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SEPARATION OF THE CONDENSATION PRODUCTS
OF β-NAPHTHALENESULPHONIC ACID AND FORMALDEHYDE
BY SALTING-OUT CHROMATOGRAPHY

SHINTARO FUDANO AND KAZUO KONISHI

Industrial Research Laboratories, Kao Soap Co., Ltd., 1334 Minato-yakushubata, Wakayama-shi (Japan)

SUMMARY

The condensates of β-naphthalenesulphonic acid and formaldehyde were separated into their component compounds by salting-out chromatography. The analytical conditions studied were as follows: column, 25 mm I.D. and 1960 mm long; resin, CM-Sephadex C-50; developing solution, a 0.005 M aqueous solution of magnesium chloride. The component compounds of the condensates were separated into at least seven peaks. The peak of the highly condensed part that could not be separated by this method was analysed using a curve resolver (Model 310, Du Pont Institute). The relationship between the proportion of component compounds and the condensation time was examined.

INTRODUCTION

The sodium salt of the condensates of β-naphthalenesulphonic acid and formaldehyde has been widely used as a dispersing agent for organic and inorganic powders. Relatively few analytical methods have been studied to examine manufacturing conditions and to control the product.

AMBLER[1] has distinguished four components from the condensates by electrophoresis. NEGORO and coworkers[2-4] have proved that the condensates have multiple (more than four) components by paper chromatography (PC). Also, HATTORI AND TANINO[5] have shown by PC that this dispersing agent consists of at least nine components. However, their methods do not comply with the requirements of the composition of the condensates or of more detailed studies of the reaction.

SARGENT AND RIEMAN[6] were first to succeed in separating a mixture of non-electrolytic organic compounds by salting-out chromatography using an ion-exchange resin that demonstrated the usefulness of salting-out chromatography as a separation method.

The present paper, one of a series[9] of studies on the application of this technique to the analysis of anionic surface active agents, describes the separation of the com-

ponent compounds of the condensates of β-naphthalenesulphonic acid and formaldehyde by salting-out chromatography.

EXPERIMENTAL

Apparatus

The columns used were 25 mm I.D. and 500 mm long and 25 mm I.D. and 1000 mm long (Sephadex column, Model K25/100, Pharmacia Fine Chemicals, Sweden). A Uvicord II detector (LKB-Produkter AB Co., Ltd., Sweden) was employed for automatically recording the elution curve, as well as for the detection. The polyethylene tube (1.8 mm O.D.) was used to connect the column with the detector. After passing through the detector, the eluate was directed to the fraction collector (Model SF-160K, Toyo-kagaku Co., Ltd.). The fraction collector and the recorder were connected electrically and a mark showing each fraction was drawn to show the elution volume. For quantitative determination of the components, a differential refractometer (Model R-4, Waters Associates Inc.) was employed as a detector. A curve resolver (Model 310, Du Pont Inst.) was used to analyse the chromatograms.

Reagents and sample

The following 11 types of resins were examined. (1) Amberlite CG-50 (100–200 mesh, weakly acidic cation exchange), (2) Amberlite CG-120 (100–200 mesh, strongly acidic cation exchange), (3) Amberlite 200 (macroreticular type), (4) Dowex 50W X2, X4 and X8 (strongly acidic cation exchange), (5) Sephadex G-50 (dextran gel), (6) CM-Sephadex C-25 and C-50 (weakly acidic cation exchange, carboxy methyl functional groups), and (7) SE-Sephadex C-25 and C-50 (strongly acidic cation exchange, sulphoethyl functional groups).

Sodium salts of the condensates of β-naphthalenesulphonic acid and formaldehyde were manufactured by our company. All reagents should be of analytical reagent grade.

Procedure

Salting-out chromatography was performed as follows. Firstly the resin was changed completely from the sodium to magnesium form using a *ca.* 1 *M* aqueous solution of magnesium chloride. Secondly, the resin was allowed to stand 4 times with 2-h intervals in contact with the eluent (0.005 *M* aqueous magnesium chloride). The resin was poured into the column (25 × 2000 mm) by the wet method, after which the resin bed in the column was settled by passing the eluent through for 5 h. One ml of sample solution (1 ml of solution contains 10 mg of sample) was carefully adsorbed on top of the resin bed; then the inside wall of the column was washed 3 times with the same eluent. The flow rate was controlled to 0.1 ml/min and the chromatogram was recorded. Moreover, the peak of the highly condensed parts which did not separate completely was analysed using a curve resolver.

RESULTS

The chromatographic conditions such as resins, salting-out reagents, eluent concentration, pH of eluent, sample size, flow rate and column temperature were

investigated. As the effect of the column temperature on the elution curve was not remarkable, the elution was carried out at room temperature (25°).

Selection of resin and salting-out reagent

For separation by salting-out chromatography, the resin and salting-out reagent are the most important factors. A preliminary examination of them was carried out. When using Amberlite CG-50, Amberlite CG-120 resin and ammonium sulphate as salting-out reagent, the separation of each component compound for a sample was very incomplete because the highly condensed parts were simultaneously eluted. One assumes that the sample molecules cannot enter the inside of the resin phase because of its high cross-linkage. Three kinds of Dowex resins having different degrees of cross-linking were examined, but they gave unsatisfactory separations. Amberlite 200 was unsuitable because the adsorption of a sample on the resin was too strong, the surface area being large. The best separation was obtained using CM-Sephadex C-50 as a Sephadex ion-exchange resin. Consequently, four kinds of Sephadex ion-exchangers and eight salting-out reagents (lithium chloride, sodium chloride, potassium chloride and ammonium sulphate as univalent inorganic salts, and magnesium chloride, calcium chloride, strontium chloride and barium chloride as bivalent salts) were carried out. The results are shown in Figs. 1 and 2. The concentration of the salting-out reagent was controlled to 1.0 M for the univalent inorganic salts and to 0.005 M for the bivalent salts. From Figs. 1 and 2, it appears that the best separation was obtained using CM-Sephadex C-50 and magnesium chloride; consequently this system was selected.

Concentration of eluent

The effect of the concentration of the eluent on the separation was investigated using magnesium chloride as a salting-out reagent. The column used was 25 mm I.D. and 500 mm long and the concentrations of the eluent tested were 0.05, 0.01, 0.005
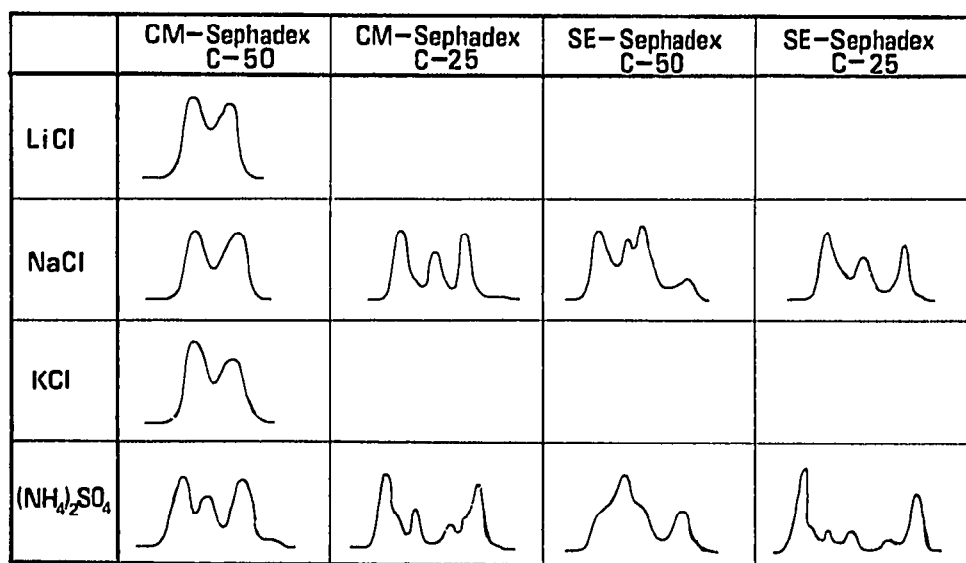
	CM-Sephadex C-50	CM-Sephadex C-25	SE-Sephadex C-50	SE-Sephadex C-25
LiCl	⌇			
NaCl	⌇	⌇	⌇	⌇
KCl	⌇			
(NH₄)₂SO₄	⌇	⌇	⌇	⌇

Fig. 1. Effect of resin and salting-out reagent on elution curve. Column, 500 × 25 mm I.D.; salt concentration, 1.0 M.

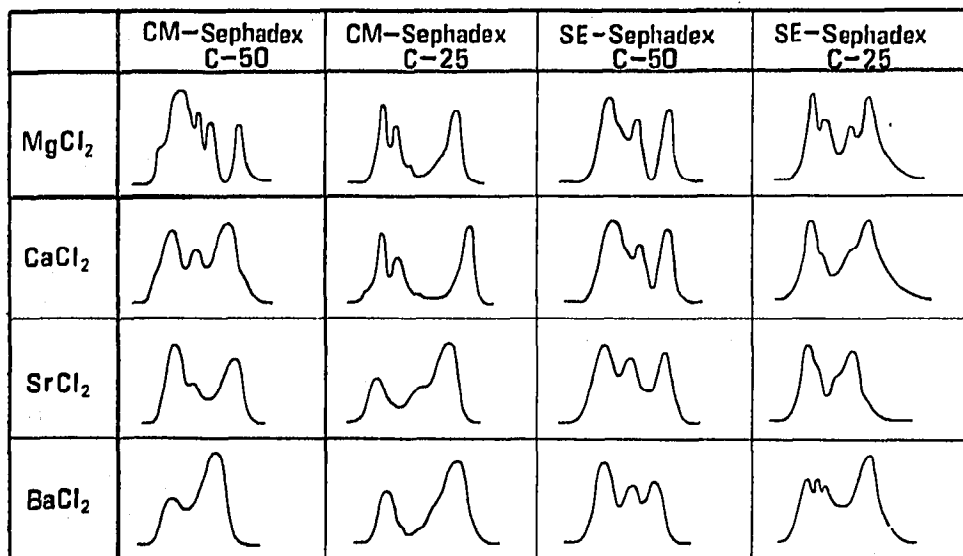|	CM—Sephadex C-50	CM—Sephadex C-25	SE—Sephadex C-50	SE—Sephadex C-25
MgCl₂				
CaCl₂				
SrCl₂				
BaCl₂				

Fig. 2. Effect of resin and salting-out reagent on elution curve. Column, 500 × 25 mm I.D.; salt concentration, 0.005 M.

and 0.001 M. The result in Fig. 3 shows that the lower the concentration of the eluent, the faster the elution of the sample. With a 0.001 M eluent, the sample eluted immediately and the separation was also incomplete. Using a 0.05 M eluent, the peaks were separated insufficiently and the sample was found to be adsorbed on the resin.
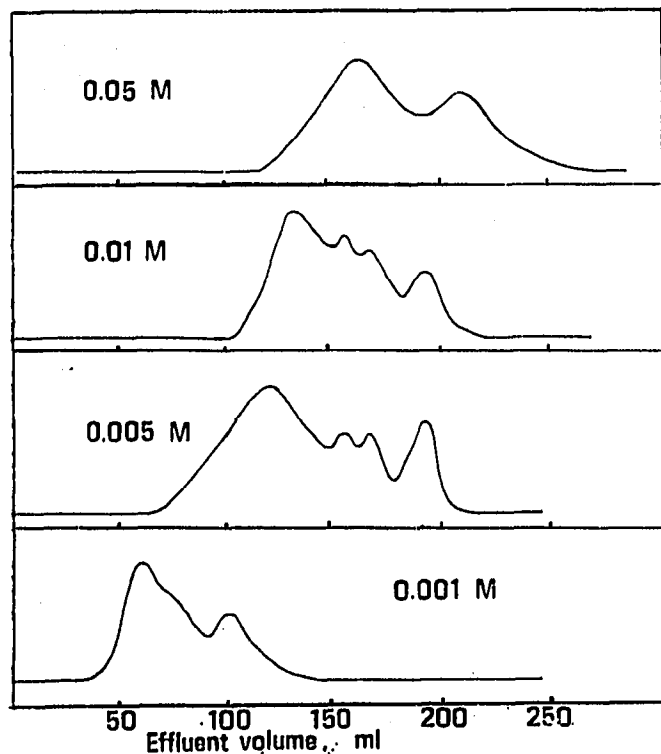
Fig. 3. Effect of concentration of eluent on elution curve. Column, 500 × 25 mm I.D.; resin, CM-Sephadex C-50; eluent, 0.005 M MgCl₂.

When the concentration was 0.005 M, separation was best. Thus, the concentration of the eluent was fixed at 0.005 M.

pH of eluent

It is assumed that the pH value of the eluent plays some role in the separation, as CM-Sephadex has ion-exchange groups. Therefore, the effect of the pH value of the eluent on the separation was studied. HATTORI AND TANINO[5] separated the same sample by PC by adding formic acid to a butyl alcohol–water system. Thus, the eluent was acidified by adding formic acid to pH 4.15 and 2.98. The pH value of the eluent was adjusted to 8.1 using magnesium hydroxide and to 9.6 using sodium hydroxide to investigate the effect of a basic eluent. In the case of the acidic eluent, the elution curve showed a worse separation than the results obtained hitherto and a considerable adsorption was found. In the basic system (pH 9.6), the separation was also incomplete and an unnatural elution curve was obtained. When the pH value of the eluent was 8.1, the best separation was obtained.

Sample size

The relationship between the separation and the sample size, ranging from 2.5 to 20 mg, was investigated. It was found that the smaller the sample size, the better the separation. A 10-mg sample was selected, considering the sensitivity of the detector and the stability of the recorder.

Flow rate

The effect of flow rate on the peak resolution and the number of theoretical plates was studied using a column 25 mm I.D. and 950 mm long. With a flow rate of 0.42 and 0.09 ml/min, the peak resolution calculated using the monomer and dimer peaks was 1.40 and 1.56, respectively, and the number of theoretical plates was 699 and 1480. These results show that the lower the flow rate, the better the separation. Consequently, the flow rate was selected at 0.1 ml/min because a very low flow rate is too time consuming.

Chromatogram and curve analysis

A sample was separated under the conditions previously described under *Procedure*. The chromatogram is shown in Fig. 4. The last elution peak is sodium β-naph-
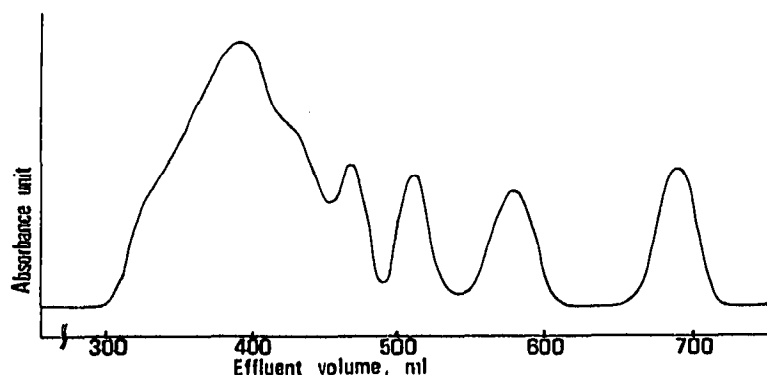

Fig. 4. Elution curve. Column, 1950 × 25 mm I.D.; resin, CM-Sephadex C-50; eluent, 0.005 M MgCl$_2$; flow rate, 0.09 ml/min; sample size, 10 mg.
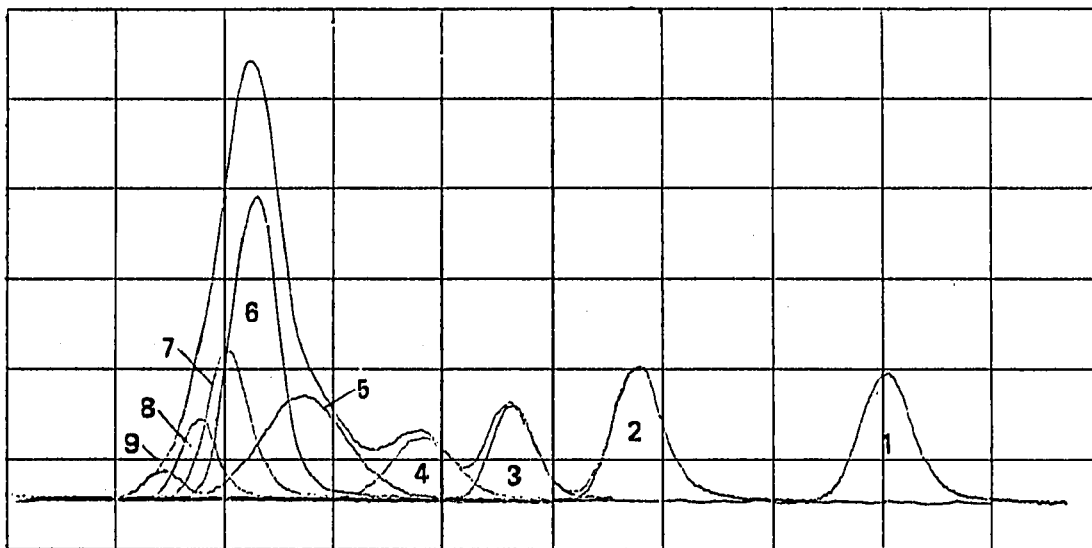
Fig. 5. Curve resolution of chromatogram. Area (%): (1) 9.4, (2) 9.6, (3) 6.0, (4) 6.0, (5) 13.3, (6) 39.5, (7) 9.8, (8) 4.7, (9) 1.6.

thalenesulphonate (monomer) and the degree of the condensation of the component compounds increases in turn toward the small elution volume. HATTORI AND TANINO[5] reported that these condensates contained nine components. Fig. 5 shows the analysis of a curve by a curve resolver according to the information given above. Furthermore, the relationship between the condensation time and the proportion of each component compound was investigated by analysing chromatograms of six
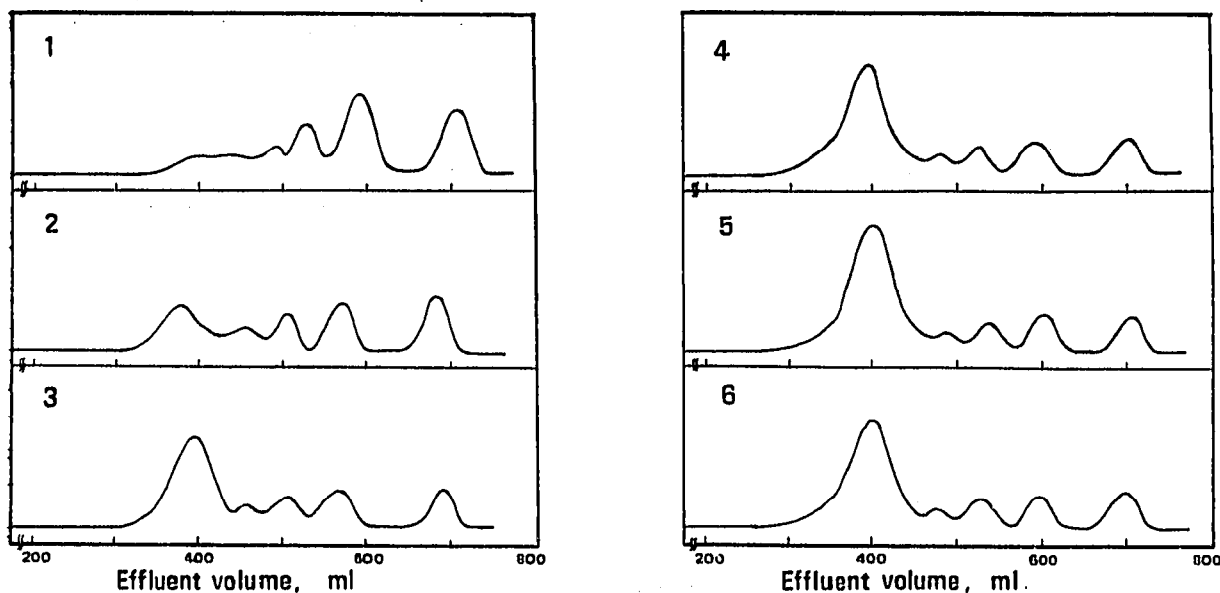

Fig. 6. Elution curve of a low condensed sample. Column, 1960 × 25 mm I.D.; eluent, 0.005 M MgCl₂; resin, CM-Sephadex C-50. Condensation time: (1) 2 h, (2) 4 h, (3) 8 h, (4) 12 h, (5) 16 h, (6) 20 h.

condensates for which the condensation time differed. The chromatograms are shown in Fig. 6. The condensation time increases with the number in the figure. Fig. 7 shows the relationship between condensation time and the proportion of each component compound using curve analysis.

DISCUSSION

When using Sephadex G-50, having no ion-exchange groups in the resins, there was no separation, showing that the sample molecules do not only separate by a molecular-sieve effect but also by a salting-out effect.

When the pH of the eluent was controlled to 9.6 by sodium hydroxide, an unnatural elution curve was obtained, probably because the effect of the sodium and magnesium ions overlaps. Magnesium hydroxide is suitable to control the pH value of the eluent because it has the same cation as a salting-out reagent.

From the curve analysis of the elution curve of the condensates, it appears that the main components of the condensates were the hexamer and heptamer (Fig. 5). This agrees with the results reported by HATTORI[7].

The reaction rate of the formation of dimer in the condensation has been resolved by HATTORI et al.[8], but the analysis of the more highly condensed parts has not been carried out as yet. From Fig. 7 it seems that the highly condensed parts are formed
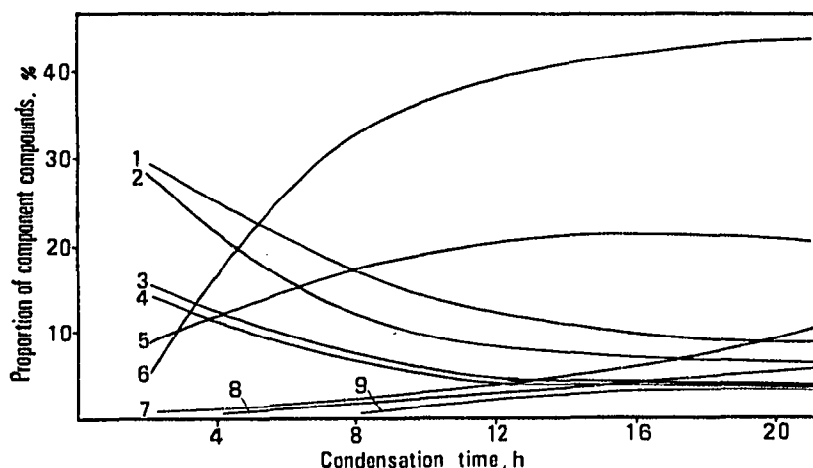

Fig. 7. Relationship between condensation time and composition ratio of each homologue. 1, 2, and 3 in the figure express monomer, dimer and trimer.

by the condensation of the low condensates with each other, because the increasing rate of hexamer and the decreasing rate of dimer are higher than the decreasing rate of monomer.

ACKNOWLEDGEMENT

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