BINDING OF LEAD AND COPPER(II) IONS TO STARCH AND AMYLOSE 2,3-DICARBOXY DERIVATIVES

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Received July 4th, 1985

The binding of Pb^{2+} and Cu^{2+} ions to starch and amylose 2,3-dicarboxy derivatives of degrees of oxidation $DO = 0{\cdot}09-0{\cdot}74$ was investigated. The interaction of the metal cations with the carboxy groups of the derivatives was evaluated in terms of the counter-ion activity, determined with ion specific electrodes. The binding is very strong; at $DO \geqq 0{\cdot}34$, lead ions are bound quantitatively (degree of association $\beta = 0{\cdot}999$), copper ions are also bound to a great extent ($\beta = 0{\cdot}94-0{\cdot}97$). The complexation of copper with the ligands at ionic strength $I = 0{\cdot}15$ mol l^{-1} was treated in terms of multiple equilibria theory. Different complexes were found in dependence on the concentration ratios of the reactants; at a high excess of COO^- groups with respect to Cu^{2+} ions, the latter are strongly bound and the number of COO^- groups binding a Cu^{2+} cation is greater than 2, whereas at a sufficiently high concentration of Cu^{2+} ions the number of COO^- groups binding a Cu^{2+} cation approaches the stoichiometric value of 2.

We have shown[1] that starch and amylose 2,3-dicarboxy derivatives bind calcium ions very strongly, even at low degrees of oxidation. With pectinates and carboxymethyl derivatives of starch and amylose with the same carboxy group contents, the binding of Ca^{2+} ions is considerably weaker. The strong binding to the 2,3-dicarboxyderivatives is due to the favourable steric arrangement of the two adjacent carboxy groups with the $C_{(2)}$ and $C_{(3)}$ carbon atoms of the oxidized D-glucose units, occurring thanks to the flexibility of the macromolecule chain in the sites of opening of the pyrane rings. A low degree of oxidation of starch and amylose may be highly desirable for some medical applications, because such substances remain partly biodegradable by amylolytic enzymes[2].

Crescenzi and coworkers[3], who studied the interaction of Ca^{2+} and Mg^{2+} ions with 2,3-dicarboxyamylose and 2,3-dicarboxycellulose by the microcalorimetry, ^1H NMR, and circular dichroism techniques, observed the formation of complexes of different compositions, the most stable of which contained four COO^- groups per M^{2+} cation. Casu and coworkers[4] examined these systems by potentiometric and conductometric titrations and found that the interaction of Ca^{2+}, Mg^{2+}, Cu^{2+}, and Fe^{2+} ions with the amylose and cellulose derivatives was well indicated by inflections on the conductometric titration curves as well as by changes in the specific

rotatory power. In both studies, preparations were used where all the D-glucose units of the polysaccharide had been oxidized to 2,3-dicarboxy derivatives.

In the present work, the effect of the degree of oxidation of starch and amylose on the binding of lead and copper ions by the corresponding 2,3-dicarboxy derivatives is studied by measuring the activities of the Pb^{2+} and Cu^{2+} counter-ions.

EXPERIMENTAL

Starch and amylose 2,3-dicarboxy derivatives of various degrees of oxidation (*DO*) were the same preparations as used in our previous work[1] where they were characterized in detail.

Water was redistilled, boiled and cooled, and was free from CO_2. Chemicals of reagent grade purity and 0·05M-KOH free from carbonate were used. The potentiometric titrations and activity determinations were carried out on an OP 208 pH-meter (Radelkis, Budapest).

Determination of Activity of Pb^{2+} and Cu^{2+} Counter-Ions

Solutions of sodium salts of starch and amylose 2,3-dicarboxy derivatives with $c_{COONa} = 5$ to 7 mmol l^{-1} were converted to the polyacids by percolation through a column of Dowex 50 W×2 (H^+) cation exchanger, and neutralized with 0·05M-KOH to pH 7·5—7·6. A 0·01M-$Pb(NO_3)_2$ or 0·01M-$Cu(NO_3)_2$ solution was added in a quantity such that the amount of the cations was equivalent to 95% of the COOK groups present ($c_{COOK} = 3{\cdot}00$ mmol l^{-1}, $c_{Pb(NO_3)_2} = 1{\cdot}420$ mmol l^{-1}, $c_{Cu(NO_3)_2} = 1{\cdot}429$ mmol l^{-1}). The ionic strength was adjusted with 0·1M-KNO_3 to $I_0 = 0{\cdot}01$ mol l^{-1}.

The copper(II) salt solution was clear, a weak colloidal haze appeared only occasionally for some samples of oxidized amylose. In the lead salt solutions, partial coagulation took place. The activities of the Pb^{2+} and Cu^{2+} ions were determined[5,6] with Crytur 82—17 and 29—17 ion specific electrodes (Monokrystaly, Turnov), respectively. A K 711 saturated calomel electrode with a double-compartment electrolyte bridge (Radiometer, Copenhagen) served as the reference electrode; the outer compartment contained 10% KNO_3 solution. The measurements were performed on constantly stirred solutions at 25·0 ± 0·1°C. For obtaining the $EMF = f(pPb, pCu)$ curves, solutions of $Pb(NO_3)_2$ and $Cu(NO_3)_2$ were used at $I = 0{\cdot}01$ mol l^{-1} (KNO_3); the tabulated Debye–Hueckel activity coefficients[7] were employed. The curves were plotted extra for each measurement series. The ionic strength of the equilibrium solutions (I), which was different from the initial ionic strength $I_0 = 0{\cdot}01$ mol l^{-1}, was calculated by an iterative procedure[5]. The activities ($a_{Pb^{2+}}$, $a_{Cu^{2+}}$) and the corrected ionic strength were used for the calculation of the free cation concentrations in the equilibrium solution.

The suspensions of the lead salts of the starch and amylose derivatives were centrifuged at 20 000 *g* for 30 min, and the total concentration of lead in solution (c_{Pb}) was determined chelatometrically with 0·01M and 0·002M Chelaton IV and $MgCl_2$ using spectrophotometric end point indication with Eriochrome Black T indicator (IF 650 nm interference filter; Carl Zeiss, Jena). The concentration of lead in the standard nitrate solution was determined likewise. The concentrations of copper in the nitrate solution and in the solutions of the copper salts of the starch and amylose derivatives were determined similarly by direct titration with 0·01M Chelaton IV in weakly ammonical medium (IF 600 nm filter, murexide indicator).

Degree of Association

The degree of association of the metal ions M^{2+} with the carboxy groups of the substance examined was determined as

$$\beta = (c_{M^{2+}} - [M^{2+}]_f)/c_{M^{2+}} , \qquad (1)$$

where $c_{M^{2+}}$ is the initial concentration of M^{2+} cations in the solution or suspension and $[M^{2+}]_f$ is the free cation concentration in the equilibrium solution.

Characterization of Equilibria in Solutions of Copper Complexes of Starch and Amylose 2,3-Dicarboxy Derivatives

The theory of multiple equilibria[8] was applied to solutions with $c_{COOK} = 3{\cdot}00$ mmol l^{-1} and different concentrations of $Cu(NO_3)_2$ in the $c_{Cu^{2+}} = 0{\cdot}2-2{\cdot}0$ mmol l^{-1} range ($I = 0{\cdot}150$ mol . l^{-1}, KNO_3). In solutions with low concentrations of the copper salt at pH > 5·0, the pH was adjusted with a very small addition of dilute HNO_3 to pH 5·00 before diluting to final volume with water. The activity of the Cu^{2+} ions was determined by using an ion specific electrode and the concentration of free Cu^{2+} ions in the equilibrium solution was calculated. The analytical straight line plot of $EMF = f(pCu)$ was also established at $I = 0{\cdot}150$ mol l^{-1} (KNO_3).

The number of binding sites of Cu^{2+} ions, n, on an oxidized D-glucose unit was calculated from the $[Cu^{2+}]_f$ values according to the formula[8]

$$r^{-1} = (nK[Cu^{2+}]_f)^{-1} + n^{-1} , \qquad (2)$$

where r is the number of Cu^{2+} ions bound to a unit of oxidized D-glucose, involving two COO^- groups, and K is the stability constant of the copper complex. The value of n was obtained by extrapolation of the function $r^{-1} = f([Cu^{2+}]_f^{-1})$ to $[Cu^{2+}]_f^{-1} \to 0$, where $r^{-1} \to n^{-1}$.

RESULTS AND DISCUSSION

The starch and amylose 2,3-dicarboxy derivatives used are characterized in this paper by the degree of oxidation of the D-glucose units (DO) and the free carboxy group content of the dry sodium salts (Table I and II); for a more detailed characterization see paper[1] (the sample numbering is identical).

The pH of the solutions was held low enough to prevent precipitation of the lead and copper salts, *viz.* pH < 6·0 for lead and pH < 5·5 for copper. For this, the polyacids were neutralized with KOH to a pH somewhat lower (pH 7·5 – 7·6) than as corresponds to the equivalence point. On the addition of the lead or copper nitrate, their hydrolysis resulted in a pH decrease such that additional pH adjustment was unnecessary; the lowest additions of $Cu(NO_3)_2$ were exceptions where the pH had to be adjusted with dilute nitric acid to pH 5·00. For the equilibrium solutions of the lead and copper salts, the pH was 5·2 – 5·6 and 5·10 – 5·38, respectively.

At $DO > 0{\cdot}3$, the starch and amylose derivatives bind Pb^{2+} cations nearly quantitatively. The trace amounts of Pb^{2+} ions found in preliminary experiments in the equilibrium solutions may have been due to the addition of the lead salt being in a slight excess over the carboxy group content. For this reason, in the present experi-

TABLE I

Binding of Pb^{2+} ions to starch and amylose 2,3-dicarboxy derivatives. $c_{COOK} = 3{\cdot}00$ mmol l^{-1}, $(c_{Pb^{2+}})_o = 1{\cdot}420$ mmol l^{-1}, $I_o = 0{\cdot}01$ mol l^{-1}

Sample No	*DO*	ϱ_{COOH} mmol g^{-1}	c_{Pb} mmol l^{-1}	$[Pb^{2+}]_f$ mmol l^{-1}	$a_{Pb^{2+}} \cdot 10^3$	$\gamma_{Pb^{2+}}$	I mol l^{-1}	β
			Starch 2,3-dicarboxy derivatives					
1	0·090	1·08 ± 0·00	1·394 ± 0·009	0·053	0·038 ± 0·009	0·027	0·0059	0·963
2	0·234	2·60 ± 0·04	1·230 ± 0·027	0·008	0·006 ± 0·001	0·005	0·0057	0·995
3	0·37	3·89 ± 0·05	0·975 ± 0·080	0·002	0·001 ± 0·000	0·001	0·0057	0·999
4	0·55	5·40 ± 0·16	0·969 ± 0·205	~0·001	<0·001	<0·001	0·0057	0·999
5	0·74	6·80 ± 0·08	0·692 ± 0·012	<0·001	<0·001	<0·001	0·0057	0·999
			Amylose 2,3-dicarboxy derivatives					
7	0·34	3·64 ± 0·12	0·929 ± 0·024	~0·001	0·0006	<0·001	0·0057	0·999
8	0·45	4·58 ± 0·09	0·784 ± 0·073	<0·001	<0·001	<0·001	0·0057	0·999
9	0·53	5·24 ± 0·05	0·827 ± 0·035	<0·001	<0·001	<0·001	0·0057	0·999
10	0·67	6·36 ± 0·14	0·655 ± 0·015	<0·001	<0·001	<0·001	0·0057	0·999

TABLE II

Binding of Cu^{2+} ions to starch and amylose 2,3-dicarboxy derivatives. $c_{COOK} = 3{\cdot}00$ mmol l^{-1}, $c_{Cu} = 1{\cdot}429$ mmol l^{-1}, $I_o = 0{\cdot}01$ mol l^{-1}

Sample No	*DO*	ϱ_{COOH} mmol g^{-1}	$[Cu^{2+}]_f$ mmol l^{-1}	$a_{Cu^{2+}} \cdot 10^3$	$\gamma_{Cu^{2+}}$	I mol l^{-1}	β
			Starch 2,3-dicarboxy derivatives				
1	0·090	1·08	0·290	0·210 ± 0·013	0·147	0·0064	0·797
2	0·234	2·60	0·114	0·084 ± 0·002	0·059	0·0059	0·920
3	0·37	3·89	0·083	0·061 ± 0·003	0·043	0·0058	0·942
4	0·55	5·40	0·056	0·042 ± 0·001	0·029	0·0057	0·961
5	0·74	6·80	0·038	0·028 ± 0·002	0·020	0·0057	0·973
			Amylose 2,3-dicarboxy derivatives				
7	0·34	3·64	0·120	0·088 ± 0·005	0·062	0·0059	0·916
8	0·45	4·58	0·087	0·064 ± 0·004	0·045	0·0058	0·939
9	0·53	5·24	0·084	0·062 ± 0·001	0·043	0·0058	0·941
10	0·67	6·36	0·041	0·030 ± 0·003	0·021	0·0057	0·971

ments the lead nitrate addition was reduced to 95% with respect to the COO^- groups ($c_{Pb^{2+}} = 1{\cdot}420$ mmol l^{-1} with respect to $c_{COO^-} = 3{\cdot}00$ mmol l^{-1}). This approach was also applied to the copper systems.

Binding of Pb^{2+} ions. Partial precipitation of the lead complexes occurred on the addition of lead nitrate to the starch and amylose derivatives (Table I). The solubility of the Pb complex decreases with increasing degree of oxidation of the D-glucose units in the polysaccharide; at the highest *DO* values, 0·74 (sample No 5) and 0·67 (sample No 10), the initial concentration of lead ions, $(c_{Pb^{2+}})_0 = 1{\cdot}420$ mmol. l^{-1}, decreased to $c_{Pb} = 0{\cdot}69$ and 0·66 mmol l^{-1}, respectively.

The binding is evaluated in terms of the free cation concentration in the equilibrium solution $[Pb^{2+}]_f$, activity $a_{Pb^{2+}}$, activity coefficient of the counter-ion $\gamma_{Pb^{2+}}$, and degree of association of the lead ions with the carboxy groups β. Owing to the strong binding of the Pb^{2+} ions to the COO^- groups, the ionic strength of the solution decreased. It should be noted that the activity coefficient in a solution of a totally dissociated lead salt at $I = 0{\cdot}0057$ mol l^{-1} is $\gamma_{Pb^{2+}} = 0{\cdot}727$. The activity coefficient $\gamma_{Pb^{2+}}$ pertains to the total lead concentration in the supernatant (c_{Pb}) after centrifugation of the suspension, whereas the degree of association β pertains to the total amount of Pb^{2+} ions added (1·420 mmol l^{-1}).

It follows from the $[Pb^{2+}]_f$, $a_{Pb^{2+}}$, $\gamma_{Pb^{2+}}$, and β values in Table I that the binding of lead to the substances examined is very strong; the lead complexes of the starch and amylose 2,3-dicarboxy derivatives at degree of oxidation $DO \geqq 0{\cdot}34$ are virtually undissociated, binding more than 99·9% of the Pb^{2+} ions present. Hence, if added in amounts lower than as corresponds to the stoichiometry of the reaction $2\,(COO^-) + Pb^{2+} \rightleftarrows (COO)_2Pb$, Pb^{2+} ions are bound quantitatively at these *DO* values. A very slight dissociation of the complex ($\alpha = 1 - \beta = 0{\cdot}037$) was only found for sample No 1, with the lowest value of $DO = 0{\cdot}09$.

Binding of Cu^{2+} ions. The starch and amylose derivative solutions remain clear after the addition of Cu^{2+} ions; no insoluble complex precipitates. In cases where a slight colloidal haze appeared, the copper concentration in the supernatant after centrifugation at 20 000 *g* was identical with its initial concentration. The results, summarized in Table II, show that Cu^{2+} ions are bound to the carboxy groups of the starch and amylose 2,3-dicarboxy derivatives very strongly, although, in contrast to Pb^{2+} ions, not quantitatively; at degrees of oxidation $DO \geqq 0{\cdot}23$, 92–97% of the total amount of Cu^{2+} ions is bound.

The dependences of the activity coefficients of the counter-ions bound to the starch and amylose derivatives on the free carboxy group contents of the latter are plotted in Figs 1 and 2; the $\gamma_{Ca^{2+}}$ values obtained previously[1] are also shown for a comparison. The activity coefficients decrease with increasing carboxy group content, which indicates that the strength of the cation binding increases with increasing average linear charge density of the macromolecule; the binding of Ca^{2+} ions to

amylose 2,3-dicarboxy derivatives at higher degrees of oxidation are an exception (Fig. 2, curve *1*, samples No 9 and 10). A considerable degradation of the amylose macromolecules took place during the preparation of the samples, as evidenced by their very low limiting viscosity numbers[1], $[\eta] = 13$ and 10 ml g^{-1}, respectively. In these low molecular weight fragments the binding of the Ca^{2+} ions is already a function of their polymerization degree. The $\gamma_{Ca^{2+}}$ values for amylose derivatives at lower degrees of oxidation (samples No 7 and 8) are consistent with the other $\gamma_{M^{2+}}$ values. It is clear that the degradation of the amylose molecule did not affect the binding of Cu^{2+} and Pb^{2+} ions to its 2,3-dicarboxy derivatives. 2,3-Dicarboxy derivatives of starch and of amylose bind Pb^{2+} ions virtually equally strongly; this is also true of Cu^{2+} ions, but at a different quantitative level.

With respect to the $\gamma_{M^{2+}}$ values (Figs 1 and 2), the affinity of the carboxy groups of the starch and amylose 2,3-dicarboxy derivatives for the divalent cations decreases in order $Pb^{2+} \gg Cu^{2+} > Ca^{2+}$. The very strong binding of the Pb^{2+} and Cu^{2+} cations to these derivatives, even at low degrees of oxidation of the polysaccharides, can be ascribed to the pair of adjacent carboxy groups with the $C_{(2)}$ and $C_{(3)}$ atoms of the oxidized D-glucose units in the macromolecule. Owing to the flexibility of the

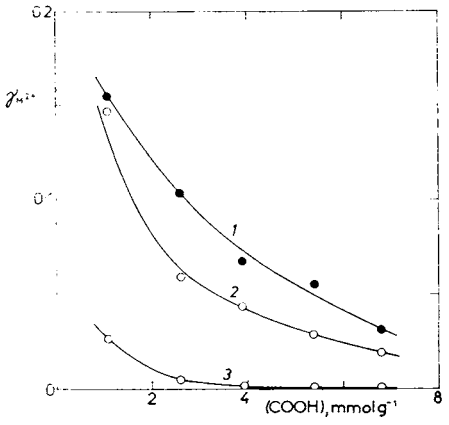

FIG. 1

Dependences of the activity coefficients of Ca^{2+} (*1*), Cu^{2+} (*2*), and Pb^{2+} (*3*) counter-ions in solutions of starch 2,3-dicarboxy derivatives on the carboxy group contents of the substances. $c_{COOMo.5} = 2{\cdot}84$ to 3·00 mmol l^{-1}, $I_0 = 0{\cdot}010$ mol l^{-1}. (COOH) is the carboxy group content of the dry sodium salt, mmol g^{-1}

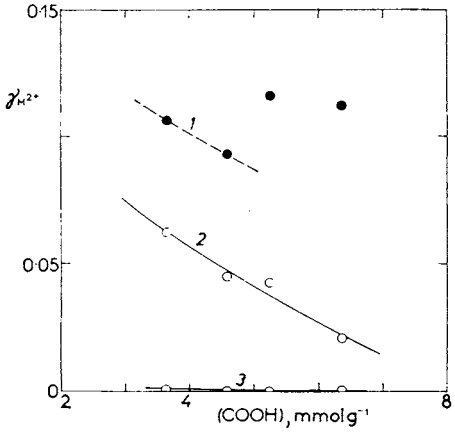

FIG. 2

Dependences of the activity coefficients of Ca^{2+} (*1*), Cu^{2+} (*2*), and Pb^{2+} (*3*) counter-ions in solutions of amylose 2,3-dicarboxy derivatives on the carboxy group contents of the substances. $c_{COOMo.5} = 2{\cdot}84$ to 3·00 mmol l^{-1}, $I_0 = 0{\cdot}010$ mol l^{-1}. (COOH) is the carboxy group content of the dry sodium salt, mmol g^{-1}

macromolecule chain resulting from the opening of the pyrane rings of the D-glucose units, these groups can assume a steric arrangement very favourable for the binding of the divalent cations of lead and copper.

Complex Formation Between Cu^{2+} Ions and Starch and Amylose 2,3-Dicarboxy Derivatives

The measurements of the activity of Cu^{2+} ions with an ion specific electrode, which can be performed with a high accuracy[6], were employed for a more detailed study of the binding of Cu^{2+} ions to the starch and amylose derivatives. With respect to the multiple equilibria theory, the experiments were performed at a higher ionic strength, $I = 0{\cdot}15$ mol l^{-1}; owing to the competitive interaction of the K^+ ions added, the binding of the Cu^{2+} ions is then somewhat weaker and the concentration of free copper(II) ions in solution somewhat higher, which affects favourably the accuracy of analysis. The pH of the equilibrium solutions was adjusted to 5·0 for preventing the formation of basic copper salts.

The interaction of Cu^{2+} ions with the starch and amylose derivatives (Figs 3 and 4, respectively) was evaluated by the multiple equilibria theory (Eq. (*2*)). In case that a single complex is formed, the function $r^{-1} = f([M^{2+}]_f^{-1})$ is linear, and the number of binding sites n on the macromolecule (its suitable segment) can be evaluated. This is the case with Ca^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} ions interacting with potassium pectinates of various degrees of esterification[9-12] and Cu^{2+} ions interacting with oligogalacturonates[6]. The macromolecule segment involving one oxidized D-glucose unit, with two carboxy groups, was taken as the basis for the calculation of the concentration of the ligand. At a stoichiometric binding of the Cu^{2+} ions to the carboxy groups, the number of sites at this segment will be $n = 1{\cdot}0$.

The plots in Figs 3 and 4 are not linear; instead, convex curves are obtained for all the samples with the different degrees of starch or amylose oxidation. The lower the r^{-1} value, the higher amount of Cu^{2+} ions is bound to a ligand. Clearly, the most stable complexes are formed at the smallest additions of Cu^{2+} ions to the starch or amylose derivative, where a cation can interact with several carboxy groups. The departure of the curves from the axis of abscissas decreases markedly with increasing degree of oxidation of the polysaccharide. This documents, in agreement with the data of Tables I and II, increasing Cu^{2+} binding strength with increasing *DO* value, *i.e.*, with increasing average linear charge density of the macromolecule.

The r^{-1} value at high concentrations of free copper ions in the equilibrium solution, *i.e.*, at the lowest $[Cu^{2+}]_f^{-1}$ values, approaches a value of approximately $r^{-1} \sim 1$. For a more accurate determination, the $r^{-1} = f([Cu^{2+}]_f^{-1})$ function was plotted in detail in the range of high concentrations of free Cu^{2+} ions in the equilibrium solution for samples where a sufficient volume of data were available (Figs 5 and 6). For the starch derivatives (Fig. 5), the plot for samples No 1 and 3 (curves *5*

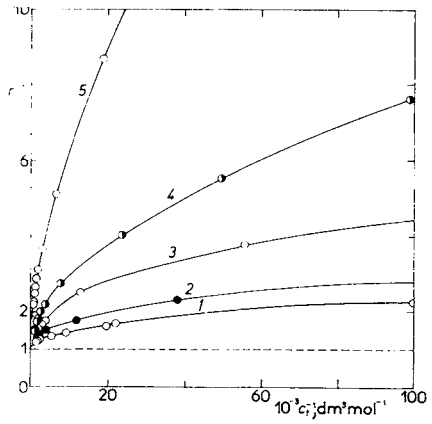

FIG. 3

Binding of Cu^{2+} ions to starch 2,3-dicarboxy derivatives. Function $r^{-1} = f(c_f^{-1})$, where $c_f = [Cu^{2+}]_f$. $I = 0{\cdot}15$ mol l^{-1}. *DO:* *1* 0·74, *2* 0·55, *3* 0·37, *4* 0·23, *5* 0·09 (Table I)

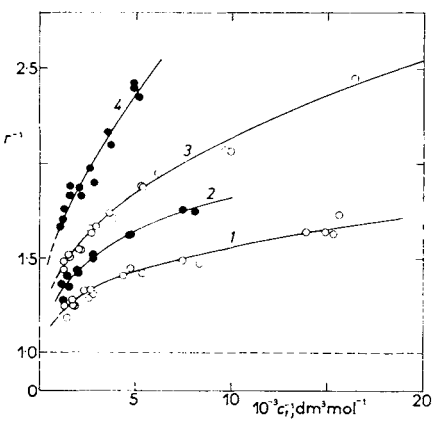

FIG. 4

Binding of Cu^{2+} ions to amylose 2,3-dicarboxy derivatives. Function $r^{-1} = f(c_f^{-1})$, where $c_f = [Cu^{2+}]_f$; $I = 0{\cdot}15$ mol l^{-1}. *DO:* *1* 0·67, *2* 0·53, *3* 0·45, *4* 0·34 (Table I)

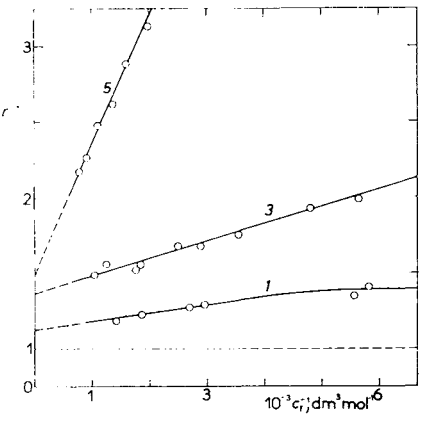

FIG. 5

Binding of Cu^{2+} ions to starch 2,3-dicarboxy derivatives at higher concentrations of free Cu^{2+} ions in the equilibrium solution. Function $r^{-1} = f(c_f^{-1})$, where $c_f = [Cu^{2+}]_f$. $I = 0{\cdot}15$ mol l^{-1}. *DO:* *1* 0·74, *3* 0·37, *5* 0·09 (Table I)

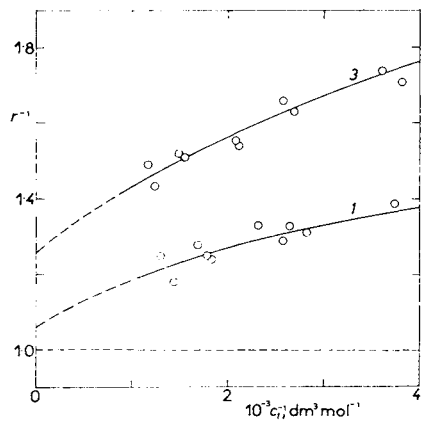

FIG. 6

Binding of Cu^{2+} ions to amylose 2,3-dicarboxy derivatives at higher concentrations of free Cu^{2+} ion in the equilibrium solution. Functions $r^{-1} = f(c_f^{-1})$, where $c_f = [Cu^{2+}]_f$; *DO:* *1* 0·67, *3* 0·45 (Table I)

and 3, respectively) is linear, so that the abscissa intercept ($r^{-1} = n^{-1}$) could be determined reliably enough and the stability constant K of the complex could be calculated. For sample No 5 (curve *1*), the stability constant only pertains to the region of $[Cu^{2+}]_f^{-1} \leqq 41\ mmol^{-1}$. For the amylose derivatives (Fig. 6), the function $r^{-1} = f([Cu^{2+}]_f^{-1})$ retains a slightly convex shape, so that the stability constant cannot be determined; the value of n was determined by extrapolation, with an absolute error of approximately $\pm 0{\cdot}05$.

The values of n and K are summarized in Table III. The n values increase with increasing degree of oxidation of the starch and amylose, and approach the theoretical value of $n_0 = 1$ corresponding to the stoichiometric interaction of the Cu^{2+} ions with the COO^- groups (one Cu^{2+} cation per 2 carboxy groups). At the lowest degree of oxidation of starch, $DO = 0{\cdot}09$, n rather approaches 0·5, a value corresponding to the interaction of one Cu^{2+} ion with four COO^- groups. With regard to this low degree of oxidation, two units of oxidized D-glucose (a total of four COO^- groups) from two different macromolecules seem to interact with a Cu^{2+} ion. Although because of the convex shape of the plots in Figs 3 and 4 the n value cannot be determined accurately enough for low concentrations of the free copper ions in the equilibrium solution, it is clear that a Cu^{2+} ion forms here complexes with a greater number of COO^- groups than as corresponds to the stoichiometry. This is consistent with the results of the work by Crescenzi, Casu and coworkers[3,4], who assume that, *e.g.*, a Ca^{2+} cation forms complexes with one, two, and four oxidized D-glucose units[13].

TABLE III

Characteristics of Cu complexes of starch and amylose 2,3-dicarboxy derivatives in solutions with higher concentrations of free Cu^{2+} ions. $I = 0{\cdot}15\ mol\ l^{-1}$

Sample No	*DO*	*n*	log *K*
	Starch 2,3-dicarboxy derivatives		
1	0·09	0·67	3·24
3	0·37	0·73	4·08
5	0·74	0·89	4·32
	Amylose 2,3-dicarboxy derivatives		
8	0·45	0·80	—
10	0·67	0·94	—

The measurements were performed at pH 5·0, under conditions preventing the formation of basic copper salts. At lower pH, however, the dissociation of the carboxy groups is partly suppressed. This decrease in the concentration of COO^- groups then corresponds to the somewhat increased r value, which in turn leads to an n value higher than as calculated based on the initial concentration of the COO^-K^+ groups. On the other hand, the strong binding of the divalent cations shifts the dissociation equilibrium in the opposite direction, thereby partly eliminating the effect of the lower pH. The values of $n = 0{\cdot}89$ and 0·94 determined for the starch and amylose derivatives of the highest degrees of oxidation ($DO = 0{\cdot}74$ and 0·67, respectively) are somewhat lower than the expected value of 1·00, for which effect the reduced dissociation of the carboxy groups at the lower pH of the equilibrium solution may be partly responsible (see refs[6,12]).

It can be concluded that at high degrees of oxidation of starch or amylose and at sufficiently high concentrations of Cu^{2+} ions in the equilibrium solution, a Cu^{2+} ion is bound stoichiometrically to two COO^- groups, whereas at a higher excess of COO^- groups and/or at a low degree of oxidation of the polysaccharide, the complexation involves more carboxy groups per Cu^{2+} cation.

Thanks are due to Mrs A. Košková for experimental cooperation.

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Translated by P. Adámek.