Analysis of High Molecular Substances by Chromatography. I. Paper Chromatography of Polyethylene Oxide

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Introduction

Studies of the molecular weight distribution of a variety of high polymers, which would be highly interesting, are at present impracticable because of the limitations and tediousness of conventional fractionation procedures. The recent success of paper chromatography in the separation of closely related chemical species in the fields of the organic and inorganic chemistry and the brilliant result of chromatographic "frontal analysis" of high polymers by S. Claesson,(1) together with its defects, have directed our attention to the possibility of applying paper chromatography to the analysis and fractionation of high polymers, referring to the case of partition chromatogaphy of nitrocellulose suggested by M. C. Brooks and R. M. Badger.(2)

The principal difficulties encountered in chromatography of high polymers are supposed to be: (1) difficulty in detection of the chromatograms, because polymers have in general neither color nor color reaction, (2) difficulty in the development under the condition of equilibrium of the solute between the fixed and mobile phases, because the velocity of establishing the equilibrium of high polymers is in general very slow compared with the ordinary flow rate of the developing solvent, (3) difficulty in preventing the unexpected disturbance of the solvent flow, which makes the chromatographic separation imperfect, especially in case of high polymers consisting of the continuous homologues. Overcoming these difficulties mentioned above, S. Claesson has first succeeded in the adsorption chromatography of polymers employing the frotal analysis of Tiselius. The results obtained, however have some defects related to the chromatography of adsorption type and especially to frontal analysis of homologues. That is, there is no determinate means to know whether the distribution in molecular weights is the only factor which dominates the distribution of polymer species suggested by the chromatograms obtained and the method, although very useful for analytical purposes, seems unavailable for fractionating purposes of polymers.

We have chosen polyethylene oxide of relatively low molecular weight as the first example of the hydrophilic polymers, and investigated the chromatographic separation with the use of the usual technique of paper chromatography. The difficulties and defects mentioned above have been overcome by a new technique of color development of polyethylene oxide, the selection of a suitable developing solvent, and the general features of paper chromatography.

Experiment

(A) Sample: Commercial polyethylene oxide, "carbowax 1,000", "carbowax 4,000" and "carbowax 6,000" of Carbide and Carbon Chemicals Corp. without special purification were used as the fractionated polyethylene oxide samples, which have the general properties as shown in Table 1.

Table 1

Properties of Carbowaxes

Name	Freezing Rrange (°C.)	Solubility in Water (% by weight)
Carbowax 1,000	35—40	70
〃 4,000	50—55	62
〃 6,000	58—62	50

Before the preparation of the sample solutions the crushed samples were dried for seven days ("1,000") and three days ("4,000" and "6,000") in a desiccator over calcium chloride to constant weights because they are somewhat hygroscopic.

(B) Development: With the use of micropipette 0.5 μl. of 2% aqueous solution of carbowax was put in the diameter of 3-5 mm. on filter paper strip (Toyo Roshi No. 3, for the quantitative analysis, 45 cm.×1 cm.), which beforehand has been dried for one day in a desiccator over calcium chloride in order to hold the constant amount of adsorbed water and to make the developed spot sharper. The position of the original

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(1) S. Claesson, *Arkiv Kem. Mineral. Geol.*, **26** A, No. 24, 1 (1949).

(2) M. C. Brooks and R. M. Badger, *J. Am. Chem. Soc.*, **72**, 1705 (1950).

spot was 8 cm above the surface of the developing solvent when set up in the apparatus, at which the flow rate of the solvent becomes stationary. Six strips were placed in a desiccator over calcium chloride until the original spot dried up and set up in the apparatus⁽³⁾ as shown in Fig. 1, which was held in the thermostatt of 25±0.1°C, and developed upwards with solvent for 4-6 hrs. making the distance between the solvent front and the position of the original spot 12-15 cm. The longer time interval between the putting of the sample on the paper and the development has a tendency to leave more parts of the sample of higher molecular weight at the original point.

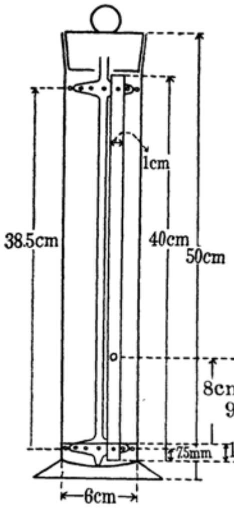

Fig. 1.—Apparatus for development.

(C) Color development: The strips developed were dried in room atmosphere, sprayed with 1/30% ethanolic solution of fluorescein, and dried naturally again. The presence of polyethylene oxide on the paper was detected as a white spot on a yellow background. The limiting amount and concentration of detection of this method were approximately 0.5 μg. polyethylene oxide per 0.5 μl water, as shown in Fig. 2. This value, however, was affected by the amount of impurity in the paper, which may cause some interference in the yellow color development of fluorescein on the paper.

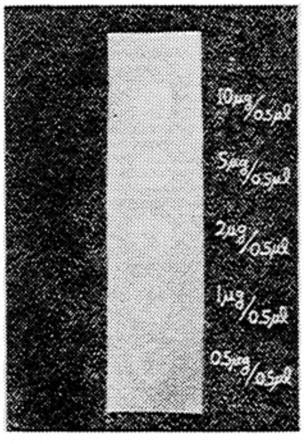

Fig. 2.—Limiting amount and concentration of detection.

Results and Discussions

The developing solvent benzene-chloroform system was at first employed, which has been investigated by G. V. Schultz et al.⁽⁴⁾ as the solvent useful for the partition fractionation of polyethylene oxide, water being used as the parent solvent. Because of the lack of affinity with the paper, however, this solvent was not suitable for the developing solvent in paper chromatography. Among various solvents involving butanol as the principal component the butanol-ethanol-water system was found to be the most suitable for the paper chromatography of polyethylene oxide. The R_f values obtained with the use of this solvent are given in Table 2, in the case of the single samples. The symbol x corresponds to the concentration of ethanol in a mixture consisting of 100 parts of butanol, 100 parts of water and x parts of ethanol in volume. The upper layer was taken for developing in case the system separates into two layers at 25±0.1°C. The system consists completely of one phase for x values larger than 40. The R_f values are calculated as

Table 2

R_f Values of Single Samples

x	$(R_f \pm \Delta R_f)\times 100$		
	Carbowax 1,000	Carbowax 4,000	Carbowax 6,000
0	22±0(22)	3±0(9)	0±0(4)
5	28±0(20)	2±0(6)	0±0(4)
10	38±0(22)	5±0(8)	0±0(4)
15	42±1(15)	7±0(10)	0±0(4)
20	50±1(16)	9±0(11)	0±0(6)
25	58±1(16)	17±0(13)	0±0(5)
30	63±2(14)	33±1(14)	6±1(10)
35	67±2(8)	46±2(8)	25±2(10)
40	76±2(10)	56±2(9)	36±2(8)
50	77±1(6)	71±2(5)	59±1(5)
60	77±1(8)	73±2(6)	66±2(6)
70	77±1(7)	76±3(5)	74±2(5)

$$R_f = (l_1 + l_2)/2l \qquad \text{(cf. Fig. 3)}$$

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(3) cf. K. Otozai, *J. Japan. Chem.*, **4**, 411 (1950).

(4) G. V. Schultz and E. Nordt, *J. prakt. Chem.*, **155**, 115 (1940).

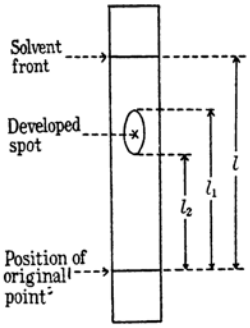

Fig. 3.

and the numerical values in brackets represent the values calculated as $(l_1-l_2)/2l$, the latter of which would correlate to the proper divergence of the spot during chromatography and the distribution of the molecular weight of polyethylene oxide used. These figures are the mean values in several experiments.

The relations between R_f values of single samples and the concentrations of ethanol in the solvent are given in Fig. 4. Furthermore the values in Fig. 4 were rearranged in Fig. 5, in which the values of the average molecular weight $\bar{M}$ of each sample were obtained by the viscosity measurement of aqueous solutions at 20±0.01°C, using the K_m constant 1.8×10^{-4} for 0.5% solution.(4)

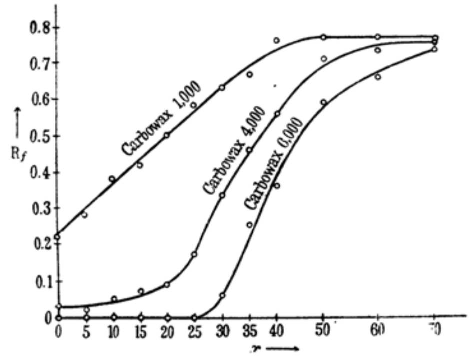

Fig. 4.—Relations between R_f and concentration of ethanol.

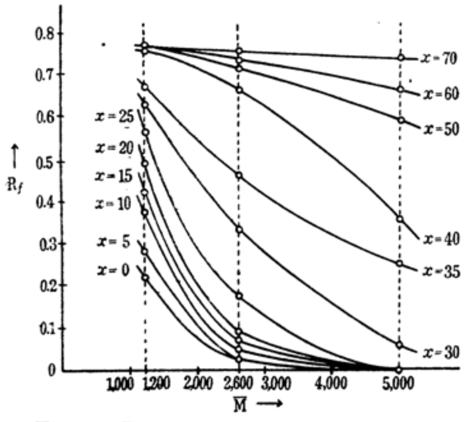

Fig. 5.—Relations between R_f and the average molecular weight.

From the form of the curves in Fig. 5 the most suitable solvent for the separation of three samples is that of $x=35$, because this solvent gives approximately linear and steep curve. A demonstration of the practical separation of the three samples by the use of this solvent is shown in Fig. 6 together with the individual developments of single samples for comparison, and R_f values obtained were shown in Table 3. which represent the good independency of each sample in paper chromatography.

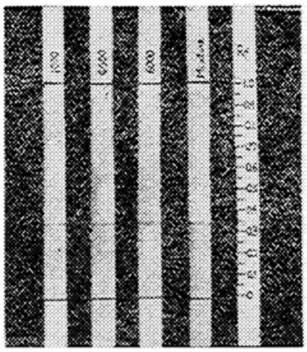

Fig. 6.—Separation of three components.

Table 3

Comparison of R_f Values of Single and Mixed Samples

$(R_f\pm\Delta R_f)\times100$

	Carbowax 1,000	Carbowax 4,000	Carbowax 6,000
Single	67±2(8)	46±2(8)	25±2(10)
Mixed	68±3(9)	45±3(7)	28±3(7)

Using the curve ($x=35$) in Fig. 5 as a working curve one can readily determine the average molecular weight of polyethylene oxide, although it is only relative, in the range of 1,000-5,000, where the molecular weight is determined only with difficulty by every known method at present. Thus employing paper chromatography a new simple relative method of determination of the average molecular weight, and a possibility of a simple method of determination of distribution function, and finally a possibility of fractionation of hydrophilic polymers have been proposed, using polyethylene oxide of molecular weight 1,000-5,000 as an illustration.

Summary

(1) It was found that polyethylene oxide on paper can readily be recognized with fluorescein as an adsorption indicator.

(2) It was found that paper chromatography can be applied also for polyethylene oxide in the range of the average molecular weight 1,000-5,000, as the first case for hydrophilic polymer, in the same manner as low molecular weight substances.

(3) It was found that paper chromatography gives a simple new relative method of determination of the average molecular weight of polyethylene oxide in the range mentioned above, where all the other conventional methods encounter serious diffiiculties.

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