Adsorption 11: 131–138, 2005

Lysozyme Adsorption onto Different Supports: A Comparative Study

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Received July 21, 2004; Revised June 2, 2005; Accepted August 2, 2005

Abstract. The interaction between proteins and solid surfaces has been investigated. The aim of this work is to compare three different materials (hydroxyapatite, polystyrene with core-shell structure (PE-CS) and a functionalized styrene divinylbenzene copolymer) to be used as adsorbents for lysozyme, known as a "hard" protein. Tests were performed according to an experimental design in order to compare the effects of pH, lysozyme and phosphate buffer concentration onto the adsorbed amount of protein. A 2^3 factorial design and a cross design, which was performed in triplicate, were used to distinguish the most important variables and to infer about the interaction between them. Hydroxyapatite showed the best performance—higher adsorbed amount of lysozyme and smaller dispersion (72.2 ± 0.9 mg/g). However, PE-CS can be regarded as a promising support as high amounts of lysozyme are adsorbed onto this material with relatively small dispersion.

Keywords: adsorption, lysozyme, hydroxyapatite, polystyrene, amberlite

1. Introduction

Biomolecules separation has been intensively studied over the last decades (Barroug et al., 1989; Kandori et al., 1997; Kawasaki et al., 2003). This is partially due to the high importance of biomolecules for medical, pharmaceutical, chemical, dentistry and other industrial purposes. Liquid chromatography is one of the classical methods used to separate proteins. So, the interaction between proteins and solid surfaces is the basis of this process.

Proteins are long chains of amino acids. Depending on the nature of the amino acids, protein charge in solutions can be positive or negative due to the amino or carboxyl groups, respectively. Lysozyme is a globular protein and is known for its antibiotic activity. It can be found in the body fluids and hen egg-white. Lysozyme is a basic protein since its isoelectric point is 11.1. It also displays a relatively large hydrophobic patch on its surface and despite its recognized hardness on hydrophilic surfaces, it remains unclear whether lysozyme compromises its structural integrity upon adsorption onto hydrophobic surfaces (Weaver and Carta, 1996).

Many materials have been investigated to be used as support in chromatographic columns as ceramics (Kandori et al., 1997), polymers (Skidmore et al., 1990) and composites (Weaver and Carta, 1996). Hydroxyapatite (Hap) is one of the most important adsorbents designed for proteins separation. Besides, it is the major mineral component of bones and teeth and this similarity with hard tissues can elucidate the mechanism of adsorption of some proteins onto them.

The study of adsorption of proteins like lysozyme onto hydroxyapatite surfaces is not recent. Barroug et al. (1989) presented a study about the adsorption of hen egg-white lysozyme at 20°C onto synthetic and commercial apatites. The influence of pH (5.5–8), ionic

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strength, addition of calcium and phosphate ions to solution on the amounts of adsorbed protein was investigated. The maximum adsorbed amount observed was 55 nmol/m^2. This corresponds to a loosely packed monolayer. It was concluded that the adsorption of lysozyme on apatites is driven by electrostatic interactions and may be considered as a surface charge neutralisation.

Later, Barroug et al. (1992) presented a paper in which the adsorption of lysozyme onto calcium-deficient hydroxyapatites was evaluated. The adsorption of hen egg-white lysozyme was measured at ambient temperature in 1×10^{-3} mol/L KNO_3 aqueous solutions buffered at pH 6.8 with 2×10^{-3} mol/L phosphate buffer. It was observed that lysozyme adsorbs onto hydroxyapatite according to a mechanism involving specific adsorption sites, by which surface concentration and affinity seem to increase together with the specific surface of the adsorbent. Despite the result presented in the aforementioned work, it was observed that electrostatic interactions are of minor importance, except when Hap is positively charged. In that case, electrostatic repulsion can prevent positively charged lysozyme molecules from accessing the Hap surface.

The adsorption of lysozyme onto various synthetic hydroxyapatites was investigated by Kandori et al. (1997). The adsorption isotherms of lysozyme onto these hydroxyapatites exhibited the pseudo-Langmuir type. It was found that only 1 to 29% of the particle surface was covered by lysozyme molecules. The study provides the evidence that lysozyme specifically adsorbs onto phosphate ions on *ac* or *bc* faces of Hap.

Recently, Kawasaki et al. (2003) studied the adsorption of proteins from saliva onto hydroxyapatite. In order to compare the results, the adsorption behavior of some model proteins, like human serum albumine, lysozyme, β-lactoglobulin and ovalbumin was also evaluated. The high-affinity adsorption of lysozyme reflected electrostatic attraction between the positively charged protein and the negatively charged hydroxyapatite surface at pH 7. Lysozyme showed adsorption saturation value of 1.2 mg/m^2, corresponding to a full monolayer of molecules onto hydroxyapatite. It was also observed that the presence of Ca^{2+} reduced the adsorption of lysozyme in hydroxyapatite. Saliva proteins have low adsorption affinity. The results suggest that an acquired pellicle in tooth surface in an oral environment contains a significant fraction of positively charged proteins. Thus, electrostatic forces are expected to play a minor role in subsequent interactions at the tooth surface (as bacterial cells).

On the other hand, polymeric materials, as ion exchange resins, have also been applied for chromatographic purposes. The advantages of this kind of material are large specific area, versatility and relative cheapness. As an example, Skidmore et al. (1990) compared the adsorption behavior of lysozyme and BSA onto the strong cation exchanger S Sepharose FF. Two different models were developed. The first model was based on a single lumped kinetic parameter. The second model considers the individual transport process occurring prior to the adsorption reaction, taking into account diffusion across the liquid film surrounding individual particles and diffusion within the ion-exchange particle itself. Experiments were performed at 25°C, pH 5.0 in acetate-acetic acid buffer in batch agitated tanks and packed-bed modes. For lysozyme, both models allowed for good fit of the available data. In the case of BSA, however, the agitated tank profile was consistent with the pore diffusion model.

Arai and Norde (1990) pointed out that lysozyme behaves like a "hard" particle, having a large structure stability and, therefore, a strong internal coherence. As an example, it adsorbs onto polystyrene hydrophobic surfaces with high affinity, even under conditions of electrostatic repulsion.

Weaver and Carta (1996) used lysozyme as a test protein to characterize the equilibrium and transport properties of two commercial adsorbents suitable for protein chromatography at elevated flow rates. One of these materials, POROS 50, is a macroporous adsorbent based on a styrene-divinylbenzene copolymer. The other material, known by the trade name Hyper D, is a composite comprising high-porosity polystyrene-coated silica particles whose pores have been filled with a functionalized polyacrylamide hydrogel. Both materials possess a strong cation exchange functionality and are suitable for adsorption of positive proteins. Tests were performed at 23°C, pH 6.5 with a 10 mmol/L NaH_2PO_4 aqueous buffer. The gel-composite material has a capacity which is more than 60% higher than that of the macroporous exchanger. This result reflects the different way in which adsorption is performed: through surface area in the case of macroporous adsorbent and through the space-filling capacity of the functionalized hydrogel in the case of the gel-composite media.

Although intensively studied, the protein adsorption mechanism onto hydrophilic and hydrophobic

materials is not clear yet. Most studies comprise different kinds of materials, operational conditions and protein models. The aim of this study is to evaluate lysozyme adsorption onto three different adsorbents: hydroxyapatite, polystyrene with a core-shell structure (PE-CS) and a functionalized styrene-divinylbenzene copolymer (Amberlite IRA 120) using experimental design as a tool. The effect of pH, phosphate buffer and initial protein concentration on the adsorbed amounts of lysozyme onto the adsorbents were investigated. The main objective is to verify interactions between the variables and determine quantitatively the best adsorbent for a specific experimental condition.

2. Theoretical Framework

2.1. Langmuir Isotherm

Although originally developed for gas adsorption onto solid surfaces, Langmuir isotherm has been used to describe the adsorption behavior of proteins onto solid/liquid interfaces (Weaver and Carta, 1996; Barroug et al., 1992; Kandori et al., 1997). The widespread use of this model is due to its simplicity. The isotherm is expressed as:

$$\frac{q}{q_{\mathrm{m}}} = \frac{bC}{1 + bC} \tag{1}$$

where q is the adsorbed amount, q_m is the maximum adsorption capacity, b is Langmuir adsorption constant and C is equilibrium concentration of the adsorbed species. As the uptake of proteins generally involves heterovalent interactions with the adsorbent, the assumptions of the mathematical derivation of the Langmuir theory are violated. However, the expression can be applied successfully for fitting of experimental data if the parameters are empirically determined at each operational condition (Weaver and Carta, 1996).

2.2. Experimental Design

An experimental design is a collection of experimental tests where purposeful changes are made to the input variables of a process in order to observe and identify the corresponding changes in the output response. The objectives may include the determination of the variables that exert the largest influence on the response or definition of experimental conditions where the response is near the nominal requirement and/or response variability is small. Thus, experimental design methods may be used to improve performance or to obtain a process that is robust or insensitive to external sources of variability.

Certain special types of factorial designs are very useful for process development and improvement. One of these is a factorial design with k factors, each at two levels. Because each complete replicate of the design has 2^k runs, the arrangement is called a 2^k factorial design. These designs have greatly simplified data analysis and form the basis of many other useful designs (Montgomery, 1991).

Factorial designs are used in this work because these experimental plans allow for independent and simultaneous analysis of the most important linear and nonlinear effects that the independent variables exert on the response variables. In order to perform the statistical analysis, empirical models are normally used to represent the relationships between the set of response variables and the set of independent design variables. This should not be seen as a major drawback, especially during the initial stages of an experimental investigation, when very little is known about the relative importance of the design variables on the analyzed responses and when theoretical models cannot be formulated *a priori*, as in the analyzed problem. For these reasons, empirical statistical analysis is performed first for screening of the most important effects caused by the design variables on the response variables and afterwards the standard Langmuir Isotherm (Eq. (1)) is used to provide physical interpretation for the obtained experimental results.

3. Experimental

3.1. Materials

Both hydroxyapatite (Hap) and polystyrene with core-shell structure (PE-CS) were synthesized in our laboratories. Hap (Ca/P molar ratio equals 1.55 and 59.4 m^2/g of BET area) was used without further treatment. PE-CS was added to ethanol/water solutions to remove air of the lattices. First, PE-CS was washed with ethanol; afterwards, with ethanol/water solutions (50:50 wt%); and finally, with pure water. The commercial cation exchange resin used was Amberlite IRA 120 (A120), from Vetec. It was converted to sodic form by addition into NaOH 50 wt% aqueous solution for 24 h. Hen egg-white lyophilized lysozyme was supplied by USB. All other chemicals were reagent grade. High purity water was used for preparing solutions.

Table 1. Range of investigation of each variable.

Variable	Minimum	Central	Maximum
Phosphate buffer concentration (mmol/L)	0	5	10
pH (−)	6	8	10
Lysozyme concentration (g/L)	0.2	0.5	0.8

3.2. Experimental Design

The adsorption of lysozyme was evaluated with an experimental design. The effects of pH, phosphate buffer and initial protein concentration on the adsorbed amounts of lysozyme were investigated. Variable ranges are presented in Table 1.

In order to compare variables during the parameter estimation step, variables were normalized. Thus, −1 corresponds to the minimum value, 0 is the middle of the range and 1 is the higher value.

For each adsorbent, tests comprised a 2^3 factorial and a cross design, which was performed in replicates in order to quantify experimental deviations. Experimental design performed is presented in Table 2. The proposed experimental design allows for independent determination of the main linear and quadratic effects and of the binary interactions.

Table 2. Experimental design for each support.

Experimental conditions	Phosphate buffer	pH	Lysozyme concentration
1	−1	−1	−1
2	−1	−1	+1
3	−1	+1	−1
4	−1	+1	+1
5	+1	−1	−1
6	+1	−1	+1
7	+1	+1	−1
8	+1	+1	+1
9*	−1	0	0
10*	+1	0	0
11*	0	−1	0
12*	0	+1	0
13*	0	0	−1
14*	0	0	+1

*In triplicates.

3.3. Protein Adsorption Tests

For each experimental condition, 0.15 g of adsorbent (Hap, PE-CS or A120) was transferred to a test tube and mixed with 13 mL of protein solution prepared by dissolving lysozyme in a proper solution. Then, pH value was adjusted with 0.01 mol/L NaOH or HCl solutions. The system was closed and shaked at 200 rpm for 2 hours. Temperature was set to 23°C. The suspension was centrifuged and supernatant was analyzed. Bradford method (Bradford, 1976) was used to quantify protein equilibrium concentration. This is a colorimetric method used to quantify the protein content in a sample. The adsorbed amount of protein was determined by a mass balance.

3.4. Modelling Adsorption Process

Experimental data obtained was evaluated with Statistica software (Statsoft, 1995), including variance analysis. Models were developed to describe a mathematical relationship between variables and the amount of adsorbed lysozyme.

4. Results and Discussion

4.1. Adsorption Behavior of Each Support

In order to compare the adsorbents performance, the adsorbed amount of lysozyme in each test is presented in Table 3. As experimental conditions from 9 to 14 were carried out in triplicate, mean values are presented.

By comparing all the supports, hydroxyapatite showed the higher adsorbed amount (72.2 mg/g) in pH 8.0, 5 mmol/L of phosphate buffer solution and 0.8 g/L of protein concentration (experimental condition 14). This value is the same saturation value observed by Kawasaki et al. (2003), which was equal to 1.2 mg/m^2, corresponding to a full monolayer onto Hap. The higher adsorbed amount of lysozyme onto PE-CS and A120 was 37.4 and 11.9 mg/g, respectively.

4.2. Variance Analysis

As the experimental cross design was replicated, variances were computed for each one of the six experimental conditions (from 9 to 14) and compared by performing the *F*-test in pairs. Thus, it was possible to test the hypothesis of variance equality. Variances

Table 3. Amount of adsorbed lysozyme for each support (mg/g).

Experimental conditions	Adsorbent		
	Hap	PE	A120
1	5.1	12.0	5.0
2	19.2	35.8	5.7
3	13.5	17.0	5.7
4	40.2	37.4	11.9
5	13.6	11.6	2.3
6	22.4	20.3	4.5
7	21.4	15.6	5.1
8	44.6	32.3	6.8
9*	11.8	18.8	8.2
10*	42.6	23.1	10.5
11*	19.9	19.0	2.6
12*	36.8	22.7	9.0
13*	19.2	15.0	4.8
14*	72.2	32.8	11.6

*Mean values.

Table 4. Variances for each experimental condition of cross design $(mg/g)^2$.

Experimental conditions	Adsorbent		
	Hap	PE	A120
9	0.41	6.84	26.52
10	0.01	6.08	209.85
11	0.30	18.94	9.82
12	0.09	0.25	3.24
13	0.02	1.39	0.04
14	4.66	13.10	14.34

for each operational condition are presented in Table 4.

Table 4 shows that the increase in lysozyme concentrations also increases adsorption variability since variances of experimental condition 14 are always higher than in experiment 13. This may be due to the surface hydrophobicity and protein hardness.

It is also noticeable that decreasing pH causes an increase on variance for PE-CS and this is the main cause of the variability in this system. It means that protein is more regular closer to its isoelectric point.

For A120, however, it should be noted that the increase on phosphate buffer concentration seems to be the main source of variability.

Table 5. Variances for each adsorbent $(mg/g)^2$.

Adsorbent	Variances
Hap	0.92
PE	7.77
A120	43.97

According to the *F*-test, variances of each adsorbent are essentially equivalent to each other with 2 degrees of freedom and confidence level of 95%. So, from this point on, it is assumed that the variances are constant and equal to the mean variances for each support, as presented in Table 5.

The *F*-test was then performed to compare variances between different adsorbents. According to the test, variances obtained for each adsorbent are not equivalent with 12 degrees of freedom and 95% of confidence. This indicates that the adsorption mechanism is different depending on the chemical nature of the support, as we could expect. It is also noticeable that hydroxyapatite has the smaller variance, proving that it is the best adsorbent. This feature is specially important for industrial purposes.

4.3. Mathematical Models

In order to determine the main variables for each system, a model for the adsorbed amount of lysozyme was developed, as presented below:

$$q = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1^2 + a_5x_2^2 + a_6x_3^2 + a_7x_1x_2 + a_8x_1x_3 + a_9x_2x_3 \quad (2)$$

Variables x_1, x_2 and x_3 correspond to phosphate buffer concentration, pH and lysozyme initial concentration, while a_i $(i = 1, 2, \ldots 9)$ are parameters of the model.

As observed previously, Eq. (2) is used here for initial screening of the most important effects caused by the design variables on the response variables. Parameters a_0, a_1, a_2 and a_3 represent the linear effects (main effects). Parameters a_4, a_5 and a_6 represent the nonlinear quadratic effects (that lead to development of points of maximum and/or minimum in the response surface). Parameters a_7, a_8 and a_9 represent the nonlinear interaction effects (that indicate the synergy between different variables and how one design variable can influence the effect of a second one). The proposed

factorial design allows for simultaneous and independent analysis of all described effects on the performance of each analyzed adsorbent. Our main objective here is the identification of the most important variables and effects.

It must be clear that the use of Eq. (2) should be limited to the experimental region where it was developed, as it is empirical and lacks physical meaning. However, the usefulness of physically meaningful models in this case is questionable, as the chemical and physical natures of the adsorbents are very different. Therefore, the adsorption of the biomolecules occurs onto different sites and is subject to different sorts of interaction in the different experiments. Besides, little is known *a priori* about the nature of the chemical and physical interactions that are present in the studied system. For all these reasons, the use of Eq. (2) as a modeling tool is completely justifiable.

The results for the adsorbed amount of lysozyme onto Hap are presented in Table 6. Error values correspond to 95% of confidence band of a normal distribution.

For hydroxyapatite, all variables affect the adsorbed amount of lysozyme. However, lysozyme initial concentration is the main variable for this system, since a_3 is greater than a_1 and a_2. Phosphate buffer concentration and pH have the same significance level since a_1 and a_2 are similar. No interaction effects were observed. However, phosphate buffer concentration and pH (x_1 and x_2) present a nonlinear relationship with the adsorbed amount of protein.

The increase of lysozyme concentration causes the increase of the adsorbed amount since there are more molecules in the system. The same behavior is observed for phosphate buffer concentration. Probably, this is due to the fact that phosphate ions can adsorb on calcium sites of hydroxyapatites, increasing the total number of sites for adsorption of lysozyme, which is positively charged in the range of pH studied. It was already seen that lysozyme adsorbs onto phosphate ions on *ac* or *bc* faces of Hap (Kandori et al., 1997). Higher pH values raise lysozyme adsorbed amount since it is closer to its isoelectric point. These results indicate that the adsorption of lysozyme is based on electrostatic forces and hydrophobic interactions between adsorbent and lysozyme.

Table 6. Estimated parameters for the adsorbed amount of lysozyme onto hydroxyapatite.

Parameters	Values
a_0	42 ± 7
a_1	8 ± 5
a_2	8 ± 5
a_3	17 ± 5
a_4	-12 ± 8
a_5	-11 ± 8
R	0.89

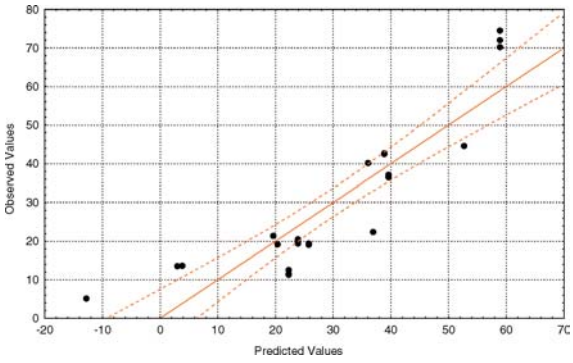

Figure 1. Observed and predicted values for lysozyme adsorption onto Hap.

It was observed that this model was able to explain 80% of variance and that the correlation coefficient between observed and predicted values was 0.89, showing that it is in good agreement with experimental data, as illustrated in Fig. 1. Dashed line corresponds to 95% of confidence band.

The results of parameter estimation of adsorbed amount of lysozyme onto PE-CS are presented in Table 7.

The main variable for lysozyme adsorption onto PE-CS is protein initial concentration, followed by pH. It was observed that phosphate buffer concentration does not affect the results. Besides that, the existence of significant nonlinear quadratic and interaction effects

Table 7. Estimated parameters for the adsorbed amount of lysozyme onto polystyrene.

Parameters	Values
a_0	22 ± 1
a_2	$2 + 2$
a_3	9 ± 2
R	0.89

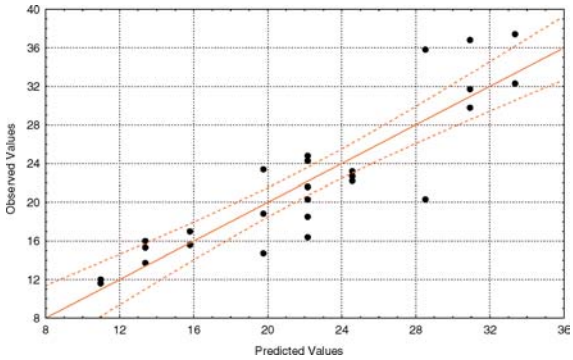

Figure 2. Observed and predicted values for lysozyme adsorption onto PE-CS.

could not be noticed. The model was able to explain 79% of experimental variance and linear correlation coefficient is 0.89, as illustrated in Fig. 2. Dashed line corresponds to 95% of confidence band. It can be concluded that linear model fits better to PE-CS, comparing it to hydroxyapatite. Electrostatic interactions play a minor role since it was observed that the adsorbed amount is greater when there is no phosphate ions, pH is 10.0 and lysozyme concentration is 0.8 g/L. This may be due to the fact that PE-CS is a hydrophobic material.

It was not possible to analyse experimental data for the adsorption of lysozyme onto A120 since variances were too high. It seems that investigated variables did not play a role on the adsorption. However, the origin of the deviations can be electrostatic and hydrophobic competitions onto the adsorbent surface, since it has hydrophobic and hydrophilic sites. In addition to it, it should be investigated whether phosphate ions and other chemicals (as NaOH and HCl, which were added to set pH values) affected the results. As ionic strength was not under control, this may be the reason for variance rise since this parameter can reduce the electrical double layer and change the adsorbed amount of lysozyme. This feature should be investigated in further works. Anyway, this is an indication that the alternative materials investigated here (Hap, PE-CS) may be better adsorbents for lysozyme than available commercial products.

Attempts to use Eq. (1) to describe the experimental data were not successful, as one might already expect and was described previously, even when parameters q_m and b were assumed to depend on the experimental conditions, as described below:

$$\frac{q}{q_{\mathrm{m}}} = \frac{(a_0 + a_1x_1 + a_2x_2 + a_3x_3)C}{1 + (a_0 + a_1x_1 + a_2x_2 + a_3x_3)C} \qquad (3)$$

In all cases, the best results were obtained when it was assumed that q did not depend on C. This probably indicates that all experimental data were obtained close to the saturation conditions, where the effect of C on q vanishes. However, as the adsorption of proteins onto surfaces depends strongly on the mechanism of adsorption (which is usually non-homogeneous) and on the surface coverage, one may also speculate that the use of the Langmuir isotherm is not appropriate in the analyzed case. Therefore, more involving physical modeling may be required for the analyzed experimental system.

5. Conclusion

Throughout the range of study of pH, lysozyme and phosphate buffer concentration, hydroxyapatite showed the best performance for lysozyme adsorption (higher adsorbed amount and smaller variance). However, PE-CS is a promising material, since low experimental deviations were observed for this support. Mathematical models showed that the adsorption of lysozyme onto Hap depends on the pH, phosphate buffer and protein initial concentration. For adsorption onto polystyrene, phosphate buffer concentration does not affect the results. For both adsorbents, interactions between the variables were not observed. Results obtained with Amberlite Ira 120 were not conclusive because it showed very high variances.

Nomenclature

a_i $(i = 1, 2, \ldots 9)$	model parameters
A120	cation exchange resin Amberlite Ira 120
b	Langmuir adsorption constant
C	equilibrium concentration of the adsobed species
Hap	hydroxyapatite
k	number of factors of a factorial design
PE-CS	polystyrene with core-shell structure
q	adsorbed amount, in mg/g
q_m	maximum adsorption capacity, in mg/g
x_n	investigated variables

References

Arai, T. and W. Norde, "The Behavior of Some Model Proteins at Solid-Liquid Interfaces 1. Adsorption from Single Protein Solutions," *Colloids Surf.*, **51**, 1–15 (1990).

Barroug, A., J. Lemaitre, and P.G. Rouxhet, "Lyzozyme on Apatites: A Model of Protein Adsorption Controlled by Electrostatic Interactions," *Colloid Surface*, **37**, 339–355 (1989).

Barroug, A., J. Lemaitre, and P.G. Rouxhet, "Influence of Crystallite Size on the Surface Properties of Calcium-Deficient Hydroxyapatites," *J. Alloys Compds.*, **188**, 152–156 (1992).

Bradford, M.M., "A Rapid and Sensitive Method for the Qualification of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding," *Analytical Biochemistry,* **72**, 248–254 (1976).

Kandori, K., M. Mukai, and A. Fujiwara, "Adsorption of Lysozyme onto Various Synthetic Hydroxyapatites," *J. Colloid Interf. Sci.*, **191**, 498–502 (1997).

Kawasaki, K., M. Kambara, and H. Matsumura, "A Comparison of the Adsorption of Saliva Proteins and some Typical Proteins onto the Surface of Hydroxyapatite," *Colloid Surface B*, **32**, 321–334 (2003).

Montgomery, D.C., *Introduction to Statistical Quality Control*, pp. 453–547, John Wiley & Sons, New York, 1991.

Skidmore, G.L., B.J. Horstmann, and H.A. Chase, "Modelling Single-Component Protein Adsorption to the Cation Exchanger S Sepharose® FF," *J. Chromatogr.,* **498**, 113–128 (1990).

Statsoft, "Statistica for Windows", Tulsa–OK, 1995.

Weaver, L.E., and G. Carta, "Protein Adsorption on Cation Exchangers: Comparison of Macroporous and Gel-Composite Media," *Biotechnol. Progr.*, **12**, 342–355 (1996).