

STOCHASTIC ANALYSIS ASSOCIATED WITH THE DIFFUSION OF β -LACTOGLOBULIN, BOVINE SERUM ALBUMIN AND *STAPHYLOCOCCUS AUREUS* IN MILK AND WHEY

Alessandra Suzin Bertan

University of Campinas, UNICAMP
School of Chemical Engineering, Campinas,
Brazil

Marco Aurélio Cremasco

University of Campinas, UNICAMP
School of Chemical Engineering, Campinas,
Brazil

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Abstract: Milk has a high biological value, making it central to the human diet. Both its complexity and nutritional richness lie in the variety of vitamins and proteins. Protein content and milk production are affected by the health of the animal, particularly when affected by mastitis, caused by bacteria, especially *Staphylococcus aureus*. Therefore, milk processing must undergo separation techniques such as microfiltration for the retention of *S. aureus* and ultrafiltration for protein separation. In these situations, it is important to understand the scientific fundamental parameters, such as protein chains representation and the molecular dispersion of the constituents of milk and its derivatives. This work represents β -lactoglobulin (β -LG) and bovine serum albumin (BSA) using a simple stochastic model, considering the representation of circular polymers. The theoretical investigation of stochastic diffusion of β -LG, BSA, and *S. aureus* in milk and whey at 25 °C is also studied. There was a greater dispersion of β -LG in relation to the other solutes, as well as a slight increase in the dispersion of such solutes in whey as diffusive medium.

Keywords: β -LG; BSA; *Staphylococcus aureus*; Mastitis; Polymer chains; Brownian motion; Stochastic diffusion.

INTRODUCTION

Milk is one of the richest foods known, with high biological value, as it contains a range of nutrients such as vitamins, minerals, and proteins (Brasil; Cabral; Silva, 2015). Milk proteins are divided into casein (around 80%) and whey proteins (around 20%). Caseins aggregate into insoluble granules called micelles and are responsible for the white color, thermal stability of the milk, and coagulation by rennet, which is central to the production of cheese (Brasil; Cabral; Silva, 2015). The other proteins found in

milk are in soluble form and are usually identified with whey (Botaro, 2007), such as β -lactoglobulin (β -LG) and bovine serum albumin (BSA). β -LG is the protein in greater quantity present in bovine whey, highlighting its antihypertensive properties, antimicrobial and immunostimulant properties, and vitamin D transporter (Kilian, 2018). BSA is a source of essential amino acids, acts in the transport of nutrients, and has an antimutagenic function. However, the casein, protein, and fat content are altered by animal health, particularly when affected by mastitis, which refers to inflammation of the mammary gland, considered the main disease that affects dairy herds, affecting the decrease in milk production and changing its characteristics (Zafalon, 2003). Among the bacteria that can be found in milk, *Staphylococcus aureus* appears as the main pathogen that causes subclinical mastitis in dairy herds (Simões et al., 2013). It is capable of growing under a wide range of environmental conditions, which can cause diseases in humans and different animal species (Gomes, 1994). Therefore, milk processing is necessary, and among the stages of such processing, we can mention the processes that involve membranes, such as microfiltration for bacteria separation (in addition to pasteurization) and ultrafiltration for protein retention, which it is necessary to have knowledge of the basic mass diffusion concepts. In this context, the present work focuses on the representation of the β -LG and BSA chains using a circular polymer model with segmented closed chains. In addition, we intend to analyze the stochastic dispersion of both proteins and *S. aureus*, translated into two-dimensional cartograms from the random displacement of the analyzed solutes.

THEORETICAL BACKGROUND AND RESULTS

REPRESENTATION OF THE B-LG AND BSA PROTEIN CHAINS

The representation of a globular or closed protein, as β -LG and BSA, can be made using a simple model that considers the representation of circular polymers by means of segmented closed chains (SCC). These, in turn, can be obtained by generating free bond chains (FBC) of the corresponding linear molecule with N segments of length L each, as illustrated in Figure 1. According to Nunes (2013), the algorithm for generating the SCC chain (Figure 1b) starts with an FBC (Figure 1a). A linear chain can be generated from a random walk of N steps, and at each step, a new point is generated, which can occupy any position within a circle of radius L , whose center resides at the previous point, as illustrated in Figure 1c for 2D linear chain. The process is repeated until the chain has N segments. The values of N and L are defined to obtain the equal length of the contour of the real molecule; L corresponds to the minimum distance required to have a segment with an orientation independent of the previous one (Nunes, 2013).

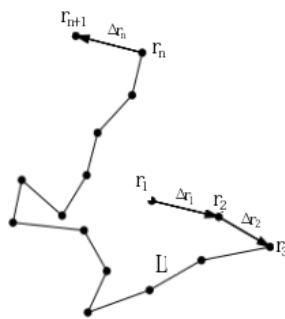
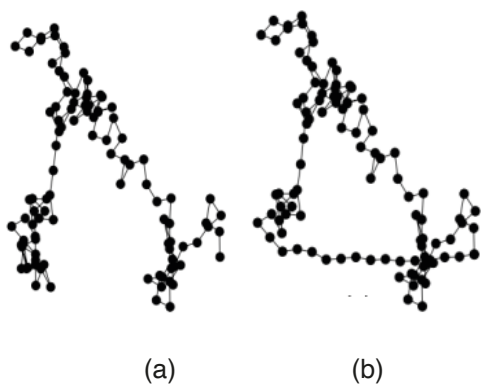


Figure 1 – Representation of the closed chain from the open chain: (a) open chain; (b) closed chain; (c) 2D random walk of an FBC chain with N segments of length L (Nunes, 2013).

In the generation of the 3D chain, the spherical coordinate system is used, in which a position vector is defined by three values: distance to the origin r , angle between the projection of the position vector, in the xy plane, and the positive axis of the xx , defined as the angle θ , and the angle between the position vector and the positive axis of the zz , angle ϕ . The relationship between these systems follows the following equation (Nunes, 2013).

$$r = (x^2 + y^2 + z^2)^{1/2} \tag{1}$$

$$\theta = \arctan 2(y, x) \tag{2}$$

$$\phi = \arccos(z/r) \tag{3}$$

$$x = r \cos \theta \text{sen} \phi \tag{4}$$

$$y = r \text{sen} \theta \text{sen} \phi \tag{5}$$

$$z = r \cos \phi \tag{6}$$

where r is a pseudorandom number between 0 and 1. The position of a new point in each segment of the chain is taken from (NUNES, 2013)

$$x_{i+1} = x_i + L \cos \theta_{i+1} \text{sen} \phi_{i+1} \tag{7}$$

$$y_{i+1} = y_i + L \text{sen} \theta_{i+1} \text{sen} \phi_{i+1} \tag{8}$$

$$z_{i+1} = z_i + L \cos \phi_{i+1} \quad (9)$$

As illustrated in Figure 1c, once the string FBC is known, point r_{k+1} must coincide with the point r_i ; then, the point r_k is obtained, considering the difference between this point and the previous one until they are at the distance L (minimum distance) requested, and so on. This distance, in turn, can be estimated by (Nunes, 2013)

$$L = r_G \left[\frac{6(N+1)}{N(N+2)} \right]^{1/2} \quad (10)$$

In the present work, it is assumed that N is coincident with the number of amino acid residues that compose the protein under analysis, and the radius of rotation comes from

$$r_G = 0,7525 \sigma_A \quad (11)$$

with the diameter of the protein, σ_A , assuming that it is equal to its Stokes-Einstein diameter. For proteins with molar mass between 14 kDa and 240 kDa, the following correlation can be used:

$$\sigma_A = 26,374 \ln(M_A) - 221,74 \quad (12)$$

with the result of σ_A in Å, using the molar mass, M_A , in Da. The protein β -lactoglobulin (β -LG) has $M_A = 18,300$ Da and consists of a sequence of 162 amino acid residues (Botaro, 2007). Bovine serum albumin (BSA) has $M_A = 66,200$ Da with 584 amino acid residues (Maciel, 2019). The diameter of protein values, the respective rotation radii, and the minimum distance values are obtained, as shown in Table 1. With N (number of amino acid residues) and L (minimum distance), and the model presented in Equations (1) to (9), it is possible to present the β -lactoglobulin (β -LG) and serum albumin (BSA) protein chains in 3D, as shown in Figure 2. It is clear that the BSA chain is much longer than that of β -LG.

Proteins	M_A (Da)	σ_A (Å)	r_G (Å)	L (Å)
β -LG	18,300	37.11	27.93	5.36
BSA	66,200	71,02	53.44	5.41

Table 1 – Characteristics of the β -LG and BSA protein chains

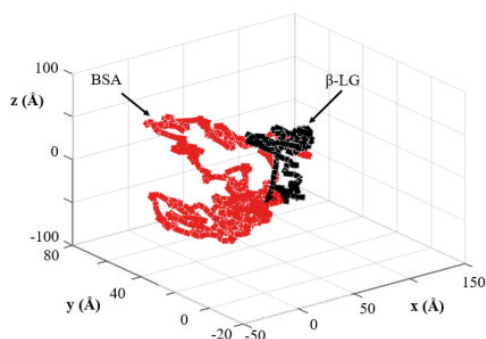


Figure 2 – Representation of the closed chains of the β -LG and BSA proteins.

STOCHASTIC DESCRIPTION OF THE SOLUTE DISPLACEMENT

To have an ordinary approach to the diffusive mechanism, guided by the Brownian displacement in liquid medium, it is assumed that *Staphylococcus aureus* and the macromolecules β -LG and BSA are diluted in the medium (milk or whey). The model for the description of the displacement of 2D solutes, by Brownian motion at viscous limit (Bertan and Cremasco, 2023), is

$$x(t) = x(t - \Delta t) + \beta_A R_i \quad (13)$$

$$y(t) = y(t - \Delta t) + \beta_A R_i \quad (14)$$

where R_i is the pseudorandom number associated with white noise relative to PDF $N(0,1)$; β_A , relative deviation obtained from

$$\beta_A = \sqrt{2D_{AB} \Delta t} \quad (15)$$

with Δt the characteristic time interval of the random step, in ns, and the diffusion coefficient of the diluted solute in the diffusive medium obtained from the Stokes-Einstein equation (Bertan and Cremasco, 2023)

$$D_{AB} = 1,465 \frac{T}{\eta_B \sigma_A} \quad (16)$$

where T, in K; η_B in cP; D_{AB} at $\text{\AA}^2/\text{ns}$. For whey, at 25 °C, $\eta_B = 1.032$ cP (deKruif and Huppertz, 2012). On the other hand, for milk, considering that the fat content of Holstein milk is equal to 3.5%, the equation can be applied (Hlavác and Božiková, 2011)

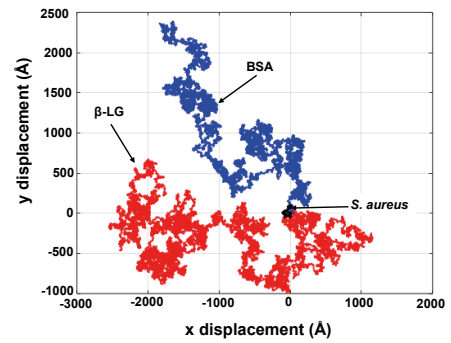
$$\eta_B = 2,2815 \exp \left[-1,921 \times 10^{-2} \left(\frac{t}{t_0} \right) \right] \quad (17)$$

with t, temperature in °C; $t_0 = 1$ °C; η_B in cP. The values of the diffusion diameter of the proteins are known and provided in Table 1. In the case of *Staphylococcus aureus*, it is presented in the globular format with a diameter between 0.5 – 1.5 μm (Simões et al., 2013). In this work, it is assumed $\sigma_A = 1.0 \times 10^4$ \AA . Considering $\Delta t = 1.0$ ns and the D_{AB} values from Equation (16), β_A values are calculated. Table 2 shows the results at 25 °C.

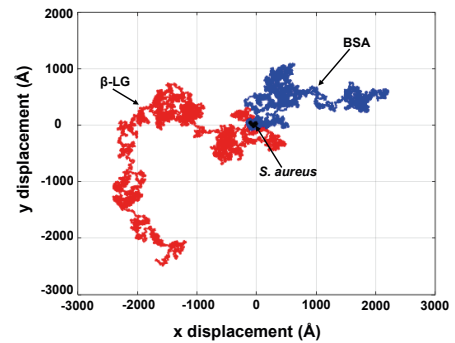
Diffusive medium \rightarrow	Whey ($\eta_B = 1.032$ cP)		Milk ($\eta_B = 1.411$ cP)	
	D_{AB} ($\text{\AA}^2/\text{ns}$)	β_A (\AA)	D_{AB} ($\text{\AA}^2/\text{ns}$)	β_A (\AA)
<i>S. aureus</i>	4.23×10^{-2}	0.291	3.83×10^{-2}	0.277
β -LG	11.11	4.777	10.32	4.543
BSA	5.96	3.453	5.39	3.283

Table 2 – Basic information to construct the

Using the algorithm found in Bertan and Cremasco (2023) and considering 10^5 steps for each medium (milk and whey), the cartograms related to the random molecular dispersion of *S. aureus* and the β -LG and BSA proteins were obtained, shown in Figure 3a, confirming the greater dispersion of β -LG, due to the lower polymer chain and, therefore, a smaller diffusional diameter. The small displacement of the bacterium is noted, as a result of its larger diameter. Figure 3b, in turn, highlights the influence of viscosity on the individual dispersion of proteins.



(a)



(b)

Figure 3 – 2D cartograms illustrating the Brownian motion of *S. aureus*, β -LG and BSA proteins at 25 °C. (a) medium: whey; (b) medium: milk.

CONCLUSION

In commercial terms, protein is one of the most valued nutrients in milk quality payments (González Noro, 2011). Proteins are natural vehicles that provide essential micronutrients (calcium and phosphorus), amino acids, and components of the immune system for the newborn. However, traditional extraction and purification techniques greatly increase the final cost of the purified compound, in addition to being able to lead to the denaturation of some of the biomolecules, reducing biological activity (Michel et al., 2015), especially with those processes that involve the use of chemicals, biological products or that involve thermal exchange. In this context, this article also examines human needs, as it understands milk while essential

food. From this viewpoint, it is essential to understand the basic science associated with technological processes, including the fundamentals of mass transfer. The diffusion coefficient is a quantity associated with the ease with which a given solute moves in a medium and is dependent on its characteristic size, which in the present work is associated with the Stokes-Einstein diameter, in view of the dilution of the diffusive medium. The greater molecular dispersion was evidenced with the reduction of the diffusional diameter

of the solute, in particular the clear greater molecular dispersion of the proteins when compared with that of *S. aureus*, indicating separation strategies, mainly by size exclusion. In view of the bacterial dimension, it can be separated by microfiltration, resulting in permeate rich in macromolecules, as is the case of proteins, which can be separated using ultrafiltration. Regardless of the separation technique, it is important to know the basic aspects of mass diffusion.

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