



The chemical reactivity control of collagen: The Hofmeister Effect revisited

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The Hofmeister effect concept was noted over a century ago. This concerns the action of certain ions in the solubility character of some colloids due to the partial breaking-up of the internal helical structure. Stabilising salt-linkages between charged protein side-groupings are broken, which in turn makes more charged groups available. In the case of collagen, these effects can be employed to gain improved reactions with tannage-related chemicals.

The tanning process first involves the isolation of collagen, being the main leather-making protein component of rawhide. This is composed as a triple helix molecular colloidal micelle species, being similar in some ways to DNA.

The actual tannage consists of chemical treatments with a variety of substances that partially and incrementally stabilise collagen. These actions provide increased resistance against biochemical agents, such as enzymes produced by microorganism, as well as to destruction by heat in an intended temperature usage context.

The process of tannage mainly involves a relative change in the physico-chemical properties of collagen, making it then useful as leather. The extent of tannage is relatively but quantitatively described by the technical term "hydrothermal shrinkage temperature" (HST). This is the temperature when shrinkage occurs with the fully water-saturated leather sample immersed in water being heated. Using these shrinkage temperature values, the extent of collagen stabilisation can be stated for different leathers. The processes involved can thus be evaluated and compared.

The Hofmeister Effect

The Hofmeister effect concept was noted over a century ago. This concerns the relative action of certain series of ions (*and chemicals*) upon the solubility properties of diverse colloidal materials – such as collagen or egg-whites – in water environments.

These effects were often described as "salting-in" or "salting-out" of solution. In the case of fibrous proteins:

- Swelling phenomena (*salting-in*) were often considered indicative as the first step towards dissolution
- De-swelling (*salting-out*) was viewed as the beginning of a potential precipitation.

With the advent of modern colloidal chemistry, the traditional craft of tanning has slowly evolved in mastering chemical efficiency in the use of materials and water consumption.

Chrome tanning, for example, which had a usage efficiency of only 65-70% on the offer before the concept of clean technology, may evolve to more than 90% nowadays. However, this may be improved significantly by the intelligent use of triple-helix destabilising agents, as described and predicted by the classical Hofmeister series studies.

The almost total absorption of chrome (*and other chemicals*) into the resulting leather is possible due to the increased reactivity and availability of the relevant chemical groups of collagen. This can be used to make better leathers, and diminish the pollution problems associated with traditional tanneries.

By further reacting collagen with Hofmeister "salting-out" or collagen stabilising agents, the previous delicate destabilisation can be reversed, bringing a still higher HST value back. Such chemicals often make-up what tanners refer to as retanning agents.

Stability of the triple helix greatly dependent on salt-bonds

Procollagen, that is the basic triple helix molecular structure, is greatly stabilised by salt bridges. These occur between cationic amino and anionic carboxyl side-groups, also from the amino acid components of each peptide strand of the triple helix.

It is said, "A high quantity of ionisable residues (*approx. 15 - 20% of all residues*) is present in every polypeptide chain. Therefore, electrostatic interactions should obviously be relevant for the stability of the triple helix conformation, as well as for biological specificity and fibril formation"⁽¹⁾.

Hofmeister series ions can cause changes in the solubility character of some colloids. This is due to the partial breaking-up of the colloid's micelle internal helical structure. As the thermodynamic stabilising salt-linkages between the charged protein side-groupings are broken, these groups are then made available. The colloid (*in this case procollagen*) thus better interacts with water as a solvent, but also shows an improved reaction with tannage-related chemicals.

Biochemists have classically referred to these types of phenomena as exposing "buried" chemical groups that are otherwise non-reactive. Tanners can use this thermodynamic



destabilisation of collagen as a tool for developing more efficient chemical processing strategies.

Sulfate, a Hofmeister coagulating anion in Cr combination tannage

It is an interesting fact that leather tanned with chromium chloride in the complete absence of sulfate has a HST value of only about 75-80°C. This value is in the same ball-park range as other well known single action tannages such as aluminum salts, formaldehyde and many vegetable extracts, and phenolic syntans. The somewhat unique coordination ability of chromium in tanning processes has been greatly over-rated as being the cause for higher HST values!

The usual highly stabilising chrome tannage - traditionally tested by boiling wet blue leather in water - does *not exist* without the additional weak Hofmeister type stabilisation conferred to the triple helix by the divalent sulfate anion tying-up adjacent cationic amino groups.

Covington⁽²⁾ has referred to the basic chromium sulfate tanning process involved as having a “link and lock” feature. This means that whereas linkages in the normal tannages provide HST values of 75°C to perhaps 85°C, the basic chromium sulfate tannage provides an additional locking mechanism that raises the HST so that these leathers can withstand the boiling test.

This increased HST of the basic chromium sulfate tanned leather is reversible if the leather is massively washed-out by an excess of chloride-containing solutions, offered in several incremental treatments. The 15-25°C HST decrease due to this removal of sulfate ion, can be reinstated - as long as the leather retains its cationic/acid character form - by treatments with sodium or potassium sulfate solutions under acid conditions. This time the chloride ion is eluted out away from the cationic amino-side groups of collagen and replaced by divalent sulfate ion.

The cationic (*positive charge*) character of the amino side chain groups determines collagen's reactivity to anionic (*negative charge*) retanning agents. The removal of sulfate (*negative charged*) as a counter-ion to the cationic amino groups, due to loss of cationic protons from the amino groups, then leads to an overall decrease in positive charge, and this detrimentally affects wet-blue leather.

If leather becomes dried-out it effectively becomes neutralised. This is due to the loss of volatile acids such as HCl and HCOOH (*their anions present from pickling*) as they are flushed

away by water vapour stream as leather dries-out. Where the wet-blue is dried-out completely, and causes a loss of positive charge, sodium sulfate can be seen as a white powder on the surface of the leather.

A surface-charge spot test using anionic/cationic dye mixtures of this defective “dry wet-blue” indicates anionic character; the cut can be as high as pH 5-6, and the leather is *no longer boil-proof*!

Classical Hofmeister Concept and collagen stability

The usual Hofmeister Series, as shown in *Panel 1*, of some ions are listed in increasing order (*left to right*) of causing “salting into solution” effect ⁽³⁾ on collagen and other colloids.

With reference to *Panel 1*, the following details should be noted:

- The chemical sites on collagen for the actions of the ions in the two series given are really very different, but Hofmeister was focusing on their relative comparative effects upon colloid solubility. At low concentration many salts have a *salting-in* effect on micellar colloidal structures, but this can reverse into *salting-out* at much higher concentrations: for this reason there is often confusion.
- Anions that are coagulating, and stabilizing of collagen, probably take up water associated with protruding charged amino groups and actually dehydrate their environment. Anions at the other end of the series do not have any dehydrating effect at all, and because they are monovalent anions, they cannot interact with several charged amino groups at one time, as the more stabilising multivalent anions do so (*towards the left*).
- Lyotropic swelling by a cation like Ca^{++} or Al^{+++} , tend to weaken the hydrogen bonds by disorienting their directionality as more water is introduced into the inner-zones of the triple helix where the cohesive forces reside. Too much water introduced can bring about the situation of the micelles of the peptide strands being diluted beneath their critical micelle concentration value. The triple helix then denatures, and collagen/leather is destroyed. HST must therefore be measured in the presence of water, since under certain circumstances, dehydration brings about the actual stabilisation of collagen.
- The symbolic > (*greater than*) should be interpreted as the

Panel 1: The classic Hofmeister Series

most stabilising
strongly hydrated anions

$\text{citrate}^{3-} > \text{sulfate}^{2-} > \text{phosphate}^{2-} > \text{F}^- > \text{Cl}^- > \text{Br}^- > \text{I}^- > \text{NO}_3^- > \text{ClO}_4^-$

$\text{N}(\text{CH}_3)_4^+ > \text{NH}_4^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{H}^+ > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{Al}^{3+}$

weakly hydrated anions

most destabilising
weakly hydrated anions

strongly hydrated anions



Panel 2 Obtaining Still More Additional Reactivity of Collagen for Chrome Exhaustion.

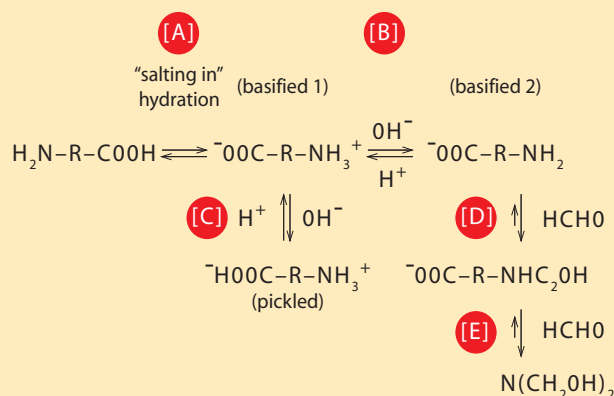
Sørensen's classical formaline titration, where (*buried*) side-group carboxyls are easier to titrate by base in the presence of formaldehyde [see PANEL 2/I, equilibrium **[B]** (that drops the effective pK_a values for $R-NH_3^+ \rightarrow R-NH_2 + H^+$ in the titration curve by 2-3 pH units) then fits into this enhancement of reactivity concept.

The equilibria between different forms for collagen under differing conditions are displaced as the reaction with formaldehyde blocks the reformation of charged amino groups. This prevents the recoupling of the collagen stabilising carboxylate-cationic amino salt-links (*enhanced by H-bonds*) in the collagen.

With reference to Panel 2/I the whole series of equilibria depicting the reactivity of collagen, are shifted towards equilibria **[D]** and **[E]** by the added formaldehyde. This allows for an easier and more complete titration by base.

In tanning practice, the old trick of adding formaldehyde (*safely replaceable by glyoxal*) to the pickle **[C]** would free more carboxyls to react with chrome polymers, because during basification, equilibrium **[B]** is driven to the right by alkali as the freed acid is neutralised. This results in a better chrome uptake. The process will thus become more efficient, so that a lower offer of chrome will still provide good tannage and less waste.

Panel 2/I Sørensen's Classical Formaline Titration Explanation Scheme⁽⁶⁾:



The equilibrium **[A]** (*displaced mainly to the right in aqueous, hydrating, media*) generates the zwitterion (*charged and reactive to water when uncoupled*) form of collagen. In this description **R** represents only one of the three peptide strands of the triple-helix forming the micellar tropocollagen colloidal aggregate. These are analogous to ordinary surfactant micelles, held together substantially by solvent water H-bonds and thus are potentially affectable by Hofmeister hydrotropic phenomena.

The interactions between the three "zwitterionic" peptide strands held-in by the collagen-water micelle, defines much of the tannery physicochemical reactions. These involve the decoupling and activation of multiple salt bonds, along and between the peptide units strands.

This action of formaldehyde occurs on complex fibrous proteins and peptides, but also for simple amino acids. This indicates that coupling salt bonds between the zwitterions can even form in amino acid solutions, if conditions are right!

This presentation, of course is a simplification. There are other interactions besides salt-bonds; but Otto used this type of simplified chemical model in explaining (*at least didactically and for optimizing tannery operations purposes*) dyeing phenomena successfully. It is also valid for many of collagen's other reactions as well. Nothing "really new" from Otto's classical isoelectric point model is being here offered, but only more widely applied.

relative coagulating enhancement. It is a curious thing that the relative orders of the ions appear in many differing phenomena, including in enzyme reaction.

The influence of specific ions in the leather making process

Anions such as naphthalene sulfonates, sulfite, trimeta-phosphate - and many more - perhaps need to be added to this original listing. These three would be placed at the left, as slightly more stabilizing than citrate, with formate located approximately between phosphate and chloride. Many high molecular anionic dyes could also be considered in this series as well, since they initially react like Hofmeister anions by forming an extra strong salt bond, augmented by three H-bonds, as their anionic sulfonic groups react with cationic peptide aminos.

The influence of aldehydes: Most of collagen's chemical reactions (*but not all!*) involve reactions with either cationic amino or anionic carboxyl groups. The tanner considers chemicals respectively as anionic or cationic in nature, thus generally

classifying the reactive products used in leathernaking. Even aldehydes are viewed as "anionic" in action, although they are themselves uncharged. This is because they effectively disable the salt bond reformation by locking-up the potentially cationic end of the salt-bond. This in turn frees anionic carboxyl's, thus lowering the over-all cationic character in further reactions.

The influence of the aluminium ion: As predicted by the Hofmeister (*cation*) series, the use of a small quantity of an aluminum salt in the pickle can carefully destabilise the triple helix, causing slight lyotropic swelling of the collagen accompanied with a slight decrease of HST. This is because there is some uncoupling of aminos from anionic carboxyl's through partial loss of helical character, thus making them more available to react with chrome. This has been reported⁽⁴⁾ as an effective chrome-saving tannage aid.

The influence of sytan-masked chrome: Perhaps the sulfonate anionic group by reacting with a cationic collagen amino causes the benefits of naphthalene and other aromatic sulfonates in the



supposedly syntan-masked chrome-tanning agents. This also would free more carboxyl groups for reaction with the usual cationic chrome tannins, or as anionic chrome complexes revert to a cationic state.

The influence of sulfate/chloride ion: Over and above the effects of sulfate within chrome tannage, the Hofmeister stabilising effects of sulfate at differing stages of tannery processing is considerable and often the cause of firmer leather than desired. This is due to less dissociation or “opening-up”. For example, when deliming with ammonium sulfate versus chloride in pickling, if sodium sulfate is used instead of sodium chloride, and even in fur tannage where aluminum chloride is wrongly deemed as “better masking” than aluminum sulfate!

The influence of the calcium ion: The greater dissociation in the liming process caused by the addition of calcium chloride (*often referred to as “opening-up of the fiber”*) - thereby increasing calcium ions in solution - is well known due to the increase in lyotropic Hofmeister swelling. The technique is no longer advocated in practice due to losses in tensile strength in the final leathers. However, the use of calcium ions does lead to greater reactivity and uptake of chemicals. For example, the application of soluble calcium salts to the surface of wet crinkled pelt can provide a controlled dyeing irregularity as sometimes demanded by fashion. There is normally a loss in strength in localised parts, but this can be acceptable for fancy goods effects and other bespoke uses when performed carefully. Shrunk grain effects from wet-blue often use soluble calcium salts as an ingredient. The fact that calcium sulfate is precipitated also lowers the HST to enhance grain shrinkage.

Delicate and controlled applications: The decoupling of stabilising salt-bonds within the helical micelle structure makes more “buried” groups available for reaction. Delicate and controlled Hofmeister reversible destabilization of the triple helix⁽⁵⁾ is thus proposed for use of collagen’s reactivity control by the tanner for many processing purposes, including new fashions creation.

Opportunities from historic observations

The reexamination of pioneering studies of observed colloidal effects, and the nature of the Hofmeister (*and the Sørensen’s formaline titration*) effects can provide us with a better understanding of collagen reactivity and stabilisation. Accordingly, this can provide us with concepts useful for development of chemical application strategies to create new fashions and run our tanneries better. 🌐

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