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The Role of Molecular Thermodynamics in Bioengineering

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This communication outlines the contribution of chemical engineering and, in particular, of molecular thermodynamics in understanding some biological events and in the development of many useful applications in bioengineering. Besides a general discussion about bioengineering and molecular thermodynamics, two examples are presented in the attempt to show a typical approach to the solution of scientific and practical problems.

Bioengineering is a typically trandisciplinary science involved both in fundamentals aspects of biophysical and biological research and technological applications devoted to the development and production of drugs, biomaterials, processes, devices and informatic techniques needed in prevention, diagnosis, therapy and rehabilitation in medical field. Besides biology, physics and chemistry, almost all branches of engineering can contribute to the development of this science through physical understanding and mathematical modelling of often complex phenomena up to carry out actual plans.

Chemical engineering plays a special role in bioengineering because of its feature to be a bridge between the macroscale of chemical processes and equipments and the microscale of molecular phenomena. In biological problems, we can recognize at least 4 size levels where chemical engineering could operate: 1) macroscopic physiological system (organism, organ etc.); 2) local cell aggregate (i.e. solid tumor); 3) cell population or single cell; 4) biomolecular structures.

It is noteworthy the role of molecular thermodynamics with its capability in understanding and describing the behaviour of many biological systems through molecular models and the tools of classical and statistical thermodynamics. Some areas where thermodynamics may support biological sciences and bioengineering are the prediction of physical and chemical properties of systems containing biomolecules [1,2], the effect of solvent, pH, temperature [3] and ionic strength on activity and selectivity of biocatalysts, the identification and right formulation of driving forces in bioprocesses, the drug delivery mediated by hydrogels etc.

In this short communication we present two examples of results we can obtain by molecular thermodynamic models. The first case refers to the evaluation of net proton charge on proteins; the other one, regarding the drug release from gels, is more interesting for biomedical applications

Net proton charge on protein molecules is a very useful information both from a fundamental and a practical point of view. Electrostatic interactions depend on this property and phenomena, such as molecule aggregation and stability

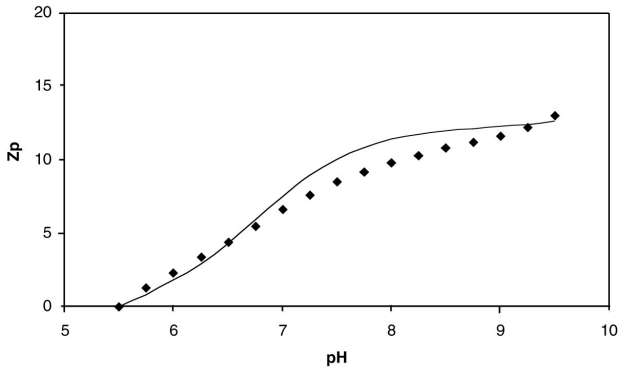

Fig. 1 - Experimental and calculated values of net proton charge of B-casein at 4°C and 0.1 M NaCl

of aggregates, solubility, salting effects, denaturation, separation by electrophoresis and ion exchange HPLC, depending on electrostatic interactions are strongly influenced by the number of charges on protein molecules. Molecular-thermodynamics allows the net proton charge, and then many of the above mentioned phenomena, to be predicted through molecular models.

The most common approach to calculate the net proton charge of a biopolymer like a protein is based on pK values of aminoacidic residues which can exchange hydrogen ions with the solution:

$$K_i = \frac{C_{H^+}\gamma_{H^+} C_{A_i}\gamma_{A_i}}{C_{HA_i}\gamma_{HA_i}} \quad (1)$$

where C and γ indicate concentrations and activity coefficients, respectively, HA_i is the acidic form of the residue and A_i its basic form. Eq. (1) allows dissociation degree of each species to be calculated at each pH and, then, electrical charge of residue *i* to be evaluated. Activity coefficients, which depend on ionic strength of the solution, account for lessening of electrostatic interactions in microenvironment of each titrable group due to the presence of electrolytes in solution. Following this approach, it is possible to define an apparent dissociation constant K_a of a polyelectrolyte depending on the degree of ionization and the ionic strength of solution.

This oversimplified model is based on the unsuitable

assumption that pK has the same value for single aminoacid and for the residue in the protein chain. Unfortunately, each group in the protein structure feels the effects of electrostatic field produced by the remainder of the molecule. Therefore, the pK of the single group (pK_{mod}) must be modified by a perturbation term ΔpK:

$$pK_i = pK_{i,mod} + \Delta pK_i \quad (2)$$

Fig. 1 shows the experimental values of net proton charge $|Z_p|$ of β-casein [4] at 4°C and 0.1 M NaCl compared with theoretical values calculated with $pK_a = pK_{mod}$. The differences between experimental and theoretical values show the weight of ΔpK term.
Several models have been proposed for the calculation of pK_a: one group of theories requires the solution of Poisson-Boltzmann (PB) equation which, however, is not available in an analytical form; the other group is based on the Manning's counterion condensation model [5,6] which assumes electrical charges spaced in the protein chain dependently on the ionization degree and deduce a predictive equation for pK_a from the minimization of free energy with respect to bound protons.
Another typical example by which we will show the usefulness of thermodynamic approach refers to the drug release form hydrogels. It is well known that significant progresses in pharmacological treatment of several diseases require both new and more effective bioactive drugs and suitable drug delivery systems that allow the drug release to be controlled in time and space, maintaining the drug level within the desired range as long as it is required, minimising the toxic side effects related with systemic administration and improving patient compliance. Among drug delivery systems, gels (i.e. three-dimensional cross-linked polymer) play a central role: in this case the release of the active compound is governed by polymer swelling, drug distribution between gel and liquid phase and their non-Fickian diffusion through the swollen matrix.
In this field, molecular thermodynamics can contribute to understand and modelize both polymer swelling and component distribution and, therefore, to design new drug delivery systems. It is worth noting that in spite of the importance of polymeric gels in medical, biological and biomedical fields, only in recent years a systematic approach to thermodynamics of phase equilibria of gel systems has been developed and many questions are still unanswered [7].
The basic idea to develop a molecular thermodynamic model for gel swelling is that hydrophilic cross-linked polymer without electric charges swells until the forces that cause the water influx are balanced by the elastic force due to the polymer chain stretching. In thermodynamic terms, equilibium occurs when:

$$\Delta\mu_w = \mu_w^{gel} - \mu_w^{bath} = \Delta\mu_{mixing} + \Delta\mu_{el} = 0$$

where mixing and elastic contribution to the swelling Dm_w are considered. The first contribution may be expressed according to Flory-Huggins model, whereas the second contribution may be evaluated on the basis of elastic network theories (Affine or Phantom models).
For gel containing fixed charges (polyelectrolyte gel), the unequal distribution of mobile ions between gel and external solvent (Donnan equilibrium) must be considered and the contribution of ion-ion interaction must be added to the chemical potential:

$$\Delta\mu_w = \mu_w^{gel} - \mu_w^{bath} = \Delta\mu_{mixing} + \Delta\mu_{el} + \Delta\mu_{ion} = 0 \quad (4)$$

In this case the gel swelling properties depend on the amount of charged monomer in the polymer and on the salt concentration in the bath.
Finally, if the fixed charge in gel are weak electrolytes, their ionization degree and, therefore, the polymer swelling depend on the solution pH. In particular, at some pH, a sharp phase transition between a swollen and a collapsed gel may occur: for example, weak basic polyelectrolyte gels are swollen at low pH, due to the electrostatic repulsion between charged groups, but they collapse at high pH when functional group are neutralized.
A deep understanding of the swelling behaviour of such a pH-sensitive gel may contribute to design "intelligent" drug-delivery systems, that release their content in response to the presence of specific molecules in the external environment. Among these systems, weak polyelectrolyte gels containing both insulin and glucose oxidase are of particular interest for self-regulating insulin delivery system: the enzyme makes the gel sensitive to the glucose concentration due to its reaction with glucose that produces gluconic acid and lowers the local pH; as a consequence the polymer swells and the insulin is released.

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