BINDING OF CALCIUM AND LEAD IONS TO CARBOXYSTARCH PREPARED BY ACTION OF NITROGEN DIOXIDE ON NATIVE STARCH AND ITS 2,3-DIALDEHYDE DERIVATIVES

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The binding of calcium and lead ions to carboxy derivatives of starch prepared by allowing nitrogen dioxide to act on native maize starch (procedure *A*) and on starch 2,3-dialdehyde derivatives of degrees of oxidation $DO_{(d.a.)} \geqq 0{\cdot}94$ (procedure *B*) was studied. The carboxy group content of the samples in the H^+ form was 4·6 – 12·1 mmol g^{-1}. The effect of alkaline medium on the stability of the carboxy derivatives and on their ability to bind and exchange cations was examined. The $Ca^{2+} \rightarrow 2\,K^+$ exchange was evaluated in terms of the decrease in the electrostatic free enthalpy $\Delta(G_{el}/N)_K^{Ca}$, determined by alkalimetric potentiometric titrations, and the binding of Pb^{2+} ions was evaluated in terms of the activity of the Pb^{2+} counter-ions determined in suspensions of Pb salts of the carboxy derivatives by means of an ion specific electrode. The IR and CD spectra revealed that the carboxystarch preparations obtained by procedure *A* contained, in addition to free carboxy groups, a considerable amount of carbonyl groups. During the conversion of the latter groups to the former, even in a weakly alkaline medium, the carboxy derivatives undergo an appreciable degradation and lose, to a great extent, their ability to bind and exchange cations. Procedure *B*, on the other hand, leads to highly selective starch and amylose carboxy derivatives, exhibiting a small amount of carbonyl groups and featuring a relative stability towards alkaline medium; their binding capacity is as high as 12 milliequivalents of cations per g of sample.

The preparation and properties of carboxy derivatives of plant polysaccharides have recently received considerable interest. Numerous studies concentrated particularly on cellulose derivatives. Allen and Cuculo[1] published a survey covering about 300 references, including some reviews. The results of these studies could be also used during the investigation of starch and amylose carboxy derivatives.

Previously we dealt with the binding of calcium, lead, and copper(II) cations to starch and amylose 2,3-dicarboxy derivatives of various degrees of oxidation[2,3]. We demonstrated that unlike polysaccharide derivatives with $C_{(6)}OOH$ carboxy groups (analogues of polyuronic acids), these derivatives exhibit a high selectivity of cation exchange also at low degrees of oxidation. The great strength of the binding of divalent cations to the derivatives is due to the favourable steric arrangement of the pair of carboxy groups involving the $C_{(2)}$ and $C_{(3)}$ carbon atoms, made possible by the flexibility of the chain of the macromolecule in sites where the pyrane rings of the D-glucose units opened during the oxidation.

The aim of our further study was to prepare carboxy derivatives of starch with the maximum attainable capacity for binding metals; the route of direct action of nitrogen dioxide on the polysaccharide was chosen. As yet, attention has been paid particularly to the preparation of starch carboxy derivatives of low degrees of oxidation which by their properties approach polyuronic acids (*e.g.*, ref.[4]); their ability to bind and exchange cations can be expected to be similar to that of carboxycellulose at low degrees of oxidation[5]. Oxidation of starch with nitrogen dioxide would be economically more convenient than multistage oxidations with other oxidants. Starch derivatives of high degrees of oxidation ($DO > 2$) can be prepared, *e.g.*, by oxidation of the polysaccharide with periodate to the dialdehyde derivative, followed by oxidation of the latter with nitrogen dioxide in an organic solvent (CCl_4) to the carboxy derivative[6]. During this process, the oxidation of the aldehyde groups is accompanied by oxidation of the primary hydroxy groups at $C_{(6)}$ to carboxy groups.

The oxidation of the primary hydroxy groups of the polysaccharide by nitrogen dioxide is not entirely selective; over a region, ketol groupings are also formed at the $C_{(2)}-C_{(3)}$ carbons .These groupings are markedly alkali-labile[7], being subject to isomerization with the formation of the endiol grouping and, subsequently, formation of new carboxy groups; the degree of polymerization of the polysaccharide decreases markedly. The mutual ratio of the carboxy, aldehyde and keto groups in the oxidized polysaccharide can be determined, for instance, by IR spectrometry combined with reduction of the substance with sodium tetrahydroborate followed by oxidation with sodium chlorite[8].

For attaining a high carboxy group content of the starch oxidized with nitrogen dioxide, the ensuing reaction stage must lead to opening of the formed lactones by the effect of alkaline medium and conversion of the carbonyl groups to carboxy groups. This treatment, however, can cause degradation of the polysaccharide macromolecule (as mentioned above), resulting in a lowered selectivity of cation exchange by the final carboxystarch product. In the present work, the binding of calcium and lead cations to carboxystarch is studied for substances with high degrees of oxidation, prepared by allowing nitrogen dioxide to act upon native starch and its 2,3-dialdehyde derivatives; particular attention is paid to the effect of alkaline medium on the ability of carboxystarch to bind and exchange these cations.

EXPERIMENTAL

The starch and amylose carboxy derivatives were prepared from maize starch (Slovak Starch Works, Boleráz, Czechoslovakia) and amylose of technical grade (AVEBE, The Netherlands), which was purified *via* its complexes with butanol, by repeated precipitation from dimethyl sulphoxide. Starch and amylose 2,3-dialdehydes of degrees of oxidation $DO_{(d.a.)} \geqq 0{\cdot}94$ were prepared by oxidation of starch with periodate[2]. The final products were free from associate salts.

The apparatus comprised an OP 208 digital potentiometer (Radelkis, Budapest), a G-222 B glass electrode, a K-401 saturated calomel electrode, and a K-711 two-compartment saturated calomel electrode with 10% KNO_3 in the outer compartment of the electrolytic bridge (all Radiometer, Copenhagen), and a Crytur 82-17 Pb-specific electrode (Monokrystaly, Turnov). The infrared spectra were scanned on a Perkin–Elmer 457 infrared spectrophotometer, the circular dichroism spectra on a Mark III instrument (Jobin Yvon, France). Solutions of 0·05M-KOH free from carbonate and 0·021M-$Ca(OH)_2$ (saturated solution) and redistilled water free from CO_2 were used.

Preparation of Starch and Amylose Carboxy Derivatives

Direct oxidation of starch with nitrogen dioxide. Nitrogen dioxide gas was allowed to act at room temperature on a thin layer of native starch over P_2O_5 in an evacuated dessicator. For sample No 1 (Table I), N_2O_4 gas was obtained by evaporation of the liquid; the time of oxidation was 48 h. For samples No 2 and 3, the N_2O_4 gas evolved slowly by reacting sulphuric acid with sodium nitrite[9] and lasted 310 h for sample No 2; a subequivalent amount of oxidant was allowed to act on the sample No 3 for 170 h, respectively. After the oxidation, the samples were freed from oxides of nitrogen with air, suspended in acetone and washed thoroughly with this solvent, and dried in a vacuum of a water jet pump at temperatures lower than 70°C.

Oxidation of starch and amylose 2,3-dialdehydes with nitrogen dioxide. Thin layers of starch and amylose 2,3-dialdehydes were oxidized with N_2O_4 at room temperature for 48 h as described

TABLE I

Characteristics of starch carboxy derivatives prepared by allowing nitrogen dioxide to act upon native starch and its 2,3-dialdehyde derivatives

Sample No	$DO_{(d.a.)}$	DC c	m_{COOH}, mmol g^{-1}			$\Delta(G_{el}/N)_K^{Ca}$ kJ $(COOH)^{-1}$	
			a	*b*	*c*	*b*	*c*
			Native starch				
1	—	1·94	4·29[d]	4·76	10·17	−3·32 ± 0·04	−0·88 ± 0·07
2	—	1·85	4·64[d]	4·77	9·67	−4·16 ± 0·39	−1·38 ± 0·20
3	—	—	—	1·93	4·65	−3·18	−1·47
			Starch 2,3-dialdehyde				
4	0·96	2·21	7·71	9·55	11·34	−9·91	−9·76 ± 0·09
5	0·98	2·40	9·11	10·47	12·14	−11·44 ± 0·54	−10·41 ± 0·04
6	1·00	2·37	10·77	—	12·01	—	−10·09 ± 0·33
7	0·94	2·23	9·40[d]	—	11·43	—	−8·88 ± 0·07
			Amylose 2,3-dialdehyde				
8	0·98	2·16	10·60	—	11·14	—	−11·06 ± 0·29

[a,b,c] Procedure labelling as in the text; [d] gel suspension.

above; N_2O_4 gas was obtained by evaporation of the liquid. All the starch and amylose carboxy derivatives (samples No 4—7 and 8, respectively) were obtained in the H^+ form.

Analytical Methods

The aldehyde group content of the dialdehyde derivatives was determined[10] with hydroxylammonium chloride, by alkalimetric determination of the released hydrochloric acid. The free carboxy group content of the samples was determined by a slow direct potentiometric titration of their solutions or suspensions with 0·05M-KOH (procedure *a*, Table I); alternatively, the determination was performed after heating the sample solution or suspension for 10 min on a boiling water bath and cooling to ambient temperature (procedure *b*).

The lactones opened and the carbonyl groups converted to carboxy groups on the action of an alkaline medium. To a 0·1% solution of sample was added KOH in an amount of 15—20 mmol . l^{-1} so that its excess after the above reactions was 8—10 mmol l^{-1} (pH 11·9—12·0). This solution was allowed to stand for 18 h in a hermetically closed vessel; clear solutions were obtained. The excess hydroxide was removed by percolation through a column of Dowex 50W×2 (H^+) cation exchanger.

The total carboxy group contents of the samples after this treatment were determined by potentiometric titration of the eluates from the cation exchanger (polyacid solutions) with 0·05M-KOH (procedure *c*). The mean relative error of determination by procedures *a*—*c* was ±1·0%.

Determination of Selectivity of $Ca^{2+} \rightarrow 2\,K^+$ Cation Exchange and Binding of Pb^{2+} Cations to Starch Carboxy Derivatives

The changes in the electrostatic free enthalpy associated with the $Ca^{2+} \rightarrow 2\,K^+$ exchange, $\Delta(G_{el}/N)_K^{Ca}$, were determined for the starch and amylose derivatives by potentiometric titrations of the polyacids in a concentration of 3·00 mmol l^{-1} with 0·05M-KOH and 0·021M-$Ca(OH)_2$ at 23—25°C (ref.[2]). The values, in kJ $(COOH)^{-1}$, are determined by the areas enclosed by the curves of the potentiometric titrations by the two titrants[11].

The activity of lead cations $a_{Pb^{2+}}$ and the corresponding activity coefficients $\gamma_{Pb^{2+}}$ in the suspensions of the lead salts of the starch carboxy derivatives were determined with a Pb-specific electrode[12]. The suspensions contained the starch carboxy derivatives in the K^+ form in a concentration $c_{COOK} = 3{\cdot}00$ mmol l^{-1} and $Pb(NO_3)_2$ in a concentration $c_{Pb^{2+}} = 1{\cdot}434$ mmol l^{-1}, *i.e.*, 96% with respect to the carboxy groups. The ionic strength of the starting solution was adjusted with KNO_3 to $I_0 = 0{\cdot}01$ mol l^{-1}. The suspension was stirred for 2 h and allowed to stand for 18 h, and the activity of lead ions was determined at 25·0°C. The ionic strength of the equilibrium solution I and the concentration of free lead ions $[Pb^{2+}]_f$ were calculated from the Pb^{2+} activity by an iterative procedure[12].

The total concentration of lead in the supernatant (c_{Pb}) after centrifugation of the starch carboxy derivative lead salt at 20 000 *g* for 20 min was determined chelometrically using spectrophotometric indication of the end point. Solutions of 0·01M-Chelaton IV and 0·01M-$MgCl_2$, Eriochrome Black T as the indicator, and an IF 650 nm filter (Carl Zeiss, Jena) were used.

The degree of association of Pb^{2+} ions with the carboxy groups of carboxystarch was calculated as

$$\beta = (c_{Pb^{2+}} - [Pb^{2+}]_f)/c_{Pb^{2+}} ,$$

where $c_{Pb^{2+}}$ is the initial concentration of lead ions in the suspension (1·434 mmol l^{-1}).

The infrared and circular dichroism spectra were measured for potassium salts of the starch carboxy derivatives. The solutions were prepared by procedure *b*, *i.e.*, by direct neutralization with

KOH after a short heating of the sample solution, or by procedure *c*, *i.e.*, by allowing alkaline medium to act upon the substances.

The infrared spectra were scanned for samples obtained by lyofilization from solutions. The circular dichroism spectra were run for solutions of 0·4 g l^{-1} concentration in a 5 mm cell at 25°C. For sample No 5 with a very low carbonyl group content, a concentration of 2·1 g l^{-1} was used and the results were related to 0·4 g l^{-1} concentration.

RESULTS AND DISCUSSION

Characterization of Starch Carboxy Derivatives

The carboxy derivatives prepared (Table I) can contain, in addition to the free carboxy groups, also lactones and carbonyl groups (aldehyde groups, keto groups). The solubility of the samples increased considerably on a short heating of the aqueous suspension on a water bath (procedure *b vs* procedure *a*). The increase in the free carboxy group content of the samples brought about by the partial opening of the lactones at elevated temperatures was 2–24% (Table I, columns *a*, *b*).

The starch and amylose carboxy derivatives were also exposed to the action of alkaline medium, which resulted in an opening of the lactones and conversion of the majority of the carbonyl groups to carboxy groups (Table I, column *c*). The carboxy group content increased considerably, particularly for samples prepared by oxidation of native starch with nitrogen dioxide, where the increase was 108–141%. The degree of carboxylation (*DC*) of the D-glucose units given in Table I, *i.e.*, the average number of carboxy groups per D-glucose unit, pertains to the total carboxy group content of the samples after applying procedure *c*.

If the action of N_2O_4 on amylose only led to oxidation of the primary hydroxy groups of the D-glucose units to carboxy groups, D-glucuronane with a maximum carboxy group content of m_{COOH} = 5·68 mmol g^{-1} would result; the situation with starch would be similar. The actual high carboxy group contents of samples No 1 and 2 after the action of KOH, *viz.* m_{COOH} = 10·17 and 9·67 mmol g^{-1}, respectively, indicate that oxidation of the secondary hydroxy groups at $C_{(2)}$ and $C_{(3)}$ of the D-glucose units to carbonyl groups also occurs to an appreciable extent. The fast oxidation of native starch with excess N_2O_4 (sample No 1, 48 h) and the very slow oxidation achieved by gradual addition of oxidant (sample No 2, 310 h) give nearly identical results. The free carboxy group content of the partly oxidized sample No 3, m_{COOH} = = 1·93 mmol g^{-1}, and its total carboxy group content, 4·65 mmol g^{-1}, indicate that during the slow oxidation with N_2O_4 the primary and secondary hydroxy groups are simultaneously oxidized. Oxidation of starch and amylose dialdehyde derivatives with N_2O_4 (samples No 4–8) resulted in a higher degree of carboxylation, *DC* = = 2·16 to 2·40, than the direct oxidation of native starch with this oxidant (Table I).

The presence of lactones and carbonyl groups in the samples and their elimination by alkaline medium were followed by IR and CD spectrometry. The IR spectrum of

sample No 1, prepared by direct oxidation of starch, exhibits, in addition to the intense $\nu_{as}(COO^-)$ band at $\sim 1\,610\ cm^{-1}$, a sharp band at $1\,735\ cm^{-1}$ due to vibrations of the carbonyl groups, $\nu(C{=}O)$, and a band at $886\ cm^{-1}$ due to the $\delta(C{-}H)$ vibrations of the aldehyde groups (Fig. 1, spectrum *1*). After the action of alkaline medium the two carbonyl bands vanish (spectrum *1'*).

The spectrum of sample No 5, prepared from starch dialdehyde of a degree of oxidation $DO_{(d.a.)} = 0{\cdot}98$, displays only weak bands at $1\,735$ and $886\ cm^{-1}$ (Fig. 1, spectrum *2*). An additional weak band appears at $1\,775\ cm^{-1}$, due to the lactone $\nu(C{=}O)$ vibrations. Because in the starting sample of starch 2,3-dialdehyde, 98% D-glucose units had been oxidized with periodate to the dialdehyde, keto groups cannot be present in the final starch carboxy derivative. Therefore, the band at $1\,735\ cm^{-1}$ corresponds to aldehyde groups. The action of alkaline medium again brings about vanishing of bands characteristic of aldehyde groups or lactones (spectrum *2'*). Sample No 4 gave spectra identical with traces *2* and *2'*.

The carbonyl $\nu(C{=}O)$ absorption at $1\,735\ cm^{-1}$ is considerably less intense than the carboxy group $\nu(COOH)$ absorption at that wavelength and than the carboxylate $\nu_{as}(COO^-)$ absorption at $1\,610\ cm^{-1}$. The circular dichroism (CD) spectra of the

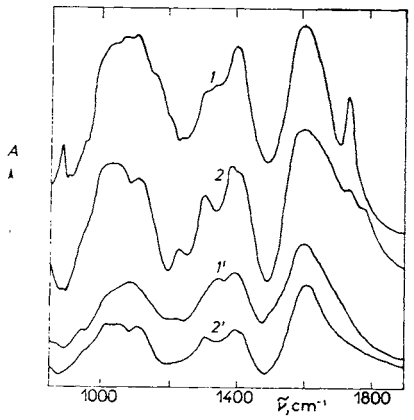

FIG. 1

Infrared spectra of starch carboxy derivatives. Samples: *1* native starch oxidized by N_2O_4 (sample No 1), *2* starch 2,3-dialdehyde oxidized by N_2O_4 (sample No 5), *1'*, *2'* samples No 1 and 5, respectively, after the action of alkaline medium

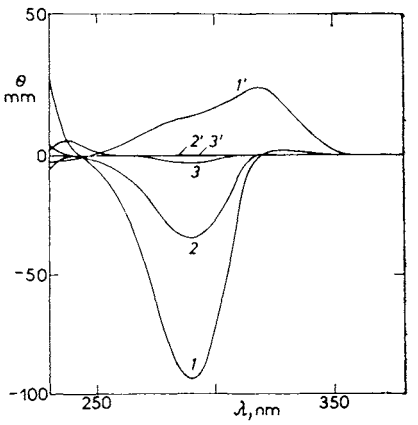

FIG. 2

Circular dichroism spectra of starch carboxy derivatives. Samples: *1*, *2* native starch oxidized by N_2O_4 (samples No 2 and 3, respectively), *3* starch 2,3-dialdehyde oxidized by N_2O_4 (sample No 5), *1'*, *2'*, *3'* samples No 2, 3, and 5, respectively, after the action of alkaline medium

substances were therefore measured over the region of $\lambda = 250-350$ nm, where the chirality of compounds with carbonyl groups manifests itself markedly. The CD spectra of samples No 2 and 3, prepared by direct oxidation of starch with N_2O_4 (Fig. 2, spectra *1*, *2*), exhibit a very intense ellipticity band at 290 nm with negative Θ values, characteristic of carbonyl compounds. The intensity of this band is approximately directly proportional to the degree of oxidation of the sample, evaluated based on its carboxy group content. (The degree of oxidation of the partly oxidized starch, sample No 3, cannot be estimated reliably from the total carboxy group content because the carboxy group distribution over the oxidized D-glucose units is not accurately known.) In the CD spectrum of the starch carboxy derivative obtained by oxidation of the 2,3-dialdehyde derivative (sample No 5), on the other hand, the band characteristic of carbonyl compounds is very weak (Fig. 2, spectrum *3*), in accordance with the IR spectrum of this sample (Fig. 1, spectrum *2*).

When alkaline medium was allowed to act on carboxystarch samples No 3 and 5, the band corresponding to carbonyl compounds vanished (Fig. 2, spectra *2'* and *3'*). For sample No 2, with a high number of carbonyl groups (spectrum *1*), spectrum *1'* was obtained, running over a positive ellipticity region (a similar spectrum was also obtained from sample No 1); this indicates that the carbonyl groups in this sample had not been eliminated completely by the alkaline medium. A detailed study of the CD spectra would be necessary for elucidation of spectrum *1'*, with a pronounced maximum at 320 nm and a hint of another band at 280 nm.

The IR and CD spectra thus give evidence that the products of direct oxidation of starch by nitrogen dioxide contain a great number of carbonyl groups, whereas in the products of oxidation of starch 2,3-dialdehyde derivatives by this oxidant the number of carbonyl groups is many times lower. In samples No 3 and 5, with moderate or low amounts of carbonyl groups, alkaline medium induced a quantitative conversion of these groups to carboxy groups. In the dialdehyde derivatives, the process involved is actually disproportionation of the aldehyde groups resulting in the formation of a primary hydroxy group and a carboxy group.

The carbonyl group content of the carboxystarch samples is closely related with their stability in alkaline medium, which can affect their ability to bond and exchange cations. The selectivity of the $Ca^{2+} \rightarrow 2\ K^+$ exchange was therefore examined both for the initial carboxystarch samples dissolved by a short-time heating of their solutions (procedure *b*) and for samples where lactones and carbonyl groups had been eliminated by alkaline medium (procedure *c*); this selectivity was evaluated in terms of the decrease in the electrostatic free enthalpy, $\Delta(G_{el}/N)_K^{Ca}$, associated with the $Ca^{2+} \rightarrow 2\ K^+$ cation exchange.

For the carboxy derivatives obtained by direct oxidation of starch with nitrogen dioxide (samples No 1–3), with a high carbonyl group content and with $m_{COOH} = 1{\cdot}93-4{\cdot}77$ mmol g^{-1}, the $\Delta(G_{el}/N)_K^{Ca}$ values are $-3{\cdot}2$ to $-4{\cdot}2$ kJ $(COOH)^{-1}$ (Table I). These values are close to the $-3{\cdot}1$ to $-3{\cdot}7$ kJ $(COOH)^{-1}$ found for pectic

acid[2,13] and somewhat higher than the $-2{\cdot}2$ kJ $(COOH)^{-1}$ found for D-mannuronane[13] and the $-2{\cdot}6$ kJ $(COOH)^{-1}$ found for carboxymethylcellulose with a degree of substitution $DC = 1$ (ref.[11]). Pectic acid bonds Ca^{2+} ions by a stronger intermolecular chelate binding, D-mannuronane and carboxymethylcellulose, by a weaker intramolecular electrostatic binding. The carboxy group content of the carboxy derivatives, samples No 1–3 increased as much as to the double on the action of alkaline medium (Table I, procedure *c*). Despite the increased charge density at the macromolecule, which should bring about an increased selectivity with respect to the cation exchange, a marked decrease in $\Delta(G_{el}/N)_K^{Ca}$ occurred. The decrease for samples No 1 and 2, with a high degree of oxidation, $-0{\cdot}88$ and $-1{\cdot}38$ kJ $(COOH)^{-1}$ is 3·8 and 3·0 times, respectively, lower than for the starting substances ($-3{\cdot}32$ and $-4{\cdot}16$ kJ $(COOH)^{-1}$, respectively). This indicates that the alkaline medium led to degradation of the starch macromolecules to such an extent that their ability to selectively bond Ca^{2+} ions was reduced considerably.

In contrast to the above samples, the carboxy derivatives prepared by oxidation of the 2,3-dialdehydes (samples No 4–8), containing carbonyl groups in small amounts only, are not alkali-labile. The $\Delta(G_{el}/N)_K^{Ca}$ values for the starting samples (*b*) and for the samples subjected to the action of alkaline medium (*c*) approach each other closely (Table I, samples No 4 and 5). The action of alkaline medium led to an increase in the carboxy group content up to 11·1–12·1 mmol g^{-1}. The $\Delta(G_{el}/N)_K^{Ca}$ values are high, $-8{\cdot}9$ to $-11{\cdot}4$ kJ $(COOH)^{-1}$, documenting a high selectivity of the samples with respect to Ca^{2+} in the $Ca^{2+} \rightarrow 2\,K^+$ cation exchange. (For a comparison: for starch and amylose 2,3-dicarboxy derivatives with $m_{COOH} = 7{\cdot}85$ and 6·80 mmol g^{-1}, the values are[2] $-8{\cdot}8$ and $-8{\cdot}7$ kJ $(COOH)^{-1}$, respectively.)

Effect of Alkaline Medium on the Ability of Carboxystarch, Prepared from Native Starch, to Bind Calcium and Lead Cations

We have shown that carboxy derivatives prepared by allowing N_2O_4 to act on native starch are alkali-labile, which is related with their high carbonyl group content. Their ability to selectively bind Ca^{2+} ions decreased considerably (Table I), in conditions pH ≈ 12·0 where KOH was in a high excess both at the beginning of the reaction (~0·02 mol l^{-1}) and at its end after 18 h at 25°C (0·01 mol l^{-1}). We followed therefore the effect of alkaline medium by adding various amounts of KOH to a 0·1% solution of carboxystarch sample No 2. After the reaction (18 h), the pH of the solution, increase in the carboxy group content of sample, and the change in the $\Delta(G_{el}/N)_K^{Ca}$ value were determined and the binding of Pb^{2+} ions to the carboxy groups was examined (Table II). The ability of the samples to bind Pb^{2+} ions was evaluated in terms of the activity coefficient of the counter-ions, $\gamma_{Pb^{2+}}$, determined in the suspensions of the lead salt of carboxystarch, and of the degree of association β of the Pb^{2+} ions with the carboxy groups of sample. Only 96% $Pb(NO_3)_2$ with respect

TABLE II

Effect of alkaline medium on carboxy starch prepared by oxidation of native starch with nitrogen dioxide (sample No 2, 1·04 ± 0·01 g l^{-1})

c_{KOH}[a] mmol l^{-1}	pH[b]	m_{COOH} mmol g^{-1}	$\Delta(G_{el}/N)_K^{Ca}$[c] kJ $(COOH)^{-1}$	c_{Pb}[d] mmol l^{-1}	$[Pb^{2+}]_f$ mmol l^{-1}	$a_{Pb^{2+}} . 10^3$	$\gamma_{Pb^{2+}}$	β
0·0	8	4·77 ± 0·01	−4·16 ± 0·39	1·017 ± 0·033	0·010	0·007 ± 0·003	0·007	0·993
1·3	8–9	5·81 ± 0·18	−2·08 ± 0·18	0·668 ± 0·006	0·049	0·036 ± 0·001	0·054	0·966
6·5	11·27	7·81 ± 0·18	−1·56 ± 0·08	0·653 ± 0·001	0·086	0·063 ± 0·005	0·096	0·940
10·9	11·79	8·38 ± 0·05	−1·36 ± 0·13	0·636 ± 0·003	0·139	0·100 ± 0·005	0·158	0·903
14·7	12·01	9·67 ± 0·01	−1·38 ± 0·20	0·533 ± 0·001	0·138	0·100 ± 0·003	0·158	0·904

[a] Excess at the beginning of reaction; [b] after reaction (18 h); [c] c_{COOH} = 3·00 mmol l^{-1}; [d] c_{COOK} = 3·00 mmol l^{-1}, $(c_{Pb})_0$ = 1·434 mmol l^{-1}, I_0 = 0·01 mol l^{-1}, I = 0·0057–0·0061 mol l^{-1}.

to the COO^- groups was added to the solution of potassium salt of carboxystarch, in order to prevent any effect of excess lead nitrate, which otherwise might occur, on the very low $a_{Pb^{2+}}$ value.

The change in the carboxy group content of the samples in dependence on the starting concentration of KOH is shown in Fig. 3 for sample No 2 with a high degree of oxidation (curve *1*) and for the partly oxidized starch sample No 3 (curve *2*). The increase in the carboxy group content arises from the opening of lactones and, particularly, from the conversion of carbonyl groups to carboxy groups. Cation exchange and acidimetric and alkalimetric titration experiments were performed and the increase in the amount of carboxy groups was found precisely equivalent to the decrease in the amount of potassium hydroxide. For sample No 2, rich in carbonyl groups, the conversion of these groups to carboxy groups was not complete in 18 h (curve *1*) even if a relatively high alkalinity of the solution (0·015M-KOH) was used. The time dependence of the carboxy group increment for this sample at the same starting alkalinity of solution was similar. For the partly oxidized starch sample (No 3), with a smaller amount of carbonyl groups, the plot (curve 2) indicates that in contrast to the previous sample, in this sample virtually all carbonyl groups were converted to carboxy groups. These conclusions are consistent with those derived from the circular dichroism spectra.

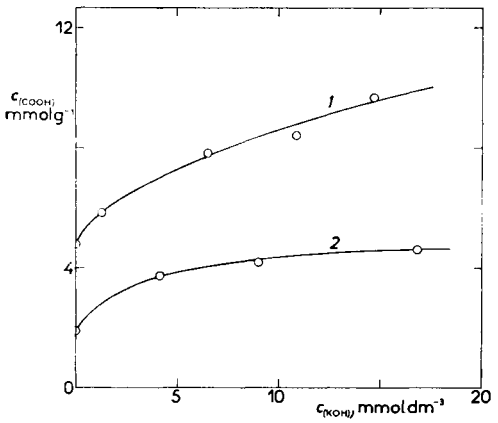

FIG. 3

Increase in the carboxy group content of starch carboxy derivatives No 2 (*1*) and No 3 (*2*) on the action od alkaline medium. Axis of abscissas: excess KOH in 0·1% carboxy starch solution at the beginning of reaction

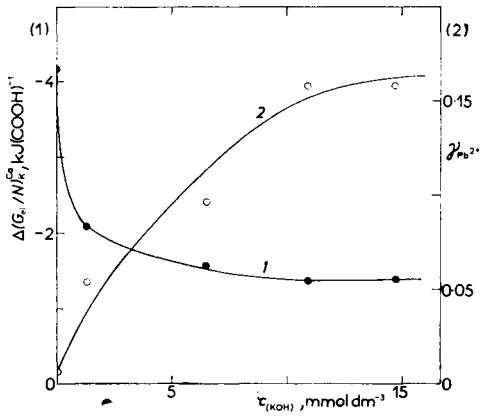

FIG. 4

Effect of alkaline medium on the ability of carboxy starch sample No 2 to bind calcium and lead cations. *1* $\Delta(G_{el}/N)_K^{Ca}$, change in the electrostatic free enthalpy on the $Ca^{2+} \rightarrow 2\,K^+$ cation exchange; *2* activity coefficient of lead counter-ions, $\gamma_{Pb^{2+}}$

Even a very small amount of alkali ($c_{KOH} = 1{\cdot}3$ mmol l^{-1}) in the carboxystarch solution brought about a considerable decrease in the $\Delta(G_{el}/N)_K^{Ca}$ value, *i.e.*, in the ability of the substance to selectively bind Ca^{2+} ions (Fig. 4, curve *1*); the change in this value becomes only little more pronounced as the starting excess of KOH is increased ($c_{KOH} \geqq 6{\cdot}5$ mmol l^{-1}). Hence, degradation of carboxystarch takes place even if the pH after the reaction is 8–9 and the action of alkaline medium results in the conversion of a small fraction of carbonyl groups only to carboxy groups.

Binding of Pb^{2+} to the starch carboxy derivative No 2 is a similar case (Table II). The starting ionic strength $I_0 = 0{\cdot}01$ mol l^{-1} decreased, owing to the binding of Pb^{2+} ions to the carboxy groups, to $I = 0{\cdot}0057-0{\cdot}0061$ mol l^{-1} in the equilibrium suspensions. The lead salt of carboxystarch coagulated partly; the total concentration of lead in the solution (c_{Pb}) decreased from the initial 1·43 mmol l^{-1} to as little as 0·63 mmol l^{-1}. The starting sample bound virtually all (99·3%) lead cations; the concentration of free Pb^{2+} ions in the suspension, $[Pb^{2+}]_f$, was as low as 0·01 mmol l^{-1}. As the starting alkalinity of the solution was raised, the ability of the final products to bind Pb^{2+} ions decreased – the activity coefficient $\gamma_{Pb^{2+}}$ increased from the initial 0·007 up to a value of 0·158 (Fig. 4, curve *2*) and the degree of association of Pb^{2+} ions with the carboxy groups β decreased from 0·993 down to 0·903.

It can be concluded that starch carboxy derivatives prepared by direct oxidation of native starch with N_2O_4 are very alkali-labile; even in weakly alkaline solutions, where only small fractions of carbonyl groups convert to carboxy groups, the ability of the substances to selectively bind calcium and lead ions reduces considerably. Hence, carboxy derivatives of starch exhibiting a high capacity and selectivity for cation exchange cannot be obtained in this way. They can be, however, prepared by a two-stage oxidation of starch, comprising oxidation with periodate to 2,3-dialdehyde derivatives and their additional oxidation with nitrogen dioxide to starch carboxy derivatives. These substances are less alkali-labile owing to the fact that the amount of carbonyl groups in them is small. Alkaline medium here does not induce a decrease in the cation exchange selectivity; highly selective starch carboxy derivatives are thus obtained, featuring a cation bonding capacity as high as 12 milliequivalents per g sample (H^+ form).

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