Julio A. Deiber[1]
Marta B. Peirotti[1]
María V. Piaggio[2]

[1]Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), Universidad Nacional del Litoral (UNL), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Santa Fe, Argentina
[2]Cátedra de Bioquímica Básica de Macromoléculas, Facultad de Bioquímica y Ciencias Biológicas, UNL, Santa Fe, Argentina

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Research Article

Interplay between electrophoretic mobility and intrinsic viscosity of polypeptide chains

The present work is motivated specifically by the need to find a simple interplay between experimental values of electrophoretic mobility and intrinsic viscosity (IV) of polypeptides. The connection between these two properties, as they are evaluated experimentally in a formulated dilute solution, may provide relevant information concerning the physicochemical characterization and separation of electrically charged chains such as polypeptides. Based on this aspect, a study on the relation between the effective electrophoretic mobility and the IV of the following globular proteins is carried out: bovine carbonic anhydrase, staphylococcal nuclease, human carbonic anhydrase, lysozyme, human serum albumin. The basic interpretation of the IV through polypeptide chain conformations involves two unknowns: one is the Flory characteristic ratio involving short-range intramolecular interactions and the other is the Mark–Houwink exponent associated with large-range intramolecular interactions. Here, it will be shown via basic and well-established electrokinetic theories and scaling concepts that the IV and global chain flexibility of polypeptides in dilute solutions may be estimated from capillary zone electrophoresis, in addition to classical transport properties. The polypeptide local chain flexibility may change due to electrostatic interactions among closer chain ionizing groups and the hindrance effect of their associated structural water.

Keywords:
Electrophoretic mobility / Flory characteristic ratio / Friction power coefficient / Global chain flexibility / Intrinsic viscosity. DOI 10.1002/elps.201100637

1 Introduction

Differences in electrophoretic mobility of polypeptide chains are relevant for their effective separation in a variety of formulated dilute solutions, at a given pH and ionic strength I. In this regard, the method of capillary zone electrophoresis (CZE) has been regularly applied to separations and physicochemical characterizations of proteins and peptides ([1–4] and citations therein). In the last years, major emphasis has been placed on the use of experimental electrophoretic mobility to investigate true hydrodynamic and electrokinetic properties of polyampholyte–polypeptide chains, mainly in what concerns to their transport properties and associated global conformational and structural parameters, which are required, for instance, in biological studies. These evaluations are possible because the experimental methods used are based on appropriate theoretical frameworks considering both hydrodynamic and electrostatic effects. In general, CZE presents several complex phenomena that must be accounted appropriately, like the charge regulation phenomenon established among different ionizing groups of amino acids residuals in the amino acid sequence (AAS), including free ions in the solvent [5–10]. The subtle coupling between chain hydrodynamic and electrostatic phenomena affecting size, shape, and effective charge of polypeptide requires a better elucidation.

In this wide framework, previous and early studies concluded that the evaluation of the ratio Z/f, between the macromolecular effective charge number Z and the chain hydrodynamic friction f, as obtained from modeling analyte electrophoretic mobility, is an important experimental result from CZE in order to understand phenomenological aspects involving hydrodynamic and electrostatic chain dynamics, in dilute solutions for different pH and I ([11–20] and many citations therein). At present, important transport properties of polypeptide chains such as the average diffusion,

Correspondence: Dr. Julio A. Deiber, INTEC, Güemes 3450, S3000GLN, Santa Fe, Argentina
E-mail: treoflu@santafe-conicet.gov.ar
Fax: +54-(0)342-4550944

Abbreviations: AAS, amino acid sequence; **BCA**, bovine carbonic anhydrase; **CG**, collapsed globule; **HC**, hybrid chain; **HCA**, human carbonic anhydrase; **HSA**, human serum albumin; **IV**, intrinsic viscosity; **LRII**, large-range intramolecular interactions; **LSZ**, lysozyme; **PE**, polyelectrolyte; **PLLCEM**, perturbed Linderstrøm–Lang capillary electrophoresis model; **SRII**, short-range intramolecular interactions; **STN**, staphylococcal nuclease

sedimentation, and friction coefficients may be estimated quantitatively in different dilute solutions from CZE models [7–9, 21–26].

In a more classical framework and still with similar purposes, the viscometric measurement of the intrinsic viscosity (IV) of charged macromolecules in solution has also been a relevant experimental tool frequently used in the studies of polypeptide chain conformations. Nevertheless, this property does not allow extracting direct electrostatic information of polypeptides because it is derived mainly from a hydrodynamic-sensitive phenomenon [27–29]. Further, the basic interpretation of the IV from the point of view that accounts chain conformations (see also below) includes two unknown, one is the Flory characteristic ratio involving short-range intramolecular interactions (SRII) and the other is the Mark–Houwink exponent associated with large-range intramolecular interactions (LRII), thus indicating the need of additional experimental information when chains in solution under study are not at the unperturbed state (the theta condition is not satisfied). Therefore, based on these conceptual aspects, here we will show that the electrophoretic mobility may provide important data concerning the coupling between hydrodynamic and electrostatic states of polypeptides to interpret better IV values. Thus, the CZE method allows one the estimation of polypeptide average friction coefficient, the value of which is then required for the estimation of average diffusion and sedimentation coefficients, and also global structural parameters and conformations apart from the whole chain electrostatic state described in part by the effective electrical charge [7–9, 26].

The present work is motivated specifically on the need of finding a simple interplay between experimental values of electrophoretic mobility and IV of proteins. The connection between these two properties, as they are evaluated experimentally in a formulated dilute solution, may provide interesting complementary information concerning the physicochemical characterization and separation of electrically charged chains, such as polypeptides. Based on this aspect, here we carry out a study on the relation between the effective electrophoretic mobility μ_p and the IV [η] of the following typical globular proteins, by using their experimental electrophoretic mobility data reported in the literature: bovine carbonic anhydrase (BCA) [17], staphylococcal nuclease (STN) [13], human carbonic anhydrase (HCA) [30], lysozyme (LSZ) [15], and human serum albumin (HSA) [6]. Their physicochemical properties obtained from theoretical and numerical characterizations are reported in [7, 9]. It is expected that a rather simple interplay between CZE and IV may help to deepen the understanding of polypeptide functional properties in biological studies. For instance, at present, it is not fully understood the mechanisms by which proteins like enzymes and antibodies display the ability to carry out both flexible motions and still maintain their fold structure in the native state [31]. In this regard, there is also a need to establish a method to estimate chain flexibility in order to identify oligopeptide segments and peptides of high antigenicity ([32–36] and citation therein). In several works, normalized scales providing the degree of flexibility-in-chain of each amino acid have been reported, where some of them were based on the atomic temperature factor (B values) evaluated from X-ray microscopy, allowing the estimation of the overall average chain flexibility ([37] and citations therein).

Here, it will be shown, mainly via basic and well-established electrokinetic theories and scaling concepts, that the IV and global chain flexibility of polypeptides in dilute solutions may be also estimated from CZE, in addition to classical transport properties. It is clear that these properties are significantly controlled by the chain friction coefficient, and consequently the effect of intrachain hydrodynamic interactions must be considered, in principle within a simple theoretical framework that is amenable to be discussed through analytical expressions. This is one of the main targets of this work by starting from the modeling of the electrophoretic mobility of these analytes, where the coupling between hydrodynamic and electrostatic effects is included.

The manuscript is organized as follows. In Section 2, first we briefly present basic equations required to estimate [η] and global flexibility of globular proteins from the method of CZE, when true physicochemical parameters and properties are available and used (mainly the chain friction coefficient f and the friction power coefficient g_f as they are defined below and in previous works [7–9, 26]). For this purpose, electrostatic and scaling considerations and concepts associated with random fractals of chains are also introduced in quite general expressions of the IV already available in the literature as derived from previous rigorous kinetic theories. Here, the hypotheses necessary to obtain results and conclusions within typical CZE experimentation ranges of polypeptides chains are presented.

In this regard, the linear CZE theory for rather low particle zeta potentials is briefly analyzed within the framework proposed above. This procedure has been already used and discussed in details [6–9, 26] for analytes having relatively small effective charge numbers and for negligible free ion convection and relaxation (see basic concepts in [22, 38–42]) thus yielding a simple model code designated perturbed Linderstrøm–Lang capillary electrophoresis model (PLLCEM) [6–9] (see also [22, 40] concerning in particular the relaxation effect of highly charged particles).

It is important to indicate that no details concerning the atomic structure of analytes are considered here. Models describing in deep these aspects may be found in the literature [21–25, 40, 43–48]. In this regard, the boundary element method to evaluate the friction and diffusion coefficients and the IV of an arbitrary shape particle is reported as one of the powerful numerical methods appropriate for these purposes. The analyses carried out in these works are certainly out of the target of the present work involving a description of the global interplay between electrophoretic mobility and IV of polypeptide chains. In fact, we work at the level of conformational scales only. Therefore, in Section 3, we show how CZE may be used to estimate the IV of several proteins, which were studied and discussed in the literature. Thus, both electrophoretic mobility and IV measurements

provide complementary information of proteins, allowing estimations of their electrokinetic parameters and chain conformations. Here, in particular, polypeptide global chain flexibility in terms of both the Flory characteristic ratio C_N and the Mark–Houwink exponent ν are studied and discussed. In fact, we show that these chain parameters may be estimated from modeling the interplay between μ_p and $[\eta]$. Finally, important conclusions are provided together with further needs for future researches.

2 Theoretical analysis

2.1 Theoretical considerations for estimating polypeptide IV from CZE

Here, the polypeptide chain is considered simply as a sequence of beads, each one with an average radius a_o and molar mass $M_m = M/N$, representing the conformation of the N amino acid residues that composes a chain of well-known AAS of molar mass M (see also, [21, 23, 24] for a more rigorous hetero-chain description within this type of framework). Therefore, it is also possible to estimate the average radius of amino acid residues $a_o = \sum_{i=1}^{N} a_i/N$, where $a_i = \{3v_i M_i/(4\pi N_A)\}^3$ is the radius of the i-amino acid residue, having specific volume v_i and molar mass M_i, as described elsewhere [7, 8, 26] (the List of Symbols is in the Supporting Information). Here, N_A is the Avogadro constant. It is also defined the total solvent–chain friction coefficient through $f = 6\pi\,\eta_s a_o\, N^{g_f}$, where η_s is the formulated solvent viscosity and $g_f \leq 1$ is the corresponding friction power coefficient [26]. The maximum friction is achieved when $g_f = 1$ for an ideal free draining chain in creeping flow with negligible intrachain hydrodynamic interactions [26,29]. In addition, $1/3 < g_f < 1$ when hydrodynamic interactions among chain units is present [9,49,50]. These limit values for both neutral homopolymer chains and polypeptide heteropolymer chains are obtained through different mechanisms. In the former chains, they relate mainly to the effect of solvent quality and temperature, while in the later ones they are due significantly to the electrical state of particles superposed to the effect of solvent and temperature. For the chain unperturbed state $g_f \approx 1/2$, while for globular polypeptides when fluctuation-attractive electrostatic interactions predominate (or when the solvent is poor for neutral chains) $g_f \approx 1/3$, yielding a collapsed globule (CG) conformation [9, 49]. On the other side, for self-avoiding random chains $g_f \approx 3/5$.

Although the IV may be measured quite simply, it is also clear that its theoretical interpretation at the molecular level is rather complex as it may be judged through a high number of publications, where rather neutral chains are under study ([27, 29, 43–48, 51, 52] and citations therein). From the chain statistical point of view, the evaluation of the IV requires the knowledge of two parameters, one is the characteristic ratio C_N describing SRII and defining local chain flexibility for a few interconnected bonds [27,29] and the other is the friction power coefficient g_f related to LRII, where solvent–chain, chain–chain, and solvent–solvent interaction energies at a given temperature are relevant [7, 9, 26, 28, 29, 53]. In particular, polypeptide hydrodynamic and electrokinetic properties are coupled through these two parameters. On the other hand, from pure hydrodynamic concepts, it is well known that the IV depends on macroscopic properties defining shape and size of the suspended particle [28, 29] as follows,

$$[\eta] = \frac{\eta - \eta_s}{C\eta_s} = \beta(v_p + \delta v_w) \tag{1}$$

In this equation, η is the polypeptide solution viscosity, δ (water mass/protein mass) is the polypeptide hydration, C is the polypeptide concentration in dilute solution, v_p is the average specific volume of the polypeptide hetero-chain calculated through $v_p = \sum_{i=1}^{N} M_i v_i / M$ [7], and v_w is the specific volume of the solvent. Consequently, the factor $(v_p + \delta v_w)$ in Eq. (1) is associated with the effective polypeptide specific volume. On the other hand, the shape factor β takes averages values according to particle shape when Brownian motion is included, at a fixed volume measured via the equivalent hydrodynamic or Stokes radius a_H, which is the sum of the polypeptide compact volume and the hydration volume. Thus, the polypeptide ionizing groups are confined in the total hydrodynamic volume $V_H = 4\pi a_H^3/3 = M(v_p + \delta v_w)/N_A$. This volume includes the protein compact volume $V_c = M v_p / N_A = 4\pi a_c^3/3$ with an equivalent compact radius a_c and the hydration volume V_w.

Taking into account that v_p and v_w may be estimated from the AAS and the solvent formulation, it is clear that for a fixed solution protocol, the corresponding IV may be evaluated as long as values of δ and β are known (see Eq. (1)). Previously, it was shown that polypeptide hydration may be estimated from CZE models [7–9, 26] (see also Section 2.3). On the other hand, data of the shape factor β are scarce. One of the few sets of well-tabulated β values, apart from those of spherical shape involving drops, bubbles, and solid particles [54] is that for spheroidal particles. It has a rather long history including some previous controversial results, which now are completely elucidated [55–61]. At present, average values of β as a function of a wide range of values of the major axis a and minor axis c of prolate spheroidal particles (alternatively the ratio $p = a/c$ may be used) are available. In this regard, one concludes that some experimental IV values may have associated these two characteristic dimensions. Here, it is important to visualize that, as a first approximation, the basic hydrodynamic characterization of proteins is obtained directly with parameters f and a_H provided by a simple CZE model as long as the polypeptide electrical potential ζ is relatively low to be able to apply Debye–Hückel approximation and Henry model [6–8, 26] (see Sections 2.3 and 3, and Figs. 1 and 2). In this regard, we found that there are many cases involving proteins and peptides where this constraint is quite valid, showing also the tendency to be within the range of application of the asymptotic Hückel equation of the electrophoretic mobility under typical CZE protocols. Therefore, the evaluation of parameters f and a_H implies that the

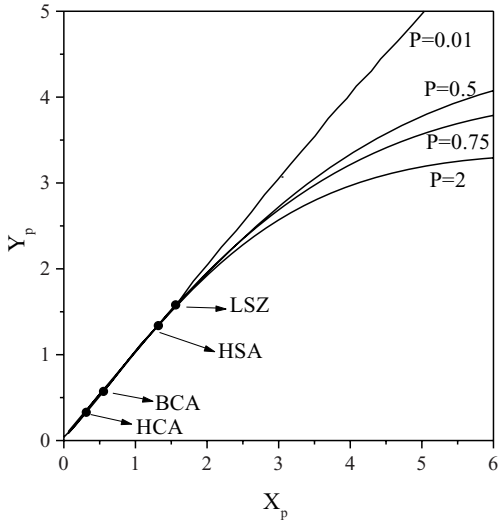

Figure 1. PLLCEM predictions (symbols) of the generalized coordinates $X_p = e\zeta/(k_B T)$ and $Y_p = 3\mu_p \eta e/(2\Omega\varepsilon k_B T)$, parametrically with $P = \kappa a_H$, for proteins BCA, HCA, HSA, and LSZ, falling in the linear CZE regime and showing that their electrophoretic mobility satisfies consistently Henry model. Full lines refer to predictions of the equivalent sphere model as computed in [64].

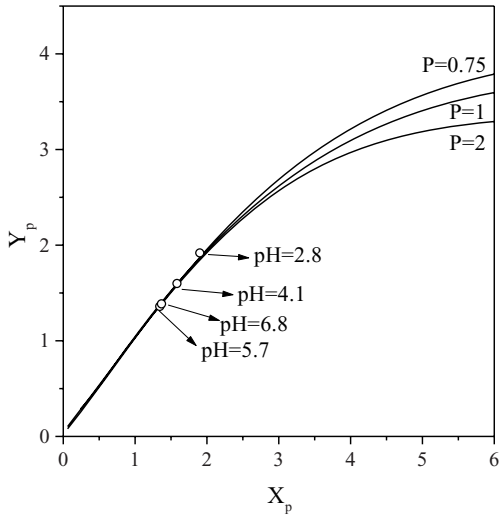

Figure 2. PLLCEM predictions (symbols) of the generalized coordinates $X_p = e\zeta/(k_B T)$ and $Y_p = 3\mu_p \eta e/(2\Omega\varepsilon k_B T)$, parametrically with $P = \kappa a_H$, for protein STN at different pH and *I*, falling in the linear CZE regime and showing that their electrophoretic mobility satisfies consistently Henry model. Full lines refer to predictions of the equivalent sphere model as computed in [64].

application of an appropriate chain or particle model is also required, which in turn shall be compatible with the average value of the polypeptide friction coefficient and shape [7]. It is then clear that in this framework several hydrodynamic particles may be adopted and determined through parameters f and a_H. In fact, there is not a unique solution taking also into account that the fluctuating shape of the particle is unknown in fine details introducing thus the need of a shape representation. In this regard, for globular proteins, the conformational structure of atoms as obtained from X-ray crystallography is one of the possible shape approximations at present [22, 40, 47, 48]. Typically in practical situations, a simple procedure is to assign spheroidal or cylindrical shapes to the hydrodynamic particle volume having the equivalent spherical volume V_H [7, 52]. The constraint is that the characteristic dimensions describing the shape shall fit the value of the average chain friction coefficient while the particle volume is constant. Alternatively, one can use a chain fractal property like the friction power coefficient defined above as described in [9, 26, 29, 62]. It is also appropriate to point out here that relevant works are available [22, 40, 43–48, 51] with particular emphasis to describe computationally the closer surface shape that relates to the actual furry protein shape in solution as inferred, for instance, from nuclear magnetic resonance and X-ray crystallographic experiments or other supposed model shapes. These types of studies are of course beyond the present proposal interconnecting the IV and the electrophoretic mobility of polypeptide chains through simple scaling relationships.

In the present framework, it is clear that the chain friction coefficient is one of the physical properties that must be considered to seek a simple interplay between electrophoretic mobility and IV of polypeptide chains. Here, we show that CZE models can provide reliable values of δ, f, and a_H to estimate the IV of polypeptide chains through Eq. (1). In fact, these parameters may be used to obtain β values of spheroidal particles simulating the actual polypeptide to estimate the IV through the direct application of Eq. (1). We apply here a similar procedure previously discussed in [7] as follows: (i) a value for $a > a_H$ is fixed with the condition that both the equivalent sphere and the prolate spheroid have the same volume to obtain $c = (a_H^3/a)^{1/2}$. (ii) Values a and c may be used to calculate the components of the prolate friction tensor according to, $f^{//} = 8\pi\eta a E_x^3/\{(1 + E_x^2)\ln[(1 + E_x)/(1 - E_x)]/2 - E_x\}$ and $f^{\perp} = 16\pi\eta a E_x^3/\{(3E_x^2 - 1)\ln[(1 + E_x)/(1 - E_x)]/2 + E_x\}$, where $E_x = (1 - (c/a)^2)^{1/2}$ is the prolate eccentricity, $f^{//}$is the friction coefficient when the particle translation is parallel to the particle rotational axis of symmetry, while $f^{\perp}$ is the friction coefficient when the particle translation is perpendicular to this axis. (iii) Then $f^{//}$, $f^{\perp}$, and f are used to evaluate α through the expression $1/f = \alpha/f^{//} + (1 - \alpha)/f^{\perp}$ with $0 < \alpha < 1$; this parameter gives the average angle $\theta = \cos^{-1}\alpha$ defined between the major prolate axis and the applied electrical field strength direction, as described in [7, 8]. Parameter α also provides the fractional weight that friction coefficients $f^{//}$ and $f^{\perp}$ have on f due to the random orientation of the particle along the electrophoretic trip. (iv) These calculations require to satisfy $a_H \approx 2a E_x/\ln\{(1 + E_x)/(1 - E_x)\}$ indicating that the average electrical potential of the prolate spheroid [42] must be approximated to that of the equivalent sphere [7, 8]. Otherwise, a new value $a > a_H$ is introduced in Step (i) above, until this constraint is satisfied. From this iterative process, values of a and c are available to determine β. Therefore, by using the hydration value δ provided by the CZE model one applies Eq. (1) to find [η]. The results

obtained from this procedure are analyzed in the Results and Discussion section for the proteins in dilute solutions of the corresponding CZE protocols at given pH, *I*, and temperature *T*, as indicated above.

2.2 Flexibility of polyampholyte–polypeptide chains at SRII and LRII

The quantitative estimation of the IV may be also accompanied by carrying out a scale analysis of the polypeptide microstructure to visualize even more the relation of this property with basic global chain parameters and conformations. Therefore, in relation to the chain configuration (AAS) considered here, each bead center is localized from the hydrodynamic friction center of the chain through the position vector $\mathbf{R}_i$, with $i = 1, \ldots, N$ [63]. Therefore, from the kinetic theory of macromolecules, generalized to some degree in a way that simple classical chain models may be comprised in this theoretical framework, one can express that the shear rate viscosity η of dilute macromolecular solutions at very low shear rates is,

$$[\eta] = \frac{\eta - \eta_s}{C\eta_s} = \frac{N_A}{6\eta_s M} < \text{tr } \mathbf{K} >_e \tag{2}$$

where $\mathbf{K} = \sum_i^N \sum_j^N \tilde{\zeta}_{ij} \mathbf{R}_i \mathbf{R}_j$ is the corresponding chain structure tensor. In this expression, $\tilde{\zeta}_{ij}$ are the modified friction coefficients referred to the chain hydrodynamic friction center, which also include the effect of preaverage intrahydrodynamic interaction between pairs of beads *i* and *j* [63]. To reach Eq. (2), the following additional hypotheses, among those belonging to basic kinetic theories, were done: (i) equilibrium preaveraged hydrodynamic interaction is assumed, indicated with subscript **e** in Eq. (2), (ii) modified effective friction tensors are diagonal and constant with component $\tilde{\zeta}_{ij}$ for the corresponding amino acids mass centers *i* and *j*, respectively, of the possible $N \times N$ ones, and (iii) phase space conformational average is indicated through $< \text{tr } \mathbf{K} >_e$. In the literature, Eq. (2) allowed the analytical study of simple macromolecular models neglecting hydrodynamic interaction, thus yielding equations for $[\eta]$ that most of them follow Staudinger rule of the IV where $[\eta] \propto N$. Thus, these equations in general do not to satisfy the expected power law $[\eta] = K N^{\nu}$. In fact, for the unperturbed chain state, it is well known that $g_f = 1/2$, $\nu = 1/2$, and $[\eta]_o = N^{1/2}$ where subscript (o) stands to place emphasis that the theta condition is satisfied. This scaling low for the IV clearly indicates that in general intrachain hydrodynamic interaction is a relevant phenomenon that cannot be neglected in the flow of dilute macromolecular solutions. Within this specific and quite complex subject, one alternative to obtain an analytical expression susceptible to simpler studies and still in the framework of preaveraging hydrodynamic interaction is to attempt a scale analysis by introducing the concepts of random fractals of chains [9]. Further, in this framework, the chain gyration radius R_g may be expressed directly as a function of the *N* amino acid residues through,

$$R_g \approx \{\cos \omega/2\}^{(2g_f - 1)} L C_N^{(1-g_f)} N^{g_f}/\sqrt{6} \tag{3}$$

where $\omega \approx 74°$ is the angle between two consecutive polypeptide virtual bonds [28] for the planar trans-chain conformation and $C_N \approx (\cos \omega/2) L_K/L > 1$ is the Flory characteristic ratio involving the Kuhn length L_K of the equivalent freely rotating chain model, and $L \approx 3.8$ Å is the virtual unit size [28, 29]. It should be observed that C_N is usually roughly evaluated in the literature with $\cos \omega/2 \approx 1$. It is relevant to point out here that Eq. (3) applies for any value of g_f and that consistently it reduces to $[\eta]_o = K N^{1/2}$ and $R_g \approx L C_N^{1/2} N^{1/2}/\sqrt{6}$ for the unperturbed chain state. Further the later expression is used to define and measure appropriately C_N without the effect of LRII at the unperturbed chain state ($g_f = 1/2$) (see, for instance [28] for the definitions and use of light scattering techniques to determine experimentally C_N).

From Eqs. (2) and (3), one obtains,

$$[\eta] = \frac{N_A}{6M\eta_s} < \sum_i^N \sum_j^N \tilde{\zeta}_{ij} \mathbf{R}_i \cdot \mathbf{R}_j >_e \approx \frac{N_A}{6M\eta_s} f R_g^2 \tag{4}$$

as long as appropriate average definitions are considered. In fact, by defining $\zeta_i^* \approx (1/N) \sum_j^N \tilde{\zeta}_{ij} \cos\theta_{ij} R_j/R_i$ with $R_i = |\mathbf{R}_i|$, which may be interpreted as the effective friction coefficient of the *i*-bead including the preaveraged hydrodynamic interaction through coefficient $\tilde{\zeta}_{ij}$, one readily finds from the second term of Eq. (4) that the effective average chain friction may be expressed, $f \approx N \sum_i^N \zeta_i^* \mathbf{R_i} \cdot \mathbf{R_i}/\sum_i^N \mathbf{R_i} \cdot \mathbf{R_i} \approx \sum_i^N \zeta_i^* \mathbf{R_i} \cdot \mathbf{R_i}/R_g^2$, to get the last term of Eq. (4). Therefore, for the purposes of a scaling analysis, Eq. (4) is combined with the basic definitions of the chain friction coefficient and gyration radius already provided above, to obtain,

$$[\eta] \approx \left(\frac{\pi N_A a_o}{6M_m}\right)\{\cos \omega/2\}^{2(2g_f - 1)} L^2 C_N^{2(1-g_f)} N^{(3g_f - 1)} \equiv K N^{(3g_f - 1)} \tag{5}$$

Interesting is the fact that this equation satisfies the expected scaling power law $[\eta] \propto N^{(3g_f - 1)}$ indicating that *N* is irrelevant in the evaluation of the polypeptide IV at the collapsed state ($g_f \approx 1/3$). Similarly, for the chain three-dimensional spatial distribution in a good solvent the limit of g_f is around 3/5 giving $\nu = (3g_f - 1) \approx 4/5$, which is also a typical value of the Mark–Houwink exponent. Further, this equation is consistent with scale analyses carried out with less details via the diffusion coefficient $D \propto 1/f$, gyrations radius R_g, chain relaxation time $\lambda \propto R_g^2/D$, and polymer contribution to the solution shear modulus, giving $[\eta] \approx \lambda/N \approx N^{(3g_f - 1)}$ [62]. It is concluded here that Eq. (5) and its prefactor *K* are able to show the structural complexity involved in a typical IV value, where chain properties such M_m, a_o, ω, C_N, and g_f are describing the effect of average mass and size of monomer units, virtual bond angle and hindrances in the SRII, and chain–solvent interaction energies associated with chain flexibility in the LRII scales, respectively. Further, one should observe that C_N takes a fixed value for neutral synthetic polymers in a given solvent and temperature giving the unperturbed chain state, and hence in typical scales analysis, the effect of this parameter on the IV is hidden in the constant *K*. This is not the case for

polyampholyte–polypeptide chains where in addition positive and negative electrical charges are also present and differences in pH and hydration values may change C_N values. Thus, one should expect that the local chain flexibility is prone to change due to electrostatic interactions among closer chain ionizing groups, solvent ions, and group hydrations due to the structural water. This will be discussed below through the estimations of C_N and ν from Eqs. (1) and (5).

Equation (5) describes the global chain flexibility within a different framework from that considered in previous works [32–37]. In fact, global polypeptide chain flexibility is visualized here as the result of both SRII and LRII, where electrostatic charges introduce modifications on C_N and $\nu = 3g_f - 1$, as illustrated in Section 3. It is relevant to point out here that one of the advantages of deriving the scaling law from the framework of kinetic theories is a quite good estimation of the prefactor K as discussed in Section 3 and inferred from Table 1.

2.3 Basic polypeptide physicochemical properties evaluated from CZE

Before illustrating the interplay between the IV and the electrophoretic mobility obtained from CZE, it is necessary to describe briefly relevant results required in Eqs. (1) to (5) as they are obtained from the PLLCEM [6–9, 26], when the effective electrophoretic mobility μ_p of a polypeptide is available for a well-specified running protocol (solvent bulk pH, ionic strength I, temperature T, electrical permittivity ε, and viscosity η_s). Additional input parameters are the polypeptide molar mass M and AAS of N amino acid residues together with the estimation of interatomic distances evaluated from the protein data bank. The description and development of this model for polypeptides in general may be found in details with the appropriate hypotheses and some limitations in [6–9, 26]. The model provides chain properties within the classical and quite linear experimentation ranges of CZE runs used for these analytes (see Figs. 1 and 2). In this regard, the PLLCEM provides the electrical state of a given analyte through total positive Z_+, total negative Z_-, effective $Z = |Z_+| - |Z_-|$, and total $Z_T = |Z_+| + |Z_-|$ charge numbers. An estimate of the equivalent hydrodynamic radius a_H is also available, and hence the polypeptide hydration is obtained from $\delta \approx [(a_H/a_c)^3 - 1](v_p/v_w)$. Also the protein hydration number $H = \delta M/18$ (number of water molecules per chain) is obtained by summing each hydration number of ionizing, polar and nonpolar groups at the local pH [8]. In fact, this model provides the pK_i values of ionizing groups yielding a shift ΔpK_i in the reference pK_i^r and the pH near molecule pH* [6]. The electrical permittivity ε' within polypeptide domain [7] is estimated from the expression $\varepsilon' = \varepsilon\delta/(1+\delta) + \varepsilon_p/(1+\delta)$, which uses the hydration as the weighing parameter between protein ε_p and solvent ε electrical permittivities. Another relevant property evaluated is the shape-orientation factor $\Omega = 6\pi\eta a_H/f$, which is the ratio $\Omega = \mu_p/\mu$ between the effective analyte mobility μ_p and the effective mobility of the equivalent sphere $\mu = eZf_H(\kappa a_H)/6\pi\eta a_H(1+\kappa a_H)$ [7,8]. In these expressions, e is the elementary charge, $f_H(\kappa a_H)$ is Henry function, $\kappa = \sqrt{2e^2 N_A I 10^3/\varepsilon k_B T}$ is the inverse of the particle screening length, and k_B is the Boltzmann constant. Here, within the PLLCEM framework, the basic characterization of polypeptides is obtained, as a first approximation, directly with parameters Ω and a_H and the charge state defined through Z and Z_T, allowing then the application of an appropriate chain model that is compatible with the average value of the chain friction coefficient. Also from the PLLCEM, one can evaluate the electrical state of the polypeptide chain through additional global structural properties such as the effective $\Delta\sigma = |Z|/N$ and the total $\sigma = Z_T/N$ charge number fractions. The importance of these global properties is that for chains in dilute solution they satisfy simple scaling relationships [9, 26, 49] suggesting at least four possible global chain conformations for a polyampholyte–polypeptide in general. Therefore, plots of $\Delta\sigma$ versus σ may delimit different chain conformational regimes (see the schematic divisions of regimes illustrated through Fig. 1 in [9] for more details). Thus, a random coil regime may destabilize into the polyelectrolyte (PE) and CG regimes. Another relevant chain destabilization is the transition from CG regime to the hybrid chain (HC) regime [9, 26].

Table 1. Intrinsic viscosity [η] of globular proteins studied here at different pH and ionic strength I

Protein	pH	I (mM)	N	p	β	C_N	$\nu = 3g_f - 1$	[η] (cm^3/g)	σ	Δσ
HCA	8.4	10	259	1.76	2.85	7.04	0.119	3.20	0.243	0.008
BCA	8.4	10	259	3.44	4.14	7.30	0.200	4.61	0.225	0.013
LSZ	8.4	8	129	3.73	4.41	7.50	0.236	4.65	0.209	0.052
HSA	9.8	110	585	2.30	3.20	6.98	0.128	3.62	0.321	0.078
STN	6.8	26	149	1.61	2.77	7.25	0.128	3.24	0.343	0.060
STN	5.7	36	149	1.35	2.64	8.15	0.200	4.70	0.355	0.076
STN	4.1	55	149	2.29	3.20	9.48	0.347	9.02	0.302	0.141
STN	2.8	5.5	149	1.94	2.96	20.71	0.713	56.93	0.241	0.202

Parameters are: number of amino acid residues N, prolate spheroid dimension ratio $p = a/c$, shape factor β, Flory characteristic ratio C_N, Mark–Houwink exponent $\nu = 3g_f - 1$, total σ, and effective Δσ charge number fractions.

3 Results and discussion

The electrophoretic mobility of proteins BCA, HCA, HSA, LSZ, and STN at the CZE protocols indicated in Table S1 may be studied through Henry model, which is the basic framework of the PLLCEM used here. The validation of this conclusion is illustrated in Fig. 1, where numerical results obtained from this model fall in the linear relationship between the generalized coordinates $Y_p = 3\mu_p\eta e/(2\Omega\varepsilon k_B T)$, $X_p = e\zeta/(k_B T)$, and $P = \kappa a_H$ as one expects when Henry model applies [6–8, 26, 64]. A similar situation is satisfied with the STN when different pHs are considered (Fig. 2). The protein characteristic parameters provided by the PLLCEM, which are required for the evaluation of Eqs. (1) and (5), are reported in the Supporting Information (Tables S1–S4). Thus, the available numerical data there allowed us to estimate p, β, ν, C_N, and $[\eta]$ as illustrated in Table 1, where the effective $\Delta\sigma$ and total σ charge number fractions are also reported.

It is shown in Table 1 that the range of IV values obtained from Eq. (1) is similar to that already found and reported in the literature for globular proteins around the native state in dilute solution at similar protocols and temperatures to those used mainly in capillary viscometric techniques evaluating $[\eta]$. For instance, the IV numerical values predicted here and shown in Table 1 for pH between 5.7 and 9.8, around protein globular native states, compare well with those reported in [52, 65–69] where IV experimental values of globular proteins are within the range 2.5–4.3 cm^3/g. This is an indication that CZE models as those proposed above can provide reliable values of δ, g_f, a_H, and f to estimate the IV of polypeptides in a consistent procedure. Also the proteins studied here with protocols that keep them in a rather native state show that the Flory characteristic ratio defining the SRII and reported in Table 1 may vary in the range $C_N \approx 7$ to 8. These values are similar to those found for synthetic polymers of high N [28, 50] where typically $C_N \approx 5$ to 9. In principle, we may conclude that the Flory characteristic ratio (chain local flexibility) of globular proteins studied here around the native state (appropriate solvent is of course required at functional pH and I) should not differ substantially from those of synthetic polymers. Nevertheless, this situation may change drastically as illustrated in Table 1 for the STN when the pH is changed, for instance, from 4.1 to 2.8 when this protein evolves from HC to PE regimes [9]. Thus, although p and β decreases while Ω increases generating a more spherical global particle when the pH is lowered (see Tables 1 and S3), one finds that these effects are not significant enough to reverse the IV to lower values. In fact, it must be taken into account that at pH 2.8 the STN transitioned to the PE state introducing changes in several properties values that validates the IV increase from 9.02 to 56.93 cm^3/g. Under these circumstances, much water is immobilized ($\delta \approx 18.48$ and $\varepsilon'/\varepsilon \approx 75$, as reported in Table S3) due to the relative high charged state in the PE regime (Table S4) giving thus a relative high Ω (less aspherical particle). Consistently, at pH 2.8, STN gets a larger radius a_H (50.34 against 26.51 Å), a very high effective charge number Z (30.15 against 20.96) promoting repulsion and hydrated chain expansion, lower Z_T (35.84 against 44.93) avoiding the tendency to chain collapse [9] due to fluctuation-attractive electrostatic interaction, higher δ (18.48 against 2.10) giving larger particle size, higher g_f (0.57 against 0.45) generating chain extension due to exclude volume effects and electrostatic high-range interaction effects, higher $\Delta\sigma$ (0.202 against 0.141) increasing particle size due to electrostatic repulsion, and higher C_N (20.71 against 9.48) decreasing local chain flexibility. Consequently the decrease in pH from 4.1 to 2.8 placing the STN in the PE regime affects both SRII and LRII. Since $\nu \approx 0.713$ at pH 2.8, it is clear that the good solvent condition ($\nu > 1/2$) has been achieved. The counterpart situation for the STN near the native state is at pH 6.8 giving $C_N \approx 7.25$ and $\nu \approx 0.128$. Thus the protein is in the HC state with $g_f \approx 0.38$ indicating the proximity to the CG state where $g_f \approx 0.33$.

It is then interesting to observe that polypeptide chains may change substantially their IV values, and hence their global flexibility (SRII and LRII) as a consequence of modifying their conformations from near CG to PE regime [9]. These effects are achieved with the STN by changing the electrical charge state through different pH values from 6.8 to 2.8, as illustrated in Table 1, where the IV jumped from 3.24 to 56.93 cm^3/g, respectively. Results thus obtained from data of CZE and Eqs. (1) and (5) are consistent with those previously reported in [9, 13] where the STN chain at pH 2.8 has a quite extended conformation. These theoretical predictions are similar from the hydrodynamic point of view to those found experimentally in [70] by studying conalbumin from pH 6.7 to 3.1, where the IV varies from 3.5 to 160 cm^3/g. Thus, our predictions are describing the right phenomenological trend for varying pH values, as it was reported experimentally in the literature.

In general, it is relevant to observe that the resulting value of a polypeptide IV, for a given AAS and electrolyte solvent, has a multivariable dependence on physicochemical properties reported in Tables S1–S4. Therefore, it is expected to find compensatory phenomenological effects on IV values for a given polypeptide AAS when chain environmental changes are introduced like for instance pH variations, as described above.

In Table 1, one also observes that when the solvent formulation is at pH = 8.4 and $I = 10$ mM, a clear distinction from the HCA and BCA (both having $N = 259$ and different AAS) is obtained. In fact, the local flexibility of HCA is a little higher than that of the BCA (C_N is 7.04 against 7.30, respectively) while the IV follows consistently this specific aspect giving IV values of 3.20 cm^3/g and 4.61 cm^3/g, respectively. These results have an explanation (see Table 1 and complementary data in Tables S1 and S2). In fact, for this particular example, all the physicochemical properties are in the expected trends: the HCA has higher M_m (112.3 against 111.9 Da), higher Ω (0.93 against 0.80, more spherical shape), and hence lower β (2.85 against 4.14), less Z (−2.07 against −3.34, less electrostatic repulsion), higher Z_T (64.03 against 59.41, major tendency to collapse), equivalently less $\Delta\sigma$ and higher σ (0.008 against 0.013 and 0.243 against 0.225,

respectively). Also HCA has lower g_f (0.37 against 0.40, more compact conformation in the CG regime). Thus, these properties indicate clearly the difference in the reported values of IV from both the physical point of view and the consistency with Eq. (5). Therefore, from these results, it is clear that the change of electrostatic charges affects both SRII and LRII among amino acid residues modifying the global chain flexibility as it may be judged from values of C_N and ν or g_f in Table 1 (see also Tables S1 and S2). It is relevant to visualize that the comparison between HCA and BCA is readily done because they have the same $N = 259$.

It is important to point out here that the primary electroviscous effect associated with the polypeptide particles studied through CZE runs (Table 1) may be considered negligible. This implies that the shape factor β of the equivalent uncharged particle in Eq. (1) is obtained directly from known values a/c as indicated in Section 2, by considering the primary electroviscous coefficient $p_1 = \beta^*/\beta - 1$ small. Here, β^* is the shape factor of the charged particle corrected by the primary electroviscous effect. In this regard, the assumption introduced in our calculations is consistent with the requirement that polypeptide case studies within the PLLCEM framework are constrained to the conditions $X_p < 2$ and $P < 3$ so that ion convection around the particle in the electrophoretic migration may be neglected (see validation of these constraints in Figs. 1 and 2). In fact, following previous rigorous studies on the evaluation of p_1 for the equivalent spherical model, where the distortion of the ionic double layer around the particle in shear flow is analyzed by including ion convection, one concludes again that as long as $X_p < 2$ and $P < 3$, the rather small correction introduced by the classical Booth theory [71] through the coefficient p_1 differs in less than 10% from rigorous results [72, 73]. This subject was analyzed and extended to hard particles of arbitrary shapes including specifically prolate spheroids by Allison [74, 75] through the numerical boundary element method, reaching thus to relevant conclusions on critical parameters at which p_1 may become important mainly for high X_p. In general, for high X_p and P, the distortion of the double layer may be significant as observed in fragments of DNA where the particle is in the PE regime with rather high electrical charge. It is also appropriate to indicate that the second electroviscous effect is not important in typical CZE runs, which are carried out under very dilute conditions. Thus, intermolecular interactions among solute units are not present. The remaining third electroviscous effect associated with intrachain electrostatic interactions due to changes in pH and I is considered in the PLLCEM through the shape-orientation factor Ω measuring the particle asphericity due to changes of the polypeptide electrical state. In addition, from the discussion above, the evaluation of the effective $\Delta\sigma$ and total σ charge fractions provides a measure of the chain tendency to adopt different conformations. Therefore, parameters Ω, $\Delta\sigma$, and σ are quantifying the third electroviscous effects in a polypeptide with given AAS when the pH and I are changed, as it is illustrated in the analysis of the STN above.

Before ending this section, it is important to indicate here that our calculations were extended to a high number of peptides where the shape-orientation factor Ω may be either lower or higher than one [8, 26]. In this regard, soft particle models like those described in [76–80] are interesting alternatives to the application of hard particle models typically used in the modeling of the CZE mobility of protein and peptides. In fact, soft particle models are able to capture, for instance, the decrease in particle mobility due to the additional flow present in a hydrophilic polymer layer surrounding a hard hydrophobic chain core. Our preliminary results indicate that polypeptides with $\Omega \neq 1$ as evaluated from the PLLCEM are susceptible to be modeled through soft particles. This subject deserves future research taking into account that the physical picture of a soft particle may assign hydrophobic and hydrophilic domains of amino acid residues in the polypeptide AAS involving also the associated water due to chain hydration.

4 Concluding remarks

The modeling of the electrophoretic mobility of polypeptides is a useful tool for appropriate interpretations of the corresponding IV values. Since this property depends on both SRII and LRII through the Flory characteristic ratio and the Mark–Houwink exponent, this work shows that the quantification of electrical charge states and conformational regimes of polypeptide chains in specific protocol solutions is required. For this purpose, the solvent–chain friction and the power friction coefficient, as obtained from the PLLCEM, are relevant to yield scaling laws relating electrophoretic mobility and IV. Global properties of polyampholyte–polypeptide chains in dilute solution are substantially more complex than those of neutral homopolymer chains. In this regard, it is evident that the chain electrostatic state introduces additional phenomena in the description of the global chain flexibility of polypeptides and further researches should place emphasis on these aspects to eliminate some limitations of the theoretical framework indicated throughout this work.

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