

# Optical rotatory dispersion and circular dichroism studies of copolyaspartates with long alkyl side chain

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Copoly(hexyl-L-aspartate-benzyl-L-aspartate), copoly(nonyl-L-aspartate-benzyl-L-aspartate) and copoly(dodecyl-L-aspartate-benzyl-L-aspartate) with various compositions were prepared by ester exchange reaction of poly( $\beta$ -benzyl-L-aspartate). Copolyaspartates of low degree of alkylation were found to take a left handed  $\alpha$  helix, similar to poly( $\beta$ -benzyl-L-aspartate), and those of high degree of alkylation changed to a right handed  $\alpha$  helix. Copoly(hexyl-L-aspartate-benzyl-L-aspartate) and copoly(dodecyl-L-aspartate-benzyl-L-aspartate) changed helix sense from left to right at 50% hexylation and 10% dodecylation, respectively. A small induced circular dichroism trough appeared around 230 nm for these two copolyaspartates above 75% alkylation, indicative of a certain ordering of the carbonyl group due to the formation of an ordered structure of a long alkyl side chain. However, copoly(nonyl-L-aspartate-benzyl-L-aspartate) of 13% nonylation exhibited the induced circular dichroism spectrum and took a left handed  $\alpha$  helix. Copoly(nonyl-L-aspartate-benzyl-L-aspartate) caused the reversal in helix sense above 70% nonylation according to a stabilizing effect of the ordered structure of the side chain.

(Keywords:  $\alpha$  helix; helix sense; optical rotatory dispersion; circular dichroism; induced circular dichroism; long alkyl side chain)

## INTRODUCTION

Polyaspartates assume an unstable  $\alpha$  helix form, since their peptide bond cannot form perfect hydrogen bonds along  $\alpha$  helices. This is due to competitive hydrogen bonding between NH groups in the main chain and CO groups in the side chain<sup>1</sup>. Conformational analysis of polyaspartates calculated by Scheraga *et al.*<sup>2,3</sup> led to the conclusion that the  $\alpha$  helix of polyaspartates is less stable compared to other polypeptides such as polyglutamates. Bradbury *et al.*<sup>4,5</sup> studied the optical rotatory dispersion of copoly(alkyl-L-aspartate-benzyl-L-aspartate). They found that the methyl group favoured the left handed nature and the longer ethyl, propyl and butyl group formed the right handed one. It is interesting to study the helix sense of copoly(alkyl-L-aspartate-benzyl-L-aspartate) with short or long alkyl side chain which favours opposite handedness to the benzyl group.

In previous papers<sup>6-8</sup>, optical rotatory dispersion (o.r.d.) and circular dichroism (c.d.) studies were performed on the helix sense of copolyaspartate with short alkyl side chain. It was found that copoly(ethyl-L-aspartate-benzyl-L-aspartate) (copoly(ELAsp-BLAsp)) and copoly(propyl-L-aspartate-benzyl-L-aspartate) (copoly(PLAsp-BLAsp)) transformed from left to right handed  $\alpha$  helix with the increase in ethylation or propylation and these two copolyaspartates with a certain composition transformed from right to left handed  $\alpha$  helix with increasing temperature<sup>6-8</sup>. One of the authors also studied the helix sense in the solid state of copolyaspartates by infra-red absorption spectrum<sup>9,10</sup>. Copolyaspartates with ethyl, butyl and hexyl groups in the side

chain, transformed the helix sense by increasing alkylation. Thus, it is interesting to examine the helix sense and stability of the  $\alpha$  helix which contains the ordered arrangement of long alkyl side chain such as nonyl and dodecyl groups.

In the present paper, the helix sense of copolyaspartate with hexyl, nonyl and dodecyl groups was examined in chloroform solution by o.r.d. and c.d. methods as a function of the degree of alkylation and temperature. The relation between the helix stability and ordering in the side chain is discussed from the viewpoint of the degree of alkylation and temperature.

## EXPERIMENTAL

### Materials

*N*-carboxy- $\beta$ -benzyl-L-aspartate anhydride (NCA) was synthesized by the Leuch method<sup>11</sup>. The NCA was polymerized in 5%(w/v) ethylene dichloride solution at room temperature for about one week, using triethylamine as the initiator. Poly( $\beta$ -benzyl-L-aspartate) (PB-LAsp) thus prepared had a viscosity average molecular weight of  $3 \times 10^4$ , determined using the viscosity-molecular weight relation<sup>12</sup>.

Copolyaspartate was synthesized by ester exchange reaction of PBLAsp<sup>9</sup>. The composition of copolyaspartate was adjusted by controlling the reaction time. The copolyaspartate obtained was poured into methyl alcohol and kept at  $\sim 0^\circ\text{C}$  for a few hours. It was filtered and dried; soxhlet extraction was then carried out by methyl alcohol for 2 days to remove unreacted hexyl,

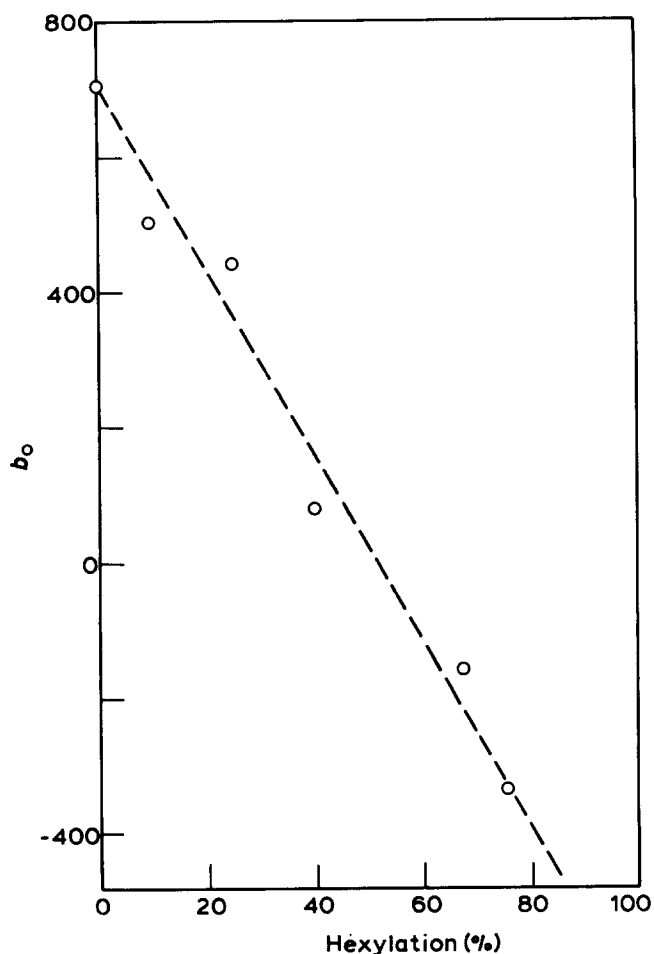


Figure 1 Moffitt parameter  $b_0$  of copoly(hexyl-L-aspartate-benzyl-L-aspartate) at 20°C against hexylation

nonyl or dodecyl alcohol. The degree of alkylation was evaluated from the proton peak of the benzyl and alkyl (hexyl, nonyl or dodecyl) group in the n.m.r. spectra measured in random coil solvent (trifluoroacetic acid).

#### Measurements

O.r.d. measurement was carried out at 20°C and in the wavelength range from 600 to 300 nm by an automatic spectropolarimeter (ORD/UV-5 manufactured by Japan Spectroscopic Co., Ltd.).

C.d. study was performed by an automatic recording spectropolarimeter with a data processor (J-40 manufactured by Japan Spectroscopic Co., Ltd.) in the temperature range from 0°C to 55°C and in the wavelength range from 260 to 219 nm. A quartz cell of 0.1 mm thick with water jacket was used, and the concentration of chloroform solution was 1% (w/v), similar to o.r.d. measurement. Ellipticity was obtained by 4 times integration using the data processor.

## RESULTS AND DISCUSSION

### Copoly(hexyl-L-aspartate-benzyl-L-aspartate)

Reduced mean residue rotation of copoly(HeLAsp-BLAsp) measured in the wavelength region from 600 to 300 nm was applied to Moffitt's equation<sup>13</sup>, and the Moffitt parameter  $b_0$  was evaluated.  $b_0$  at 20°C is shown as a function of degree of hexylation of copoly(HeLAsp-BLAsp) in Figure 1. The parameter  $b_0$  changed linearly

from positive through zero to negative value, that is, helix sense transformed from left to right handed with increasing hexylation. Thus, a hexyl-L-aspartate residue favours right handed helix, and this agrees with the previous result for the solid state copoly(HeLAsp-BLAsp)<sup>10</sup>. Linear extrapolation of the  $b_0$  parameter to 100% hexylation gives a value about -700, indicative of a stable right handed  $\alpha$  helix of poly(hexyl-L-aspartate). Figure 2 shows c.d. spectra of copoly(HeLAsp-BLAsp) of 39% hexylation at various temperatures. Molecular ellipticity  $[\theta]$  of copoly(HeLAsp-BLAsp) of 39% hexylation exhibited a peak or a trough at 222 nm, indicating a left or right handed  $\alpha$  helix, respectively. A sign of  $[\theta]$  changed from positive to negative with increasing temperature, representing the reversal in the helix sense from left to right handed  $\alpha$  helix. This is contrary to the fact that copoly(ELAsp-BLAsp) and copoly(PLAsp-BLAsp) transformed from right to left handed  $\alpha$  helix with increasing temperature. This suggests the much more favourable right handed nature of hexyl-L-aspartate residue. Smaller absolute value of  $[\theta]$  suggests less stable  $\alpha$  helix of copoly(HeLAsp-BLAsp), comparing to  $[\theta] = 38\,000$  for stable  $\alpha$  helix. C.d. spectra of copoly(HeLAsp-BLAsp) of 67% hexylation at various temperatures are shown in Figure 3. All the troughs observed indicate a right handed  $\alpha$  helix, irrespective of temperature. Molecular ellipticity of copoly(HeLAsp-BLAsp) with various hexylations at the peak or the trough ( $[\theta]_{\max}$ ) was plotted against temperature in Figure 4. Copoly(HeLAsp-BLAsp) below 25% hexylation took left handed  $\alpha$  helix in the temperature range examined. The  $[\theta]_{\max}$  of copoly(HeLAsp-BLAsp) decreased gradually with temperature. Thus, this copolymer tends to become unstable as temperature increases. As pointed out above, copoly(HeLAsp-BLAsp) of 39% hexylation caused the reversal in the helix sense from left to right handed  $\alpha$  helix by temperature increasing. However, copoly(HeLAsp-BLAsp) above 67% hexylation remained unchanged in a right handed  $\alpha$  helix, irrespective of temperature.

C.d. spectrum of copoly(HeLAsp-BLAsp) of 76% hexylation at 0°C is shown in Figure 5. A drastic change in

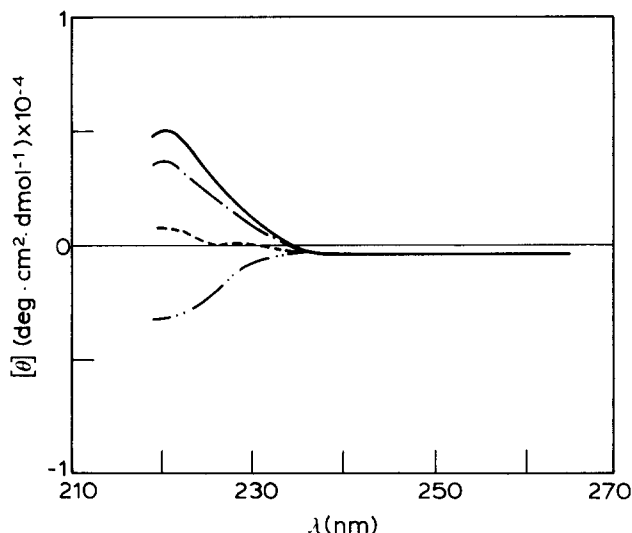


Figure 2 Circular dichroism spectra of copoly(hexyl-L-aspartate-benzyl-L-aspartate) of 39% hexylation at various temperatures; —, 0°C; ---, 20°C; ···, 40°C; - · - ·, 50°C

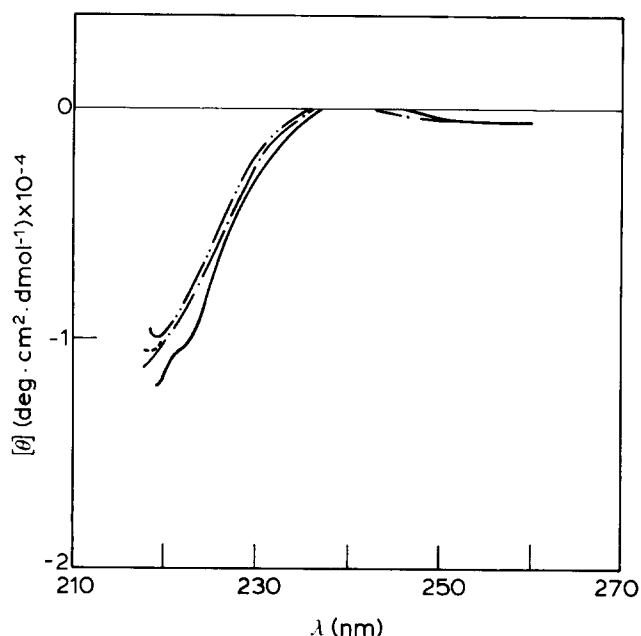


Figure 3 Circular dichroism spectra of copoly(hexyl-L-aspartate-benzyl-L-aspartate) of 67% hexylation at various temperatures; —, 0°C; ---, 20°C; - · - ·, 40°C; · · · ·, 50°C

spectrum was observed. The peak or the trough at 222 nm, characteristics of the  $\alpha$  helix, disappeared and another trough appeared around 238 nm. The Moffitt parameter  $b_0$  of copoly(HeLAsp-BLAsp) is  $-340$ , as can be seen in Figure 1 and this copolymer is in the right handed  $\alpha$  helix conformation. Further increase in hexylation is thought to make  $\alpha$  helix more stable, and simultaneous association of long alkyl side chains is likely to form ordered structure along  $\alpha$  helical axis as hexylation increases. A possible explanation for the c.d. trough at 238 nm is an induced c.d. due to poorly helically arranged carbonyl groups in the ordered alkyl side chain. It is noticeable that the helix sense of the main chain is the same as the poorly helically arranged side chain. The induced c.d. obtained here is essentially the same as the side chain induced c.d. observed for a lyotropic liquid crystal of polypeptide<sup>14,15</sup>.

#### Copoly(dodecyl-L-aspartate-benzyl-L-aspartate)

Figure 6 shows the Moffitt parameter  $b_0$  of copoly(dodecyl-L-aspartate-benzyl-L-aspartate) (copoly(DoLAsp-BLAsp)) plotted against dodecylation. In contrast to copoly(HeLAsp-BLAsp), the reversal in the helix sense took place at a low content of dodecyl group such as about 10%. This suggests that dodecyl-L-aspartate residue can break left handed  $\alpha$  helix more readily than hexyl-L-aspartate residue. The  $b_0$  parameter decreases gradually up to about 80% dodecylation and increases with further increasing dodecylation.

Copoly(DoLAsp-BLAsp) about 75% dodecylation exhibited induced c.d. due to the side chain in the vicinity of 235 nm, which is similar to the spectra of copoly(HeLAsp-BLAsp) above 76% hexylation. Figure 7 shows c.d. spectra of polyDoLAsp at various temperatures. The trough was observed only up to 40°C, in contrast to copoly(HeLAsp-BLAsp). This means the dodecyl side chain arranged in a right handed helix up to 40°C. The induced c.d. disappeared and the c.d. trough

due to right handed  $\alpha$  helix was observed at 50°C, indicative of a breakdown of only the ordered dodecyl side chain. Therefore, the ordered dodecyl side chain is thought to begin melting around 40°C.

#### Copoly(nonyl-L-aspartate-benzyl-L-aspartate)

Moffitt parameter  $b_0$  of copoly(nonyl-L-aspartate-benzyl-L-aspartate) (copoly(NoLAsp-BLAsp)) at 20°C is plotted against nonylation in Figure 8. A remarkable difference in the  $b_0$  behaviour of copoly(NoLAsp-BLAsp) from that of copoly(HeLAsp-BLAsp) and copoly(DoLAsp-BLAsp) was observed. Left handed  $\alpha$  helix predominated up to about 70% nonylation, and a reversal in the helix sense took place above 70% non-

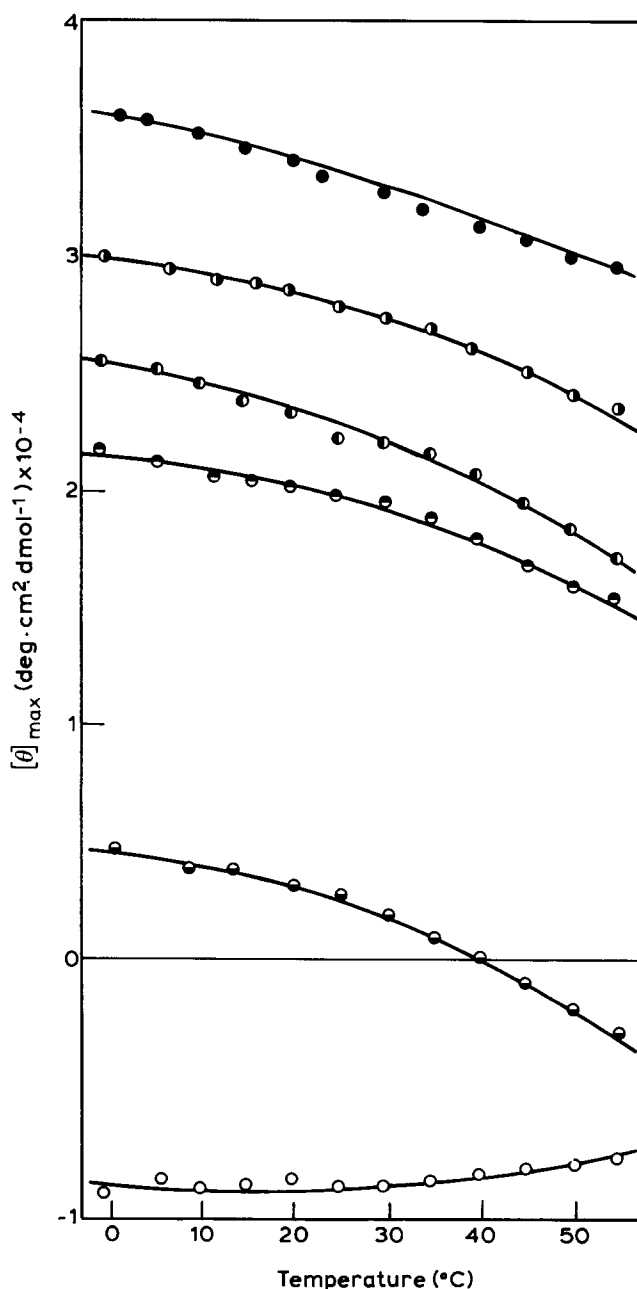


Figure 4 Molecular ellipticity of copoly(hexyl-L-aspartate-benzyl-L-aspartate) with various hexylations at 222 nm vs. temperature. (●) PBLAsp; (●) copoly(HeLAsp-BLAsp) of 9% hexylation; (⊙) copoly(HeLAsp-BLAsp) of 15% hexylation; (⊖) copoly(HeLAsp-BLAsp) of 25% hexylation; (⊕) copoly(HeLAsp-BLAsp) of 39% hexylation; (○) copoly(HeLAsp-BLAsp) of 67% hexylation

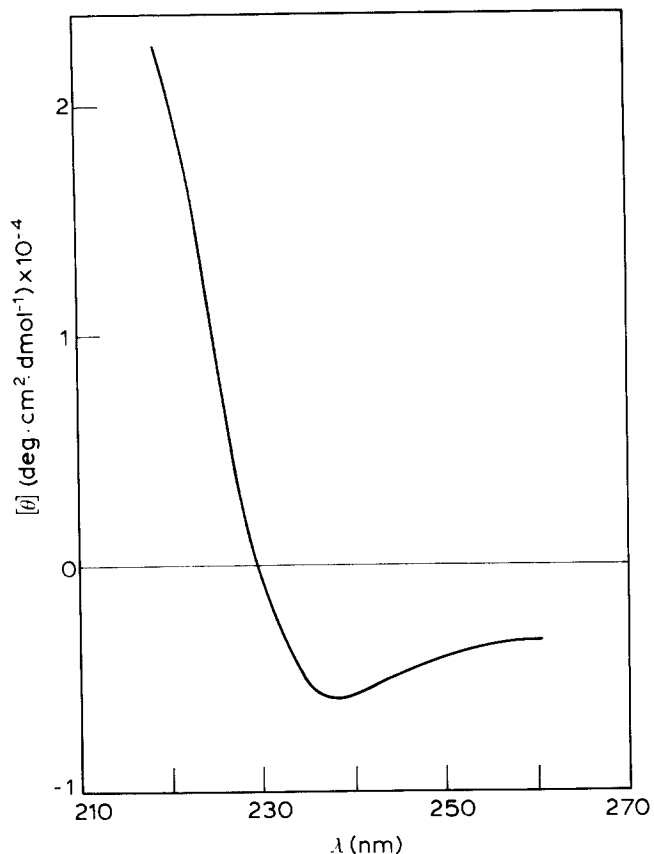


Figure 5 Circular dichroism spectrum of copoly(hexyl-L-aspartate-benzyl-L-aspartate) of 76% hexylation at 0°C

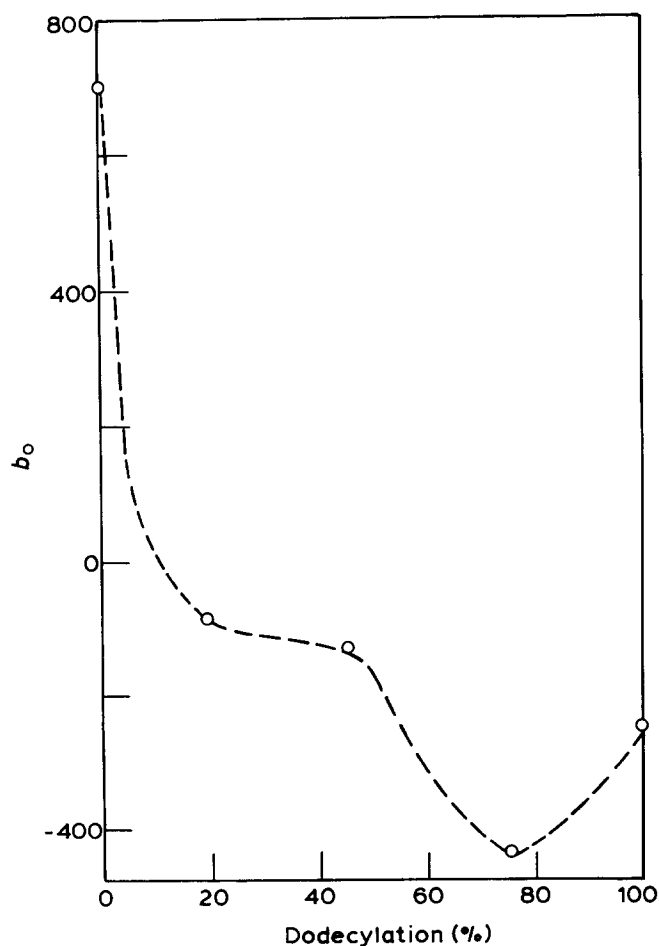


Figure 6 Moffitt parameter  $b_0$  of copoly(dodecyl-L-aspartate-benzyl-L-aspartate) at 20°C against dodecylation

ylation. Copoly(NoLAsp-BLAsp) above 70% nonylation is likely to assume much more stable right handed  $\alpha$  helix, differing from the rather unstable right handed  $\alpha$  helix of copoly(DoLAsp-BLAsp).

C.d. spectra of copoly(NoLAsp-BLAsp) of 31% nonylation at various temperatures are demonstrated in Figure 9. The c.d. peak around 232 nm was ascribed to the

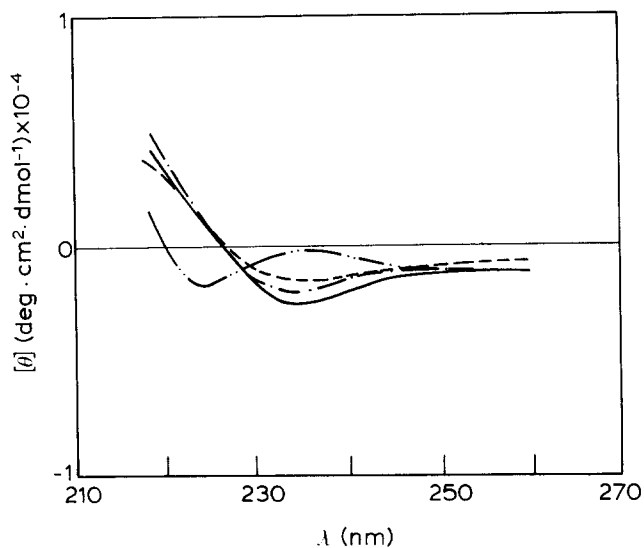


Figure 7 Circular dichroism spectra of poly(dodecyl-L-aspartate) at various temperatures. —, 0°C; ---, 20°C; - · - ·, 40°C; · · · ·, 50°C

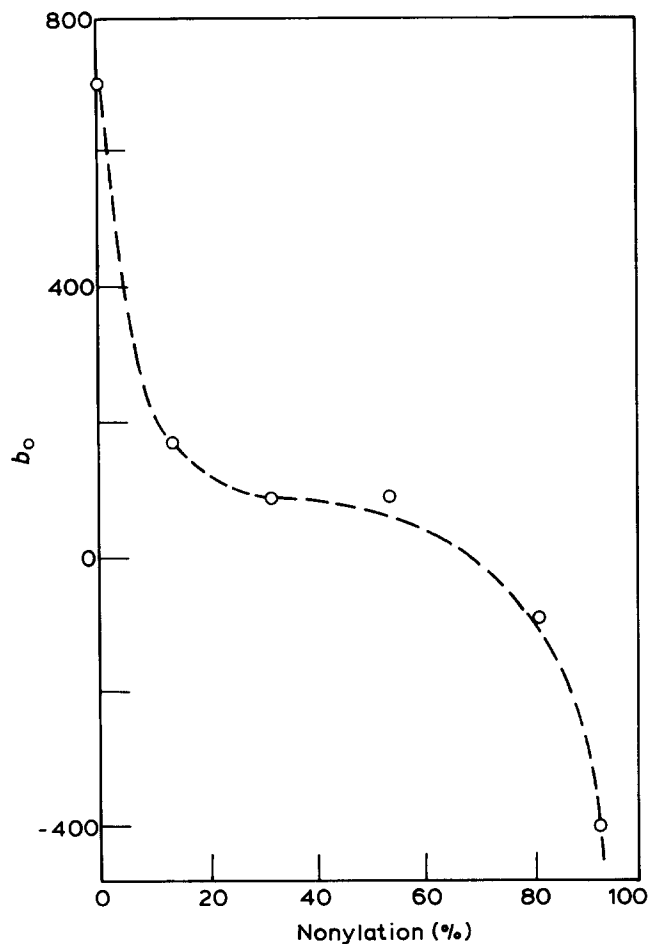


Figure 8 Moffitt parameter  $b_0$  of copoly(nonyl-L-aspartate-benzyl-L-aspartate) at 20°C against nonylation

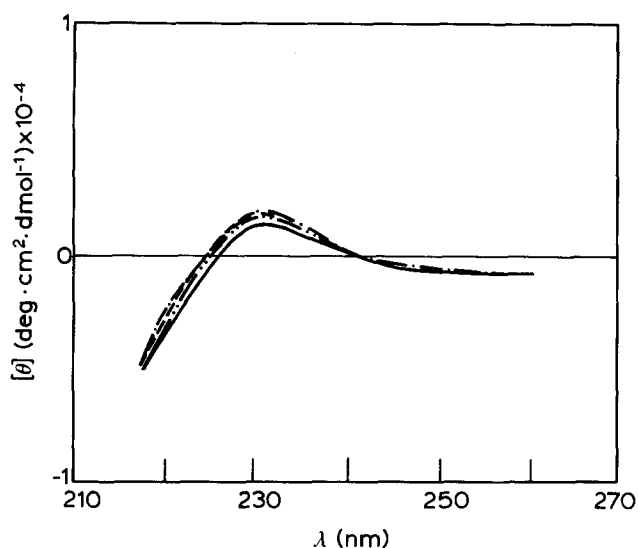


Figure 9 Circular dichroism spectra of copoly(nonyl-L-aspartate-benzyl-L-aspartate) of 31% nonylation at various temperatures. —, 0°C; ---, 20°C; - · - ·, 40°C; ·····, 55°C

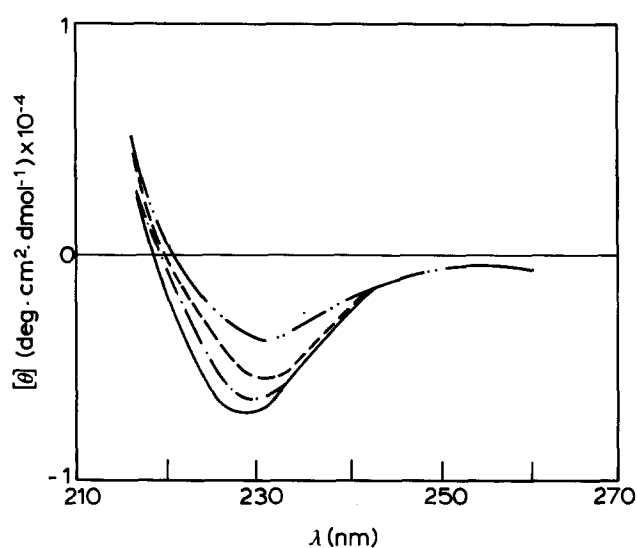


Figure 10 Circular dichroism spectra of copoly(nonyl-L-aspartate-benzyl-L-aspartate) of 92% nonylation at various temperatures. —, 0°C; ---, 20°C; - · - ·, 40°C; ·····, 55°C

induced c.d. which is due to the ordered alkyl side chain, similar to the induced c.d. of copoly(HeLAsp-BLAsp) and copoly(DoLAsp-BLAsp). Corresponding to the fact that copoly(NoLAsp-BLAsp) is in the left handed  $\alpha$  helix, as seen in Figure 8, positive molecular ellipticity ascribed to the induced c.d. was observed, indicating the left handedness of the helically ordered side chain sense. Figure 9 also shows the temperature independence of the induced c.d. peak. This suggests that the side chain nonyl group of this copolyaspartate is stable and impossible to melt within temperature range measured.

However, copoly(NoLAsp-BLAsp) of 92% nonylation exhibited an induced c.d. trough with negative molecular ellipticity, as shown in Figure 10. This fact corresponds not only to the right handed  $\alpha$  helix of this copolymer but also to the right handedness of the ordered side chain structure. In the case of 92% nonylation, the temperature dependence of the induced c.d. was observed and the absolute value of molecular ellipticity decreased with increasing temperature. This indicates less ordered side chain structure and more active molecular motion of side chain with increasing temperature although the side chain is not molten. The ordered side chain structure of copoly(NoLAsp-BLAsp) is much more stable than that of copoly(DoLAsp-BLAsp).

The result of the  $b_0$  parameter and c.d. spectra showed that the left handed  $\alpha$  helix did not transform to the right handed one and became much more stable because of the formation of the ordered side chain structure at lower nonylation. This is contrary to the case of the former two

copolyaspartates which are in the right handed  $\alpha$  helix when the ordered side chain was formed. The nonyl-L-aspartate residue is considered to be most favourable for the formation of the ordered side chain structure among the copolyaspartates with three kinds of long alkyl side chains.

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