Intrinsic Viscosity Determination by "Single-Point" and "Double-Point" Equations

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Abstract:
"Single-point" equations used for intrinsic viscosity determination are greatly used when working with synthetic polymer solution systems. In this work we have applied them to a biological macromolecule in a bovine serum albumin (BSA)/water system. Almost all single-point equations are available and errors can be lowered. However, we have detected a systematic bias in the estimations provided by "single-point" methods. To overcome it we propose a "double-point" method which gives lower estimation errors for this system. This novel method is not system specific and could be applied to other polymeric solution.

Zusammenfassung:
Single-Point-Gleichungen (Einzelpunkt-Gleichungen) zur Bestimmung der inneren Viskosität werden in hohem Maße bei der Arbeit mit Systemen synthetischer Polymerlösungen angewandt. In dieser Arbeit haben wir sie auf ein biologisches Makromolekül eines Rinder-Serum-Albumin (BSA)/Wasser-Systems angewandt. Fast alle Single-Point-Gleichungen stehen zur Verfügung und die Fehlerquoten können niedrig gehalten werden. Wir haben jedoch eine systematische Abweichung in den Schätzungen, welche die Single-Point-Methode liefert, vorgefunden. Um diese zu überwinden, schlagen wir eine Double-Point-Methode (Zweipunkte-Methode) vor, die niedrigere Schätzwerte für dieses System erlaubt. Diese neue Methode ist nicht systemspezifisch und kann auf andere Polymerlösungen angewandt werden.

Résumé:
Les équations de simple point pour la détermination de la viscosité intrinsèque sont très employées en systèmes de solutions de polymères synthétiques. Dans ce travail-ci nous les avons appliquées au système: eau-BSA (macromolécule biologique), et, nous y avons détecté un erreur systématique dans les estimations données par cette méthode. Dans ce travail, on montre graphiquement l'erreur que on peut obtenir. Pour résoudre cette difficulté on présente ici une équation pour l'estimation an utilisant une méthode de double point, d'application générale et qui diminue l'erreur commise. Cette nouvelle méthode n'est pas spécifique à ce système et elle peut être appliquée à les autres polymères en solution.

Key words: intrinsic viscosity, "single-point", "double-point", BSA

1 INTRODUCTION TO TECHNICAL FIBRE SUSPENSIONS

With rheology progress was made in understanding of structure of gels and colloidal suspensions, particularly with regard to polysaccharides and large glyconjugates. Whereas the rheology of concentrated dispersions is the considerable interest to the colloidal scientist, pharmacist or food technologist [1], of more interest to molecular biophysicist is dilute solution viscometry and the structural parameter the intrinsic viscosity [2]. The intrinsic viscosity is a viscosity measure extrapolated to infinite dilution that depends on the properties of isolated macromolecules in solution (effects of interaction have been eliminated by extrapolation). So, intrinsic viscosity provides information about molecular weight, radius of gyration, shape, specific volume, conformation, hydration, flexibility of the macromolecules. Several empirical equations relating viscosity to concentration [2 - 4] have been proposed in order to determine intrinsic viscosity $[\eta]$. The three most commonly used equations, concerning dilute solutions, are the following:

Huggins' equation [5] which relates the increment of relative viscosity (η_i) to concentration C:

$$\frac{\eta_i}{C} = [\eta]\left(1 + K_H[\eta]C\right)$$ (1)

IUPAC recommends the term "increment of relative viscosity (η_i)", instead of "specific viscosity", because it is a non-dimensional parameter that has no attributions of specific quantity, meaning:

$$\eta_i = \eta_{rel} - 1$$ (2)

We recall that

$$\eta_{rel} = \frac{\eta_s}{\eta_o} = \frac{\rho_s t_s}{\rho_o t_o}$$ (3)

where ρ is density, t is the drain time while the sub indexes s and o indicate "macromolecule solution" and "used solvent", respectively.

In an equivalent formulation, Kraemer [6] relates the logarithm of relative viscosity to concentration:

$$\frac{1}{C}ln\,\eta_{rel} = [\eta]\left(1 - K_K[\eta]C\right)$$ (4)

while Schulz-Blaschke [7] relates the first term of Huggins' equation to the increment of relative viscosity

$$\frac{\eta_i}{C} = [\eta]\left(1 + K_{SB}\eta_i\right)$$ (5)

K_H, K_K and K_{SB} represent the Huggins, Kraemer and Schulz-Blaschke non-dimensional constants, respectively, and $[\eta]$ is the intrinsic viscosity with units inverted to concentration (mL/g). The value for the intrinsic viscosity is obtained by plotting Equations 1, 4, or 5 and extrapolating to concentration zero. Upon this situation non-ideal effects and association phenomena are corrected. The name "intrinsic viscosity" is probably due to it being a function of only the macromolecule dispersed or dissolved. However, it frequently occurs that extrapolations do not have a common value at their origin ordinates. These deviations may be caused by experimental errors in the measurements or by inadequate linear extrapolations as well [8].

This extrapolation method is the most common technique used for $[\eta]$ determination, although the procedure is laborious and consumes a considerable amount of time and reagents. Because of this, several equations were developed to estimate intrinsic viscosity at one single concentration which do not require of a graphical adjustment either. They are known as "single-point" methods. "Single-point" equations assume that K_H, K_K and K_{SB} are constants and that $K_H + K_K = 0.5$, as is indicated by the combination of Equations 1 and 4 [3]. They all include the values for relative viscosity, increment of viscosity and concentration. For example, Solomon-Ciuta [9] propose:

$$[\eta] \cong \frac{1}{C}\sqrt{2\eta_i - 2\,ln\,\eta_{rel}}$$ (6)

In 1968, Deb and Chatterjee [10] suggested that:

$$[\eta] \cong \frac{1}{C}\sqrt[3]{3\,ln\,\eta_{rel} + \frac{3}{2}\left(\eta_i\right)^2 - 3\eta_i}$$ (7)

More recently, Ram-Mohan-Rao and Yassen [11] gave a simplified expression:

$$[\eta] \cong \frac{1}{2C}\left[\eta_i + ln\,\eta_{rel}\right]$$ (8)

Kuwahara [12] proposes the expression:

$$[\eta] \cong \frac{1}{4C}\left(\eta_i + 3\,ln\,\eta_{rel}\right)$$ (9)

while Palit and Kar [13] suggest

$$[\eta] \cong \frac{1}{C}\sqrt[4]{4\eta_i - 2\eta_i^2 + \frac{4}{3}\eta_i^3 - 4\,ln\,\eta_{rel}}$$ (10)

The Schulz-Blaschke equation (Equation 5) is also used for direct calculation of intrinsic viscosity [14], where it is assumed that $K_{SB} = 0.28$ (common value to several systems) [15].

$$[\eta] \cong \frac{\eta_i/C}{1 + K_{SB}\eta_i}$$ (11)

Chee [16] and Rao-Yassen [11] have examined the applicability of the "single-point" method and have found that some equations are inadequate or applicable only to some specific macromolecule-solvent systems. The present work is based on viscosity measurements of aqueous bovine serum albumin (BSA) solutions at different concentrations and pH, (data drawing on BSA conformers [17]). Following Harding [2], the results obtained from "single-point" equations are compared to the outcome from the Huggins procedure, considered one of the most popular methods for the determination of intrinsic viscosity.

2 MATERIALS AND METHODS

BSA (lyophilized and deionized powder, purity grade > 98%) was obtained from Fedesa-UNSL. Measurements were taken from fresh 2 % BSA solutions. pH was adjusted with HCl or NaOH 1N. Solutions and dissolutions were prepared with deionized water. Temperature was kept constant at 25 +/- 0.1°C using a LAUDA thermostatic bath. Determinations were done using an Ubbelohde "suspended level" viscosimeter (Schott-Geräte), with a water draining time of 123.53 s. Even though this viscosimeter works in a solution volume independent fashion and allows dissolutions, it was here used performing at least three measurements for each concentration and was later washed until the solvent draining time was recovered. Six solutions with concentrations ranging from 0.125 to 2 g/dL BSA were measured at each pH. These solutions were previously prepared from a mother solution with a concentration determined by UV-absorbency at 278 nm with a Shimadzu UV-160A spectrophotometer. Density of each solution was measured using an Anton Paar 35N densitometer.

3 RESULTS

Table 1 shows data obtained by applying the most common procedures to calculate intrinsic viscosity, namely, those based upon Equatios 1, 4 and 5, to the conformers *E*, *F*, *N* and *B* according to the Foster [18] classification. Table 2 include experimental data and error resulting from single-point Equations 6 to 11. The values in last column have been computed by using a "double-point" equation which will be described next. The values obtained by the Huggins method were taken as reference in the percentage error calcula-
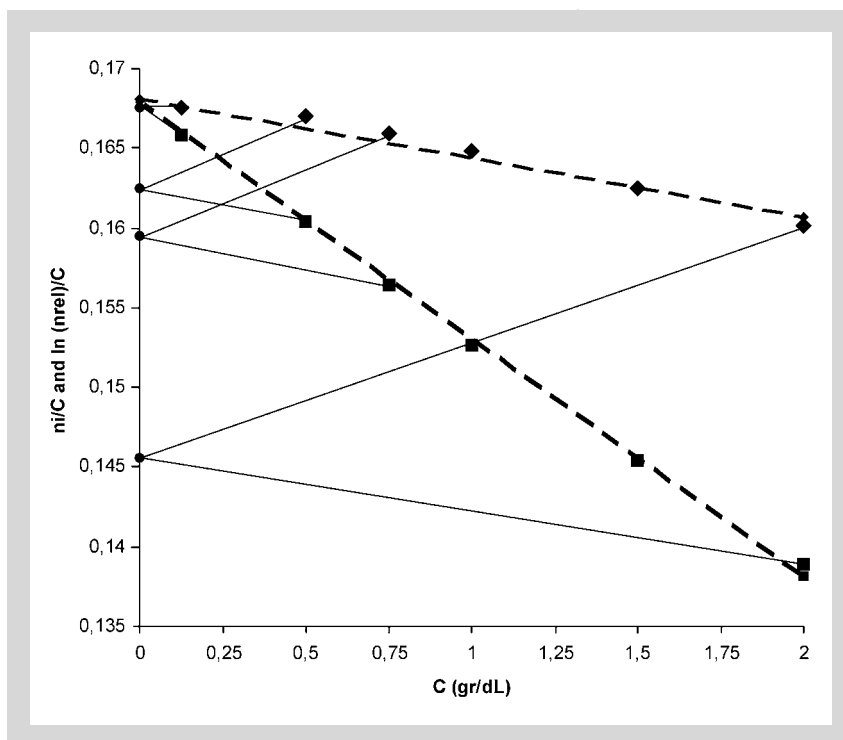
tion and the negative sign indicates that the value resulting from the application of the "single-point" method is lower than the reference value. Tables 3, 4, 5, and 6 containing similar information for other experimental conditions.

The most commonly used equation is the Salomón-Ciuta equation, which gives values with acceptable errors ranging from 1 to 15 %. Throughout the entire set of equations, determinations at pH = 8.1 present errors higher than expected and do no adjust properly. From Tables 2 to 6 it appears that none of the 'single-point' equations used provides an overall good estimation for [η]. Relative errors always increase when the concentration values become bigger. Figure 1 provides a graphical explanation for that observed behavior.

Figure 1:
Solomon-Ciuta estimation of [η] for the BSA-water system at pH = 2.71 (Dotted line: Multiple Linear Least-Squares Fits with a Common Intercept method; Solid lines: Solomon-Ciuta method at different concentrations).

Table 1:
Intrinsic viscosity at different pH values, determined by Huggins, Kraemer and Schulz-Blaschke methods.

Table 2:
Percentage errors for [η] values at pH = 2.71 with reference value [η]$_{ref}$ = 0.1687.

Conformer	E	F	physiological	N	B
pH	2.71	4.32	7.4	8.06	9.97
[η] Eq. 1	0.1687	0.0597	0.0484	0.0723	0.0654
[η] Eq. 4	0.1674	0.0596	0.0485	0.0719	0.0652
[η] Eq. 5	0.1688	0.0599	0.0486	0.0748	0.0656
K_H	-0.148	-0.959	1.579	-1.741	-0.842
K_K	0.517	1.295	-0.935	2.012	1.2
$K_K + K_H$	0.37	0.336	0.644	0.271	0.358
K_{SB}	-1.546	-1.055	1.358	-2.373	-0.951

C g/dL	η_{rel}	Chatterjee Eq. 7	Rao Eq. 8	Kuwahara Eq. 9	Palit Eq. 10	Schulz Eq. 11	Solomon - Ciuta Eq. 6	Double - Point Eq. 12
2	1.320	-11.50	-11.40	-14.54	-10.63	-12.92	-13.69	-0.83
1.5	1.243	-8.75	-8.75	-11.28	-8.24	-9.84	-10.55	-0.30
1	1.164	-5.82	-5.93	-7.74	-5.81	-6.60	-7.17	-0.18
0.75	1.124	-4.32	-4.49	-5.90	-4.67	-4.97	-5.45	0
0.5	1.083	-2.76	-2.98	-3.96	-3.66	-3.29	-3.67	-0.59
0.125	1.021	-0.81	-1.19	-1.45	-5.36	-1.26	-1.36	-1.19

C g/dL	η_{rel}	Chatterjee Eq. 7	Rao Eq. 8	Kuwahara Eq. 9	Palit Eq. 10	Schulz Eq. 11	Solomon-Ciuta Eq. 6	Double-Point Eq. 12
2	1.105	-13.65	-13.82	-14.91	-14.16	-14.18	-14.57	2.51
1.5	1.082	-9.52	-9.73	-10.63	-10.38	-10.01	-10.38	0.33
1	1.057	-6.13	-6.39	-7.04	-7.62	-6.58	-6.87	-0.33
0.75	1.043	-4.60	-4.90	-5.40	-6.67	-5.03	-5.19	-0.17
0.5	1.029	-2.98	-3.32	-3.67	-6.16	-3.41	-3.52	-0.67
0.125	1.007	-0.86	-1.35	-1.44	-15.19	-1.37	-1.34	-1.34

C g/dL	η_{rel}	Chatterjee Eq. 7	Rao Eq. 8	Kuwahara Eq. 9	Palit Eq. 10	Schulz Eq. 11	Solomon-Ciuta Eq. 6	Double-Point Eq. 12
2	1.112	12.96	12.75	11.24	12.39	12.24	11.78	-1.65
1.5	1.081	9.40	9.14	8.07	8.33	8.81	8.47	-1.03
1	1.052	6.06	5.76	5.09	4.20	5.57	5.37	0.41
0.75	1.038	4.75	4.41	3.92	2.18	4.28	4.13	1.03
0.5	1.025	3.67	3.29	2.97	-0.27	3.21	3.10	0.41
0.125	1.006	1.62	1.11	1.03	-17.01	1.09	1.03	1.03

C g/dL	η_{rel}	Chatterjee Eq. 7	Rao Eq. 8	Kuwahara Eq. 9	Palit Eq. 10	Schulz Eq. 11	Solomon-Ciuta Eq. 6	Double-Point Eq. 12
2	1.110	-25.56	-25.70	-26.68	-25.95	-26.02	-26.21	-11.09
1.5	1.086	-21.93	-22.11	73.43	-22.62	-22.36	-22.47	-2.08
1	1.063	-13.90	-14.13	-14.79	-15.10	-14.32	-14.29	-2.91
0.75	1.049	-11.12	-11.38	-50.45	-12.80	-11.53	-11.51	3.47
0.5	1.034	-6.23	-6.54	-76.73	-8.83	-6.65	-6.52	0.97
0.125	1.009	-0.60	-1.07	-98.46	-11.99	-1.10	-0.83	-0.83

C g/dL	η_{rel}	Chatterjee Eq. 7	Rao Eq. 8	Kuwahara Eq. 9	Palit Eq. 10	Schulz Eq. 11	Solomon-Ciuta Eq. 6	Double-Point Eq. 12
2	1.117	-12.86	-13.02	-14.23	-13.25	-13.43	-13.78	-2.30
1.5	1.090	-10.19	-10.39	-11.36	-10.93	-10.70	-10.87	-1.07
1	1.061	-7.11	-7.36	-8.05	-8.44	-7.56	-7.66	1.22
0.75	1.047	-4.95	-5.23	-5.78	-6.82	-5.39	-5.51	0.15
0.5	1.032	-3.18	-3.51	-3.89	-6.07	-3.61	-3.47	0.61
0.125	1.008	-0.02	-0.50	-0.60	-12.82	-0.52	-0.46	-0.31

Table 3:
Percentage errors for [η] values at pH = 4.32 with reference value [η]$_{ref}$ = 0.0597.

Table 4:
Percentage errors for [η] values at pH = 7.4 with reference value [η]$_{ref}$ = 0.0484.

Table 5:
Percentage errors for [η] values at pH = 8.06 with reference value [η]$_{ref}$ = 0.0723.

Table 6:
Percentage errors for [η] values at pH = 9.97 with reference value [η]$_{ref}$ = 0.0654.

The crosses represent the values of η_i/C and $\ln\eta_{rel}/C$ at the different concentrations levels for the BSA-water system at pH = 2.71, while the dotted lines are the adjusted lines obtained by the method introduced in [19], whose common intersection point at C = 0 provides an extrapolation estimation for [η]. Each pair of full lines passing through the points at concentrations 0.125, 0.5, 0.75 and 2 has a common intercept at C = 0 which is the Solomon-Ciuta "single-point" estimation for the corresponding concentration value obtained through Equation 6. This is one of the most used "one-point" estimation, and for the systems considered in this paper, it provides relative errors ranging from 1 to 27 %. The triangular regions determined by the full lines in Figure 1, which are all similar (a fact that can be proven rigorously), exhibit a behavior very different from that of the triangular region determined by the dotted lines which are used to get a reasonable estimation for [η]. Moreover, it is also clear now why the Solomon-Ciuta gives

worst estimations as the concentration level grows like all the other methods reported above, as indicated in Tables 2 to 6. It is worth noting that "single-point" estimators would provide good estimations for [η] whenever the rheological behavior of the solute-solvent system under study is well represented by a set of Huggins and Kraemer equations with graphic representations like those shown in Figure 2.

On the other hand, when a system does not present such an appropriate behavior, "single-point" methods will always provide estimations for [η] with poor relative errors, increasing with concentrations. Therefore, if a system has not been studied previously in order to determine which its behavior is going to be, 'single-point' methods expose oneself to get unacceptable errors in estimating [η]. As an alternative strategy we propose a 'double-point' method of estimation. In this article we propose to estimate [η] by

$$[\eta] = \frac{1}{2}\frac{C_2}{C_2 - C_1}\left(\frac{\eta_{i,1}}{C_1} + \frac{\ln\eta_{rel,1}}{C_1}\right) - \frac{1}{2}\frac{C_1}{C_2 - C_1}\left(\frac{\eta_{i,2}}{C_2} + \frac{\ln\eta_{rel,2}}{C_2}\right) \quad (12)$$

where $\eta_{rel,1}$, $\eta_{i,1}$ and $\eta_{rel,2}$, $\eta_{i,2}$ are the data gathering from levels $C_1 < C_2$ of concentrations. The estimation given by Eq. 12 coincides with the intercept at C = 0 of the line determined by the points (C_1, $1/2(\eta_{i,1}/C_1 + \ln\eta_{rel,1}/C_1)$) and ($C_2$, $1/2(\eta_{i,1}/C_2 + \ln\eta_{rel,2}/C_2)$), related to the Ram-Moham-Rao and Yassen [11] estimations based on the date obtained at concentrations C_1 and C_2 respectively. Indeed, the equation of the line joining these points is

$$[\eta](C) = \left[\frac{1}{2}\left(\frac{\eta_{i,2}}{C_2} + \frac{\ln\eta_{rel,2}}{C_2}\right) - \frac{1}{2}\left(\frac{\eta_{i,1}}{C_1} + \frac{\ln\eta_{rel,1}}{C_1}\right)\right]\frac{C_2}{C_2 - C_1}$$
$$- \left[\frac{1}{2}\left(\frac{\eta_{i,2}}{C_2} + \frac{\ln\eta_{rel,2}}{C_2}\right) - \frac{1}{2}\left(\frac{\eta_{i,1}}{C_1} + \frac{\ln\eta_{rel,1}}{C_1}\right)\right]\frac{C_1}{C_2 - C_1} + \frac{1}{2}\left(\frac{\eta_{i,1}}{C_1} + \frac{\ln\eta_{rel,1}}{C_1}\right)$$

from which we easily derive Equation 12 by evaluating [η](0). In Figures 3 and 4 we present, as we did in Figure 1, how the estimation for [η] by this method works for two different rheological behaviors. The estimations reported graphically in Figures 3 and 4, and analytically in Tables 2 to 6 below have been computed by Equation 12 using values gathered at consecutive values of concentration. For instance, the estimation of [η] reported at C = 0.75 used the values $\eta_{rel,2}$, $\eta_{i,2}$ obtained at C = 0.75 and the values $\eta_{rel,1}$, $\eta_{i,1}$

obtained at C = 0.5 as well. In the particular case of C = 0.125 where we do not have available data at a lower concentration, the reported estimation is that of Ram-Mohan-Rao and Yassen [11].

From the graphics we can see that the "double-point" method has a very consistent behavior when compared with the "Multiple Linear Least-Squares Fits with a Common Intercept method", although is even better in the case of the system dealt with in Figure 3. In the last column of Tables 2 to 6 we have included the percentage relative errors of the estimations obtained by our 'two point' method given in Equation 12 and where the observations made in the previous paragraph have been taken into account.

4   CONCLUSION

Although the suitability of 'single-point' equations has been objected, some automated viscosimeters have already incorporated them into their software. Also, there is a continued interest in verifying their applicability to different systems [15]. However, we have not found any reference towards biological macromolecule solutions. In this work we have studied the BSA-water system and pH variation. BSA is a non-linear, ramified, highly cross-linked molecule with strong internal and water interactions, in which it is soluble. BSA is globular, heart shaped at neutral pH [20] and has more extended conformations as pH changes. The intrinsic viscosity of BSA solutions changes at different pH because BSA size and shape are modified. Regarding diluted BSA solutions, viscosity dependence on concentration is unusual [17, 21].

The highest errors are observed in the run at pH 8.06, with data presenting a linear regression (R^2) of 0.983, but with a negative and very high K_H. Also, the sum of the K_H and K_K constants is more deviated from the 0.5 value. At the remaining runs, $K_H + K_K$ deviates approximately 25 % and error decreases 50 %. All runs have values of $\eta_i < 0.1$ [10] but the obtained values were acceptable. All equations show dependence on concentration and enhance their outcome at concentrations lower than 1 %, except for Equation 10 that shows a non-systematic behavior. $K_{SB} = 0.28$ was used in Equation 11 [15] although a very different value is observed in Table 1. In general, it can be stated that these equations (except Equation 10) can be used for approximate determinations of intrinsic viscosity in the system BSA-water when concentra-

tions are inferior to 0.75 %. For higher concentrations, all "single-point" estimators provide poor estimations, and a graphical explanation for this behavior is provided. Therefore, we propose a "double-point" method to estimate [η] which have given errors smaller then 3 % in all the system studied in this paper, except for the case pH = 8.6 where an error of 11 % is observed, although it may be due to two experimental points which seem to be no coherent [17]. Our method not require a graphical adjustment and is more economic than that presented in [19], which simultaneously adjust series of Huggins and Kraemer's values with two lines with a common intercept at C = 0, providing good estimates for [η], but requiring of at least four determinations.

Methods based upon "single point" equations are strongly dependent on the system under consideration, its concentration and the verification of the condition $K_H + K_K = 0.5$. On the other hand, regarding the BSA-water system (which presents different rheological behaviors), the "double-point" method of estimation performs better, exhibits less dependence on the concentration and does not require of previous knowledge about the values of K_H and K_K.
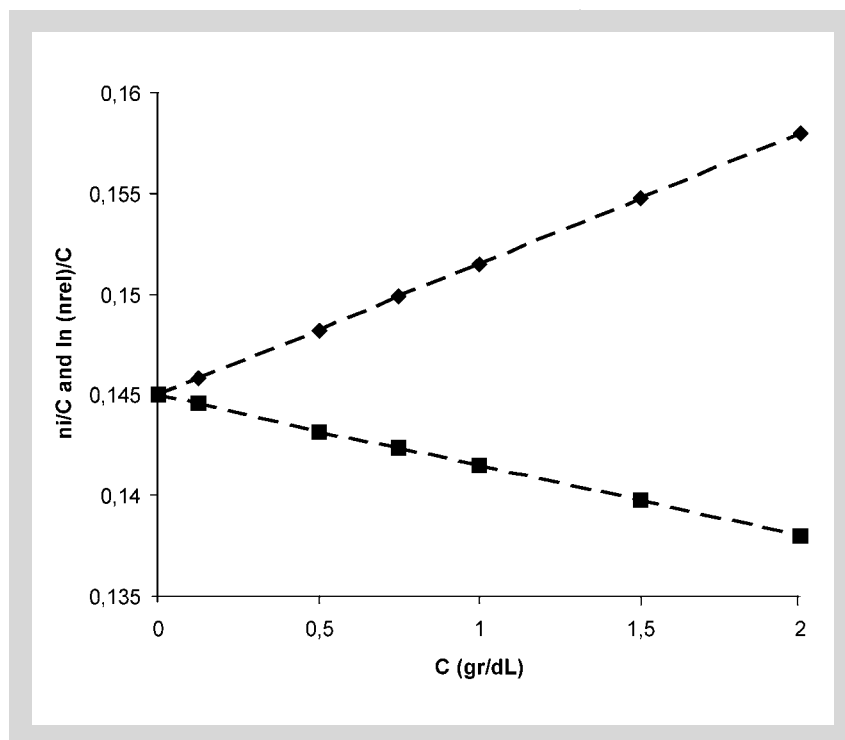
ACKNOWLEDGMENTS

Figure 2:
Graphic representation (Huggins and Kraemer's equations) of the results of viscosimetric measures of a solute-solvent system for which 'single-point' estimators provides good estimations for [Ë].
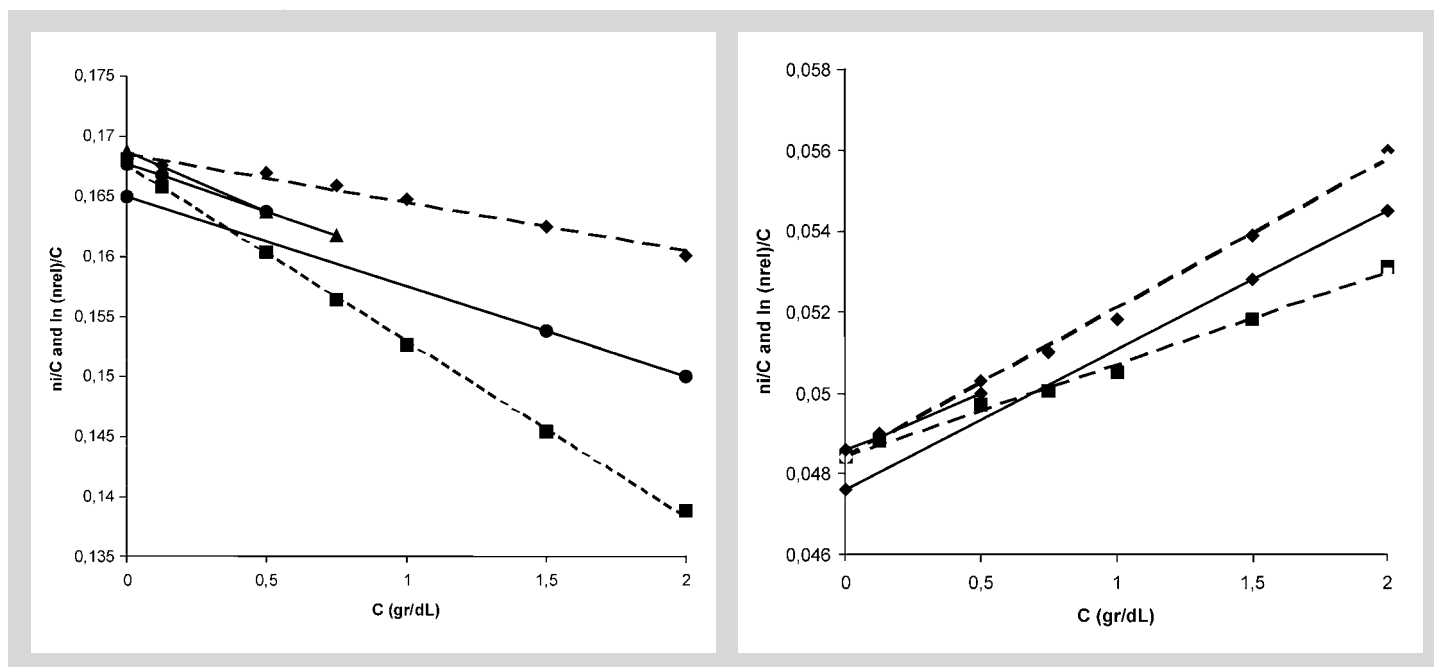
Figure 3 (left):
'Double-point' estimation of [η] for the BSA-water system at pH = 2.71 (Dotted line: Multiple Linear Least-Squares Fits with a Common Intercept method; Solid lines: Lines joining two consecutive Ram-Moham-Rao and Yassen estimations (represented by the solid squares in the graphic)). The double-point estimation is the point of each solid line just on the vertical axis.

Figure 4:
'Two-point' estimation of [η] for the BSA-water system at pH = 7.4 (Dotted line: Multiple Linear Least-Squares Fits with a Common Intercept method; Solid lines: Lines joining two consecutive Ram-Moham-Rao and Yassen estimations (represented by the solid squares in the graphic)). The double-point estimation is the point of each solid line just on the vertical axis.

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