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Original Article

Optimizing Formulation Variables of KCl Loaded Waxy Microspheres

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Abstract

Potassium chloride (KCl) is a highly water soluble substance which is usually used to prevent hypokalemia. Oil in water disperse phase encapsulation method was used to prepare KCl loaded microcapsules using beeswax and carnauba wax as a hydrophobic matrix. The morphology and characteristics of KCl loaded microspheres were studied using scanning electron microscopy (SEM), photon correlation spectroscopy (PCS) and differential scanning calorimetery (DSC). The particle size distribution of resulting microspheres was narrow and DSC diagrams showed no interaction between waxes and drug. The effective variables were determined using Plackett-Burman design followed by Response Surface methodology for optimization of these variables. Based on this analysis, the most effective parameters on drug loading were found to be beeswax/carnauba wax ratio, drug/wax ratio and rate of emulsification. Using the response surface method, the optimum values of effective parameters were found to be beeswax/carnauba wax ratio = 6.245, drug/wax ratio = 0.668 and rate of emulsification = 679.8 rpm.

Keywords: Bees wax, Carnauba was, Controlled Release, KCl, Optimizing, Waxy Microspheres

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1. Introduction

In the past few years, great attention has been paid into controlled release dosage forms, because of their potential applications in biomedical and pharmaceutical systems [1]. These formulations are usually made of a membrane, matrix, liposomes [2] or capsule. Matrix type formulations are prepared from either swellable hydrophilic polymers or non-swellable lipophilic excipients, like waxes

and lipids [3]. The commonly used materials in lipid carriers, prepared by various melt dispersion techniques are beeswax and carnauba wax. These waxes contain a wide group of chemicals such as glycerides, fatty acids, fatty alcohols and their esters. These are widely used as release retardants in the design of sustained release beads, tablets, suspensions, implants, and microcapsules. The advantages of waxes include good stability at various pH and moisture levels, well-established safe application in humans due to their nonswellable and water insoluble nature, minimal effect on food in the gastrointestinal tract, and no dose dumping [4,5].

Potassium chloride is a highly water soluble drug which is usually used to prevent or treat low blood levels of potassium (Hypokalemia) or severe potassium loss of various etiologies [6]. Reduction of side effects of KCl while prolonging its action by using a suitable sustained release dosage form is highly desirable. There are some extended release products in which KCl is encapsulated. The most popular materials which used as carriers are ethyl-cellulose [7,8], hydroxypropyl cellulose, cellulose nitrate, cellulose acetate phthalate, magnesium stearate, and microcrystalline Cellulose [9,10]. Also some of these microencapsulated products like K-DUR don't have matrix structure and in most of them coacervation method used to encapsulate KCl in microparticle [10,11]. This method is complicated and various parameters affect on encapsulation efficiency. Encapsulation of KCl by a waxy membrane composed of beeswax and carnauba wax is postulated to be a suitable procedure. However, the high water solubility of KCl could impress the amount of initial drug loading. Various techniques of microencapsulation, utilized for the production of multiparticulates, will affect the encapsulation efficiency [12,13]. Toxicity of the organic solvent residues in the final microparticles and the use of potentially toxic monomers are major problems in the conventional microencapsulation process [13].

The oil-in-water meltable disperse-phase encapsulation method is a simple and useful procedure avoiding harmful organic solvents [14,15]. It is easier and more economical to work with aqueous media instead of oils or organic solvents with regard to agglomeration of microarticles, viscosity of the external phase, recovery of the organic phase, and clean-up requirements of the final product [16]. The encapsulation efficiency of hydrophilic drugs in this method were enough high [17]. In this technique, the melted wax is first mixed with drug and then emulsified into an external heated aqueous phase containing an emulsifier which is heated 5^0C above the

melting point of waxes. The obtained emulsion then cools to room temperature, while its droplets solidify into microparticles. The drug:wax ratios were significantly high (from 1:1 to 1:4) with the drug loading in the range of 15–40% [18]. The use of aqueous media, the inertness of the waxes, reproducibility with respect to size distribution and its simplicity are the main advantages of this procedure. [19,20]. However, microparticles characteristics are greatly affected by processing and formulation variables, such as type of wax and their ratio [21], emulsification stirring speed [19,22], type and amount of emulsifier, and time [24,25,26]. Kamble et al. [4] have shown that the encapsulation efficiency will increase due to an increase in the speed of agitation up to a certain level of agitation above which it favors decrease in the desired yield.

The first aim of this study was to encapsulate potassium chloride in a mixture of beeswax and carnauba wax as insoluble lipid matrices. An attempt has been made to increase the overall incorporation of this highly water soluble drug in hydrophobic matrix by using a proper mixture of waxes. The screening of significant variables and optimizing their levels were the second principal objective of this study. In the first step, Plackett-Burman design was used to screen the effective variables on the drug loading. Then, Response Surface Methodology (RSM) was used for further optimization of the selected significant variables. The morphology and characteristics of KCl loaded microspheres were studied using scanning electron microscopy (SEM), photon correlation spectroscopy (PCS) and differential scanning calorimetery (DSC). In vitro drug release study was also performed and the release parameters of different kinetic models were evaluated.

2. Materials and Methods

2.1. Materials

Beeswax was purchased from Fluka Chemical Co. (USA) and carnauba was purchased from Sigma–Aldrich Chemical Co. (USA). Hydrochloric acid was purchased from Scharlau (Barcelona), potassium chloride and polysorbate 80 (Tween 80) were both purchased from Merck (Germany). All other reagents were analytical grade and were used as received.

2.2. Preparation of Microparticles

The waxy microparticles were prepared by a melt dispersion method. Specified amount (2 g) of both beeswax and carnauba wax were weighed out and transferred to a 100 mL beaker and melted over a heated water bath. Specified amounts of KCl was added to the molten wax and stirred well with a magnetic

stirrer to get a uniform suspension of drug in the molten wax.

Double distilled water (60 mL) was poured into a 100 mL beaker in a water-bath at 90^{o}C and stirred at 300 rpm with magnetic stirrer. Specified amount of emulsifier (Tween 80) was added to double distilled water to obtain a uniform suspension. Then, this suspension was poured into the pre-prepared mixture of wax and drug. Final mixture was stirred for several times with an overhead propeller stirrer to obtain oil in water emulsion. Then stirring was stopped and the beaker was put into 4^{o}C water bath for 5 minutes, until microparticles formed and solidified. The microparticles were separated with centrifugal force, washed with 30 mL double distilled water and then freeze-dried under vacuum (VaCo 5, Zirbus Technology, Denmark).

2.3. Drug Loading

The drug loading of KCl loaded microspheres was determined as the mass ratio of entrapped drug to the total mass of microparticles. For this purpose, 50 mg of microparticles was weighted and added to phosphate buffer solution (PBS, pH=7.4); which was gently stirred for 24 h. Then the suspension was sonicated with ultrasound vehicle (Dr. Hielscher, Up 400 S) to assure that the entire entrapped drug has released. After that, the mixture was filtered and the solution was analyzed with atomic absorption (Philips, PU 9100X) to determine the KCl concentration. The percent of drug loading was calculated according to the following equation:

DL% = (mass of entrapped KCl) / (total mass of microparticles) (1)

2.4. Particles Morphology

The physical appearance and surface structure of microparticles were determined using scanning electron microscopy (SEM, 30 XL Phillips, the Netherlands). The samples were prepared on aluminum stabs and coated with gold prior to examination by SEM.

2.5. Size Measurement

Microparticles mean diameter and polydispersity index were determined using photon correlation spectroscopy (PCS) (Malvern Instrument, NANO-ZS). The analysis was performed at a temperature of 25^{0}C using samples appropriately diluted with ultra purified water.

2.6. Differential Scanning Calorimetry (DSC)

DSC (DSC- 200, DSC-Netzesch Co.) experiments were carried out to characterize the physical state of drug and waxes in microparticles. Ten milligrams of Beeswax, Carnauba wax, Tween 80 and KCl loaded waxy microparticles were separately placed

Table1. Plackett–Burman design with coded values along with observed results for drug loading. (The parameters are: X_1=BW/CW, X_2=D/W, X_3=Rate (rpm), X_4=Time (min), X_5=Emulsifier (mL))

Trial	Parameters with actual value					Experimental Results (Drug loading)
	X_1	X_2	X_3	X_4	X_5	
1	3.000	0.3	1000	20	0.10	10.66
2	3.000	0.3	600	10	0.20	11.39
3	0.333	0.3	600	10	0.10	11.10
4	3.000	0.5	1000	10	0.20	20.98
5	0.333	0.5	1000	20	0.10	9.56
6	0.333	0.5	600	10	0.10	21.04
7	0.333	0.3	1000	20	0.20	5.11
8	0.333	0.5	1000	10	0.20	17.64
9	3.000	0.5	600	20	0.20	21.74
10	3.000	0.3	1000	10	0.10	11.73
11	3.000	0.5	600	20	0.10	19.73
12	1.630	0.4	800	15	0.15	12.55
13	0.333	0.3	600	20	0.20	9.973
14	1.630	0.4	800	15	0.15	12.650

in aluminum pans and hermetically sealed. An empty aluminum sealed pans was used as a reference. The heating rate was set at 5^0C/min, nitrogen served as purge gas at a flow rate of 50 mL/min at 5 bars, and the system was cooled down by liquid nitrogen. The thermograms of samples were recorded and peak temperatures and phase transition range of the prepared samples were determined.

2.7. Statistical Design

Since there are many factors determining an acceptable microparticle formulation, therefore the Plackett–Burman screening design was used to determine the significant factors. The selected factors were Beeswax/Carnauba wax ratio, drug/wax ratio, emulsification time, rate of emulsification and amount of emulsifier. Each of these factors was evaluated at tow levels with a zero level as a center point (Table 1). The factor ranges were selected based on prior knowledge about the system under study. These variables were screened with a fourteen run Plackett–Burman design shown in Table 1. The design was constructed using MINITAB software (Version 15).

A systematic optimization procedure was carried out using response surface methods (RSM), in order to estimate the values of the most important factors leading to the best compromise between Beeswax/Carnauba wax ratio, drug/wax ratio and rate of emulsification on one hand, and high drug loading on the other. The rest of the factors

were kept at a constant level. A central composite design (CCD) was employed for applying the RSM. A 14-factorial central composite experimental design, with six axial points (α=SQRT (3)) and six replications at the center points (n_0 = 6) leading to a total number of 20 experiments, was employed for the optimization of the parameters.

2.8. Release Study

To study the release profile of KCl from the optimized formulation obtained by Response Surface design (Table 7), 50 mg of dried microparticles was placed into dialysis bag and immersed in 50 mL of HCl (pH=1.2) at 37±0.5°C for 2h then HCl was replaced with PBS (pH=7.4) and release was continued for 6h; while stirring at 150 rpm. At specified time intervals, 1 mL of samples was taken out and replaced by 1 mL of fresh solution. The cumulative concentration of released KCl was determined by measuring adsorption of the dissolution medium. Two kinetic models were used to evaluate the mechanism of drug delivery from obtained microparticles. Power-law model

$$M=K_p t^n, \quad (2)$$

and Higuchi square root of time model

$$M=K_H t^{1/2}, \quad (3)$$

where M is the cumulative drug released at time t, and K_p and K_H are release rate constants.

3. Results and Discussion

3.1. Determination of Effective Parameters

The Plackett–Burman designs can identify main parameters from large number of suspected contributing parameters for the desired response variables. Therefore, these designs are extremely useful in preliminary studies where the aim is to identify variables that can be fixed or eliminated in further investigation [26]. The design analyzes the input data and presents a rank ordering of the variables with magnitude of effect and designates signs to the effects to indicate whether an increase in factor value is advantageous or not [27]. Plackett-Burman

Table 2.The least-squares fit and coefficient estimates (significance of regression coefficients).

Term	Effect	Coef.	T	p
Constant		14.221	20.61	0.000
BW/CW	3.632	1.816	2.63	0.034
D/W	8.456	4.228	6.13	0.000
Rate	-3.217	-1.609	-2.33	0.052
Time	-2.853	-1.427	-2.07	0.077
Emulsifier	0.502	0.251	0.36	0.727
Center Point		-1.621	-0.89	0.404

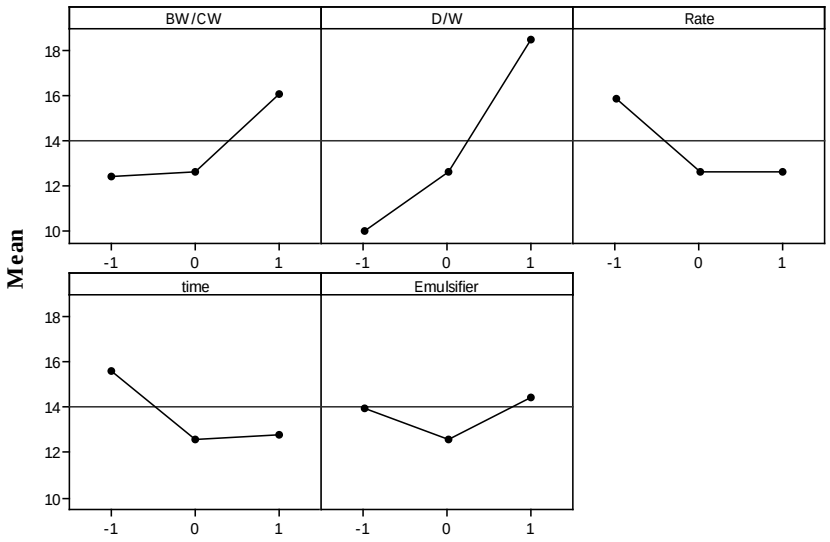


Figure 1. Main effect plot of parameters on drug loading.

experimental design is based on the first-order model. This model describes no interaction amongst factors and it is used to screen and evaluate the important factors that influence drug loading [26]. The factors that have confidence level above 95% are considered the most significant factors that affect the drug loading [28].

In this study, the effects of five variables on drug loading were evaluated (Table 1). These variables were screened with a twelve run Plackett–Burman design as shown in Table 1. Two center points were added for the variables that could be assigned numerical values. The significance of each coefficient was determined via Student's *t*-test and by p-values (Table 2). Parameters evidencing p-values less than 0.05, which means significant effects on the responses, were selected for further optimization studies. So, the beeswax/carnauba wax ratio (BW/CW) and the drug/wax ratio (D/W) showed a positive significant effect on drug loading ($p<0.05$), while the rate of emulsification showed a negative effect and be less significant ($p<0.052$).

Fig. 1 shows that the drug loading efficiency increased when the amount of beeswax and KCl in the formulation increased. On the other hand, increasing the time and rate of stirring during the emulsification process resulted in reduction of the drug loading efficiency (Fig. 1), as noted by Varshosaz & Keihanfar [25].

In agreement with the work of Bodmeier et al. [23], the obtained results showed that the emulsifier content had no significant effect on the response (Table 2 and Fig. 1). Emulsifier has dual function on drug loading. Increasing the emulsifier content will decrease the surface tension of solution which results in the size reduction of wax droplets.

Table 3. ANOVA for the experimental results of the Plackett–Burman design.

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Main Effects	5	310.328	310.328	62.0655	10.78	0.003
Curvature	1	4.504	4.504	4.5036	0.79	0.404
Residual Error	7	39.982	39.982	5.7117		

The later will lead to an increase in the diffusion rate of drug from the waxy droplets into the external solution. On the other hand, a thin layer of emulsifier may be formed on the surface of microparticles which acts as a barrier for drug diffusion into the external phase [16].

The value of the coefficient of determination (R^2 = 0.8873) indicated that 88.73% of the variability in the response could be explained by the linear model (Table 3).

Table 4.Central composite design matrix for the experimental design of KCl loaded waxy microparticles with corresponding results.（The parameters are: X_1=BW/CW, X_2=D/W, X_3=Rate (rpm).

Trial	Parameters with actual value			Experimental results (Drug loading)
	X_1	X_2	X_3	
1	4.335	0.600	700	14.00
2	6.245	0.500	600	12.50
3	1.665	0.400	500	9.80
4	4.335	0.600	500	13.00
5	4.335	0.400	700	10.00
6	3.000	0.500	600	11.20
7	1.665	0.400	700	8.05
8	1.665	0.600	700	9.60
9	3.000	0.668	600	12.00
10	3.000	0.500	600	10.76
11	3.000	0.500	432	11.80
12	3.000	0.500	768.18	9.05
13	3.000	0.500	600	12.03
14	3.000	0.500	600	12.40
15	0.755	0.500	600	10.00
16	1.665	0.600	500	10.25
17	3.000	0.500	600	11.81
18	4.335	0.400	500	11.05
19	3.000	0.500	600	10.96
20	3.000	0.332	600	9.50

3.2. Optimization of Drug Loading

CCD was usually used for optimization of the parameters which were found to be important for drug loading. Based on the screening results of Plackett–Burman design, beeswax/carnauba wax ratio, drug/wax ratio and rate of emulsification were selected as the independent input variables. The other parameters were kept constant. A central composite design was employed to analyze the interactive effect of these parameters and to arrive at an optimum condition. The parameters were coded according to the following equation:

$$X_i = \frac{x_i + x_i^*}{\Delta x_i} \qquad i=1,2,3,\ldots \qquad (4)$$

where X_i is the coded value, x_i is the actual value of the i^{th} test variable; x^*_i is the value of x_i at the center point of the investigated area and Δx_i is the step size.

The range of the variables determined by screening results, Theexperimental design protocol (Minitab 15), and corresponding results are presented in Table 4. The coefficients of the regression model are selected as one constant, three linear, three quadratic and three interaction terms (Table 5). The significance of each coefficient was determined by p-values (Table 6).

Based on the p-values, it can be concluded that the first order main effects of beeswax/carnauba wax ratio, drug/wax ratio, stirring rate, and also the second order of stirring rate are all significant. Therefore, a small variation in their level will result in a considerable change in drug loading. On the other hand, no significant effect for interaction of parameters was observed.

The analysis of variance was used to test the significance and adequacy of the correlation. The Fisher variance ratio or the F-value (S^2_r/S^2_e) is a suitable estimate of how well the factors explain the variation in the

Table 5.The least-squares fit and coefficient estimates (significance of regression coefficients) for central composite design.

Term	Regression coefficient	t- Value	
Constant	11.5282	43.055	0.000
BW/CW	1.0657	5.999	0.000
D/W	0.8900	5.010	0.001
Rate	-0.5180	-2.916	0.015
BW/CW*BW/CW	-0.1075	-0.622	0.548
D/W*D/W	-0.2843	-1.644	0.131
Rate*Rate	-0.3992	-2.308	0.044
BW/CW*D/W	0.4938	2.127	0.059
BW/CW*Rate	0.2938	1.266	0.234
D/W*Rate	0.3937	1.696	0.121

Table 6. Analysis of the central composite design results for drug loading.

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Regression	9	37.073	37.073	4.1193	9.56	0.001
Linear	3	29.994	29.994	9.9979	23.20	0.000
Square	3	3.199	3.199	1.0662	2.47	0.121
Interaction	3	3.881	3.881	1.2936	3.00	0.082
Residual Error	10	4.310	4.310	0.4310	----	----

data about their mean. The greater the F-value is from unity, the more certain it is that the factor explains adequately the variation in the data about their mean, and the estimated factor effects are real [29]. The analysis of variance of the regression demonstrated that the correlation is highly significant, as is evident from the Fisher's F-test (Fmodel = 23.20). The goodness of fit with this linear expression was confirmed by the determination coefficient (R^2). In this case, the value of the determination coefficient (R^2 = 0.8965) indicates that 89.6% of the variability in the response could be explained by the correlation.

The experimental results of the CCD were fitted with a second order polynomial equation. The values of regression coefficients were calculated and the fitted equation (in terms of coded values) for prediction of drug loading was obtained as follow:

$$DL\% = 11.5282 + 1.0657x_1 + 0.89x_2 - 0.518x_3 - 0.1075x_1^2 - 0.2843x_2^2 - 0.3992x_3^2 + 0.4938x_1x_2 + 0.2938x_1x_3 + 0.3937x_2x_3 \quad (5)$$

where x_1 , x_2 , and x_3 are beeswax/carnauba wax ratio, drug/wax ration and rate of stirring, respectively. By the differentiation of the above equation, the maximum drug loading can be obtained at: x_1 = 1.682, x_2= 1.682, x_3= 0.798 (Table 7). The predicted optimal drug loading corresponding to these values was calculated to be 15.36. To confirm the accuracy of the correlation, the maximum drug loading were determined experimentally, using the above optimized parameters. These triplicate experiments

Table7. Optimized results of KCl loaded waxy microparticles.

Variables	Coded level	Real Value
BW/CW	1.682	6.245
D/W	1.682	0.668
Rate	0.798	679.8
Predicted Responses	15.36	14.95

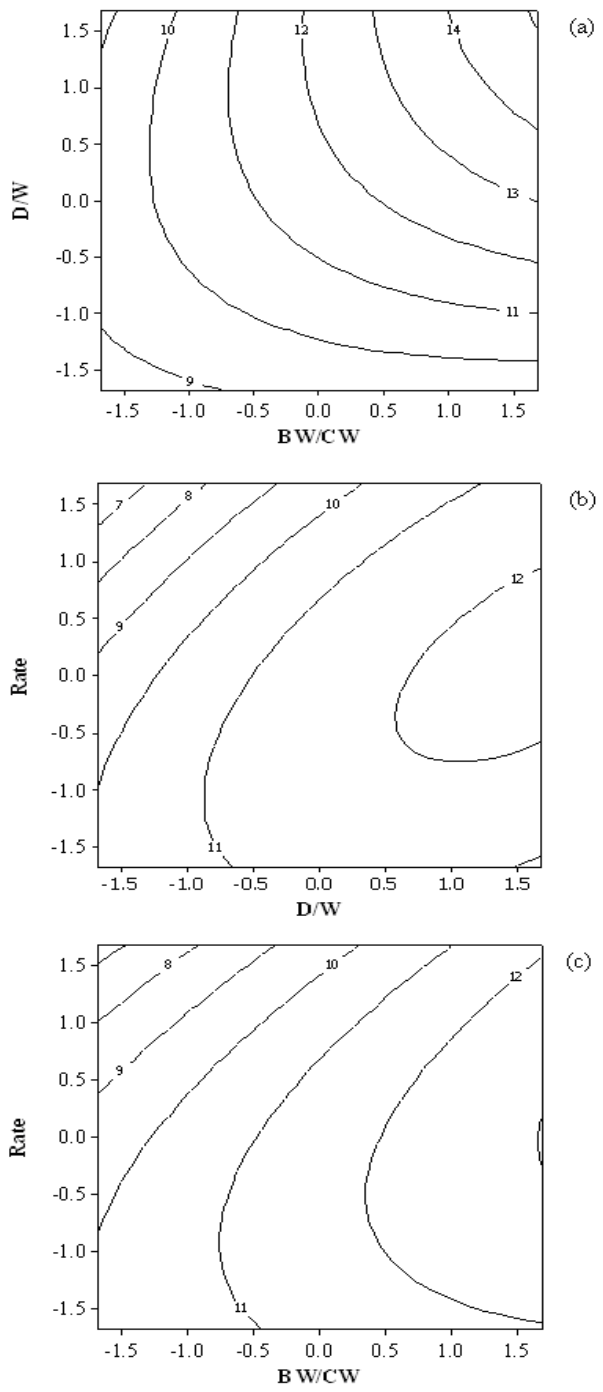
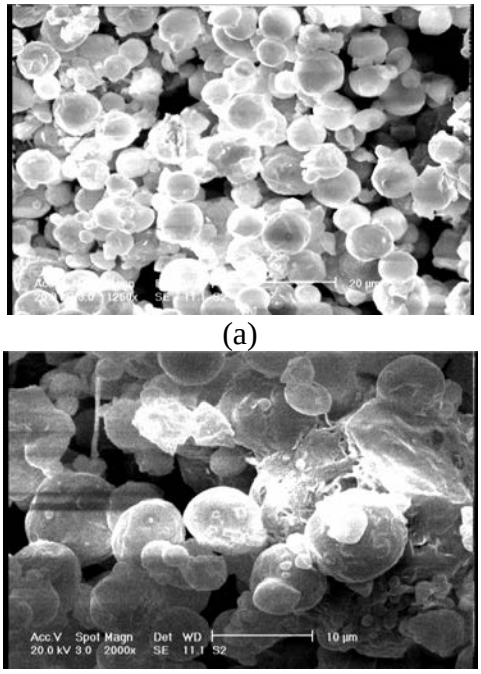


Figure 2. Contour plots of the drug loading (a) for the interaction between BW/CW and D/W, and (b) for the interaction between D/W and Rate, and (c) for the interaction between BW/CW and Rate at zero level of other parameter.

yielded an average value of 14.95±0.5 for maximum drug loading. The good agreement between the predicted and experimental results verifies the validity of the correlation and the existence of the optimal point. The encapsulation efficiency of these formulations was about 63-70 %.

The 2D contour plots which are the graphical representations of the regression equation were generated to visualize the interaction of the variables and to locate the optimum level of each variable for maximum response. Each contour plot for drug loading represents the different combinations of two test variables at one time while keeping the third variable at their respective zero level. The three pairs of contour plots are shown in Fig. 2. The interactions between the variables can be inferred from the shapes of the contour plots. Circular contour plots indicate the negligible interaction between the variables, while the elliptical ones indicate the evidence of the interactions [30]. Beeswax/carnauba wax ratio and drug/wax ratio are found as the important factors for drug loading (Table 5). Hence, a weak interaction between these two factors is inevitable. As it can be seen, both of them cause an increase in drug loading at the higher concentrations (Fig. 2a). Since, there is not any interaction between



(a)

(b)

Figure 3. SEM micrograph of KCl loaded micropaticles prepared at run 4(a) andand run 6 (b) in Plackett-Burman design.

beeswax/carnauba wax ratio or drug/wax ratio and stirring rate, their contour plots are nearly linear at low drug loading and circle at high drug loading (Fig.2b).

3.3. Microparticles Characterization

The structure of waxy microparticles prepared according to the formulations of run 4 and 6 of Plackett-Burmann design is shown in Fig. 3. Preparation conditions seemed smooth and the obtained microparticles were rigid and spherical.

Size distribution and polydispersity of waxy microparticles in emulsion state is shown in Fig. 4. The number average diameter and their polydispersity was 4.24 µm and 0.01% respectively.

In drug formulation studies, DSC analysis is usually used to evaluate the behavior of crystalline phases as well as the possible interactions between the drug and the carrires. The DSC thermograms corresponding to beeswax, carnauba wax, Tween 80, and those for KCl loaded microparticles are shown in Fig. 5. As would be expected the Tween 80 thermogram displayed no peak at this ranges of temperature, But the DSC thermograms of beeswax and carnauba wax showed a single melting peak at 67^0C and at 87^0C, respectively. The DSC thermograms of KCl

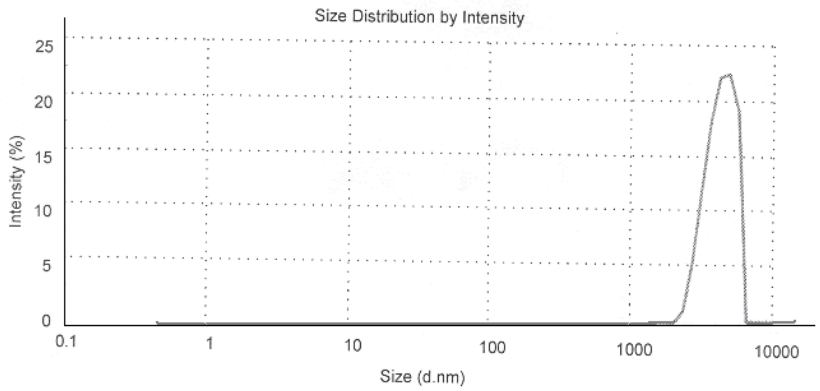


Figure 4. Size distribution of microparticles prepared at run 7 in Plackett-Burman design.

loaded microparticles prepared in Run 4 showed four endotherms, tow large peaks at 41.8^{0}C and 51.2^{0}C attributing to the phase transition and the melting temperature of beeswax; and two small peaks around 76.5^{0}C and 79.6^{0}C attributing to the carnauba wax behavior (Fig. 5). The large peaks appeared near the melting point of beeswax, since its mass ratio in this sample was higher than carnauba wax. As could be seen in thermogram of Run 4, the smaller peaks near melting point of carnauba was resulted from the dispersion of carnauba wax in bees wax that cause to lossing of natural crystalline structure of carnauba wax.

The DSC thermogram of microparticles produced in Run 8 is given also in Fig. 5. The narrower and sharper peak at higher temperature (81.2^{0}C) indicates Carnauba wax, while the other peaks in lower temperature (39.5^{0}C and 51.1^{0}C) are Beeswax. As shown in Fig. 5, combination of waxes decreases the melting point of both waxes in the structure of microparticles.

This result qualitatively illustrates that Beeswax and Carnauba wax are crystalline.

3.4. Release Study

Fig. 6, shows the resulting release profiles of KCl from optimized formulation (Table 7) in HCl (pH=1.2) for 2 hr followed by PBS (pH=7.4) for 6 hr. As it can be seen, the release pattern was biphasic, comprising an initial burst effect followed by a sustained continuous phase. The initial burst effect was relatively high; more than 25% of the incorporated drug released within 15 min. This behavior could be assigned to the immediate dissolution and release of the accumulated drug on the surface of microparticles [31,32]. During storage of KCl loaded waxy particles in refrigerator (4 ^{o}C) structure of waxes changed and the extent of crystallization increased with holding time (as shown in Fig. 5). This transformation

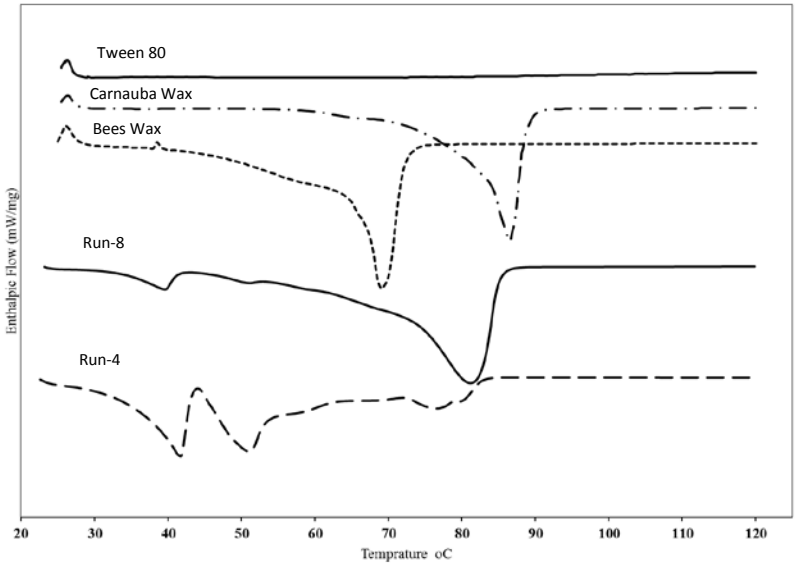


Figure 5. DSC thermogram of raw materials and KCl loaded waxy micropaticles were prepared in Run 4 and 8 in Plackett-Burman design. The graphs were replaced vertically for better visualization.

pushed KCl molecules out of crystals and leads to accumulation of KCl in outer surface. The dissolution efficiencies of 480 min (DE480) which were calculated from the area under the dissolution curves are listed in Table 8. The DE480 values were about 673.87, 582.36, and 577.22 for samples of run-4, run-8, and run optimum, respectively.

The data obtained from in vitro drug release studies was fit into two different kinetic models and their parameters such as release constants and regression coefficient (R^2) were calculated (Table 8) [33,34]. Considering the high initial burst effects, the amount of drug released between 0-30 min time points was not included in this analysis. As shown in Table 8, the experimental data can be well described by Higuchi model, which indicates diffusion controlled mechanism [17,35] for KCl release from waxy microparticles. Tian et al. showed that increases in emulsification stirring rate led to

Table 8. Dissolution efficiency at 480 min (DE_{480}) and release mechanism of correlation coefficient of KCl from waxy microparticle

Run	DE_{480}	Power law			Higuchi Model	
		$M/Mt = K_p t^n$			$M = K_H t^{1/2}$	
		K_p	n	R^2	K_H	R^2
4	673.87±35.25	0.74	0.12	0.9862	0.12	0.9538
8	582.36±42.15	0.66	0.08	0.7854	0.05	0.9477
Optimum	577.22±28.50	0.59	0.29	0.9862	0.22	0.9778

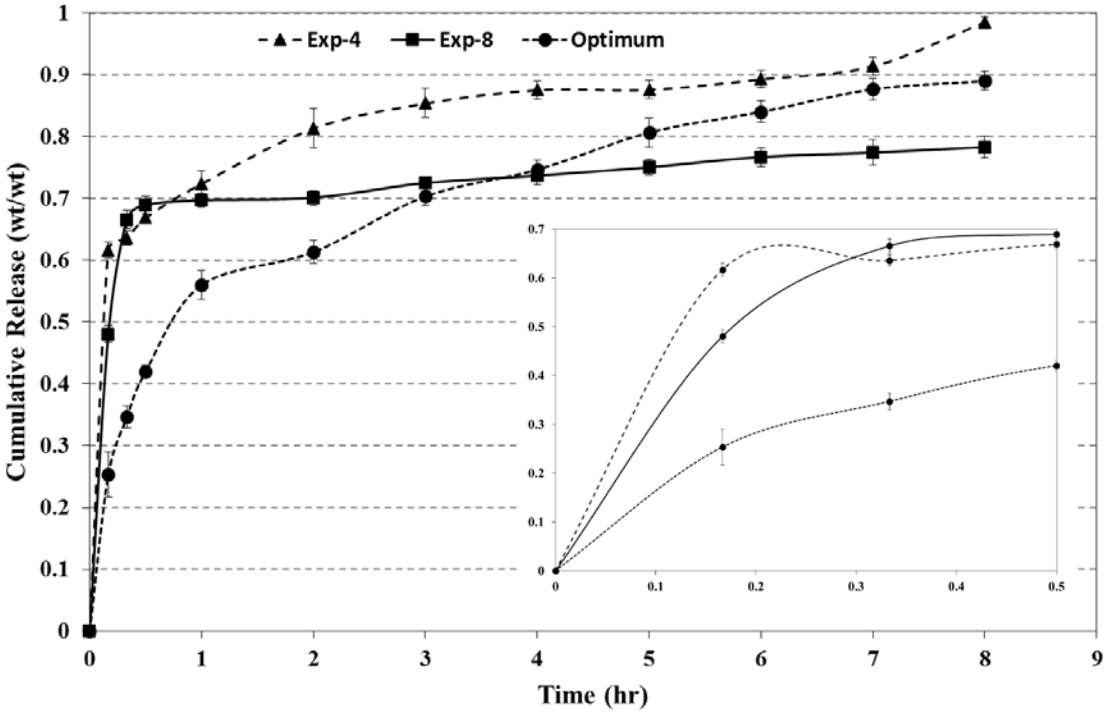


Figure 6. The release profiles of KCl in HCl buffer solution (pH=1.2, T=37±0.5°C)for 2 hours followed by phosphate buffer solution (pH=7.4, T=37±0.5°C) from microparticles prepared in▲:Run 4,■**:** Run 8of Table 2 and ●:Run Optimum.

increases in release rate that are in accordance whit our release results [37]. Increasing in stirring rate could be resulted to formation of smaller particles and eventuate the decreasing in diffusion path for drug to migrate from the inner part of particles to outer surface. Decreases in diameter led to increases in the surface area of the microparticles in contact with the dissolution medium led to greater wetting of the microparticles and higher rate of diffusion [36].In optimum samples the drug:wax ratio is more than other two samples and shows a vague higher release rate which is in accordance whit Zhang et al., reports [35]. Considering the slight solubility of beeswax and complete insolubility of carnauba wax [3] in aqueous media, water penetration into microparticles and hence their swelling rate is low enough to confirm the obtained results [3]. In some commertial products release kinetics govern by zero order model because of gelatin coated which were used as outer layer on microcapsules [10].

4. Conclusion

In this study a sustained drug delivery system for KCl, based on beeswax and carnauba wax developed. Melt dispersion technique was used to produce KCl loaded waxy microparticles. The effective variables were determined using Plackett-Burman design followed by Response Surface methodology for optimization of these variables. The obtained results indicated that the initial drug loading as well as the beeswax/carnauba wax ratios could adjust the initial burst effect and release profiles of highly soluble drugs loaded in lipidic microparticles. Beeswax/carnauba wax ratio, drug/wax ratio and rate of emulsification were selected as the most effective parameters on drug loading. Using the response surface method the optimum values of the effective parameters were found to be beeswax/carnauba wax ratio = 6.245, drug/wax ratio = 0.668 and rate of emulsification = 679.8 rpm. The release pattern for optimized formulation was biphasic, comprising an initial burst effect followed by a sustained continuous phase. Kinetic modeling of released data indicated that diffusion (Higuchi model) was the predominant release mechanism.

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