THE USE OF CHITOSAN ON WOOL SHRINK-RESISTANCE

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Abstract

Due to the natural propensity of wool to felt or shrink when agitated in an aqueous or moist environment, shrink-resist finishes have become essential for wool garments, but traditional methods (chlorine+ resin) are no longer environmentally acceptable.

Chitosan, possible substitute of synthetic polymers in wool finishing, can be basically sorbed on wool by means of ionic bonds. However, under the conditions where chitosan is water soluble (pH < 6), the wool surface acquires a predominant positive charge which tends to reduce the attraction for chitosan.

To promote the formation of new anionic groups on wool to enhance the subsequent chitosan sorption, two different pretreatments have been carried out:
A) hydrogen peroxide under alkaline conditions, and **B)** sorption of an anionic surfactant (sodium lauryl sulphate).

Shrink-resist properties of wool treated with chitosan are influenced by the pH of the hydrogen peroxide pretreatment as well as by the sorption of the sodium lauryl sulphate in the range of pH below 6.

Keywords: Chitosan, wool, shrink-resistance, AOX, hydrogen peroxide, anionic surfactant.

Introduction

Since chlorination is known to produce substantial pollution with adsorbable organo halogens (AOX) in textile waste water (1), shrink resist treatments, like the widely used chlorine/Hercosett process, are no longer environmentally acceptable and accordingly has to be appropriately substituted. Moreover, the current trends towards the employement of natural products have focused the attention to the natural polymers.

Chitosan, which is water soluble at pH < 6.3 in the form of cationic polyelectrolyte, could be regarded as a substitute of synthetic polymers in wool finishing. However, in this range of pH wool acquires a predominant positive charge which makes difficult the subsequent electrostatic attraction between chitosan and wool.

Therefore, before chitosan treatment, it would be convenient to increase the presence of anionic groups in wool surface in order to enhance chitosan sorption. This could be achieved through either **A)** an oxidative treatment with hydrogen peroxide to promote the formation of cysteic acid in the wool or **B)** by a treatment of wool with an anionic surfactant (2).

Traditionally, wool has been hydrogen peroxide bleached under alkaline conditions but the development of a weakly acidic activated hydrogen peroxide bleaching system, permits effective bleaching time and less fiber damage (3).

It is well kown that when wool is treated in aqueous solution in the presence of an anionic surfactant, the sorption of the surfactant is highly dependent of the pH of the bath (4). At acid pH carboxilic and basic groups are protonated and the wool fibre exhibit an increasing electropositive charge which permits the electrostatic link with the anionic

surfactant. At alkaline pH wool acquires a global electronegative charge and the sorption of the anionic surfactant takes place by means of hydrophobic interactions (5).

In order to enhace the sorption of chitosan, the aim of this work consists of to modify previously the surface charge of the wool fibre by means of either an oxidative pretreatment or the sorption of an anionic surfactant. In this paper the study of the influence of the pH of the pretreatment bath (**A:** hydrogen peroxide or **B:**anionic surfactant) on the shrink-resist properties ow wool subsequently trated with chitosan, is presented.

Materials and methods

Wool: Knitted wool fabric, cover factor 1.28(tex/mm), supplied by IWS.
Chitosan (Chit): low molecular weight, Mr 70,000, supplied by Fluka, was used without further purification.
Hydrogen peroxide: by exhaustion at a liquor ratio 30:1, 18ml/l H_2O_2 33% w/v.
Sodium lauryl sulphate (SLS): by exhaustion in aqueous solution at a liquor ratio 50:1, 50°C for 120 min. SLS concentration in the treatment bath: 0.3% w/v.
Chitosan Treatments: Chitosan was dissolved in distilled water containing acetic acid (3g/l). Treatments were carried out by exhaustion. Liquor ratio 20:1, 25°C, 60 min. Treated samples were let to dry at room temperature.
Staining: a staining technique to obtain a qualitative assessment of the presence of chitosan in wool fibres was used. Chitosan trated wool samples were stained with the dye C.I.Reactive Red 180 at a liquor ratio 60:1 at 50°C for 5 minutes. Then, samples were rinsed with water and allowed to dry in air.
Microscope observations: wool samples were observed in a Polyvar 2 transmission light microscope.
Felting shrinkage: IWS Test Method 31. After 2 cycles 5A on a Wascator FOM 71 washing machine. When the area shrinkage is less than 8% , wool can be considered as "machine washable".
Wetting time: was measured as the time for complete immersion of a fabric sample (2cmx2cm) dropped flat onto the surface of distilled water.
SLS content in the treatment bath was determined by the two-phases titration method (6).

Results and discussion

A) Hydrogen peroxide pretreatment

It is well kown that when wool is treated with H_2O_2 , either at alkaline or acidic conditions, the oxidation of the disulphide bonds takes place giving rise to cysteic acid groups which increase the presence of anionic groups on wool. Thus, the adsorption of the chitosan on wool could be enhanced and consequently the shrinkage of wool could be avoided.

The influence of the type of the hydrogen peroxide activation (alkaline or acid) on the shrink-resistance and wetting time properties, was determined on wool only pretreated with hydrogen peroxide and also on wool pretreated with hydrogen peroxide and then treated with 1% chitosan. The results are shown in Table I. For comparative purposes wool samples with any kind of treatment (untreated) and samples only treated with chitosan were also included in this table.

Table I.-Influence of the pH of the H_2O_2 pretreatment on the area shrinkage measured on wool treated with chitosan, after one and two washing cycles (1c and 2c) and on the wetting time (W.T.)

H_2O_2 TREATMENTS	SHRINKAGE (%)		W.T. sec
	1 c	2 c	
pH 9.0, 60°C, 2h	17	34	62
id+1% Chit.	1	4	11
pH 9.0, 70°C, 1h	12	32	51
id+1% Chit	5	10	9
pH 5.5, 50°C=>80°C, 1h	30	47	>900
id+1% Chit	28	44	28
Untreated wool	27	50	>900
id+1% Chit	20	36	122

The oxidative pretreatment at **alkaline pH**, confers a small degree of shrink-resistance to wool, but the subsequent presence of chitosan provides shrink-proofing properties.

The **alkaline H_2O_2 pretreatment** confers a certain hydrophilic character to wool (low W.T. values). The subsequent presence of chitosan increases the hydrophilicity on wool surface.

In order to assess the presence of chitosan in wool, **microscope observations** of cross-sectioned fibres, previously dye stained, show that chitosan diffusion takes place only when fibres have been pretreated with H_2O_2 at alkaline pH. However, independently of the kind of the pretreatment, the surface of the wool fibres are partially coated with chitosan.

Table II.-Influence of temperature of the alkaline H_2O_2 pretreatment on the area shrinkage and on the wetting time

TREATMENTS	SHRINKAGE (%)		W.T. sec
	1 c	2 c	
H_2O_2 50°C	23	44	120
id+1% Chit	10	17	16
H_2O_2 60°C	26	39	85
id+1% Chit	7	12	6
H_2O_2 70°C	19	32	61
id+1% Chit	5	6	7
Untreated wool	29	50	>900
id+1% Chit	18	35	125

The higher the temperature of the H_2O_2 pretreatment the higher the shrink-resistance conferred by chitosan.

The wetting time decreases as the temperature increases.

Considering the alkaline H_2O_2 treatment as the best pretreatment to promote an effective chitosan sorption in wool, the influence of the temperature and the chitosan concentration on area shrinkage and wetting time were also determined (Table II and Table III, respectively)

Table III.- Influence of chitosan concentration on the area shrinkage and on the wetting time.

TREATMENTS	SHRINKAGE (%)		W.T.
	1 c	2 c	sec
H_2O_2	12	32	51
H_2O_2+1% Chit	5	6	5
H_2O_2+0.75% Chit	2	14	4
H_2O_2+0.5% Chit	9	16	2
H_2O_2+0.25% Chit	14	25	3
UT+1% Chit	17	33	13
UT+0.75% Chit	13	32	56
UT+0.5% Chit	32	52	177
UT+0.25% Chit	30	49	287
Untreated wool	30	49	>2000

As was expected, the concentration of chitosan influences wool shrinkage, even on unpretreated wool (UT). The higher the chitosan concentration the lower the area shrinkage

This influence is very important when wool has been pretreated with H_2O_2.

From all these results it can be concluded that the presence of chitosan on wool pretreated with H_2O_2 at alkaline pH, enhances both the shrinkage prevention and the hydrophilicity of the wool surface.

B) Sodium lauryl sulphate (SLS) pretreatment

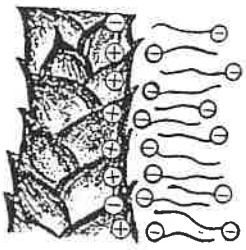
Since wool exhibits cationic character in the range of pH values suitable for chitosan solubilization, the presence of the anionic surfactant previously adsorbed in the wool could enhance the subsequent chitosan sorption.

It has been reported (7) that the interaction SLS-wool is primarily based on electrostatic links between their opposite charged groups. Due to the hydrophobic nature of wool, the electrostatic bonds alone could not explain the whole phenomena and in consequence, both ionic and hydrophobic links must be taken into account when considering the adsorption process. Once a first layer of anionic surfactant is ionically linked to wool surface, a second step in the adsorption takes place in which hydrophobic bonds between the hydrocarbon chain of the surfactant and hydrophobic sites of wool fibre are implicated.

The influence of pH in the SLS aqueous treatment influences the way how the surfactant is sorbed in wool (4).

Ionic and cooperative adsorption

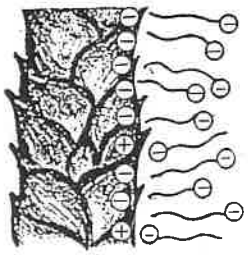
pH 2.2 →



In acid medium the amino groups in the wool are protonated and a first layer of SLS is ionically linked to wool surface, then a second layer of SLS is hydrophobically bonded to the previously sorbed hydrocarbon chains. The fibre acquires a predominant negative surface charge.

Hydrophobic adsorption

pH 11.8 →



In basic medium wool fibres exhibit a global electronegative charge and the surfactant is linked to them by means of hydrophobic bonding. Therefore the surface charge of the fibre is also negative.

The **adsorption kinetics of the surfactant on wool** were determined on wool samples pretreated with SLS in aqueous solution at different pH. The amount of SLS sorbed in wool for the different treatment times is shown in Fig.1.

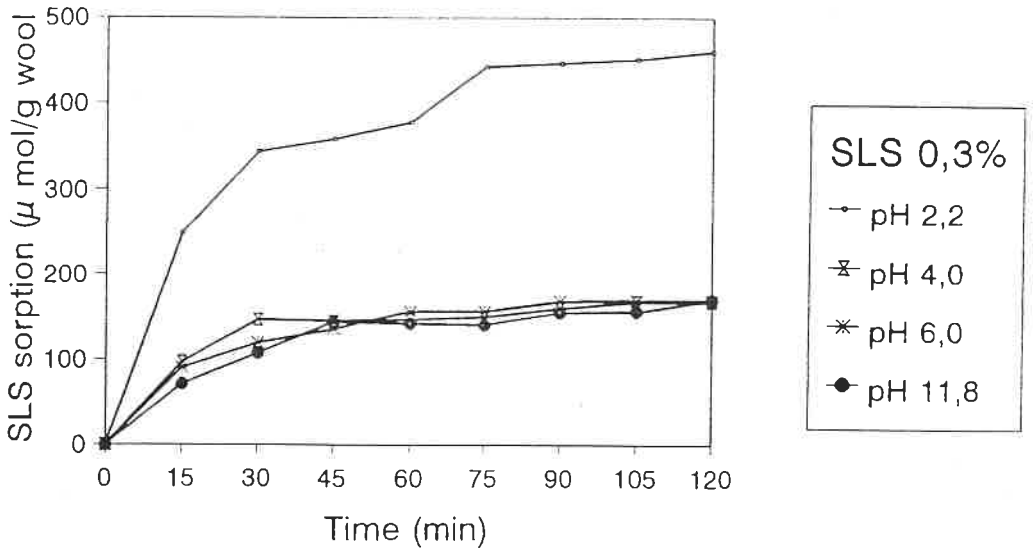


Fig.1- Adsorption kinetics of SLS on wool

At acid pH, due to that ionic and hydrophobic links are involved, a considerable adsorption of SLS on wool has occurred.

After SLS were sorbed in wool, all samples were treated with 0.75% chitosan and the **percentage of area shrinkage** after 2 cycles washing test were determined, as is shown in Fig.2.

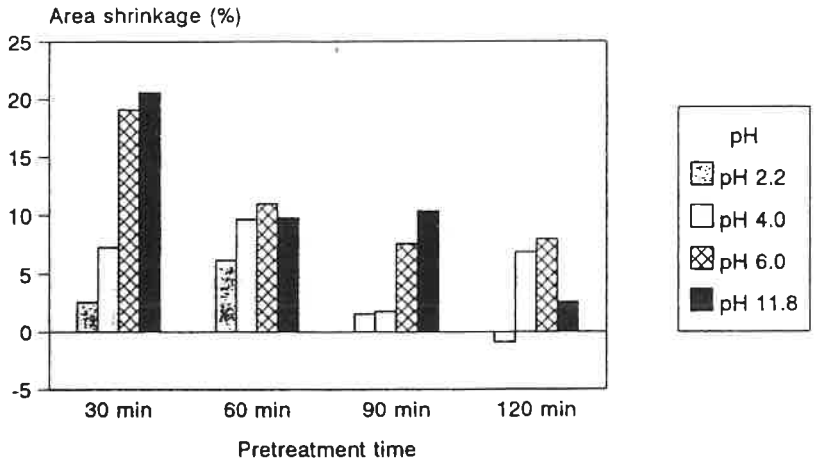


Fig.2.-Percentage of area shrinkage obtained after 2 cycles 5A on wool samples pretreated with SLS (0.3%) at different pH and different times, and all of them treated with chitosan (0.75%).

The lowest area shrinkage corresponds to the treatments carried out at acid pH and the treatment time hardly influences on the area shrinkage. When the treatment has been carried out at basic pH, the influence of the time of the treatment is evident, the longer the time the lower the area shrinkage. Similar behaviour can be noticed for pH 6.

Conclusions

* The shrink resist properties of wool treated with chitosan can be enhanced by means of a pretreatment of wool either with hydrogen peroxide or anionic surfactant (SLS).
* The alkaline H_2O_2 pretreatment promotes the diffusion of chitosan into the wool fibre.
* The sorption of the anionic surfactant on wool is influenced by the pH of the aqueous bath.
* "machine washable wool" has been achieved on all those treatments where the shrinkage percentages obtained after 2 cyles washing test has been lower than 8%.

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FINE STRUCTURE OF CHITIN FILAMENTS

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Abstract: Fine structure parameters: kind od crystalline struccture, occurrance of crystalline spherulitical aggregations, degree of crystallinity, average crystallite sizes, overall, -crystalline, -amorphous orientation of chitin filaments obtained from di-butyrylchitin precursor filaments vere investigated.

Keywords: Chitin filaments, fine structure.

Materials and methods

The chitin filaments investigated were obtained by alkaline hydrolysis of precursor di-butyrylchitin (DBCH) filaments (1). The utilized DBCH filaments were spun from 22 % acetone solution of polymer with intrinsic viscosity[η]=1,28 using dry method of spinning.The average thickness of the filamets was ca 40 μm.

The hydrolysis was conducted in the following manner: - water solution of NaOH with concentrations of 0,939 M and 1,25 M were used as hydrolysis medium. Hydrolysis reaction was carried out at 25° C. Weighed samples of DBCH filaments in loose state were put in excess of NaOH solution for a definite period, then they were taken out, washed to remove any trace of alkali, dried and weighed again. The scheme of hydrolysis reaction is as follows :

$$C_8H_{11}NO_5(C_3H_7CO)_2 + 2NaOH \text{ -------} > C_8H_{11}NO_3(OH)_2 + 2C_3H_7COONa$$
$$(M = 343) \qquad (M = 203)$$

The efficiency of chitin reconversion was checked threefold, - by means of IR spectroscopy, analyzing the disapearance of 1740 cm^{-1} absorption band referring to the ester bonds in DBCH filaments, - by means of elementaly analysis of the increase of nitrogen contents after the hydrolysis and by ascertaining the weight losses of DBCH filaments after the hydrolysis. As evidence of the alkaline hydrolysis efficiency dates referring to the chitin contents and to the diminishing of 1740 cm^{-1} absorption band in DBCH are presented in Table 1 and 2. For study purposes filaments with a contents of chitin above 92 % were utilized.

Results and discussion

Kind of crystalline structure

Chitin deprived of protein inclusions reveals strong ability to crystallize. In the natural state the degree of crystallinity may reach 0,55 - 0,60.

Until now there have been recognized 3 polymorphic modifications of crystalline chitin - α, β and γ. They differ among them in respect to the crystal system and to the parameters of the lattice unit cell. These differences are a result of differences in the manner how adjacent molecules are arranged in the lattice. Chitin chains occurring in the lattice in sheets may be arranged all in a parallel or antiparalel manner ; - in the case of α- chitin all antiparallel , in the case of β - chitin all parallel. The arrangement of chains in the γ- modification is characterized by an alternative appearance of 2 sheets with parallel arrangment followed by 1 sheet with antiparallel arrangement.

The parameters of unit cells for particular polymorphic crystalline forms of chitin are presented in Table III.

The α polymorphic form is more common than β and γ. It is characterized by a strong chemical stability. This is caused by greatest possible number of inter-, as well as intramolecular hydrogen bonds. From point of view of the manner in which the adjacent molecules are arranged in lattice a chitin reveals resemblance to the cellulose II.

In turn β - chitin, the second in prevalance, is characte-rized by a lower stability than the α form. This is due to a minor number of hydrogen bonds. An evidence of lower stability manifest itself in the possibility to transform β into α form in strong solution of HCl. The reduced, as compared to α form number of hydrogen bonds opens the possibility to link permanently water molecules what in turn means that chains in the lattice might be regarded as chitin monohydrates. From point of view of the manner in which chains are arranged in the lattice β chitin is similar to cellulose I. This resemblance is a reason why Gardner and Blackwell consider the transformation chitin β into α as analogous to the conversion cellulose I into II. β chitin occurs as a rule in the form of large crystalline fibrils of ribbon - like morphology.

The crystalline structure of investigated c h i t i n and DBCH precursor filaments was recognized on the base of X- ray examination. The stated angular localization of the diffraction peaks and the corresponding interplanar spacings for both kind of filaments are shown in Table IV.

In the case of c h i t i n filaments the angular positions of 4 diffraction peaks is in full agreement with the monoclinic lattice of the β crystalline chitin proposed by Dweltz (11). The unit cell is characterized by parameters a = 4,8 A, b = 13,4 A, c = 10,3 A, γ = 112 degr . The very probable constitution of the lattice is shown in Fig.1.

In the case of DBCH filaments the angular position of 5 diffraction peaks is, as it was previosly recognized by the author, agrees with a orthorhombic latttice with unit cells characterized by parameters a = 4,4 A, b = 13,4 A, c = 10,3 A (direction of the molecule axis).

The relatively small number of difraction peaks in the diffraction paterns of both kind of filaments proves that the lattices are relatively defective i. e. low perfect in geometrical regularity.

Crystalline spherulitical aggregations

The performed polarization - interference microscope examination carried out on large picture magnification exhibited for both kinds of investgated filaments the absence of Maltese Cross brightenings, characteristic for the occurance of spherulitical crystalline aggregations.Such kind of pictures testifyies to the homogenous crystalline-amorphous structure,which comprises only crystallites of the fringed - fibrylar morphology and a lack of spherulites.

Degree of crystallinity and average lateral crystallite sizes

The rebuilding of the lattice taking place in result of alkaline hydrolysis of DBCH filaments is accompanied by a change of the crystallinity degree and an alteration of the average lateral crystallite size.

The established values of the degree of crystallinity and the average lateral size of crystallites for both kind of filaments are shown in Table V.

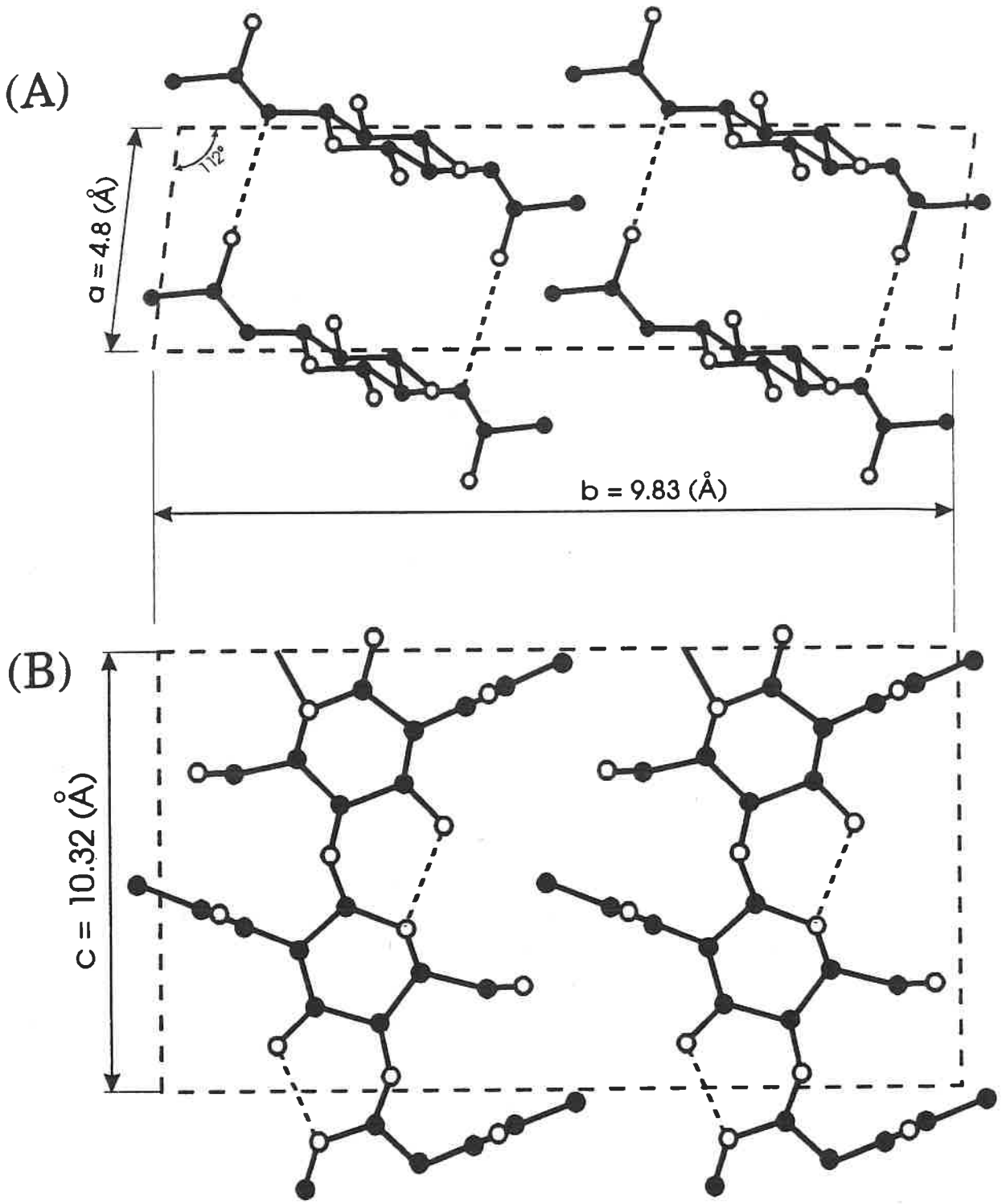

Fig. 1 Visualization of the lattice of β-chitin in studied filaments. (A) - projection on ab plane (B) - projection on bc plane

Table I. Chitin content after alkaline hydrolysis of DBCH filaments ascertained on the base of elementary analysis for nitrogen.

Order Number	Reaction Time (min)	Contents N (%)	Chitin Contents (% mol)	Hydrolysis Terms
1	0	4,08	0	Excess of 1.25 M NaOH temp.25^{0}C DBCH with (η)=1.28
2	60	5,31	56,0	
3	90	5,69	70,0	
4	150	6,14	83,0	
5	360	6,37	97,0	

Table II. Chitin content after alkaline hydrolysis of DBCH filaments ascertained on the base of the weighing method.

Order Number	Reaction Time (min)	Weight loss (%)	Chitin Contents (% mol)	Concen-tration of NaOH (M)	Temp (^{o}C)
1	60	21,3	52,2	1,25	25
2	90	28,9	70,8		
3	150	36,4	89,2		
4	240	37,7	92,4		
1	60	15,8	38,7	0,938	25
2	90	24,4	59,8		
3	150	34,4	84,3		
4	420	40,5	99,2		

Table III Parameters of the chitin lattice unit cells.

Polymorphic Form	Unit cell parameters				Cell Volume (Å^{-3})	Number of NAG residues on 1 cell	Density d_{cr} (g/cm^3)	Authors
	a (A)	b (A)	c (A)	γ (deg)				
α - modyfic	4,76	18,85	10,28	90^0	922,38	4	1,461	(2)
	4,74	18,86	10,32	90^0	922,57	4	1,461	(3)
β- modyfic	4,70	10,50	10,30	$\approx$90^0	508,30	2	1,3260	(4)
	4,80	9,83	10,32	112^0	451,39	2	1,4130	(5)
	4,85	9,26	10,38	97,5^0	462,32	2	1,4580	(6,7,8)
	4,57	9,60	10,30	90^0	451,90	2	1,4919	(9)
γ- modyfic	8,90	17,0	10,25	90^0	1550,82	6	1,304	(10)

Table IV X-ray Diffraction Data of Chitin Filaments.

Reflection Intensity	Bragg. Angle 2θ (degree)	Interplanar Spacing d_{hkl} (Å)	Miller Indices (hkl)
Strong	8,8 ÷ 9,0	9,11	(010)
Weak	12,5 ÷ 12,8	7,00	(220)
Very Strong	19,5	4,42	(100)
Medium	26,5	3,40	(110)

X-ray Diffraction Data of DBCH- Filaments.

Reflection Intensity	Bragg. Angle 2θ (degree)	Interplanar Spacing d_{hkl} (Å)	Miller Indices (hkl)
Strong	6,6 ÷ 7,0	13,4	(010)
Very Weak	12,3	7,2	(240)
Strong	19,8 ÷ 20,2	4,4	(100)
Very Weak	24,6	3,7	(120)
Very Weak	42,0	2,1	(210)

Table V Degree of crystallinity and average lateral crystallite sizes.

Kind of filament	Degree of crystallinity		Lateral crystallite size	
	X - Ray	Densitometric	D_{100} (Å)	D_{010} (Å)
DBCH Precursor	0,13	0,23	18,2	54,5
Chitin	0,26	0,33	27,0	55,5

Table VI Crystalline and amorphous orientation parameters of Chitin and DBCH Filaments

Kind of filament	Crystalline orientation				Orient. Plate Effect.	Amorphous Orientation			
						Dichroic Ratio-R		Orientation Function F_{IR}	
	$\langle\cos^2\rho_{110}\rangle$	$\langle\cos^2\rho_{010}\rangle$	$\langle\cos^2\rho_{100}\rangle$	Hermans orientation function- fx	$\frac{\langle\cos^2\rho_{010}\rangle}{\langle\cos^2\rho_{100}\rangle}$	1455cm^{-1}	1375cm^{-1}	1455cm^{-1}	1375cm^{-1}
DBCH*	–	0,2073	0,1911	0,4024	1,085	1,22	1,19	0,067	0,061
Chitin	0,1703	0,2045	0,1796	0,6355	1,138	1,04	1,04	0,0137	0,0130

* - established after method of the authors - G.Urbańczyk et all. -The Structure and Selected Properties of Dry - and Wet-Spun Butyrylochitin Filaments; -Text Res.Journal in press.

The presented results give evidence that the alcaline hydrolysis of DBCH filaments leads to a noticeable increase in filament crystallinity. One can conclude that this increase proceeds as a result of growth of lateral average crystallite size, especially in the direction perpendicular to the (1 0 0) lattice planes.

Internal orientation

The values of parameters characterizing the crystaline and the amophous orientation are presented in Table VI .

As one can see the crystalline orientation of chitin filaments is better, but on the contrary, the amorphous orientation is worse than for the precursor DBCH filaments.

It can be assumed that such a report of results is a consequence of recrystallisation occuring during the alkaline hydrolysis of DBCH. The increase in the amount of crystallized polymer refers first of all to the best ordered chains of the noncrystalline fraction. The remaining noncrystalline fraction consists of worse ordered chains.

Additionally the lower amorphous orientation in chitin filaments may also be caused by a very probably disorientation effect occuring in the amorphous phase during the alcaline hydrolysis.

The crystalline orientation in both kind of filaments is characterized by a weak but differentiated so called plate effect. In the case of chitin filaments the plate effect is more pronounced. This provides indirect information about the morphology of the crystallites. From the larger values of the plate effect it may be inferred that in the case of chitin filaments the crystalline phase consits of stronger shaped, ribbon like, crystallites than in DBCH filaments.

Conclusions

1. The c h i t i n filaments differ essentialy in respect to their crystalline structure and noticeably in respect to their internal orientation from the precursor DBCH filaments.
2. The crystalline structure of the c h i t i n filaments is characterized by a monoclinic lattice of the β crystalline form of chitin with unit cell a=4,80 A, b=9,83 A, c=10,3 A, γ=112 degr., whereas the DBCH filaments by an orthorhombic lattice with unit cell a=4,4 A, b=13,4 A, c=10,3 A. The crystalline densities are correspondigly 1,4130 g/cm^3 and 1,8758 g/cm^3.
3. C h i t i n filaments are more crystalline and contains crystallites of bigger average lateral sizes than DBCH filaments.
4. C h i t i n filaments exhibit a better crystalline, but on the contrary, a worse amorphous orientation than the precursor DBCH filaments.

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CHITIN FILAMENTS - THEIR BASIC PROPERTIES

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Abstract

Chitin filaments obtained after alkaline hydrolysis of di-butyrylchitin (DBCH) precursor filaments were investigated. The selected physical and physico-chemical properties were studied..

The characterization of physical properties included the appraisal of density, mechanical, thermal, electrical and optical properties. In the area of physico-chemical properties, moisture regain,swelling and dyeability were examined.

The results obtained were confronted with results refering to filaments from di-butyrylchitin precursor .

Keywords: Chitin, di-butyrylchitin, filament, properties.

Materials and methods

Materials

The Chitin filaments obtained by means of alkaline hydrolisis of di-butyrylchitin precursor filaments and di-butyrylchitin precursor filaments [1] were investigated.

Methods

The selected physical and physico-chemical properties were studied.

The characterization of physical properties included the appraisal of density ,mechanical, thermal electrical and optical properties. In the area of physico-chemical properties, moisture reagain, swelling and dyeability were examined.

The samples density were measured using the gradient column technique in the mixture of toluene and CCl_4 at 20 ^{0}C.

The investigations into the mechanical properties comprised measurements of rupture force, tensile strenght, elongation at break and initial modulus. To assess the type of deformation at stretching the course of the stress-strain curves were analyzed. The appraisal conducted on the 1111 INSTRON table universal testing instrument.

The appraisal of thermal properties was based on thermograms from DSC technique. They were obtained on a Du Pont 990 thermoanalyzer.

The electrical properties of filaments were evaluated on the basis of assigned values of volume resistivity ρ .

Within the appraisal of the optical properties the refraction indices were assessed. These examinations were conducted on a BIOLAR-PI polarization - interference microscope, utilizing a monocromatic light λ= 0,580 μm at 20 ^{0}C.

Moisture regain was determined after the conditioning of the filaments in normalized air conditions.

The swelling ability of filaments in water and physiological salt solution was measured during the observation under the microscope at the temperature 20 ^{0}C.

The dyestuffs sorption ability of the fibres was evaluated on the basis of the total dyestuff quantity which was absorbed by filaments investigated. It was verified for different classes of dyes as direct, acid, reactive (hot and cold) and disperse.

Results and discussion

Mechanical Properties.

The results of measurements on single filaments are presented in Table 1 and Fig.1 Fig.2. The results testifies to that in chitin filaments occurs as a result of alkaline hydrolysis of DBCH a decrease of tensile strength σ,-of elongation at break ε and the initial modulus E. Such kind of changes refers to filaments conditioned at 20 % RH as well as at 65 % RH.One can notice that mechanical properties of chitin filaments due to their high hygroscopicity are strongly dependent on their state of humidity. This is especially drastic in the case of elongation at break .

The Fig.1 proves that the water content of chitin filaments influences very strongly the stress-strain curve,while for the DBCH precursor the changes are relatively small (Fig.2)

Table 1 Mechanical Properties of Chitin and DBCH Filaments

Kind of filament	Relative Humidity (%)	Rupture Force F (N)	Tensile Strength		Elongation at Rupture		Initial Modulus	
			σ (MPa)	Cv	ε (%)	Cv	E (GPa)	Cv
DBCH	20	0,155	112,4	5,4%	36,2	4,7%	2,6	3,5%
	65	0,135	97,9	6,1%	45,7	3,6%	1,8	2,4%
Chitin	20	0,093	77,1	13,2%	4,6	15,7%	2,2	16,0%
	65	0,070	58,0	2,3%	42,2	13,1%	1,0	9,1%

Cv - Coefficient of Variation

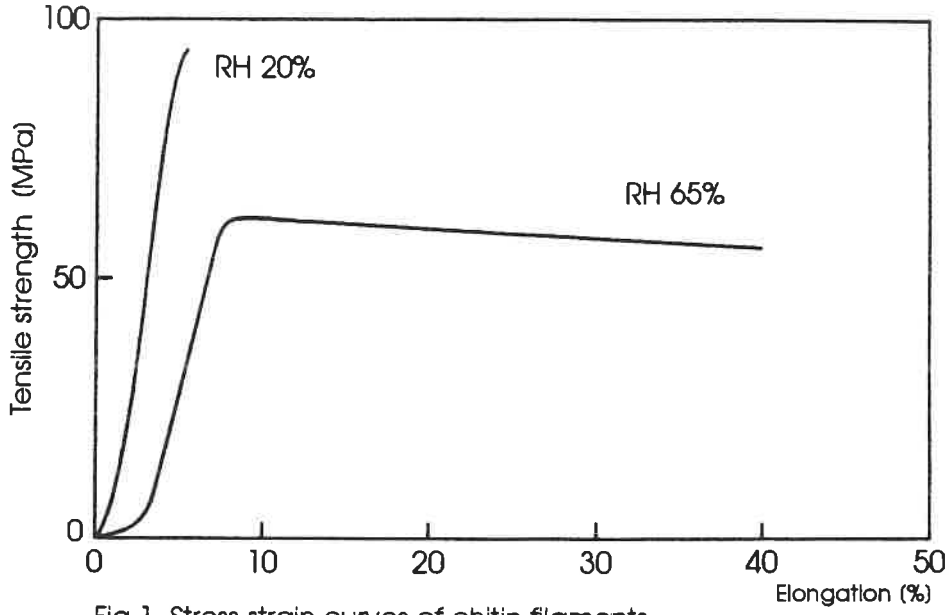

Fig 1 Stress-strain curves of chitin filaments

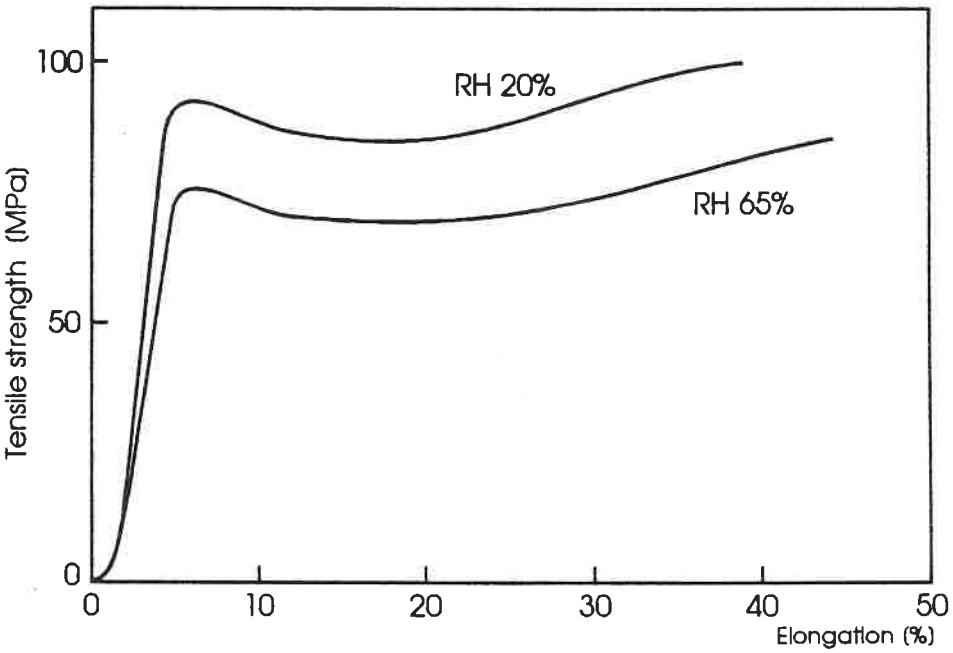

Fig 2 Stress-strain curves of DBCH filaments

Thermal properties.

DSC thermograms of chitin and precursor filaments are depicted in Fig.3, Fig.4. The thermograms show differences.In general it may be inferred that chitin filaments exhibit greater thermal stability. The temperatures of specific thermal transitions are higher than for DBCH filaments. Thus, the first endothermic minimum, reflecting the initial stage of glass transition with reference to molecules in the amorphous phase which are strongly entangled and are not connected with crystalline regions, appears in the case of chitin filaments at 100°C, for DBCH filaments at 80-90°C. Further the second endothermic minimum which can be due to the thermal decomposition of crystalline regions starts at 330^0 C for chitin and at 280^0 C for DBCH filaments.

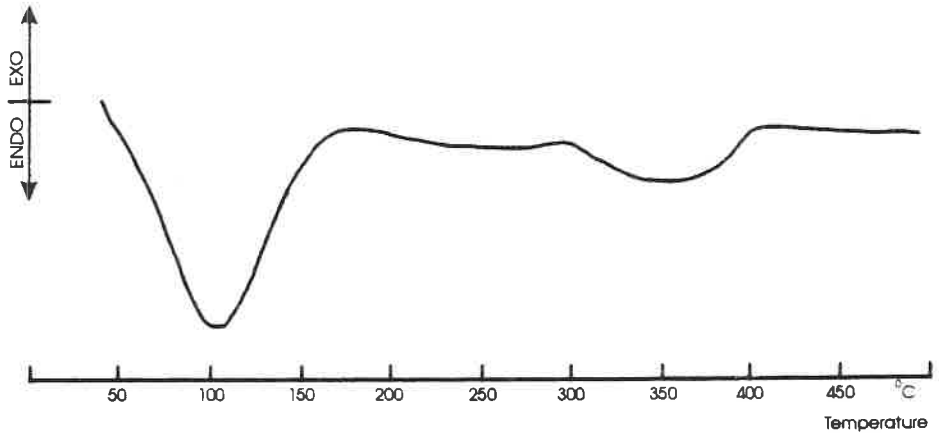

Fig. 3. DSC Thermogram of Chitin Filaments

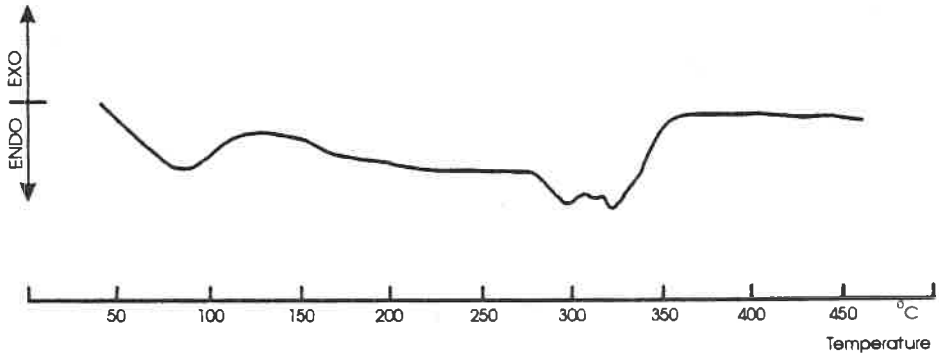

Fig. 4. DSC Thermogram of DBCH Filaments

Electrical Properties.

The ascertained results of electrical volume resistivity for chitin and precursor filaments are presented in Table 2 .
The achieved results prove that chitin filaments as compared to precursor DBCH filaments are characterized by a better electrical conductivity. This pertains to lower as well as to higher values of humidity.

Table 2 Electrical Volume Resistivity ρ_v of Chitin and DBCH Filaments.

Kind of Filament	Measuring Terms			
	25% RH T= 23°C	$\delta\rho$	65% RH T= 23°C	$\delta\rho$
DBCH	$8{,}1 \times 10^8$ (Ω m)	$2{,}0 \times 10^8$	$4{,}6 \times 10^8$ (Ω m)	$1{,}2 \times 10^8$
Chitin	$1{,}3 \times 10^8$ (Ω m)	$0{,}3 \times 10^8$	$4{,}8 \times 10^7$ (Ω m)	$0{,}6 \times 10^7$

$\delta\rho$ - mean value error of ρ_v

Density and Optical Properties.

Density values and the refractive indices are collected in Table 3.As one can see there is a good correspondence between the density and light refraction ability for both kinds of filaments. The higher density of chitin filaments are fully in line with their higher degree of crystallinity.

Table 3 Density and refractive index of Chitin and DBCH filaments

Kind of Filament	Density (g/cm^3)	Refractive Index λ = 0,589 µm 20^0C
DBCH	1,2190	1,4928
Chitin	1,3767	1,5283

Moisture Regain, Swelling and Dyeability.

The measured moisture regain and swelling values are presented in Table 4 .The stated higher values of moisture regain and swelling for chitin than for DBCH filaments can be explained by the differences in the number of hydrophilic OH groups in the absorbing water amorphous phase of both filament types.The large amount of such groups in chitin enhances the water sorption and there fore leads to higher swelling.
The chitin filaments differ in respect to their dyeability from precursor DBCH filaments. They reveal a very good dyeability with direct dyestuffs and cold as well as hot reactive dyestuffs, while DBCH filaments do not exhibit such an ability. A common feature of both kind of filaments consists in good dyeability with disperse dyestuffs.

Table 4 Moisture Regain and Swelling of Chitin and DBCH Filaments.

Kind of Filament	Moisture Regain at 65% RH, 20^0C (%)	Swelling in Water at 20^0C - (%)	Swelling in Soluttion of Physiological Salt at 20^0C - (%)
DBCH	4,8	35,4	40,0
Chitin	10,9	39,8	57,9

Conclusion

1. The physical properties of the chitin filaments are described by tensile strength 77,1 and 58,0 MPa., elongation at rupture 4,6 and 42,2 % , initial modulus 2,2 and 1,0 GPa. correspondingly for RH=20 % and RH=65%, - by volume resistivity 1,3 x 10^8 and 4,8x10^7 Ωm, respectively for RH=25 % and RH=65 %, - by density 1,3767 g/cm^3, - by refractive index 1,5283.
2. The physico-chemical properties of the chitin filaments i.e.the swelling ability and dyeability are as follows:swelling in water 39,8%, swelling in solution of physiological salt 57,9%, very good dyeability by application of direct , reactive and disperse dyestuffs.

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Effect of Chitosan from Shrimp, Squid and Crab on the State of Water and Denaturation of Myofibriliar Protein during Frozen Storage

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Abstract

During frozen storage the proteins of fish meat are expose to changes, among these changes to avoid or to retard denaturation properties are the most important to keep a high quality product. A decrease in water retention, gel forming ability, and lipid-emulsifying capacity, as well as by a toughening of texture and increased dryness of a fish meat are characteristics of the protein denaturation.

Chitosans were extracted from crab, squid and shrimp, and changes in the amount of unfrozen water in fish myofibrils in presence of 5% of chitosan associated with freezing were analyzed by a differential scanning calorimetry (DSC). Ca-ATPase as an indicator of denaturation of fish myofibrils was simultaneously measured. The amount of unfrozen water increased by the addition of chitosan. When chitosan was not added to myofibrils, the amount of unfrozen water rapidly decreased during frozen storage, whereas the decrease was moderate in myofibrils with chitosan. Meanwhile the decrease in Ca-ATPase activity during frozen storage was a similar tendency to that of the amount of unfrozen water. Chitosan from squid was the most effective of all three species. The results obtained show that chitosan from the three species had a suppressive effect on denaturation of myofibrils during frozen storage and the denaturation of myofibrils might be surpresed by the addition of chitosan, since chitosan constructed the water molecules resulting in an increase in the amount of unfrozen water.

Keywords: Chitosan, Ca-ATPase, myofibrils, DSC, frozen storage

The thermal analysis has been introduced as an efficient tool for studying the chemical and physicochemical reaction involved in change of the state of various matters The methods of differential scanning calorimetry (DSC) are based on the measurement of endothermic or exothermic heat to and from the sample as the temperature is raised continuously.

Materials and Methods

The samples of Japanese fan lobster (*Ibacus ciliatus*), spear squid (*Doriteuthis blekeri*) and Japanese swimming crab (*Portinus trituberculatus*),

were purchased in the Nagasaki fish market. Chitin was isolated from these three species using the method of Hackman[1] slightly modified. The samples were pulverized and pass through a 40 mesh sieve.

Preparation of chitosan

The samples of spear squid, Japanese swimming crab and Japanese fan lobster were cleaned by washing and scraping under running water, then they were crushed and inmerse in 20 volumes of 2N HCl solution for 48 hours (the solution was changed every 12 hr.). The precipitated was washed with distilled water and adjust to pH 7.0. Then 20 volumes of 1N NaOH were added (this solution was changed every 6 hours) during heating at 98°C for 36 hours. The samples were washed with distilled water and the pH was adjusted at 7.0 and finally lyophilized to obtain chitin. Then, 20 volumes of 60% NaOH were added to the chitin powder (150 g) while heating at 130 °C for 3 hours. The sample is washed until get a pH 7.0. acetic acid solution (10%) was added and the sample was stirring for 12 hours. Centrifuged at 10,000 x g, for 30 minutes and the precipitate was washed with distilled water until reach pH 7.0. Finally the precipitate was lyophilized and a white chitosan powder produced.

The average molecular weight of chitosan was determined by viscosity measurement in a 0.1 M acetic acid, 0.2 M sodium chloride solution, by means of Robert's viscosity law[2]. The average degree of polymerization was calculated from the viscosity average molecular weight.

The degree of Acetylation was determined by spectrophotometry using Muzzarelli's[4] method and also by potentiometric titration according with Broussignac[5] method. The values obtained for the three species were 0.18 for squid, 0.14 for shrimp and 0.17 for crab.

Preparation of fish myfibrils

Fish myofibrils were prepared from lizardfish (*Saurida wanieso*) meat. After heading and eviscerating, the fish was washed in ice water. The skin and bones were removed with a meat separating machine. Fish meat was washed in 5 volumes of 0.1M KCl adjusting the pH to 7.0 with 20mM tris-maleate buffer. This washed process was repeated three times. After excess water was pressed out, the resulting residue was homogenized with volume of the same buffer as above. The suspension thus obtained was filtered through a nylon net (#16) and 1% triton x-100 was added then was kept standing at 5°C for 30 minutes, then was centrifuged at 1,400 x g for 10 minutes. After being washed again with 5 volumes of the same buffer as stated earlier, the residue was centrifuged at 1,400 for 10 minutes. The washing treatment with the same buffer was repeated until the supernatant became clean. The residue was suspended

in 5 volumes of cold distilled water and centrifuged first at 3,800 x g for 10 minutes and then at 9,200 x g for 20 minutes to produce the myofibrils

Determination of the moisture content.

The moisture content was determined as the loss of water in a sample by oven drying at 105°C for 16 to 18 hours.

Preparation of the samples

For the preparation of the differential scanning calorimeter (DSC) sample, one part of myofibril was mixed with 5% of chitosan extracted from Japanese fan lobster, spear squid and Japanese swimming crab. These myofibrils were adjusted to pH 7.0 and 1 g of sample was placed in a microtube and frozen at -25°C. Samples were taken out for analysis of the unfrozen water at different periods of time.

Analysis of unfrozen water

For analysis of the unfrozen water a differential scanning calorimeter (DSC) model SSC-5200 Analysis System with plotter type SP-520 from Seiko Electronic Industry, Co., was used. A sample of about 20 mg was placed in an aluminum cell of Al_2O_3 was used to balance the heat capacity of the sample of the cell. The sample was frozen at -40°C using liquid nitrogen gas. The sample was then heated to 25°C at a rate of 1°C per minute. The amount of total water in the sample was measured by drying at 105°C while the amount of unfrozen water in the sample was calculated by subtracting amount of free water from amount of total water.

Analysis of Ca-ATPase

Analysis of Ca-ATPase activity were analyzed according to the method of Katoh[3] indicated by micromoles per minute of the inorganic phosphorous released by a milligram of myofibrils protein in the presence of 1 mM ATP, 100 mM KCl and 5 mM $CaCl_2$ at pH 7 with 25 mM tris-maleate buffer. The concentration of protein of fish myofibrils was determined by the Biuret method with bovine serum albumin as a standard.

Results and Discussions

The effects of chitosan from the shells of these three different crustaceans and the difference in concentration of chitosan on the unfrozen water of myofibrils during frozen storage at -25 °C are shown in figs. 1-3.

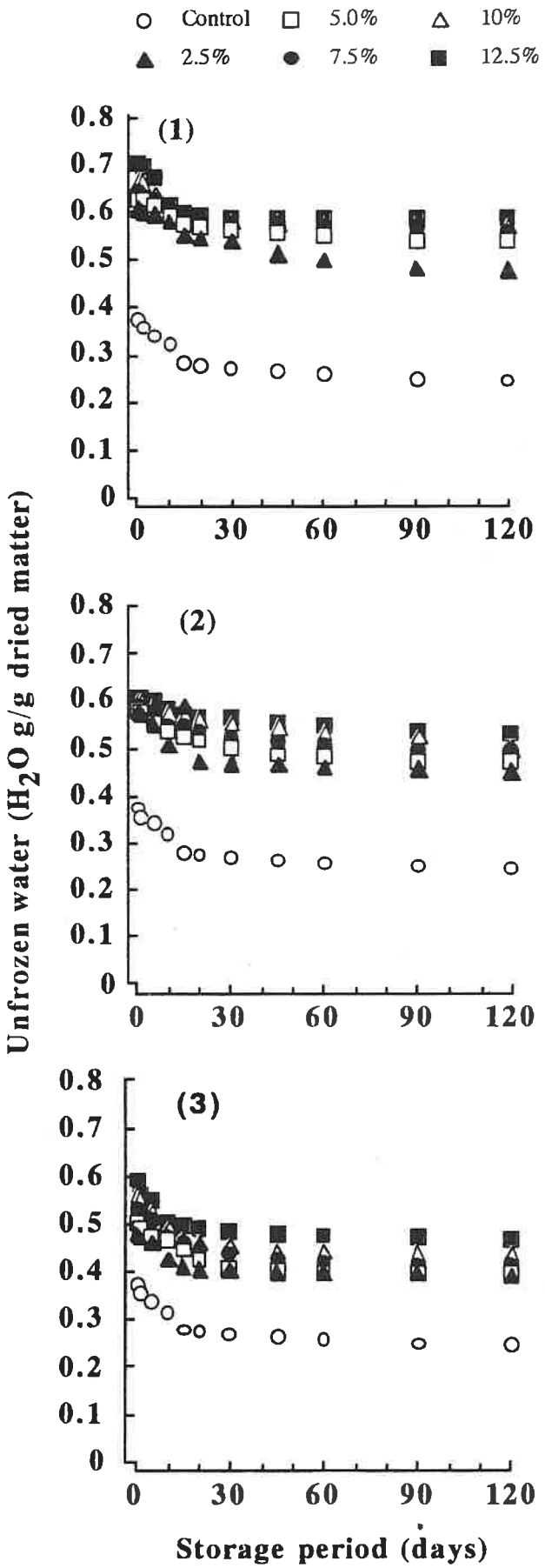

Fig.1. The unfrozen water to show effect of various concentration of chitosan from 1) spear squid 2) Japanese fan lobster, and 3) Japanese swimming crab.

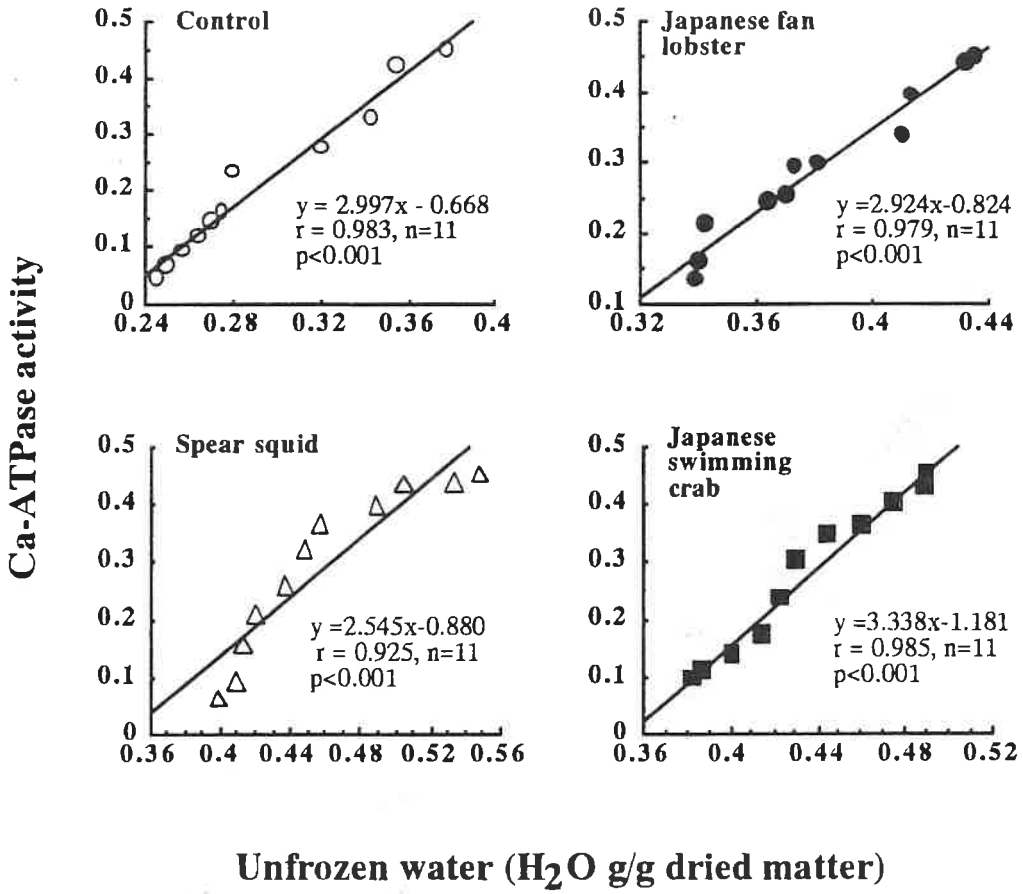

Fig. 2. Correlation between Ca-ATPase activity and unfrozen water and unfrozen water of various concentration of chitosan from different crustaceans.

Frozen storage is an excellent process for preventing putrefaction and autolysis of fish muscle, however, deterioration can not be avoided when the storage period is lengthened. This is because when fish has been frozen for a long period, the functional properties such as emulsifying, lipid binding, water holding and gel forming capacities are decreased. Based on the theory above, this research investigated the effect of chitosan extracted from different sources on the unfrozen water and Ca-ATPase activity of myofibrils during frozen storage. Results show that during frozen storage chitosan had a highly significant effects on the unfrozen water. During frozen storage the amount of unfrozen water decreased rapidly to the 20 days and then gradually to 120 days. The amounts of unfrozen water from the three Chitosans show that spear squid unfrozen water is bigger than Japanese fan lobster and Japanese swimming crab following this order. Furthermore, the quantity of unfrozen water at various concentrations of chitosan from this three species shows that at higher concentrations, there will be more unfrozen water. denaturation in proteins has been monitored by following the change in Ca-ATPase activity. Results in this research showed that during frozen storage chitosan can suppress the decreased of Ca-

ATPase activity, the ability of suppression varying by the source that was extracted.

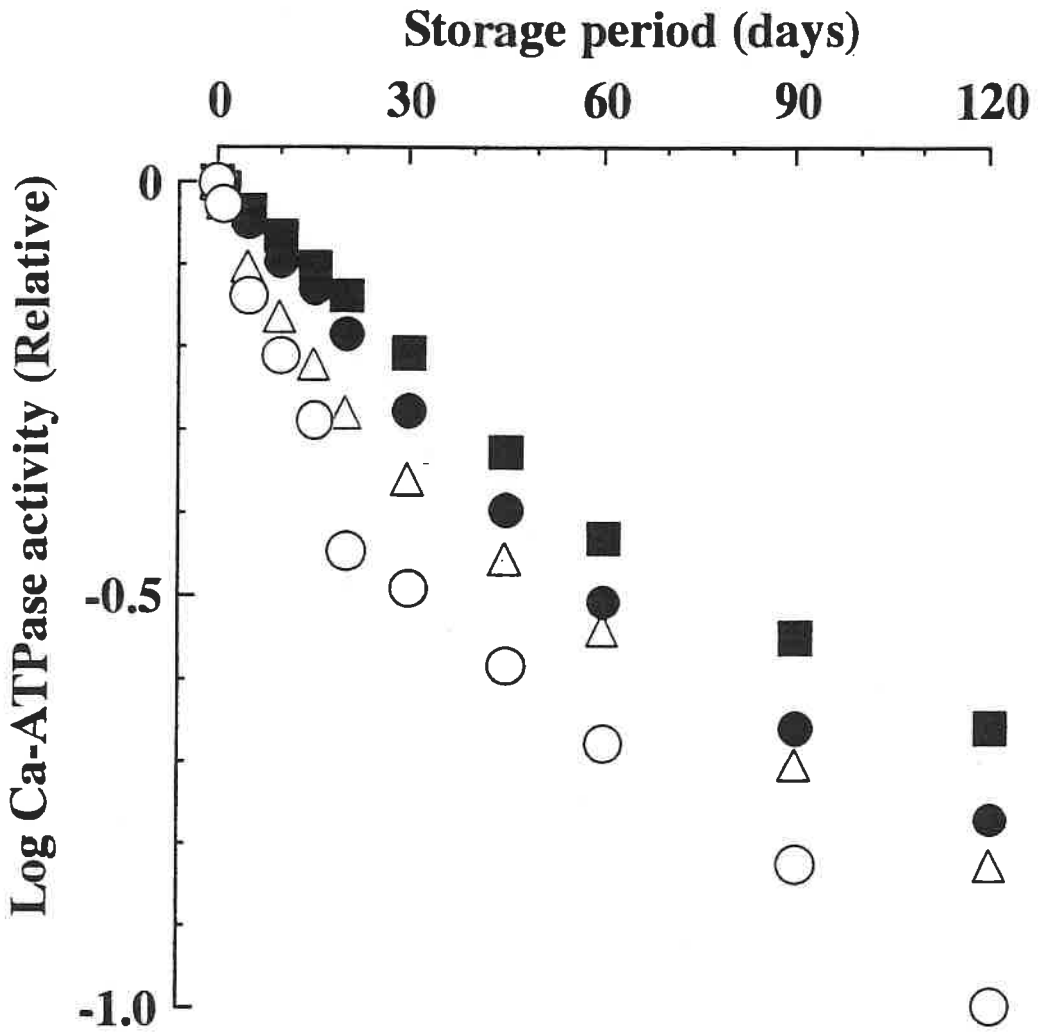

Fig. 3. The Ca-ATPase activity to show the effect of various chitosan 5% added to lizardfish myofibrils during frozen storage. ○ Control, △ Spear squid, ● Japanese fan lobster and ■ Japanese swimming crab.

According with these results. The figure shows that there is a close correlation between Ca-ATPase activity and the amount of unfrozen water. This fact suggest that since chitosan causes the water molecules to be more constitutive, resulting in an increase in the amount of unfrozen water, denaturation of myofibrils might be suppressed by chitosan.

Table 1. Chemical properties of chitosan extracted from spear squid, Japanese fan lobster and Japanese swimming crab

	Japanese fan lobster	Spear squid	Japanese swimming crab
Ash content (%)	3.5	1.0	6.4
Lipids (%)	0.17	0.06	0.13
Proteins*1 (%)	0.03	0.04	0.04
MW*2	$1.06x10^5$	$1.80x10^5$	$2.05x10^5$
DP*3	$4.80x10^2$	$3.03x10^2$	$2.77x10^2$
DA*4	0.18	0.14	0.17

*1 According with Lowry's method.
*2 Viscosity average molecular weight.
*3 Average degree of polymerization.
*4 Degree of acetylation.

The quality of the chitosan obtained in this research can be seen in the table above. A direct relation between the suppressive effect on the denaturation of fish myofibrils and the degree of acetylation was observed. A low concentration of ashes of the chitosan extracted from spear squid could have been also very important factor to have an increase in this suppressive effect on the denaturation of fish myofibrils though the ashes content in the chitosan extracted from the Japanese swimming crab were higher than the chitosan extracted from Japanese fan lobster.

Conclusions

1. Chitosan from the three species had a suppressive effect on denaturation of myofibrils during frozen storage.

2. There is a direct relation between the degree of acetylation and the suppressive effect on denaturation of myofibrils

3. Chitosan from spear squid was the most effective of all three species followed by chitosan from Japanese swimming crab and Japanese fan lobster.

4. Chitosan constructed the water molecules resulting in an increased in the amount of water.

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Blue Chitin Column — A New Efficient Technique for Concentrating Mutagens/Carcinogens in Environmental Waters

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Abstract

A major obstacle in monitoring mutagens/carcinogens in environmental waters (river, lake, and sea) lies in the difficulty for preparing suitable samples for bioassays. Minute amounts of numerous organic compounds are present in waters. Non-selective concentration of organics from such samples inevitably results in obtaining a mixture of all classes of compounds. Such samples may give false negative mutagenic responses in the Ames test due to masking components, such as fatty acids, in them. This undesirable situation may be overcome by confining the target to a specific class of compounds. We have focused our attention to mutagens with polycyclic structures; there is ample evidence that humans are exposed to polycyclic carcinogens, such as heterocyclic amines and polycyclic aromatic hydrocarbons. We have developed an adsorbent selective to polycyclics, cellulose bearing copper phthalocyanine trisulfonate as a ligand (blue cotton/blue rayon), and have used it extensively in monitoring water-borne mutagens and carcinogens. We have now discovered that chitin is a suitable powdery support for the blue pigment to be used as a column form. With the use of a short column of blue chitin, the concentration can be done in a quantitative manner. A water sample from Katsura River, Kyoto, collected in January, 1996, showed 888 ± 27 revertants per plate with *Salmonella typhimurium* TA98, +S9, at 500 ml equivalent. A linear dose-response was observed. For comparison, conventional XAD-2 resin column concentration was done for the same lot of water sample, and the mutagenicity found was about 1/2 that of the blue chitin assay.

Keywords: Blue chitin column, Blue rayon, XAD-2, Mutagens in river, Adsorption of polycyclics to Cu-phthalocyanine trisulfonate

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Mutagenicity in surface water is of concern if the water is to be used for preparing drinking water. There are two techniques practiced currently for concentrating mutagens from river water,

which is often used in Japan as the source of drinking water; the XAD-resin column and the blue-rayon adsorption. The conventional XAD-resin column concentration will collect almost all organic substances[1] whereas the blue rayon technique, a technique using copper phthalocyanine trisulfonate(*cpt*)-cellulose as an adsorbent hung in the flowing water, collects polycyclic type compounds selectively[2]. The blue-rayon hanging method is easy to execute and therefore is suitable for qualitative screening of the river-water mutagenicity. We have recently devised a new technique, blue(*cpt*)-chitin column concentration[3] which allows highly efficient quantitative concentration of polycyclics from river water.

In the present work, we compare the efficiency of these methods in concentrating mutagens from waters of the Katsura River in Kyoto and of the Asahi River in Okayama City. Previous studies on the mutagenicity of these rivers have shown that the water concentrates, either by the XAD-resin column or by blue-rayon adsorption, show activity in the Ames test almost exclusively in *Salmonella typhimurium* strain TA98 with metabolic activation[4-7] Using this bioassay as a measure, the efficiency of mutagen concentration was evaluated for the three techniques, the blue-chitin column, blue-rayon hanging, and the XAD-2 column. A full account of this work is given in ref. 8.

Materials and Methods

Reagents Blue chitin, chitin (poly-*N*-acetylglucosamine) bearing covalently linked copper phthalocyanine trisulfonate (*cpt*) as ligand, was prepared by dyeing chitin (Wako Chemicals Co., Osaka, Japan) with C.I. Reactive Blue 21 as described previously[3]. A column of blue chitin consists of a Waters Sep-Pak cartridge filled with 0.12 g blue chitin (containing 5 mmole *cpt*; column size 9 mm i.d. x 16 mm). This column is now commercially available (Funakoshi Co., Hongo 2-9-7, Bunkyo-ku, Tokyo 113). Blue rayon in nets bound to a wooden plate, equipped with an anchor and a wedge for stabilization in the river stream, was obtained from U-Tech Co. (Okayama, Japan). Methanol-ammonia used throughout this work for elution of mutagens from blue-chitin and blue-rayon was a mixture of methanol and concentrated ammonia (25%) at 50:1 (v/v). Amberlite XAD-2 resin was a product of Organo (Tokyo, Japan). Before use, the resin was washed extensively with methanol, acetonitrile and diethyl ether in a Soxhlet extractor as described in the literature[1] The washing needed a period of 3 days. The resin was stored in methanol.

Mutagenicity assay The Ames test using *Salmonella typhimurium* strains TA98 and TA100 was carried out by the

preincubation method[9] as described previously[7]. The colony numbers were determined by manual counting.

Concentration procedures Blue-chitin column procedure was described in our previous paper[3]; however, after investigating various conditions, we established the following treatment as the optimum. Thus, immediately before use, the column was washed with water (10 ml x 2), methanol-ammonia (10 ml x 3), and water (10 ml x 3), the passage speed being 5 ml/min. River water (1 liter) was passed through the column at a speed of 20 ml/min by use of a Waters Sep-Pak concentrator (Nihon Waters, Tokyo). The column was then washed with 20 ml water, and eluted with 100 ml methanol-ammonia (5 ml/min). The methanol-ammonia was evaporated to dryness under reduced pressure, the residue was taken up in methanol (about 2 ml), the methanol solution was transferred to a sterilized glass tube for mutation assays, the methanol was evaporated to dryness, and the residue was dissolved in 0.1 ml dimethylsulfoxide and subjected to the Salmonella mutation assay.

Blue-rayon hanging for 24 h in the river water was done as detailed in our previous publication[5] XAD-2 resin concentration using a column of 15 mm i.d.x 150 mm was performed according to Junk et al.[1]: elution of mutagens from the column was done with 300 ml twice-distilled diethyl ether.

Results and Discussion

Comparison of blue-chitin and XAD-2 resin column concentrations of river water mutagens The mutagenicity of the Katsura was very high, which had been anticipated from previous experiences[2] and both blue-chitin and XAD-2 gave more than 2000 revertants per 1-liter of water. The colony numbers found with the blue-chitin technique (mean ± s.d., 2655 ± 76) were significantly higher than those found with the XAD-2 concentration (2210 ± 30, $p<0.001$ (Student's t-test)). For the 0.5-liter equivalent blue-chitin concentrates of the Katsura River water, mutagenicities in *Salmonella typhimurium* TA98 + and - S9, and TA100 + and - S9 were determined: His^+ revertant numbers (solvent control numbers) were 1095 (35) for TA98 +S9; 114 (14) for TA98 -S9; 273 (106) for TA100 +S9; and 186 (141) for TA100 -S9. As we observed previously[2], the TA98 +S9 activity was the most prominent among these mutagenicities in this river water.

In the second series of experiments, the Katsura River water samples collected on 31 January 1996 were analyzed again with blue-chitin and XAD-2 column concentrations, this time with more extensive comparisons including dose response measurements. As Table 1 shows, again the blue-chitin technique was more efficient

than the XAD-2 column; about 2-fold higher mutagenicities were observed for the blue-chitin concentrates at each dose examined. Also, the reproducibility of the colony numbers among three independent processings was remarkably high in the blue-chitin method.

Table 1 Comparison of blue-chitin and XAD-2 resin column techniques for concentrating Katsura River mutagens

Method[a)]	No. of revertants per plate (*S. typhimurium* TA98, +S9)		
	100	200	500
	(ml river-water equivalent)		
Blue chitin			
Column-*1*	170	305	853
Column-*2*	167	341	929
Column-*3*	152	304	956
Mean ± s.d.	163 ± 10	317 ± 21	913 ± 53
XAD-2			
Column-*1*	115	168	558
Column-*2*	100	157	397
Column-*3*	90	123	302
Mean ± s.d.	102 ± 13	149 ± 23	419 ± 129

Background-control revertant numbers: Solvent only plates 21, 28; blue-chitin columns charged with distilled water (1 liter) Column-*4* 38, -*5* 44, -*6* 40; XAD-2 columns charged with distilled water (1 liter) Column-*4* 74, -*5* 73, -*6* 64; redistilled diethyl ether (300 ml, evaporated to dryness) Sample-*1* 30, -*2* 28, -*3* 33.

a) Each column was charged with 1-liter sample of Katsura River water collected on January 31, 1996, near Miyamae Bridge. The column processings were carried out on January 31 for XAD-2, and on February 2 for blue chitin.

Comparison of the blue-chitin concentration with the blue-rayon hanging method For the Asahi River water, which was much lower in mutagenicity than that of the Katsura, we compared the efficiency of blue-chitin column and blue-rayon hanging. These two methods were applied to the water at the same time at the same spot. The results given in Table 2 show that the blue-chitin concentrates exhibited dose-dependent positive mutagenicity and that the blue-rayon hanging gave more than 2-

times greater mutagenicity than that found for the 5-liter blue-chitin concentrates. Again, highly reproducible results were obtained for blue-chitin column concentrates.

Table 2 Detection of Asahi River mutagens by blue-rayon hanging and blue-chitin column techniques

Method[a]		No. of revertants per plate (*S. typhimurium* TA98, +S9)	
		Found	Mean ± s.d.
Blue-rayon hanging[a]			
Sample-*1*		637	
Sample-*2*		490	563 ± 74
Sample-*3*		563	
Blue chitin[b]	Vol. of water		
Column-*1*	2.5 liter	177	
Column-*2*	2.5 liter	157	166 ± 10
Column-*3*	2.5 liter	165	
Column-*4*	5 liter	246	
Column-*5*	5 liter	264	253 ± 10
Column-*6*	5 liter	249	
Solvent only		27	

a) 0.5 g Blue rayon in a net, 3 nets on one floating wooden plate, were hung in Asahi river, at Ezaki, Okayama City, from May 15, 1996, for 24 h. The 3 blue-rayon samples (0.5 g each) were processed on the next day.
b) Water collected on May 15 at the site of the blue-rayon hanging was processed on the next day. Two blue-chitin cartridges connected in a siries were used as a column.

Some organics, such as long-chain fatty acids, which are ubiquitously present in the environment, are known to strongly inhibit the Salmonella assays of mutagens[10] The lower mutagenicity we observed in the XAD-2 concentrates of the Katsura River water samples (Table 1) may be ascribable to the presence of such inhibitory substances in them. Alternatively, it is possible that the affinity of polycyclic mutagens present in this sample to XAD-2 was lower than the affinity to the *cpt*-adsorbents.

Features of the two versions of the *cpt*-based concentration techniques for river water monitoring are summarized in Table 3. As it shows, the blue-chitin column and blue-rayon hanging have their own unique features. Both in the present experiment and in the one we performed previously[3], the 0.5 g blue-rayon hung in Katsura River was capable of collecting about 10-liter equivalent mutagens. Therefore, with one blue-rayon hanging kit commercially available, which is equipped with 6 bags of 0.5 g blue-rayon, 50-100 liter-equivalent mutagens can be collected by a 24 h hanging. It should be noted that an approximately equal efficiency was noted for blue-rayon hanging in the Asahi River (Table 2). These methods are easy to carry out, without any laborious pre-washing requirements. In combination with appropriate mutagenicity bioassays, the blue-chitin and blue-rayon techniques are expected to be useful in wide-scale screening of environmental polycyclic mutagens.

Table 3 Features of blue-chitin and blue-rayon techniques for monitoring river water mutagens

	Blue-chitin column	Blue-rayon hanging
Quantitativeness:	Quantitative	Semiquantitative
Time for work:	3-6 h work-up	24 h hanging; 2-3 h work-up
Site-visit number:	1	2
Other features:	Spot sampling	1-Day exposure sampling
	Experiments repeatable with stored water samples	Suitable for collecting large amounts of target substances
		Water transportation to laboratory unnecessary

Acknowledgments

This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture.

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AFFINITY OF A CROSS-LINKED CHITOSAN DERIVATIVE FOR ORGANOCHLORINATED XENOBIOTICS IN FRESHWATER

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Abstract

The cross-linkage of chitosan by glutaraldehyde aliphatic chain and its secondary reduction by $NaBH_3CN$ (« CHT201 derivative ») increase the adsorption properties of this polymer for PCBs (PolyChlorinated Biphenyls) and PCP (PentaChloroPhenol). To complete the study of the applications of this attractive chitosan derivative for the elimination of other types of organochlorinated xenobiotics from freshwater, the affinity of this reduced cross-linked chitosan derivative for several aromatic (HCB, pp'DDE) and aliphatic (dieldrin, lindane) organochlorinated pesticides has been evaluated. The organochlorinated hydrocarbon adsorption rate is higher for the chitosan derivative (CHT201) than for the unmodified chitosan. Moreover, the affinity of the CHT201 derivative is strongly higher for aromatic (HCB, pp'DDE) than for aliphatic (dieldrin, lindane) organochlorinated pesticides. A non-woven filter based on a cross-linked chitosan derivative was also tested for recovering organochlorinated hydrocarbons from freshwater.

Keywords: chitosan, cross-linked chitosan, waste water, organochlorinated pesticides, water scavenging, adsorbing agent, non-woven filter

Introduction

On the basis of the structure and properties of chitosan, high performance adsorbents based on chemically modified chitosan have been developed to improve the sorption capacity of this polymer in waste water treatment and in potable water purification, especially for removing organochlorinated xenobiotics from contaminated stream water. In spite of legal regulations and controls of these organochlorinated xenobiotic use, they are responsible for an ubiquitous contamination of the global ecosystem due to their high lipophility, their low degradability by chemical, microbial and metabolic tranformations. As a consequence, they accumulate in living organisms and up food chains by means of the well-known bioaccumulation processes. These xenobiotics continue to enter the aquatic environment by means of atmospheric transport and effluents originating from discharges and contaminated soils.

In previous works, Thomé *et al.* (1, 2, 3) and Weltrowski *et al.* (4) have shown that the cross-linkage of chitosan by glutaraldehyde aliphatic chain and its secondary reduction by $NaBH_3CN$ (« CHT201 derivative ») increase the adsorption properties of this polymer for several organochlorinated xenobiotics, i.e., PCBs (PolyChlorinated Biphenyls) and PCP (PentaChloroPhenol). According to the PCB and PCP sorption efficiency experiments performed with powder of this chitosan derivative (CHT201), it appeared that the optimal particle size is the granulation range $170<G<100$ mesh (2, 4)

In order to complete the study of the applications of this attractive chitosan derivative (CHT201) for the elimination of organochlorinated xenobiotics from freshwater, this paper reports further investigations on the affinity of CHT201 for aromatic and aliphatic organochlorinated pesticides. The aromatic organochlorinated pesticides tested were HCB (HexaChloroBenzene, fungicide) and pp'DDE (a DDT metabolite). The aliphatic organochlorinated pesticides tested were dieldrin (cyclodiene insecticide) and lindane (γ-HCH, i.e. γ-HexaChloroycloHexane, insecticide). To evaluate the CHT201 adsorption rate in a dynamic system which is more realistic to develop useful filtration cartridges, the experiments have been performed in flow-through system. Moreover, to increase the ease of manipulations, the treatment space reduction and the decrease of head loss which occured inevitably with the CHT201 powder cartridges, the organochlorinated pesticide adsorption efficiency of a non-woven reactive filter, based on a cross-linked chitosan derivative (K8WOT filter) developed earlier (3, 4, 5), has been tested.

Materials and methods

Chemicals

Chitosan originating from crustacean cuticle was purchased from the PROTAN Society (Practical grade) and from WATER AND OIL TECHNOLOGIES Inc. The cross-linkage of chitosan with glutaraldehyde and the secondary reduction by $NaBH_3CN$ was performed by chitosan treatment as described earlier (1, 2). The non-woven reactive filter based on a cross-linked chitosan derivative (K8WOT filter) was obtained by the procedure previously described (5). All solvents (n-hexane, acetone, methanol) were of Pesticide grade. These solvents and the analytical pure organochlorinated pesticides (HCB, pp'DDE, lindane and dieldrin) were obtained from PROMOCHEM (Wesel, Germany).

Experimental Procedure

The filtration of organochlorinated pesticide spiked water was performed by means of a flow-through system .

- chitosan powder filter: For each organochlorinated pesticide (O.C. pesticide), 2 liters of spiked water (pH 7.5) were passed through a column filled with 100 mg or 250 mg of CHT201 (170<G<100 mesh) as described by Thomé *et al.* (2, 3). The flow rate was 5 $ml.min^{-1}$ which correspond, according to the type of column used for the different chitosan weight, to a superficial velocity of 17.7 and 8.84 $ml.min^{-1}.cm^{-2}$, respectively. The O.C. pesticide concentrations in spiked water were 4 $\mu g.l^{-1}$ for 100 mg column and 10 $\mu g.l^{-1}$ for 250 mg column to maintain a constant ratio between the xenobiotic concentration and the CHT201 weight. The O.C. pesticide concentrations were measured in the effluents every 250 ml. The breakthrough volume was determined for 90% to 95 % adsorption rate on CHT201

- K8WOT filter: For each O.C. pesticide, 4 to 9 liters of spiked water (10 $\mu g.l^{-1}$) were passed at a constant flow rate (20 $ml.min^{-1}$) through ten layers of K8WOT filter (± 1 g of chitosan derivative) placed in a stainless stell filtration cell as described by Weltrowski *et al.* (5). The filtration cell surface was 9.62 cm^2 and the flow rate was 20 $ml.min^{-1}$ i.e. a superficial velocity of 2.03. $ml.min^{-1}.cm^{-2}$. The O.C. pesticide concentrations were measured in the effluents every 250 ml.

Organochlorinated pesticide extraction from residual contaminated water

The O.C. pesticide extraction, gas chromatography analysis and O.C. pesticide quantification were carried out according to the procedures described in previous works (1, 2, 6)

Results and Discussion

Organochlorinated Pesticide Binding Ability of CHT201 powder

The evolution of O.C. pesticide concentrations measured in effluents, as a function of the spiked water volume filtered on CHT201 powder cartridge, are presented for aromatic and aliphatic O.C. pesticides in figures 1 and 2, respectively.

From these figures, it appears that the CHT201 sorption efficiency is quite different according to the pesticide chemical structure. Indeed, for aromatic O.C. pesticides (fig. 1), the breakthrough volume for an adsorption rate of 95 % is 1.5 liters for HCB and the CHT201 adsorption efficiency is still 100 % after filtration of 2 liters of pp'DDE spiked water.

The figure 2 shows clearly that CHT201 derivative remains rapidly uneffective for aliphatic pesticides, lindane and dieldrin. Indeed, for a 90 % adsorption rate, the breakthrough volumes are very low, less than 50 ml for lindane and 300 ml for dieldrin.

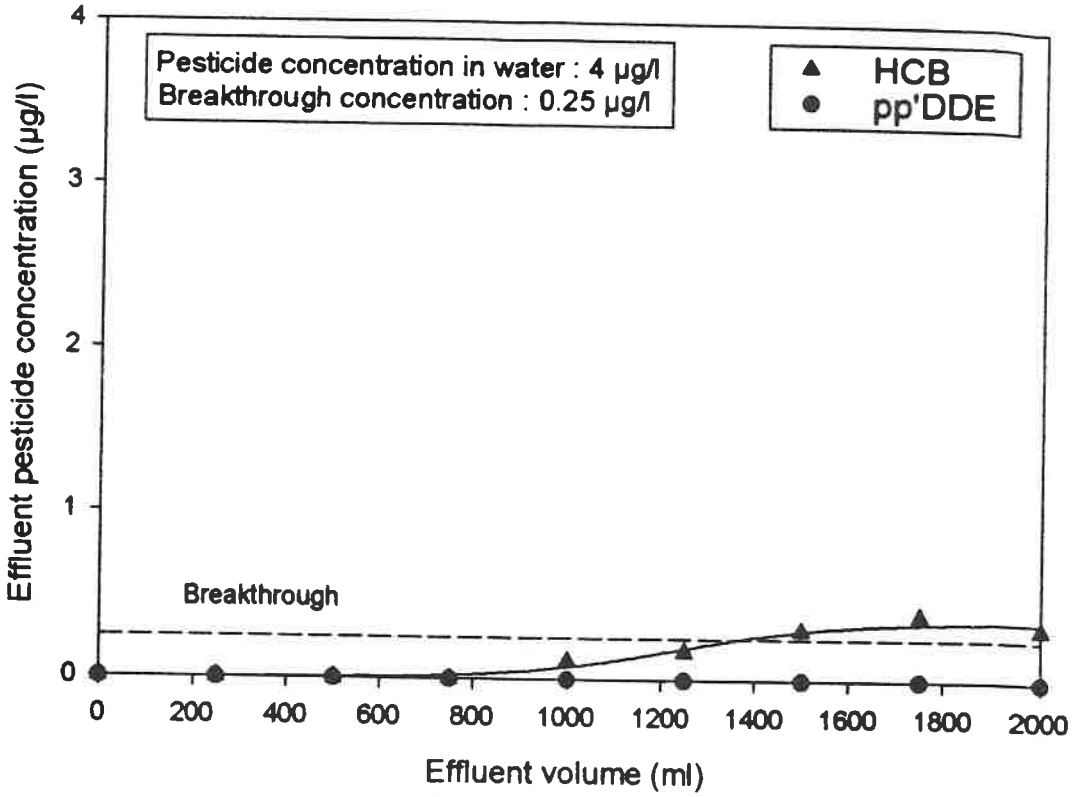

Figure 1: Breakthrough curves obtained for aromatic organochlorinated pesticides after filtration through a column filled with 100 mg of CHT201 powder (170<G<100 mesh).

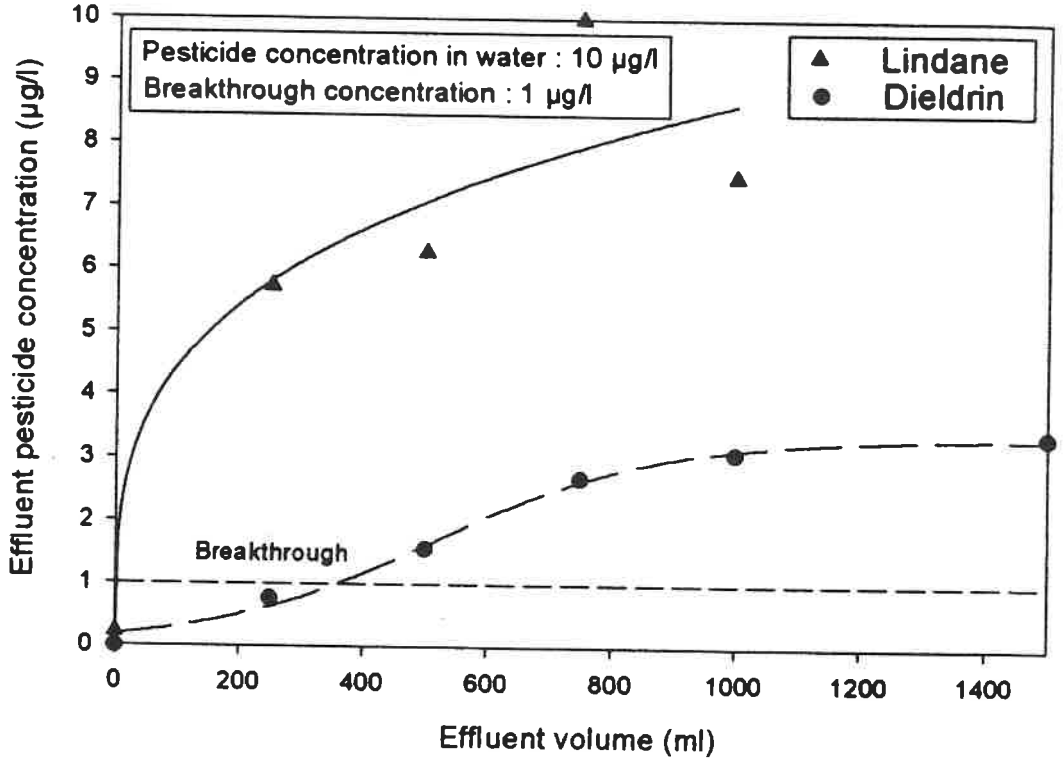

Figure 2: Breakthrough curves obtained for aliphatic organochlorinated pesticides after filtration through a column filled with 250 mg of CHT201 powder (170<G<100 mesh).

In previous works (1, 2, 3), we have shown that CHT201 powder in flow-through system allowed high recovery of PCBs. If this chitosan derivative displays a similar powerful adsorption efficiency for aromatic pesticides, it is uneffective for aliphatic pesticides. Moreover, the O.C. pesticide adsorption efficiency of CHT201 powder in flow-through system remains generally lower than for PCBs with the exception of pp'DDE whose chemical structure is close to that of PCBs.

Organochlorinated Pesticide Binding Ability of K8WOT non woven filter

The figure 3 and 4 show the evolution of the O.C. pesticide concentrations in the effluent as a function of the water volume filtered through the K8WOT filter.

For the aromatic pesticides (HCB and pp'DDE), the K8WOT adsorption efficiency is similar to that observed with CHT201 powder filter. Indeed, the breakthrough volume for 95 % adsorption rate of HCB is 1.5 liters whereas the pp'DDE adsorption rate is still 100 % after filtration of 9 liters of spiked water. However, the amount of the chitosan derivative is about ten times higher for the K8WOT filter (± 1 g of cross-linked chitosan) than for the CHT 201 powder filter (100 mg). Moreover, when compared with the PCB adsorption efficiency of K8WOT filter observed earlier (3), the HCB elimination from contaminated water is relatively low. Indeed, the breakthrough volume for 99 % adsorption rate of PCB101 (a pentachlorobiphenyl) was 25 liters for the same amount of cross-linked chitosan on K8WOT filter.

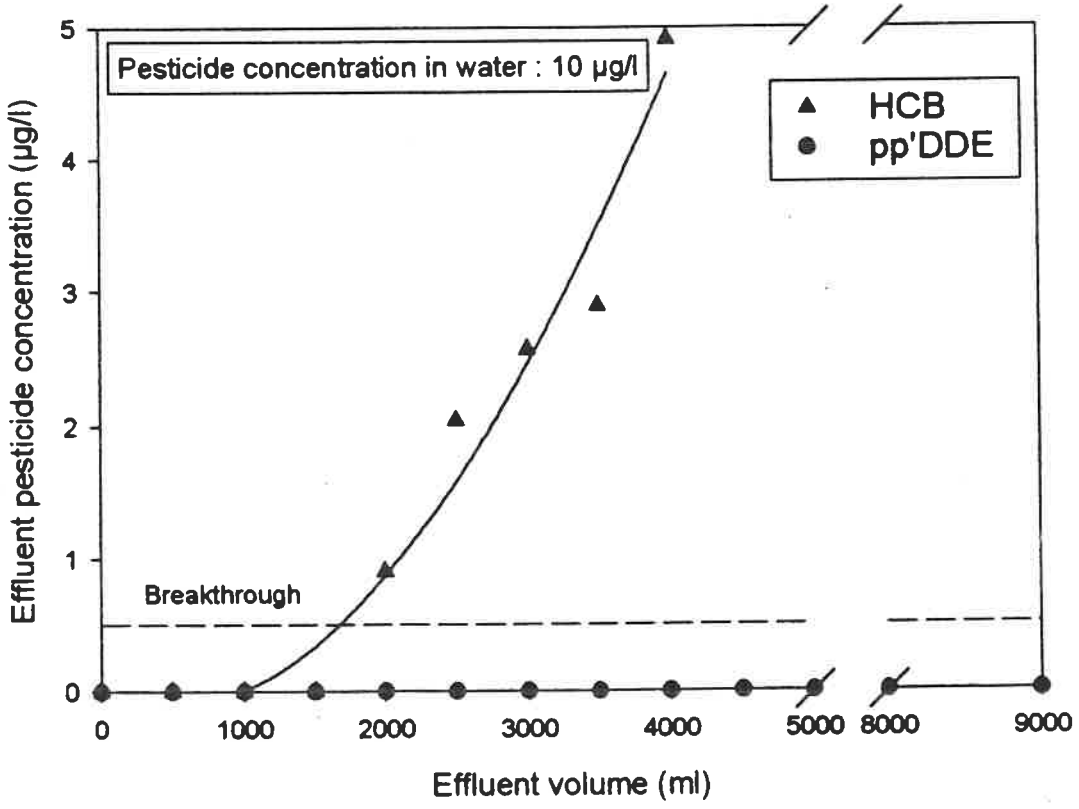

Figure 3: Breakthrough curves obtained for aromatic organochlorinated pesticides (HCB and pp'DDE) after filtration through the K8WOT filter.

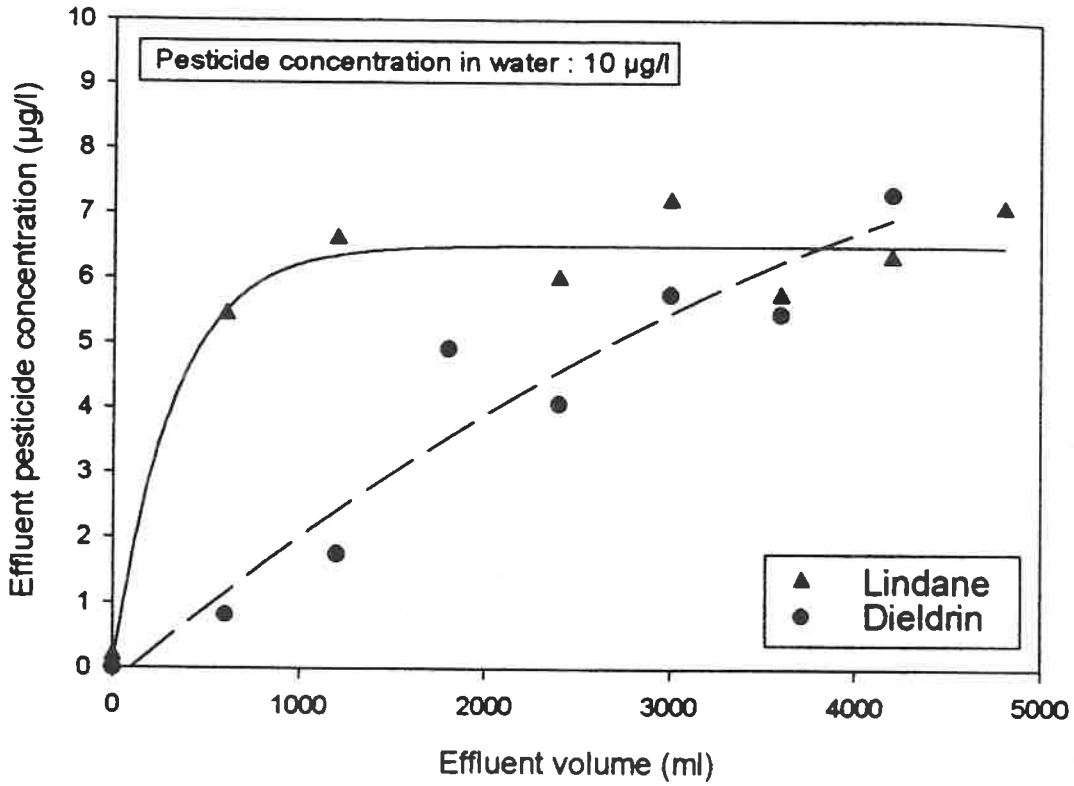

Figure 4: Breakthrough curves obtained for aliphatic organochlorinated pesticides (lindane and dieldrin) after filtration through the K8WOT filter.

For the aliphatic O.C. pesticides (lindane and dieldrin), this K8WOT filter remains largely uneffective (figure 4). After filtration of 1 liter for lindane and 4 liters for dieldrin, the K8WOT adsorption rate is only 30 %.

Conclusions

The study of the organochlorinated pesticide adsorption efficiency of chitosan derivative (CHT201) confirms that the cross-linkage of chitosan by glutaraldehyde aliphatic chain and the secondary reduction by $NaBH_3CN$ improves greatly the chitosan adsorption properties for organochlorinated xenobiotics. The CHT201 reduced cross-linked chitosan derivative has a higher adsorption efficiency for aromatic organochlorinated xenobiotics (PCBs, PCP, HCB and pp'DDE) than for the aliphatic ones (lindane and dieldrin). Moreover, CHT201 remains largely uneffective for recovering lindane from contaminated water. The non-woven reactive filter based on cross-linked chitosan (K8WOT filter) appears also as a powerful organochlorinated xenobiotic adsorbent but a little less efficient than the CHT201 powder. However, the K8WOT filter facility of manipulation and of regeneration by a simple chemical treatment related to the treatment space reduction and the decrease of head loss which occur inevitably with the CHT201 powder filter make of the K8WOT filter one of the most efficient tool to ensure organochlorinated xenobiotic recovery from contaminated stream water.

Acknowledgments.

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Development of Amphoteric Flocculants and Strong Metal Uptaking Agents Through Chemical Modifications of Chitosan

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Abstract

Amphoteric flocculants which function both as polycationic flocculants in acidic aqueous solutions and also as polyanionic flocculants in alkaline aqueous solutions were developed through graftcopolymerization of alkenoic acids and alkenedioic acids onto chitosan. Those copolymers were also found to be far superior to pure chitosan in removing various kinds of contaminants sweeping whole pH range of test solutions. Those copolymers were also found to be extraordinarily strong metal uptaking agents comparing with pure chitosan. Eventually those copolymers are expected to be very useful in the processes for production of ultrapure water or metal free water for the use of advanced technology and industry and also useful in recovering precious metals and radioactive elements.

Introduction

The author could affirm that chitosan was an excellent flocculant and metal uptaking agent through many experiments with many and various kinds of used or wastewater and test solutions. But the author has found that the efficiency and power of flocculation and metal uptake rate by chitosan are remarkably dependent upon pH values of the test solutions within a fixed time. Further more, chitosan has become more precious these days as its use or usage has been increased and expanded in many ways.

The author tried to find out a way for the elevation of value or worth of chitosan uses- a way of more precious and economical use.

The author modified chemical structures of chitosan through graft-copolymerization of elaborately selected monomers(alkenoic acids and alkenedioic acids) onto chitosan to raise contaminant removing efficiencies and metal uptaking rates so that chitosan could be used in more effective and valuable way.

Materials and Methods

· CHITOSAN

All chitosan used in this experiment was prepared in the same batch in

the author's laboratory to keep reactions and experimental conditions constant and it was found to be DA degree : around 89%, viscosity : 1400 cps by the Brookfield L-type viscometer with 1% of chitosan in 5%(v/v) acetic acid solution.

· GRAFT-COPOLYMERIZATION

The conditions of graft-copolymerization are:

amount of chitosan : 2g/batch, amount of each monomer : 1.7422g/batch, conc. of ceric ammonium nitrate(CAN) : 5.0×10^{-3}moles/batch

· Flow sheet of graft-copolymerization

dissolving mixture of chitosan and monomer in dilute acetic acid solution → swelling → adding reaction initiator → reaction for more than 3hrs → precipitation in acetone → filtration → washing off homopolymer or other contaminants → vacuum drying

The states of graft-copolymerization were identified through IR spectra checking and observation of SEM graphs.

The graft-copolymers would be positively charged on free amino groups of chitosan molecules when they are dissolved in acidic aqueous solutions whilest the copolymers would be negatively charged with the carboxylic groups introduced through graft-copolymerization when they are dissolved in alkaline aqueous solutions. Thus the graft-copolymers play in amphoteric ways sharply corresponding with instantly changing characteristics of the test solutions.

· SYMBOLS for MONOMERS and GRAFTED CHITOSAN developed in the author's laboratory

Cs : no grafted and pure chitosan	PAm : polyacrylamide, a synthetic polymer
CsAc : chitosan grafted with acrylic acid	CsAm : chitosan grafted with acrylamide
CsMa : chitosan grafted with maleic acid	CsFa : chitosan grafted with fumaric acid
CsIa : chitosan grafted with itaconic acid	CsCa : chitosan grafted with citraconic acid

RESULTS and DISCUSSION

The experimental scheme for this study is composed of the primary treatment system(dose and sedimentation process) and the secondary

treatment system(beads column filtering process) as shown in Fig.1

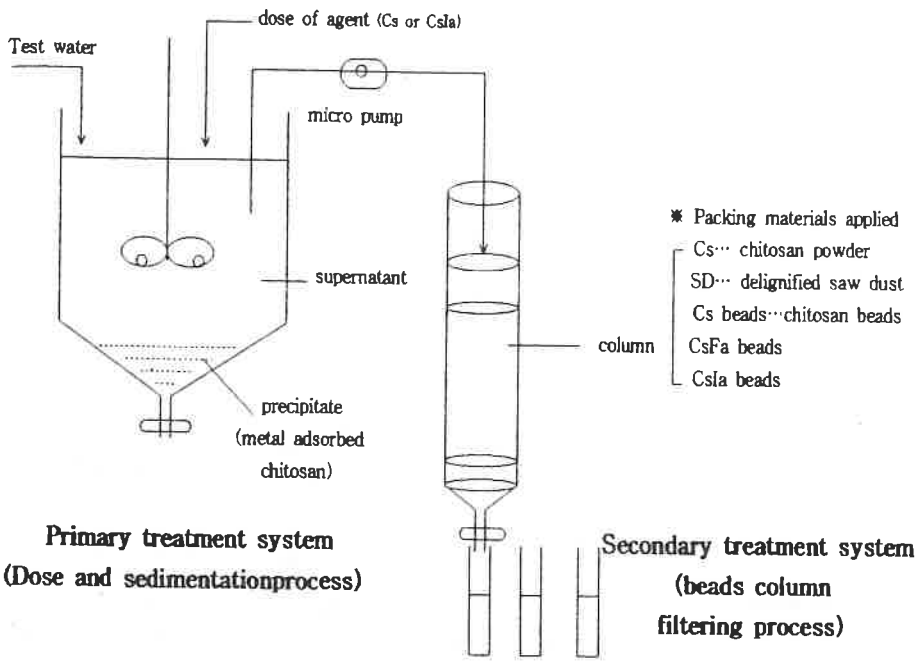


Fig.1 Primary and Secondary Treatment Systems

In to the primary treatment system, the raw test water is introduced and most contaminants are removed as much as possible by doses of agents (chitosan and grafted chitosan) to form flocs and settle down, and then the supernatant formed in the primary treatment tank is introduced into the secondary treatment system where the remained contaminants(mainly metal ions and some other substances) are removed.

The supernatant in the sedimentation tank and the eluent from the beads columns are subjected to chemical analysis procedures separately.

1. Flocculation ability and COD removal

Pure chitosan(Cs) and one of graft-copolymers of chitosan, CsFa were subjected to sedimentation and transmittance tests with kaoline suspension to evaluate their flocculation power. The CsFa demonstrates its remarkable superiority to pure chitosan in flocculation efficiency sweeping whole pH range of the test solution. Particularly in alkaline pH range, the grafted chitosan remarkably increases in flocculation power whilest the pure chitosan decreases(Fig.2 and Fig.3)

These phenomena also appeared in COD removal of various kinds of wastewater as shown in Fig.4

The phenomena of such extraordinary increase in flocculation power and COD removal rates by the graft-copolymers in alkaline aqueous solutions are evidently caused by the rich carboxylate ions introduced into chitosan molecules through graft-copolymerization.

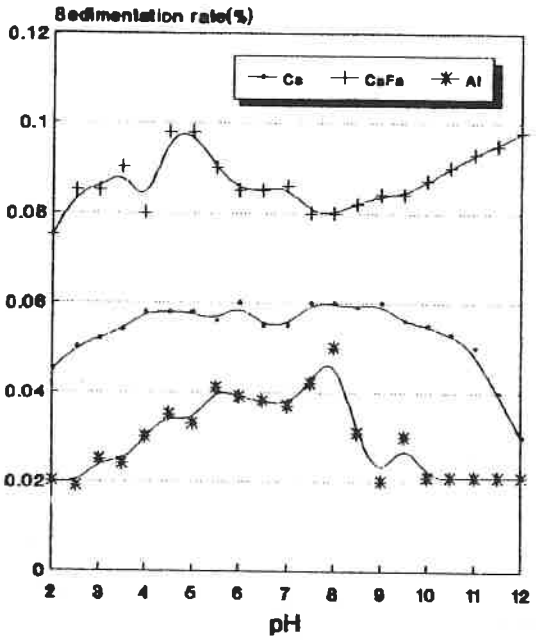


Fig. 2. Sedimentation tests with 0.4%(w/w) kaoline suspension vs pH of the test solution (in acidic)
· dosage of each agent : 40ppm · Al : alum(dose:100ppm)

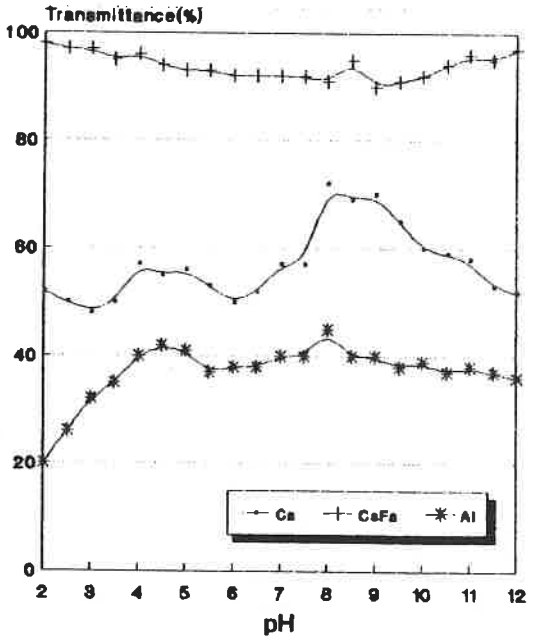


Fig. 3. Transmittance of supernatants after sedimentation of 0.4%(w/w) kaoline suspension vs pH of the test solution
· dosage of each agent : 40ppm · Al:alum (dose:100ppm)

Thus the grafted chitosans function as polyanionic flocculants in alkaline aqueous solutions while they function as polycationic flocculants with free amino groups of chitosan molecules in acidic aqueous solution—amphoteric flocculants. The branched chemical structure of the graft-copolymers of chitosan and the increased MWs of them favor the Stoke's law and coverage of particles of contaminants and eventually bring out extraordinarily high contaminant removal rates sweeping whole pH range of the test solution leaving big gaps among them(Fig.2, 3, 4, 5)
The order of removal rate is : PAm < Cs < CsAm < CsAa < CsMa

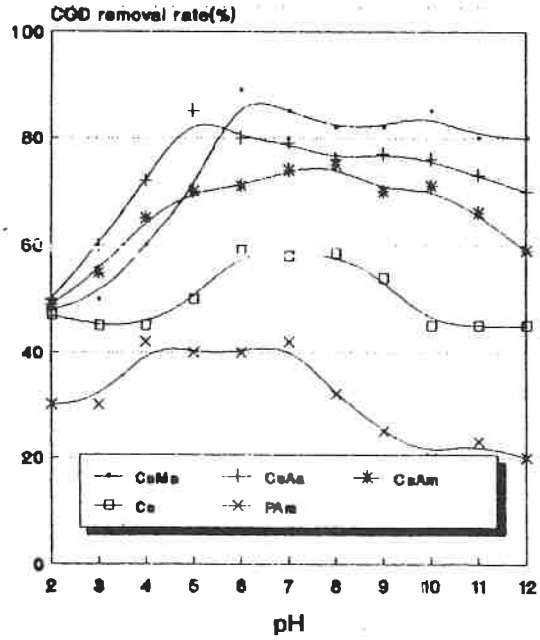


Fig. 4. COD removal vs pH values of a skin/leather process wastewater by the flocculants.
· original COD of the wastewater : 13,500 ppm

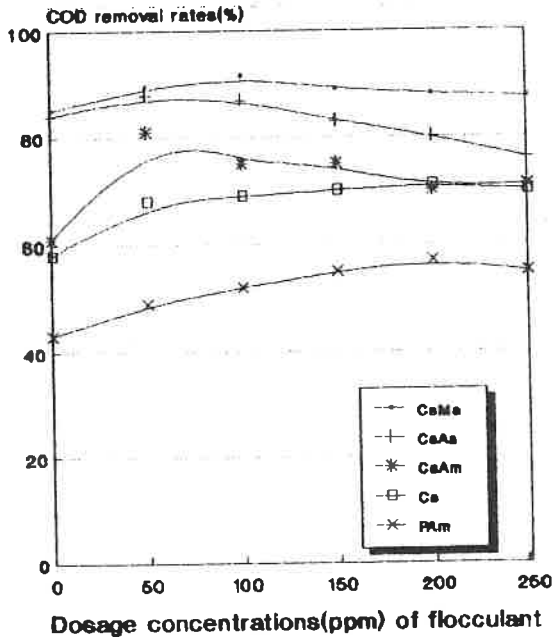


Fig. 5. COD removal vs dosage of the flocclants at their appropriate pH values of the water
· original COD of the wastewater : 13,500 ppm

Fig.5 shows dose effect of the test flocculants. This suggests that the grafted chitosans are required extremely small amount to achieve the same COD removal rate accomplished by pure chitosan.

2. Metal uptake tests

(A) In Primary Treatment(dose and sedimentation process)

The test solution was prepared by mixing many species of metal ions (Pb^{2+}, Cu^{2+}, Cd^{2+}, Cr^{6+}, Fe^{3+}, Hg^{2+}, Al^{3+}, Zn^{2+}, $Ca^{2+}$and Mn^{2+}) and introduced into primary treatment system, in which doses of each agent were applied into the test solution with thorough agitation to form ppt. vs pH values of the test solutions.

Metal concentrations remained in the supernatant were determined by the ICP analysis. Removal rates are sharply dependent upon pH values of the solution.

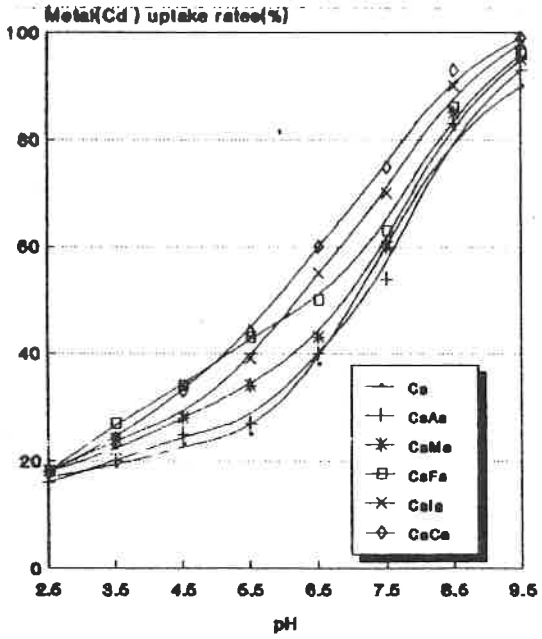


Fig. 6. Cd^{2+} uptake profiles by the agents VS pH values in dosage processes.

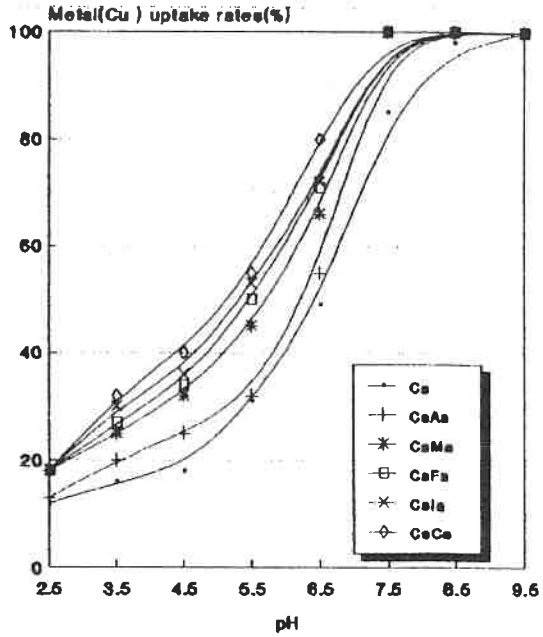


Fig. 7. Cu^{2+} uptake profiles by the agents VS pH values in case of addition of kaoline in dosage processes.

(Dosage concent of each agent : 40ppm, each metal concent :20ppm)

Profiles of Cd^{2+} and Cu^{2+} uptake rates by the agents are shown in Fig. 6. and Fig. 7 respectively. Most of other metals showed the similar profiles with those metals. In all test cases, all the graft-copolymers strongly dominate pure chitosan in metal uptake rates sweeping whole pH range of the test solutions. The order of metal uptake rates by the agents are not changed with kind of metals. The order of metal uptake rates is

$$Cs < CsAa < CsMa < CsFa < CsIa < CsCa.$$

(B) In Secondary Treatment (Beads Column Filtering Process)

Chitosan and grafted chitosan were shaped into beads and treated with glutaraldehyde to have them bridged to keep them from dissolving in acidic solutions. Those beads were packed into columns as filtering kits.

Life time of chitosan beads columns were examined with change of primary treatment conditions. When the raw test solution(mixture of several kind of metal ions) was introduced directly into Cs column, saturation curve intersects the Q(L) axis at the very early stage(4.6L).

But when the raw test solution was treated with Cs dose in the primary treatment system, the saturation curve by Pb^{2+} ions intersects the Q(L) axis far later (17.0L). That means the life time of the column was elongated 4.7times longer when the primary treatment system was applied. If CsIa was used instead of Cs in the primary treatment, the life time of the column was prolonged almost 4.8times longer(Fig. 8)

Also in the case of Cu^{2+} removal through the same routine as of Pb^{2+}, almost similar life time values for the column were obtained.(Fig. 9)

Those experimental results suggest how important and critical the primary treatment is for the effective use of beads columns. It is critically necessary to remove contaminants(metals) as much as possible in the primary treatment system before applying the beads columns.

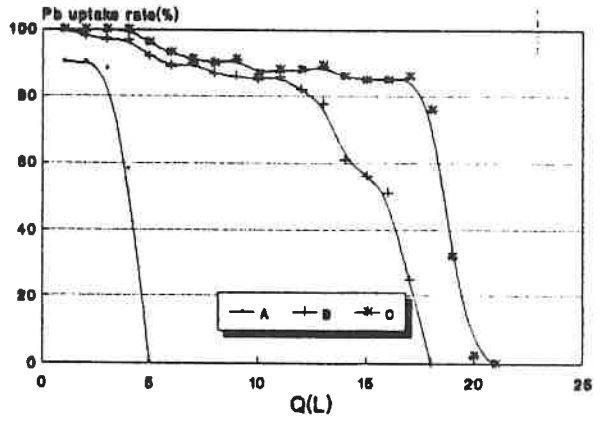


Fig. 8. Pb^{2+} Saturation curves of chitosan beads columns in Pb^{2+} uptake tests. Solution : mixture of Pb^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, (each conc. of metal ions : 5ppm), Flow rate : 1 ℓ/day, Amt. of packing material : 10g(dry weight)

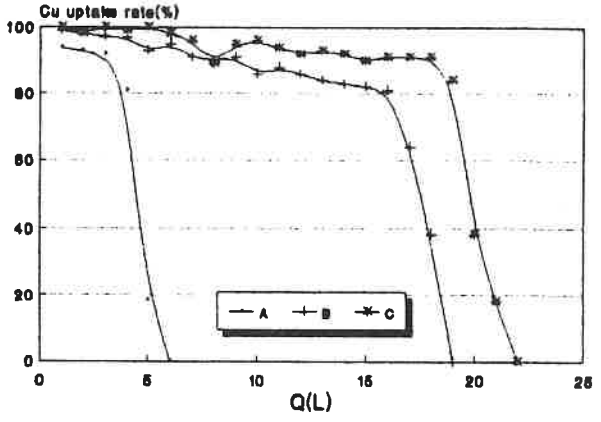


Fig. 9. Cu^{2+} Saturation curves of chitosan beads columns in Cu^{2+} uptake tests. Test solution : mixture of Pb^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, (each conc. : 5ppm) Flow rate : 1 ℓ/day, Amt. of packing material : 10g(dry weight)

A : direct introduction of test solution into column without primary treatment.

B : introduction of test solution into column after Cs dosage in primary treatment

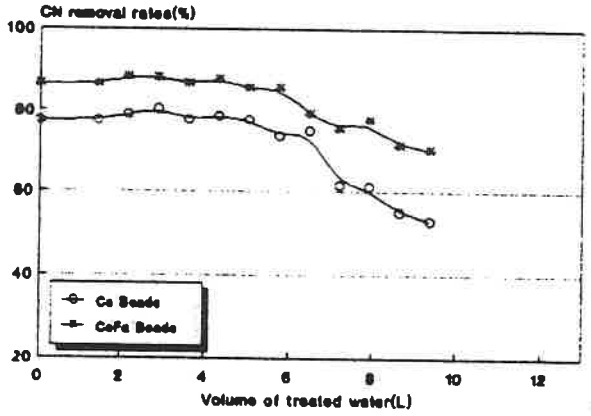
C : introduction of test solution into column after CsIa dosage in primary treatment.

Finally metal removal efficiencies by Cs beads column and one of the graft-copolymers of chitosan, CsFa beads column were compared and the beads of the graft-copolymer of chitosan was proved to be far more effective than that of pure chitosan in CN^- and metal removal.

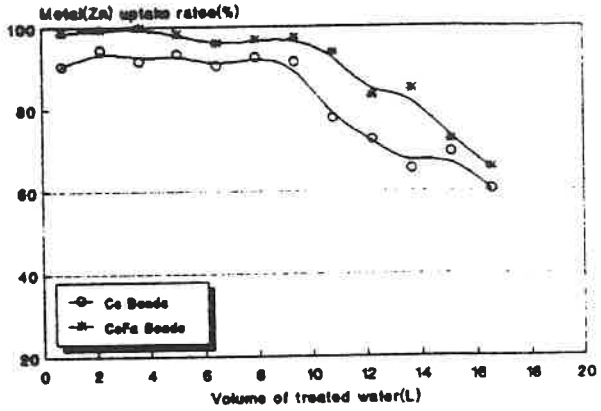
This time Cs dosage was commonly applied in the primary treatment system for the both cases.

Even in beads form, the grafted chitosan demonstrates its outstanding contaminant removal power overwhelmingly dominating pure chitosan beads. The grafted chitosan exhibits its superiority in removing cyanide to pure chitosan as in the case of Zn^{2+} removal(Fig. 10, 11).

In the case of beads columns, the grafted chitosan beads demonstrate their outstanding metal uptake power strongly dominating that of chitosan as in the case of dose and sedimentation process(primary treatment system).



10. CN^- uptake profiles



11. Zn^{2+} uptake profiles

(The raw wastewater was primarily treated by Cs dose process)

Practical application with Raw wastewater from metal plating factory

Regeneration of beads

The bridged beads of chitosan and grafted chitosan were easily regenerated with treatment of hydrochloric acid solution(pH3) without any damage and could be used many times.

CONCLUSION :

Very effective amphoteric flocculants and strong metal uptaking agents were developed step by step through graft-copolymerizations of deliverately selected alkenoic and alkendioic acids onto chitosan.

1. In flocculation efficiency tests

Through sedimentation and transmittance tests with kaoline suspension and COD and other contaminants removal tests with practical wastewater, the graft derivatives of chitosan demonstrate their high efficiencies strongly dominating pure chitosan with amphoteric effect and molecular advantages(MW increase and branched structure).

The order of removal efficiency is

PAm < CsAm < CsAa < CsMa (dicarboxylic acid is superior)

2. In metal uptake tests

The graft-copolymers of chitosan were proved to be remarkably excellent metal uptaking agents which are far superior to pure chitosan in both dose-and-sedimentation process and beads-filtering-process.

The order of uptake rates is

Cs < CsAa < CsMa < CsFa < CsIa < CsCa

(dicarboxylic acids and MW advantageous)

3. Regeneration of the used beads

The used beads were easily regenerated by washing with hydrochloric acid solution(pH 3)

Thus the graft-copolymers of chitosan are expected to be useful for the partial process for production of ultrapure and metal free water for the use of advanced technology and industry and also useful in recovering precious metals and radioactive elements.

4. The prospects for the use of graft-copolymers of chitosan

Remarkable solubility of the graft-copolymers in wide pH of water and alcoholic water enable them more useful and more applicable in many ways and fields.

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