, with the higher molecular weight gelatins yielding the stronger films (important in photography). The strength of all gelatin films, though, is decreased under conditions of high humidity due to the water solubility of the glue. This is a disadvantage in a final glue joint, but the re-wettability provides an advantage at other times, for instance, in the development of photographs.

The collagen molecules in the viscous glue solution begin to interact as it cools to the temperature of the surface on which it is spread. As this cooling and initial interaction between collagen molecules occurs, a tackiness in the glue develops, allowing the surfaces on which the glue is spread to adhere to each other. This is followed by formation of a rigid gel as the collagen molecules are still further attracted to each other. The whole process is accelerated when water diffuses into the required porous surface, bringing the glue molecules into still more intimate contact. This contact fosters strong interaction between the molecules of the surfaces to be glued and the adhesive, and between the adhesive molecules.

This rapidly developed tackiness of animal glues as they pass from solution to a gel has been found useful in a number of applications such as attaching cloth to wood. In bookbinding, animal glues are used to glue cloth and leather to form book covers, and for glueing the edges of the pages that form the spine. Where the glue must remain flexible, as in bookbinding, plasticizers such as glycerol, sorbitol and sugar syrups also have been added. In other cases where a thicker glue which will not run is needed, fillers such as clay, and powdered bone (sometimes to 50% weight) are added. Animal collagen also has been used as a binder for organic and inorganic particles to make molded forms such as doll heads. If the glue is spread out and dried rapidly before gelling can occur, a later wetting with water will restore tackiness and allow gel formation to proceed. This property is utilized in the manufacture of paper tapes. Animal glues have also been used as a size for textiles and paper because of their ability to form a strong film and adhere well.

Animal glues may also be modified by adding to them a compound (e.g., paraformaldehyde) which decomposes into formaldehyde with heat. The formaldehyde which is formed then generates cross-links between collagen molecules, and the glue may be used as a thermosetting adhesive. Formaldehyde may also be used to make glue films insoluble by using the formaldehyde as an after treatment under alkaline conditions, where it rapidly undergoes oxidation-reduction (Cannizzaro-type) reactions with other aldehydes present in the collagen.

Not surprisingly, disrupted collagen in the form of gelatin glue undergoes many of the same degredative reactions mentioned previously for skin and bone. When separated and modified, these molecules are even more susceptible to the effects of the environment. Spread as a thin film on the surface of paper as a sizing or emulsion, the gelatin reacts to changes in relative humidity and to light. Water produces hydrolysis and amino acids such as tryptophan are vulnerable to degradation by ultraviolet light. Additives used to modify the properties of the gelatin film are usually small polar molecules which may themselves undergo degradation, promote it, or enter into reaction with collagen. These events all weaken intermolecular bonding and promote chain cleavage. Alterations in pH have effects similar to those mentioned before and promote hydrolysis. The presence of metals (e.g., copper salts) catalyze the alteration of amino acids. The presence of oxygen will promote oxidation of other amino acids (e.g., those containing hydroxyl groups). Each of these actions in combination lead to deterioration of intermolecular crosslinks and the collagen molecule itself. This eventually leads to a loss of adhesive or film forming properties, a loss of water and a gain in brittleness. Eventually the chain lengths are below that required for good cohesion and adhesion, and the gelatin cracks and powders.

In addition, a number of insects feed selectively on protein material such as gelatin and glue. Under conditions of high humidity and temperature, the growth of microorganisms may also be encouraged. These not only produce staining, but they exist by secreting extracellular enzymes which digest the protein. Metabolic by-products are produced as well, many of which are acidic or reactive in other ways and may further enhance degredation.

FISH GLUE

Fish glue was a popular by-product when cod fishing and the preparation of salt cod formed an important part of the New England economy. It was extracted directly from de-salted cod skin and other fish wastes with hot, slightly acidic water. Fish glue does not form a gel at room temperature. Compared to the gelatins from mammalian sources, fish glue posesses much weaker "gelling properties", due to the lower molecular weight of the collagen (on the order of 30,000 to 60,000).

It was the first commercially available liquid glue and has been in use for more than 100 years. Fish glue is more sensitive to heat and enzymes than mammalian collagen and is very water soluble. The normal pH range of this material falls between 5 and 8, depending on the processing involved. It also is degraded rapidly at pH's below 3 or above 9. It can be made water insoluble by the addition of such salts as aluminum or iron sulfates or acid chromates, which oxidize the protein. The addition of aldehydes such as glyoxal, formaldehyde or gluteraldehyde will crosslink these proteins, as mentioned before, rendering them insoluble.

Below room temperature at about 40^{o}F, fish glue solution sets rapidly to a gel, which can be reversed on warming. Although fish glue is quite brittle when dry, it can be plasticized with a glycol or glycerine. It has been used as a wood adhesive and as a photo-resist coating when mixed with a sensitizer.

Fish glue also has been used in conservation. Here the "fish glue" is a special one, made from sturgeon swim bladders or sounds. Glue made from this source yields a very pure form of collagen, the swim bladder being the only tissue composed entirely of collagen. The bladders are usually collected, dried, then heated in water for several hours just below boiling before straining out the finished product. Sturgeon glue has been mixed with polyvinyl alcohol and plasticizers such as glycerol. Although it is rare, Russia being the primary source, it has been used to conserve icons, objects and paper. It is subject to the same deteriorative factors as were mentioned before.

CASEIN

Casein is the major protein found in milk. A glue utilizing this protein is manufactured from skim milk by precipitating casein with acids such as hydrochloric, lactic or sulfuric. Since acid precipitation is not a precise method, casein glues usually contain significant amounts of impurities found in milk. A typical casein glue formulation would contain about 80-90% protein, 0.1-3% butterfat, 0-4% lactose, 7-10% water, 0-3% organic acids and 1-4% inorganic materials. Casein is a phosphoprotein in which phosphorous in the form of either phosphate or pyrophosphate is bound as an ester to the hydroxyl group of serine in the polypeptide. These also are able to form crosslinks between the protein molecules. The word casein denotes a group of closely related proteins and not a homogenous single protein. The phosphorous linkages can be disrupted by bases and the proteins are subject to attack by microorganisms, hydrolysis by acids, bases and enzymes. The actions of any of these result in a loss of adhesive strength. The caseins have an isoelectric point of about pH 4.6, at which point they are quite insoluble in water.

When water is lost from the casein solution (by evaporation or by diffusion into a porous surface) gel formation occurs and the glue sets. It has poor water resistance however, and calcium hydroxide is sometimes added to increase its water resistance (the addition of a base, though, will increase alkaline hydrolysis). Formaldehyde may also be used as before. Casein may be plasticized with glycerol, sugar or agar. It has been used industrially as a paper coating. It also has been used as a pigment and ground binder for works of art.

ALBUMIN

This glue is obtained by rapidly drying the serum from slaughterhouse blood under a vacuum at low temperature in order to prevent coagulation, since coagulated serum is both water insoluble and unsuitable for use as an adhesive. The addition of a small amount of base to the glue mixture improves its adhesive qualities. Heat will denature and insolubilize these proteins, rendering them very water-resistant. As with casein, albumin is still subject to attack by fungi and bacteria. Albumin has been used primarily to glue wood and to manufacture plywood. It was used in the past as a coating to fasten light-sensitive particles to the surface of photographic prints.

REFERENCES

The following is a partial list of references consulted for this section. The relevant portions of each volume may be used for further readings on the subjects which they cover.

Cantarow, A. and Schepartz, B. (1957) Biochemistry. Philadelphia: W.B. Saunders.

Fruton, J.S. and Simmonds, S. (1958) General Biochemistry, 2nd Edition. London: J. Wiley & Sons.

Patrick, R.L., ed. (1967) Treatice on Adhesion and Adhesives, Vol. 1. New York: Marcel Dekker.

Reed, R., ed. (1966) Science for Students of Leather Technology. London: Pergamon Press.

Skeist, I., ed. (1977) Handbook of Adhesives. New York: Van Nostrand-Reinhold.