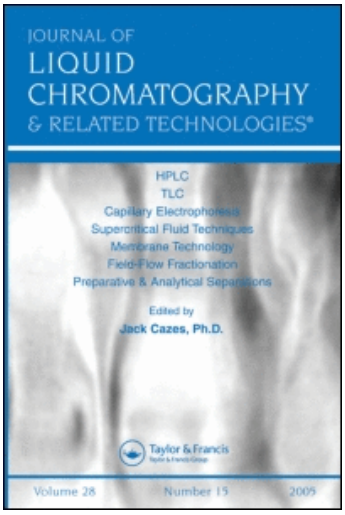

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COMPUTATIONAL CHEMICAL ANALYSIS OF NEWLY DEVELOPED GUANIDINO-PHASE FOR QUANTITATIVE ANALYSIS OF SACCHARIDES IN LIQUID CHROMATOGRAPHY

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ABSTRACT

A guanidino-phase was synthesized from a propylamine bonded phase. This new bonded phase had higher retention capacity for saccharides than the original amino phase, and saccharides were quantitatively recovered. The computational chemical analysis of the retention mechanism indicated that electrostatic forces may contribute to the retention on the guanidino phase, and that a combination of electrostatic force and hydrogen-bonding may contribute to this on amino phase. The poor recovery from amino phase may be due to the formation of glycosides.

INTRODUCTION

Frequently used packing materials for separation of saccharides are ion-exchangers in borate buffer,[1] aqueous sodium hydroxide,[2] or aqueous acetonitrile,[3] propylamine-bonded packing materials in aqueous acetonitrile,[4] and amide-bonded packing materials in aqueous acetonitrile.[4] Among them, propylamine-bonded packing materials have been the most commonly used because they are easily handled, but the recovery of saccharides from the packing material is very poor, probably due to formation of N-glycosides or their Amadori rearrangement products. This can be explained by the chromatographic behavior and reactivity of 2-deoxysaccharides easily converted to N-glycosides in vitro.[5]

Protamine-bonded packing material has been synthesized from propylamine-bonded packing materials and protamine, which is a basic protein containing about 70% arginine.[6] The recovery of saccharides is excellent from the column, but the retention capacity was not satisfactory. Therefore, we directly modified the amino-phase to synthesize the guanidino-bonded phase. In biochemical research, amino-groups of proteins have been converted to guanidino groups at moderate conditions.[7] This reaction method was applied for synthesis of the guanidino phase; the guanidino bonded phase was studied for liquid chromatographic behavior towards saccharides. The retention of saccharides on the guanidino phase was further analyzed by computational chemistry.

EXPERIMENTAL

Reagents and Materials

An NH2P-50 column (150 x 4.6 mm I.D., 5μm) packed with a pentaethylenehexamine-bonded vinyl alcohol copolymer gel[8] was purchased from Showa Denko (Tokyo, Japan). S-Methylisothiourea sulfate was obtained from Fluka Chemie AG. Monosaccharides were obtained from Wako (Osaka, Japan). HPLC-grade acetonitrile was obtained from Kanto-Kagaku (Tokyo, Japan). Water was Milli-Q grade water.

Preparation of the Guanidino-Bonded Column

A guanidino-bonded column was prepared by delivery of a 0.5M S-methylisothiourea sulfate solution. The flow rate was 0.5 mL/min and the

reaction temperature was 80°C. The reaction process was monitored from the retention time of glucose in aqueous acetonitrile. The retention time of glucose reached a constant after two days of reaction. Therefore, 1400 mL S-methylisothiourea solution was necessary.

Chromatography of Saccharides

The liquid chromatograph consisted of a DGU3A degassing unit, an LC-9A pump, a LC-10A RID, a CR6A integrator (Shimadzu, Kyoto, Japan) and a Model 7125 injector (Rheodyne, CA, USA). The water bath, Model Minder H, was from Taiyo Kagaku, Tokyo, Japan.

Computational Chemical Calculation

The computer used for the calculations was a Macintosh 8100/100, and the software was CAChe™ from Sony-Tektronix (Tokyo, Japan). The chemical calculation was performed without modification of the programs. The geometry of a molecule, created using CAChe™ molecular editor, was first optimized using molecular mechanics calculations.

The properties used for the calculation were bond stretch, bond angle, dihedral angle, improper torsion, van der Waals, electrostatic and hydrogen bond. The cut-off distance for van der Waals interaction was 9Å.[9] Cricket-Graph™ from Computer Associates was used for data handling.

RESULTS AND DISCUSSION

The retention times and recoveries of saccharides listed in Table 1 were measured in aqueous 80% acetonitrile before and after modification of an NH2P-50 column. The effect of anions was measured, after modification, to phosphate, sulfate and borate forms.

The retention capacity of the hydroxy form of the guanidino column was about three times that of the original amino column, and that of the phosphate and sulfate-forms was about 110%. The capacity of the phosphate form was 120% of that of the amino phase. However, the saccharides were quantitatively recovered from the guanidino column. Such recovery was not achieved from the amino column. An example of chromatography on the phosphate form guanidino column is shown in Figure 1.

Table 1

Retention Factors (k) and Relative Recovery (r, %) On and From Amino Acid and Guanidino Phases

Saccharides	Amino-Phase			Guanidino-Phase									
	k	r Fru*,[1]	r Glc*,[2]	k OH*,[3]	r OH*,[3]	k COOH*,[4]	r COOH*,[4]	k SO_4*,[5]	r SO_4*,[5]	k PO_4*,[6]	r PO_4*,[6]	k B_2O_4*,[7]	r B_2O_4*,[7]
Arabinose	2.07	10.1	12.0	5.91	92.1	2.28	75.2	2.24/2.85	84.9	2.82	82.9	2.32	93.6
Deoxyglucose	8*	8*	8*	2.71	107.2	2.22	57.3	1.79	94.6	2.54	86.3	1.89	102.7
Deoxyribose	8*	8*	8*	1.29	59.1	1.22	13.9	1.00	65.5	1.36	74.3	3.25	9*
Fructose	2.61	100.0	118.4	10.54	58.6	2.94	101.5	3.04	97.1	3.55	102.0	5.28	9*
Galactose	3.79	21.1	25.0	10.30	92.0	3.74	83.0	4.08/5.06	88.6	5.52	81.3	3.70	93.7
Glucose	4.02	84.4	100.0	9.94	100.0	4.30	100.0	4.21/4.84	100.0	5.56	100.0	3.73	100.0
Maltose	9.47	58.0	68.7	---	---	---	---	---	---	---	---	---	---
Mannose	3.35	15.5	18.4	9.57	103.8	3.23	84.4	3.54	89.0	4.27	91.5	3.82	92.4
Ribose	1.78	10.3	12.2	5.18	90.7	1.64	82.0	1.65	85.7	1.82	89.9	2.64	79.0
Sucrose	7.21	106.1	125.7	---	---	---	---	---	---	---	---	---	---
Xylose	2.21	42.1	49.8	6.99	95.5	2.43	87.5	2.18/2.69	87.1	2.79	87.2	2.68	86.8

*All measurements were made on a 15 cm x 4.6 mm ID column in 80% aq. acetonitrile, flow rate 1.0 mL/min using an RI detector; however, eluent for borate-form guanidino-phase[7] was 70% aq. acetonitrile and the flow rate for amino column was 0.6 mL/min. [1] Recovery was calculated as fructose standard. [2] Recovery was calculated as glucose standard. [3] Hydroxy-form guanidino-phase; [4] Carboxy-form guanidino-phase; [5] Sulfate-form guanidino-phase, some saccharides showed two peaks; [6] Phosphate-form guanidino-phase; [7] Borate-form guanidino-phase; [8] Peaks were too small and retention times were close to system peaks; [9] Peaks were too flat.

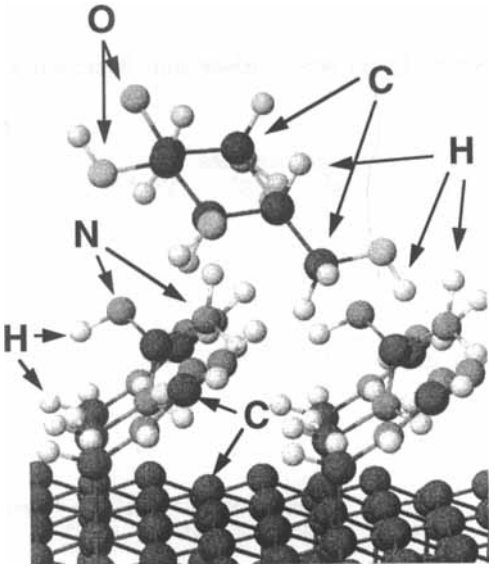

Figure 1. Glucose adsorbed on guanidino phase, optimized by MM2 calculation.

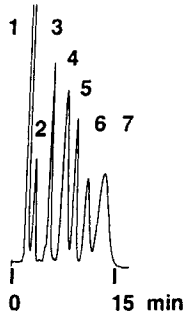

Figure 2. Chromatogram of saccharides in 82% aqueous acetonitrile. 1 & 2: solvent peak, 3: ribose, 4: xylose + arabinose, 5: fructose, 6: mannose, 7: glucose + galactose.

Previously, the elution order of saccharides from an amino column was successfully analyzed by molecular mechanics calculation of computational chemistry. The retention factors were basically related to summation of calculated van der Waals and hydrogen bonding energies after subtraction of

Table 2

Energy Values Optimized by Molecular Mechanics Calculation

Compounds	Monosaccharides FIN[1]	HB[2]	ES[3]	VW[4]	Complex With Amino-Phase ES[6]	HB+VW[6]
Arabinose	16.02	-2.81	6.05	1.87	-0.37	-16.54
Deoxyribose	8.48	-1.69	-1.03	1.29	---	---
Fructose	20.64	-2.28	12.08	1.31	-0.58	-17.59
Galactose	17.26	-2.39	10.22	3.64	1.28	-25.92
Glucose	16.09	-2.62	9.28	3.60	2.39	-28.12
Mannose	16.15	-3.01	9.21	4.00	-0.18	-21.97
Ribose	17.03	-1.96	7.41	1.38	-0.74	-22.83
Xylose	16.67	-1.93	6.41	1.44	0.757	-16.56

	Complex With Guanidino-Phase FIN[1]	HB[2]	ES[3]	VW[4]	ES[5]	HB+VW[5]
Arabinose	1590.3	-12.92	-272.65	253.32	-0.18	-10.62
Deoxyribose	1582.56	-10.56	-280.34	251.74	-0.78	-10.37
Fructose	1593.63	-12.94	-267.57	252.99	-0.12	-10.94
Galactose	1588.33	-13.15	-271.41	255.53	-3.11	-10.83
Glucose	1587.03	-12.47	-270.94	252.77	-1.69	-12.64
Mannose	1587.18	-13.92	-270.73	253.61	-1.42	-13.26
Ribose	1589.26	-11.80	-273.42	254.36	-2.30	-8.81
Xylose	1589.69	-11.81	-273.93	252.82	-1.82	-10.47
Guanidino-Phase	1584.93	-5.92	-278.52	257.87	---	---

[1] Final energy, Kcal/mol; [2] Hydrogen bond energy, Kcal/mol; [3] Electrostatic energy, Kcal/mol; [4] van der Waals energy, Kcal/mol; [5] Complex form electrostatic and hydrogen plus van der Waals energy, Kcal/mol; [6] Complex form electrostatic and hydrogen plus van der Waals energy, Kcal/mol on amino-phase.

the individual energy of saccharides from the molecular interaction energy.[10] The same method was applied to study the retention mechanism of saccharides on the guanidino column. The original amino phase consisted of 368 carbons, 30 nitrogens, and 318 hydrogens. The molecular weight was 5,154. The amino groups were converted to guanidino groups. The final guanidino phase consisted of 316 carbons, 36 nitrogens and 71 hydrogens, due to the computer capacity and the size of guanidinyl group. The molecular weight was 4367. The adsorption form of glucose on the guanidino phase is shown in Figure 2.

The retention factors were related to their final, hydrogen bonding, electrostatic, and van der Waals energy values as calculated by MM2. After subtraction of the individual energy of saccharides from the molecular interaction energy listed in Table 2, the retention factor showed a high correlation with the electrostatic energy values of saccharides, with a correlation coefficient of 0.892 (n = 8). Although aminoguanidine has been reported to react with D-Glucose,[11] the weak interaction in chromatographic condition resulted in a high recovery of saccharides from this guanidino column. Some saccharides showed two peaks. This may be due to on-column isomerization of saccharides in liquid chromatography.[12] The retention time on the borate-form column was too long and the theoretical plate number was poor, due to formation of borate-esters.[13] Therefore, the borate-form column can be used for only specific separation. If the retention form in chromatography and the surface structure of adsorbent are known, the difference of retention factors can be predicted from energy values calculated by computational chemistry.

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