Title	水溶液中におけるポリ-S-カルボキシエチル-L-システインのβ-ランダムコイル転移と静電相互作用
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DOCTORAL DISSERTATION

Title : Charge Induced β-Random Coil Transition of Poly-S-Carboxyethyl-L-Cysteine in Aqueous Media.

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SUMMARY

As a new protolytic polypeptide capable of forming β-structure in aqueous media, Poly-S-carboxyethyl-L-cysteine, a higher side-chain homologue of poly-S-carboxymethyl-L-cysteine, has been prepared from poly-S-carbobenzoxyethyl-L-cysteine with hydrogen bromide in chloroform or acetic acid. The polymer is found to be in the β -structure of an antiparallel arrangement of polypeptide chains in solid films, both in acid and salt forms, when examined by infrared measurements. Aqueous solutions of the polymer have been investigated by measurements of optical rotatory dispersion and circular dichroism as well as by infrared spectra in D_2O. These properties show sharp changes around pH 5.5, as the pH of solution is varied. At higher ionization or higher pH the polymer is randomly coiled, but at lower ionization it is in the β-structure. The β-structure is characterized by a negative circular dichroic band at 223 mμ and a positive dichroic band at a wavelength lower than 200 mμ, and by a peak around 210 mμ in the rotatory dispersion curve, and further by a negative b_o value - 140°.

Potentiometric titration curves have been determined for aqueous solutions of the polymer which is subject to the β-coil transition by the change of pH. Reversibility and time dependence of titration curves are examined by different methods, in order to establish the conditions for obtaining equilibrium titration curves. The β-coil transition is manifest at some region on the equilibrium titration curve, if pH - log $(\alpha/1-\alpha)$ is plotted against α. For an assumed pK_o

value, 4.0, the non-electrostatic free energy change for the β-coil transition is evaluated from the extrapolation of observed titration curves and is found to depend on ionic strength and polymer concentration. Generally, the non-electrostatic free energy change for the β-coil transition is much higher than that for the helix-coil transition. The Henderson-Hasselbach plot of the titration curve yields clearer distinction between β-structure and random coil, and permits to estimate the content of β-structure at a given pH. From the dependence of the β-content on both ionic strength and polymer concentration, it is concluded that at a given degree of ionization β-structure is more stabilized if ionic strength and polymer concentration are higher. This conclusion is supported by the dependence of the optical rotatory properties on ionic strength and polymer concentration. The dependence on polymer concentration may be attributed to the formation of intermolecular β-structure in solutions. Comparison of β-content with circular dichroism measurements leads to a value of about -10,000 for $[\theta]_{223}$ for pure β-structure.

Precipitation which occurs at low ionizations and, especially, at high ionic strengths does not reveal any discontinuous change on the titration curve.

It is also concluded that the precipitation takes place at a certain stage of the β-coil transition rather than at the stage when the transition to β-structure is completed.

Counter ion activity of poly-S-carboxyethyl-L-cysteine neutralized to various degrees with sodium hydroxide is measured in salt-free aqueous solutions, using a Na-glass electrode. Activity coefficient of sodium counter ion for β-structure is considerably smaller than that for random coil, if compared at the same degrees of neutralization. The effect of conformational change on the activity coefficient of counter ion becomes obscured as polymer concentration decreases. The dependence of the activity coefficient on polymer concentration is different for different conformations. On increasing polymer concentration, the activity coefficient of counter ion decreases for random coil whereas it increases for β-structure. For β-structure it is concluded that the activity coefficient of counter ion is almost independent of the degree of neutralization. This particular behavior may be a characteristic feature of β-structure.

Counter ion activity in salt-free polyelectrolyte solution is also evaluated from the data of potentiometric titration. The coincidence of the results obtained from two different methods is good for the case of random coil state. This coincidence implies that the additivity rule holds for the random coil of the present polymer in its approximate sense.

The effect of precipitation on the titration behavior is not observed in the present investigations. This behavior seems an extraordinary one and is expected to afford a characteristic feature of β-structure. This and other characteristic features derived from present investigations are useful informations when we construct a model for β-structure in solution. However it is rather difficult to carry out this attempt at the present stage of our knowledge. As a preliminary attempt, the effect of precipitation on the titration behavior is treated in a systematic manner in the framework of thermodynamics. A requirement for the thermodynamic properties of the system is derived for the case that the effect of precipitation is not observed.

Introductory Part.

Summary of the Studies of β-Structure in Synthetic Polyamino Acids*

1. Models for β-Structure.

During 1951-1953, Pauling and Corey proposed several models for possible three dimensional structure of polypeptides. The essential feature of their works resided in that their models were based on by now quite rigid stereochemical restrictions which had been established from their extensive studies on simple peptides and amino acids. Among their models the β-structure was also included.[4-6]

In their model, β-structure consists of nearly extended polypeptide chains joined together side by side through peptide hydrogen bonds. So in β-structure polypeptide chains form a two dimensional pleated sheet and bulky side chains stick out alternately upward and downward nearly normal to the plane of sheet.

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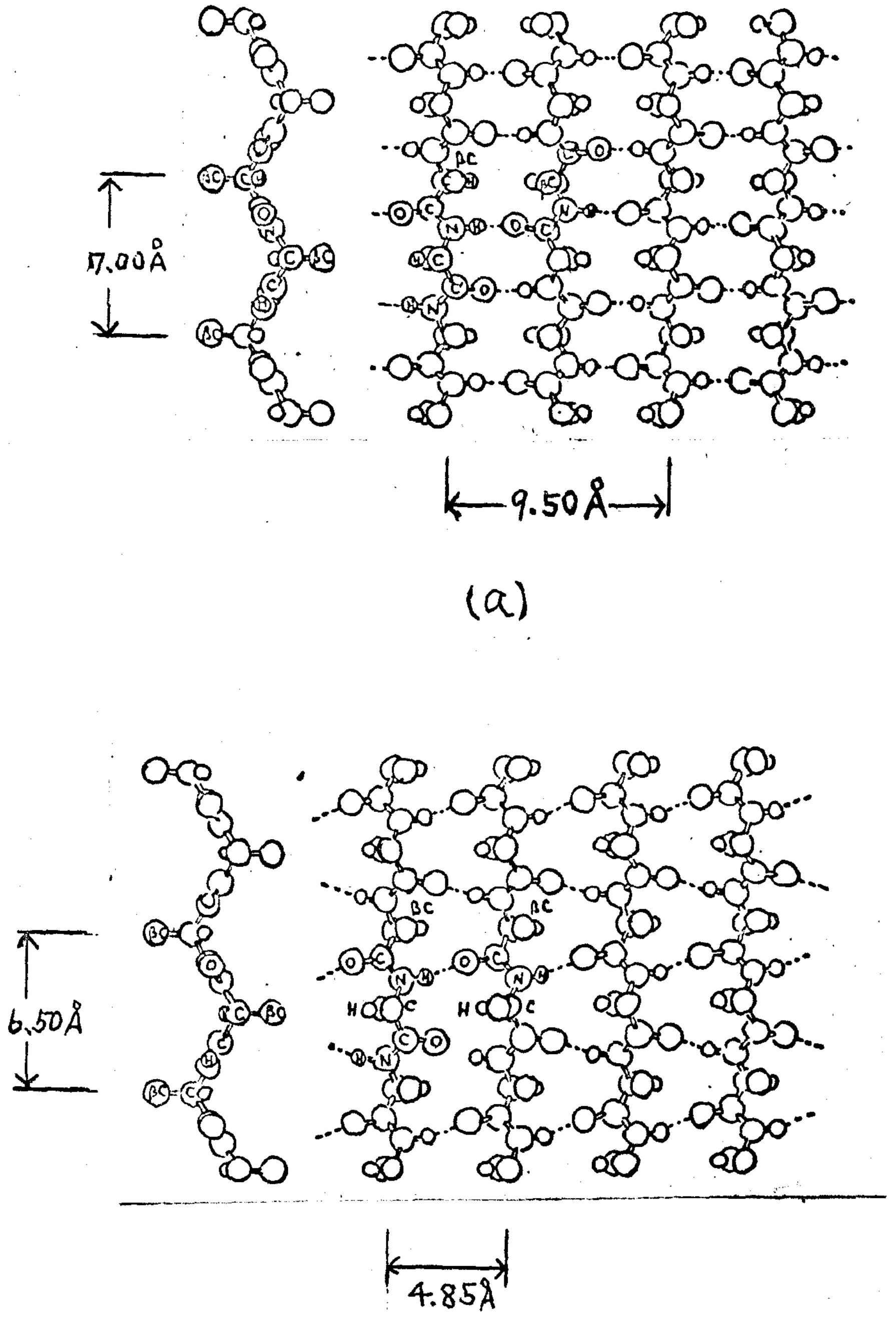
* The complete and extensive review of the conformational studies on polypeptides is, of course, beyond the scope of this dissertation. Here we confine ourselves only to the β-structure of homopolypeptides. Proteins and other structures of synthetic polypeptides (α-helix and other helical structures including poly-L-proline I and II and polyglycine II) as well as synthetic copolypeptides are omitted thoroughly. Concerning these subjects many excellent reviews are available.[1-3]

At this stage, two different types of arrangement are possible whether adjacent chains are parallel or antiparallel. Both pleated sheets thus formed may interact further with one another, due to van der Waals force between side chains of different sheets, and form a three dimensional aggregate. The dimensions proposed by Pauling and Corey are the followings.[5]

Along the polypeptide chain, two residues are involved in one repeat. The distance of repeat is 6.50 A and 7.00 A for parallel and antiparallel arrangement respectively. Usually y axis is taken parallel to this direction. In the plane of sheet, x axis is taken to be perpendicular to y axis. The distance of repeat along this direction is 4.85 A and 9.50 A for parallel and antiparallel arrangement respectively. Since two residues are involved in one repeat in the latter case, the separation becomes about twice of the former. The direction of hydrogen bond is nearly parallel to x axis. The remaining z axis is perpendicular to the plane of pleated sheet. The direction of side chains (more precisely, the direction of C_α-C_β bond) is nearly parallel to this axis. The distance of repeat is dependent on the size of side chains. The schematic representations of these two pleated sheets are given in Fig. 1.

Aside from these pleated sheets, fully extended configuration is also possible if side chains are short enough not to bring the steric hindrance between them in adjacent chains. In this structure, the distance of repeat along y axis is 7.23 A[6], and side chains are tilted by about 35° from the normal to the plane of sheet. Two dimensional sheet formed in this case is no longer pleated but is a plane, and



(a)

(b)

(Pauling and Corey, 1953)

Fig. 1. Two pleated sheets composed of L-amino acid residues with (a) antiparallel and (b) parallel chain arrangements. (This figure is provided by courtesy of Dr. S.Ikeda.)

all peptide groups become coplanar forming a plane of sheet. From the consideration of linearity of hydrogen bond, antiparallel arrangement is more favored in this fully extended configuration. This structure has been assigned to Nylon 66[9], but its existence in synthetic polyamino acids ha~~ve~~s not been reported so far.

2. Classification of β-structure.

As mentioned in the preceding section, we notice that there are two types of β-structure,parallel or antiparallel arrangement. In addition we have two more categories to classify β-structure.

The first one arises only when we examine the oriented specimen such as oriented films or fibers in X-ray diffraction studies or infrared measurements. In such a case, the direction of polypeptide chains relative to the direction of orientation(fiber axis) is one of the characteristics of the β-structure. If polypeptide chains are parallel to fiber axis, it is designated as parallel-β , whereas if perpendicular, it is said to be cross-β.

The second category resides in the point whether the adjacent chains in a pleated sheet belong to the same polymer molecule or not. There is a possibility that a polypeptide chain may transversely folded and form its own pleated sheet with antiparallel arrangement. This small pleated sheet is a polymer molecule itself. These small pleated sheets associate with each other by hydrogen bonds formed intermolecularly between edges and thus form a large pleated sheet. Moreover, stacking of sheets may develop a three dimensional aggregate. The β-structure thus formed is almost indistinguishable from that previously described. However, the small pleated sheets are formed intramolecularly, this type of β-structure is called as intramolecular β-structure. It should be noted that even in the intramolecular β, intermolecular association both in two and three dimensional ways are essential to produce β-structure.

From the discussion of this section, it is clear that we must specify two or three(in the case of oriented specimen) terms to describe β-structure in consistent way.

3. Experimental detection of β-structure.

Evidence for the presence of β-structure has been chiefly made with X-ray diffraction studies and infrared measurements. Measurements of optical rotatory properties also afford valuable informations about the conformations of polypeptides. However, the relations between the observed quantities in rotatory properties and conformations are not so satisfactory as the former two techniques.'This is especially true in the theoretical aspect. In the case of α-helix,however, such relations have been firmly established experimentally. Contrary to this, experimental establishment of such relations has been a main purpose in the study of β-structure.

(a) X-ray diffraction study.

The important features in the diffraction pattern needed for the assignment of β-structure are following spacings. 4.7-4.9 A; This spacing corresponds to the distance of repeat or pseudorepeat along x axis. 3.2-3.5 A; This spacing corresponds to pseudorepeat along y axis. 1.0-1.2 A; This spacing arises because there is a strong pseudorepeat in one-sixth of the two residue period along y axis. The remaining spacing is side chain spacing, which is very strong but varies with the size of side chains.

With a uni-directionally oriented specimen of the fiber axis parallel to the meridian axis of diffraction pattern, spacings of 3.2-3.5 A and 1.0-1.2 A are observed on the meridian, whereas other two spacings are observed on the equator, if parallel-β is to be the case. In the case of cross-β these relations are reversed.

Thus X-ray diffraction method is useful to detect β-structure and further to determine whether 'parallel' or 'cross'. However, the distinction between parallel and antiparallel arrangement is very difficult, since scattering powers are similar for the unlike chains in the latter case. Therefore, this distinction has been suggested indefinitely from the examination of the repeat along y axis, which is different in two arrangements according to Pauling-Corey's proposal.

(b) Infrared spectroscopy.

At earlier stage of the conformational studies of polypeptides, infrared measurements were made in parallel with X-ray diffraction on the same samples. In this way structural features became more clearly elucidated and validities of proposed models were examined more definitely when compared with the mere use of either technique. This way of investigation further provided the correspondence between chain conformations and the characteristic absorption bands of peptide group. This was worked out by the Courtaulds group especially by Elliott.[7] The frequencies of amide I and II corresponding to various conformations are as follows(in cm^{-1}):

amide I 1652-1657(α-helix) 1628-1632(β-structure) 1661-1665(random coil)
amide II 1545-1551(α-helix) 1521-1526(β-structure) 1539-1551(random coil)
From these figures one will notice that this technique is especially suitable for the study of β-structure.

An attempt to give a theoretical ground to this correspondence was made by Miyazawa.[8,9] He considered that in the case of amide I and II, the vibrational motions were rather localized in each peptide group. In these circumstances, the effect that these oscillators are confined in a regular manner within a polymer chain or in a pleated sheet can be treated by perturbation. This treatment leads to the splitting of absorption bands analogous to the exciton splitting in electronic transitions in a molecular assembly. The new levels created by perturbation are characterized with the differences in phase between adjacent groups along y axis(δ) and along x axis(δ'). So any of these new levels is characterized with its frequency $\nu(\delta,\delta')$. With the aid of selection rule[10], the numbers of infrared active bands originating from each unperturbed band are two for parallel and three for antiparallel arrangement. These bands are $\nu_y(0,0)$ and $\nu_{x-z}(\pi,0)$ for parallel arrangement, and $\nu_x(\pi,0)$, $\nu_y(0,\pi)$ and $\nu_z(\pi,\pi)$ for antiparallel arrangement. Subscripts represent the polarization of band in zeroth order. Under the approximation of the nearest neighbor interaction and the assumption of identical interaction energy for both chain arrangement, these five bands can be specified with three parameters. In the case of amide I, $\nu_{x-z}(\pi,0)$ and $\nu_x(\pi,0)$ are the strongest bands for each arrangement, since the transition moment is nearly parallel to x axis, and moreover they must be equal under these circumstances described above. So observed band around

1630 cm^{-1} is naturally assigned to either of them. The unperturbed frequency, ν_0, may be taken from the data for random coil. The last condition for the specification is taken from the data for Nylon 66, in which the interaction along y axis is negligible and thus the difference of the observed frequency from ν_0 is considered to represent only the interaction along x axis. With these three conditions the frequencies of the remaining four bands become calculable.

In this way the presence of an additional weak band with polarization parallel to y axis in either arrangement was concluded by Miyazawa. The frequency was calculated to be 1685 cm^{-1} and 1645 cm^{-1} for antiparallel and parallel arrangement respectively.

On the other hand, a band near 1690 cm^{-1} was reported with β-structure but its assignment had not been established.

Since frequency, intensity and polarization were in good agreement between the observed and calculated bands, the band near 1690 cm^{-1} was assigned by Miyazawa to the amide I $\nu_y(0,\pi)$ component of the antiparallel arrangement.

By virtue of contributions made by Elliott and Miyazawa, infrared measurements have become a powerful tool for the detection and examination of β-structure without complementary information from X-ray diffraction. This technique also enables us to determine whether 'parallel' or 'cross' if infrared dichroism is observed with oriented films. Moreover the determination of chain arrangement can be made in more definite manner.

With respect to practical applications, infrared measurements have some advantages. For instance, this technique is applicable to the studies of solution phase. This takes an important role in connecting the informations obtained in both phases, solid and solution.

To conclude this section, it is worth while to point out that every β-polypeptide so far examined shows the band near 1690 cm^{-1}. Therefore we have no example of β-structure with parallel chain arrangement as yet, so long as judged from infrared measurements.[24]

(c) Optical rotatory properties.

Polypeptides have an asymmetric structure with long range order when they are in regular conformations. This asymmetry produces a new contribution to the optical rotatory power of the structure aside from the intrinsic one inherent to asymmetric α-carbon atom of each residue.

Moffitt and Yang have proposed a famous equation for analysis of the optical rotatory dispersion of polypeptides.[11] This Moffitt-Yang equation is written as follows.

$$[m'] = \frac{M_o}{100}\frac{3}{n^2+2}[\alpha] = \frac{a_o\lambda_o^2}{\lambda^2-\lambda_o^2} + \frac{b_o\lambda_o^4}{(\lambda^2-\lambda_o^2)^2} \qquad (1)$$

Here notations are;

$[m']$; residue rotation, $[\alpha]$; specific rotation, M_o; molecular weight per residue or residual weight, n; refractive index of solvent, λ; wave length in mμ, and λ_o, a_o, and b_o; parameters determinable from dispersion data. The parameter b_o has been established experimentally to be conformation dependent in the α-helix, whereas a_o depends on conformation, side chain, and solvent.

At the same time of the proposal of the equation, Moffitt also gave a theoretical basis for the equation.[12] One of his results, the splitting of absorption band in helical structure, has been proved experimentally.[13,14] However, later works revealed that his theory concerning optical activity was incorrect and insufficient with some respects. One of them is the use of inadequate procedure to evaluate the rotational strength.[15] A general expression has been given and discussed by Tinoco in this respect.[16] Another one is the neglect of the contribution from n-π* transition. This was treated by Schellman and Oriel[17], and they were successful to give a reasonable conclusion.

In the case of β-structure, Moffitt-Yang equation has been shown to hold for this conformation experimentally. However the interpretation of b_o or its correlation with several types of β-structure described earlier is not successful. Some theoretical studies have been made.[18-21] These results are not consistent with each other, except for that in the case of antiparallel arrangement there are virtually two dichroic bands experimentally accessible, one is negative located near 220 mμ, due to n-π* transition, and another one is positive and larger than the former in magnitude, located at a somewhat shorter wave length than 200 mμ.[18,19]

4. Summary of the studies in solid state.

Conformational studies of synthetic polypeptides in solid state have shown that the conformation of a polypeptide is determined rather severely by its intrinsic properties, such as chain length and kind of amino acid of which they are composed. Though some exceptions have been reported against the generalization of this statement.[13,23] In such examples conditions for preparation of solid samples are shown to affect the conformations.

Short chain poly-γ-benzyl-L-glutamate was firstly shown to be in β-structure,[42] whereas high molecular weight samples of the polymer have been known as a representative of α-helix. It has been considered that β-structure is the most stable conformation in any short chain polypeptide, except for that of proline. This arises because α-helix becomes less stable if chain length is decreased , since α-helix has eight unbonded sites capable of hydrogen bonding at its ends. Thus short chain polypeptides form a group in which β-structure is the most stable conformation.

The relation between conformations and kinds of amino acid residue has been established.[22] Following amino acids are reported as β -forming ones. These are L-valine,L-isoleucine,L-serine,L-cysteine,L-threonine, and L-allothreonine and their derivatives. The first two amino acids are characterized with the disubstitution by alkyl groups on the β-carbon atom. The remaining amino acids have, in common, a hetero atom, oxygen or sulfur, attached to the β-carbon atom. Since the polypeptides composed of one of these amino acids are always in β-structure irrespective of their chain lengths, the preference of this particular conformation is supposed to be due to intrinsic properties inherent to these amino acids. These intrinsic properties have not been elucidated as yet, but in the case of valine or isoleucine, it is most likely that steric hindrance due to side chain causes unfavorable condition for the formation of α-helix. The exceptions to this group are poly-S-benzylthio-L-cysteine, which has been reported to be in ω-helix,[25] and poly-S-carbobenzoxy-L-cysteine Form II.[26]

Aside from these two groups, one is of short chain and another is of intrinsic nature, there are some examples which have been reported to be in β-structure. These are polyglycine I[1], β-structure of poly-L-alanine[1], of poly-L-lysine[13], and of poly-β-n-propyl-L-aspartate[27], sodium poly-L-glutamate[28], and poly-L-lysine hydrohalide[29]. These examples form a third group.

To conclude this section, it should be mentioned that the most stable conformation of a polypeptide in solid state should not be considered as that for an isolated chain. The most stable conformation in solid state is determined by interactions between chains as well as intrachain interactions.

A list of materials so far reported is given in the followings.

List of materials so far reported on which investigations in solid state have been made.

Poly-L-alanine; 1*

Poly-β-n-propyl-L-aspartate; 27*

Poly-S-benzyl-L-cysteine; 22 and 57*

Poly-S-carbobenzoxy-L-cysteine; 26*

Poly-S-carbobenzoxymethyl-L-cysteine; 30 and 31

Poly-S-carbobenzoxyethyl-L-cysteine; this dissertation

Poly-S-carboxymethyl-L-cysteine; 32

Poly-S-carboxyethyl-L-cysteine and its sodium salt; this dissertation

Poly-S-methyl-L-cysteine; 22

Sodium poly-L-glutamate; 28

Polyglycine I; 1*

Poly-L-isoleucine; 33

Poly-L-lysine; 13

Poly-L-lysine hydrohalide; 29,34*

Poly-L-serine; 35* and 36

Poly-O-acetyl_-L-serine; 36*,37*, and 38

Poly-O-benzyl-L-serine; 35* and 45

Poly -O-acetyl-L-threonine and Poly-O-acetyl-L-allothreonine; 39

Poly-L-valine; 22,41, and 40*

Poly-γ-benzyl-L-glutamate; 27,42, and 43.

* Results of X-ray diffraction studies are given.

5. Summary of the Studies in Solution.

a) Studies in Organic Solvents.

Studies of β-structure in solution have been made commonly in organic solvents. Moreover, suitable solvents for this conformation are so few that binary solvent systems have been employed, in which one component is a polar one (dichloroacetic acid(DCA) and trifluoroacetic acid are of common use) whereas another one is a less polar solvent freely miscible with the former (usually chloroform or ethylene dichloride is choosen for this purpose). In these cases, the β-random coil transitions have been investigated with the change in composition of binary solvent systems.

a)-1. Short Chain Poly-γ-benzyl-L-glutamate(PBLG).[42-44]

The first report of the existence of β-structure in solution was made with this example.[42] The extensive study by Wada et al. showed that β-structure in dioxane or ethylene dichloride was enhanced with increasing the polymer concentration.[44] Quantitative study by means of infrared measurements made it possible to evaluate the fraction of this conformation. Moreover, the values of dispersion parameters in Moffitt-Yang equation could be obtained for pure β-structure. These results are $a_o^\beta = 840°$ and $b_o^\beta = 420°$ respectively.

a)-2. Derivatives of Serine.[35,36,38,45]

Acetyl and benzyl derivatives have been investigated extensively.[36,45] In both cases, mixed solvent system, DCA-$CHCl_3$, was employed. With decreasing the volme fraction of DCA, these polypeptides were shown to undergo random coil- β transitions.

With this conformational change, a_0 was shown to increase largely. However, the behavior of b_0 was different in two cases. With the formation of β-structure, b_0 increased (about 200°) , as in the case of short chain PBLG, in the case of poly-O-benzyl-L-serine.[45] On the other hand, b_0 increased slightly with the formation of β-structure of poly-O-acetyl-L-serine (about 60°).[36] Further discrepancy was reported that in spite of the substantially equal intrinsic viscosities in DCA of both polymers, 'inter-β' was assigned to the former, whereas 'intra-β' was suggested to the latter. Both conclusions were drawn from the concentration dependence of optical rotation or infrared absorption.

a)-3. Derivatives of Cysteine.[30,31,41,46,47,57]

Only one example,poly-S-carbobenzoxymethyl-L-cysteine, has been examined extensively.[30,31,46] The large increase of a_0 and either small increase or no change of b_0 have been reported with the formation of β-structure. However, the changes in intrinsic viscosity accompanying the transition were in opposite direction between our study[30] and other two investigations.[31,46] Moreover, 'inter-β' was suggested by us from the concentration dependences of both optical rotation and infrared absorption. Contrary to this, 'intra-β' was assigned by Anufrieva et al., from the determination of molecular weight by light scattering.[31] Solvent systems employed were different in these two cases, DCA-$CHCl_3$ was used in our study,whereas DCA-$C_2H_4Cl_2$ or TFA(trifluoroacetic acid)-$C_2H_4Cl_2$ was emplyed by Anufrieva et al. Intrinsic viscosity in DCA,which is supposed to represent molecular weight, was 0.10 for our sample ,which was considerably smaller than that employed by the latter investigators(0.25).

In the case of this polypeptide, it is most likely that different β-structure arise from the difference in molecular weight.

a)-4. Poly-O-acetyl-L-threonine and Its Isomers.[39]

Poly-O-acetyl-L-threonine has been shown to be soluble in pyridine and 2-chloroethanol in addition to polar solvents such as DCA and TFA. The conformation of this polypeptide in pyridine has been confirmed to be 'intra-β'. Increase of intrinsic viscosity in pyridine than that in DCA suggested that intermolecular association occured substantially. Two other solvent systems, 2-chloroethanol-dichloromethane and DCA-$CHCl_3$, were employed for further studies. In both solvent systems, with decrease in the content of polar component, the formation of β-structure occurs accompanying a considerable increase of a_o. The behavior of b_o is somewhat different in these two solvent systems. In the former solvent system, b_o remains almost constant, whereas it slightly increases in the latter solvent system (about 80°). The verifications of 'intra-β' for three cases have been made with the concentration dependence of optical rotation.

Similar results have been obtained for the following isomers, poly-O-acetyl-L-allothreonine, poly-O-acetyl-D-threonine, and poly-O-acetyl-D-allothreonine.

b) Studies in Aqueous Solutions.

Studies of β-structure in aqueous solutions were difficult until recently, because we had not suitable materials for the purpose. At present, we have only few examples of β-structure in aqueous solutions.

b)-1. Poly-L-Lysine.[13,48-50]

It has been shown that α-helical poly-L-lysine can be converted to β-structure by heating the solution above 50°C.[13] Thus formed β-structure of poly-L-lysine had been sole example of this conformation in aqueous solution for long time until 1966. Ultraviolet absorption,[13] circular dichroism,[49,50] and optical rotatory dispersion[48,50] have been reported on this polypeptide. Sarkar and Doty found a new β-structure formed by the addition of sodium dodecyl sulfate to the neutral solution of poly-L-lysine.[50] Thus formed β-structure exhibits somewhat different character in respect to optical rotatory prpperties from that induced in alkaline solution by heating.[50,95]

b)-2. Poly-L-Serine.[35,51,36]

As to the solubility of this polypeptide in aqueous media, inconsistent statements have been made.[35,36] Recently, a sample with short chain length was reported to be soluble in water, and infrared measurements in D_2O showed the presence of β-structure.[51] Measurements of circular dichroism have been reported also.[51]

b)-3. Poly-S-Carboxymethyl-L-Cysteine.[32,52-54]

Two examples previously described,poly-L-lysine in alkaline solution and poly-L-serine, are not polyelectrolyte. Although poly-L-lysine has dissociable side chains, reversible potentiometric titration accompanying β-coil transition can not be expected from the fact that in this example β-structure is induced in somewhat irreversible nature. As to the β-structure of this polypeptide formed in detergent solution, potentiometric titration has not been attempted so far.

Since potentiometric titrations afford useful informations concerning the conformational transition, as have been proved in the helix-coil transitions, it is desirable if we have such examples in the study of β-structure.

Poly-S-carboxymethyl-L-cysteine is sole example in this respect. It has been shown that this polypeptide can undergo β-coil transition depending on the pH of the solution.[32,52] Optical rotatory properties have been also investigated.[32,53,54]

With these three β-structures in aqueous solution except for that of poly-L-lysine in detergent solution, the reported optical rotatory properties are in good, though not complete, agreement with one another. Common features of circular dichroism and optical rotatory dispersion in far ultraviolet region are as follows. In circular dichroism, we have two bands, one is positive and larger than another in magnitude, located between 195-198 mμ, another one is negative, located between 217-227 mμ. Optical rotatory dispersion curves are characterized with a large peak at 205-210 mμ and a small trough at 230-242 mμ, though magnitude of the latter trough varies between wider range. These common features are in good agreement with that postulated from theories for the case of antiparallel arrangement (section 3C). Further discussions concerning the small differences present between reported data will be given in later part. The decrease of b_o, or increase of $-b_o$, with the formation of β-structure is also a common feature of these polypeptides in aqueous solutions. On the contrary, the β-structure of poly-L-lysine formed in detergent solution shows small decrease of b_o (about 30°), and the trough around 230 mμ of optical rotatory dispersion curve is largely diminished compared with other examples.

An example must be added finally. Epand and Scheraga investigated the optical rotatory properties of poly-L-valine in the form of copolypeptide with poly-DL-lysine HCl.[55] They showed that almost same results were obtained as that reported on the β-strcture of poly-L-lysine in alkaline solution.

c) Concluding Remarks to This Section.

It is easily seen that almost all studies described in this section have been made to establish the optical rotatory properties of β-structure. From this point of view, the followings may be regarded as experimentally established conclusions.

(1) Circular dichroism and optical rotatory dispersion in far ultraviolet region.

These curves can be distinguished from those of α-helix. First, there are two dichroic bands rather than three in the latter case. Second, the magnitudes of both bands are rather small. The molar ellipticity of these are about $2\text{-}3 \times 10^4$ and $-5\text{-}20 \times 10^3$, whereas in α-helix at the same wave length regions are 10^5 and -4×10^4 respectively.[56] Thus optical rotatory properties in far ultraviolet region can serve as a tool for the determination of β-structure.

(2) The dispersion parameter a_o increases with the formation of β-structure.

(3) As to the dispersion parameter b_o, it decreases by about -150°

in aqueous solutions, whereas it increases in organic solvents.

Aside from these established relations, there remain several ~~di~~ discrepancies unsolved between various investigations. These are summarized below.

(1) The changes of b_0 accompanying the transition are in opposite direction in orgnic solvents and in aqueous solutions. In this connection, further investigations on β-structure of poly-L-lysine in detergent solution may afford some valuable informations.

(2) The magnitudes of the changes of b_0 in organic solvents are not coincident among the reported investigations.

(3) There are also inconsistencies between the reported changes of intrinsic viscosities accompanying the formation of 'intra-β'.

(4) It has not been elucidated as to what conditions are necessary for the preference of either type of β-structure, inter or intra.

All these characteristics described above will be easily seen from Tables I and II, where the representative data are tabulated.

TABLE I

Material	Poly-O-acetyl-L-threonine			
Solvents	Pyridine	Pyridine	$DCA-CHCl_3$	2-Chloroethanol-Dichloromethane
$[\eta]$ in DCA (dl/g)[a]	0.14	0.10	0.14	0.10
Δa_o[b]	400[f]-395	-330	400-700	290-160
Δb_o[b]	-50[f]-150	-0	-50-0	no change
$\Delta[\eta]$(dl/g)[b]	increase[f] (up to 0.38)	increase[f] (up to 0.16)		
Assigned β-Structure	intra-β	intra-β	intra-β	intra-β
Reference	39	39	39	39

Abbreviations used here are; DCA; dichloroacetic acid, TFA; trifluoroacetic acid, EDC; ethylene dichloride.

[a] Intrinsic viscosity in DCA is considered to be a measure of molecular weight of the sample.

[b] Difference from random coil state is taken to represent the characteristic features of β-structure.

[c] Value for complete β-structure.

[d] Measured in TFA.

[e] Reduced viscosity at $C_p=0.5\%$.

[f] In this case, difference is taken from the random coil state in DCA.

TABLE I (continued)

Summary of the representative data of the studies of β-structure in organic solvents.

Material	Short Chain PBLG	Poly-O-acetyl-L-serine	Poly-O-benzyl-L-serine	Poly-S-carbobenzoxymethyl-L-cysteine		
Solvents	Dioxane or EDC	DCA-$CHCl_3$	DCA-$CHCl_3$	DCA-$CHCl_3$	DCA-EDC or TFA-EDC	DCA-EDC
$[\eta]$ inDCA (dl/g)[a]		0.12[d]	0.13[e]	0.10	0.25	0.43
Δa_o[b]	840[c]	200-680	200-800	-730-380	-800-500	-730-400
Δb_o[b]	420[c]	662-0	-40-150	-80-0	-70-0	no change
$\Delta[\eta]$(dl/g)[b]		increase		increase (up to 0.45)	decrease (down to 0.05)	decrease (down to 0.08)
Assigned β-Structure	inter-β∞	intra-β	inter-β	inter-β	intra-β	
Reference	44	36	45	30	31	46

TABLE II

Summary of the representative data of the studies of β-structure in aqueous solutions.

Material	Poly-L-lysine in alkaline solution			Poly-L-lysine in detergent		Poly-S-carboxymethyl-cysteine			Poly-L-serine
a_o			-340	174		-225			
b_o			-152	- 9		-130			
Cotton Effects									
λ_{peak}	205		205	205			210		
$[m']_{peak}$	29,000		23,000	26,000			23,000		
λ_{trough}	230		230	230			242		
$[m']_{trough}$	-6,200		-6,400	-2,000			480		
Circular Dichroism									
λ_{peak}		195			197			198	197
$[\theta]_{peak}$		28,000			4,100			15,000	23,000
λ_{trough}		217	218		217		226	227	222
$[\theta]_{trough}$		-19,000	-23,000		-1,200		-6,600	-6,000	-5,000
Refernces	48	49	50	50	95	32	53	54	51

Charge Induced β - Random Coil Transition of Poly-S-Carboxyethyl-L-Cysteine in Aqueous Media

Acknowledgment

The author would like to express his sincere thanks to Professor Toshizo Isemura of Osaka University, who has introduced him to this field of study and encouraged him throughout the course of this study. The author is indebted very much to Dr. Shoichi Ikeda of Nagoya University for valuable advices and discussion throughout the course of this study. The author is also indebted to Professor Fumio Oosawa of Nagoya University for valuable discussion about the analysis of the counter ion activity. Finally the author wishes to thank his wife Junko and his parents, Kenzo and Asako for their encouragement.

CONTENTS

1. Introduction and Scope of the Present Investigations.

Although considerable amount of data have been accumulated in the study of β-structure in organic solvents, little has been elucidated about the β-coil transition. This situation arises because we can not treat theoretically the transition induced by the changes of solvent compositions. For any theoretical analysis, we must describe the system with activities of components instead of solvent compositions. However evaluation of the activity in three component system,such as polypeptide-dichloroacetic acid-chloroform for example, is considerably difficult. Therefore no such a study has been reported. If temperature induced β-coil transition is possible, situations become different. Then studies of the transition in organic solvents may afford more valuable informations. However such transitions have not been realized so far. Therefore a possible development in this respect will be sought in the study of the transition induced by change of pH in aqueous solutions. For,in principle, a theoretical treatment becomes possible in such a case. Of course some ambiguities are present concerning the concept of single ion activity. Moreover,the treatment of the interactions between multi-charged ions inevitably involves some difficulties. Thus at present rather extensive investigations on the β-coil transition induced by pH change are necessary. For this purpose,of course, a new polypeptide example is not always necessary. We have a suitable example,poly-S-carboxymethyl-L-cysteine,for this purpose,as described in section I-5(b). However previous investigations on this polypeptide have examined mainly its optical rotatory properties. Moreover,the results from potentiometric titration studies should be accepted with reservations. For, in the experiments of these studies, some complicated situations are present,on which discussions will be given in detail in later part. Nevertheless appropriate attentions have not been paid for these situations in previous investigations.

Aside from the aspect of conformational transition, the optical rotatory properties of β-structure have not been clearly characterized in the case of organic solvents. This situation is expected to be improved if we measure the optical rotatory properties in far ultraviolet region. Investigations in aqueous solutions have proved it to be the case. Although characteristic features inherent to β-structure have been deduced from these measurements,however,there are also several differences among reported data,as to positions or magnitudes of dichroic bands. These differences are shown in Table II. Therefore it is desirable to add a new example and to increase data in this respect.

With these purposes we have synthesized a new polypeptide, poly-S-carboxyethyl-L-cysteine which is a higher side-chain homologue of poly-S-carboxymethyl-L-cysteine,and investigated its conformational transition induced by change of pH.

Since theoretical treatment of β-coil transition in polyelectrolytes is a very difficult one,we made our efforts to provide experimental data. To obtain the data corresponding to equilibrium state, several precautions were taken. The effects of both ionic strength and polymer concentration on conformational transition were investigated rather extensively.

In the next section synthesis of the polymer is described. Then conformational studies on the polymer in aqueous media will be given. The investigations are made on optical properties; optical rotatory dispersion in visible and ultraviolet wave length regions, circular dichroism, and infrared absorption. The conformation in solid state is also examined by infrared measurements.

In section 3, potentiometric titration of the polymer will be discussed. Reversibility and time dependence of titration curves are examined by different methods, in order to establish the conditions for obtaining equilibrium curves.

In section 4, activity coefficient of sodium counter ions is given, which is measured in salt-free polyelectrolyte solution using a Na-glass electrode. Activity coefficient of counter ions in salt-free solution is also estimated from the data of potentiometric titration. The comparison of the results obtained from two methods is discussed. The effect of conformational change and concentration dependence of activity coefficient are also discussed.

In the final section, the effect of precipitation on the titration behavior is treated in the framework of thermodynamics. This way of treatment has the advantage in the present investigation that a specified model is not necessary for the treatment.

2. Conformational Studies of Poly-S-carboxyethyl-L-cysteine in Aqueous Solutions.

Synthesis.

S-Carboxyethyl-L-cysteine.[58] In 2 l. of 2 N NaOH were added 175 g of L-cysteine HCl monohydrate and 168 g of β-bromopropionic acid at a temperature not exceeding 30°C. After 50 minutes stirring at 30°C, the solution was cooled and concentrated H_2SO_4 was added until pH 2.5 at a temperature lower than 20°C. The crystal was filtered, washed with cold water and dried. It was recrystallized twice from a neutral solution by adding concentrated H_2SO_4. Yield. 80%.m.p.,214-215°C.

S-Carbobenzoxyethyl-L-cysteine.[59] Two hundred g of S-carboxyethyl-L-cysteine was suspended in a mixture of 168 g of benzyl alcohol and 160 g of 70% H_2SO_4, and vigorously stirred at 70°C for an hour. After evaporated in vacuo for 2 hours, the clear solution was poured into 192 g of $NaHCO_3$ in 400 ml. of ice water and the crystal was collected by filtration. It was recrystallized five times from boiling water. Yield. 35%. m.p.,203°C.

S-Carbobenzoxyethyl-L-cysteine anhydride.[59] After dried over P_2O_5 overnight, 20 g of S-carbobenzoxyethyl-L-cysteine was suspended in 400 ml. of dry dioxane, and phosgene was bubbled in for 45 minutes at 40°C. Nitrogen was passed through the clear solution for 2 hours and the solution was evaporated up in vacuo at 50°C. The oily syrup was tritiated with petroleum ether. and the supernatant was discarded by decantation. The oil was dissolved in ethyl acetate and reprecipitated with petroleum ether. The procedure was repeated five times. Yield. 15 g.

Poly-S-carbobenzoxyethyl-L-cysteine(PZEC).[59] Thirty g of the oil of the N-carboxyanhydride was dissolved in dioxane or methylene chloride and polymerized by addition of triethylamine or sodium methoxide. After several days, a large amount of petroleum ether was added and the precipitates was washed with dioxane and ethyl ether. Yield. 12 g. Anal. Calculated for $(C_{13}H_{15}O_3NS)_x$; C,58.84%;H,5.71%;N5.28%;S,12.08%. Found; C, 58.58%;H, 5.71%;N, 5.27%;S, 11.98%. Conditions for polymerization and some of the properties of the obtained PZEC are listed in Table III. Poly-S-carboxyethyl-L-cysteine(PSCEC). Five g of PZEC suspended in 400 ml. of chloroform or glacial acetic acid which had been saturated with hydrogen bromide was stirred at 0 to 10°C for more than 20 hours. The suspension gradually swelled and turned to be viscous. Dry air was passed through the mixture for more than 3 hours and acetone was added. The white precipitate was centrifuged,washed with acetone until the supernatant became colorless. The polymer was washed with ethyl ether and dried. Yield. 2 g. Anal. Calculated for $(C_6H_9O_3NS)_x$; C, 41.13%;H, 5.19%; N, 8.00%;S, 18.30%. Found; C,41.27%;H, 5.12%;N, 8.14%;S,17.88%. Some data for the preparation and of properties of poly-S-carboxyethyl-L-cysteine samples are given in Table III.

Poly-S-carboxyethyl-L-cysteine is soluble in dimethyl sulfoxide and alkaline water. Ultraviolet spectra of the polymer were examined in aqueous solutions of pH 7. The amount of the remaining benzyl groups was estimated on the basis of an assumed value for the molar extinction coefficient,200,at 280 mu. It was found that the debenzylation was complete at 20 hours. Infrared spectra of the polymer in solid also confirmed this result.

TABLE III

Synthesis of Poly-S-carboxyethyl-L-cysteine

Poly-S-carbobenzoxyethyl-L-cysteine					Poly-S-carboxyethyl-L-cysteine			
Sample Number	Solvent	Initiator	[A]/[I]	[η]* (dl/g)	Sample Number	Debenzylation		[η]** (dl/g)
						Solvent	Reaction Time	
B622A	CH_2Cl_2	CH_3ONa	250	0.35	B622AW	CH_3COOH	22 hrs.	0.17
H920	CH_2Cl_2	$(C_2H_5)_3N$	50	0.18	H920W	CH_3COOH	24 hrs.	0.17
H1030	CH_2Cl_2	$(C_2H_5)_3N$	50	0.15	H725AW1	$CHCl_3$	24 hrs.	
H725A	CH_2Cl_2	CH_3ONa	50	0.10	H725AW5	$CHCl_3$	30 hrs.	
H725B	CH_2Cl_2	$(C_2H_5)_3N$	50	0.14	H725BW	CH_3COOH	8 hrs.***	0.08
H615	Dioxane	CH_3ONa	50	0.08	H615W1	$CHCl_3$	4 hrs.	insoluble
H622B	Dioxane	CH_3ONa	100	0.12	H615W2	$CHCl_3$	5.5 hrs.	insoluble
H622C	Dioxane	CH_3ONa	100	0.08	H615W3	$CHCl_3$	21 hrs.	0.09

* Measured in dichloroacetic acid at 25°C.

** Measured in 0.2M NaCl solution at 25°C and pH=7.

*** Remaining benzyl groups were estimated to be about 15%.

The samples of polymer thus obtained were dialyzed against distilled water for a week and precipitated by the addition of 1 N HCl. The precipitate was collected by centrifugation and washed with water, acetone and ethyl ether. The crystal was dried over P_2O_5 in vacuo until no more loss of weight.

Experimental.

Material and Preparation of Solutions. A sample of the polymer used in this work was B622AW. The sample H920W gave substantially identical results.

Concentrated,0.025 and 0.050 N, solutions of the polymer were prepared on the weight basis. Dilute solutions,0.005 and 0.010 N, were prepared in the following way. The polymer sample dissolved in alkaline salt-free water was dialyzed against distilled water and then passed through a cation exchange column containing Amberlite IR-120. To the eluate were added 0.2 N NaOH and NaCl to give desired polymer concentration and degree of neutralization as well as ionic strength. Polymer concentration of stock solutions was determined by titration in the presence of sufficient NaCl. Throughout the present section, the pH of solution or the degree of neutralization of polymer will be given in order to specify the state of ionization of polymer in aqueous solutions. The degree of neutralization is shown to be substantially equal to the degree of ionization under the conditions examined,as far as the added salt is present. In salt-free aqueous solutions,however,the degree of ionization is generally higher than the degree of neutralization at pH less than about 5 or at the degree of neutralization lower than about 0.1.

Apparatus. Infrared spectra were measured on a JASCO Model DS-402 G spectrophotometer at 20°C. Solid films were cast from dimethyl sulfoxide or aqueous solution of the polymer on NaCl or AgCl plates. Spectra of D_2O solutions of the polymer were measured in a cell having adjustable thickness of KRS-5 windows and compensated against D_2O of the same thickness.

Circular dichroism and optical rotatory dispersion were measured on a JASCO ORD/UV 5 spectropolarimeter with a CD attachment at 25°C. Measurements were performed with 0.1 and 1 mm cells from 260 to 200 mμ and with 1 and 5 cm cells for visible region. The signal-to-noise ratio in the circular dichroic spectra was better than 4 at 220 mμ. Visible optical rotatory dispersion was analyzed by means of the Moffitt-Yang equation (1) with λ_0 = 212 mμ. Dispersion of the refractive index of water was taken into considerations. But the effect of the presence of added salt was ignored.

Measurements of pH were performed as will be described in the following section.

Results.

Conformation of the Polymer in the Solid State.

Infrared spectra of the polymer films in both acid (H) and salt (Na) forms are shown in Fig. 2. The acid form was obtained from a dimethyl sulfoxide solution and the salt form was from a salt-free aqueous solution at a degree of neutralization unity. Frequencies of characteristic absorption bands are listed in Table IV.

The amide I band is located around 1630 cm^{-1} to 1635 cm^{-1} together with a weak band at 1695 cm^{-1}. This clearly indicates that both forms are in the β-structure with an antiparallel arrangement of adjacent chains. While the acid form has a band at 1720 cm^{-1} due to the unionized carboxyl groups, the salt form has two strong bands at 1570 and 1405 cm^{-1} charadteristic of ionized carboxyl groups.

Optical Rotatory Dispersion.

Ultraviolet rotatory dispersion of the polymer in aqueous solutions is illustrated in Fig.3(a)-(c). At neutral pH where the polymer is almost fully ionized, the rotation is negative at all wavelength regions. And Cotton effects occur around 225 and 200 mμ. Two troughs are manifest at 236 and 207-210 mμ. With lowering pH, the trough around 210 mμ turns to be a peak, while the other trough at 236 mμ remains almost unaltered. At acid pH lower than 6, for example in Fig.3(b), Cotton effects occur with a trough at 236 mμ and a peak at 210 mμ.

The Cotton effects observed for the polymer at neutral pH are quite similar to those for the random coil poly-S-carboxymethyl-L-cysteine, both in position and shape. On the other

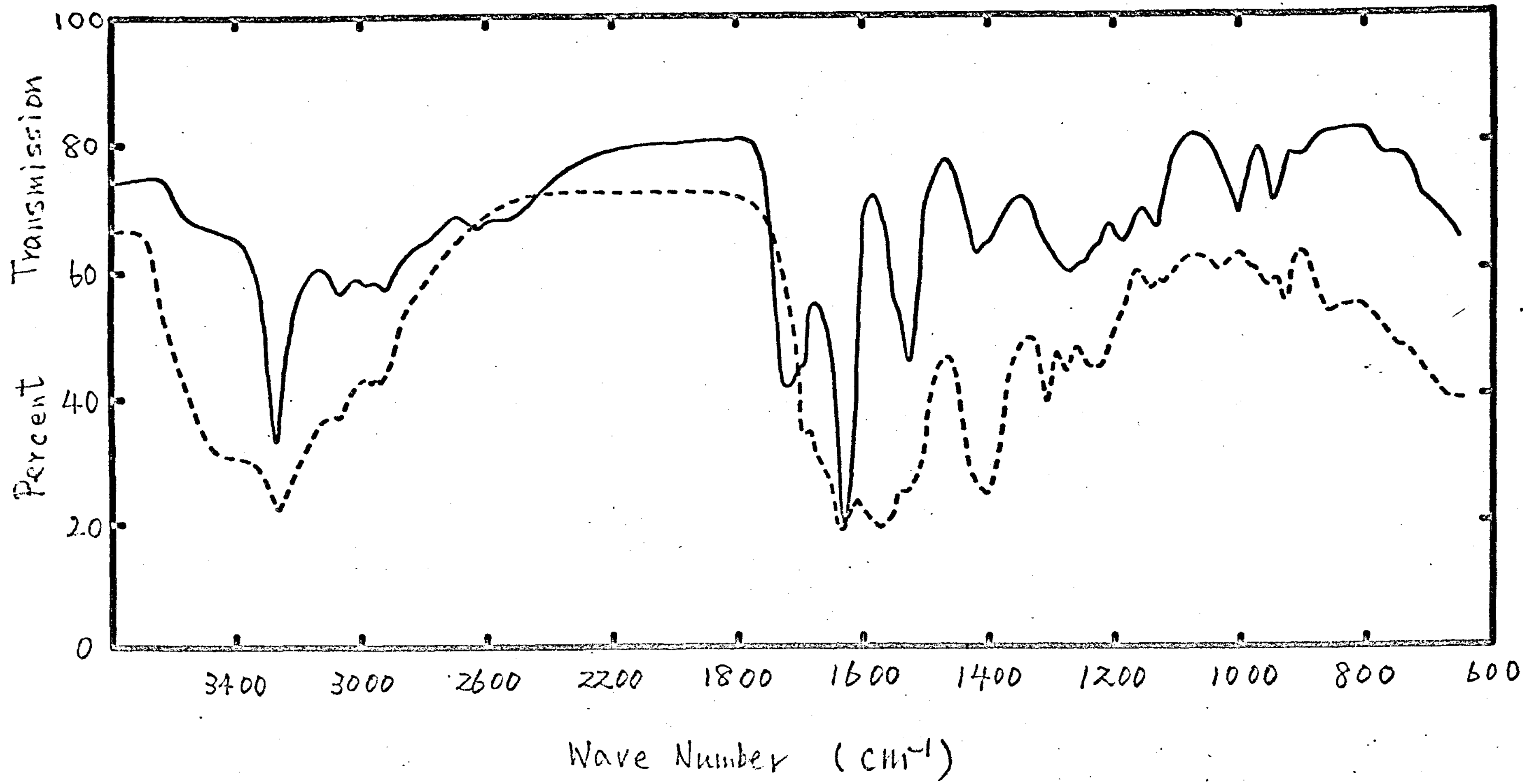


Fig. 2. Infrared spectra of poly-S-carboxyethyl-L-cysteine in solid films.

——— ; Acid form cast from a dimethylsulfoxide solution.

- - - ; Sodium salt form cast from a salt-free aqueous solution with degree of neutralization unity.

TABLE IV

Characteristic Absorption Bands of PSCEC in Different Forms

Assignment	Wave number (cm^{-1})	
	Acid (H) form	Salt (Na) form
ν_{O-H} Adsorbed water	3400 (sh)	3600 (s)
Amide A	3280 (vs)	3260 (vs)
Amide B	3080 (w)	3060 (w)
$\nu_{C=O}$ Carboxyl	1718 (s)	
Amide I (0,π)	1698 (sh)	1695 (sh)
Amide I (π,0)	1631 (vs)	1635 (vs)
ν_{C-O} antisymmetric Carboxylate		1570 (vs)
Amide II	1527 (s)	1520 (sh)
ν_{C-O} Symmetric Carboxylate		1405 (vs)

II (0,π)

vs; very strong; s, strong; w, weak; sh, shoulder

Fig.3 Ultraviolet rotatory dispersions of the polymer.

Polymer concentration; 0.005 N.

(a) Ionic strength 0 molal.

(a) $\alpha = 0$, pH =4.22, (b) $\alpha = 0.150$, pH =5.56,
(c) $\alpha = 0.205$, pH =5.74, (d) $\alpha = 0.402$, pH =6.08,
(e) $\alpha = 0.489$, pH =6.17, (f) $\alpha = 0.701$, pH=6.66 ,
and (g) $\alpha = 0.903$, pH =7.52.

(b) Ionic strength 0.030 molal.

(a) $\alpha = 0.389$, pH =5.50, (b) $\alpha = 0.452$, pH =5.59,
(c) $\alpha = 0.549$, pH =5.67, (d) $\alpha = 0.645$, pH =5.79,
(e) $\alpha = 0.768$, pH =6.13, and (f) $\alpha = 0.896$, pH =6.98.

(c) Ionic strength 0.15 molal.

(a) $\alpha = 0.460$, pH =5.10, (b) $\alpha = 0.499$, pH =5.16,
(c) $\alpha = 0.558$, pH =5.21, (d) $\alpha = 0.606$, pH =5.25,
(e) $\alpha = 0.684$, pH =5.39, and (f) $\alpha = 0.864$, pH =6.05.

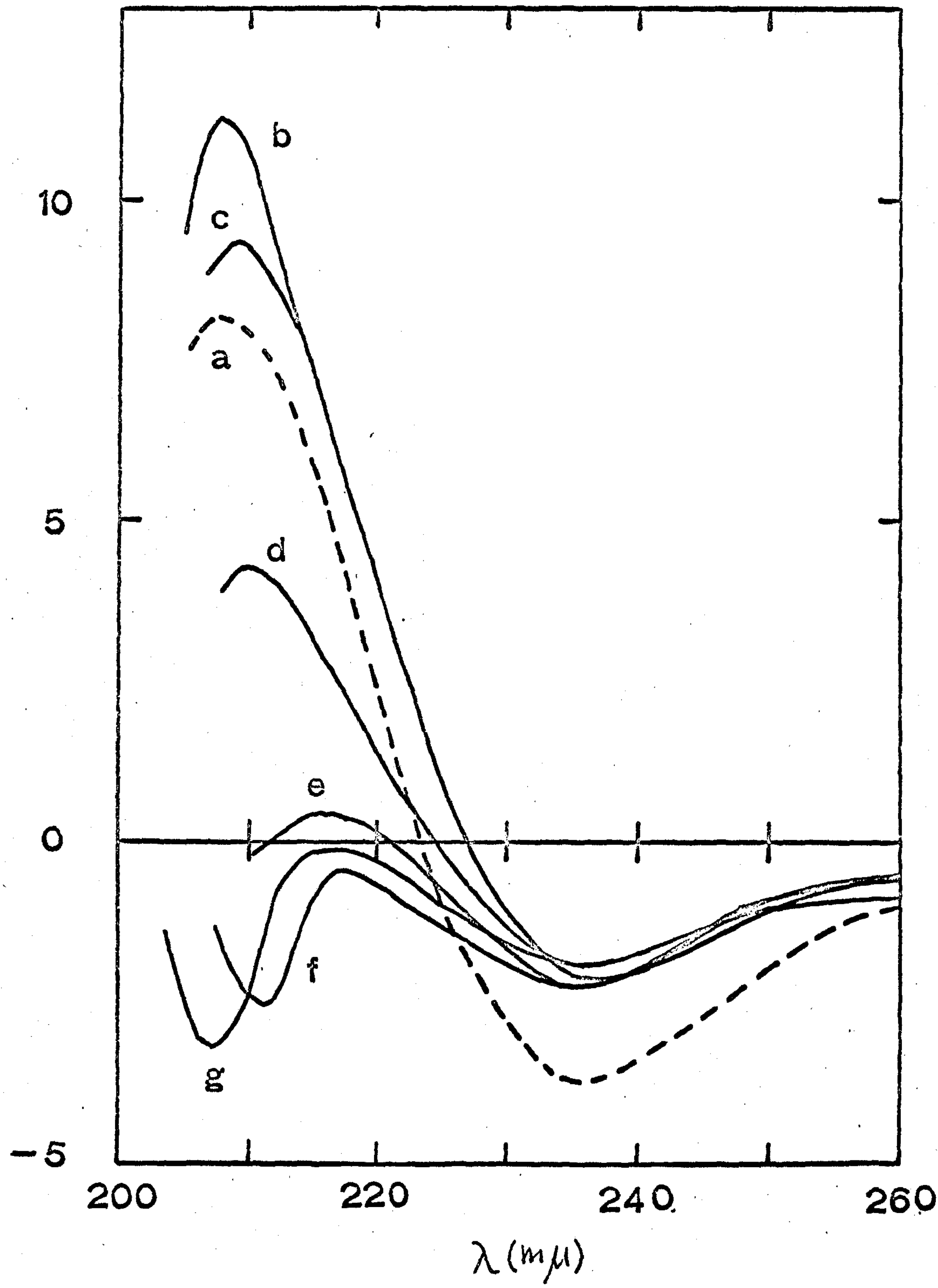


Fig. 3 (a)

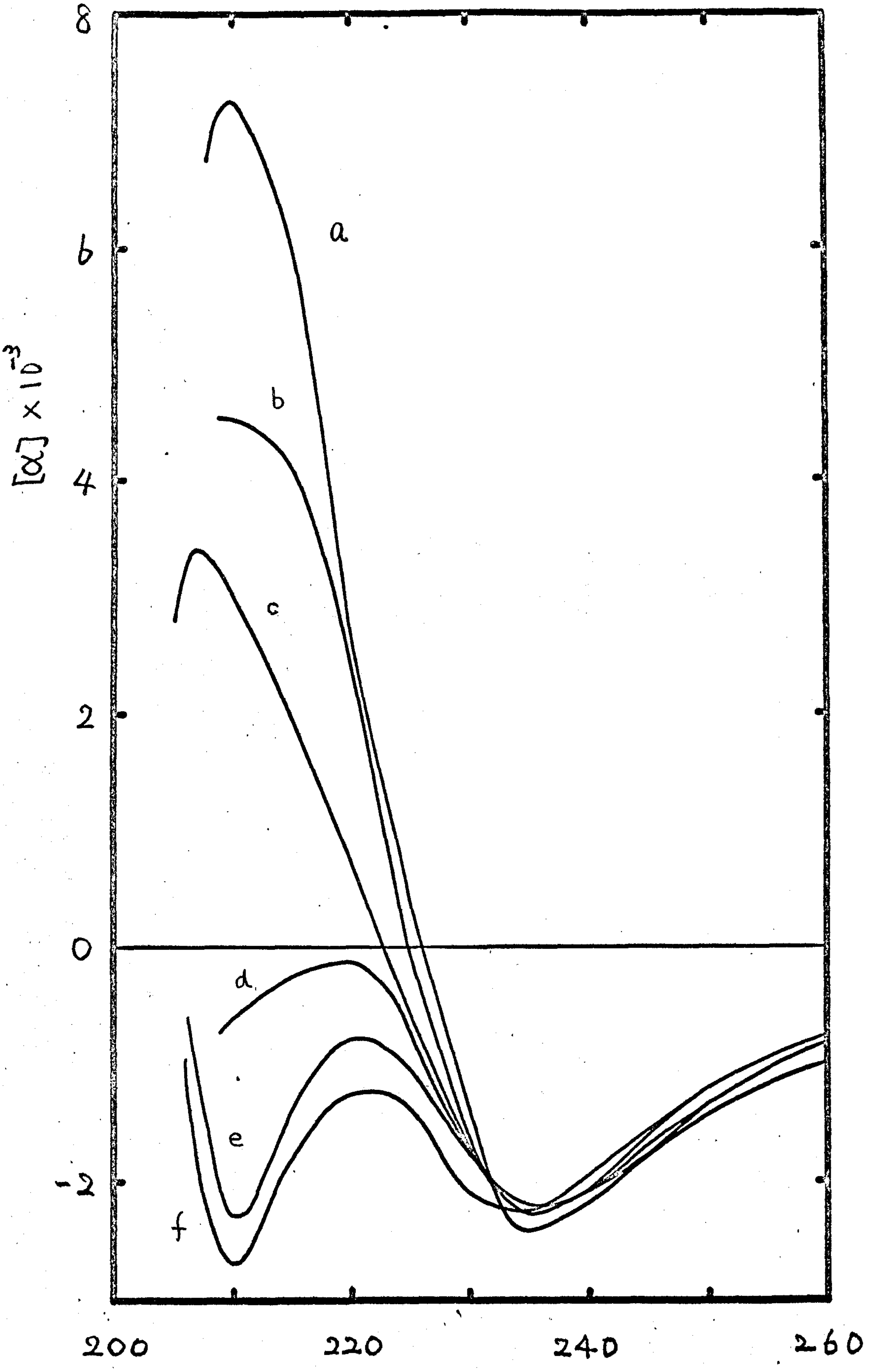


Fig. 3 (b)

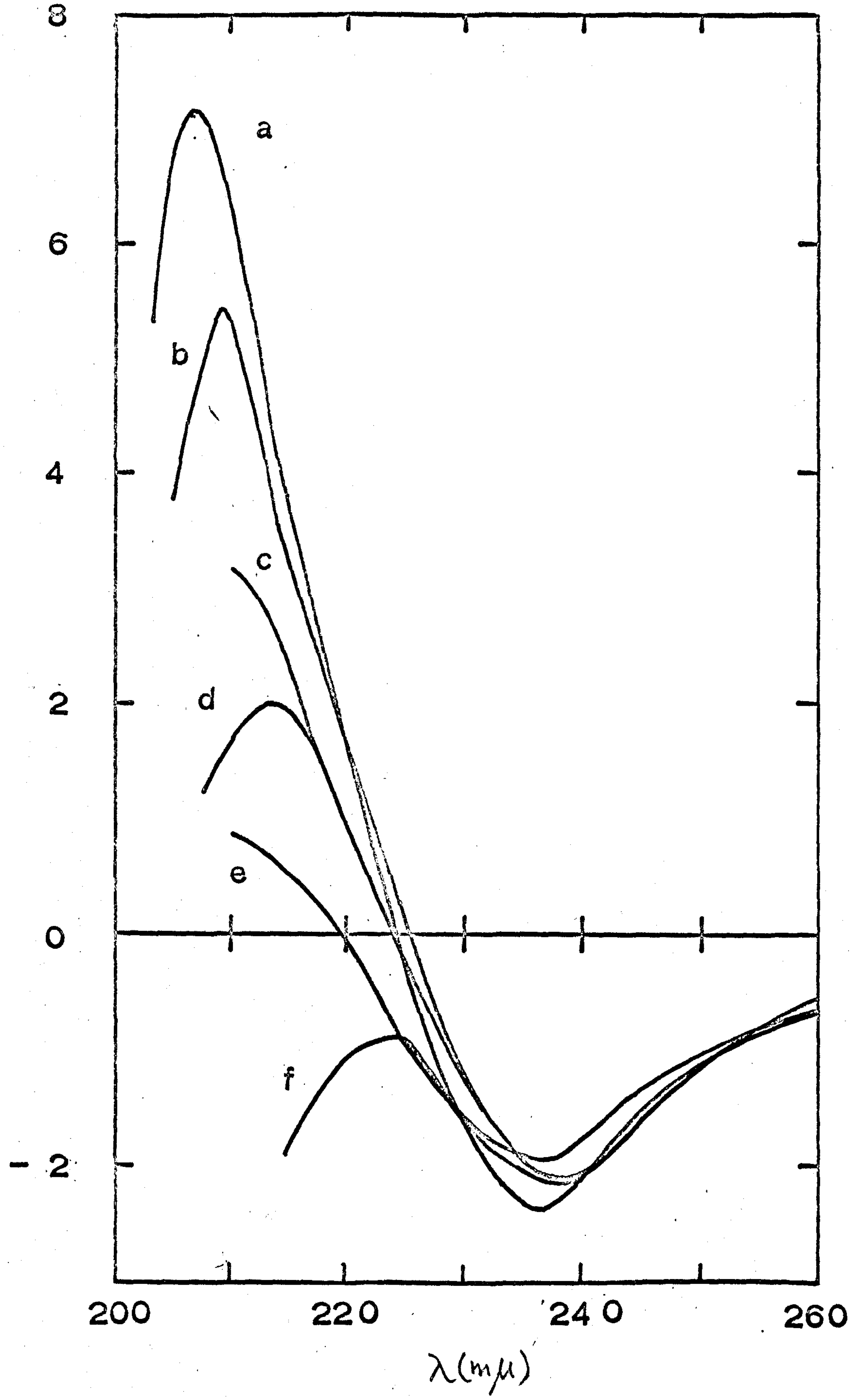


Fig. 3 (c)

hand,the Cotton effects of the polymer at acid pH are quite similar to those for β-structure previously reported. So it is clear that a coil-β transition is induced by a change of pH or degree of ionization of Poly-S-carboxyethyl-L-cysteine in aqueous solutions. The positions and magnitudes of the observed Cotton effects of this polymer are tabulated in Table V. The magnitude of the peak at 210 mμ is considerably smaller than other examples,but the position is of the same range. The position of the trough(236 mμ) is about a midpoint between that for poly-L-lysine or silk fibroin[60](230 mμ) and that for poly-S-carboxymethyl-L-cysteine(242 mμ). This trough has been shown to be conformation dependent in every case of previous investigations. With the formation of β-structure,this trough was largely reduced in the case of poly-L-lysine or silk fibroin,whereas it was enhanced in the case of poly-S-carboxymethyl-L-cysteine. It should be noted that this trough is almost insensitive to conformations in ~~this~~ present polypeptide.

In the visible region from 450 to 270 mμ, the specific rotation is again negative at any pH,ionic strengths and polymer concentrations, but dextrorotation increases with decreasing pH. Rotatory dispersion in this region obeys the Moffitt-Yang equation,and the parameters, a_o and $-b_o$,generally increase with decreasing pH. Fig.4 shows the values of a_o and b_o as a function of degree of neutralization. The b_o value of the polymer at neutral pH is not equal to zero,but has a small negative value,-30°, and it decreases down to -140° at the most acid pH.at lower polymer concentration. The latter is of the same magnitude as those of the other water-soluble β-structure (see Table II).

TABLE V

Cotton Effects of Poly-S-carboxyethyl-L-cysteine.

(a) β-Structure

m_s (molal)	α	pH	λ_{peak} (mu)	$[m]'_{peak}$	λ_{trough} (mu)	$[m]'_{trough}$
0	0	4.22	207	10,800	236	-4,900
0	0.100	5.34	211	11,900	235	-3,000
0	0.150	5.56	208	14,800	236	-3,000
0	0.205	5.74	210	12,100	236	-2,700
0.030	0.389	5.50	210	9,600	235	-3,300
0.150	0.460	5.08	207	9,400	236	-3,200

(b) Random Coil

m_s (molal)	α	pH	λ_{trough} (mu)	$[m]'_{trough}$	λ_{trough} (mu)	$[m]'_{trough}$
0	0.900	7.65	206	-4,000	235	-2,500
0.030	0.896	6.98	210	-3,600	235	-2,900

Fig. 4 Dispersion parameters in the Moffitt-Yang equation determined from the wavelength region 270 - 450 mu.

(○), b_o and (○-), a_o .

(a) Polymer concentration; 0.005 N.

Ionic strength (in molal);

0 (○) and 0.030 (◑).

(b) Polymer concentration; 0.010 N.

Ionic strength (in molal);

0 (○), 0.010 (◑), 0.020 (⦶), 0.050 (⊖), 0.100 (◑), and 0.200 (◒).

(c) Polymer concentration; 0.025 N.

Ionic strength (in molal);

0 (○), 0.050 (◑), and 0.110 (⦶).

(d) Polymer concentration; 0.050 N.

Ionic strength (in molal);

0 (○), 0.050 (◑), and 0.110 (⦶).

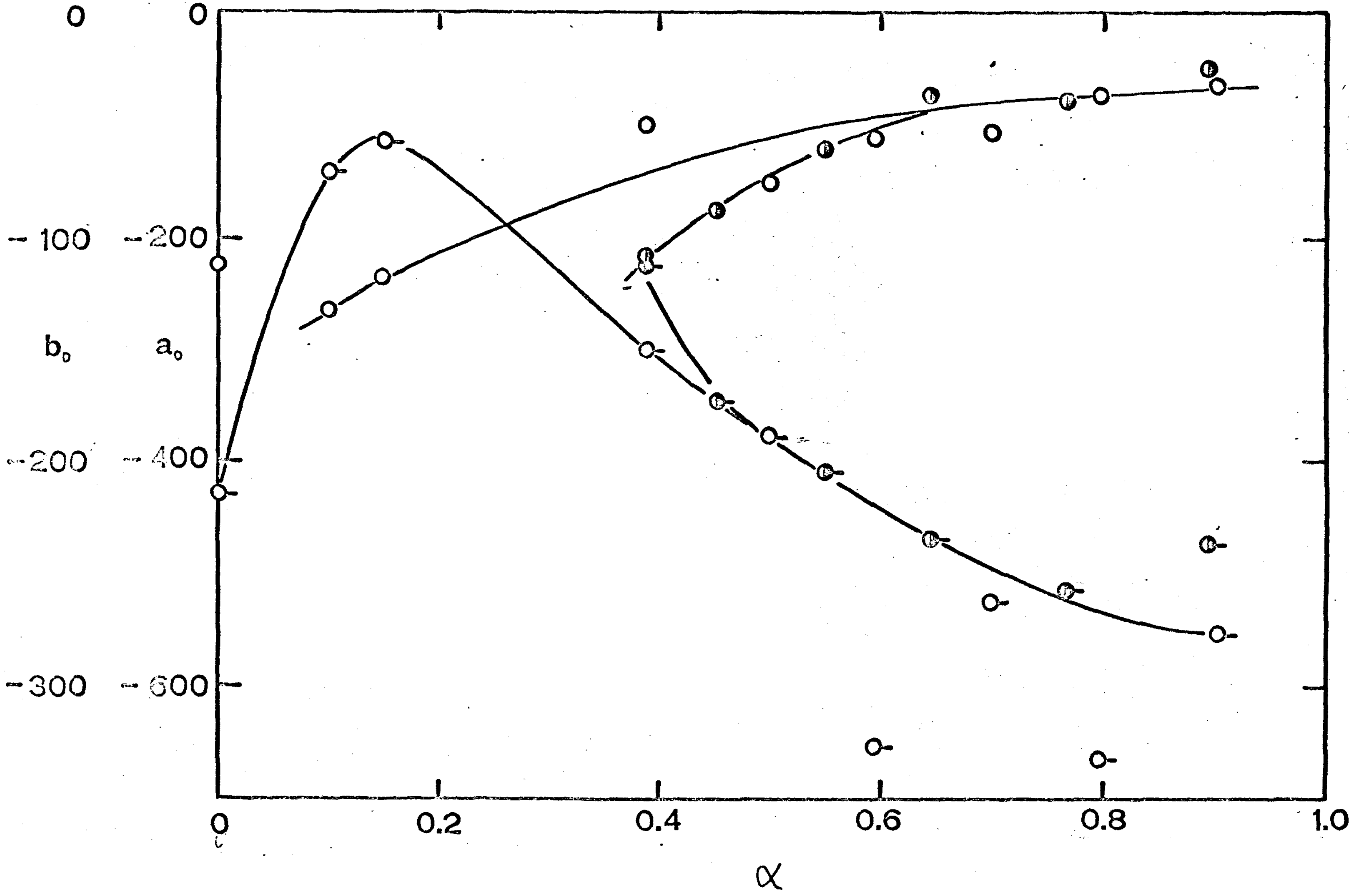


Fig. 4 (a)

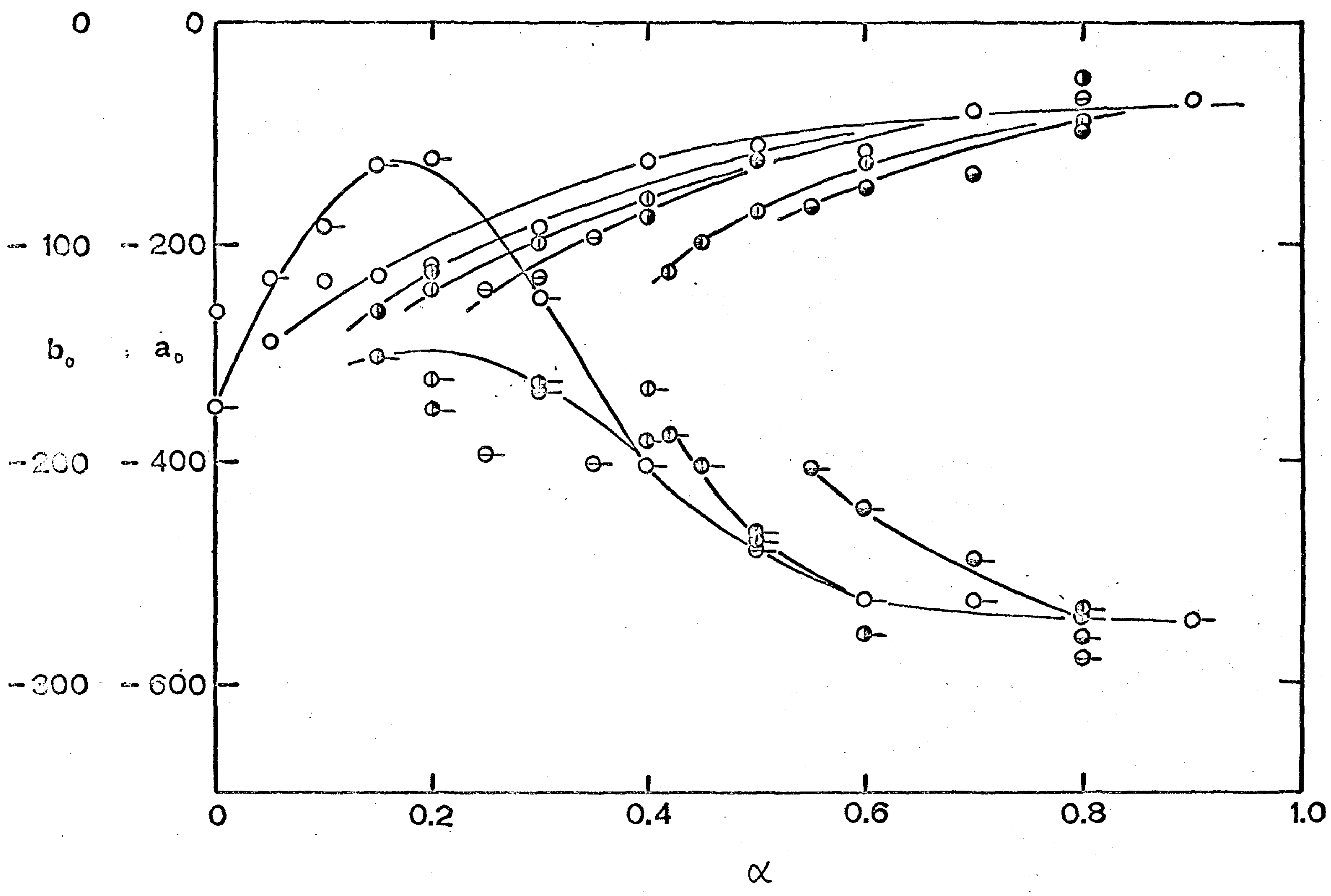


Fig. 4 (b)

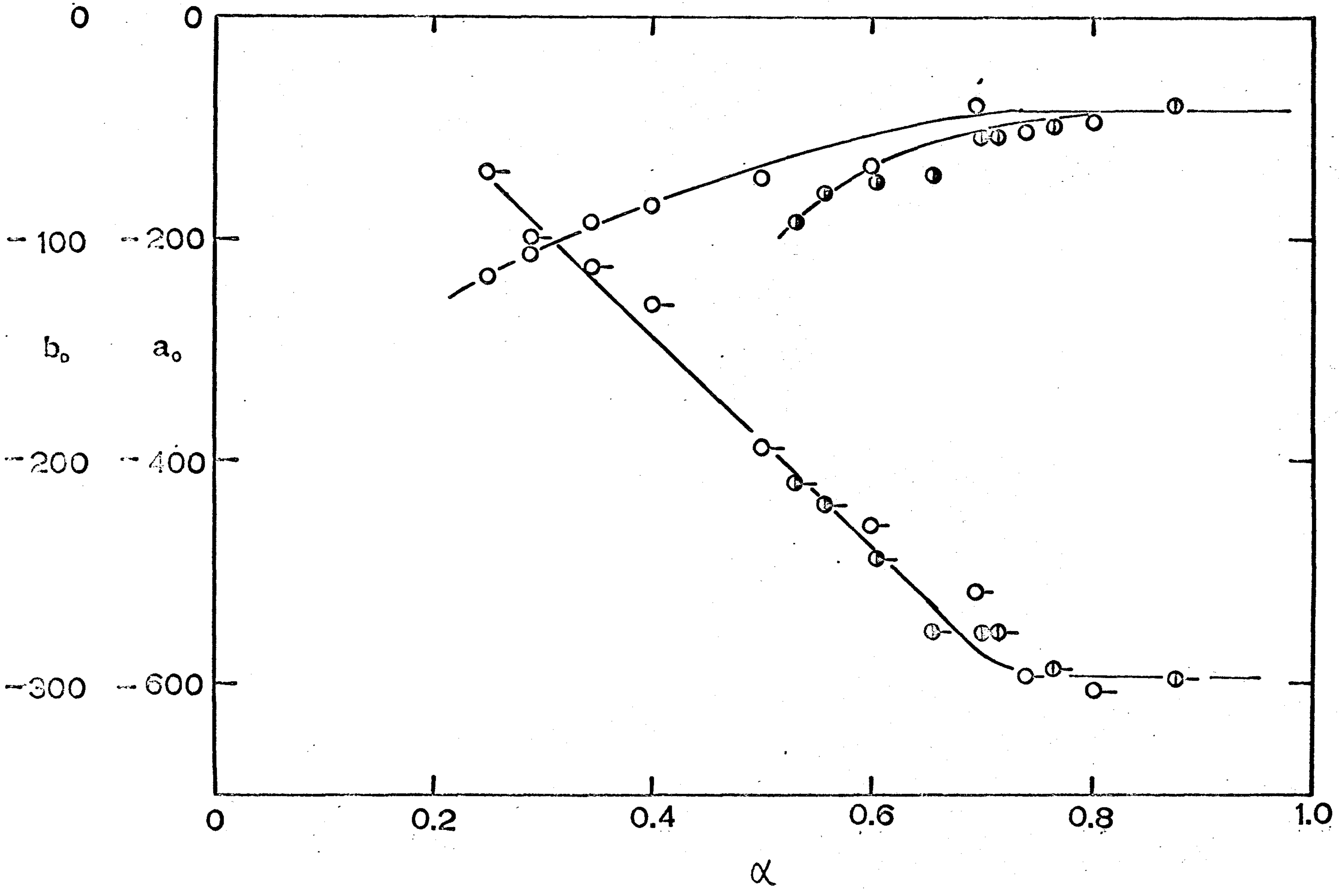


Fig. 4 (c)

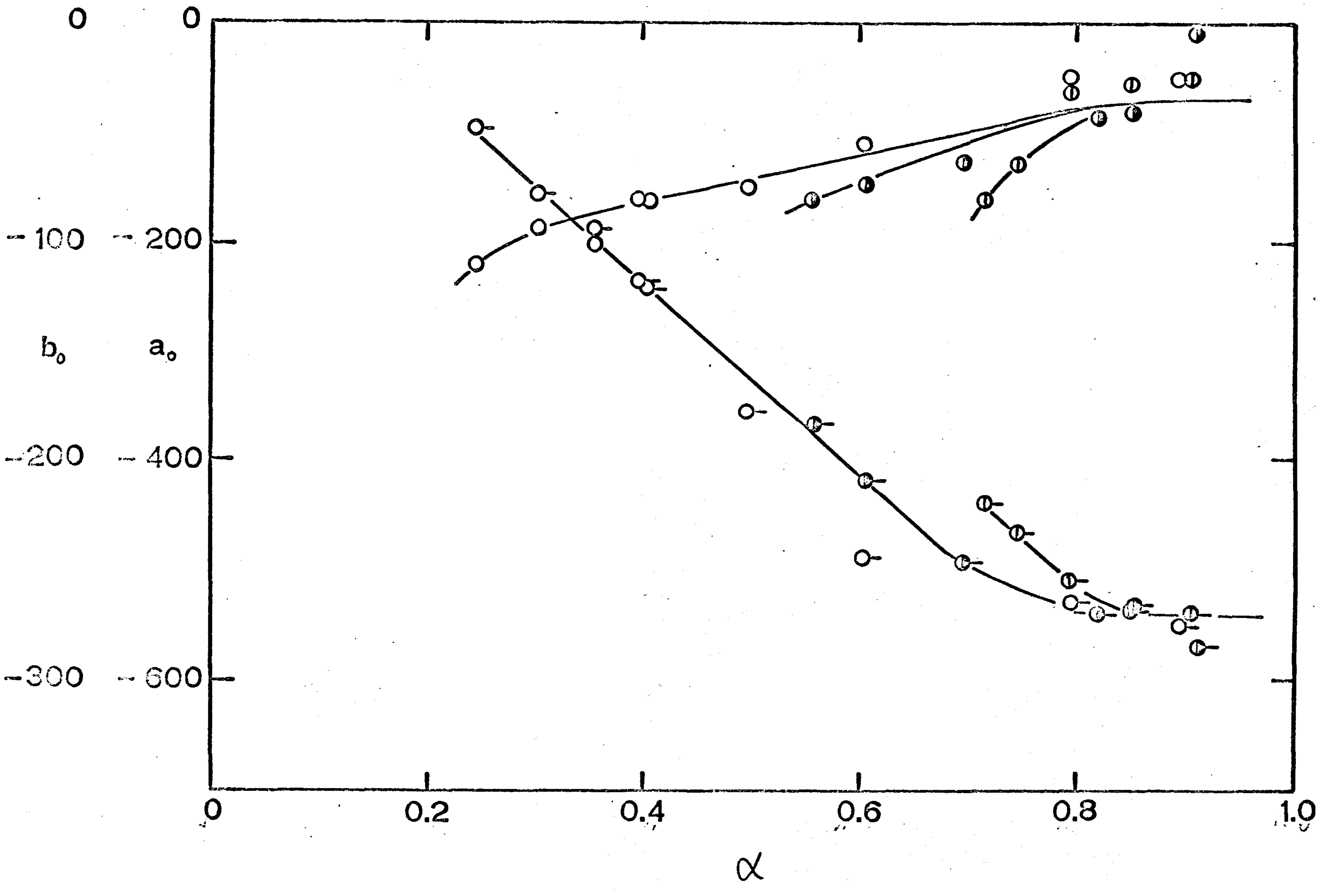


Fig. 4 (d)

For lower polymer concentration with no added salt, considerable decrease of a_0 is observed in the region where degree of neutralization is extremely low. Under the same conditions, the magnitude of the peak at 210 mμ is also reduced. These results together with a large trough found for the solution with zero degree of neutralization (dashed line in Fig.3(a)), suggest that this trough is more sensitive to the environment of the peptide chromophore including the extent of association, rather than to conformations.

Circular Dichroism

Circular dichroism of the polymer in aqueous solutions is shown in Fig.5. A single negative dichroic band is manifest at 223 mμ and its magnitude changes with the pH of the solution. The minimum molar ellipticity, $[\theta]_{223}$, is plotted as a function of pH in Fig.6. This clearly indicates the occurence of a pH induced coil-β transition around pH 5.5. The transition pH lowers with increasing ionic strength, as has been observed in the conformational transitions of other acidic polypeptides.[61,67] Hydrogen ion titration of the polymer, which will be discussed in the succeeding section in some detail, shows that the polymer is more ionized at a given pH as the ionic strength is higher. Increase in ionic strength introduces the transition of the polymer at higher degree of neutralization. Thus the β-structure of the polymer is more stable at a given degree of ionization as the ionic strength is higher, owing to the shielding effect of ionic atmosphere around the charged groups. This may be confirmed in more direct way, if we plot the data against degree of neutralization.

Fig. 5. Circular dichroism of the polymer in aqueous solutions. Polymer concentration 0.050 N; ionic strength 0 molal. (a) α= 0.248, pH =5.55, (b) α = 0.302, pH =5.60, (c) α = 0.394, pH =5.67, (d) α = 0.498, pH =5.74, and (e) α = 0.898, pH =7.04.

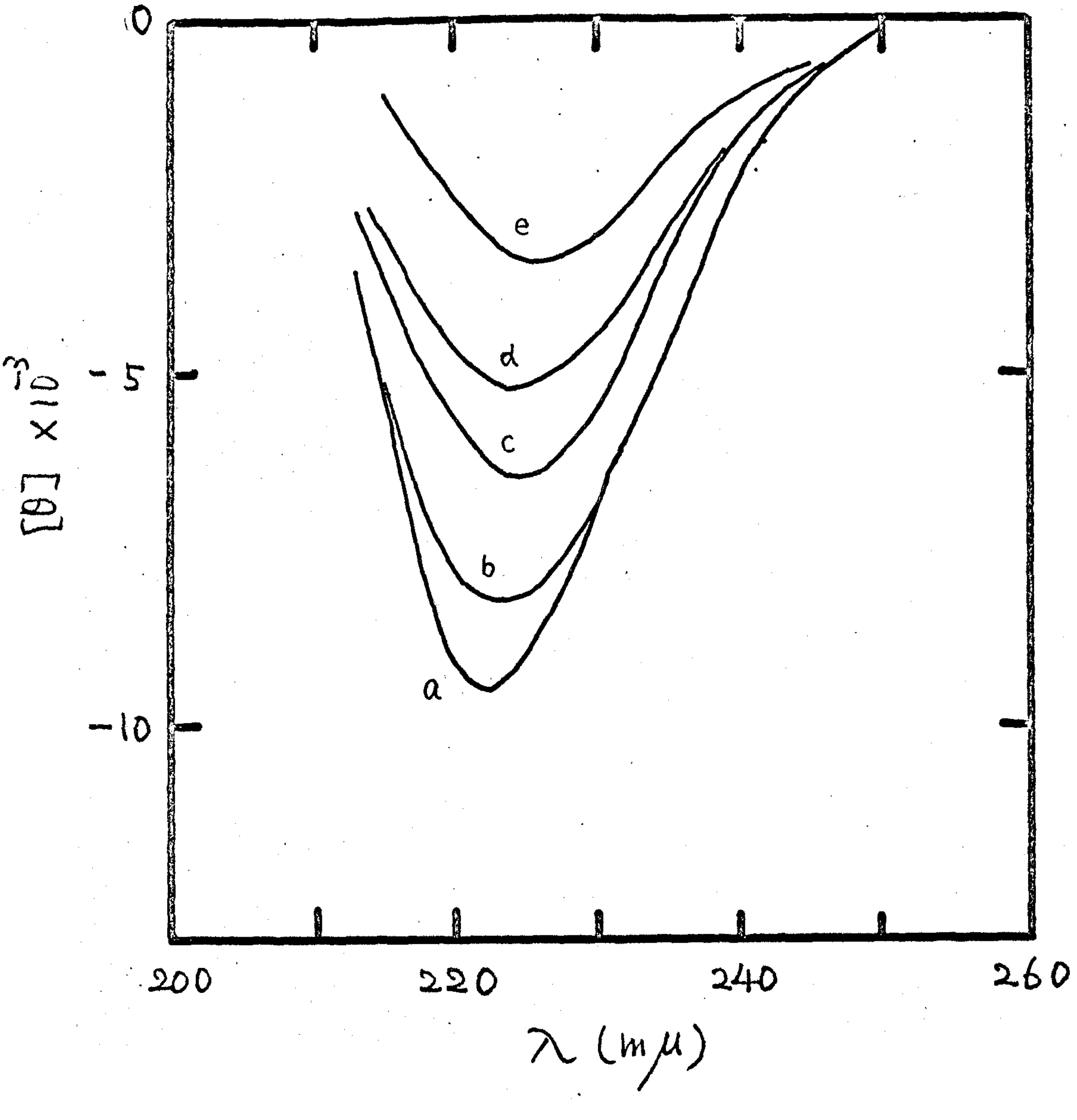


Fig. 5

Fig. 6. Effect of ionic strength on the transition.

Polymer concentration, 0.010 N.

Ionic strength (in molal);

0 (○), 0.010 (◑), 0.020 (⦶), 0.050 (⊖), and 0.200 (◒).

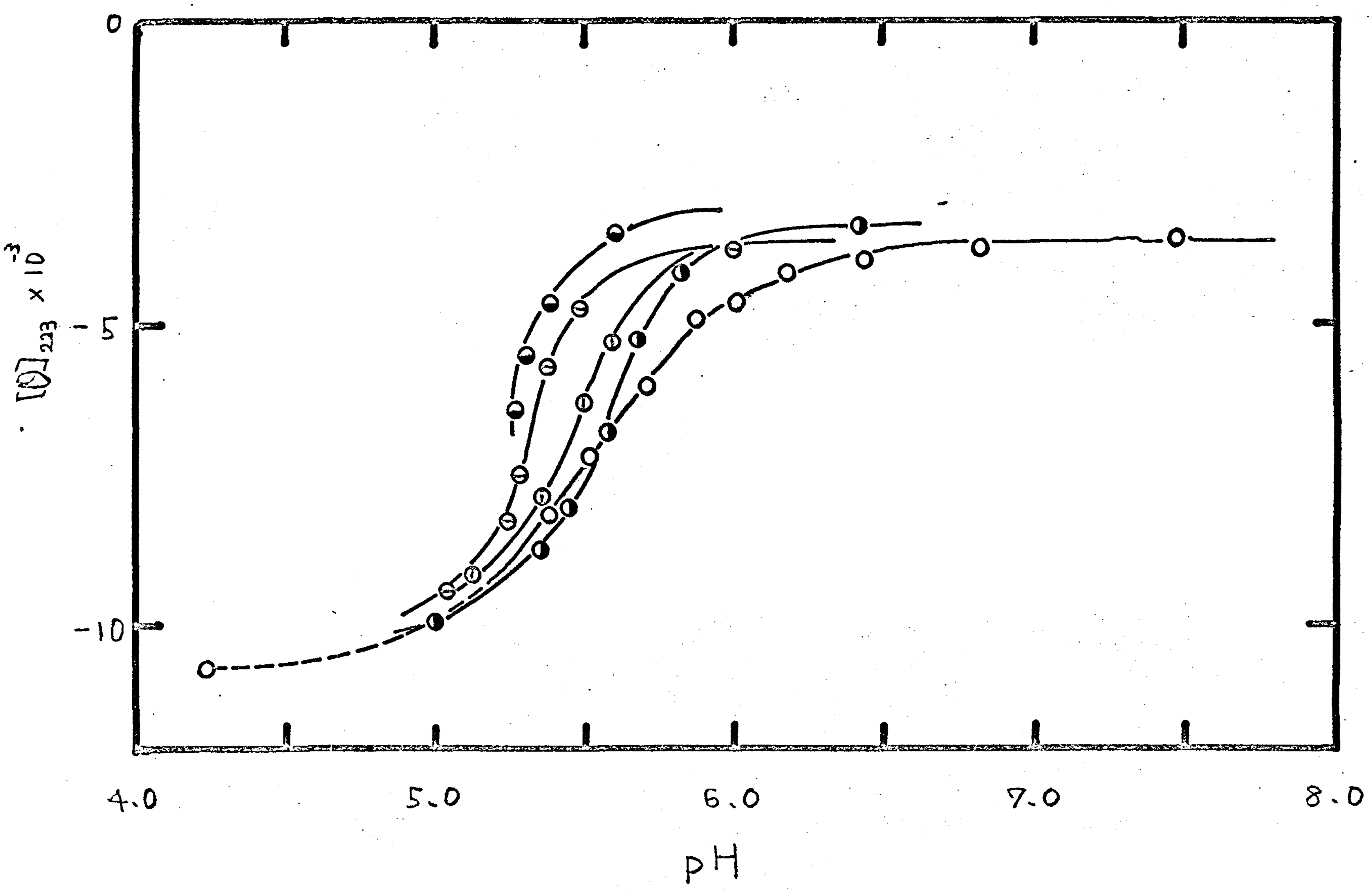


Fig. 6

Such plots are given in Fig. 7. From this figure, it is also noticed that the transition takes place at higher degree of neutralization in more concentrated polymer solutions, although the effect of polymer concentration on the transition pH is rather ambiguous. However, the effect of polymer concentration on the transition is largely obscured in the presence of added salt.

The position of the negative dichroic band is 223 mμ and very close to that of poly-L-serine(222 mμ) rather than that of poly-L-lysine or silk fibroin(217 mμ) and of poly-S-carboxymethyl-L-cysteine(226 mμ). The magnitude(10,000-12,000) is an intermediate one between that of poly-L-lysine(20,000) and that of poly-S-carboxymethyl-L-cysteine(6,000) or of poly-L-serine(5,000) and close to that of silk fibroin(8,000). As compared with that of poly-L-lysine or of poly-S-carboxymethyl-L-cysteine, the magnitude of this negative dichroic band is more conformation-dependent in this polypeptide.

Rotatory dispersion of the polymer suggests that while a negative circular dichroic band exists around 223 mμ at any pH, the random coil must have another negative band but the β-structure should have a positive band, each at a wavelength shorter than 200 mμ. These features are consistent with the direct observations of circular dichroism previously reported.

In Fig. 8, the relation between b_o and $[\theta]_{223}$ is shown. A linear relation seems to hold approximately. Thus b_o can be used as an alternative conformation-dependent parameter.

Fig. 7. Effect of ionic strength on the transition.

(a) Polymer concentration 0.005 N.

Ionic strength (in molal);

0 (○), 0.030 (◑), and 0.150 (⦶).

(b) Polymer concentration 0.010 N.

Ionic strength (in molal);

0 (○), 0.010 (◑), 0.020 (⦶), 0.050 (⊖), 0.100 (◐), and 0.200 (◒).

(c) Polymer concentration 0.025 N.

Ionic strength (in molal);

0 (○), 0.050 (◑), and 0.110 (⦶).

(d) Polymer concentration 0.050 N.

Ionic strength (in molal);

0 (○), 0.050 (◑), and 0.110 (⦶).

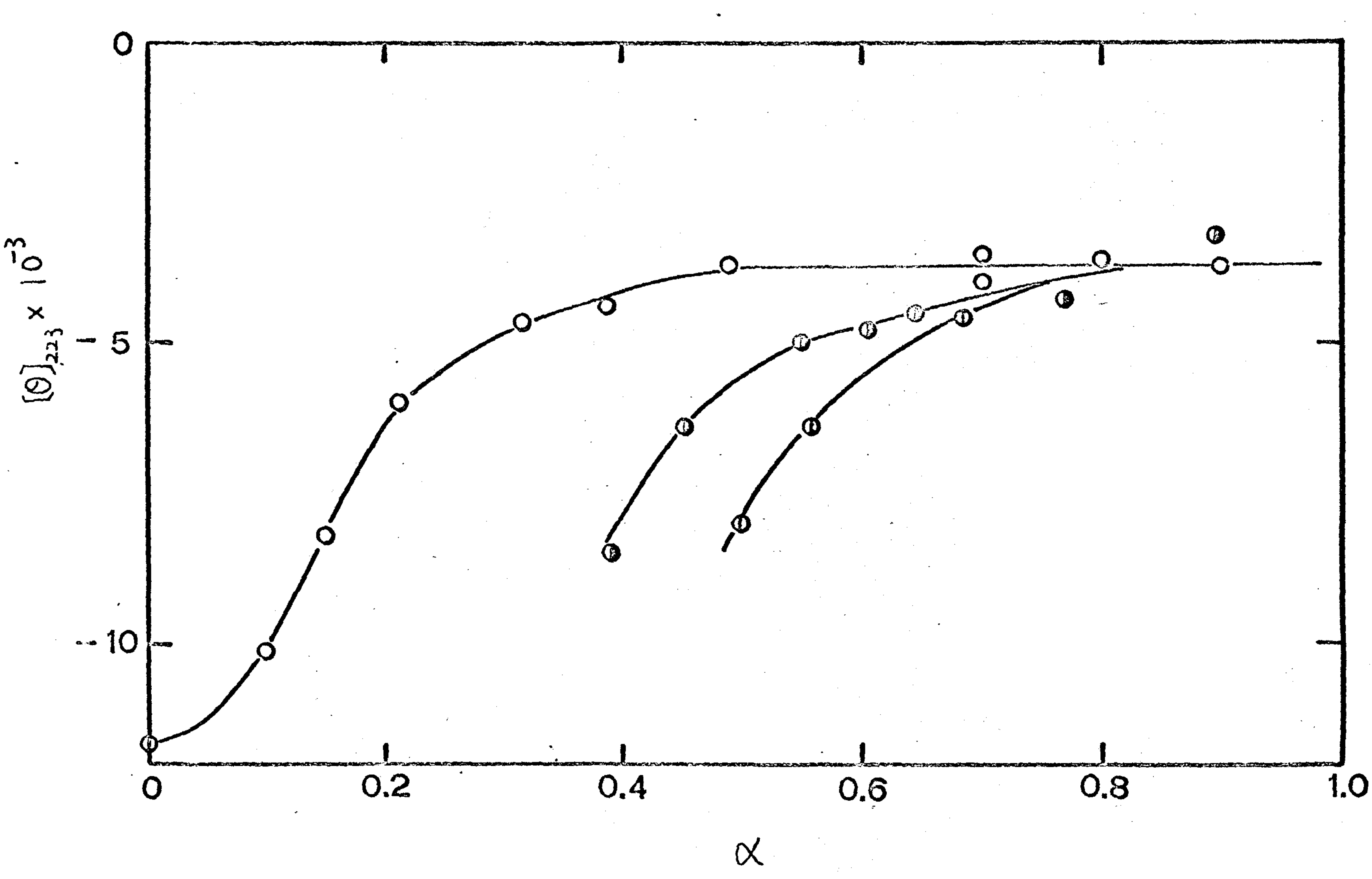


Fig. 7 (a)

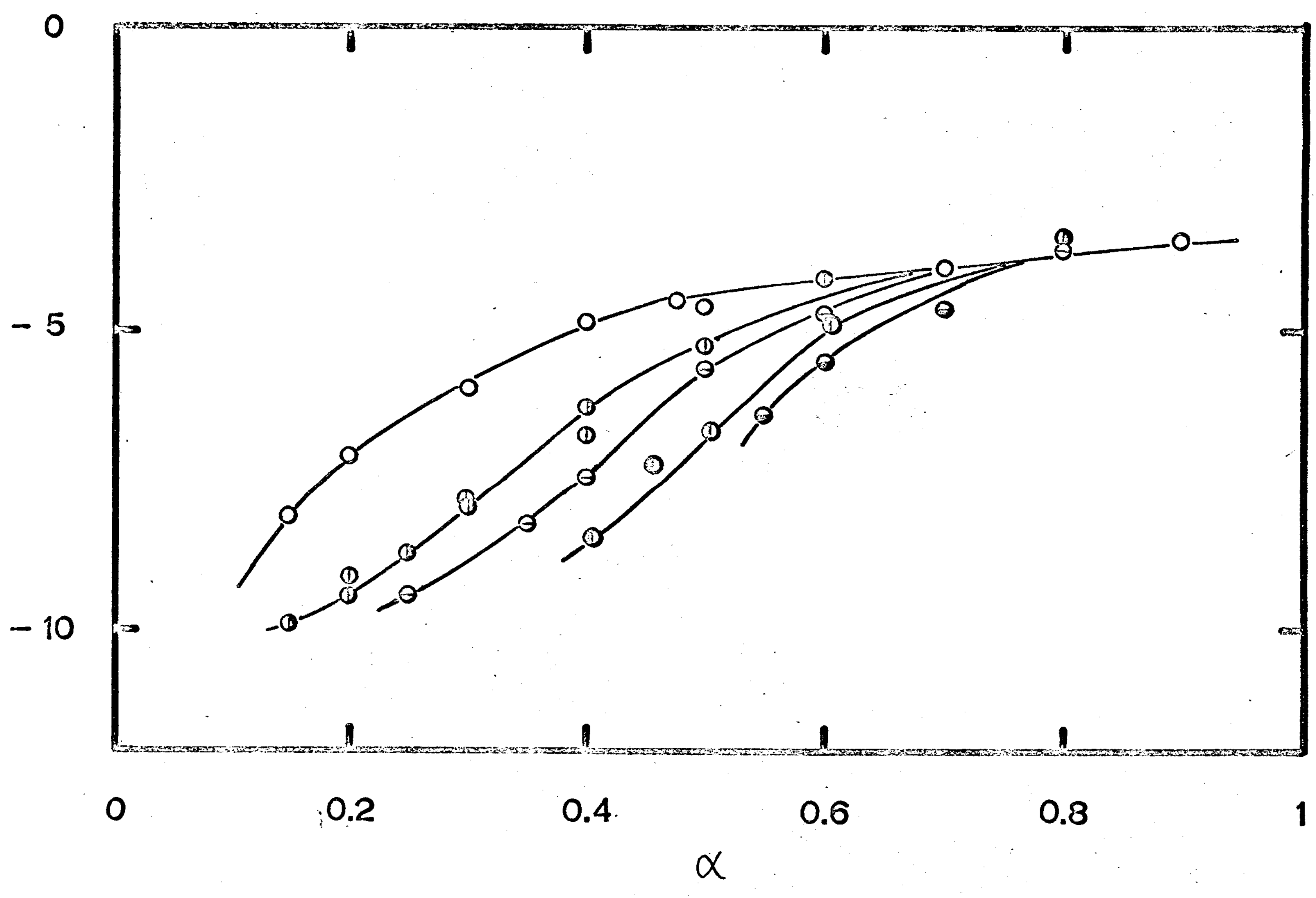


Fig. 7(b)

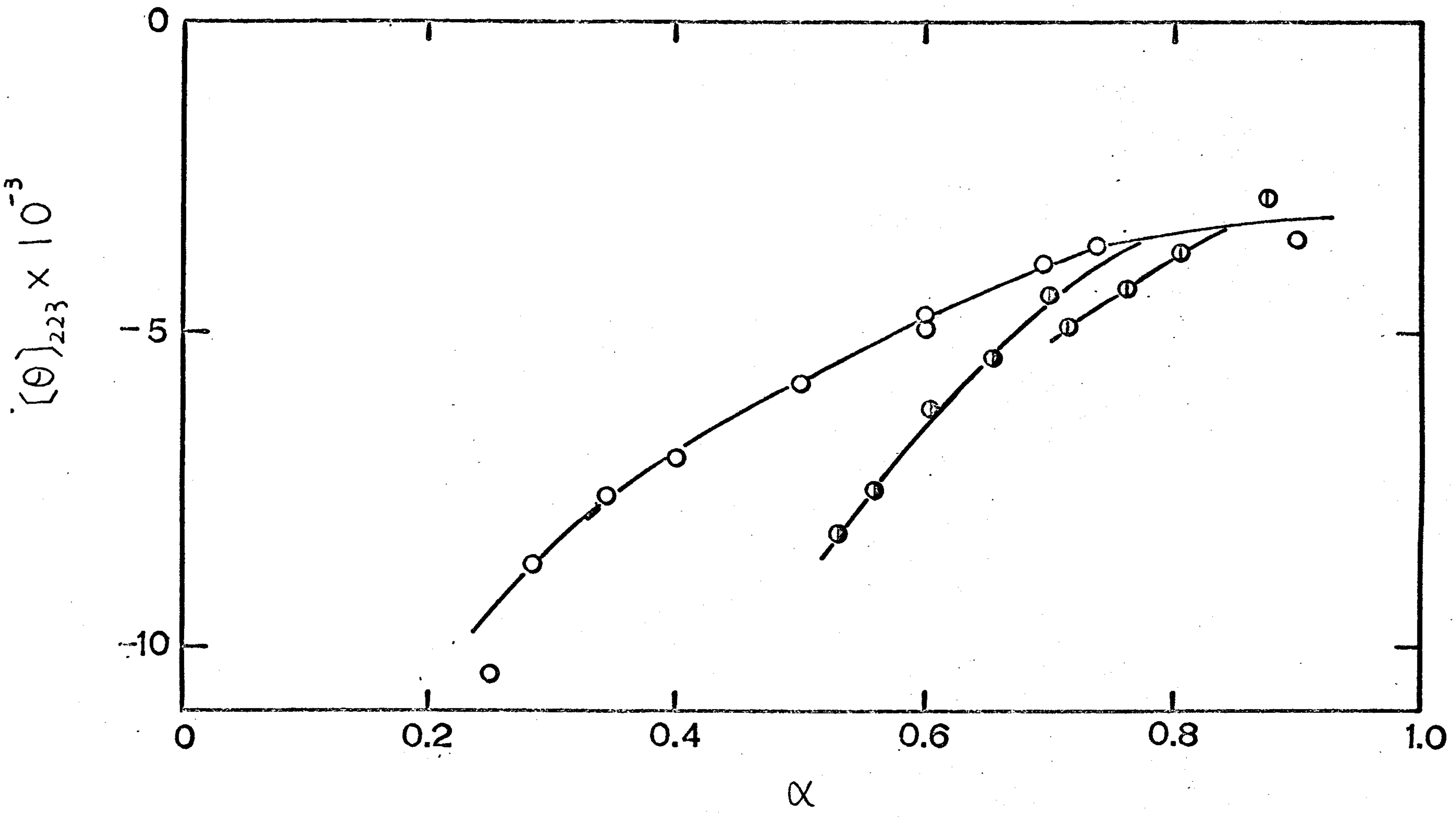


Fig. 7 (c)

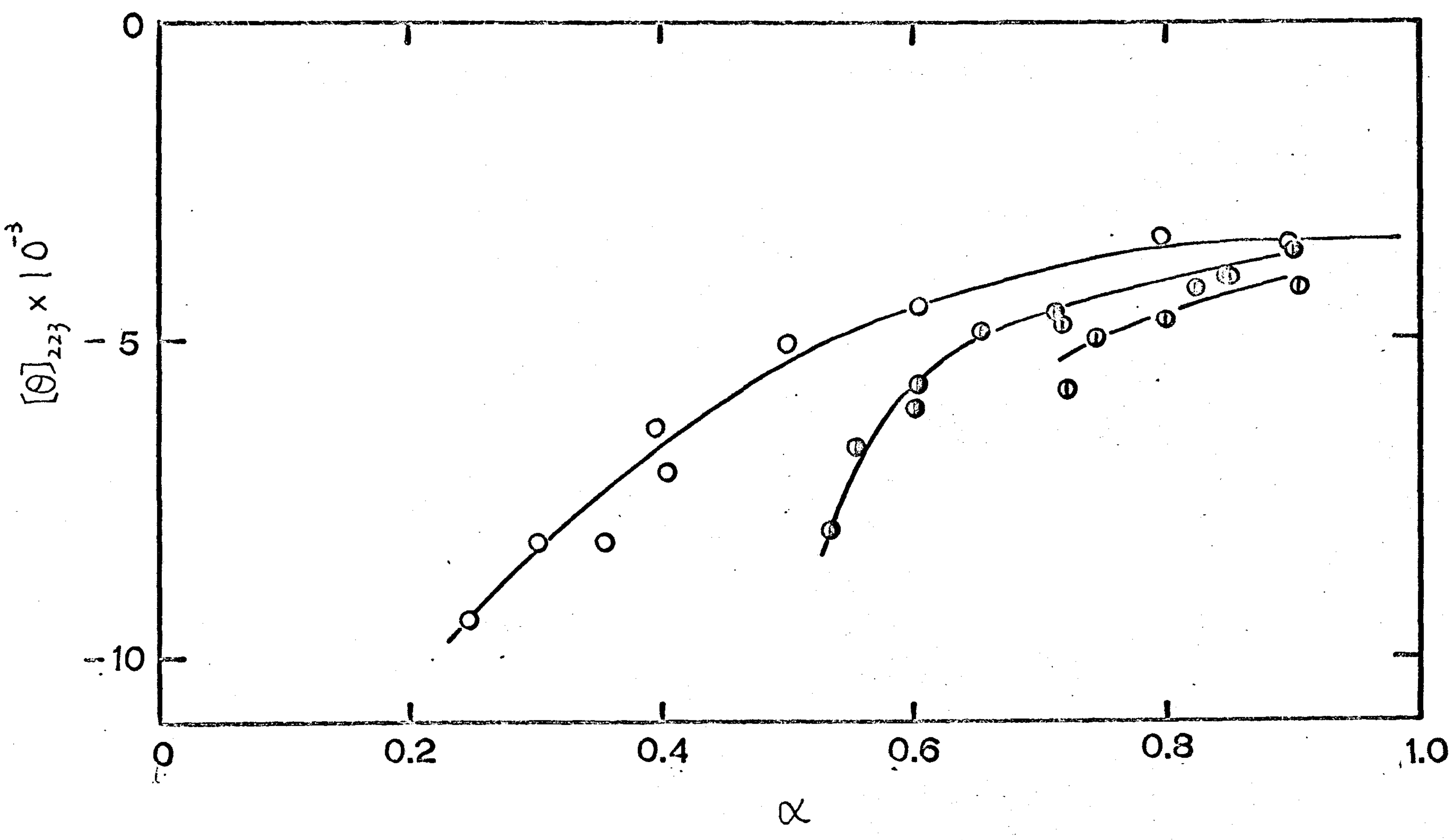


Fig. 7 (d)

ig. 8. Relation between b_o and $[\theta]_{223}$.

(a) Polymer concentration, 0.005 N.

Ionic strength (in molal);

0 (○), and 0.030 (◑).

(b) Polymer concentration, 0.010 N.

Ionic strength (in molal);

0 (○), 0.010 (◔), 0.020 (⦶), 0.050 (⊖), 0.100 (◑), and 0.200 (◒).

(c) Polymer concentration, 0.025 N.

Ionic strength (in molal);

0 (○),0.050 (◑), and 0.110 (⦶).

(d) Polymer concentration, 0.050 N.

Ionic strength (in molal);

0 (○),0.050 (◑), and 0.110 (⦶).

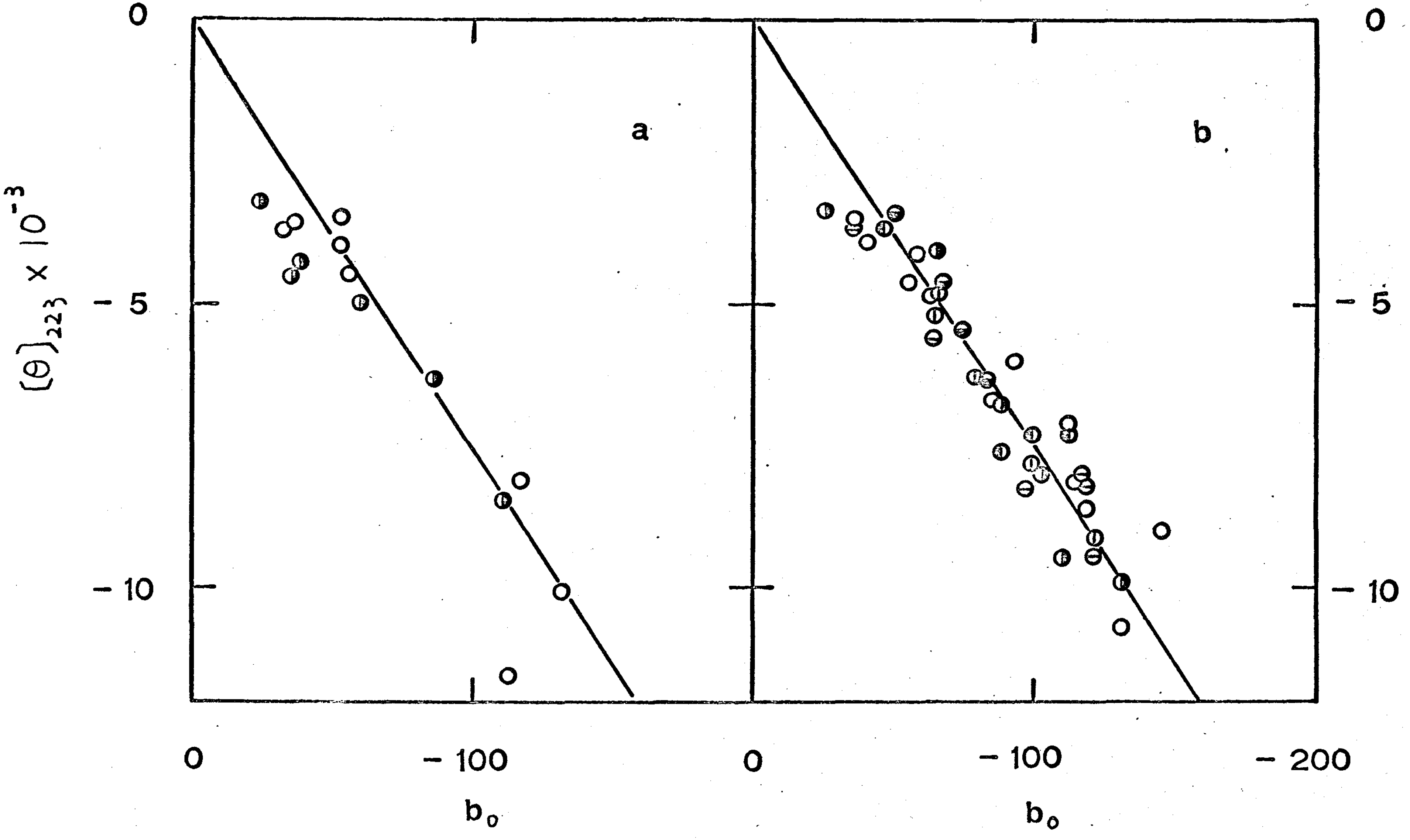


Fig. 8

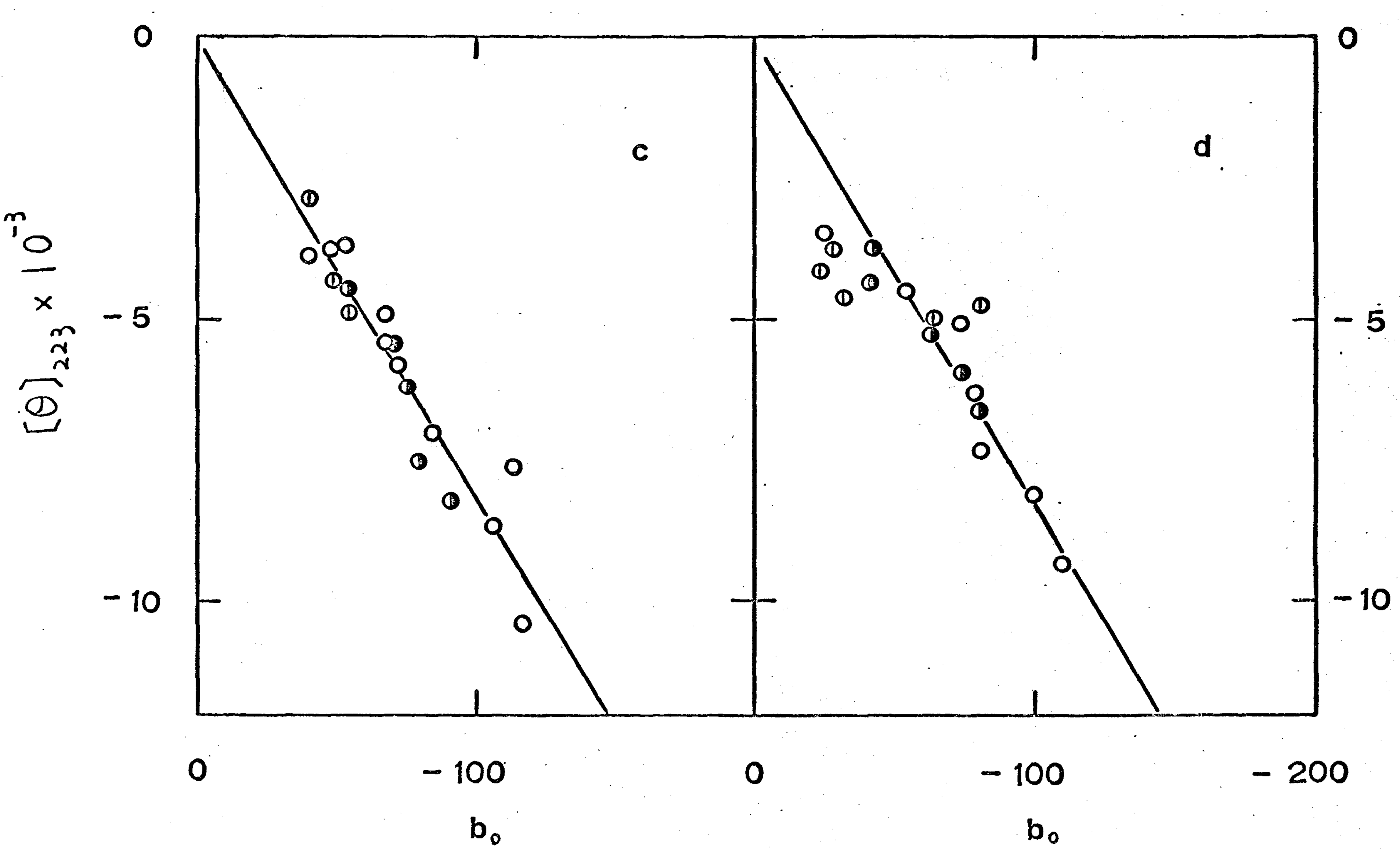


Fig. 8

Infrared Spectra of the Polymer in D_2O Solutions.

Infrared spectra of the polymer in D_2O solutions are shown in Fig.9. The spectra obtained at pD higher than 6 are not distinguishable from one another. They have an amide I band at 1650 cm^{-1},which can be assigned to random coil. However,the amide I band shifts to 1620 cm^{-1} at pD 5.7, suggesting the formation of β-structure. These results confirm the conclusion derived from rotatory properties that β-structure is stable at acid pH,while random coil is stable at neutral pH.

At intermediate pD,the intensity of the two bands,1650 and 1620 cm^{-1},gradually changes as the transition proceeds from random coil to β-structure. The transition pD is about 5.8 for 0.025 N polymer without added salt. It is also found that the transition takes place at higher degree of neutralization as ionic strength or polymer concentration is higher. This is coincident with the results deduced from the rotatory measurements. The effect of deutration on the stability of α-helix was ascribed to the shift in pH or the difference in titration behavior, for poly-L-glutamic acid and poly-L-lysine in H_2O and D_2O respectively,[62] but the effect on β-structure has not been worked out.

Discussion.

The conformation of poly-S-carboxyethyl-L-cysteine in aqueous solutions was shown to be dependent on pH or the state of ionization. The coil form stable at neutral pH is characterized by a negative circular dichroic band at 223 mμ and another negative band at a wavelength shorter than 200 mμ. The $[\theta]_{223}$ value for random coil is independent of polymer concentration as well as of ionic strength,which also supports

Fig. 9. Infrared spectra of the polymer in D_2O solutions.

Polymer concentration, 0.025 N.

Ionic strength, 0 molal.

Cell length 30 μ.

———— ; $\alpha = 0.345$, pD =5.73.

—·—·— ; $\alpha = 0.504$, pD =5.84.

- - - ; $\alpha = 0.605$, pD =5.97.

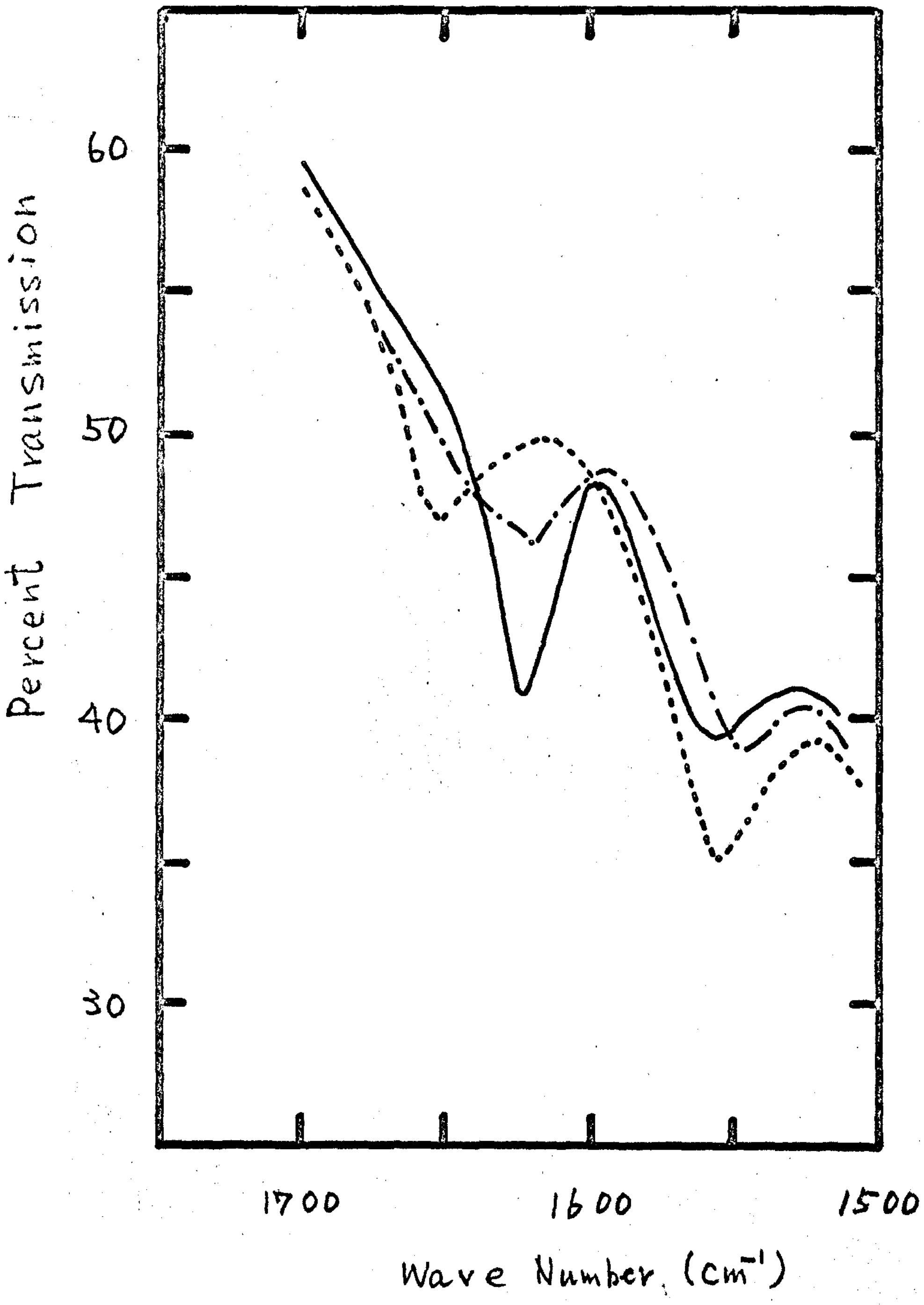

60
50
40
30
Percent Transmission
1700
1600
1500
Wave Number (cm⁻¹)

Fig. 9

the presence of isolated random coil chains.

The rotatory dispersion of the β-structure has a trough at 236 $m\mu$ and a peak at 210 $m\mu$, which corresponds to circular dichroism with a negative band at 223 $m\mu$ and a positive band at a wavelength shorter than 200 $m\mu$. The Moffitt parameter, b_o, is negative. These characteristics are common to those of β-structures formed by water-soluble polypeptides.

The β-structure of poly-S-carboxyethyl-L-cysteine is disrupted by electrostatic repulsion due to the ionization of side chain carboxyl groups. The transition occurs around pH 5 to 5.5. Summary of the dependence of the rotatory parameters on pH together with the degree of ionization is given in Fig.10 for two cases. This figure exhibits the profiles of the transition more clearly. The $[\theta]_{223}$ value is dependent on the degree of neutralization and ionic strength as well as on polymer concentration, when the polymer conformation departs from random coil. Even at the most acid pH or at the lowest degree of neutralization attained without phase separation, the $[\theta]_{223}$ value is influenced by ionic strength as well as polymer concentration. The β-structure is more stabilized with increasing polymer concentration at a given ionic strength. This strongly suggests that the β-structure is formed through intermolecular association.

It is pertinent to note here the solubility or the precipitation of the polymer at the acid pH where the polymer is mostly in β-structure. The lowest degree of neutralization attained or probably the critical degree of neutralization, α^*, for phase separation is shown as a function of both ionic strength and polymer concentration in Fig.11. This indicates that the precipitation takes place at a higher degree of neutralization as ionic strength is higher. The phase diagram also shows

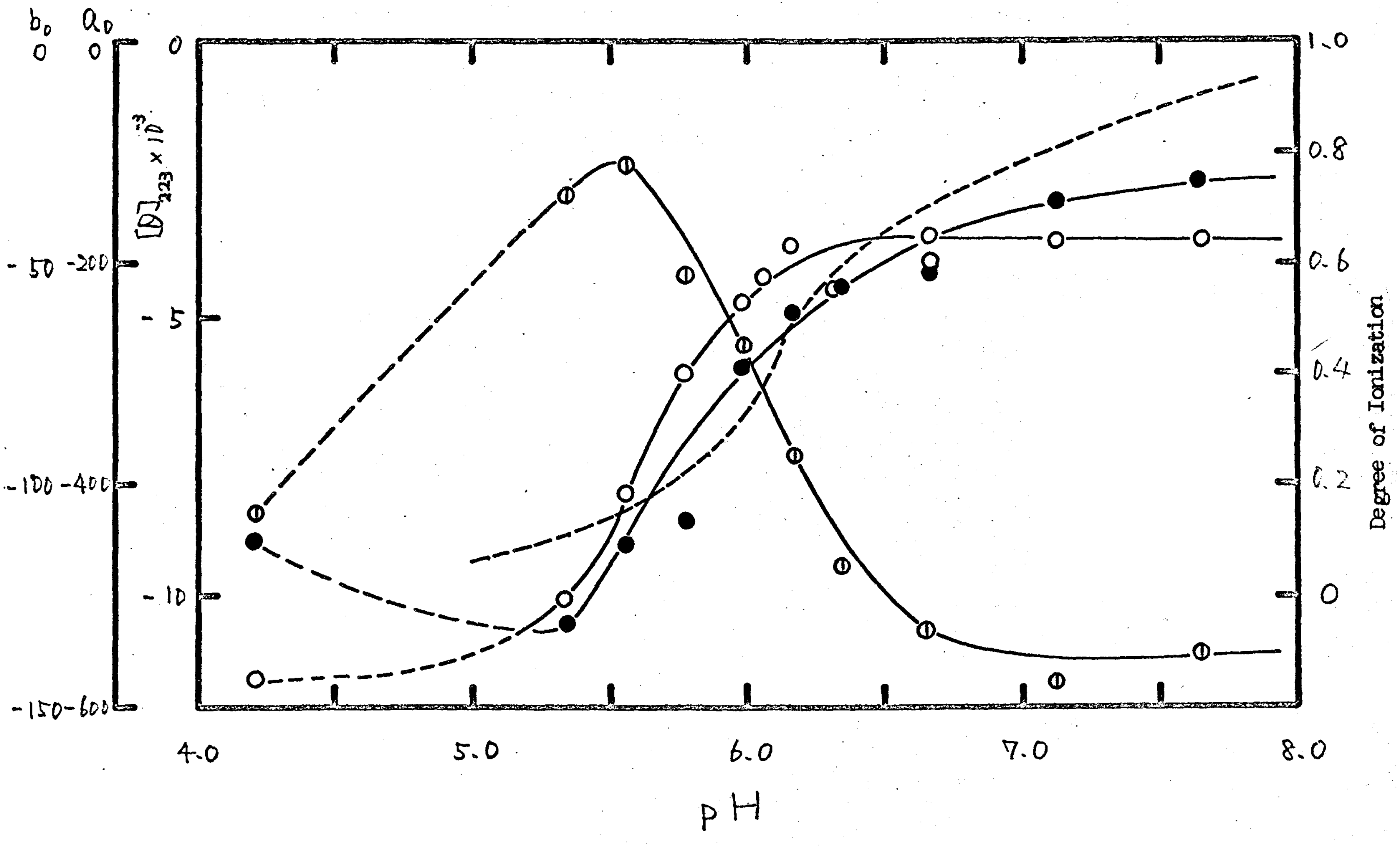
Fig. 10. Dependence of rotatory parameters on pH.

(a) Polymer concentration, 0.005 N.
Ionic strength, 0 molal.

(b) Polymer concentration, 0.050 N.
Ionic strength, 0.050 molal.

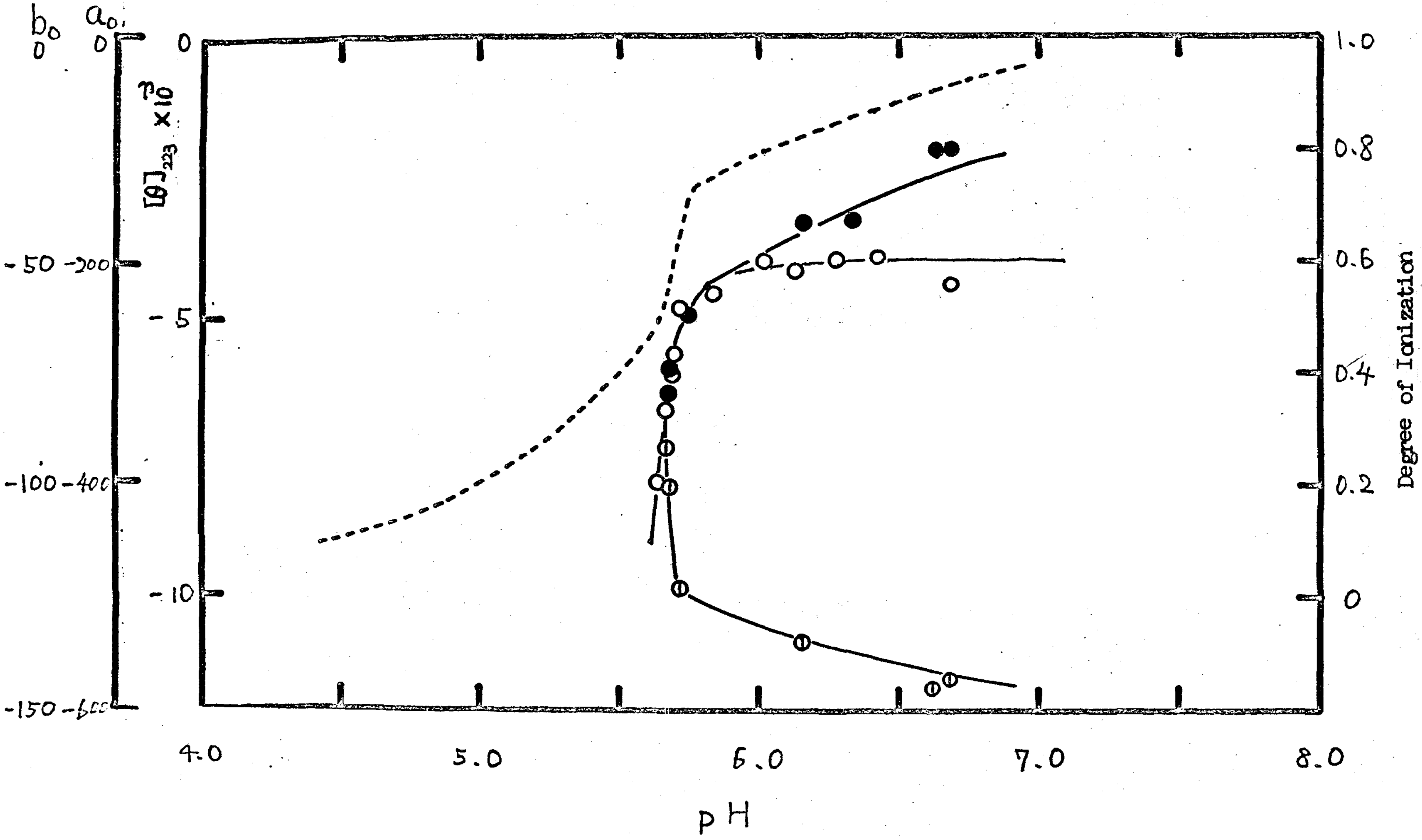
(○), $[\theta]_{223}$; (●), b_o ; (◐), a_o.

Dashed line represents degree of ionization obtained from titration.



b₀
a₀
0
0
-50
-200
-100
-400
-150
-600
$[\theta]_{223} \times 10^{-3}$
0
-5
-10
1.0
0.8
0.6
0.4
0.2
0
Degree of Ionization
4.0
5.0
6.0
7.0
8.0
pH

Fig. 10 (a)



b_0
a_0
0
0
0
$[\theta]_{223} \times 10^{-3}$
-50 -200
-5
-100 -400
-10
-150 -600
1.0
0.8
0.6
0.4
0.2
0
Degree of Ionization
4.0
5.0
6.0
7.0
8.0
pH

Fig. 10 (b)

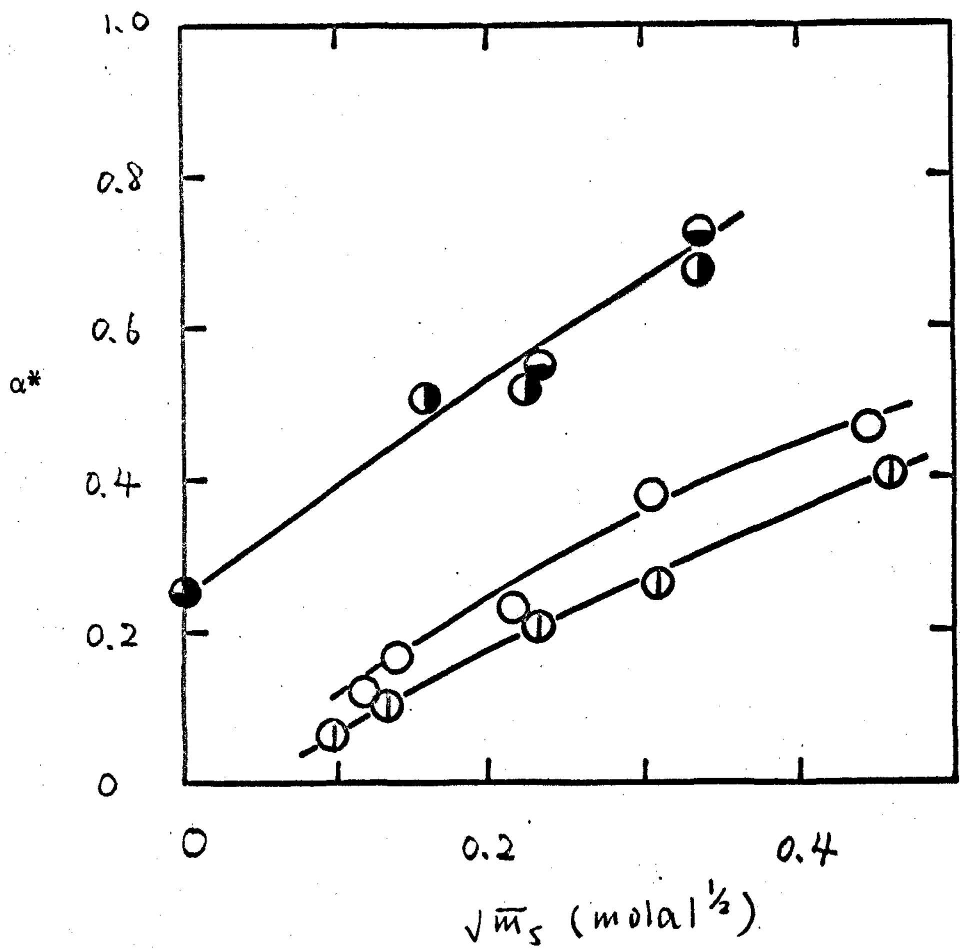


Fig. 11. Plots of the critical degree of neutralization against the square root of ionic strength. Polymer concentrations; 0.005 (⦶), 0.010 (○), 0.025 (◑), 0.050 N (◒).

that the critical degree of neutralization is higher for more concentrated polymer solutions. The precipitates would have a non-zero,finite degree of neutralization,which will be discussed in detail in the following sections.

Fig.12 shows the value of $[\theta]_{223}$ as a function of degree of neutralization where the phase separation occurs. This indicates that the precipitation takes place at a certain stage of the β-structure formation, provided that the $[\theta]_{223}$ value is taken to represent the content of β-structure in solution. But the conversion from random coil to β-structure is clearly more perfect at lower ionic strength and at lower polymer concentration. Conversely,in the limit of very high ionic strength or, more effectively,of very high polymer concentration, the β-structure would not be formed in solution before precipitation occurs,that is, the polymer would be stable in solution only in highly charged and randomly coiled form.

For a charge induced β-coil transition to occur, a significant change in the charge density on a polyelectrolyte chain must be accompanied. Since a single extended polymer chain has a charge density almost equal to that of a randomly coiled chain,[63] β-structure formed by an ionized polypeptide must be either intramolecularly or intermolecularly hydrogen bonded, in which a hydrogen bonded sheet has a very high charge density as compared with a single isolated chain. The β-structure of the present polymer would be intermolecular if small size of the sample as judged from its intrinsic viscosity was taken into account. It is also probably that the sheets stack together to give a three dimensional aggregate.

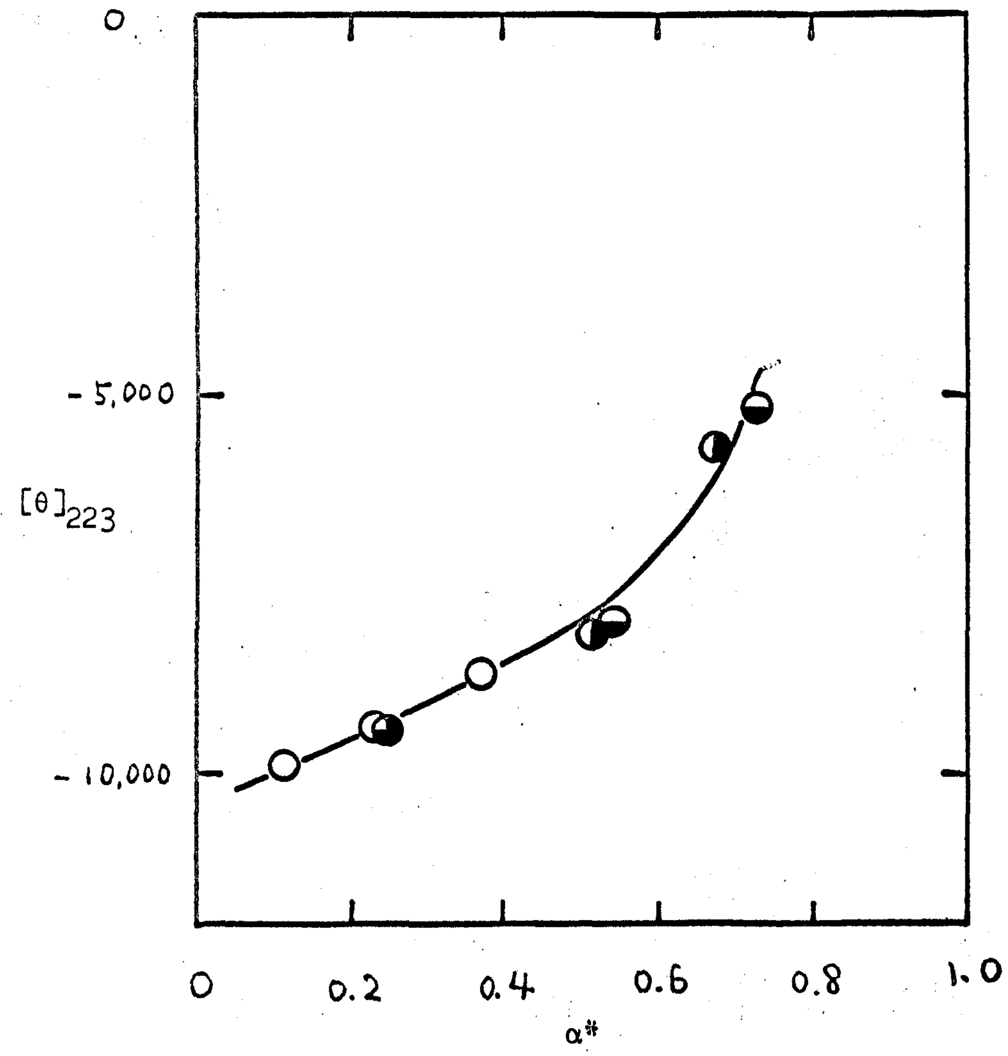


Fig. 12. Values of $[\theta]_{223}$ at different critical degrees of neutralization.

Polymer concentrations; 0.010 (○), 0.025 (◑), and 0.050 N (◒).

3. Potentiometric Titration of Poly-S-carboxyethyl-L-cysteine.

Introduction.

Potentiometric titration has been a useful and important technique in the studies of thermodynamic properties of polyelectrolyte solutions. Moreover, this technique has revealed somewhat detailed aspects concerning the conformational transition of polypeptides. Charge induced conformational change in general has been treated theoretically,[64-66] and the application to the helix-coil transition has been made.[61,67]

In the case of α-helix, the charge induced conformational transition is effected by the fact that it has a larger charge density than random coil with the same charge. Thus with increasing the charge on a polypeptide chain, the free energy due to electrostatic interaction increases more rapidly for α-helix than for random coil. Eventually, the charge repulsions become of such magnitude that they disrupt the hydrogen bonded helical structure converting it to a random coil.

Since the β-structure is somewhat extended conformation,[4,5] so the charge density of a polypeptide chain in this conformation will not be so larger than that of random coil. However in β-structure, polypeptide chains are hydrogen bonded side by side to give a two dimensional pleated sheet and the interactions between side chains cause stacking of these sheets to form a three dimensional aggregate. Therefore the charge density of the resultant sheet or aggregate may be expected to be extremely large. Thus the charge induced β-coil transition is expected to occur depending on the pH of solutions.

In the study of titratory behavior of β-structure, however, there lie three problems to be examined before we proceed to discuss anything from the titration.

One of them is that of phase separation. Since β-structure has so limited solubility, we can not discuss the whole aspect of β-coil transition unless the region under phase separation is also included and analyzed as well as the region under a single solution phase. For this purpose, the partitions of polymer, salt, alkali and water between two phases must be examined.

The second one is the change of the observed pH values with time. In contrast to the helix-coil transition, where the transition depends mainly on the intramolecular interactions, the β-coil transition depends strongly on the intermolecular interactions, that is association. In almost all cases, longer or shorter, the time dependence of optical rotatory properties has been reported with the formation of β-structure.[68] The time scale encountered is quite different for both types of measurements. In optical measurements, it usually takes several hours before measurements after the preparation of solutions. On the other hand, in the experiments of potentiometric titration, the time interval between two successive addition of alkali or acid is of order of several minutes. So whether ordinary way of titration is applicable to the present case or not must be examined.

The last one is of the reversibility, which has close interrelations with the former twos. For, the reversibility may be strongly affected by the phase separation, and the discussions of reversibility inevitably related to the period examined.

Because of these three problems, there have not been reported the titration properties of β-structure. Recently the potentiometric titrations of poly-S-carboxymethyl-L-cysteine were reported.[69,96] However the treatments presented there were limited, since the situations mentioned above were not taken into consideration.

In the present investigation, we examined both the reversibility of titration curves and the time dependence of pH. From these experiments we were able to obtain the titration curves corresponding to equilibrium ones. The last point to be examined, phase separation, still remains unsolved in the present study.

In this section, charge-induced β-coil transition of poly-S-carboxyethyl-L-cysteine is investigated from the standpoint of titration behavior. To reveal the conformational transition in more detail, the dependence of titration upon polymer concentration is also examined.

EXPERIMENTAL.

A) Method.

Potentiometric titrations were performed in two ways. One was a usual method, where a continuous titration was possible and designated here as C method. In this method, a polymer solution (7 to 10 g) with zero degree of neutralization and containing no added salt, was placed in a cell. Sodium chloride was added to produce a specified salt concentration, and this resulted precipitations in most cases. Then solutions of alkali were added to the system by means of a micro syringe(0.5 ml.) under the atmosphere of nitrogen. The system was stirred magnetically, and the cell and water of thermostat were grounded to earth. The values of pH were read about five minutes after each addition of alkali. Almost constant value was obtained after a minute.

The another method was as follows. The system (ca. 5g) with specified compositions was placed in a sealed vessel and maintained at 25°C for more than a day. Then the pH of the system was measured. The period of more than a day was proved to be sufficient to attain a constant value of pH in most cases examined. This method thus gave a discrete titration, and was designated here as D method.

Measurements were performed at 25 ± 1°C. The pH meter used was Hitachi-Horiba Model P, with a glass electrode and a calomel electrode as a reference electrode. The electrodes were calibrated using phosphate buffer of pH 6.86. After the experiment,pH of the buffer was again measured and the data were discarded if the value differed from that measured before the experiment by more than 0.03. The observed values of pH in phthalate buffer (pH 4.00) were always in the range between 4.01-4.03.

B) Preparation of Systems.

Systems were prepared also in two ways. One way was such that was described in C method. The polymer solutions with zero degree of neutralization were prepared by passing the nutral salt free polymer solutions through the ion exchange column containing Amberlite IR 120. Then sodium chloride and alkali(if D method was to be employed) were added to produce the specified compositions. The polymer concentrations of the stock solutions were determined by the end point of the titration in the presence of sufficient salt.

The another one was such that the weighed polymer powders were placed in a vessel and then water,salt,and alkali were added to desired composition. This way was employed for higher polymer concentrations, 0.050 N and 0.025 N. The systems thus prepared were always titrated with D method.

The polymer sample used for titration was B622AW. Conformational studies were carried out with this sample. The another sample H920W was also investigated in this study and almost the same results were obtained.

In this study, sodium chloride was used throughout as an added salt. Sodium hydroxide (0.2 N) and hydrochloric acid (0.5 N) were employed throughout as titrants in the forward and back titrations respectively.

c) Estimation of Degree of Ionization.

Degree of ionization, α, is related to degree of neutralization, β, as follows.

$$\alpha = \beta + (C_{H^+} - C_{OH^-}) / C_p \tag{2}$$

where C_p, C_{H^+} and C_{OH^-} denote the concentration of polymer, hydrogen ion and hydroxyl ion respectively. The quantities C_{H^+} and C_{OH^-} were obtained approximately by the titration of salt solution with the same ionic strength as the polymer solution. The approximation involved here is such that the activity coefficient of hydrogen ion is not affected by the presence of macro ion. In solutions containing both macro ion and added salt, the activity coefficient of hydrogen ion is determined mainly by its ionic atmosphere. So the approximation is expected to be valid. However, in the case of no added salt, this ionic atmosphere is no longer present and hydrogen ions are subject to large electrostatic field produced by macro ions. Therefore in this situation, the validity becomes doubtful.

RESULTS

Reversibility

To check the reversibility of the titration, the coincidence of the forward and the back titrations was examined with C method. The results are shown in Fig.13. These results showed that the precipitation point was different in two cases. The difference was as large as 0.5-0.9 pH unit. Thus the titration curve showed a hysteresis, but the difference of pH was small in contrast to the large difference in the precipitation points. In the region where the hysteresis was observed, the system existed in two phases in the case of the forward titration, whereas in the back titration still remained in a single solution phase. However this hysteresis was proved to be reduced to less extent, if longer period was adopted between two successive addition of HCl in the back titration. To make clear this situation, the following experiments were undertaken.

At first, the system (C_p=0.005 N, m_s=0.050 molal) was brought into the state of complete random coil. This was effected by the D method and the degree of dissociation of the system was adjusted to be 0.80 . The pH was measured and the coincidence with C method was confirmed. Then to this solution HCl was added to give the final state. After the equilibration of the system at 25°C for more than a day, the pH was again measured. This value may be considered to represent the value of the back titration with D mathod. The results are shown by triangles in Fig.13(a). This figure shows us the complete coincidence between the forward and the back titrations.

Fig. 13. Reversibility and time dependence of the titration curves.

Solid lines; forward with the C method.

Dotted lines ; backward with the C method.

Open circles; forward with the D method.

Triangles; backward with the D method.

Chain lines; forward with the D method without added salt.

(a) Polymer concentration, 0.005 N.

Ionic strength (in molal);

(a) 0, (b) 0.010, (c) 0.018, (d) 0.054, (e) 0.096, and (f) 0.211.

(b) Polymer concentration, 0.010 N.

Ionic strength (in molal);

(a) 0, (b) 0.014, (c) 0.019, (d) 0.048, (e) 0.093, and (f) 0.197.

Arrows represent points where precipitates dissolve or appear.

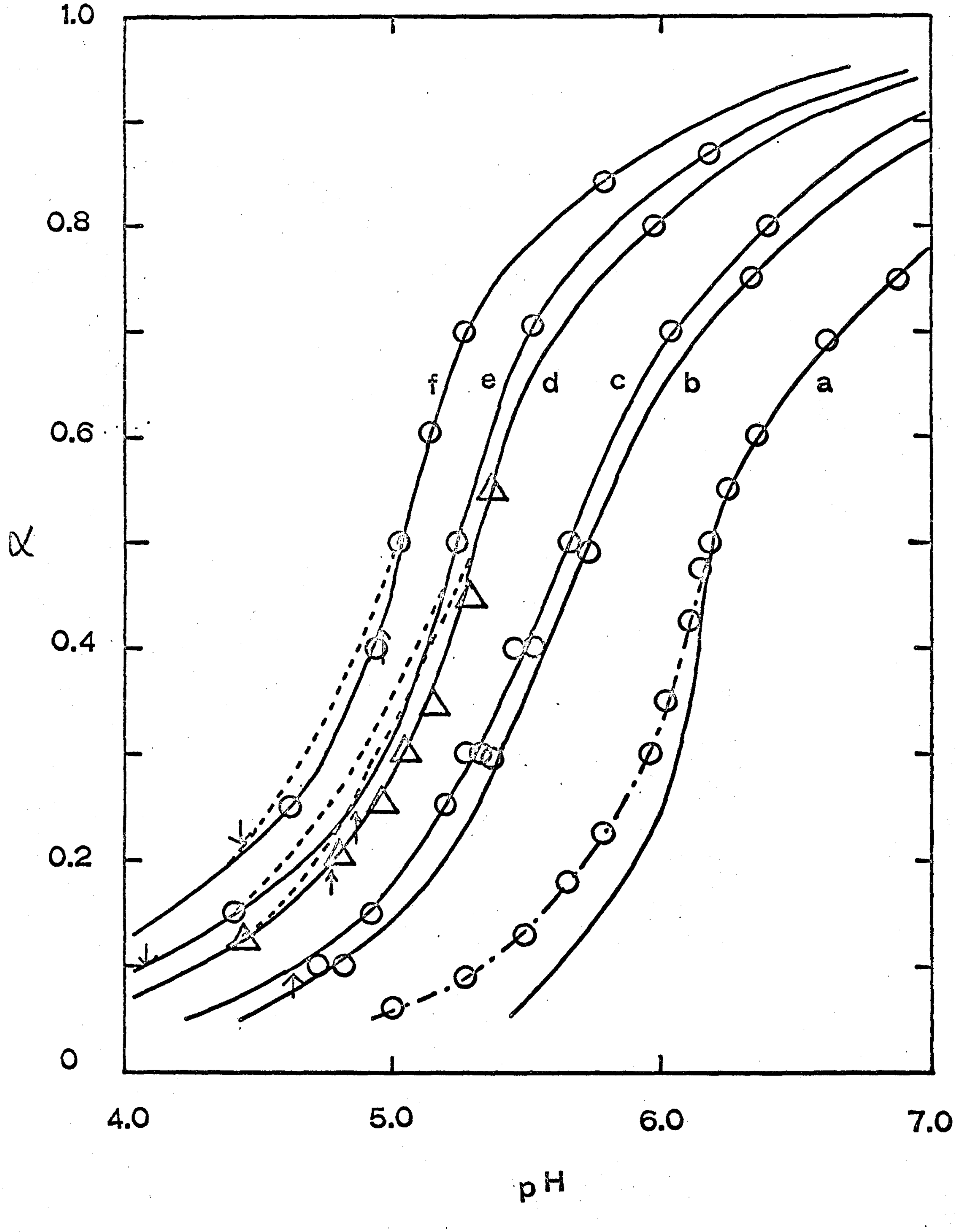


Fig. 13 (a)

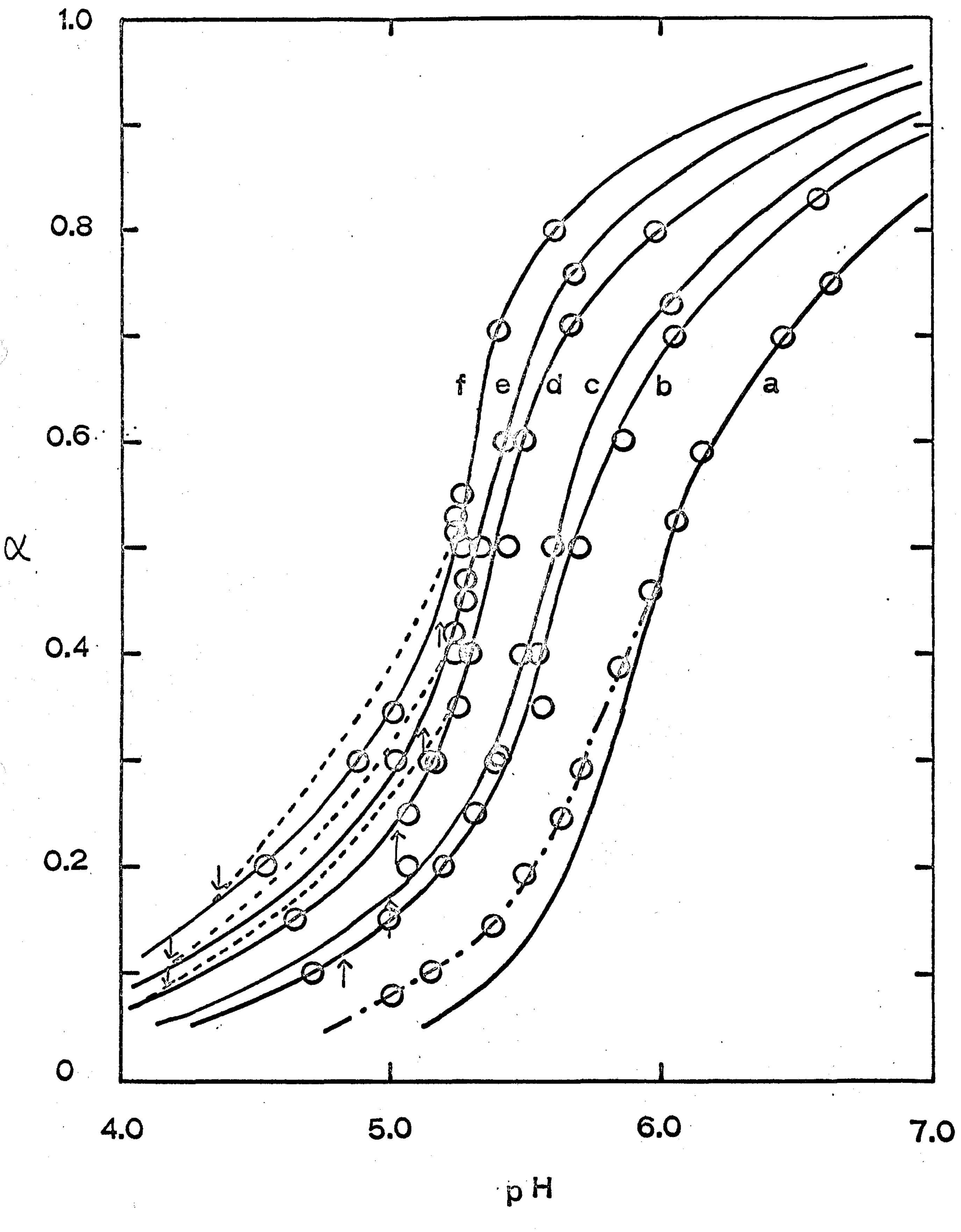


Fig. 13 (b)

Change of PH with Time

Though the C method gave almost constant pH readings, the change of pH with longer time scale was examined. This was done by the check of the coincidence between C and D methods. For the system containing salt, the coincidence was satisfactory. In the case of no added salt,however, D method gave a considerably lower value than C method, though this difference was restricted to those outside the random coil region. These results are also shown in Fig.13.

In summarizing these two experiments, the reversibility and the time dependence of pH, we may conclude the followings.

The D method gave an equilibrium value. Except for the no added salt system, the C method also gave the same results. The observed hysteresis with the C method seemed to be responsible for the back titration. With longer period between two successive addition of acid, the back titration becomes closer to, and finally coincides with the forward one or those given by the D method. For higher polymer concentration(0.050 N and 0.025 N), D method was employed throughout. Thus all data presented in this paper are considered to be equilibrium values.

For the system with no added salt and higher polymer concentration (0.050 N and 0.025 N), the values $pH - \log\frac{\alpha}{1-\alpha}$ did not tend to pK_0 but increased slightly with decreasing α to zero. These features may be responsible for the shortage of time for the equilibration of the system under these conditions. If it is to be the case, the data concerning these state can not be considered to be equilibrium ones.

The period of the equilibration was three days in these cases, and longer period than this caused the change of the concentrations of the components , perhaps due to the evaporation of water. In any way, whether the state of equilibrium was attained in these cases or not could not be decided. On the other hand, the possibility that α did not be correctly assessed can not be excluded also.

Modified Plots.

It is well known that potentiometric titration of polyelectrolytes are well described by the following equation.[70-72]

$$pH = pK_o + \log \frac{\alpha}{1-\alpha} + \frac{0.434}{RT} \frac{\partial G^{el}}{\partial \nu} \qquad (3)$$

Here G^{el} is the electrostatic free energy of a polyelectrolyte chain having ν charges. With degree of polymerization, x, ν can be written as follows; $\nu = \alpha x$. K_o and α represent the intrinsic dissociation constant of dissociable sites and degree of ionization respectively. In the present case, charge-independent contribution is also included in G^{el}. This contribution arises from that the conformational equilibrium between β-structure and random coil is present in the system. Moreover, if the two conformations have different intrinsic dissociation constants, the term pK_o in eq.(3) should be regarded as representing a suitable average quantity.

Examples of modified plots, $pH-\log\frac{\alpha}{1-\alpha}$ vs. α , are given in Fig.14. In these Figures a titration curve shows a maximum and a minimum for most cases and thus exhibits a conformational transition. From the conformational studies of the previous section, it is confirmed that the polymer is in β-structure at low degree of ionization, and on increasing pH, electrostatic repulsion prevails the stabilizing forces (hydrogen bonds and hydrophobic interactions) and finally the polymer becomes a random coil at high degree of ionization. However, the assignments of pure random coil and pure β-structure regions are shown to be rather difficult if compared with the helix-coil transition. The extrapolation to zero degree of ionization from random coil region is long and arbitrary.

Fig. 14. Plots of pH - $\frac{\alpha}{1-\alpha}$ vs. α.

Solid lines; experimental data.

Dashed lines; Extrapolated curves corresponding to either β-structure or random coil.

(a) Polymer concentration, 0.005 N.

Ionic strength (in molal);

(a) 0, (b) 0.010, (c) 0.018, (d) 0.054, (e) 0.096, and (f) 0.211.

(b) Polymer concentration, 0.010 N.

Ionic strength (in molal);

(a) 0, (b) 0.014, (c) 0.019, (d) 0.048, (e) 0.093, and (f) 0.197.

(c) Polymer concentration, 0.025 N.

Ionic strength (in molal);

(a) 0, (b) 0.025, (c) 0.050, and (d) 0.110.

(d) Polymer concentration, 0.050 N.

Ionic strength (in molal);

(a) 0, (b) 0.050, and (c) 0.110.

Arrows represent precipitation points.

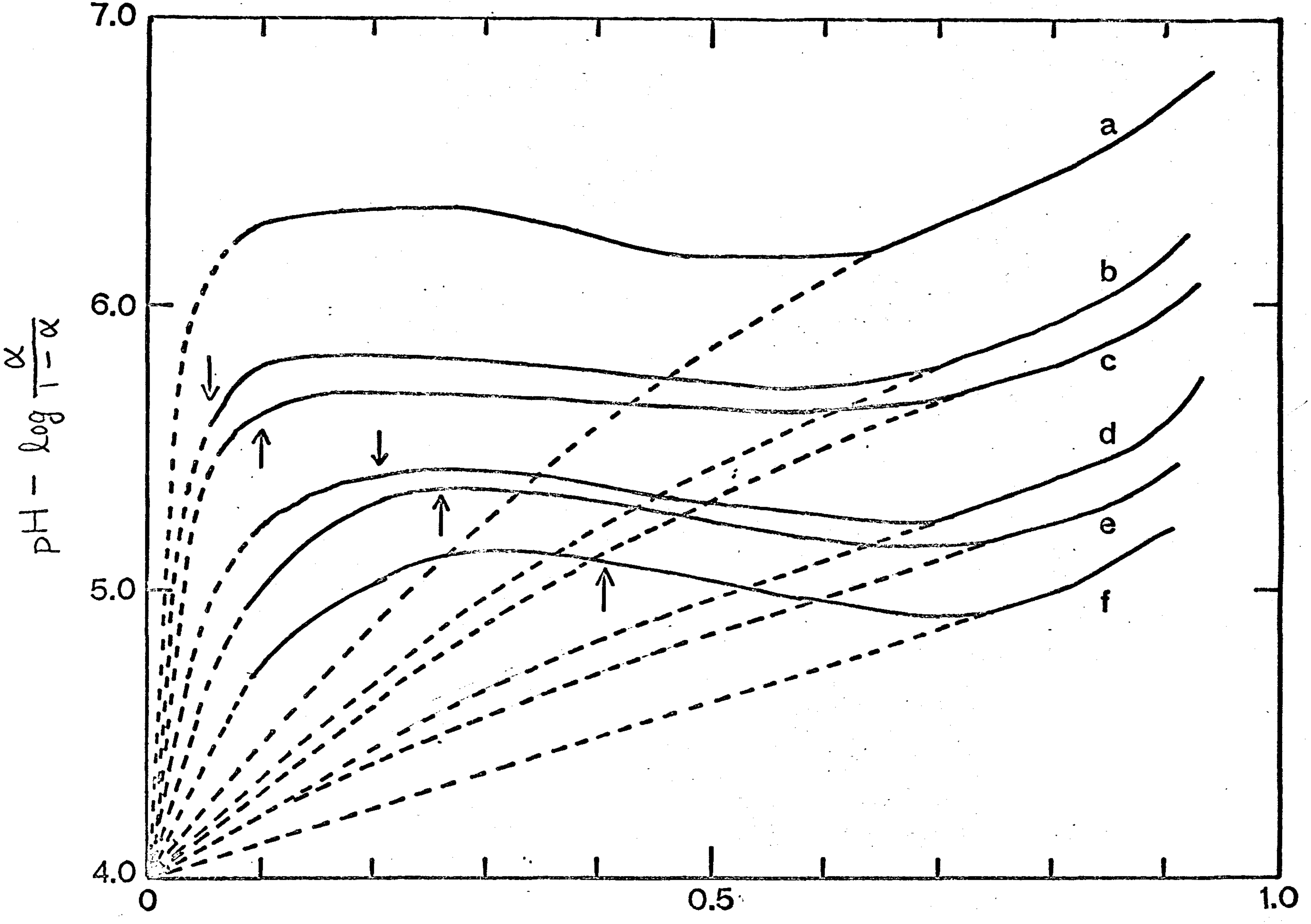


Fig. 14 (a)

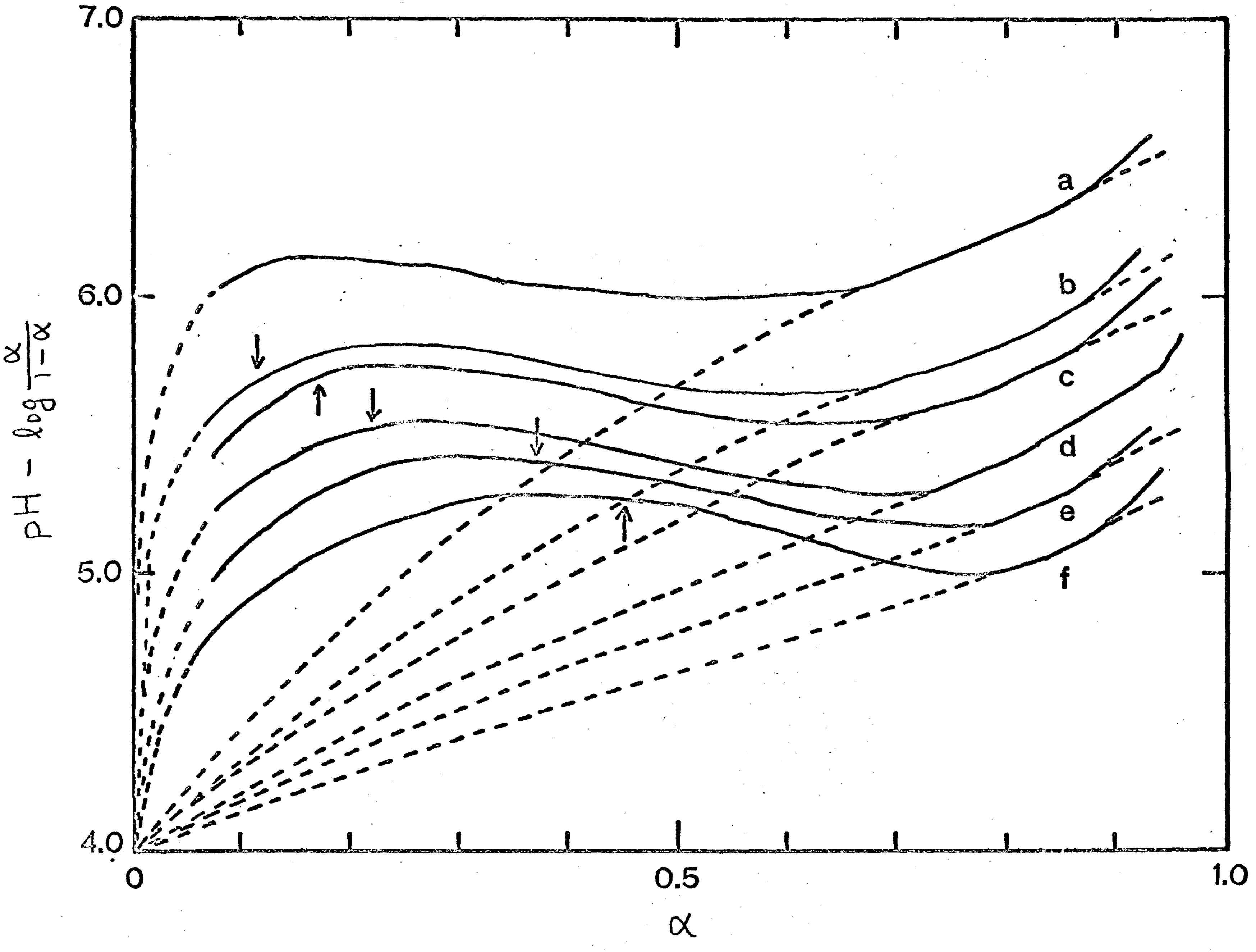


Fig. 14 (b)

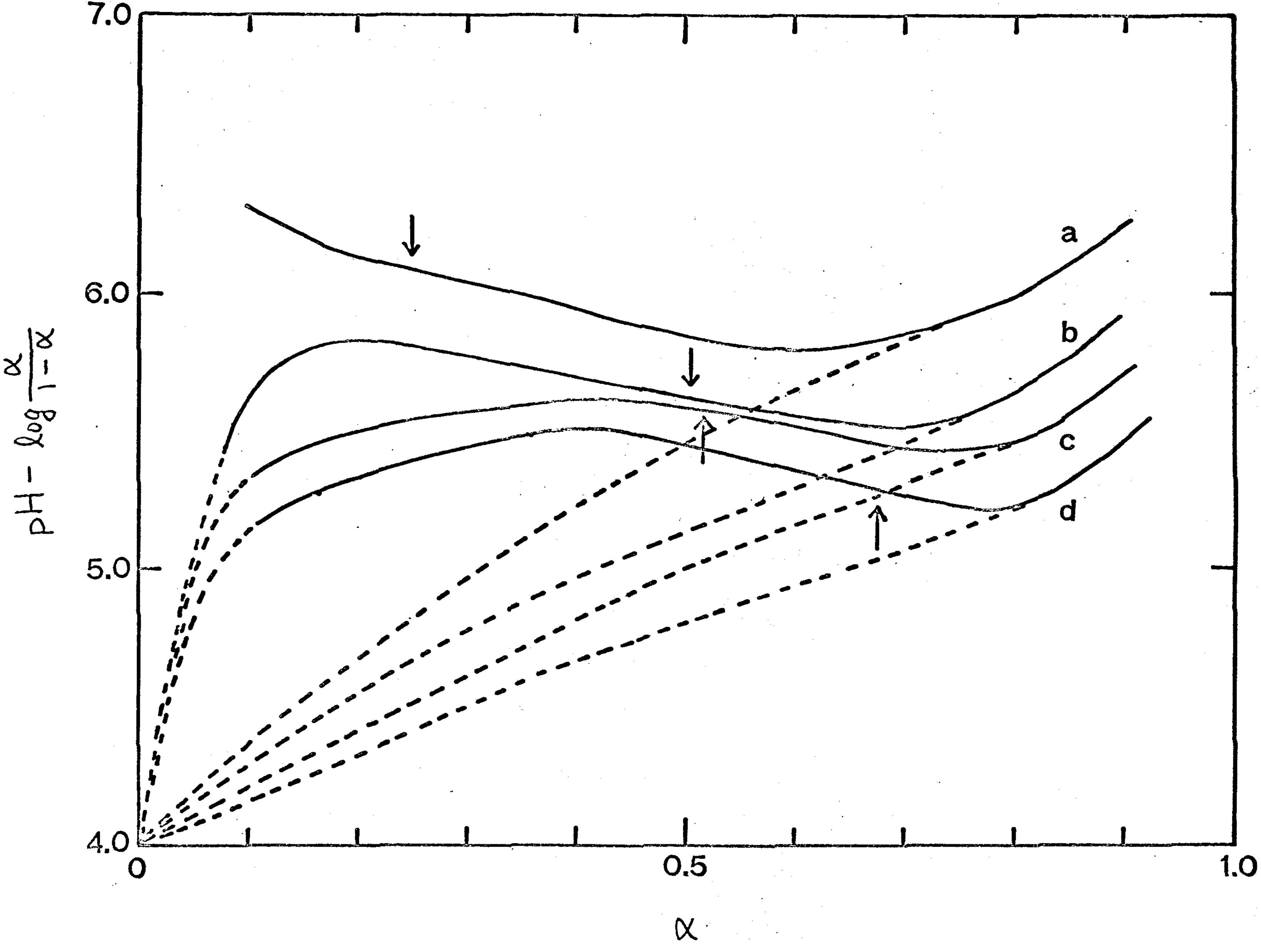


Fig. 14 (c)

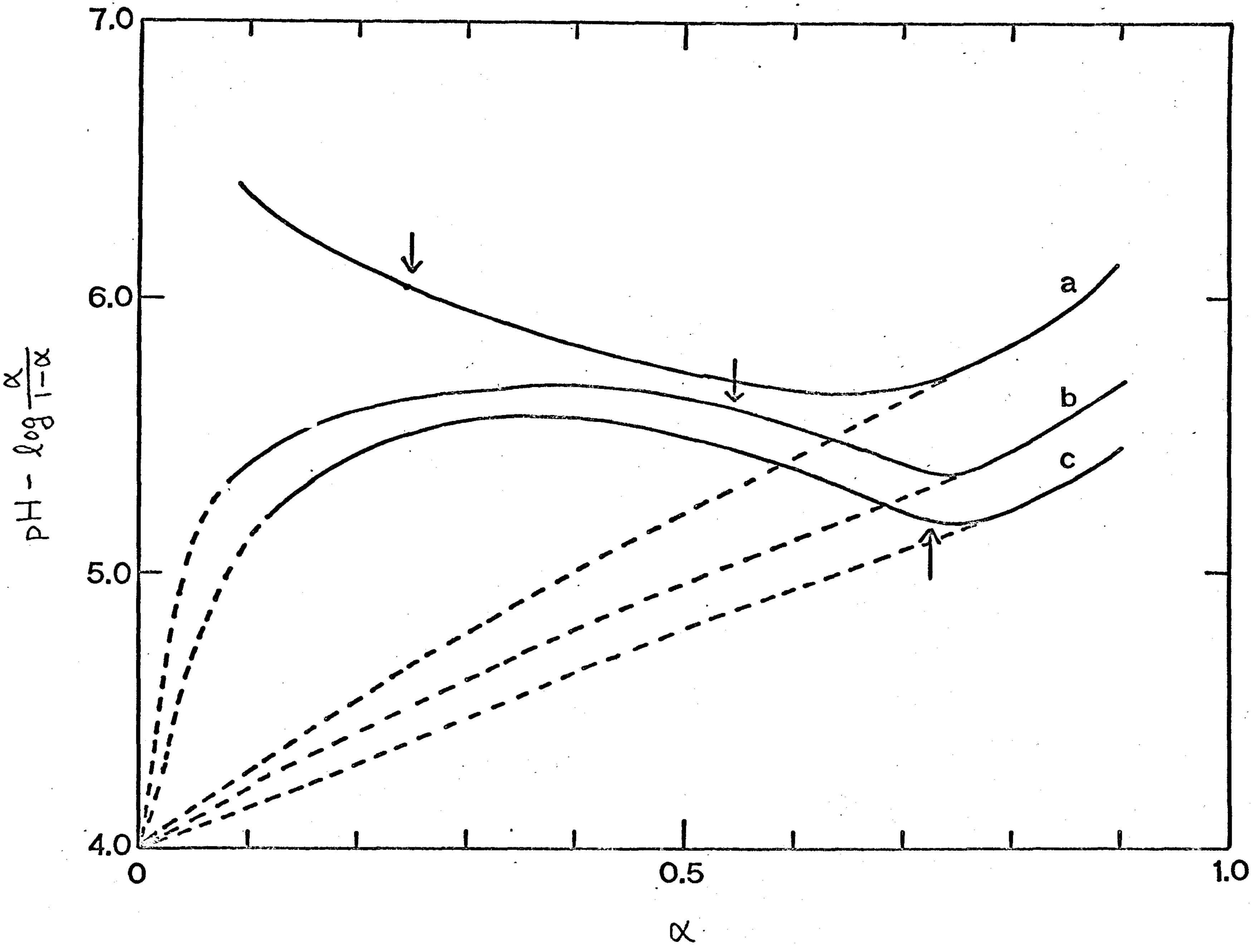


Fig. 14 (d)

Therefore in the case of helix-coil transition, the extrapolation has been mainly made from helical region. In the present case, the β region is represented by a more curved line than helical region, so the extrapolation from β region is also arbitrary. With these situations, the determination of the intercept or pK_o in eq.(3) was not successful. For the values of pK_o between 3.8 and 4.2, we can draw curved lines consistent for all data obtained (except for the no added salt cases with high polymer concentrations). Therefore we tentatively assume the value of pK_o to be 4.0 for this polypeptide. This value is low compared with that of poly-L-glutamic acid(4.5)[67], but is higher by unity than that of poly-S-carboxymethyl-L-cysteine(3.0)[69].

Titrations of the monomer, S-carboxyethyl-L-cysteine, were also carried out. The values of pK_2 of the monomer was found to be 4.0.

It must be stated that the possibility can not be neglected that two conformations, random coil and β-structure, have different values of pK_o, although we have determined pK_o in the above extrapolation assuming that both conformations have the same intrinsic dissociation constant.

Extended Henderson-Hasselbach Equation.

The titration curves may be analyzed in another way using the extended Henderson-Hasselbach equation,

$$pH = A + B \log\frac{\alpha}{1-\alpha} \qquad (4)$$

where A and B are the functions of m_s and C_p but independent of α. Examples of the Henderson-Hasselbach plots, $\log\frac{\alpha}{1-\alpha}$ vs. pH, are shown in Fig.15. This plot gave two straight lines on both sides of higher and lower pH regions,corresponding to random coil and β-structure respectively. It may be easily seen that the transition is exhibited more clearly in these plots than in the modified plots. So the assignments of three regions, random coil,β-structure,and transition region,were made with these plots. Even in this plot, in the case of low ionic strength, the region corresponding to pure β-structure is so narrow that we can not get the corresponding straight line. The obtained values of parameters A and B are listed in Table VI. The parameter A is larger in β-structure than that in random coil, and is seen to be a linear function of log m_s for both cases from Fig.16. Contrary to this, the dependence of B on both ionic strength and conformation is not clear and seems to be independent of these factors.

Although the extrapolations in the modified plots were made with the aid of the extended Henderson-Hasselbach equation, the values of pH given by this equation were somewhat higher at low α region. So drawing a curve through these points gave an unreasonable extrapolation. This clearly expresses the limit of the validity of the linear approximation in the extended Henderson-Hasselbach plots.

Fig. 15. The Henderson-Hasselbach plots.

(a) Polymer concentration, 0.005 N.

Ionic strength (in molal);

(a) 0, (b) 0.010, (c) 0.018, (d) 0.054, (e) 0.096, and (f) 0.211.

(b) Polymer concentration, 0.010 N.

Ionic strength (in molal);

(a) 0, (b) 0.014, (c) 0.019, (d) 0.048, (e) 0.093, and (f) 0.197.

(c) Polymer concentration, 0.025 N.

Ionic strength (in molal);

(a) 0, (b) 0.025, (c) 0.050, and (d) 0.110.

(d) Polymer concentration, 0.050 N.

Ionic strength (in molal);

(a) 0, (b) 0.050, and (c) 0.110.

Arrows represent precipitation points.

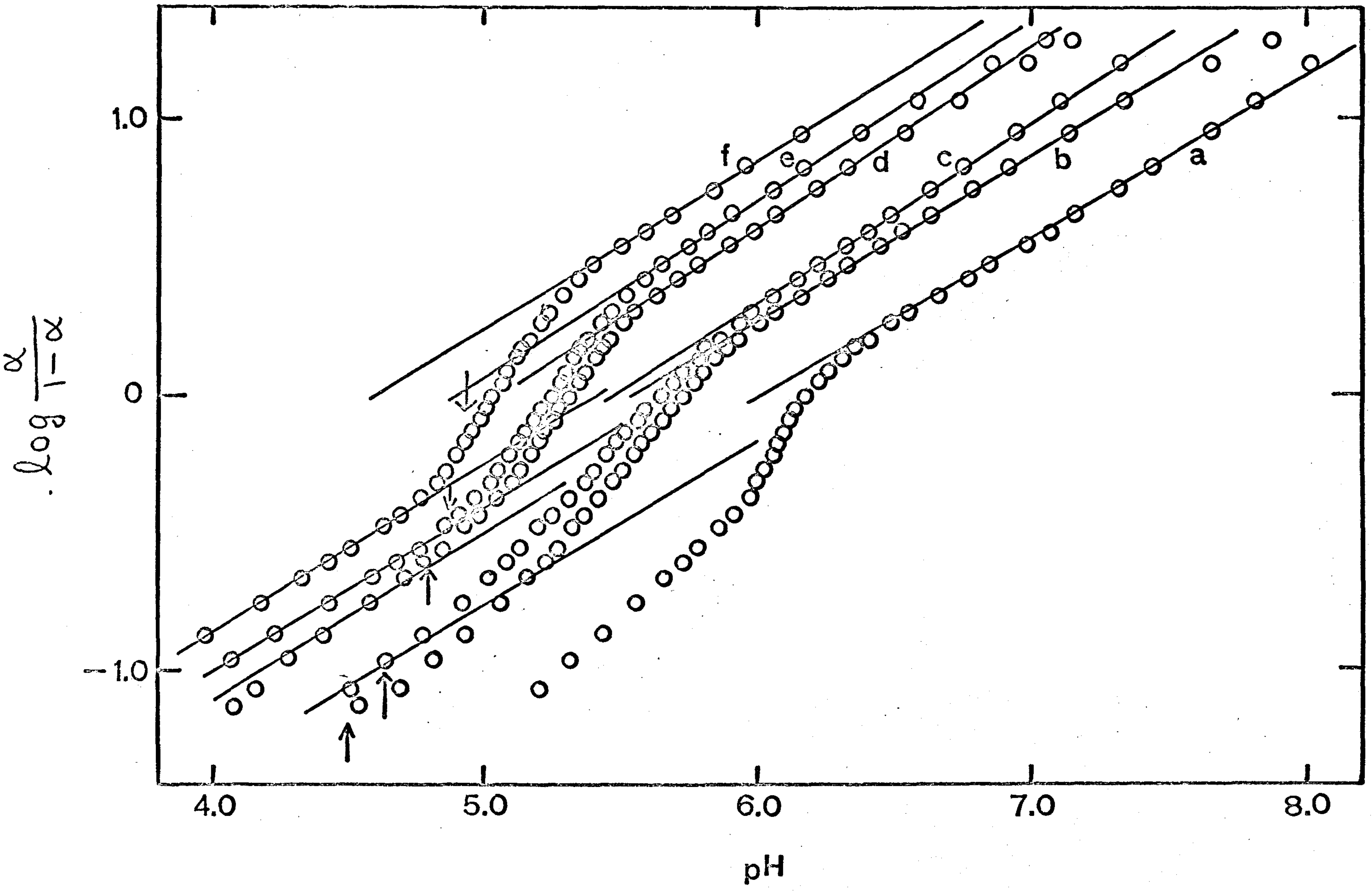


Fig. 15 (a)

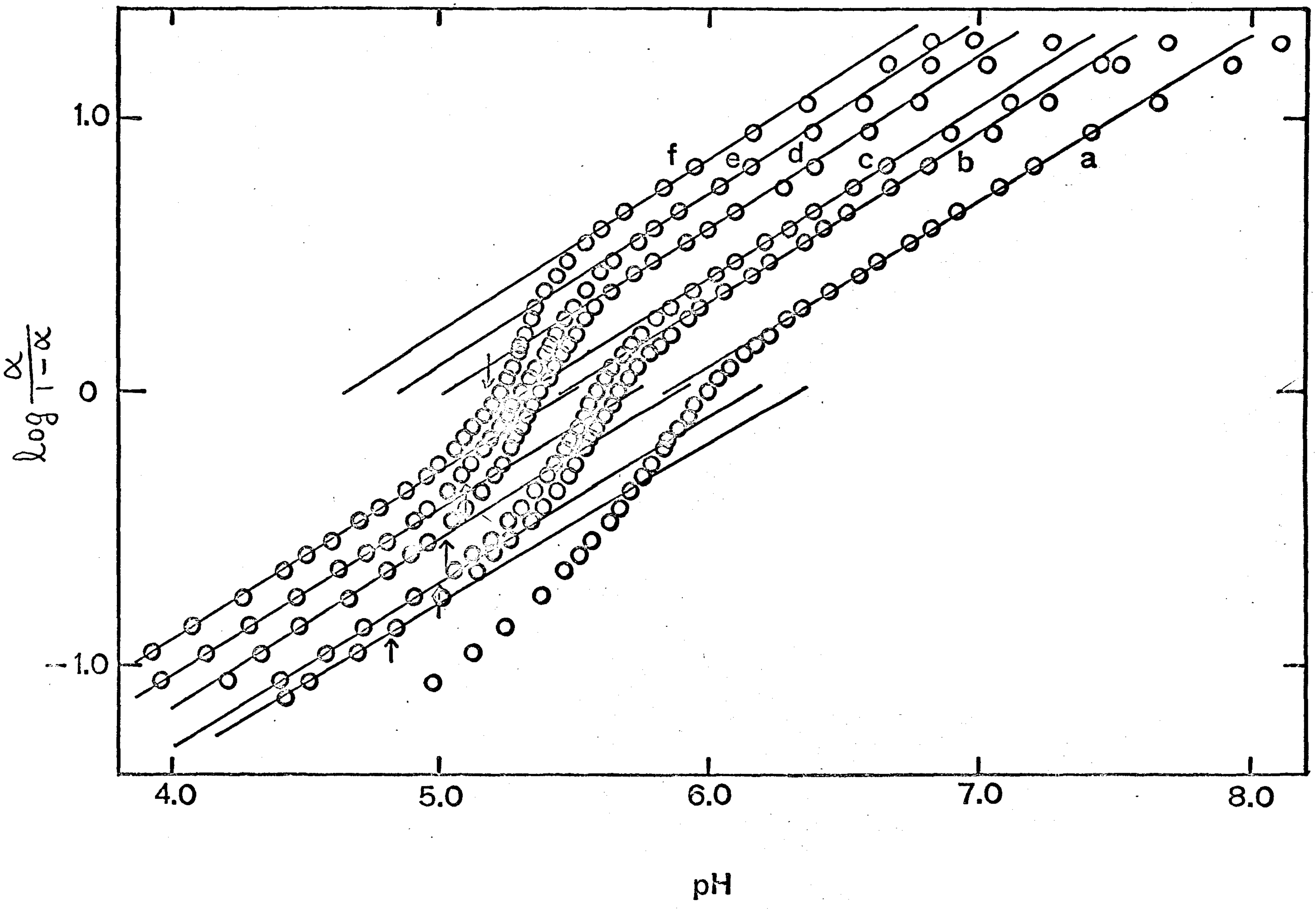


Fig 15 (b)

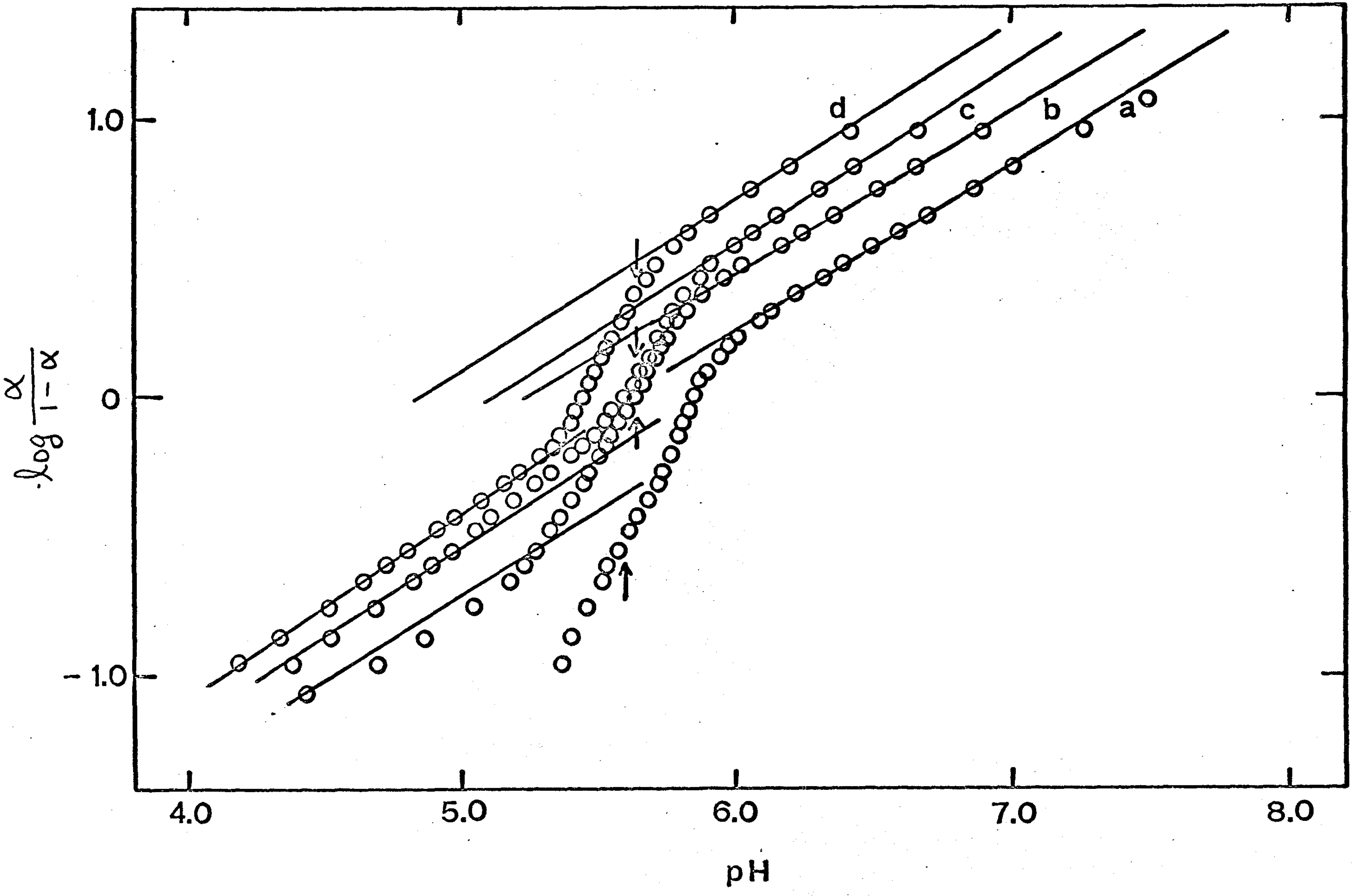


Fig. 15 (c)

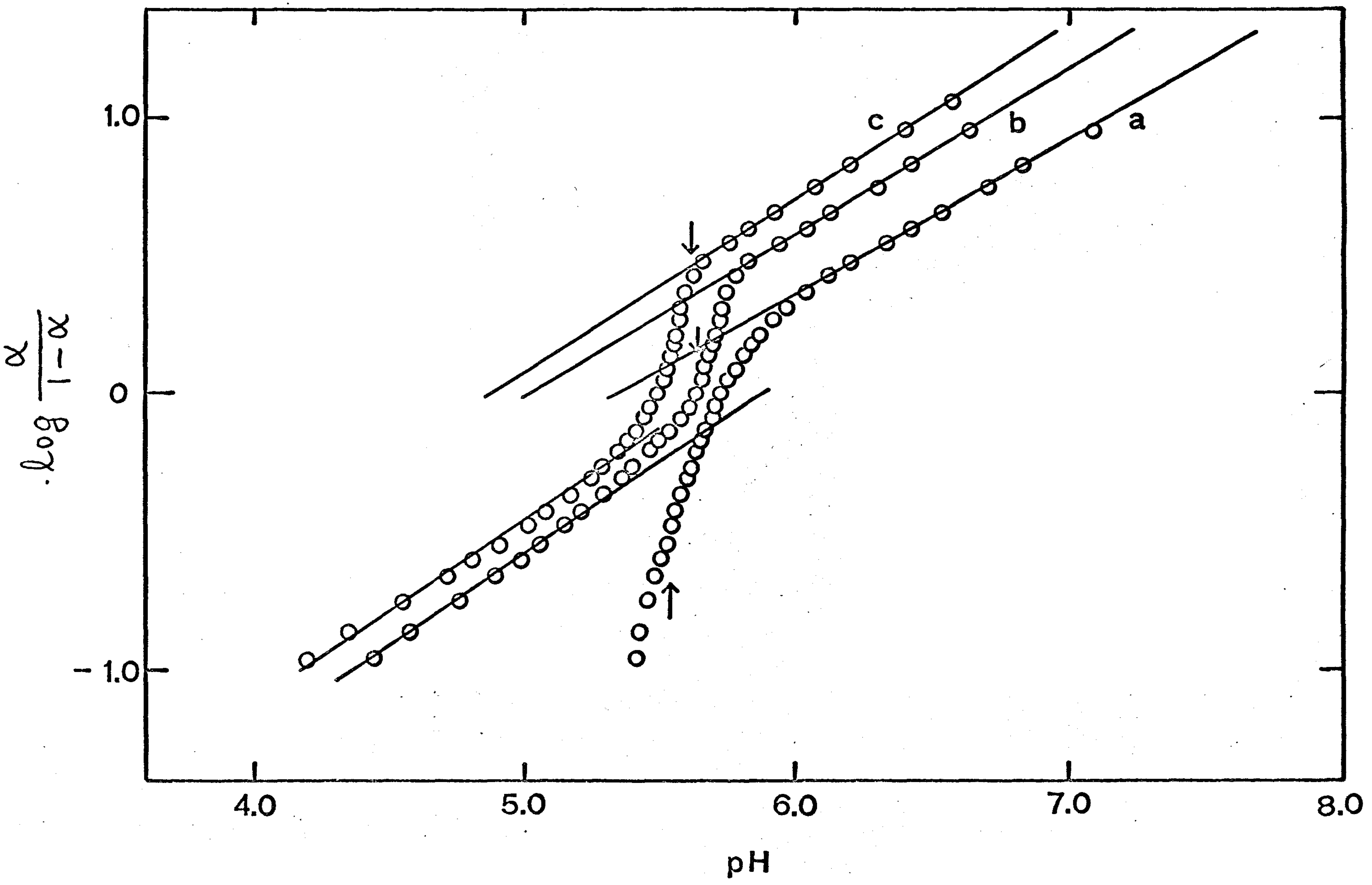


Fig. 15 (d)

TABLE VI

Parameters in Extended Henderson-Hasselbach Equation.

		Random Coil		β-Structure	
C_p(N)	m_s (molal)	A	B	A	B
0.005	0	6.01	1.72		
	0.010	5.53	1.69		
	0.020	5.47	1.56	6.29	1.71
	0.050	5.06	1.54	5.81	1.66
	0.100	4.90	1.56	5.71	1.71
	0.200	4.60	1.65	5.42	1.67
0.010	0	5.84	1.65		
	0.010	5.46	1.62	6.34	1.73
	0.020	5.33	1.61	6.17	1.66
	0.050	5.04	1.60	5.88	1.63
	0.100	4.85	1.58	5.72	1.66
	0.200	4.65	1.59	5.49	1.63
0.025	0	5.59	1.68		
	0.025	5.23	1.72	6.17	1.64
	0.050	5.16	1.59	5.86	1.59
	0.110	4.85	1.62	5.63	1.51
0.050	0	5.34	1.79		
	0.050	5.01	1.67	5.88	1.52
	0.110	4.88	1.59	5.69	1.52

Fig. 16 (a). Dependence of the parameter A in the extended Henderson-Hasselbach equation on ionic strength.

(a) β-structure. (b) Random coil.

Polymer concentrations;

(⦶) 0.005, (○) 0.010, (◐) 0.025, and (◑) 0.050 N.

Fig. 16 (b). Dependence of the parameter B in the extended Henderson-Hasselbach equation on ionic strength,

(a) β-structure. (b) Random coil.

Polymer concentrations;

(⦶) 0.005, (○) 0.010, (◐) 0.025, and (◑) 0.050 N.

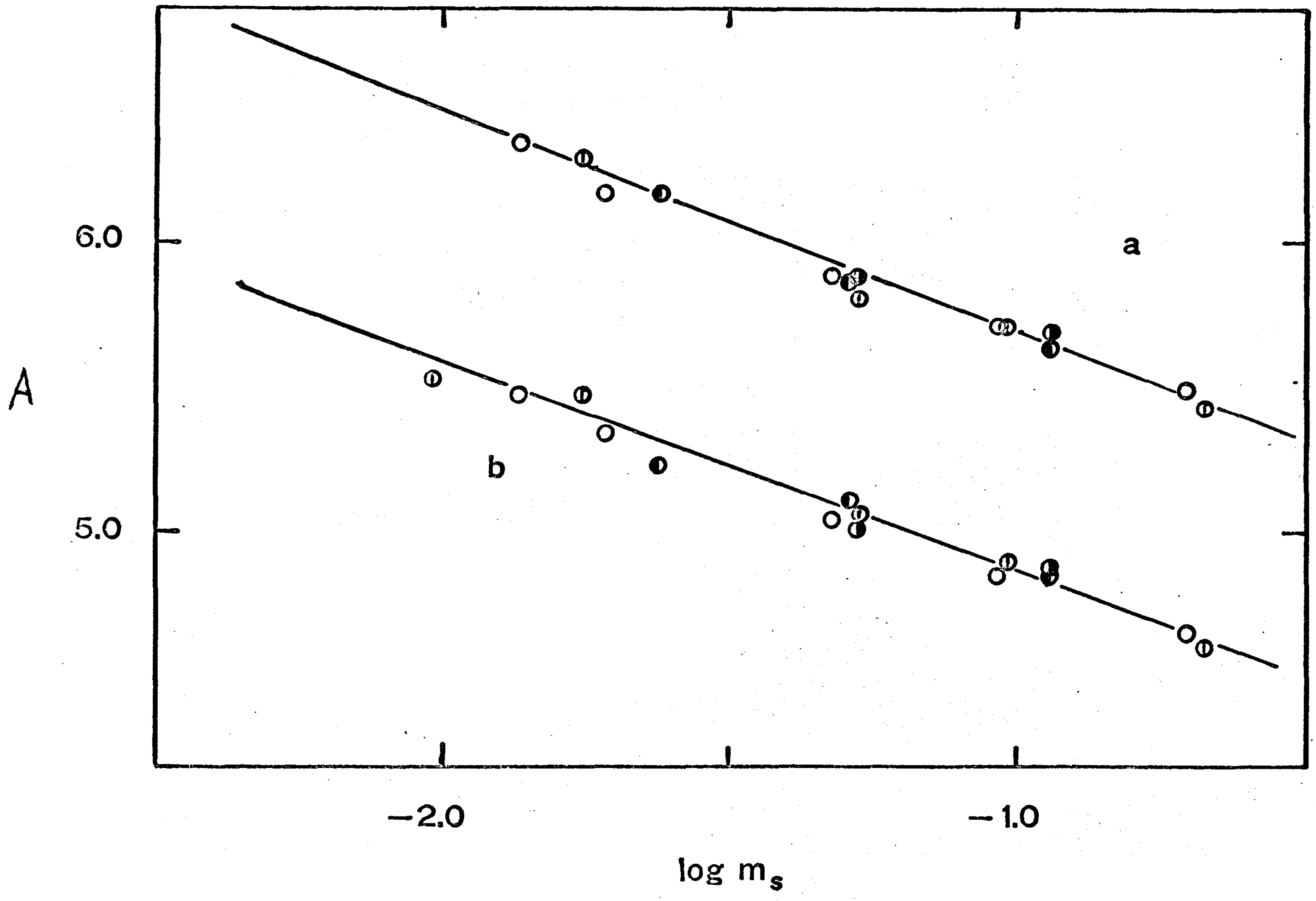


Fig. 16 (a)

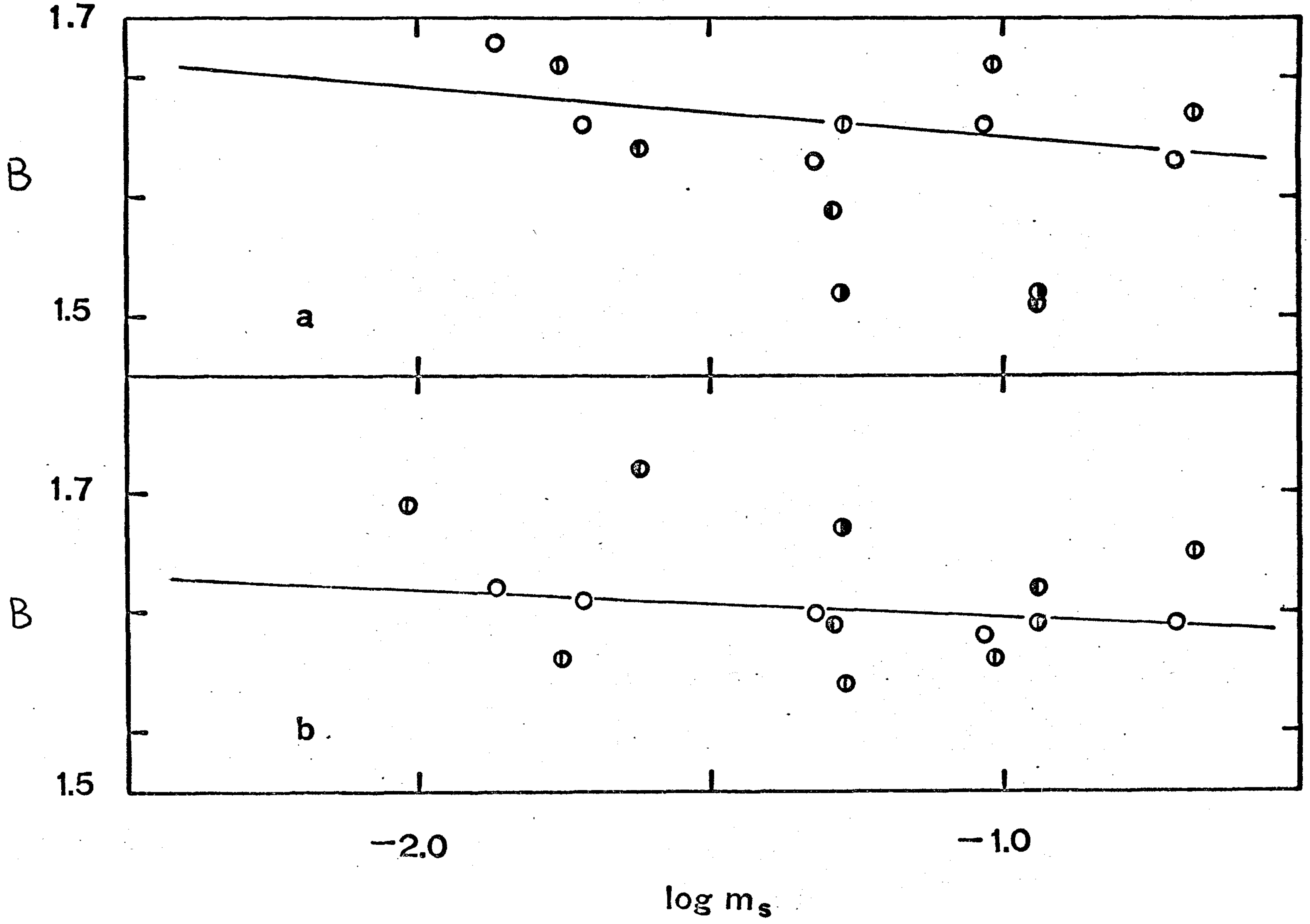


Fig. 16 (b)

DISCUSSION

Nonelctrostatic Free Energy Change for β-Coil Transition.

In the modified plots the area bounded by pure β curve, experimental data, and pure coil curve has been shown[64,67] to yield ΔG , the non-electrostatic free energy difference per mole of residue between β - structure and random coil. As mentioned earlier, both extrapolation and intercept (pK_o) in modified plots are somewhat arbitrary, so obtained ΔG must be taken as such. Errors in pK_o alter the magnitude of ΔG , but affect little its dependence on polymer concentration or ionic strength. On the other hand errors in extrapolations influence both magnitude and the dependence. From Figure 14 extrapolations are shown to be fairly good for higher ionic strength (more than 0.05 molal), whereas in water they are quite arbitrary, so ΔG in water is unreliable. In Figure 17, ΔG is plotted against $\sqrt{m_s}$ for various polymer concentrations. At the polymer concentration of 0.005 N, ΔG is 815 cal mole^{-1} in water and reduces to 470 cal mole^{-1} at higher ionic strength (0.2 molal). Contrary to the case of helix-coil transition, ΔG increases with polymer concentration. At the ionic strength of about 0.1 molal, ΔG increases from 480 cal mole^{-1} to 760 cal mole^{-1} corresponding to the change in polymer concentration from 0.005 N to 0.050 N. These figures are quite large compared with that of poly-L-glutamic acid at the same condition, (ca. 160 cal mole^{-1}).[67] This difference may be ascribed to the hydrophobic interaction between side chains in β-structure, which will be weaker in the case of α-helix

If hydrophobic interaction plays an important role to stabilize

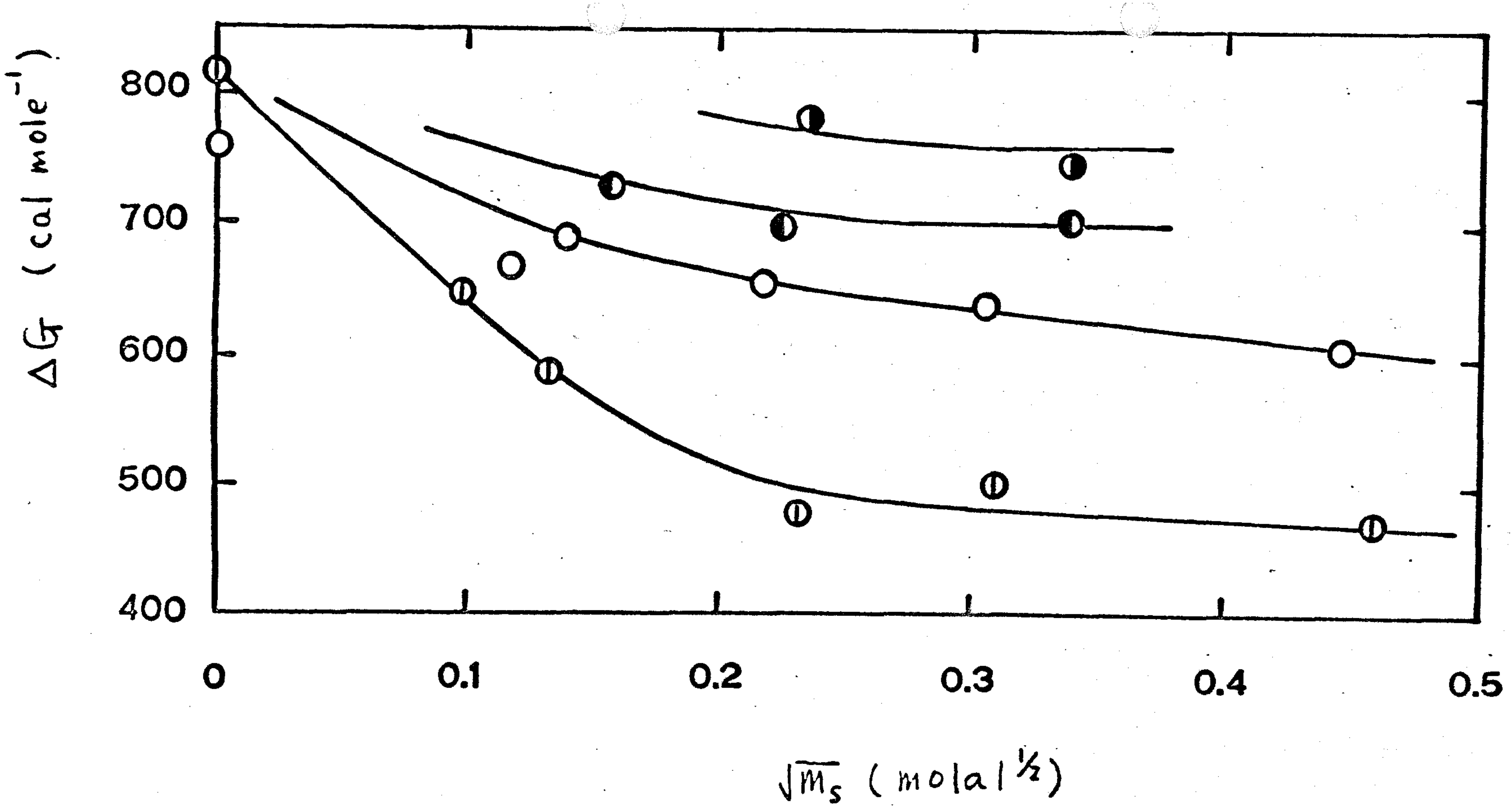


Fig. 17. Non-electrostatic free energy change for β-coil transition.

Polymer concentrations; (◐) 0.005, (○) 0.010, (◐) 0.025, and (◑) 0.050 N.

the β-structure, it is interesting to see the contribution of methylene group to this interaction. With the same polymer concentration(0.010 N) and ionic strength (0.1 molal), ΔG for poly-S-carboxyethyl-L-cysteine ([η] = 0.17 dl./g in 0.2 M NaCl) is 630 cal $mole^{-1}$, whereas that for poly-S-carboxymethyl-L-cysteine ([η] = 0.23 dl./g in 0.2 M NaCl) is 510 cal $mole^{-1}$.[69] Therefore, roughly speaking, with introducing a methylene group into side chain β-structure is stabilized by about 120 cal $mole^{-1}$.

It should be pointed out that in the case of helix-coil transition ΔG is identical to the standard free energy change, since cratic term RT ln c_p has the same magnitude for both conformations. In the case of β-coil transition, we don't know the dependence of non-electrostatic free energy on polymer concentration in the β-structure. Thus ΔG expresses the nonelectrostatic free energy difference in this case.

However, the difference in ΔG, 120 cal $mole^{-1}$, due to the increase of a methylene group, represents the difference of the standard free energy change accompanying β-coil transition between two different β-structures formed by different polymers, because the comparison is made at the same polymer concentration.

The Content of β-Structure.

In the case of charge induced conformational transition, it is known that in the transition region of the transition the concentrations of polymer in each conformation can be calculated from the titration curves.

In the present case, it was shown that the Henderson-Hasselbach plot permits to distinguish the regions of pure β-structure,pure random coil, and the transition on a titration curve. Consequently, at a given pH, the degree of ionization, of pure β-structure,α_β, and of pure random coil, α_c, can be estimated from this plot. Then the content of β-structure, f_β, at a given pH will be given by

$$f_\beta = (\alpha_c - \alpha)/(\alpha_c - \alpha_\beta) \qquad (5)$$

When the system is under phase separaton, it would be possible that the degree of ionization of the β-structure in solution differs from that of the precipitates. However the value of α_β obtained from the Henderson-Hasselbach plots may be generally regarded as the average degree of ionization of the β-structure, in solution and in precipitates. So the content of β-structure obtained from eq.(5) depends on the assumption that the polymers in the precipitates are in β-structure. If we take into consideration the results of the conformational studies on solid state of the present polymer, this assumption will be quite reasonable. The content of β-structure thus obtained is plotted in Fig.18 against degree of ionization.

In the case of helix-coil transition, it was shown that the helical content can be obtained from spectroscopic data as well as from titration. The coincidence between them has been reported to be very good.[67]

Fig. 18. Conformational titration curves.

(a) Polymer concentration, 0.005 N.

Ionic strength (in molal);

(a) 0.018, (b) 0.054, (c) 0.096, and (d) 0.211.

(b) Polymer concentration, 0.010 N.

Ionic strength (in molal);

(a) 0.019, (b) 0.048, (c) 0.093, and (d) 0.197.

(c) Polymer concentration, 0.025 N.

Ionic strength (in molal);

(a) 0.025, (b) 0.050, and (c) 0.110.

(d) Polymer concentration, 0.050 N.

Ionic strength (in molal);

(a) 0.050, and (b) 0.110.

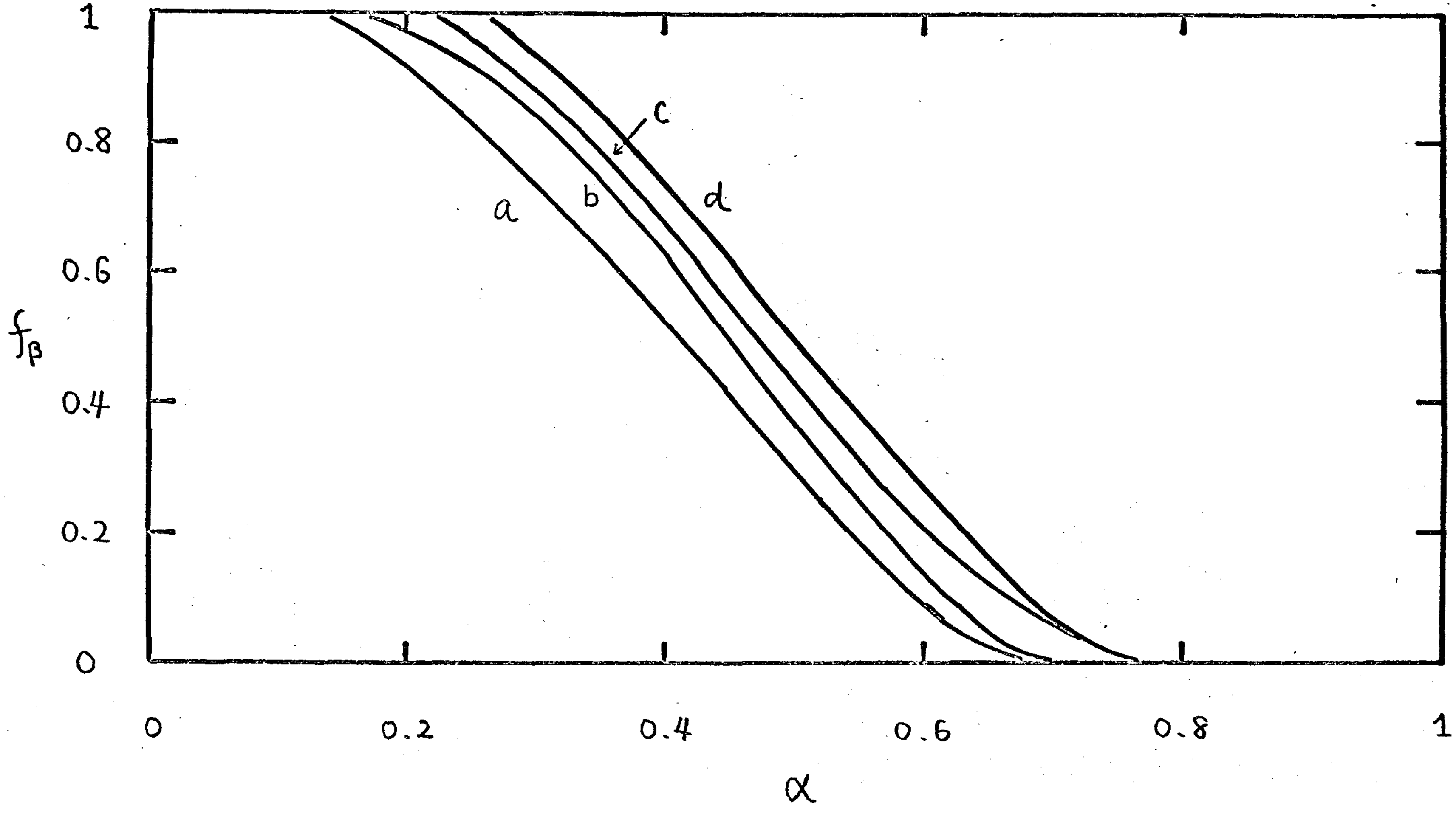


Fig.18(a)

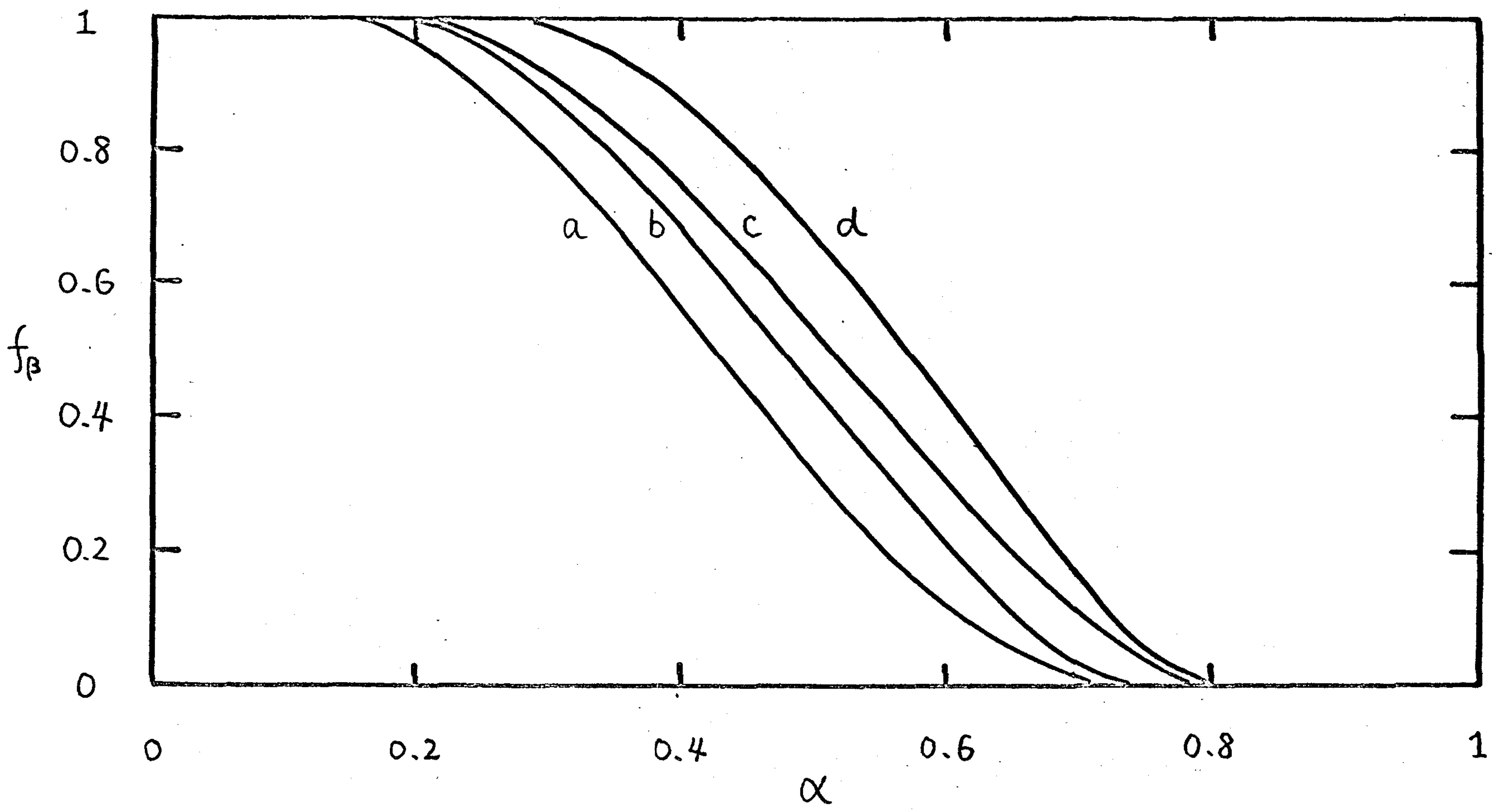


Fig. 18 (b)

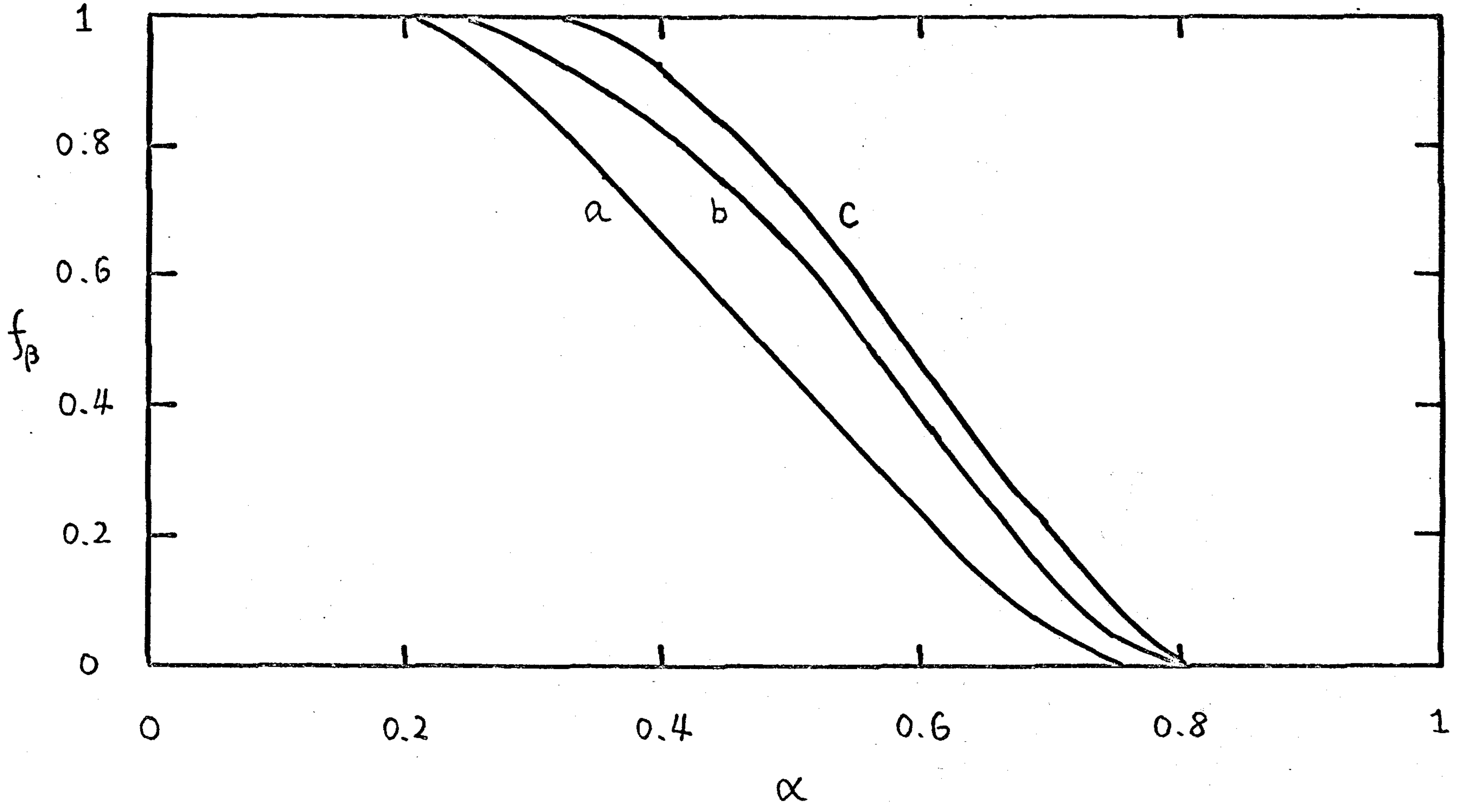


Fig. 18 (c)

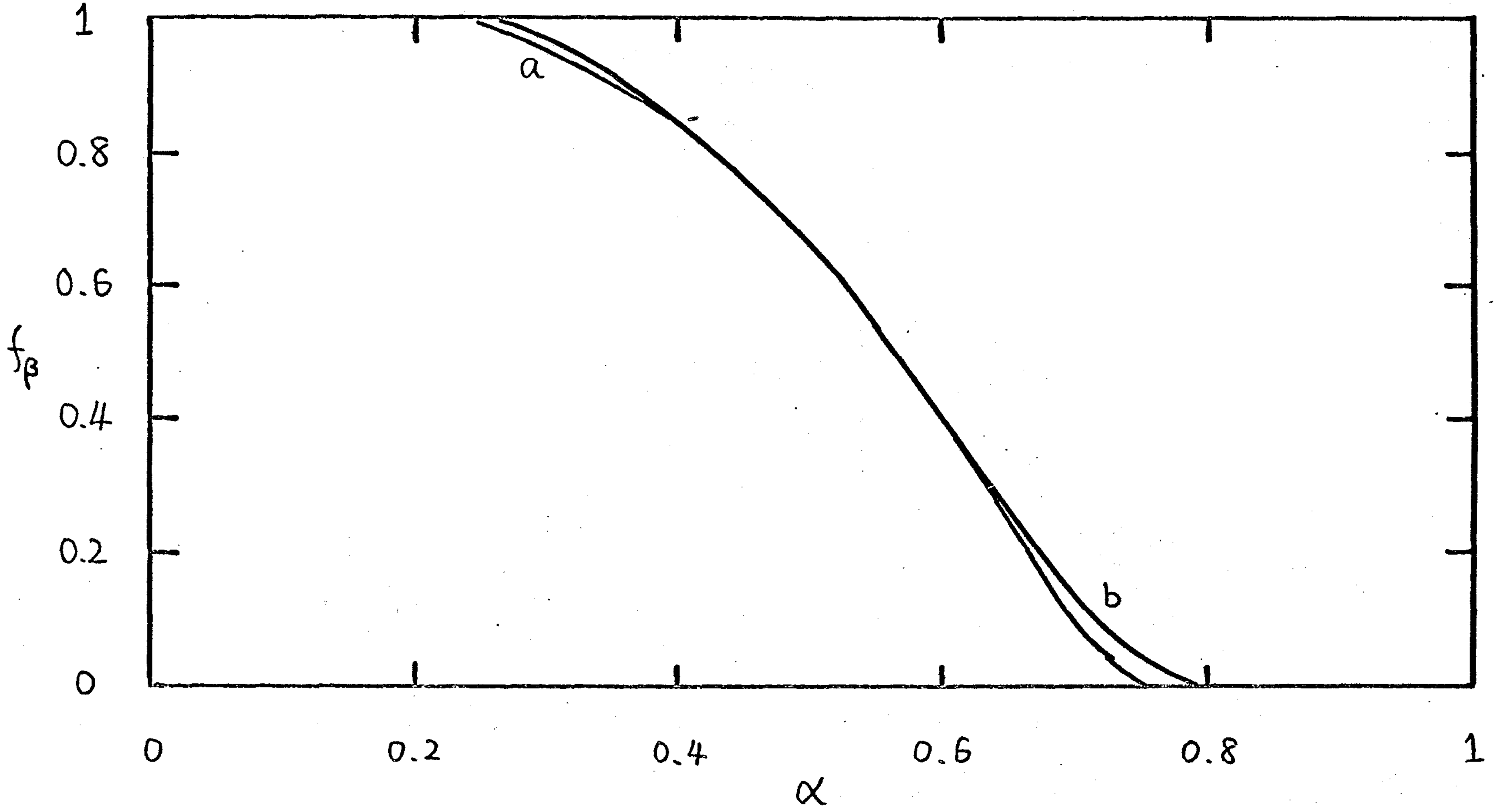


Fig. 18 (d)

Contrary to this, in the case of β-structure-random coil transition, the state of pure β-structure is generally inaccessible to the spectroscopic measurements, since the phase separation occurs before the pure β-structure is attained in solution. Of course exceptions to the generalization of this statement are present in some special conditions, extremely low polymer concentration and low ionic strength. The very situation involved in the statement makes the potentiometric titration be a more important measurement than those of spectroscopy in the study of β-coil transition.

Since the content of β-structure can be estimated only from titration data, we examine the relation between f_β and the conformation-dependent optical properties. In Fig. 19, $[\theta]_{223}$, the magnitude of the negative circular dichroic band at 223 mμ, is plotted against f_β. This dichroic band has been shown to be dependent on conformation in Section 2. A linear relation between f_β and $[\theta]_{223}$ is shown to hold approximately, and the value of $[\theta]_{223}$ for pure β-structure is shown to be about -10,000 deg.cm/decimole for the present polypeptide.

Fig. 19. Relation between β-content, f_β, and $[\theta]_{223}$.

(a) Polymer concentration, 0.005 N.

Ionic strength (in molal); (◑) 0.030, (⦶) 0.150.

(b) Polymer concentration, 0.010 N.

Ionic strength (in molal);

(◑) 0.010, (⦶) 0.020, (⊖) 0.050, (◐) 0.100, and (◒) 0.200.

(c) Polymer concentration, 0.025 N.

Ionic strength (in molal); (◑) 0.050, and (⦶) 0.110.

(d) Polymer concentration, 0.050 N.

Ionic strength (in molal); (◑) 0.050, and (⦶) 0.110.

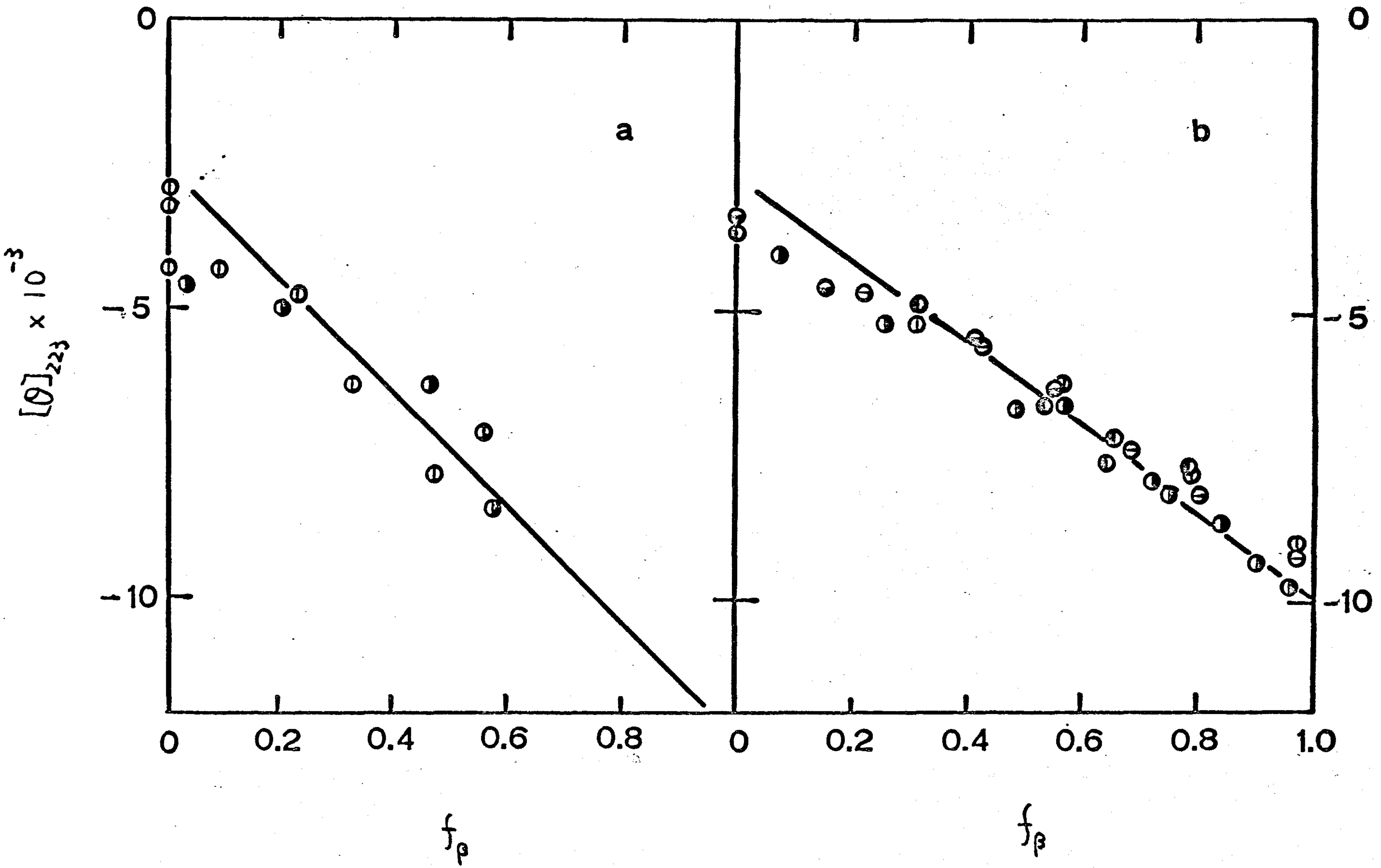

a
b
0
-5
-10
0
-5
-10
$[\theta]_{223} \times 10^{-3}$
0
0.2
0.4
0.6
0.8
0
0.2
0.4
0.6
0.8
1.0
f_β
f_β

Fig. 19

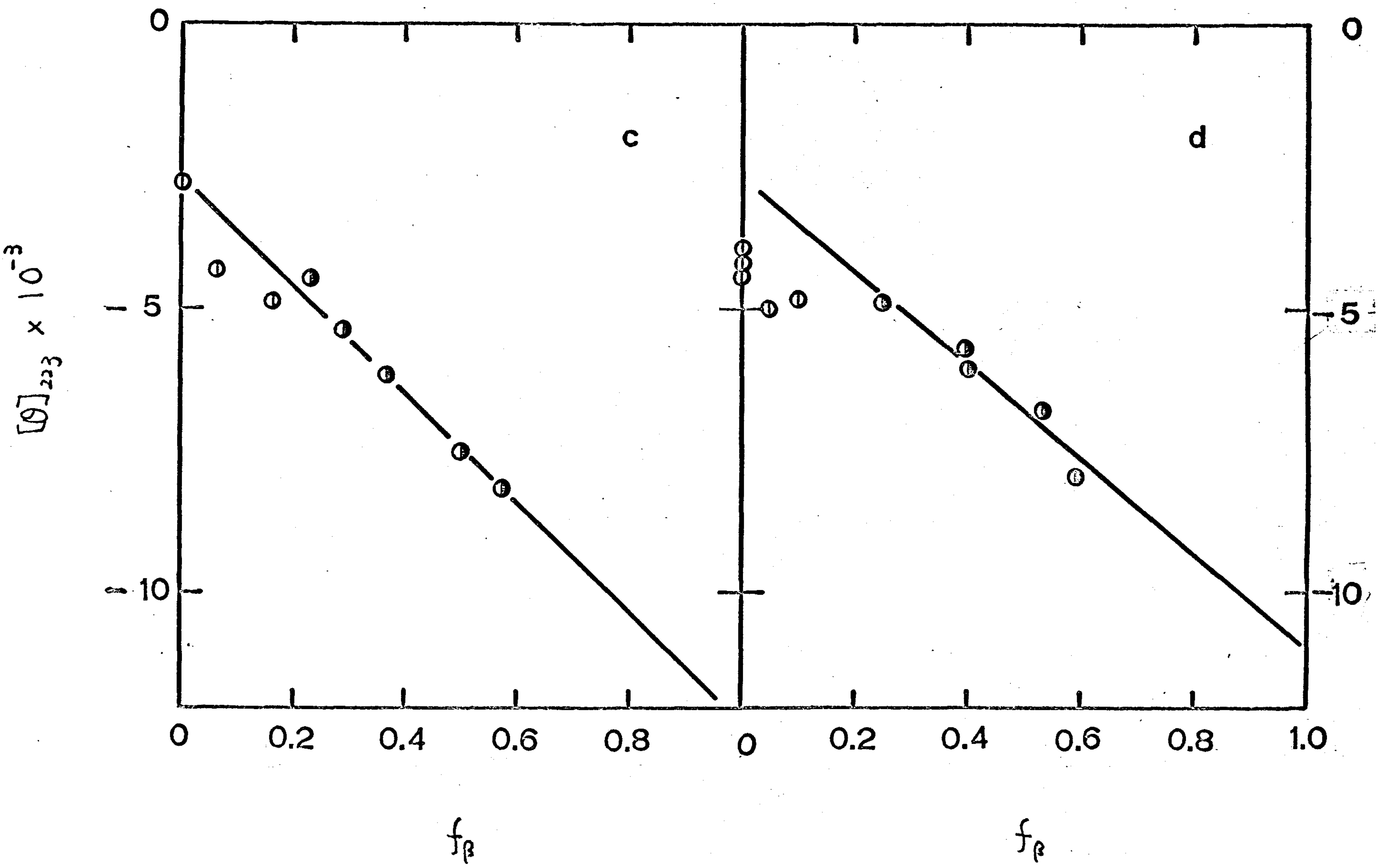

c
d
$[\theta]_{223} \times 10^{-3}$
0
5
10
0
5
10
0
0.2
0.4
0.6
0.8
0
0.2
0.4
0.6
0.8
1.0
f_β
f_β

Fig. 19

Phase Separation

A few comments may be added here on the potentiometric titration accompanying phase separation, which was first investigated by Linderstrøm-Lang[73]. The Linderstrøm-Lang's treatment was later extended by Shatkay and Michaeli[74], who derived a more general relation. From these theories it could be expected to derive that a discontinuous change should occur on a titration curve whenever phase separation occurs, if the degrees of ionization are different in both phases. This behavior can be seen on the titration curves measured by Shatkay and Michaeli, for which the degree of ionization was found to be zero in the precipitates.

In the present system containing poly-S-carboxyethyl-L-cysteine and added salt, the precipitates are separated out from the solution at low ionizations, where the polymer is mostly or partly in the β -structure. However, the titration curve connects continuously before and after visible precipitates are formed. This extraordinary behavior encountered in the present system can not be properly interpreted unless the investigations on the state under phase separation is made.

4. Sodium Counter Ion Activity of Poly-S-carboxyethyl-L-cysteine.

Introduction

Counter ion activity is a measure of the interactions between counter ions and polyions. Electrostatic interaction has been considered to play an important role in determining counter ion activity. This interaction is strongly dependent on the electrostatic potential of the domain where distributions of polymer segments are rather dense. This potential, in turn, is determined by the charge density of that domain in terms of the Poisson equation. Therefore the charge density on a polyion and polymer concentration are the most important quantities to determine the thermodynamic properties of polyelectrolyte solutions without added salt. Generally, the charge density is dependent on chain conformation as well as on degree of neutralization. For randomly coiled polyelectrolyte, however, it has been shown that the electrostatic interactions of polyions with their counter ions can be well described by a rod model.[63] So in this case, the charge density is determined by molecular structure, such as distance between neighboring dissociable sites, and becomes linearly proportional to degree of neutralization irrespective of real chain configuration.

Counter ion activity coefficient has been shown to increase with decreasing degree of neutralization. On the other hand, the dependence of the activity coefficient on polymer concentration has been shown to be not determined uniquely.[75] The dependence on polymer concentration was treated theoretically and interpreted due to the difference in charge density .[76]

In the case of charged polypeptides, however, conformatioanl changes usually occur accompanying the change in pH of solutions. Since different conformations have different charge densities at the same degree of neutralization, activity coefficient of counter ions is expected to reflect the effects of conformational transitions.

Counter ion activity has been investigated on several polypeptides.[77-81,97] The dependence of the activity coefficient on polymer concentration has been chiefly examined for the case of fully neutralized polypeptides.[77-80] The effect of conformational change has been also examined,[81,97] and it has been found that the change of activity coefficient manifests the helix-coil transition.[97]

In the present investigation, counter ion activity of poly-S-carboxyethyl-L-cysteine neutralized to various degrees with sodium hydroxide is investigated in two different ways. The counter ion activity of the polymer in salt-free solutions is determined either directly from the e.m.f. measurements of concentration cells formed across a Na-glass electrode, or from the potentiometric titrations at different ionic strength by means of a theory recently developed by Oosawa[82] based on the assumption of the additivity rule.*

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* The additivity rule has been established empirically.[83-85] According to this rule, counter ion originating from a polyelectrolyte and mobile ions originating from added salt make independent contributions to colligative properties of polyelectrolyte solutions with added salt. Theoretical investigations on this subject have been carried out by several authors.[87-90,98]

In the previous sections, the present polypeptide has been shown to undergo the β-random coil transition depending on the pH of the solutions. At high degree of neutralization, polymer chains are in random coil conformation. On reducing degree of neutralization, polymer chains become more densely coiled and hydrogen bonds between peptide groups may begin to be formed resulting in the pleated sheets. These pleated sheets may further associate in terms of the hydrophobic interaction between side chains of different pleated sheets, to give three dimensional aggregates,i.e. β-structure. As degree of neutralization is more reduced , association into both two and three dimensional ways becomes more prevailing, and the transition to β-structure becomes more complete. This conformational transition can be detected in terms of optical properties. For example, the residue ellipticity of the negative circular dichroic band at 223 mμ, $[\theta]_{223}$, has been shown to vary from about -3,500 to about -10,000 when random coil are transformed into β-structure.

In the present study, the activity coefficients of counter ions are obtained corresponding to random coil conforamtion, the transition region, and β-structure. The dependence of the activity coefficient on both degree of neutralization and polymer concentration are examined for the three different states. For random coil state, the result will be compared with those of other polypeptides previously reported. This comparison will make it clear whether randomly coiled polypeptides can also be described by a rod model in respect to counter ion activity.

On the other hand, the results obtained for β-structure will be expected to afford valuable informations concerning the electrostatic properties of this particular conformation.

Experimental

Materials.

The polyelectrolyte samples employed were poly-S-carboxyethyl-L-cysteine B622AW and H920W. Reagent grade sodium chloride was used without further purification. The value of e.m.f. of water used (pH>6.0) was always larger, in magnitude, than that corresponding to the sodium chloride solution having pNa of 4.0.

E.M.F. Measurements.

The activity of sodium ions was determined by the measurements of e.m.f. of the concentration cells after the method of Ise et al.[91,92] Calibrations of a Na-glass electrode (Horiba No.1582) were carried out with seven sodium chloride solutions. The activity of sodium ions in these reference solutions was in the range from 1.0 to 1×10^{-4} molal. A linear relation between observed e.m.f. value and logarithm of the activity was always obtained with Nernstian slope for the range where the activity was larger than about 2.5×10^{-3} molal. Thus the activity of sodium ions could be precisely determined by the glass electrode only when it was larger than this value. This situation prevented us from the determination of the activity at low degree of neutralization α, if polymer concentrations were low. At high polymer concentration, measurements were not undertaken for the region of α smaller than 0.25, where precipitates appeared.

The values of the slope of the calibration data obtained from more than ten times calibrations were in the range from 58.8 to 59.9 mV/pNa. Theoretical value for the slope is 59.2 mV/pNa at 25.0°C. An example of the calibration data is illustrated in Fig.20.

Before and after a series of measurements calibrations with seven reference solutions were made. Before and after a measurement of each sample solution e.m.f. of a dilute reference solution (pNa is larger than 3.0) was measured. If two e.m.f. values of the reference solution differed more than 2mV, the data were discarded.

The measurements of e.m.f. were carried out with a Hitachi-Horiba Model F-5X pH meter by using its expanded scale and connected with a Hitachi Model QPD-53 recorder. The precision of the measurements was ±1 mV. The corresponding error in activity coefficient was about 8.7%, if the relative error in concentration was assumed to be 1%, which was the maximum value in the present investigation. Thus if activity coefficient observed is 0.80 (the maximum value appearing in the present measurements), the corresponding error is 0.07 or ±0.035.

The reproducibility was very good for the measurements of a solution (within 1 mV), whereas the reproducibility for the measurements of several solutions with the same compositions was not good and sometimes exceeded the range originating from the precision of e.m.f. measurements.

Observed e.m.f. values increased, in magnitude, with time and reached stationary values after about ten minutes. When contamination of KCl from calomel electrode began to occur, the e.m.f. value suddenly decreased to a large extent and thus it could be checked.

No change of e.m.f. values was observed upon the addition of HCI to sodium chloride solutions, if the activity of sodium ions was higher than 10^{-3} molal and pH was higher than 5.0. In the present study, the

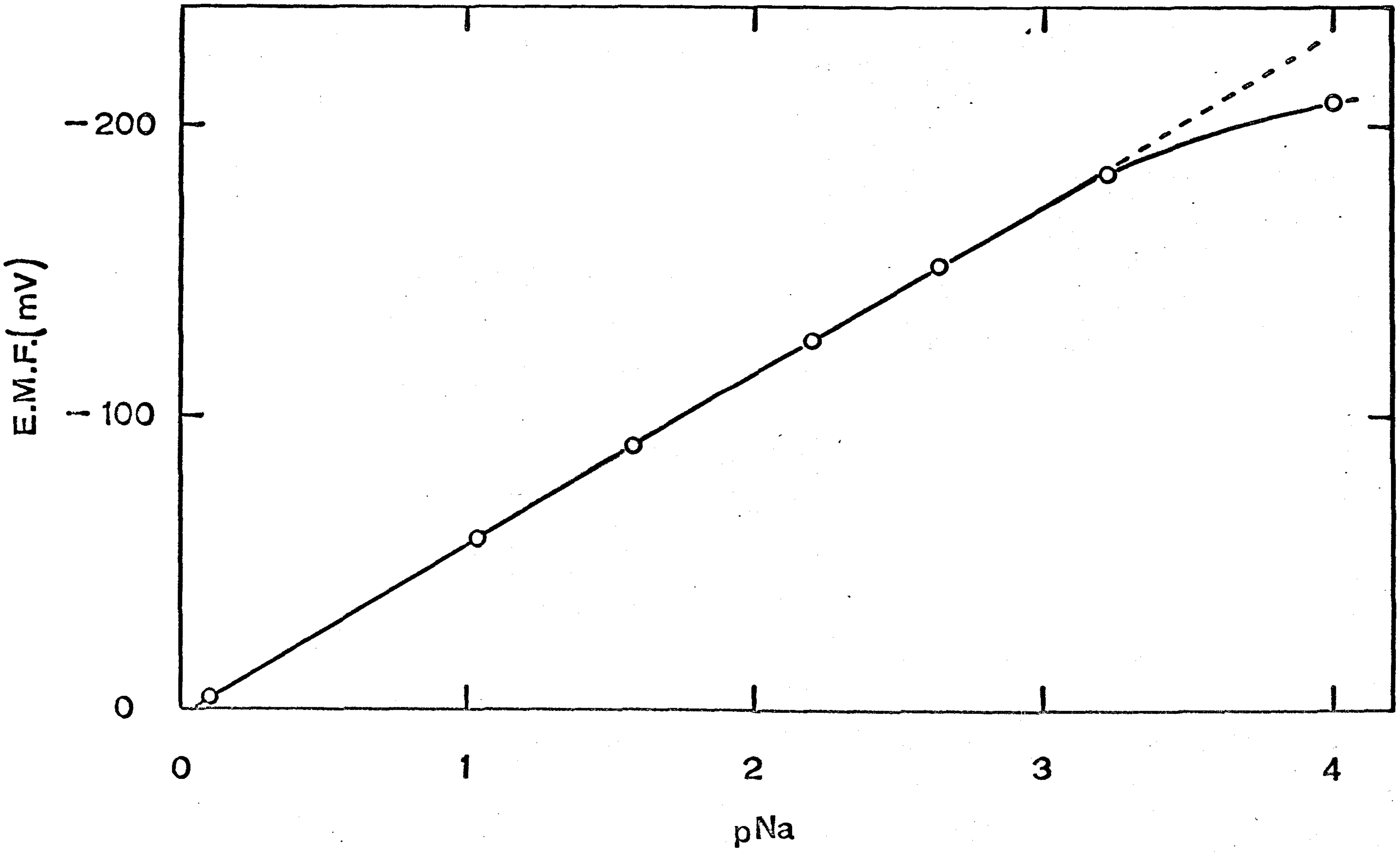


Fig. 20. Calibration of the Na-glass electrode. ——— ; Observed data. - - - ; Theoretical line.

pH region of the polymer solutions examined was always higher than 5.5 and the effect of pH on e.m.f. measurements was negligible.

The e.m.f. was measured at 25±0.5°C. Solutions were maintained at the same temperature before measurements for two or three days. E.m.f. measurements were performed under the atmosphere of nitrogen passed through a concentrated sodium hydroxide solution and through distilled water.

Concentrations of polymer and added salt, denoted by m_p and m_s respectively, are expressed in molality scale. The polymer concentrations examined were 0.005,0.010,0.025,and 0.050 monomolal,i.e., residue mole per 1 kg of water.

RESULTS

Activity Coefficient from E.M.F. Measurements.

In Figures 21-24 the activity coefficients of sodium ions in salt-free solutions, γ_+, are represented by circles. Figures 21 and 22 show that two samples examined gave essentially identical results.

In random coil state where degree of neutralization α is considerably high, γ_+ increases with decreasing α. Further decrease of α causes the conformational transition, nevertheless γ_+ still increases. At low polymer concentrations, as shown in Figures 21 and 22, the transition from random coil to β-structure is far from completed at the lowest α attained in the e.m.f. measurements. Thus the dependence of γ_+ on α scarcely exhibits the effect of the conformational transition. On the other hand, as shown in Figure 24, the e.m.f. measurements cover the entire region of the conformational transition for high polymer concentration, though the pure β-structure region is not involved. In this case, the activity coefficient clearly decreases with decreasing α, if β-structure is considerably formed. In Figure 24, γ_+ reaches a value of 0.44, which is the minimum value attained without phase separation. It may be safely assumed from the data of circular dichroism that the fraction of β-structure is not smaller than 90% at this state(m_p=0.050 monomolal and α=0.25). Thus the activity coefficient for β-structure is 0.44 or somewhat smaller at this state.

From the measurements of e.m.f., counter ion activities both for random coil and the transition region are obtained.

For random coil conformation, the activity coefficient is found to be dependent on both degree of neutralization and polymer concentration.

Fig. 21-24. Activity coefficients of sodium ions.

Data from e.m.f. measurements; (○) B622Aw, and (◑) H920W.

Dashed lines; data obtained from titrations.

Vertical line represents an approximate degree of neutralization below which the transition from random coil to β-structure occurs.

Polymer concentrations;

0.005 N (Fig.21), 0.010 N (Fig.22), 0.025 N (Fig.23), and 0.050 N (Fig.24).

A dashed line represented by (b) in Fig.23 represents the activity coefficient obtained from the integration along a line (b) in Fig.26(c), and can be regarded as representing the upper limit for the activity coefficient.

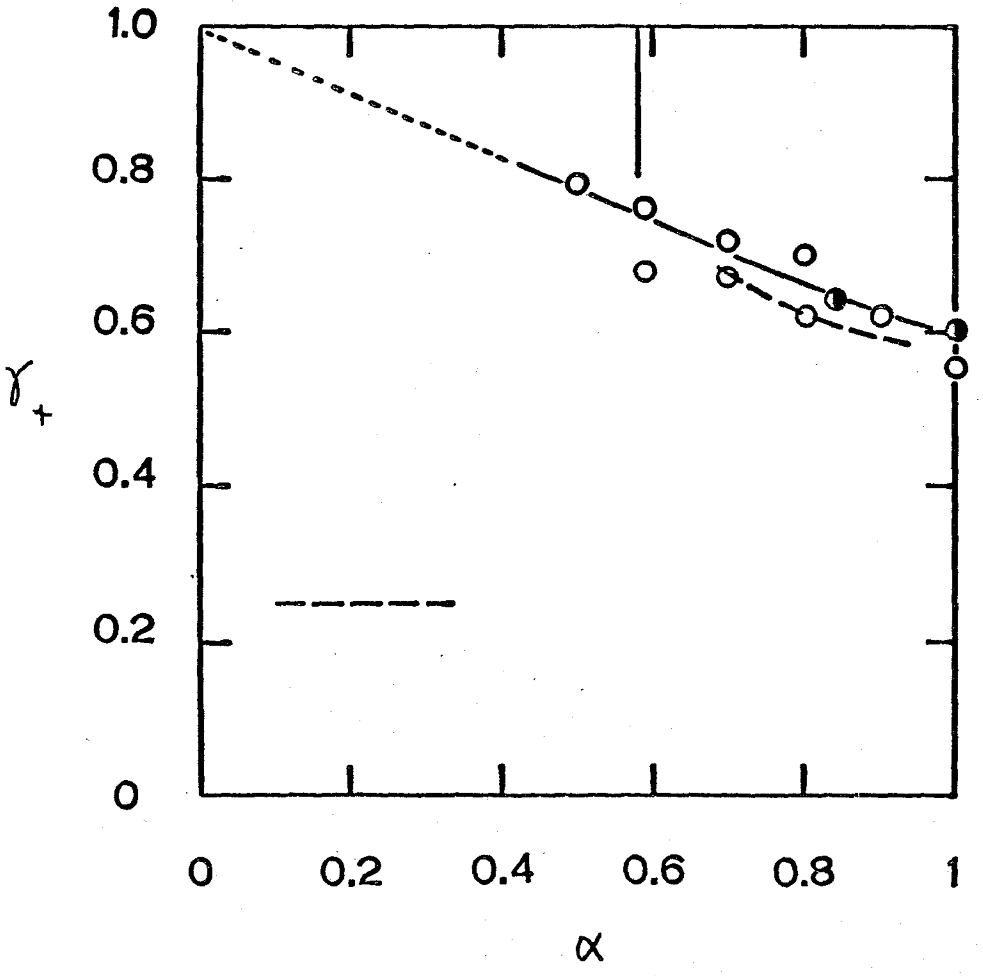


Fig. 21

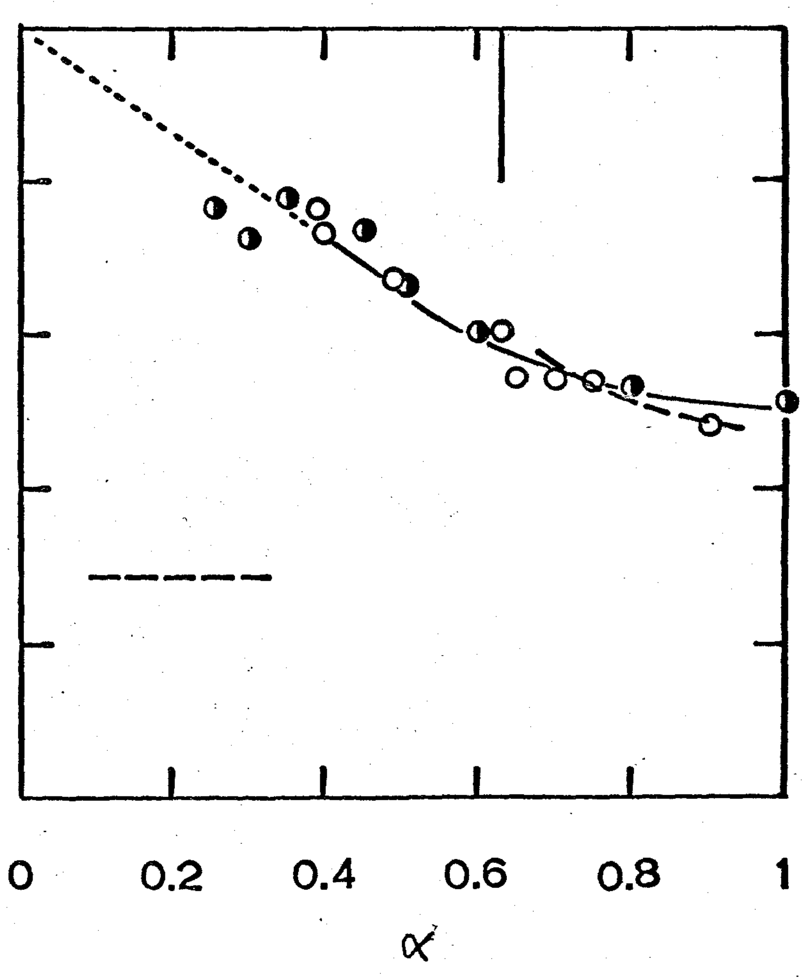


Fig. 22

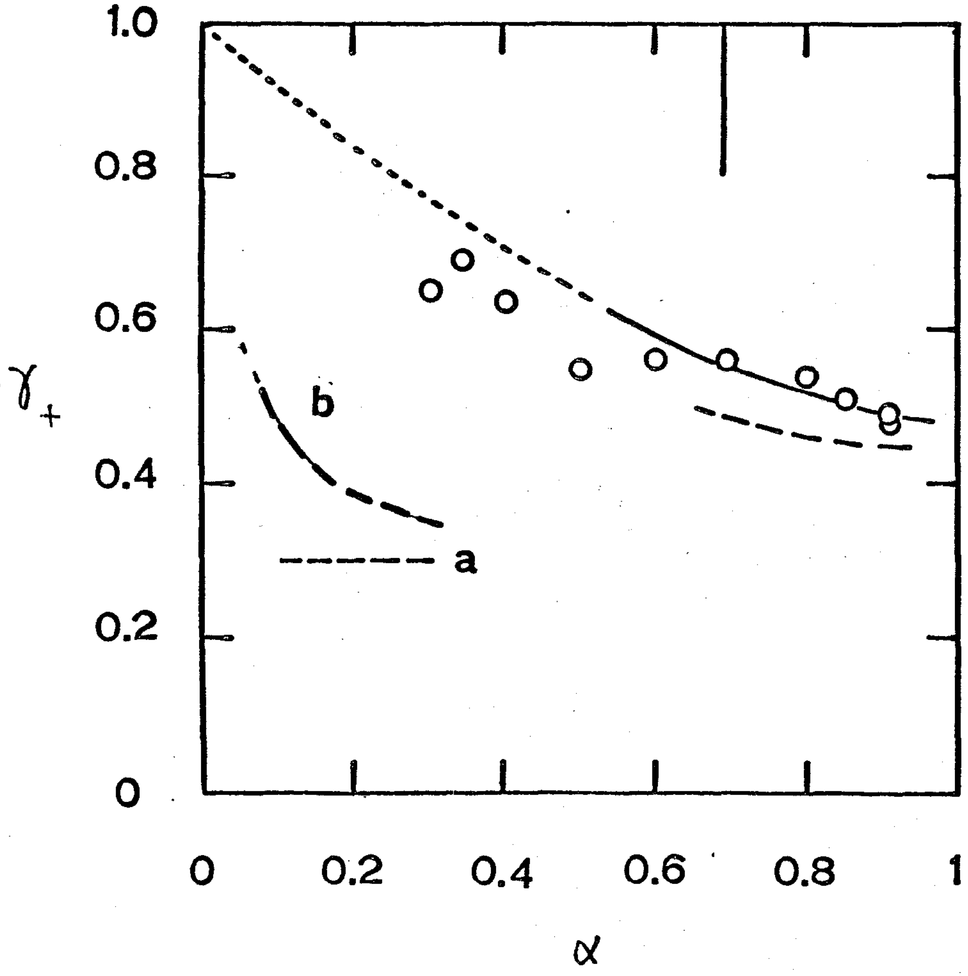


Fig. 20

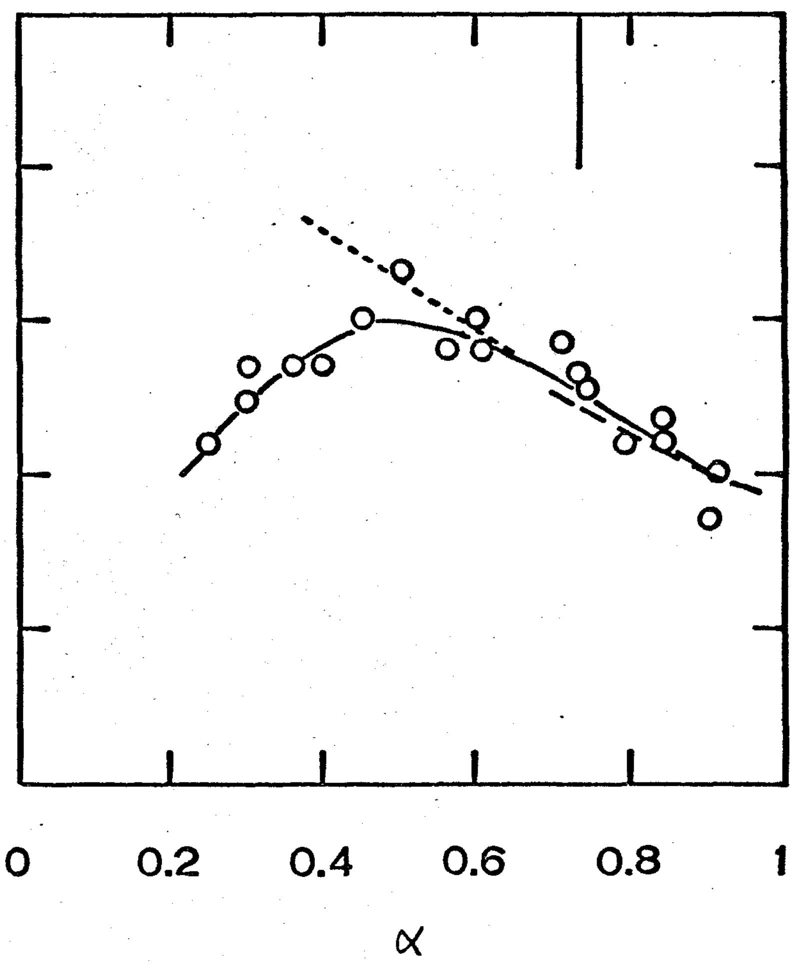


Fig. 24

A common behavior that γ_+ increases with decreasing α is well exhibited in all polymer concentrations examined. In Figure 27, the activity coefficient for fully neutralized polymer is plotted against polymer concentration. With increasing m_p, γ_+ decreases rather sharply at first, but the decrease becomes gradual if m_p is higher than 0.010 monomolal. Thus the activity coefficient decreases with increasing polymer concentration. This dependence on polymer concentration is also observed for other degrees of neutralization ao long as the polymer is in random coil conformation.

For the transition region, degree of neutralization affects the activity coefficient in two different ways. From Figure 24 it is clear that the activity coefficient decreases with the formation of β-structure. Thus on reducing degree of neutralization the transition to β-structure is promoted and consequently the activity coefficient may decrease. On the other hand, the electrostatic interaction between charged sites and counter ions becomes diminished due to the decrease of α and this effect may cause the increase of activity coefficient. The observed dependence of γ_+ on α in Figures 21-24 is regarded as a resultant of the two effects. From these figures it may be concluded that the activity coefficient decreases with decreasing α if the transition to β-structure proceeds to considerable extent and vice versa.

As compared with the two atates, random coil and the transition region, the activity coefficient for β-structure was not well elucidated in the present e.m.f. measurements. It should be noted, however, that a value of 0.44 is obtained as the activity coeffiicent for β-structure.

Activity Coefficient from Potentiometric Titration.

Potentiometric titration data are taken from the results of the preceding section(Section 3).

In Figure 25(a)-(d), values of pH are plotted against logarithm of salt concentration. Linear relations are shown to hold both for random coil and β-structure. But this linearity no longer holds for the transition region. For a state corresponding to the transition region, however, we can estimate the pH values which would be obtained if conformational transition would not to occur but polymers were in either conformation at the same conditions. These hypothetical pH values can be estimated in terms of the extrapolation in Henderson-Hasselbach plots of titration data. Straight lines in Figures 25(a)-(d) have been drawn for random coil state (upper five lines) and for β-structure (lower five lines), with the aid of these hypothetical pH values if necessary.

According to Oosawa,[82] the change of pH with salt concentration can be given by the following equation.

$$\mathrm{pH}\ (m_s,\alpha,m_p) - \mathrm{pH}\ (0,\alpha,m_p) = -\ a \log[\ 1 + (\langle\gamma_{0s}\rangle m_s/\gamma_+\alpha\ m_p)] \tag{6}$$

Here a is defined as follows.

$$a = 1 - \frac{1}{\langle\gamma_{0s}\rangle}\frac{\partial(\gamma_+\alpha)}{\partial\alpha} \tag{7}$$

In the above equations, $\langle\gamma_{0s}\rangle$ denotes the activity coefficient of sodium ions in sodium chloride solutions, averaged over the concentration range from 0 to m_s. According to eq.(6), the pH change is proportional to the logarithm of the salt concentration, if the ratio $\langle\gamma_{0s}\rangle m_s/\gamma_+\alpha\ m_p$ is large compared with unity. The values of salt concentration, m_s^*, where the straight lines in Figures 25(a)-(d) meet the observed pH values in no added

Fig. 25. Dependence of pH on salt concentration.

Random coil; upper five lines.

Degree of neutralization (from top to bottom);;

0.90, 0.85, 0.80, 0.75,and 0.70.

β-structure; lower five lines.

Degree of neutralization (from top to bottom);

0.30, 0.25, 0.20, 0.15, and 0.10.

(○); Experimental data corresponding to either random coil or β-structure.

(●); Experimental data corresponding to transition region.

(⦶); Intercepts obtained by extrapolation of straight lines. (see text)

(◑); For this point, the value of pH corresponding to pure random coil in no added salt case was evaluated in terms of the extended Henderson-Hasselbach equation.

Polymer concentrations; (a) 0.005, (b) 0.010, (c) 0.025, (d) 0.050 N.

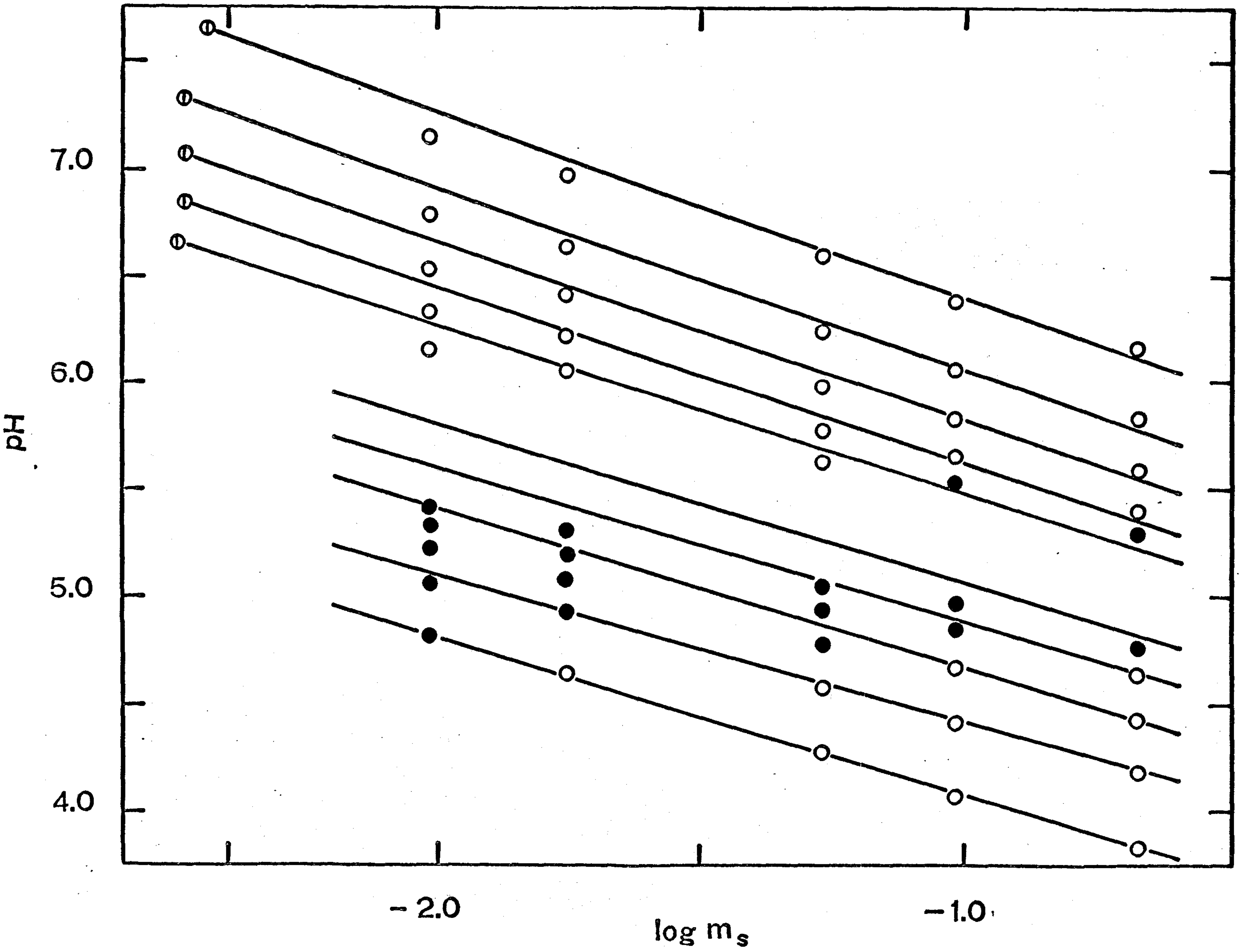


Fig. 25 (a)

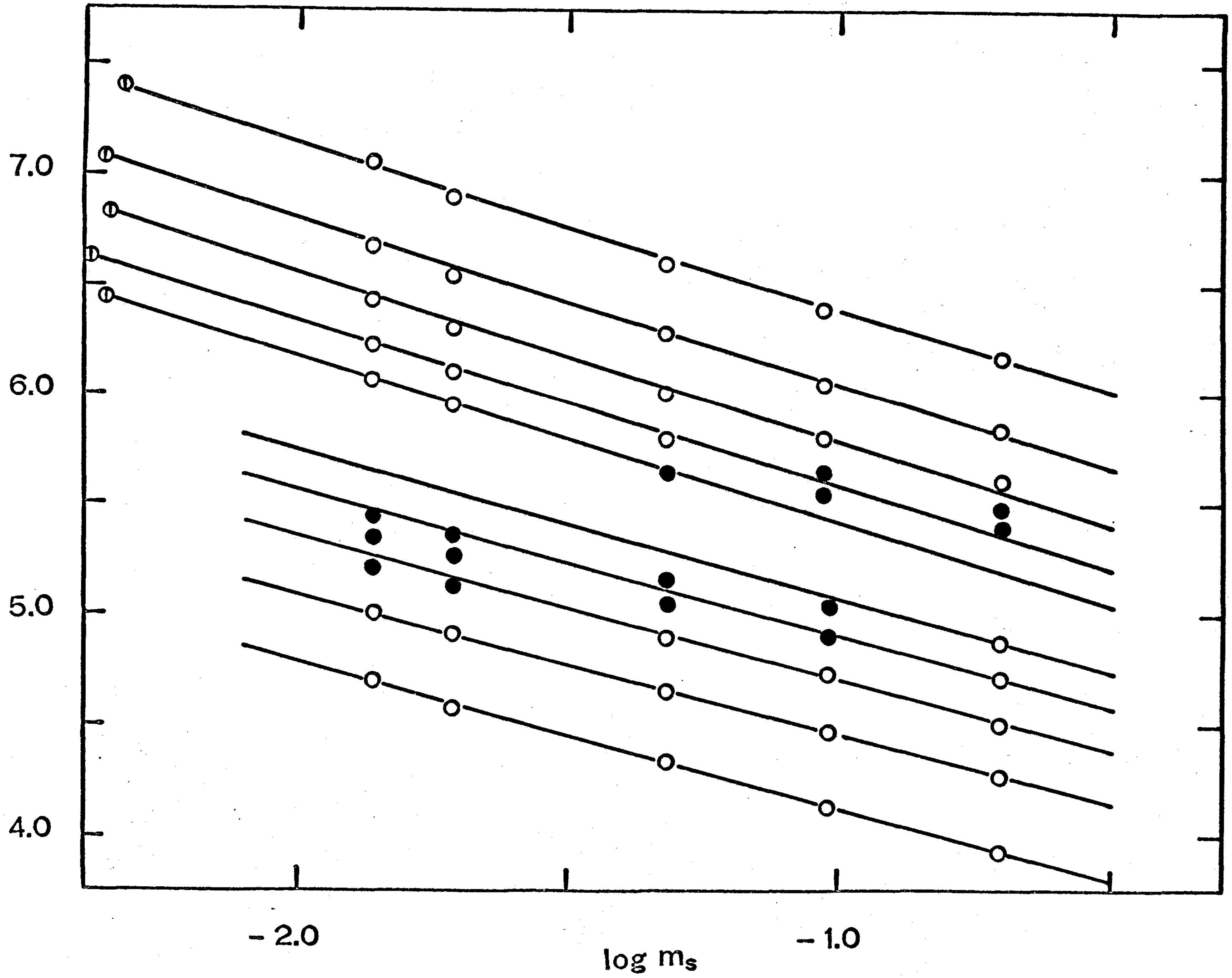
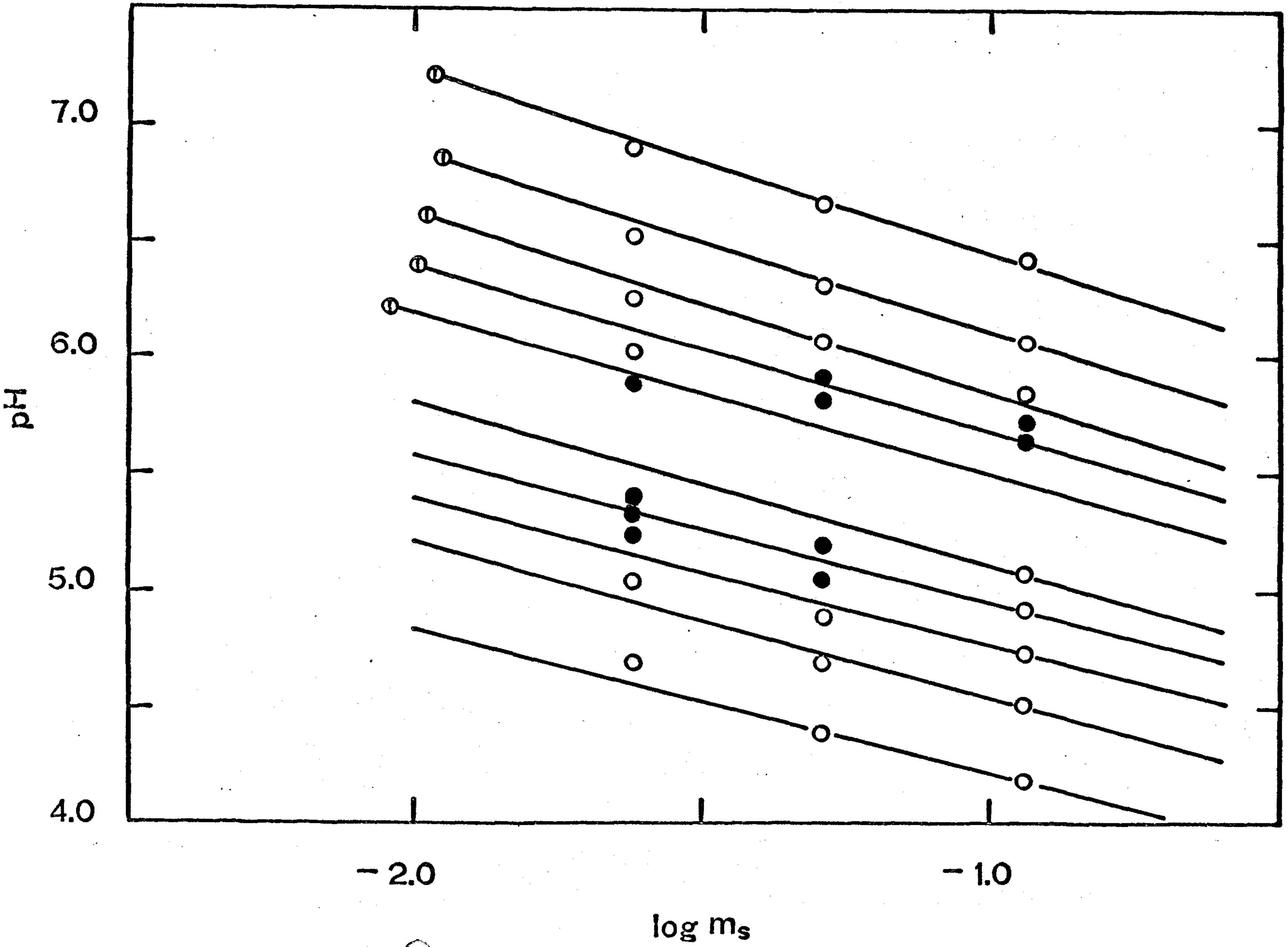


Fig. 25 (b)



○

Fig. 25 (c)

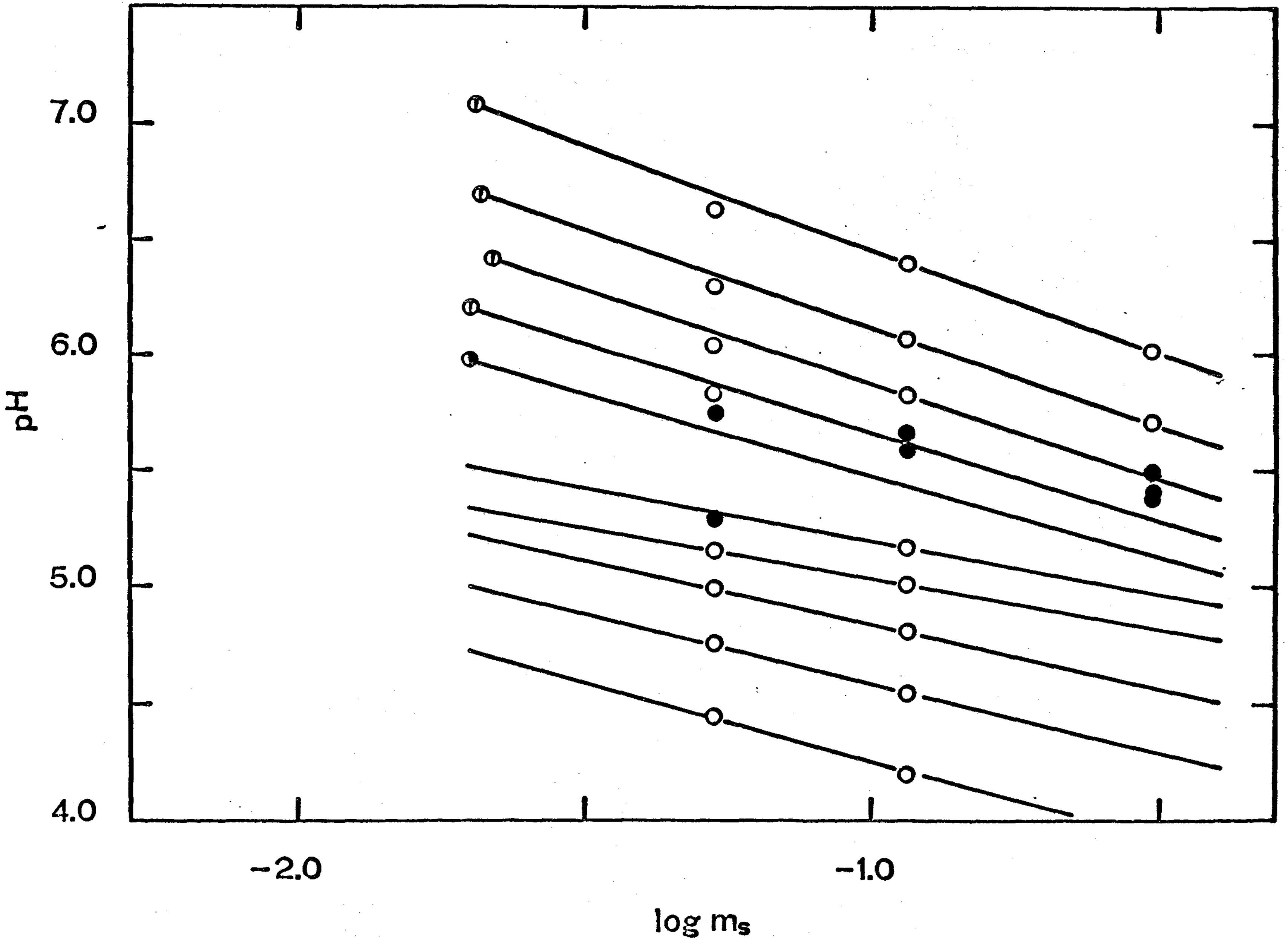


Fig. 25 (d)

salt cases are shown to satisfy the following relation.[82]

$$\langle\gamma_{0s}\rangle m_s^* = \gamma_+ \alpha\, m_p \tag{8}$$

From this equation γ_+ can be obtained and these results are also shown in Figures 21-24 with a dashed line drawn for high α region. In the present study the region of pure β-structure can not be discriminated if added aslt is absent. So the applicability of this procedure is restricted to random coil region.

However, we can derive an alternative procedure to evaluate γ_+. Integration of eq.(7) yields,

$$\gamma_+ = \frac{\langle\gamma_{0s}\rangle}{\alpha} \int_0^{\alpha} (1 - a)\, d\alpha \tag{9}$$

Thus from the slope, a, we can obtain γ_+ in terms of eq.(9). This procedure is applicable even when the data in the case of no added salt are not available. As seen from Figures 25(a)-(d), for random coil state we can get the slopes only when α is higher than 0.70. So the integration in eq.(9) could not be performed because the extrapolation of $\langle\gamma_{0s}\rangle(1 - a)$ to $\alpha=0$ is quite difficult. On the other hand, for β-structure, this procedure is a useful one since the previous procedure in terms of eq.(8) is not applicable. In Figures 26(a)-(d), $\langle\gamma_{0s}\rangle(1 - a)$ or equivalently $\partial(\gamma_+\alpha)/\partial\alpha$ is plotted against α. In the limit that α approaches zero this quantity is expected to tend to unity. However, for β-structure, $\langle\gamma_{0s}\rangle(1 - a)$ is shown to be almost constant and independent of α. Therefore in the evaluation of γ_+ for β-structure in terms of eq.(9), the integration was made along the horizontal line obtained. In this approximation γ_+ is constant and coincides with $\langle\gamma_{0s}\rangle(1 - a)$. The results are also shown in Figures 21-23 with a dashed line drawn for low α region.

Fig.26. Relation between $<\gamma_{0s}>(1 - a)$ and α.

(a) Polymer concentration 0.005 N. $<\gamma_{0s}> = 0.89$

(b) Polymer concentration 0.010 N. $<\gamma_{0s}> = 0.89$

(c) Polymer concentration 0.025 N. $<\gamma_{0s}> = 0.91$

(d) Polymer concentration 0.050 N. $<\gamma_{0s}> = 0.86$

Curves (a) and (b) in Fig.26(c) represent the alternative paths for the integration involved in eq.(9). (see text)

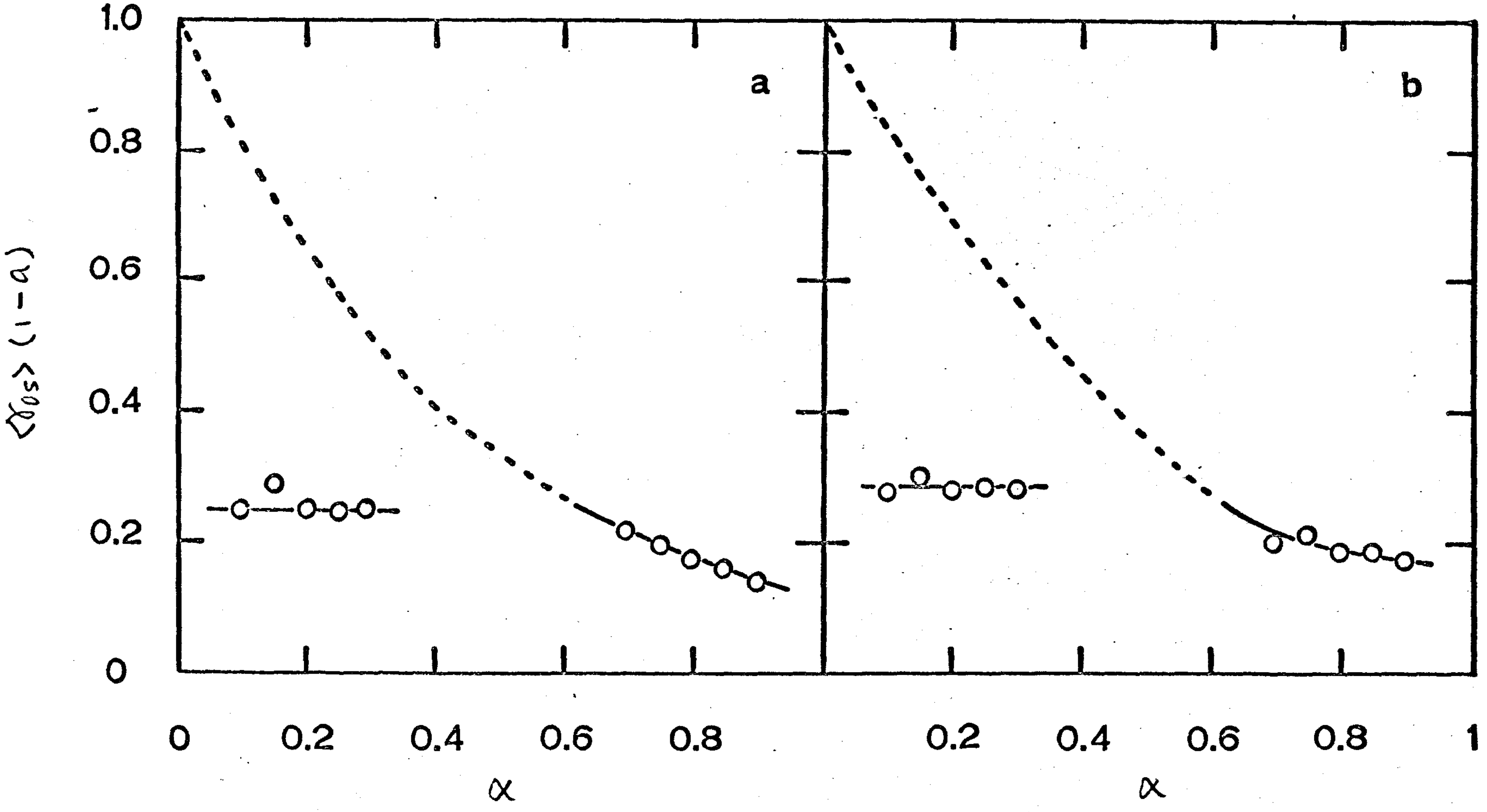

1.0
0.8
0.6
0.4
0.2
0
$\langle\gamma_{0s}\rangle(1-\alpha)$
a
b
0
0.2
0.4
0.6
0.8
1
α
α

Fig. 2b

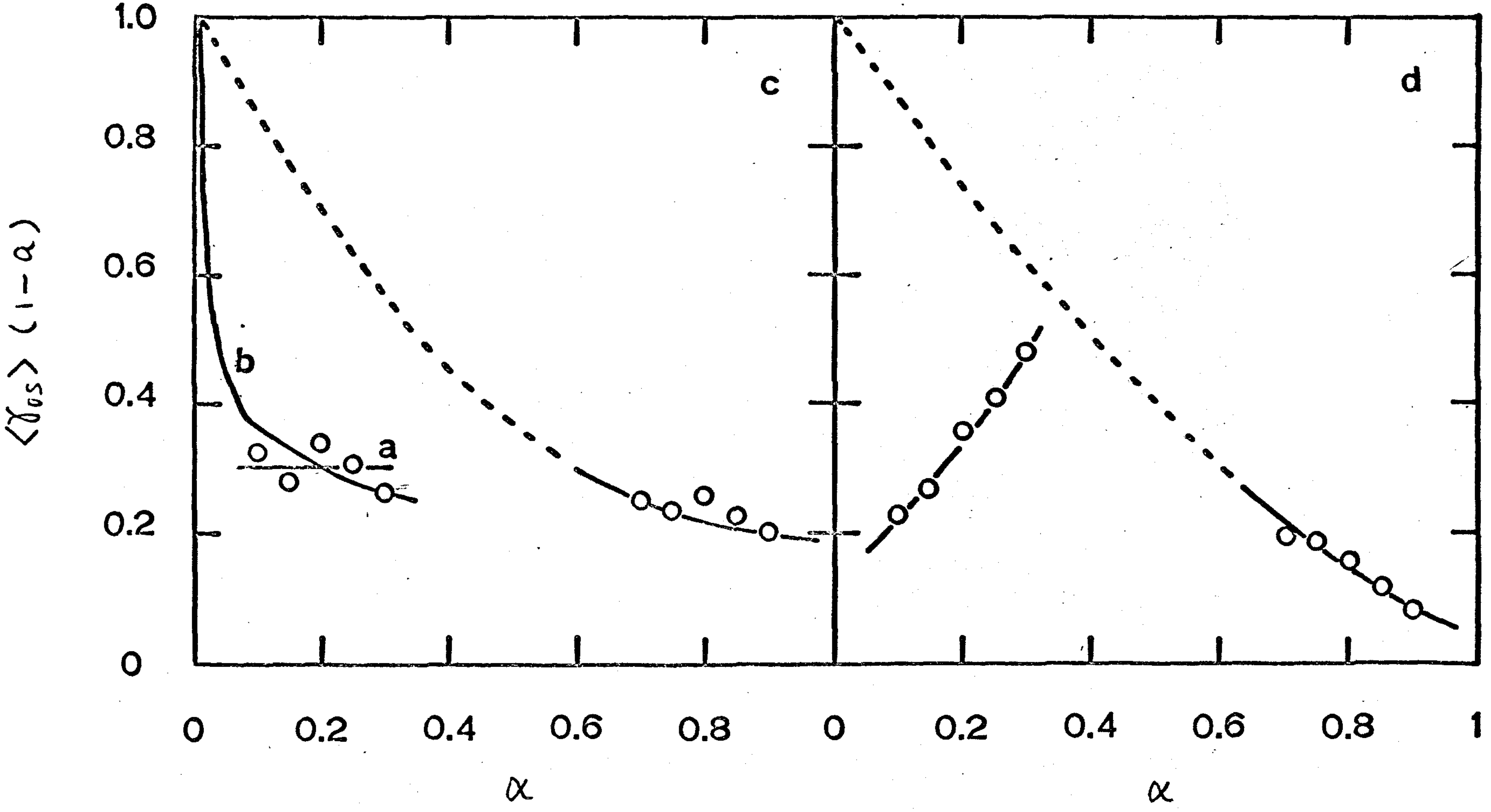

1.0
0.8
0.6
0.4
0.2
0
$\langle \gamma_{os} \rangle (1-\alpha)$
0
0.2
0.4
0.6
0.8
0
0.2
0.4
0.6
0.8
1
α
α
a
b
c
d

Fig. 2b

It should be stated that at the polymer concentration of 0.050 monomolal, the available data of titration for low α region are so few that the results are somewhat unreliable. For this reason the value of γ_+ for β-structure at this polymer concentration has not been evaluated.

From the potentiometric titrations the counter ion activities both for random coil state and β-structure are obtained. However this method informs us nothing about the behavior of the activity coefficient in the transition region.

For random coil conformation, the results agree well with those from e.m.f. measurements. This can be seen from Figures 21-24.

For β-structure the activity coefficient is extremely small if we compare with that for random coil at the same degree of neutralization. This conclusion is consistent with that derived from e.m.f. measurements. The activity coefficient is found to be almost independent of degree of neutralization. However, this behavior is observed for relatively low degree of neutralization ($\alpha<0.3$). Moreover, the dependence on degree of neutralization was not obtained at the polymer concentration of 0.050 monomolal. In Figure 27 the activity coefficient for β-structure is also plotted against polymer concentration. These data correspond to the state that α is 0.25. The result obtained from e.m.f. measurements is represented with a half-filled circle. The data corresponding to other polymer concentrations are obtained from titrations and represented with filled circles. From this figure it is seen that the activity coefficient increases when polymer concentration is raised. This conclusion should be accepted, however, with the reservations described below. First, the dependence shown in Figure 27 corresponds to a degree of neutralization of 0.25. For other degrees of neutralization, the same conclusion is obtained for the concentration range up to 0.025 monomolal. Second, the values of activity coefficient shown in Figure 27 are of approximate nature. The value at the polymer concentration of 0.050 monomolal is somewhat larger than true one, since the transition from random coil to β-structure is shown to be incomplete under the condition (m_p=0.050 and α=0.25). For other values obtained from titrations, they are smaller than true ones because of the approximation used in the integration involved in eq.(9). An estimation is made to evaluate the errors due to this approximation. The result gives that the maximum value of the activity coefficient consistent with experimental data represented in Figures 26(a)-(c) is about 0.40 for each polymer concentration. Taking into account these two reservations described above, the possibility can not be neglected that counter ion activity coefficient is almost insenitive to polymer concentration.

Finally, it should be noted that the results of the activity coefficient obtained from potentiometric titration are based on the assumption of the additivity rule, experimental verification of which has not been worked out on the present polypeptide-added salt system.

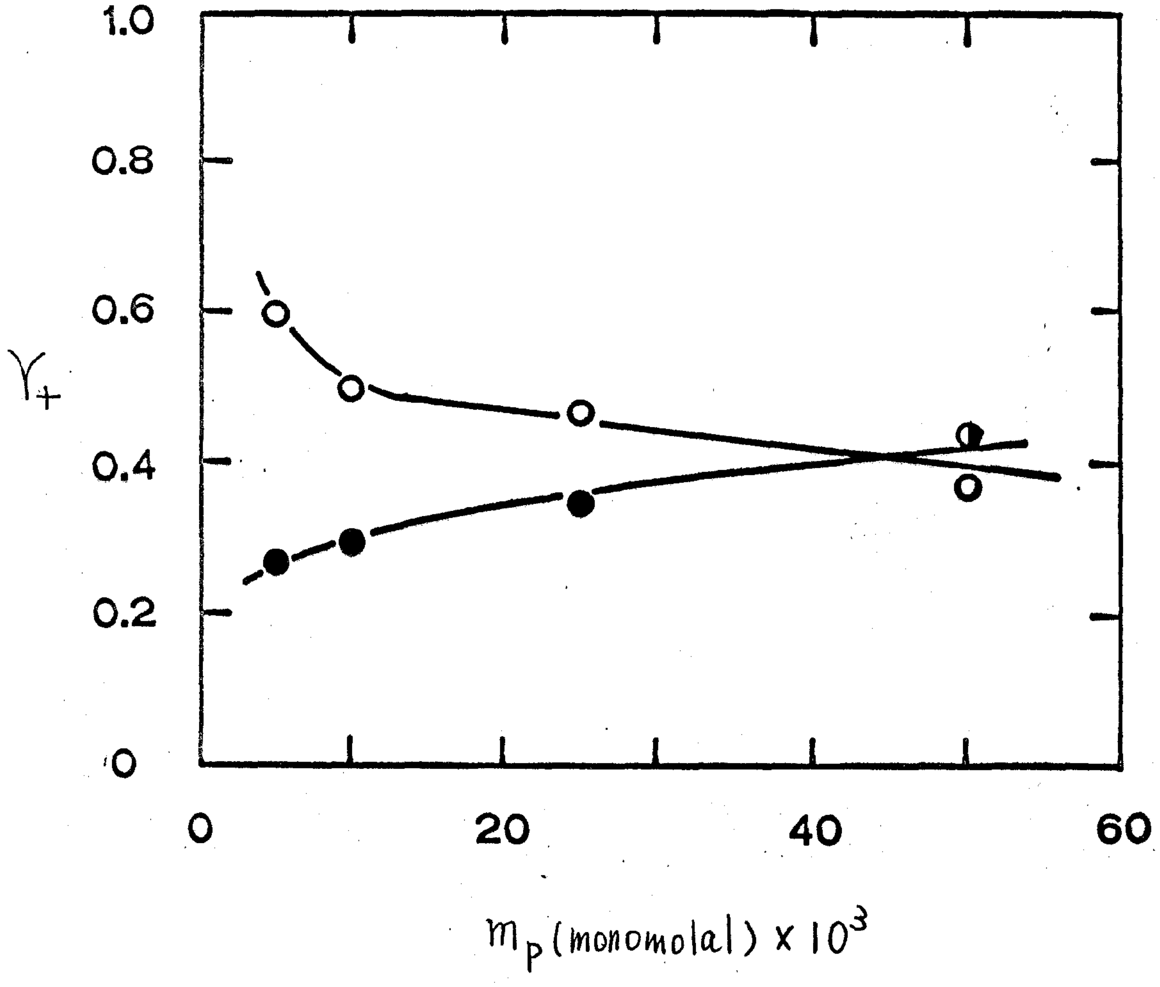


Fig. 27 Concentration dependence of activity coefficients.

(○); Sodium salt (α =1), random coil.

(◑); β-structure (α =0.25), obtained from e.m.f. measurements.

(●); β-structure (α =0.25), obtained from titrations.

DISCUSSION

The Additivity Rule.

As shown in Figures 21-24, for random coil conformation the values of activity coefficient obtained from potentiometric titrations are in good agreement with those from direct measurements. This coincidence confirms that counter ion activity has been correctly determined in present study and further suggests that the additivity rule holds for the present system if polymers are in random coil conformation.

In the transition region, pH does not change in proportion to logarithm of salt concentration. This behavior implies that the additivity rule is not satisfied for the system in this region.

For β-structure, no confirmation that the additivity rule is satisfied is present in contrast to the case of random coil conformation. However, as illustrated in Figure 27, the values of activity coefficient obtained from titration (filled circles) are consistent with the value from e.m.f. measurements (half-filled circle). Moreover the linearity between pH and logarithm of salt concentration is satisfied for this particular conformation. Taking into account these results, we can assume that the rule holds for β-structure.

Activity Coefficient for Random Coil.

As compared with the data on other random polypeptides previously reported, the observed dependence on polymer concentration is similar to that of poly-L-cysteate.[77,78] On the other hand, the magnitude of activity coefficient obtained in the present study is smaller by about 0.1 than those of other examples, if compared in the concentration range higher than 0.010 monomolal.[77,78,80,81]

If activity coefficient of counter ions in randomly coiled polypeptide can be described by simple interionic electrostatic interactions, then, present polypeptide should have a larger activity coefficient. This arises because the separation of charged site from main chain is larger in the present polypeptide than in others previously examined,[77-81] and all polypeptides have the same separation between adjacent charges along main chain in their fully extended configurations. Therefore the small value of activity coefficient obtained should be considered as the results of effects other than simple interionic electdostatic interactions, so long as a rod model is valid for this interaction.

The present polypeptide has side chains which are of more nonpolar nature than those of other polypeptides previously examined.[77,78,80,81] When this polypeptide is brought into aqueous media, the structure of water becomes more ordered due to the presence of nonpolar side chains, and the hydrophobic interaction between side chains becomes significant. After the reasoning developed by Frank[99], counter ions should be strongly salted-out by the present polymer than by other polypeptides. This effect is, however, expected to result in a raising of activity coefficient of counter ions. Therefore the effect that the present polypeptide serves as a strong structure-maker can not explain the observed lowering of counter ion activity coefficient.

However, an interpretation becomes possible, if we take into consideration the hydrophobic interaction between side chains. This interaction is expected to produce some local regions on a polypeptide chain, where several side chains are brought into close contact with each other. This local micelle may be further stabilized by hydrogen bonds between peptide groups involved in it. Since every side chain is negatively charged, the local micelle can not be stable unless counter ions are bound to it, to some extent, to reduce the electrostatic repulsion between charged sites. This binding causes a decrease of activity coefficient of counter ions. Thus we can interprete the small activity coefficient obtained in the present study in terms of the idea of the local micelle. Moreover, the observed dependence of activity coefficient on polymer concentration can be also explained by this idea.

However, the idea of local micelle is no longer than an explanation. Moreover, it is not clear, at present, whether the idea of local micelle is consistent with the additivity rule which is suggested to hold approximately for random coil conformation in the present study.

Activity Coefficient for β-Structure.

The characteristic features of counter ion activity coefficient for β-structure obtained in the present study may be the consequences of the two properties inherent to this conformation. These are large surface charge density and the counter ion binding between the pleated sheets.

A rough calculation shows that the surface charge density of the pleated sheet is about five to six times larger than that of random coil at the same degree of ionization, if the latter is estimated in terms of a rod model. This large surface charge density of the pleated sheet is undoubtedly a cause for the small activity coefficient obtained.

On the other hand, it should be noted that many side chains are incorporated in the inner region of β-structure. Since a state that charged site is located within nonpolar environment is unfavorable, every charged site of the pleated sheet except for that located at the surface of a three dimensional aggregate may probably bind a counter ion to neutralize its charge. It is certain, therefore, that the average numbers of association in both two dimensional (number of polymer chains in a pleated sheet) and three dimensional (number of the pleated sheets in an aggregate) ways and their dependences on both degree of neutralization and polymer concentration are essential factors in determining the electrostatic properties of β-structure. However, no investigations have been worked out in these respects, so further discussion becomes difficult.

Relations between two expressions for potentiometric titration.

Equation (6), togather with Equations (7) and (8), can be regarded as an equation describing potentiometric titration. The extended Henderson-Hasselbach equation(Equation(4)), on the other hand, also serves for this purpose. It is pertinent to derive relations connecting these two equations.

Recalling that the parameter A represents the pH when $\alpha=1/2$, then from eq.(6) the following results.

$$A(m_s) - A(0) = -a(\alpha=1/2)\log[1 + b(\alpha=1/2)m_s] \tag{12}$$

Thus A is postulated to vary linearly with log m_s, as has been illustrated in Fig.16(a). The slope is now shown to represent, $-a(\alpha=1/2)$.

From eqs.(4) and (6) it is easily shown that,

$$B\log\frac{\alpha}{1-\alpha} = pH(m_s,\alpha) - A(m_s) = -a\log[1 + bm_s] - A(m_s) + pH(0,\alpha). \tag{13}$$

Substituting eq.(12) into $A(m_s)$ yields,

$$B\log\frac{\alpha}{1-\alpha} = -a\log[1 + bm_s] + a(\alpha=1/2)\log[1 + b(\alpha=1/2)m_s] + pH(0,\alpha) - A(0) \tag{14}$$

Since $B(0)\log\frac{\alpha}{1-\alpha} = pH(0,\alpha) - A(0)$, (15)

$$[B(m_s) - B(0)]\log\frac{\alpha}{1-\alpha} = \log\frac{[1 + b(\alpha=1/2)m_s]^{a(\alpha=1/2)}}{[1 + b\ m_s]^{a}} \tag{16}$$

Equations (12) and (16) are the desired relations.

5. Effect of precipitation on the behavior of potentiometric titration.

In the potentiometric titration of polyelectrolytes it is frequently observed that precipitates separate out when charges on a polyion become sufficiently small. It has been observed experimentally that considerable changes occur in the titration curve if precipitation occurs. On the other hand, in the potentiometric titration of the present polypeptide, no appreciable change is observed under the state of phase separation. This particular behavior is pointed out in section 3. This apparent inconsistency arises from the fact that the effect of precipitation on the titration behavior has not been examined systematically. It is desirable, therefore, to examine the effect in a systematic way and to make clear the necessary conditions for the absence or the presence of the changes due to the precipitation.

Derivation of the equation for potentiometric titration is made in this section after the reasoning of Harris and Rice.[72]

In this section it is also intended to include the case where two different conformations of the polyelectrolyte are present in the solution phase.

The effect of polydispersity is not considered in the present treatment.

Theory

Let us consider a polyelectrolyte solution containing four components: solvent, e.g.,H_2O; polyacid; alkali,e.g., NaOH ; and simple salt,e.g., NaCl. Let us denote the numbers of moles of these components in the solution as n_w,n_p, n_a, and n_s respectively. Here it should be noted that instead of taking the partially neutralized polyions as an independent component we define the polyacid and alkali as two independent components. Moreover, counter ions from alkali and those from simple salt are assumed to be of the same species.

Since we consider the changes at constant temperature and pressure, these two variables are not involved explicitly in the present treatment.

Gibbs free energy and equilibrium condition.

Let us denote the number of ionized sites per polyion as i and the chemical potential of hydrogen ion as μ_H. At equilibrium <i> or u_H must be determined by the set of three variables$(n_p/n_w,n_a/n_w,n_s/n_w)$. The equilibrium condition is

$$(\partial G/\partial i)_{n_p,n_a,n_s,n_w} = 0 \qquad (17)$$

or

$$(\partial G/\partial \mu_H)_{n_p,n_a,n_s,n_w} = 0 \qquad (18)$$

Here G denotes the Gibbs free energy of the solution.

We divide the Gibbs free energy into two parts;

$$G = G^{non} + G^{el} \qquad (19)$$

G^{el} represents the free energy arising from the electrostatic interactions between charged particles. However the self-energy of the polyion, the electrostatic interaction between charged sites in a polyion, is also included in G^{el}.

The chemical potential of any component or species can be written as the sum of the two terms corresponding to equation(19); $\mu = \mu^{non} + \mu^{el}$.

Chemical potential of polymer component.

The chemical potential of polymer component, μ_p, can be written in terms of the constituting ionic species,

$$\mu_p = \mu_{p-<i>} + <i>\mu_H \tag{20}$$

The two terms of the chemical potential of polyion can be written explicitly as follows.

$$\mu^{non}_{p-<i>} = \mu^{o}_{p-<i>} + RT \ln n_p \tag{21}$$

$$\mu^{o}_{p-<i>} = \mu^{o}_{p} - <i>\mu^{o}_{H} - <i>RT \ln K_o + (RT\ )[<i>\ln(<i>/P) + (P - <i>) \ln(P-<i>)/P] + \mu^{con}_{p-<i>} \tag{22}$$

Here P denotes the number of dissociable sites per polymer molecule and K_o denotes the intrinsic dissociation constant for the site. The standard chemical potential of the fully protonated species, μ^{o}_{p}, is independent of <i> but $\mu^{o}_{p-<i>}$ is not so.

For ordinary polyelectrolytes the term $\mu^{con}_{p-<i>}$ is essentially zero. However, if two or more conformations are present in the solution, then the term $\mu^{con}_{p-<i>}$ must be added.

At this stage we briefly consider the case that two conformations a and b are present in the solution. Let f_a be the fraction of the a-conformation, that is, $f_a n_p$ is the number of the polymer in the a-conformation. The average quantities in the solution can be written as follows.

$$\mu^{non}_{p-<i>} = f_a \mu^{non}_{p-<i>_a} + f_b \mu^{non}_{p-<i>_b} \tag{23}$$

and

$$\langle i \rangle \; = \; f_a \langle i \rangle_a \; + \; f_b \langle i \rangle_b \tag{24}$$

where $\langle i \rangle_a$ and $\langle i \rangle_b$ represent the number of ionized sites per polymer molecule in each conformation.

The non-electrostatic part of the chemical potential of polyion in each conformation is given by eqs.(21) and (22), if we replace n_p by $f_a n_p$ or $f_b n_p$ and $\langle i \rangle$ by $\langle i \rangle_a$ or $\langle i \rangle_b$ and omit the term μ_p^{con}. Substituting these expressions into eq.(23) we obtain;

$$\begin{aligned} \mu^{non}_{p-\langle i \rangle} = \; & RT(f_a \ln f_a + f_b \ln f_b) + RT \ln n_p + f_a \mu_p^\circ(a) + f_b \mu_p^\circ(b) \\ & - \langle i \rangle \mu_H^\circ - RT[f_a \langle i \rangle_a \ln K_{o_a} + f_b \langle i \rangle_b \ln K_{o_b}] \\ & + RT\,[\, f_a \langle i \rangle_a \ln(\langle i \rangle_a/P) + f_a(P-\langle i \rangle_a)\ln(P-\langle i \rangle_a)/P \\ & + f_b \langle i \rangle_b \ln(\langle i \rangle_b/P) + f_b(P-\langle i \rangle_b)\ln\,(P-\langle i \rangle_b)/P] \end{aligned} \tag{25}$$

The term, $f_a \mu_p^\circ(a) + f_b \mu_p^\circ(b)$, represents the standard chemical potential of the fully protonated species when two conformations coexist in the same ratio as in the actual solution. Since $\mu_p^\circ(a)$ differs from $\mu_p^\circ(b)$, the term, $f_a \mu_p^\circ(a) + f_b \mu_p^\circ(b)$, is a function of $\langle i \rangle$ and thus inadequate for the standard chemical potential of the fully protonated species. Instead we define μ_p° as $f_a^\circ \mu_p^\circ(a) + f_b^\circ \mu_p^\circ(b)$, where f_a° and f_b° denote the fraction of each conformation when $\langle i \rangle = 0$. Then we get $\mu^{con}_{p-\langle i \rangle}$ from equations (22) and (25).

$$\begin{aligned} \mu^{con}_{p-\langle i \rangle} = \; & (f_a - f_a^\circ)\mu_p^\circ(a) + (f_b - f_b^\circ)\mu_p^\circ(b) + RT(f_a \ln f_a + f_b \ln f_b) \\ & RT[\, f_a \langle i \rangle_a \ln(\langle i \rangle_a/\langle i \rangle) + f_b \langle i \rangle_b \ln(\langle i \rangle_b/\langle i \rangle) \\ & f_a(P-\langle i \rangle_a)\ln(P-\langle i \rangle_a)/(P-\langle i \rangle) + f_b(P-\langle i \rangle_b)\ln(P-\langle i \rangle_b)/(P-\langle i \rangle)] \end{aligned} \tag{26}$$

The term, $RT\langle i \rangle \ln K_o$, in eq.(22) should be regarded as $RT(f_a \langle i \rangle_a \ln K_{o_a} + f_b \langle i \rangle_b \ln K_{o_b})$ when conformational equilibrium is present.

Equation for potentiometric titration.

It is easily shown that the equation (17) reduces to the following relation.

$$(\partial\mu_{p-<i>}/\partial<i>)_{n_p,n_s,n_w} + \mu_H = 0 \qquad (27)$$

Replacing the variable <i> by μ_H we obtain,

$$(\partial\mu_{p-<i>}/ \partial\mu_H)_{n_p,n_s,n_w} + \mu_H(\partial<i>/\partial\mu_H)_{n_p,n_s,n_w} = 0 \qquad (28)$$

Combining the equations (20) and (28) yields,

$$(\partial\mu_p/\partial\mu_H)_{n_p,n_s,n_w} = <i> \qquad (29)$$

Equation (27) or (29) is an equation for potentiometric titration. Substituting the explicit expression for $\mu^o_{p-<i>}$ given in eq.(22) into eq.(27), the familiar expression results.

$$pH = pK_o + \log (\alpha/1-\alpha) + (0.434/RT)[(\partial\mu^{el}_{p-<i>}/\partial<i>) + (\partial\mu^{con}_{p-<i>}/\partial<i>)] \qquad (30)$$

Here α is the degree of ionization and is given by <i>/P.
When this equation is compared with the equation (3) introduced in section 3, thermodynamic meaning of the quantity G^{el} is clearly understood. If conformational equilibrium is present in the solution G^{el} must contain the non-electrostatic contributions as clearly exhibited in eq.(30).

It should be noted that conformational equilibrium demands the equality of the chemical potentials of the polymer component in two conformations. This condition is written as follows.

$$\mu_p(a) - \mu_p(b) = \mu_{p-<i>_a} - \mu_{p-<i>_b} + (<i>_a - <i>_b) \mu_H = 0 \qquad (31)$$

Thus it is clear that the chemical potentials of polymer ion in different conformations are not equal unless their degrees of ionization are the same.

Phase separation.

Next we consider the system under the phase equilibrium between solution and precipitates. Let us denote the quantities concerning the precipitates by superscript 'ppt'. By the term "precipitates" we imply that solvent component can be negligible in the precipitate phase. Moreover we assume that the precipitates contain no salt component. The precipitates treated here are thus two component system containing polymer and alkali components. In this case chemical potentials of the precipitates are determined by single variable, such as $<i>^{ppt}$. For the solution phase, the state is uniquely determined by n_p, n_a, n_s, and n_w. Since total number of the polymer and the alkali components, n_p^t and n_a^t, as well as n_s and n_w are given quantities, we can specify the state of the whole system if two additional parameters, $<i>^{ppt}$ and n_p, are known. These two quanities are determined from the condition for phase equilibrium.

These relations are;

$$\mu_p^{ppt}(<i>^{ppt}) = \mu_p(n_p, n_s, n_w, <i>) \tag{32}$$

$$\mu_a^{ppt}(<i>^{ppt}) = \mu_a(n_p, n_s, n_w, <i>) \tag{33}$$

Differentiation of eq.(32) with respect to μ_H gives,

$$(\partial\mu_p^{ppt}/\partial\mu_H)_{eq} = <i> + (\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H}(\partial n_p/\partial\mu_H)_{n_s,n_w,eq} \tag{34}$$

where the suffix 'eq' represents the differentiation under the phase equilibrium.Another condition, eq.(33), can be converted into the following relations if we utilize the reasonable approximations that $(\partial\mu_a/\partial\mu_H)_{n_s} = (\partial\mu_a^{ppt}/\partial\mu_H^{ppt}) = -1.$

$$\mu_H = \mu_H^{ppt} \quad \text{and} \quad \mu_+ = \mu_+^{ppt} \tag{35}$$

For the precipitate phase an expression analogous to equation (27) holds,

$$(d\mu_{p-<i>^{ppt}}/d<i>^{ppt}) + \mu_H^{ppt} = 0 \tag{36}$$

From equations (34),(35), and (36) we obtain,

$$<i>^{ppt} = <i> + (\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H} (\partial n_p/\partial\mu_H)_{n_s,n_w,eq} \tag{37}$$

This is a fundamental relation for the system under phase separation. This eqation gives a relation between the degrees of ionization of precipitates and solution.

Since we are interested in the state of phase separation, the term $(\partial n_p/\partial\mu_H)_{n_s,n_w,eq}$ should be different from zero. Therefore $<i>^{ppt}$ should differ from $<i>$ unless the term $(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H}$ is zero. For the particular case that $(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H} = 0$ is satisfied, the degrees of ionization of the precipitates and solution become identical to the average degree of ionization of the whole system, α^s.

$$\alpha^{ppt} = <i>^{ppt}/P = <i>/P = \alpha = \alpha^s$$

Here α^s is defined as follows.

$$n_p^t \alpha^s = (n_p^t - n_p)\, \alpha^{ppt} + n_p\, \alpha \tag{38}$$

Using equation (38) we rewrite equation (37) in the following form.

$$- n_p^t (<i>^s - <i>^{ppt}) = (\partial\mu_p/\partial \ln n_p)_{n_s,n_w,\mu_H} (\partial n_p/\partial\mu_H)_{n_s,n_w,eq} \tag{39}$$

The left-hand side of this equation is a function of μ_H only. If the term $(\partial\mu_p/\partial \ln n_p)_{n_s,n_w,\mu_H}$ can be written in the form $N(n_p)M(\mu_H)$, then this differential equation can be solved and n_p is obtained as a function of μ_H. For example, when the term $(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H}$ can be written as RT/n_p, equation (39) reduces to the following equation, which is identical to that derived by Shatkay and Michaeli.[74]

$$n_p^t (<i>^s - <i>^{ppt}) = (\partial n_p/\partial pH)_{n_s,n_w,eq}\ 0.434 \tag{40}$$

Effect of precipitation on the titration behavior.

In the case of potentiometric titration with separation of phases, we measure the pH of the solution phase as a function of α^s rather than of α. The pH of the solution phase is determined by eq.(27), so eq.(30) still holds for the solution phase. We consider the change of the titration curve expressed in the type of the plot; $pK = pH - \log(\alpha^s/1-\alpha^s)$ vs. α^s. We consider the hypothetical state of the system which might be obtained if phase separation were not present. The degree of ionization of this hypothetical solution would be α^s, and pH would be given as $pH(n_p^t,\alpha^s)$. For the actual solution, which is under the phase equilibrium with precipitates, the degree of ionization, α, is in general, different from α^s and the number of the polymer in the solution, n_p, is always different from n_p^t.

The difference of pK between two states at a given α^s, which is designated as J, may afford a quantitative measure for the effect of the precipitation on titration behavior.

$$J = \mu_H(n_p,n_s,n_w,\alpha) - \mu_H(n_p^t,n_s,n_w,\alpha^s) \tag{41}$$

If J is zero the effect of precipitation will not be observed and vice versa. It is necessary, therefore, to examine the various cases in a systematic manner and to find under what conditions J becomes zero or not.

(A) $(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H} \neq 0$

This condition is equivalent to $\alpha \neq \alpha^s$. Of course this conclusion is not true if eq.(35) becomes inadequate.

(1) $(\partial\mu_H/\partial n_p)_\alpha = 0$ or $(\partial\alpha/\partial n_p)_{\mu_H} = 0$

In this case μ_H becomes independent of n_p and $J = \mu_H(\alpha) - \mu_H(\alpha^s)$. Since μ_H is a single-valued function of α, J is always different from zero.

(2) $(\partial\mu_H/\partial n_p)_{\alpha,n_s,n_w} \neq 0$ or $(\partial\alpha/\partial n_p)_{\mu_H,n_s,n_w} \neq 0$, and $\mu_H(n_p,\alpha) \neq \mu_H(n_p^t,\alpha^s)$. In this case, J is obviously not zero.

(3) $(\partial\mu_H/\partial n_p)_{\alpha,n_s,n_w} \neq 0$ or $(\partial\alpha/\partial n_p)_{\mu_H,n_s,n_w} \neq 0$, and $\mu_H(n_p,\alpha) = \mu_H(n_p^t,\alpha^s)$. In this case, J is obviously zero.

It is necessary, however, to examine the requirement imposed on the thermodynamic properties of the system in order tosatisfy the relation $\mu_H(n_p,\alpha) = \mu_H(n_p^t,\alpha^s)$. This equation can be transformed into the following equation.

$$\int_{n_p^t}^{n_p} (\partial\langle i\rangle/\partial n_p)_{\mu_H}\, dn_p = \langle i\rangle - \langle i\rangle^s \qquad (42)$$

Here the integration is made at constant μ_H. Substituting eq.(29) into the left-hand side of eq.(42) we get

$$\int_{n_p^t}^{n_p} (\partial^2\mu_p/\partial\mu_H\partial n_p)\, d\,n_p = \langle i\rangle - \langle i\rangle^s \qquad (43)$$

On integration of the left-hand side and rewriting the right-hand side in terms of eq.(38) we obtain,

$$(\partial\mu_p/\partial\mu_H)_{n_p}\Big|_{\mu_H,n_p} - (\partial\mu_p/\partial\mu_H)_{n_p}\Big|_{\mu_H,n_p^t} = \frac{n_p^t - n_p}{n_p}\left(\langle i\rangle^s - \langle i\rangle^{ppt}\right) \qquad (44)$$

Thus we have arrived at the conclusion that effect of precipitation disappears when the chemical potential of the polymer component in the solution phase satisfies the eq.(44).

(B) $(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H} = 0$

This condition is equivalent to $\alpha = \alpha^s$.

(1) $(\partial\mu_H/\partial n_p)_{\alpha,n_s,n_w} = 0$ or $(\partial\alpha/\partial n_p)_{\mu_H,n_s,n_w} = 0$

In this case u_H becomes independent of n_p, and J is zero.

(2) $(\partial\mu_H/\partial n_p)_{\alpha,n_s,n_w} \neq 0$ or $(\partial\alpha/\partial n_p)_{\mu_H,n_s,n_w} \neq 0$

In this case $\mu_H(n_p,\alpha)$ should differ from $\mu_H(n_p^t,\alpha^s)$, so J is not zero.

It is pertinent to point out the necessary consequence of the condition $(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H} = 0$. This condition implies that polymers in the solution phase form a complex which is composed of sufficient number of polymers and thus its chemical potential is independent of polymer concentration.

Summary of the present analysis is given in Table VII.

Application of the present results.

(1) For ordinary polyelectrolytes, it has been established that titration behaviors are independent of polymer concentration if salt concentration is not smaller than that of counter ions originating from alkali. Therefore the condition $(\partial\mu_H/\partial n_p)_{\alpha,n_s,n_w} = 0$ is always satisfied. Since the condition $(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H} \neq 0$ is an ordinary one, we see at once that most polyelectrolytes fall into the class A-1. Therefore we arrived at the position to explain why the effect of precipitation is so commonly observed.

It should be noted here that the system involving the helix-coil transition also falls into the class A-1. For, it has been shown that helix-coil transition is independent of polymer concentration, which is a natural consequence of the fact that intramolecular interaction plays an essential role in the transition.

Table VII

Effect of precipitation on potentiometric titration.

	$(\partial\mu_H/\partial n_p)_{\alpha,n_s,n_w} = 0$ or $(\partial\alpha/\partial n_p)_{\mu_H,n_s,n_w} = 0$	$(\partial\mu_H/\partial n_p)_{\alpha,n_s,n_w} \neq 0$ or $(\partial\alpha/\partial n_p)_{\mu_H,n_s,n_w} \neq 0$	
		Eq.(44) is not satisfied.	Eq.(44) is satisfied.
$(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H} \neq 0$ $[\alpha \neq \alpha^s]$	$J \neq 0$ (A-1)	$J \neq 0$ (A-2)	$J = 0$ (A-3)
$(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H} = 0$ $[\alpha = \alpha^s = \alpha^{ppt}]$	$J = 0$ (B-1)	$J \neq 0$ (B-2)	

(2) If observed titration behavior in the present polymer can be regarded as that corresponding to the case J=0, then present system falls into the class of either A-3 or B-1. Since β-coil transition of the present polymer depends on polymer concentration, the term $(\partial\mu_p/\partial n_p)_{n_s,n_w,\mu_H}$ may be considered to differ from zero. However, a possibility also present that the term becomes essentially zero for the polymer concentration range higher than a certain value.
On the other hand, the term $(\partial\mu_H/\partial n_p)_{\alpha,n_s,n_w}$ is different from zero for the solution in which conformational equilibrium is present between β-structure and random coil, whereas this term becomes zero for the solution in which either conformation is predominant.
So if the precipitation takes place at the stage that the transition to β-structure is considerably completed, then the system falls into the class B-1. Conversely, if the precipitation occurs under the state in which conformational equilibrium can not be neglected, then the system is considered to belong to the class A-3.

The results of the present analysis can not be utilized for the present system, since an explicit expression for the chemical potential of the polymer component in solution has not been derived. For this purpose, it is necessary to treat the problem in statistical mechanical way interms of a proper model for β-structure in solution.

However the present analysis is informative in some respects. For example, it can be concluded from the present analysis that association equilibrium of polymer must be present in solution phase if precipitation causes no observable effect on potentiometric titration.

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