

## A PREFORMULATION STUDY ON INTERACTIONS BETWEEN DOXORUBICIN HYDROCHLORIDE AND BOVINE SERUM ALBUMIN

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Received: 09 Sep 2017, Revised and Accepted: 10 Oct 2017

### ABSTRACT

**Objective:** The objective was to study the effect of concentrations of doxorubicin hydrochloride (Dox) and bovine serum albumin (BSA) in a sample on fluorescence intensity, UV absorbance, refractive index and optical rotation.

**Methods:** A circumscribed central composite statistical design with 2 factors, 5 levels, and 13 runs was selected for the study. According to that influence of both in interaction was measured by fluorescence intensity, UV absorbance, refractive index and optical rotation and were analyzed by the design expert software.

**Results:** It was observed that concentration of BSA alone was significantly affecting the fluorescence intensity and optical rotation of samples. Dox alone was having a significant effect on UV absorbance at 280 nm. In the case of a refractive index, both Dox and BSA were having a significant effect. But the effect of BSA was much pronounced than that of Dox on refractive index.

**Conclusion:** Interaction studies between BSA and Dox would be beneficial as they are commonly used in combination with tumor-targeted delivery. The interaction was observed that in a linear model for a wide range of concentration of both. So it will be useful to determine the interaction of unknown concentration.

**Keywords:** Fluorescence intensity, UV absorbance, Optical rotation, Refractive index

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DOI: <http://dx.doi.org/10.22159/ijap.2017v9i6.22470>

### INTRODUCTION

Drug-excipient interaction studies are very important [1, 2]. There are many drug-excipient interaction studies reported for conventional dosage forms such as tablets and capsules [3]. But nowadays a good number of reports are there on bovine serum albumin (BSA) and doxorubicin hydrochloride (Dox) for tumor targeted delivery [4-7]. In this scenario, it would be interesting to carry out a study on interactions between Dox and BSA.

In this study, the effect of concentrations of Dox and BSA in a sample of fluorescence intensity, UV absorbance, refractive index and optical rotation was evaluated. There four dependent factors or responses are seldom considered in any reported work on BSA and Dox. Statistical evaluation using the design of experiments is a simple and powerful tool to determine the effect of independent formulation factors on various dependent factors [8]. Here we checked the effect of concentrations of Dox and BSA on some

dependent factors such as fluorescence intensity, UV absorbance, optical rotation and refractive index.

### MATERIALS AND METHODS

#### Materials

Doxorubicin hydrochloride (Dox) and bovine serum albumin (BSA) was obtained from Sigma-Aldrich Co. (MO, USA). Reagent grade I water (Millipore, Molsheim, France) was used for the study.

#### Evaluation of the effect of concentrations of Dox and BSA

A circumscribed central composite statistical design with 2 factors, 5 levels, and 13 runs was selected for the study using Design-Expert 8.0.0.6 software (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 for central composite design

Independent factors		Levels				
Factor code	Factor	-1.414	-1	0	+1	+1.414
A	Dox concentration, (mM)	0.15	0.3	0.65	1.0	1.14
B	BSA concentration, (mM)	0.01	0.05	0.15	0.25	0.29
Dependent factors (Responses)						
Response code	Response					
R1	Fluorescence intensity (mAU)					
R2	UV absorbance at 280 nm					
R3	Refractive index					
R4	Optical rotation (°)					

The coded and actual values for the selected central composite experimental design matrix were as given in table 2. BSA and Dox solutions were prepared and stored in dark (protected from light) at 4 °C.

The samples were prepared by mixing and subjected to evaluation.

#### Fluorescence intensity

The fluorescence intensity of the samples was determined at an excitation wavelength of 280 nm and an emission wavelength of 347 using a spectrofluorophotometer (Shimadzu RF-5301PC spectro-fluoro-photometer, Shimadzu Scientific Instruments Inc., Maryland, U. S. A).

Table 2: The central composite experimental design matrix

Run	Coded values		Actual values	
	Dox concentration	BSA concentration	Dox concentration (mM)	BSA concentration (mM)
1	-1	-1	0.30	0.05
2	1	-1	1.00	0.05
3	-1	1	0.30	0.25
4	1	1	1.00	0.25
5	-1.414	0	0.15	0.15
6	1.414	0	1.14	0.15
7	0	-1.414	0.65	0.01
8	0	1.414	0.65	0.29
9	0	0	0.65	0.15
10	0	0	0.65	0.15
11	0	0	0.65	0.15
12	0	0	0.65	0.15
13	0	0	0.65	0.15

Dox: Doxorubicin hydrochloride, BSA: Bovine serum albumin

**UV-Vis spectrophotometry**

The UV absorbance of sample at 280 nm was determined using a UV-visible spectrophotometer (Agilent Cary 100, Agilent Technologies, Santa Clara, CA, United States) equipped with Cary WinUV software.

**Refractive index**

Refractive index was determined by Refractometer (Abbat 350, Anton Paar India Pvt. Ltd., Haryana, India) at 589 nm and 20.0 °C temperature.

**Optical rotation by polarimetry**

Optical rotation of samples was determined by Saccharimeter (Sac-i, Atago India Instruments Pvt Ltd., Mumbai, India) with the modified

validated procedure using 1 cm path length quartz cuvette at 589 nm (D-line of sodium lamp at visible wavelength).

**RESULTS AND DISCUSSION**

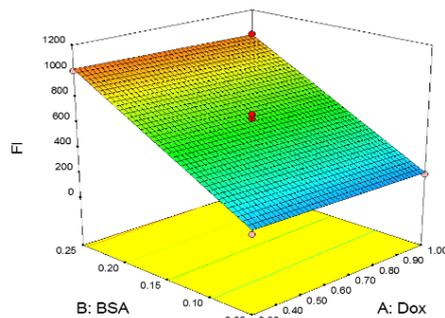
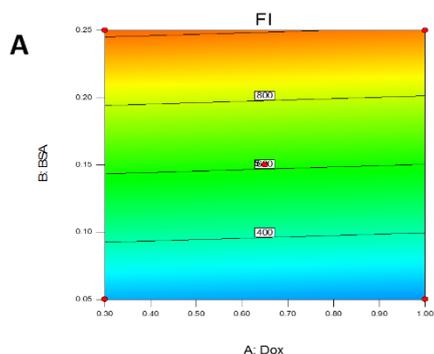
**Evaluation of the effect of concentrations of Dox and BSA**

The influence of doxorubicin hydrochloride and BSA concentration with the analytical parameters were determined by evaluating the all the 13 batches proposed by the experimental design.

Table 3 displays the results obtained for various experimental runs suggested by the software. The contour and response surface plots are shown in fig. 1.

Table 3: Results obtained for various experimental runs

Run	Responses			
	Fluorescence intensity	UV absorbance	Refractive index	Optical rotation
1	200.84	1.3331	-0.009	0.028
2	189.939	1.3331	-0.019	0.107
3	1003.238	1.3341	-0.046	0.035
4	995.874	1.3342	-0.044	0.113
5	697.932	1.3336	-0.025	0.022
6	631.287	1.3336	-0.016	0.131
7	43.009	1.3328	0	0.076
8	1127.806	1.3343	-0.045	0.071
9	633.12	1.3336	-0.02	0.093
10	574.659	1.3336	-0.02	0.074
11	598.399	1.3336	-0.013	0.092
12	670.098	1.3336	-0.013	0.077
13	593.322	1.3336	-0.022	0.076



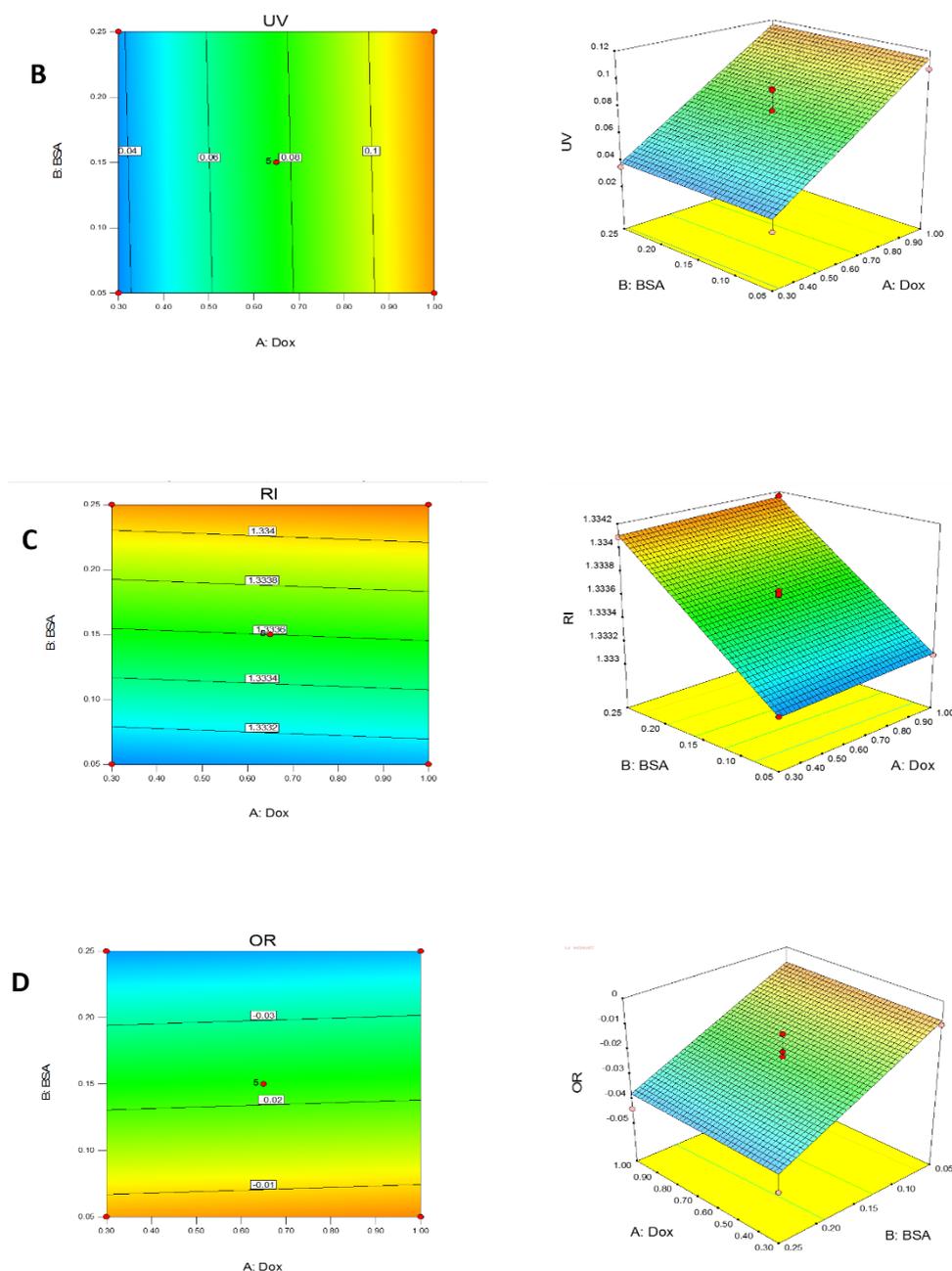


Fig. 1: Contour and response surface plots of (A) Fluorescent intensity, (B) UV absorbance, (C) Refractive index and (D) Optical rotation

**Effect on fluorescence intensity**

The Analysis of variance (ANOVA) table for the response surface linear model is given in table 4. The model was found to be significant whereas lack of fit was found to be not significant. The predicted R-square value of 0.9784 was comparable with the adjusted R-Square value of 0.9857. Adequate precision value of 59.804 was acceptable. Here the effect of BSA alone was significant. This was evident from the contour and response surface plots obtained for fluorescence intensity (fig. 1A). In the contour plot it can be seen that the iso-value curves are almost parallel to the x-axis of Dox and perpendicular to the y-axis of BSA. The results implied that Dox almost have no effect on the fluorescence intensity whereas BSA has much effect on fluorescence intensity. A similar observation is also seen in the response surface plot. The surface level does not

change much on change on changing Dox but increased significantly on increasing BSA. Thus increased BSA concentration caused increased fluorescence intensity.

**Effect on UV absorbance at 280 nm**

The ANOVA table for the response surface linear model is given in table 5. The model was found to be significant whereas lack of fit was found to be not significant.

The predicted R-square value of 0.9176 was comparable with the adjusted R-Square value of 0.9325. Adequate precision value of 26.967 was acceptable. Here the effect of Dox alone was significant. This was evident from the contour and response surface plots obtained for UV absorbance at 280 nm (fig. 1B). In

the contour plot, it can be seen that the iso-value curves are almost perpendicular to the x-axis of Dox and parallel to y-axis of BSA. The results implied that BSA almost have no effect on the UV absorbance whereas Dox has much effect on UV absorbance.

A similar observation is also seen in the response surface plot. The surface level does not change much on change on changing BSA but increased significantly on increasing Dox. Thus increased Dox concentration caused increased UV absorbance at 280 nm.

**Table 4: ANOVA for response surface linear model for fluorescence intensity**

Source	Sum of squares	df	Mean square	F Value	p-value prob>F
Model	1.236E+006	2	6.180E+005	413.21	<0.0001
A-Dox	1582.46	1	1582.46	1.06	0.3279
B-BSA	1.234E+006	1	1.234E+006	825.36	<0.0001
Residual	14955.71	10	1495.57	---	---
Lack of Fit	9224.50	6	1537.42	1.07	0.4956
Pure Error	5731.21	4	1432.80	---	---
Cor Total	1.251E+006	12	---	---	---

**Table 5: ANOVA for response surface linear model for UV absorbance**

Source	Sum of squares	df	Mean square	F value	p-value prob>F
Model	0.012	2	6.053E-003	83.94	<0.0001
A-Dox	0.012	1	0.012	167.82	<0.0001
B-BSA	4.394E-006	1	4.394E-006	0.061	0.8100
Residual	7.211E-004	10	7.211E-005	---	---
Lack of Fit	3.759E-004	6	6.265E-005	0.73	0.6549
Pure Error	3.452E-004	4	8.630E-005	---	---
Cor Total	0.013	12	---	---	---

**Effect on refractive index**

The ANOVA table for the response surface linear model is given in table 6. The model was found to be significant whereas lack of fit was found to be not significant. The predicted R-square value of 0.9981 was comparable with the adjusted R-Square value of 0.9985. Adequate precision value of 185.242 was acceptable. Here the effect both Dox and BSA was significant. But the effect of BSA was much pronounced than that of Dox. This was evident from the contour and response surface plots obtained for refractive index (fig. 1C). In the

contour plot, it can be seen that the iso-value curves are more or less perpendicular to y-axis of BSA thus implying its significant effect on the refractive index. A slight inclination of iso-value curves are noted towards the higher values of Dox concentration along the x-axis. This implied that increased Dox concentration causes a slight, but statistically significant, increase in refractive index. But an increased in BSA concentration caused drastic increase in refractive index. A similar observation is also seen in the response surface plot. The surface level drastically changed with BSA concentration whereas slightly with Dox concentration.

**Table 6: ANOVA for response surface linear model for refractive index**

Source	Sum of squares	df	Mean square	F value	p-value prob>F
Model	2.239E-006	2	1.119E-006	3968.16	<0.0001
A-Dox	4.950E-009	1	4.950E-009	17.55	0.0019
B-BSA	2.234E-006	1	2.234E-006	7918.77	<0.0001
Residual	2.821E-009	10	2.821E-010	---	---
Lack of Fit	1.341E-009	6	2.234E-010	0.60	0.7236
Pure Error	1.480E-009	4	3.700E-010	---	---
Cor Total	2.241E-006	12	---	---	---

**Effect on optical rotation**

The ANOVA table for the response surface linear model is given in table 7. The model was found to be significant whereas lack of fit was found to be not significant. The predicted R-square value of 0.6437 was comparable with the adjusted R-Square value of 0.7626. Adequate precision value of 13.247 was acceptable. Here the effect of BSA alone was significant. This was evident from the contour and response surface

plots obtained for optical rotation (fig. 1D). In the contour plot, it can be seen that the iso-value curves are almost parallel to x-axis of Dox and perpendicular to y-axis of BSA. The results implied that Dox almost have no effect on the optical rotation whereas BSA has much effect on optical rotation. A similar observation is also seen in the response surface plot. The surface level does not change much on change on changing Dox but increased significantly on increasing BSA. Thus increased BSA concentration caused increased optical rotation.

**Table 7: ANOVA for response surface linear model for optical rotation**

Source	Sum of squares	df	Mean square	F value	p-value prob>F
Model	1.976E-003	2	9.880E-004	20.28	0.0003
A-Dox	2.794E-006	1	2.794E-006	0.057	0.8156
B-BSA	1.973E-003	1	1.973E-003	40.49	<0.0001
Residual	4.873E-004	10	4.873E-005	---	---
Lack of Fit	4.141E-004	6	6.901E-005	3.77	0.1098
Pure Error	7.320E-005	4	1.830E-005	---	---
Cor Total	2.463E-003	12	---	---	---

**CONCLUSION**

The effect of concentrations of Dox and BSA on some dependent factors such as fluorescence intensity, UV absorbance, optical rotation and refractive index were studied. A circumscribed central composite statistical design with 2 factors, 5 levels, and 13 runs was selected for the study. From the results, it was observed that concentration of BSA alone was significantly affecting the fluorescence intensity and optical rotation. Dox alone was having a significant effect on UV absorbance at 280 nm. In the case of a refractive index, both Dox and BSA were having a significant effect. But the effect of BSA was much pronounced than that of Dox on refractive index.

**ACKNOWLEDGEMENT**

K. C. Ajithkumar gratefully acknowledges Kerala State Council for Science, Technology and Environment (KSCSTE), Thiruvananthapuram, India, for sanctioning student project (No. 14/SPS 57/2016/KSCSTE, Dated 02.04.2016).

**CONFLICT OF INTERESTS**

Declared none

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