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Selective complex formation of saccharides with europium(III) and iron(III) ions at alkaline pH studied by ligand-exchange chromatography

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ABSTRACT

At high pH, saccharides become negatively charged by deprotonation of one or several hydroxylic groups and they are highly and selectively retained by ligand-exchange chromatography. The systems consist of a sulphonated polystyrene strong cation exchanger in europium(III) or iron(III) form and sodium hydroxide as mobile phase. The degree of complex formation is dependent on solute character and concentration, metal ion and pH, the reaction being of second order as confirmed by breakthrough studies. Rapid desorption of the solutes is performed by the introduction of an acidic mobile phase. Monosaccharides, and especially sugar alcohols, are selectively retained by a column in Fe(III) form whereas all saccharides are strongly retained as Eu(III) complexes, e.g., the capacity factor for the breakthrough of 10 μM glucose, in 0.1 A4 NaOH as mobile phase, was ***ca.*** 3500 The systems are proposed to be highly selective for the analysis of sugars.

INTRODUCTION

Carbohydrates are polar compounds and difficult to retain in reversed-phase LC systems (for a review, see ref. 1) and the low capacity factors limit the number of compounds to be separated. The development of gas-liquid chromatographic (GLC) methods included the conversion of the sugars into volatile derivatives such as acetates [2] or trimethylsilyl ethers [3] and borohydride reduction [4]. The introduction of capillary columns with highly polar solid phases coupled with mass spectrometry [5] considerably improved the resolution and the confirmation of identification. Normal-phase partition chromatography has been used with polar solid phases, e.g., silica or diol, amine, amide or cyano-functionalized silica, and a high content of acetonitrile in the mobile phase [6]. Refractive index or UV absorption around 200 nm is usually employed, resulting in low sensitivity and limitations for mobile phase additives.

Most saccharides have pK_a values in the range 12-14 [7] and they can be separated by ion chromatography using strongly alkaline solutions. This separation mode, in combination with pulsed electrochemical detection, has been shown to offer high selectivity and sensitivity in the analysis of sugars, polyols and related compounds [8]. Sugars have also been separated by ligand-exchange chromatography using ion exchangers in different metal forms, calcium being the most commonly used [9]. Recently, carbohydrates [10] have been found to form strong complexes at alkaline pH with rare earths, yttrium and uranyl metal ions loaded on to a sulphonated polystyrene cation exchanger. The effects of mobile phase additives and temperature were studied [11] and the methodology was utilized for the bioanalysis of the diastereomeric glucuronides of almokalant from human urine [12].

In this study, saccharides were used as solutes in

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order to investigate the influence of the anomeric hydroxylic group on complex formation compared with glycosides [10]. The retention mechanism is described as a ligand-exchange reaction and retention and isotherm studies were performed by frontal analysis using pulsed electrochemical detection.

EXPERIMENTAL

Instrumental

The LC instrumentation consisted of an LKB (Bromma, Sweden) Model 2150 HPLC pump, a Rheodyne Model 7125 injector with a 20-μl loop, a Rheodyne Model 7000 switching valve (the valves were equipped with Tefzel alkali-resistant rotor seals), a pulsed electrochemical detection (PED) system with a gold working electrode and pH reference electrode (Dionex, Sunnyvale, CA. USA), a Kipp & Zonen BD 40 recorder and a Haake (Berlin-Steglitz, Germany) Fe water-bath for thermostating the chromatographic systems. The program for PED detection was E_1 = 0.40 V, t_1 = 0–500 ms and integration for 300-500 ms, E_2 = 0.90 V, t_2 = 510–590 ms and E_3 = -0.30 V, t_3 = 600-650 ms. E_2 and E_3 are used to remove adsorbed compounds oxidatively and to reduce the gold oxide formed, respectively, in order to minimize fouling of the working electrode.

Chemicals and column packing

All test solutes, except for the metal chloride salts (Janssen Chimica, Beerse, Belgium) were obtained from Sigma (St. Louis, MO, USA) and used as received. Sodium hydroxide solutions were prepared from 1 *M* Titrisol solution (Merck, Darmstadt, Germany) and kept in a plastic bottle. The column packing was Hitachi-Gel 3011-S 10–12 μm, a spherical and macroporous sulphonated polystyrene resin in H^+ form, kindly provided by Hitachi (Tokyo, Japan).

Column preparation

The ion exchanger was packed with water as slurry medium in a polished stainless-steel column (21 × 4.6 mm I.D.). It was converted into the metal form by a 500-μl injection of the metal salt (2 *M*). The excess of metal ions was rinsed off the column by washing with 0.3 A4 morpholinoethansulphonic acid (MES) buffer (pH 5.0) for 20 min. The metal ions were held firmly by the resin and not eluted during the separation process. MES does not form complexes with the metal ions [10] and was used to elute the solutes rapidly from the resin after the breakthrough. The column was equilibrated with 10 ml of 0.1 A4 NaOH solution prior to each run.

RESULTS AND DISCUSSION

Principle and retention model

Saccharides are weak acids with pK_a values between 12 and 14 [7] and the negatively charged saccharides, S^-, form complexes with the electrostatically immobilized metal ions, R $M(OH)_2$, at pH > 11 [10]. Hydroxide ions compete for complex formation with the metal ions according to a ligand-exchange reaction and assuming one solute molecule and one metal ion in the complex [10]:

$$R \cdot M(OH)_2 + S^{x-} \xrightarrow{K_S} R\ M(OH)_{2-x} \cdot S + xOH^- \quad (1)$$

The capacity factor is

$$k' = q K_S^* [R\ M(OH)_2]/[OH^-]^x \quad (2)$$

where q is the phase ratio in the column and K_S^* is the apparent thermodynamic exchange constant; $K_S^* = K_S[1/(1 + 10^{pK_a - pH})]$. C_s, the concentration of solute on the solid phase, is calculated accordingly and a linear relationship *versus* hydroxide concentration is obtained:

$$\ln C_s = \ln(K_S^*[R \cdot M(OH)_2][S^{x-}]) - x\ln[OH^-] \quad (3)$$

The concentration of the metal ions on the cation exchanger was about 200 μmol per column and the mass of the dry packing material in the H^+ form, m, was 0.16 g, *i.e.,* 1.25 mmol of metal ions bound per gram of exchanger.

Breakthrough curves

Retention and isotherm studies were performed by the breakthrough (BT) technique. The BT curve (Fig. 1) is characterized by pronounced tailing, which was asumed to be caused by slow distribution kinetics. The complexation on the solid phase for chelating resins is often a slow process and, occasionally, such reactions have been shown [13] to be of approximately second order. The BT curve was normalized. by setting $BT_{1/2}$ = 0.5, with respect to

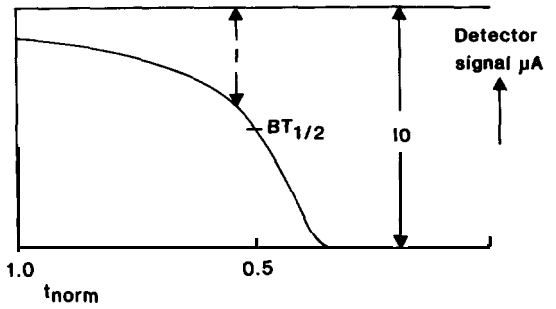

Fig. 1. Typical breakthrough curve. Mobile phase: 0.1 m*M* glucose in 0.1 M NaOH.

the time axis. $BT_{1/2}$ is defined as the time necessary for the detector signal to reach half of its maximum value. The effluent was monitored by PED. The equation for a simple second-order kinetic reaction:

$$-dI/I^2 = kdt \quad (4)$$

yields after integration and rearrangement

$$I_0/I = I_0kt + \text{intercept} \quad (5)$$

where I_0 is the concentration at $t = 0$ and I that at time t, k is the rate constant and the intercept is obtained when $BT_{1/2}$ is chosen as the starting point for calculation. A plot of I_0/I *versus t (i.e.,* the value for normalization) was linear (Fig. 2). As a consequence, the distribution to the solid phase was concentration and time dependent. C_s was calculated from the BT curve according to

$$C_s = V_{BT}\, C_m/m \quad (6)$$

where V_{BT} is the BT volume minus the void volume, C_m the mobile phase concentration and m the amount of dry resin in H^+ form in the column (g/l). After BT, the solutes were desorbed by introduction of 0.3 *M* MES buffer (pH 5.0) for 10 min, followed by sodium hydroxide solution as the mobile phase until the eluent from the column was alkaline, i.e., the metal ions were converted into the corresponding hydroxides.

Carbon dioxide is taken up by the alkaline mobile phase, forming carbonate, which complexes with the metal ions and, hence, the mobile phase was prepared on a daily basis and protected by soda-lime tubes.

Influence of hydroxide concentration

The effect on solute distribution to the solid phase, C_s in µmol/g, versus the hydroxide concentration was investigated using glucose and sorbitol as solutes for the Eu(III) and the Fe(III) systems, respectively (Fig. 3). The initial increase in C_s is caused by the increasing degree of ionization of the sugars and subsequent complex formation with the metal ions. The decrease in C_s at high hydroxide concentrations is caused by the competition from hydroxide for complex formation and the maxima in C_s will be dependent on the pK_a values (glucose = 12.35 and sorbitol = 13.5 [7]). According to eqn. 3, ln C_s was plotted *versus* ln [OH-] for sorbitol, glucuronic acid and glucose using the column in the Eu(III) form (Fig. 4). The slopes were -0.42, − 0.42 and − 1.13, respectively.

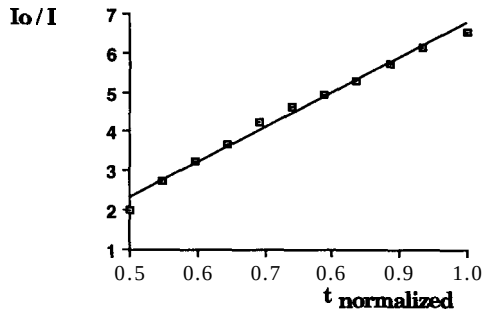

Fig. 2. Plot of the breakthrough curve kinetics according to a second-order reaction (eqn. 5).

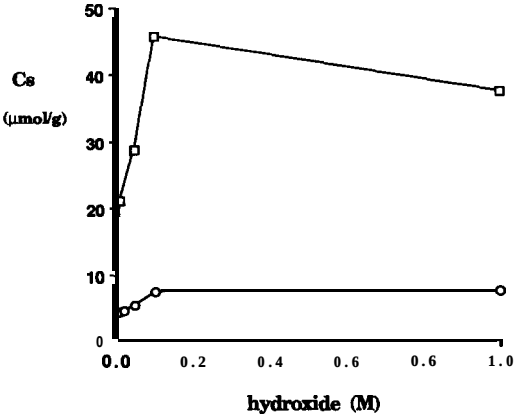

Fig. 3. pH dependence of C_s for (0) 1 .O m*M* glucose and (0) 1 .O m*M* sorbitol using the cation exchanger in europium(III) and iron(III) form, respectively, and sodium hydroxide as mobile phase.

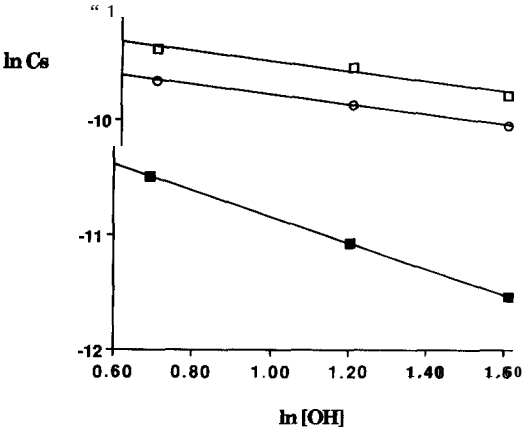

Fig. 4. Hydroxide competition on complex formation for (□) sorbitol, (0) glucuronic acid and (■) glucose on the europium (III)-loaded column plotted according to the retention model (eqn. 3).

Solute structure

The influence of solute structure on complex stability was studied by the BT technique and the results are given in Table I. All solutes were highly retained on the Eu(III) column. Di-, tri- and tetrasaccharides were retained to about the same extent (but less than the monosaccharides), possibly indicating the importance of steric effects on complex formation.

Substitution for a carboxylic acid at C-6 (sugar acids) increased the complex stability whereas an amino group (amino sugars) at C-2 gave an 80% reduction, probably owing to a decrease in the acid dissociation constants.

The sugar alcohols showed a different order in complex stability compared with their related monosaccharides (e.g., xylitol-xylose, sorbitol–glucose). There was no relationship between the number of hydroxylic groups and the complex stability. As shown previously, the pK_a values is of fundamental importance (the sugar alcohols are approximately 1–10% as acidic as the corresponding monosaccharides [7]) and, most likely, the configuration of the solutes and the ability to orient the hydroxyl groups toward the metal ion are of major significance. In spite of the pK_a differences, they were retained to a similar extent as the monosaccharides, which might to be due to the more flexible and open-chain structure of the sugar alcohols, thus facilitating the complexation.

TABLE I

INFLUENCE OF STRUCTURE ON RETENTION IN THE Eu(III) SYSTEM

Column: Hitachi 301 I-S, 21 × 4.6 mm I.D. in Eu(III)-form. Mobile phase: 0.1 A4 NaOH and 1.0 m*M* saccharide. C_s was measured at the inflection point with the baseline using frontal analysis.

No.	Solute	C_s (μmol/g)
Monosaccharides		
1	Ribose	158
2	Tagatose	119
3	Fructose	99.8
4	Sorbose	79.4
5	Lyxose	78.9
6	Galactose	74.2
7	Mannose	66.4
8	Xylose	55.2
9	Arabinose	46.9
10	Glucose	45.8
11	Fucose	40.8
Disaccharides		
12	Sucrose	38.4
13	Gentiobiose	37.4
14	Maltose	30.4
15	Melibiose	30.4
16	Lactose	28.9
17	Cellobiose	23.8
Trisuccharide		
18	Raffinose	30.2
Tetrasaccharide		
19	Stachyose	30.2
***Sugar** acids*		
20	Galacturonic acid	77.7
21	Glucuronic acid	70.4
***Amino** sugars*		
22	Galactosamine	18.9
23	Glucosamine	7.9
Sugar alcohols		
24	Xylitol	110
25	Sorbitol (glucitol)	104
26	Galactitol	94.8
27	Arabitol	80.2
28	Mannitol	74.8
29	Ribitol (adonitol)	41.1
Micellaneous		
30	myo-Inositol	43.9
31	β-Cyclodextrin	15.6

The results on the Fe(III)-loaded column are displayed in Table II. The complex stabilities were one to two orders of magnitude lower compared with

TABLE II

INFLUENCE OF STRUCTURE ON RETENTION IN THE Fe(III) SYSTEM

Column: Hitachi 3011-S, 21 × 4.6 mm I.D. in Fe(III)-form. Mobile phase: 0.1 *M* NaOH and 1.0 m*M* saccharide. C_s was measured at the inflection point with the baseline using frontal analysis.

No.	Solute	C_s (μmol/g)
Monosaccharides		
1	Tagatose	5.41
2	Ribose	5.20
3	Sorbose	1.98
4	Glucose	0.31
Disaccharide		
5	Sucrose	0.10
Sugar acid		
6	Glucuronic acid	0.00
Sugar alcohols		
7	Sorbitol (glucitol)	7.28
8	Xylitol	4.06
9	Galactitol	3.23
10	Mannitol	1.98
11	Arabitol	1.88
12	Ribitol (adonitol)	1.09

the Eu(III) column. This is certainly caused by the strong complex formation of hydroxide with the Fe (III) ion, i.e., competition. Changes in selectivity

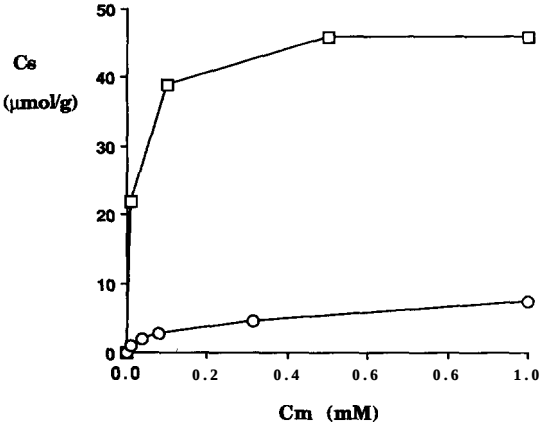

Fig. 5. Distribution isotherms for (□) glucose and (0) sorbitol on the Eu(III) and Fe(III) columns, respectively. Solute in 0.1 *M* NaOH as mobile phase.

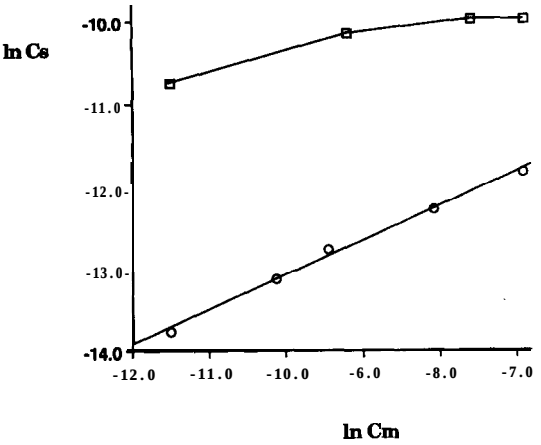

Fig. 6. Isotherms from Fig. 5 according to the retention model (eqn. 3). Symbols as in Fig. 5.

[*cf.*, the Eu(III) system] were observed for the tagatose-ribose, sorbitol-xylitol and arabitol-mannitol pairs and they were probably due to differences in ionic radius and electronic properties of the metal ions. Unexpectedly, glucuronic acid eluted with the front. As shown previously [10], few compounds were retained on the Fe(III) column and, tentatively, such a system may provide selective clean-up possibilities and subsequent separation of sugar alcohols and monosaccharides from complex matrices, e.g., biological fluids.

Distribution isotherms

The isotherms for glucose and sorbitol using the Eu(III) and Fe(III) systems, respectivley, were obtained from BT studies using 0.1 *M* NaOH as mobile phase and are shown in Fig. 5. C_s was calculated from eqn. 6. Ln C_s *versus* ln C_m was plotted according to the retention model (eqn. 3) and the results are shown in Fig. 6. The non-linearity observed for the glucose-Eu(III) system was due to the very strong complex formation and, hence, solid-phase saturation, i.e., the distribution became independent of the mobile phase concentration. Saturation effects have been previously obtained at ln $C_s > -10.5$ [10]. The linear concentration range was probably at the low-to sub-micromolar level and because of the high capacity factors obtained (k'_{BT} for 10 μM glucose was cu. 3500), this was not studied further. Most of the other saccharides studied were retained to an even greater extent. The

magnitude of the complex formation constant for the sorbitol–Fe(III) system was, however, suitable and a linear relationship was obtained.

CONCLUSIONS

Saccharides were shown to form more stable complexes than the corresponding glycosides at alkaline pH with Eu(III) and Fe(III) metal ions electrostatically immobilized on a strong cation exchanger. All sugars were highly retained with the Eu(III) system whereas the Fe(III) system was selective for monosaccharides and sugar alcohols. The ligand-exchange reaction, studied by frontal analysis and being of second order, was metal ion, pH and concentration dependent. Rapid elution of the solutes was performed by introduction of an acidic mobile phase. Using this technique off- or on-line in a coupled column separation system, selective separation and isolation of saccharides from complex matrices is proposed.

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