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Preparation Characterisation and Antibacterial Properties of Sucrose-1-Naphtylacetic Acid Adduct

Abstract
A modified sucrose has been used as a carrier of a biologically active compound for the antibacterial finishing of textiles. Sucrose functionalised with chloroacetate groups was obtained by reacting sucrose with chloroacetyl chloride using pyridine as a catalyst and N-methyl-2-pyrrolidone as a solvent. Based on the results of elemental analysis, FTIR, ^{1}H-NMR and ^{13}C-NMR spectra, the structure of the product was proposed. The coupling of model bioactive carboxylic acid (1-naphtylacetic acid) to sucrose functionalised with chloroacetate groups was carried out by reaction with potassium salt. The hydrolysis in the heterogenous phase of sucrose-1-naphtylacetic acid adduct showed that the release of the bioactive compound from application of the nonwoven is dependent on pH values as well as on the medium's temperature value. An analysis of the antibacterial activity of one of the obtained adducts of sucrose-1-naphthylacetic acid towards Escherichia coli was also performed.

Key words: *sucrose, sucrose functionalisation, chloroacetate groups, bioactive carboxylic acid, antibacterial activity.*

Introduction

Sucrose is produced at a very high scale from renewable resources, and it is an attractive molecule for chemical transformation. Literature reports [1,2] mainly described the ester derivatives of sucrose. Sucrose esters find important applications in a variety of industrial processes, including their use as biodegradable surfactants in the food and cosmetic industry, as plant growth inhibitors.

The development of possibilities for applying this disaccharide is connected with the modification of its properties, among other things, by chemical modification.

Recently, much attention has been paid to specialty compounds of useful materials [3]. Compounds of low molecular mass are used as a biocidal coating, widely used to prevent the growth of micro-organisms on the surface of materials (e.g. antifouling paints, used to protect submerged structures in sea water) [4]. At the present time, the protection is achieved by leaching bioactive molecules from the coating. Making textiles antibacterial by methods such as coating with bioactive compounds has been described in papers [5,6].

Controlled-release polymeric systems, in which bioactive compounds are pendantly bound to a polymeric backbone via covalent bonds of limited stability in biological environments, have emerged as one approach which promises to solve the problems accompanying the use of biologically-active agents [7,8]. A gradual release of the bioactive agent can be achieved by hydrolytic or enzymatic cleavage of the linking bond. In this connection, sucrose has been used as the bioactive agent carrier.

In this paper, we report the applicability of pendant chloroacetate groups previously linked to sucrose in the coupling of model bioactive carboxylic acid (1-naphthylacetic) by reaction with potassium salt. A study of the hydrolysis of the resulting adduct in the heterogeneous phase was also made, in order to evaluate the release of the bioactive acid. The efficacy of antibacterial activity towards *Escherichia coli* was also determined for the selected adduct.

Experimental

Materials

Sucrose trade product (available on the market) was used without additional purification. N-methyl-2-pyrrolidone (NMP) (Aldrich), dimethyl sulfoxide (DMSO) (Merck) and tetrahydrofurane (THF) (Aldrich) were purified by distillation and then stored above Merck 4 Å molecular sieves. Chloroacetyl chloride (Aldrich) was purified prior to use by distillation under reduced pressure. Pyridine (POCh) was refluxed over CaH_2 under a nitrogen atmosphere and then distilled. 1-naphtylacetic acid (Fluka), was used without further purification. Potassium salt of 1-naphtylacetic acid was obtained by dissolving 0.01 mol of the acid in 10 cm^3 of chloroform, then neutralised with 0.01 mol of KOH dissolved in 8 cm^3 of

Figure 1. *Scheme of the synthesis of sucrose modified with chloroacetate groups of different degrees of substitution.*

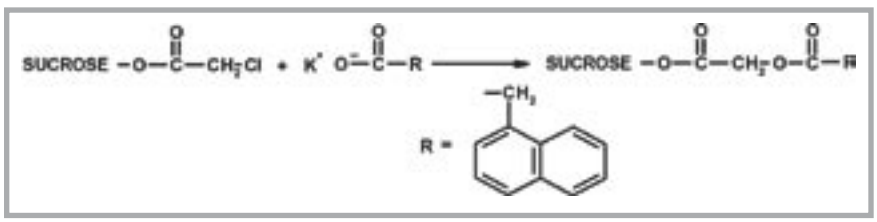

Figure 2. *Scheme of the coupling of bioactive carboxylic acid to sucrose functionalised with chloroacetate groups by using the potassium salt of 1-naphtylacetic acid.*

Table 1. Characteristic data of the ^{1}H- and ^{13}C-NMR spectra of sucrose-naphthylacetic adduct.

Groups	δ, ppm	
	^{1}H-NMR	^{13}C-NMR
-CO-$\underline{CH_2}$-O-	4.40 - 4.75	37.5
-CO-$\underline{CH_2}$-$C_{10}H_7$	3.80 - 4.35	59.0
- $C_{10}H_7$	7.3 - 8.0	122 - 132
-$\underline{CO}$-CH_2-$C_{10}H_7$	-	167

ethyl alcohol. The product was precipitated by being poured into 150 cm^3 of dry acetone. After filtration, the salts were dried in vacuum for a few days at 50°C in the presence of phosphorus pentoxide to a constant weight. Polypropylene (PP) non-woven (from Cenaro) with a surface weight of about 30 g/cm^2 was washed several times in distilled water and then in ethanol. Finally, the non-woven was dried, and cut into rectangular 5×6 cm pieces. The samples were then padded by immersing them in a 20% solution of sucrose-naphthylacetic acid adduct in DMSO, and dried to a constant weight at 50°C under reduced pressure. The adduct content in dried samples was 40% by weight.

Esterification of sucrose with chloroacetyl chloride

Sucrose 5 g (0.117 mol of group -OH) was dissolved in 25 cm^3 NMP by gradual heating to the temperature of 60-70°C. The solution obtained was then cooled to room temperature. Equimolar concentrations (0.126 mol) of pyridine and chloroacetyl chloride were added while stirring to the solution which had been earlier cooled to 0°C. After 24 h the pyridine hydrochloride obtained was filtrated off, and the esterification product was isolated by precipitation using distilled water as the precipitant. It was purified by re-precipitation using THF as solvent and water as the precipitant, then dried under reduced pressure at 40°C to constant weight. The degree of substitution (DS) determined from elemental analysis (Cl, 23.01%) of the sucrose modified with chloroacetyl chloride was about 4.25.

Reaction of chloroacetylated sucrose with potassium salt of 1-naphtylacetic acid

The chloroacetylated sucrose 2.1 g was dissolved in 20 cm^3 DMSO at room temperature. The calculated amounts of potassium salt of 1-naphtylacetic acid (3.7 g; 0.018 mol) was added while stirring. All the reactions were performed at a constant temperature and under intense stirring for ca. 5 h. The product obtained was isolated by precipitation using distilled water as the precipitant, and then washed with ethyl alcohol to remove the unreacted potassium salt of acid. All samples were purified by re-precipitation, using chloroform as the solvent and distilled water as the precipitant, and then dried in vacuum at 50°C to constant weight.

Study of heterogenous hydrolysis of sucrose-naphthylacetic acid adduct

The PP non-woven samples with the applied sucrose-naphthylacetic acid adduct were placed in conical flasks with an aqueous solution of NaOH (pH=11-13). The flasks were then put into a water bath heated to the prescribed temperature. At fixed intervals, solution specimens were taken from the liquid above the padded non-woven samples. The homogenous solution contained the released bioactive agent, which was quantitatively determined by UV spectroscopy at the absorption wavelength of 1-naphthylacetic acid (λ=281 nm) using the previously determined calibration curves (aqueous solution of sodium hydroxide as the solvent). Tests were performed at various

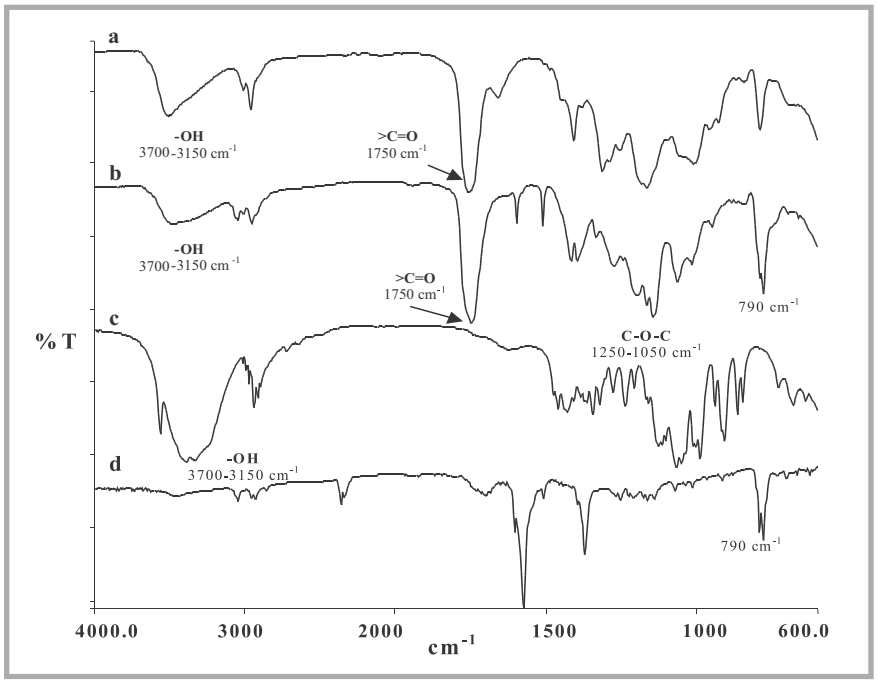

Figure 3. Spectra FTIR of: a - adduct of sucrose-1-naphthylacetic acid, b - chloroacetylated sucrose, c - sucrose, d - potassium salt of naphthylacetic acid.

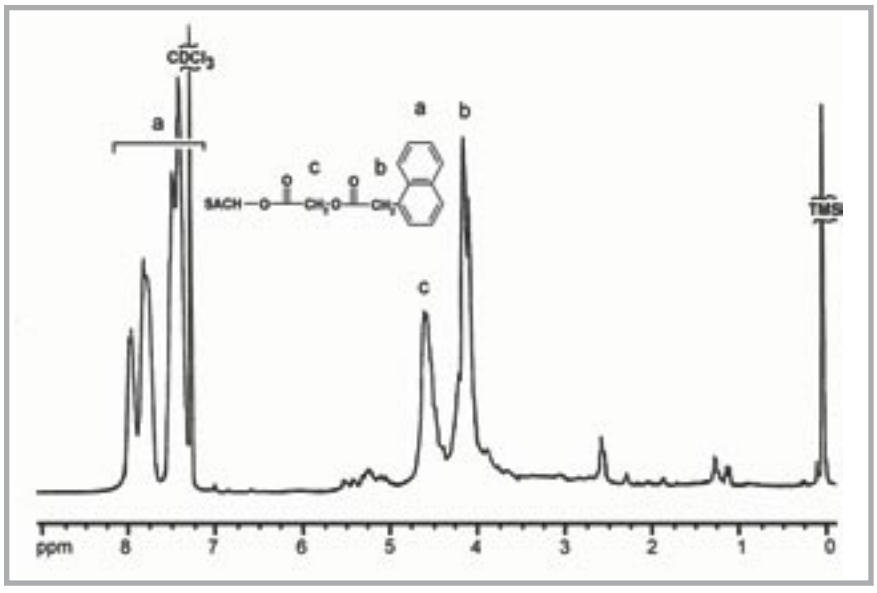

Figure 4. ^{1}H-NMR spectrum of adduct of sucrose-1-naphthylacetic acid in $CDCl_3$.

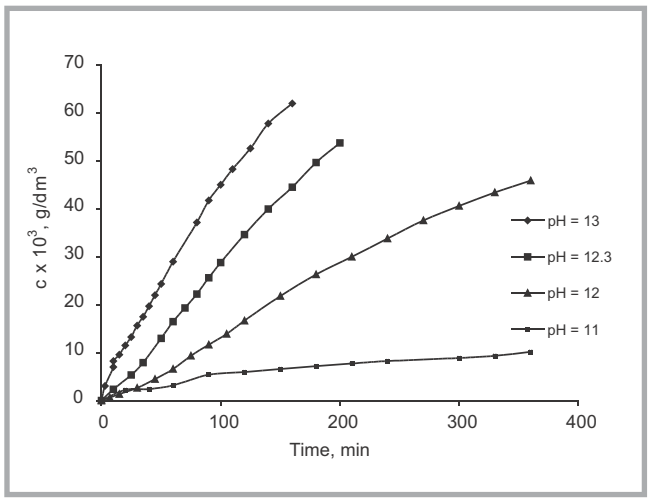

Figure 5. The release of the bioactive compound with naphthylacetate sucrose derivative, depending on pH value of reaction environment.

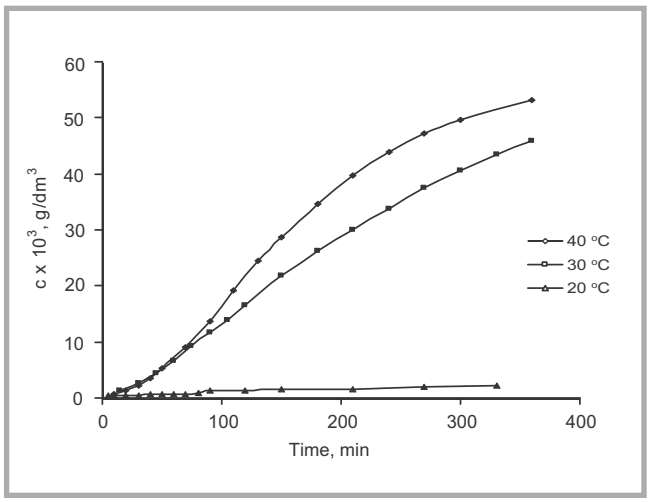

Figure 6. Biocide release from naphthylacetate sucrose derivative, depending on changing temperature of reaction environment.

temperatures and various pH values of the reaction environment.

Measurements

Infrared spectra were recorded using a Perkin-Elmer 2000 (FTIR) instrument. ^{13}C-NMR and ^{1}H-NMR spectra were obtained using a Bruker DPX 250 MHz spectrometer with $CDCl_3$ and DMSO-d_6 as solvents and TMS as an internal reference. The UV-VIS spectra were obtained using a Perkin Elmer UV/VIS Lambda 2 spectrometer. The Japanese Industrial Standard (JIS L 1902:1998 'Testing method for antibacterial textiles') was used to assess the antibacterial efficiency of the sucrose derivatives produced with bioactive naphthylacetic groups applied onto a PP non-woven. The test method was performed using a gram-positive strain of *Escherichia coli* (ATCC 11229- American Type Culture Collection). The germs counted on the non-woven with the applied sucrose-1-naphthylacetic acid adduct and those on a reference sample were determined after a 24 h incubation period. Antibacterial activity (quantitative test) was determined at the Microbiological Laboratory of the Institute of Chemical Fibres in Łódź.

Results and Discussion

Sucrose modified with chloroacetate groups of different degrees of substitution were synthesised in a homogeneous medium by using the method followed in the bromoacetylation of poly(vinyl alcohol) [9] according to the reaction presented by the scheme in Figure 1. The coupling of bioactive carboxylic acid to sucrose functionalised with chloroacetate groups was carried out by using the potassium salt of 1-naphtylacetic acid according to the reaction presented by the scheme in Figure 2.

The ^{1}H- and ^{13}C-NMR spectra of the sucrose-naphthylacetic adduct show the characteristic bands of the pendant bioactive groups, as can be seen in Table 1. The structure of the resultant product was confirmed by FTIR, ^{1}H and ^{13}C-NMR spectroscopies. Example spectra from FTIR and ^{1}H-NMR are presented in Figures 3 and 4.

The IR spectra (Figure 3a) of partially-modified sucrose with chloroacetate groups (DS=4.25) show the characteristic bands of the pendant groups at 1750 cm^{-1} (>C=O) and 790 cm^{-1} (-CH_2-Cl). The relative intensities of these bands seem to depend on the extent of modification in the polymer. Moreover, in spectra (Figure 3b) of the adduct sucrose-1-naphthylacetic groups, an absorption band appears at 790 cm^{-1}, which results from scissoring vibration bands >C=C< and C-H in the naphthyl ring. The ^{1}H-NMR spectrum (Figure 4) of the same modified sucrose shows the characteristic bands at 7-8 ppm, which can be assigned to the protons of the naphthyl ring.

Analysis of heterogeneous hydrolysis of the sucrose-naphthylacetic acid adduct at various temperatures and various solution pH values was also performed. Analysing the curves presented in Figures 5 and 6, it turns out that in standard conditions the release of the bioactive compound with sucrose derivatives spread onto polypropylene non-woven is dependent on pH values as well as on the temperature value of the medium. Significant differences in the rate of biocide release into the solution were observed. The higher these values are, the quicker is the release of the bioactive compound.

The efficacy of the antibacterial activity of the sucrose-naphthylacetic acid derivative produced was also analysed. Table 2 presents the results of the bioactivity of sucrose derivatives with naphthylacetic groups. Samples of PP non-woven with spread layers of sucrose-naphthylacetic acid adduct (40% by wt. of the non-woven) were analysed. The data given in Table 2 was compared to those of a cotton sample used as reference material. Tests of unpadded PP non-wovens were also carried out. Such a standard, untreated PP non-woven shows a positive

Table 2. Results of bacteriological tests on Escherichia coli. Tests were repeated three times.

Sample symbol	Time, h	Quantity of bacteria on a sample	Bacteriostatic activity	Bactericidal activity	Antibacterial activity [10]
Cotton standard	0	7.9×10^4	-	-	-
Cotton standard	24	1.9×10^8	-	-	-
PP standard	24	5.4×10^7	0.6	-2.8	slight
Tested sample	24	$<2.0\times10^1$	7.0	3.6	strong

value of bacteriostatic activity. Despite its bacteria growth inhibiting action (0.6 - very low), this PP non-woven shows no bactericidal action. The bacteria are not killed; on the contrary, they reproduce themselves. Tests with the PP non-woven samples padded with sucrose-naphthylacetic acid adduct confirm not only the inhibition of bacteria growth, but also the total destruction of bacteria. The high values of bacteriostatic and bactericidal activities indicate the efficiency of the sucrose-naphthylacetic acid adduct produced.

Conclusions

As the result of esterification of sucrose with chloroacetyl chloride, using pyridine as catalysts and N-methyl-2-pyrrolidone as the solvent, sucrose with chloroacetate groups was produced. The presence of chloroacetate groups was used to obtain the adduct with bioactive carboxylic acid during the reaction with potassium salt. On the basis of the results of the adduct heterogenous hydrolysis, it was stated that the rate of biocide release depends on pH and temperature. Quantitative tests of the bacteriological activity of the sucrose-naphthylacetic acid adduct, applied onto a polypropylene non-woven, show high bacteriostatic and bactericidal activities on *Escherichia coli*.

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CORRECTION

In issue No. 4/2004 (48) of Fibres & Textiles in Eastern Europe in the paper of Dr Danuta Ciechańska 'Multifunctional Bacterial Cellulose/Chitosan Composite Materials for Medical Applications' on page 72, we printed by mistake Table 6 without its numerical content. The correct content of Table 6 is printed below. We apologise to the Author of this article and to our readers for this mistake and for any inconvenience that may have been brought about.

Editor-in-Chief

Table 6. *Bioactivity tests for modified bacterial cellulose (bacteria count after 24h incubation)*

Symbol of sample	*Escherichia coli* (ATCC 11229)			*Staphylococcus aureus* (ATCC 6538)		
	Total bacteria number, CFU	Bacterio-static activity	Bactericidal activity	Total bacteria number, CFU	Bacterio-static activity	Bactericidal activity
Unmodified bacterial cellulose	5.2×10^8	-	-	3.2×10^8	-	-
MBC/O	$< 2.0 \times 10^1$	8.7	3.4	2.9×10^6	2.0	-1.8
MBC/M	$< 2.0 \times 10^1$	7.4	3.7	1.3×10^7	1.4	-2.4