Instantaneous shape and segmental density of flexible linear macromolecules: 1. Direct observation of tagged poly(*cis*-isoprene) molecules in untagged matrix

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Monomolecular suspensions of tagged poly(*cis*-isoprene) in untagged poly(*cis*-isoprene) or polyisobutylene were prepared by two methods. Ultra-thin slices of the materials were examined by transmission electron microscopy to reveal that the instantaneous shape and segmental density of individual molecules are neither symmetrical nor Gaussian. Each macromolecule appears as an association of high density regions intermixed with regions of lower segmental density. The high density regions tend, on the average, to be closer to the geometrical centre of the macromolecule. Nevertheless, when averaged over all angles and/or a large population of observable molecules, the shape and segmental density of the molecules become spherical and Gaussian. The size of the tagged molecules (M_v = 954 000) as measured on the electron microscope image plates, is in rather good agreement with their sizes as obtained from light scattering and solution viscosity measurements.

INTRODUCTION

Conformational properties of linear flexible polymer chains are generally characterized by distribution functions derived from various random flight models[1-25]. Initially, the resultant random coils were assumed to be spherically symmetrical[1,2,6] but later treatments led to various ellipsoidal shapes[9,10,18,25]. Independent of whether the segmental distribution is assumed to be spherical[1,2,6,7] or instantaneously aspherical[8-10,12-25], the resultant segment-clouds all have a common denominator. This is a single maximum segmental distribution increasing smoothly and monotonically from the molecular perimeter towards its centre or thereabouts. In general, the molecules in random flight treatments are depicted as possessing both shape and segmental distribution symmetry, albeit not necessarily spherical.

It would be most interesting to find whether the above characteristics hold true each instant for each macromolecule or whether they are a result of an averaging process of a large population of molecules, each averaged over space and time.

In this paper we present our observations on samples prepared essentially in the concentrated solution regime, after which the solvent was rapidly removed. In part 2 of this series additional samples, prepared in the solid state and at various concentrations at the concentrated solution regime, will be described. In these two papers the tagged polymer is polyisoprene while the untagged matrix is polyisoprene or polyisobutylene. In part 3 of this series similar observations obtained from partly iodized polystyrene suspended in untagged polystyrene will be described.

In this paper we shall present electron microscope photographs of individual macromolecules, tagged to make them visible, suspended in a matrix in such a way to remain as close as possible to their unperturbed state. In this fashion we may be able to compare the observed shape and segmental density distribution of macromolecules with the calculated Gaussian model.

There are several reports in the literature in which individual flexible linear macromolecules were observed by means of electron microscopy[26-31]. In all of these, however, the observed molecules were suspended in air or vacuum during preparation so that they tended to collapse into dense spheres. It is clear, therefore, that the shape and dimensions of the observed macromolecules are not a true representation of their shape and size in solution, the melt or the glass. Rigid macromolecules do not collapse when prepared for replication in the microscope or when exposed to air, but remain stretched out[32,33]. These are, however, out of the scope of this work. Electron microscopy replication techniques for flexible molecules all suffer from the same drawback of exposing the molecule to be later observed, to air or vacuum. The energetics of such a molecule are affected by the exposure, making it unrepresentative of its shape and size in the unexposed state.

EXPERIMENTAL

The polymer to be tagged used in this study was poly(*cis*-isoprene) (PIP) of M_v = 954 000, obtained by precipitation from cyclohexane in acetone from PIP of M_v = 700 000. For this study only the high molecular weight (M) fraction was tagged, but for the purpose of studying the process of demixing and phase separation, to be published in part 2 of the series, the whole polymer was tagged.

As untagged polymeric matrix either the high-M (M_v = 954 000) PIP or polyisobutylene (PIB) of M_v = 990 000 (Exxon Chemicals' Vistanex L-80) were used. In both instances, the attainment of a monomolecular dispersion of tagged in untagged polymer required a concentration not higher than 2.5% tagged polymer in the untagged matrix. At higher concentrations the majority of the tagged molecules were trapped in various stages of aggregation and phase separation, even though individual tagged molecules were still to be seen.

Two procedures of sample preparation were employed, one for tagged in untagged PIP (Method A) and the other for tagged PIP in PIB (Method B).

Method A

A solution of 0.095% high-M PIP in benzene was prepared with gentle warming and stirring. Upon complete dissolution the solution was exposed, with gentle stirring, to OsO_4 vapours for a period of 24 h. At that point the sealed vessel, in which the exposure took place, was opened, the solution retrieved and the polymer precipitated in a large excess of methanol. After careful washing and vacuum drying, the dark grey tagged polymer was redissolved in benzene. This solution was added to a solution of untagged high-M PIP in benzene, to obtain a solution of 2% total polymer, and 2.25% tagged PIP in untagged PIP. After stirring for several minutes to attain uniformity, the resultant solution was cast into shallow Teflon®-lined trays and the solvent removed as quickly as possible in forced air draft while the trays with the solutions were maintained at the temperature range of $40° < T < 45°C$. At this rate of solvent evaporation a solid film, albeit not fully dried, was obtained within several seconds. The thickness of such films ranged usually between 0.3 and 0.6 mm when dry.

Method B

A mixture of high-M PIP and PIB having the desired polymer ratio, say 1% PIP, was dissolved in cyclohexane to yield a solution of about 1% total polymer concentration. The solution was exposed, with stirring, to OsO_4 vapours for 24 h in a sealed vessel. Then the solution was cast into shallow Teflon®-lined trays and the solvent was removed as fast as possible in forced air draft while maintaining the temperature of the trays at room temperature (r.t.). Here again films of 0.3 to 0.6 mm thickness were obtained within seconds, although they were completely dry only after several hours.

In both methods the molar ratio of double bonds to OsO_4 was not smaller than 5:1. The films dried in the forced air draft were readily handled by hand. They were then exposed for several hours to vacuum of about 3 mmHg at r.t. The samples were then prepared for examination with a transmission electron microscope using a Reichert OMU3-FC2 microtome. The ultra-thin (500 to 900 Å) frozen sections were prepared at cutting temperatures of $-120°$ to $-160°C$ for the sample, and $-100°$ to $-120°C$ for the knife. A receiving medium of 60:40 dimethyl sulphoxide/water was used.

The bulk of the solid films is comprised of uncrosslinked rubbery material and tends to be tacky. At low temperatures the receiving medium is of rather high viscosity. This does not allow the sections to float away and, because of their tackiness, they tend to clump together. Therefore, each section was retrieved as it was cut, using a single fibre brush, and placed in an ethanol/water solution; then it was mounted on a 200 mesh nickel grid. The samples were examined with a Hitachi HU-11C transmission electron microscope at 75 kV using a low beam current. The samples tended to contract or shrivel in the electron beam, so an area was focused, then at low second condenser lens setting the adjacent area was moved into view and quickly photographed, maintaining a fixed exposure time for all exposures. Several photographs were taken from each grid and several grids were prepared from each sample. The photographs presented in this paper are true representatives of the corresponding samples.

To study the density distribution in the photographed particles, scans across many of their images on the glass electron image plates were performed with the aid of a Joyce, Loebl and Co. Ltd, double-beam recording microdensitometer MK III CS, operating in the 0–3 range of optical density to ensure linearity in the transfer of the intensity from the electron image plate to the densitometer chart. The shape of the scanned area at each instant was an oblong 0.9 x 0.3 mm in size. This oblong was moving on the electron image plate in one direction, leaving behind a scanned path 0.9 mm wide. Since the microdensitometer averages the optical density in the instantaneously scanned oblong, fine details that were observed in the printed photographs were often smeared out. Most affected by the densitometer averaging process were such details as fine strands and cilia which were visible in several printed photographs and almost or completely invisible in the densitometer scans. The densitometer scans were performed only on regions that were neither underexposed nor overexposed, to eliminate artifacts due to non-linearity in the response of the electron image plates, or excessive scattering, as the case may be. The relation between the electron density in particle in the sample and the optical density of their images on the electron image plates is considered to be linear[34] in the intermediate density range used for the densitometer scans. The linearity was also evaluated and found satisfactory by measuring the optical density changes in several electron image plates that were exposed for various times while holding all other variables constant.

The unperturbed R_G of the tagged PIP, as calculated[35] from the molecular weight of $M_v = 954\,000$, is 340 ± 20 Å, leading to an equivalent sphere diameter of about 875 Å. This is about the thickness of the ultra-thin microtomed sections, resulting in whole and parts of tagged molecules being observed in the sections. When the molecules are completely random and do not possess any shape or segmental distribution symmetry, then the exact shape of the parts of the tagged molecules remaining in the microtomed section is unimportant. Observation of many such parts of molecules will still yield the desired information concerning the instantaneous shape and segmental distribution of the whole molecules. On the other hand, if the molecules do have a shape and segmental distribution symmetry, then the knowledge of the shape of the observed part is of great importance in the determination of the segmental density in the parent molecule. A discussion of this point will be presented in part 2 of this series.

Light scattering dissymmetry measurements on the high-M tagged PIP were performed in a Brice–Phoenix Universal Light Scattering Photometer. The solutions were filtered through 0.22 μm filters prior to the measurements. The solvent of choice was benzene, which is essentially isorefractive with the untagged PIP.

Solution viscosities in cyclohexane and benzene were measured with Cannon–Ubbelohde glass viscometers at 25°C. Gel permeation chromatography (g.p.c.) scans were obtained in a Waters Associates Liquid Chromatograph model M-6000.

RESULTS AND DISCUSSION

The intrinsic viscosities, $[\eta]$, of the tagged and untagged

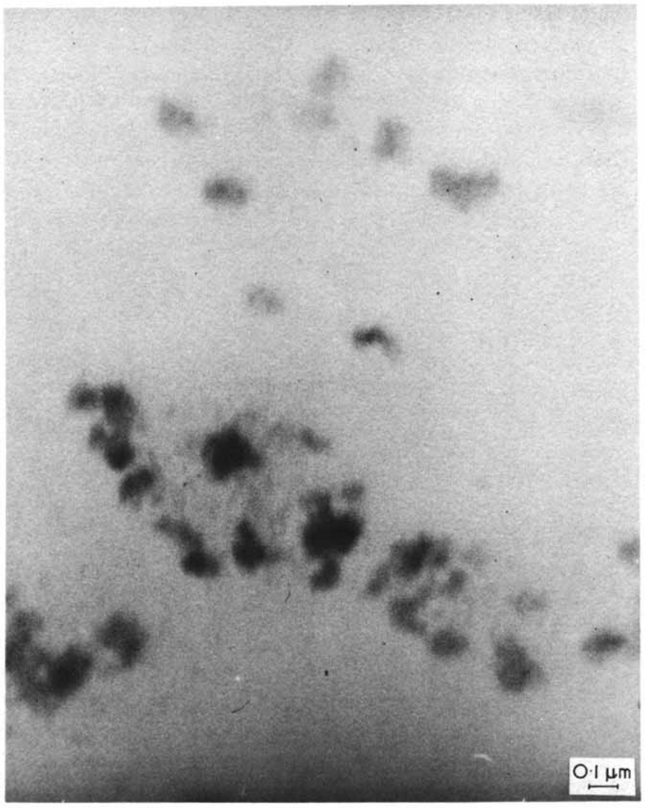

Figure 1 Tagged poly(*cis*-isoprene) molecules of M_v = 954 000 in untagged poly(*cis*-isoprene) of the same molecular weight. Sample prepared according to Method A

high-*M* PIP in cyclohexane were determined to be 4.6 and 4.7 dl/g, respectively. The similar viscosity values indicate that no significant measure of intramolecular or intermolecular crosslinking took place during the tagging procedure; or that they, somehow, counterbalanced one another, resulting in no change in viscosity. In benzene the viscosity values of the polymer were about 4% smaller than in cyclohexane. From the intrinsic viscosity, molecular weight and unperturbed R_G of 340 ± 20 Å were calculated, using data in ref 35.

Light scattering measurements of tagged high-*M* PIP gave a dissymmetry number of 1.36, yielding a radius of gyration of 380 ± 30 Å (corresponding to about 1000 Å as the diameter of the equivalent sphere), where the scatter is dependent largely on the polydispersity of the polymer sample[36,37]. The dissymmetry number of the untagged polymer was practically unity, reflecting the fact that benzene is essentially isorefractive with PIP. The observed R_G of the tagged PIP is slightly larger than the unperturbed R_G calculated for the tagged and untagged PIP from viscosity measurements. This indicates that the species observed by light scattering are individual tagged molecules and not aggregates thereof.

Several dozen electron micrographs were obtained and analysed. These were typical of the hundreds of areas observed in the microscope. The choice of a particular area to be photographed was based on its fulfilling three requisites: (a) being typical of the sample; (b) having high enough contrast so that clear photographs may be obtained, and, (c) the observed area of the microtomed sample having sufficient mechanical strength not to shrivel or burst upon exposure to the electron beam. The Figures presented in this paper are a small, yet typical, sampling of the preparations.

The matrix PIB polymer exposed to OsO_4, and the unexposed PIP, were completely featureless and essentially electron transparent, as is expected from untagged rubbery polymers of the thickness used. The backgrounds in *Figures 1* to *4* are typical in that respect.

Figures 1 and *2* are those of visible OsO_4 tagged high-*M* PIP suspended in transparent untagged PIP according to Method A above. The size of the major entities cover the range 500 to 1500 Å in averaged diameter. The minor entities, much smaller in size, are also tagged PIP and may belong to either the low-*M* tail of the tagged PIP or be fragments sectioned from high-*M* tagged PIP in the process of microtoming the samples.

One should note first that the average size of the major entities, 1000 Å, is in good correspondence with the sizes determined for both the untagged and the tagged PIP by means of solution viscosity and light scattering. Noting that the tagged PIP molecules in solid untagged PIP are of unperturbed size or very close to it, and those in PIB may also be of about the same size, one may conclude that the observed major entities are single macromolecules of tagged PIP.

Large areas of the samples, stretching over tens of microns in every direction, showed visible entities as in *Figures 1* and *2*. Had the major entities been merely aggregates of low-*M* tagged molecules, then the question may arise as to where did the high-*M* tagged molecules disappear to? G.p.c. indicated that less than 10% of the high-*M* PIP was of low-*M* ($M_v \leqslant 210000$). It is inconceivable that the large majority of the high-*M* polymer would preferentially migrate over very large distances and vanish from the observed areas, to be replaced by aggregates of low-*M* polymer fortuitously having the correct size expected from the high-*M* individual

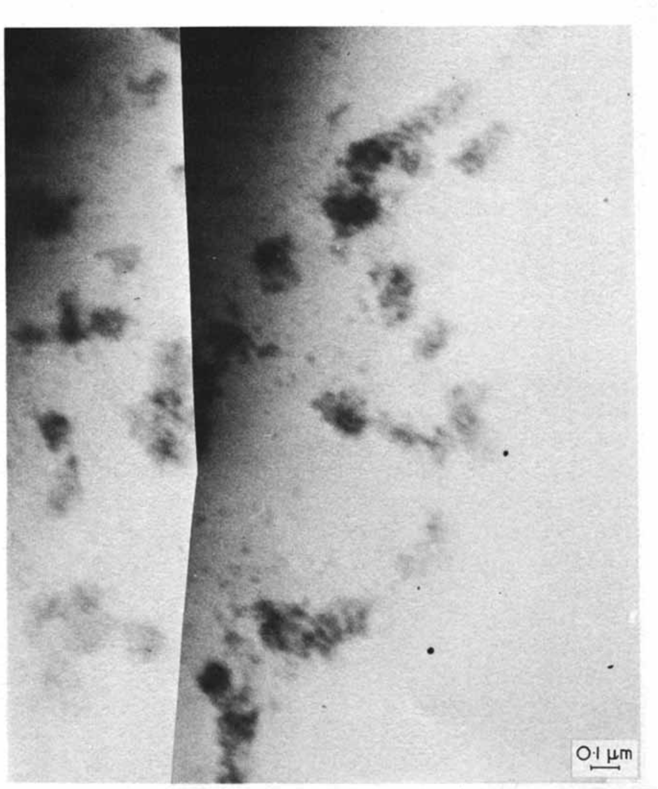

Figure 2 Tagged poly(*cis*-isoprene) molecules of M_v = 954 000 in untagged poly(*cis*-isoprene) of the same molecular weight. Some material from the lower tail end of the molecular weight distribution, or fragments of high-*M* molecules, are visible. Sample prepared according to Method A

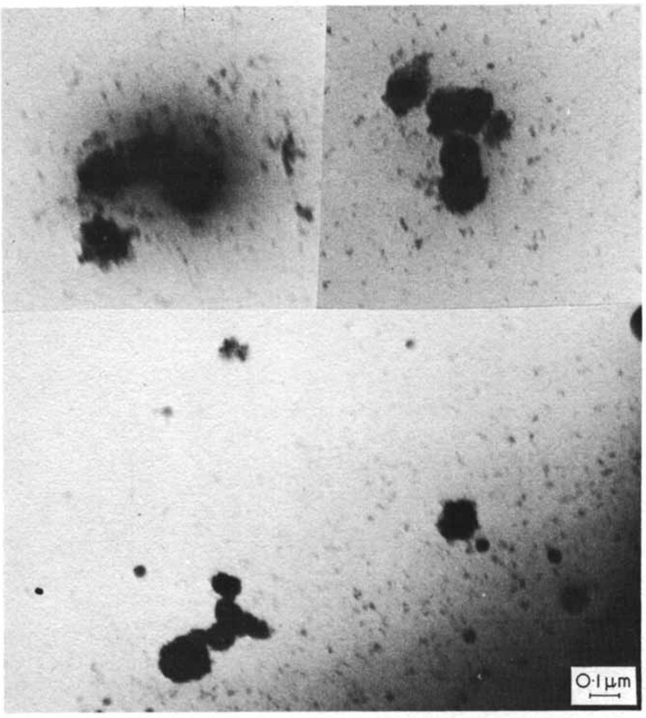

Figure 3 Tagged poly(*cis*-isoprene) molecules of M_v = 954 000 in untagged polyisobutylene of M_v = 990 000. Sample prepared according to Method B and exposed to OsO_4 vapours at 1% poly(*cis*-isoprene) in polyisobutylene and 1% total polymer concentration in cyclohexane solution

molecules. One must, hence, conclude that the observed major entities are indeed the individual macromolecules of tagged PIP of M_v = 954 000.

The tagged macromolecules are neither spherical nor have any other shape symmetry. Their segmental distribution is neither uniform nor characterized by a monotonic increase in density upon moving from the molecular periphery towards the centre. The segmental distribution appears to be an aggregation of high density regions intermixed with regions of lesser density. The high density regions are, on the average, closer to the centre of the molecules than to their perimeters.

Now, our preparative procedure called for a fast removal of the solvent from the polymer-containing solutions, (during the exposure to OsO_4 vapours the solutions were, however, at equilibrium). Because of the rather high concentration of total polymer in the solutions, they turned out to be rather viscous prior to solvent removal. The stripping of solvent increased the solutions' viscosity a great deal and very fast. In this fashion, we believe, single tagged macromolecules were trapped first in a very viscous matrix of solvent and untagged polymer, and later in untagged polymer alone. Therefore, we believe that the observed asymmetrical shape and segmental distribution are the shape and segmental distribution of the macromolecules at the point of exposure to OsO_4 vapours, or a close approximation thereof. This belief is also supported by the following. (1) The observed sizes of the tagged molecules are in reasonably good agreement with their sizes as calculated from solution viscosity and light scattering measurements. (2) The shape of individual molecules or their aggregates, appear not to be deformed as a result of solvent removal during the solidification of the films: the tagged chains seem to be permanently 'fixed' by the tagging procedure. (3) The averages of many densitometer scans across such molecules yield distributions that are symmetrical and, under certain conditions, approaching Gaussian densities. (4) Significant differences exist between samples having significant differences in their total polymer concentration in solution. (5) Exposure of solid films to OsO_4 vapours, after the removal of the solvent, resulted in markedly different morphologies from when solutions were first exposed to OsO_4 vapours and the solvent subsequently removed. (6) Partly iodized polystyrene suspended in uniodized polystyrene also yielded aspherical macromolecules having the correct size. The last four points will be discussed in detail in parts 2 and 3 of this series.

Figure 3 is a typical electron micrograph of tagged PIP embedded in untagged PIB, prepared according to Method B above. Here the concentration of PIP in PIB was 1% and the total polymer concentration in the solution was also 1%. Notice that the tagged PIP molecules lack, in general, shape and segmental density symmetry. This is similar to the case of tagged in untagged PIP. On the other hand, the tagged PIP molecules in PIB appear to be more compact and not so spread out as in the former case. This is, most probably, due to demixing of the two polymers, PIP and PIB, in solution.

When one reduces the amount of solvent in the system before exposing the solution to OsO_4 vapours, the initial steps in the process of phase separation may be observed. *Figure 4* is typical of a system of 1% PIP in PIB and total polymer concentration of 5% in the solution at the time of exposure to OsO_4. Here we see that in the same field of vision two molecular morphologies coexist. One is the non-symmetrical shape observed previously in dilute tagged PIP in PIP or PIB. The other is macromolecules that turned spherical and, apparently, possess segmental distribution symmetry. At higher concentration, of either the total polymer in solution or PIP in PIB, the spherization and phase

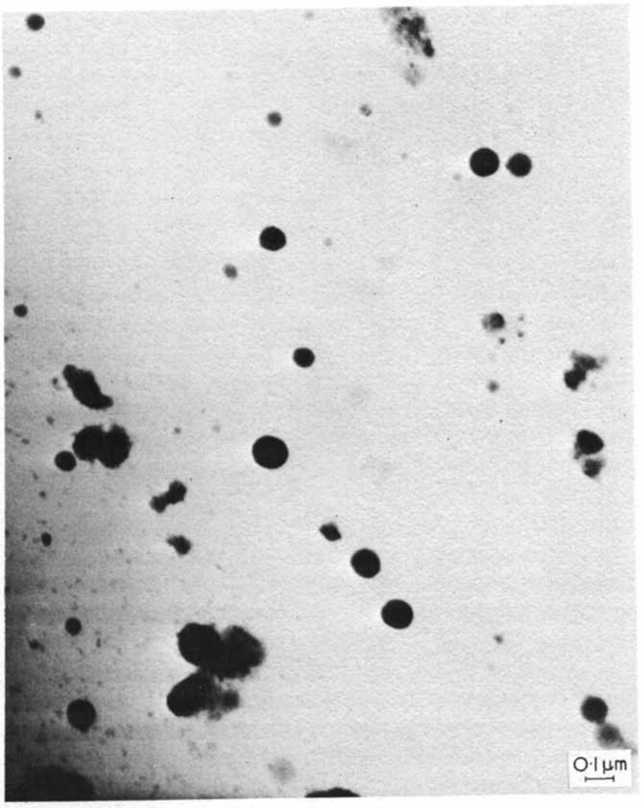

Figure 4 Same system as in ***Figure 3***, but the solution exposed to OsO_4 vapours was of 5% total polymer concentration

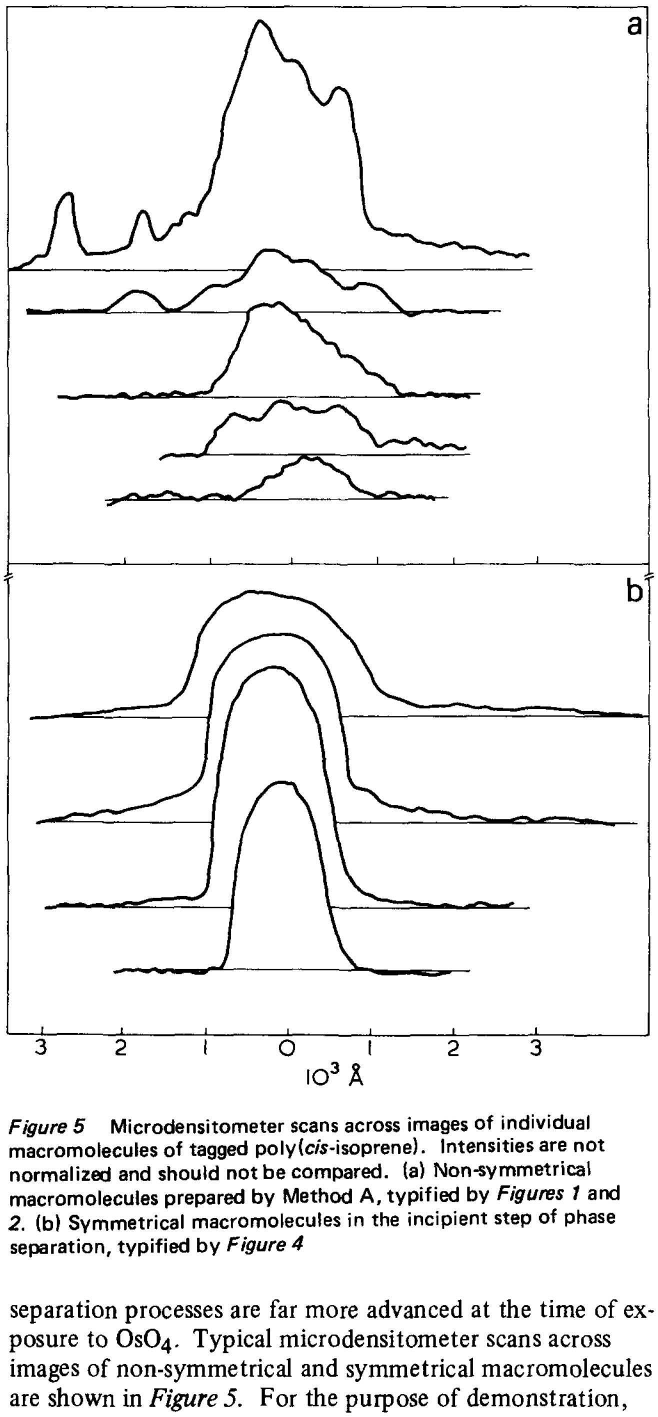

Figure 5 Microdensitometer scans across images of individual macromolecules of tagged poly(*cis*-isoprene). Intensities are not normalized and should not be compared. (a) Non-symmetrical macromolecules prepared by Method A, typified by *Figures 1* and *2*. (b) Symmetrical macromolecules in the incipient step of phase separation, typified by *Figure 4*

separation processes are far more advanced at the time of exposure to OsO_4. Typical microdensitometer scans across images of non-symmetrical and symmetrical macromolecules are shown in *Figure 5*. For the purpose of demonstration, the largest individual molecules in the photographs were chosen for the densitometer scans. Smaller individual molecules, averaging about 1000 Å in diameter, were scanned at random, yielding the same conclusions regarding shape and segmental density distribution. A more detailed analysis of the change from non-symmetry to symmetry, in the process of demixing and the consequent phase separation, will be presented in part 2 of this series. The densitometer scans are not normalized relative to one another, so that the intensities of the individual scans must not be compared with one another.

Infrequently, tagged PIP molecules of the billowing shape shown in *Figure 6* were observed in samples obtained from dilute solutions. Such molecules give one the impression as if they were trapped while in motion, probably in a convective flow of the solution. The subsequent removal of solvent failed to visibly affect the shape and size of the molecules, as was the case in all the preparations in this study.

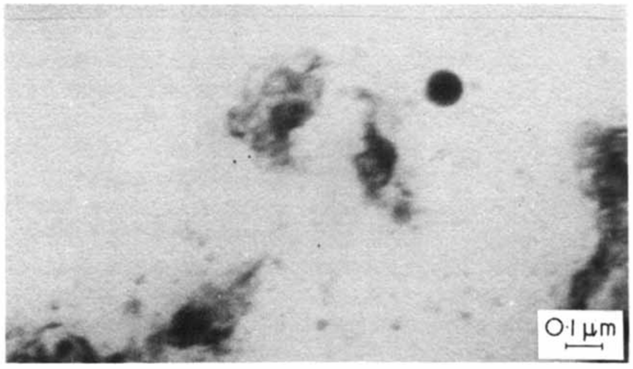

Figure 6 Several tagged poly(*cis*-isoprene) molecules apparently billowing in some local flow in the solution. No effect of subsequent solvent removal is noticeable

CONCLUSIONS

The instantaneous shape and segmental distribution of flexible linear unperturbed macromolecules, as obtained from tagged molecules trapped in untagged matrix, are not symmetrical, and in particular, not spherical. The appearance of each macromolecule is as regions of high segmental density intermixed and held together by regions of lower segmental density. The regions of high segmental density, non-symmetrical in themselves, tend to cluster, on the average, closer to the geometrical centre of the macromolecule. The measured size of the molecules is in a rather good agreement with the sizes of the tagged and untagged molecules as obtained from light scattering dissymmetry and solution viscosity.

Even though unsymmetrical on an instantaneous scale, when the shape and segmental distribution of the macromolecules are averaged over all angles and conformations, and averaged over a large population of visible macromolecules, spherically symmetrical molecules of Gaussian segmental distribution are obtained.

In the incipient state of demixing, molecules belonging to the minor phase tend to attain spherical symmetry of both shape and segmental density.

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