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Physico-chemical studies of polymeric carriers

6. Ultracentrifugal molecular weight determination of a polyacid

Éva Csákvári, László Korecz, Mária Azori, and Ferenc Tüdős*

Central Research Institute for Chemistry of the Hungarian Academy of Sciences,
P.O. Box 17, H-1525 Budapest, Hungary

SUMMARY

Ultracentrifugal studies were made on poly/1-vinyl-2-pyrrolidone-co-maleic acid/ samples eliminating the polyelectrolyte character of the polyacid. Molecular weight values were evaluated from sedimentation-diffusion equilibrium measurements. Good correlation was found between weight average molecular weight values obtained from the equilibrium measurements and those evaluated viscometrically, applying the Mark-Houwink equation determined earlier.

INTRODUCTION

Recently, the evaluation of physico-chemical properties of aqueous solutions of polyelectrolytes has become of interest for biologic applications (1-3). Particular emphasis is put on the determination of real molecular weight values, as in one respect, the molecular weight dependence of the biologic activity was supposed and confirmed in a number of cases (e.g. 4-8), while on the other hand, exact methods applicable for charged macromolecules are still missing. In addition, the utilization of estimated molecular weight values proved to be insufficient for pharmacological purposes, since the error in estimation may exceed even an order of magnitude, and molecular limits can be stipulated in both directions. A considerable loss in biologic activity can be caused by decreasing molecular weight, while increased toxicity appears applying polymeric drugs with relatively high molecular weight. Furthermore, these effects are generally present within the same order of magnitude.

Special interest has been devoted to polycarboxylic acids, specifically to copolymers of maleic anhydride. The most extensively studied and reviewed one is DIVEMA (the copolymer of divinyl ether and maleic anhydride), considered as potential antitumor, antibacterial, antifungal, anticoagulant, hemostatic, interferon inducing agent (1,5,6,9). In spite of the presumed application area of pyran copolymer, as well as the attempts focussed on its physico-chemical characterization, the difficulties still have not been overcome (5,6).

The alternating copolymer of maleic anhydride with N-vinylpyrrolidone has got some advantages over DIVEMA, such as structural homogeneity, decreased toxicity when hydrolysed (10-12), etc. In order to obtain reliable molecular weight values, we aimed at establishing proper conditions for poly/1-vinyl-2-pyr-

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* To whom offprint requests should be sent

rolydone-co-maleic acid/, later on: P/NVP-co-MAc/, to exhibit no polyelectrolytic properties. The general practice of the screening of the charges along the chains by simple electrolytes is unacceptable, since this procedure acts as a source of additional theoretically unsolved questions (13).

Instead, it was found appropriate to suppress the dissociation of the polyacid by using a strong, low molecular acid as solvent. In previous papers of this series it was shown, that P/NVP-co-MAc/ exhibits nonelectrolyte character in dilute aqueous HCl solution (0.0085 M) at 25 °C, thus the common techniques (light scattering, viscometry) became applicable for the characterization of the copolymer (10,14).

The aim of the present work is to study the sedimentational-diffusional features of P/NVP-co-MAc/ in order to open new possibilities of molecular weight determination of the copolymer and also of related polyacids by applying the conception of suppressed dissociation (10).

EXPERIMENTAL

Sedimentation-diffusion equilibrium studies were performed at 25 °C in a Beckman-Spinco Model E-HT ultracentrifuge equipped with an electronic speed control. An-H titanium rotor was applied. The cell,was equipped with a Kel-F six-channel centerpiece and quartz windows. The revolution ranged between 12000 and 24000 rpm. Schlieren optics was used with photographic registration.

Phoenix Precision Instrument 60 type differential refractometer and A.Paar DMA 50 type digital precision densitometer were applied for the evaluation of the refractive index increment and the partial molar volume of the polymer, respectively.

The viscometric measurements were made in an Ubbelohde viscometer.

Hydrochloric acid solution (pH = 2.1) was used as solvent. The concentration of the P/NVP-co-MAc/ solutions were in the range of $6\cdot10^{-3} - 16\cdot10^{-3}$ g$\cdot$cm^{-3}.

RESULTS AND DISCUSSION

The calculation method applied for the determination of weight average molecular weight M_w^{SE} is based on the one described in ref.(15). The apparent molecular weight is defined as:

$$M_{app}^{SE} = \frac{2RT}{(1 - V\rho)\,\omega^2}\,\frac{d\ln c}{dr^2}$$

where V is the partial specific volume of the solute, ρ is the density of the solvent, ω is the angular velocity, r is the distance from the centre of rotation, R and T are the gas constant and the absolute temperature, respectively.

Straight lines were obtained plotting $\ln c + k$ versus r^2 for the evaluation of M_{app}^{SE} values, as it is illustrated on Fig.1.

The molecular weight of the copolymer was obtained by extrapolation of the apparent molecular weight values determined for solutions of different concentrations according to the equation:

$$\frac{1}{M^{SE}_{app}} = \frac{1}{M^{SE}_{w}} + Bc_{0}$$

where c_0 is the initial concentration of the solution, and B is a constant. Linear relationship was found in all cases with positive slope, showing nonelectrolyte properties of the polyacid under the given conditions, as represented in Fig.2.

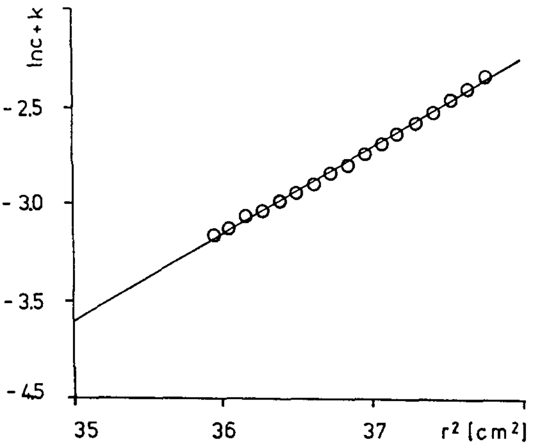

Fig.1. A plot of ln c versus r^2 for the evaluation of the apparent molecular weight of a P/NVP-co-MAc/ sample in HCl at pH 2.1

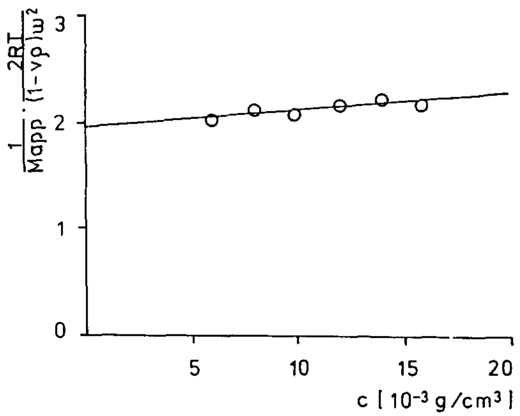

Fig.2. Extrapolation of the apparent molecular weight values of a P/NVP-co-MAc/ sample for infinite dilution; solvent: 0.0085 M HCl (pH 2.1).

Molecular weight values evaluated from sedimentation-diffusion equilibrium measurements are listed in Tab.1 together with those, obtained viscometrically, applying the Mark-Houwink equation determined earlier by light scattering and viscometry on fractionated P/NVP-co-MAc/ samples (10,14). The intrinsic viscosity values /determined as described in (14)/ are also given in Tab.1. The linearity of the reduced viscosity vs. concentration function is shown in Fig.3, proving the fulfilment of the requirement for calculating the intrinsic viscosity.

Tab.1. Intrinsic viscosity and molecular weight values of P/NVP-co-MAc/ samples measured viscometrically and by ultracentrifuge in HCl at pH 2.1

$[\eta]$	M_w^V	M_w^{SE}
0.10	11000	10000
0.12	14000	14000
0.12	14000	12000
0.14	17000	16000
0.16	22000	22000
0.20	31000	31000
0.25	45000	48000

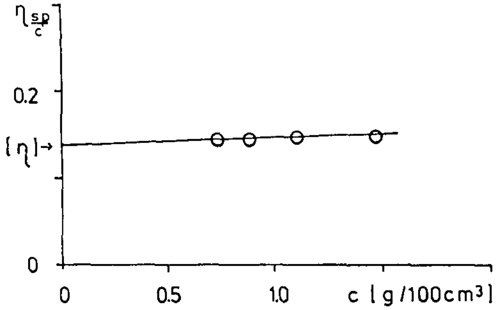

Fig.3. A plot of reduced viscosity versus concentration for the evaluation of the intrinsic viscosity of a P/NVP-co-MAc/ sample in HCl at pH 2.1

Good correlation was found between the M_w values obtained by the two different methods, as it can be seen in Fig.4, where a slight difference appeared between the theoretical and the experimental linear regression functions. The correlation factor is 0.998 in the latter case.

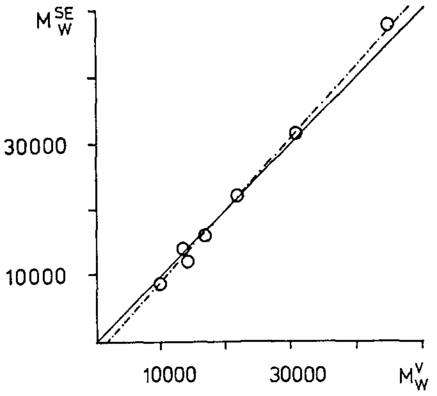

Fig.4. Comparison of molecular weight values of P/NVP-co-MAc/ samples, determined in HCl at pH 2.1 by ultracentrifugation M_w^{SE} and by viscometry M_w^V, (the solid line is the theoretical and the dotted line is the experimental correlation function).

A further analysis of the data is given in Fig.5, where it is obvious, that the difference in M_w^{SE} and M_w^V values occur within the accuracy of the methods applied.

It can be concluded, that the conception of suppressing the dissociation of the polyacid (10) could be verified by the good correlation found, since different physical characteristics of the solute serve for the determination of M_w in the two methods applied. Expectedly, the given mode of the determination of the molecular weight can be used also for related polyacids, soluble and hydrolytically stable in aqueous acidic medium and showing structural homogeneity. It should be noted, that the determination of the required amount of the added acid is of great importance, since at higher than needed con-

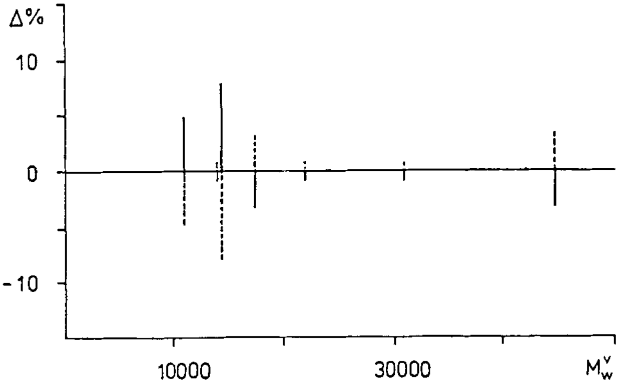

Fig.5. Deviation of M_w^{SE} (dotted lines) and M_w^V (solid lines) values of P/NVP-co-MAc/, related to the mean M_w data.

centration of the added acid, partial coagulation of the polymer can be expected due to compact coil formation. This phenomenom occured presumedly in the case of pyran copolymer studied at pH 1.5 (6).

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