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Modeling particle size of polyacrylic acid-copper(II)-bovine serum albumin ternary complex in salt solution

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Particle size and zeta potential of polyelectrolyte-protein binary conjugate and polyelectrolyte-metal-protein ternary complexes are among the most important factors affecting biopharmaceutical behavior such as bioavailability, dissolution and immune toxicity of these complexes. For this purpose, in the current study, the interactions between binary and ternary anionic polyelectrolyte, "polyacrylic acid (PAA)", BSA and copper ions in different salt concentrations were investigated by zetasizer analysis. The particle size of poly(acrylic acid)-copper (II)-bovine serum albumin (BSA) ternary complex in a salt solution was modeled using extensive regression methods to Partial Least Squares (PLS) regression from conventional multiple linear regression to Partial Least Squares (PLS) regression.

Keywords: Particle size, zeta potential, binary and ternary complex, ridge regression, LASSO, Elastic Net, PLS.

Introduction

Bioconjugations and biocomplexes of synthetic polyelectrolytes (PE) with biomacromolecules, such as protein, have attracted much attention in the last decades for its great advantages in biotechnology, medicine, and pharmacy field. Polyelectrolytes of synthetic origin have been found to increase immuno response to the immunizing antigen and to produce an adjuvant effect. The use of PE as a carrier, which is firmly linked to microbial and viral antigens to form a stable complex (or conjugate), not only increased by several orders of magnitude the immune responsiveness of the organism but also afforded effective immune protection.

Water-soluble polyelectrolytes (PEs)-protein binary conjugates and PE-metal-protein ternary complexes as the functional biopolymer systems represent a specific class of these conjugates and complexes that have been the subject of many studies in the application areas. The copper complexes interact with DNA, leading to chemically induced cleavage of DNA and, thus, antitumor activity. The mode of action is probably related to the binding of PE-copper(II)-bovine serum albumin (BSA), which is likely to leave some potential donor atoms free and these free donor atoms enhance the biological activity[1–5].

Zetasizer measurements such as zeta potential, particle size and mobility have been used to explain the effect of ions on coagulation in blood. Zetasizer measurements provide valuable properties of particles or molecules in liquid medium. These characteristics directly affect bioavailability, dissolution and immunotoxicity of molecules. Zeta potential is the electrostatic potential at the electrical double layer surrounding a nanoparticle in solution and automatically calculated by the analyzer using the Smoluchowski equation[6,7].

Multiple regression models investigate the effects of independent variables on a dependent variable, and covers model building and prediction. In this study, the regression methods of Ridge, Least Absolute Shrinkage and Selection Operator (LASSO), Elastic Net, and PLS following the classical multiple regression were conducted using SAS 9.2 to evaluate the models of the particle size of poly(acrylic acid)-copper(II)-bovine serum albumin (BSA) ternary complex in a salt solution. The effects of particle sizes and zeta potential of binary complex as PAA-BSA and ternary complex as PAA-

Cu(II)-BSA were investigated systemically increased salt solution into account.

Materials and method:

Polyacrylic acid was freshly synthesized by radical polymerization of acrylic acid in the presence of benzoilperoxide as initiator in toluene solution and fractionated[8]. PAA-BSA conjugate was synthesized in the presence of polyacrylic acid, BSA, (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride as conjugate, PBS in DMSO. PAA-Cu(II)-BSA complex was obtained in the presence of PAA, BSA and $CuSO_4.5H_2O$ as metal salt.

The particle size of BSA, PAA-BSA and PAA-Cu(II)-BSA in water were measured via Brookhaven 90 Plus/BI-MAS (Multi Angle Particle Sizing) and electrophoretic mobility and zeta potential measurements of all solutions were also determined by Brookhaven Zeta Potential Analyzer in the different salt concentrations at physiological temperature (37°C).

Results and discussion

The particle size, mobility and zeta potential of BSA (Table 1), binary conjugate, PAA-BSA (Scheme 1(a); Table 2) and ternary complex, PAA-Cu(II)-BSA (Scheme 1(b); Table 3) were measured in the different salt concentrations via Zetasizer. The results given as received.

According to Tables 1, 2 and 3, particle size and zeta potential of solutions were not changing in the concentrated salt solution. BSA, PAA-BSA and ternary complex, PAA-Cu(II)-BSA have negative mobility values. It means that these molecules have negative charge in salt solution.

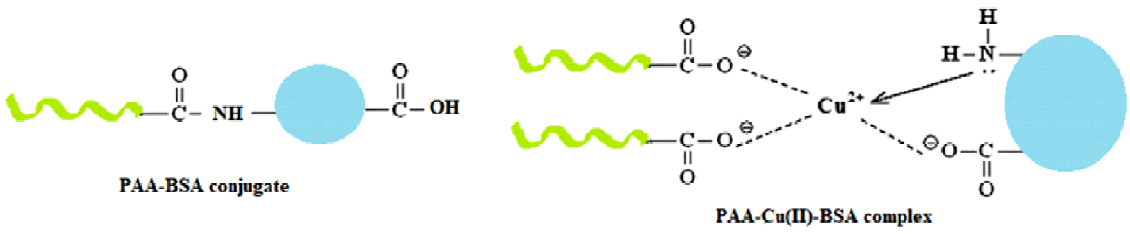
We want to predict the particle size of poly(acrylic acid)-copper(II)-bovine serum albumin (BSA) ternary complex in a salt solution. Firstly, the multiple regression model was estimated using Ordinary Least Squares (OLS). Even with a remarkable high coefficient of determination (R^2 = 0.9501), the model was found insignificant [Root Mean Square Error (RMSE) = 22.71694, F value = 7.62, p = 0.1201] implying that all the slope coefficients were also insignificant. This model suffered from the multicollinearity problem as well as small sample size. The two of Variance Inflation Factor (VIF) values were found as 5.74093 and 9.27297 for [NaCl] and particle size of BSA which were greater than the recommended cut-off value of 5 for VIF. Further, fifth and sixth condition indices were 54.85859 and 86.39285 which were considerable greater than 30.

Table 1. Salt effect on the Zetasizer measurements of BSA

[NaCl] (*M*)	Particle size (nm)	Mobility	Zeta potential (mV)
0	470	–1.33	–16.97
0.001	480	–1.86	–23.78
0.002	490	–2.04	–26.12
0.005	500	–1.67	–21.40
0.008	530	–1.80	–23.05
0.01	540	–1.80	–23.07
0.02	600	–1.62	–20.71
0.05	610	–1.54	–19.67

Table 2. Salt effect on the Zetasizer measurements of PAA-BSA conjugate

[NaCl] (*M*)	Particle size (nm)	Mobility	Zeta potential (mV)
0	290	–1.66	–21.24
0.001	210	–1.70	–21.79
0.002	240	–2.02	–25.90
0.005	250	–2.05	–26.24
0.008	300	–1.41	–24.08
0.01	310	–1.91	–24.48
0.02	320	–1.89	–24.23
0.05	325	–2.03	–25.92



Scheme 1. The chemical structure of water soluable (a) binary conjugate and (b) ternary complex.

Table 3. Salt effect on the Zetasizer measurements of PAA-Cu(II)-BSA complex

[NaCl] (*M*)	Particle size (nm)	Mobility	Zeta potential (mV)
0	490	–2.51	–32.18
0.001	480	–2.40	–30.78
0.002	485	–2.02	–25.81
0.005	430	–1.78	–22.74
0.008	445	–1.68	–21.48
0.01	440	–1.71	–21.84
0.02	395	–1.82	–23.27
0.05	335	–1.98	–25.28

Ridge regression is a regularization method designed to improve the estimation of in linear models possibly shrink OLS regression coefficients with standards errors but increase Mean Square Error (MSE). Ridge regression produces biased parameter estimates but attempting smaller variance[9].

Ridge parameter was selected as 0.03 using Ridge trace graphic considering both of the stability of regression coefficient and acceptable levels of VIF values. As seen from Table 4, the OLS regression coefficients were regularized through Ridge regression providing smaller standard errors, while a not remarkable increase in RMSE (= 23.0049) of Ridge regression.

Table 4. OLS and Ridge estimates

	OLS		Ridge	
Coefficients	Estimate	Std. error	Estimate	Std. error
Intercept	725.48906	190.21950	721.157	169.685
[NaCl]	–1374.10496	1235.14068	–1451.13	942.08
Particle size of BSA	–0.62849	0.48977	–0.53949	0.33919
Zeta potential of BSA	–2.39129	4.29243	–1.85758	3.69812
Particle size of PAA-BSA	0.18758	0.43972	0.09875	0.34816
Zeta potential of PAA-BSA	1.89220	3.45836	2.10953	3.30329

Ridge regression and LASSO regression are the two main types of regularization. LASSO simultaneously estimates regression coefficients and selects variables efficiently[10]. The Elastic Net regression has the advantages of the LASSO and Ridge regression combing their strengths and diluting the disadvantages. It balances LASSO for parsimonious model to Ridge for shrinkage coefficients of correlated variables[11].

LASSO and Elastic Net models were estimated based on the minimization of four-fold cross validation Predicted REsidual Sum of Squares (PRESS) measure. Table 5 shows LASSO and Elastic Net regression results. LASSO estimated rather parsimonious model selecting only [NaCl] among independent variables whereas Elastic Net included Particle Size of BSA to model in addition to NaCl; both of the models had also intercept terms. Remarkable differences were found between them in terms of R^2 and RMSE values. Even though the prediction performance of LASSO with R^2 value of 0.2436 was poor, Elastic Net's R^2 value of 0.9117 was comparable to OLS. RMSE values of LASSO and Elastic Net regression were 51.06613 and 19.10743, respectively.

Table 5. LASSO and Elastic Net estimates

Coefficients	LASSO	Elastic Net
Intercept	443.371614	647.555716
[NaCl]	–454.9261	–1611.256045
Particle size of BSA	na	–0.360774
Zeta potential of BSA	na	na
Particle size of PAA-BSA	na	na
Zeta potential of PAA-BSA	na	na

na: Not selected to the regression model.

PLS regression is a popular method especially in cases of multicollinearity, violations of distributional assumptions, and small sample size[12]. To determine the optimum number of factors, PLS model was used PRESS statistic that is obtained from the cross-validation process. From Table 6, one can find that one factor explains 89.6287% of the variance of particle size of PAA-Cu(II)-BSA. This suggests that this single factor should be kept for the final model. Nonlinear Iterative Partial Least Squares (NIPALS) algorithm was performed to obtain the scores for single factor.

Table 6. Percent variation accounted for by PLS factor

Number of	Model effects		Dependent variables	
extracted factor	Current	Total	Current	Total
1	55.128	55.128	89.628	89.628

As seen Table 7, the prediction results with single component, we have PRESS 0.6120, and R^2 = 0.8962. The cross-validation analysis confirms that: the model with one PLS factor achieves the absolute minimum PRESS, and it is significantly better than the model with different factors.

Table 7. Cross validation results

Number of extracted factors	Root mean PRESS
0	1.142857
1	0.612011
2	0.744026
3	0.872202
4	0.918241
5	21.0053
Minimum root mean PRESS	0.612011
Minimizing number of factors	1

As seen Table 8, the absolute value of a regression coefficient and Variable Importance for Projection (VIP) represent the importance each predictor has in the prediction of the response. According to the regression coefficients and VIP, [NaCl] has the biggest influence on the particle size of PAA-Cu(II)-BSA. The particle size of BSA has a great contribution in predicting particle size of the PAA-Cu(II)-BSA. The zeta potential of BSA and particle size of PAA-BSA, and zeta potential of PAA-BSA had smaller coefficients and smaller VIP's. As a result of the prediction results with one component, the residual ranged from 0.000 to 0.29 and all of them are low. The PLS regression model explains 89.6287% of the variation of the particle size of PAA-Cu(II)-BSA, and 55.1280% of the variation in the independent variables of [NaCl], particle size of BSA, zeta potential of BSA, particle size of PAA-BSA, zeta potential of PAA-BSA.

Table 8. The regression coefficient and VIP values of independent variables

Label	Standardized regression coefficients	VIP
[NaCl]	–0.3466907983	1.324257314
Particle size of BSA	–0.3404001547	1.294598471
Zeta potential of BSA	–0.1064162280	0.482857667
Particle size of PAA-BSA	–0.2426472660	0.968161112
Zeta potential of PAA-BSA	0.1520059352	0.632352669

Conclusions

It were modeled the particle size of poly(acrylic acid)-copper(II)-bovine serum albumin (BSA) ternary complex in a salt solution by using [NaCl], particle size of BSA, zeta potential of BSA, particle size of PAA-BSA, and zeta potential of PAA-BSA as independent variables. Under the data circumstances of small sample and evident collinearity, multiple regression model was insignificant but providing high coefficient of determination. This motivated us to use regularization methods to model the particle size of poly(acrylic acid)-copper(II)-bovine serum albumin (BSA) ternary complex in a salt solution by Ridge regression, LASSO and Elastic Net. Further, PLS regression was also considered to evaluate its prediction performance. LASSO results gave the most parsimonious model selecting [NaCl] as single predictor, consequently prediction performance was poor. Ridge regression shrank regression coefficient and got standard errors smaller providing similar results to OLS with an insignificant model. Elastic Net and PLS regression performed well and they were comparable. The prediction performance of Elastic Net was superior in terms of the largest accounted variance of particle size of PAA-Cu(II)-BSA, and the smallest RMSE.

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