Studies of Mono and Divalent Cations Effects on Hair Immunization*

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Abstract

Within the studies on the phenomenon of alkali-induced hair immunization, it has been observed that the hair immunization process arising from the action of dibasic alkalis takes place differently than immunization using monobasic alkalis. On the basis of these observations, our work investigated the influence of mono and divalent cations on the hair immunization mechanisms. In our experimental design, fresh salted bovine hides were exposed to mono and divalent alkalis and salts at different concentrations for various time periods. After treatment, the hides were exposed to reducing agents in order to verify the effectiveness of each cation in promoting immunization. We observed that immunization occurs with dibasic cations, but that both a minimum exposure time and a proper alkalinity are required. The appearance of inter and intra polypeptide chain links was investigated by comparing non-immunized and immunized hair using MIR and FIR spectroscopy, ICP chemical analysis and DSC. The chemical and the instrumental analyses indicate that the exposure of hair to divalent cations in alkaline medium, unlike the monovalent cations, induces the formation of cross-links in the keratin structure and promotes immunization. These investigations contribute to confirmation that the immunization process occurs only in the presence of divalent cations. Research work is in progress in order to further understand the nature and the mechanisms of these complex interactions.

Resumen

Dentro de los estudios sobre el fenómeno de inmunización del pelo inducida por álcalis, se ha observado que el proceso de inmunización del pelo provocado por la acción de álcalis dibásicos ocurre de manera distinta que la inmunización usando álcalis monobásicos. En base a estas observaciones, nuestro trabajo investigó la influencia de cationes mono y bivalentes en los mecanismos de la inmunización del pelo. En nuestro diseño experimental, pieles bovinas saladas frescales fueron expuestas a los álcalis y a sales mono y bivalentes en diversas concentraciones por varios períodos de tiempo. Después del tratamiento, las pieles fueron expuestas a los agentes reductores para verificar la eficacia de cada catión en promover la inmunización. Observamos que la inmunización ocurre con los cationes di-básicos, pero un tiempo mínimo de exposición y una alcalinidad apropiada son requeridos. El aspecto de las uniones dentro y entre las cadenas polipépticas fue investigado comparando el pelo no-inmunizado y el pelo inmunizado por medio de espectroscopía infrarroja media (MIR) y lejana (FIR), el análisis químico de plasma inductivo (ICP) y Calorimetría Diferencial de Barrido (DSC). Los análisis químicos e instrumentales indican que la exposición del pelo a los cationes bivalentes en medio alcalino, diferente de los cationes monovalentes, induce a la formación de reticulaciones en la estructura de la queratina y promueve la inmunización. Estas investigaciones contribuyen a la confirmación que el proceso de la inmunización ocurre solamente en la presencia de cationes bivalentes. El trabajo de investigación está en marcha para entender más sobre la naturaleza y los mecanismos de estas interacciones complejas.

Introduction

In the traditional hair destruction (burning) process, breaking down of the -S-S- bonds that characterize the keratin structure of the hair is accomplished by the use of a reducing agent (sodium sulphide or sulphydrate) accompanied by the use of an alkali to hydrolyse the -SH groups of the reduced proteins and promote their subsequent solubilization. Traditionally, this

* A Technical paper based on a presentation at the 102nd annual meeting of the American Leather Chemists Association at the Hyatt Regency, Milwaukee, WI, on June 23, 2006

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Manuscript received July 28, 2006 and accepted for publication April 18, 2007

alkaline hydrolysis is accomplished by the use of calcium hydroxide (lime). The hair, previously exposed to lime for even a few hours, is no longer attacked by sulphides in concentrations that would bring about the speedy destruction of untreated hair. This phenomenon, known as hair immunization, has been the object of numerous studies for nearly 100 years. Most theories on immunization are based on the premise that the first reaction of keratin with alkali is a hydrolytic splitting of the disulfide bridge, which originates sulphenic acid and a thiol group (suphydryl) according to the reaction:

$$P\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}P + H_2O \xrightarrow[\text{alkali}]{} P\text{-}CH_2\text{-}SOH + HS\text{-}CH_2\text{-}P$$

(P=polypeptide chain).

On the basis of these reaction products, several mechanisms have been postulated for interpreting the formation of new cross-links that would provide keratin with chemical resistance[1-5]. While some authors[3] report that, independently from the type of alkali, the exposure of the hair to moderate alkali concentrations for a few hours induces immunization, it has been also indicated that the action of dibasic alkalis, such as calcium hydroxide and barium hydroxide, takes place differently from that resulting from sodium hydroxide. This is because the divalent cations, differently from the monovalent cations, are supposed to be able to link the fragments of the initial hydrolysis of the disulfide bridge[2].

Starting from the studies of McKay[2], who conducted his investigations on hair samples, our work investigated the coupled effect of alkalinity and cations on the immunization phenomenon by using fresh salted bovine hides that were exposed to mono and divalent alkalis and salts at different concentrations for various time periods. After treatment, the hides were exposed to reducing agents in order to verify the effectiveness of each cation in promoting immunization. There were a total of five experiments in this study. The appearance of the new, inter and intra polypeptide chain links was investigated by comparing non-immunized and immunized hair using MIR and FIR spectroscopy, ICP chemical analysis and DSC.

EXPERIMENTAL

The laboratory scale immunization tests were conducted on pieces cut from the butt of heavy cattle fresh salted skins (32+ kg). The process runs were conducted in cylindrical stainless steel laboratory drums (35 cm diameter, 20 cm length) rotating in a temperature controlled bath (room temperature, 25-27°C), each loaded with two pieces of skin (average weight 250 g). The hide samples, previously soaked (Table I), were drummed in 500 ml solutions of different alkalis and salts at different concentrations and for various periods. The experimental procedures and conditions are reported as follows.

Immunization tests at different concentrations of mono and divalent alkalis

Low alkali concentrations

A preliminary investigation was conducted by drumming the samples for 5 hours at room temperature (25-27°C) in 1 g/l

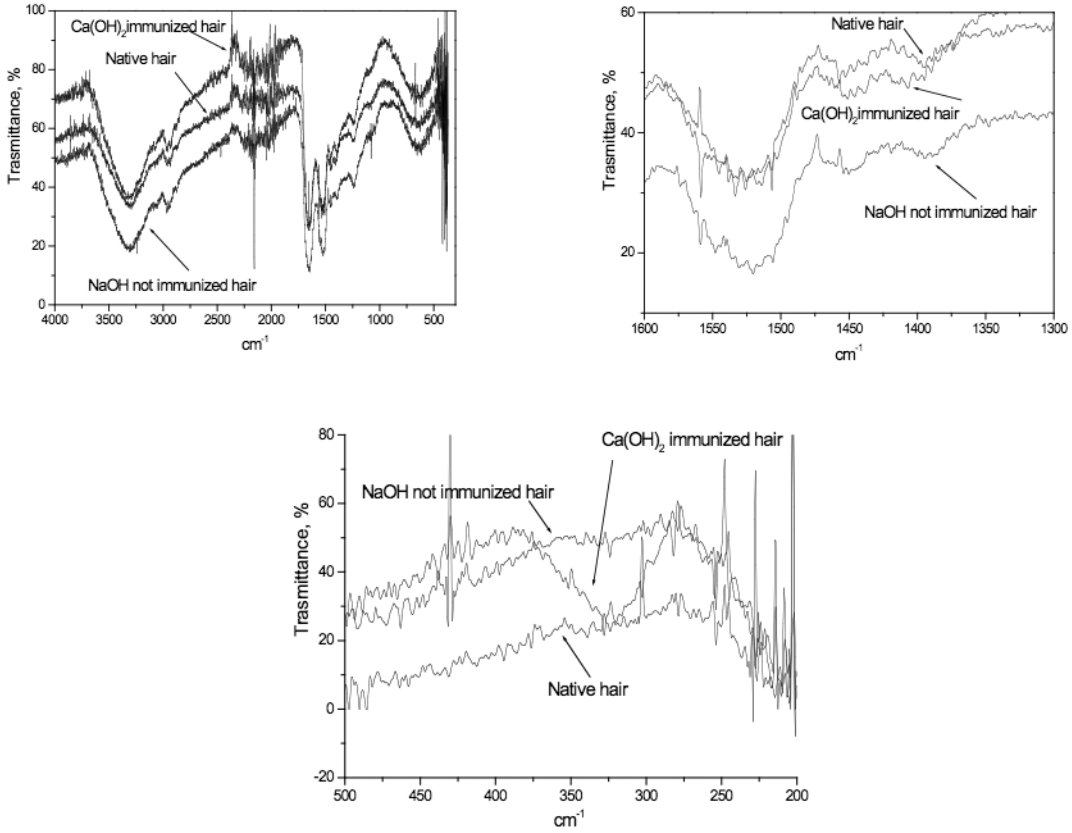

Figure 1: IR spectra of the immunized and not immunized hair

TABLE I
Steps of the Soaking Process
(offers: wt.% based on salted weight)

Desalting		
Water 25°C	200%	
Antibacterial	0.2%	
Surfactant	0.1%	40 min
Drain		
Soaking		
Water 25°C	100%	
Basifying agent (MgO content: >96 wt. %)	0.5%	
Antibacterial	0.3%	
Polyfosfate	0.2%	
Surfactant	0.2%	40 min
Automatic drumming for 24 h (5 min of rotation/h)		
Drain		

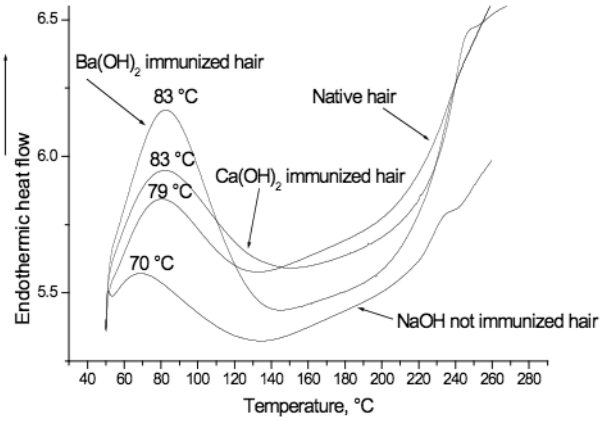

Figure. 2: DSC curves for the native and the treated hair samples.

solutions of $Ca(OH)_2$ or NaOH (0.2 g/100 g of raw salted hide). After exposures to the alkaline medium, Na_2S commercial flakes were added to the float (9 g/100 g of raw salted hide, 45 g/l concentration in the bath) in order to verify the occurring of the immunization phenomenon.

Higher alkali concentrations

After the preliminar runs, two sets of trials in which the exposure times to alkalis were varied between 1 and 5 hours; then prolonged to 6, 8 and 10 hours were performed. After exposure to the alkaline medium, commercial Na_2S flakes were added to the bath. The tests were performed at room temperature by using mono (NaOH, KOH) and divalent ($Ca(OH)_2$, $Ba(OH)_2$) alkalis at such concentrations as to obtain equimolar concentrations of the respective cations. As reference concentration we assumed the lime and the Na_2S concentrations typically used in the industrial hair burning floats, both close to 30 g/l (6 g/100 g of raw salted hide). In order to emphasize the immunization phenomenon, we increased these concentrations by 50% (9 g/100 g of raw salted hide).

The time necessary for the reducing agent to promote the detachment of the hair was recorded and the state of the hair was determined by visual observation.

Immunization in the presence of mono and divalent cations

Exposure at low alkalinity

In a first test of this pair, the hide samples were exposed to solutions of calcium sulfate or sodium sulfate (same equimolar concentrations of Ca^{++} and Na^{+} ions as in the tests at higher concentration of alkalis) for 8 hours. After exposure to each salt solution, commercial Na_2S flakes (Na_2S concentration 45 g/l, 9 g/100 g of raw salted hide) were added to each bath.

Exposure at high alkalinity

In the second test the samples were exposed to a NaOH solution (4 g/l to achieve pH 12) for 2 hours before adding calcium sulfate and sodium sulfate in the same concentration as the first test. Afterwards, commercial Na_2S flakes (Na_2S concentration 45 g/l, 9 g/100 g of raw salted hide) were added and the immunization observed.

Instrumental characterizations

The native and the treated hair were observed by IR analysis with a Perkin-Elmer Spectrum GX equipped with a MIR (medium infra-red) and a FIR (far infra-red) detectors, as well as by DSC analysis (powdered hair was dried under vacuum at 50°C, airtight aluminum capsule, heating rate 10°C/min) with a Perkin Elmer Pyris 1 thermoanalyzer. The chemical analysis of the hair, previously washed (a first washing with 100 ml HCl 10 vol. %/g of hair followed by 3-5 washings with 100 ml distilled water/g of hair until neutral pH) and dissolved in hot concentrated nitric acid (20 ml HNO_3 65% w/w/g of hair), was performed by a ICP-AES with a Perkin Elmer 400.

Results and Discussion

While for some authors hair immunization is due, independently from the type of alkali, to the exposure of the hair to moderate alkali concentrations for a few hours[3], it has been also indicated that the action of dibasic alkalis, such as calcium hydroxide and barium hydroxide, takes place differently from that resulting from sodium hydroxide, since the divalent cations, differently from the monovalent cations, are supposed to be able to link the fragments of the initial hydrolysis of the disulfide bridge[2]. In order to investigate the coupled effect of alkalinity and cations on the immunization process, the following tests were performed.

Immunization tests at different concentrations of mono and divalent alkalis

Low alkali concentrations

After 5 hours drumming at low alkali concentration (1g/l), the pH of the bath was close to 8 when using $Ca(OH)_2$ and 12 when using NaOH. Despite the prolonged exposure time, no immunization was observed independently of the use of mono or divalent alkali. The hair was in fact completely burned in the

TABLE II
Immunization Tests by Exposure to Different Alkalis

Exposure time to alkali (hours)	Time required for unhairing (hours) and visual observation of the state of the hair			
	$Ca(OH)_2$ 45 g/l (9 g/100 g of raw salted hide)	NaOH 24 g/l (4.8 g/100 g of raw salted hide)	$Ba(OH)_2$ 35 g/l (7 g/100 g of raw salted hide)	KOH 34 g/l (6.8 g/100 g of of raw salted hide)
1	2 Hair detached and reduced in short-length fragments	2 Hair detached and reduced in very short length fragments	2 Hair detached and reduced in short-length fragments	1 Hair detached and reduced in very short length fragments
2	2 Hair detached and reduced in short-length fragments	2 Hair detached and reduced in short-length fragments	2 Hair detached and reduced in short-length fragments	2 Hair detached and reduced in short-length fragments
3	4 Hair detached and reduced in longer-length fragments	2 Hair detached and reduced in very short length swelled fragments	2 Hair detached and reduced in very short length swelled fragments	2 Hair detached and reduced in very short length swelled fragments
4	4 Hair detached and reduced in longer-length fragments	3 Hair detached, reduced in very short length swelled fragments	3 Hair detached and reduced in longer-length fragments	3 Hair detached, reduced in very short length swelled fragments
5	5 Hair detached but not fragmented, intact	3 Hair detached, reduced in very short length swelled fragments	4 Hair detached but not fragmented, intact	3 Hair detached, reduced in very short length swelled fragments
6	5 Hair detached but not fragmented, intact	3 Hair detached, reduced in very short length swelled fragments	not done	not done
8	15 Hair not detached and intact, complete immunization	3 Hair detached, reduced in very short length swelled fragments	not done	not done
10	15 Hair not detached and intact, complete immunization	3 Hair detached, reduced in very short length swelled fragments	15 Hair not detached and intact, complete immunization	not done

15 minutes following the introduction of the reducing agent. From this preliminar tests we may observe that a) divalent cations at relatively low pH/concentration or b) movalent cations at relatively low concentration are not adequate to produce immunization.

Higher alkali concentrations

The tests were performed at higher pH/concentration of different mono and divalent alkalis. Due to the higher concentration of the alkali in the bath, the pHs of the solutions were in the range 12-13. The results of the tests are summarized in Table II. When the exposure was limited to 5 hours, the reducing agent was able to detach the hair independently from the alkali used, though the time necessary to achieve the detachment of the hair increased as the exposure time to alkali increased. We may conclude that 5 hours was not sufficient to promote the complete immunization of the soft keratins of the hair root. A different behavior of the divalent alkalis is evidenced by the longer time necessary for the reducing agent to detach the hair. Besides, when using the divalent alkalis the detached hair appeared more resistant (more intact and less swelled) to the effect of the reducing agent. These results indicate that the exposure to divalent alkalis, even not sufficient to prevent the detachment of the hair, induced a more pronounced resistance of the hair shaft to the attack of the reducing agent in comparison with the exposure to monovalent alkalis.

We observed (Table II) that complete immunization (hair not detached and intact) occurred only when the hides were exposed for times longer than 5 hours to the divalent alkalis, at the concentrations used. From these tests we may conclude that a) the increase of concentration of monovalent alkalis does not produce immunization and that b) hair immunization by divalent alkalis may be due to the increase of concentration as well as to the increase of pH.

In order to decouple the effect of pH from the effect of the cation used the following tests were performed.

Immunization in the presence of mono and divalent cations

Exposure at low alkalinity

In a first test the hide samples were exposed to solutions of calcium sulfate or sodium sulfate for 8 hours. This time, on the basis of the previous tests, was sufficient to allow complete immunization. We observed that the pH of the baths ranged between 7 and 9. In both cases, following the introduction of the reducing agent no immunization was observed since the hides were completely unhaired and the hair was rapidly burned.

We may thus confirm that the divalent cations, even at relatively high concentration, do not produce immunization at low pH.

Exposure at high alkalinity

In a second test the samples were exposed to a NaOH solution for 2 hours before adding calcium sulfate and sodium sulfate in the same concentration as the first test. While the sample treated with Na+ cations were unhaired within 3 hours from the addition of Na_2S, in the presence of Ca^{++} cations only a partial immunization was observed since unhairing occurred only after 8 hours from the application of the reducing agent. This result reveals that the immunization of Ca^{++} ions is more effective when the divalent cations interact with the keratin structure contemporary with the adequate alkalinity.

Following the results of all tests, we may conclude that the hair immunization is the result of the interaction between the keratin structure and divalent cations in the presence of an adequate degree of alkalinity.These observations may be considered a starting point to further define the ranges of either concentrations of divalent cations or pH in which the immunization phenomenon occurs and further experimental work is in progress regarding this aspect.

Final Experiment and Results

Investigations on the effects induced by divalent cations in the keratin structure

The above experimental observations are in agreement with the hypothesis, reported in literature[2], that the divalent cations, different from the monovalent cations, are able to link the fragments of the initial hydrolysis of the disulfide bridge, thus promoting resistance of the keratin structure to the action of the reducing agents. In order to support this hypothesis, different chemical and instrumental analyses were performed on the immunized and not immunized hair, and compared with the native hair.

A screening test was performed on lime-immunized haired hide that was exposed to a concentrated HCl solution for 1 hour (acid was used in excess to neutralize the residual lime, the pH of the solution was close to 1) in order to promote the dissolution of the calcium ions not permanently linked into the keratin structure. Afterwards, the hide was treated with 45 g/l Na_2S solution. Despite a partial conversion of sulphide ions to hydrogen sulphide due to the reaction of Na_2S with HCl, the high concentration of the reducing agent assured in any case the presence of effective sulphide ions in solution. After 5 hours of exposure to the reducing solution, the immunization state (hair not detached and intact) was not modified.

This result indicates that a stable link, resistant to the attack of a strong acid medium, is formed between non-ionisable calcium and keratin structure. This observation is confirmed by the of the ICP-AES analyses of the native hair and of the hair after prolonged exposure to $Ca(OH)_2$ (immunized), NaOH (not immunized) and $Ba(OH)_2$ (immunized). As reported in Table III, a significant increase of calcium and barium content was observed in the $Ca(OH)_2$ and $Ba(OH)_2$ - immunized hair samples, while the NaOH - not immunized sample was characterized by a sodium content similar to that of the native hair. Since the samples were washed with HCl solution and water (as reported in the experimental section) before analysis, the results obtained confirms that the divalent cations are chemically fixed into the keratin structure, while the monovalent, present in their ionisable form are easily removed by acid washings.

TABLE III
ICP-AES Analyses of the Native and Treated Hair

	Na	Ca	Ba
Native hair	1.17	0.17	0
10 hours exposure to $Ca(OH)_2$ - immunized hair	not done	12.3	not done
10 hours exposure to NaOH - not immunized hair	1.13	not done	not done
10 hours exposure to $Ba(OH)_2$ - immunized hair	not done	not done	0.46

Fig. 1 shows the IR spectra of the native hair and of the hair after prolonged exposure to NaOH (not immunized) and to $Ca(OH)_2$ (immunized). While the native and the NaOH exposed hair showed similar IR spectra, the $Ca(OH)_2$ exposed hair displayed a shift of the peak in the 1400 cm^{-1} region and the occurrence of a peak in the 350-300 cm^{-1}.

DSC investigation is still in progress. Preliminary DSC curves of the native hair and of the hair after prolonged exposure to $Ca(OH)_2$ (immunized), NaOH (not immunized) and $Ba(OH)_2$ (immunized) are shown in Fig. 2. Even after drying under vacuum at 50°C, the powdered hairs show broad endothermic peaks in the temperature range 70-83°C. This event may be attributed to the removal of loosely bound water, but an endothermic peak in this range has been reported also for the glass transition of native keratin in wool[6]. A second endothermic peak, observed in the range 230-250°C, may be related to melting/denaturation of the keratin crystalline phase[7]. The peaks of the treated hair are shifted with respect to the native hair. The peaks of the immunized hairs move to higher temperatures indicating a higher thermal stability, while the peaks of the not-immunized hair move to lower temperatures with reference to the native hair. This behaviour may be explained by a ligand binding effect of the divalent cations in the protein matrix[8,9].

The chemical and the instrumental analyses indicate that the exposure of hair to Ca^{++} cations (and to divalent cations in general) in alkaline medium, differently from the monovalent cations, induces the formation of new cross-links in the keratin structure and promotes immunization.

Conclusions

We observed that immunization does occur with divalent cations, but that both a minimum exposure time of 5 hours (to promote the hair shaft immunization) and 8 hours (to promote the complete immunization of hair shaft and root)

together with proper alkalinity (pH 12-13) are required. The chemical and the instrumental analyses indicate that the exposure of hair to divalent cations in alkaline medium, unlike the monovalent cations, induces the formation of new cross-links in the keratin structure and promote immunization. Research work is in progress in order to further understand the nature and the mechanisms of these complex interactions.

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