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Application of Response Surface Methodology and Central Composite Design for the Optimization of Metformin Microsphere Formulation using Tangerine (*Citrus tangerina*) Pectin as Copolymer

Adenike Okunlola[1*] and Olawunmi Lateefat Akindele[1]

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author AO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors AO and OLA managed the literature searches, analyses of the study and author AO managed the experimental process. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To prepare metformin microspheres by ionic gelation using novel pectin from the peel of tangerine, *Citrus tangerina* (Rutaceae), as a copolymer with sodium alginate.
Study Design: Central composite design and response surface methodology.
Place and Duration of Study: Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria, between September 2015 and February 2016.
Methodology: Central composite design and response surface methodology were applied to evaluate the interactive effects of three variables: Percent of pectin in polymer blend, X_1, (50 to 75% w/w), curing time, X_2, (10 to 30 min) and concentration of chelating agent (calcium chloride), X_3, (5 to 10% w/v) on entrapment and dissolution time (t_{90}).
Results: Entrapment efficiency was 59.10 to 94.90% and t_{90} was 6.52 to 10.50 h. X_1 and X_3 had significant effects on entrapment and t_{90} ($p < 0.0001$). The interactions between X_2 and X_3 was

**Corresponding author: E-mail: nabidogun@yahoo.com;*

significant at p = 0.033 for entrapment. The correlation coefficients (R^2 = 0.9557 and R^2(Adj) = 0.9158 for entrapment; R^2 = 0.9677 and R^2 (Adj) = 0.9387 for t_{90}) showed that the regression model represented the experimental data well. The optimization of the analyzed responses demonstrated that peak conditions for obtaining desired maximum responses, entrapment (102.66%) and t_{90} (12.27 h), were 82.91% w/w pectin; 36.33 min curing time and 7.50% w/w of calcium chloride.

Conclusion: Tangerine pectin could serve as a cheaper alternative polymer for the formulation of microspheres.

Keywords: Central composite design; metformin microspheres; response surface methodology; tangerine pectin; entrapment; dissolution time.

1. INTRODUCTION

Pharmaceutical applications require highly reproducible dosage and the controlled release of active agents, which cannot be achieved with conventional powders and granules [1]. One of the approaches currently explored in delivering therapeutic agents to their target sites in a controlled release manner is the use of microspheres as carriers for drugs. Microspheres are characteristically free flowing, consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having particle size of 1-1000 µm [2]. They constitute an important part of the novel drug delivery system by virtue of their small size and efficient carrying capacity. Polymers used in microspheres can be classified into synthetic and natural polymers. Natural polymers can be obtained from different sources such as proteins, carbohydrates and chemically modified carbohydrates. Pectin (derived from the Greek word meaning "congealed and curled) is a structural heteropolysaccharide initiated in the primary cell walls of terrestrial plants, particularly in the non-woody parts as well as in the middle lamella between plant cells where it helps to bind the cells together [3]. Pectin is a complex polysaccharide comprising mainly esterified D-galacturonic acid residues in a chain. The acid groups along the chain are largely esterified with methoxyl groups in the natural product [4]. The amount, structure and chemical composition of the pectin differ between plants, within a plant over time and in different parts of a single plant. The traditional, commercial sources of pectin have been citrus peel and apple pomace [5]. Citrus peel has often been the preferred material for pectin manufacture due to its high pectin content and good color properties. Generally, lemon and lime peel are the preferred sources of citrus pectin. More recently other sources of pectin are beginning to find usefulness as materials for industrial application [5-7]. Tangerine (*Citrus tangerina*), Family Rutaceae, is an orange-coloured citrus fruit in which the peel is very thin, with very little bitter white monocarp which makes them usually easier to peel and to split into segments. The peel of tangerine which is usually discarded as a by-product of the citrus fruit-processing, offers a good source of pectin and an opportunity to convert waste to wealth. The ability of pectin to rapidly form viscous solutions and gels on contact with aqueous media has been exploited by the pharmaceutical industry in its wide application as a carrier in oral controlled release formulations.

Metformin is the only drug of the class biguanide antidiabetics presently used. They differ markedly from sulfonylureas because they cause little or no hypoglycaemia in non-diabetic subjects and even in diabetics episodes of hypoglycaemia due to metformin are rare. They do not stimulate beta cells and has been reported to improve lipid profile as well in type 2 diabetics [8,9]. The absorption window of metformin hydrochloride is predominantly in the small intestine. The drug has a short half-life of about 3 hours. The immediate release formulations of metformin that are available in the market require repeated administration 2 – 3 times daily and are associated with high incidence of side – effects [10]. In order to optimize drug therapy and improve patient compliance, a time independent drug release is desirable in sustained release formulations. Thus to prolong drug release of metformin hydrochloride, the development of a microsphere formulation would be a better alternative to the conventional dosage form [11].

Response surface methodology is a collection of mathematical and statistical techniques useful for analysing and optimizing the response of multivariate systems. Central composite design-response surface methodology (CCD-RSM) is the preferred method to further optimize the

formulations of metformin microspheres with a nonlinear model to fit the experimental data [12,13]. When a combination of several independent variables and their interactions affect the desired responses, the accuracy can be fully demonstrated, and the adequacy of the proposed model can be revealed using the analysis of variance (ANOVA) [14].

Thus the focus of this research was to optimize the preparation of the microspheres of metformin using a novel polymer, tangerine pectin in different blend combinations with sodium alginate prepared at different curing times and using different concentrations of the chelating agent, calcium chloride. The total polymer concentration, drug: polymer ratio, stirring speed and dropping rate were all kept constant. The CCD-RSM was then used to optimize the formulation by choosing entrapment efficiency and time taken for 90% dissolution (t90) as the evaluation indicators.

2. MATERIALS AND METHODS

2.1 Materials

Tangerine fruits were purchased from a local market in Ibadan, Oyo State. Metformin obtained from Zydus Cadila Healthcare Ltd. (Ahmedabad, India). Sodium alginate was obtained from S.D. Fine Chem. (Mumbai, India).

2.2 Methods

2.2.1 Extraction of pectin

The peels obtained from mature tangerines were cut into pieces, washed with distilled water and then air-dried. Fifty grams of the dried peels was transferred into a 1000 mL beaker containing 500 mL of water. Hydrochloric acid 1N (2.5 mL) was added to give a pH of 2.2 and then boiled for 45 minutes. The peels were then removed from the extracts by filtering and the cake collected was washed with 250 mL of boiled water and then allowed to cool to 25°C to minimize heat degradation of pectin. The extracted pectin was precipitated by adding 200 mL 95% acetone to 100 ML of the extracted pectin with thorough stirring. It was then left for 30 min to allow the pectin float on the surface. The gelatinous pectin flocculants was then skimmed off. The extracted pectin was purified by washing in 200 mL acetone. The pectin obtained was air dried and blended to powder [3].

2.2.2 Characterization of tangerine pectin

2.2.2.1 Qualitative tests

Solubility of dry pectin in cold and hot water: Pectin samples were separately placed in a conical flask with 10 ml of 95% ethanol added followed by 50 mL distilled water. The mixture was shaken vigorously to form a suspension which was then heated at 85 – 95°C for 15 min [15].

Solubility of pectin solution in cold and hot alkali (NaOH): To 1 ml of 0.1 N sodium hydroxide, NaOH, 5 ml of pectin solution (0.25% w/v) was added and then heated at 85 – 90°C for 15 min. The pH of the above solution was determined using pH meter.

2.2.2.2 Quantitative tests

Equivalent weight determination: Pectin sample (0.5 g) was weighed into a 250 mL conical Volume of flask. Ethanol (5 mL) was added to form a mixture. Ethanol. Sodium chloride (1g) was added to the mixture. The solution of pectin was made by adding 100 mL distilled water and few drops of phenol red indicator. The solution was titrated slowly (to avoid possible de-esterification) with 0.1 M Sodium hydroxide.

Equivalent weight was calculated using the equation below:

$$\frac{\text{Weight of Pectin Sample}}{[\text{Volume of Alkali (cm3)} \times \text{ Molarity of Alkali}]} X\ 100 \quad (1)$$

Methoxyl content determination: To the neutralized solution of the pectin (obtained during the equivalent weight determination by the saponification of the pectin followed by titration of the liberated acid), 25 mL of 0.25 M sodium hydroxide NaOH was added. The mixture was stirred thoroughly and allowed to stand for 30 mins at ambient temperature. The percentage content was calculated using the equation below:

$$Methoxyl\ content\ (\%) =$$

$$\frac{(\text{Volume of alkali (cm3)x Alkali Weight})}{\text{Weight of Pectin Sample (mg)}} X\ 100 \quad (2)$$

2.2.2.3 Morphology

The morphology of tangerine pectin was observed using a scanning electron microscope

(Hitachi SU8030 FE-SEM Tokyo, Japan) at an accelerating potential of 5.0 kV. All samples were sputter-coated with Osmium tetra oxide prior to examination using the Osmium plasma coater (OPC 60 X1, Japan). The size of the pectin particles was determined using an optical microscope.

2.2.2.4 FT-IR analysis

Tangerine pectin was mixed with the KBr powder (1% w/w of the amount of KBr) and triturated in an agate mortar for 5 minutes. The die-set was assembled and the powder-mixture was transferred into it. The powder was pressed for 2 min (Thermo Qwik Handi-Press P/N 0016-125) to form a thin and transparent pellet. Analysis by FTIR was carried out in transmission mode. Transmission spectra were recorded using at least 64 scans with 8 cm1 resolution in the spectral range 4000—400 cm-1.

2.2.3 Preparation of metformin microspheres containing tangerine pectin by the ionic gelation technique

Microspheres were prepared from a hot gel blend of pectin and sodium alginate to obtain various blend compositions of tangerine pectin-sodium alginate having a total polymer concentration of 2% w/v and appropriate quantity of metformin was added such that the ratio of total polymer to drug was 1:1. The drug-polymer dispersion was then added drop-wise at a standardized rate of 2 ml/min into 100 ml of calcium chloride in a 250 ml beaker, while stirring at 500 rpm. Curing was done for the various time duration to produce spherical microspheres. The formed microbeads were collected by decantation, washed repeatedly with distilled water followed by drying at 40ºC for 24 hours in an oven (GallenKamp Moisture Extraction Oven -Model: BS 250 GallenKamp Co., UK) to obtain discrete microspheres.

2.2.4 Characterization of microspheres

2.2.4.1 Scanning electron microscopy

The morphology of the microspheres were observed using a scanning electron microscope (Hitachi SU8030 FE-SEM Tokyo, Japan) at an accelerating potential of 5.0 kV.

2.2.4.2 FT-IR analysis

Pectin, sodium alginate, pristine drug and drug-loaded microspheres were analyzed by FTIR (FTIR-Thermo Nicolet Nexus 870 Madison, WI, USA) in transmission mode. Transmission were recorded using at least 64 scans with 8 cm-1 resolution in the spectral range 4000 cm-1.

2.2.4.3 Entrapment efficiency

Drug-loaded microspheres (50 mg) were accurately weighed, crushed and suspended in 10 mL of phosphate buffer, pH 6.8. After 24 hours, the solution was filtered and the filtrate was appropriately diluted using phosphate buffer, pH 6.8 and analyzed using UV/VIS spectrophotometer (Spectrumlab 752s UV-VIS spectrophotometer, No 752S12090, China) at 282 nm. The drug entrapment efficiency (E) was calculated using the formula:

$$E\ (\%) = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} \times 100 \quad (3)$$

2.2.4.4 Drug release study

The *in vitro* dissolution studies were carried out using the paddle method (USP XXXVI), rotated at 50 rpm in 900 ml of phosphate buffer, pH 6.8, maintained at 37±0.5°C. Samples (l0 ml) were withdrawn at different intervals and replaced with equal amounts of fresh medium. Each sample was diluted and the amount of metformin released was determined at wavelength of 225 nm, using a UV Spectrophotometer (Spectrumlab 752s UV-VIS spectrophotometer, No 752S12090, China). Determinations were done in triplicates.

2.2.5 Experimental design and optimization

In this study, Response Surface Methology (RSM) was used for the optimization of process ariables in combination with the factorial experimental design of Composite Central Design (CCD). RSM is a useful method for studying the effect of several variables influencing the responses by varying them simultaneously and carrying out a limited number of experiments. The CCD is an effective design that is ideal for sequential experimentation and allows a reasonable amount of information for testing the lack of fit while not involving an unusually large number of design points [16].

In the present study, a CCD was employed for determining the optimum conditions for the preparation of metformin microspheres using tangerine pectin. The statistical software Minitab 16 Software USA, (Minitab Inc., USA) was used

for design of experiments, regression and graphical analyses of the data obtained, and statistical analysis of the model to evaluate the analysis of variance (ANOVA). Percent composition of pectin in the pectin-alginate blend, curing time and calcium chloride concentration were chosen as three independent variables in the formulation process. Accordingly, the CCD matrixes of 20 experiments for the full design of two factors were used for building quadratic models. The experimental data obtained from the CCD model experiments can be represented in the form of the following equation:

$$Y_i = f(y) = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{i=1}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (4)$$

Where Yi is the predicted response used to relate to the independent variable, k is the number of independent variables (factors) Xi (i = 1, 2, 3); while β is a constant coefficient and βi, βij and βii the coefficient of linear, interaction and square terms respectively and ε is the residual error [17]. Multivariate regression analysis with model equation (4) was carried out on the data using to yield equation (5) which was used to optimize the product responses.

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X^1_1 + \beta_{22} X^1_2 + \beta_{33} X^1_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon \quad (5)$$

The model developed for each determination was then examined for significance and lack-of-fit, while response surface plots were designed with the same software. The quality of the polynomial model was expressed by the coefficient of determination, namely, R^2 and R^2 (adj). The statistical significance was verified by the f test [18]. According to the obtained experimental data, the levels of the three main parameters investigated in this study are presented in Table 1.

3. RESULTS AND DISCUSSION

3.1 Characterization of Pectin

3.1.1 Qualitative test

The results of the qualitative tests on tangerine pectin revealed it is insoluble in cold water (25°C) but soluble in hot water (> 90°C). The pH of 0.25% w/v solution of tangerine pectin was 6.0±0.02, indicating it is weakly acidic.

3.1.2 Quantitative test

The methoxyl content of the tangerine pectin was 18.41%. This classifies tangerine pectin as a low methoxyl pectin (degree of esterification < 50%). Such low methoxyl pectins do not require high levels of sugar or low pH to initiate gelation, but gel in the presence of divalent cations such as calcium, known to form associations between sequences of charged species on adjacent chains [19].

3.1.3 Morphology

The SEM image of tangerine pectin is shown in Fig. 1. The granules were irregular in shape with a mean size of 92.43±7.34 µm.

3.1.4 FTIR analysis

The FTIR spectrum of tangerine pectin is also shown in Fig. 1. Absorptions were observed at 1740 (-C=O); 1608 (-COO-); 1430 (CH); 1300 (CH2, CC); 1035 cm-1 [20]. Tangerine pectins belong to a class of carboxyl polysaccharides which differ from neutral polysaccharides with an intense band at 1740 cm-1. The stronger absorptions at 1608 cm-1 (-COO-) confirms that it is a low methoxyl pectin.

3.2 Characterization of Metformin Hydrochloride Microspheres

3.2.1 Morphology

Scanning electron image for the pectin-based metformin microspheres is shown in Fig. 2. This revealed that the metformin microspheres containing tangerine pectin were near spherical in shape with a size ranging from 653±13.68 to 1108±20.6 µm.

3.2.2 FTIR analysis

The FTIR spectra of pectin, sodium alginate, metformin and metformin microbeads shown in Fig. 2 suggest that there was no interaction between metformin and the extracted pectin and the drug was well entrapped into the copolymer blend.

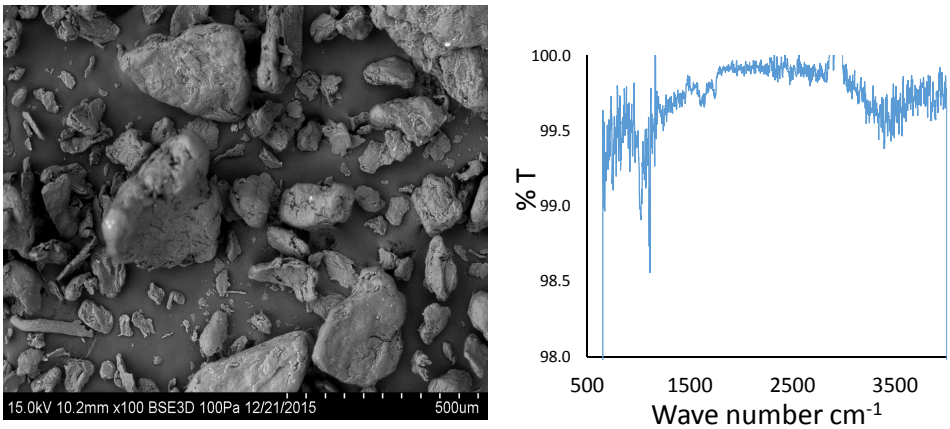


Fig. 1. (i) SEM image and (ii) FTIR spectra of tangerine pectin

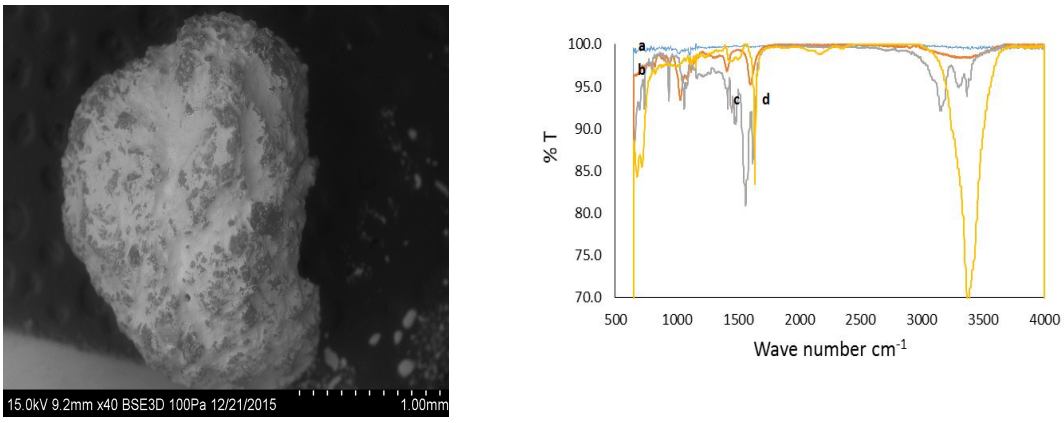


Fig. 2. (i) SEM of metformin and (ii) FTIR spectra of: (a) Tangerine pectin; (b) Sodium alginate; (c) Metformin and (d) Metformin microspheres

3.2.3 Dissolution profile

The dissolution plots of eight batches of metformin microspheres are presented in Fig. 3. From the dissolution plots, the time taken for 90% drug release (t_{90}) were determined.

3.3 Optimization of Process of Metformin Microsphere Formulation using RSM-CCD

The levels of the three main parameters investigated in this study are presented in Table 1. The levels were selected based on results from preliminary studies. From CCD and experimental results, RSM was used to optimize formulation process design factors (independent variables) [21]. The statistical combinations of variables in coded and actual values along with the predicted and experimental responses are presented in Table 2. Table 2 depicts a complete 2^3 factorial design with four center points in cube, and six axial points and two center points in axial. The number of experiments required (N) is given by the expression 2^k (2^3 = 8; star points) + 2 k (2 x 3 = 6; axial points) + 6 (center points; 6 replications). For the response surface method involving CCD, a total of 20 experiments was conducted for the three factors at five levels with the replicates at the center point.

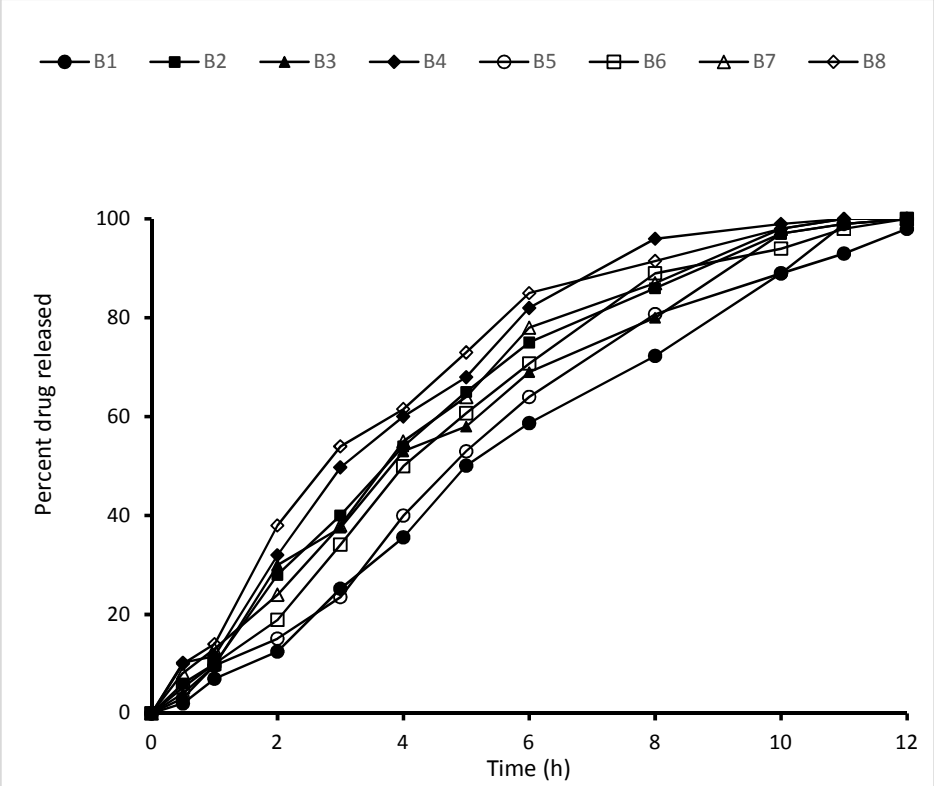


Fig. 3. Dissolution profile of metformin microspheres, Batches 1 - 8

Polynomial regression modelling was performed on the responses of the corresponding coded values of the three different process variables and the results were evaluated. The regression equation characterizing the influence of the three variables on entrapment and t90 were obtained using equations 7 and 8 respectively:

$$\text{Entrap} = 68.75 + 8.79X1 + 0.06X2 + 5.82X3 - 0.44X1X2 + 1.37X1\ X3 - 2.52X2X3 + 3.10X1\ 2 + 2.12X2\ 2 + 1.52\ X32 \quad (7)$$

$$t90 = 7.26 + 0.81X1 - 0.01X2 + 0.76X3 - 0.09X1X2 + 0.12X1\ X3 - 0.09X2X3 + 0.44X1\ 2 + 0.48X2\ 2 + 0.46X32 \quad (8)$$

A positive sign indicate a synergistic effect whereas a negative sign indicate an antagonistic effect.

3.3.1 Statistical analysis and validation of model

The analysis of variance (ANOVA) values for the quadratic regression model obtained from CCD are presented in Table 3. Statistical test was carried out using the Fisher's test for ANOVA. The models were observed to be statistically significant at 95% confidence level, with F-value of 23.97 and 33.31 for entrapment and t90 respectively, and very low probability P value of < 0.0001. The interactions between X2 and X3 was significant at p = 0.033 for entrapment. The values of the determination coefficients, R2 and R2 (Adj), which measure the model fitting reliability for the models were calculated to be R2 = 0.9557 and R2 (Adj) = 0.9158 for entrapment; R2 = 0.9677 and R2 (Adj) = 0.9387 for t90. This suggests that approximately 95.57% and 96.77% of the respective variance is attributed to the variables and indicates high significance of the models [22]. Furthermore, good agreement between experimental and predicted values of response variables shown in Table 2 confirm the adequacy of the regression models. Lack of fits was not statistically significant for both entrapment and t90 (p = 0.084 and 0.921 respectively), indicating good fit of the model and suggesting that there is no systematic variation that may be unaccounted for in the hypothesized model.

Table 1. Experimental range and levels of the independent variables

Variables	Factor	Range and level				
		-α	-1	0	+1	+α
Pectin: alginate (% w/w)	X_1	41.48	50.00	62.50	75.00	83.52
Curing time (min)	X_2	3.18	10.00	20.00	30.00	36.82
$Cacl_2$ concentration (% w/v)	X_3	3.30	5.00	7.50	10.00	11.70

Table 2. Central composite design experiments and experimental results

Design point	Independent variables coded			Real values of independent variables			Observed entrapment %	Predicted entrapment %	Observed t_{90} (h)	Predicted t_{90} (h)
	X_1	X_2	X_3	X_1 (% w/w)	X_2 (min)	X_3 (%w/v)				
1	+1	-1	+1	75.00	10.00	10.00	94.90	94.38	10.50	10.52
2	-1	+1	+1	50.00	30.00	10.00	70.10	69.13	8.60	8.49
3	+1	+1	-1	75.00	30.00	5.00	80.78	79.24	8.55	8.58
4	-1	-1	-1	50.00	10.00	5.00	59.10	59.26	7.15	6.99
5	+1	+1	+1	75.00	30.00	10.00	91.50	88.58	10.10	10.16
6	-1	-1	+1	50.00	10.00	10.00	75.00	73.18	8.60	8.47
7	+1	-1	-1	75.00	10.00	5.00	77.35	74.96	8.55	8.56
8	-1	+1	-1	50.00	30.00	5.00	68.12	65.28	7.50	7.39
9	0	0	-1.682	62.50	20.00	3.42	60.61	63.28	7.20	7.28
10	-1.682	0	0	42.09	20.00	7.50	60.75	62.75	6.90	7.15
11	0	+1.682	0	62.50	36.33	7.50	71.55	74.85	8.60	8.63
12	0	-1.682	0	62.50	3.67	7.50	73.20	74.66	8.50	8.60
13	+1.682	0	0	82.91	20.00	7.50	90.15	92.31	9.99	9.87
14	0	0	+1.682	62.50	20.00	11.50	80.75	82.84	9.80	9.85
15	0	0	0	62.50	20.00	7.50	70.80	68.75	7.44	7.26
16	0	0	0	62.50	20.00	7.50	69.45	68.75	7.40	7.26
17	0	0	0	62.50	20.00	7.50	69.37	68.75	7.45	7.26
18	0	0	0	62.50	20.00	7.50	66.55	68.75	6.52	7.26
19	0	0	0	62.50	20.00	7.50	66.66	68.75	7.38	7.26
20	0	0	0	62.50	20.00	7.50	70.50	68.75	7.37	7.26

Table 3. ANOVA regression model for entrapment and dissolution time (t_{90})

Source	Degrees of freedom	Sum of squares	Mean square	F value	P value
Entrapment					
Mode	9	1787.33	198.59	23.97	0.000
X_1	1	1055.22	1055.22	127.35	0.000
X_2	1	0.04	0.04	0.01	0.943
X_3	1	461.87	461.87	55.74	0.000
X_1^2	1	138.75	138.75	16.74	0.002
X_2^2	1	64.88	64.88	7.83	0.019
X_3^2	1	33.40	33.40	4.03	0.072
$X_1 X_2$	1	1.52	1.52	0.18	0.677
$X_1 X_3$	1	15.10	15.10	1.82	0.207
$X_2 X_3$	1	50.75	50.75	6.12	0.033
Residual	10	82.86	8.29		
Lack of fits	5	65.63	13.13	3.81	0.084
Pure error	5	17.23	3.45		
Dissolution time (t_{90})					
Mode	9	24.90	2.77	33.31	0.000
X_1	1	8.94	8.94	107.61	0.000
X_2	1	0.001	0.0010	0.01	0.914
X_3	1	7.95	7.95	95.80	0.000
X_1^2	1	2.85	2.85	34.27	0.000
X_2^2	1	3.34	3.34	40.23	0.000
X_3^2	1	3.10	3.10	37.33	0.000
$X_1 X_2$	1	0.07	0.07	0.85	0.379
$X_1 X_3$	1	0.11	0.11	1.36	0.271
$X_2 X_3$	1	0.07	0.07	0.85	0.379
Residual	10	0.83	0.08		
Lack of fits	5	0.17	0.03	0.25	0.921
Pure error	5	0.66	0.13		

The relationship between predicted and experimental values are shown in Fig. 4. A high correlation (R2 = 0.9992 and 0.9994 for entrapment and t90 respectively) was observed between the predicted and experimental values indicating reasonable agreement. This suggests that the data fit well with the models and show good estimate of responses for the system in the experimental range studied. The actual value is the measured result from a specific run, and the predicted value is evaluated from the independent variables in the CCD model [23].

Figs. 5 and 6 show the residual plots for entrapment and t90 respectively. The normal probability plots indicate good validity for the approximation of the quadratic regression model. In the plots of residual versus predicted values for the responses, points of observed runs were scattered randomly within the constant range of residuals across the graph. Thus, it revealed no obvious pattern and unusual structure, indicating the adequacy of the model.

3.3.2 Interactive effects of factors influencing entrapment and t_{90}

To understand the impact of each variable, three dimensional (3D) plots were made for the estimated responses which were the bases of the model polynomial function for analysis to investigate the interactive effects of two factors. These are presented in Fig. 7.

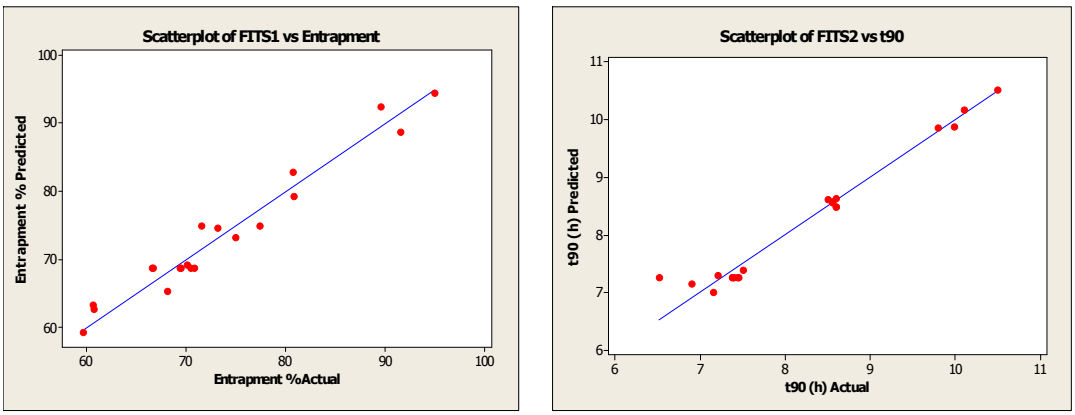
Fig. 7a (i) represents the effects of varying percent content of pectin in polymer blend (% w/w) and curing time (min), X1X2, on entrapment at constant calcium chloride concentration, 7.50% w/v. It can be observed that the percent content of pectin has greater effect on entrapment than curing time. Curing time of 3.67 to 10.00 min resulted in a decrease in entrapment but as the curing time increased from 10 to 36.33 min, entrapment increased. Maximum entrapment was 94.90% at curing time 20.00 min and pectin concentration 82.91%w/w. Fig. 7a (ii) shows the effect of both percent content of pectin in polymer blend (% w/w) and calcium chloride concentration (%w/v), X1X3, on entrapment, keeping curing time constant at 20.00 min. Entrapment was observed to increase with increase in percent content of pectin (% w/w). Entrapment was initially almost constant at calcium chloride concentration of 3.42 to 5.00% w/v followed by a reduction between 5.00 to 7.50% w/v calcium chloride concentrations. However when increased from 7.50% w/v to

11.50% w/v, entrapment was observed to be greatly enhanced. Fig. 7a (iii) shows the interactive effects of curing time (min) and calcium chloride concentration (% w/v), X2X3, on entrapment, keeping percent content of pectin in polymer blend constant at 62.50 % w/w. There was a slight increase in entrapment as calcium chloride concentration increased between 3.42% w/v and 7.50% w/v followed by higher entrapment values as the concentration of chelating gent increased from 7.50 to 11.50% w/v. Entrapment was also observed to increase with increase in curing time. The maximum entrapment of 94.90% occurred at 36.33 min of curing time and 7.50% w/v of calcium chloride concentration.

Fig. 7b (i) represents the effects of varying percent content of pectin in polymer blend (% w/w) and curing time (min), X1X2, on dissolution time, t90, at constant calcium chloride concentration, 7.50% w/v. A significant positive impact is detectable; t90 was observed to increase with increase in percent pectin from 42.09 to 82.91% w/w. On the other hand, t90 initially reduced as curing time increased from 3.67 to 20 min but later increased at curing time increased beyond 20 min. Maximum t90 was observed at curing time 30 min and percent pectin 82.91% w/w. Fig. 7b (ii) shows the effect of both percent content of pectin in polymer blend (% w/w) and calcium chloride concentration (% w/v), X1X3, on t90, keeping curing time constant at 20.00 min. Between 3.42 and 5.00% w/w calcium chloride concentration, t90 initially reduced slightly but was observed to greatly reduce as calcium chloride concentration increased beyond 5.00% w/w. Percent pectin exhibited a more powerful effect than that of calcium chloride with t90 increasing with increase in percent pectin. The maximum time for dissolution was 10.52 h at percent pectin concentration 82.91% w/w and calcium chloride 7.50% w/v. Fig. 7b (iii) shows the interactive effect of curing time min and calcium chloride concentration %w/v on t90 at constant percent content of pectin 62.50% w/w. With increase in curing time from 3.67 to 30 min, t90 increased. There was a slight increase in t90 as calcium chloride concentration increased from 3.42 to 7.50% w/v. Above 7.50% w/v, t90 increased only slightly. According to this figure, maximum t90 was obtained at curing time 36.33 min and 7.50 %w/v concentration of calcium chloride.

3.3.3 Optimization of formulation process and model verification

The optimization process was carried out to determine the optimum value of entrapment efficiency and dissolution time t90, using the Minitab 16 Software USA, (Minitab Inc., USA). The desired goal for each operational condition (percent content of pectin in polymer blend (% w/w), curing time (min) and calcium chloride concentration) was chosen "within" the studied range. The Response Optimizer in Minitab was used to identify the combination of input variable



(a) **(b)**

Fig. 4. The actual and predicted (a) entrapment % and (b) t_{90} h

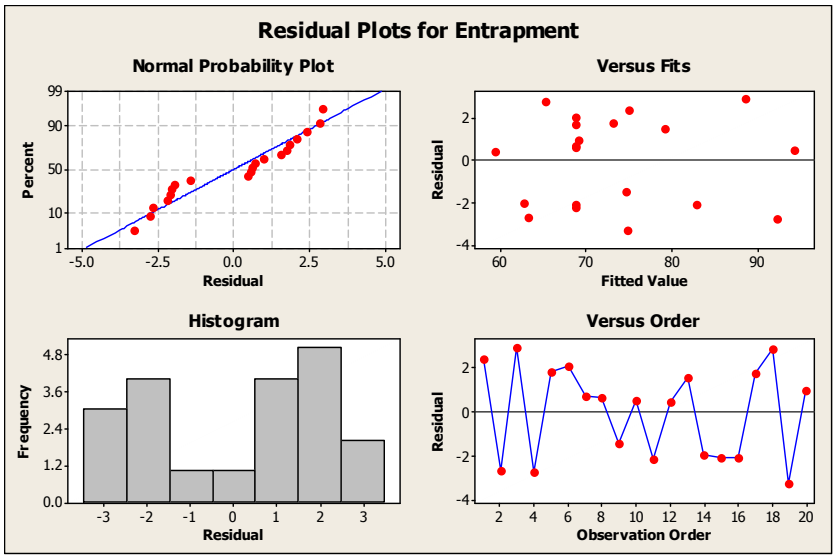


Fig. 5. Residual plots for entrapment

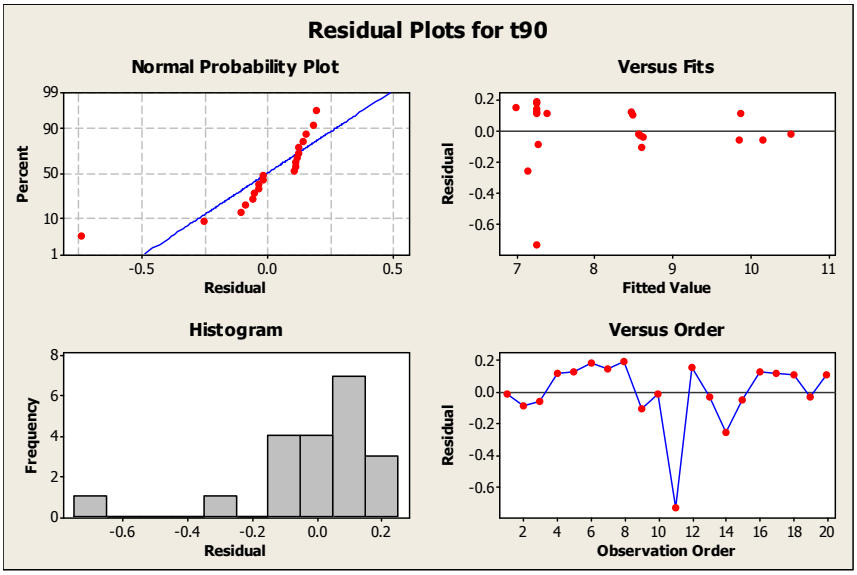
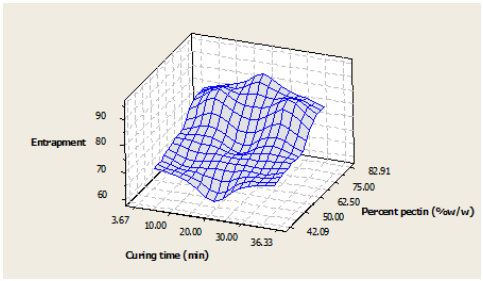
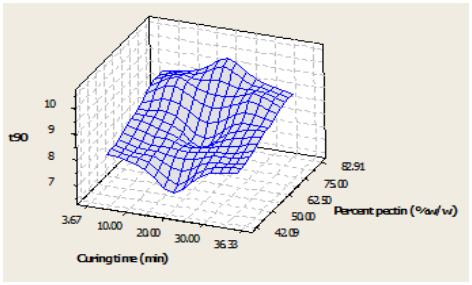


Fig. 6. Residual plots for dissolution time (t_{90})

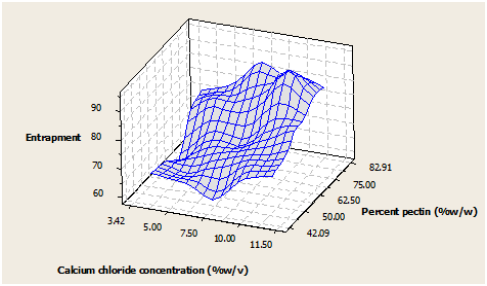
settings that jointly optimize a single response or a set of responses. This provided optimal solutions for the input variable combinations, along with optimization plots shown in Fig. 8. The optimization plot is interactive and input variable settings can be adjusted on the plot to search for more desirable solutions. Using the Response Optimizer, the maximum response (arcsin) for entrapment (%) and t90 (h) respectively were 102.66 and 12.27 respectively, with a desirability of 1. In order to verify the optimizer suggested values, confirmation runs were conducted. The values of the input parameters from the response optimizer suggestion were first calculated and tests were conducted under the optimized conditions identified from RSM. The maximum entrapment and t90 were 99.85% and 11.75 h respectively. Hence the predicted and experimental values under optimized conditions are in close agreement.

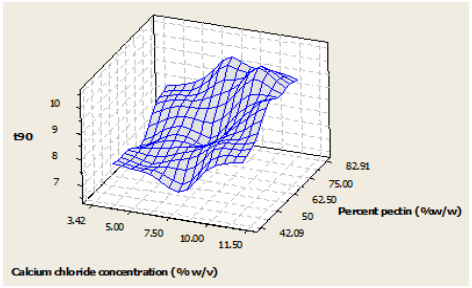
i



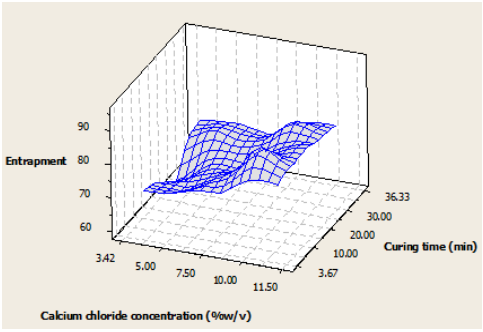


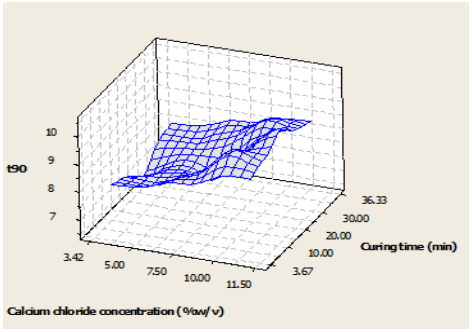
ii





iii*





(a) **(b)**

Fig. 7. 3D surface plots (a) Entrapment and (b) t_{90}

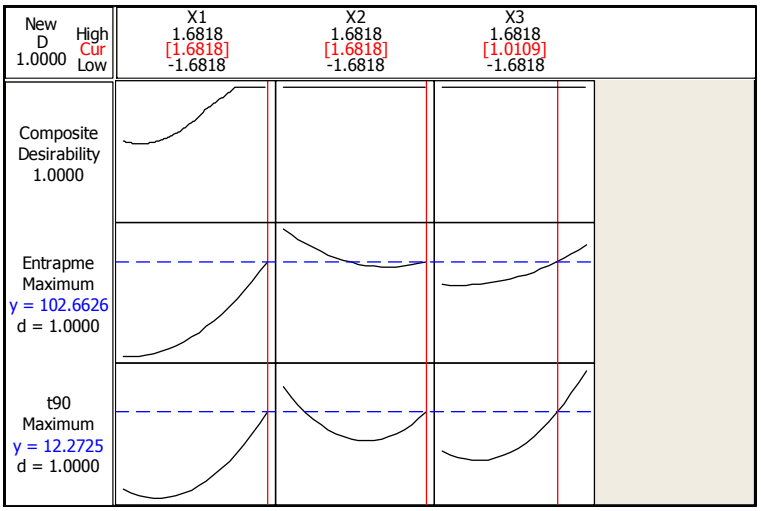


Fig. 8. Optimization plots for input variable combinations

4. CONCLUSION

Central composite design and Response surface methodology proved to be reliable and powerful tools for modeling, optimizing and studying the interactive effects of the three variables (percent pectin in the pectin-alginate blend; curing time and calcium chlorides concentration) on the entrapment efficiency and dissolution time of metformin microspheres containing a novel polymer, tangerine pectin. It is also evident that the combination of two or more input variables played a greater role in enhancing entrapment and prolonging dissolution time. One of the main contributors to high entrapment and t90 values is the percent pectin in the polymer blend. A highly significant regression quadratic model equation was obtained by analyzing the data of a 23 factorial design. The predicted values were found to be in agreement with the experimental values [R2 = 0.9557 and R2 (Adj) = 0.9158 for entrapment; R2 = 0.9677 and R2 (Adj) = 0.9387 for t90], defining the propriety of the model in the optimization of the process for the formulation of metformin microspheres. The maximum predicted and actual entrapment were respectively 102.66% and 99.85% while the maximum predicted and actual t90 were respectively were 12.27 h and 11.75 h. Tangerine pectin is therefore suitable as a copolymer for the formulation of microspheres for controlled release which can serve as a cheaper substitute to synthetic polymers in drug delivery.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Robinson JR, Gurny R, Junginger HE. Rationale of bioadhesion / mucoadhesion and bioadhesion possibilities and future trends. Wissenchaftliche Verlag Gesellschaft Sci. 1990;1(2):13-28.
2. Vasir JK, Tambwekar K, Hang S. Bioadhesive microspheres as controlled drug delivery system. Int. J. Pharm. 2003; 255:13-32.
3. McGready RM. Extraction of pectin from citrus peels and conversion of pectin acid. 2nd Ed. Academic Press, New York 1996;4:167-170.
4. May CD. Industrial pectins: Sources, production and applications. Carbohydr. Polym. 1990;12(1):79–99. DOI: 10.1016/0144-8617(90)90105-2
5. Sriamornsak P. Chemistry of pectin and its pharmaceutical uses: A review. J. Pharm. Pharmac Sci. 1998;44:207–228.
6. Boonrod D, Kamonrad R, Hataichanoke N. Extraction and physicochemical characteristics of acid-soluble pectin from raw papaya (*Carica papaya*) peel. Chiang Mai J Sci. 2006;33(1):129-135.
7. Pinheiro E, Silva I, Gonzaga L, Amante E, Teofilo R, Ferreira M, Amboni R. Optimization of extraction of high-ester pectin from passion fruit peel (*Passiflora edulis-flavicarpa*) with citric acid by using response surface methodology. Bioresource Technol. 2008; 99:5561-5566.
8. Glueck CJ, Fontaine RN, Wang P. Metformin reduces weight, centripetal obesity, insulin, leptin, and low-density lipoprotein cholesterol in nondiabetic, morbidly obese subjects with body mass index greater than 30. Metabolism. 2001; 50:856–861. DOI: 10.1053/meta.2001.24192
9. Kusaka I, Nagasaka S, Horie H, Ishibashi S. Metformin, but not pioglitazone, decreases postchallenge plasma ghrelin levels in type 2 diabetic patients: A possible role in weight stability? Diabetes Obes Metab. 2008;10:1039–1046. DOI: 10.1111/j.1463-1326.2008.00857.x
10. Marathe PH, Wen Y, Norton J, Greene DS, Barbhaiya RH, Wilding IR. Effect of altered gastric emptying and gastrointestinal motility on metformin absorption. Br J Clin Pharmacol. 2000;50(4):325-332.
11. Okunlola A, Patel RP, Odeku OA. Evaluation of freeze-dried pregelatinized Chinese yam (*Dioscorea oppositifolia*) starch as a polymer in gastroretentive metformin microbeads. J. Drug Del. Sci.and Tech. 2010;20(6):457-465.
12. Dutta M, Jayanta KB. Statistical optimization for the adsorption of acid fuchsin onto the surface of carbon alumina composite pellet: An application of

response surface methodology. Int J Environ Sci Tech. 2012;5(1):42–53.
13. Jain M, Garg VK, Kadirvelu K. Investigation of Cr (VI) adsorption onto chemically treated *Helianthus annuus*: Optimization using response surface methodology. Bioresource Technol. 2011; 102(2):600–605.
14. Rosales E, Sanromán MA, Pazos M. Application of central composite face-centered design and response surface methodology for the optimization of electro-Fenton decolorization of Azure B dye. Environ Sci Pollut R. 2012;19(5): 1738–1746.
15. Fishman ML, Coffin DR, Konstance RP, Onwulata CI. Extrusion of pectin/starch blends plasticized with glycerol. Carbohy Polym. 2000;41:317-325.
16. Özer A, Gürbüz G, Çalımlı A, Körbahti BK. Biosorption of copper (II) ions on *Enteromorpha prolifera*: Application of response surface methodology (RSM). Chem. Eng. Journal. 2009;146:377–387.
17. Moghaddam SS, Alavi Moghaddam MR, Arami M. Coagulation/flocculation process for dye removal using sludge from water treatment plant: Optimization through response surface methodology. J Hazard Mater. 2010;175:651–657.
18. Kayan B, Gözmen B. Degradation of Acid Red 274 using H2O2 in subcritical water: application of response surface methodology. J Hazard Mater. 2012;201–202:100–106
19. Oakenfull D. Gelling agents. CRC Crit. Rev. Food Sci. Nutr. 1987;26:1–25. (CRC critical reviews in food science and nutrition)
20. Schulz H, Baranska M. Identification and quantification of valuable plant substances by IR and Raman spectroscopy. Vibration. Spec. 2007;43:13–25.
21. Myers RH, Montgomery DC. Response surface methodology: Process and product optimization using designed experiment, 2nd edition, Wiley, New York; 2002.
22. Montgomery DC. Design and analysis of experiments: Response surface method and design. John Wileyand Sons Inc., New Jersey; 2006.
23. Kayan B, Gozmen B. Degradation of acid red 274 using H2O2 in subcritical water: Application of response surface methodology. J. Hazard Mater. 2012;(201-202):100-106.

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