

Bromoacetylation of polysaccharides

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Preparation of bromoacetyl and acetyl-bromoacetyl derivatives of cellulose and starch is described. The derivatives were prepared by treatment of the suitably activated starting materials (powdered cellulose, and cellulose or starch cross-linked with epichlorohydrin) with bromoacetyl bromide in dioxan or acetic acid.

Depending upon the reaction conditions the bromoacetyl and mixed derivatives showed a degree of substitution in the range of $DS_{\text{bromoacetyl}}$ 0.04—0.64 and DS_{overall} 0.32—1.0, respectively.

The prepared derivatives are white, powdered materials suitable for chemical binding of amino acids, proteins, enzymes and the like.

Описан способ приготовления бромацетил- и ацетил-бромацетил- производных целлюлозы или крахмала действием бромистого ацетилбромидна на подходящим способом активированный исходный продукт (порошок целлюлозы, целлюлозу и крахмал шитый с эпихлоргидрином) в реакционной среде диоксана или уксусной кислоты.

В зависимости от параметров реакции имеют приготовленные производные целлюлозы $DS_{\text{бромацетил}}$ от 0,04—0,64 и смешанные производные $DS_{\text{полное}}$ 0,32—1,0.

Приготовленные вещества — порошкообразные, белого цвета и имеют свойства, удобные для химического присоединения аминокислот, протеинов, энзимов и т. п.

Derivatized polysaccharides have been frequently used recently as support materials for biologically active molecules [1]. Besides their preserved specific properties an important quality of support-bonded enzymes, antibiotics, and immunosorbents [2] is their insolubility in water so that they can be easily removed from the reaction medium without their biological activity being affected. In this way a number of difficulties encountered when water-soluble enzymes are used can be eliminated [3, 4]. Although cellulose itself can be used as a support material, cellulose chemically modified by etherification or esterification is more frequently used. A support material of the latter type is bromoacetylcellulose which is well suitable for binding trypsin, chymotrypsin, ribonuclease, thioethanol, phenylalanine, polylysine, globulin serum *etc.* [5, 6].

The protein molecules are bonded to bromoacetylcellulose through chemical linkages which, depending on pH, are formed between the functional groups present in the protein and those in the cellulose support material. Thus, for instance, sulfhydryl and amino groups react at pH 6 and 8, respectively.

The objective of the present work was to find the optimum conditions of the bromoacetylation of cellulose and starch. The effect of the various activation procedures of the starting materials and the temperature and duration of the reaction upon the quality of the final product has been investigated.

Experimental

Starting materials

The following starting materials were used: powdered cellulose, DP 820, swelling volume 7.6 cm³/g; powdered cellulose cross-linked with epichlorohydrin [7] containing one cross-linkage per eight glucose units, swelling volume 5.2 cm³/g; starch cross-linked with epichlorohydrin [8] containing one cross-linkage per two glucose units, swelling volume 3.5 cm³/g.

Analytical methods

The determination of bromine was carried out by the method of *Schöniger* [9]. For the determination of acetyl and bromoacetyl groups a sample of esterified cellulose was saponified with an excess of alcoholic sodium hydroxide solution which was then back-titrated with 0.1 N sulfuric acid. For the determination of DP a sample of bromoacetylcellulose was saponified with sodium methoxide, the regenerated cellulose was nitrated [10] and the viscosity of the nitrate was determined using an Ubbelohde-type viscometer. The swelling volume was determined in a 10 ml calibrated tube by reading the volume after two-hours swelling of the cellulose material in water.

Activation of cellulose

Prior to the bromoacetylation with bromoacetyl bromide samples of powdered cross-linked cellulose were treated for approx. 1 hr with solvents summarized in Table 1. Treatment with every following solvent was preceded by filtration, the last solvent being the reaction medium.

Bromoacetylation of cellulose

Except when otherwise stated the basic reaction conditions comprised the treatment of activated cellulose with an 1.8 molar excess of bromoacetyl bromide at 10°C for 2 hrs at a solvent—substrate ratio 1:8. The effect of the varied reaction conditions upon the course of the reaction can be seen from Figs. 1—5. The respective results are summarized in Tables 3—5.

Cellulose and starch bromoacetates

The air-dry polysaccharide (10 g) was dispersed in water and allowed to swell for 1 hr. After filtration the sample was washed with glacial acetic acid and allowed to stay in this solvent for additional 1 hr. The acetic acid was filtered off, the sample washed with dioxan and left in this solvent for 1 hr. The sample was sucked, transferred to a three-necked flask and dioxan was added to the total volume 80 ml. The mixture was cooled to 10°C and, under stirring, bromoacetyl bromide (10 ml) was added dropwise while the formed hydrogen bromide was sucked off. The temperature of the reaction mixture was kept at 10°C for two hours and then ice-water (200 ml) was added. The obtained white product was filtered, thoroughly washed with water, saturated sodium bicarbonate solution, water, and finally with acetone. The bromoacetate thus obtained was dried at 40°C.

Cellulose and starch acetate-bromoacetates

The activation of the substrate with water and acetic acid without the use of dioxan for washing out the solvents and the bromoacetylation reaction was carried out as described above.

Table 1

Effect of various activation procedures upon the quality of cross-linked bromoacetylcellulose (Subsequent bromoacetylation was carried out at 30°C and at a substrate-solvent ratio 1 : 6)

Experiment	Activation with	% Br in the product	Colour and consistency
1	Water, ethanol, acetone, dioxan, bromoacetic acid	30.2	White
2	Water, ethanol, acetone, benzene, bromoacetic acid	29.4	White
3	Water, ethanol, acetone, bromoacetic acid	26.6	White
4	Water, ethanol, acetone, dioxan	8.6	White
5	Water, ethanol, acetone, benzene	3.2	White
6	Water, ethylenediamine, ethanol, dioxan	11.7	Yellow, semisolid
7	Water, dimethylamine, dioxan	0	White
8	Water, diethylamine, dioxan	0	White
9	Water, dioxan, pyridine	—	Black oil
10	Water, acetic acid, tetrahydrofuran	36.7	Black
11	Water, dimethylformamide	0	White
12	Water, acetic acid, dimethylformamide	21.0	Yellow
13	Water, acetic acid, carbon tetrachloride	13.0	White
14	Water, acetic acid	20.0	White
15	Water, acetic acid, dioxan	22.0	White
16	Acetic acid	2.9	White
17	Dioxan	0	White.

Results and discussion

Cellulose and starch bromoacetates were prepared by treating the suitably activated starting materials in an organic solvent with bromoacetyl bromide while, to prevent the hydrolysis of the glycosidic linkages, the formed hydrogen bromide was constantly removed from the reaction.

Due to its particular supermolecular structure natural cellulose is a poorly reactive polymer and, normally, any chemical reaction thereof must be preceded by a so-called activation process [11]. Ideally, the solvent in which the reaction is carried out should, at the same time, play the role of the activator. An efficient activation of cellulose is achieved by its swelling in water. Bromoacetylation, however, cannot be done in the presence of water and, therefore, the swollen starting polysaccharide was rid of water by means of organic solvents. The order of the solvents was chosen in such a manner that the polar liquid was gradually replaced with a less polar solvent, the measure of the polarity being ϵ . Various activation procedures which preceded bromoacetylation are summarized in Table 1. The first three activations (activation with bromoacetic acid) afforded white product with a high content of bromine.

Table 2

Comparison of the original bromoacetylation procedure with the present modification

Procedure	Activation hrs	Reaction time hrs	Cellulose- -bromoacetic acid ratio moles per mole	Cellulose- -bromoacetyl bromide ratio moles per mole	Bromine-content in the final product %
Original [13]	20	6—8	7.45	13.55	20.0
Modified [12]	2	1—2	0	1.8	22.0

Considering the content of bromine, the quality of the preparation (colour, consistency) and the economy of the reaction, the superior means of the activation of cellulose is the swelling in water followed by the replacement of water with acetic acid or dioxan (Procedure 14 and 15). The working conditions are given in the Experimental and constitute the objective of a patent [12].

The importance of water in the swelling of cellulose is obvious from the results of Procedure 16 and 17 where the bromoacetylation was carried out under the conditions identical to those of Procedure 14 and 15 except for the swelling in water.

It can be seen from the data in Table 2 that, compared to the procedure elaborated by *Patchornik* [13], the new procedure makes the preparation of bromoacetylcellulose less time-consuming. As a result of the efficient activation of cellulose the reaction time could be shortened by 24 hours, the activation with bromoacetic acid could be omitted and the consumption of the reagent — bromoacetyl bromide was cut by the factor of 7.5. The thus obtained product contains the same percentage of bromine.

The determination of the most suitable method of activation of cellulose was followed by the determination of the optimum conditions of bromoacetylation in acetic acid or dioxan. Fig. 1 shows the effect of the amount of the derivatization reagent (0.4—3.5 moles per mole of cellulose) upon the content of bromine in the final product.

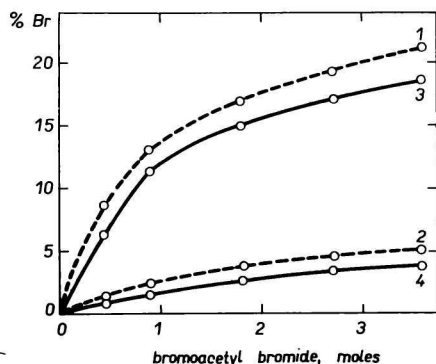


Fig. 1. Effect of the amount of the reagent upon the bromine-content in the bromoacetylcellulose (substrate-solvent ratio 1 : 6).

1. Cross-linked cellulose reacted in acetic acid; 2. powdered cellulose reacted in acetic acid; 3. cross-linked cellulose reacted in dioxan; 4. powdered cellulose reacted in dioxan.

x = moles of bromoacetyl bromide.

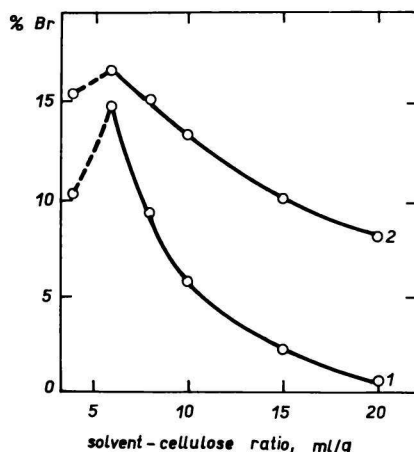


Fig. 2. Effect of the volume of the reaction mixture upon the bromine-content in cross-linked bromoacetylcellulose.

1. Reaction in dioxan; 2. reaction in acetic acid.

x = solvent-substrate ratio, ml/g.

Judging the final products by its content of bromine, its colour and consistency, the optimum molar substrate—reagent ratio in the bromoacetylation of cellulose is approx. 1.8. When their ratio is higher than 3, a gradual discolouration of the product is observed.

The relation between the volume of the reaction mixture and the content of bromine in the final bromoacetylcellulose can be seen from Fig. 2. From the preparative point of view the most convenient reaction volume is 60—80 ml of the solvent per 10 g of air-dry cellulose. Using less than 50 ml of the solvent per 10 g of the air-dry substrate the reaction mixture becomes too thick, less homogeneous, and the content of bromine in the final product somewhat decreases.

The effect of the reaction medium and the temperature upon the results of the bromoacetylation of powdered cellulose, and cross-linked cellulose and starch are summarized in Tables 3—5. When the bromoacetylation is carried out in acetic acid the products contain both bromoacetyl and acetyl groups.

It is known that acetic acid itself acetylates cellulose to some extent. Fibrous cellulose treated with acetic acid contains approx. 6% of acetyl groups, and when regenerated cellulose is treated with the same reagent the product contains approx. 20% of acetyl groups. Thus, when the bromoacetylation of cellulose or starch is conducted in the presence of acetic acid, acetylation, catalyzed by the formed hydrogen bromide, takes place at the same time.

The relative abundance of the bromoacetyl and acetyl groups in the derivatized polysaccharides was determined by a combination of saponification, by which the overall substitution with both acetyl and bromoacetyl groups was determined, and the determination of bromine [9], from which the content of bromoacetyl groups was calculated. During the saponification of cellulose and starch bromoacetates with sodium hydroxide solution at elevated temperature the hydrolysis of α -carbon in the bromoacetyl groups may also take place to some extent [14]. Consequently, this side reaction leads to a higher consumption of

Table 3

Bromoacetylation of activated powdered cellulose in acetic acid or dioxan

Reaction medium	<i>t</i> °C	Weight increment, %		Content of bromine %	Bromoacetyl groups %	Acetyl groups %	DS _{bro}	DS _{acetyl}	DS _{overall}	DP	Swelling volume cm ³ /g
		calculated	found								
Acetic acid	10	11.2	8.1	2.0	3.05	6.8	0.04	0.28	0.32	220	7.0
	20	13.1	9.0	2.3	3.6	8.5	0.05	0.37	0.42	171	5.3
	30	14.6	10.5	2.6	4.0	8.8	0.06	0.38	0.44	144	5.2
Dioxan	10	4.5	2.0	2.8	4.4		0.06		0.06	226	7.8
	20	5.3	3.6	3.2	5.0		0.07		0.07	174	6.6
	30	6.9	4.3	3.6	5.6		0.085		0.085	168	6.0

Table 4

Bromoacetylation of activated cross-linked cellulose in acetic acid or dioxan

Reaction medium	<i>t</i> °C	Weight increment, %		Content of bromine %	Bromoacetyl groups %	Acetyl groups %	DS _{bro}	DS _{acetyl}	DS _{overall}	Swelling volume cm ³ /g
		calculated	found							
Acetic acid	10	37.2	32.7	15.4	24.0	3.4	0.43	0.17	0.60	3.0
	20	46.1	36.5	17.5	26.7	4.9	0.52	0.27	0.79	3.2
	30	54.7	42.3	19.4	29.6	5.8	0.61	0.38	0.99	3.6
Dioxan	10	17.0	14.1	9.4	14.4		0.22		0.22	4.0
	20	39.4	34.6	18.4	28.6		0.53		0.53	4.0
	30	47.5	40.7	21.4	32.6		0.63		0.63	3.8

Table 5

Bromoacetylation of activated cross-linked starch in acetic acid or dioxan

Reaction medium	t, °C	Weight increment, %		Content of bromine %	Bromoacetyl groups %	Acetyl groups %	DS _{bromoacetyl}	DS _{acetyl}	DS _{overall}	Swelling volume cm ³ /g
		calculated	found							
Acetic acid	10	14.1	4.6	2.5	4.9	7.8	0.06	0.33	0.39	5.0
	20	34.7	5.2	7.8	12.0	13.75	0.21	0.65	0.86	4.8
	30	36.3		8.0	12.4	14.5	0.22	0.69	0.91	4.8
Dioxan	10	3.0	0.7	2.2	3.4		0.04		0.04	5.0
	20	5.2	0	2.9	4.6		0.07		0.07	5.0
	30	6.0	0	3.5	5.4		0.08		0.08	5.0

sodium hydroxide. To avoid the thus caused distortion of the bromoacetyl group determination the amount of bromides formed in this manner was determined argentometrically [15] and subtracted from the overall consumption of sodium hydroxide.

The content of bromine and of acetyl groups in the final product increases with the reaction temperature (Tables 3—5). For the preparation purposes the reaction should be conducted at 10°C since the thus obtained white product is more stable. The product prepared at the temperatures above 30°C are of a poor quality.

The observed weight increments (Tables 3—5) are lower than those corresponding to the determined degree of bromoacetylation and acetylation. This can be attributed to the above-mentioned hydrolysis of the glycosidic linkages of the starting polysaccharides affected by the liberated hydrogen bromide. The extend of hydrolysis was deduced from the decrease of the degree of polymerization of powdered cellulose vs. reaction temperature (Table 3) and the duration of the reaction (Fig. 3).

It is evident that the higher reaction temperature results in a more pronounced degradation of the starting material and that most of this degradation occurs during the first 30 minutes of the reaction. The change of DP is proportional to the increase of $DS_{\text{bromoacetyl}}$, *i.e.* to the amount of the liberated hydrogen bromide.

The course of the bromoacetylation in dioxan can be seen from Fig. 4 and the kinetic curves of the bromoacetylation in acetic acid are given in Figs. 5*a* and 5*b*. It follows that $DS_{\text{bromoacetyl}}$ of the derivatized cellulose and cross-linked cellulose is almost the same regardless whether a simple or a mixed derivative was prepared, *i.e.* in the case of cellulose the reaction medium has little effect upon $DS_{\text{bromoacetyl}}$. On the contrary, bromoacetylation of starch in acetic acid results in 3.4 times higher $DS_{\text{bromoacetyl}}$ compared to the similar reaction conducted in dioxan.

Comparison of the results of bromoacetylation of activated and nonactivated starting materials (Figs. 4 and 5*a*) shows the importance of a suitable activation without which, in most of the shown examples, the bromoacetylation does not take place at all. An exception is the bromoacetylation of cross-linked cellulose in acetic acid ($DS_{\text{bromoacetyl}}$ 0.06).

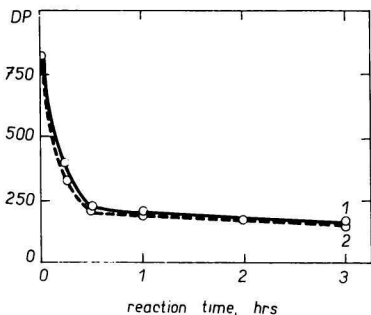


Fig. 3. Decrease of DP of powdered cellulose during bromoacetylation at 20°C.

1. Reaction in dioxan; 2. reaction in acetic acid.

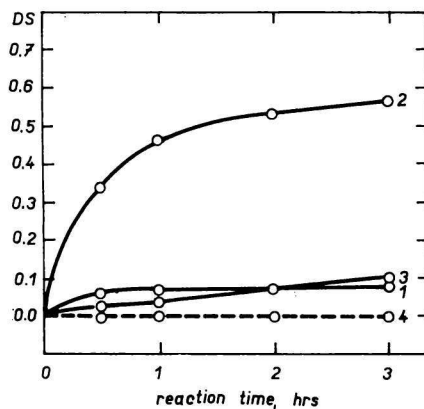


Fig. 4. Kinetics of the bromoacetylation in dioxan at 20°C.

1. Activated powdered cellulose; 2. activated cross-linked cellulose; 3. activated cross-linked starch; 4. nonactivated substrates.

In addition, an important factor in the bromoacetylation is the nature of the starting material. From the point of view of the achieved DS yield and the quality of the product the most suitable starting material is cross-linked cellulose. This is attributed to the better sterical accessibility of the hydroxyl groups in the cross-linked material, as a result of the introduced hydroxyl groups present in the hydroxypropyl cross-linkages. The cross-linkages take apart the individual linear cellulose macromolecules, weaken the hydrogen bonds which block the reaction centra of the noncross-linked material and the effected cross-linking introduces additional hydroxyl groups into the molecule. The used cross-linked cellulose contained one secondary hydroxyl group per eight glucose units and one primary and one secondary alcoholic hydroxyl group per thirty glucose units formed as a result of occurred substitution, instead of cross-linking.

These factors showed themselves in such a manner that when the cross-linked cellulose was used as the starting material DS 0.35 was achieved compared to DS 0.07 achieved with the noncross-linked starting material. Moreover, in the preparation of the mixed derivative from the cross-linked cellulose bromoacetylation takes place predominantly, whereas when noncross-linked cellulose is treated in the same manner acetylation predominates.

The difference in the results (DS, yield) of bromoacetylation of cross-linked starch and cellulose are attributed not only to the different shape of the macromolecules but also to the different degree of cross-linking of the starting materials. Acetylation of starch occurs easily and, under the identical reaction conditions, in the product obtained from the cross-linked starch the found $DS_{\text{acetyl}} = 0.65$. DS_{acetyl} achieved in the product obtained from powdered cellulose was 0.37 and 0.27, respectively (Tables 3—5). A disadvantage of the starch bromoacetates and acetate-bromoacetates are poor rheological properties resulting in high resistance during filtration.

An important property in the biochemical usage of cellulose and starch derivatives is, besides the type and the amount of the functional groups, stability and other physical properties, their swelling volume. The observed results show (Tables 3—5) that, in agreement with Flory's theory, the cross-linked materials swell less than do the noncross-linked ones and that the degree of cross-linking affects markedly this property [16].

The prepared bromoacetyl and acetyl-bromoacetyl cellulose derivatives show, in most cases, a lower swelling volume than the starting materials from which they were prepared.

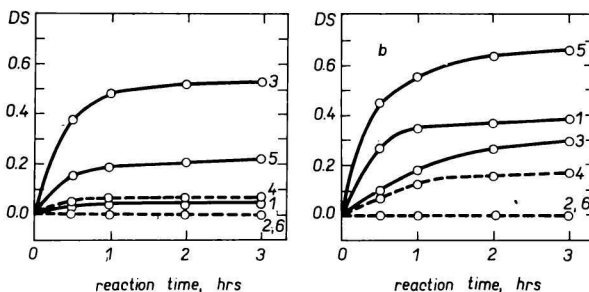


Fig. 5. Kinetics of the bromoacetylation in acetic acid at 20°C.

a) $DS_{\text{bromoacetyl}}$; b) DS_{acetyl} .

1. Activated powdered cellulose; 2. nonactivated powdered cellulose; 3. activated cross-linked cellulose; 4. nonactivated cross-linked cellulose; 5. activated cross-linked starch; 6. nonactivated cross-linked starch.

On the other hand, bromoacetylation of starch results in an increased swelling volume of the derivative (Tables 3—5). The mixed cellulose derivative shows a lower swelling volume than bromoacetylcellulose prepared under identical conditions which is attributed to the presence of the hydrophobic methyl groups [17].

In conclusion it can be stated that the procedure described makes it possible to prepare bromoacetyl and acetyl-bromoacetyl cellulose derivatives suitable as support materials for proteins.

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