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Fabrication and evaluation of citrate stabilized zinc oxide quantum dots

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ABSTRACT

The triumph of quantum dots (QDs) in biomedical applications roused the scientists to further extensive research in this area. Citrate stabilized zinc oxide QDs are useful in drug and gene surface loading. Response surface quadratic models are well reported to study the influence of independent parameters on dependent parameters. The objective of the present study was thus to carry out evaluation of citrate stabilized zinc oxide QDs by employing a statistical experimental design. From the results it was concluded that the particle size, polydispersity index and zeta potential data does not fit into the response surface quadratic design model. A comparison of the prepared citrate stabilized zinc oxide quantum dots was done with respect to particle size, polydispersity index and zeta potential. Though a response surface statistical modeling was not possible with the selected range of independent factors in the study, the results could be of use in choosing constraints for independent variables in further studies towards tailored citrate stabilized zinc oxide QDs with desired values for particle size, polydispersity index and zeta potential.

Keywords: Zinc oxide, Quantum dots, Box-Behnken design

INTRODUCTION

The triumph of quantum dots (QDs) in biomedical applications roused the scientists to further research in this area. At present QDs are used as carriers and labeling therapeutics [1]. Zinc oxide QDs have now been well reported for biomedical applications [2-4]. Citrate stabilized zinc oxide QDs are useful in drug and gene surface loading. Preparation of citrate stabilized zinc oxide QDs with tailored particle size, polydispersity index and zeta potential would be much useful. Response surface quadratic models are well reported to study the influence of independent parameters on dependent parameters (such as particle size, polydispersity index and zeta potential) [5,6]. The objective of the present study was thus to carry out evaluation of prepared citrate stabilized zinc oxide QDs by employing a statistical experimental design. The feasibility of fitting the obtained data into response surface quadratic model was as also studied.

EXPERIMENTAL SECTION

Materials
Zinc acetate dihydrate (Zn $(Ac)_2.2H_2O$) and lithium hydroxide monohydrate ($LiOH.H_2O$) were purchased from Sigma-Aldrich Co., (MO, USA). Ethanol (99.9%) was purchased from Jiangsu Huax Co., Ltd., Jiangsu, China.

Preparation of citrate stabilized zinc oxide quantum dots
Here a modified sol-gel synthesis method was adopted. Zinc acetate dihydrate (Zn $(Ac)_2.2H_2O$), lithium hydroxide monohydrate ($LiOH.H_2O$) and ethanol were used as precursor materials to synthesize ZnO QDs. Selected concentration of $Zn(Ac)_2.2H_2O$ in ethanol was refluxed at a selected temperature range for 20 minutes to get clear solution and then cooled to room temperature. Prepared a selected concentration of $LiOH{\cdot}H_2O$ in ethanol and was added drop wise to the zinc acetate solution in the ratio of 1:2 with stirring. The pH of the solution was measured between 11~12. 0.01 M of trisodiumcitrate dihydrate ($C_6H_5Na_3O_7{\cdot}\ 2H_2O$) was further added to the solution during continuous stirring. The resulting solution was centrifuged and washed with ethanol and water [7].

Evaluation of citrate stabilized zinc oxide quantum dots
A Box-Behnken statistical design with 3 factors, 3 levels, and 17 runs was selected for the statistical evaluation using Design-Expert software (Design-Expert 7.0.0, State-Ease Inc, Minneapolis, USA). This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. The independent and dependent variables are listed in Table 1.

Table 1: Variables and their constraints in Box-Behnken design

Variables	Constraints	
	Lower limit	*Upper limit*
Independent variables		
A = Temperature (°C)	30	80
B = $Zn(Ac)_2.2H_2O$ (mg/20 mL)	18.3	54.9
C = $LiOH.H_2O$ (mg/20 mL)	8.39	25.17
Dependent variables	***Goals***	
R1 = Particle size (nm)	Minimize	
R2 = Polydispersity index	Minimize	
R3 = Zeta potential (mV)	Maximize	

The coded and actual values for the selected central composite experimental design matrix for the formulation of zinc oxide quantum dots were as given in Table 2. The data obtained from the design was evaluated using Design-Expert software (Design-Expert 7.0.0, State-Ease Inc, Minneapolis, USA).

Table 2: Box Bhenken design matrix for the optimization of citrate stabilized ZnO QDs

Exp. Run	Coded values			Actual values		
	Temperature	ZAD	LHM	Temperature (°C)	ZAD (mg/20 mL)	LHM (mg/20 mL)
1	0	-1	1	55	18.3	25.17
2	-1	0	1	30	36.6	25.17
3	-1	0	-1	30	36.6	8.39
4	0	0	0	55	36.6	16.78
5	0	1	-1	55	54.9	8.39
6	1	0	1	80	36.6	25.17
7	-1	1	0	30	54.9	16.78
8	0	0	0	55	36.6	16.78
9	0	0	0	55	36.6	16.78
10	0	0	0	55	36.6	16.78
11	1	1	0	80	54.9	16.78
12	1	-1	0	80	18.3	16.78
13	0	0	0	55	36.6	16.78
14	1	0	-1	80	36.6	8.39
15	0	1	1	55	54.9	25.17
16	0	-1	-1	55	18.3	8.39
17	-1	-1	0	30	18.3	16.78

ZAD = Zinc acetate dihydrate; LHM = Lithium hydroxide monohydrate

The polynomial equation generated by this experimental design is as follows.

$$R = C_0 + C_1A + C_2B + C_3C + C_4AB + C_5AC + C_6BC + C_7A^2 + C_8B^2 + C_9C^2$$

Where R is the dependent variable, C_0 is the intercept, C_1 to C_9 are the regression coefficients, and A, B and C are the independent variables.

Particle size and polydispersity index

Mean particle size and polydispersity index were determined by dynamic light scattering technique. The obtained quantum dots were dispersed in ultrapure water and were taken in a cuvette. Its size and size distribution were determined using Zetasizer Nano ZS Particle Sizer (Malvern Instruments Ltd, Worcestershire, United Kingdom) at 25 °C.

Zeta potential

Zeta potential was determined by the electrophoretic mobility of quantum dots in U-type tube at 25 °C, using Zetasizer Nano ZS Particle Sizer (Malvern Instruments Ltd, Worcestershire, United Kingdom).

RESULTS AND DISCUSSION

Preparation of citrate stabilized zinc oxide quantum dots

Citrate stabilized zinc oxide quantum dots were prepared by precipitation method. All the 17 batches proposed by the experimental design yielded quantum dots and were evaluated for particle size (PZ), polydispersity index (PDI) and zeta potential (ZP).

Evaluation of zinc oxide quantum dots

The data obtained for the experimental design are displayed in Table 3.

Table 3 Box-Behnken experimental design data for citrate stabilized ZnO QDs

Exp. Run	Independent factors			Dependent factors		
	Temperature (°C)	ZAD (mg/20 mL)	LHM (mg/20 mL)	PZ ± SD (nm)	PDI ± SD	ZP ± SD (mV)
1	55	18.3	25.17	896.6 ± 12.5	0.887 ± 0.042	-2.32 ± 0.11
2	30	36.6	25.17	378.7 ± 18.5	0.041 ± 0.002	-1.29 ± 0.07
3	30	36.6	8.39	649.6 ± 14.3	0.717 ± 0.035	-2.28 ± 0.22
4	55	36.6	16.78	996.4 ± 15.4	0.932 ± 0.045	-1.96 ± 0.15
5	55	54.9	8.39	1139 ± 8.2	0.223 ± 0.013	-4.52 ± 0.27
6	80	36.6	25.17	1735 ± 6.3	1.000 ± 0.08	-3.38 ± 0.33
7	30	54.9	16.78	513.8 ± 11.4	0.592 ± 0.047	-2.64 ± 0.23
8	55	36.6	16.78	932.4 ± 14.6	0.834 ± 0.041	-2.03 ± 0.14
9	55	36.6	16.78	972.4 ± 13.2	0.934 ± 0.074	-1.89 ± 0.15
10	55	36.6	16.78	1002 ± 13.8	0.873 ± 0.06	-1.93 ± 0.11
11	80	54.9	16.78	1041 ±11.2	0.481 ± 0.02	-5.83 ± 0.46
12	80	18.3	16.78	1983± 12.6	0.934 ± 0.074	-2.32 ± 0.20
13	55	36.6	16.78	945.5 ± 14.8	0.889 ± 0.053	-1.91 ± 0.11
14	80	36.6	8.39	4202 ± 8.4	0.824 ± 0.042	-5.78 ± 0.51
15	55	54.9	25.17	2301 ± 11.2	0.165 ± 0.008	-3.75 ± 0.33
16	55	18.3	8.39	621.2 ± 12.6	0.518 ± 0.031	-2.85± 0.22
17	30	18.3	16.78	1037 ± 11.3	0.750 ± .045	-2.91 ± 0.14

ZAD = Zinc acetate dihydrate; LHM = Lithium hydroxide monohydrate; PZ= Particle size; PDI = Polydispersity index; ZP = Zeta potential; SD = Standard deviation (n = 3)

Effect of independent factors on particle size (R1)

Table 4 displays the analysis of variance (ANOVA) data for the response. The Model F-value of 1.25 implied that the model was not significant relative to the noise. There was a 39.34 % chance that a Model F-value this large could occur due to noise. In this case A is significant model term. The Lack of Fit F-value of 1813.99 implies the Lack of Fit was significant. A negative Pred R-Squared implied that the overall mean was a better predictor of the response than the current model. Adeq Precision measures the signal to noise ratio and the obtained ratio of 4.688 indicated an adequate signal. Fig. 1 shows the effect of independent factors on particle size.

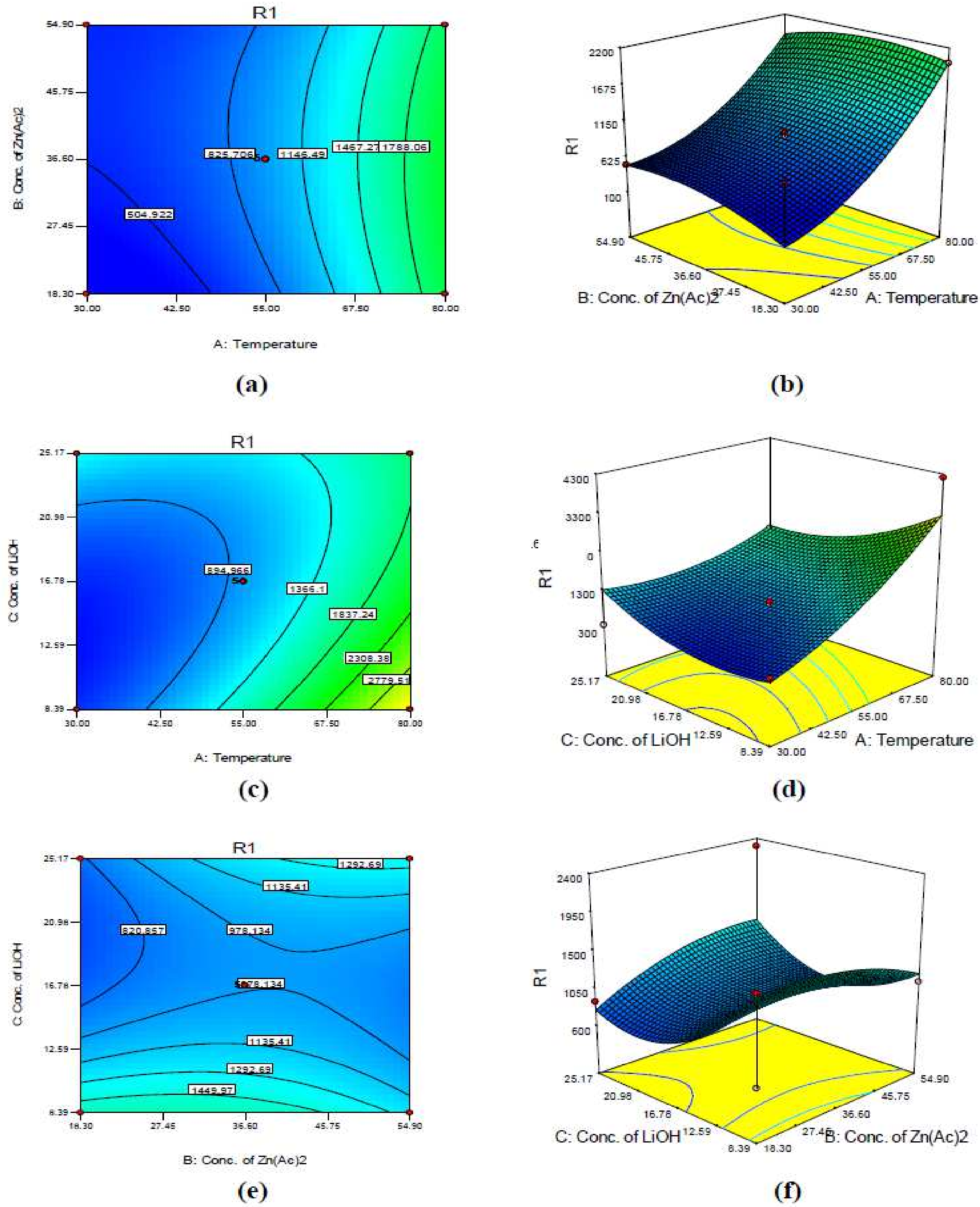

Figure 1: Contour and response surface plots for particle size (R1)
(a) Contour plot of factor B vs. A against R1; (b) Response surface plot of factor B vs. A against R1;
(c) Contour plot of factor C vs. A against R1; (d) Response surface plot of factor C vs. A against R1;
(e) Contour plot of factor C vs. B against R1; (f) Response surface plot of factor C vs. B against R1.

Table 4: Analysis of variance (ANOVA) for response surface quadratic model (Response 1- Particle size)

Source	Sum of Squares	df†	Mean Square	F Value	p-value (Prob > F)
Model	8.178E+006	9	9.087E+005	1.25	0.3934
A-Temperature	5.091E+006	1	5.091E+006	7.00	0.0332
B- Conc. of Zn(Ac)$_2$	26106.13	1	26106.13	0.036	0.8551
C- Conc. of LiOH	2.114E+005	1	2.114E+005	0.29	0.6065
AB	43848	1	43848.36	0.060	0.8131
AC	1.206E+006	1	1.206E+006	1.66	0.2389
BC	1.965E+005	1	1.965E+005	0.27	0.6193
A^2	4.808E+005	1	4.808E+005	0.66	0.4430
B^2	1.132E+005	1	1.132E+005	0.16	0.7050
C^2	7.919E+005	1	7.919E+005	1.09	0.3315
Residual	5.093E+006	7	7.275E+005		
Lack of fit	5.089E+006	3	1.696E+006	1813.99	<0.0001
Pure error	3740.39	4	935.10	-	-
Cor total	1.327E+007	16	-	-	-

†df = degrees of freedom

Effect of independent factors on polydispersity index (R2)

Table 5 displays the analysis of variance (ANOVA) data for the response. The Model F-value of 3.98 implied the model was significant. In this case B, C^2 were significant model terms. The Lack of Fit F-value of 42.47 implied that the Lack of Fit was significant. A negative Pred R-Squared implied that the overall mean is a better predictor of the response than response surface quadratic model. Adeq Precision measures the signal to noise ratio and the obtained ratio of 7.105 indicatesd an adequate signal. Fig. 2 shows the effects of independent factors on polydispersity index.

Table 5: Analysis of variance (ANOVA) for response surface quadratic model (Response 2- Polydispersity index)

Source	Sum of Squares	df†	Mean Square	F Value	p-value (Prob > F)
Model	1.19	9	0.13	3.98	0.0412
A- Temperature	0.16	1	0.16	4.87	0.0631
B- Conc. of Zn(Ac)$_2$	0.33	1	0.33	9.95	0.0161
C- Conc. of LiOH	4.465E-003	1	4.465E-003	0.13	0.7251
AB	0.002	1	0.022	0.65	0.4456
AC	0.18	1	0.18	5.45	0.0523
BC	0.046	1	0.046	1.37	0.2804
A^2	3.664E-005	1	3.664E-005	1.00E-003	0.9745
B^2	0.17	1	0.17	5.07	0.0591
C^2	0.25	1	0.25	7.52	0.0288
Residual	0.23	7	0.033	-	-
Lack of fit	0.23	3	0.075	42.47	0.0017
Pure error	7.097E-003	4	1.774E-003	-	-
Cor total	1.43	16	-	-	-

†df = degrees of freedom

Effect of independent factors on zeta potential (R3)

Table 6 displays the analysis of variance (ANOVA) data for the response. The Model F-value of 12.55 implied the model was significant. In this case A, B, C, AB, A^2, B^2, C^2 were significant model terms. The Lack of Fit F-value of 186.35 implied that the Lack of Fit was significant. The Pred R-Squared of 0.0723 was not as close to the Adj R-Squared of 0.8666 as one might normally expect. This may indicate a large block effect or a possible problem with the model and/or data. Adeq Precision measures the signal to noise ratio and the obtained ratio of 11.348 indicated an adequate signal. It was concluded that the particle size data does not fit into the response surface quadratic design model. Fig. 3 shows the effects of independent factors on particle size.

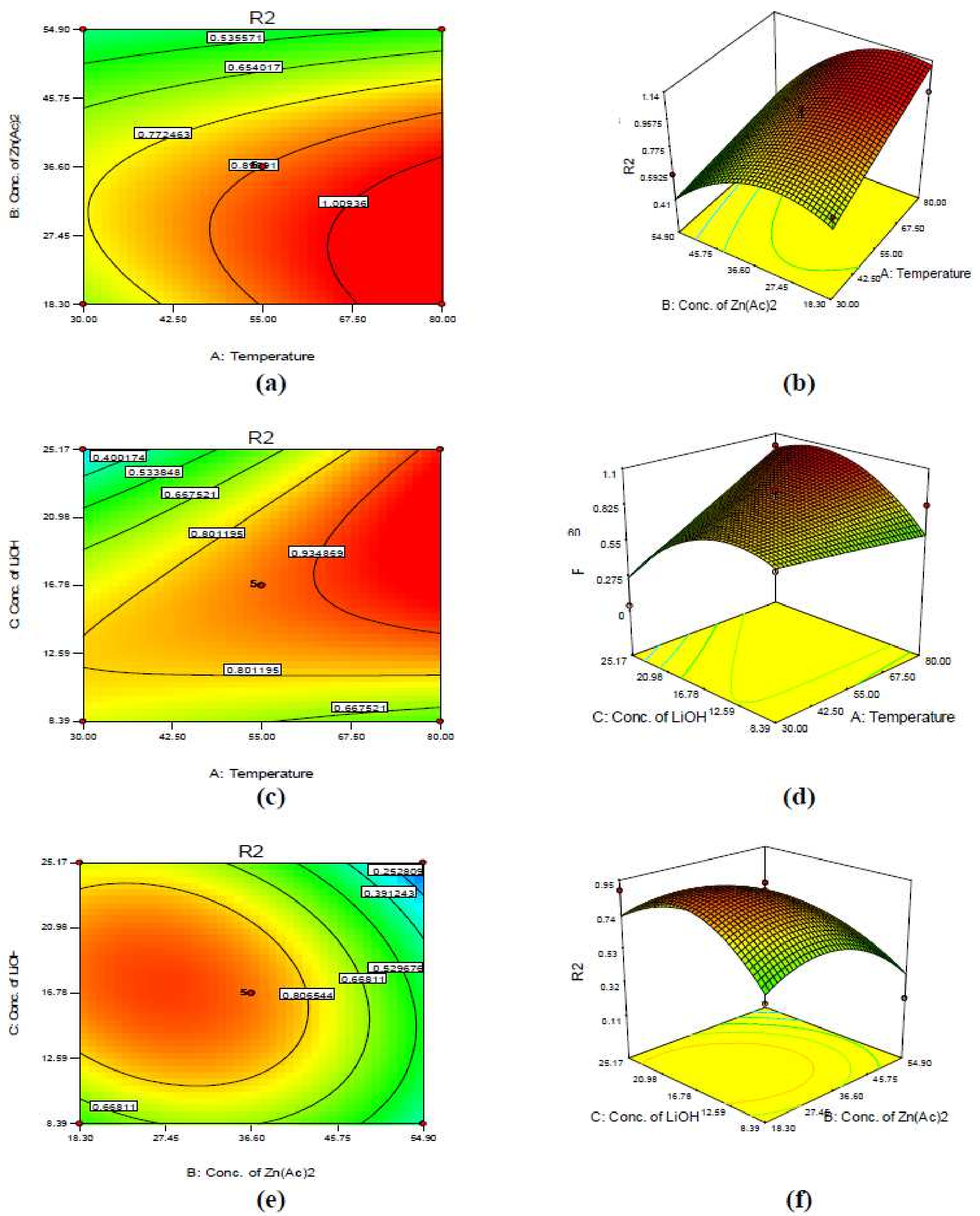

Figure 1: Contour and response surface plots for zeta potential (R2)

(a) Contour plot of factor B vs. A against R2; (b) Response surface plot of factor B vs. A against R2;
(c) Contour plot of factor C vs. A against R2; (d) Response surface plot of factor C vs. A against R2;
(e) Contour plot of factor C vs. B against R2; (f) Response surface plot of factor C vs. B against R2.

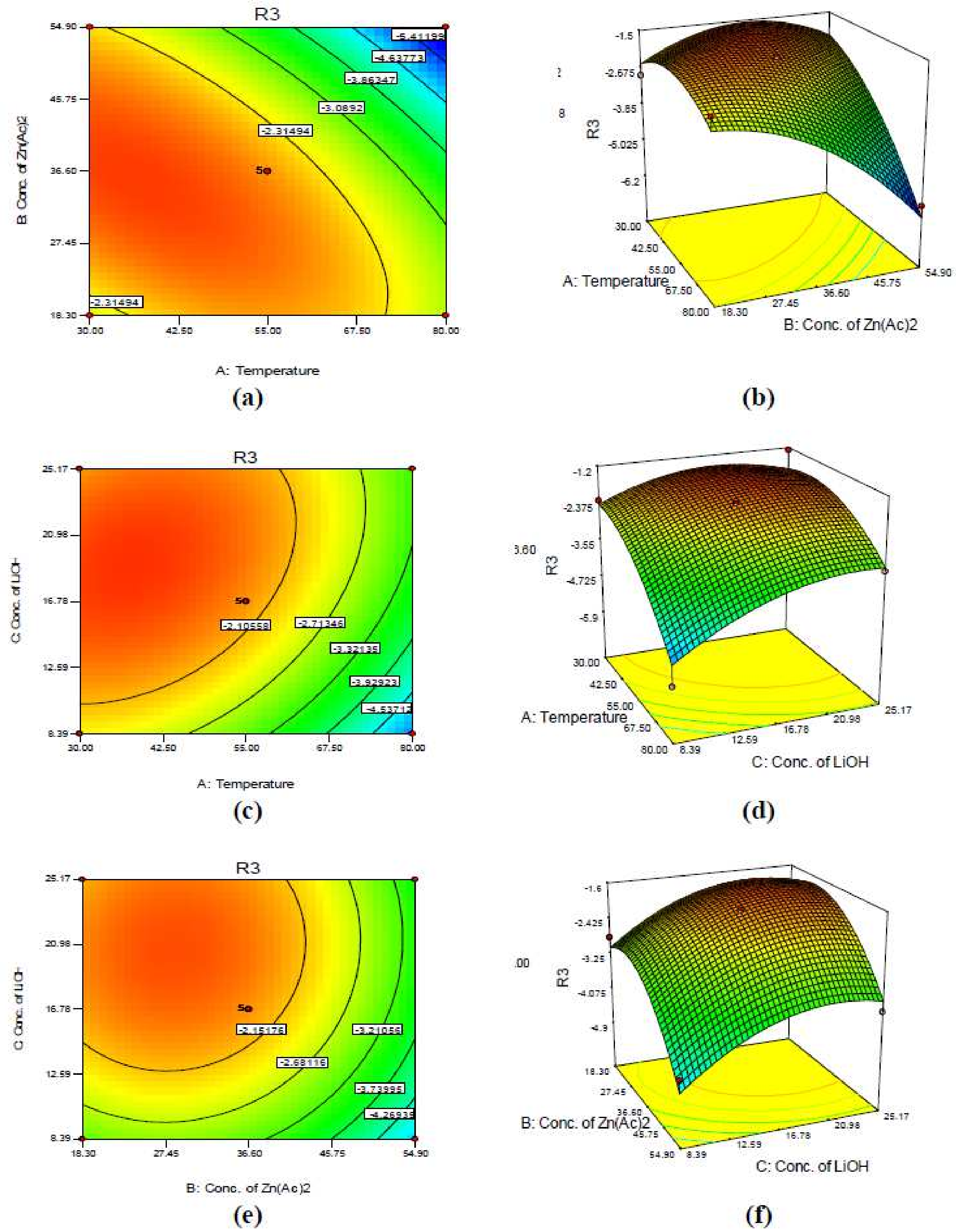

Figure 3: Contour and response surface plots for zeta potential (R3)
(a) Contour plot of factor B vs. A against R3; (b) Response surface plot of factor B vs. A against R3;
(c) Contour plot of factor C vs. A against R3; (d) Response surface plot of factor C vs. A against R3;
(e) Contour plot of factor C vs. B against R3; (f) Response surface plot of factor C vs. B against R3

Table 6: Analysis of variance (ANOVA) for response surface quadratic model (Response 3- Zeta potential)

Source	Sum of Squares	df†	Mean Square	F Value	p-value (Prob > F)
Model	27.07	9	3.01	12.55	0.0015
A- Temperature	8.38	1	8.38	34.98	0.006
B- Conc. of $Zn(Ac)_2$	5.02	1	5.02	20.96	0.0025
C- Conc. of LiOH	2.75	1	2.75	11.47	0.0116
AB	3.57	1	3.57	14.90	0.0062
AC	0.50	1	0.50	2.07	0.1931
BC	0.014	1	0.014	0.060	0.8134
A^2	1.79	1	1.79	7.46	0.0293
B^2	2.90	1	2.90	12.08	0.0103
C^2	1.45	1	1.45	6.05	0.0435
Residual	1.68	7	0.24	-	-
Lack of fit	1.67	3	0.56	186.35	<0.0001
Pure error	0.012	4	2.980E-003	-	-
Cor total	28.75	16	-	-	-

†df = degrees of freedom

CONCLUSION

In the present study efforts were carried out to statistical evaluation of fabricated citrate stabilized zinc oxide quantum dots and further response surface modeling of the dependent variables. From the results it was concluded that the particle size, polydispersity index and zeta potential data does not fit into the response surface quadratic design model. A comparison of the prepared citrate stabilized zinc oxide quantum dots was done with respect to for particle size, polydispersity index and zeta potential. Mean particle size and PDI were found to be in the range of 378.7 – 4202 nm and 0.041 – 1.00 respectively. The zeta potential was negative and comparable for all quantum dots and was in the range of -5.83 – -1.29 mV. The magnitude of zeta potential was low for all samples. Though a response surface statistical modeling was not possible with the selected range of independent factors in the study, the results could be of use in preparation of quantum dots with desired values for particle size, polydispersity index and zeta potential. The results could also be of use in choosing constraints for independent variables in further studies towards tailored citrate stabilized zinc oxide quantum dots with desired values for particle size, polydispersity index and zeta potential.

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