CHROM. 4764

GEL PERMEATION CHROMATOGRAPHY OF THE LINEAR MONOMER AND OLIGOMERS IN POLYAMIDES

SADAO MORI
Laboratory of Chemistry, Faculty of Engineering, Yamanashi University, Kofu (Japan)
AND
TSUGIO TAKEUCHI
Department of Synthetic Chemistry, Faculty of Engineering, Nagoya University, Nagoya (Japan)

(Received April 3rd, 1970)

SUMMARY

The linear monomers and oligomers in nylon 6, nylon 66, and nylon 12 were separated and determined by gel permeation chromatography using Sephadex LH-20 as the gel and 0.05 *N* HCl–methanol as eluant. The water–ethanol extractables of the polyamides were reacted with 2,4-dinitrofluorobenzene and the reaction solution was chromatographed. The effluent was channeled into a flow cell and the absorbance of the DNP-derivatives of the linear monomer and oligomers separated was measured continuously by a spectrophotometer in the 370 to 450 mμ range. The cyclic monomers and oligomers did not interfere with the separation and determination of the linear monomer and oligomers. Higher oligomers, up to the octamer of nylon 6, the tetramer of nylon 66, and the hexamer of nylon 12, were separated and determined. As little as 0.01 μmole of the DNP-derivatives has been determined. A linear relationship was obtained between the elution volume and the logarithm of the molecular weight of monomer and oligomers from which that of the dinitrophenyl group had been subtracted.

INTRODUCTION

In order to study the kinetics of polymerization of caprolactam or polycondensation of the bifunctional compound such as diamine-dicarboxylic acid salt or ω-amino acid, it is necessary to know the linear monomer and oligomer content in the equilibrium product, as well as cyclic monomer and oligomer content. The linear monomer and oligomers are formed by the hydrolysis of the amide group of the corresponding cyclic monomer and oligomers or of the polyamide, and by the amide-interchange reaction of the polyamide. They remain in the final polymerization product in low concentrations. In addition, ε-aminocaproic acid (linear monomer of nylon 6) is used, in some cases, instead of water as the initiator for the polymerization of caprolactam to nylon 6.

Paper chromatography has been widely used in the separation of ε-aminocaproic acid and the linear oligomers of nylon 6 (refs. 1–4). HERMANS and co-workers[1] separated the aminocaproic acid from the alcohol extractables of nylon 6 on a paper chromatogram by use of a suitable eluant, and evaluated the spot visually against known aminocaproic acid standards after developing it with ninhydrin. The concentration of aminocaproic acid in the equilibrium polymer ranged from 0.0008 to 0.005 mmole per g of polymer[5]. The linear monomer, dimer and the higher oligomers were separated by paper chromatography (PC) with a solvent mixture of *sec.*-butanol–20% aq. ammonia (80 : 20) and the concentration of each component on the paper chromatogram was determined colorimetricaly by extracting the component from the paper into water and by developing the color of the component with sodium hypochlorite solution[2]. The components of the initial phase products of the polymerization of ε-caprolactam were examined and separated into linear monomer to pentamer fractions with isobutanol–acetic acid–water (4 : 1 : 1) on the paper chromatogram[3]. Thin-layer chromatography (TLC) was also possible[6]. The linear monomer and oligomers of nylon 66 have been separated in a similar manner by PC[7,8].

The methods described above are complicated, time-consuming and give poor reproducibility for the purpose of quantitative analysis of each component. ROBINSON[9] has reported a polarographic procedure for the analysis of aminocaproic acid in nylon 6. The aminocaproic acid was reacted with formaldehyde to form the Schiff base and polarographic reduction of the Schiff base was carried out in an alkaline medium. This procedure has very good possibilities for the analysis of the linear monomers of other types of polyamide, but in the case cited caprolactam and oligomers included in the water extractables depressed the wave for the Schiff base. Furthermore, the linear dimer and higher oligomers could not be detected by ROBINSON's procedure.

Gel permeation chromatography has enabled us to separate the cyclic monomers and oligomers in nylon 6 and nylon 66 and each component was determined continuously by UV spectrophotometry[10]. The linear monomer and oligomers could not be detected in these experiments because of their low concentration in the polymers and low molar absorption coefficient at 210 mμ.

Since its introduction in 1945 by SANGER[11], 1-fluoro-2,4-dinitrobenzene (DNFB) has frequently been employed as an analytical reagent in studies involving proteins, peptides, amino acids, and amines. Various techniques for identifying the 2,4-dinitrophenyl (DNP)-derivatives have been reported[12]. KESNER and co-workers[13] have separated ether- and water-soluble DNP-derivatives of amino acids, peptides and amines by means of silica gel column chromatography with a gradient elution system. The absorbance of the effluent was measured at 340 mμ. SATAKE and co-workers[14] have reported the automatic analysis of amino acids and peptides based on dinitrophenylation with DNFB which was used for the colorimetry instead of ninhydrin[14]. The DNFB reaction has been applied to the determination of amine end groups in nylon 6, by reacting nylon fiber heterogeneously[15] or homogeneously[16] with DNFB.

The method described in this paper gives quantitative results for the linear monomers and oligomers in nylon 6, nylon 66, and nylon 12. The linear monomers and oligomers are converted into the DNP-derivatives with DNFB and their separation

accomplished by means of gel permeation chromatography. The gels used in this study were Sephadex LH-20 (Pharmacia) which was used with organic solvents and Bio-Beads S-X2 (Bio-Rad Lab.) made of cross-linked polystyrene. The effluent was passed through a flow cell from which the absorbance was continuously recorded. The previous isolation of the cyclic monomer and oligomers from the extractables was not necessary.

EXPERIMENTAL

Materials

The gels used were Sephadex LH-20 (particle size 25–100 μ, Lot. No. 1152) and Bio-Beads S-X2 (particle size 200–400 mesh, control No. 6604). The solvent regains were determined in our laboratory. The eluant was a 0.05 *N* hydrochloric acid solution in methanol for LH-20 and a 0.05 *N* hydrochloric acid solution in methanol–benzene (1 : 1) for Bio-Beads. DNFB and ε-aminocaproic acid (ACA) (linear monomer of nylon 6) were commercial products of reagent grade. A sodium borate–sodium phosphate buffer solution[14] was prepared as follows: 0.1 *M* sodium borate solution was mixed with 0.8 *M* mono-sodium dihydrogen phosphate solution until the pH of the mixture was near 8.5. The linear dimer, trimer and tetramer of nylon 6 were synthesized according to VAN DER WANT's method[17,18]. The higher linear oligomers of nylon 6, the linear monomer and oligomers of nylon 66 and nylon 12 were obtained by extracting them from the corresponding polymers.

Packing

Packing of the gels in the columns was carried out according to the method previously reported[10]. Columns and fittings were also identical.

Dinitrophenylation

A 10-mg portion of ACA was weighed into a 30-ml round-bottomed flask and 700 mg of DNFB (50-fold molal excess), 12 ml of ethanol, 1 ml of the buffer solution, and 2 ml of water were added. The flask was attached to a water-cooled condenser and the solution was refluxed on a water bath for 1 h. After cooling, the solution was transferred to a 25-ml volumetric flask and made up to volume with 0.05 *N* HCl–methanol. DNP-oligomers of nylon 6 (dimer to tetramer) were prepared in the same manner, adding 50-fold molal excess of DNFB-reagent.

Both linear and cyclic monomers and oligomers were extracted from 10 g of the corresponding polymers with 85% ethanol and the extract solution was evaporated nearly to dryness. The dried extract was transferred to a 30-ml round-bottomed flask and 500 mg of DNFB, 6 ml of ethanol, 0.5 ml of the buffer solution and 0.5 ml of water were added. After the reaction, the mixture was transferred to a 10-ml volumetric flask and diluted to volume.

Elution

A 1-ml portion of the solution of the DNP-derivatives was placed on the column. A constant flow rate was kept at 30 ml/h for the Sephadex columns and 20 ml/h for the Bio-Beads column. Blue Dextran 2000 (Pharmacia), methylated with dimethylsulphate[19], was also applied in order to determine the void volume (V_0)

of the columns. The other conditions and procedures were as described in a previous report[10].

Detection

The effluent was delivered to a flow cell adapted to the Hitachi spectrophotometer Model 124 equipped with an absorbance recorder. The absorbance of the linear monomer and oligomer dinitrophenylates in the effluent was recorded continuously. The wavelength was selected in the range of 370 mμ and 450 mμ according to their concentration and elution position.

Calibration

Calibration curves were plotted from prepared solutions of DNP-ACA. The solutions were prepared so that the concentration of DNP-ACA varied within the range expected. Injection of the solutions was made in duplicate at each wavelength. The calibration curves were prepared by plotting the peak area (unit: absorbance × ml) *vs.* the concentration of DNP-ACA (mmole/l) for each wavelength.

RESULTS AND DISCUSSION

The reaction rate of DNFB with amine groups in ethanol depends upon the water content and the pH. The rate increases with increasing water content up to about 15% by weight[16], and also increases ten-fold on increasing the pH value by 1 unit[14]. The dinitrophenylation procedure described under EXPERIMENTAL was developed in the light of these considerations. Under the prescribed conditions, the reaction was complete in 1 h. If the refluxing was stopped in 30 min, the conversion rate was not satisfactory—only about 70%.

The UV and visible absorption spectra of DNP-ACA and the reagent blanks (dinitrophenol, dinitrophenetole, and unreacted DNFB) in 0.05 *N* HCl–methanol are shown in Fig. 1. The maximum molar absorption coefficient (ε) of DNP-ACA in

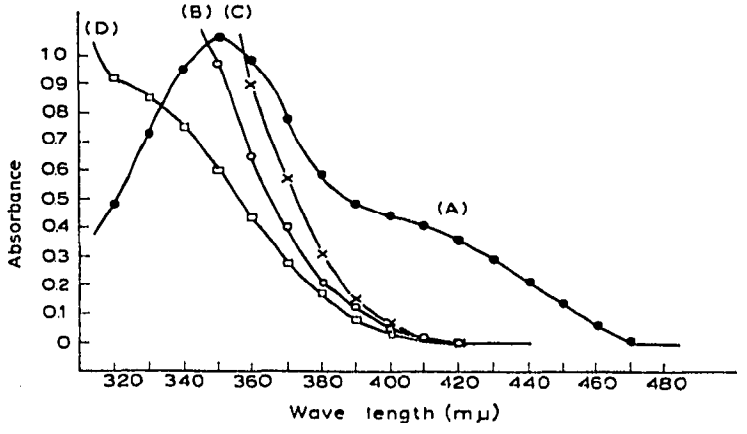

Fig. 1. Spectra of DNP-ACA and reagent blanks. (A) 22.5 mg (0.076 mequiv.) of DNP-ACA in 1250 ml of 0.05 *N* HCl–methanol. (B) 700 mg of DNFB, 12 ml ethanol, and 200 mg $NaHCO_3$ were added and refluxed for 1 h and then diluted to 1250 ml with 0.05 *N* HCl–methanol. (C) 700 mg of DNFB, 12 ml of water, and 1 ml of the buffer solution were added and refluxed for 1 h and then diluted to 1250 ml with 0.05 *N* HCl–methanol. (D) 600 mg of DNFB in 1250 ml of 0.05 *N* HCl–methanol. Cell length: 1 cm; reference: 0.05 *N* HCl–methanol.

TABLE I

THE MOLAR ABSORPTION COEFFICIENTS OF DNP-ACA

Wavelength (mμ)	*M.A.C.*[a] ($l\ cm^{-1}\ moles^{-1}$)
360	16 050
370	12 700
380	9 530
390	7 870
400	7 220
410	6 660
420	5 890
430	4 740
440	3 950
450	2 210

[a] Measured in 0.05 *N* HCl–methanol.

0.05 *N* HCl–methanol was measured as 17 400 at 351 mμ. This wavelength could be employed for measurement of DNP-derivatives, if no blank materials elute near the DNP-derivatives. The molar absorption coefficients of DNP-ACA in 0.05 *N* HCl–methanol were also measured in the range of 360 and 450 mμ and listed in Table I. The absorbance of the reagent blanks decreases on the acid side, and in the range 420–450 mμ the effect of the reagent blanks is negligible. A 0.05 *N* HCl–methanol solution was thus employed as an eluant. Provided that the elution peaks of DNP-derivatives and the reagent blanks do not overlap, the behavior of the reagent blanks deserves little consideration. In the chromatography as described later, the influence of the reagent blanks must be considered. The choice of 410 mμ (Fig. 3) is a compromise between the sensitivity and magnitude of the reagent blanks. The molar absorption coefficients of DNP-oligomers were considered to be almost identical to that of DNP-ACA.

The column constants showing the column performance for Sephadex LH-20 and Bio-Beads S-X2 are listed in Table II. The elution volume, 143.5 ml, on the

TABLE II

COLUMN CONSTANTS

	Column		
	LB	*LC*	*S*
Type of gel	LH-20	LH-20	S-X2
Column head; cm	91	142	90
Dry gel weight, a; g	41.7	65.1	65
Total volume of gel bed, V_t; ml	174	271	172
Void volume, V_0; ml	72.5	113	50
Inner volume, V_i; ml	71	111	62
Volume of gel phase, V_x; ml	101.5	158	122
Volume of gel matrix, V_g; ml	30.5	47	60
Solvent regain, S_r; ml/g of dry gel	1.7	1.7	0.95
Bed volume per g dry gel; ml	4.2	4.2	2.6

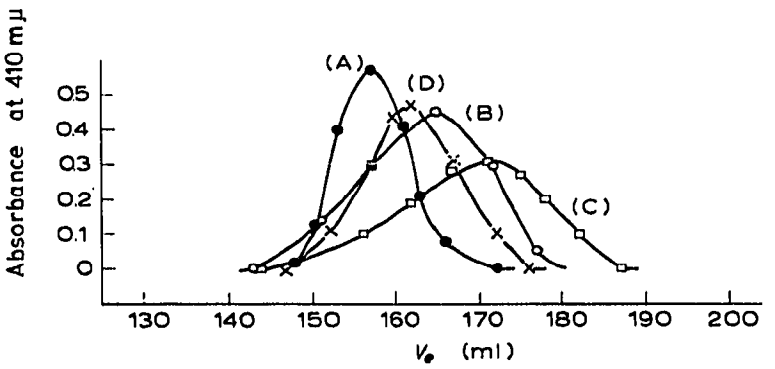

Fig. 2. Elution behavior of DNP-ACA in several solutions on the LB column. Sample volume injected: 1 ml. (A) A 15 mg portion of DNP-ACA was dissolved in 25 ml of ethanol. (B) A 9.8 mg portion of ACA and 1 ml of the buffer solution were refluxed with DNFB in ethanol and diluted to 25 ml with ethanol. (C) A 14.9 mg portion of DNP-ACA and 10 ml of the buffer solution were mixed and diluted to 25 ml with ethanol. (D) A 10.1 mg portion of ACA and 1 ml of ammonia (28%) were refluxed with DNFB in ethanol and diluted to 25 ml with ethanol.

LB column represents a K_d value of unity. If a solute has a higher K_d value than unity, it implies that adsorption (or something else to retard the elution) is taking place between the solute and the gel matrix. The elution behavior of DNP-ACA in the presence of electrolyte on the LB column is shown in Fig. 2. The elution volume of DNP-ACA is between 157 and 171 ml and the K_d value is higher than unity. An electrolyte, such as sodium borate (buffer solution), sodium bicarbonate, retards the elution of DNP-ACA by 8 to 14 ml and in addition broadens the peak. The neutralization of the alkali with hydrochloric acid was not efficacious. The use of ammonia instead of alkali was effective in eliminating the influence of the electrolyte. Without use of alkali, buffer solution, or ammonia the conversion rate of ACA to DNP-ACA was only about 77%, even after refluxing for 1 h. The effect of the electrolyte will be discussed separately[20].

The elution curves of the linear monomer and oligomer of nylon 6, nylon 66, and nylon 12 on the LH-20 column are shown in Fig. 3. Peaks representing unreacted DNFB, dinitrophenol, and dinitrophenetole appear before and after the DNP-ACA (Fig. 3A) and are not shown. Fig. 3 demonstrates good resolution in the fractionation of a mixture of the linear monomer and oligomers. The higher oligomers (up to the octamer of nylon 6, the tetramer of nylon 66, and hexamer of nylon 12) were separated and determined by use of the LC column (LH-20, gel bed 142 cm). The elution volumes of the linear monomer and oligomers on each gel column are presented in Table III. The elution volume of DNP-ACA is large in comparison with the value of $V_0 + V_t$ in Table II. This result shows that the elution of the DNP-derivatives was retarded by adsorption or some other mechanism. A linear relationship can be seen (Fig. 4) between the elution volume and logarithm of the molecular weight of the raw monomer and oligomers minus that of the dinitrophenyl group. The relationship between elution volume and molecular weight of DNP-derivatives will be discussed in detail in a separate paper[20].

The linear monomer and oligomers of nylon 66 are generally classified into three types from their end groups as follows: diamine type (which has two amine groups in both ends), dicarboxylic acid type (two carboxylic acids), and amino acid type (an amine group and a carboxylic acid group). In Fig. 4, the plot of the

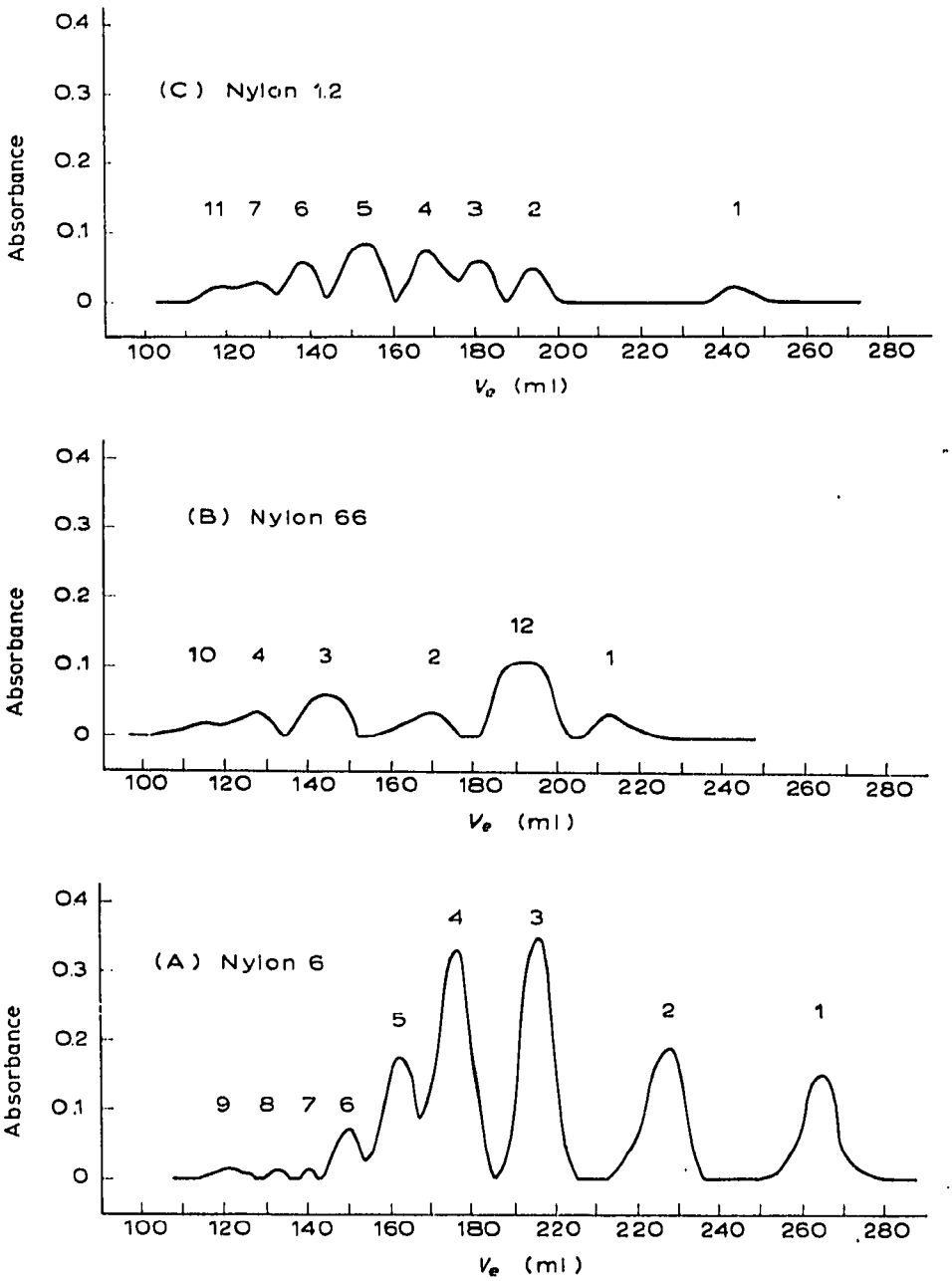

Fig. 3. Elution chromatograms of the DNP-derivatives of linear monomer and oligomers in polyamides on the LC column (LH-20). (A) Absorbances were measured at 370 mμ for oligomers and 450 mμ for the monomer. (B) Absorbances were measured at 410 mμ. (C) Absorbances were measured at 410 mμ for oligomers and 450 mμ for the monomer. 1 = monomer; 2 = dimer; 3 = trimer; 4 = tetramer; 5 = pentamer; 6 = hexamer; 7 = heptamer; 8 = octamer; 9 = nonamer and higher; 10 = pentamer and higher; 11 = octamer and higher; 12 = diamine type monomer of nylon 66.

elution volumes of the monomer and oligomers of nylon 66 fitted the positions for the molecular weight of the amino acid type and a linear relationship was obtained. From this result it was concluded that the linear monomer and oligomers of nylon 66 are of the amino acid type. A solute which eluted between the monomer and the dimer

TABLE III

THE ELUTION VOLUMES (V_e) (ml) OF THE DNP-DERIVATIVES OF LINEAR MONOMER AND OLIGOMERS

Oligomer	*Nylon 6*				*Nylon 66*			*Nylon 12*		
	Column			*Mol. wt.*	*Column*		*Mol.*[a] *wt.*	*Column*		*Mol. wt.*
	LB	*LC*	*S*		*LB*	*LC*		*LB*	*LC*	
Monomer	165	264	114	131	124	212	244	153	242	215
Dimer	130	227	102	244	102	170	470	116	193	410
Trimer	115	195	64	357	88	143	696	102	180	605
Tetramer	103	176	59	470	78	127	922	91	167	800
Pentamer	95	162		583				83	153	995
Hexamer	88	150		696				78	137	1190
Heptamer	82	140		809					127	1385
Octamer	72	132		922						
Higher	~75	114 ~128								

[a] The molecular weights of the linear monomer and oligomers of nylon 66 were calculated by assuming that they have both amino and carboxyl groups as end groups.

of nylon 66 (in Fig. 3B 113 ml on Column LB and 193 ml on Column LC) is looked upon as the linear monomer of the diamine type (molecular weight 342).

Peak areas were calculated from the elution chromatograms of the dinitrophenylated extractables of each polyamide, and the concentration (mmole/l) of each DNP-derivative was determined from the calibration curves. The content of each monomer or individual oligomer in the polymer was also calculated on a weight percentage basis from the molecular weight of corresponding monomer or oligomer and the weight of the polyamide from which the monomer and oligomers were extracted. The results are shown in Table IV. Consistent results were obtained with known mixtures of the monomer and oligomers, provided that the conditions for

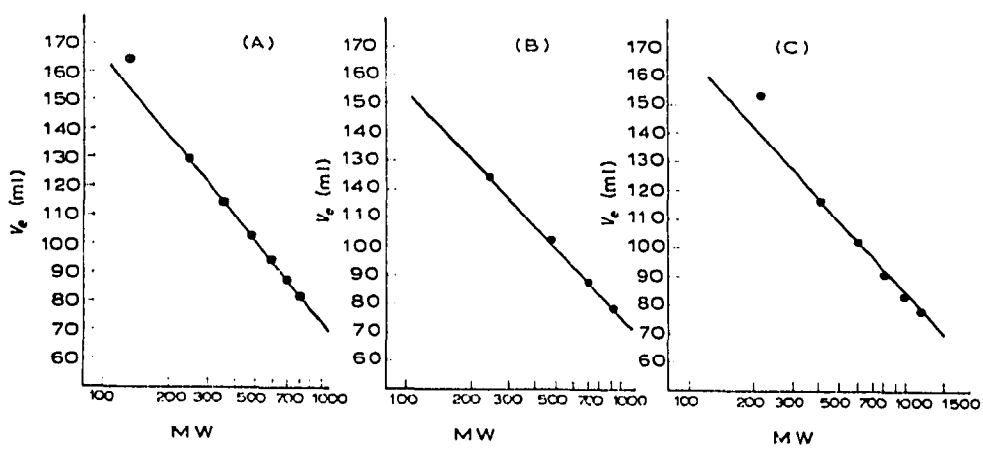

Fig. 4. Correlations between elution volume and molecular weight of the linear monomer and oligomers in polyamides on the LB column (LH-20). (A) nylon 6; (B) nylon 66; (C) nylon 12. Molecular weight of dinitrophenyl group is not included.

TABLE IV

PER CENT OF LINEAR MONOMER AND OLIGOMERS IN THE POLYAMIDES
1×10^{-2} wt.% *vs.* polymer.

	Nylon 6	*Nylon 66*	*Nylon 12*
Monomer	3.6	0.9	1.0
Dimer	2.0	2.5	0.9
Trimer	4.8	5.2	1.5
Tetramer	4.2	3.4	5.4
Pentamer	1.5		9.8
Hexamer	0.9		4.0
Heptamer	0.1		2.0
Octamer	0.1		
Diamino monomer		2.2	
Sum	17.2	14.2	24.6

dinitrophenylation and for the separation of the DNP-derivatives were held constant. An identifiable peak at 410 mμ is obtained with as little as 0.01 μmole of the DNP-derivatives. The amount of the higher oligomers (hexamer to octamer) is less than that of the lower oligomers. According to Flory's theory, the content of higher oligomers would be expected to increase with increasing the degree of polymerization. From the results in Table IV it would appear that the higher oligomers are barely extracted from the polymer. The content of the linear monomer in nylon 6 and nylon 12 is much more than that of the linear dimer. This fact gives some clue as to the mechanism of the polymerization; the oligomers greater than the dimer are considered to be a part of the polymer; on the other hand, the monomer acts as a catalyst for the polymerization and will be an intermediate to the polymer in the polymerization process.

Dinitrophenol and dinitrophenetole are formed by reaction of DNFB with water and ethyl alcohol in the dinitrophenylation process as by-products and they are included in the reaction products in addition to unreacted DNFB. The molar absorption coefficients of dinitrophenol and unreacted DNFB decrease with decreasing pH value and thus methanol acidified with HCl was employed as an eluant. The elution volumes of the various constituents on the LC column are as follows: 257 ml for unreacted DNFB, 278 ml for dinitrophenol, and 320 ml for dinitrophenetole. These reagent blanks interfere, therefore, with the absorption measurement of the DNP-ACA which elutes between unreacted DNFB and dinitrophenol. The interference can be eliminated by the measurement at higher wavelengths such as 420 $\sim$ 450 mμ and if necessary the addition of ammonia after the dinitrophenylation of the mixture of monomer and oligomers could be considered when the dinitrophenol and unreacted DNFB would be converted to dinitroaniline. The elution order of these reagent blanks is in contrast to their molecular weights. According to NILSSON[21], the estrogenic isoflavones in red clover are effectively separated on Sephadex G-25 in 0.1 *M* ammonium hydroxide and daidzein, genistein, formonetin, and biochanin A are eluted in this order. Daidzein where a hydrogen atom is substituted for the hydroxyl group in genistein elutes earlier than genistein, and biochanin A containing a methoxy group in place of the hydroxyl group in genistein elutes later than genistein. His result is therefore consistent with ours.

In the course of the chromatography of the solution of reagent blanks, unknown solutes were measured before the peak of unreacted DNFB and after the peak of dinitrophenol by the measurement of absorption at 370 mμ. The wavelength of 410 mμ was therefore selected for the measurements after due consideration of the sensitivity and the magnitude of the reagent blanks. The wavelength range of 440–450 mμ was used, if necessary, to reduce the absorption which appeared near the peak concerned due to the reagent blanks when the absorbance of the DNP-monomer of nylon 6 and nylon 12 was measured.

The swelling of Bio-Beads S-X2 with methanol–benzene (1 : 1) solvent was so incomplete that separation above the DNP-trimer of nylon 6 was incomplete.

ACKNOWLEDGEMENTS

The authors were indebted to Toray Ind., Inc. for supplying nylon 12 samples and to Miss K. KOBAYASHI for her technical assistance.

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