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Modified chitosans carrying sulfonic acid groups

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The sulfonic acid function was introduced into chitosan by reacting it with 5-formyl-2-furansulfonic acid, sodium salt, under the mild conditions of the Schiff reaction, thus avoiding polymer degradation and *O*-substitution. The reaction of chitosan (degree of deacetylation 0·58) with 5-formyl-2-furansulfonic acid, sodium salt produced a viscous solution that, upon hydrogenation, yielded *N*-sulfofurfuryl chitosan sodium salt. Infrared spectrometry, alkalimetry and elemental analysis provided evidence that the degree of substitution was 0·26. Circular dichroism measurements on solutions showed multiple Cotton bands in the pH interval 7·1–8·3, while at lower and higher pH values just one negative band was observed, thus providing indication of the polyampholyte nature of *N*-sulfofurfuryl chitosan. The ^{13}C-NMR and FTIR spectra showed typical signals of furane carbons. Metal ion solutions at concentrations in the range 0·1–5·0 mM, pH ~6, promoted precipitation of metal ion complexes of *N*-sulfofurfuryl chitosan, with most effective removal from the solutions for Cu(II), Pb(II) and Ni(II). Sulfoethyl *N*-carboxymethyl chitosan was also synthesized from 2-chloroethanesulfonic acid in organic media: the sulfur content was similar (3·7%) in both polymers.

INTRODUCTION

In contrast to chitin and chitosan sulfate esters, which have been widely studied in view of their anticoagulant activity and general interest as polyampholytes (Focher *et al.*, 1986; Hirano *et al.*, 1986, 1991; Naggi *et al.*, 1986; Okiei *et al.*, 1986; Gorbacheva *et al.*, 1990, 1991), chitosans carrying sulfonic acid groups appear to have been scarcely studied, in spite of early work on sulphoethyl chitosan synthesis (Nud'ga *et al.*, 1974; Muzzarelli, 1977).

By using 2-chloroethanesulfonic acid sodium salt in alkaline organic media, Nud'ga *et al.* (1974) obtained sulfoethyl chitosan with a degree of substitution of 0·11–0·35 and a sulfur content of 1·39–5·32%. Substitution involved both the *O*-6 and *N*-2 positions. Sulfoethyl chitosan films had antithrombogenic properties. Scarce solubility was observed for degrees of substitution higher than 0·30 (Nud'ga *et al.*, 1974).

The author and co-workers have recently reported on a modified chitosan obtained from 5-hydroxymethyl furfural, via reductive amination (Delben *et al.*, 1992). The presence of the hydroxymethyl furan moiety was found to impart solubility to chitosan and to make it more susceptible to the action of lysozyme. Furan-based aldehydes suitable for the chemical derivatization of chitosan include 5-hydroxymethyl-2-furfural, glucosyloxymethyl furfural, 5-chloromethyl furfural, 3-(2-furyl)acrolein and 5-formyl-2-furansulfonic acid, sodium salt, besides 2-furaldehyde itself. These compounds are easily available, being produced from common sugars.

Apart from being used as polymer intermediates (Gandini, 1990), these furanoid compounds are widely used in other ways. For example, 2-furaldehyde is used in the synthesis of chemotherapeutic agents (Sherman, 1962; Ebetino *et al.*, 1963), and hydroxymethyl furfural derivatives are used as pharmaceuticals, surfactants, cosmetics (Kuhn & Dury, 1954; Moeller *et al.*, 1975; Lichtenthaler *et al.*, 1991), perfumes and fragrancies (Proserpio, 1985). Bifunctional derivatives of 2-furaldehyde have been described (Musau & Munavu, 1990).

5-Formyl-2-furansulfonic acid is an attractive intermediate for the sulfonation of chitosan because the modification would be conducted via Schiff base formation, i.e. under mild conditions rather than the very harsh conditions demanded by the use of 2-chloroethanesulfonic acid.

The presence of a sulfonic acid group on the furane ring is expected to impart solubility and polyampholyte behavior to chitosan, and to affect its chelating ability

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and biological activity. As a continuation of works from the author's laboratory on *N*-carboxymethyl chitosan sulfate esters (Muzzarelli *et al.*, 1982), the present article describes the preparation and the characterization of *N*-sulfofurfuryl chitosan and sulfoethyl *N*-carboxymethyl chitosan.

EXPERIMENTAL

Materials

Preparation of N-*sulfofurfuryl chitosan*
Chitosan from antarctic krill, *Euphausia superba*, having a degree of deacetylation of 0·58 and an average molecular weight of 465 000 Da (Muzzarelli *et al.*, 1987) was used. 5-formyl-2-furansulfonic acid, sodium salt was purchased from Aldrich. Chitosan powder (10 g, 180–350 μm) was suspended in water (250 ml) and a solution containing 5-formyl-2-furansulfonic acid, sodium salt (6·5 g in 250 ml) was added to the slurry. Stirring was protracted overnight and a viscous solution was obtained, having pH 9·0. A 1% solution was obtained by dilution with water (500 ml). Sodium borohydride (3 g) was slowly added over a 5-h period; pH was raised to 10 or higher by careful addition of sodium hydroxide. The modified chitosan was isolated by insolubilization with methanol (2 liters) and acetone (1 liter), and carefully washed with methanol and ether.

Preparation of sulfoethyl N-*carboxymethyl chitosan*
Finely milled, water-insoluble *N*-carboxymethyl chitosan (20 g) obtained from crab chitosan (Muzzarelli *et al.*, 1989) was suspended in isopropanol (200 ml), then NaOH (30%, 31·25 ml) was added under stirring at 5–10°C over 1 h. The mixture was diluted with isopropanol (200 ml) and the temperature was brought to 80°C. 2-Chloroethanesulfonic acid, sodium salt was added in portions (14·37 g), and heating was protracted for 3 h. Hydrochloric acid was added to neutrality and the product was filtered and washed with ethanol until chloride was no longer detected.

Methods

Circular dichroism spectra were recorded with a Jasco spectropolarimeter model J-500-A. For infrared spectroscopy, polysaccharide powder was ground with IR grade potassium bromide in an agate mortar, and spectra were recorded with an FTIR Perkin-Elmer spectrometer, on translucent discs obtained by pressing the ground material. The ^{13}C-NMR spectra were obtained at 35°C with a 20-MHz Varian CFT-20 spectrometer equipped with a 10-mm probe. The solutions were prepared in D_2O (45 mg/ml) and their pH was adjusted to less than 5 by adding DCl. Kontron Uvikon 810 or Uvikon 860 spectrophotometers were used with far-UV cuvettes (10 mm path length). For the alkalimetric measurements, the samples (0·5 g) were dissolved in 0·1 M hydrochloric acid (50 ml) and titrated with 0·1 M sodium hydroxide under nitrogen. Both reagents contained 0·1 M sodium chloride. Additions were made at time intervals to permit accurate readings, especially in the central part of the pH interval. Atomic absorption spectrometric analyses were performed with a Perkin-Elmer 2380 spectrometer, equipped with flame and graphite atomizers, according to standard methods; the samples were mineralized with nitric acid in a microwave oven.

RESULTS AND DISCUSSION

Chemical identity

The introduction of the sulfonic acid function in the chitosan macromolecule via the Schiff reaction with 5-formyl-2-furansulfonic acid and sodium salt yielded chitosan derivatives perfectly soluble over an extended pH range including alkaline values.

The elemental analysis done on *N*-sulfofurfuryl chitosan gave an indication that the degree of acetylation was 0·42 ± 0·04, the degree of substitution was 0·26 ± 0·02, the rest of the amino groups being in the free form; moisture was 7% (Table 1). Confirmation of these data was obtained by titrating the polysaccharide (0·5 g) dissolved in 0·1 M HCl (50 ml) containing NaCl (0·1 M). The alkalimetric titration was done under nitrogen with 0·1 M NaOH in the presence of NaCl (0·1 M). Based on the difference between the inflection points, 13·3 ml of 0·1 M NaOH were necessary to titrate the totality of primary and secondary amino groups.

The presence of the sulfonic acid function was revealed by bands at 1220, 800 and 655 cm^{-1} in the infrared spectrum of *N*-sulfofurfuryl chitosan. The spectrum also showed distinct bands centered at 2876, 2910 and 2960 cm^{-1} due to the furfuryl group, whilst this part of the spectrum for the chitosan itself exhibited a wide absorption band centered at 2900 cm^{-1}. It is known that sodium borohydride does not hydrogenate the furane and thiophene double bonds.

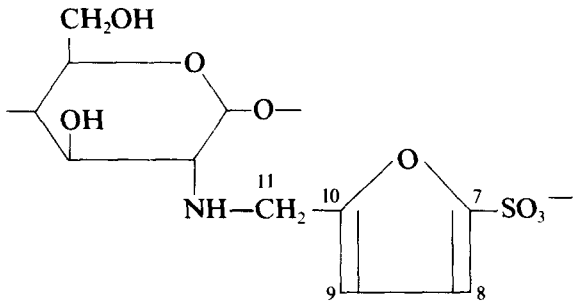
Table 1. Elemental analysis data and degrees of substitution for 2-sulfofurfuryl chitosan

Elemental analysis (%)	
C, 42·4; O, 43·2; H, 6·3; N, 6·0; S, 3·7	
Degrees of substitution (%)	
Acetylation,	42 ± 4
Sulfofurfuryl,	26 ± 2
Free amine,	32 ± 2 (33 ± 2 by alkalimetry)
Moisture,	7 ± 1

Table 2. ^{13}C NMR chemical shifts of *N*-sulfurfuryl chitosan and parent chitosan in D_2O (50 mg/ml) with added DCl to lower the pD value below 5·0

Assignment	Chemical shifts (δ ppm)	
	Parent chitosan	*N*-sulfofurfuryl chitosan
C-1	101·2	99·0
C-2	59·2	58·6
C-3	73·7	72·7
C-4	80·1	80·0
C-5	78·2	77·4
C-6	63·1	63·1 63·9
C-7		154·6
C-8		117·4
C-9		115·4
C-10		148·0
C-11		46·4

Signals for $-CH_3$ and C = O of the acetyl group were also present in both spectra.



The presence of the heterocyclic component was also revealed by ^{13}C-NMR spectrometry (Table 2); signals at 154·6, 148·0, 117·4 and 115·4 ppm being typical of furane carbon atoms. In the methylene region, a signal (absent in chitosan) at 46·3 ppm could be assigned to C-11; an analogous signal was found in the spectrum of *N*-carboxymethyl chitosan (Muzzarelli *et al.*, 1982).

The optical characteristics of the *N*-sulfofurfryl chitosan solutions, studied by UV-vis spectrophotometry and by circular dichroism spectropolarimetry, appeared to be pH-dependent.

Ultraviolet spectra of *N*-sulfofurfuryl chitosan in buffer solutions were recorded over a wide pH range against the same buffer; a single absorption band was recorded at acidic and alkaline pH values, while two distinct bands were present in nearly neutral solutions.

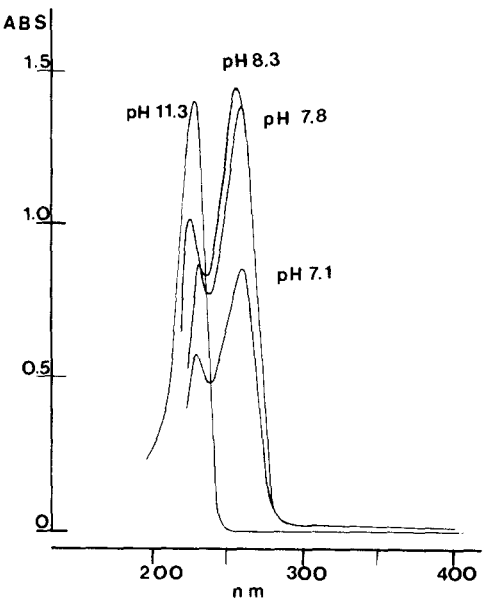


Fig. 1. Ultraviolet spectra for *N*-sulfofurfuryl chitosan at pH values 7·1, 7·8, 8·3 and 11·3.

The data are listed in Table 3 and presented in Fig. 1. Circular dichroism spectra showed the negative Cotton band at 205–210 nm which is typical for glycosaminoglycans (Cowman *et al.*, 1981), in agreement with data on glucosamine oligomers (Domard, 1986), and on *N*-carboxymethyl chitosan (compare Fig. 5 in Muzzarelli & Tanfani, 1982); as a point of difference at pH 3·5, the negative band at 210 nm did not shift in the case of *N*-carboxymethyl chitosan, whilst it shifted to 228 nm in the case of *N*-sulfofurfuryl chitosan (Fig. 2). Evidence of pH-dependent alterations in the polymer conformation was also given by circular dichroism spectroscopy. In fact, when going from low to high pH values, new Cotton bands appeared in the spectra as indicated in Table 4. In agreement with the spectrophotometric data, in the pH interval 7·1–8·3 novel positive Cotton bands at 228–230 nm were observed, indicative of conformational changes due to polyampholyte behavior and internal salt formation.

Chelating ability

Solutions of *N*-sulfofurfuryl chitosan, when treated with 0·1–5·0 mM Ni(II), Cu(II) and Pb(II) solutions, yielded insoluble metal chelates. Dilute solutions of

Table 3. Maximum wavelengths for bands in the ultraviolet spectra of solutions of *N*-sulfofurfuryl chitosan in various buffers

	Acetate			Phosphate			Carbonate		
pH value	3·55	3·80	4·20	7·10	7·80	8·30	9·70	10·50	11·30
nm	236	233	232	196	198	195	224	223	227
				226	226	221			

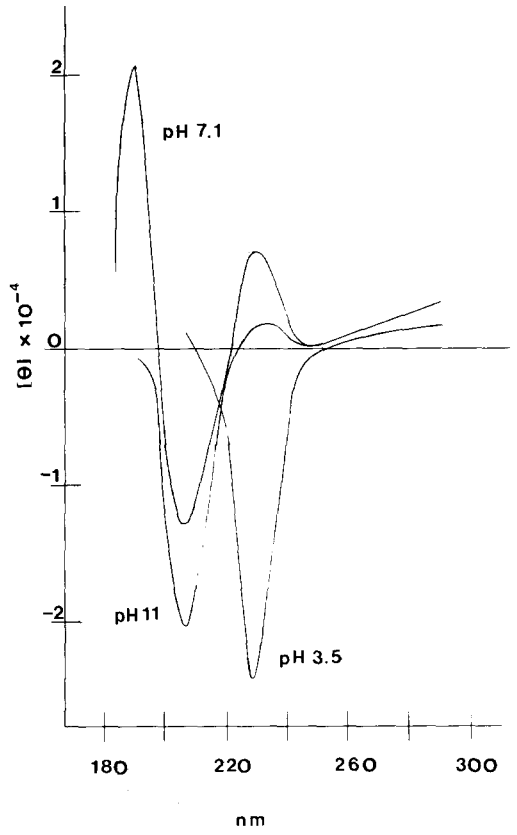


Fig. 2. Circular dichroism spectra for 0·5% solutions of *N*-sulfofurfuryl chitosan in water, at pH values 3·5, 7·1 and 11·0. (Degrees centimeter^2 decimole^{-1} *vs* nanometers.)

Cr(III), up to 0·5 mM, and solutions of Co(II) up to 5 mM, did not produce precipitates.

In order to detect the quantitative precipitation of *N*-sulfofurfuryl chitosan, advantage was taken of the optical characteristics illustrated above: when the typical absorption band at 225 nm (pH 4·2) of *N*-sulfofurfuryl chitosan was not detected by spectrophotometry in the supernatant, precipitation of *N*-sulfofurfuryl chitosan was considered quantitative. In that case, the element was determined by atomic absorption spectrometry in the precipitate, and the capacity for the polymer in terms of mg metal per g polymer was calculated. The data for nickel, lead and copper are in Table 5. Results can be summarized as follows: aliquots of the available chromium precipitated with partial amounts of the polymer at pH 5·5–6·5; in no case was precipitate observed with cobalt; complete polymer precipitation was obtained with 4–5 mM nickel or copper solutions (pH 5·1–7·1), partial precipitation with more dilute solutions; complete polymer precipitation was obtained with 3–5 mM lead solutions (pH 5·6–5·7), opalescence at lower concentrations. Metal chelation followed therefore the order Cu(II) > Pb(II) > Ni(II) ≫ Cr(III) > Co(II).

Polyelectrolyte complex formation

Unusual polyelectrolyte complexes are formed by *N*-sulfofurfuryl chitosan upon treatment with chitosan.

Table 4. Maximum wavelengths for Cotton bands in the circular dichroism spectra of solutions of *N*-sulfofurfuryl chitosan at various pH values

	pH value								
	3·55	3·80	4·20	7·10	7·80	8·30	9·70	10·50	11·30
Negative band (nm)	228	225	220 210	208	210	206	208	208	208
Positive band (nm)			198	190 232	190 230	230	230	230	230

Table 5. Capacity of *N*-sulfofurfuryl chitosan for transition metal ions (mg/g) calculated for quantitative precipitation of the polysaccharide[a]

Polymer (mg)	Nickel concentration (mM)					Lead concentration (mM)					Copper concentration (mM)								
	1	2	3	4	5	1	2	3	4	5	0·1	0·2	0·3	0·4	0·5	1	2	3	4
5	NO	TU	P	P	P	TU	P	319	623	130	P	P	57	62	67	80	72	88	70
10	NO	TU	P	212	76	TU	TU	194	438	149	P	P	55	/	51	48	72	43	41
15	NO	P	P	127	64	TU	TU	36	208	185	P	P	43	44	45	59	64	52	44
20	NO	P	P	86	41	TU	TU	93	127	149	P	P	40	40	43	49	58	/	40
25	NO	P	P	63	78	TU	TU	95	98	175	P	P	27	39	40	45	58	53	37

[a]P, partial precipitation; NO, no precipitate; TU, light turbidity. Precipitates were obtained upon mixing a metal ion solution (50 ml) of specified concentration, with a quantity of *N*-sulfofurfuryl chitosan in the range 5–25 mg, for 24 h at pH close to 6. The salts used were nickel sulfate, lead nitrate and copper sulfate.

Upon mising chitosan acetate salt solution (1 g liter) and *N*-sulfofurfuryl chitosan sodium salt solution (1 g liter) and rapidly raising the pH, clear alkaline solutions, stable for at least one day, were obtained. Immediate precipitation occurred only when the chitosan was 5-fold excess (Table 6). In view of the fact that the pH value for these mixtures was over 11, at which no chitosan solution can be prepared with the use of common reagents, *N*-sulfofurfuryl chitosan appears to be an attractive polyelectrolyte for keeping chitosan in alkaline solutions, which possessed film-forming ability.

Table 6. Conditions for the production of water-soluble polyelectrolyte complexes of chitosan and *N*-sulfofurfuryl chitosan at pH 12

N-Sulfofurfuryl chitosan, 1 g/liter (ml)	Chitosan acetate, 1 g/liter (ml)	NaOH, 1 M (ml)	Precipitate
1	29	1	present
5	25	1	present
10	20	1	opalescence
15	15	1	absent

Sulfoethyl *N*-carboxymethyl chitosan

The influence of the molar ratio, reaction time and temperature on reaction yield, elemental composition and solubility was studied. The data in Table 7 show that a high molar ratio (1:1) leads to high sulfur contents, accompanied, however, by a low degree of solubility in acidic media. Isopropanol, molar ratio 0·7, 80°C and 3 h seem to be the most acceptable set of conditions: the elemental analytical data for a typical preparation under these conditions are presented in Table 8.

5-Formyl-2-furansulfonic acid, sodium salt is a

Table 7. Elemental analysis for sulfoethyl *N*-carboxymethyl chitosan, sodium salt prepared from *N*-carboxymethyl chitosan (0·5 g) suspended in organic solvents (5 ml) in the presence of 30% NaOH (0·625 ml) under various conditions

2-Cloroethane sulfonic acid sodium salt,		Temperature (°C)	Time (h)	Nitrogen (%)	Sulfur (%)	Solubility at 20°C.		Reaction yield factor[b]
Grams	Molar ratio[a]					Water	Acetic acid 3%	
Isopropanol								
0·290	0·7	70	3	4·22	2·30	98·4	95·9	1·19
0·290	0·7	80	3	3·96	3·58	98·9	94·4	1·23
0·290	0·7	70	4	4·53	3·97	99·1	89·9	1·22
0·415	1·0	70	3	4·37	3·50	99·3	77·5	1·26
0·415	1·0	80	3	4·45	5·10	98·8	37·8	1·33
0·415	1·0	70	4	4·42	4·68	99·2	65·8	1·27
0·415	1·0	60	4	4·94	3·83	98·9	89·7	1·16
0·415	1·0	60	3	5·11	2·18	91·8	89·8	1·18
n-*Propanol*								
0·290	0·7	70	3	4·3	3·58	96·9	98·9	1·14
0·290	0·7	80	3	4·86	2·64	99·4	91·2	1·20
0·290	0·7	70	4	4·77	2·67	99·1	96·7	1·21
0·415	1·0	70	3	4·62	2·98	97·1	97·1	1·19
0·415	1·0	80	3	4·57	4·37	99·0	53·5	1·22
0·415	1·0	70	4	4·94	3·83	98·9	89·7	1·16
0·415	1·0	60	4	5·19	2·65	95·4	98·6	1·12
0·415	1·0	60	3	5·54	1·94	91·6	98·7	1·08
Dioxane								
0·290	0·7	70	3	4·65	3·48	96·8	98·7	1·14
0·290	0·7	80	3	5·08	2·97	98·7	92·5	1·15
0·290	0·7	70	4	4·77	2·67	99·1	96·7	1·21
0·415	1·0	70	3	4·62	2·98	97·7	97·1	1·19
0·415	1·0	80	3	4·57	4·37	99·0	53·5	1·22
0·415	1·0	70	4	5·02	4·36	99·1	70·6	1·22
0·415	1·0	60	4	5·06	3·01	96·3	98·5	1·11
0·415	1·0	60	3	5·39	2·72	94·6	98·6	1·11

[a]The molar ratio of the reagent to the polymer unit.
[b]Reaction yield factor is the ratio of the product weight to the *N*-carboxymethyl chitosan weight.

Table 8. Elemental analysis for crab chitosan, *N*-carboxymethyl chitosan and sulfoethyl *N*-carboxymethyl chitosan sodium salt (conditions given on second line of Table 7)

Chitosan	C	H	N	S	Solubility at 20°C (%)	
					Water	Acetic acid 3%
Chitosan	36·10	5·90	6·20	—	7·1	98·7
N-Carboxymethyl chitosan	38·97	6·60	6·03	—	8·5	49·1
Sulfoethyl *N*-carboxymethyl chitosan	32·74	5·56	4·73	3·49	98·7	97·0

convenient reagent for the introduction of the sulfonic acid function into chitosan, where the arm is a furfuryl moiety linked to the glucosamine unit nitrogen. This compound dissolves chitosan and reacts under mild conditions which do not introduce polysaccharide chain degradation. The modified chitosan thus obtained contains 3·7% sulfur, which is comparable to the sulfur content recorded for sulfoethyl chitosan and for sulfoethyl *N*-carboxymethyl chitosan. The *N*-sulfofurfuryl chitosan was obtained from krill chitosan having a low degree of deacetylation (0·58): it might be possible that higher degrees of substitution could be obtained with more extensively deacetylated chitosans. The novel modified chitosan can be easily identified and studied by optical means, as well as by NMR spectrometry. Work on its biological significance is in progress.

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