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Oligomeric separation of alkylphenol ethoxylate surfactants on silica using aqueous acetonitrile eluents

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Abstract

Alkylphenol ethoxylate surfactants can be separated by high-performance liquid chromatography on Spherisorb silica using pH 3 buffer–acetonitrile eluents. Increasing the organic content of the eluent leads to more rapid elution, but evidence is produced to suggest that hydrogen bonding is the chief retention mechanism and the separation is an example of a “pseudo-reversed-phase” process.

Keywords: Mobile phase composition; Surfactants; Alkylphenol ethoxylate surfactants; Ethoxylates; Triton X-100

1. Introduction

During a recent study of the analytical chemistry of alkylphenol ethoxylate surfactants (APEs) it was observed that when such compounds were injected on to high-performance liquid chromatography (HPLC) columns containing silica, complete binding took place when aqueous buffer solutions were used as the eluent. Addition of methanol or acetonitrile permitted elution to take place and in this respect the separations were a form of “pseudo-reversed-phase” chromatography [1]. Subsequently, investigation has revealed that on one particular HPLC silica (Spherisorb) the chromatography can be adapted to permit the separation of individual oligomers of the surfactant mixtures.

Commercial APEs are usually based on nonylphenol or *tert*-octylphenol and the ethoxylated derivatives used as non-ionic surfactants are complex

mixtures with the ethoxylate repeat group ranging between values $n=3$ to $n=50$. HPLC has been used extensively to achieve analytical separations of oligomeric mixtures of APEs with normal-phase separations on silica [2], aminopropyl-silica [3], diol-silica [4] or cyano-silicas [5], and reversed-phase separations on C_{18} [6] and C_1 [7] modifications. As far as we know, no previous publications have ever reported APE separations on silica using the aqueous eluents usually associated with reversed-phase chromatography.

The complex chemistry of silica surfaces can result in several competing separation mechanisms in HPLC, but adsorption processes are usually maximised under normal-phase conditions, and hydrophobic interactions dominate under reversed-phase conditions. Although it has been noted that mobile phases containing water can produce weak reversed-phase retention on silica [8,9], there have been few practical applications of this phenomenon. The major application of separations on silica using eluents

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usually associated with reversed-phase chromatography is for the analysis of basic drugs. It was in that context that Cox and Stout [1] used the term “pseudo-reversed-phase”, in order to mark out the conditions as anomalous. Basic drug separations on silica were first investigated in depth in the pioneering studies by Jane published in 1975 [10] and have been investigated subsequently by many workers [11,12]. Ion exchange on ionised silanol groups, and hydrophobic interactions with siloxane groups appear to be the dominant separation mechanisms. Size exclusion separations have also been shown to occur on silica under aqueous eluent conditions [13], but by definition such methods are dependent upon the pore structure of the silica matrix rather than its surface chemistry, which is usually rendered as inactive as possible.

In the case of the separation of APEs on silica a quite different retention mechanism seems to operate. The long ethoxylate chains characteristic of this class of non-ionic surfactant apparently hydrogen bond to silanol groups very strongly in completely aqueous eluents and only weaken as the proportion of organic modifier is increased.

2. Experimental

A Varian “Star” fully automated HPLC system was used for these studies. The detector was either a Varian 9065 linear diode array detector operated with the display channel derived from the diode monitoring at 273 nm or 220 nm, or alternatively a Jasco FP-920 fluorescence detector with an excitation wavelength (λ_{ex}) of 230 nm and emission wavelength (λ_{em}) of 302 nm [14]. Stainless-steel columns (12.5 cm \times 4.5 mm I.D.) were packed with the following proprietary 5- μ m silicas: (1) Lichrosorb Si 60, (2) Lichrosorb Si 100, (3) Spherisorb S5w (batches DO5113 and F05187), (4) Hypersil (batch 5-395).

The physical characteristics of these silicas are given in Table 1. The columns were thermally equilibrated in a Spark Holland “Mistral” column oven and the temperature calibration was confirmed with a narrow-range mercury thermometer.

Table 1
Physical chemical data for the 5- μ m HPLC-grade silicas^a

Silica	Surface area (m ² g ⁻¹)	Pore diameter (nm)	Pore volume (cm ³ g ⁻¹)
LiChrosorb Si 60	500	6	0.75
LiChrosorb Si 100	300	10	0.75
Spherisorb	220	8	0.44
Hypersil	170	12	0.60

^a Data derived from Ref. [15].

In addition, one column was packed with Spherisorb silica which had been silanol-deactivated by using the following procedure: 5 g of silica was dried at 100°C for 24 h and then refluxed with 10 ml of chlorotrimethylsilane in 50 ml of toluene (sodium dried). Before refluxing commenced the mixture was subjected to vacuum to draw air from pores in the silica. After refluxing for 1 h the product was filtered through a fritted glass funnel and washed with toluene and then hexane (sodium dried). The product was dried at 100°C overnight before being slurry-packed into the column blank.

Eluents were prepared by mixing pH 3 or pH 7 sodium phosphate buffers (0.014 M) with acetonitrile. For isocratic elution a buffer–acetonitrile ratio of 75:25 (v/v) was most appropriate, but eluents of different proportions were investigated. Gradient elution starting with eluents of buffer–acetonitrile (80:20, v/v) through to (50:50, v/v) offered superior oligomer separations. Methanol and tetrahydrofuran were also investigated as alternative organic modifiers. Flow-rates of 1 ml min⁻¹ were used in all the work. Solutions of surfactants were prepared in water or methanol. The surfactants studied were either Tritons (based on *tert.*-octylphenol) or Synperonics (based on 4-nonylphenol). Concentrations were typically 0.1–10 mg ml⁻¹. Marker compounds with 3 and 9 ethoxylate units were synthesised using *tert.*-octylphenol as the hydrophobe. (These are subsequently referred to as 3 EO and 9 EO).

The non-retained volume was determined by injecting a solution of sodium nitrate and monitoring the breakthrough with the detector set at 220 nm.

3. Results

It was found that unless the concentration of organic modifier in the aqueous eluents exceeded 15–20% the retention of even the lower oligomers of APEs was considerable (e.g. $k' > 10$). Tetrahydrofuran, methanol and acetonitrile were studied as modifiers but superior separations were achieved with the latter solvent. At the same concentration of organic modifier to buffer, oligomers were more strongly retained on Spherisorb at pH 3 than at pH 7 (see Table 2). In the light of these findings, pH 3 buffer–acetonitrile mixtures were the only combinations studied in depth. Of the four silicas studied only Spherisorb gave good oligomer separations, and in Fig. 1 chromatograms of a Triton X-100 mixture eluted with pH 3 buffer–acetonitrile (75:25) are shown on each silica. Superior separations resulted when gradient elution were used and Fig. 2 shows the same test mixture separated on silica using gradient elution conditions [A=pH 3 buffer–acetonitrile (80:20), B=pH 3 buffer–acetonitrile (50:50); A→B over 12 min. B→A for 5 min].

All oligomer separation was lost when the Spherisorb silica was trimethylsilylated. Table 3 shows retention time data for various test compounds on Spherisorb silica and the trimethylsilylated prod-

uct under the gradient elution conditions just described. In Fig. 3, Fig. 4 and Fig. 5 are shown $\log k'$ values as a function of eluent composition, ethoxylate chain length and temperature.

4. Discussion

When we first observed the separation of APEs on Spherisorb silica we assumed that it was a size exclusion phenomenon modified by adsorption processes. Synthesis of the 3 EO and 9 EO oligomers showed that the elution sequence ranged from lower to higher oligomers and hence, it could not be size exclusion. Fig. 4 showed that under isocratic conditions the retention increased proportionally to the ethoxylate chain-length, thereby indicating that it was this part of the surfactant molecules that interacted with the silica. Complete loss of oligomer separation after trimethylsilylation of the silica suggested that it was the silanol groups which were the interactive part of the stationary phase. The pH comparisons are also consistent with a silanol-induced adsorption process as more free silanols would be expected to be present at pH 3 than at pH 7 due to suppression of the ionisation of these groups.

The substantial increase in retention time associated with retention on the trimethylsilylated silica, together with very similar increases shown by the parent phenols as well as the APEs, suggest that the hydrophobic portion of the molecule was the dominant moiety in producing retention once the silica surface was trimethylsilylated. On the unmodified silica it was the hydrophilic portion of the molecule which provided the main retention site. For example, as shown in Table 3, there was a slight difference in the retention of the free phenols on silica (e.g. $k' = 0.80$ and 1.04 for *tert*-octylphenol and nonylphenol, respectively), but when the same compounds were linked to the 9 EO group there was virtually no difference in the retention of the two surfactant oligomers (e.g. $k' = 3.90$ and 3.94 , respectively). On the TMCS-treated silica, however, these two oligomers were well resolved (e.g. $k' = 7.88$ and 8.72 , respectively) suggesting that the phenolic hydrophobe was exerting a strong effect. The break in the linearity of the $\log k'$ curves in Fig. 3 could not be

Table 2
Retention data for Triton X-100 oligomers under gradient elution conditions with pH 3 and pH 7 buffers

Gradient 1			Gradient 2	
pH 3 buffer and acetonitrile			pH 7 buffer and acetonitrile	
<i>n</i>	t_r (min)	k'	t_r (min)	k'
5	6.6	2.7	5.5	2.1
6	7.2	3.0	6.2	2.4
7	7.7	3.3	6.7	2.7
8	8.3	3.6	7.2	3.0
9	8.8	3.9	7.7	3.3
10	9.4	4.2	8.3	3.6
11	9.9	4.5	8.8	3.9
12	10.5	4.9	9.3	4.2
13	11.1	5.2	9.8	4.5
14	11.7	5.5	10.4	4.8
15	12.3	5.8	11.0	5.1
16	12.9	6.1	11.5	5.4

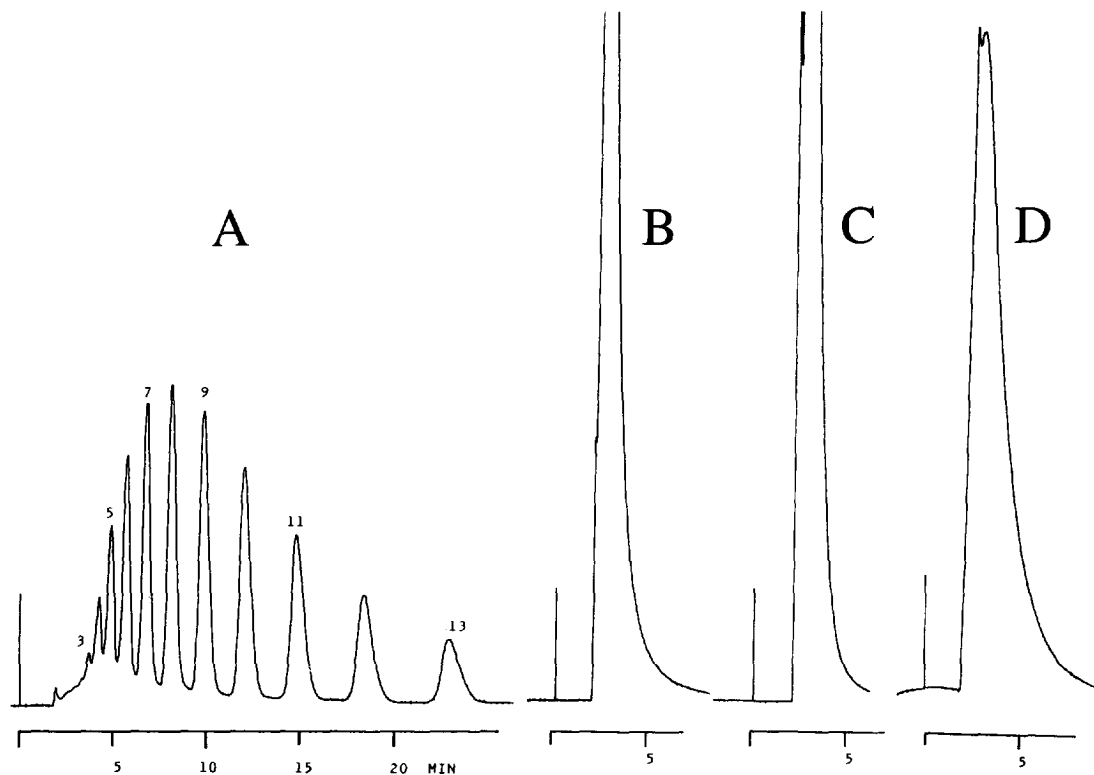


Fig. 1. Chromatograms of Triton X-100 (1 mg/ml) on silica (A) Spherisorb 5 μm , 80 \AA ; (B) Hypersil 5 μm , 120 \AA ; (C) LiChrosorb Si 60 5 μm , 60 \AA ; (D) LiChrosorb Si 100 5 μm , 100 \AA , under isocratic elution conditions, pH 3 buffer-acetonitrile (75:25), with fluorometric detection $\lambda_{\text{ex}} = 230 \text{ nm}$, $\lambda_{\text{em}} = 302 \text{ nm}$; Att. = 128; Rec. = 10 mV.

explained, but it is worth bearing in mind that ethoxylates may be subject to conformational changes in solution. For example, Melander et al. had studied the separation of APEs on C_{18} and C_8 modified silicas [16], and suggested that the surfactant molecules might display two different conformations which they designated zigzag and meandering. The linearity displayed in Fig. 4 and Fig. 5 is perhaps indicative of one major retention mechanism providing the basis of APE oligomer separations and, since silanol and ethoxylate groups are undoubtedly involved, hydrogen bonding seems the most likely explanation for the retention process. As the acetonitrile content of the eluent was increased binding between the surfactant oligomers and the silica weakened, and more rapid elution resulted. Such a separation pattern displays a superficial resemblance

to reversed-phase chromatography. That it is, in fact, an example of a “pseudo-reversed-phase” separation becomes apparent when it is found that the most strongly retained oligomers are those with the greatest hydrophilic character.

Reversed-phase behavior is characterised by a decrease in retention as the mobile phase polarity decreases (i.e. the proportion of organic modifier added to the aqueous eluent increases), and the surfactant separation conforms to this pattern. On the other hand in reversed-phase chromatography the solutes elute in a sequence in which the most polar compounds elute before the less polar. Water solubility studies on alkylphenol ethoxylate oligomers show that their solubility, and by inference their polarity, increases as the length of the ethoxylate group increases [17]. Hence the observed elution

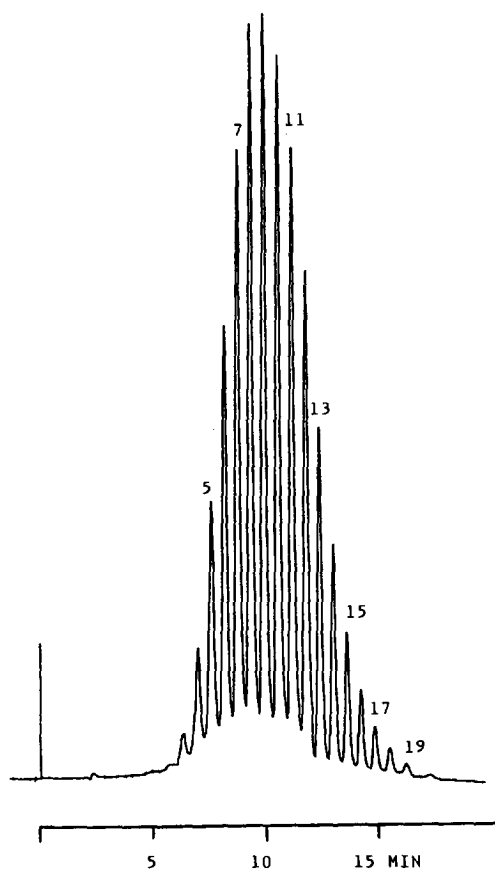


Fig. 2. Chromatogram of Triton X-100 (1 mg/ml) on Spherisorb silica 5- μ m under gradient elution conditions. A = pH 3 buffer-acetonitrile (80:20), B = pH 3 buffer-acetonitrile (50:50); A \rightarrow B over 12 min, B \rightarrow A for 5 min with fluorometric detection λ_{ex} = 230 nm, λ_{em} = 302 nm; Att. = 128; Rec. = 10 mV.

sequence on Spherisorb silica is the reverse of what would be expected for a reversed-phase separation. These separations therefore present a paradox which we are currently unable to explain; they may be an example of "pseudo-reversed-phase" chromatography but they bear little similarity to those investigated by Cox and Stout [1].

Another puzzling feature of our study is the difference between Spherisorb and the other silicas. Fig. 1 demonstrates the remarkable superiority of Spherisorb over the other HPLC silicas. The physical data for these silicas shown in Table 1 provide little clue as to why one should so out-perform the others,

Table 3

Retention data of APE oligomers and the precursor phenols on Spherisorb silica and its trimethylsilylated derivative using gradient elution conditions

Test compound	Silica		TMCS silica	
	t_r (min)	k'	t_r (min)	k'
<i>tert</i> -Octylphenol	3.2	0.80	14.4	7.0
Nonylphenol	3.7	1.04	15.5	7.6
Triton X-100 ($n=9$)	8.8	3.90	16.0	7.88
Synperonic NP10 ($n=9$)	8.9	3.94	17.5	8.72

Conditions

Columns 12.5 cm \times 4.5 mm I.D.

Gradient conditions A=pH 3 buffer-acetonitrile (80:20, v/v)

B=pH 3 buffer-acetonitrile (50:50, v/v)

A \rightarrow B over 12 min; B \rightarrow A over 5 min

Flow-rate 1 ml min⁻¹

Detection Fluorescence at λ_{ex} 230 nm, λ_{em} 302 nm

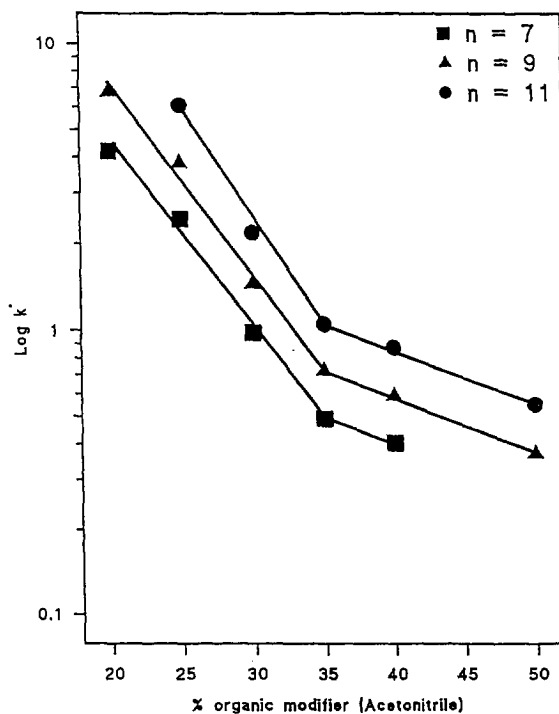


Fig. 3. Variation of log k' value with the percentage organic content of the eluent at 20°C for different Triton X-100 oligomers.

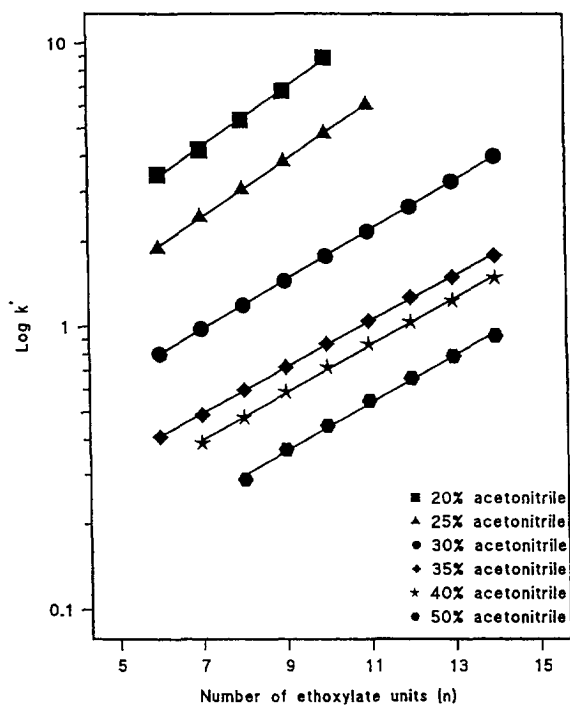


Fig. 4. Variation of $\log k'$ value with the number of oligomer repeat units at 20°C.

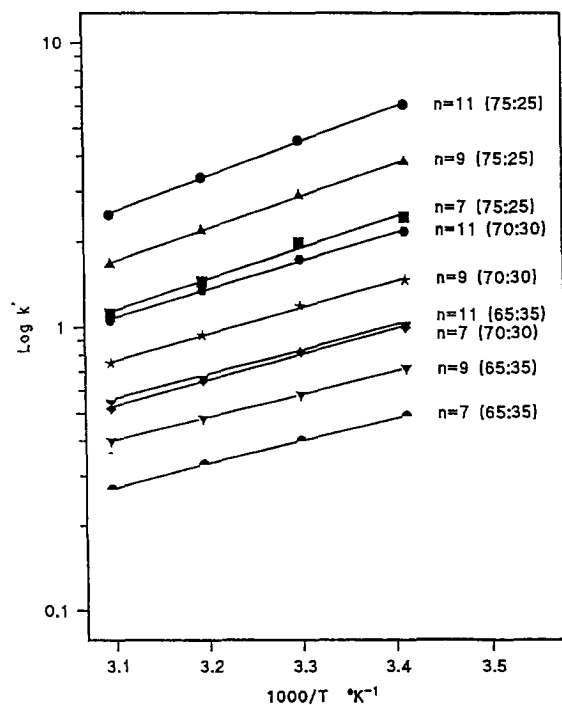


Fig. 5. Variation of $\log k'$ value with $1000/T$ (T = temperature of the separation in K^{-1}) for different Triton X-100 oligomers.

although the Spherisorb had a noticeably lower pore volume than the other silicas tested. Under the conditions used all the silicas would be expected to be fully hydrated, which should maximise the concentration of silanol sites relative to siloxanes. Nevertheless, the complexity of the silica surface is notorious and we were unable to understand why Spherisorb should be such an effective separator of APE oligomers. What would appear to be beyond dispute is that such surfactants offer a convenient probe to provide a greater insight into silica surfaces.

The findings reported above were made in a study that extended over three years and during this period several different samples of Spherisorb silica were purchased and assessed. Despite this only two different batches (Batch Numbers D05113 and F05187) were designated by the manufacturer. Both batches gave identical separations which was reassuring as in basic drug separations batch to batch variation was noted by earlier workers [12].

As mentioned previously oligomeric separations of APEs under reversed-phase and normal-phase conditions have been reported extensively. The elution sequence was usually the same as we observed on silica, although one publication described a reverse elution sequence [18]. The major analytical advantages which we have found with the "pseudo-reverse-phase" conditions for separating APEs on silica were that the column system was very robust, and that many interferences found in aqueous trade effluents were non-retained and hence were chromatographically resolved from APEs. A single column was used for the analysis of trade effluent extracts for over a year, with minimal change to the retention characteristics. Other surfactants e.g. anionic and cationic compounds were found to elute before the APEs and did not complicate the interpretation of the chromatograms. A wide variety of commercial APE surfactants were tested and all eluted under the gradient conditions described earlier

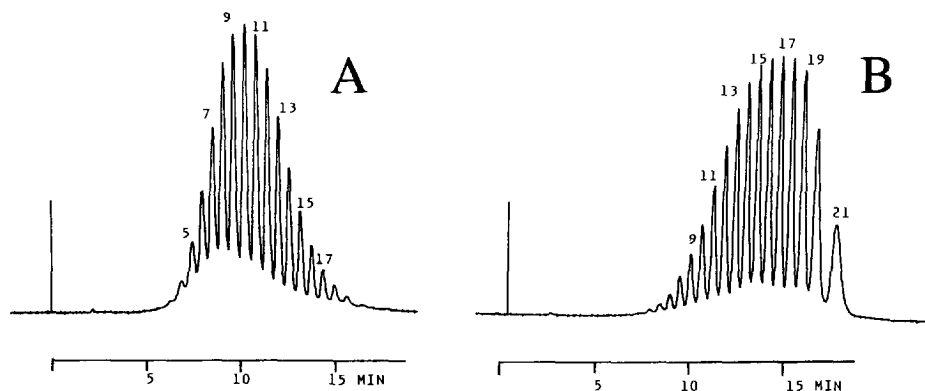


Fig. 6. Separations of 4-nonylphenol ethoxylate oligomers in commercial surfactants A = Synperonic NP10 (0.3 mg/ml); B = Synperonic NP20 (1 mg/ml). Gradient elution as in Fig. 2.

(see Fig. 6), although a somewhat longer gradient would be preferable to provide good separation of oligomers where n is greater than 20.

5. Conclusions

Eluents containing from 20–50% of acetonitrile in aqueous pH 3 phosphate buffers permitted oligomeric mixtures of alkylphenol ethoxylate surfactants to be separated on Spherisorb silica. The retention mechanism is believed to primarily involve hydrogen bonding.

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