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Synthesis and viscometric characterization of poly(*N*,*N*-dimethylacrylamide) for DNA separations

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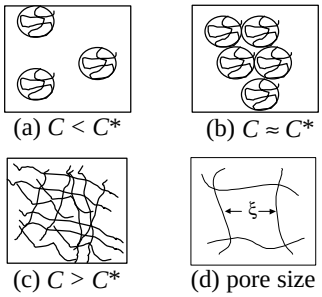
We present the synthesis and purification of poly(*N*,*N*-dimethylacrylamide) (PDMA) ranging from about 300 up to 800 kDa (M_v), for DNA separation applications. The synthesis was carried out in aqueous solution using TEMED, and APS as radicalar initiator. Molecular weight is determined by applying the Mark-Houwink-Sakurada equation to the measured intrinsic viscosity. We compared three different plots of viscosity dependence on the polymer concentration: Huggins, Kraemer and Martin approaches. In addition, we present the use of a numerical interactive calculation to determine the intrinsic viscosity by solving Martin equation from the flow time of a single solution. This approach were shown to be valid in the whole studied range of PDMA molecular weight and concentration.
From the intrinsic viscosity we were able to calculate the critical concentration for each sample, from which it's also possible to determine the pore size for one entangled solution, as a function of the concentration. (These are important parameters to enhance read length in DNA sequencing).

Introduction

Eletrophoretic separation of nucleic acids in polymeric solutions is an important separation technique in molecular biology. The advent of capillary electrophoresis as a much more efficient analytic method strongly increased the throughout in many applications, for instance, in DNA sequencing, protein analysis, and others. In the last decade, several efforts were made in order to improve this technique. Many of them stimulated by the Human Genome Project, an international consortia that intended to sequence the whole human genome.
The use of water solutions from entangled linear polymer chains as sieving matrices for nucleic acids separations in capillary electrophoresis (DNASCE), increased the efficiency in the one-base resolution separation of oligonucleotides, required for DNA sequencing. This replaceable separation matrices and the development of capillary arrays allowed the construction of full automated and high-throughout sequencers that are presently used all over the world in many sequencing projects. Beside the base calling software and the fluorescent tags used, the sieving matrix is the critic point to achieve large read lengths and resolution.

For DNASCE, the polymer solutions are used in the semi-diluted regime, where the linear polymer chains are completely entangled, creating a sieving net with a characteristic average pore size. The size of this pore, is then described by an average diameter, the so called "blob" size, ξ. (Fig. 1).



(a) $C < C^*$ (b) $C \approx C^*$ (c) $C > C^*$ (d) pore size

Fig. 1. The concentration regimes of polymer solutions: (a) bellow the overlap threshold; (b) near the critical point; and (c) above the overlap threshold. (d)Blob size: the average diameter of the pore formed by entangled polymer chains.

Above the overlap threshold, the blob size does not depend on the molecular weigh, but on the concentration of the polymer. By knowing the

concentration of the overlap threshold, *C**, one can also calculate the blob size dependence on concentration and adapt the system to obtain larger read lengths.

Since the entangled polymer solutions were suggested to be used in DNASCE for the first time[1], several different polymers have been studied[2,3]. So far, linear poly(acrylamide) (LPA) allowed the longest read lengths ever reached, from tens of bases to thirteen hundred bases in about one hour[4]. However, this polymer requires the use of treated capillaries to avoid electroosmotic flow (EOF) and DNA interactions with the capillary wall. The polymer poly(*N,N*-dimethylacrylamide) (PDMA) presents the useful property of self coating of the capillary wall, suppressing the EOF, and allowing the use of untreated capillaries. These capillaries are much more cheap and can be reconditioned, extending the working life time.[5]

It is well established by experimental studies and models of DNA migration mechanisms[6] that larger read lengths in DNA sequencing are reached when the system obeys specific ratios between the oligonucleotide lengths and the so called blob size of the sieving matrix. Therefore, in order to get longer read lengths when applying synthetic polymers in DNASCE, one might want to know the average molecular weight, the molecular distribution, the overlap threshold, and the blob size of the sieving matrix.

The techniques usually applied for polymer molecular weight determination are laborious and not so common in most molecular biology laboratories. Viscometry seems to be the simplest and also the cheapest of all commonly used methods and does not require sophisticate apparatus. Even so, it is necessary to measure the flow time of pure solvent and several dilute polymer solutions at different concentrations. Molecular weight determination is then achieved by applying the Mark-Houwink-Sakurada equation (eq.1) in which K_{MHS} and a are characteristic constants for each polymer-solvent pair in a given temperature range, and $[\eta]$ is the intrinsic viscosity.

$$[h] = K_{MHS}\,\overline{M}^{a} \qquad \textbf{eq. 1}$$

In this work we describe the synthesis and purification of PDMA ranging from about 300 up to 800 kDa (M_v) and compare three different plots of viscosity dependence on polymer concentration. In addition, we present the use of numerical interactive calculation to determine the intrinsic viscosity by solving Martin equation from the flow time of a single solution. From the intrinsic viscosity we were able to calculate the critical concentration for each sample. Such a parameter allows the calculation of the pore size for one entangled solution, as a function of the polymer concentration.

Experimental

PDMA synthesis was carried out in 10 mL glass flasks sealed with a rubber septum tighten by an aluminum ring. *N,N*-dimethylacrylamide (DMA) (Aldrich) was previously distilled under reduced pressure, and solutions were prepared in the adequate concentration, 10, 15 or 20% v/v, in ultrapure water. Reaction vials were then degassed using vacuum and ultrasound. The reaction flasks were maintained under nitrogen at atmospheric pressure. Polymerization reaction was initiated by adding the given amount of *N,N,N',N'*-tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) with a microsyringe (0.5, 1.0 or 2.5μmol/mL DMA; TEMED to APS ratio were 1:1mol). The vials were left to react for 24h at 7ºC, without stirring. The purification of the polymer was made by two consecutive precipitation in acetone, dissolution in water, and freeze-drying. Diluted solutions were prepared in ultrapure water. Viscometric characterization was carried out in an Ubelohde dilution viscometer with a 0.63mm diameter capillary (Schott 53110). The flow times were recorded by the data acquisition apparatus AVS 310 (Schott Geräte).

Results and Discussion

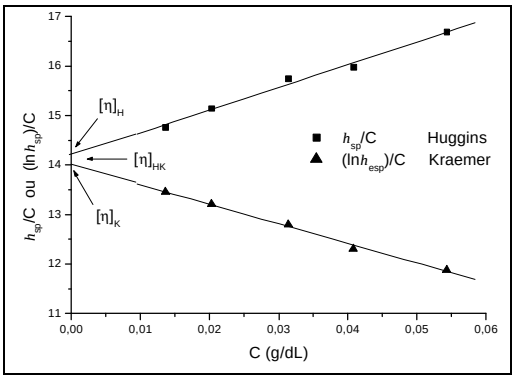
The intrinsic viscosity of six different samples were determined by plotting the viscosity dependence on polymer concentration, and subsequent graphic extrapolation to infinite dilution. Linear regression of experimental data are usually based on Huggins (eq.2), Kreamer (eq.3), and Martin (eq.4) plots. The former is based on theoretical description for polymer chains in solution, and the others are empirical relations.[7]

$$\frac{\eta_{sp}}{C} = [\eta]_H + K_H[\eta]_H^2 C \qquad \textbf{eq. 2}$$

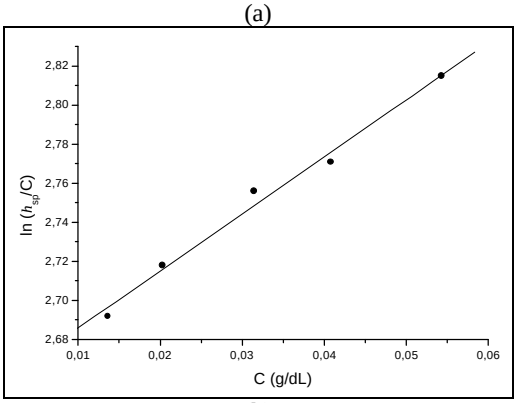
$$\frac{\ln \eta_r}{C} = [\eta]_K + K_K[\eta]_K^2 C \qquad \textbf{eq. 3}$$

$$\ln\left(\frac{h_{sp}}{C}\right) = \ln[h]_M + K_M[h]_M C \qquad \textbf{eq. 4}$$

The three methods were applied to the samples for evaluation of fitting accuracy. By linear fit for each plot, Martin, Huggins and Kraemer constants, K_M, K_H, K_K, were determined for PDMA aqueous solutions. The dependence of the lines' slope on the polymer molecular weight was shown to be negligible in the studied molecular weight range. The Huggins coefficient, K_H , which is used as a measure of solvent quality[7], was found to be 0.23, pointing that water is a good solvent for PDMA.



(a)



(b)

Fig. 2. (a) Huggins and Huggins-Kraemer plots and (b) Martin plot.

Examples of the obtained results are given in *Tab. 1*, where N is the degree of polymerization, and C* is the polymer concentration at the overlap threshold, calculated according to reference 8. Molecular weight determined by the three graphic methods are in good agreement. From viscometric data one can also determine the average pore size for a solution in a given concentration, or the minimum concentration required to achieve the desired pore size for DNA sequencing, for example.[8]

Tab. 1. Intrinsic viscosity and subsequent molecular weight obtained by different plots.

Sample	$[\eta]_H$	$\mathbf{M_{V\,H}}$	$[\eta]_{HK}$	$\mathbf{M_{V\,HK}}$	$[\eta]_{Mg}$	$\mathbf{M_{V\,Mg}}$	$N_{\mathbf{Mg}}$	$\mathbf{C^*_{Mg}}$
	dL/g	kDa	dL/g	kDa	dL/g	kDa		g/dL
F	11.528	**628**	11.484	**625**	11.554	**630**	6.351	0.130
C	13.858	**788**	13.816	**785**	13.915	**792**	7.990	0.108
E	14.188	**811**	14.092	**805**	14.253	**816**	8.231	0.105

Mg stands for Martin plot, H to Huggins and HK to Huggins-Kraemer plot.

The Martin equation can be written as an implicit function of [η] (eq. **5**), which can be solved numerically by interactive calculation. By knowing the K_M constant, determined from Martin plot (*Fig. 2*, K_M = 0,20514), one can calculate the intrinsic viscosity from one single diluted solution, as a single point determination method.

$$[h] = \frac{\left(\frac{t_s}{t_0}\right) - 1}{C \exp[K_M \times [h] \times C]} \qquad \textbf{eq. 5}$$

Martin-numeric approach was also shown to be valid in the whole studied range of PDMA molecular weight and concentration. Results obtained for different concentrations are summarized in table 2.

Tab. 2 Martin-numeric single point determination for solution concentration range.

C	$[\eta]_{Mn}$	$\mathbf{M_{V\,Mn}}$
0.0543	14.244	**815**
0.0408	14.188	**811**
0.0314	14.353	**823**
0.0203	14.271	**817**
0.0136	14.190	**811**

Mn regarding to Martin-numeric approach.

Conclusions

By varying either, the APS/DMA ratio, and DMA concentration, it was possible to obtain polymers with different average molecular weights, ranging from about 300 to 800 kDa. The use of Martin-numeric approach allows fast determination of Molecular weight from a single solution, using simple viscometric apparatus. The MHS equation (eq.1) and relations for critical concentration and pore size determination were included in the program that calculates the intrinsic viscosity. The parameters Mv and C* can then be easily obtained just by feeding the program with the PDMA concentration and the flow times of the solution and pure solvent.

Acknowledgment

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References

1 - P.D. Grossman, D.S. Soane, *Biopolymers*, 1991, 31, 1221.
2 - M.A. Quesada, *Current Opinion in Biotechnology*, 1997, 8, 82.
3 - A.E. Barron, M.N. Albarghouthi, *Electrophoresis*, 2000, 21, 4096.
4 - E. Carrilho, M. C. Ruiz-Martinez, J. Berka, *et. al. Analytical Chemistry,* 1996, 68, 3305.
5 - C. Heller, *Electrophoresis*, 1998, 19, 3114.
6 - J.L. Viovy, *Reviews on Modern Physics*, 2000, 72, 813.
7 - Jimmy W. Mays, in *Modern Methods of Polymer Characterization*, H.G. Barth and J. W. Mays Ed.; John Wiley, 1991.
8 - C. Heller, *Electrophoresis*, 2001, 22, 629.