Manipulating Chemical Reactivity of Collagen in Tannery Processing*

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Abstract

The control of the chemical reactivity (astringency) of collagen towards the chemicals used in the tannery is essential not only for controlling penetration and ultimate fixation, but conforms an effective methodology approach towards achievement of near stoichiometric completion of the reactions involved and thus can diminish the needless waste caused by the usual excessive offer of chemicals that is often needed to force reactions towards completion because of lack of reactivity enhancement in collagen. In the current ecological witch-hunt, only the most efficient tanners will survive.

Resumen

El control de la reactividad (astringencia) del colágeno hacia los productos químicos utilizados en la Tenería es fundamental no solo en el control de penetración y fijación de tales, sino que proporciona metodología en el alcance al objetivo de finiquitar estequiométricamente las reacciones involucradas y por ende disminuir desperdicios causados por ofrecimiento de excesos innecesarios de productos para forzar a su conclusión las reacciones debido a la falta de reactividad del colágeno. En la presente cacería de brujas causada por histeria ecológica, solo los curtidores más eficientes sobreviviremos.

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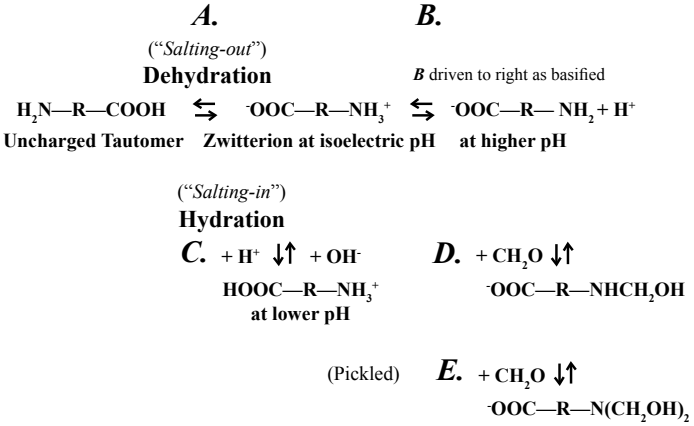
INTRODUCTION AND DISCUSSION

Collagen reacts with tannery chemicals mainly, although not exclusively, through the ionizable side-groups present on the three polypeptide components of the triple helix that are associated with water, likely structured by forces thermodynamically analogous to those that conform surfactant-water micelles. The cationic amino and anionic carboxyl side-groups arising from the formation of the hydration-stabilized Zwitterion tautomer present in proteins, amino acids and peptides, will form saline links in leather/collagen that are involved at differing hierarchical dimension levels in affecting the final properties of leather. Salt solutions screening-out of electrostatic forces, as described by the Debye-Hückel theory, can decouple longer ranged electrostatic saline links affecting the chemical properties of even insoluble fibrous proteins. Manipulation of the reactivity of amino and carboxyl side-groups is a way to control an offered chemical's penetration, as well as its ultimate fixation, since pelt and leather behave chemically similarly to an amphoteric ion exchange resin system: *At more "astringency", less penetration — at less "astringency", more penetration.*

A Classical Chemical Reactivity model for amino acids, peptides and proteins:

Collagen's chemical reactivity can be summarized by the following diagram expressing the different forms that peptides can take, based on arrays of connected chemical equilibriae classically used for explaining the observed titration curves of proteins, amino acids, and peptides, in the presence of formaldehyde.[1] This slightly modified scheme presented, by inclusion of the effect of ionic strength on hydration and dehydration, is suggested as a guide for tanners of the chemistry observed and studied by classical peptide biochemists like **Sørensen** and **Hofmeister**, over a century ago:



The hydration of peptides, (here illustrated by a simple amino acid as in the case of **—R—** being then a small group), yields the multipolar Zwitterion. In fibrous proteins the Zwitterion tautomer consists of various ionized amino and carboxyl side-groups with differing pKa values, distributed along each of the polypeptide chains composed by peptide-linked amino acids. These three peptide chains are inter-joined by *water* enhanced H-bonds, into the spiral helix structure called tropocollagen. These ionized side groups are important because of additionally stabilizing the triple helix over and above the basic stabilization provided by the H-bonds residing within the triple helix, self-organizing water micelle structure. They also serve as the reactive sites for most of the *anionic* and *cationic* chemicals employed by tanners to make leather. The amino and carboxyl side-groups serve to form *compound* saline (electrostatic) and H-bonds (bridging electronegative elements) combined salt-links, which can then give collagen additional hydrothermal stability. These relative *long-ranged* ionic saline compound bonds are also involved at the higher dimensional level hierarchical structures that are involved in formation of fibrils, fibers and even the layered mats of fibers that define many of the macro-properties of the final resulting leathers.

Rotation of Titration Curves of Peptides (etc.) Suggests Astringency Control:

The equilibrium labeled as ***A*** and displayed for a simple amino acid as an example, but can be visualized as applicable for a fibrous protein as a long chain of peptide linked amino acids with their attached anionic and cationic side-groups. Note that a *mid-series Hofmeister effect* (combined cation and anion), non-lyotropic, non-swelling salt such as NaCl, KCl, etc. at lower ionic strengths, would start to first *screen-out* the longer ranged salt-bonds interactions between more distantly interacting collagen triple helices, yielding the separated saline bond components. Such screening would result in separated and loose charged amino and loose ionized carboxyl side groups, sticking-out into the solvent in the vicinity of each of the three polypeptide chains, **-R-.** This peptide configuration for even undissolved fibrous proteins would be referred to as being *"salted-in"* or as *"peptisiert"* by older German tanners. At much higher salt concentrations, equilibrium ***A*** is partially reversed to the left, as the protein is dehydrated and there are *fewer* polar *Zwitterions* present. The titration curves of many proteins, amino acids and peptides, performed under increasing ionic strength reveal interesting ways to manipulate collagen's reactivity:

Ovalbumin illustrates many of the most characteristic features of protein titration curves. The first titration data on this protein (the first studied from this point of view) were published in 1898 by Bugarszky and Lieberman, and it was the first to be analyzed in terms of the Lang model (Cannan et al., 1941; Cannan, 1942).

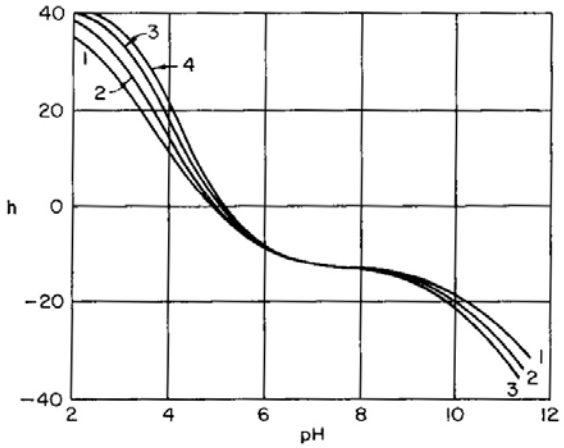


Figure 7. – Titration curves of ovalbumin. The numbered curves correspond to (1) 0.033 M KCl; (3) 0.667 M KCl. (From Cannan, Kibrick and Palmer, 1941.)

The point being here presented is that increasing ionic strength, by causing increased electrostatic screening, can at first affect the extent of anionic/cationic reactive groups available (salting-in), and then as higher salinity values are reached, by reversal of the hydration (salting-out) that *had* enabled charges to initially form, *reduce the* offered chemical's *astringency* by discharge of the peptide side-groups and thus can encourage an added charged chemical's penetration. In order for this method of reactivity control in the tannery to be at all practical, very *low floats* and adequate drums are required to avoid excessive salinity in tannery effluents.

This gross ionic strength of the solution's effect on collagen's Zwitterion fraction, establishing hydration/dehydration extent, affects concurrently both anionic and cationic sites of the Zwitterion configuration, but is essentially separate from each of the Hofmeister Series effects for *specific ions* that can lyotropically either *swell* or *dehydrate* the *interior* of collagen's triple helix; their properties described according to their relative position in each of the series. Either cationic amino side-groups or anionic carboxyl side-groups may or may not be available because of other reasons, such as pH or tannage. Osmotic pH effects caused by Donnan's forces occurring at the very extreme pH values and in the *total* absence of salts are here not discussed, as they can be avoided when not required! These last osmotic phenomena occur at higher hierarchical dimension level than that of the triple helix. Thus general gross ionic strength effect may be combined with *specific* Hofmeister series anions and cations to reach desired astringency situations for obtaining optimum conditions for offering specific tannery chemicals.

We can assert by the behavior of simple amino-acids and peptides, in which *strong* dehydration (by salts) would bring about the reversal of Zwitterion formation made feasible by peptide *hydration.* Discharging the Zwitterion tautomer causes "salting- out."[2] A typical generalized titration curve under increasing salinity for proteins is presented and illustrates a clock-wise rotation about the isoelectric point for ovalbumin[3], which is very similar in general shape to those curves obtained both for simple amino acids and for still more complicated proteins such as gelatin, the building blocks for tropocollagen.

The pH at the inflexion point of a titration curve, is interpreted as the definition of the particular side-group Pka, that shifts with higher salinity, in the case of carboxyl groups (pH values: 2-5) towards higher pH values, and in the case of the acid amino groups (pH values: 9-11) towards lower pH values. This is demonstrated by the displayed clockwise rotation about the isoelectric point of the titration curves by progressive gross saline dehydration of amino-*acids, peptides and polypeptides*. Alpha-helical micelle structures are more complicated to consider, as they sometimes can display as well as rotation, a horizontal displacement of the isoelectric point itself by ionic strength variations caused probably by some Hofmeister Ions Series multi charged members, fixing on the gelatin-like polypeptide chain components and thus altering the overall charge. Nevertheless the clockwise curve rotation trend features caused by simple ionic strength (NaCl, KCl) variation are clearly discernable in both hemoglobin and in collagen's titration curves. The author believes the explanation for the rotation of the titration curves of peptides, as evidenced by the changes in the values of the pKa's of the peptide's ionized side-groups, is due to diminution of the Zwiterion tautomer concentration by salt dehydration:

Ka (amino) = $[H^+][^-OOC—R—NH_2] \div$ **[Zwitterion]**
from equilibrium ***B***

Ka (carboxyl) = $[H^+]$**[Zwitterion]** $\div [HOOC—R—NH_3^+]$
from equilibrium ***C***

Note that the reversal of equilibrium ***A*** by dehydration towards the uncharged tautomer decreases the Zwitterion concentration and thus the results on the shifts of the pKa values are congruent with the observed rotation of the titration curves as ionic strength varies.

The titration curves for *ordinary aqueous amino acids* are similarly affected by increasing ionic strength (salinity) and would be also consistent with the proposed explanation based on partial reversal of the tautomeric equilibrium towards the uncharged species.

The Use of These Concepts in Developing Dyeing Strategies:

A light ionic strength medium can affect the protein's reactive status by *decoupling;* by means of Debye-Hückel screening-out of those Zwitterion-formed saline bonds spanning the longer ranges, as "*salting-in*" of the fibrous protein occurs. The *"salting-out"* of solution caused by *additional* added salt (at above approximately 10 βe in our non-lyotropically, non-swelling NaCl example) begins to reverse the Zwitterion formation equilibrium by its charge destabilization (through the dehydration of the Zwitterion tautomer in the equilibrium ***A***). Thus *"salting-in"* colloidal reactivity effects can be displayed by insoluble fibrous proteins involved at relatively *lower salinities*. At *higher saline* (let's say 6-8 **β**e Na_2SO_4) concentrations of many such lyotropic, Hofmeister deswelling salts (sulphate is such a specific coagulating anion), there can be produced not only the gross dehydration and a strong "salting-out" effect, but an additional electrostatic *"masking"* of remaining cationic aminos. This is, in this author's opinion, the real basis behind an operational dyeing strategy proposal, by the celebrated Dr. Rosenbusch (of Hoechst's oxidative beam-house fame) and stated by Dr. Heidemann in his book.[4] This novel method for increased dye penetration conditions was based on initiating the dyeing process at *very low* floats, adding the powdered dyestuff undissolved, at relatively high pH (see pH effect on equilibrium ***B***), and at cooler temperatures (to additionally encourage colloidal dye-anion aggregation, similar as that as occurs with surfactants or "self-masking"; an astringency-reducing processes in its own right). The resulting salinity due to the ubiquitous presence of Glaubber (Na2SO4) salt in the chemicals *then widely used*, would cause enough inactivation of collagen/leather reactivity through Zwitterion discharge by general dehydration, and/or by sulfate "masking" Hofmeister electrostatic inactivation of the astringent cationic amino side-groups. Cationic amino groups are the ones astringent to sulphonic-group bearing anionic dyes. This allows the colloidal aggregated dyestuff anions to penetrate easier, the electrostatic less-active, amphoteric collagen fiber matrix. Subsequent use of a large (~250%) float of hot and acid water (after the desired penetration) reverses the low astringency conditions, and causes a most efficient dye fixation. Wool dyers use without any ecologic regrets, large amounts of Glaubber salt to help penetration and leveling in their much longer float dyeing processes.

Dr. Otto also reported on his knowledge of the effect of higher ionic strength has on enhanced anionic dye penetration in chrome tanned leather.[5] Specifically he reports that Stubbings and Strauss got complete and rapid penetration of chrome-tanned leather by offering commercial anionic dyes from a 5% common salt solution float.

Concepts Applicable to Developing Tanning & Retanning Strategies:

Equilibrium ***C*** (downwards by acid) would suggest that very good penetration of cationic chrome tannins that would occur by the initiation of tannage in the pickle. Good penetration of the initially anionic sulphate-masked chrome complexes also occurs since chrome reacts by complexation with proton-unblocked carboxyls; because the expected dehydration of collagen by the customary 6-7 βe pickle NaCl salinity, would tend to discharge both carboxyl and amino groupings of the Zwitterion tautomer of collagen, as well as by the extra sodium sulphate present in the commercial chrome product "*specifically* blocking" or "masking" cationic amino sites. Certainly equilibrium ***B*** would be driven to the right by basifying and making more *anionic* carboxyl groupings available and hence the substrate would be more reactive towards chrome or aluminum cationic tannage as basification is executed. This pH caused shift of ***B*** towards the right would also result in collagen/leather configuration with better penetration properties towards an anionic dyestuff.

"First Aid" Emergency Method for Improving Chrome Use Efficiency:

But there is much more to be explained by this complicated appearing (but simply applicable!) series of equilibriae shown, arising out of the classic explanations as to why the titration curves for *proteins, amino acids and peptides* are so radically changed in the presence of a little formaldehyde! The observed pKa of the acid on the amino group on the right side of equilibrium ***B*** is shifted to *lower pH values,* by 2-5 pH units, by just the mere presence of formaldehyde. The formaldehyde presumably shifts the whole interconnected series of *all equilibriae* towards ***D*** and ***E***. This reasoning is the basis for an old tanner's trick of placing small amounts of formaline in the pickle to improve chrome exhaustion as the formaldehyde (today easily replaceable by the work-place *safe* glyoxal) blocks uncharged amino groups from interacting *through the solution,* by very long ranged electrostatic saline links, with anionic carboxyls present both near and far, *within* and *in-between* tropocollagen units! This use of an aldehyde, in effect then "unburies" *carboxyls* otherwise effectively inert to added chrome when they are involved in a salt-link association with amino groups, and *frees* them to be able to react with cationic chrome. The fact that the same pattern in the titration curves is seen to occur to *simple amino acids* in solution, as well as in the case of *fibrous proteins* (even wool!), indicates that very long range saline linkages can form at much greater distances than those involved in possible *chemical* cross-linking at the ordinary *molecular-scale* distances involved in the case of other (not inverse square law) much shorter-range chemical bonding regimes, such as covalent, coordinated, H-bonded, van-der-Waals, etc., etc.

Glyoxal pretreatment will also tend to be an anionic dye-leveler and a penetration enhancer for the same reason of discouraging the formation of dye-astringent charged amino groupings. The action of aldehydes on scar-tissue dyeing by its "camouflage" action is also due to this cationic amino deactivation chemistry, as many highly reactive cationic amino groups are normally present in scar tissue. The shifting of the pKa (of ***B***) by aldehyde is in the same direction as that caused by general salt dehydration, thus decreasing collagen's astringency to dyes, is in the same sense as that observed as by reversal of ***A***.

The Importance of the Saline Links Determining Collagen's "Astringency:"

Salt-bonds have been postulated by Dr. T. Taeger *et al.*[6], from BASF, to substantially contribute to help stabilize collagen/leather's triple helix tertiary micro structure at this dimensional hierarchy: "*…a high quantity of ionizable residues (approx. 15 - 20% of all residues) are 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."*

Such salt-bonds between *anionic* side group carboxyls and *cationic* charged amino side-groups on each of the distinct three primary peptide strands comprising tropocollagen may very well be the very same type of chemical "handles" that anchor the grain layer to the corium. The reason why some leathers become too stiff is that fibers can stick together by means of salt-bonds that form when leather dries (unless partially disabled by blockage of cationic amino sites of half a salt-bond by an anionic fat liquor!). The multiple consequences on the resulting properties of leather due to compound salt-bonds that occur at different hierarchical levels of dimensions, albeit by related chemistries, thus also need to be well considered by the tanner.

Bienkiewicz states: "*Among the collagen functional groups of greatest importance for tanning reactions are carboxylic and amino groups, whereas in the last group we may consider .....histidine and arginine."* [7]

Hydrothermal Stability "Buries" Potentially Reactive Groups:

There are considerable closer-ranged salt linkages occurring between amino and carboxyl groups of the same triple helix structure, that is, *between* (interhelical) the three gelatine strands, however, being primarily held together by (water-aided) H-bonds. The inertness of collagen to offered chemicals and referred in the classic biochemical literature as due to "buried groups", can be thought of as phenomenon caused by the triple helix's "thermodynamic stability induced steric hindrance". Thus a delicate partial destabilization of the triple helix is caused by input of the right kind of *energy* such as ultrasound or maximization of Hinsch's index value of mechanical drumming effect; and/or by the intelligent offer of Hofmeister swelling ions or other chemicals that can bring about increased collagen reactivity. By the *destabilization* freeing-up of some of the component elements of the salt linkages (somewhat akin to 19th Century term "peptization" or "salting-in"), which we then wish to react with a specific tannery chemical, one can help obtain better uptake of a specific chemical that might even lead to better leather, but certainly to *less waste and effluent pollution*.

Hofmeister Lyotropic Cation – Destabilization of Collagen by Aluminum:

Aluminum is such a *highly hydrated* Hofmeister lyotropic swelling cation.[8] It has been reported that *small* amounts of aluminum sulphate or chloride in the pickle can cause a very remarkable drop (~25°C) in the hydrothermal shrinkage temperature[9], that is to say, there is a very fundamental geometric and *molecular destabilization* of the triple helix as the hydrated aluminum cation introduces excessive water *into the interior of the micelle triple helix* structure itself, as it interacts presumably with available carboxyl side-groups. This excess hydration (*lyotropic swelling*) of the triple helix presumably "*dilutes*" internal H-bonds and disorients their action, thus weakening the overall collagen micelle structure. This type of operation when lyotropic swelling agents are used, has to be carefully done in order not to subceed the analogue of the *CMC* (critical micelle concentration) value by excessive interhelical hydration dilution, or else collagen's irreversible denaturation takes place by formation of completely separated gelatin strands.

Thus proposal of using soluble aluminum salts to constitute a *"chrome-saver" type of chemical* came about because of the resulting great increase of collagen's reactivity towards chrome tannins when aluminum ion was previously offered, which was reported by Sykes and Covington in great detail.[10] In this work, this increase of astringency of collagen towards chrome tannins has been attributed to a "reorientation" of the carboxyl groups of the three peptide strands composing the triple helix, by the previous action of aluminum. Whether this explanation is the most properly phrased answer or not; the fact is that such a Hofmeister lyotropic swelling cation *is causing helicoidal distortion*. *That then helps* to fix more chrome on the additional liberated carboxylic sites. Could it be then that as more carboxyl-amino salt links are broken-up by the strain imposed by the triple helix's geometrical lyotropic swelling distortion, then more chrome can fix? This idea describes the kind of operational strategy to be taken against the "thermodynamic stability induced steric hindrance" concept being proposed, to get better reactivity. Gustavson reported over half-a-century-ago on how collagen destabilization (treated with "lyotropes" and also fully heat-

denatured as well) fixed much more chrome than regular pickled pelt[11], presumably as carboxyls are being "unburied" out of decoupled saline bridges by just the plain straining of the structure.

The Use of Chrome to Make Collagen More Reactive to Anionic Chemicals:
What about the converse case? Would there be increased reactivity of cationic amino groups expectable by the blockage of potential reactive carboxyl groups, by the use of cationic metal tannage? Would that procedure on leather/collagen analogously cause greater availability of freed charged amino groups which could then increasingly react with anionic (retanning) chemicals? The remarkable enhancement of anionic dyeing by metal tannage is certainly true and well known by all tanners. Dr. Otto explained this phenomenon as due to the tanning metal making the leather's surface more cationic, but the notion of the disruption of the saline bond being broken-up by tannage, to yield *more* reactive cationic aminos, applies just as well by its own merit. Metal tannage is then the cationic chrome reactivity-aid inverse analogue to the Sørensen formaldehyde titration reaction causing increased reactivity towards chrome, but acting instead on the anionic carboxyl end of the saline link, to increase collagen's overall reactivity to anions!

The Astringency Control Concept Being Offered Is:
Any chemical that by chemically blocking either member of collagen peptide saline-link ($R\text{--}NH_3^+ {}^*{}^-OOC\text{--}R'$-) components, whether anionic or cationic, *makes the remaining unblocked member* of the salt link ("*astringent*") reactive. Of course pH and other factors such as ionic strength must always be considered as well, such as described by the included classical biochemical interconnected equilibriae series.

By the use of a Hofmeister lyotropic *deswelling* multivalent anion such as sulphate, citrate, sulfite, a β-naphthalene sulphonate (syntan), polyphosphate, thiosulphate, carbonate, etc. that can interact with cationic amino-side groups, all of which then result in the activation of previously associated anionic carboxyls towards cationic chrome complexes, then results in better chrome tannage. For this very same reason, ("link and lock" enabled) chrome sulphate gives a much *better* tannage that just plain chrome chloride. Such chemicals, by prying-apart and blocking a saline-link, involving two oppositely natured tanning chemicals, is often referred to as yielding *"a combination tannage",* and often much better overall chrome exhaustion does result as well. Sulphonate *syntan-masked chrome* tannage or retannage results in additional favorable *leathering* characteristics that would fall into this last category. Today, any aid enabling appreciable *reduction* of chrome waste will become a tanner's survival necessity!

SUMMARY

The use of this concept is for enhancing reactivity synergistically through a surgically selective reaction with an offered chemical fixing on a specific cationic or anionic anchoring side group on collagen, which then keeps the *saline link* from easy recoupling. The liberated opposite charged group of the salt bond thus remains ready for chemical reaction with an appropriate charged offered chemical. This selective augmentation of reactivity principle is in fact employed almost subconsciously by most tanners. By our experience honed "instincts", we emphatically insist that any tannery chemical be first classed as either being anionic or cationic before we even think of employing it; this implies the tacit working acceptance and use of this reactivity control concept. Other than aldehydes (that are never-the-less *always* classed as being "anionic"!), sulphochlorinated paraffins, and perhaps chloro-silane containing resins; most other tannery chemicals are considered as belonging either to one or the other main two families: *Anionic* or *Cationic*.

This presentation is not affording *anything* really new, but is only just a more extensive application of Dr. Otto's original viewpoint of selective isoelectric point manipulation being used not only as part of his dyeing methodology, but becoming a basis for a generalized tannery chemical reactive control operational concept, without any need of delving into the controversial ultimate nature of tannage!

Many different factors such as *pH*, *temperature*, *ionic strength* of the medium, *dielectric properties* of the medium (by using solvent fat liquors at low float), *mechanical drumming effect* at low float, input of *high frequency* sound, presence of Hofmeister *swelling/coagulating* effects chemicals, *"peptization"* by saline screening, aldehyde tannage consequences, etc, *can* and *must have to* be decisively employed by today's tanner to obtain desired optimal reactivity (astringency) conditions in order to properly control tannery reactions. The temporary, man-made, delicate *destabilization of the triple helix*, such as caused by highly lyotropic aluminum ions and other chemicals, is then also of required usage, to overcome the thermodynamically stability induced *"steric hindrance"* of collagen towards reactions, to get better controlled overall astringency. These topics all need as well to be intelligently considered to make good leather and *waste less* chemicals. The groundbreaking mechanical drumming effect studies of BASF's Harald Hinsch of the sixties brought about the modern plant engineering concept of a drum as being a *chemical reactor*, (instead of just a one-step-up motorized pit!) that uses mechanical energy and friction generated temperature to achieve better chemical reactivity control of collagen by the resulting controlled energy input. The trend towards the use of a more modern drum as reactors, such as Canguilones, undoubtedly will

affect how efficiently we will offer our anionic and/or cationic tannery chemicals in up-to-date technology recipes to solve our wasted chemical problems.

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REFERENCES

1. Edwin J. Cohn and John T. Edsall, Proteins, *Amino Acids and Peptides*, 2nd Printing, Reinhold P. Corp. ACS Monograph #90, 1943, p.136-139, p.187, p. 447.
2. Edwin J. Cohn and John T. Edsall, *Proteins, Amino Acids and Peptides*, 2nd Printing, Reinhold P. Corp. ACS Monograph #90, 1943, p. 468-471 and p.608-609.
3. Hans Neurath, *The Proteins*, Academic Press, 2nd Ed. N.Y. 1964, p 201 Hans Neurath, *The Proteins*, Academic Press, 2nd Ed. N.Y. and London, 1964, p 201.
4. Dr. Eckhart Heidemann, *Fundamentals of Leather Manufacture*, Eduard Roether KG, Darmstadt, 1993, p 440 (bottom).
5. Dr. Gerhardt Otto, *Mechanism of Leather Dyeing*, chap 32, p 25-26 in: F. O'Flaherty, W.T. Roddy and R.M. Llolar, *The Chemistry and Technology Of Leather*, Krieger Pub. Co., Malabar, Fla, ACS Monograph # 134 vol III, 1962.
6. Uwe Freudenberg , Sven H. Behrens , Petra B. Welzel *, Martin Müller *, Milauscha Grimmer , Katrin Salchert , Tilman Taeger , Kati Schmidt , Wolfgang Pompe and Carsten Werner. Electrostatic Interactions Modulate Conformation of Collagen. *Biophysical Journal* **92**, 2108, 2007.
7. Krystof Bienkiewicz, *Physical Chemistry of Leather Making*, original English edition, Robert Krieger Publishing Co. Malabar (Fla. 32950), 1983, pgs. 256 & 321.
8. Dr. Martin Chaplin, www.lsbu.ac.uk/water/hofmeist.html.
9. George D. McLaughlin, Edwin R. Theis, *The Chemistry of Leather Manufacture*, Reinhold P. Corp.ACS Monograph #101, 1945, pgs. 317& 320.
10. Covington & Sykes; *JALCA* **79**,.72, 1984.
11. K.H. Gustavson, *The Chemistry Of Tanning Processes*, Academic Press Inc. 1956, p 52-53.