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Characteristic Properties of *N*-Carboxybutyl Chitosan

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ABSTRACT

N-Carboxybutyl chitosans obtained from levulinic acid (4-oxo-pentanoic acid) and five crustacean chitosans (heteropolymers of N-acetylglucosamine and glucosamine) have been instrumentally characterized and found to have degree of N-carboxyalkylation 0·27. They dissolve in water and in water–ethanol mixtures without the need of any acid and give more viscous solutions than the corresponding chitosans. Their compatibility with other polymers and with salts has been surveyed, and the soluble chelates of Cr(III) and Pb(II) have been studied. The bacteriostatic activity of the N-carboxybutyl chitosans, together with other favorable properties, such as the viscosifying action, the enhanced film-forming ability, the moisturizing effect and the stabilization of emulsions, make these novel modified chitosans most suitable as functional cosmetic ingredients.

INTRODUCTION

The use of chitosan solutions finds limitations in the need for an organic acid capable of dissolving chitosan powder. For a number of applications, especially cosmetic formulations, accompanying anions are undesirable and dissolution in excess acid is not practicable.

A remarkable common feature of various series of modified chitosans is their tendency to form gels and to exhibit unusual rheological properties, so that a new kind of limitation against the use of chitosan solutions in the pH range 2–7 has been encountered. For instance, modified chitosans obtained by reacting anhydrides with chitosan are in the form of more or less rigid and insoluble gels, such as those carrying fatty acid groups (Saito *et al.*, 1982), the *N*-acetyl to *N*-tetradecanoyl series (Hirano & Ohe, 1975; Fuji *et al.*, 1980; Kurita *et al.*, 1988), the *N*-(2′-acetoxybenzoyl) chitosan (Hirano & Ohe, 1984) and a variety of *N*-acyl chitosans including *N*-isovaleryl chitosan (Moore & Roberts, 1981). Chitosans modified with phthalaldehydes are insoluble (Hirano &

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Takeuji, 1983), and *N*-alkyl chitosans obtained from aldehydes do not give homogeneous solutions (Muzzarelli *et al.*, 1983), in contrast to *N*-carboxybenzyl chitosans from phthalaldehydic acids (Muzzarelli *et al.*, 1982). Chitosans carrying sugar units are more soluble, but form rigid or elastic gels at certain concentrations or in slightly acidic media (Yalpani & Hall, 1984).

Levulinic acid was not extensively used to dissolve chitosan; it did not appear in the first published list of 70 salts of deacetylated chitin (Rigby, 1936) or in more specialized articles such as that by Gross *et al.* (1983). However, levulinic acid is reported by Muzzarelli (1977), as a quotation of the work of Merrill (1936), who classified chitosan levulinate as 'a salt of monobasic aliphatic acid'. It was not until recently that Muzzarelli (1985) provided a better understanding of the reaction of levulinic acid and chitosan, and described the resulting ketimine and its reduction product.

Levulinic acid (4-oxo-pentanoic acid, $CH_3COCH_2CH_2COOH$) is obtained from fructose (Horvat *et al.*, 1985) and can be easily handled (Sunjic *et al.*, 1984*a*,*b*). It is a metabolic product of the human body (Adamovich *et al.*, 1981); it has been proposed as an ingredient for hair cosmetics (Kanebo Ltd., 1984, 1985; Morita, 1985), as an antioxidant for use in foods (Yi & Kim, 1982) and as a carrier for drugs (Ganem, 1986).

N-Carboxybutyl chitosan obtained from chitosan and levulinic acid under reducing conditions possesses a number of interesting characteristic properties, and the purpose of this paper is to describe them and to propose a cosmetic grade of *N*-carboxybutyl chitosan as a cosmetic functional ingredient.

MATERIALS AND METHODS

Instrumentation and chitosans

The experimental techniques and the chitosans used were those already described by Muzzarelli *et al.* (1983, 1985, 1987). An additional shrimp chitosan was obtained from Chito-Bios, Ancona, Italy. The preparation process, the chitosan derivatives named *N*-carboxybutyl chitosans and the trade name Evalsan are the property of Chito-Bios.

Preparation conditions

A suspension of finely milled chitosan (360 g, 100–250 μm) in water (4 liters) was prepared and a 50% solution of levulinic acid was added

(molar ratio levulinic acid/glucosamine unit, 1·5). After dissolution of the chitosan powder, sodium borohydride (60 g) was added. The final pH of the viscous solution was 6·0. The solution was then dialysed against distilled water for 3 days and lyophilized.

RESULTS AND DISCUSSION

Degree of substitution and molecular weight

Five chitosans from crabs, shrimps and krill were submitted to laser light scattering spectrometry to measure their weight average molecular weights. Larger aliquots of the same samples were used for the synthesis of the corresponding *N*-carboxybutyl chitosans. It is important to note that the molecular weights of the chitosans and corresponding *N*-carboxybutyl chitosans can be compared provided that the two compounds have gone through the same physical treatment, with particular attention to the milling step, which can alter the molecular weight. The Zimm plot for *N*-carboxybutyl chitosan obtained from a crab chitosan is in Fig. 1.

Results are listed in Tables 1 and 2, and show that the average molecular weight increase for all five of the chitosans studied is 19–21% and corresponds to degrees of *N*-carboxyalkylation in the range 0·26–0·28. These results are based on the polymer concentration determined after freeze-drying the solutions, and on the degrees of *N*-acetylation determined by first derivative UV spectrophotometry on the parent chitosans. The results show that all of the chitosans tested react to a significant extent with levulinic acid under the experimental conditions adopted and

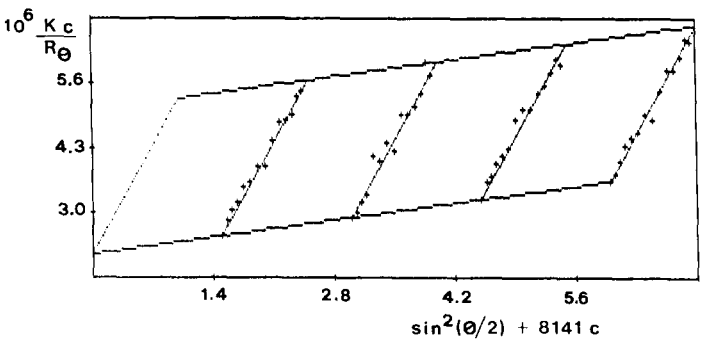

Fig. 1. Typical Zimm plot for the *N*-carboxybutyl chitosan obtained from the Protan chitosan. Solutions contained 0·25, 0·50, 0·75 and 1·00 mg/ml, in the presence of 0·2 M NaCl. Measurements were taken at 10° intervals between 30 and 150°C.

TABLE 1
Properties of *N*-Carboxybutyl Chitosans

Parent chitosan			*N-Carboxybutyl chitosans*				
Name	*Wt av. mol.wt (amu)*	*Degree of deacetylation (%)*	*Wt av. mol.wt (amu)*	*Radius of gyration (nm)*	*2nd virial coeff. (g^{-2} mol cm^3) × 1000*	*Viscosity (mPa s)*	*Mol.wt. ratio*
Katakura	627 660 ± 34 485	85·0	750 170	89·8	1·3	72	1·19
Rybex	464 990 ± 27 840	58·0	555 000	76·9	0·52	—	1·19
Chito-Bios	405 000 ± 25 000	87·3	491 750	80·4	1·03	43	1·21
Protan	384 500 ± 13 500	82·0	459 850	78·2	0·98	40	1·19
Bioshell	191 260 ± 13 390	86·8	229 000	80·8	—	17	1·20

Diluted chitosan solutions (0·1–1·0 g/liter in 1% acetic acid with 0·2 M acetate).
Diluted Evalsan solutions (0·1–1·0 g/liter in 0·2 M sodium chloride).

TABLE 2
Degrees of Substitution and Average Molecular Weights for the Polymer Units of *N*-Carboxybutyl Chitosans Obtained from Various Chitosans under the Same Conditions

Chitosan	*Degree of substitution at C-2*			*Average mol. wt for polymer unit*
	$-NHCH(CH_3)[CH_2]_2COOH$	$-NHCOCH_3$	$-NH_2$	
Chito-Bios	0·27	0·13	0·60	195
Katakura	0·28	0·15	0·57	197
Rybex	0·28	0·42	0·30	240
Protan	0·26	0·18	0·56	196
Bioshell	0·27	0·13	0·60	195

that all the *N*-carboxybutyl chitosans contain a certain proportion of free glucosamine. This finds an explanation in the very mild conditions adopted, namely, room temperature (20°C), dilute chitosan solutions (10 g/liter), small molar ratio levulinic acid/glucosamine unit (1·5) and relatively short reaction time (30 min). These conditions provide the advantage, among others, of preserving the integrity of the polymer chain.

Infrared spectrometry

Spectra were recorded on samples preliminarily dialyzed and then either isolated with acetone in the presence of 1 M HCl (protonated samples) or obtained by freeze-drying the solutions at pH 6·0. The protonated *N*-carboxybutyl chitosans showed bands at 1700 cm^{-1} (-COOH), 1600–1680 (protonated amine and acetamide), 1500 (protonated amine, this band being present in the protonated chitosan as well) and 1380–1450 (alkyl groups).

N-Carboxybutyl chitosans isolated at pH 6·0 showed bands at 1600–1670 cm^{-1} (amide), 1550 (overlapping of primary and secondary amines, amide and carboxylate anion) and 1400 (alkyl groups and carboxylate). Because levulinic acid itself exhibits the -COOH band at 1700 cm^{-1}, the corresponding band in the protonated *N*-carboxybutyl chitosans is very close to the broad band at 1600–1680, while for glutamate glucan and for *N*-carboxymethyl chitosans it appears more distinctly at 1730 cm^{-1}, i.e. at the same wave number as the acids from which they are obtained (Muzzarelli *et al.*, 1982, 1985). Some of the spectra are reported in Fig. 2.

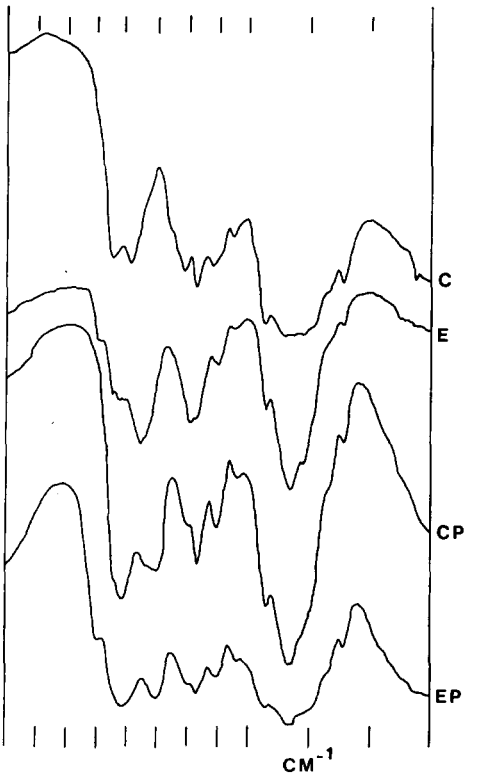

Fig. 2. Infrared spectra (600–2000 cm^{-1}) for Chito-Bios chitosan (C), *N*-carboxybutyl chitosan (E), protonated chitosan (CP) and protonated *N*-carboxybutyl chitosan (EP), providing evidence of the substitution at the amino group.

^{13}C NMR spectrometry

The spectra for dialyzed *N*-carboxybutyl chitosan obtained at pH 6·0 showed the presence of the signal for the methyl group at 24 ppm, in addition to signals at 33 ppm assigned to the N–CH group and at 35 and 40 ppm for the methylene group. A signal at 178 ppm for the carboxyl group was also present (Fig. 3). Assignments were made by correlation criteria and analogy with those reported for *N*-decanoyl and *N*-stearoyl chitosans by Saito *et al.* (1982), with those for levulinic acid (Sunjic *et al.*, 1984*a*) and for chitosan (Yalpani & Hall, 1984; Focher *et al.*, 1986).

The NMR and IR spectra are not against the formation of heterocycles. Kitano *et al.* (1975) indicate that reductive amination of levulinic acid leads to formation of pyrrolidone. Leonard (1956) also indicates that 4-amino derivatives of levulinic acid readily form lactams and 5-methylpirrolidinones.

X-Ray diffraction spectrometry

The effect of functionalized alkyl chains on the crystallinity of *N*-carboxybutyl chitosan was examined by X-ray spectrometry. Freeze-

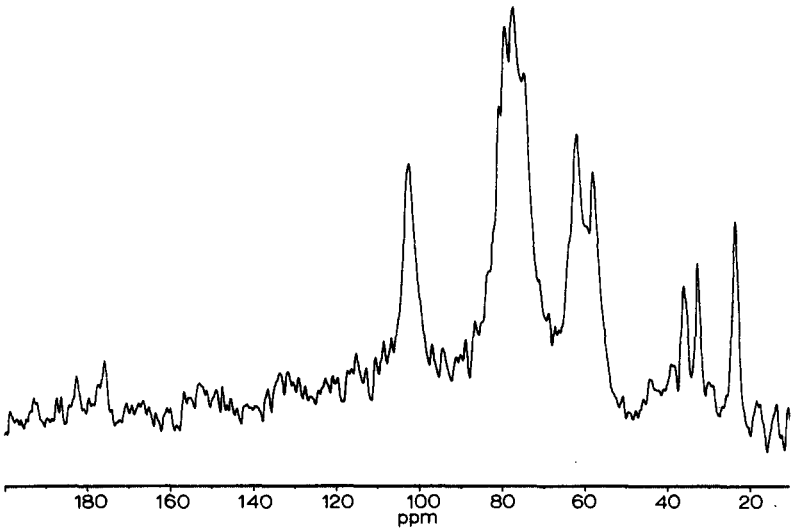

Fig. 3. ^{13}C NMR spectrum of the *N*-carboxybutyl chitosan obtained from krill chitosan, showing signals in the 25–40 ppm region due to the *N*-substitution.

dried *N*-carboxybutyl chitosans did not show any crystalline peak, thus indicating their amorphous state. Because freeze-dried *N*-hexyl chitosan was found to possess a certain degree of crystallinity and limited solubility (Muzzarelli *et al.*, 1983), as confirmed by Kurita *et al.* (1988), it seems that a properly sized chain bearing the carboxyl group is convenient to impart solubility to chitosan by depressing inter- and intrachain interactions. However, the simplified isolation procedure adopted here might be responsible for the absence of diffraction bands in the spectrum.

Viscometry

As indicated previously (Muzzarelli *et al.*, 1986), it is possible to establish a relationship between the viscosity data and the weight average molecular weights, not only for chitosans but also for classes of modified chitosans. To demonstrate the general validity of the adopted instrumental approaches and to permit a comparison of chitosan and Evalsan™, viscosity and weight average molecular weights were determined for 14 chitosans. The results (Fig. 4) fit straight lines and permit the rapid identification of the molecular weight based on double readings with the Haake Rotovisco viscometer.

The relationship between the molecular weights of the various *N*-carboxybutyl chitosans and the viscosity of their respective solutions (0·5%) is described in Fig. 5; *N*-carboxybutyl chitosans are more viscous

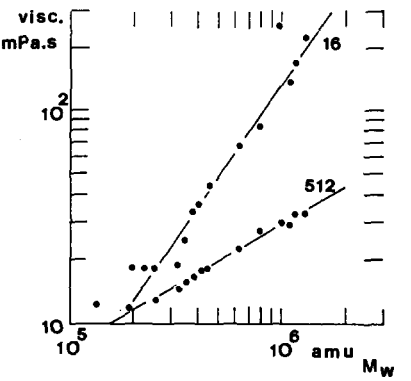

Fig. 4. Relationship between molecular weight (by laser light scattering (lls)) and viscosity of 0·5% solutions (measured at 16 and 512 rpm with the Rotovisco system) for a number of chitosans of various origins.

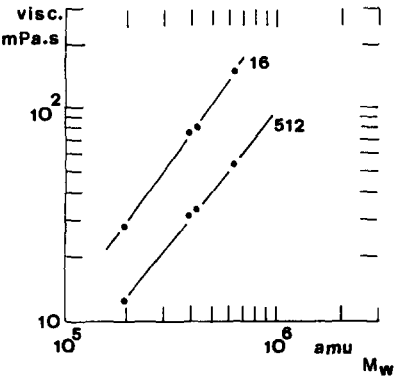

Fig. 5. Relationship between molecular weight and viscosity of 0·5% solutions (determined at 16 and 512 rpm with the Rotovisco system) for four *N*-carboxybutyl chitosans.

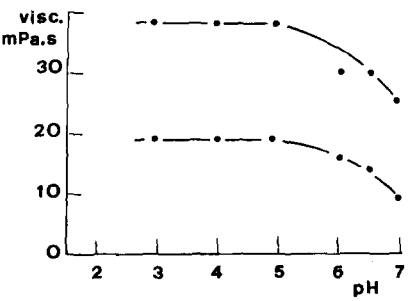

Fig. 6. Dependence on pH of the viscosity of 0·5% *N*-carboxybutyl chitosan solutions at 20°C.

than the corresponding chitosans. While the data for this figure have been obtained at pH 5·0, it was found that the viscosity of *N*-carboxybutyl chitosans is practically independent of the pH value in the range 2·9–5·5 (Fig. 6). While the solutions in the acidic pH range are clear, limpid and colorless for all the *N*-carboxybutyl chitosans tested, at pH 8·5 and higher, *N*-carboxybutyl chitosans become insoluble and precipitate as white, voluminous products.

The viscosity of the *N*-carboxybutyl chitosans was found to be less temperature dependent than in the case of other modified chitosans. It was also verified that the *N*-carboxybutyl chitosan solutions exposed to air and light in uncapped vials for 4 weeks did not develop any mold and their viscosity drop was less than 10%. Every day distilled water was added to compensate for the water loss due to evaporation (room temperature, 20–25°C). Degradation of the polymer due to microbial growth could be macroscopically detected during the fifth week of exposure.

Scanning electron microscopy

N-Carboxybutyl chitosan retains the film-forming ability of chitosan; by slow evaporation of aqueous and water–alcohol solutions, membranes were obtained. The freeze-dried product, isolated from dialysed solutions, showed an aspect where filaments predominated (Fig. 7), this being a characteristic property that distinguishes lyophilized *N*-carboxybutyl chitosan from other lyophilized substituted chitosans.

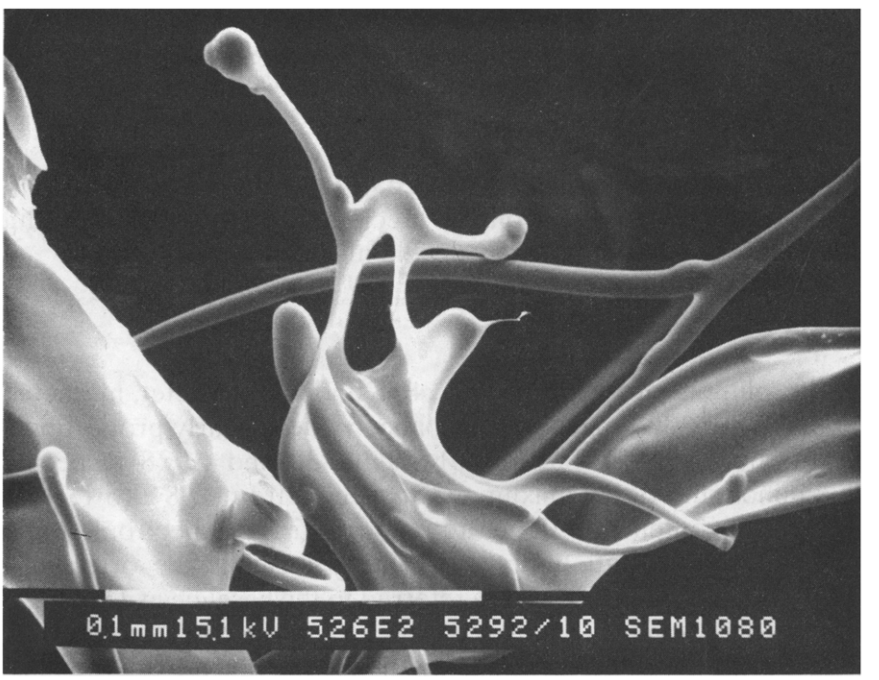

Fig. 7. Scanning electron microscopy of freeze-dried *N*-carboxybutyl chitosan, showing its film-forming ability (× 526).

Compatibility with other polymers

Evalsan™ is fully compatible with animal gelatin and promotes its gelation; the resulting mixture (1:1) is slightly less viscous than the gelatin itself. Excellent compatibility of Evalsan™ has also been observed with poly(acrylic acid) (PAA) (Aldrich), Sandopan-DTC (Sodium trideceth-7 carboxylate, Sandoz), poly(vinylpyrrolidone) and poly(vinylpyrrolidone)-poly(vinylacetate) copolymer (Henkel). On the other hand, precipitates and gels are observed with carboxyvinyl polymers such as Carbopol 940 (Goodrich), carboxymethyl guar (Lamberti), Acrysol (Rohm & Haas), carrageenan Gelatan (Comiel) and sodium alginate Kelgin F (Kelco). It is interesting that PAA from the same manufacturer (Aldrich) was recently used by Chevasit *et al.* (1988) to produce insoluble polyelectrolyte complexes of chitosan. It would seem that *N*-carboxybutyl chitosan is less prone than chitosan to reaction with PAA even though it forms insoluble complexes with Acrysol, a cosmetic grade PAA.

Compatibility with salts

N-Carboxybutyl chitosans are compatible with many salts. Among the sodium salts tested (1 g/liter), chloride, hydrogen carbonate, acetate, sulfate, disodium hydrogen phosphate and nitrate do not render *N*-carboxybutyl chitosan insoluble when added in 1:1 v/v ratio to its solutions (5 g/liter, pH 5·9). Sodium carbonate and lauryl sulfate produce precipitates upon mixing; sodium tungstate produces a gel. Mg(II), Ca(II), Cr(III) and Co(II) do not promote precipitation of insoluble products, while Fe(II), Ni, Cu, Zn, Ag and Cd yield insoluble metal chelates.

Spectrophotometry

Evalsan solutions at 5·8, 17·5, 29·2, 40·8 and 52·5 mg/liter, measured in the range 180–300 nm, gave absorbance values linearly proportional to concentration, i.e. 0·194, 0·356, 0·531, 0·732 and 0·928 respectively, with a minor shift of the maximum wavelength from 188·2 to 189·2 nm. The Evalsan content can therefore be quantified by spectrophotometry in the said concentration range. Linearity includes the origin of the coordinates, if measurements are referred to the baseline at 180 nm. The following are examples of three distinct behaviors of *N*-carboxybutyl chitosan with transition metal ions, studied in the range 180–400 nm.

Copper chelation. *N*-Carboxybutyl chitosan–Cu(II) dilute solutions (less than 40 mg/liter) were clear and suitable for spectrometry, showing

a decrease of the 199·8 nm peak and a new peak typical of the soluble chelate at 257·7 nm at pH 5·4–5·9.

Lead chelation. The samples containing Evalsan and lead ions were clear for days after preparation. In the presence of lead, the peak at 199·0 nm disappeared completely; a new peak at 228·0 nm was detected, whose intensity was proportional to the lead concentration, and for which the maximum position was slightly shifted from 228·8 to 236·9 nm. Results are shown in Table 3.

Chromium(III) chelation. All mixtures were clear for days after preparation and showed a progressive disappearance of the peak at 207·4 nm with increasing Cr(III) concentration, as shown in Table 4. No new peak was detected.

TABLE 3
Spectrophotometric Data on Evalsan–Pb(II) Solutions

Pb(II)		*R*	*Absorbance*
mg	*mmol*		
0·75	0·0036	0·22	0·790
1·05	0·0051	0·32	0·934
1·50	0·0072	0·45	1·082
3·00	0·0145	1·91	1·278
3·60	0·0174	1·09	1·365
4·50	0·0317	1·36	1·464

Evalsan with average molecular weight for the repeating unit 195 was used as a 3·5 g/liter solution and Pb(II) as a 1 g/liter solution. The indicated amount of Pb(II) was diluted with Evalsan (0·9 ml containing 3·15 mg, i.e. 0·016 mmol) and the mixture was diluted to 6·0 ml with distilled water. *R* is the molar ratio Pb(II)/Evalsan unit.

Cosmetic formulations

Evalsan™ has been included in cosmetic formulations, to demonstrate its compatibility with other ingredients and its functional properties.

A toothpaste was formulated as follows (data are in % by weight): Evalsan™ (Chito-Bios), 25; hydroxyethyl cellulose, 1; Gram (Rohm & Haas), 0·25; glycerol, 25; calcium phosphate, 40; silica gel (Grace), 1; saccharose monopalmitate (Biochim), 2; mint extract or sage extract (Gattefossé). This paste showed perfect consistency over a 9-month period, did not deteriorate and was used daily by several persons. It

TABLE 4
Spectrophotometric data on Evalsan–Cr(III) Solutions

Cr(III)		*R*	*Absorbance*
mg	*mmol*		
0·25	0·0048	0·17	1·908
0·50	0·0096	0·34	2·123
0·75	0·0144	0·52	1·953
1·00	0·0192	0·69	1·817
1·25	0·0240	0·87	1·734
1·50	0·0288	1·04	1·684

Evalsan with average molecular weight for the repeating unit 195 was used as a 12 g/liter solution and Cr(III) as a 1 g/liter solution. The indicated amount of Cr(III) was diluted with Evalsan (0·45 ml containing 5·4 mg, i.e. 0·0277 mmol) and the mixture was diluted to 6·0 ml with distilled water. *R* is the molar ratio Cr(III)/Evalsan unit.

showed antibacterial and hemostatic effects, thus exerting favorable action for the prevention of periodontopathies.

A hand and body cream (water-in-oil emulsion) was prepared as follows (data are in % by weight): Xalifin (Vevy), 7; cetyl alcohol, 2; oleyl oleate, 10; Karité, 2; olive oil, 0·5; Evalsan, 38; water, 40; EDTA, 0·05; Katon CG, 0·18; Gram, 0·25; perfume. This cream did not deteriorate over a 6-month period of use by 20 individuals who appreciated its moisturizing effect and softening action on skin exposed to detergents, chemicals and salt water. The cream was also used in the presence of bacterial and fungal infections with good results, e.g. in dermatitis and in decubitus ulcers.

CONCLUSIONS

The chemical derivative of chitosan whose general characteristics have been presented in this article is of particular value because its preparation is simple and straightforward and leads to products of consistent properties irrespective of the origin of the chitosan used. The degree of carboxyalkylation obtained under the mild conditions that preserve the high molecular weight of the parent chitosan imparts peculiar and attractive characteristic properties: Evalsan™ is easily soluble in water

and the viscosity of its solutions is not pH-dependent in a very important pH range for most applications; it forms a number of soluble chelates, among them the Cr(III) derivative, and it is practically indifferent to high concentrations of magnesium and calcium.

While Evalsans™ retain a number of biological activities found in chitosan, such as the wound-healing ability, the film-forming ability and the cationic character, a number of novel and peculiar properties, namely its bacteriostatic action, its powerful viscosifying ability for emulsions and gelatins and its moisturizing and reparative capacity, qualify Evalsan™ as an effective and functional ingredient for cosmetic preparations.

REFERENCES

Adamovich, G. G., Timakin, N. P., Bykova, M. Y., Lykagin, V. V. & Konovalova, L. M. (1981). *Gig. Sanit.*, **11**, 75.

Chavasit, V., Kienzle-Sterzer, C. & Torres, J. A. (1988). *Pol. Bull.*, **19**, 233.

Focher, B., Massoli, A., Torri, G., Gervasini, A. & Morazzoni, F. (1986). *Makromol. Chem.*, **187**, 2609.

Fuji, S., Kumagai, H. & Noda, N. (1980). *Carbohydr. Res.*, **83**, 389.

Ganem, H. (1986). Fr. Patent 2,567,757.

Gross, P., Konrad, E. & Mager, H. (1983). *Parfum. Kosmetik*, **64**, 367.

Hirano, S. & Ohe, Y. (1975). *Agric. Biol. Chem.*, **39**, 1337.

Hirano, S. & Takeuji, M. (1983). *Int. J. Biol. Macromol.*, **5**, 373.

Hirano, S. & Ohe, Y. (1984). *Carbohydr. Polym.*, **4**, 15.

Horvat, J., Klaic, B., Metelko, B. & Sunjic, V. (1985). *Tetrahedron Lett.*, **26**, 2111.

Kanebo Ltd. (1984). *Jap. Kokai Tokkyo Koho*, **59**, 164,768.

Kanebo Ltd. (1985). *Jap. Kokai Tokkyo Koho*, **60**, 28,913.

Kitano, M., Tanimoto, F. & Okabayashi, M. (1975). *Chem. Econ. Engin. Rev.*, **7**, 25.

Kurita, K., Ishiguro, M. & Katajima, T. (1988). *Int. J. Biol. Macromol.*, **10**, 124.

Leonard, R. H. (1956). *Ind. Engin. Chem.*, **48**, 1330.

Merrill, W. J. (1936). US patent 2,047,218.

Moore, G. K. & Roberts, G. A. F. (1981). *Int. J. Biol. Macromol.*, **3**, 337.

Morita, S. (1985). *Jap. Kokai Tokkyo Koho*, **60**, 197,615.

Muzzarelli, R. A. A. (1977). *Chitin*, Pergamon Press, Oxford.

Muzzarelli, R. A. A. (1985). Ital. patent 1,190,380; US patent appl. 875,482; Eur. patent appl. 0,212,688 A3.

Muzzarelli, R. A. A., Tanfani, F., Emanuelli, M. & Mariotti, S. (1982). *Carbohydr. Res.*, **107**, 199.

Muzzarelli, R. A. A., Tanfani, F. & Emanuelli, M. (1983). *J. Membr. Sci.*, **16**, 295.

Muzzarelli, R. A. A., Tanfani, F., Emanuelli, M. & Bolognini, L. (1985). *Biotechnol. Bioengin.*, **27**, 1115.

Muzzarelli, R. A. A., Jeuniaux C. & Gooday, G. W. (Eds) (1986). *Chitin in Nature and Technology*, Plenum Press, New York.

Muzzarelli, R. A. A., Lough, C. & Emanuelli, M. (1987). *Carbohydr. Res.*, **164,** 433.
Rigby, G. W. (1936). US patent 2,040,879.
Saito, H., Tabeta, R. & Hirano, S. (1982). Proc. 2nd Int. Conf. Chitin and Chitosan. Japanese Soc. Chitin, Tottori, p. 71.
Sunjic, V., Horvat, J., Klaic, B. & Horvat, S. (1984*a*). *Kem. Ind.*, **33,** 593.
Sunjic, V., Horvat, J. & Klaic, B. (1984*b*). *Kem. Ind.*, **33,** 599.
Yalpani, M. & Hall, L. D. (1984). *Macromolecules,* **17,** 272.
Yi, S. H. & Kim, D. H. (1982). *Hanguk Sik. Kwa. Chi.*, **14,** 265.