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Biomedical and Pharmaceutical Applications of Alginate and Chitosan

ØYVIND SKAUGRUD, ANNIKEN HAGEN, BERIT BORGERSEN AND MICHAEL DORNISH*

Pronova Biomedical, Gaustadalleen 21, N-0371 Oslo, Norway

Introduction

Biopolymers from marine sources have been studied and utilized in commercial applications and product development for a number of years. Pharmaceutical and medical uses of biopolymers have gained interest. Alginates and chitosan can be utilized as hydrophilic drug carriers and as matrix materials. In addition, these polysaccharides have potential as rate-controlling excipients in drug release systems. One of the most interesting applications of alginate is in the development of alginate-immobilized cells as artificial organs. For example, the potential use of alginate-encapsulated pancreatic islet cells for the treatment of type I diabetes could be a useful treatment for a large number of patients. This application, however, puts strict requirements on the encapsulation system as well as on the purity and documentation of the alginate used. Chitosan has shown promise in the development of non-parenteral delivery systems for challenging drugs. For example, chitosan salts have been shown to increase the transport of drugs across the nasal epithelial surface.

These, and other applications will be pursued in the near future. Such applications will require highly purified polymers with documented safety profiles. In addition, polymers used in pharmaceutical applications will also have to be acceptable to regulatory authorities. The characterization of key parameters for each polymer will be necessary.

Description of alginate and chitosan

ALGINATE

Alginate, extracted from brown algae, is a linear polymer composed of two uronic acid monosaccharides: D-mannuronic (M) and L-guluronic (G) acid linked by $\beta(1\rightarrow4)$ and $\alpha(1\rightarrow4)$ glycosidic bonds. The two monomers are arranged in homopolymeric blocks,

*To whom correspondence may be addressed.

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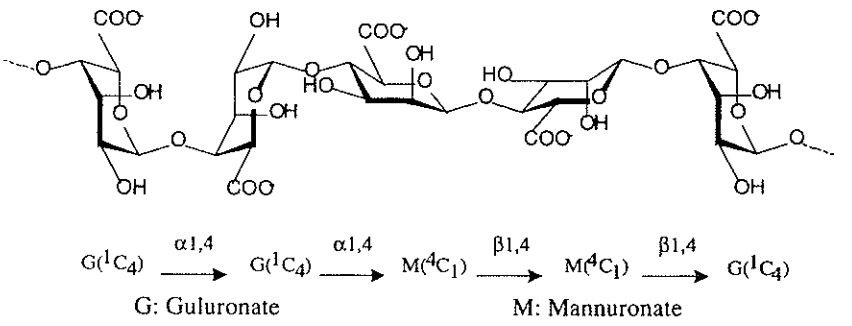

Figure 1. Chemical structures of alginate.

M-blocks and G-blocks as well as sequences containing both monomers, MG-blocks (*Figure 1*).

Alginates isolated from different algae can vary both in monomer composition and block arrangement (*Table 1*), and these variations are also reflected in the properties of the alginate.

Whilst viscosity depends mainly on molecular size, the affinity for cations and the gel forming properties are mostly related to the block structure of repeating guluronic acid residues. When two guluronic acid residues are adjacent in the polymer, they form a binding site for polyvalent cations. The content of G-blocks is the main structural feature contributing to gel strength and stability of the gel. Reactivity with calcium, causing gel formation, is a direct function of the average length of the G blocks occurring in the polymer chain (*Figure 2*) (Smidsrød and Draget, 1996; Smidsrød and Skjåk-Bræk, 1990)

CHITOSAN

Chitosan is a high molecular weight cationic polysaccharide derived from crustacean shells by deacetylation of naturally occurring chitin. Chitosan is also a linear polymer which is composed of glucosamine and *N*-acetyl glucosamine units linked

Table 1. Typical values for M (mannuronate) and G (guluronate) content in seaweed used for alginate production

Seaweed	M/G	%M	%G	%MM	%GG
Laminaria hyperborea (stem)	0.45	30	70	18	58
Laminaria hyperborea (leaf)	1.22	55	45	36	26
Laminaria digitata	1.22	55	45	39	29
Macrocystis pyrifera	1.50	60	40	40	20
Lessonia nigrescens	1.50	60	40	43	23
Ascophyllum nodosum	1.86	65	35	56	26
Laminaria japonica	1.86	65	35	48	18
Durvillea antarctica	2.45	71	29	58	16
Durvillea potarum	3.33	77	23	69	13

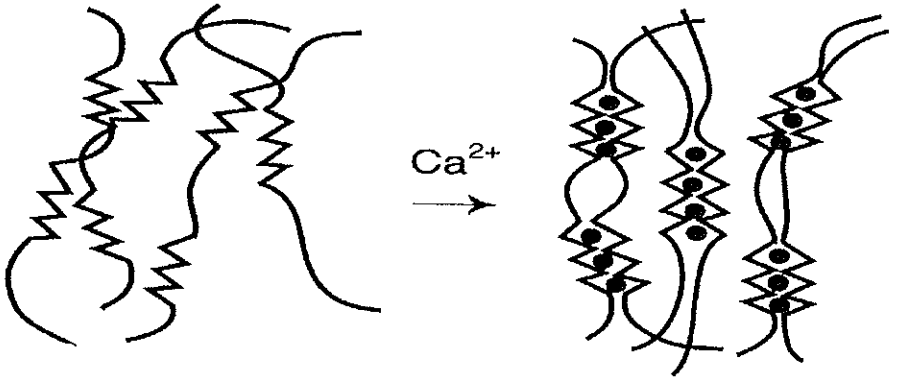

Figure 2. Crosslinking of alginate G-blocks with calcium.

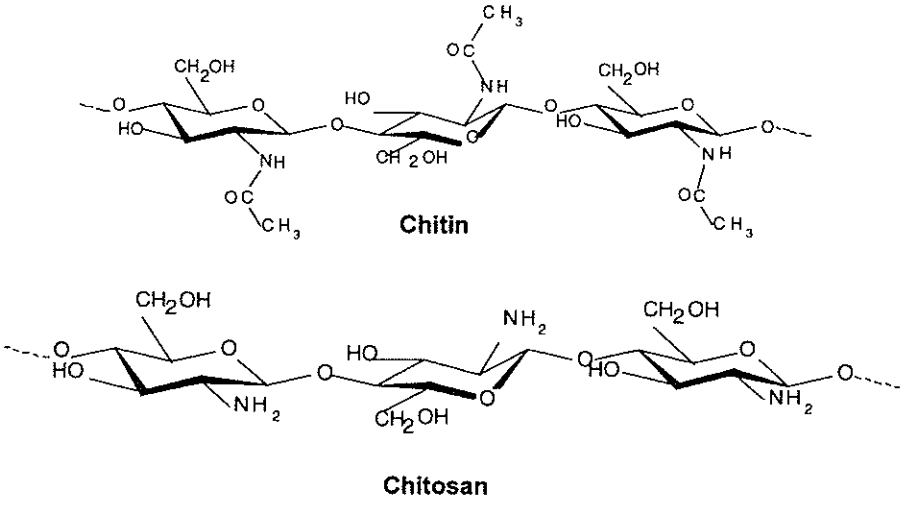

Figure 3. Chemical structure of chitin and chitosan.

in a β(1→4) manner (*Figure 3*). The glucosamine and *N*-acetyl glucosamine can theoretically be arranged by a similar sequential structure as M and G in alginate. Most often, commercial products will have a random distribution of the remaining *N*-acetyl glucosamine units after deacetylation. The ratio between glucosamine and *N*-acetyl glucosamine is referred to as the degree of deacetylation. In solution, chitosan salts will carry a positive charge through protonization of the free amino group on glucosamine. Reactivity with negatively charged surfaces is a direct function of the positive charge density of chitosan. The cationic nature of chitosan gives this polymer a mucoadhesive property (Skaugrud, 1995; Allan *et al.*, 1984; Li *et al.*, 1992).

Production

Commercial grades of alginate and chitosan have traditionally been processed from marine sources. Alginate constitutes the structural material of brown seaweed and kelp and is extracted from various species of these plants through a process whereby calcium alginate is converted into the soluble sodium alginate. Filtration and purification steps are performed followed by precipitation as calcium alginate and/or alginic acid before the final alginate salt is made. For chitosan manufacture, protein and calcium salts are removed from the exoskeleton of crustaceans by treatment with alkali and acids. The chitin is deacetylated by use of concentrated NaOH and elevated temperature (Skaugrud and Sargent, 1990; No and Meyers, 1995).

Technical grades of alginate and chitosan are further purifed and tailor-made for use in pharmaceutical and biomedical applications by using various chemical treatment and filtration steps. Microfiltration is used to remove insoluble compounds while ultrafiltration removes low molecular weight compounds. For pharmaceutical use in particular, the endotoxin content is reduced to a level acceptable for use of these polymers in humans. Of additional importance is the specification of the range of molecular weight distribution and the monomer composition of these polymers. To meet regulatory requirements, the materials are manufactured in compliance with

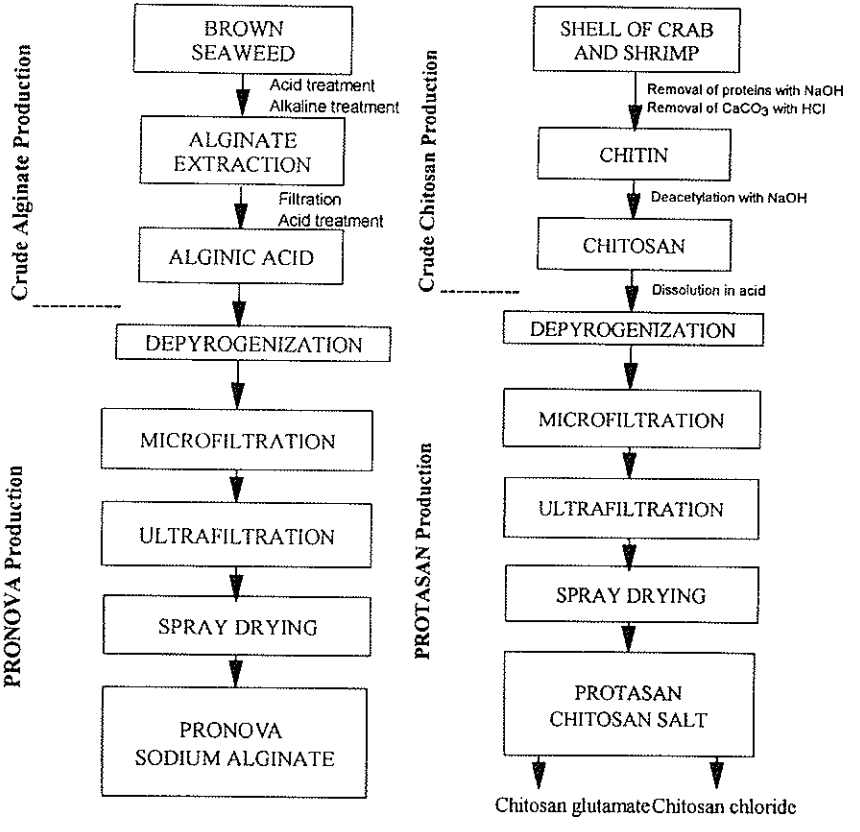

Figure 4. Production scheme for PRONOVA™ sodium alginate and PROTASAN™ chitosan salts.

'Good Manufacturing Practice' (GMP) guidelines. Basic toxicological data and polymer properties are documented in 'Drug Master Files' (DMF).

While small quantities of polymers can be produced relatively easily at laboratory scale, large scale production must meet the supply requirements for commercial applications. The scale-up process is not always straight forward and the documentation required to satisfy the regulatory authorities can be considerable. Pronova Biomedical's experience with large scale production of alginate and chitosan together with experience in characterizing key parameters has made these biopolymers commercially available in standard and ultrapure grades. Currently sodium alginate is being produced under the PRONOVA™ trademark and the chloride and glutamate salts of chitosan are being produced under the PROTASAN™ trademark (*Figure 4*).

Characterization

COMPOSITION AND SEQUENTIAL STRUCTURE

The composition and sequential structure of alginate and chitosan can be determined by high resolution ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy (NMR). For alginate, techniques have been developed to determine the monad frequencies as well as diads and triads. Based on such measurements, parameters such as M/G ratio, G-content with consecutive G>1, and average length of blocks of consecutive G units can be calculated (*Figure 5*) (Grasdalen, 1983). For chitosan, the degree of deacetylation can be detected by ^{1}H- and ^{13}C-NMR (Vårum *et al.*, 1991a,b).

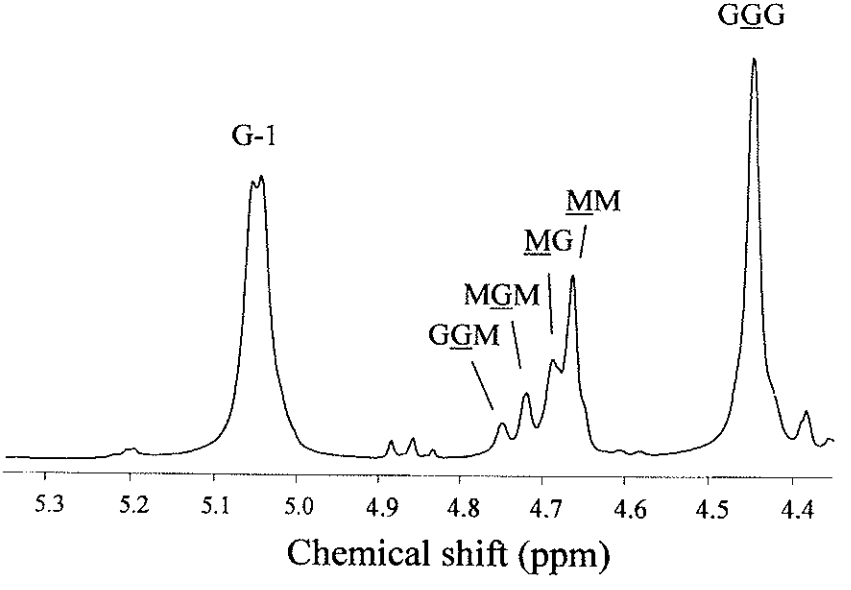

Figure 5. Typical ^{1}H-NMR spectrogram of PRONOVA™ UP LVG alginate.

MOLECULAR WEIGHT AND POLYDISPERSITY

Commercial alginates, like polysaccharides in general, are polydisperse with respect to molecular weight (M). Therefore, the given M of an alginate always represents an average of all of the molecules in the population. The most common ways to express molecular weights are as the number average ($\overline{M}_n$) and the weight average ($\overline{M}_w$). The two averages are defined by the following equations:

$$\overline{M}_n = \frac{\sum_i N_i M_i}{\sum_i N_i} \qquad \text{and} \qquad \overline{M}_w = \frac{\sum_i w_i M_i}{\sum_i w_i} = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}$$

where N_i = number of molecules having a specific molecular weight M_i
w_i = weight of molecules having a specific molecular weight M_i

In a polydisperse molecule population the relation $\overline{M}_w > \overline{M}_n$ is always valid. The coefficient $\overline{M}_w/\overline{M}_n$ is referred to as the polydispersity index, and will typically be in the range 1.5–3.0 for commercial alginates. More methods exist for determination of molecular weights. The most common ones in use are calculations based on intrinsic viscosity and light scattering measurements.

SOLUBILITY

Sodium alginate is soluble in water, but will precipitate as alginic acid at low pH. The pK_a values of guluronic and mannuronic acid are 3.6 and 3.3, respectively. The solubility of chitosan depends, to a certain extent, on the molecular weight and degree of deacetylation (*Figure 6*). Dissolution of chitosan, however, requires an acidic

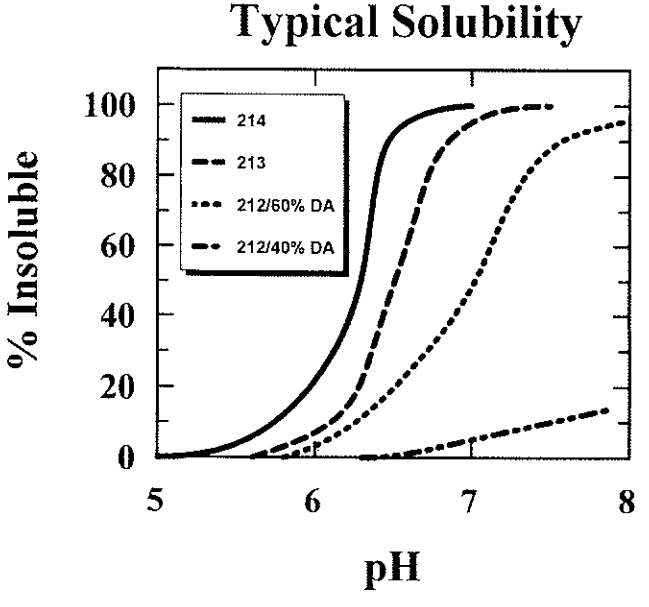

Figure 6. Solubility of PROTASAN™ chitosan. The degree of deacetylation increases from 40% and 60% for type 212, through to approximately 85% for 213 to 95% for type 214.

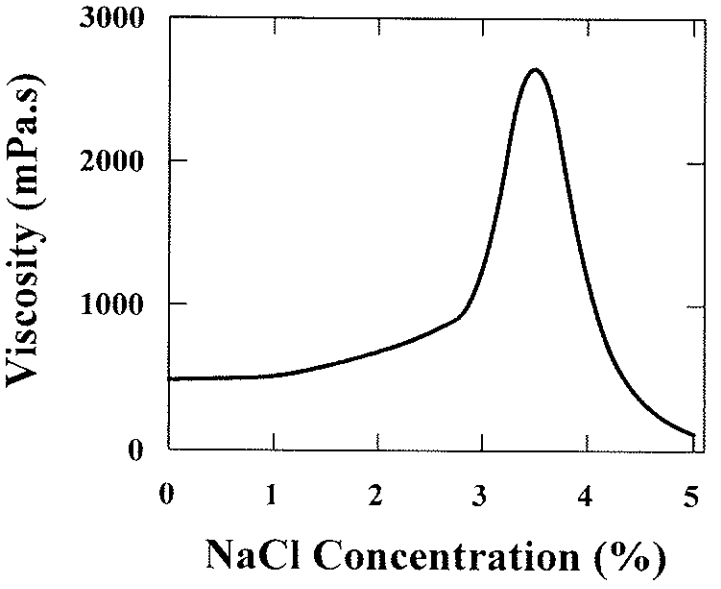

Figure 7. Alginate viscosity as a function of ionic strength .

environment. PROTASAN™ grades contain a stoichiometric amount of an appropriate acid integrated with chitosan as a counterion and will easily dissolve in water. The apparent pK_a value of the amino group of the glucosamine moiety is 6.5. In aqueous media at acidic pH, the chitosan molecule will be highly positively charged.

VISCOSITY

Viscosity is a function of the molecular weight of the biopolymer and its conformation in solution. Under different conditions the flow characteristic (rheology) of the alginate or chitosan solution will vary as, for example, on varying the ionic strength of the polymer solution (*Figure 7*). *Figure 8* illustrates the dependency of viscosity following increases in concentration for two chitosans.

POLYELECTROLYTIC PROPERTIES OF PROTASAN™

The apparent pK_a-value of the amino group of the glucosamine moiety is 6.5. In aqueous media at acidic pH, the chitosan molecule will be highly positively charged. The repelling effect of each positively charged deacetylated unit on neighboring glucosamine units will result in an extended conformation of the polymer in solution. The addition of salt will reduce this effect, resulting in a more random coil conformation of the molecule. At higher ionic strength a salting-out effect will occur, precipitating the chitosan from the solution. This is shown as a reduction in solution viscosity in *Figure 9*.

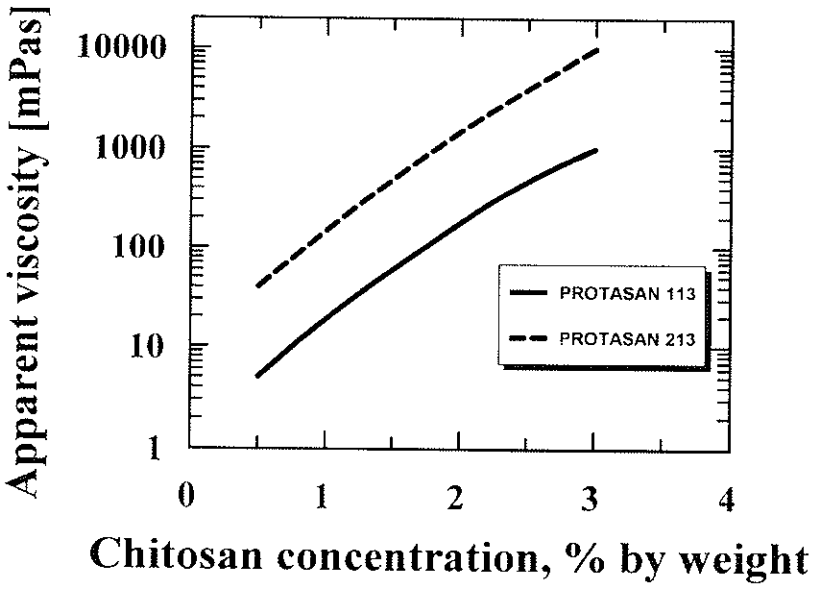

Figure 8. Chitosan viscosity as a function of concentration.

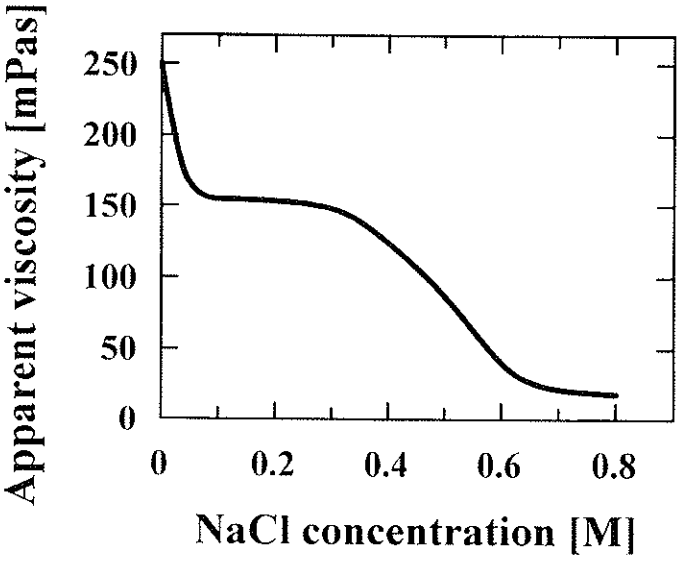

Figure 9. Viscosity of chitosan as a function of ionic strength.

Applications

A general overview of the characteristics of alginate and chitosan which can be used in biomedical and pharmaceutical applications is shown in *Table 2* (Skaugrud, 1995; Allan *et al.*, 1984; Li *et al.*, 1992).

Table 2. Characteristics of alginate and chitosan useful for medical and pharmaceutical applications

Feature	Benefits	Alginate	Chitosan
Solubility	Control of dissolution in the gastrointestinal tract	+++	+++
Gel	Immobilization agent for drug delivery systems	+++	+
Electrolyte	Bioadhesive agent for drug delivery systems	–	+++
Swelling	Tablet disintegration. Matrix for drug delivery systems	+++	++
Film/fibre	Wound treatment. Encapsulation agent for drug delivery systems	+++	+++
Chelation	Cation binding	++	+
Viscosity	Suspensions	++	–
Biological	Immune Response	+++	+
Properties	Anti-microbial effect	–	+++

CELL IMMOBILIZATION WITH ALGINATE

The technique to immobilize cells, particularly pancreatic islet cells, in calcium alginate matrices was developed by Lim at the end of the 1970's (Lim and Sun, 1980). By coating the bead with polycations like poly-L-lysine, poly-L-ornithine, or chitosan, the strength of the surface coating as well as the capsule porosity can be controlled (*Figure 10*) (Skjåk-Bræk and Espevik, 1996).

NASAL DELIVERY OF CHALLENGING DRUGS

Polar drugs are not well absorbed across the nasal mucosa. These drugs include low molecular weight compounds as well as biotechnology products such as polypeptides

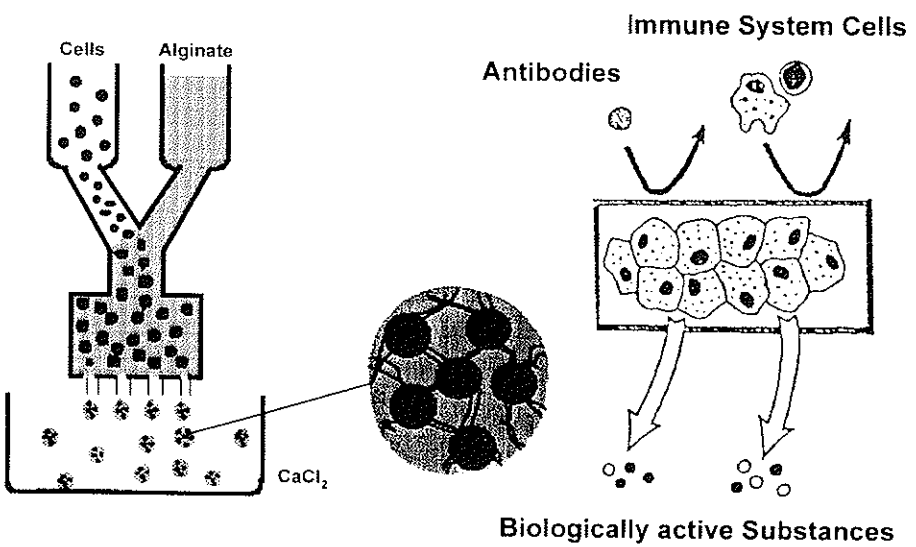

Figure 10. Encapsulation of cells with alginate.

and proteins. Solutions and powder formulations based on chitosan salts of a molecular weight of greater than 100 kD and with a defined degree of deacetylation have been found to enhance the delivery of nasally administered polypeptides such as insulin, calcitonin, LHRH analogues, growth hormone as well as non-peptide polar compounds (Illum *et al.*, 1994; Aspden *et al.*, 1996, 1997).

Regulatory considerations

The underlying documentation of the GRAS ('Generally Recognized As Safe') status of alginate goes back prior to 1972 (21CFR 184.1724). In the US *Pharmacopoeia* alginate is still described as a poly mannuronate, even though the existence of guluronate and the block forming structure of alginate is now well-known. For chitosan no US monograph exists yet, however, a European monograph is under evaluation.

For pharmaceutical and biomedical applications of alginate and chitosan to be successful, regulatory issues will have to be addressed. There are three main areas in this respect which must be dealt with:

- characterization and functionality
- product reproducibility
- toxicology and long term safety

Safety is one of the biggest issues for the commercialization of alginate and chitosan for human medical applications. The development of new biomedical products involves several different aspects such as the property of the biopolymer, production quality and quantity, and clinical effects. The applications of alginate and chitosan to human studies will also involve regulatory approval by national and international authorities, such as the FDA in the United States. Regulatory issues that are important in the commercialization of alginate and chitosan for biomedical uses are (*Table 3*): Characterization and functionality, specifications of the product, and analysis using validated methods. Stability of the compound is of prime importance. Reproducibility of the manufacture of the compound is very important, and this is ensured under a series of GMP guidelines. Documentation of not only the manufacture but also specifications and safety of the product is described in a 'Drug Master File' (DMF), both in the US and in Europe. Drug Master Files form the backbone of documentation that is required for registering a product, either as an active drug or as an excipient. Finally, toxicology and safety covering basic studies and application-specific studies must be documented.

Table 3. Regulatory Issues

Characterization and Functionality:	Product specification Validation of analytical methods Stability Studies
Manufacture:	Reproducibility (GMP) Documentation (DMF)
Toxicology and safety:	Basic studies Application-specific studies

As examples we now consider ultrapure alginate (PRONOVA™) and ultrapure chitosan (PROTASAN™). The reader is refererred to the two articles by Dornish *et al.* (1997a, b).

SAFETY AND TOXICOLOGY OF PRONOVA™

Pharmacokinetics of sodium alginate in mice. In spite of various uses of alginate in the biomedical and pharmaceutical field for more than 50 years, no extensive toxicology documentation is yet available in the public domain. Pharmacokinetic studies of PRONOVA™ alginate have been carried out and the *Figures 11–14* and *Table 4* show the results following various bolus injections of a radiolabeled alginate (Hagen *et al.*, 1995 and unpublished results).

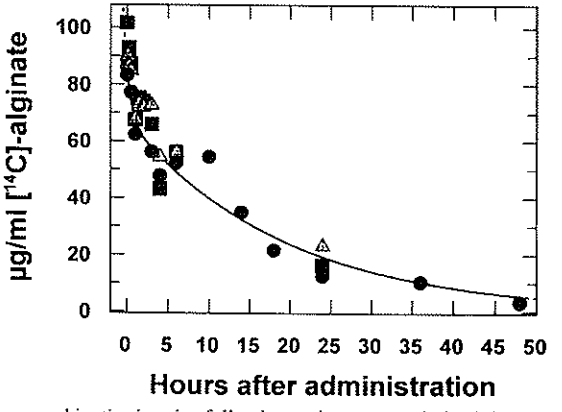

Figure 11. The pharmacokinetics in mice following an intravenous bolus injection of 100 mg alginate. The profile appears to be biphasic, indicating a 2-compartment model. The initial half-life ($t_{½}$) is approximately 4 hours, while the secondary $t_{½}$ appears to be about 22 hours.

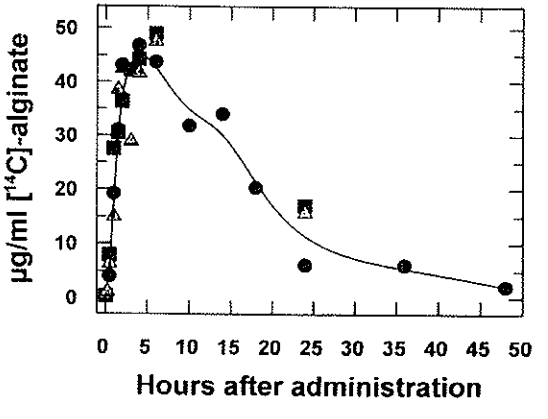

Figure 12. The pharmacokinetics in mice following an intraperitoneal bolus injection of 100 mg alginate. The absorption reaches a maximum after 5–6 hours. Thereafter, the serum concentration declines with an apparent half-life of about 12.5 hours. The elimination following intraperitoneal administration may also occur in a biphasic fashion, similar to intravenous administration.

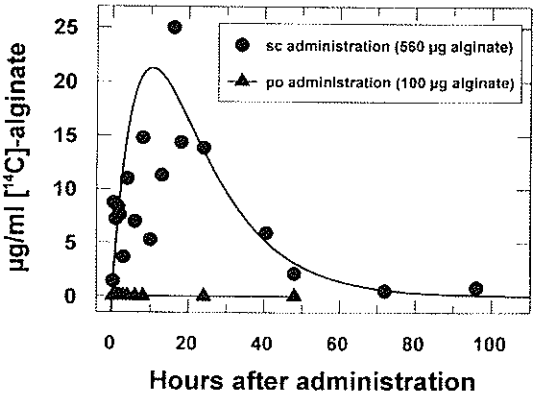

Figure 13. The pharmacokinetics in mice following oral and subcutaneous administration of alginate.

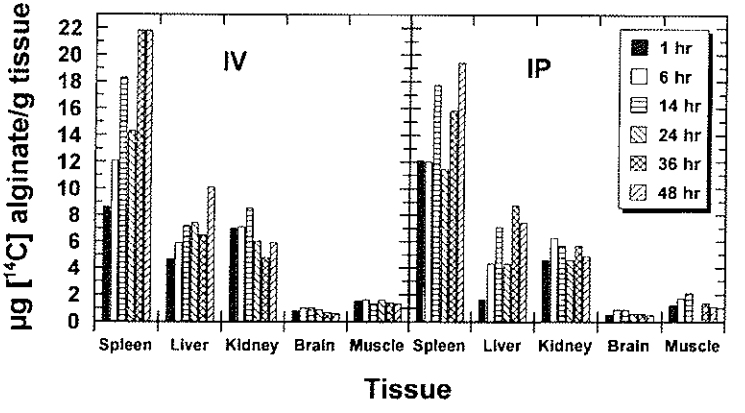

Figure 14. Tissue distribution studies of intravenous and intraperitoneal administrated alginate. Only very small amounts of alginate were found in brain and muscle tissue, while the liver and the kidney contained moderate amounts. The spleen, not surprisingly, appeared to concentrate the alginate over a 48 hours period.

Table 4. Pharmacokinetic parameters following various administrations of [^{14}C] alginate.

Pharmokinetic Parameter	Intravenous, (IP)	Intraperitoneal, (IV)	Subcutaneous, (SC)	Oral (per oral), (PO)
Area under the Curve (µg h ml^{-1})	1253	550	69	0
Volume of Distribution (l)	1.09	1.0	–	–
Clearance (l/h)	0.08	0.09	–	–
Bioavailability (%)	100	44	5	0

Calculations were performed by using the MK Model version 5.0 program (Biosoft, Cambridge, UK).

Table 5. Specifications of PROTASAN™ used in the studies

Specification	UP G 110	UP G 210	UP Cl 110
Batch number	705-583-04	610-583-07B	310-490-01
Apparent viscosity	2.8 mPa.s	91 mPa.s	12 mPa.s
Intrinsic viscosity	0.7 dl/g	6.9 dl/g	ND
Degree of deacetylation	83%	83%	87%
Acid content	41% glutamic	40% glutamic	19% chloride
Loss on drying	4.9%	4.5%	4.6%
Insolubles	<0.1%	<0.1%	<0.1%
Heavy metals	<27 ppm	<26 ppm	<30 ppm
Endotoxin content	195 EU/g	625 EU/g	250 EU/g
Microbiology	< 1 cfu/g	<1 cfu/g	<1 cfu/g

UP: Ultrapure grade. G: glutamate salt. Cl: Chloride salt.

SAFETY AND TOXICOLOGY OF PROTOSAN™

We review here some of the results reported in Dornish *et al.* (1997a,b). Specifications for the chitosan glutamate or chitosan chloride used are shown in *Table 5*. The 'UP' designation indicates an ultrapure quality. The G stands for glutamate while Cl indicates a chloride salt. The 110 is a low viscosity chitosan while 210 indicates a medium viscosity. The chitosan salts were made up in physiological saline solution (0.9% NaCl), or in cell culture medium. Safety and toxicological studies were performed in accordance with applicable guidelines.

Oral toxicity studies were performed in rats which received daily administration of chitosan glutamate solutions up to 600 mg/kg/day for 13 weeks. The results summarized in *Table 6* indicate no toxicological effects were seen in any group. No real change in body weight was found between treated and control animals. Blood chemistry was also comparative between the four groups.

Table 6. Oral toxicity of PROTASAN™ UP G 210. Daily oral administration (gavage) to rats for 13 weeks

Group Number	Group Designation	Dose level (mg/kg/day)	Dose volume (ml/kg/day)	Dose conc. (mg/ml)	Results
1	Control	0	10	0	No toxicological effects seen in any group
2	Low dose	100	10	10	
3	Intermediate	300	10	30	
4	High dose	600	10	60	

Gavage: Gastric incubation

Table 7. Intravenous toxicity of PROTASAN™ UP G 210 & UP G 110. One single intravenous administration to rats

Group Number	Group Designation	Dose level (mg/kg)	Dose conc. (mg/ml)	Results
1	Low viscosity (UP G 110)	25	5	No toxicological effects seen in any group
2	Medium viscosity (UP G 210)	25	5	

Table 8. Intraperitoneal toxicity of PROTASAN™ UP G 210 & UP Cl 110. One single intraperitoneal administration to rats

Group Number	Group Designation	Dose level (mg/kg)	Dose conc. (mg/ml)	Results
1	Low dose	100	5	No toxicological
2	Intermediate dose	250	15	effects seen in
3	High dose	500	25	any group

An intravenous (IV) study was performed as a limit study investigating the effect of 25 mg/ml. Higher doses were toxic to animals. The cause of toxicity is most likely the aggregation of red blood cells by binding to chitosan resulting in blockage of capillaries. As seen in *Table 7*, no toxicological effects were seen at this dose level.

IP (intraperitoneal) studies showed that a bolus injection of up to 500 mg/kg chitosan glutamate or chitosan chloride does not result in any toxicological effects evaluated seven days after injection (*Table 8*). There was no change in body weight relative to control animals and no changes in physical or macroscopic appearance of the organs.

Two hypersensitization studies using PROTASAN™ UP G 210 were performed. A Magnusson and Kligman test (see Method B6 in Annex V of EEC Commission Directive 92/69 (1922) and as described in OECD Guidelines for Testing of Chemicals (1992) No. 406.) of the sensitizing potential in guinea pigs is a study in which the compound to be tested is intradermally injected to small areas on the back of the guinea pigs either with or without Freund's adjuvant. A topical application of a highly concentrated chitosan glutamate solution was given as the challenge at a separate site to the induction. Dichloronitrobenzene was used as the positive control. The results in *Table 9* show no hypersensitive reaction on application of the chitosan glutamate.

Anaphylactic shock is an antibody-medicated reaction leading to mortality. Guinea pigs were injected subcutaneously with chitosan glutamate as an induction. The challenge was given as an intravenous injection 21 days later. The positive control was ovalbumin which, on challenge, caused death in 8 of 10 animals. There was no mortality in the chitosan-treated groups and body weights were normal. There was some cyanosis directly after injection of chitosan salts, but this condition cleared by four hours after injection.

Table 9. Hypersensitization studies with PROTASAN™ UP G 210

Study	Induction	Challenge	Results
Magnusson and Kligman	1 mg/ml UP G 210 Intradermal 2 × 0.1ml –Freund's –0.9%NaCl 50:50 NaCl/Freund's	60 mg/ml UP G 210 Topical 0.5ml 48 hr.	No mortality. Body wt. not affected Irritation during induction No delayed hypersensitization (Positive control: 10/10 with DCNB)
Anaphylactic Shock	10 mg/kg UP G 210 Subcutaneous Days 1 and 8	20 mg/kg IV Day 21	No mortality in treated groups. Body wt. not affected. Cyanosis in 5/10, normal after 4hr. (Positive control death in 8/10 using ovalbumin)

Table 10. Nasal irritancy of PROTASAN™ UP G 210. Three daily intranasal administrations to the rat for one week

Group Number	Group Designation	Dose level (mg/rat/day)	Dose volume (µl/rat/day)	Dose conc. (mg/ml)	Results
1	Control	0	3 × 100µl	0	Congestion in all groups.
2	Low dose	1.5	3 × 100µl	5	Increase mucus, not dose related.
3	High dose	3	3 × 100µl	10	No toxic effect on ciliated cells

Table 11. Other safety studies with PROTASAN™

Study	Dose	Schedule	Results
Ames test	Up to 5000 mg/plate UP G 210	±S9 activation TA98, TA100, TA1535, TA1537 & TA102 strains of *Salmonella*	No significant increases in the number of revertants observed. 5000µg/plate toxic to TA98 & TA1537
In vitro cell survival (Colony forming assay)	Up to 1 mg/ml	24 hr exposure 3T3 and V79 cells	Little effect on cell survival.

One of the prime applications of chitosan salts is in the field of drug delivery. The bioadhesive properties of chitosan can be used in nasal applications (Illum *et al.*, 1994). Safety of nasally administered chitosan glutamate was determined by treating rats with 0.5 or 1% chitosan glutamate solutions three times a day for seven consecutive days (*Table 10*). Sections of the nasal cavities were stained with hematoxylin and eosin. Histological examination of the nasal mucosa indicated some increase in the thickness of the mucus layer in chitosan-treated animals (*Figure 15*). Goblet cells increased the production of mucus, not unlike the reaction occurring in other nasal irritation reactions. This reaction could also be due to the treatment technique itself. One of the most important findings, however, was that ciliated cells appeared normal, there was no de-ciliation of these cells.

The Ames test is an evaluation of the mutagenic potential of a compound. In this study chitosan glutamate was incorporated into the dishes used for culturing various strains of *Salmonella*. The study also evaluated the potential of an S9 mitochondrial extract to metabolize the chitosan into a mutagenic compound. The results in *Table 11* show that chitosan glutamate induced no mutagenic effect up to a concentration of 5000 µg incorporated in each dish. The lack of mutagenicity was irrespective of the presence of an S9 mixture.

In vitro studies have shown that chitosan salts induce very little toxicity to cultured cells. In *Table 11*, the effect of a 24 hour incubation with chitosan salts on the mouse embryonal cell line 3T3 and the Chinese hamster lung cell line V79 were tested. Neither of these cell lines are considered cancerous or malignant.

Conclusions

The evaluation of ultrapure chitosan salts has shown these compounds to be well

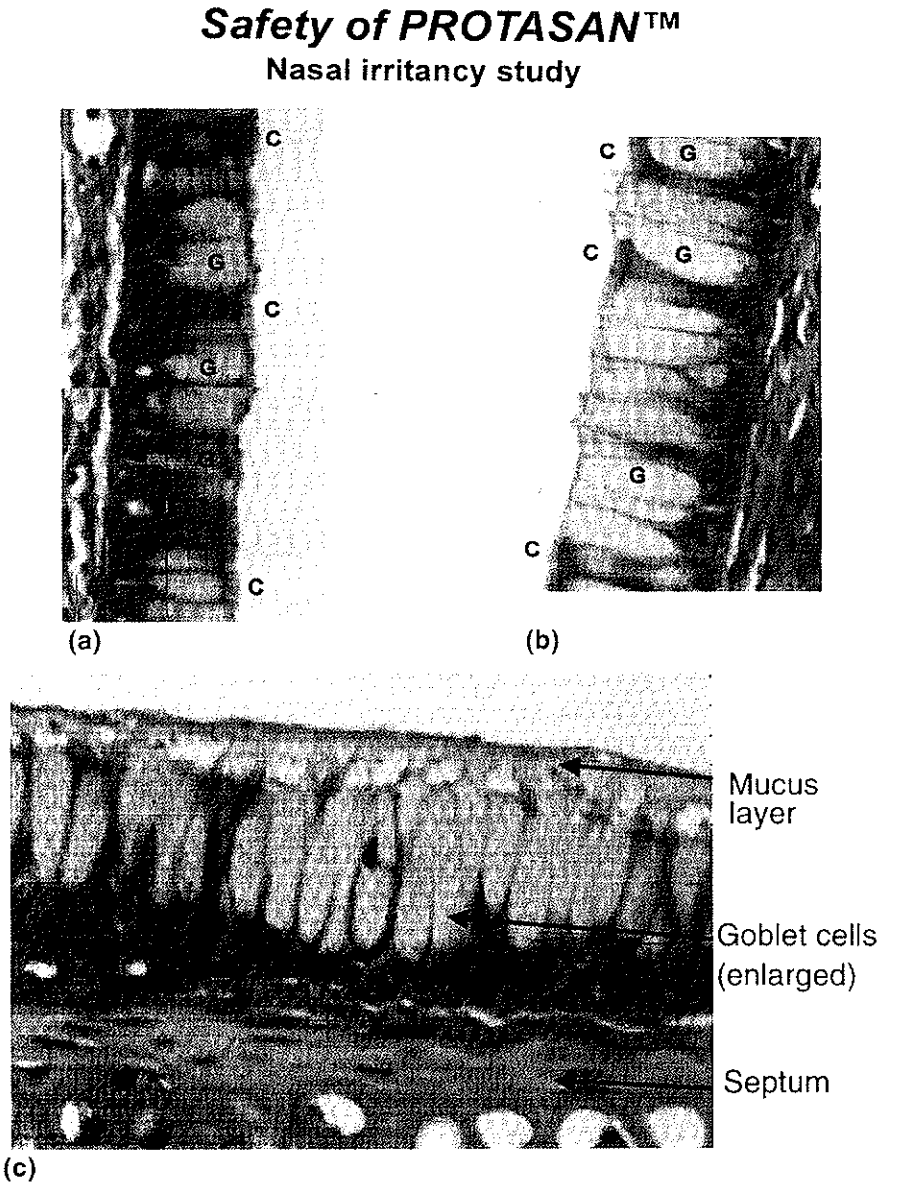

Figure 15. Micrographs showing the effect of chitosan glutamate on rat nasal epithelia. (a) control, (b) and (c) after 'high dose' of 1 mg/rat in right nostril, 3 times a day for a 7 day period. G: Goblet cells, C: Ciliated cells.

tolerated in safety and toxicology studies. These data are important for a further evaluation of the usefulness and applicability of chitosan in biomedical and pharmaceutical applications. In addition, safety studies of the types presented here are necessary for regulatory approval of the use of chitosan salts in humans.

Oral applications of chitosan have been previously reported by others and the safety of orally administered chitosan has been reviewed by Weiner (1992) and Hirano *et al.*

(1990). In work involving PROTASAN™ we have also shown that long-term (13 week) administration of chitosan glutamate had no deleterious effect in rats. Further toxicological evaluation in animals and man have shown that chitosan glutamate has no deleterious effects on the nasal mucosa nor on mucociliary transport (Aspden, 1997a,b). These findings are of importance for the development of chitosan in nasal drug delivery systems.

When chitosan was introduced as an industrial product in the early 1970's, the field of wound healing played a significant role in the initial commercial development of the biopolymer. For new pharmaceutical and biomedical applications of chitosan to be successful, studies like the ones presented here will be of importance. Moreover, regulatory issues, such as production process validation, quality control and product stability, will have to be addressed. In addition to characterization and functionality, the commercial manufacture of chitosan products for pharmaceutical use must also include product reproducibility and safety. PROTASAN™ salts are manufactured in accordance with GMP guidelines in order to ensure quality control and documentation for commercial grades of water-soluble chitosan salts.

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