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Characterisation of C_{18}-bonded silicas for solid-phase extraction by solid-state NMR spectroscopy

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Abstract

^{29}Si and ^{13}C cross-polarisation magic-angle-spinning (CP-MAS) NMR spectroscopy have been used to characterise a total of seven C_{18}-bonded silicas used for solid-phase extraction. Wide variations were observed in the extraction properties of these silicas for basic analytes such as the drug propranolol. These differences depended upon the degree of carbon loading of the phases and whether or not endcapping had been performed. The different properties of the phases appear to correlate well with the differences observed using CP-MAS NMR. This demonstrates the utility of such techniques as a means of further understanding the underlying mechanisms responsible for the observed extraction properties of the phases.

1. Introduction

The use of silica based materials for sample preparation in the form of solid-phase (or liquid–solid) extraction (SPE, LSE) is now well established, particularly in the fields of biomedical and environmental analysis. With the widespread and increasing use of these products it has become clear that there can be wide variations in extraction properties for nominally similar packings (*e.g.* C_{18}) obtained from different manufacturers. Further, batch-to-batch variation has also been noted for materials produced by the same manufacturer. Whilst this situation is by no means unique, and indeed such differences have been widely discussed for liquid chromatography packings (where they have also been exploited as a useful source of chromatographic selectivity) it nevertheless merits study as a means of improving our understanding of the extraction behaviour of SPE phases. For some time we have been interested in the extraction of basic drugs (particularly β-blockers), where it is suspected that a mixed mode of extraction onto C_{18}-bonded silica (involving an ion-exchange interaction with residual silanols) occurs [1–4]. Recently we have investigated the SPE of a range of β-blockers onto a number of C_{18}-bonded silicas produced by the same manufacturer but with different degrees of carbon loading and endcapping [5]. Clear differences were apparent in the extraction behaviour of these phases under the conditions employed, with extraction efficiency decreasing with increasing carbon loading/endcapping. Such results provided a further indication of the likely importance of residual silanols in explaining the SPE of the analytes. Accordingly we have undertaken a further study in which the phases have been investigated using solid-state NMR. This

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approach has previously been found to be of value in the study of the bonded phases used for HPLC [5–11]. Here, ^{29}Si and ^{13}C cross-polarisation magic-angle-spinning (CP-MAS) NMR spectroscopy have been utilised to characterise the various bonded silicas in order to attempt to better understand the mechanisms responsible for the SPE results obtained with these materials.

2. Experimental

2.1. *Cartridges*

Three types of C_{18}-bonded cartridges, without endcapping, were used. These corresponded to the ODS [5% nominal carbon loading (CL)], ODS1 (14%CL), and the ODS2 (16% CL). Four types of C_{18}-bonded and endcapped cartridges were studied corresponding to the ODS3 (10.5% CL), ODS4 (14% CL), ODS5 (18% CL) and the ODS6 (22% CL). The cartridges were obtained from Whatman (Maidstone, UK). Elemental analysis gave values of 3.8, 11.6, 16.9, 10.7, 13.6, 20.4 and 21.5% for the carbon loadings of the ODS, ODS1, ODS2, ODS3, ODS4, ODS5 and ODS6 phases, respectively.

2.2. *Extraction procedure*

The extraction procedure used for [^{14}C]propranolol has been described in detail elsewhere [5]. Briefly, aqueous samples (0.2 *M* sodium acetate buffer, pH 5) were applied to columns, preconditioned by washing with methanol. Following sample application the columns were washed with water and then acetonitrile. The retained material was then eluted from the cartridges with methanol–triethylamine acetate (0.1 *M*, pH 7) (80:20, v/v). Radioactivity in column eluates was determined by scintillation counting.

2.3. *CP-MAS NMR spectroscopy*

Details of the solid-state NMR spectroscopy are as follows: NMR measurements were performed on a Bruker MSL 200 spectrometer on samples of the various C_{18}-bonded silicas (2–300 mg) in double bearing rotors of zirconium oxide. Magic-angle spinning was carried out at a rate of 3500 Hz. The spectra were recorded using a flip-back pulse sequence with pulse lengths of 5–7 μs and a repetition rate of 1 s. For each spectrum 2000 scans were accumulated. ^{29}Si CP-MAS NMR spectra were recorded with a contact time of 5 ms. For ^{13}C CP-MAS NMR spectra contact times of 3 ms were used. All NMR spectra were externally referenced to liquid tetramethylsilane.

3. Results and discussion

Typical results for the extraction of propranolol on the SPE materials studied here are shown in Fig. 1 [5]. Samples were applied at pH 5 to ensure the ionisation of the analyte and thus promote ionic interactions with the silanols, followed by an acetonitrile wash (to elute compounds retained only by reversed-phase partition). Under these conditions the higher the carbon loading and degree of endcapping the lower the extraction efficiency and the more easily the compound was eluted from the column at the wash step. Similar results were obtained for a number of other β-blockers [5]. As we have discussed elsewhere [1–3,5], such results are most easily explained by postulating an ionic interaction with residual silanols on the silica surface. Increasing the degree of coverage with ODS groups coupled with endcapping would therefore be expected to affect such interactions by both decreasing the number of available silanols and also reducing access to them as a result of steric effects. The investigation of these modified silicas using CP-MAS NMR might therefore be expected to reveal differences between the various SPE phases. Previously we have successfully used such techniques to characterise bonded phases for HPLC, [7–9,11–12] and the resonances for the various groups present on the surface of the silica have been assigned (see Fig. 2). The ^{29}Si CP-MAS NMR spectra for each of these phases are shown in Figs. 3 (unend-

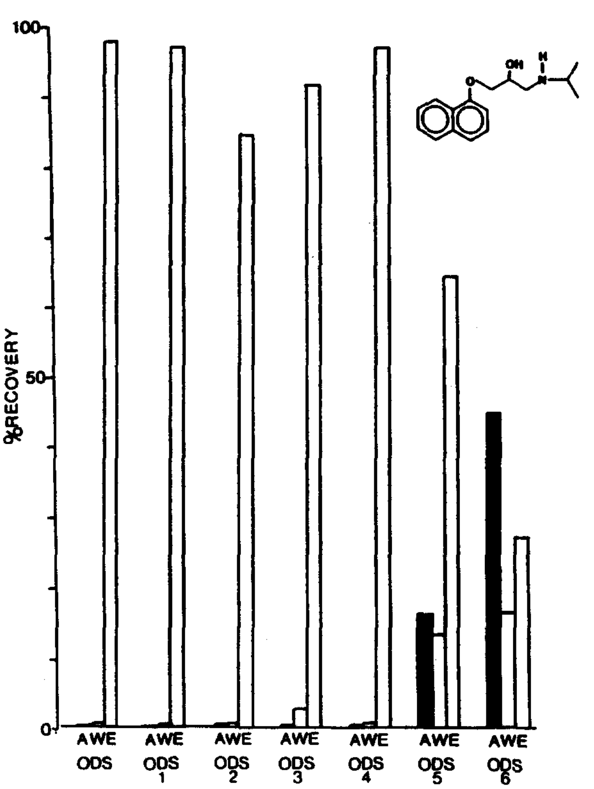

Fig. 1. Typical results for the solid-phase extraction of [^{14}C]propranolol from aqueous buffer on to the various C_{18}-bonded silicas showing the proportion of the applied radioactivity recovered at each stage of the extraction. A = Application step; W = wash step; E = elution step. Each value is the mean of two determinations.

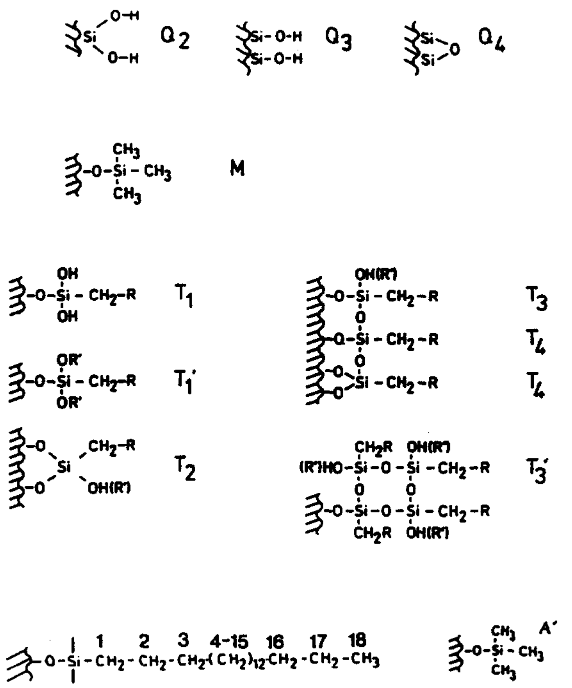

Fig. 2. The various types of groups likely to be found on the surface of C_{18}-bonded silicas. The lettering (Q_2 etc.) is used in the subsequent CP-MAS NMR spectra.

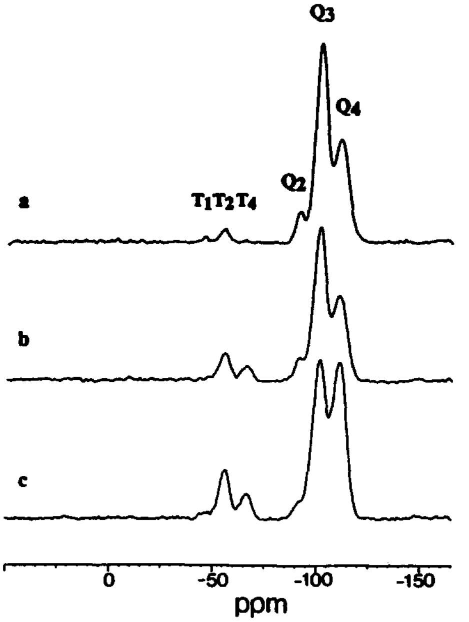

Fig. 3. ^{29}Si CP-MAS NMR spectra of the unendcapped C_{18}-bonded silicas. (a) ODS; (b) ODS1; (c) ODS2. See Fig. 2 for key to signal assignments.

capped) and 4 (endcapped) respectively. The spectra show three groups of peaks. The first group, from −90 to −110 ppm, result from the surface silicon atoms present on the silica gel. The resolution of the peaks is sufficient to distinguish between the silanediols [−90 ppm, Si(OH)2, Q_2], silanols [−100 ppm, Si(OH), Q_3] and siloxanes (SiOSi, −110 ppm, Q_4). The second group of peaks, covering the range −48 to −66 ppm indicate that the silica gel has been modified by reaction with a trifunctional alkylsilyl group. The exact chemical shift of the signal being dependent on the degree of cross-linking. Thus monodentate bonded and non-cross-linked silicons appear at −48 ppm (T_1), bidentate at −56 ppm (T_2) and fully cross-linked

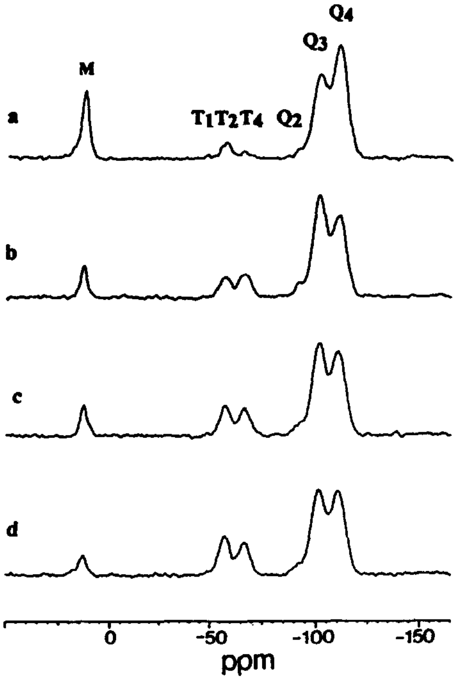

Fig. 4. ^{29}Si CP-MAS NMR spectra of the endcapped C_{18}-bonded silicas. (a) ODS3; (b) ODS4; (c) ODS5; (d) ODS6.

species at −66 ppm (T_4). The endcapped silicas show a further signal at +13 ppm corresponding to the so-called M group of a monofunctional alkylsilyl, in this case that of the trimethylsilyl (TMS) group used for endcapping.

Given that the spectra for all of the various phases were recorded and processed under the same conditions the intensities of the peaks of the M and T groups can be compared. In the case of the unendcapped phases the comparison is simplified by the fact that only ODS (T) groups are present, and therefore a higher carbon loading causes an increase in the intensity of the T-group family of peaks. Clearly, in the case of those silica gels with a low carbon loading, such as the ODS material (nominal 5% carbon loading), and in the absence of endcapping, a considerable number of residual silanols remain available (Fig. 3a) for interaction with a basic analyte such as propranolol. As the spectra show (Fig. 3a–c), with increasing carbon loading (*e.g.* ODS1 and ODS2 with a nominal loading of 14 and 16% carbon, respectively) the proportion of these silanols was steadily reduced. However, without endcapping large numbers are still present. Where the silica gel has been endcapped the number of TMS groups (M groups) must also be considered. However, the M and T groups have similar dynamic behaviours and thus their ratio can readily be determined. For the ODS3 phase some 70% of all the groups bonded to the silica are in fact M groups. For the remaining endcapped silicas (ODS4, 5 and 6) the ratio of TMS to ODS groups ranges from 20 to 30%.

Comparison of the intensities of the Q_2, Q_3 and Q_4 peaks in the ^{29}Si spectra allows an assessment of the numbers of residual silanols to be made. Thus as a first approximation it can be seen that, as the carbon loading increases, the intensity of the Q_2 and Q_3 groups decreases. The intensity of the Q_4 peak (−110 ppm), which corresponds to the Si–O–Si of the siloxanes should remain unchanged, as these groups take no part in bonding with ODS or TMS groups. Nevertheless the intensity of the Q_4 peak does fall as the carbon loading is increased. This is a consequence of the CP-MAS NMR experiment in which signal intensity is proportional to the number of ^{1}H nuclei in the immediate vicinity of the silicon atoms. Modification of the silica, causing a reduction in the number of silanols, therefore results in a decrease in the intensity of the siloxane signals. In general the results obtained are consistant with these expectations. The ODS2 phase does, however, provide an exception to this trend, giving the highest Q_4 peak of all the phases. Furthermore the intensity of the Q_3 peak for the silanols is also higher in this material than in the ODS1 phase, despite the ODS2 material having a higher carbon loading. It is not easy to rationalise this behaviour. It is possible that some other magnetisation mechanism(s) are operating (*e.g.* through space from the ODS chain to the surface silicon atoms) or some other dynamical behaviour of the silica gel is responsible. Alternatively some further treatment of the silica may have been undertaken (*e.g.* heating or acid treatment), or another type/batch of silica may have been used for the manufacture of the ODS2 material. The reduction in the number of silanols present on the surface of the ODS3 material, an endcapped material which has a carbon loading of 10.5% is however, quite marked. As noted above the amount of TMS relative to ODS on this material is quite high. At the higher carbon loadings obtained with the ODS4 (14%), ODS5 (16%) and ODS6 (22%) the endcapping with TMS appears to have been less efficient and more silanols are detectable on these phases than on the ODS3 silica. This perhaps results from steric factors, due to the higher ODS coverage, preventing the endcapping reagent from gaining access to the silanols.

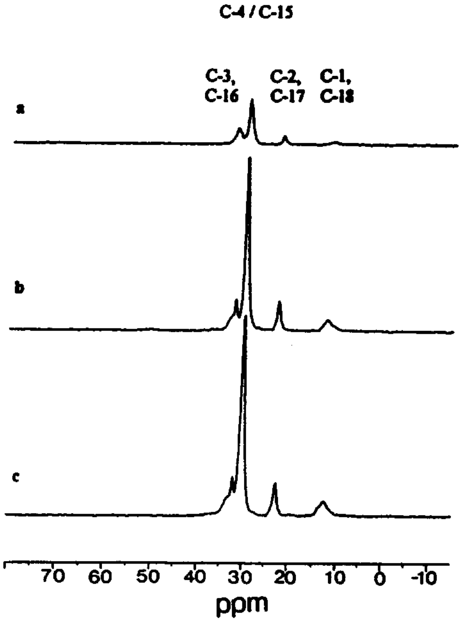

Fig. 5. ^{13}C CP-MAS NMR spectra of the unendcapped silicas. (a) ODS; (b) ODS1; (c) ODS2. See Fig. 2 for key to signal assignments.

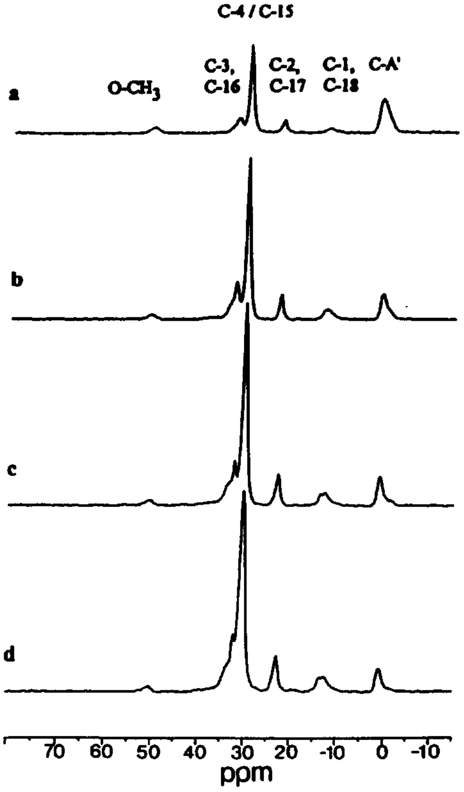

Fig. 6. ^{13}C CP-MAS NMR spectra of the endcapped silicas. (a) ODS3; (b) ODS4; (c) ODS5; (d) ODS6. See Fig. 2 for key to signal assignments, O–CH3 is residual methanol.

The ^{13}C CP-MAS NMR spectra reveal a range of peaks between 0 and +50 ppm (Figs. 5 and 6). Many of the carbon atoms of the ODS chains have similar chemical shifts and are not resolved in these spectra. For the ODS chains the signal assignments are as follows: C-1 12 ppm, C-18, 14 ppm; C-2 and 17, 22.5 ppm, C-4 to C-15, 30 ppm and C-3 and C-16, 32 ppm. The endcapped phases also show resonances for the methyl carbons of the TMS groups (C-A′) which appear at +1 ppm. A further peak at +50 ppm results from the presence of adsorbed methanol. The ^{13}C spectra show a good agreement between carbon loading and peak intensity. With the higher carbon loading the integral of peaks for the ODS chain increases, and the peak intensity of the C-A′ groups also increases in proportion to the degree of endcapping. Thus, the ^{29}Si and ^{13}C CP-MAS NMR results show clear differences between the seven C_{18}-bonded phases examined, and differences in the SPE properties of these phases for β-blockers have also been demonstrated [5]. The question to be addressed therefore is, can the differences in extraction properties be reconciled with the NMR spectra of the phases?. Clearly the presence of silanols on the surface of these phases is consistent with a role in an ionic extraction mechanism, and the reduced number available on the more highly loaded and endcapped phases is also consistent with the extraction characteristics of these phases. However, this is clearly not the complete explanation as significant numbers of silanols are still present even at the very highest carbon loadings. Indeed it is interesting to note that the material which had the fewest residual silanols was the ODS3 and not the ODS6 material. However, despite the lower number of silanols present reasonably efficient extractions were obtained on ODS3 but not on the ODS6 phase. This result is explicable not merely in terms of numbers of silanols but also access to them. In fact close examination of the data do show a trend for the extractions of propranolol and a number of other β-blockers to be less good on the ODS3 material than on either ODS2 or ODS4 silicas, with small but significant loss of material from the ODS3 column at the wash step [5]. The relatively low loading of C_{18} groups on the ODS3 phase probably means that, whilst the overall number of silanol groups is lower than on the other endcapped phases, steric factors do not prevent the ready interaction of the analyte with such silanols as remain. In contrast it seems likely that on the ODS6 phase the access of the analytes to the silanols present on the silica surface might be significantly reduced, both as a result of steric factors and the need for the analyte, which will be positively charged under the conditions used, to penetrate the highly lipophilic C_{18} layer.

4. Conclusions

Solid-state CP-MAS NMR spectroscopy revealed clear differences between a number of different silicas used for SPE. These differences appear to relate to the observed extraction behaviour of the phases suggesting that CP-MAS NMR may be a useful tool for the investigation of these types of phenomena. Given these results it would seem that it would be highly desirable for such information to the generated by the manufacturers of SPE (and indeed bonded phases for chromatography) as part of the quality control process. In this way batch-to-batch variations might become more easily predicted.

Further experiments to explore the utility of CP-MAS NMR in the study of SPE are continuing.

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