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Journal of Chromatography A, 676 (1994) 355–359

JOURNAL OF CHROMATOGRAPHY A

Stationary phase complexation of polyethers: separation of polyethers with amino-bonded silica gel

Tetsuo Okada*, Toshinori Usui

Faculty of Liberal Arts, Shizuoka University, Shizuoka 422, Japan

Received 23 February 1994

Abstract

Polyethers are retained on a common amino-bonded column by forming complexes with protonated amino groups, and then separated according to the complex formation ability. The fact that counter anions strongly affected the retention indicates that the retention mechanism involves not only complexation with the ammonium groups but also ion-pair formation with counter anions. The practical separation of crown ethers and polyoxyethylene chains involved in a variety of non-ionic surfactants is reported.

1. Introduction

The separation of polyethers is of practical importance because they are often used as hydrophilic groups in various surface-active agents [1]. Apart from practical analysis, the separation of polyethers has some interesting features, *e.g.*, their ability to form complexes with hard metal ions can be evaluated when an ion-exchange resin is used for the separation [2–5] and the partitioning of acyclic polyethers to reversed-phase stationary phases reflects the conformational changes of polyether chains [6,7] and the complexation [8], etc. We therefore believe that it is worth developing a novel separation method for polyethers not only to expedite their practical analysis but also to elucidate the polyether chemistry itself.

Amino groups, which in general have been utilized for the separation of sugars [9,10] as functional groups anchored on the stationary phase, donate lone-pair electrons to hydroxyl groups in sugars in their unprotonated forms. Once amino groups have been protonated, they are expected to play the role of cationic sites and to be complexed by polyethers. Amino-bonded silica gel can therefore differentiate polyethers according to the complexation ability with the corresponding ammonium ion. In this paper, we present preliminary results and describe the practical application of amino-bonded silica gel to the separation of some polyethers.

2. Experimental

The chromatographic system was composed of a Tosoh CCPM computer-controlled pump, a Thomastat thermostated water-bath, a

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00280-M

Rheodyne injection valve equipped with a 100-μl sample loop and a Tosoh UV-8020 UV–visible detector. The separation column was a Tosoh TSKgel NH_2-60 stainless-steel column (250 mm × 4.6 mm I.D.) packed with 3-aminopropylsilica gel of 5-μm particle size and was used after the amino groups had been completely protonated with a moderately acidic solution (HCl, etc.). Complete protonation was confirmed by measuring the pH of the effluent from the column. Once the amino groups had been protonated, it was not necessary to take disprotonation into account with neutral mobile phases because of the high pK_a values of aliphatic amines. This was verified by the unchanged retention of a particular polyether over a long period; if disprotonation takes place, the retention of polyethers should decrease because the amino group itself did not act as a Lewis acid and was not complexed by any polyethers. The separation column was immersed in water thermostated at 25°C. Methanol, which has been known as a medium favourable for polyether complexation, was used as a mobile phase.

Reagents were of analytical-reagent grade. Methanol was distilled twice. Distilled, deionized water was used throughout. Polyoxyethylenes (POE) were labeled with 3,5-dinitrobenzyl chloride, if necessary. Crown ethers were synthesized according to the literature [11].

3. Results and discussion

3.1. Choice of counter anion

The primary retention mechanism of polyethers on protonated amino-bonded silica gel is their complexation with the ammonium ion sites in the stationary phase. Unfortunately, the solvation of ammonium ions has not been well investigated in comparison with other simple cations. However, it is possible to predict roughly the solvation of an ammonium ion. The similarity of the complexation behaviour and the crystalline ionic size of ammonium ion to those of potassium and rubidium ions implies a resemblance of solvation [12,13], except that the former is a stronger hydrogen-bond donor than the latter.

It has been reported that the complexation of polyethers with a cation is affected by the counter anion. However, in most reported instances, effects of anions are classified into two major types: (1) hydrogen bonding between the anion and a ligand influences the complexation [13]; (2) in low-permittivity solvents, ion-pair formation is related to the complexation. However, our results clearly indicated that the stationary phase complexation is influenced by the nature of the counter anion despite the use of a polar protic solvent.

Table 1 shows the effects of counter anions on

Table 1
Variation of k' of crown ethers with counter anions in the protonated amino-bonded column

Crown ether[a]	k'						
	SO_4^{2-}	AcO^- [b]	Cl^-	Br^-	I^-	SCN^-	ClO_4^-
B15C5	0.98	1.35	1.62	2.88	5.13	6.45	7.08
B18C6	28.2	42.7	31.6	–[c]	–[c]	–[c]	–[c]
DB18C6	4.47	6.46	5.75	11.22	29.5	34.7	40.7
DB21C7	3.39	5.13	3.39	6.61	17.0	24.5	26.3
DB24C8	0.49	0.62	0.10	0.79	1.28	2.82	2.88
DB30C10	0.23	0.33	0.093	0.54	1.45	2.14	2.75

Methanol was used as the mobile phase.

[a] Abbreviations: B15C5 = benzo-15-crown-5; B18C6 = benzo-18-crown-6; DB18C6 = dibenzo-18-crown-6; DB21C7 = dibenzo-21-crown-7; DB24C8 = dibenzo-24-crown-8; DB30C10 = dibenzo-30-crown-10.

[b] Acetate.

[c] Not determined because of extremely strong retention.

the stationary phase complexation of crown ethers as changes in capacity factors. Once the amino groups in the stationary phase have been protonated, they cannot be disprotonated with either pure methanol or methanol containing a neutral salt. The replacement of a counter anion was therefore possible in the usual manner, *i.e.*, by passing the solution of a salt of the anion of interest through the column. It is obviously that large "hydrophobic" anions enhance the stationary phase complexation, whereas small ions, in other words strong hydrogen bond acceptors, lower the retention (use of the term "hydrophobic" is not strictly appropriate; however, as bulky less hydrated ions such as ClO_4^- usually show the hydrophobic properties in water, this term has been used here). This phenomenon will be related to the retention mechanism. Although details are being studied in our laboratory and will be reported in the near future, it appears that ion-pair formation in the stationary phase is strongly related to the mechanism. In the practical sense, ClO_4^- will be the best choice because the retention is the strongest of the counter anions tested (strong retention can be modified by the addition of various components to the mobile phase, whereas it is difficult to alter weak retention by modifying the mobile phase).

3.2. Separation of polyethers

Separation of POE chains contained in nonionic surfactants is of general and practical interest. Therefore, the present method was first applied to this aspect. As is known, the complexation ability of POE is enhanced by the chain length; in complexation with an alkali metal ion, the complexation ability of POE is almost exponentially enhanced with increasing chain length [3]. Such complexation behaviour strongly indicates a need for gradient elution. Various gradient modes can be considered, *e.g.*, the addition of a solvent unfavourable for the complexation (*e.g.*, water is an example of such solvents), increasing the temperature because the complexation is exothermic [5] and the addition of an appropriate cation to the mobile phase [2]. Of these three methods, we selected the last one because of the flexibility of the choice of the cation; as we quantitatively know the complexation of polyether with a cation employed in the mobile phase, we can easily control the retention of polyethers by both selecting an appropriate cation and varying the concentration of the cation.

Fig. 1 shows the separation of POE chains contained in Triton X-100, where ClO_4^- was chosen as the counter anion because of the strong retention ability of the ClO_4^--form stationary phase. Therefore, a perchlorate salt should be used in the gradient solution to keep the stationary phase in the ClO_4^- form. Of various perchlorate salts, we selected potassium perchlorate for this purpose, because K^+ is one of the best cations for polyether complexation, especially for acyclic polyether complexation, and thus permits the facile control of the retention. UV detection can be utilized to detect the elution of POE oligomers in Triton X-100, and therefore gradient elution is applicable in this case. However, other POE-based surfactants such as Brij 35 do not necessarily involve an effective chromophore. A common method, derivatization with 3,5-dinitrobenzoyl chloride, was used in such cases. Figs. 2–4 show the separation of POE chains contained in Brij 35 and other

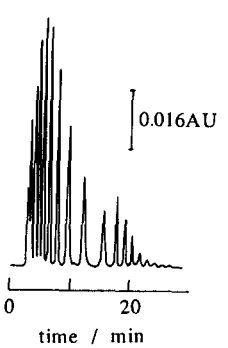

Fig. 1. Separation of POE oligomers contained in Triton X-100. Stationary phase in ClO_4^- form. Sample concentration, 1 mg/ml. Mobile phase, methanol (0–15 min)→5 m*M* $KClO_4$ in methanol (25 min). Detection, UV at 280 nm.

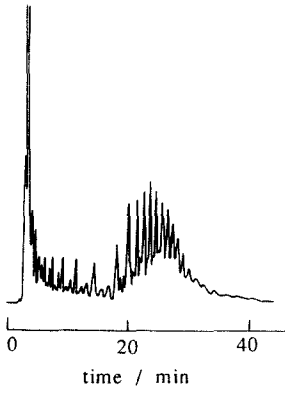

Fig. 2. Separation of POE oligomers contained in Brij 35 [trade-name of POE(23)dodecyl ether]. Stationary phase as in Fig. 1. POE was dinitrobenzylated in advance. Sample concentration, 1 mg/ml. Mobile phase as in Fig. 1. Detection, UV at 250 nm.

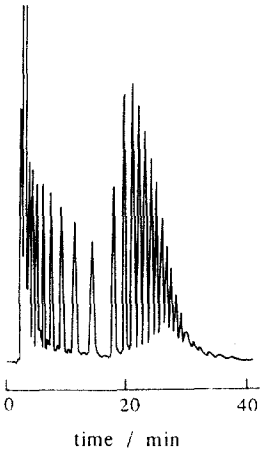

Fig. 4. Separation of POE oligomers contained in POE(20)S. Conditions as in Fig. 2.

POE-based surfactants bearing C_{16} and C_{18} hydrophobic chains.

Some small peaks other than main series of POE chains are visible. Although complete identification was difficult, the comparison of retention times obtained from Figs. 2–4 indicates that these small minor peaks are given by POE chains having different hydrophobic chains. This speculation implies that this separation method can be used for the separation in terms of both hydrophobic and POE chain levels.

Finally, an example of the separation of crown ethers is shown in Fig. 5. Such a separation will not often be needed, but is interesting from a fundamental viewpoint. The selectivity clearly correlates with those of solution complexation with alkylammonium salts; the elution order in DB30C10 < DB24C8 < B15C5 < DB21C7 < DB18C6 < B18C6, and though it was a rough determination, our electrophoretic research [14] indicated that the complexation constants with an ethylammonium ion are $24 < 25 < 25 < 94 < 170 < 370$ in the same order. This indicates that

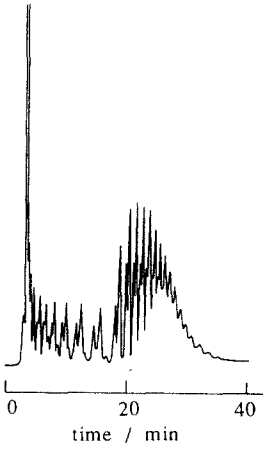

Fig. 3. Separation of POE oligomers contained in POE(20)C. Conditions as in Fig. 2.

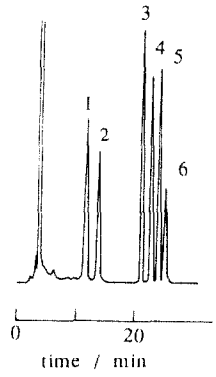

Fig. 5. Separation of crown ethers. Sample concentration, $2.5 \cdot 10^{-5}$ *M*. Mobile phase, methanol (0–25 min)→5 m*M* $KClO_4$ in methanol. Detection, UV at 280 nm. Peaks: 1 = DB30C10; 2 = DB24C8; 3 = B15C5; 4 = DB21C7; 5 = DB18C6; 6 = B18C6. Abbreviations are given in Table I.

the primary retention mechanism is complexation of polyethers with protonated amino groups in the stationary phase. In addition, effects of counter anions should be elucidated, in future work.

Acknowledgements

We thank Tosoh for the gift of the separation column. This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Education, Science and Culture, Japan.

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