BIODEGRADABLE BLENDS BASED ON CHITOSAN AND POLY(VINYL ALCOHOL) (PVA) WITH SORBITOL AND SUCROSE

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Abstract

The physical properties of chitosan/PVA/plasticizer blends, prepared by solvent casting technique, were investigated. It was shown that the observed thermal properties (melting point, heat of fusion) for PVA underwent a substantial decrease proportional to the plasticizers' content. The percentage elongation and CO_2 and water vapour permeability of chitosan/PVA blends were found to increase (up to 50% of the original values) with an increase in plasticizer content. On the contrary, a substantial decrease was observed in tensile strength and modulus. The semiempirical models, applied for calculating the mechanical properties and the CO_2 permeability, were rather in satisfactory agreement with the experimental values, apart from a certain variation which should be probably attributed to differences in molecular weight, percentage crystallinity and percentage of hydrolyzed or deacetylated material (PVA and chitosan, respectively).

Keywords: chitosan, Poly(vinyl alcohol) PVA, blend, plasticizer, CO_2 permeability, mechanical properties

Introduction

Most commercially available PVA is produced from poly(vinyl acetate) (PVAc). PVA has found many applications in

pharmaceuticals, cosmetics and in the paper and in the food industry either alone or in blends. Chitosan is the deacetylated product of chitin. Chitin is the second, next to cellulose, most abundant polysaccharide in nature. The production of chitin is possible primarily as a secondary activity related to the marine food industry. Chitosan has been used in very wide range of applications such as prevention of water pollution by chelating heavy metals or radioactive isotopes, membrane separation, in medicine and biotechnology and in the food areas either as food packaging material because of its antimicrobial action or as dietary fiber and a potential medicine against hypertension thanks to its scavenging action for chloride ions.
Numerous publications reported on improving the selectivity of chitosan membranes by manipulating the chain flexibility of chitosan. Blending of chitosan with hydrophilic polymers as well as chemical crosslinking of chitosan or blends appears to be a promising approach [1-3].
Previous studies on chitosan/PVA blends were focused either on studying the plasticizing effect of water on these blends or on the crosslinking and swelling of these blends [4]. The aim of this investigation is to study the synergistic plasticizing action of several low molecular weight compounds such as sorbitol and sucrose in conjunction with water on the structure and on the mechanical properties and CO_2 permeability of plasticized chitosan/PVA blends.

Materials and methods

Materials

Chitosan was purchased from Seigakaku Corporation (Tokyo, Japan). PVA, sorbitol, and sucrose were purchased from Wako Chemicals Industries Ltd (Japan).

Preparation of films

Two separate solutions of PVA (2%w/w) in water and chitosan (2% w/w) in 2% acetic acid were prepared. The PVA solutions were then added, under vigorous stirring and heating, to the chitosan solution and then the plasticizer was added and mixed to the solution for 10-15 mins till dispersed.
Plexiglass plates with an enclosed framing area (30cm×30cm) were levelled and cleaned. The solution was cast in a circular area in the central part of the plates and then spread uniformly. Films were dried for approximately 48 hrs at ambient conditions and

then placed in chambers at various relative humidities (21%, 53% and 74%).

CO_2 Sorption Apparatus

Both equilibrium sorption and kinetic sorption experiments were carried out by using a gravimetric method. The measurements were taken with a Sartorius (Germany) electrobalance (1μg sensitivity) properly enclosed in a glass chamber with connections through a vacuum line to a CO_2 cylinder, a mercury pressure gauge and a Pirani/Penning vacuum gauge. Constant pressure throughout the sorption experiments on polymers was maintained by adding a 12L glass reservoir to the vacuum system. Polymer samples of known weight (700-950 mg) were suspended in a light aluminum pan by thin glass fibers close to the lower end of a 20cm hung down tube. In order to prevent any buildup of static charges in the tube a radioactive material (500μCPO210) was used. The entire system, comprising the electrobalance and the vacuum system, was enclosed in a thermostated chamber (25±0.50℃). At the early stages of the experiment the system was fully evacuated by using the liquid nitrogen trap and continuously pumping in order to degas the sample and remove any volatile material. The evacuation continued till constant weight and then, the system, apart from the electrobalance, was charged with CO_2 (completely free from water) to various pressures. The sorption process started when CO_2 entered the electrobalance chamber. The sample mass was continuously recorded with an IBM compatible PC. The initially recorded linear increase of sample mass versus square time was finally leveled off to a plateau value which corresponded to the equilibrium sorption levels.

Gas Permeability (GP) measurements

The measurements of permeability of blends to N_2 & CO_2 was carried out as described in previous publications.

Results and discussion

Mechanical Properties

Tensile strength, tensile modulus and percentage elongation for chitosan/PVA/H_2O with or without polyols were measured (Table 1). Since it is a two component blend (chitosan/PVA) in order to calculate the tensile modulus it could be roughly as a composite material.

$$E_{blend} = V_{PVA} E_{PVA} + (1 - V_{PVA}) E_{chitosan} \quad (1)$$

$E_{blend} = 1 / ((V_{PVA} / E_{PVA}) + (1 - V_{PVA}) / E_{chitosan}$ (2)

By using $E_{PVA} = 0.2$ GPa and $E_{chitosan} = 1.2$ GPa for non-plasticized homopolymers, we get 0.7 GPa and 0.34 GPa from equations (1) & (2), respectively. Property changes with composition are related to the degree of compatibility. In particular, the moduli of blends as a function of composition can be also expressed by the modified rule of mixtures:

$E = w_1 E_1 + w_2 E_2 + \beta_{1\,2} w_1 w_2$ (3)

where E is the modulus of the blend, E_1 and E_2 are those of components, w_1 *and* w_2 stand for the corresponding weight fractions. The $\beta_{1\,2}$ is an empirical parameter usually calculated from eq. (4)

$\beta_{1\,2} = 4E_{1\,2} - 2E_1 - 2E_2$ (4)

By using 0.78 Gpa and 0.1 Gpa for plasticized (10% sorbitol) chitosan and PVA, respectively, we get $\beta_{1\,2} = -0.38$. Comparison of calculated tensile moduli, with experimental values shows a reasonable agreement.

CO_2 Equilibrium Sorption

Although several studies of gas sorption in polymers at temperatures below Tg showed that in many cases sorption isotherms rather deviate from Henry's law and their behaviour conforms satisfactorily to the dual mode sorption model, our experiments showed that Henry's law is applicable, at least at low pressures, where only the initial linear segment of the total isotherm is examined.

Since the gas sorption (CO_2 in our case) occur only in the amorphous areas, it is essential to calculate the volume fraction of the amorphous phase (a) in the blends. The chitosan/PVA blend is a semicrystalline and very clearly defined melting peaks of PVA are detected at higher than 25% PVA content.

$a = 1 - r X_c / r_c$ (5)

where ρ is the density of the blend, ρ_c is the 100% crystalline taken as 1.34g/cm^3 and X_c was calculated from the determined value for 100% crystalline PVA 140.35 J/g.

In a mixed amorphous phase system, volume additivity is assumed and the PVA amorphous phase volume fraction can be calculated as follows:

$\phi_1 = V_{1\,a} / (V_{1\,a} + V_2)$ (6)

where $V_{1\,a}$ is the volume of the amorphous PVA and V_2 is the

volume of chitosan, both expressed per unit mass of blend. Although chitosan is a semicrystalline polymer according to the WAXDP, in terms of DSC/DTA chitosan is regarded as amorphous. Values of the specific volumes for amorphous PVA and chitosan were taken as 0.7874 c m^3/g and 0.689 c m^3/g (Table 2).

Table 1. Tensile strength (TS, MPa), Tensile modulus (TM, GPa) and percentage elongation (%E) of plasticized chitosan/PVA blends.

PVA	Chitosan	Sorbitol	Water	TS (MPa)	TM (MPa)	%E
45.0	45.0	5	5	58±5	305±20	26±4
42.5	42.5	10	5	47±3	270±18	37±5
40.0	40.0	15	5	35±5	214±15	45±6
37.5	37.5	20	5	19±2	135±17	61±7
32.5	32.5	30	5	10±1	60±8	75±9
PVA	Chitosan	Sucrose	Water			
45.0	45.0	5	5	64±7	346±30	25±2
42.5	42.5	10	5	55±6	295±188	30±2
40.0	40.0	15	5	40±5	250±24	33±3
37.5	37.5	20	5	28±2	173±13	37±4
32.5	32.5	30	5	17±3	97±7	40±3

Table 2. Thermal and volumetric properties of chitosan/PVA blends.

Wt% PVA	ΔH_m (J/g)	X_c	p(g/ c m^3)	α	ϕ_1
100	81.9	0.578	1.307	0.436	1
75	46.0	0.324	1.3096	0.683	0.662
50	28.0	0.198	1.3122	0.806	0.385
25	0	0	1.3147	1	0.257
0	0	0	1.3173	1	0

Table 3. Calculated sorption parameters for CO_2 in chitosan/PVA blends at 25°C.

Chitosan	PVA	K_D (c m^3(STP)/(m^3 atm)	K_D/α c m^3 (STP)/(cm^3 atm)	K_D/α 10^{-6}c m^3 (STP)/(cm^3 Pa)
0	100	0.0789	0.1810	1.78
25	75	0.18180	0.2660	2.61
50	50	0.2222	0.2757	2.70
75	25	0.3500	0.3500	3.43
100	0	0.2700	0.4250	4.17

The equilibrium sorption isotherms for CO_2 in PVA, chitosan and PVA/chitosan (50/50) are linear and are shown in Figure 6. The 50/50(PVA/chitosan) blend isotherms lie between the two polymer components. This linear behaviour can be expressed in terms of Henry's law as follows:

$$C = K_D p \qquad (7)$$

where C is the equilibrium concentration of the penetrant, p stands for the penetrant pressure at equilibrium and K_D is the Henry law constant. The Henry's law constants were obtained from the slope of straight lines (Table 3).

In the case of a miscible blend, by applying the ternary solution theory, the blend solubility coefficient can be analyzed in terms of its polymer components:

$$\ln K_D = \varphi_1 \ln K_{D1} + \varphi_2 \ln K_{D2} + (B V_3/RT)\ \varphi_1 \varphi_2 \qquad (8)$$

where R is the universal gas constant, B is the interaction energy density, V_3 stands for the molar volume of the penetrant molecules and the subscripts 1 and 2 indicate the two components.

If B = 0 the relation between In K_D and blend composition is linear. By taking $V_3 = 55$ c m^3/mol for CO_2, non linear regression analysis of the data gives B=-18.81 J/c m^3 which is in satisfactory agreement with previous publications.

Conclusions

The tensile strength decreased proportionally to the plasticizer content whereas the percentage elongation increased considerably, particularly in the case of sorbitol. The carbon dioxide sorption curves were shown to obey Fickian equation. The low levels of CO_2 should be probably attributed to the high level of hydrogen bonding in the plasticized or non-plasticized blends. From the semiempirical equations lower values were obtained, compared to the actual values, for CO_2 sorption and permeability, maybe because of strong intermolecular interactions.

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USE OF CHITIN AND CHITOSAN BEING ELECTROCHEMICALLY OBTAINED FROM SEA AND FRESHWATER CRUSTACEA AS SORBENTS OF HEAVY METALS IONS

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Abstract

The problem of purifying waste water from ions of heavy metals is extremely vital for industrial regions of Russia where metallurgic, galvanic, leather tanning and other factories that produce in large quantities effluents with high content of Copper, Chromium, Plumbum, Cadmium etc are concentrated.

Traditional sorbents of inorganic (zeolites) and organic (activated carbon, synthetic sorbents) nature used for the extraction of ions of heavy metals are somewhat lacking in sorption capacity and selectivity. Therefore, search for the sorbents of new type is in process. In this connection, use of natural polysaccharide - chitin being contained in sea and fresh water crustacea (crab, krill, shrimp gammarus etc) as well as its deacetylated derivative chitosan merits shrewd attention.

Use of wastes of one industry for the disposal of the wastes of other industries is very attractive and economically reasonable.

The sorption capacity of chitin and chitosan for the ions of heavy metals is studied perfectly well (Kurita K. And others, 1979; Muzzarelli R.A.A., 1969, 1978; Domar A., 1987; Hirano S. And others, 1982). Detailed surveys have demonstrated their high activity relative to ions of transient and post- transient elements , whereas alkali and alkaline-earth metals practically never bind by these polysaccharides .

Further research into the sorption capacity of chitin and chitosan depending upon the source of raw material being used for their production, as well as the manufacturing process itself , conditions of sorption etc., represent a certain interest and the present study is devoted to these problems.

Keywords : Chitin, chitosan, gammarus, shrimp, electrochemical method, sorption capacity, heavy metals.

Materials and methods

Scientists from the Research and Design Institute for the Development and Operation of Fishing Fleet "GIPRORYBFLOT" in collaboration with their colleagues from the Institute of High-molecular Compounds of the Russian Academy of Sciences, St.Petersburg, have carried out studies of the sorption properties of chitin and chitosan being obtained from fresh water shrimp *gammarus* and sea water shrimp in accordance with new technology based upon electrochemical treatment of crustaceous raw material [Maslova. G. And others, 1996].

The sorption capacity of chitin and chitosan in relation to heavy metals has been illustrated by the example of extraction of $Cu^{(2+)}$ ions from cutting emulsion and of $Cr^{(3+)}$ ions from chromium chloride solution. The efficiency of chitin and chitosan sorption capacity has been defined in accordance with pH-medium, concentration of heavy metal ions in stock solutions, rate of chitin deacetylation (RD), kind of raw material which was used to get these polymers.

The sample of cutting emulsion under investigation had pH=8.0 and content of $Cu^{(2+)}$ ions being 98.5 mg/100 ml.

Sorption of $Cu^{(2+)}$ ions was tested on dissolved solutions containing 0.958 mg of $Cu^{(2+)}$ (1 ml of cutting emulsion was added into 20 ml of distilled water) and on concentrated solution - 20 ml of pure cutting emulsion containing 19.7 mg of $Cu^{(2+)}$ ions .

Content of copper and chromium was defined by means of complexation titration. The number of sorbed ions was calculated using the difference between their concentration in initial sample (solution, emulsion) and number of ions left in the filtrate after sorption.

Results and discussion

Fig. 1 shows the isothermal curves of $Cu^{(2+)}$ ions sorption from cutting emulsion with the use of chitin (Curve 1) and chitosan (Curve 2) being obtained from *Gammurus* . On the ordinate the values of copper absorbed by the sorbent are plotted in percents from initial number.

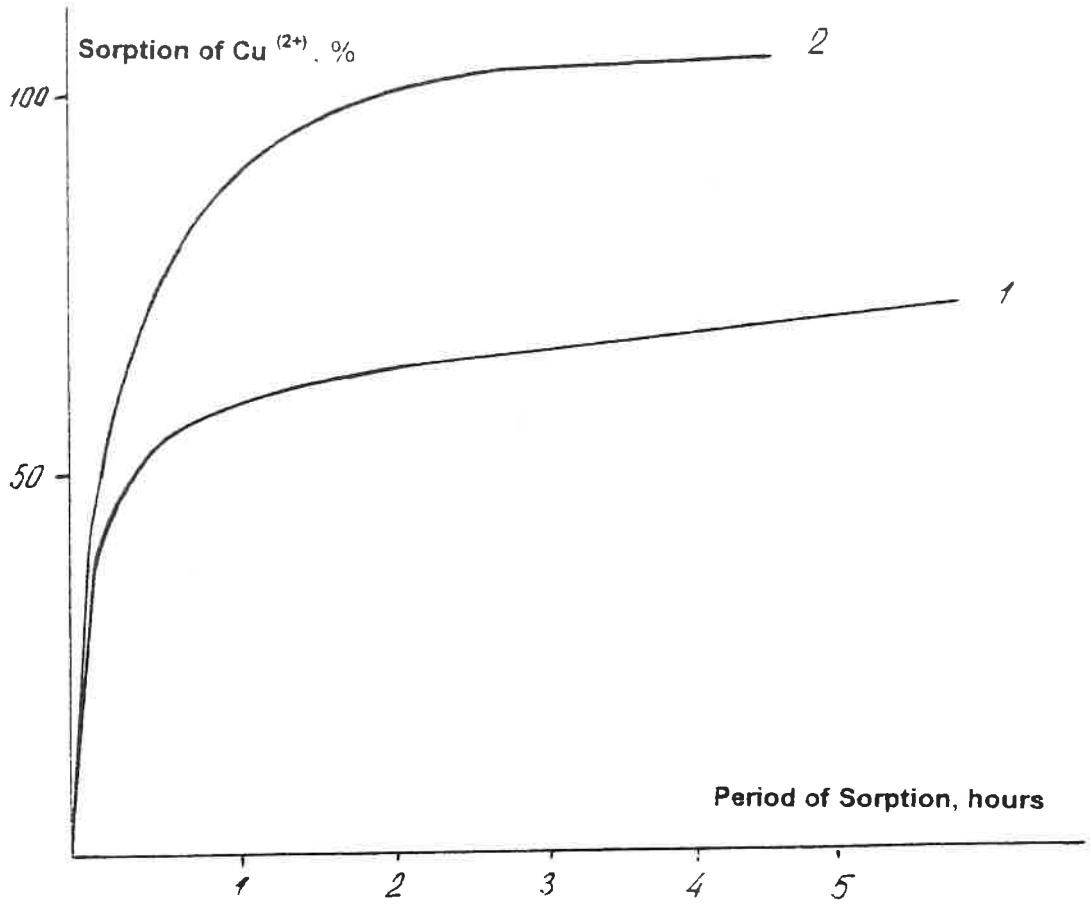

Fig. 1 Isothermal curves of $Cu^{(2+)}$ ions sorption from cutting emulsion with the use of chitin (1) and chitosan (2) being obtained from *Gammurus*
Conditions of sorption : T = 20°C, pH = 8.0, static mode

Absolute values of the amount of Copper absorbed are given in Table 1.

Table 1. Sorption of $Cu^{(2+)}$ ions from cutting emulsion by means of *gammarus* chitin (1) and chitosan (2)

Sorbent	Amount of Copper (mg) absorbed for the period of : (min)						
	10	30	60	120	180	240	300
Chitin	0.33	0.39	0.45	0.46	0.71	0.72	0.70
Chitosan	0.66	0.88	0.96	0.99	-	-	-

The results achieved show that both sorbents absorb $Cu^{(2+)}$ ions but their rate of sorption differ. Chitin extracts Copper at lower rate and its isotherm of sorption reaches saturation in three hours, extraction being 73 %. Chitosan sorbs copper more intensively - it needs 10 minutes to extract 70 % of ions and in two hours complete sorption of Copper present in the sample takes place . Consequently, to define the total exchange capacity of chitosan an experiment using higher copper content (20 ml of stock cutting emulsion was taken for sorption) was performed. The total exchange capacity (TEC) of chitosan was nearly one order of magnitude higher than that of chitin. (Table 2).

Table 2. Total exchange capacity of sorbents for $Cu^{(2+)}$ ions

Sorbent	Total exchange capacity (mg-equ/g)
Chitin	0.28
Chitosan	1.99

Note : Sorption took place at pH = 8.0. Concentration of $Cu^{(2+)}$ ions in the sample was 19.7.

Kinetics of copper ions sorption by two samples of chitosan obtained from different raw material has been studied as well. Experiments were performed on *gammarus* chitosan having the rate of deacetylation RD = 0.75. The fig. 2 illustrates kinetics of sorption of copper ions from cutting emulsion with copper ions concentration in the sample being 19.7 mg by these two versions of chitosan.

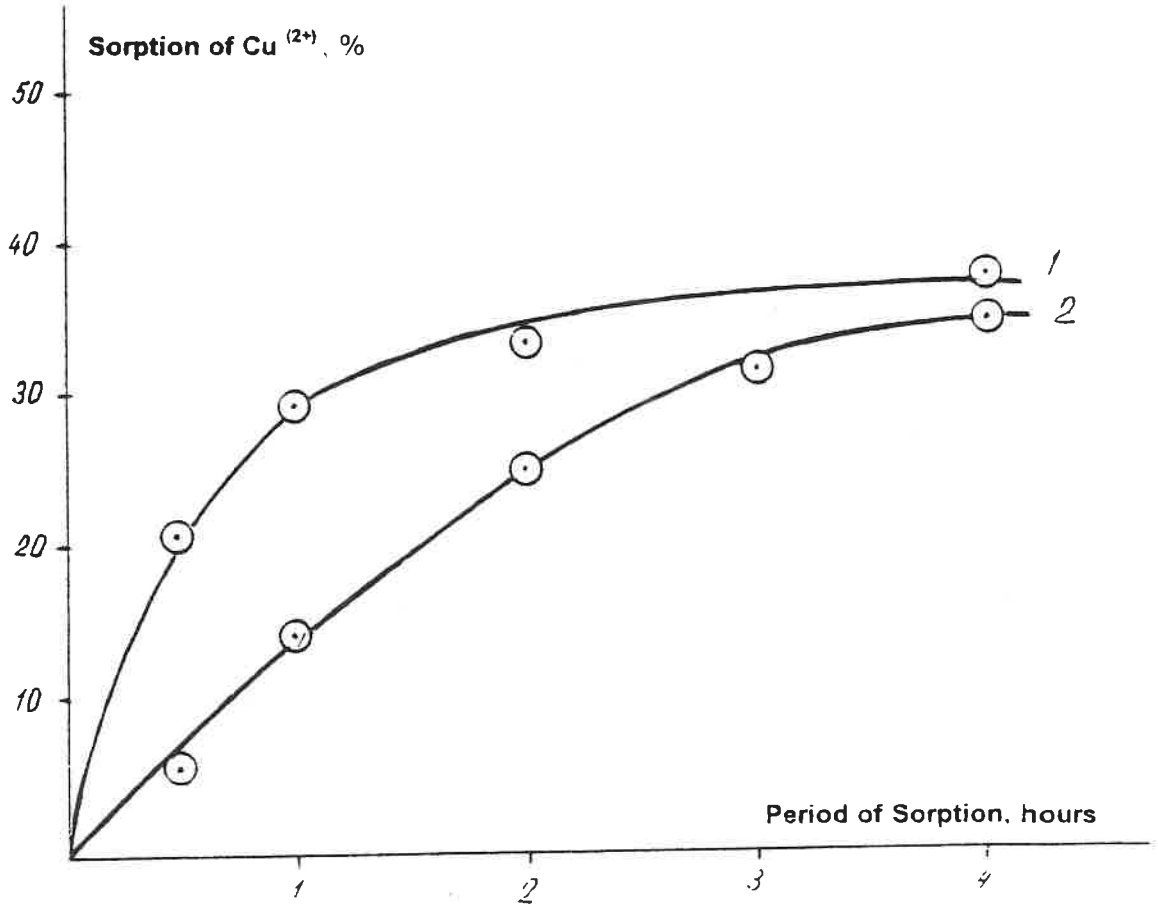

Fig. 2 **Kinetics of copper ions sorption by chitosan obtained from different `raw material**
1 - gammarus chitosan **2** - shrimp chitosan
Conditions of sorption : T = 20°C, pH = 8.0, static mode

It is evident that gammarus chitosan has higher rate of sorption in comparison with shrimp chitosan, but the saturation points of both sorbents are very close to each other. This difference can be explained by the morphological structure of samples : gammarus chitosan has porous and fibrous structure being easily accessible for the ions of copper, whereas shrimp chitosan has harder textured flaked structure. The morphology of samples has greater influence upon the rate of sorption than the rate of deacetylation . Despite higher deacetylation rate shrimp chitosan sorbs less copper and its total exchange capacity is lower than that of gammarus chitosan which is seen from the Table 3.

Table 3. Sorption of Cu (2+) ions from cutting emulsion by chitosan obtained from different raw material

Sorbent	Amount of copper (mg) absorbed within (min)				
	30	60	12	180	240
Gammarus chitosan RD = 0.59	3.7	5.2	5.9	5.9	6.6
Shrimp chitosan RD = 0.75	1.0	2.5	4.5	5.6	6.2

Note : Concentration of the ions of copper in the sample - 19,7 mg
Total exchange capacity of shrimp chitosan 1.96 mg-equ/g

In the Table 3 absolute amounts of absorbed copper are given. The total exchange capacity is calculated according to the amount of copper being sorbed within 4 hours.

To study the sorption of chromium from water solutions of chromium chloride a sample of gammarus chitin having the rate of deacetylation of 0.15 was used.

Since the sample of chitin being used showed low sorption capacity (only 15% of the total amount of chromium present in the solution was extracted) an attempt was made to activate the process by additional treatment with the solution of ammonium sulphate. In this case 21% of total amount of chromium was extracted.

It is possible to boost sorption by treating chitin with the mixture of 0.1 M of sulphurous acid and of 0.1 M ammonium sulphate. The amount of sorbed chromium made 39%.

Thus , the sorption capacity of gammarus chitin varies from 0.15 to 0.39 mmol/g with relation to the ion of $Cr^{(3+)}$ which is very close to the sorption capacity of crab chitin being obtained by traditional alkali-and-acid method. To study the sorption of $Cr^{(3+)}$ by chitosan a sample having deacetylation rate (RD) of 0.79 was taken. The kinetics of sorption were taken for pH-values equal to 3.5 and 6.3. In connection with the fact that at pH = 3.5 chitosan dissolves in the solution of chromium chloride we used to add after a certain period of time ammonia to activate alkali reaction, washed sorbent off with water and defined the amount of chromium in the filtrate. As chitosan does not dissolve at pH = 6.3 there was no need to alkalize solution.

The results achieved are illustrated by the fig. 3. The kinetic curves are similar for the both of pH-values. It is clearly demonstrated that the maximum amount of chromium is extracted during the first hour of reaction (85% at pH = 3.5 and 80% at pH = 6.3). The peak of sorption takes place after four hours of reaction and makes 95 % at pH = 3.5 and 86% at pH = 6.3). It turns out that further extending of the sorption period does not influence the amount of chromium extracted. Similar pattern has been observed in case of the $Cr^{(6+)}$ ions being sorbed by the crab chitosan. Lower sorption capacity of chitosan at pH = 6.3 could be caused by the presence in the solution of phosphate ions which significantly hampers sorption.

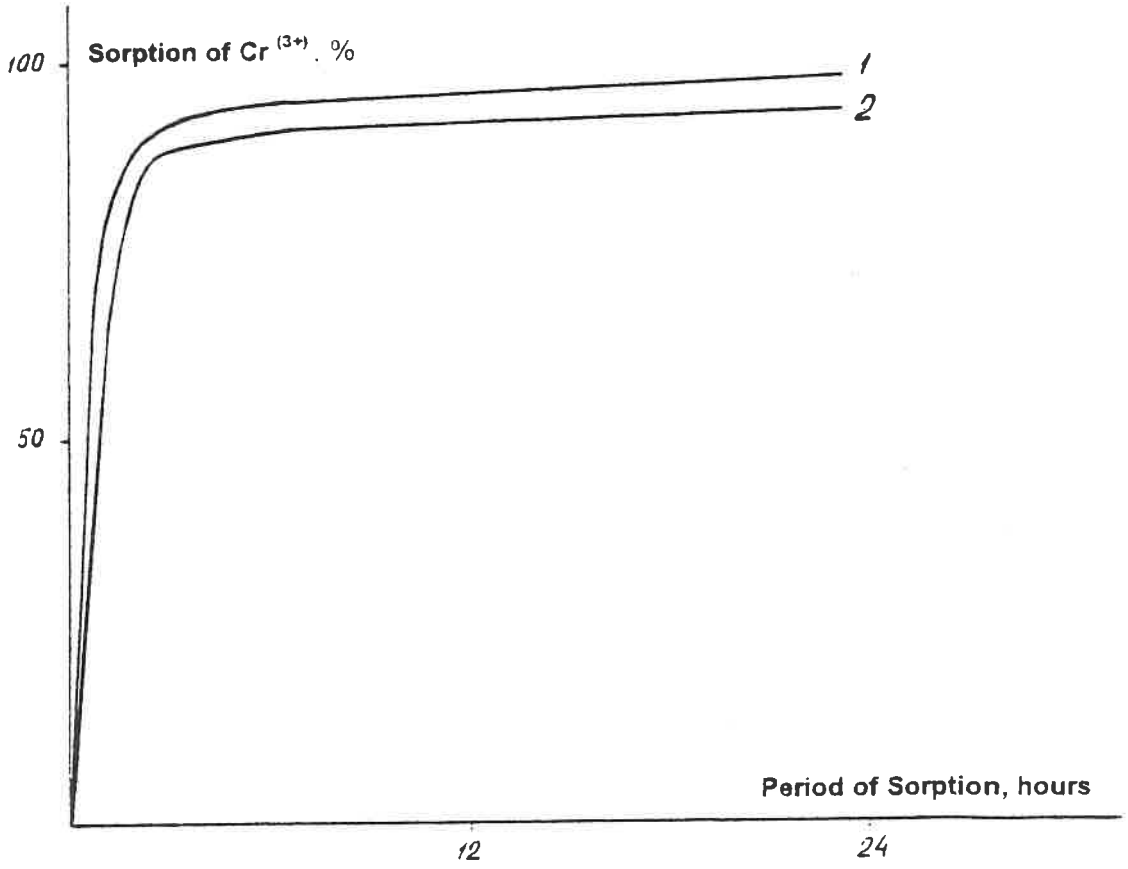

Fig. 3. Kinetics curves of the sorption of chromium by gammarus chitosan at different pH-values.

Conditions of sorption : T = 20°C ; 0.01M solution of $CuCl_3$; pH = 6.3 created by phosphate buffer

1 - pH of 3.5 solution 2 - pH of 6.3 solution

Conclusion

The results achieved allow to conclude that electrochemically obtained crab and gammarus chitin can be used as sorbents of $Cu^{(2+)}$ and $Cr^{(3+)}$ ions though their exchange capacity is not high and total extraction of copper and chromium ions requires large amount of sorbent. Partially deacetylated chitosan rapidly and totally sorbs ions of copper and chromium and could be used to purify cutting emulsion and industrial effluents containing ions of these heavy metals. Taking into consideration that sorption of chromium and copper by electrochemically obtained from gammarus and shrimp chitin and chitosan is similar to the sorption performed with the use of chitin and chitosan obtained from crab, krill etc. it is possible to suppose that ions of other transition metals will be similarly sorbed by the biopolymers under investigation.

Thus the study has demonstrated that electrochemically obtained chitin and chitosan in particular can be used to extract ions of heavy metals.

It is worth mentioning that gammarus based chitosan due to the specific morphological structure of fibres and its physical and chemical properties [Maslova G and others, 1996] has higher sorption capacity which makes it highly usable in particular cases.

With the purpose to draw up recommendations for the practical use of gammarus based chitosan we continue to work under further investigation of its sorption capacity in relation to ions of other heavy metals and on creation of the mostly optimal form of its use as well (i.e. pellets, membranes, filter beds).

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Effect of Acetyl Group Content on the Miscibility of Blends of Chitosan with Poly(ethylene oxide)

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Miscibility of blends of poly(ethylene oxide) (PEO) with two commercial chitosans, which have F_A values of 0.25 and 0.02 respectively, was studied by means of mechanical properties, solubility, FTIR and SEM . The results indicated that specific interactions between chitosans and PEO macromolecules exist, which are mainly due to the formation of intermolecular hydrogen bonds between bridge oxygens in the PEO main chains and amine groups on the chitosan chains. Both commercial chitosans are partially miscible with PEO, which dispersed in the continuous chitosan phase as a separate component. The interfacial interactions of chitosan having F_A = 0.25 with PEO is stronger and greater than that of chitosan having F_A =0.02 with the same PEO, and the maximum interaction of blends was observed at 50% PEO content for the former and at 20% for the later, because the size of PEO phase is smaller and finer in the former than in the latter due to the more amorphous morphology of chitosan having F_A value of 0.25.

Keywords: chitosan; acetyl group content; poly(ethylene oxide); blends; miscibility

There have been some reports dealing with polyblends of chitin/chitosan with other natural or synthetic polymers. For example, cellulose/chitin blend fibre[1] prepared by mixing alkaline chitin and alkaline cellulose to improve Young's moduli and the dyeability, polyamide-6/chitin blend prepared using 85% phosphoric acid as cosolvent[2], whose blend product with 50/50 of two polymers can be processed by using extrusion, injection or compression molding at around 210-215 ^{0}C, cellulose/chitosanblend films prepared using trifluoro acetic acid and chloral/ dimethylformamide as cosolvent respectively to improve the mechanical properties and solute permeability of respective polymer and the stability of chitosan[3-5], poly(vinyl alcohol) (PVA)/chitosan film with good dyeability and membrane used as pervaporation of ethanol and water[6,7], nylon-4/chitosan blends which have good mechanical property and retain the excellent chelating ability of chitosan[8]. Recently chitosan and poly(ethylene oxide)(PEO) blends, have been reported for the preparation of membranes for haemodialysis[9] and semi-IPNs for pH-sensitive drug delivery[10]. PEO is a synthetic water-soluble polymer that is also nontoxic and biocompatible. It is therefore of interest to study chitosan and PEO blends in detail.

Zhao et al[11)] have studied the changes of melting point of PEO/chitosan[0.16] blends by DSC. They found that the melting point of blends decreased gradually with increasing chitosan content when the chitosan content was less than 50% and then increased as the chitosan content increased above 50% level. Combined with the morphological changes of fractured sections of blends observed by SEM, they concluded that compatibility was dependent on the composition of the blends, being miscible when the chitosan content was below 50%, and above this composition, the blends were phase separate. However, Angelora et al[12] reported that the melting point of PEO/chitosan[0.02] blends tended to decrease on increasing the chitosan proportion over the whole range of PEO/chitosan ratios. There was no phase separation in PEO/chitosan blend films.

This work is an attempt to fully study the miscibility of PEO with two chitosans having different F_A values to obtain a more complete understanding about blends of chitosan and PEO.

Experimental

Materials

Commercial chitosan[0.25] was supplied by Pronova Biopolymer and was purified twice by dissolving in 0.1 M CH_3COOH and precipitating with NaOH. The value of F_A and the Limiting Viscosity Number [η] in 0.2 M CH_3COOH/0.1 M CH_3COONa were determined by the dye adsorption[14] and the dilution method[15] respectively and found to be 0.25 and 1300 ml/g.

Commercial chitosan[0.02] was obtained from Aber Technologies and was purified once following the same way as above. The values of F_A and [η] in 0.2 M CH_3COOH/0.1 M CH_3COONa were 0.02 and 700 ml/g respectively.

PEO powder was purchased from the Aldrich Chemical Company. Its stated viscosity average molecular weight was 4×10^5.

Preparation of chitosan/PEO blend films

Chitosan and PEO were each dissolved in 0.1 M CH_3COOH aqueous solution overnight. Chitosan and PEO solutions were then mixed in different weight percentage ratio and the mixtures were stirred overnight at room temperature. The degassed and well mixed solutions then were cast on a glass plate and evaporated at room temperature.

Analysis of Miscibility

Tensile Strength: The tensile strength of the films was measured with a 1122 Instron Tensile Strength Tester at 20 ^{0}C and 65% relative humidity. All sample films were prepared by casting solutions at concentrations of 1-2% (w/v). The dried films were cut into 25 mm × 100 mm pieces with a thickness of 20 μm. The test speed was 10 mm/min.

Solubility and stability of films in neutral water: The chitosan, PEO and their blend films were weighed accurately and immersed in 100 ml methanol for 24 hours to remove acetic acid in films, then taken out and dried for 12 hours at room temperature and weighed again. The dried films were then immersed in distilled water for 8 hours, and the remained films were taken out and washed with water and methanol, then dried for 24 hours and finally weighed for the third time to determine the amount of PEO extracted from blends. All films used were the same samples used in the tensile strength measurements above.

FTIR measurements: FTIR spectra of the films were obtained using a Perkin-Elmer 1600 Series FTIR Instrument. The specimen films were prepared as described above except that the concentration of solution was 0.5% instead of 1-2%. The dried blend films were treated by immersing films in methanol for 24 hours to remove acetic acid and dried for 12 hours. The pure chitosan films were also obtained by treatment of dried chitosan films with 0.1 M NaOH aqueous solution over night, followed by washing in distilled water and drying at room temperature.

Scanning Electron Microscopy(SEM): Solutions of chitosans, PEO and their well stirred mixtures were cast on microscope glass coverslips (⌀19mm). After drying, the films with slips were immersed in methanol for two days, then taken out and immersed in distilled water for one day, and then dried at room temperature. The dried films were mounted on a SEM specimen holder and coated with gold-palladium. The surface morphology of the blend films was observed with a Cambridge Stereoscan 600 Instrument at an accelerating voltage of 20 kv.

Results and discussion

Preparation of blend films

It is known that chitosan has excellent film-forming property. Chitosan films with good transparency and high mechanical strength are easily formed by casting chitosan solutions, but PEO films are normally opaque because of its highly crystalline structure. According to the procedures described above, two series of blends with different ratios of chitosan and PEO were prepared by casting well mixed solutions of them using two commercial chitosans. All the blend films were flexible, but the appearance of the two series differed. Although the transparency of the films decreased in both with increase in PEO content, this decrease was less pronounced in the series of chitosan[0.25]/PEO blends. For example, in this series the films were still transparent at a PEO content of 50%, while at that composition the chitosan[0.02]/PEO blend film was translucent.

Tensile strength of blend films

The tensile strength of chitosan, PEO and their blend films was measured. Figure 1 gives the dependence of the tensile strength of the films on the blend ratios. Figure 2 indicates more clearly the increase of tensile strength of chitosan/PEO blend films over the linear additivity with the blend ratio.

The tensile strength of chitosan[0.25] is less than that of chitosan[0.02], although the molecular weight of the former is much greater than that of the latter, showing the mechanical property of chitosan is quite dependent on its F_A value as a number of properties of chitosan.

As can be seen, the tensile strength of blend films is obviously greater, over the entire blend ratio range, than that would be predicted from simple linear additivity. This suggests the presence of specific interaction between chitosan and PEO and, since the enhancement of the tensile strength is greater for the chitosan[0.25]/PEO blend films than for the chitosan[0.02]/PEO blend films, that these specific interactions are greater between chitosan[0.25] and PEO than between chitosan[0.02] and PEO. Furthermore the greatest enhancement of tensile strength occurs at 50% PEO content for the former and at 20% PEO content for the latter, indicating that these are the compositions within the respective series at which these chitosan-PEO interaction are at a maximum.

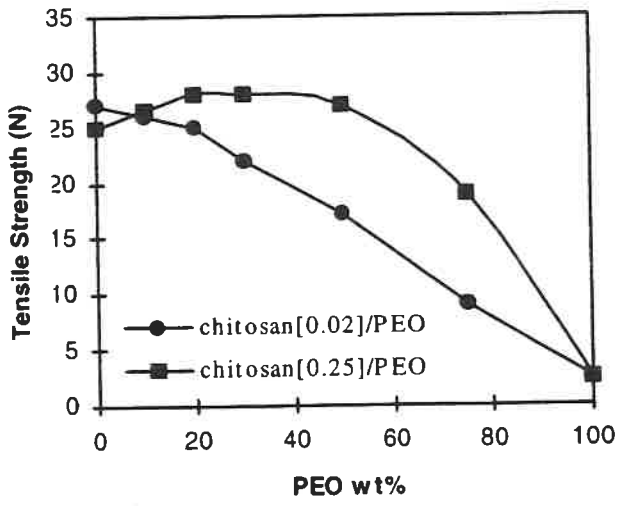

Figure 1 Tensile strength of chitosan/PEO blend films *versus* blend ratio

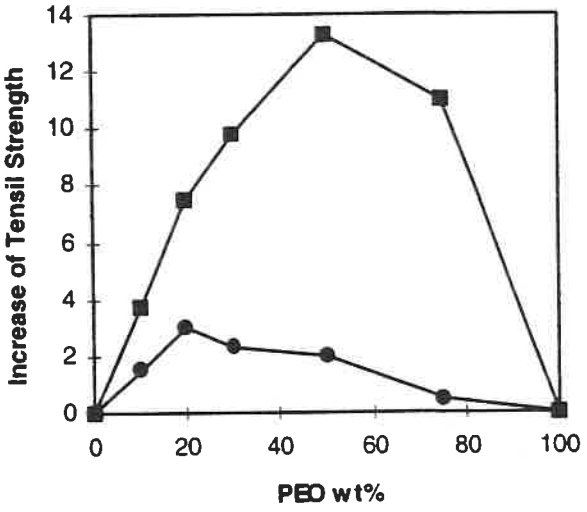

Figure 2 Increase of tensile strength over the linear additivity *versus* blend ratio

Solubility and stability of blend films in neutral water

As chitosan is naturally insoluble water at neutral pH but PEO is easily soluble in water, the solubility and stability of blend films were studied in water to test specific interactions between two chitosans and PEO. The studies showed that all dried films of chitosan and chitosan/PEO blends were highly swollen or soluble in distilled water, because some acetic acid was adsorbed by the amine groups of chitosan in the form of amine salts. In this work, it was found that methanol can be efficiently used to dissolve and remove acetic acid left in films, and the films treated with methanol was less or more swollen but insoluble in water. Table 1 and Table 2 show that almost all of acetic acid was removed after immersing in methanol for 24 hours. Table 1 and Table 2 also show that the PEO in blend films treated in this way can not be extracted completely by treatment with distilled water, although pure PEO film dissolved completely in water after less than 1 hour. Apparently at least 5% PEO remained in the blend films after immersing in water for 8 hours. It is interesting to note that the maximum PEO remained in blend films is 14% at 20% PEO content for chitosan[0.02]/PEO blends, but is 24% at 50% PEO content for chitosan[0.25]/PEO. These results further showed that there are specific interaction between PEO and the two commercial chitosans and that the maximum interaction between them happens at 20% PEO content for chitosan[0.02]/PEO, and at 50% PEO content for chitosan[0.25]/PEO.

On the other hand, the appearance of the extracted films was different for the two series blends. Chitosan[0.02]/PEO blend films containing up to 20% PEO became semi-transparent and cloudy while those containing from 30%-75% PEO became totally opaque, on extraction of the PEO with water. In comparison, all the chitosan[0.25]/PEO blend films retained their transparency after the extraction treatment.

Table 1 Extraction behaviour of PEO from blends with chitosan[0.02]

Blend ratios (Chitosan/PEO)	100/0	90/10	80/20	70/30	50/50	25/75	0/100
Original weight of film (g)	0.3136	0.3651	0.3433	0.3450	0.2650	0.3361	0.2633
Weight after treatment with methanol (g)	0.2182	0.2841	0.2656	0.2688	0.2223	0.3114	0.2571
Weight after treatment with water (g)	0.2153	0.2766	0.2496	0.2010	0.1228	0.0946	0
Remaining film weight (%)	99	97	94	75	55	30	0
Remaining PEO in film (%)		7	14	5	5	5	0
Appearance of remaining films	+	±	±	-	-	-	

+: Transparent; ± : Sem-itransparent; -: Opaque with white colour

Table 2 Extraction behaviour of PEO from blends with chitosan[0.25]

Blend ratios (Chitosan/PEO)	100/0	90/10	80/20	70/30	50/50	25/75	0/100
Original weight of film(g)	0.3271	0.2767	0.2786	0.3056	0.3420	0.3263	0.2633
Weight after treatment with methanol (g)	0.2821	0.2378	0.2562	0.2606	0.2920	0.3131	0.2571
Weight after treatment with water (g)	0.2828	0.2259	0.2231	0.1962	0.2158	0.0964	0
Remaining film weight (%)	100	95	87	75	74	31	0
Remaining PEO in film (%)		5	7	5	24	6	0
Appearance of remaining films	+	+	+	+	+	+	

+: Transparent

FTIR measurement of blend films

The frequency of free amine band of chitosan[0.02]/PEO blends shifts up gradually from 1583 cm^{-1} to 1594 cm^{-1} when the ratio of chitosan/PEO increases from 100/0 to 80/20, then shifts down if further increasing PEO content, and the maximum shift is 11 units at 20% PEO content . Similarly the blend of chitosan[0.25]/PEO has a tendency of frequent shift of 18 units from 1578 cm^{-1} to 1596 cm^{-1}, but the maximum shift is 18 units at 50% PEO content. These results are very agreement with the discussions about mechanical property measurement and solubility and stability test of chitosan/PEO blend films.

The shift of frequency of the free amine band is because of the formation of intermolecular hydrogen bonds between oxygen -O- in PEO repeat unit and the side group -NH in chitosan repeat unit. Though there are two hydroxyl groups –OH in each chitosan repeat unit (on C_3 and C_6 positions respectively), the frequency assigned to O-H stretching did not have any obvious and regular change, which occur at 3450 cm^{-1} for chitosan[0.02] and 3367 cm^{-1} for chitosan[0.25]. This is because -OH on C_3 position prefers to form intramolecular hydrogen bonds with O(5) oxygen atom with adjacent repeat unit , and C(6) O-H prefer to form interchain hydrogen bonds with O(6) in adjacent chain and inramolecular hydrogen bonds with C(2)N-H.

Morphology of blend films

The SEM results indicate that in neither series are the chitosan/PEO blends homogeneous morphological structures. Instead the PEO forms a dispersed phase in the continuous chitosan phase, even at PEO contents as high as 75%. For the chitosan[0.02]/PEO blend films having 50% and 75% PEO, the individual dispersed elements are very large compared to those in the

chitosan[0.25]/PEO blend films having equal concentrations of PEO. This explains the difference in appearance after extraction in water.

As we know, chitosan is normally obtained from chitin by deacetylation with NaOH solution, especially for commercial chitosan. Chitin is a tight crystalline natural polymer, and the crystalline structures are broken down gradually during deacetylation. The crystalline peaks, $2\theta=9.2^0$ and 19.1^0, almost disappear and chitosan is almost amorphous when deacetylation reaches about 80-90%, however, at about 95% deacetylation, chitosan is again crystalline and the peak positions moves to $2\theta=11.2^0$ and 20.4^0, indicating the formation of new crystalline structures[16]. In the current work, the commercial chitosan[0.02] is an almost complete deacetylated chitosan whose structure is more crystalline and tight than that of chitosan[0.25]. Hence the PEO is more easily to mix with chitosan[0.25] and disperse in its matrix.

Conclusions

Both commercial chitosan[0.25] and chitosan[0.02] are partially miscible with PEO[poly (ethylene oxide)] over the whole range of mixing ratios due to specific interactions between chitosan and PEO molecules. The specific interactions are because of the formation of intermolecular hydrogen bonds between chitosan and PEO. The specific interactions in chitosan[0.25]/PEO blends are greater than that in chitosan[0.02]/PEO blends, and the maximum interaction happens at 50% PEO content for the former but at 20% PEO content for the latter. This is because the PEO component is better dispersed in the continuous chitosan[0.25] phase than in the chitosan[0.02] phase due to the more amorphous structure of chitosan[0.25] compared with chitosan[0.02].

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Relation between Mechanical Properties of a Chitosan Film and Content of Hydroxyapatite

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Abstract

Periodontal surgical procedures based on the principle of guided tissue regeneration have been extensively investigated and both non-biodegradable and resorbable film have been developed and used in such procedures. It would be desirable if films used in guided tissue regeneration are not only resoluble but also would have components that are osteoconductive. A composite film containing chitosan and hydroxyapatite may potentially have these features.

Hydroxyapatite in the form of particles has been used in the treatment of periodontal bony defects,for preservation of alveolar bone following tooth extraction and for augmentation of edentulous alveolar ridges that had undergone extensive resorption[1,2]. A problem with hydroxyapatite implants is difficulty in localization due to tendency of the particles to migrate from the implantation site. A biodegradable film which is also osteoconductive and which could be molded over the implanted particles limiting their migration would have obvious advantages.Chitosan is polysacchatride which can be extracted from crustacean. Chitosan is biocompatible and biodegradable. Several studies on this material have been published[3-11]. Synthetic hydroxyapatite is biocompatible and osteoconductive. Chitosan bonded hydroxyapatite self hardening paste was evaluated with regard to its osteoconductive properties. Radiographic examination revealed that a bone like irregular radiopacity appeared in the region of the embedded paste. This was judged histopathologically as formation of bone tissue with chondral tissue. These date suggest that the chitosan bonded hydroxyapatite self hardening paste has osteoconductive properties and may, therefore, prove clinically useful as a bioactive bone substitute.A composite film is being developed by mixing chitosan sol with hydroxyapatite particles[12].The resulting mixture can then be made into films that are characterized by their elasticity. Although these film become inelastic upon sterilization they will regain their elasticity upon rehydration due to the hydrophilic properties of chitosan.

The present study investigated the effect of adding various amounts of hydroxyapatite to chitosan sol on certain physical properties of the composite material.

Keywords: Chitosan, Hydroxyapatite, Film, Bone Osteoconductive

Materials and Methods

Chitosan sol was made by dissolving 0.5 g chitosan (Kauyo Chemical) in a solution of 0.5 g malonic acid (Nacali Tesque) in 10 ml physiologic salt solution. Varying amounts of hydroxyapatite (HA) (Mitsui Toatsu) with average particle diameter of 10 μm were then mixed with 11 g of the chitosan sol. The amounts of the HA were 0,2.0,3.0,4.0 and 5.0 g per 11 g (a future use abbreviated 0, 2/11, 3/11, 4/11 and 5/11) The mixtures were kneaded manually with a spatula to make a paste. The paste was neutralized with 5 % polyphosphate solution of 50 ml.

The paste was then used to prepare samples for evaluation of various physical properties. For evaluation of shrinkage and for measuring tensile strength and elongation, the paste was spread into petri dishes to form film 10 mm thick, 30 mm long and 7 mm wide. For each property and at each HA concentration, the test were done on five freshly set films and five films that were allowed to dry at room temperature for 24 hours and then subsequently rehydrated. For evaluation of shrinkage about after neutralized and after dried film. The paste was spread into petri dishes to form film 1.0 mm thick and 95 mm diameter.

1. after neutralized film

A - A'/ A X100 = shrinkage % (A : before neutralized diameter of petri dish, A' : after neutralized diameter of chitosan film)

The freshly membrane to cut 1.0 mm thick, 30 mm long and 7 mm wide and measured shrinkage of dried film.

2. after dried film

B - B' / B X100 = shrinkage % (B : before dry wide of film B' : after dried wide of film)

Shrinkage was assessed by estimating difference in the diameter of the film from that of the petri dish. Each measurement was repeated five times. Tensile strength and elongation were tested by using a Universal testing machine (Imada) at cross-head speed 5 mm / minute.

Hardness was measured using freshly set specimens as well as specimens that were allowed to dry at room temperature for 24 hours and then subsequently rehydrated. The dimensions of the specimens were 10 mm thick, 10 mm long, 10 mm wide. The test was done by using a JIS A type machine (Teclock) . Five fresh and five rehydrated specimens at each HA concentration were tested and the results were the average of five measurements.

Calcium ion dissolution was evaluated using test specimens 2 g in weight. The specimens were immersed in 70 ml physiologic saline and shaken at 37· C for 2 weeks at the rate of 100 times per

minute. The solution was then filtered and the filtrate was analyzed for calcium ion concentration using an ion meter (Toa Electronics). Three samples were tested at each HA concentration in the paste.

Morphologic studies were also done using an X-ray microanalyses (Nippon Electronics JCXA 733). This X-ray microanalyses be able to use a nalysis and scanning electron microscope. Scanning was conducted at 15 kv on gold coated surface of freshly prepared films as well as films that were shaken in physiologic saline at 37· C for two weeks at a rate of 100 times/ minute.

Results

Shrinkage of both freshly set film and dry film decreased with increase in the amount of HA (Figure 1). Greatest shrinkage occurred in film without HA. The least shrinkage was observed at the 5/11 level. The Hardness for hydrated dry films was greater than that of the freshly set films (Figure 2). For both freshly set and rehydrated dry specimens hardness was greatest at the 4/11 but it decreased at the 5/11. With freshly set films the greatest tensile strength was observed at the 4/11 level. There was a tendency for decrease in elongation with increase in the amount of HA in both the freshly set a nd the rehydrated dry specimens (Figure 3). Elongation was not different between the freshly set and the dry rehydrated films (Figure 4).

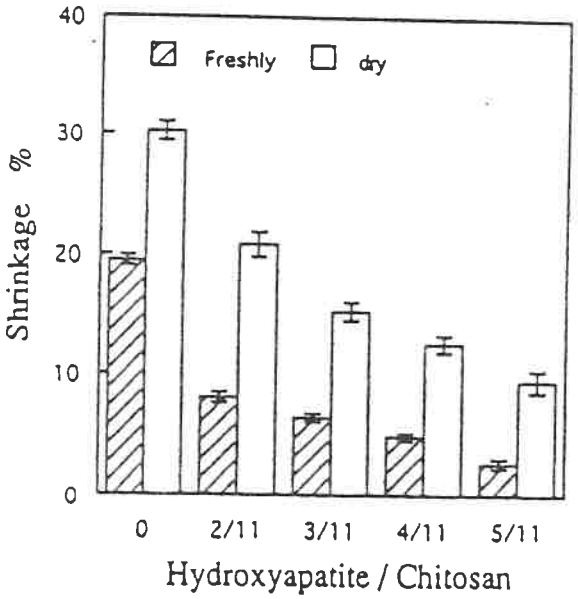

Fig.1 Relationship between shrinkage and hydroxyapatite content $p<0.01$
▨Freshly set film □Rehydrated dry film

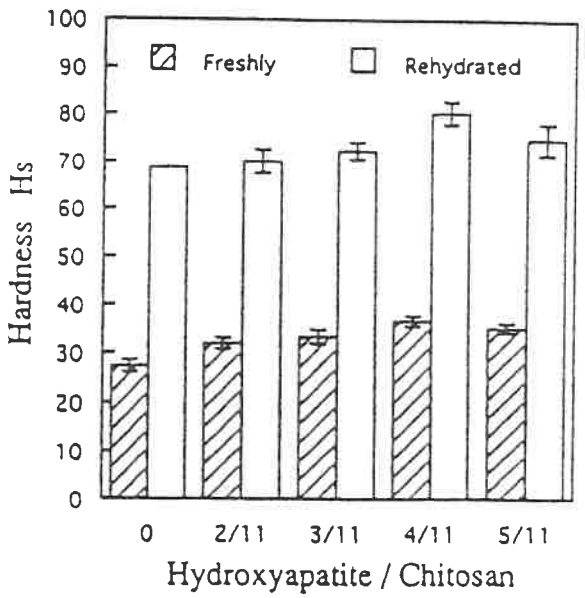

Fig.2 Relationship between hardness and hydroxyapatite content $p<0.01$
▨Freshly set film □Rehydrated dry film

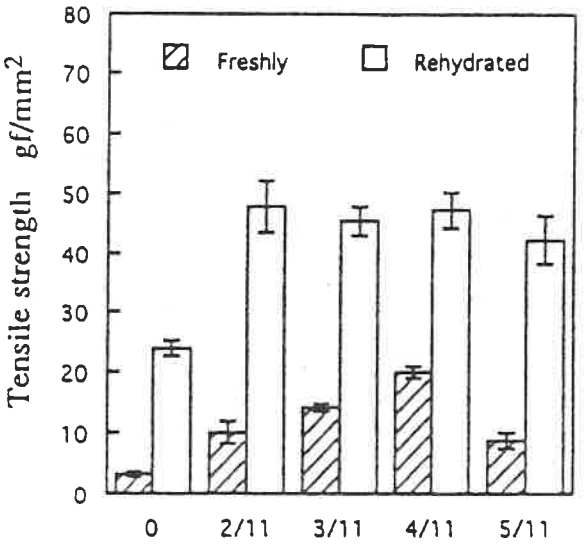

Fig.3 Relationship between tensile strength and hydroxyapatite content $p<0.01$ ▨ Freshly set film □ Rehydrated dry film

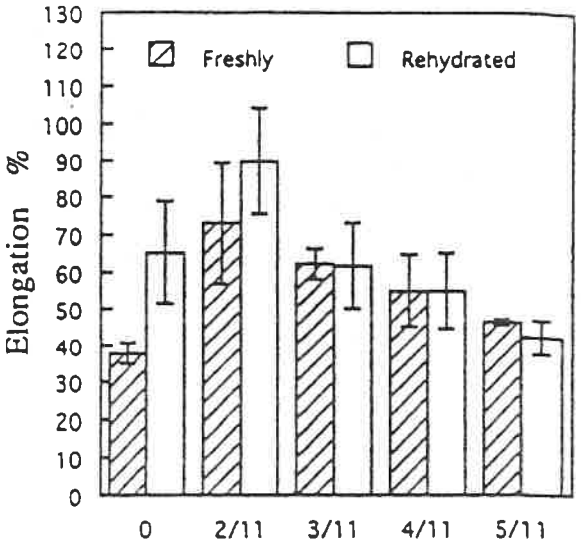

Fig.4 Relationship between elongation and hydroxyapatite content $p<0.01$ ▨ Freshly set film □ Rehydrated dry film

Figure 5 illustrates the finding for calcium ion release. The maximum amount of calcium was released at the 4/11.

Morphology examination of freshly set specimens revealed decreased in surface irregularities with increased HA level. After shaking in physiologic saline for two weeks some chitosan dissolution occurred and the specimen surface became porous.

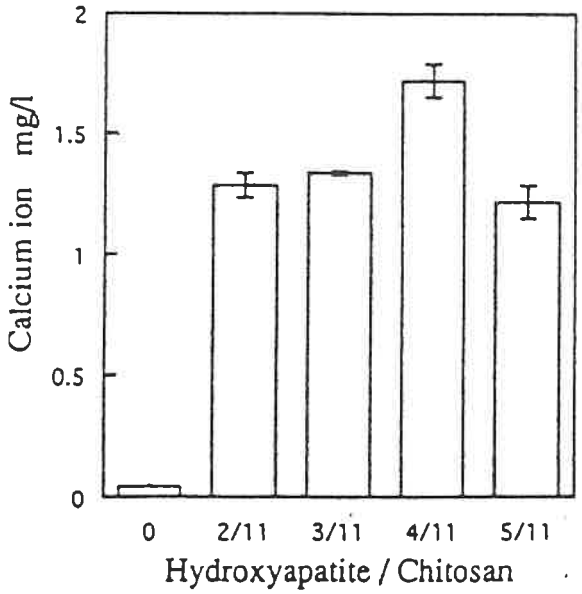

Fig.5 Quantity of Ca ion released $p<0.01$

Discussion

The present study was designed to investigate various physical properties of a composite chitosan calcium hydroxyapatite film. Shrinkage of the film decreased when the HA content of the film was increased. This can be attributed to increase in the contacting surface between the HA particles with increase in the HA content.

Hardness and tensile strength of rehydrated dry composite material were greater than those of freshly set material.

The ion dissolution test indicated that calcium ions were released from the composite. Maximum calcium ion release occurred at a HA concentration of 4//11 of chitosan sol. However, calcium ion release decreased when HA level was increased to 5/11 chitosan sol. This phenomenon can be attributed to decrease in the contacting surface area of HA writhe a physiologic saline solution with increase in HA level to 5 g. Morphologic observation showed numerous pores on the surface of composite at 2/11, 3/11 and 4/11. However, when the level of HA was increased to 5/11 the porosity decreased, thus decreasing the contacting surface between HA and the physiologic saline solution.

The finding of the present study indicates that the ratio HA to chitosan sol of 4/11 is optimum for preparation of a composite film which can be of value in guided tissue regeneration. A histologic study is currently being conducted to investigate the biological responses to the composite material.

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SWELLING AND PERMEABILITY OF CHITOSAN/CARBOXYMETHYL CELLULOSE POLYELECTROLYTE COMPLEX MEMBRANES: EFFECT OF pH AND Ca^{2+} IONS

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Abstract

The membranes of the polyelectrolyte complex (PEC) of chitosan (CHI) and carboxymethyl cellulose (CMC) readily absorb water reaching a maximum swelling degree after which they slowly shrink until a pH dependent equilibrium value is attained. The contraction stage follows a first order kinetics. The increase in conductivity observed during contraction is controlled by the diffusion of ions and exhibits Fickian behaviour. The membranes immersed in aqueous solutions of Ca^{2+} salts exhibit a volume collapse upon continuous increase of Ca^{2+} concentration. These materials are also pH-sensitive: at low and high pH values (pH<3; pH>11) they show high swelling values, whereas at intermediate pH they experience moderate swelling. The permeability of the membranes to phenylalanine varies with the pH of the medium. In HCl solution it is observed that the swelling degree and the permeability coefficient are both greater than in NaOH solutions. A decrease in permeability is observed by the addition of $CaCl_2$ in basic medium.

Keywords: chitosan, carboxymethyl cellulose, polyelectrolyte complex, membrane swelling, permeability.

Materials and methods

Chitosan (Protan, Mv = 6.9×10^5, DD = 82%) and carboxymethyl cellulose sodium salt (BDH, Mv = 7.7×10^4, SD = 0.7) were used as purchased. Deionized water was used in all experiments. All other reagents were analytical grade.

Membrane formation. CHI (1g) was dispersed in water (25 mL) with agitation and glacial acetic acid (5 mL) was added until dissolution. CMC (1.7 g) was dissolved in water (25 mL). Both solutions were allowed to stand overnight. The solutions were then mixed and HCl (1.5 mL) was added in order to obtain an homogeneous solution. Then 10% (w/v) aqueous NaOH was added dropwise under vigorous agitation until the desired pH value at 25°C. The PEC formed as a finely dispersed hydrogel was poured into a sintered glass filter (ϕ = 8.5 cm) and allowed to decant and dry for a few days. A transparent, rigid and brittle membrane was obtained which was easily separated from the filter.

Swelling experiments. Completely dried membranes were weighed and immersed in water at pH 5.5 (approx.1 L) at 25°C. Water was changed regularly in order to eliminate the low molecular weight electrolytes diffusing out of the

membrane. This process was monitored with a Hydromat LM 302 conductimeter.

The water uptake, *W*, was calculated by measuring the weight gain of the membrane after carefully wiping its surface. It is reported as the weight of water sorbed by the membrane per gram of dry sample. All experiments were performed using three replicates, and the swelling degrees reported are average values.

The calcium ion concentration was determined by atomic absorption spectrometry in a Philips SP-9 equipment at $\lambda = 422.7$ nm.

Permeability experiments. A glass cylinder of 3.4 cm diameter with the lower end closed with the PEC membrane was partially immersed in a 400 mL beaker containing 0.01 mol/L aqueous NaOH (or HCl as indicated). The cylinder contained 20 mL of the same solution as the beaker and it was immersed in the latter until the level of both liquids coincided. The solutions in both compartments were constantly agitated. Then 0.44 g of DL-β-phenyl-α-alanine (Phe) were added to the external solution. The instant at which Phe had completely dissolved was taken as zero time.

The Phe concentration in the internal compartment was determined at different times by taking an aliquot of the solution and measuring the absorbance at 258 nm. The aliquot was returned immediately after making the spectrophotometer reading.

Results and discussion

The CHI/CMC PEC is obtained by mixing together equivalent amounts of CMC and chitosan hydrochloride. The reaction leading to PEC formation is as:

$$\sim\!\!\sim COOH + {}^{+}NH_3\sim\!\!\sim \rightleftharpoons \sim\!\!\sim COO^{-}\,{}^{+}NH_3\sim\!\!\sim + H^{+}$$

The degree of complexation, θ, -defined as the ratio between the saline bonds concentration to the concentration of any of the polyelectrolytes- is strongly dependent on the pH of formation of the PEC [1,2]. Complex formation and its consequent precipitation occurs very rapidly and as a result, the polymer chains of the PEC become trapped in arrangements that are not the equilibrium ones.

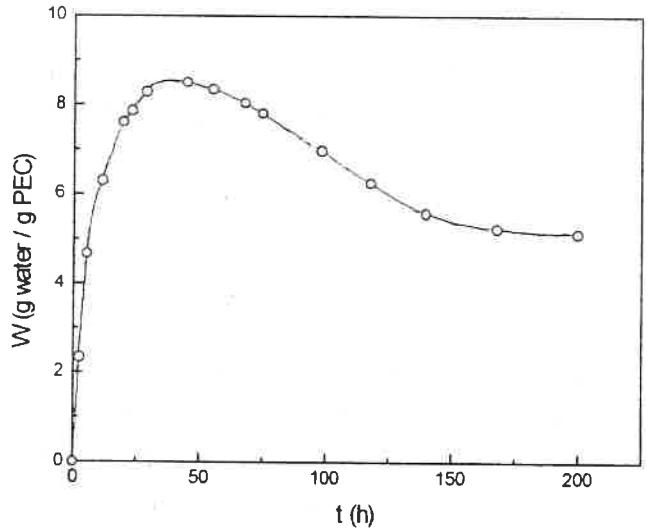

Fig. 1 *Typical swelling curve of a CHI/CMC PEC membrane in water at 25°C (pH = 5.5).*

Because of its ionic character the PEC swells considerably when immersed in water, as it can be appreciated in Fig. 1. In the maximum swelling state the macromolecular chains composing the PEC network possess the necessary mobility in order to rearrange themselves adopting more favoured conformations. The contraction experienced by the PEC membrane after maximum swelling (Fig. 1) can then be explained if one considers that at the pH at

which the experiment was performed (pH 5.5), the free carboxylic groups of the CMC chains are mostly as sodium carboxylate and the amino groups of chitosan are protonated, so that new salt bonds can be formed through the following reaction:

$$\sim\!COO^-Na^+ + Cl^-{}^+NH_3\sim \rightleftharpoons \sim\!COO^-{}^+NH_3\sim + Na^+ + Cl^-$$

The segmental mobility on the swelling state must be sufficient to allow the attainment of the required spatial arrangements for this reaction to proceed [3] As a result of this, θ increases in time, giving the sustained contraction observed in Fig.1.

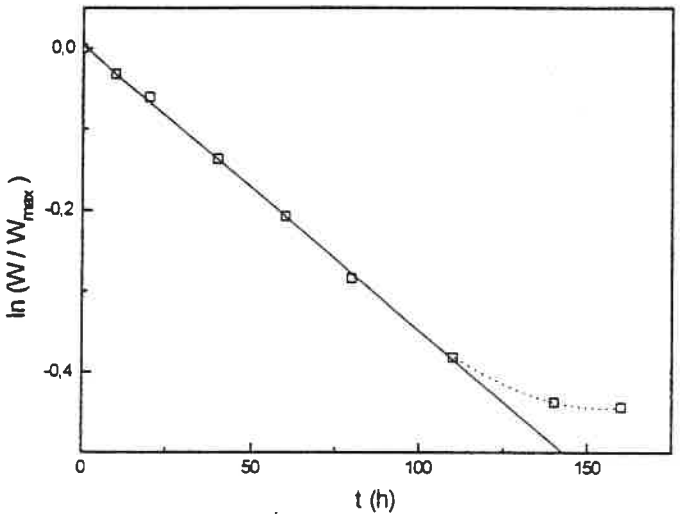

Fig. 2 *Time dependence of the logarithm of the swelling degree of a CHI/CMC PEC membrane relative to the maximum swelling degree during contraction.*

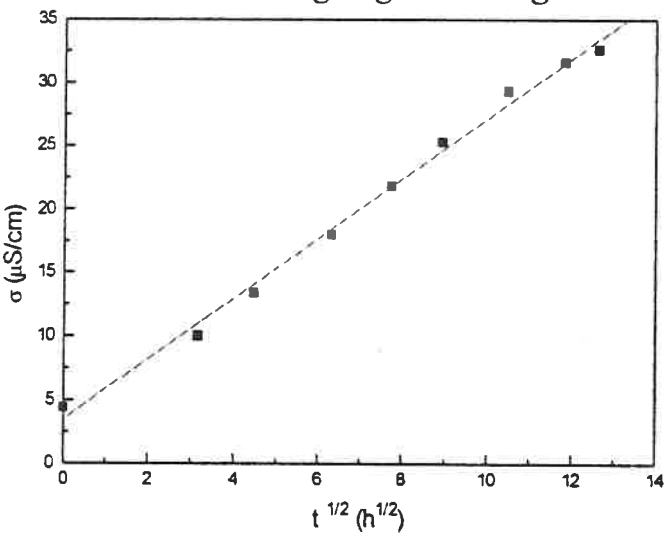

Fig. 3 *Water conductivity vs. $t^{1/2}$ for a CHI/CMC PEC membrane during the contraction stage.*

This reaction is favoured by the migration of Na^+ and Cl^- ions towards solution. In fact the shrinkage of the membrane is accompanied by a simultaneous increase in the conductivity of the solution with time resulting from this migration.

The membrane contraction follows a first order kinetics until beyond the first 100 min -corresponding to more than 80% of the total contraction- which is manifested by the excellent linearity of the ln (W/W_{max}) vs. t diagram in Fig. 2. This first order kinetics indicates that the dominant process during membrane contraction is the interpolymer reaction leading to salt bonds formation. Each salt bond formed gives rise to a new crosslink in the macromolecular network, with the consequent increase in its retractile force. However, the increase in the number of crosslinks also causes a decrease in the segmental mobility of the macromolecular chains, until a point at which the relaxation of the PEC chains becomes the limiting step of the process. This is the cause of the deviation of the first order behaviour observed when contraction reaches 80%.

On the other side, the increase in conductivity of the solution is controlled by

the diffusion of the ions outside the membrane. The linear dependence of the conductivity vs. $t^{1/2}$ diagram of Fig. 3 shows that this diffusion process follows a Fickian behaviour.

Influence of the pH of the medium. PEC membranes at their maximum swelling were immersed in water at different pH and constant ionic strength (I) and allowed to stand for 200 hours in order to reach equilibrium. The values of the ratio of the swelling degree at equilibrium, W, to maximum swelling, W_{max}, are shown in Fig. 4.

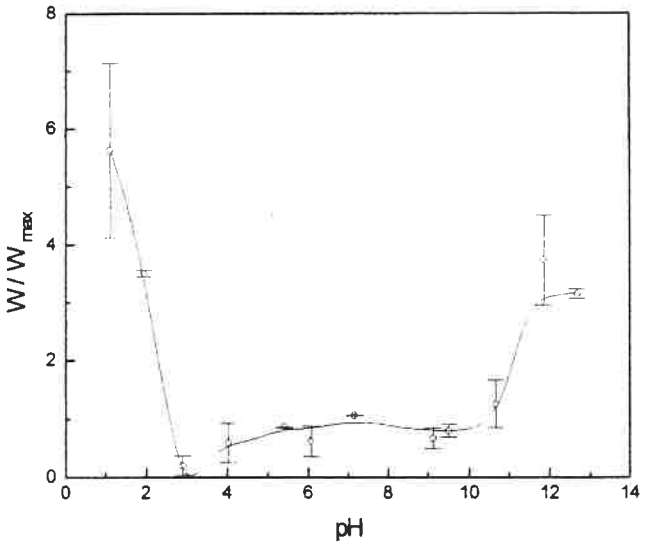

Fig. 4 *Relative swelling degree of CHI/CMC PEC membrane at equilibrium when immersed in solutions at different pH values. I = 0.05.*

At pH values below 3 or above 11 a considerable increase in swelling is produced. This can be explained if one considers that when the membrane is placed in strongly acid or basic media dissociation of the interchain bonds takes place according to:

$$\sim\!\sim NH_3^+ + \sim\!\sim COOH \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} \sim\!\sim NH_3^+ {}^-OOC\sim\!\sim \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} \sim\!\sim NH_2 + \sim\!\sim COO^-$$

This bond dissociation decreases the crosslinking degree of the complex thus allowing a greater swelling of the membrane. At intermediate pH values the PEC experiences moderate swelling. Membranes of the CHI/Pectin PEC exhibit a similar dependence of W on pH [4].

Influence of salts. The contraction after maximum swelling was also followed by placing membranes at maximum swelling in 0.001 mol/L aqueous solutions of NaCl, KCl, $NaNO_3$ and Na_2SO_4, respectively. The contraction process was almost the same as in water independently of the nature of the cation or the anion.

However, when the external solution contained $CaCl_2$ a volume collapse was observed. The magnitude of this effect can be appreciated in Fig. 5, where it is shown in a logarithmic scale the final swelling (W_∞) reached after contraction as a function of Ca^{2+} concentration for membranes formed at three different pH values. In the three cases a similar behaviour is observed, but as the pH of formation of the membrane increases the contraction becomes more pronounced. It is interesting to note that for very high salt concentrations the membranes swell again.

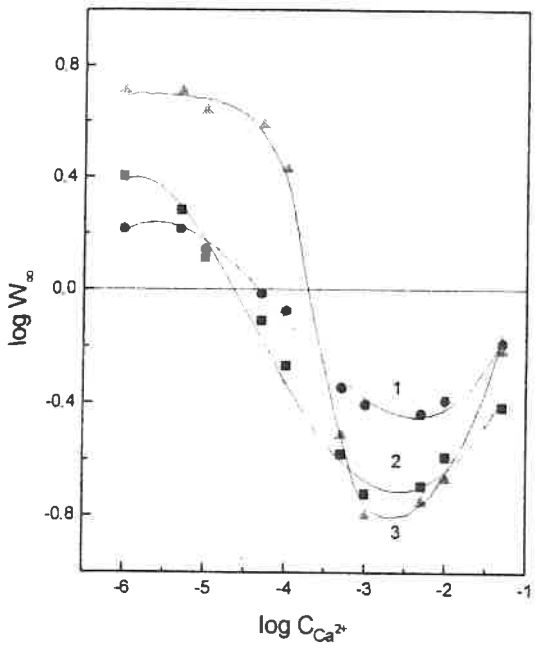

Fig. 5 *Logarithmic plot of the final swelling (W_∞) vs. Ca^{2+} concentration for CHI/CMC PEC membranes formed at three different pH values. (1) 4.43; (2) 5.35; (3) 5.70.*

This indicates the existence of a specific effect of Ca^{2+} on the PEC's free carboxylate groups, possibly by the formation of Ca^{2+} complexes with fixed ligands of the network. This complexation can be visualised as the formation of new crosslinks, which would increase the retractile force of the PEC's macromolecular network, thus provoking its contraction. The effect increases with increasing the pH of formation of the membranes, since at higher pH there exists a greater amount of free carboxylate groups due to the lesser completion of the PEC's formation reaction.

A similar effect has been reported for swelling of gels of acrylic acid/acrylamide copolymers immersed in Cu^{2+} solutions [5]. The increase in swelling of the CHI/CMC PEC observed at high $CaCl_2$ concentrations is due to the fact that under these conditions the Ca^{2+} concentration in the gel is so high as to produce Bjerrum type ionic associations, thus prevailing positively charged calcium-carboxylate pairs. The electrostatic repulsion between such ion pairs opposes the formation of the complex [5].

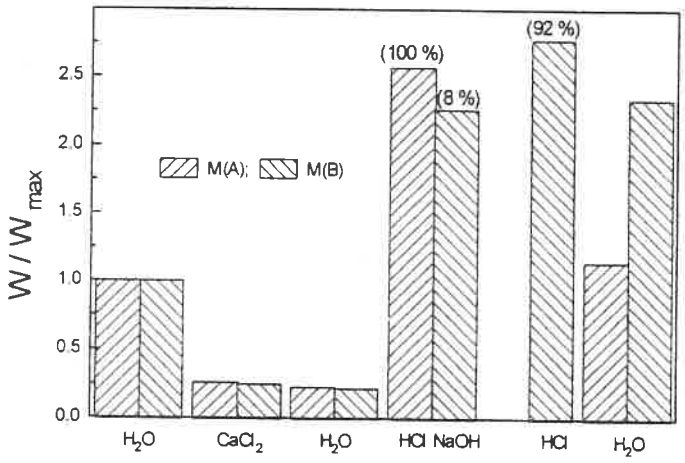

Fig. 6 *Variation of the relative swelling degree for two membranes subjected to different treatments. The amount of Ca^{2+} removed is given at the top of the bars.*

The crosslinking ability of Ca^{2+} may be used to control the velocity of solute fluxes through the membranes as well as their permselectivity by varying the degree of swelling as a result of changing the environmental conditions.

The latter is illustrated in the following experiment. PEC membranes that were previously swelled in water up to the maximum swelling were immersed in 2×10^{-3} mol/L $CaCl_2$ solutions. The characteristic contraction was observed, as represented in the bar diagrams of Fig. 6. Then, they were washed and placed again in water without experiencing any appreciable change in their swelling degree. Afterwards some membranes (represented as **M(A)**) were immersed in 2×10^{-2}

mol/L HCl, while others (designed as **M(B)**) were placed in 2×10^{-2} mol/L NaOH. It was observed that while the membranes in acid medium release all the calcium retained, the ones placed in basic medium release only 8% of the initial calcium content. If these **M(B)** membranes are then placed in 2×10^{-2} mol/L HCl they accuse a further increase in swelling, liberating all the remaining calcium. When **M(A)** membranes were eventually placed back into water, they returned to their initial swelling, showing a reversible behaviour. However, **M(B)** membranes remained highly swelled after being returned to water. This histeresis is explained by the drastic basic/acid treatments experienced by **M(B)** membranes which may have caused irreversible changes leading to amore loose network structure.

Permeability of the membranes. The permeability of the CHI/CMC PEC to Phe in two different media, acid and basic, was studied for two membranes prepared at different pH (designed as M1 and M2) which as a result of this exhibited different initial maximum swelling. The variation of the swelling degree of the membranes in both media was simultaneously followed.

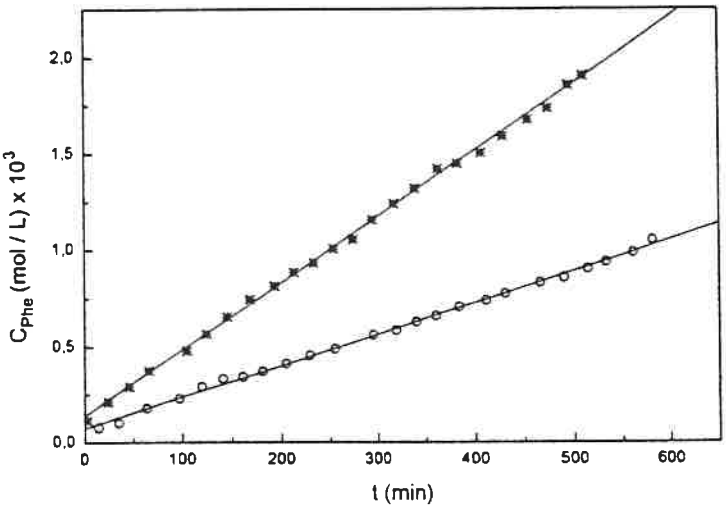

Fig. 7 *Phenylalanine concentration in the internal compartment of the transport cell as function of time for membrane M1 in (*) acid and (o) basic media.*

The evolution in time of the amount of Phe passing through membrane M1 for the two pH values studied is shown in Fig. 7. It becomes clear that the membrane immersed in acid solution possess a higher permeability to Phe than the membrane in basic medium. This is in agreement with the swelling of membrane M1 in these media, since the membrane in acid pH has a greater swelling (W = 6.0) than in basic pH (W = 4.5). The greater pore size of the membrane at lower pH, facilitates the diffusion of Phe.

The permeability coefficient, *P*, of the membranes was determined on the basis of the expression derived for the pseudostationary diffusion across a membrane in a closed transport cell as described elsewhere [6,7]. The *P* values obtained for the membranes studied are reported in Table 1. The permeability is higher in the membrane with greater initial swelling (M2), as a result of its greater pore size. The values found for the permeability coefficients are comparable to those reported for polyacrylic membranes used in the controlled release of insulin [8].

$CaCl_2$ ($[Ca^{2+}] = 0.001$mol/L) was added during one permeability experience. A decrease in the permeability coefficient from 3.15×10^{-6} to 1.8×10^{-6} cm^2/s, with the simultaneous decrease in swelling was obtained when the addition was performed in basic medium. However, when the addition took place in acid medium

no change was observed in the swelling degree of the membrane nor in its permeability coefficient. This once more corroborates the effect of calcium on the free carboxylate groups of the PEC, since in acid medium they are not dissociated, and therefore do not interact with Ca^{2+}.

Table 1. *Permeability to Phe of two CHI/CMC PEC membranes.*

Membrane	W* (g H_2O/g PEC)	P_{HCl} (cm^2/s)	P_{NaOH} (cm^2/s)
M1	2.7	2.51×10^{-6}	1.19×10^{-6}
M2	5.0	3.14×10^{-6}	2.08×10^{-6}

**These values correspond to the maximum swelling state in water.*

Conclusions

The water uptake capacity of the CHI/CMC PEC membranes is susceptible to changes in the pH of the medium, especially at low and high pH values (pH<3; pH>11). The contraction of the membranes induced by Ca^{2+} ions can be explained by the development of new crosslinks resulting from the formation of Ca^{2+} complexes with fixed ligands of the network. This variation of the swelling degree with pH and Ca^{2+} concentration can be utilised for controlling the permeability of the CHI/CMC PEC membranes.

Acknowledgements

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Medical and veterinary applications of chitin and chitosan

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Summary. The applications of chitin and chitosan to wounded human tissues has been experimented only in recent years. The advantage over traditional dressings is the regular histoarchitetcture of the regenerated tissue and the absence of visible scar. The beneficial effects of chitins and chitosans in the healing of damaged tissues in humans and animals derive from the hydrolytic activities of certain enzymes, mainly lysozyme and N-acetylglucosaminidase, present in the body fluids.

Introduction

Chitin based tissues are absent in the human body, but N-acetylglucosamine, the repeating unit of chitin, and chitobiose are present in glycosaminoglycans and glycoproteins [1].
In many situations the administration of chitin derivatives to the human body would potentially be helpful in alleviating disease, preventing sickness or contributing to good health. Modified chitins have actually been administered to humans in form of dressings for wounded soft and bone tissues, anticholesterolemic dietary foods and items for the controlled delivery of drugs. Moreover antibacterial, antimethastatic, antiuricemic, antiosteoporotic and immunoadjuvant activities are reported in recent literature [2-7].
The availability of biodegradable and non toxic materials capable of activating host defences to prevent infection and to accelerate the healing of the wound is desired. Several pre-clinical studies on chitin and chitosan biomaterials show that they are endowed of biochemical significance not encountered in cellulose, starch and other polysaccharides [8]. The biological roles of chitosans applied to human tissues are summarised in Table 1.

Preparation of biomaterials

Chitin-based products for wound dressing include regenerated chitin powders, chitin non-woven fabric (NWF), porous beads, lyophilised soft fleeces, gel-forming lyophilised soft fleeces and gauzes, laminated sheets, transparent films, microspheres and associations with other polymers such as cellulose, collagen, keratin, chondroitin sulphate, polyester, poly(tetrafluorourethylene), polyurethane and polyethylene terephtalate. These products are sterilised with ethylene oxide gas or by γ-irradiation. Chitin and chitosan products are shown in Tables 2 and 3, respectively.

Ordered reconstruction of soft tissues

In general chitin based products provide improved healing of surgical wound by first intention in all cases. Activated macrophages and fresh neutrophils are more abundant than in the controls; fibroblast activation is also intense, and in no case suppuration or

microbial proliferation was reported. Vascularisation and the presence of regular cellular elements provide smooth scars.

The hydrolytic actions of lysozyme and N-acetyl-β-D-glucosaminidase, which make available chitooligomers capable not only of macrophage activation and favourable influence on collagen deposition but also of being incorporated into extracellular matrix components, are the key factors which explain the activities exerted by chitosans in the rebuilding of physiologically valid tissues. Hyaluronic acid synthesis in fresh wounds seems to be enhanced, with the consequence of reducing the risk of scar formation and related complications such as keloids, intraperitoneal adhesion and intestinal strictures [9]. In fact, recent evidence points to the presence of DG42 protein (a chitooligomer synthase) during embryogenesis, thus producing chitooligomers capable to act as primers in synthesis of hyaluronic acid. Overexpression of DG42 in mouse cells gives the synthesis of chitooligomers; hyaluronan synthase preparations also contain chitin synthase activities. On the other hand most preparations of hyaluronan have chitooligomers at their reducing end core region. Thus chitooligomers would act as templates for hyaluronan synthesis (Fig. 1).

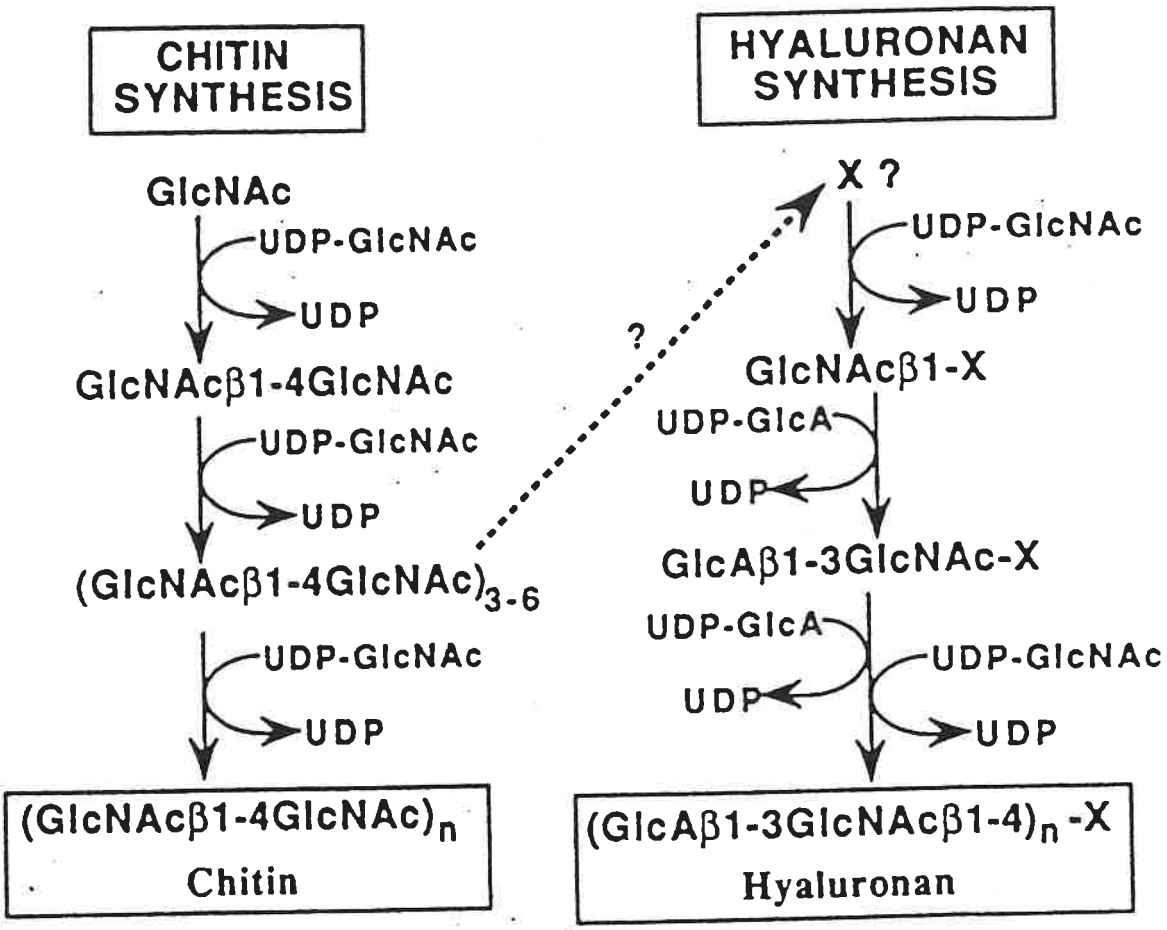

Figure 1. The known and proposed biosynthetic pathways for hyaluronan and chitin highlight the similarities and differences. The X indicates the currently unknown primer for hyaluronan synthase action. The dotted line indicates the possibility that small chitin oligosaccharides might act as primers for hyaluronan synthesis. From A. Varki, *Proc. Natl. Acad. Sci.* USA, 93, 4523-4525 (1996).

Various chitosans were found to induce TNF-α production from human monocytes [10]. Their ability to induce TNF-α was dependent on their molecular weight. Lipopolysaccharides and water soluble chitosans recognise a binding site on monocytes

Table 1. BIOLOGICAL ROLES OF CHITOSANS APPLIED TO HUMAN TISSUES

* **Ordered reconstruction of the connective tissue**
  - osteoinduction
  - healing of meniscal lesions
  - healing of ulcers
  - healing of surgical wounds and donor sites
    - neoangiogenesis and opposition to the regression of vascularization
    - correct assembly and orientation of collagen fibrils
* **Immunostimulation**
  - stimulation of macrophages : production of interferon, TNF and interleukin
* **Stimulation of migration of stromal cells, including fibroblasts**
  - chemotactic action
* **Growth factor entrapment**
  - molecular recognition
  - lectin-type activity of TNF
* **Enzymatic biodegradability**
  - hydrolytic depolymerization to oligomers by lysozyme and lipase
  - hydrolysis to monomers by N-acetylglucosaminidase
* **Rebuilding of the extracellular matrix**
  - N-acetylglucosamine
  - glucosamine
* **Mucoadesion and increase of the epithelial permeability**
  - molecular recognition
  - reaction with sialic acid units in glycoproteins
  - specific interaction with apical membranes of the epithelial cells
  - tight-junction opening
* **Antimicrobial activity-**
  reaction with teichoic acids, and formation of polyelectrolyte complexes
  - chelation of metals present in metalloenzymes
  - alteration of the bacterial adhesion
  - inhibition of the enzymes that link glucans to chitin
* **Dietary significance**
  - anticholesterolemic activity
  - antiulcer agent
  -contol of overweight

Table 2. APPLICATIONS OF CHITIN BIOMATERIALS

FORM	VETERINARY CLINICAL CASE
Cotton	Abscess, bite wound, countused wound, decubitus ulcer
Sponge	Decubitus ulcer, abscess, arthitis, Contused wound, Surgical dead space (Tumor resection, inguinal ernia, lacerated wounds, alveolitis), Maxillosinusectomy
Composite with NWF	Fetlock deformity, skin defect, Prosthesis of subcutaneous tissue
Powder	Contused wound, bite wound, Surgical wound, excision of a keratosis ulcer, trauma, amputation
Film	Dermatomed wound, Fresh burn, artificial skin

Table 3. APPLICATIONS OF CHITOSAN BIOMATERIALS

FORM	CLINICAL CASE
PLAIN	
Cotton	Abscess, Bite wound, Countused wound, Lacerated wound, Gangrenous mastistis, Surgical infection, Decubitus ulcer
Sponge	Experimental surgical wound
Composite with NWF	Contused wound, Skin defect
Fine Powder	Mastitis, Abscess, Contused wound, Lacerated wound, Bite wound, Mammary tumor, Stomatitis
Film	Experimental surgical wound, Cornea injury
MODIFIED	
Chitosan solution	Experimental surgical wound, Burn, Dermatitis
5-Methyl pirrolidinone chitosan	Bone, Decubitus ulcer, Dermo-epidermal explant
N-Carboxybutyl chitosan	Meniscus
Dicarboxymethyl chitosan	Bone

which involves CD14, a receptor of lypopolysaccarides present in monocytes and macrophages. These data suggest that CD14 receptor involved in cytokine production recognises chitosan probably due to its similarity with the saccharide portion of lipid A notwithstanding the 1-6 anhydroglucosidic bond (instead of 1-4 for chitosan).

Skin substitutes [11-13] and materials for nerve and meniscus regeneration were developed [14-18].

Biomedical efficacy on the wound microenvironment

Mesenchymal cells: Mesenchymal cells in the presence of chitosan create a three-dimensional network where each compartment is well-represented. These elements have generally undifferentiated aspects and sometimes show an endothelial attitude [19].

Anti-inflammatory action: Chitin accelerates the first phase of wound healing where inflammation is accompanied with infiltration of mononuclear and polymorphonuclear cells without any uncomfortable side effects such as high temperature and dolor [20]. The observation of enhanced local antiphlogistic activities by administration of chitosan were explained by the data on the activation of inflammatory cells [21].

Polymorphonuclear (PMN) cells: In vivo histological studies on animal models are highly suggestive of the fact that the chitosan fibres constitute a stimulant for migration of PMN and mononuclear cells [21]. Transmission electron microscopy identifies the presence of many leukocytes in the specimens after 14 day post-implantation, showing poor healing processes (i.e. fibroblast proliferation and collagen deposition) that characterise tissue repair at this time in an animal model [19].
In order to clarify the effects of these polysaccharides on animal PMN the cell migration and the chemiluminescence tests clearly indicated that PMN cells interact with these preparations by opsonisation with serum complement [22] and suggest that there are no receptors interacting with N-acetyl-D-glucosamine (GlcNAc) and D-glucosamine monomers on the surface of PMN cells [23].
In vivo, after the application of chitin or chitosan to a wound it has been frequently observed a moderate amount of exudate on the wound surface [21]. This may indicate a signal of good response for a wounded body, because the chitin or chitosan particle opsonised by exudate enhance the phagocytic activity of PMN cells.

Macrophages: Peritoneal macrophages obtained from some laboratory animals are activated by chemically modified chitin [24-26]. Chitosan activate macrophages for tumoricidal activity and for the production of interleukin-1 (IL-1). Moreover, chitosan shows immunopotentiating activity which is desirable for drug carriers to be administered to tumour bearing host, whose immunities are depressed [25]. Chitosan has an in vivo stimulatory effects on both macrophage nitric oxide (NO) production and chemiotaxis [27]. Nitric oxide synthase catalyses the conversion of L-arginine to L-citrulline and nitric oxide [28]. Inducible nitric oxide synthase is widely distributed in mammalian tissues and cells, while endothelial cNOS and neuronal cNOS are constitutively expressed. The NO production catalysed by various isoforms of NOS plays a major role in diverse physiological functions and pathological conditions.
Similarly, cyclooxygenases are inducible and constitutive. The inducible COX-2 is expressed primarily in macrophages, endothelial cells, fibroblasts and smooth muscle cells. Both enzymes are capable of producing active oxygen intermediates: superoxide, O_2^- may interact with NO to form peroxynitrite, ONO_2^-. Taken together inducible COX-2 and NOS-2 may have deleterious effects (up to the shock), therefore specific drugs about inflammation and tissue injury are being studied (enzyme inhibitors).

In consideration of the high reactivity of chitosan (a primary amine) with NO and ONO_2^-. leading to depolymerisation of the biopolymer and stabilisation of the reactive oxygen species, it may be speculated that these enzymes and products are involved in the wound treatment with chitosans [29].

Effect on angiogenesis and on endothelial cells : Morphological examination performed at the site of lesion administered with chitin showed an increased vascularization during wound healing [24]. On the histological appearance in chitin/NWF composite implanted sites the newly formed granulation tissue around the chitin/NWF composite actively invaded the composite with new blood vessels, but not in the control [21,31].

Cytokines: It is well known that the granulation is accelerated by interleukin-1 (IL-1), Tumor Necrosis Factor-alpha (TNF-α) or Fibroblast Growth Factor (FGF) and suppressed by interleukin-4 (IL-4) or Tumor Necrosis Factor-gamma (TNF-γ) released by macrophages, lymphocytes, fibroblasts [32]. In particular IL-1 e TNF-α produced by macrophages [33] are known to activate fibroblasts [34]. Peritoneal macrophages obtained from laboratory animals are activated by chitin [24,25]. The amount of IL-1 in the exudate taken from around the areas where chitin-NWF was implanted increased two fold in comparison with that of control medium [31].

Fibroblasts, collagen production and tensile strength: Scar formation which depends on both continued synthesis and catabolism of collagen is a serious problem in the wound healing process [35].
Fibroblast proliferation increases at the site in contact with chitin sponge [20]. A mild fibroblasts activation is observed in the neighbouring area around the fibres after implantation of chitosan in experimental animals [21] and minimum scar formation remains in the wound. Several reports documenting an acceleration of tensile strength in the presence of chitin [36,37] suggest that N-acetylglucosamine may serve as substratum for a reinforcement of wounded tissues without excessive inflammatory reaction, because tensile strengths were significantly accelerated without an increase in collagen synthesis [38]. However, recent studies show that in the chitin treated lesions many histiocytes invaded the wounds and fine collagen fibres were produced. Histiocytes might be induced by chitin and might promote the proliferation of fibroblast which produce fine collagen [39], little histiocyte invasion and thick collagen fibres were observed in the controls [40]. Therefore, in the chitin treated wounds synthesis of collagen will accelerate in the early wound healing process, but synthesised collagen will be degraded very conveniently to an appropriate amount in the subsequent healing stages. The degradation of collagen is initiated by a variety of collagenases from granulocytes [41], macrophages [42], epidermal cells, neutrophils and fibroblasts [35,43]. Since inflammatory accumulation at the site of contact with chitin sponge is observed, the excess collagen may be degraded by this chitin-induced inflammatory cells [44].
Chitosan could be considered a primer on which a normal tissue architecture is organised. Collagen fibres shows a clear tendency to maintain a well defined orientation. The progressive deposition of collagen fibres starts from the proximity of fibroblasts that seem to guide the extracellular-oriented deposition of these fibres [19] .

Granulation: Granulating tissue can generally be divided into two types, healthy and unhealthy granulating tissue [45]. The healthy one develops only in the absence of foreign bodies (bacteria, debris and so on) and its formation, closely related to angiogenesis, is a very important factor in wound healing [35]. As chitin was found to promote the formation of granulating tissue with angiogenesis, chitin could induce healthy granulating tissue in the early stage of wound healing. In the case that granulating tissue did not develop, general conditions were serious and contamination of the wounds severe [44].

However, the effect of chitin on the mechanism of the formation of granulating tissue are still unclear.
Chitosan provides a non-protein matrix for three dimensional tissue ingrowth and is able to promote tissue growth and differentiation in tissue culture [46]. Furthermore chitosan was used as a treatment for subcutaneous infections including cutaneous erosion and ulcerations and for the acceleration of granulated tissue formation. Chitosan was superior to chitin in its effect on the acceleration of granulation tissue [21].

Epidermisation and keratin production: In the treatment of human burns chitin acts as an excellent wound remedy for pain, good epidermisation without scar and any functional disturbances [44,47]. In the treatment of purulent digit disease with chitosan epidermisation was also observed with a minimum convalescence periods without antibiotic administration [21,48].
Chitin might promote keratin production and facilitate rapid and effective regeneration of oral mucosa [39].

Immunological and antibacterial activity: Chemically modified chitins including partially deacetylated and carboxymethlyated chitins were found to have potent immunological activities [24-26,48].
The protection of the host against bacterial infection is stimulated by chitin [48]. The effectiveness of chitosan bacteriostatic properties were tested against bacterial strains and a common skin fungus. Powdered chitin, chitosan or whole crab shell were not effective in any of the tests, but solution of chitosan in acetic acid inhibited the bacterial strains and the fungus [49]. The mechanism underlying the inhibition of bacterial growth, is though to be that the cationically charged amino-group may combine with anionic components such as N-acetylmuramic acid, sialic acid and neuraminic acid, on the cell surface, and may suppress bacterial growth by impairing the exchanges with the medium, chelating transition metal ions and inhibiting enzymes.

Degradation: It has been shown that the effect of treatment of burns with either low or high molecular weight chitosan is statistically different from the no treatment situation [50]. It is also clear that the size of the chitosan molecule plays some role with the low molecular weight being the most effective. The chitosan macromolecule acts as a controlled sources of low molecular weight amino-sugar then the release rate is inversely proportional to the molecular weight [51]. There is of course, evidence that D-glucosamine has a minor effect on the rate of healing of surgical incisions [52].
In many surgical tissue defects, it is desirable for the material buried to be biodegradable and to be replaced by native organisms. Chitin is degraded by some enzyme such as lysozyme [4] and chitinase [8,52].
Lysozyme plays the major role in the degradation of chitin in vivo: oligomers are further hydrolysed to GlcNAc, a common aminosugar in the body, which enters in the innate metabolic pathway to be incorporated into glycoproteins or to be excreted as carbon dioxide [11]. In clinical use, a rapid degradation of chitosan has been experienced in the treatment of purulent lesions and trauma. The difference between the clinical and experimental implantation is whether the inflammatory reaction has already started or not. The mechanism of chitosan degradation in animal tissue is unknown, but wound exudate might be an important triggering factor [21].

Regeneration of bone tissue
Several studies dealing with the reconstruction of the periodontal tissue with chitosan were a prelude to the discovery of the osteoinductive property of chitosan [53,54]. Surgical wounds from wisdom tooth avulsions were treated with freeze-dried methylpirrolidinone chitosan which promoted osteoinduction. Methylpirrolidinone chitosan was progressively

depolymerised by lysozyme and was no longer detected 6 months after surgery. Methylpirrolidinone chitosan was found useful in apicectomy as well. None of the patients reported adverse effects over 3 years of observation [55].

The existence of osteoprogenitor cells with the capacity to produce bone in the wound site, offers the possibility of regenerating the periodontal, peri-implant and alveolar ridge bone tissue simply with the aid of chemical mediators from chitosan.

Bone forming colonies were identified histochemically and found to be 6.2 ± 1.2 colonies per well in the presence of chitosan (3.6 ± 0.6 in the controls); chitosan therefore potentiates the differentiation of osteoprogenitor cells and facilitates the formation of bone. Chitosan may function as a substitute that enhance osteoblast differentiation and migration [56].

Bone defects surgically produced in sheep and rabbit models were treated with freeze-dried imidazolyl chitosan and methylpirrolidinone chitosan [55-59]. The histological examination performed 60 days after surgery showed a considerable presence of neoformed bone tissue as compared to control, originating from the pre-existing bone as well as from the periosteum. The cationic nature and the chelating ability of the methylpirrolidinone chitosan apparently favoured the mineralization. Endosteal-periosteal and bone marrow osteoblast-like precursors, stimulated by growth factor entrapped in the coagulum-polysaccharide mixture, gave rise to intramembranous bone formation. Ultrastructural examination showed that bone osteoid formation was followed by mineralization. Osteoinduction was also observed in rabbit endochondral bones [60].

Experimental studies were performed in order to evaluate the possibility of improving bone tissue reconstitution with the association of calcium phosphate with chitosan. Microscopic analysis of the legs treated with this modified chitosan showed the presence of an osteogenic reaction moving from the rim of the surgical lesion toward the centre. The surgical hole was completely occluded and in the core of the defect only mesenchymal-type tissue was observed. This central fibrous area was irregularly bordered with neoformed trabeculae. In control legs, dense fibrous tissue surrounded the surgical lesion, and a blood coagulum was observed. Macroscopic analysis evidenced an irregular rimmed area smaller than the surgical defect, filled with a tissue without histoarchitectural characteristic of bone tissue. In control femurs the hole had not changed much in shape and size since surgery and the area lacked in bone tissue.

Finally, a study, carried out in an osteoporotic experimental model, was performed in view of the evaluation of the pattern of bone regeneration in the presence of BMP linked to chitosan. This association is intended for the synergistic potentiation of the respective biological effects BMP was released from the chitosan matrix where it has been incorporated as a consequence of the biodegradation of chitosan. Morphometric and morphological analysis shows that the association. of BMP with chitosan improves the bone tissue regeneration in a surgical bone defect This result seems important insofar it shows the validity of the biochemical approach to the therapeutical correction of various affections in the elderly, such as osteoporosis [61].

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APPLICATIONS OF CHITIN AND CHITOSAN AS FIBER AND TEXTILE CHEMICALS

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Abstract

U.S. consumers spend $250 billion (US) annually on textile goods. Americans purchase one billion pair of trousers, 2.4 billion shirts and blouses and 450 million sweaters each year. Whereas the world fiber per-capita consumption is 7.4 kg, the U.S. per-capita consumption of fibers is 35.4 kg. The US textiles complex purchased $13 billion (US) in chemicals, fibers and dyes in 1996. These activities represent significant opportunities for the application of chitin and chitosan as textile chemicals. A number of uses for chitin and chitosan in the textile industry have been demonstrated in the US and many other countries as well. These polymers have a number of features attractive for these end-uses. The biodegradability and anti-microbial properties of these materials are well known. However chitin is compatible with a number of fiber forming processes, particularly those employed to produce cellulosic fibers, such as rayon and lyocell. These polymers also have an affinity to cotton and wool and have been employed as surface coatings to alter characteristics such as friction, moisture adsorption, and the dye affinity of these fibers. Chitin and chitosan are also readily converted directly to fibers, but which have primarily been used for special applications such as medical textiles and as component of wound dressings. The use of chitin and chitosan as textile auxiliaries to decolorize textile waste water is discussed.

Keywords: Antimicrobial, auxiliaries, chitin, chitosan, coatings, fabric, fiber, finishes, nonwovens, textiles

Introduction

The textile industry is second to the automotive industry among the basic manufacturing industries of the U.S. Textile goods are considered to be any article composed of fibers, such as apparel, home furnishings including carpets and industrial goods such as tire cord. In order to manufacture these goods, the U.S textile industry purchased $13 billion worth of chemicals, fibers and dyes. The inclusion of chitin and chitosan as specialty chemicals for this industry would seem to hold considerable promise if the economic balance of cost and value-in-use can be made. A wide range of naturally occurring polysaccharides are now commercially available and widely used in the textile industry. Alginates, carrageenan, cellulose, dextrans, pectin, and starch represent a wide range of properties and products. Chitin and chitosan which are also commercially available, but under utilized relative to cellulose and starch, differ from all of the above in an important aspect. This is the quality to exhibit basic rather than acidic characteristics.

This basicity gives chitosan, in particular, its unique properties, which are described in this article. Further, the β-1,4 linkage found in these polysaccharides, readily lends itself to the formation of microfibrils, fibers and films, which have useful mechanical and physical properties as well.

Chitin is considered the second most plentiful organic resource on the earth next to cellulose, and is present in marine invertebrates, insects, fungi and yeasts [1]. Chitin is essentially a homopolymer of 2-acetamido-2-deoxy-β-D-glucopyranose, although some of the glucopyranose residues are in the deacetylated form as 2-amino-2-deoxy-β-D-glucopyranose. When chitin is further deacetylated to about 50% it becomes soluble in dilute acids and is referred to as chitosan.

In order to describe and classify the many applications of chitin/chitosan to textile products and processes, a further description of the textile industry, or more properly, the textile, fiber and apparel complex is necessary [2,3]. U.S. consumers spend more than $250 billion annually on textile goods. These textile goods contained 46.3 billion kilograms of fiber and represented a per capita fiber consumption of 35.4 kg. With only 5 percent of the world's population and 14 percent of the world's textile mill output, the U.S. consumes about 20 percent of the world's textiles and receives more than 21 percent of the world's textile and apparel imports.

Chitin and chitosan have a number of attributes that make them attractive for a wide number of textile applications. Many of these attributes coincide with other applications of chitosan and chitin in the agricultural, biomedical, paper and food industries for example[1]. The proceedings of the various international conferences on chitin and chitosan also review many of these fields [4-6]. The chemical nature of these polysaccharides are well known and was recently described by Roberts [7]. The chemical versatility of chitosan is key to the wide variety of its textile uses. The chemistry of chitosan is similar to that of cellulose but also reflects the presence of a primary aliphatic amine. Many of the chemical processes devised for cellulose are also successful with chitosan. Chitosan reacts readily with carbonyl compounds, such as acylation with acid anhydrides, to form a ride range of ester and amide products. The water solubility or dispersibility of many of the chemical derivatives of chitin/chitosan is necessary in many cases inorder to have an environmentally friendly process.

Mechanical properties such as toughness, flexibility, and tensile strength are typically associated with many textile end-uses. These mechanical properties are frequently found with linear, semi-crystalline and formable polymers such as chitosan. The demonstrated antimicrobial properties of chitosan has also led to the possibility of fibers with potential hygienic and medical textile applications[8]. We can also assume that much of the chitin/chitosan utilized by the textiles complex will be incorporated into consumer goods which are ultimately disposable and then the biodegradative nature of these materials assumes importance.

The application of chitosan as a textile chemical is conveniently categorized into three topics: the primary production of man-made fibers; textile fiber finishes and coatings; and textile chemical auxiliaries (process aids). Textile finishes refer to those compositions applied to the surface of a fiber during a process subsequent to extrusion or to modify the properties of a natural fiber such as cotton or wool. This would also include the coating of fabrics to produce laminated or barrier-type materials. Textile auxiliaries are those chemicals or materials used as process aids during the manufacture of a fiber, yarn or fabric. The production of all textile fibers and the finishing of all textile products

requires the use of textile auxiliary chemicals. Examples of textile auxiliaries applications involving polymeric materials includes viscosity building agents and binders for print pastes. Also the use of dye scavengers during the laundering of textiles or to decolorize process waste water after the dyeing of a textile describes the use of textile auxiliaries.

Chitin and the Production of Man-Made Fibers

There have been extensive investigations of chitin and chitosan as fiber and film formers [9]. These fibers and films could be useful as membranes [10], nonwovens [11], papers [12], medical gauze, sutures and wound dressings [13-15]. Chitosan and chitin are converted to fibers by dissolving the polymer in a spinnable solvent and coagulating the solution in a bath, by the wet spinning process. A number of solvent systems for chitin and chitosan have been described. Ziabicki [16] has provided a general overview of the wet spinning process. Wet spinning involves the extrusion of a spin solution directly into a coagulation bath. The fiber may travel over rollers to be drawn or attenuated in length. One or two additional baths can be used for solvent removal and washing. The dimensions of the spinneret, the composition of the coagulation bath and of the solvent system as well as the draw ratio are the most important spinning parameters.

Thor and Henderson were first in making chitin xanthates [17], the standard technology for the production of viscose rayon from cellulose. The xanthate process involves first the steeping of chitin in cold concentrated sodium hydroxide followed by shredding of the chitin. The shredded chitin is then mixed with carbon disulfide and water. This is followed by extrusion of the now solubilized chitin xanthate derivative. Cellulosic rayon fibers produced by the viscose process have also been modified by the addition of chitin or chitosan. Reports indicate that good anti-microbial properties are imparted to these blended rayon fibers [18]. These antimicrobial properties are discussed below in the Textile Finish section.

Austin suggested in 1975 that a series of organic solvents containing acids are direct solvents for chitin. Such systems included chloroethanol and sulfuric acid or trichloroacetic acid in methylene chloride [19]. A number of patents followed upon this work issued to Unitika Co. (Japan) [20]. The tensile strengths of fibers from these halogenated solvent systems ranged from 1.67 to 3.2 g/den and with elongations of 8.7 to 27.3%. Though these are reasonable tenacities for the dry state, the wet tensile strength of these fibers are undesirable. This may also be a reflection of the copolymeric structure of these polymers, being a mix of N-acetylated and N-deacetylated residues. The distribution of these groups is expected to be essentially random, and hence impede the development of extensive crystallinity in these fibers. It has been shown that chemically cross-linking chitosan fibers improves the wet strength [21].

Another approach to spinning chitosan fibers was to make use of the liquid crystalline nature of chitosan. These liquid crystalline phases have been described as early as 1959 when Marchessault [22] suspended microcrystalline chitin particles. Ogura [23] reported evidence of chitosan mesophases. Chitosan solutions ranging from 30 to 90% by weight in 10% aqueous acetic acid were prepared and observed. Sakurai [24] spun chitosan fibers from lyotropic liquid crystalline solutions. Chitosan was dissolved in anhydrous formic acid to give a 5% wt solution. The solution was allowed to concentrate by evaporation, to 35% wt. The fibers from these solutions had a relatively high density of 1.46 g/cm^3 and a tenacity of 3.8 g/den with a 3.5% elongation to failure and an initial

modulus of 181 g/den.

A third approach to obtaining high value fibers from chitosan involved the spinning of liquid crystalline solutions of chitosan derivatives. In general, polysaccharide esters are easily dissolved in a variety of simple solvents. For example secondary cellulose acetate is readily soluble in acetone. Many cellulose derivatives are well known to form liquid crystalline solutions, which are precursors to high strength fibers, such as the DuPont fiber Kevlar®. The use of acetate esters has been an attractive process. Extensive work has been reported by Tokura and his coworkers on the properties of acetylated chitins and chitosans, [25]. Trends involving the degree of esterification and the tensile properties were demonstrated. However, tenacities ranged upto only 1.8 g/den. A series of patents, though demonstrated that with mixed esters of formate and acetate much higher tensile properties could be obtained [26-29]. These polymers are then dissolved at high solid levels in trichloroacetic acid and methylene chloride to yield liquid crystalline solutions. The as-spun fibers are easily converted back to chitin or chitosan by hydrolysis. Tenacities of regenerated fibers ranged up to 7 g/den with an initial modulus of 194 g/den. These are some of the highest tensile values reported to date for chitin or chitosan filaments.

Another series of useful solvents are based on lithium salts dissolved in amides [30]. Typically N,N dimethylacetamide (DMAC) or N-methyl-2-pyrrolidone (NMP) is used with 5% LiCl. These solvents are very useful for the synthesis of chitin derivatives in an anhydrous system with no competing active hydrogens[31]. Fibers are easily wet spun from this system by coagulation in an alcohol bath, although it is difficult to wash the lithium salt out. Tenacities of upto 4.25 g/den have been reported [9].

The direct spinning of chitosan from aqueous acetic acid has also received considerable attention. Of course, without further treatment these fibers will redissolve at a pH below 5.5. East and Qiu[32] have reported the spinning of these fibers. Tenacities of upto 1.8 g/den have been achieved. While these are not particularly strong fibers, they are easily processed from aqueous solvents and caustic coagulation baths.

Table 1. Qualitative Properties of Sheath Core Chitin Fibers [33].

GROUP	CORE FIBER	REMARKS
Complete and Homogeneous coating	Cotton, rayon,glass and alginate.	High fiber surface bond polarity aids binding
Discontinuous and uneven coatings	Polyester and nylon	Polysester coated better than nylon
Poor binding to core fiber surface	Kevlar, polypropylene,	Loose film only observed over the bundle of core fibers

The formation of sheath core fibers has been described by Allan and Lopez-Dellamary [33]. Many of the contemplated uses of chitin do not involve the use of its bulk properties. Also, the relative high cost and low tensile strength of pure chitin fibers has impeded their commercial development. Chitin coated fibers is an approach to avoid some of these problems. Allan and Lopez-Dellamary [33] prepared an array of coated natural and synthetic fibers by applying chitin to the surface of these fibers from a

N,Ndimethyl-acetamide / lithium chloride solvent system. These fibers were prepared for further evaluation as biomedical nonwoven fabrics, because of the wound healing properties associated with chitin [8]. The coatings ranged from homogeneous with good binding to discontinuous coatings to coatings with little binding to the substrate fiber, see Table 1 above.

Textile Finishes

The birth of a textile good is said to occur when the so-called greige good is taken from the mill and is finished. Finishing is generally considered to be any operation which improves the usefulness of a fabric after it leaves the loom or knitted machine. The art of chemically finishing fabrics to render them more useful or functional is constantly undergoing changes as new materials and methods and needs are developed. Examples of only recent developments in the use of chitin and chitosan as chemical finishes will be discussed here.

Chemical finishes, as applied to synthetic fibers, were developed for several needs. First, non-permanent finishes are applied to textiles during their manufacturer to facilitate their processibility. These are customarily removed during a subsequent scouring step. The largest use of finishes, though, involves the need to over come the limitations of many man-made fibers. The demands of comfort, appearance, ease of wear, washing and processing drives the development of these finish materials for the modification of the relatively few synthetic fiber types, e.g., polyester, nylon, polyolefins, cellulosics and acrylics. These demands are also true for natural fibers (mostly cotton), which still represent 50% of world fiber consumption.

Most finishes are intended to boost fabric weight, smooth fabric surfaces, improve drape, create surface effects and repel water, oil, soil and microbes. Finishes are used most widely on cellulosics, nylons, and the natural fibers such as cotton and wool. General chemical finishes include: softeners; stabilization resins; bodying agents; water repellents; flame retardants; mildewproofers, rotproofers and bactericides; shrinkproofers; anti-stats; and no-soil finishes [34].

Chitosan has many of the necessary chemical qualities to function as a textile finish. For example a number of authors describe the sorption-desorption behavior of chitosan on to fibers such as cotton and wool inorder to modify surface properties. It should also be evident that a polymer such as chitosan may simultaneously satisfy several functions as a textile finish.

The use of chitin and chitosan as antimicrobial finishes and additives to fiber and textile products has resulted in a number of recent patents and reports. Closely related to this antimicrobial property is the wound healing acceleration of chitin [35]. Both of these properties are apparently related to the release of soluble deoxy-amino sugars from the chitin/ chitosan matrix by enzymatic hydrolysis and to the binding properties of polycationic chitosan with cell walls and enzymes. The suggested uses for these fibers includes: wound dressings [36], medical textiles [37], sanitary absorbents [38], non woven cellulosic fabrics [33], the treatment of fiber materials for skin protection and deodorant effects on synthetic fibers [39,40], on cotton [41] , on polyesters and polyamides [42], and on wool [43] have recently been reported.

The antimicrobial activity of chitosan is also enhanced by combining it with other bactericides. For example, the use of silver lactate [44], silver ceramics [45], metal oxides

of Group Ib, IIa, IIb, IIIb and/or IVb elements[46] and with iodine [47] is described. In fact, the chitosan-iodine complex was reported to be more effective than the widely used bactericide betadine (povidone-iodine), plus provide a gradual timed release effect.

The antimicrobial activity and its mechanism was recently discussed by Ueno et.al. [48]. They studied several of the mechanisms that have been proposed for the antimicrobial activity of chitosan. The mechanisms they cite are: a reduction of bacterial metabolism by the adsorption and stacking of chitosan polymer chains on the bacterial cell wall and the blockage of DNA transcription by chitosan. Interestingly the antimicrobial activity against E. coli was greater for the high molecular weight chitosan oligomers, while the low molecular weight fraction exhibited little antimicrobial activity.

The finishing of wool fibers was recently reported by Julia et.al [49]. The presence of chitosan on wool has been demonstrated to improve dyeability, colorfastness and also contribute to the shrink proofing of this type fabric [50,51]. They noted that polymers and surfactants are often used together in industrial formulations to make the most of their different properties. In the case of chitosan, which can form aggregates with surfactants [52], Julia et.al. [49] demonstrated that the absorption of surfactant was strongly influenced by the presence of chitosan in the bath.

Masri et.al. [51] reviewed and evaluated the deposition of chitosan on wool as a means of shrinkage control. Interfacial deposition of polymers on wool fabric to cover the fiber scales is an accepted method of imparting laundering shrinkage resistance. Their work demonstrated a one step application process using a chitosan solution containing either blocked cross-linkers or a slow reacting unblocked cross-linkers. The choice of cross-linker influences the cure conditions and kinetics, giving some flexibility to adapting the process to existing equipment.

The wrinkling of cotton and other cellulosic fibers is considered an aesthetic problem and extensive work on the cross-linking of cellulose has been carried out. Typically methylol derivatives of ethylene/urea compounds are used with Lewis acid catalysts. These systems typically result in damaging and weakening of the fibers though. Adding chitosan to these compositions was reported to minimize this damage for the typical pad- bake application of these finishes [53].

In the manufacture and dyeing of cotton fabrics, many dyeing problems are encountered [54]. In many cases, the cotton does not absorb dye uniformly which creates small lightly colored, almost white spots. This is the result of the presence of small groups of immature cotton fibers, known as neps. Mehta and Combs [54] and Rippon [55] evaluated the deposition of chitosan in solution by exhaustion methods onto cotton. This pretreatment of cotton prior to dyeing resulted in the covering of the neps by direct dyes and reactive fixable Indosol dyes. The pretreatment was not as effective for reactive dyes, though.

Coated fabrics with high water vapor permeability but also waterproofness are of considerable interest to consumers. The key to this interest is the comfort such a "breathable" fabric has. Hydrophillic block copolymers of polyurethanes/polyethers are widely used for coating fabrics. Yen and Cheng [56] have described the effects of adding chitin to polyurethane coated fabrics. Their results showed that adding chitin powder to the hydrophillic polyurethane resin (PTMG-PEG/MDI/1,4BD) resulted in an increase in the water vapor permeability of such coated fabrics. The addition of 3% w/w chitin into the coating led to a 73% increase in water vapor permeability. However, large decreases in tensile strength and strain to failure were observed. Chitin was also reported as an

additive in coated fabrics by Asahi Kasei Co. [57]. Their research indicated that polyamino acid modified polyurethane containing chitin had better water vapor permeability, antimicrobial properties and gave a better hand to the coated fabrics. It should also be noted that polyurethanes are one of the few synthetic polymers to mildew, as the urethane linkage also occurs in nature.

Chitosan as a Textile Auxiliary

Textile auxiliaries are those chemicals used as process aids involved in the manufacture of any textile product. The production of fibers and filaments frequently requires the use of lubricants and fiber stabilizers. The compositions employed as textile finishes are frequently blends and reaction products involving many components. Salts, acids, bases, surfactants, polymers, polyelectrolytes, bleaches are but a few examples.

There is an extensive literature on the interactions of chitosan with dyestuffs. See, for example Parisher et.al [1] and Kim et.al. [58] for lists of references. The well known affinity that chitosan has for textile dyestuffs has primarily lead to the consideration of chitin and chitosan as scavengers for fugitive dyes and the decolorization of process waste water.

Textile wet processing operations produce high volumes of effluent waste water of varied composition, often containing salt plus organic surfactants, solvents and dyes. Textile effluents usually contain only very small amounts of dye, however they are highly detectable. Since water is a critical and expensive resource in many regions many methods are been developed to remove color. Several difficulties are encountered in the removal of dyes from wastewaters. By design, dyes are highly stable molecules, made to resist degradation by light, chemical, biological, and other exposures. Commercial dyes are usually mixtures of large complex and often uncertain molecular structures and properties. The physical adsorption of these colors has the advantage of not generating toxic side-products or utilizing toxic reagents such as ozone or chlorine. A number of workers have evaluated the dye absorption performance of chitin and chitosan [59-61]. Chitosan has a high affinity for a wide range of dye classes. In fact, the only class of dyes chitosan has low affinity for are the cationic basic dyes. In particular, many of the dyes designed for cellulose, such as the directs and reactive dyes, have a very high affinity for chitosan. This is explained by noting that the repeat structure of chitosan is very nearly that of cellulose for which these dyes were designed and that many of these dyes carry acidic groups, particularly the sulfate group, in order to solubilize the dye.

The success of chitin and chitosan in this application will be dependent upon a number of factors. Key will be the availability of a low cost "technical grade" chitosan that will be competitive with other low cost adsorbents such as carbon black. The disposal of the spent adsorbent must also be considered. There are some situations where process water is recycled by means of ultrafiltration and reverse osmosis. In these cases the concentrate would be quite advantageous for decolorization, due to its high dye content. Much work can yet be done to create greener textile processes by focusing on source reduction of color and improved waste treatment.

An interesting consumer product is described in a recent patent for a laundry aid which will scavenge fugitive dyes during laundering [62]. Frequently, new textile garments tend to lose dyes not fixed to the textile, during the first several home launderings they undergo, which may stain other items in the wash. A laundry aid was prepared by

coating a wood pulp/polyester fabric with a finish containing chitosan and other dye transfer inhibitors. When this article is added to the laundry it then prevents these fugitive dyes from staining other fabrics that may be present.

Another laundry aid, prepared by immobilizing cellulase enzymes in a chitosan gel has been described [63]. Cellulase enzymes help remove small lint particles from the surfaces of cellulosic fibers, which form from abrasion during wear. The presence of these small fibrils on the fibers tends to make them dull in appearance. By immobilizing the enzymes it was possible to make a product which is conveniently added to a home laundering machine. These enzymes may also act as fabric softeners.

Chitosan was also recently investigated as an auxiliary in a print paste [64] capable of producing high dyestuff fixation of reactive dyestuffs on rayon. When chitosan was added in small amounts to urea based print paste, it was then possible to greatly reduce the urea content of the paste. The addition of 2% w/w chitosan to the paste allowed the urea content to be reduced from 30% to 7.5% on weight of the paste. Cyclodextrins were also found to produce a similar effect.

Conclusions

Clearly, many applications of chitin and chitosan as textile chemicals have been demonstrated to be technically feasible. Many of these applications have been or will shortly be commercialized. However, the textile chemical industry's use of polymeric materials is dominated by a number of very large volume and inexpensive materials. Some are natural based materials such as cellulose and starch, but a large number are inexpensive petro-chemical based compositions. By copolymerizing a variety of monomers, synthetic polymer properties are cheaply and easily customized, creating a strong business position for these materials.

Studies must continue to determine high value-in-use applications for chitin and chitosan. The anti-microbial, biodegradable and dye affinity properties of these materials appear to lead to the most promising applications at this time. As chitin and chitosan become more readily available as commodity, bulk chemicals many other economical applications will undoubtedly arise.

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