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Chitosan in Cosmetics: Technical Aspects when Formulating

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Abstract
A high-grade chitosan made from shrimp shells has viscosity-building and antimicrobial properties that support its use in cosmetic emulsions and gels.

In this article, we explore formulation issues when using chitosan in cosmetic products. The chitosan used here is a commercially available, high-grade chitosan made from shrimp shells by a 75-90% deacetylation process. We will discuss its viscosity-building and antimicrobial properties, and its use in cosmetic emulsions and gels.

Chitin and Chitosan

Chitin and chitosan are well-known natural biopolymers found in many organisms, including crustaceans, insects, spiders, fungi and algae. In fact, chitin is one of the most common natural polysaccharides, second only to cellulose.

Chitosan is a derivative of chitin, usually extracted from the exoskeletons of crabs, lobsters and shrimps at an industrial level. It is a polymeric N-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose) well known for its numerous and interesting biological properties for which potential applications include cosmetic ingredients, wound-healing materials, food and feed additives, biomedical and pharmaceutical materials and agricultural materials [1-6]

Chitosan, a positively charged (cationic) polymeric product, is obtained industrially when chitin is heated at very high temperatures in sodium hydroxide. This positive charge, produced by double free electrons on the nitrogen atom of the NH_2 group, is an exception in biology. It allows chitosan to react with all the negatively charged biological surfaces (e.g. skin, hair) and bind them tightly by ionic interactions. In addition to this bioadhesive quality, the product possesses remarkable film-forming properties, hence its applications in cosmetic science and medicine. The polymer, characterized by its non-toxicity, can thus be used in the cosmetic industry as a surfactant and viscosity builder, an antistatic agent, an emollient and a moisturizer. The potential fungicidal and bactericidal properties of chitosan are currently widely explored and such properties would confer to chitosan additional potential applications in this field.[7-10]

Last year there were more than 2000 applications using chitosan and its derivatives; these applications were either commercialized or in development.

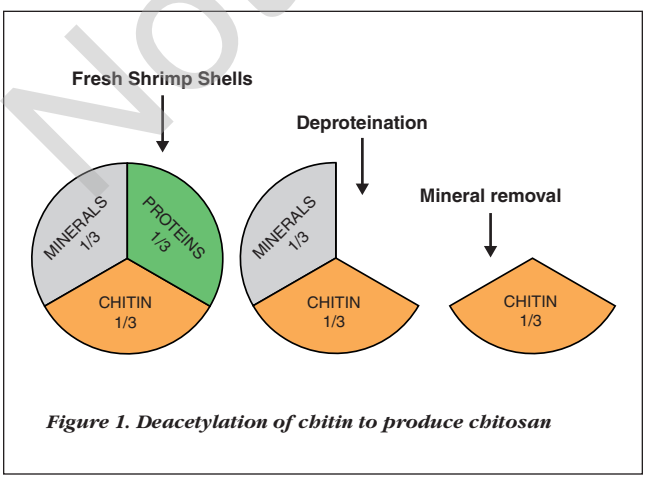

Figure 1. Deacetylation of chitin to produce chitosan

Chitosan Preparation

Factors affecting chitosan end product: Chitosan is obtained by alkaline or enzymatic deacetylation of the acetamido groups of chitin, as shown in Figure 1. It becomes soluble in slightly acidic solutions and its solubility varies according to the conditions of the reaction and the degree of deacetylation performed.

The properties of chitosan are further affected by the physical state and quality of the chitin as well as by its initial pre-treatment. Finally, other relevant factors include the careful control of all process conditions, especially during the removal of by-products such as proteins and minerals.

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Raw materials: The most important source of chitosan for industrial purposes is crustacean shells, with shrimp shells proving to be the best raw material in terms of both availability and consistent quality. Moreover, some manufacturing aspects give the shrimp shells several competitive advantages. For example, fewer chemicals are required for chitosan purification because of the shell structure of shrimp. In addition, shrimp shell hydrolyzes more quickly, has lower levels of minerals and pigmentation, and dissolves more easily than shells of other crustaceans. Those manufacturing advantages are exploited in the preparation of commercially available, high-grade chitosans.

Appropriate controls on other factors must be carefully monitored in order to insure chitosan powders of the highest quality possible. Those factors include the site of the fishing grounds harvested, and handling by the fishing fleets and processing plants on land.

Degree of deacetylation: After chitin is prepared, chitosan is obtained by removing the $CO\text{-}CH_3$ radical, a process called deacetylation. In this operation, chitin is heated in suspension in concentrated sodium hydroxide. The end product is flocculent chitosan. While chitin is not soluble in either acid or sodium hydroxide, chitosan is soluble in low acidic environments.

After deacetylation, chitosan can be either dried and crushed into a powder, or added to a low-acid solution. The level of solubility depends on the degree of deacetylation as well as the actual purity of the product.

Pure and Clear Chitosan (PCC)

The present article aims to explore the formulation issues when using a specific form of cosmetic high-grade chitosan,[a] which we will call Pure and Clear Chitosan (PCC).

PCC is prepared from the exoskeleton of shrimps. Fresh shrimp shells contain equal percentages (approximately 33% each) of proteins, minerals, and chitin. The process begins with the extraction of the proteins and minerals from the carapaces. The chitin obtained looks like wet "corn flakes" and is of a light beige color. Deacetylation produces the chitosan. We prepare the PCC at three viscosities, depending on the degree of deacetylation, as shown in Table 1.

As previously mentioned, several factors might impair the quality of the final chitosan product and the lot-to-lot consistency when produced at an industrial level. PCC is manufactured in-line with the shelling chain to insure appropriate process control from the very beginning. This allows control on the different operating parameters such as the quality of the reagents used, their concentration, the reaction duration, the reaction temperature as well as the gaseous environment, all of which are all critical factors, which, if not controlled, might impair the quality of the final product.[7]

The inter-lot reproducibility might be determined by several analytical parameters. The commonly used parameters are the

[a] *Marine Biopolymer from Atrium Biotechnologies Inc.*

Table 1. Viscosity characteristics of three pure and clear chitosans, depending on degree of deacetylation

	Deacetylation	Viscosity
Viscosity characteristic	(%)	(cps)
Medium viscosity (MVPCC)	>85	<100
High viscosity (HVPCC)	80-90	1000-2000
Very high viscosity (VHV PCC)	75-85	3000-5000

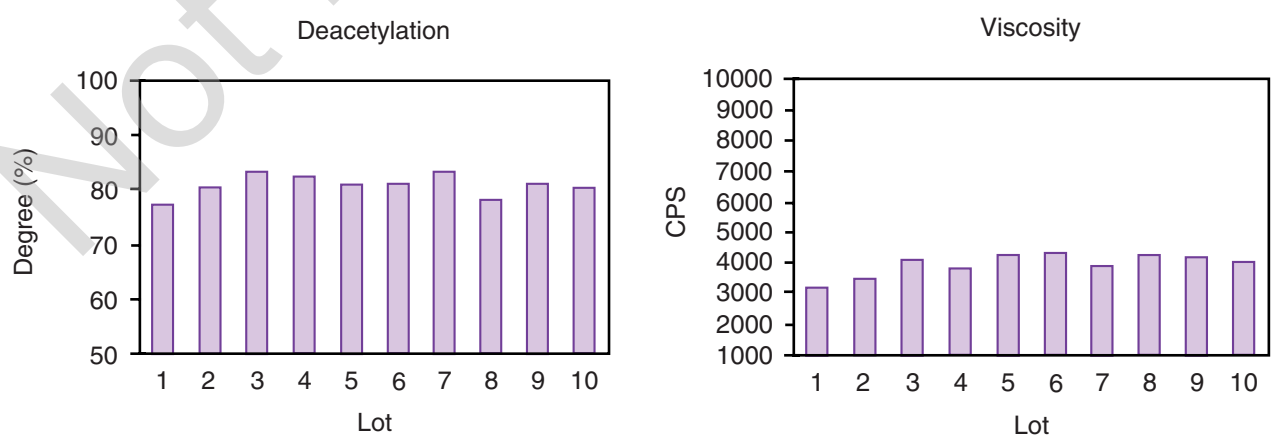

Figure 2. Lot-to-lot consistency of deacetylation degree and viscosity for ten lots of PCC

degree of deacetylation, the viscosity of a slightly acidic solution comprising a known concentration of chitosan, and the determination of the remaining contaminants (such as proteins and minerals). Figure 2 shows the lot-to-lot consistency of the deacetylation degree and viscosity for the VHV PCC.

PCC in Cosmetic Formulations

Within the range of polymer lengths made commercially available for PCC, the MV and HV grades of PCC were more likely to fit into formulations offering film-forming properties and potential salutary skin effects. Higher polymer length VHV PCC would be more effective in producing gels and supplementing the viscosity of creams and lotions. These higher viscosity products also function as stabilizers in emulsion products whereas the lower viscosity grades may act as polyelectrolytes to destabilize certain emulsions.

To better evaluate some of the potential chitosan applications in formulating cosmetics, as well as to establish their limitations, we performed several trials in the laboratory to evaluate the following:

- Turbidity measurements of MV, HV and VHV grades of PCC in aqueous solutions.
- Effect of selected concentrations of VHV PCC on viscosity of emulsions.
- Effect of PCC's degree of deacetylation on a hair gel's ability to provide curl retention.
- Antimicrobial properties of PCC.

Table 2. Turbidity measurements of aqueous 1% PCC solutions

PCC aqueous solution	Turbidity NTU	pH
Medium viscosity (MV PCC)	3.0	3.23
High viscosity (HV PCC)	5.0	3.01
Very high viscosity (VHV PCC)	5.0	3.02

Table 3. Viscosity results with different grades of PCC in 1% and 2% aqueous solutions (pH between 3.0 and 3.3)

PCC aqueous solution	Viscosity* cps
1% Medium viscosity (MV PCC)	100
1% High viscosity (HV PCC)	1,000
1% Very high viscosity (VHV PCC)	3,000
2% Very high viscosity (VHV PCC)	41,000

* Spindle 6; rpm 20

Turbidity of Solutions

As already mentioned, common commercial preparations of chitosan vary dramatically in their quality. Any residual chitin, or other impurities from the shell, will cause a cloudy or hazy appearance in aqueous solutions of chitosan. Some commercial products will even contain large flakes and particulates that no amount of acid neutralizer or heat will solubilize. The recalcitrant chitin stubbornly resists dissolution.

Pure versions of chitosan, even those of moderate deacetylation, will dissolve in acid solutions and remain soluble below pH of approximately 6. Solutions and gels of pure and clear chitosan give crystal-clear aqueous solutions, as shown by nephelometry measurements in Table 2.

Viscosity of Emulsions

Chitosan acts very much like a cationic carbohydrate. In a sense, it mimics soluble cellulose derivatives. In emulsion formulations, the viscosity increases with polymer length and concentration. When we use very high viscosity PCC there is an exponential rise in emulsion viscosity. Table 3 shows the variations in viscosity according to the grades of PCC used and their concentrations in solution.

Because chitosan has a very high cationic charge density, there is the potential for incompatibility with anionic and crypto-anionic ingredients. Amphoteric systems can be compatible but, again, the order of addition and pH when combined will affect the results. Therefore, in emulsion formulations we focused on nonionic and cationic systems.

Preliminary experiments show that VHV PCC dramatically increases viscosity in a nonlinear fashion in simple formulations, as depicted in Figure 3 for the formulations described in Table 4.

As shown in Figure 4 for a cationic system, such as the formulations in Table 5, the effect of VHV PCC on viscosity was far less dramatic than with the previous nonionic system. A 1% concentration in the cationic system produced a lotion, whereas in the nonionic system it produced a cream.

If the subject ingredient is anionic, there will be a strong charge associa-

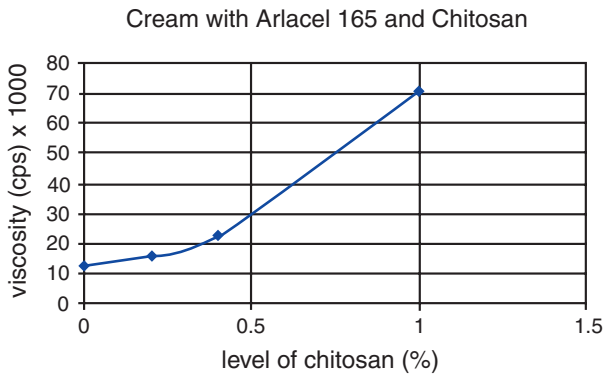

Figure 3. Effect of concentration of VHV PCC on viscosity of a nonionic sample formulation (lotion/cream)

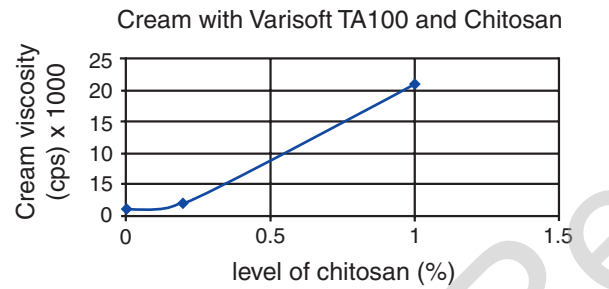

Figure 4. Effect of concentration of VHV PCCon viscosity of a cationic sample formulation (cream)

tion. For instance, a carbomer solution or gel will completely precipitate with chitosan. Likewise, anionic surfactants and other polymers will combine with chitosan. Proteins, because of their amphionic nature, will combine strongly above their isoionic point, where they are primarily anionic; but, they can also be compatible below it, for example, at low pH where they are cationic like the chitosan. However, the order of addition will affect the final outcome because many of these interactions are not reversible.

Curl Potential of Gels

Because of the exponential viscosity increase with increasing concentration of VHV grades of PCC and the film forming ability of all polymer lengths, these natural cationic polymers lend themselves to the formulation of gels for skin and hair. Elegant gels can be based on the VHV grade and these have marked slip on the skin upon application. When they dry down, they have smooth, silky-feeling films with very minimal interim tack.

The lower-polymer-length varieties are especially useful to supplement other cationic polymers, adding both hold and anti-static properties. In a qualitative study, we compared the curl retention potential of MV PCC, HV PCC, and PVP in a gel formulation (Table 6). Both grades of PCC gave an effect comparable to that of PVP on curl retention when combined with Polyquaternium-10, as shown in Figure 5 and Table 7. We also noted a much better static control and ease of dry comb, which should be repeated in a quantified test. The use of 0.5% and 1.0% of HV PCC in the same gel formulation also improved curl retention.

Antimicrobial Properties

The anti-microbial activity of chitosan is well observed on a wide variety of micro-organisms including fungi, algae and some bacteria. This activity constitutes another potential advantage of chitosan when used in cosmetic formulations. However, the anti-microbial action is influenced by intrinsic and extrinsic fac-

Table 4. Nonionic lotions and cream used in viscosity tests

	Lotion 1	Lotion 2	Lotion 3	Cream	
A	Water *(aqua)*	81.00%	80.50%	80.00%	78.50%
	VHV PCC	0.00	0.20	0.40	1.00
	Glycolic acid, 70% soln	0.00	0.30	0.60	1.50
	Preservative (Phenonip, NIPA)	1.00	1.00	1.00	1.00
	Butylene glycol	5.00	5.00	5.00	5.00
B	Glyceryl stearate (and) PEG-100 stearate (Arlacel 165, ICI)	3.00	3.00	3.00	3.00
	Cetyl alcohol (Lanette 16, Cognis)	2.00	2.00	2.00	2.00
	Octyl palmitate	3.00	3.00	3.00	3.00
	Aloe vera oil	3.00	3.00	3.00	3.00
	Sunflower *(Helianthus annuus)* seed oil	2.00	2.00	2.00	2.00
		100.00	100.00	100.00	100.00

tors, among which are the following: the type of chitosan (e.g., plain or derivative); degree of chitosan polymerization; host natural nutrient constituency; substrate chemical and/or nutrient composition; and environmental conditions (e.g., substrate water activity and/or moisture).[8,9,12,13]

To better evaluate the anti-microbial potential of a commercially available chitosan product, we added a concentration of 0.1% VHV PCC to a culture broth previously inoculated with different bacterial or yeast strains. After an incubation of 22 hours, aliquots of the culture broth were spread on a Petri dish to allow count of individual colony forming units (cfu). The trials were performed according to the procedure of Jung et al.[13] We calculated a % Growth Inhibition according to the following formula:

$$\% \text{ Growth Inhibition} = \frac{\text{cfu control} - \text{cfu sample}}{\text{cfu control}} \times 100$$

where cfu sample is the cfu after adding 0.1% VHV PCC.

Table 5. Cationic lotions used in viscosity tests

		Lotion 1	Lotion 2	Lotion 3
A	Water *(aqua)*	80.00%	79.50%	77.50%
	VHV PCC	0.00	0.20	1.00
	Glycolic acid, 70% soln	0.00	0.30	1.50
	Glycerin	5.00	5.00	5.00
B	Distearyldimonium chloride (Varisoft TA100, Th Goldschmidt)	5.00	5.00	5.00
	Cetyl alcohol (Lanette 16, Cognis)	3.00	3.00	3.00
	Octyl palmitate	3.00	3.00	3.00
	Petrolatum	3.00	3.00	3.00
C	propylene glycol, diazolidinyl urea, methylparaben, Propylparaben (Tristat D2, TRI-K)	1.00	1.00	1.00
		100.00	100.00	100.00

Table 6. Hair gels used in curl retention tests

		Gel B	Gel C	Gel D
A	Water *(aqua)*	67.45%	71.95%	67.45%
	MV PCC	3.00	0.00	0.00
	HV PCC	0.00	0.00	3.00
	Glycolic acid, 70% soln	4.50	0.00	4.50
	PVP (PVP -K30, ISP)	0.00	3.00	0.00
B	Polyquaternium 10, 1.5% soln	25.00	25.00	25.00
	Preservative (Kathon CG, Rohm & Haas)	0.05	0.05	0.05
		100.00	100.00	100.00

Table 7. Curl retention results of the tested hair gels. The negative control is treatment with distilled water. For formulations B, C and D, see Table 6.

	Hair length (inches)			
Measurement sequence	Untreated Dist. water (Sample A)	Treated Gel B (Sample B)	Treated Gel C (Sample C)	Treated Gel D (Sample D)
Before treatment	9.0	9.0	9.0	9.0
After treatment, drying, and removal of rollers	4.0	4.5	4.75	3.75
16 hours after removal of rollers	5.75	4.25	5.25	3.75
After combing, 17 hours after removal of rollers	6.0	5.5	6.0	4.75
After 30 min steam exposure, 42 hrs after removal of rollers	8.8	8.7	8.3	8.2

The results (Table 8) confirm the antimicrobial properties of PCC on several micro-organisms and thus, its wide range of potential use for those specific applications.[11,13] Even though the exact mechanism of action has not been characterized, several possibilities have been put forward. Because chitosan is known to interact and chelate some minerals necessary for bacterial growth, chelation might be one mechanism explaining the anti-microbial potential of chitosan. Some laboratories have also suggested that the chitosan can interact directly with the structural wall (external envelope) of the bacterium due to the opposing charges attraction. Destabilization of the structural wall integrity and/or blockage of nutrient exchanges may lead to the leakage of the enzymatic machinery and nutrients, causing bacterial death.

Technical Overview

The high cationic charge and high polymer length of all the available PCCs govern most of the properties of interest to the formulator. This material is able to thicken various personal-care products, both single-phase aqueous-based and multiphase emulsion systems.

After removing the curlers

After drying tresses and then combing them out

After exposing the tresses to steam from a beaker for 30 min, and then combing them out

After drying the steamed tresses and then combing them out

Figure 5. Curl retention potential of a negative control (A) and a hair gel formulation containing MV PCC (B), PVP K30 (C) or HV PCC (D). The negative control is treatment with distilled water. For formulations A, B and C, see Table 6. The unlabelled tress on the left represents untreated equivalent virgin hair of comparable length.

The complexion phenomenon can be a benefit or a detriment to a product, depending on many factors but centering on the overall pH-dependent ionic nature of the components. In fact, any pH modification will produce a conformational change of the molecule. As an example, in a weak ionic density environment (acidic pH), the PCC will stretch and the viscosity of the solution will increase. If higher pH cosmetic formulations have to be prepared, the use of salt might be useful and its addition will increase the pH of the solution while minimizing variations in the viscosity.

Conclusions

High purity and clear chitosans can work in a variety of skin- and hair-care applications wherein their physical and chemical benefits are complemented by their natural origin.

Since the polymer itself acts to suspend particles and retains most of its viscosity at higher temperatures, it does have a stabilizing effect in some formulations. However, the high cationic nature may be responsible for complexing and gelling some emulsion systems leading to a creaming phenomenon at high temperature that may or may not show up over extended room temperature storage. It is thus essential to target the final cosmetic product use prior to formulating so the appropriate grade of PCC can be chosen and optimize compatibility between formulations' ingredients.

The final arbiter will always be relevant controlled testing, but pure and clear chitosan will be effective in many cases.

References

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1. CA Stone et al, Healing at skin graft donor sites dressed with chitosan, *Br J Plast Surg* 53(7) 601-606 (2000)
2. H Ueno et al, Accelerating effects of chitosan for healing at early phase of experimental open wound in dogs, *Biomaterials* 20(15) 1407-1414 (1999)
3. S Hirano, Chitin biotechnology applications, *Biotechnol Annu Rev* 2 237-258 (1996)
4. A Denuzière, D Ferrier and A Domard, Interactions between chitosan and glycosaminoglycans (chondroitin sulfate and hyaluronic acid): Physicochemical and biological studies, *Ann Pharm Fr* 58(1) 47-53 (2000)
5. YW Cho et al, Water-soluble chitin as a wound healing accelerator, *Biomaterials* 20(22) 2139-2145 (1999)
6. G Kratz et al, Heparin-chitosan complexes stimulate wound healing in human skin, *Scand J Plast Reconstr Surg Hand Surg* 31(2) 119-123 (1997)
7. M-C Martini and M Seiller, chap 9, in *Actifs & additifs en cosmétologie*, Paris, Lavoisier (1992) pp 125-146
8. S Senel et al, Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery, *Int J Pharm* 193(2) 197-203 (2000)
9. O Felt et al, Chitosan as tear substitute: A wetting agent endowed with antimicrobial efficacy, *J Ocul Pharmacol Ther* 16(3) 261-270 (2000)
10. A Begin and MR Van Calsteren, Antimicrobial films produced from chitosan, *Int J Biol Macromol* 26(1) 63-67 (1999)
11. RG Cuero, Antimicrobial action of exogenous chitosan, *EXS 87* 315-333 (1999)
12. S Roller and N Covill, The antifungal properties of chitosan in laboratory media and apple juice, *Int J Food Microbiol* 47(1-2) 67-77 (1999)
13. B-O Jung et al, Preparation of amphiphilic chitosan and their antimicrobial activities, *J Applied Polymer Science* 72 1713-1719 (1999)

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