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The influence of properties of packing materials upon the recovery of biological substances isolated from urine by solid-phase extraction*

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Abstract: A series of packing materials with alkyl phase chemically bonded to silica gels of various porosity have been prepared. These packings have been used to isolate the test substances 5-hydroxyindole-3-acetic acid (5-HIAA) and serotonin (5-HT) from urine. The influence of the support porosity, structure of chemically bonded phase, length of alkyl chain, and coverage density on the recovery of isolated substances was studied.

Keywords: *Chemically bonded phase; liquid chromatography; solid-phase extraction; hydroxyindoles; isolation from urine.*

Introduction

Solid-phase extraction (SPE) on off-line columns has become the most popular method for isolating substances present in biological materials, e.g. urine, blood, serum, tissue [1–3]. Development of this method has been made possible by the employment of packing materials with chemically bonded phases (CBPs). These packing materials are now widely used in routine clinical, pharmaceutical and biochemical analysis [1–3].

Many factors influence the recovery rates of substances isolated by SPE from biological materials, and as a consequence, also the reproducibility of the analyses. Among these factors are the packing selectivity, the height of the packing bed, and the choice of eluent [4].

Little work has been published on the effect of the porous structure of the silica gel, the length of the alkyl chain, and coverage density of CBP upon the recoveries obtained with off-line SPE columns. These problems are discussed in the present work.

Serotonin (5-HT) and 5-hydroxyindole-3-acetic acid (5-HIAA) were used to test the prepared packings. These substances belong to the group of hydroxyindoles [5, 6], and are routinely determined in biological material. Our interest was to study the influence of the chemical character of these substances upon their recovery. Whilst 5-HIAA and 5-HT have similar molecular diameters, they have widely differing pK_a values, e.g. 4.7 for 5-HIAA and 9.8 for 5-HT.

Experimental

Chemically bonded phase (CBPs) were prepared from two different types of silica gels, namely Kiesegel Si-60 (E. Merck, Darmstadt, FRG) and SG-100 prepared in the Polymer Institute of the Slovak Academy of Sciences (Bratislava, Czechoslovakia) [7]. The characteristics of these underivatized materials are listed in Table 1.

The following organosilanes were used as modifiers: octadecyldimethylchlorosilane (MC_{18}), dodecyldimethylchlorosilane (MC_{12}), octyldimethylchlorosilane (MC_8), hexyldimethylchlorosilane (MC_6), trimethylchlorosilane (MC_1) and octadecyltrichlorosilane (TC_{18}) and were purchased from Wacker GmbH (München, FRG). The chemical modifications of the surface of the silica gels were made according to the method proposed by Buszewski [8, 9].

Analytical-grade chemicals used for SPE and

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Table 1
Physico-chemical properties of the packing materials used for SPE

Number	Type of packing	Fraction	Porosity S_{BET}	V_p	D	Type of CBP	Coverage P_c	α_{RP}
1	Kiesigel Si-60	40–63	351	0.89	8.6	Bare	—	—
2	SG-100	45–60	196	2.10	23.0	Bare	—	—
3	Si-60-MC_{18}	40–63	—	—	—	M	18.45	2.92
4	SG-100-MC_{18}	45–60	—	—	—	M	15.75	4.20
5	SG-100-TC_{18}		—	—	—	T	13.21	3.72
6	SG-100-MC_{18}		—	—	—	M	8.46	2.02
7	SG-100-MC_1		—	—	—	M	3.68	5.63
8	SG-100-MC_6		—	—	—	M	8.46	5.06
9	SG-100-MC_8		—	—	—	M	9.52	4.66
10	SG-100-MC_{12}		—	—	—	M	11.79	4.32

Where: S_{BET}, specific surface area ($m^2\ g^{-1}$); D, mean pore diameter [nm]; V_p, pore volume ($cm^3\ g^{-1}$); M, "monomeric" structure of CBP; T, "polymeric" structure of CBP; P_c, percent of carbon (%C); α_{RP}, concentration of alkylsylyl ligands on the support surface ($\mu mol\ m^{-2}$).

for chromatography were obtained from Merck. Water was double distilled. The mobile phase consisted of 0.15 M sodium phosphate buffer (pH 4.2)–methanol (87:13%, v/v).

The test mixture solution consisted of 5-HIAA (2 $\mu g\ ml^{-1}$) (Merck) and 5-HT (1 $\mu g\ ml^{-1}$) (Koch-Light Labs., Colnbrook, UK) dissolved in water. The SPE columns were prepared by packing 2-ml plastic extraction tubes with the appropriate materials to a bed height of 2 cm. The isolation of 5-HT and 5-HIAA from the test mixture solutions and from urine was carried out according to the procedure described previously [10].

The column for HPLC analysis (125 × 4.6 mm i.d.) was prepared in our laboratory and packed with a material having a high coverage density of mono- C_{18} phase (α_{RP} = 4.2 $\mu mol\ ml^{-2}$) obtained on the spherical silica gel with 5-μm particles [11].

Chromatographic measurements were made using a liquid chromatograph system consisting of a pump Model M-6000A (Waters Assoc., Milford, CA, USA), an injection valve Model 7125 (Rheodyne Co., Berkeley, CA, USA) and electrochemical detector, Model 460 (Waters Assoc.) with a glass carbon electrode working at a potential of +0.55 V versus an Ag/AgCl reference electrode. Peak heights and retention time were calculated with help of a CI 4100 computing integrator (Milton Roy, Staffordshire, UK).

The physico-chemical characterization (by porosimetry, elemental analysis and ^{29}Si CP/MAS NMR measurements) of the packing before and after chemical modification was performed as described previously [9, 11].

Results and Discussion

Effect of the support porosity

The pore diameter of the silica gel used as the basis for commercial SPE packings is about 6 nm [4]. This silica gel was compared with material having wide-pores (D = 23 nm, Table 1, packings 1 and 2). Both silica gels were subjected to chemical modification with the monosilane (MC_{18}) under identical reaction conditions [8, 9].

The coverage density was found to be greater for wide-pore silica gel (Table 1, packing 3 versus packing 4), presumably as a result of the better penetration of MC_{18} silane molecules into the pores and hence more effective blockage of accessible surface silanol groups [12].

The recoveries of 5-HIAA and 5-HT isolated from the test mixture solution (Table 2) were higher on the column with the wide-pore material (packing 4). Similar results were obtained when isolating the substance from urine (Table 2). This is due to the lower mass transfer in packing with small pores diameters (packing 3). Moreover, these pores could be blocked by the film of CBP [12, 13]. The difference in recovery values between standards from test mixture solution and from urine may be explained by the presence of other substances in the biological samples.

Effect of the structure of CBP

Figure 1 shows typical ^{29}Si CP/MAS NMR spectra of packings 2, 4 and 5 (Table 1), and possible surface texture before and after chemical modification. From this figure, it appears

Table 2
Comparison of recoveries (%) and relative standard deviations (RSD, %) for 5-HIAA and 5-HT isolated from test mixture solution and urine

Number of packing	Recovery from mixture solution				Recovery from urine			
	5-HIAA	RSD	5-HT	RSD	5-HIAA	RSD	5-HT	RSD
3	90.0	±2.4	31.9	±4.2	86.3	±3.6	23.4	±5.6
4	92.5	±2.3	41.3	±5.2	92.8	±3.1	38.7	±6.4
5	80.7	±5.8	64.8	±4.7	79.5	±8.6	56.1	±3.4
6	62.8	±3.5	76.1	±3.4	61.2	±4.8	75.3	±3.9
7	28.3	±5.2	3.5	±0.4	15.2	±4.2	1.7	±0.5
8	74.6	±4.8	14.5	±0.9	54.3	±5.3	6.3	±2.1
9	82.8	±3.7	33.4	±2.6	77.1	±4.1	23.4	±4.8
10	86.3	±3.1	48.4	±2.3	80.6	±3.9	43.1	±5.2

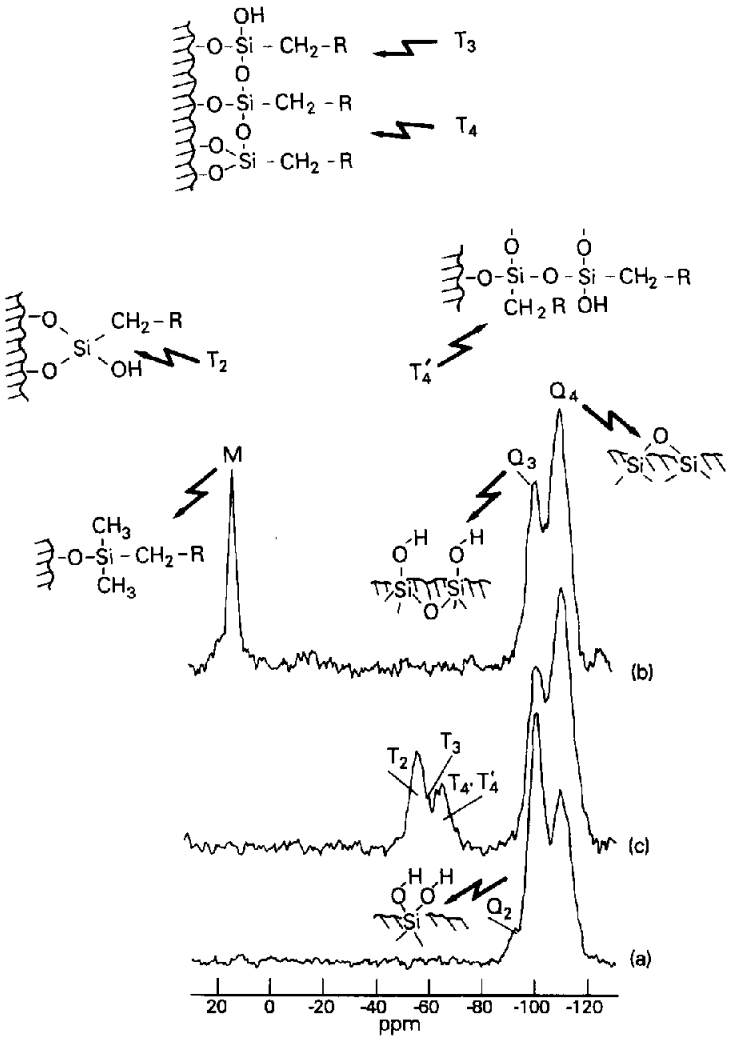

Figure 1
^{29}Si CP/MAS NMR spectra: (a) unmodified packing 2, (b) modified packing 4 by MC_{18} silane, (c) modified packing 5 by TC_{18} silane (see Table 1) and possible surface structure before and after chemical modification.

that formation of monofunctional structure of CBP is only possible (M = +14.439 ppm) as a result of chemical modification by MC_{18} silane (Fig. 1b). These modifications changed the concentration of silanol groups [Q_2 — vicinal (δ = −91 ppm); Q_3 — free (δ = −100 ppm) and Q_4 — siloxane (δ = −108 ppm)] in relation to the bare silica (packing 2, Table 1,

Fig. 1a) [11, 14, 15]. Similarly the support surface changed after chemical modification by TC_{18} silane (packing 5, Fig. 1c). On the surface of this packing, cross-linked (polymer) structure of CBP was formed with residual hydroxyl and/or silanol groups [9, 11, 14, 15].

The recovery of 5-HIAA and 5-HT from packings 4 and 5 (Table 2) when applied as a standard test mixture solution and as a urine sample showed that higher recovery was obtained for 5-HIAA on packing 4 but for 5-HT the higher recovery was obtained on packing 5. This is probably due to the different chemical characters of 5-HIAA and 5-HT which mean that different isolation mechanisms are observed (5-HIAA is an organic acid, $pK_a = 4.7$ but 5-HT is a typical organic base, $pK_a = 9.8$). Possibly, the higher recovery for basic substances may be obtained on the packing with greater number of unblocked silanol groups. In contrast, for substance with acidic character the hydrophobic packing would be more suitable. The same conclusion also appears from comparison of the relative standard deviations (RSD) (Table 2).

Effect of the coverage density

Information as to the influence of residual silanol groups on the recovery rate may be gained by comparing two packings that differ only in alkyl chain coverage density (Table 1, packing 6, with $\alpha_{RP} = 2.02$ μmol m^{-2} versus packing 4, with $\alpha_{RP} = 4.20$ μmol m^{-2}). One finds that the recovery rate for 5-HT is enhanced by lower alkyl coverage density, while the opposite is true of 5-HIAA (Table 2). The recovery of 5-HT on bare (unmodified) silica gel (packing 2), however, was only 23.2%, indicating that blockage of part of the population of silanol groups (those of higher energy) is necessary [16, 17], since these silanols irreversibly adsorbed serotonin. It is possible that the mobility of the alkyl chains may also have some influence upon the recovery rates.

Effect of alkyl chain length

A series of packings with different alkyl chain lengths were prepared (packings 7–10, Table 1). From the data listed in Table 1, it is apparent that with increasing length of the alkyl chain, the coverage density of CBP decreased. The α_{RP} values are high and are very close to the theoretical possible [18]. This situation is advantageous for the isolation of 5-HIAA, but less suitable for 5-HT.

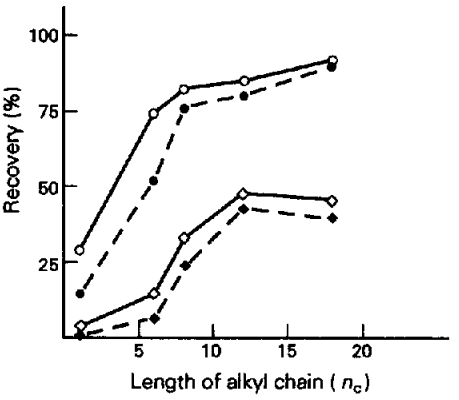

Figure 2
Relationship of 5-HIAA (○ and ●) and 5-HT (◇ and ◆) recoveries versus length of alkyl chain of CBP. Data from test mixture solution (continuous line), and from urine solution (dotted line).

Figure 2 shows the correlation between the recovery and the length of bonded alkyl chains (n_c). It is apparent that the best recoveries of 5-HIAA are obtained using packing 4 (Table 1). Packing with shorter alkyl chains showed lower recovery rates despite higher α_{RP} values (packing 7, Tables 1 and 2). Similar results were found for the isolation of 5-HT. Here, maximum recovery was obtained with packing 10. It is possible that the use of an MC_{12} packing with lower coverage density may result in improved recovery rates, because of better mass transfer.

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