Determination of Flory's Parameter Φ for Proteins Based on the Modified Universal Calibration of the Gel Permeation Chromatography

Anastasios Dondos

University of Patras, Department of Chemical Engineering, 26504 Patras, Greece

Received May 9, 2007
Revised Manuscript Received July 11, 2007

In a recent article[1] we have shown that we have a good applicability of the modified universal calibration of gel permeation chromatography (GPC)[2] in the case of native proteins. In this article[1] we have used the results of GPC obtained on proteins in three different laboratories.[3−5] In order to show that the modified universal calibration is of general applicability, we study here the results of GPC coming from a number of other different laboratories. Moreover, we will show here that the modified universal calibration presents a good applicability even in the case of denatured proteins.

The goal of this note is not to provide a way to measure the molecular mass of unknown proteins but to propose a method in order to obtain the Φ values of native proteins, and from these values we will discuss, in the end of this note, why the Flory's factor varies so such among native proteins; the principal parameter affecting the Φ value could be the compactness of the protein.

According to the modified universal calibration of GPC[2] we plot $\log([\eta]M/\Phi)$ versus elution volume, expressing the hydrodynamic volume of the molecules by $[\eta]M/\Phi$. In the "classical" universal calibration[6] the hydrodynamic volume is expressed by $[\eta]M$ considering Φ as a constant. Nevertheless, the value of Flory's parameter Φ is only constant for the synthetic linear polymers which do not present a draining effect or an important polarity ($\Phi = 2.6 \times 10^{23}$ in cgs). In the case of nonlinear polymers,[7,8] polar polymers,[9−11] and nonpolar polymers presenting a draining effect,[12] the value of Φ is different, in general, from the value of 2.6×10^{23}, and the "classical" universal calibration presents failures.

The value of Φ for a given native protein is calculated considering that in the same elution volume, all polymers, when they do not present interactions with the GPC columns, must present the same hydrodynamic volume. We have based[1] our estimation on the GPC results of Miller et al.[3] obtained with fractions of polystyrene (PS) and different proteins, using the same columns. We have estimated[1] the deviations of the points obtained with different proteins from the curve obtained with the PS fractions in the "classical" universal calibration[3] ($\log[\eta]M$ vs elution volume), and we have calculated[1] the Flory's parameter for nine proteins. More precisely, in the same elution volume of this calibration we must have the same hydrodynamic volume for the polystyrenes (PS) and proteins (Pr), and according to the Fox−Flory theory[13] we must have $[\eta]_{PS}M_{PS}/2.6 \times 10^{23} = [\eta]_{Pr}M_{Pr}/\Phi_{Pr}$. From this relation we have obtained the value of Flory's parameter for the proteins. We have now calculated the Flory's parameter for another six native proteins investigated in the article of Miller et al.,[3] according to the procedure described in the previous article,[1] and the results for the 15 proteins are given in Table 1.

Table 1. Calculated Values of Flory's Parameter for 15 Native Proteins

no.	protein	$\Phi\ 10^{-23}$ (cgs)
1	insulin	6.8
2	lysozyme	6.1
3	hemoglobin	5.0
4	chymotrypsinogen	4.6
5	trypsin	4.6
6	pepsin	3.5
7	catalase	3.0
8	ribonuclease	2.6
9	ovalbumin	2.6
10	myoglobin	2.6
11	transferrin	2.1
12	cytochrome *c*	2.0
13	γ-globulin	2.0
14	serum albumin	1.2
15	β-lactoglobulin	1.2

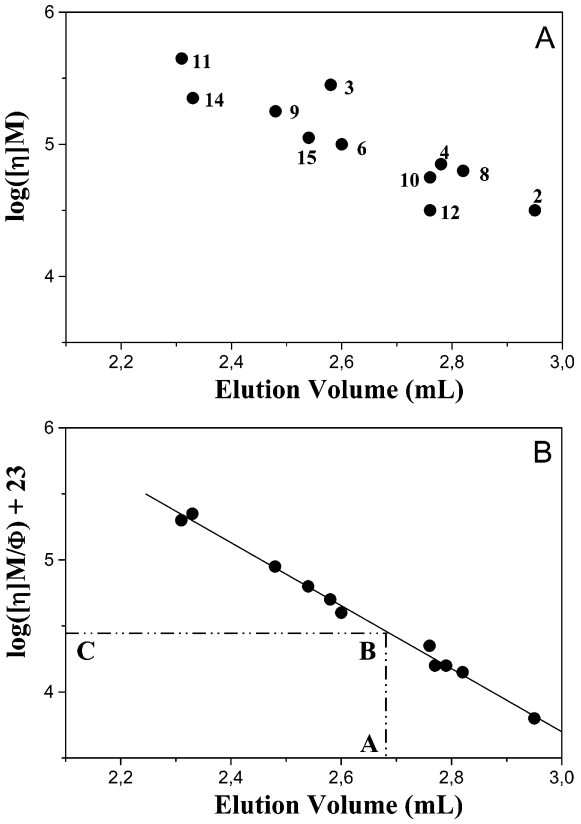

Figure 1. (A) GPC "classical" universal calibration of 11 native proteins. Numbers correspond to the numbers of Table 1 and identify the proteins. (B) GPC modified universal calibration of the same proteins (results from ref 14). Experimental conditions: apparatus Waters 6000A; column, Intel model 730; eluent, 0.1 M sodium acetate, 0.1 M sodium sulfate, pH 5.0; stationary phase, diol-bonded silica gel. For the letters A, B, and C see the end of the text.

Although the authors of the articles from which we have taken the GPC results, as Miller et al.,[3] say that they have not observed any notable interactions between the proteins and the columns, we can say that some minor errors have been interfered in the values of Φ in Table 1.

10.1021/bm700508y CCC: $37.00
Published on Web 08/15/2007

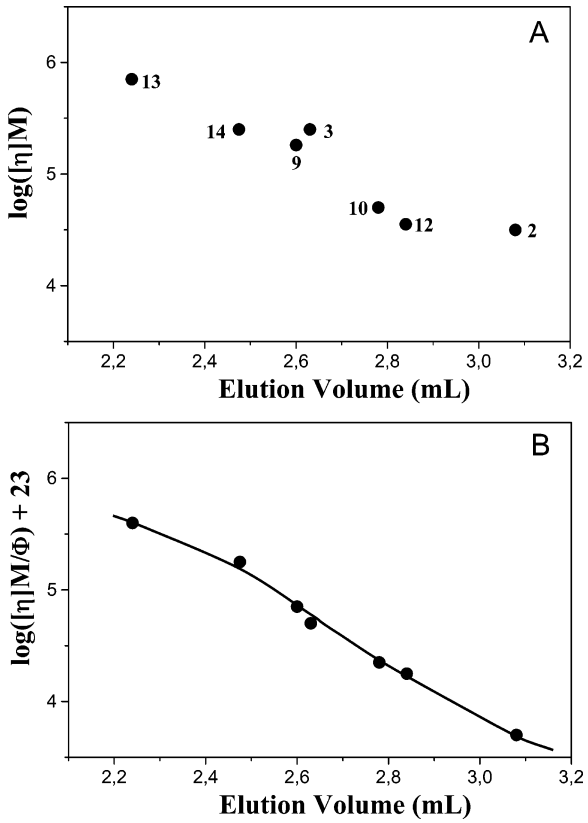

Figure 2. (A) GPC "classical" universal calibration of seven native proteins. Numbers correspond to the numbers of Table 1 and identify the proteins. (B) GPC modified universal calibration of the same proteins (results from ref 15). Experimental conditions: apparatus Waters 6000A; column, twine-80-coated diphenylsilica; eluent, 0.02 M phosphate buffer containing 0.1 M Na_2SO_4, pH 6.8.

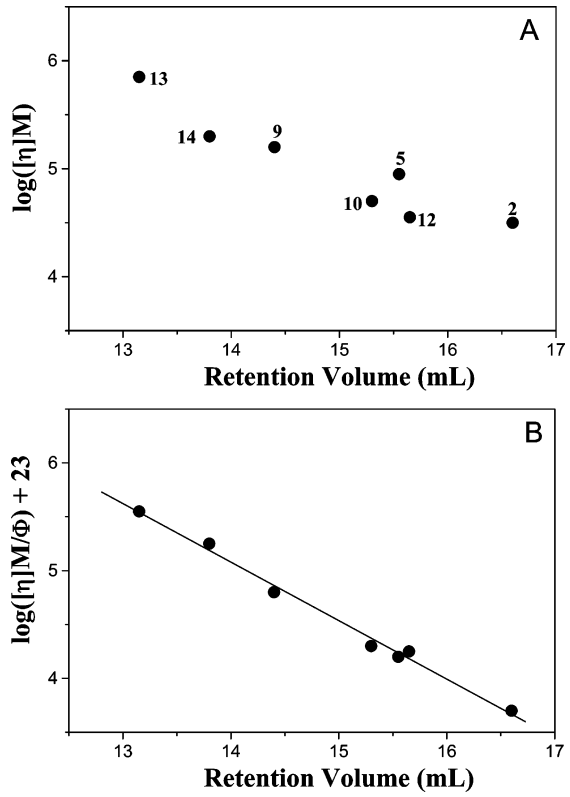

Figure 3. (A) GPC "classical" universal calibration of seven native proteins. Numbers correspond to the numbers of Table 1 and identify the proteins. (B) GPC modified universal calibration of the same proteins (results from ref 16). Experimental conditions: column, glyceryl−propyl-bonded silica gel; eluent, 0.1 M phosphate buffer + 0.2 M NaCl, pH 7.0.

We present, in the following, the application of the "classical" universal calibration of GPC[6] (hydrodynamic volume $\sim [\eta]M$) and the modified universal calibration[2] (hydrodynamic volume $\sim [\eta]M/\Phi$ according to the Fox−Flory theory[13]), for four groups of proteins. The values of Φ, necessary for the second presentation, are the values given in Table 1. The values of the intrinsic viscosity, $[\eta]$, of each protein, if they are not given in the article from which the GPC results are obtained, are found in other articles of the literature.

In Figure 1A we present the "classical" universal calibration and in Figure 1B the modified universal calibration for a group of 11 proteins. These proteins have been studied by Schmidt et al.[14] We can see that the points obtained in Figure 1A with the proteins do not lie in the same curve. This indicates that the hydrodynamic volume is not correctly expressed by $[\eta]M$. On the contrary, when $\log([\eta]M/\Phi)$ versus elution volume is plotted, the points obtained with the same proteins lie approximately in the same curve (Figure 1B). This shows that we have an applicability of the modified universal calibration, or in other words, it is correct to express the hydrodynamic volume of proteins by $[\eta]M/\Phi$ with the values of Φ as it is calculated by the described procedure in ref 1.

The results of GPC obtained by Chang[15] are presented in Figure 2A according to the "classical" universal calibration and in Figure 2B according to the modified universal calibration. Also with these proteins we have a good applicability of the modified universal calibration (Figure 2B).

A linear plot was assumed in most cases for the GPC calibration, but this is debated in the literature, so in Figure 2B (and in Figure 4C) a small deviation from linearity was preferred.

In Figure 3A we present the application of the "classical" universal calibration using the results of Mori et al.,[16] while with the same results we present the application of the modified universal calibration in Figure 3B. We can see that only the second calibration presents a good application.

Corbett and Roche[17] have studied in GPC not only a certain number of native proteins but also the polypeptides derived from these proteins after their denaturation with 6.0 M GuHCl. For the denatured proteins we have used a value for Φ equal to 1.33×10^{23}. This value is obtained from the relation

$$\Phi = (0.52 \times 10^{23})a^{-2.32} \qquad (1)$$

established for the polymers presenting a draining effect.[12] In the above relation a is the exponent in the Mark−Houwink−Sakurada (MHS) representation ($\log[\eta]$ vs $\log M$). The denatured proteins are considered as polymers which constitute a homologous series,[18] and we can apply with these products the MHS equation. The value of the exponent a in the MHS representation is equal to 0.666,[18] and from eq 1 we obtain $\Phi = 1.33 \times 10^{23}$.

In Figure 4A we present the "classical" universal calibration for the native and denatured proteins studied by Corbett and Roche.[17] The points obtained with the denatured proteins lie on the same curve, and this is expected because for these proteins we have the same value for Φ. On the contrary, the points obtained with the native proteins do not lie in the same curve, as in the case of native proteins of the preceding systems (Figures 1A, 2A, and 3A). Plotting in Figure 4B $\log([\eta]M/\Phi)$ versus elution volume taking the values of Φ from Table 1 for native proteins and considering the denatured proteins as polymers which do not present a draining effect ($\Phi = 2.6 \times$

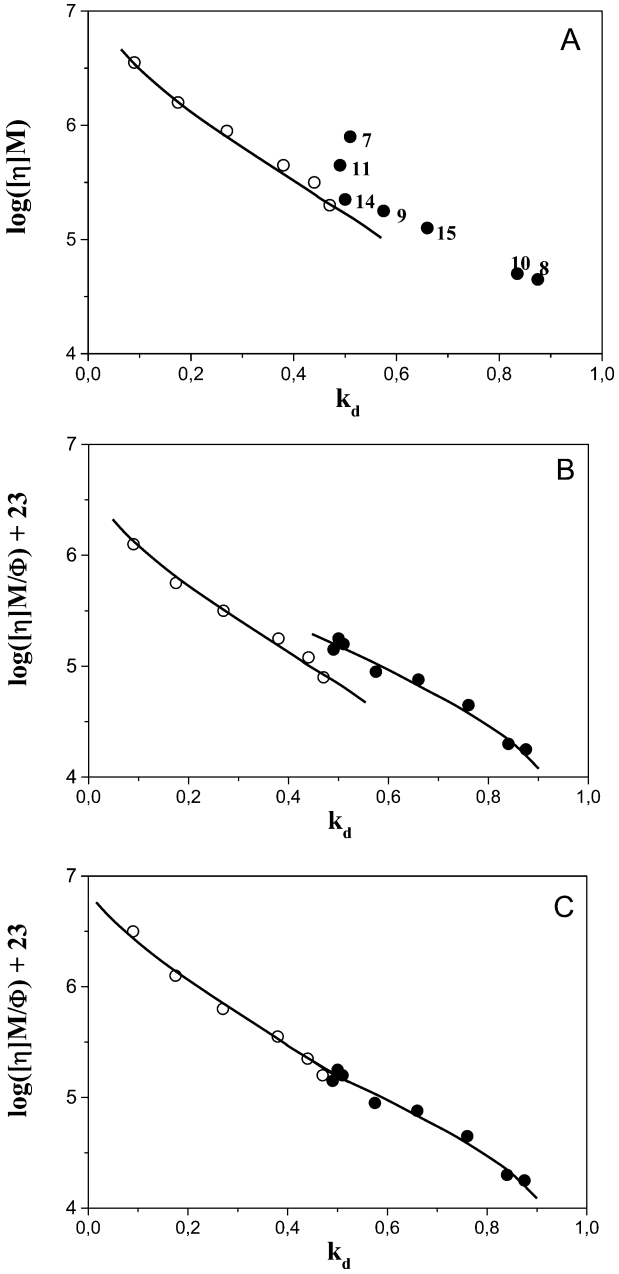

Figure 4. (A) GPC "classical" universal calibration of six denatured proteins (○) and eight native proteins. (●) Numbers correspond to the numbers of Table 1 and identify the native proteins. (B) GPC modified universal calibration of the same denatured (○) and native (●) proteins. The Φ value for the denatured proteins is equal to 2.6×10^{23}. (C) GPC modified universal calibration of the same denatured (○) and native proteins (●). The Φ value for the denatured proteins is equal to 1.33×10^{23} (results from ref 17). Experimental conditions: native proteins in 0.2 M NaCl in TSK 3000W column; denatured proteins in 6 M GuHCl and the same column.

10^{23}) we obtain two distinct curves. If now we apply the modified universal calibration considering the denatured proteins as polymers presenting a draining effect ($\Phi = 1.33 \times 10^{23}$, from eq 1) we obtain a unique curve with these polypeptides and the native proteins (Figure 4C).

We must indicate that we have also a good applicability of the modified universal calibration using the results obtained on proteins by Fukano et al.[19] and Ahmed and Modrek.[20]

Assuming this validity of the modified universal calibration, the proposed methodology for calculating the Φ_{Pr} value of a given protein is the following:

First, with some proteins of Table 1 we established, under given conditions, the modified universal calibration curve, as, for example, in Figure 1B. Second, we measure the elution volume of the given protein under the same experimental conditions; this defines point A in Figure 1B. Third, we draw the straight line which is parallel to the *y*-axis in Figure 1B to intersect the curve representing the modified universal calibration; this defines point B in Figure 1B. The value of point B in the *y*-axis of Figure 1B defines point C, whose value is the hydrodynamic volume of the protein. Knowing $[\eta]_{Pr}$ and M_{Pr} of this protein we obtain the value of Φ_{Pr} from the relation $C = [\eta]_{Pr}M_{Pr}/\Phi_{Pr}$ which provides an estimate of the protein's compactness.

Finally, let us present some comments about the compactness of the proteins. Most of the proteins of Table 1 present a value of the Flory's parameter Φ which is equal or higher than 2.6×10^{23}. This means that these products present a low permeability to the solvent as the semipermeable synthetic polymers ($\Phi = 2.6 \times 10^{23}$) or a very compact conformation as it is, in general, considered for the proteins ($\Phi \geq 3 \times 10^{23}$). The values of Φ for hemoglobin, lysosyn, and insulin tend to the value of Φ presented by the very compact star polymers with 19 branches[8] ($\Phi = 8.5 \times 10^{23}$). For the serum albumin and β-lactoglobulin, on the contrary, the obtained values of Φ are very low ($\Phi = 1.2 \times 10^{23}$) indicating that these proteins present a free draining effect as the wormlike polymers and the denatured proteins examined here ($\Phi = 1.33 \times 10^{23}$). The native proteins, contrary to the denatured proteins, present intramolecular bonds which lead to compact conformations. We are obliged to accept that we have some drained parts in the periphery of the protein molecules[18] or some of proteins present binding cavities favoring an important draining. These cavities are indeed observed in the case of the last two proteins of Table 1.[21]

References and Notes

(1) Dondos, A. *J. Chromatogr., A* **2006**, *1127*, 183−186.
(2) Dondos, A.; Papanagopoulos, D. *J. Polym. Sci., Part B: Polym. Phys.* **2003**, *41*, 707−710.
(3) Miller, N. T.; Feibush, B.; Karger, B. L. *J. Chromatogr*. **1985**, *316*, 519−536.
(4) le Maire, M.; Viel, A.; Moller, V. *Anal. Biochem*. **1989**, *177*, 50−56.
(5) Frigon, R. P.; Leypoldt, J. K.; Uyeji, S.; Henderson, L. W. *Anal. Chem*. **1985**, *55*, 1349−1354.
(6) Grubisic, Z.; Rempp, P.; Benoît, H. *J. Polym. Sci.* **1967**, *B5*, 753−759.
(7) Roovers, J.; Toporowski, P.; Martin, J. *Macromolecules* **1989**, *22*, 1897−1903.
(8) Papanagopoulos, D. *J. Polym. Sci., Part B: Polym. Phys.* **2005**, *43*, 2388−2391.
(9) Dondos, A. *J. Polym. Sci., Part B: Polym. Phys.* **2006**, *44*, 1106−1113.
(10) Dubin, P. L.; Tecklemburg, M. M. *Anal. Chem.* **1985**, *57*, 275−279.
(11) Mori, S. *Anal. Chem.* **1989**, *61*, 530−534.
(12) Dondos, A.; Staikos, G. *Colloid Polym. Sci.* **1995**, *273*, 626−632.
(13) Fox, T. G.; Flory, P. J. *J. Phys. Colloid Chem*. **1949**, *53*, 197−212.
(14) Schmidt, D. E.; Giese, R. W., Jr.; Cornon, D.; Karger, B. L. *Anal. Chem*. **1980**, *52*, 177−182.
(15) Chang, J. P. *J. Chromatogr.* **1984**, *317*, 157−163.
(16) Mori, S. In *Size Exclusion Chromatography*; Mori, S., Barth, H. G., Eds.; Springer: Berlin, 1999; Chapter 11.3.2.
(17) Corbett, R. J. T.; Roche R. S. *Biochemistry* **1984**, *23*, 1888−1894.
(18) Tanford, C. *Adv. Protein Chem.* **1968**, *23*, 121−281.
(19) Fukano, K.; Kamiya, K.; Sasaki, H.; Hashimoto, T. *J. Chromatogr.* **1978**, *166*, 47−54.
(20) Ahmed, F.; Modrek, B. *J. Chromatogr.* **1992**, *599*, 25−33.
(21) Peyre, V.; Lair, V.; Andre, V.; le Maire, G.; Kragh-Hansen, U.; le Maire, M.; Moller, J. V. *Langmuir* **2005**, *21*, 8865−8875.

BM700508Y