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Solid state NMR and extraction studies on "phenyl"-bonded stationary phases used for solid phase extraction

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Abstract

The solid phase extraction (SPE) and elution of [^{14}C]-propranolol from aqueous buffer has been studied for a range of phenyl-bonded SPE materials. Differences were noted in the recovery of the analyte using methanol–water eluents depending upon the manufacturer and whether or not phase had been endcapped. Efficient recoveries of [^{14}C]-propranolol were only achieved when triethylamine was added to the eluting solvent as a competing base. Solid state cross polarisation/magic angle spinning (CP/MAS) NMR spectroscopy was used to characterise the phases further, which revealed differences in endcapping between materials as well as differences in the type and extent of cross-linking.

Keywords: Solid state NMR; Solid phase extraction; Phenyl bonded phases; ^{29}Si NMR; ^{13}C NMR; Propranolol

1. Introduction

With the increasing application of solid phase extraction (SPE) methods to the analysis of drugs in biological fluids, it has become clear that nominally similar products from different manufacturers can have quite different extraction properties. Such differences may be particularly apparent when the extraction mechanism employed depends upon interactions with residual silanols on the silica surface rather than the "bulk" bonded phase. We have therefore undertaken a series of studies aimed at both characterising SPE materials, and gaining an understanding of the underlying extraction mechanisms involved in SPE [1–7]. As part of this work, we have recently performed experiments involving the use of ^{13}C and ^{29}Si solid state cross polarisation/magic angle spinning (CP/MAS) NMR to characterise a number of C18-bonded SPE silicas of differing character (endcapped and non-endcapped, different carbon loadings, etc.) [4]. The solid state NMR results were then related to the extraction properties of these phases for the beta-blocking drug propranolol. Here we describe similar studies on a range of "phenyl"-bonded phases from a number of different manufacturers.

2. Materials and methods

2.1. Chemicals and reagents

[^{14}C]-propranolol-[2(*R*,*S*)-1-isopropylamino-3-(1-naphthyloxy)-2-propanol] was synthesised in the radiochemical laboratory at Zeneca

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pharmaceuticals at a radiochemical purity of greater than 95%.

Methanol was of HPLC grade (Fisons, Loughborough, UK). Triethylamine and acetic acid were of analytical grade (Fisons).

2.2. Cartridges

Phenyl-bonded cartridges (each containing 100 mg of bonded phase) were obtained from JT Baker Ltd. (lot 7095-01) (Reading, UK), Varian Assoc., (lot 081479) (Walton-on-Thames, UK) and International Sorbent Technologies Ltd (IST) (lots 361-2-01, an endcapped phenyl phase, and 360-2-1, a non-endcapped phenyl phase) (Hengoed, UK).

2.3. Extraction experiments

The basic extraction protocol adopted for all of the four phenyl-bonded phases involved the solvation of the phase by washing with methanol (1 ml), water (1 ml) and finally the application buffer (0.2 M sodium acetate, pH 5) (1 ml). The [^{14}C]-propranolol (2.5 μg ml^{-1}) was then applied in aqueous buffer (1 ml), and the cartridge washed with a further 1 ml of the application buffer. The cartridge was then eluted with mixtures of either methanol–water (1 ml) or methanol–triethylamine (TEA) acetate (1 ml, pH 7, 0.1 M), prepared by mixing 0.1 M triethylamine with acetic acid, of increasing eluotropic strength (see text for details). Radioactivity recovered in the application, wash and elution steps was then determined by scintillation counting of the eluates.

2.4. Scintillation counting

Scintillation counting was performed by mixing the eluates from the columns, collected directly in 20 ml plastic scintillation vials, with 10 ml of Ready Value scintillation fluid (Beckman). Samples were analysed on either a Packard TRI CARB 1900 CA (Packard) or a Beckman LS 1801 (Beckman) liquid scintillation counter with quench correction.

2.5. Solid state NMR

^{29}Si CP/MAS solid state NMR spectroscopy was performed on a Bruker MSL 200 spectrometer on samples of the various phenyl-bonded silicas (200–300 mg) in 7 mm double bearing rotors of zirconium oxide. Magic angle spinning was carried out at 3000 Hz. The spectra were recorded using a phase-cycling pulse sequence with pulse length of 7 μs and a repetition rate of 1 s. For each spectrum a total of 8K scans were accumulated. The contact time was 5 ms.

^{13}C CP/MAS NMR spectra were obtained using a Bruker ASX 300 instrument. The samples (100 mg) were packed in 4 mm double bearing rotors of zirconium oxide. High speed magic angle spinning was carried out at a rate of 10 000 Hz. The spectra were recorded using a phase-cycling pulse sequence with pulse lengths of 4 μs, a repetition rate of 1 s and a contact time of 3 ms. Each spectrum was the result of 4400 scans.

The quantification of the observed signals was performed by directly comparing the areas of the peaks (obtained by peak deconvolution).

All solid state NMR spectra were externally referenced to liquid tetramethylsilane.

3. Results

3.1. Solid state NMR

We have previously used solid state CP/MAS NMR (^{13}C and ^{29}Si) to characterise a number of different C18-bonded silicas used for SPE [6]. These experiments gave readily interpretable spectra that were typical of this type of phase [8]. However, to date, no such solid state NMR studies have been reported for phenyl-bonded silicas. We therefore proceeded to characterise fully the four phenyl-bonded phases from the various manufacturers using both ^{13}C and ^{29}Si CP/MAS NMR techniques as described below.

3.2. ^{13}C CP/MAS NMR spectroscopy

The ^{13}C CP/MAS NMR spectra obtained for the four phases under investigation are shown in Figs. 1(a)–1(d). The four spectra shown here reveal that three of the phases (a–c, the Varian and the two IST products) look similar, whilst the JT Baker phase (d) is very different. In the case of phases a–c a group of aromatic peaks appear in the range 125–135 ppm. In these materials, where the phenyl ring is bonded directly to the silica surface, the following assignments can be made. The signal at 131 ppm corresponds to the carbon atom bonded to the silica; the ortho-carbon atoms are found at 134 ppm and the meta-carbons at 127 ppm; the

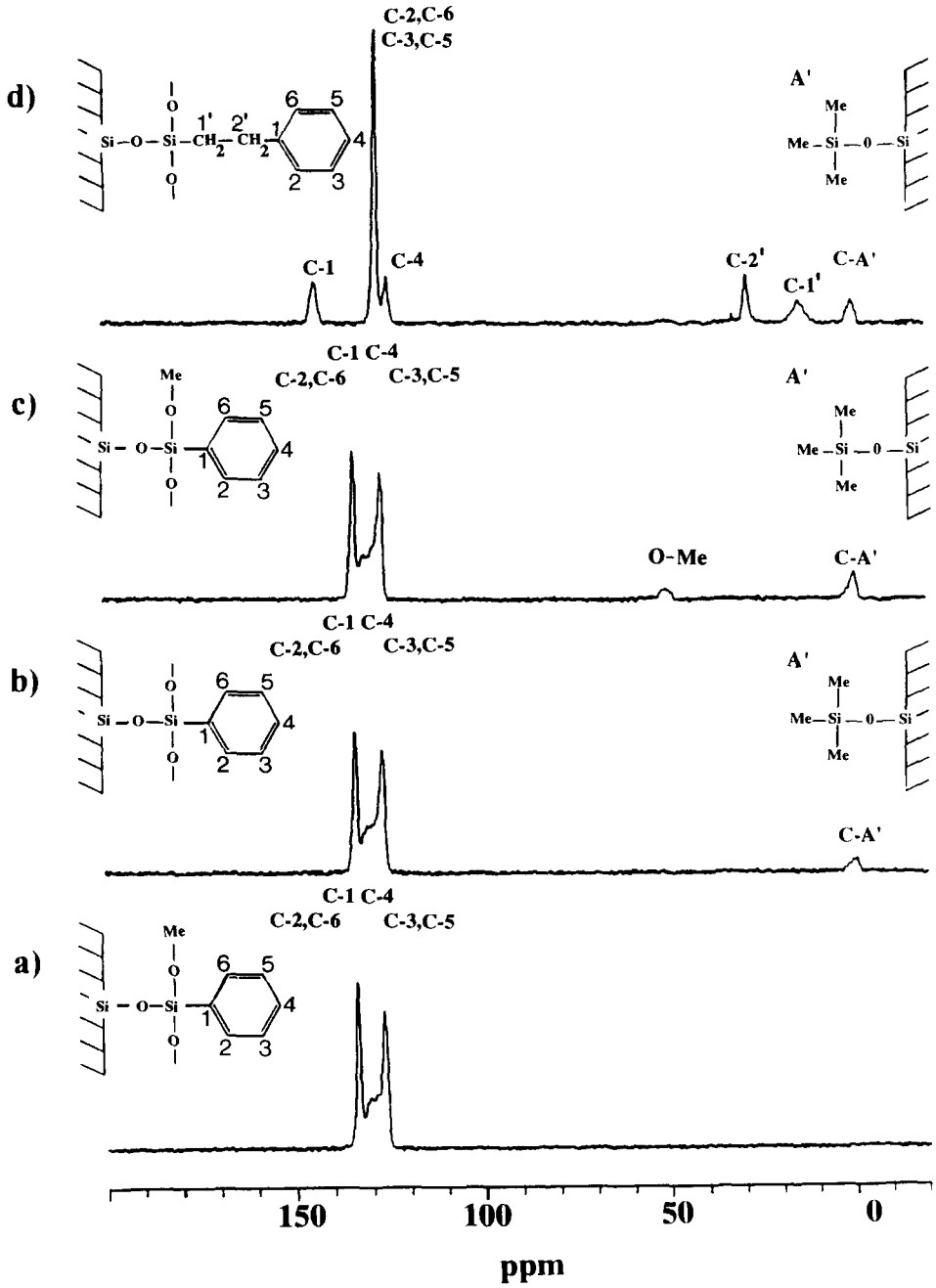

Fig. 1. ^{13}C CP/MAS NMR spectra obtained for (a) IST phenyl, (b) IST phenyl endcapped, (c) Varian phenyl and (d) JT Baker phenethyl SPE phases.

para-carbon atom appears as a shoulder at approximately 129 ppm [9]. Some differences between the ^{13}C spectra of phases a–c are apparent in the aliphatic region of the spectrum. Thus, both b and c show ^{13}C peaks characteristic of trimethylsilyl groups used for endcapping the residual silanols, whilst phase a shows no evidence of endcapping. The observation of these signals for phases a and b is consistent with the claims of the manufacturer that the materials have been endcapped. In addition, for phase c, a signal at 50 ppm was observed which is consistent with the presence of small amounts of methoxy groups of the phenyltrimethoxysilane starting material. In contrast to the spectra obtained from phases a–c, that obtained from phase d shows peaks at 144, 125, 127.5, 28, 14, and 0 ppm. The signal at 0 ppm is probably due to the presence of the endcapping trimethylsilyl groups, whilst the resonances at 14 and 28 ppm appear to be those of an ethyl spacer via which the phenyl

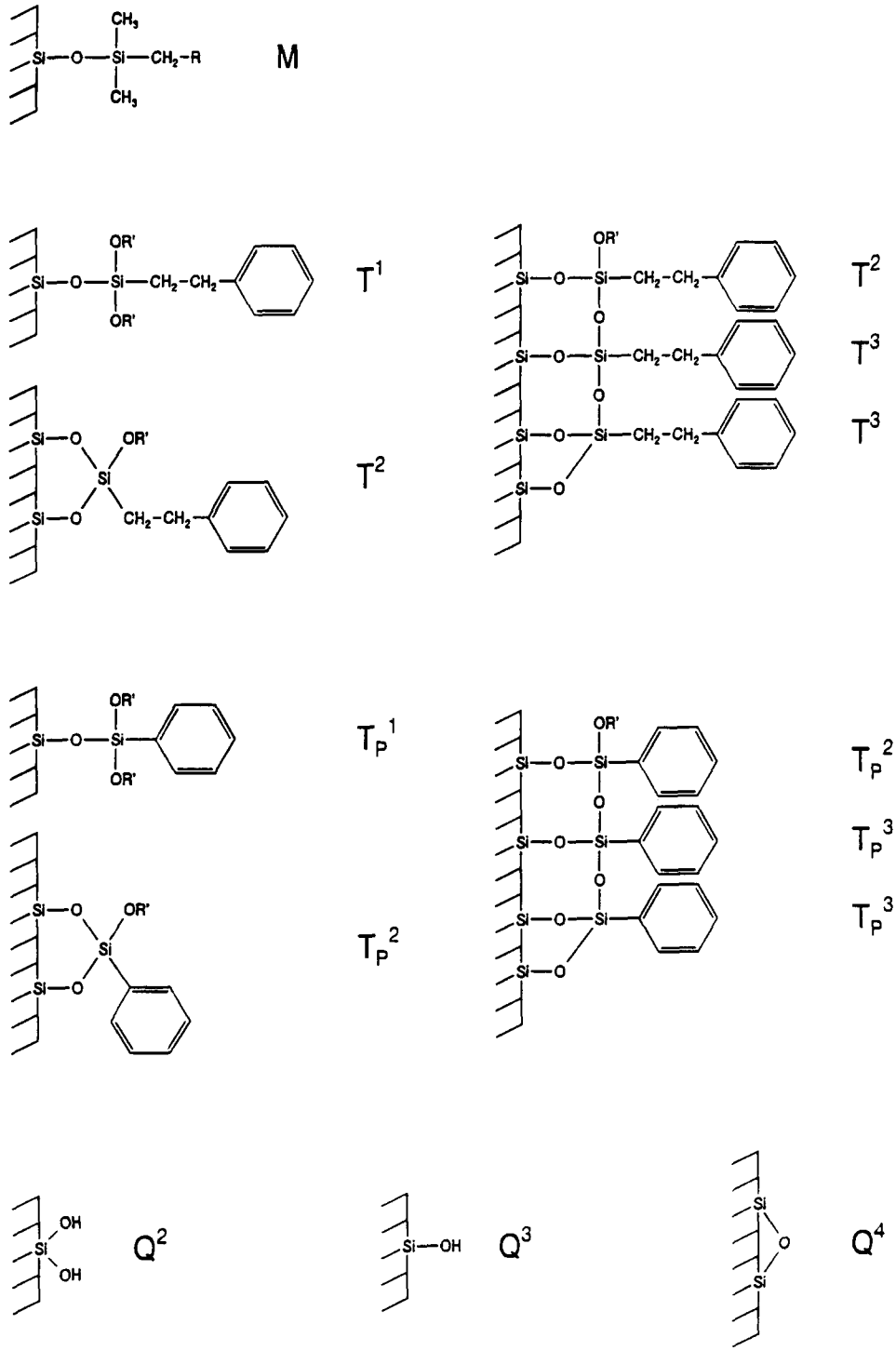

Fig. 2. Key to the structural features detected by ^{29}Si CP/MAS NMR.

group is attached to the silica. The resonances for the aromatic carbon atoms can be assigned as follows. The signal at 144 ppm is that of the carbon bonded to the ethyl spacer, with the ortho- and meta-carbons showing an identical chemical shift of 127.5 ppm. The para-carbon for this phase is observed as the highest field signal at 125 ppm.

3.3. ^{29}SiCP/MAS NMR spectroscopy

The ^{29}Si CP/MAS NMR spectra of the four phenyl-bonded phases under investigaton are shown in Fig. 3 (Fig. 2 provides a key to the nomenclature used below and in Fig. 3). All four spectra show typical resonances for silanediols (Q2), silanols (Q3) and siloxanes (Q4) [8].

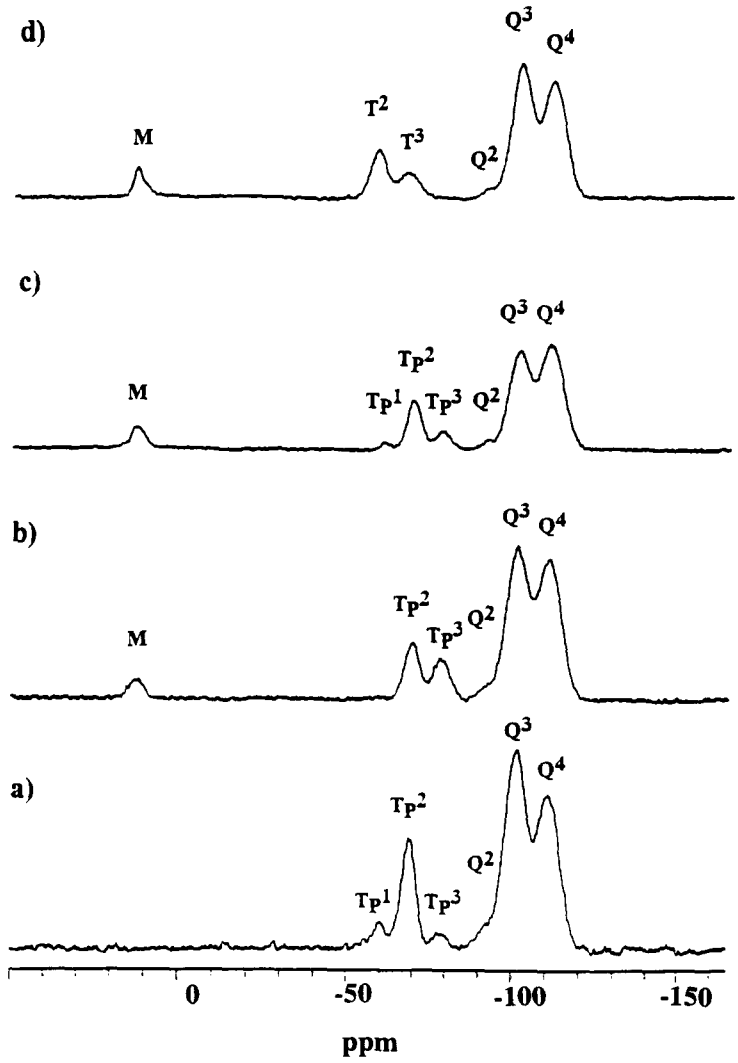

Fig. 3. ^{29}Si CP/MAS NMR spectra obtained for (a) IST phenyl, (b) IST phenyl endcapped, (c) Varian phenyl and (d) JT Baker phenethyl SPE phases.

In addition, phases b–d show a signal at +13 ppm (M), which is consistent with the presence of the endcapping trimethylsilyl group detected in the ^{13}C NMR experiments. In the spectra of phases a–c, peaks (labelled $T_p^{1,3}$) are observed which are due to the various bonded phenyl moieties and their degree of cross-linking (see Fig. 2) [10]. The spectra clearly show significant differences between phases a–c with respect to their degree of cross-linking, with phase b showing the highest degree of cross-linking. These results are summarised in Table 1. The greater extent of cross-linking of phase b compared to phase a (both manufactured by IST) probably results from additional heat treatment during endcapping.

Phase d, where the phenyl groups are bonded to the phase via an ethyl-spacer rather than directly as in the case of the other three phases, shows two resonances, at −58 and −66 ppm respectively (T^2 and T^3), due to the presence of partially and totally cross-linked trialkoxysilyl groups. Like phase b, phase d shows a high degree of cross-linking.

The spectra of all four phases reveal the presence of residual silanols, even where endcapping has been performed (phases b–d) to reduce their numbers. From these spectra, it would appear that the material with the smallest number of residual silanols was phase c. Predictably, the material which had not been endcapped (phase a) had the highest number of residual silanols.

Table 1

Phase	Relative amount				Degree of cross-linking
	M	T_p^1	T_p^2	T_p^3	
(a) ISP Ph Lot 360-2-01	–	20%	69%	11%	63%
(b) IST Ph ec. Lot 361-2-01	16%	2%	45%	37%	81%
(c) Analytichem Intern. Ph ec. Lot 081479, Part 608101	24%	7%	66%	27%	73%
	M	T^1	T^2	T^3	
(d) J.T. Baker Ph ec. Lot 7095-01	20%	–	45%	35%	81%

See Fig. 2 for key; ec = endcapped.

3.4. Solid phase extraction properties

To characterise the extraction properties of these phenyl-bonded phases, experiments were performed in which [^{14}C]-propranolol was extracted from aqueous buffer and then recovered from the cartridges using different elution protocols. Following the extraction step, attempts were first made to elute the adsorbed radiolabel using methanol–water mixtures of increasing eluotropic strength (from 0 to 100% methanol). Such conditions should have been sufficient to elute any propranolol retained on the cartridges by simple hydrophobic "reversed-phase" interactions. However, the recoveries of [^{14}C]-propranolol were poor. On the Bond Elut and JT Baker phases, the overall recovery of radiolabel after the final elution step with 100% methanol was less than 5%. In the case of the IST sorbents, significant elution of the adsorbed propranolol was detected from the endcapped phase, beginning with the 60 and 80% methanol elution steps, eventually achieving a recovery of about 40% of the adsorbed radiolabel with 100% methanol. Recoveries from the non-endcapped IST material were also higher than from the Bond Elut or JT Baker materials, approaching about 25% with pure methanol as eluent. These results are summarised in Fig. 4(a). The poor overall recoveries of radiolabel achieved when elution was attempted with methanol–water alone is typical of the results obtained for the elution of propranolol from C18-bonded silica gel. This behaviour is thought to be due to the interaction of bases such as propranolol with residual silanols, and can be overcome by the inclusion of a competing base in the eluent. These observations led us to perform experiments on the phenyl-bonded phases where TEA was added to the eluting solvents as a competing base in an attempt to increase recoveries of propranol from the cartridges.

The effect of using methanol–TEA mixtures of increasing eluotropic strength is illustrated in Fig. 4(b). Employing methanol–TEA mixtures with 80% or more methanol enabled quantitative recoveries of the adsorbed [^{14}C]-propranolol from all four cartridge types. Differences between the phases were, however, apparent. Some recovery of the radiolabel was observed from the non-endcapped IST phase (a) with as little as 10–20% of methanol in the eluent. In contrast, the endcapped material from the same manufacturer (b) and that of the other endcapped phenyl phase (c) did not show any recovery until the eluting solvent contained about 40% methanol. The radiolabel was only recovered from the JT Baker phenethyl phase when the proportion of methanol in the eluate reached 60%.

4. Discussion

All four phenyl-bonded phases demonstrate different elution properties for [^{14}C]-propranolol, a situation reminiscent of the variety that is observed for C18-bonded materials from different manufacutrers (e.g. see Refs. [1–3]). Such diversity is undoubtedly valuable as a source of different extraction selectivities. However, such results once again emphasise the potential dangers of attempting to substitute one phase for another in a developed procedure.

Elution of propranolol was only efficiently achieved in the presence of buffers containing triethylamine, irrespective of the manufacturer. This behaviour is probably best explained in

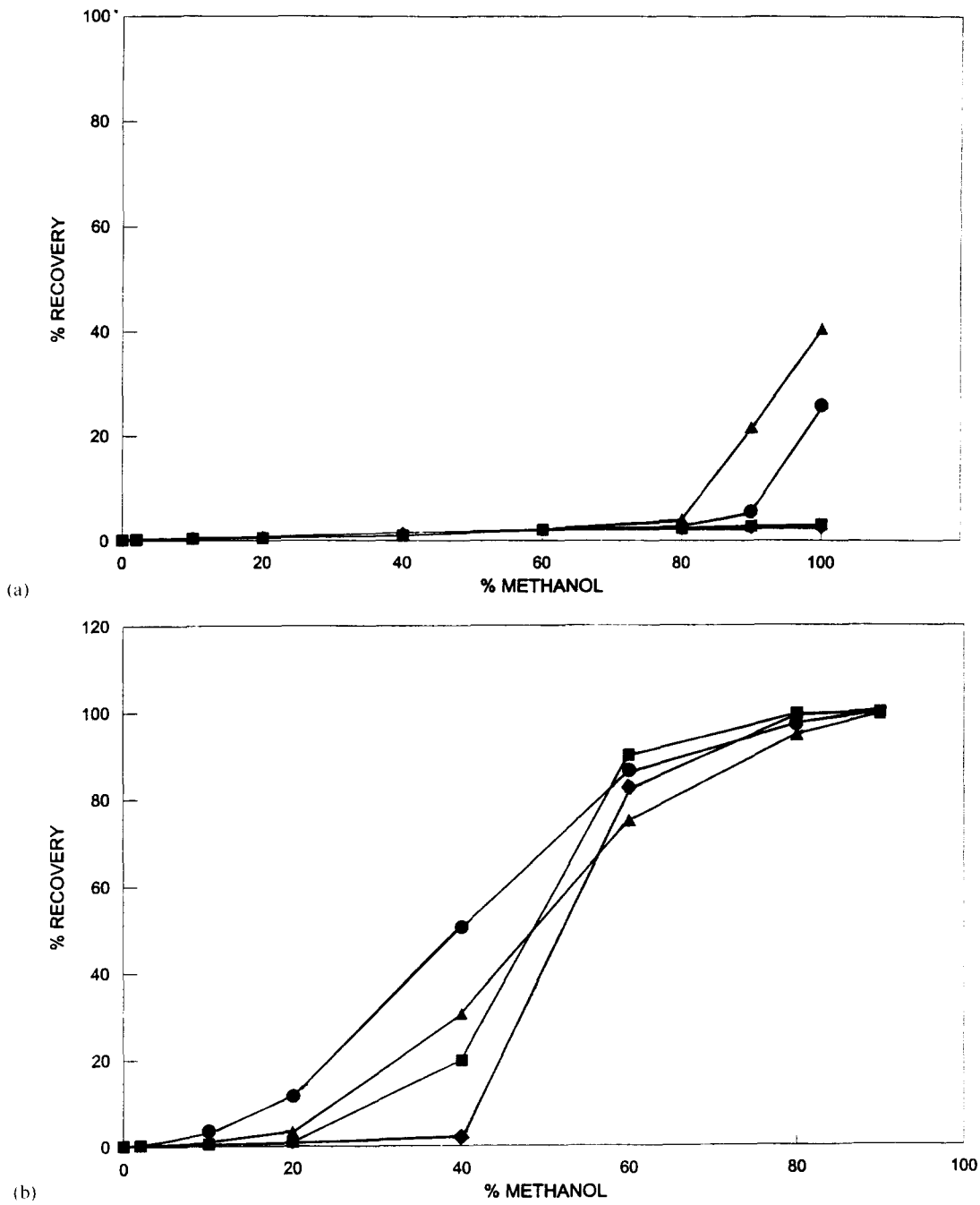

Fig. 4. Cumulative elution-recovery profiles for [^{14}C]-propranolol from IST phenyl (●), IST phenyl endcapped (▲), Varian phenyl (■), and JT Baker phenethyl (◆) SPE phases following extraction from aqueous buffer using (a) methanol–water and (b) methanol–triethylamine.

terms of ionic interactions with surface silanols, as has been postulated to explain similar results for C18-bonded materials. The evidence from ^{29}Si CP/MAS NMR spectroscopy is that numerous residual silanols are still present on these phases, even after endcapping. The extraction of this analyte from buffer under conditions (pH 5) that would ensure the ionisation of the secondary amino group also implies the involvement of silanols. It is noteworthy that significant, but incomplete, recoveries of propranolol were obtained from the two IST phenyl-bonded phases with methanol–water eluents, with the highest recoveries seen with the endcapped material. This perhaps implies a lower level of silanol involvement in the retention of propranolol on these phases. In general, the elution profiles for the three phenyl phases

with methanol–TEA were similar to those obtained with a low carbon loaded C18-bonded phase [4]. The phenethyl-bonded phase was rather different in that significantly higher concentrations of methanol were required in order to elute the analyte. Whether this reflects a difference in selectivity due to the ethyl-spacer group or a difference resulting from the manufacturing process used cannot be determined on the basis of these experiments.

Many other differences between the phases were shown by the CP/MAS NMR data, but it is not yet possible to relate all of these to the extraction properties observed.

5. Conclusions

The four phenyl-bonded phases studied here showed large differences in extraction/elution properties for nominally similar materials from different manufacturers. This result is similar to those when different C18-bonded materials were examined [1–4]. Both ^{13}C and ^{29}Si CP/MAS NMR proved valuable as a method of characterising these phenyl and phenethyl phases, in particular showing features such as residual silanols and endcapping, and the degree of cross-linking of the bonded phase. The extraction mechanism for propranolol on phenyl-bonded silica gel was probably, at least in part, an ionic interaction with the residual silanol groups shown by the ^{29}Si CP/MAS NMR to be present on the surface of all four phases.

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