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COMPLEXES OF COPPER(II), CALCIUM, AND OTHER METAL IONS WITH CARBOHYDRATES: THIN-LAYER LIGAND-EXCHANGE CHROMATOGRAPHY AND DETERMINATION OF RELATIVE STABILITIES OF COMPLEXES

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ABSTRACT

Thin-layer ligand-exchange chromatography with sodium, magnesium, aluminium, calcium, chromium(III), iron(III), nickel, copper(II), zinc, strontium, cadmium, and barium as the central atoms has been investigated. With copper(II) as the central atom, the method is a simple, inexpensive, and speedy means of resolution of mixtures of carbohydrates not easily achieved by other methods. The molar ratios of complexed to uncomplexed polyhydroxy compounds, which give an indication of the relative stabilities of the complexes, are calculated from the chromatographic migration rates. For a particular compound, this ratio is, in general, greatest for the complex with the copper(II) ion.

INTRODUCTION

Polyhydroxy compounds have long been known to act as ligands in the formation of co-ordination complexes of alkali[1], alkaline-earth[1], and several polyvalent[2,3] metal ions. The metal-centred structures may carry a negative[3,4] or positive charge. Cationic complexes are formed in aqueous solutions of magnesium[5], calcium[5], copper(II)[6], strontium[5], and barium[5] acetates. Complexes have been investigated by polarimetry[1], electrophoresis[4–7], n.m.r. spectroscopy[8], potentiostatic titration[9], and chromatography[5,10,11]. Recently, chromatography employing a column of an ion-exchange resin in the lanthanum form has been described[12]. Some conclusions regarding the structure and the stability constants of complexes have been drawn from these investigations. For a particular chelating molecule, *e.g.*, D-gluconic acid, the stability constants of transition-metal ion complexes generally seem to be greater than those of alkali and alkaline-earth metal ion complexes[13]. The current interest in "metal conjugation"[14–16] and in the role of trace elements in the pathogenesis and treatment of some diseases[17] prompts us to report further work on cationic complexes with metal ions.

In aqueous solutions, the metal ions form[18a] the octahedral aqua ion, or aqua

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complex, *e.g.*, $[Cu(H_2O)_6]^{2+}$. The process of complex formation in aqueous solution is essentially a displacement of one set of ligands, *i.e.*, water molecules of the aqua complex, by another set, *e.g.*, a diol (Eq. *1*).

$$[Cu(H_2O)_6]^{2+} + R(OH)_2 \rightleftharpoons [Cu(H_2O)_4R(OH)_2]^{2+} + 2H_2O \qquad (1)$$

Ligand-exchange chromatography[19,20] (l.e.c.) involves this type of ligand-exchange reaction when such complex-forming ions as $[Cu(H_2O)_6]^{2+}$ are immobilised, *e.g.*, on ion-exchange resins or silica gel. Potential ligands, $R^1(OH)_2$ and $R^2(OH)_2$, are then distributed between this stationary phase (*s*) and water, the mobile phase (*m*), in a ratio which is related[21] to their stoichiometric stability constants (*K*). Potential ligands will migrate as sharply defined zones if the equilibrium of Eq. *1* is established rapidly when compared with the chromatography time-scale. Applications of l.e.c. in the carbohydrate field, although not always recognised as such, using either h.p.l.c. apparatus[10] or the more time-consuming, open-column method[6,11,22] are already recorded. We have used the latter method[6] for the separation of selected pairs of polyhydroxy compounds on Amberlite IR-120(Cu^{2+}) resin. In our continuous effort[23] to extend the range of simple, inexpensive, and speedy methods for the resolution of mixtures of polyhydroxy compounds, our present method of choice was thin-layer ligand-exchange chromatography employing copper as the central atom. For comparison, the complexes with sodium, magnesium, aluminium, calcium, chromium(III), iron(III), nickel, zinc, strontium, cadmium, and barium ions were also investigated.

TABLE I

R_F VALUES OF D-GLUCOSE, D-GLUCITOL, AND D-MANNITOL ON THIN LAYERS IN VARIOUS METAL-ION FORMS, COMPLEXATION COEFFICIENTS, AND PERCENTAGES OF COMPLEXED POLYHYDROXY COMPOUNDS

Metal ion	D-*Glucose*			D-*Glucitol*			D-*Mannitol*		
	R_F	k	*Percentage complexed*	R_F	k	*Percentage complexed*	R_F	k	*Percentage complexed*
Na^{+a}	0.88	0.05	4.8	0.83	0.12	10.7	0.82	0.13	11.5
Mg^{2+}	0.93	0	0	0.91	0.02	2.0	0.91	0.02	2.0
Al^{3+}	0.84	0.10	9.1	0.79	0.17	14.5	0.81	0.14	12.3
Ca^{2+}	0.92	0.01	1.0	0.66	0.40	28.6	0.77	0.20	16.7
Cr^{3+}	0.82	0.13	11.5	0.81	0.14	12.3	0.71	0.30	23.1
Fe^{3+}	0.85	0.09	8.3	0.71	0.30	23.1	0.81	0.14	12.3
Ni^{2+}	0.88	0.05	4.8	0.81	0.14	12.3	0.82	0.13	11.5
Cu^{2+}	0.80	0.16	13.8	0.02	45.30	97.8	0.18	4.14	80.5
Zn^{2+}	0.92	0.01	1.0	0.88	0.05	4.8	0.89	0.04	3.8
Sr^{2+}	0.93	0	0	0.69	0.34	25.4	0.80	0.16	13.8
Cd^{2+}	0.92	0.01	1.0	0.88	0.05	4.8	0.90	0.03	2.9
Ba^{2+}	0.87	0.06	5.7	0.72	0.29	22.5	0.80	0.16	13.8

[a]The R_F values on thin layers in the Na^+-form of all compounds listed in Table II are >0.75 (D-talose).

TABLE II

R_F VALUES OF POLYHYDROXY COMPOUNDS ON Ca^{2+}- AND Cu^{2+}-FORMS OF THIN LAYERS, COMPLEXATION COEFFICIENTS, AND PERCENTAGES OF COMPLEXED POLYHYDROXY COMPOUNDS

Compound	*Ca^{2+}-form*			*Cu^{2+}-form*		
	R_F	k	*Percentage complexed*	R_F	k	*Percentage complexed*
D-Arabinose	0.86	0.08	7.4	0.76	0.22	18.0
D-Lyxose	0.86	0.08	7.4	0.67	0.38	27.5
D-Ribose	0.60	0.54	35.1	0.30	2.09	67.6
D-Xylose	0.92	0.01	1.0	0.76	0.22	18.0
D-Allose	0.64–0.84	<0.45	<31.0	0.39	1.37	57.8
D-Galactose	0.90	0.03	2.9	0.79	0.17	14.5
D-Glucose	0.92	0.01	1.0	0.80	0.16	13.8
D-Gulose	0.49–0.92	<0.89	<47.1	0.40–0.59	<1.31	<56.7
D-Mannose	0.91	0.02	2.0	0.63	0.47	32.0
D-Talose	0.62	0.49	32.9	0.14	5.61	84.9
L-Galactose, 6-deoxy-	0.85	0.09	8.3	0.78	0.19	16.0
L-Mannose, 6-deoxy-	0.89	0.04	3.8	0.69	0.34	25.4
D-Fructose	0.84	0.10	9.1	0.65	0.42	29.6
Kojibiose	0.79–0.94	<0.17	<14.5	0.87	0.06	5.7
Laminaribiose	0.93	0	0	0.87	0.06	5.7
Maltose	0.94	0	0	0.87	0.06	5.7
Cellobiose	0.94	0	0	0.88	0.05	4.8
Isomaltose	0.94	0	0	0.92	0.01	1.0
Gentiobiose	0.94	0	0	0.88	0.05	4.8
Lactose	0.95	0	0	0.87	0.06	5.7
Lactulose	0.78–0.93	<0.19	<16.0	0.77	0.20	16.7
β-D-Galactopyranoside, methyl	0.90	0.03	2.9	0.78	0.19	16.0
α-D-Glucopyranoside, methyl	0.91	0.02	2.0	0.81	0.14	12.3
methyl 2-amino-4,6-*O*-benzylidene-2-deoxy-				0	>90	>99
methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-				0	>90	>99
α-D-Mannopyranoside, methyl	0.83	0.12	10.7	0.75	0.23	18.7
methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-				0	>90	>99
D-Galacturonic acid	0.82	0.13	11.5	0–0.15	>90	
D-Glucuronic acid	0.86	0.08	7.4	0.02	45.30	97.8
D-Mannuronic acid	0.74	0.25	20.0	0	>90	>99
D-Gluconate, sodium				0	>90	>99
L-Ascorbic acid				0.01	91.59	98.9
Glycerol	0.77	0.20	16.7	0.70	0.32	24.2
Erythritol	0.78	0.19	16.0	0.56	0.65	39.4
L-Threitol	0.73	0.27	21.3	0.37	1.50	60.0
D-Arabinitol	0.80	0.16	13.8	0.28	2.31	69.8
Ribitol	0.86	0.08	7.4	0.56	0.65	39.4
Xylitol	0.71	0.30	23.1	0.05	17.52	94.6
Allitol	0.87	0.06	5.7	0.42	1.20	54.5
D-Altritol	0.78	0.19	16.0	0.23	3.03	75.2
1-deoxy-	0.79	0.17	14.5	0.30	2.09	67.6

TABLE II *(continued)*

Compound	*Ca^{2+}-form*			*Cu^{2+}-form*		
	R_F	k	*Percentage complexed*	R_F	k	*Percentage complexed*
Galactitol	0.67	0.38	27.5	0.13	6.12	86.0
1-deoxy-L-	0.70	0.32	24.2	0.28	2.31	69.8
D-Glucitol	0.66	0.40	28.6	0.02	45.30	97.8
4-*O*-α-D-glucopyranosyl-	0.86	0.08	7.4	0.30	2.09	67.6
4-*O*-β-D-glucopyranosyl-	0.67	0.38	27.5	0.46	1.01	50.2
D-Mannitol	0.77	0.20	16.7	0.18	4.14	80.5
L-Mannitol, 1-deoxy-	0.77	0.20	16.7	0.31	1.99	66.6
D-Talitol, 1-deoxy-	0.84	0.10	9.1	0.49	0.89	47.1

RESULTS AND DISCUSSION

Separation of compounds

Tables I and II show the observed R_F values (see below for real migration-rates, R_F') of compounds on a mixture of silica gel and IONEX-25 SA in the various metal-ion forms, layered onto POLYGRAM sheets. The H^+-form of the thin layers furnished R_F values in the absence of any metal ion. As R_F <0.93 would indicate complex formation, the results (Tables I and II) show that complex formation can be readily detected by thin-layer l.e.c. The method is therefore complementary to electrophoresis in the appropriate electrolyte. Of the metal ions listed in Table I, the copper(II) ion complexes with D-glucose, D-glucitol, and D-mannitol to the greatest extent, and these complexes have been compared with those of calcium.

Although complex formation with calcium ions in these circumstances is not very selective, mixtures of selected compounds, *e.g.*, talose and other, ribose and other, and some aldoses and their reduction products, can be satisfactorily resolved by this method. The results obtained also serve as more realistic indicators as to the separations that may be feasible on larger scale[11] l.e.c. employing calcium as central atom than the suggested[11,24,25] electrophoretic mobilities.

The R_F values on the Cu^{2+}-form of the thin layer, in contrast to those on the Ca^{2+}-form, have a much wider range. Therefore, complex formation with copper(II) ions seems to be more selective than with calcium ions. The simplicity of thin-layer l.e.c. with copper as the central atom, which has the added advantage that water is the only solvent used, renders it a speedy and inexpensive means of resolving mixtures of polyhydroxy compounds not easily achieved by other methods. For example, the members within the groups of tetritols, pentitols, and hexitols can be distinguished. Monosaccharides, and in at least two cases also disaccharides, can be separated from their reduction products. Where the identity of a monosaccharide might be in doubt, *e.g.*, arabinose (R_F 0.76) or xylose (R_F 0.76), it can be unambiguously identified after reduction, *e.g.*, to arabinitol (R_F 0.28) or xylitol (R_F 0.05). Similarly, whereas

maltose and cellobiose cannot be distinguished by their R_F values, their anomeric reduction products, 4-*O*-α- (maltitol) and 4-*O*-β-D-glucopyranosyl-D-glucitol (cellobiitol), respectively, can be resolved. It is anticipated that the range of possible resolutions is far greater than is reported here. Further, and as indicated for l.e.c. using calcium ions, the R_F values of compounds in thin-layer l.e.c. with copper(II) ions serve as indicators for the separations that may be achieved in larger scale l.e.c.

Stabilities of complexes

The chromatographic distribution* coefficient, K', of a polyol, P, can be defined[26] by Eq. *2*.

$$K' = x_{P_m}/x_{P_s}\,\zeta, \tag{2}$$

where x_{P_m} and x_{P_s} are the mole fractions of the polyol in the mobile and stationary phase, respectively, *i.e.*, $x_{P_m} + x_{P_s} = 1$, and where the phase ratio, ζ, accounts for the amount of the mobile phase (V_m) and of the stationary phase (V_s), *i.e.*, $\zeta = V_m/V_s$. In general[26],

$$1/K' = \zeta(1/R_F' - 1), \tag{3}$$

and, if ζ and R_F' are known, K' may be calculated. That this approach is justifiable here follows from a consideration[27] of the R_F values of a series of substituted styrenes (on thin layers of silica gel impregnated with silver nitrate) in terms of $\Delta G = -RT\ln K'$ and the Hammett relationship $\log K' - \log K_0' = \sigma\rho$. The value for the reaction constant, ρ, thus obtained was in close agreement with that obtained from distribution measurements (*i.e.*, complexed and uncomplexed styrenes) in free solution. Unfortunately, it is difficult to determine reliably the phase ratio, ζ, in thin layers. However, combining Eq. *2* (after rearranging) with Eq. *3* gives the ratio in Eq. *4*, namely, a complexation coefficient, *i.e.*, the molar ratio of the complexed to the uncomplexed polyol. The percentage of complexed polyol, $100k/(k + 1)$, can therefore be calculated without knowledge of the stoichiometry of the complexation reaction.

$$k = x_{P_s}/x_{P_m} = 1/R_F' - 1 \tag{4}$$

In Eqs. *3* and *4*, R_F' represents the real migration rate relative to the solvent front, which is related[26] to the observed migration rate, R_F, by Eq. *5*.

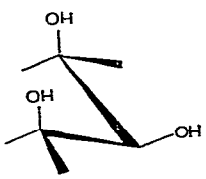
$$R_F' = \xi R_F \tag{5}$$

Usually[26], ξ is ~1.1–1.2. From the observed R_F values on the thin layers in the H^+-form, ξ was determined as 1.08. Tables I and II give the values of k calculated from Eq. *4*, as well as the percentages of the complexed polyols. It is appreciated that the values of k would have a more significant meaning if the stoichiometry of the complexation reactions under the conditions of thin-layer l.e.c. were known.

*In ref. 26, the relationship is developed for the process of adsorption. The process of ligand exchange on an immobilised metal ion can be considered analogously.

However, the complexation coefficients, k, serve as reasonable measures of the relative stabilities of complexes.

The following conclusions can be drawn. All of the compounds examined formed complexes to some extent with copper(II) ions. For a particular compound, the extent of complex formation is greatest with copper(II) ions. In general, alditols form stronger complexes than do monosaccharides. Amino sugars form very strong complexes with copper(II) ions, offering a means of separating them from other sugars. The results with D-ribose, D-gulose, and D-talose indicate that strong copper(II)-ion complexes are formed when the relative disposition of three hydroxyl groups can approximate structure **1**, a tridentate arrangement which has already been noted to react with periodate[28], molybdate[29], tungstate[30], and benzeneboronic acid[31,32], as well as calcium[8] and europium[24] ions. In the series of alditols, there are similar trends in the sets of calcium and copper complexes, *e.g.*, the complexation coefficients seem to be dependent on the number of *threo*-1,2-diol groups (or the presence of a *threo-threo*-1,2,3-triol group). It is, however, unlikely that the polyol ligands in the calcium and copper complexes have identical conformations, as the d^9 configuration makes Cu(II) subject to the Jahn–Teller distortion[18b]. Also, the stoichiometry of the complexation reaction with copper(II) ions* may be different from that with calcium ions, for which a 1 : 1 complex has been proposed[25]. Further work on the complexation reactions with copper(II) ions and the structures of the copper complexes will be reported elsewhere.



1

EXPERIMENTAL

POLYGRAM IONEX-25 SA-Na sheets (Macherey-Nagel GmbH, Düren) were immersed in deionised water until the thin layers were completely wet. Separate sheets were then immersed for 1 h in the following aqueous solutions: 0.1M HCl, 0.05M $Mg(AcO)_2$, 0.1M $AlCl_3 \cdot 6\ H_2O$, 5% $Ca(AcO)_2$, 0.1M $Cr(AcO)_3$, 0.1M $FeCl_3$, 0.1M $Ni(AcO)_2 \cdot 4\ H_2O$, 5% $Cu(AcO)_2 \cdot H_2O$**, 0.05M $ZnCl_2$, 0.1M $SrCl_2$, 0.1M

*[With Harjagbir Gill and Shirley Sylvester]. The Job plots[33,34] of changes of optical rotation and absorbance (at 765 nm) of solutions (pH 5.5) containing D-glucitol and copper(II) acetate (combined, constant concentration 0.25M) showed maxima corresponding to a complex containing copper and D-glucitol in the ratio 3:1. A similar ratio was found for the copper complex[3] of D-glucitol formed at pH 12.

**Ready-for-use Carbohydrate TLC plates SIL/IONEX-25 Cu are available from Machery-Nagel, P.O. Box 307, D-5160 Düren, Germany, or its agents.

$CdCl_2 \cdot 2.5\ H_2O$, and 0.05M $BaCl_2$. The sheets were washed several times with deionised water and then dried in air at room temperature for 24 h. Compounds were spotted and the chromatograms developed (~1–1.5 h). Except for the acids, compounds were located by treating the dried sheets with a saturated solution of potassium permanganate in acetone, when, within ~2 min, bright-yellow spots appeared on a purple background. As both colours fade after ~10 h, the position of the compounds was marked at once. Acids were detected with ethanolic 5% H_2SO_4 at ~100°.

On the layers in the H^+-form, the following compounds had the R_F values indicated: D-arabinose, 0.92; D-ribose, 0.92; D-xylose, 0.94; D-galactose, 0.92; D-glucose, 0.92; D-mannose, 0.93; D-fructose, 0.93; galactitol, 0.94; D-glucitol, 0.90; and D-mannitol, 0.93. Average $R_F = 0.93$; *i.e.*, $\xi = 1/0.93 = 1.08$.

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