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Influence of surface carbon coverage of C_1 (TMS) stationary phases on the separation of nonylphenol ethoxylate ethoxymers

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Abstract

The determination of surfactants in surface waters is required owing to their toxicity to aquatic micro-organisms and potential as endocrine disrupters. We have previously reported a method for the simultaneous separation of linear alkyl benzene sulfonates (LAS) and nonylphenol ethoxylates (NPEO) by high-performance liquid chromatography using a C_1 (TMS) column. In this earlier work we discussed some problems with the resolution of individual ethoxymers from NPEO using C_1 columns from different manufacturers. Here, we postulate that this phenomenon may be linked to carbon coverage of the C_1 (TMS) stationary phases and study this utilising both elemental (bulk) analyses and surface specific analyses by X-ray photoelectron spectroscopy. Data obtained indicate that for the simultaneous separation of the LAS homologues and ethoxymers of NPEO, the stationary phase must have some trimethylsilyl groups bound to the surface of the silica in order to achieve separation of the LAS homologues, however the degree of surface coverage must not be greater than ca. 0.5 $\mu mol/m^2$ in order to achieve adequate resolution of the NPEO ethoxymers. These data support earlier evidence for a ''pseudo'' reversed-phase mechanism for this separation.

Keywords: Stationary phases, LC; Carbon coverage; X-Ray photoelectron spectroscopy; Nonylphenol ethoxylates

1. Introduction

Nonylphenol ethoxylates (NPEO) are a class of non-ionic surfactants that find use in various industrial cleaning processes. Their determination is important as recent work by Jobling and Sumpter [1] has shown that NPEO and their associated degradation products are weakly oestrogenic in nature. This work stemmed from results published by Soto et al. [2] that show nonylphenol (a biodegradation product of NPEO) demonstrates an oestrogenic response with breast cancer cells. Furthermore, Sharpe and co-workers [3,4] have linked these compounds, along with other environmental pollutants, to the apparent decrease in sperm production and an increase in sexual reproductive problems observed throughout the Western Hemisphere.

We have recently reported a method for the simultaneous determination of linear alkylbenzene sulfonates (LAS) and NPEO in surface water [5]. This method is based on the use of an isocratic high-performance liquid chromatography (HPLC) separation using a C_1 (trimethylsilyl, TMS) stationary phase. It allowed the separation of the two classifications of surfactants; with separation by alkyl chain length for the anionic alkylbenzene sulfonates

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and by ethoxymer chain length for the non-ionic alkylphenol polyethoxylates. In this earlier work we commented on the difficulties in reproducing the separation on C_1 columns other than the Spherisorb S5 C_1 column supplied by HiChrom UK. Subsequently we have noted that the separation can no longer be reproduced on columns obtained from this source. Hence we have investigated possible reasons for this variation in column performance.

Previous studies of column performance have tended to rely on chromatographic properties such as retention time, capacity factor and efficiency to provide data on silica stationary phases [6–9]. Bulk carbon analyses [10] and conventional infrared (IR) and nuclear magnetic resonance (NMR) techniques [11–13] have also been employed however the most important chromatographic interactions are thought to occur on the surface of the silica stationary phase. Consequently, highly surface-specific techniques, such as X-ray photoelectron spectroscopy (XPS), have been employed recently to study the outermost regions (first few nm) of bonded-silica stationary phases [14–17]. These studies have shown that the atomic percentages obtained from the surface region of silica stationary phases can be related to the alkyl chain length of the bonded species and also to the surface coverage of the species [17]. In this work, we use both surface atomic percentages obtained via XPS and bulk carbon percentages to calculate surface coverage of the C_1 (TMS) alkyl species and relate these values to the chromatographic performance of the phases, with particular reference to separation of anionic and non-ionic surfactants.

2. Experimental

2.1. Materials

Synperonic NP9 (an NPEO surfactant with an average of nine ethylene oxide units) was a gift from ICI Materials Research Centre (Wilton, Middlesborough, UK). All solvents used were HPLC grade (Fisher Scientific, Loughborough, UK).

2.2. High-performance liquid chromatography

Chromatographic conditions were as reported previously [5].

Spherisorb S5 C_1, 150 mm×4.6 mm I.D. HPLC columns were obtained from HiChrom (Reading, UK). A Supelcosil LC-1 C_1 (TMS), 150 mm×4.6 mm I.D. HPLC column was purchased from Supelco (UK). A Hypersil C_1 SAS, 250 mm×4.6 mm I.D. HPLC column was obtained from Hypersil (Runcorn, UK). Subsequent to HPLC analyses, the columns were washed with HPLC-grade water and methanol, the end fitting removed from each column and the silica stationary phase removed. The alkyl bonded silica was then dried in an oven, to give a dry free-flowing powder sample.

2.3. Elemental analysis

Bulk carbon analysis was performed by Medac (Brunel Science Centre, Egham, UK).

2.4. XPS instrumental parameters

All X-ray photoelectron spectra were produced on a VG ESCALAB Mk II using Al Kα X-rays ($h\nu$= 1486.6 eV). The X-ray gun was operated at 14 kV and 20 mA, giving 280 W. A wide scan spectrum (0–1000 eV) and high-resolution spectra of the Si 2p (95–120 eV), C 1s (275–300 eV), In 3d (435–460 eV) and O 1s (520–545 eV) regions were recorded for each sample. The analyser was operated in fixed transmission mode with a pass energy of 20 eV for both survey scans and narrow scan data. With this instrument all parameters such as the X-ray gun, analyser, etc., were computer controlled with the exception of the sample position, which was controlled manually. VGS 5250 software used on a PDP 11/53 data system was used for data acquisition and analysis. Quantification was performed using the software, and associated sensitivity factors, supplied with the data system. Note that In 3d scans were not included in quantification but were used to indicate the degree of sample coverage of the sample stub.

2.5. XPS sample preparation

Samples for XPS analysis were prepared using an ''indium mirror'' technique. A small square (ca. 2×2 mm) of indium foil (0.25 mm thickness, 99.99% purity; Aldrich, Poole, UK) was applied to a heated XPS sample stub, mounted on a hot plate. Upon melting the indium was spread over the stub, using a

scalpel blade, until a mirrored surface was achieved. This surface was then pressed into the silica sample. After cooling, the stub was shaken to remove any excess silica.

3. Results and discussion

The separation developed by Scullion et al. [5] was, to the authors' knowledge, the first HPLC method for the simultaneous separation of anionic and non-ionic surfactants that featured resolution of both the LAS homologues and NPEO ethoxymers (Fig. 1a). The method was derived from two previous methods for the separation of these classes of surfactant; the resolution of LAS homologues on a C_1 column by Castles et al. [18], and the method by Wang and Fingas [19] for the separation of NPEO ethoxymers using a C_1 column. The new, simultaneous method was produced on the Spherisorb S5 C_1 column (batch No. 1317) supplied by HiChrom. Repeating the method on the Supelcosil LC-1 C_1 (TMS) column purchased from Supelco demonstrated inferior resolution of the NPEO ethoxymers (Fig. 1b). A similar situation was described by Wang and Fingas [19] for the separation of octylphenol ethoxylate ethoxymers on Spherisorb and Supelco C_1 stationary phases.

As previously described, the Spherisorb column from the later batch of packing material (batch No. 1334) was also unable to achieve the same resolution of NPEO ethoxymers as that obtained on batch 1317 (Fig. 1c). In addition, the Hypersil SAS C_1 column was also unable to achieve adequate resolution of NPEO ethoxymers (Fig. 1d).

To investigate this phenomenon, the carbon loading of each of the stationary phases was studied, as it was postulated that this may influence the resolving capabilities of the phases. Bulk carbon loading and surface specific carbon loading were both examined.

The percentage bulk carbon loading data obtained by elemental analysis are shown in Table 1. These data are in good agreement with a previous study by Brown et al. [16] who quoted a figure of 2.14% carbon for bulk carbon analysis of a C_1 phase from Hypersil. This equates well with the figure of 2.03% obtained in this work for the Hypersil column, indicating that any contamination of the phase through use is minimal.

The pattern of results produced by elemental analysis suggests an obvious trend that equates well with the ability of the different alkyl bonded phases to resolve the NPEO ethoxymers. Spherisorb S5 C_1-1317, which produced the best resolution of all of the phases examined, had the least carbon by bulk analysis. Next, in order of increasing percentage carbon, was the phase from Supelco that was also able to separate the ethoxymers but not with the resolution of the Spherisorb S5 C_1-1317. The other two phases, both of which were unable to resolve the ethoxymers of NPEO, showed the highest carbon content. Therefore, it would seem from these results that the phases with the least amount of carbon (by elemental analysis) produced the superior separation.

As the stationary phases under investigation had been produced by a range of manufacturers using differing base silicas, it was also necessary to take the surface area of the silicas into account whilst studying surface coverage. Using Eq. (1) [20], the data for surface coverage, shown in Table 1, were produced. It can be seen that the same pattern for level of coverage occurs when the surface area is considered, with Spherisorb S5 C_1-1317 showing the lowest coverage, at 0.42 $\mu mol/m^2$.

$$N\,(\mu mol/m^2) = \frac{10^6 P_c}{1200 n_c - P_c(M-1)} \cdot \frac{1}{S} \qquad (1)$$

where N is the surface coverage ($\mu mol/m^2$); P_c is the percent carbon of the bonded phase; n_c is the number of carbons in the bonded silane molecule; M is the molecular mass of the bonded silane molecule; and S is the specific surface area of the non-bonded silica (m^2/g).

To further probe the characteristics of each phase, XPS was utilised to investigate the surface carbon loading of the phases. As XPS analyses will probe to a depth of 5–6 nm for this type of sample, the atomic percentages obtained give an indication of the composition of the chromatographically important outer layers of the silica particles. Narrow scan spectra, for oxygen and carbon, obtained for the various stationary phases are shown in Figs. 2 and 3. It can be seen that the narrow scans show two peaks for each element. For the oxygen scans the peak with a binding energy of ca. 537 eV is due to oxygen on the surface of the silica. In the case of the carbon scans, the peak with a binding energy of ca. 287 eV

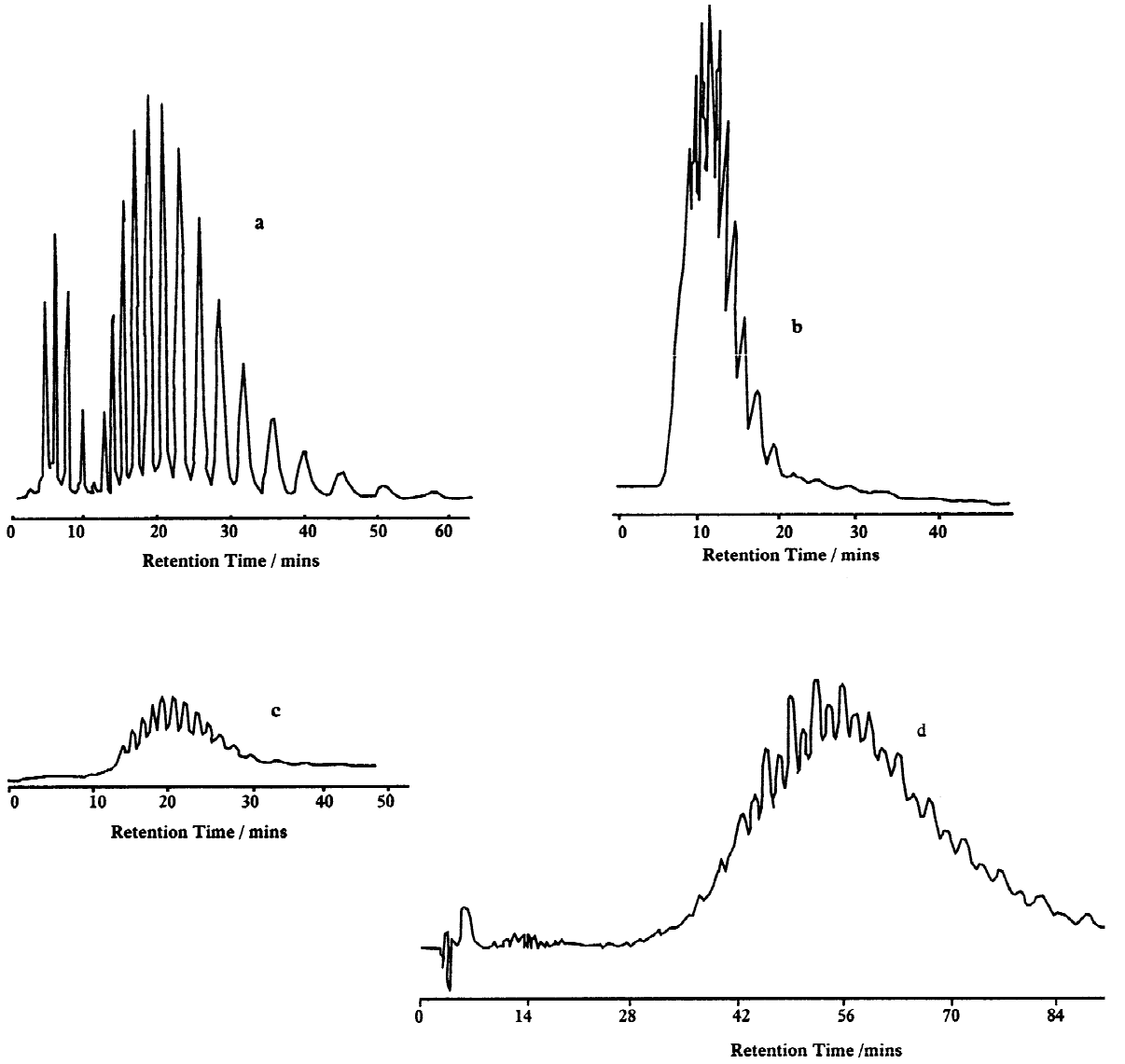

Fig. 1. HPLC chromatograms of Synperonic NP9 produced on (a) older batch (1317) of Spherisorb column [5], (b) Supelco column, (c) later batch (1334) of Spherisorb column and (d) Hypersil column. In (a) peaks arising from linear alkylbenzene (LAS) homologues can be observed between t_r 3 and 11 min and the resolved pattern of NP9 ethoxymers between t_r 12 and 60 min. In (b)–(d) unresolved envelopes of NP9 ethoxymers only are observed, LAS was not injected in these cases.

is due to carbon on the silica surface. The other peak in these scans is due to oxygen and carbon, respectively, on the surface of the indium used in sample preparation. This was identified by running scans on a blank indium mirror, without the addition of silica.

The atomic percentages determined for each of the three elements investigated are shown in Table 1. The carbon/silicon ratios of each of the stationary phases examined are between 0.1 and 0.2; figures which show good agreement with those published by Linton et al. [15] in their study of TMS silica stationary phases by XPS. This correlation supports the previously stated supposition that there is no evidence of contamination of the phases from usage.

Table 1
Bulk and surface specific data produced for four C_1 (TMS) stationary phases

Sample	Bulk carbon[a] (%)	Surface area, S[b] (m^2/g)	Surface coverage, N[c] ($\mu mol/m^2$)	Surface coverage, N[d] ($\mu mol/m^2$)	Data from XPS analyses			
					% O	% Si	% C	C/Si
Spherisorb S5 C_1, batch 1317	0.33	220	0.42	6.1	55.3	40.5	4.2	0.10
Spherisorb S5 C_1, batch 1334	1.45	220	1.89	10.2	56.2	37.4	6.4	0.17
Supelco LC-1	0.93	170	1.55	6.9	59.1	37.2	3.7	0.10
Hypersil SAS	2.03	170	3.45	14.0	54.8	38.5	6.7	0.17

[a] Data obtained by elemental analysis as described in the Experimental section.
[b] Values given in Ref. [21].
[c] Data calculated using Eq. (1) [20] and bulk % C data obtained by elemental analysis.
[d] Data calculated using Eq. (1) [20] and % C data obtained by XPS analysis.

The atomic percentages obtained by XPS are representative of the surface composition of the silica stationary phases and hence, the percentage carbon determined by XPS is thought to be a better representation than bulk carbon of the alkyl-bonded phase available for chromatographic interaction with an

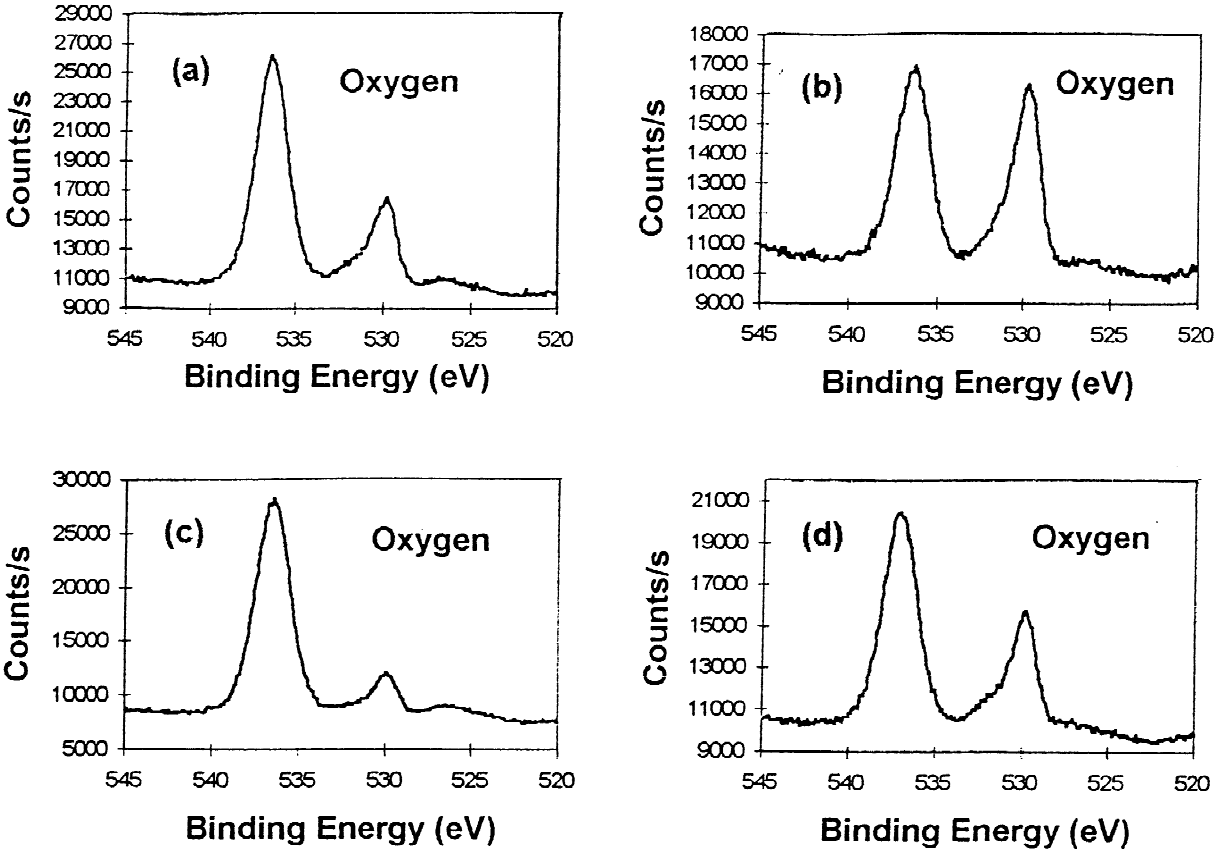

Fig. 2. Oxygen XPS narrow scan spectra for (a) Spherisorb S5 C_1-1334 stationary phase, (b) Spherisorb S5 C_1-1317 stationary phase, (c) Hypersil SAS stationary phase and (d) Supelcosil LC-1 stationary phase.

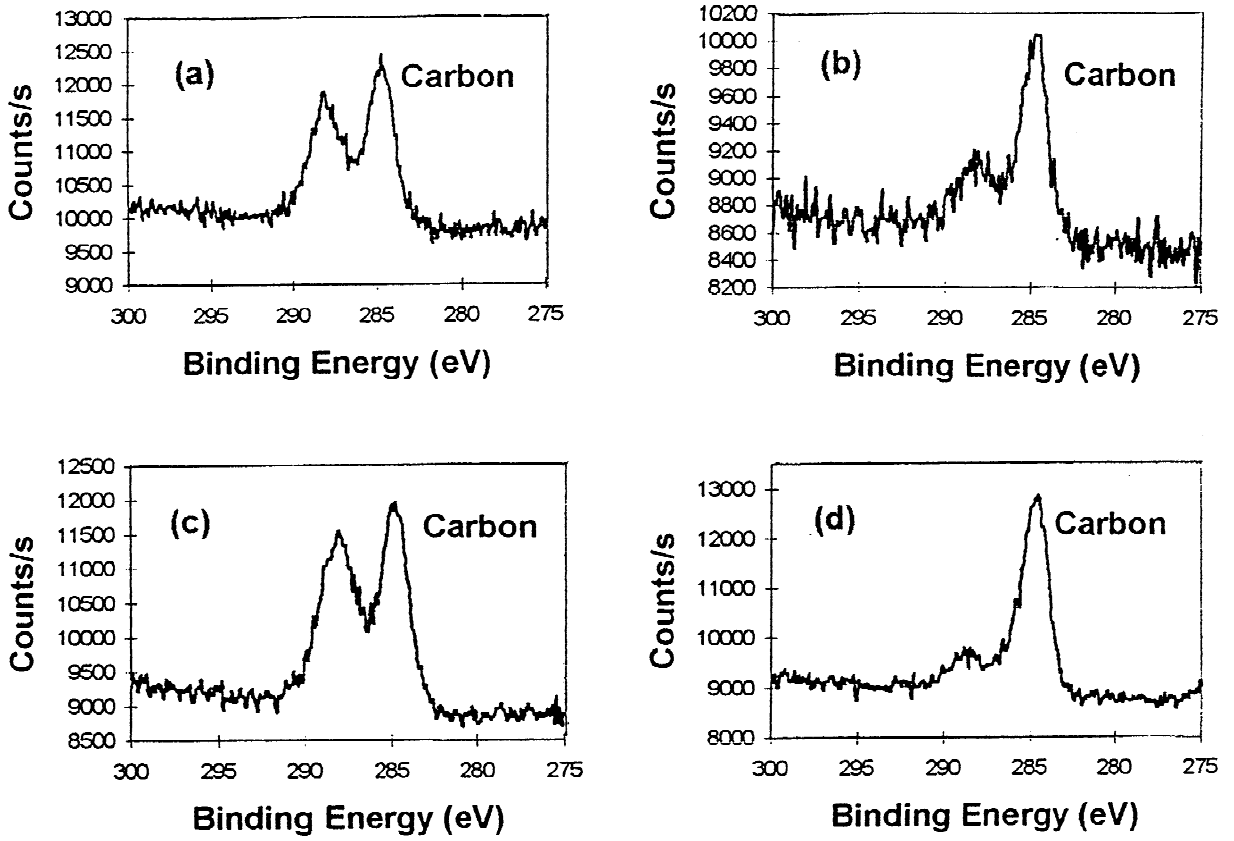

Fig. 3. Carbon XPS narrow scan spectra for (a) Spherisorb S5 C_1-1334 stationary phase, (b) Spherisorb S5 C_1-1317 stationary phase, (c) Hypersil SAS stationary phase and (d) Supelcosil LC-1 stationary phase.

analyte species. A comparison of the percentage carbon determined by XPS with the percentage bulk carbon as determined by elemental analysis shows a different pattern for the four phases studied. It is not surprising that the levels of carbon identified are different because the bulk carbon data display the amount of carbon as a percentage of total sample, whereas the XPS data is as a percentage of the outer layers (5–6 nm) of the alkyl bonded silica particles. Nevertheless, the XPS results do not follow any sensible pattern in relation to the chromatographic performance of the phases. However, since these values do not take into account the surface areas of the base silicas they may be misleading.

The best approximation of surface coverage by the alkyl bonded phase is believed to be achieved by incorporating the surface area (supplied by the manufacturers [21]) of the non-bonded silica with the percentage carbon measured by XPS. To facilitate this, Eq. (1) was used [20]. Although the equation was developed to allow alkyl coverage to be calculated using bulk carbon percentages, it was postulated that it can also be used with surface specific data to give an indication of relative alkyl coverage. The absolute figures produced must be used with caution, however the trend produced, taking surface area into account, can be seen to be comparable with the trends obtained from the bulk data. That is, the Spherisorb S5 C_1-1317 phase also has the lowest relative ''surface'' alkyl coverage, when compared with the other phases studied. The calculated surface carbon coverage is seen to increase as the NPEO resolving power of the various phases decreases. (As expected, the surface carbon

values are much higher than the bulk figures as the alkyl moiety is preferentially bound to the surface of the silica).

It appears, therefore, that for the separation of NPEO on a C_1 (TMS) column low surface coverage of the alkyl moiety gives the best results. This observation is difficult to account for by invoking a purely reversed-phase mechanism for this separation. The separation would appear to involve both an adsorption and a partition mechanism. Evidence for the adsorption mechanism lies in the fact that the most strongly retained species are the most hydrophilic (longest ethoxylate chain) and by the data presented here that show the column with the lowest surface coverage of alkyl material produces the best separation. The fact that increasing the concentration of the organic component of the mobile phase decreased retention times would suggest a partition mechanism. Ibrahim and Wheals have reported the separation of NPEO on a non-bonded silica column [22]. Their separation showed very similar characteristics to the separation achieved here on earlier batches of the Spherisorb S5 C_1 (TMS) column. The authors described their separation as ''pseudo-reversed-phase'', justifying the statement by the fact that, as in this work, it uses typical reversed-phase eluents, but the most strongly retained oligomers were the most hydrophilic. Interestingly, Ibrahim and Wheals also found their separation worked best on Spherisorb silica, compared with Hypersil and LiChrosorb silicas. The mobile phase used was an acetonitrile–phosphate buffer (pH 3) gradient, which again is very similar to that used here.

In reversed-phase or partition chromatography, the most strongly retained solutes are the most nonpolar. In the separations described here, the most strongly retained ethoxymer has the longest ethoxylate chain. Ibrahim and Wheals [22] and Wang and Fingas [19] have suggested that if polarity is based on hydrophilicity, then the most strongly retained ethoxymer, that is, the most hydrophilic, is also the most polar. On this basis, the order of elution achieved here is not characteristic of a reversed-phase mechanism.

Ibrahim and Wheals also found that, after trimethylsilylation of the surface of the Spherisorb silica, resolution of the NPEO ethoxymers was dramatically reduced [22]. This suggests the major retention mechanism in this separation to be hydrogen bonding between surface silanols and the ethoxylate groups on the analyte molecule. This is feasible, as separation is seen to be on the basis of ethoxylate chain length and not alkyl chain length. (Separation of alkylphenol ethoxylate surfactants by alkyl chain length has been shown on C_{18} and C_8 columns by several groups, e.g., Marcomini et al. [23]).

The carbon loading results presented here, along with the findings of other groups [19,22], would seem to indicate that the separation of NPEO on a C_1 column is not a function of the trimethylsilyl groups, but in fact, separation is achieved on the remaining unreacted silanol groups. Indeed, incorporation of trimethylsilyl groups, above a certain concentration, is seen to exhibit a negative effect on the resolution of NPEO ethoxymers.

It must be remembered, however, that in the original work by Scullion et al. [5] LAS homologues were separated along with the NPEO ethoxymers. These LAS homologues were separated by alkyl chain length, a separation which is very typical of a reversed-phase mechanism, the homologue with the shortest alkyl chain eluting first. A reversed-phase separation such as this could not take place on a purely silica stationary phase; the trimethylsilyl moiety bonded to the silica surface on the C_1 phase must be the factor that effects the separation of the LAS homologues. This is supported by the fact that all of the phases used in this study were capable of adequate resolution of the LAS homologues. It would seem therefore, that for the simultaneous separation of the LAS homologues and ethoxymers of NPEO a C_1 stationary phase must be used. The phase must have trimethylsilyl groups bound to the surface of the silica in order to achieve separation of the LAS homologues, but the degree of coverage by the trimethylsilyl moiety must not be greater than ca. 0.5 $\mu mol/m^2$ (calculated from the bulk carbon value) in order to achieve adequate resolution of the NPEO ethoxymers. Results from other studies mentioned above would also suggest that only Spherisorb silica is able to provide this resolution of ethoxymers. The reason for this is unknown at present.

4. Conclusion

Four C_1 (TMS) HPLC stationary phases have

been analysed by elemental analysis and XPS in order to determine the reason for the inability of recent batches of Spherisorb S5 C_1 material to resolve NPEO ethoxymers.

The elemental analysis data for bulk carbon showed good agreement with the work of other authors [16]. These data indicated that the Spherisorb S5 C_1 (batch No. 1317) material obtained from HiChrom (the only phase shown to be capable of resolving the NPEO ethoxymers) contained the lowest percentage of carbon and the lowest surface coverage of the TMS moiety. The more recent batch of the Spherisorb material (batch No. 1334) exhibited a much higher percentage of carbon. While Supelcosil LC-1 stationary phase purchased from Supelco showed a degree of separation, the resolution achieved was inferior to that achieved by the Spherisorb S5 C_1-1317 material; a higher value for percentage carbon was determined by elemental analysis for this phase, along with a higher surface coverage by the TMS moiety.

The percentage carbon determined by XPS provides an alternative representation of carbon available for chromatographic interaction to bulk methods as its surface specificity means that only surface carbon is examined, i.e., carbon which is chromatographically important. Data from the XPS analyses did not produce the same pattern as that seen by elemental analysis. However, when taking the surface area of the base silicas into account, the results did show a similar trend to the bulk elemental results. That is, the XPS results showed that the stationary phase that exhibited the best resolution of NPEO ethoxymers also had the lowest surface coverage of the alkyl moiety.

The results suggest that the separation actually takes place on the unreacted silica sites, and that the presence of the trimethylsilyl groups have a negative effect on the separation of the NPEO ethoxymers. However, since the separation of the LAS homologues is on the basis of alkyl chain length, it has to be assumed that it is the trimethylsilyl groups that effect the separation in this case, and are therefore essential if the separation is to remain simultaneous. In order to investigate this further, work is currently taking place to study the effect of different coverages of the trimethylsilyl moiety bonded to a single base silica, on the simultaneous surfactant separation.

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