A COMPARATIVE STUDY OF THE ANOMALOUS VISCOSITY OF A HIGH MOLECULAR WEIGHT POLYELECTROLYTE AND THYMUS NUCLEIC ACID

by

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Certain biological macromolecules[1] such as tobacco mosaic virus, hyaluronic acid[2] and in particular myosin[3] and thymus nucleic acid (T.N.A.)[4] have a viscosity which even in very dilute solutions varies greatly with the rate of shear. This viscous anomaly is of the same order of magnitude as that found in certain relatively concentrated solutions of colloids where it results from the formation of network-like aggregates which are broken down in streaming. This behaviour is accurately described as structural viscosity and has been treated quantitatively in a number of cases[5]. Changes of viscosity with shear of a very much smaller order of magnitude have been found in solutions of synthetic high polymers and many attempts have been made to study these cases theoretically[6, 7], but only the more recent treatment by KUHN AND KUHN[8] appears to be satisfactory and in accord with experiment. These authors showed that for asymmetric particles which are randomly coiled and easily deformed the viscosity is independent of shear, while for particles which resist deformation a shear-dependence is to be expected. However, even with rigid rod-like particles the viscosity would not decrease as a result of orientation to less than half on going from zero to high rates of shear. Variations of this order have been observed for polymers the molecular weight of which does not exceed a few hundred thousand[9, 10, 11] and show clearly that orientation of the molecules is not sufficient to explain the magnitude of the anomaly found with molecules such as T.N.A. for which a different mechanism must be operative. It is difficult to determine this for such systems since the size and shape of the T.N.A. molecule in solution has not yet been established. The object of this investigation was therefore to see if a shear-dependence of this magnitude could be obtained in a synthetic polyelectrolyte of very high molecular weight. For this purpose polymethacrylic acid (P.M.A.) and its sodium salt are well suited since their structure in solution have been determined by KUHN, KÜNZLE AND KATCHALSKY[12]. It was found that P.M.A. of average molecular weight $2 \cdot 10^5$ showed the shear dependence as predicted by theory[8], but a sample with an average molecular weight of $2 \cdot 10^6$ showed a viscous anomaly as great as or greater than that found for T.N.A. The viscosity of this synthetic material was studied in detail and compared with similar data for T.N.A.

EXPERIMENTAL

Materials. The P.M.A. of average molecular weight $2 \cdot 10^6$ was prepared by irradiating a 10% solution of methacrylic acid in water with U.V. from a mercury arc for 12 to 15 hrs; the solution set to a stiff gel which was broken up and dissolved in water (this took 2–3 days). The exact concentration was determined by a potentiometric titration with sodium hydroxide. Solutions of different degree of ionisation were prepared by adding a quantity of sodium hydroxide determined from the titration curve. The concentration of all solutions is expressed in normality, the polymer has an equivalent weight of 86 and a 1 N solution therefore corresponds to 8.6 g of P.M.A. in 100 ml. A degree of ionisation of 60% was used in most of the experiments since the pH of such a solution is approx. 7.2.

A sample with an average molecular weight of $2 \cdot 10^5$ was prepared by irradiating pure methacrylic acid and dissolving the solid P.M.A. obtained. The average molecular weight was determined by esterifying the P.M.A. with diazo methane to produce polymethyl methacrylate[13] and determining the viscosity of the latter in chloroform using the constants measured by BAXENDALE, BYWATER AND EVANS[14].

Determination of viscosity. Measurements were made in a horizontal capillary viscometer similar to that described by TSUDA[15].

The rate of flow was varied by applying different and constant pressures from an air reservoir compressed by a pump. The capillary used throughout this work had a radius (γ) of 0.034 cm, length 10 cm. The average rate of shear (β) was given by $\beta = \frac{8}{3} \cdot \frac{V}{\pi \gamma^3 t}$ where V is the volume of liquid flowing through the capillary in time t. All viscosities are expressed either as specific viscosity (ηsp) $= \frac{\eta\text{solution}}{\eta\text{solvent}} - 1$ or as reduced viscosity $\frac{\eta\text{sp}}{c}$ where c is the normality of the solution. In a capillary viscometer the rate of shear varies from zero at the centre to a maximum value at the walls of the tube and the method is therefore not ideally suited for determining shear dependence. It would be better to study these solutions in a Couette viscometer but it is not possible to cover the very wide range of viscosities observed in these solutions on changing the rate of shear with anyone set of cylinders and using such an instrument for this investigation would therefore be difficult. The use of a capillary viscometer can further be justified by the fact that nearly identical curves for ηsp versus β are obtained with capillaries of different dimensions so that the rate of shear calculated represents a significant average.

RESULTS

Influence of charge

As already indicated in the introduction, marked viscous anomaly occurs only in solutions of the high molecular weight P.M.A. (see Fig. 1) and it is this material which is used throughout this investigation. KUHN *et al.*[12] showed that with P.M.A. of molecular weight $5.4 \cdot 10^4$ the viscosity is increased 130 times when 50% of the –COOH groups are converted to –COO⁻ groups by the addition of alkali. These authors showed conclusively that the addition of alkali brings about an uncoiling of the molecule owing to the repulsion of the ionised groups. In dilute solutions the polymer is already fully extended when 50% of the groups are ionised and can then be considered as a fully extended chain. On the addition of more alkali than that required to ionise 50% of the groups the viscosity remained constant and finally fell again as complete neutralisation was

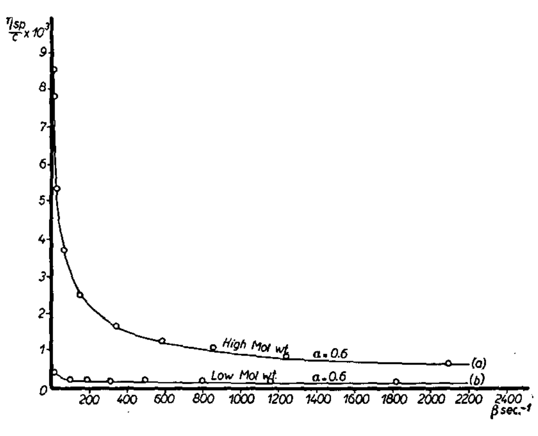

Fig. 1. Viscosity at different rates of shear of 60% ionised P.M.A. a. Average molecular weight $2 \cdot 10^6$, concentration 0.003 N; b. Average molecular weight $2 \cdot 10^5$, concentration 0.006 N.

approached. The viscosity maximum results from an increase in the ionic concentration of the solution as the pH rises which decreases the repulsion between the groups and allows a limited degree of coiling of the chain to take place. An exactly comparable effect is obtained with the high molecular weight P.M.A. investigated here. On increasing the degree of ionisation both the absolute viscosity and the change of viscosity with shear increase markedly (see Figs. 1 and 2) although not to quite the same extent as found by KUHN *et al.* (1948).

Fig. 2 shows that the influence of ionisation on the viscosity of P.M.A. is qualitatively similar at all rates of shear but that the effect is quantitatively much more pronounced at low rates of flow.

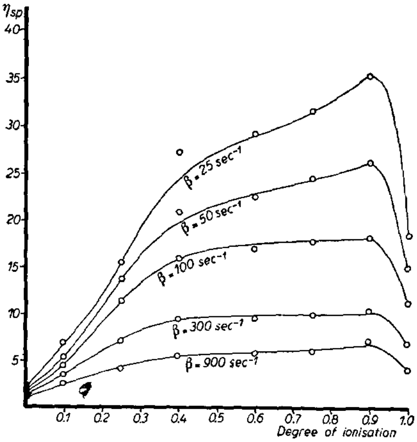

Fig. 2. Influence of degree of ionisation on viscosity of 0.006 *N* P.M.A. at different rates of shear.

Influence of added electrolyte

KÜNZLE[16] as well as HERMANS AND OVERBEEK[17] showed that the viscosity of ionised P.M.A. was very greatly reduced on the addition of electrolyte. This effect was quantitatively interpreted by KÜNZLE[16] as a coiling up of the extended molecule due to a reduction of the repulsion of the charged groups on increasing the ionic strength of the solution. Fig. 3 shows that both the anomaly with flow and the magnitude of the viscosity of the high molecular weight P.M.A. with 60% of its groups ionised is decreased on the addition of electrolyte, that 10^{-3} *M* KCl already exerts a pronounced effect and that in 0.1 *M* KCl the viscosity of the ionised P.M.A. is similar to that of the unionised P.M.A. in the absence of electrolyte. The viscosity of unionised P.M.A. is also reduced on the addition of salt (see Fig. 4) and the flow anomaly almost completely disappears. In 0.1 *M* KCl the viscosity of unionised P.M.A. could only just be distinguished from water in the viscometer used.

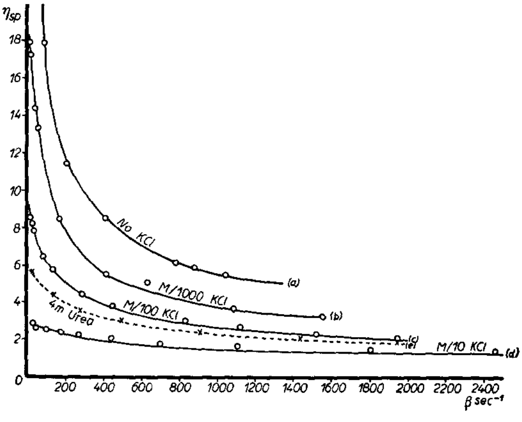

Fig. 3. Influence of adding electrolyte and urea on viscosity at different rates of shear of 0.006 *N* P.M.A. (60% neutralised). a. No salt added; b. 10^{-3} *M* KCl added; c. 10^{-2} *M* KCl added; d. 10^{-1} *M* KCl added; e. 4 *M* urea added (broken line).

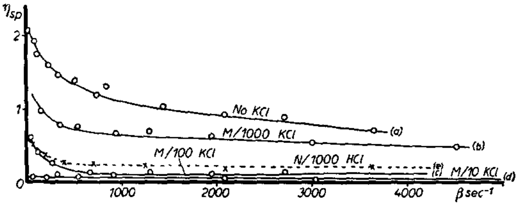

Fig. 4. Influence of adding electrolyte on viscosity at different rates of shear of unionised P.M.A. a. No salt; b. 10^{-3} *M* KCl added; c. 10^{-2} *M* KCl added; d. 10^{-1} *M* KCl added; e. 10^{-3} *N* HCl added (broken line).

The marked reduction in the viscosity of unionised P.M.A. on increasing the ionic strength of the solution only occurs in the high molecular weight P.M.A. and was not observed by KÜNZLE[16]. The most probable explanation seems to be that the molecule changes its shape from an unstretched statistical coil to a condensed solvent-impermeable particle on going from a good solvent (*i.e.* pure water) to a bad solvent (0.1 M KCl). Similar effects are well known in solutions of non-polar high polymers in organic solvents, and for example the contraction of the polystyrene molecule in benzene on adding methanol has been followed quantitatively by light scattering[18]. The fact that the contraction on adding electrolyte occurs only with the very high molecular weight P.M.A. is in accord with the treatment develop by KUHN[19] which shows that the concentration of polymer within a statistical coil increases with increasing molecular weight and predicts that self condensation should occur more readily with samples of high than low molecular weight.

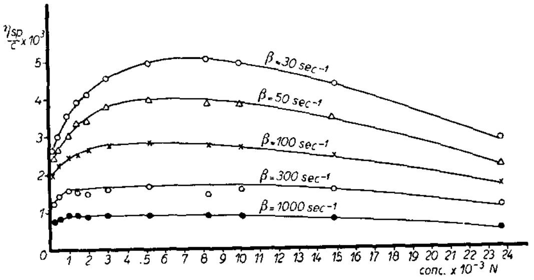

Fig. 5. Relationship between reduced viscosity and concentration of P.M.A. (60% neutralised) at different rates of shear.

The influence of concentration

KUHN *et al.*[12] found that P.M.A. with 40% of its carboxyl groups ionised exists in dilute solution (less than $1.25 \cdot 10^{-3}$ N P.M.A.) as a fully extended chain. On increasing the concentration of P.M.A. the reduced viscosity fell gradually and in a $2 \cdot 10^{-2}$ N solution was half its maximum value. This decrease results from the increase in ionic strength which allows a limited amount of coiling of the chain. When the solution is sufficiently dilute for the chain to be fully extended no change in reduced viscosity occurs on further dilution.

The high molecular weight P.M.A. (60% neutralised) behaves rather differently; at high rates of shear the reduced viscosity is virtually independent of concentration (see Fig. 5) whereas at low rates of shear the reduced viscosity passes through a pronounced maximum. Fig. 6 shows clearly that the shear dependence is greatest for intermediate concentration (approx. $5 \cdot 10^{-3}$ N) and becomes less at both higher and lower concentrations.

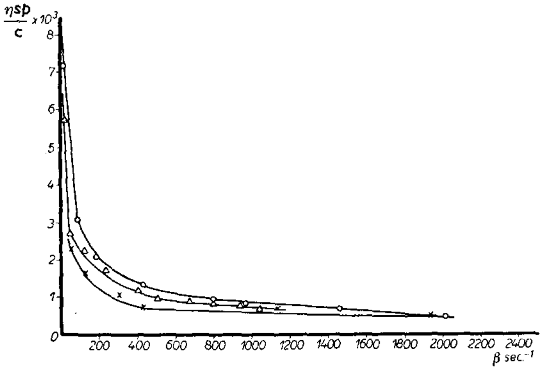

Fig. 6. Viscosity at different rates of shear of 60% neutralised, P.M.A. a. 0.0052 N (○——○); b. 0.0149 N (△——△); c. 0.00025 N (×——×).

It should be emphasized that the relationship between reduced viscosity and concentration for both high and low molecular weight P.M.A. is entirely different from that found originally by KERN AND BRENNEISSEN[20] and later also by FUOSS AND STRAUSS[10] for polyvinyl pyridine. In this and related materials the reduced viscosity increases with dilution at a rate which increases as zero concentration is approached and MACCLAY AND FUOSS[21] believe that this behaviour is typical of all polyelectrolytes a generalisation not supported by the data for P.M.A.[12] Unlike P.M.A. polyvinyl pyridine probably does not form fully extended chains under electrostatic repulsion because of VAN DER WAALS interaction and this may be the reason for the unusual dependence of the reduced viscosity on concentration.

DISCUSSION

Inspection of the results shows that it is not possible to obtain an absolute value for the viscosity of ionised P.M.A. by extrapolating either to zero or infinite rate of shear. The viscosity at low rates of shear increases so rapidly that any extrapolation is clearly impossible and even at rates of shear of 4000 to 5000 sec^{-1} the viscosity gives no indication of flattening out. This behaviour is illustrated in Fig. 7 from which it can be seen that the simple inverse relationship between η and β which obtains with many colloids[5] does not apply to P.M.A. and that moreover no extrapolation to infinite rate of shear is possible. It is questionable therefore whether numerical values of viscosity can be used for the determination of the dimensions of the molecule in systems such as the one studied.

The shear-dependence or more simply the value of the viscosity at low rates of shear of P.M.A. can be reduced in two ways: (1) by decreasing the chain length, *i.e.*, reducing the molecular weight, and (2) by changing the shape of the molecule. This last process can take place in two distinct stages with the high molecular weight P.M.A.; on decreasing the electrostatic repulsion within the molecule, by reducing the degree of ionisation or increasing the ionic strength of the solution, the molecule coils and still gives rise to solutions having a smaller viscosity which still show some dependence on rate of shear. The random coil can on changing the properties of the solvent condense to a tightly packed particle of very low and completely Newtonian viscosity. It is not possible therefore to conclude from a reduction in the shear-dependence of the viscosity that degradation of the macromolecule has taken place since a change in shape can produce the same effect; a similar conclusion was reached by LAWRENCE *et al.*[1], when studying the influence of adenosine triphosphate on myosin.

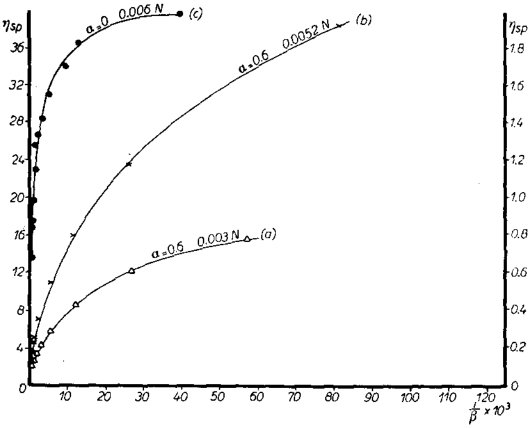

Fig. 7. Relationship between viscosity and $\frac{1}{\text{rate of shear}}$ for a. 0.003 *N* P.M.A. (60% neutralised) Viscosity scale left axis; b. 0.0052 *N* P.M.A. (60% neutralised) Viscosity scale left axis; c. 0.006 *N* P.M.A. (un-ionised) Viscosity scale right axis.

Molecular interaction and anomalous viscosity

The decrease of the reduced viscosity and its shear dependence on dilution (see Figs. 5 and 6) suggests that the anomalous viscosity results from molecular interaction between the extremely long P.M.A. molecules. It seems probable that the anomalous viscosity in dilute solutions of extended macromolecules is essentially similar to that found in relatively concentrated solutions of colloids and that it results from the existence of a reversible network of interlocking macromolecules which is progressively broken down as the rate of flow increases (compare BUTLER AND JAMES[25]). A simple calculation (assuming the chain-length corresponding to each P.M.A. monomer unit to be 2.6 A) shows that if each molecule of P.M.A. is to have a complete sphere of rotation then for a molecular weight of 10^6 the concentration must not exceed $1.2 \cdot 10^{-5}\%$; this concentration varies inversely as the square of the chain length (*i.e.* molecular weight for a fully extended molecule) and molecular interaction is therefore powerfully favoured on increasing the molecular weight.

The shear-dependence decreases as the molecule coils up (*i.e.* on decreasing charge or increasing ionic strength) because the interaction between molecules is reduced. This does not become negligible however even in a completely coiled molecule since unionised P.M.A. (see Fig. 4) still exhibits a viscous anomaly and this cannot be attributed to uncoiling during flow since for a highly flexible molecule this would not lead to a shear-dependence[8]. However, when the random coil collapses the solution becomes completely Newtonian (see Fig. 4), since the tightly packed particles are then widely separated and cannot interact.

To examine whether the network of macromolecules which is broken down during flow results only from mechanical interaction or if the molecules are held together at points of intersection by secondary forces such as hydrogen bonds the action of urea on the viscosity was examined (see Fig. 3). Since urea was shown by STEINHARDT[22] and by ALEXANDER AND STACEY[23] to dissociate hydrogen bonded aggregates of proteins and dyes respectively its profound influence on the viscous anomaly of P.M.A. can probably also be explained in terms of the breakdown of intermolecular links. The suggestion that the anomalous viscosity studied here is not produced merely by the interaction of macromolecules but by the formation of a network held together by secondary forces is further supported in that a solution of methylated P.M.A. in chloroform shows Newtonian behaviour in dilute solutions (*i.e.* $< 0.02\%$). This polymethyl methacrylate solution and unionised P.M.A. in water have approximately the same size and shape of a statistically coiled molecule and the mechanical interaction in the two systems is therefore similar but only in the latter case where intermolecular hydrogen bonds can be formed is the solution non-Newtonian. Probably the explanation advanced here for the anomalous viscosity of P.M.A. can similarly be applied to T.N.A. where there are numerous possibilities for the formation of intermolecular hydrogen bonds between extended chains (*cf.* [24] and [25]) especially since the effect of urea is the same in the two systems[4].

Similarity between P.M.A. and T.N.A.

JORDAN[26] amongst others has advanced the view that the physico-chemical behaviour of T.N.A. in solution may be determined by its polyelectrolyte character. The major stumbling block in this hypothesis has been the fact that the synthetic polyelectrolytes studied hitherto did not show a pronounced anomalous viscosity and they also had an entirely different relationship between reduced viscosity and concentration.

The viscous behaviour of the high molecular weight P.M.A. reported in this paper is strikingly similar to T.N.A. In both systems the reduced viscosity initially increases with concentration (compare Fig. 5 and [26, 27]), the viscosity is reduced on decreasing the degree of ionisation (compare Fig. 2 and [28]), and the shear dependence of the viscosity is decreased both by increasing the ionic strength and by the addition of urea (compare Fig. 3 and 4, [28]). As a first approximation therefore T.N.A. can be considered by analogy as a chain the shape of which is determined by the electrostatic interaction between ionised phosphate groups. In neutral solutions this repulsion is sufficient to render it fully extended but if its extension is reduced by the addition of electrolyte or back titration of the charged groups the molecule coils up and becomes less asymmetric. Quantitatively the effect of salt on the shear-dependence of the viscosity is less marked with T.N.A. than P.M.A., probably because the T.N.A. with its bulky side chains is more rigid and the statistical element (*i.e.* the average chain-length whose orientation is independent of the neighbouring chain element) is comparatively long[29]. If this is so the dimension of the statistical coiled molecules of T.N.A. will be larger and more asymetric than those of P.M.A. of the same chain-length. Accordingly the interaction between fully coiled molecules of T.N.A. will be greater than with P.M.A. and the relative effect of adding salt will be less.

The fact that T.N.A. still shows some streaming birefringence in the presence of salt[4, 28] does not necessarity invalidate this hypothesis as has been claimed by SCHWANDER AND SIGNER[30], since statistically coiled particles may still be anisotropic[31] and show streaming birefringence even when their viscosity is Newtonian[8, 31].

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SUMMARY

It has been shown that the viscosity of aqueous solutions of the sodium salt of polymethacrylic acid (P.M.A.) of high (*i.e.* $2 \cdot 10^6$) but not of low (*i.e.* $2 \cdot 10^5$) molecular weight shows a very pronounced shear-dependence. The viscous anomaly is decreased by (1) reducing the degree of ionisation of the polymer, (2) increasing the ionic strength, (3) addition of urea. From the relationship between viscosity and concentration it is concluded that the anomalous viscosity results from the reversible formation of a network of chains which is progressively broken down by increasing rate of shear.

The viscous behaviour of P.M.A. and thymus nucleic acid (T.N.A.) is qualitatively very similar and by analogy it is proposed that the shape of the T.N.A. molecule in solution is determined by the electrostatic repulsion of the phosphate groups. The reduction of this repulsion (*e.g.* by adding salt) leads to a coiling of the molecule resulting in decreased interaction and hence a decrease in the shear-dependence (*i.e.* a reduction in viscosity at low rates of shear).

RÉSUMÉ

Les auteurs ont montré que la viscosité de solutions aqueuses du sel de sodium de l'acide polyméthacrylique (P.M.A.) de poids moléculaire élevé (c.à.d. $2 \cdot 10^6$) mais non de poids moléculaire faible (c.à.d. $2 \cdot 10^5$) montre une dépendance prononcée du cisaillement. L'anomalie de viscosité est diminuée 1. par réduction du degré d'ionisation du polymère, 2. par accroissement de la force ionique, 3. par adjonction d'urée. De la relation entre la viscosité et la concentration les auteurs concluent que la viscosité anormale résulte de la formation réversible d'un réseau de chaînes, qui est progressivement rompu lorsque le cisaillement s'accroît.

Au point de vue de la viscosité le comportement du P.M.A. est très semblable à celui de l'acide nucléique de thymus (T.N.A.); pour des raisons d'analogie les auteurs suggèrent l'idée que la forme de la molécule de T.N.A. en solution serait déterminée par la répulsion électrostatique des groupes phosphate. La réduction de cette répulsion (p.e. par addition de sel) conduit à un pelotonnement de la molécule; l'interaction s'en trouve diminuée et de ce fait aussi la dépendance du cisaillement (c.à.d. qu'une réduction de la viscosité a lieu lorsque le cisaillement est faible).

ZUSAMMENFASSUNG

Es wurde gezeigt, dass die Viskosität wässriger Lösungen des Natriumsalzes der Polymethacrylsäure (P.M.A.) von hohem Molekulargewicht (d.h. $2 \cdot 10^6$) aber nicht von niedrigem Molekulargewicht

(d.h. $2 \cdot 10^5$) eine ausgesprochene Abhängigkeit vom Strömungsfall zeigt. Die Viskositätsanomalie wird vermindert 1. durch Verminderung des Ionisationsgrades des Polymeren, 2. durch Erhöhung der Ionenstärke, 3. durch Zugabe von Harnstoff. Aus dem Verhältnis zwischen Viskosität und Konzentration wird geschlossen, dass die abnormale Viskosität auf die reversibele Bildung eines Netzwerkes von Ketten zurückzuführen ist, welche allmählich bei steigender Strömungsfall abgebrochen werden.

Was die Viskosität betrifft, so ist das Verhalten von P.M.A. und Thymusnukleinsäure (T.N.A.) qualitativ sehr ähnlich; aus Analogiegründen wird vorgeschlagen, dass die Form der T.N.A.-Molekel in Lösung durch die elektrostatische Abstossung der Phosphatgruppen bedingt sein könnte. Die Verminderung dieser Abstossung (z.B. durch Salzzugabe) führt zu einer Knäuelung der Molekel, welche eine Herabsetzung der gegenseitigen Wirkung und daher der Abhängigkeit der Viscosität von der Strömungsfall (d.h. Verminderung der Viskosität bei niedriger Strömungsfall) zu Folge hat.

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