

BOSWELLIA PAPYRIFERA RESIN AS MICROENCAPSULATING AGENT FOR CONTROLLED RELEASE OF DICLOFENAC SODIUM: FORMULATION, EVALUATION AND OPTIMIZATION STUDY

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ABSTRACT

Objective: The present study is aimed to investigate the resin from local olibanum (*Boswellia papyrifera*) as a wall material for microencapsulation using diclofenac sodium as a model drug.

Methods: Microcapsule formulations were prepared by solvent evaporation method with varying polymer to drug ratio, stirring rate, temperature and dispersed phase volumes and their effects on percentage yield, particle size, encapsulation efficiency and release rate were evaluated.

Results: The preliminary experiments revealed that the polymer to drug ratio and stirring rate significantly affect both the encapsulation efficiency and release rate while the other factors only affect one of the responses. Therefore, the effects of polymer to drug ratio and stirring rate on the encapsulation efficiency and release rate of the microcapsules were further studied and optimized by central composite design. The optimal conditions were obtained at 3.7:1 polymer to drug ratio and 1200 rpm stirring rate. Under these conditions, the encapsulation efficiency and release rate were 26.45 % and 27.87 h^{-1/2}, respectively. The optimum formulation also provided discrete, spherical and freely flowing microcapsules. The *in vitro* drug release exhibited minimum burst release with sustained release for 12 h. The kinetic study showed the optimized formulation followed Higuchi square root kinetic model with non-Fickian diffusion release mechanism.

Conclusion: The results of this study showed that the resin of *B. papyrifera* could be used as a potential alternative wall material for microencapsulation.

Keywords: Olibanum resin, Diclofenac sodium, Solvent evaporation method, Microcapsules, Encapsulation efficiency, Controlled release, Release kinetics.

INTRODUCTION

Microencapsulation is the process in which small droplets or particles of liquid or solid material are surrounded or coated by a continuous film of polymeric materials [1]. Microencapsulation has received considerable attention in pharmaceutical and biomedical applications, specifically in achieving sustained/controlled release objectives [2]. Polymers which are used for coating play important role in controlling drug release from microcapsules [3]. Despite the successful development of many synthetic polymers for use in the manufacture of controlled release dosage forms, natural polymers still remain attractive and are extensively investigated because of their bio-compatibility, low toxicity and environmental "friendliness" [2, 4].

Olibanum or frankincense is an oleo-gum-resin harvested from several different trees belonging to the genus *Boswellia*, a member of the Burseraceae family [5]. It is a complex mixture composed of about 5-9 % highly aromatic essential oil (mono- and sesquiterpenes), 65-85% alcohol soluble resin (diterpenes, triterpenes), and the remaining 6-30% water-soluble gum (polysaccharides) [6]. Olibanum is phytotoxically safe raw material used in several industries including food, flavour, liquor and beverage, cosmetics, perfumery, pharmaceutical and others [7].

Diclofenac sodium (DS), a phenylacetic acid derivative, is a nonsteroidal anti-inflammatory drug (NSAID). Its plasma half-life is about 1-2 h and its usual oral dose is 75 to 150 mg daily in divided doses [8]. On the basis of its pharmacokinetic properties, diclofenac sodium is a suitable candidate for controlled release by microencapsulation. Hence, the objective of this study was to evaluate the resin of *Boswellia papyrifera* as a wall material for microencapsulation using diclofenac sodium as a model drug.

MATERIALS AND METHODS

Diclofenac sodium powder and reference standard were received as a generous gift from Addis Pharmaceutical Factory

(APF). Olibanum (*Boswellia papyrifera*) was purchased from the Ethiopian Natural Gum Processing and Marketing Enterprise (NGPME). Sodium carboxy methyl cellulose (NaCMC) (Dhariyal Polymers Pvt. Ltd. Gujart, India) was donated by Cadila Pharmaceuticals PLC. Ethanol and potassium dihydrogen orthophosphate (UNICHEM, China), dichloromethane, hydrochloric acid, sodium hydroxide and Tween 80 (BDH Ltd., Poole, England) were all used as received.

Preparation of resin fractions of olibanum

The olibanum obtained was first dried in an oven (Kottermann@ 2711, Germany) at 60 °C for 4 h, powdered in a grinder and passed through a mesh having pore size of 224 µm. In order to extract the resin, the powdered olibanum oleo-gum-resin was stirred with ethanol (90%) for 2 h. The ethanol slurry was filtered through Whatman No. 1 filter paper. The filtrate was concentrated to a thick paste by evaporation of ethanol at 80 °C and hydrodistilled using distillation apparatus for 3 h to isolate essential oil. The paste was finally dried in oven (Kottermann@ 2711, Germany) at 40 °C [9, 10].

Preparation of microcapsules

The preparation of microcapsules was based on the emulsion solvent evaporation method described elsewhere [11] with slight modifications. A weighed amount of olibanum resin (0.8 g, 1.6 g, 2.4 g, 3.2 g and 4 g) was dissolved in dichloromethane (10 ml, 20 ml and 30 ml); to this 0.8 g diclofenac sodium was added under magnetic stirring (Arex, Velp Scintifica, Europe) and the mixture was blended for 15 min. Then, the suspension was slowly dispersed in 200 ml water containing NaCMC (0.5% w/v). The system was maintained under agitation (300 rpm, 500 rpm, 800 rpm and 1200 rpm), at different temperatures (25 °C, 30 °C and 40 °C), to allow the complete evaporation of the solvent. The resulting microcapsules were filtered with vacuum filtration, washed three times with distilled water and air-dried over a period of 24 h.

Characterization of microcapsules

Microscopy

The formation of microcapsules during preparation was observed using an optical microscope (Leitz Dialux 22, Germany), connected with digital camera (Leica DFC 280, USA).

Particle size and size distribution

Particle size of microcapsules was determined by optical microscopy using calibrated eyepiece micrometer [12]. From each batch of microcapsule, 300 particles were measured and the average particle size was determined using Equation 1:

$$\text{Average particle size} = \frac{\sum nd}{\sum n}$$

Eq. 1 where n is the total number of microcapsules observed and d is midpoint of the size range.

Density and flow properties

Bulk and tapped densities were assessed by the conventional tapping method using a 250 ml graduated measuring cylinder (Equations 2 and 3):

$$D_b = \frac{M}{V_b} \quad \text{Eq. 2}$$

where D_b is bulk density (g/cm^3), M is mass of sample in grams, and V_b is volume of microcapsules in cm^3 .

$$D_t = \frac{M}{V_t} \quad \text{Eq. 3}$$

where D_t is tapped density (g/cm^3) and V_t is final volume of particles after 500 tappings.

The Carr's index of each formulation was calculated from the bulk and tapped densities according to Equation 4:

$$\text{Carr's index (\%)} = \frac{(D_t - D_b)}{D_t} \times 100 \quad \text{Eq. 4}$$

The angle of repose was measured by using the fixed funnel method and calculated by Equation 5:

$$\text{Angle of repose } (\theta) = \tan^{-1} \left(\frac{h}{r} \right) \quad \text{Eq. 5}$$

where r is the base radius and h is pile height.

Percentage yield

The percentage yield of microcapsules was calculated as the ratio of the mass of microcapsules obtained at the end of the process to the mass of initially added drug and polymer (Equation 6) [13]. The experiment was done in triplicate.

$$\text{Yield (\%)} = \frac{\text{Weight of microcapsules}}{\text{Total expected weight of drug and polymer}} \times 100$$

Eq. 6

Encapsulation efficiency

One hundred milligrams of microcapsules were accurately weighed and crushed in a clean mortar. Twenty milligrams of the crushed microcapsules were transferred into a volumetric flask containing 100 ml of pH 6.8 phosphate buffer. The solution was then stirred at

900 rpm for 2 h. The solution was filtered and absorbance readings of diclofenac sodium were taken at 276 nm [14]. The encapsulation efficiency was estimated using Equation 7. All the formulations were analyzed in triplicate ($n=3$).

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100$$

Eq. 7

In vitro drug release

In vitro dissolution studies were performed using USP type I dissolution apparatus (ERWEKA, DT600, Germany) at 50 rpm. Microcapsules equivalent to 20 mg of diclofenac sodium were placed in the basket. The dissolution was done in HCl for the first 2 h then changed to pH 6.8 phosphate buffer for the next 10 h. Tween 80 (0.1% w/v) was used to increase the wettability of the water insoluble drug in the medium. The temperature was maintained at 37 ± 0.5 °C. Samples of 10ml were withdrawn at different time intervals (2, 3, 4, 6, 8, 10 and 12 h). Each sample withdrawn was replaced with an equal volume of fresh dissolution medium at 37 °C to maintain sink condition. Each of the sample solutions were analyzed for the drug content at 276 nm using UV/Visible Spectrophotometer (CECIL CE 1021, England).

Drug release kinetics

The rate and mechanism of release of diclofenac sodium from the prepared microcapsules were analyzed using the methods described elsewhere [15]. Accordingly, the release data were fitted to: zero-order equation, first-order equation, Higuchi square root equation, Hixson-Crowell cube root equation and Korsmeyer-Peppas model.

Analysis of release data

Dissolution efficiency $DE_t^{\%}$ [16] after 12 h of release test was used to compare the results of dissolution tests of different formulations:

$$DE_t^{\%} = \frac{\int_0^t y dt}{y_{100} t} \times 100 \quad \text{Eq. 8}$$

where y is the drug percent dissolved at time t and DE is defined as the area under the dissolution curve up to a certain time, t , expressed as a percentage of the area of the rectangle described by 100 % dissolution in the same time.

Experimental design

Based on the preliminary studies, the polymer to drug ratio and stirring rate were found to be the critical factors which affect the response variables (encapsulation efficiency and release rate). Thus, systematic optimization was carried out using response surface methodology for estimating the effect of the independent variables on the dependent variables. Central composite design (CCD) with five coded levels as shown in Table 1 was used to describe the nature of the response surface in the optimum region. According to this design, the total number of treatment combinations was $2^k + 2k + n_0$, where k is the number of independent variables and n_0 is the number of repetitions of experiments at the centre point [17]. For two factors, a total of 13 experiments ($2^2 + (2 \times 2) + 5$) were carried out and their observations were analyzed using Design-Expert 8.0.4 software. The experimental runs were performed in random in order to minimize the effects of uncontrolled variables that may introduce bias into the measurements.

Statistical analysis

The results were treated statistically using Origin 8 software (OriginLab Corporation, MA, and USA). One way analysis of variance (ANOVA) was applied for comparison of all results. Each test was done in triplicate and the results are reported as mean and standard deviation. A p value < 0.05 was considered statistically significant.

Table 1: Experimental levels of the independent variables for optimizing *B. papyrifera* resin microcapsule formulations of diclofenac sodium.

Variables	Levels				
	- α	-1	0	+1	+ α
Polymer: drug (w/w)	1.38	2	3.5	5	5.62
Stirring rate (rpm)	355.03	500	850	1200	1344.99

$\alpha=1.414$

RESULTS AND DISCUSSION

Formation of microcapsules

Formulations were prepared by varying polymer to drug ratio and whether the given ratio produced microcapsules was checked. The formulation which was prepared at 1:1 (polymer: drug ratio) did not form any microcapsules. This might be due to the low polymer

concentration which was insufficient to coat the particles. However, ratios from 2:1 to 5:1 provided microcapsules (Figure 1).

Effect of polymer to drug ratio

There was a statistically significant increase ($P < 0.05$) in particle size and encapsulation efficiency as the polymer to drug ratio increased from 2:1 to 5:1 (Table 2). The increase in particle size may be attributed to an increase in viscosity of the internal phase with increasing polymer to drug ratio which makes it more difficult to disperse in the external phase during emulsification, resulting in larger microcapsules [18]. This increase in viscosity of the internal phase also restricts the migration of the drug to the continuous phase that increases the entrapment efficiency [19]. The cumulative release profiles of diclofenac sodium from microcapsules prepared at different polymer to drug ratios were significantly different, as shown in Figure 2a. This can be explained by increased thickness of the coating with increased polymer concentration [20].

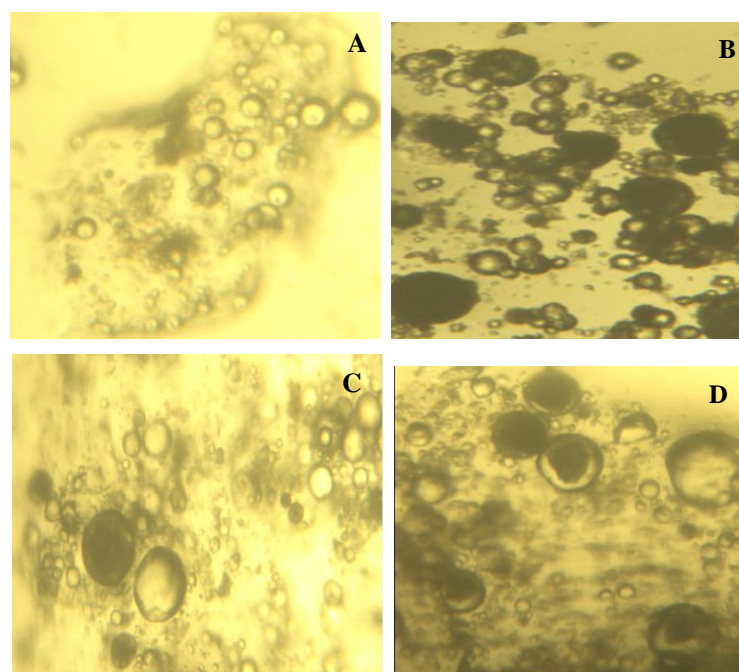


Fig. 1: Microscopic photos ($\times 100$) of diclofenac sodium microcapsules prepared at 2:1 (A), 3:1 (B), 4:1 (C) and 5:1 (D) polymer-drug ratios.

Effect of stirring rate

It was observed that lower stirring speed of 300 rpm was not sufficient to produce microcapsules, and a huge coalesced mass was obtained. However, microcapsules were formed at 500 rpm and above (Table 2). This is in part attributed to the inadequate agitation of the media to disperse the inner phase into discrete droplets within the

bulk phase [20]. Increasing stirring rate was found to significantly decrease average particle size, encapsulation efficiency and percentage yield. The smaller particle size at higher stirring rate could be a result of more intense mixing which disperses the solidifying droplets in the bulk emulsion during the microcapsules formation leading to smaller droplets [21]. This decrease in particle size also significantly influenced the drug release rate in 12 h (Figure 2b).

Table 2: Effects of various formulation and process parameters on response variables

Factor	Response variable			
	Yield (%) \pm SD	Particle size (μm) \pm SD	EE (%) \pm SD	Cumulative % drug released at 12 h \pm SD
Polymer to drug ratio [25°C, 10 ml, 500 rpm]*				
1:1	-	-	-	-
2:1	58.4 \pm 1.26	288.6 \pm 2.78	20.6 \pm 3.52	98.2 \pm 1.07
3:1	60.8 \pm 1.45	316.2 \pm 1.32	36.3 \pm 1.96	93.6 \pm 1.41
4:1	61.4 \pm 0.86	408.0 \pm 3.14	42.9 \pm 0.82	74.5 \pm 2.22
5:1	63.7 \pm 0.82	413.0 \pm 1.47	52.0 \pm 1.63	68.3 \pm 1.53
Stirring rate [25°C, 10 ml, 3:1]*				
300 rpm	-	-	-	-
500 rpm	60.8 \pm 1.45	316.2 \pm 1.32	36.3 \pm 2.25	93.6 \pm 1.41
800 rpm	57.1 \pm 1.31	294.7 \pm 2.19	29.0 \pm 1.52	97.5 \pm 1.83
1200 rpm	44.7 \pm 0.90	290.3 \pm 1.25	25.2 \pm 2.37	102.7 \pm 1.15
Temperature [10 ml, 3:1, 500 rpm]*				

25°C	60.8 ± 1.45	316.2 ± 1.32	36.3 ± 2.25	93.6 ± 1.41
30°C	55.7 ± 2.03	322.6 ± 0.29	30.0 ± 1.63	92.0 ± 2.00
40°C	-	-	-	-
Volume of dispersed phase [25 °C, 3:1, 500 rpm]*				
10 ml	60.8 ± 1.45	316.2 ± 1.32	36.3 ± 2.25	93.6 ± 1.41
20 ml	46.6 ± 3.75	290.5 ± 2.40	34.6 ± 2.35	96.8 ± 2.03
30 ml	42.0 ± 1.02	286.1 ± 3.10	32.0 ± 2.83	98.1 ± 1.12

NB. '*' represent the values at which other factors were kept constant while the factor under study was varied.

'-' indicate factor levels at which microcapsules were not formed.

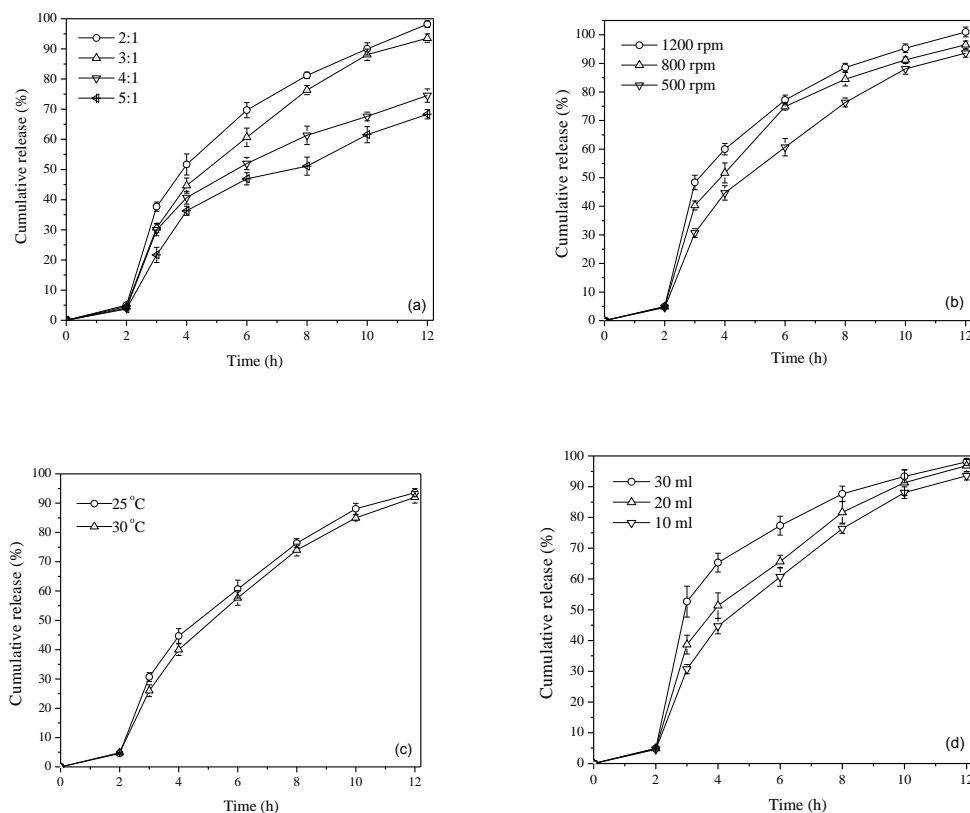


Fig. 2: Effect of polymer to drug ratio (a), stirring rate (b), temperature (c) and volume of dispersed phase (d) on the *in vitro* drug release of diclofenac sodium microcapsules.

Effect of Temperature

The effect of temperature was evaluated by preparing formulations at 25 °C, 30 °C and 40 °C. Large aggregates were formed at 40 °C. At the boiling point of the solvent (40 °C), the process could result in a very rapid solidification of microcapsules with insufficient mixing time to reduce the droplet sizes [19]. Increasing temperature from 25 °C to 30 °C resulted in increased particle size from 316.2 to 322.6 μm (Table 2). This might be attributed to the faster solidification process at higher temperature [21]. The encapsulation efficiency decreased significantly ($P < 0.05$) as the temperature increased. This could be explained by the decrease in the viscosity of the dispersed phase with increasing temperature which enhances the diffusion of the drug to the continuous phase, thus causing decreased encapsulation efficiency. On the other hand, increasing the temperature did not produce a significant effect on drug release (Figure 2c).

Effect of volume of dispersed phase

Varying volumes of dichloromethane were used in the preparation of the dispersed phase. Increasing the volume of dispersed phase resulted in significant decrease in particle size ($P < 0.05$). This could be attributed to the decreased viscosity of the internal phase allowing the droplets to distribute as agitation is applied [22]. On

the other hand, the decrease in encapsulation efficiency was not significant ($P > 0.05$) as the volume of the dispersed phase varied from 10 ml to 30 ml. The decrease in viscosity due to increased organic phase resulted in reduced barrier ability to the drug subsequently leading to loss of drug to the continuous phase [23]. The influence of the volume of dispersed phase on the drug release is shown in Figure 2d. It is evident that drug release rate was increased considerably when volume of dispersed phase increased. This is expected because smaller particle size, with increased volume of dispersed phase, provides higher surface area for dissolution [23, 24].

Effect of pH

The results of the *in vitro* dissolution tests indicate that the release of diclofenac sodium in 0.1 N HCl was generally lower compared to those in pH 6.8 phosphate buffer in all formulations studied. Less than 5% of the drug was released in the acidic pH. This is because in acidic media, diclofenac sodium is present mostly in its free acid form, which is even less soluble than the salt form. As the pH of the medium increases, however, the solubility of the active ingredient increases due to the contribution from the ionized form [25].

Optimization study

The results of the preliminary experiments revealed that the polymer to drug ratio and stirring rate significantly affect both the encapsulation efficiency and release rate while the other factors only affect one of the responses. Therefore, the effect of polymer to drug ratio and stirring rate were further studied with central composite design (CCD). The other factors, temperature and volume of dispersed phase, were kept constant at 25 °C and 10 ml, respectively.

Since the drug release from diclofenac sodium microcapsules in 0.1 N HCl was negligible, the *in vitro* drug release study for the optimization process was performed only in pH 6.8 phosphate

buffer. The *in vitro* drug release of the 13 formulations were found to be slow and sustained over 12 h except for formulations F3 (2:1, 1200 rpm), F5 (1.38:1, 850 rpm) and F8 (3.5:1, 1344.97 rpm) (Figure 3). Formulations F3, F5 and F8 showed an initial burst release of 50%, 40% and 48%, respectively in 1 h. The burst release is most probably due to the smaller particle size which occurs because of higher stirring rate that provided larger surface area for dissolution. The smaller polymer concentration in cases of F3 and F5 may also have contributed to the burst release since smaller polymer concentration leads to decreased coat thickness.

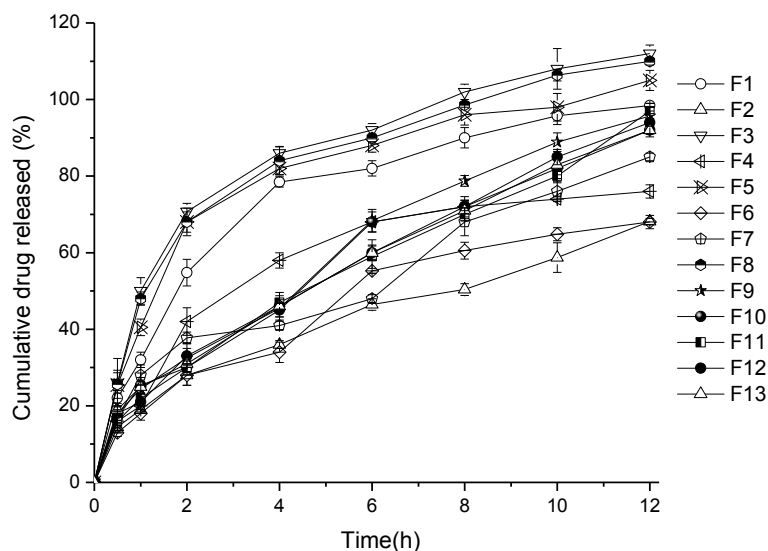


Fig. 3: Effect of polymer to drug ratio and stirring rate on the *in vitro* drug release from diclofenac sodium microcapsules

Dissolution profiles of all the formulations were compared using dissolution efficiency and results of ANOVA from the dissolution efficiency values of the formulations revealed that there was a significant difference ($p < 0.05$) in release profiles of the formulations. These differences in release profiles evidenced that changes in values of the investigated formulation variables had significant influence on the pattern of release and hence optimization was required to achieve a controlled drug release over predetermined duration.

On subjecting all the 13 formulations to the release kinetic models, the majority of the formulations exhibited best fit to Higuchi square root model with high linearity of $R^2 \geq 0.929$ except for formulations F1, F3, F5, F7 and F8 (Table 3). Since majority of the formulations exhibited best fit to the Higuchi square root model, it was selected for the optimization of the release rate. According to Higuchi model for 90-100% drug release in 12 h, the release rate should be 26- 30 $h^{-1/2}$. Therefore, the optimization was done by targeting the drug release rate within this range.

Table 3: Rate constants and correlation coefficients of the drug release kinetic model fittings for diclofenac sodium loaded microcapsules.

Formulation	Zero order		First order		Higuchi equation		Hixson-Crowell	
	Slope	R ²	Slope	R ²	Slope	R ²	Slope	R ²
F1	6.110	0.839	-0.135	0.968	27.23	0.941	-0.255	0.974
F2	4.399	0.980	-0.034	0.986	18.61	0.990	-0.100	0.988
F3	6.811	0.861	-0.170	0.973	30.02	0.945	-0.379	0.934
F4	5.155	0.822	-0.048	0.913	23.06	0.929	-0.132	0.884
F5	6.019	0.806	-0.159	0.987	28.54	0.916	-0.300	0.962
F6	4.964	0.936	-0.039	0.962	21.31	0.974	-0.114	0.959
F7	5.650	0.966	-0.056	0.974	23.86	0.973	-0.151	0.980
F8	6.852	0.878	-0.203	0.946	30.05	0.953	-0.371	0.969
F9	5.977	0.903	-0.149	0.863	26.16	0.978	-0.260	0.973
F10	5.887	0.945	-0.124	0.857	25.30	0.986	-0.236	0.956
F11	5.791	0.941	-0.112	0.872	24.94	0.986	-0.223	0.957
F12	5.650	0.916	-0.121	0.806	24.34	0.960	-0.229	0.917
F13	5.615	0.916	-0.121	0.803	24.18	0.959	-0.228	0.916

The release rate and encapsulation efficiency results obtained from the 13 formulations that were prepared as per the experimental

design are shown in Table 4. These results were input into the Design-Expert 8.0.4 software for the optimization analysis.

Table 4: Experimental design matrix for diclofenac sodium microcapsule formulations in terms of both actual and coded factor levels and response parameters

Formulation	Point type	Factor		Response	
		Polymer to drug ratio	Stirring rate (rpm)	EE (%)	Release rate (hr ^{-1/2})
F1	Factorial	2:1 (-1)	500 (-1)	20.58	27.23
F2	Factorial	5:1 (+1)	500 (-1)	52.00	18.61
F3	Factorial	2:1 (-1)	1200 (+1)	18.45	30.02
F4	Factorial	5:1 (+1)	1200 (+1)	46.095	23.06
F5	Axial	1.38:1 (- α)	850 (0)	16.24	28.54
F6	Axial	5.62:1 (+ α)	850 (0)	56.98	21.31
F7	Axial	3.5:1 (0)	355.03 (- α)	40.00	23.86
F8	Axial	3.5:1 (0)	1344.97 (+ α)	22.61	30.05
F9	Central	3.5:1 (0)	850 (0)	30.65	26.16
F10	Central	3.5:1 (0)	850 (0)	24.70	25.30
F11	Central	3.5:1 (0)	850 (0)	28.95	24.94
F12	Central	3.5:1 (0)	850 (0)	27.13	24.34
F13	Central	3.5:1 (0)	850 (0)	29.32	24.18

Selection of mathematical models

The best fitting mathematical model was selected based on the comparisons of several statistical parameters, including multiple correlation coefficient (R^2) and adjusted multiple correlation coefficient (adjusted R^2). Accordingly, the selected model for drug encapsulation efficiency is quadratic. The goodness of fit of the model was checked by determination coefficient (R^2) of 0.9725. In this case, the R^2 value indicates that 97.25% of the variability in response could be explained and only 2.75% of the total variation cannot be explained by the model. The adjusted determination coefficient (adj. $R^2 = 0.9528$) was also satisfactory for confirming the significance of the model. On the other hand, the selected model for release rate is linear. The determination coefficient (R^2) is 0.8880 which indicates 88.80% of the variability in the response could be explained by the model. Although the closer the value of R^2 to 1 indicates a better prediction of the models, for a good fit of a model, the correlation coefficient should be a minimum of 0.80 [26]. The predicted R^2 of 0.7749 is in reasonable agreement with the adjusted R^2 of 0.8656.

It is also necessary to check the fitted models to ensure that they provide adequate approximation to the real system [27]. ANOVA table has been used to summarize the test for significance of regression model, test for significance for individual model coefficient and test for lack-of-fit [28]. As shown in Table 5, models of both responses were significant. The ANOVA result also revealed that the main effects, polymer to drug ratio and stirring rate, were significant model terms for linear model of release rate whereas both the main effects, polymer to drug ratio and stirring rate, and quadratic effect of polymer to drug ratio were significant model terms for quadratic model of encapsulation efficiency. The lack of fit

test was insignificant for both models indicating the models are adequate to describe the observed data (Table 5). The value of adequate precision (signal to noise ratio) of 26.459 for encapsulation efficiency and 18.043 for release rate obtained were very high compared to the desirable value of greater than 4. Therefore, with evidence of the adequacy checking tests, it was concluded that the selected models were fairly accurate and could be used for further analysis. Thus, the final polynomial equations of response variables in terms of coded coefficients of the factors were developed as:

$$\text{Encapsulation efficiency } (Y_1) = +29.30 + 14.59X_1 - 4.08X_2 + 4.09X_1^2 \quad \text{Eq. 9}$$

$$\text{Release rate } (Y_2) = +25.20 - 3.23X_1 + 2.00X_2 \quad \text{Eq. 10}$$

where X_1 is polymer to drug ratio, X_1^2 is second order effect of polymer to drug ratio and X_2 is stirring rate.

Positive sign before a factor in polynomial equations represents that the response increases with the factor. On the other hand, a negative sign means that the response and factors have reciprocal relation [29]. It can be observed from Equations 9 and 10 that the polymer to drug ratio has a positive effect on encapsulation efficiency and negative effect on release rate which indicates increasing polymer concentration increases encapsulation efficiency and decreases release rate. On the contrary, increasing stirring rate decreases encapsulation efficiency and increases release rate. Furthermore, for both responses polymer to drug ratio has a greater effect than stirring rate as the coefficient before the factors are larger for polymer to drug ratio in both cases. These phenomena can be clearly seen in 2D contour and 3D response surface plots in Figures 4 and 5.

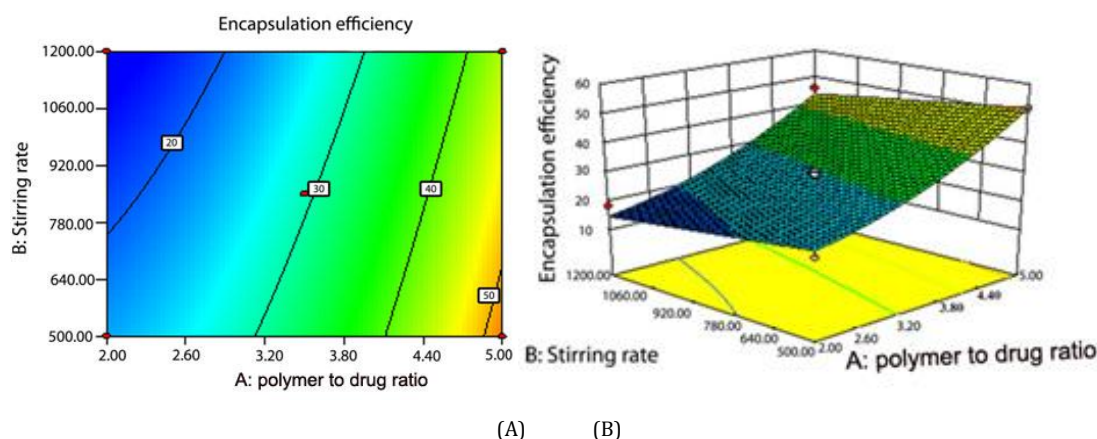


Fig. 4: Contour plot (A) and surface response plot (B) showing effect of polymer to drug ratio and stirring rate on encapsulation efficiency.

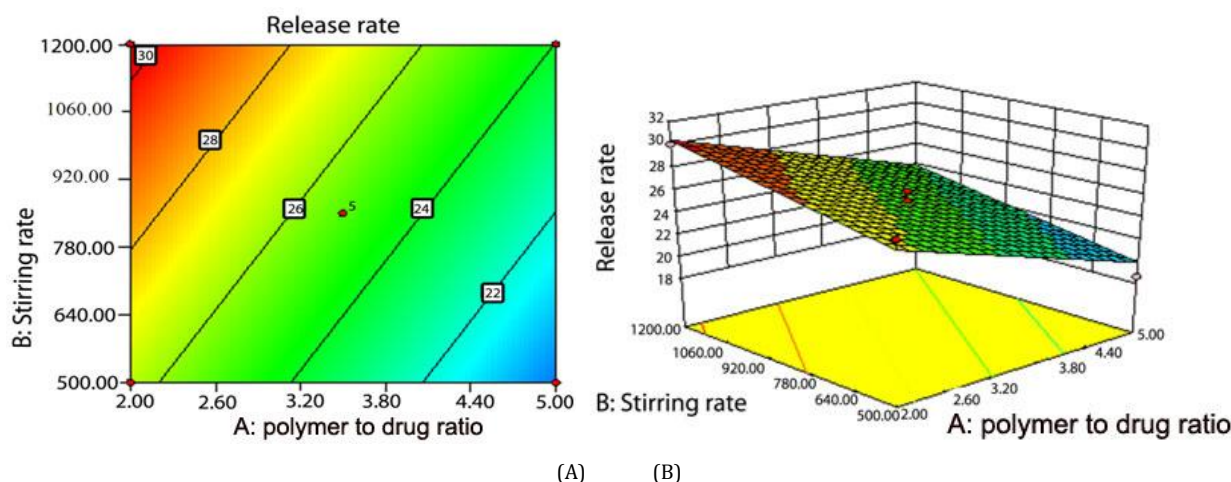


Fig. 5: Contour plot (A) and surface response plot (B) showing effect of polymer to drug ratio and stirring rate on release rate.

Table 5: Summary of ANOVA results of response surface quadratic model for encapsulation efficiency and surface response linear model for release rate.

Response	Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Encapsulation efficiency	Model	1953.51	3	651.17	74.52	<0.0001	Significant
	Polymer: drug(X_1)	1701.78	1	1701.78	194.76	<0.0001	Significant
	Stirring rate (X_2)	133.07	1	133.07	15.23	0.0036	Significant
	X_i^2	118.65	1	118.65	13.58	0.0050	
	Residual	78.64	9	8.74			
	Lack of fit	57.44	5	11.49	2.17	0.2368	Insignificant
	Pure error	21.20	4	5.30			
	Core total	2032.15	12				
Release Rate	Model	115.21	2	57.61	39.63	<0.0001	Significant
	Polymer: drug(X_1)	83.24	1	83.24	57.27	<0.0001	Significant
	Stirring rate(X_2)	31.98	1	31.98	22.00	0.0009	Significant
	Residual	14.53	10	1.45			
	Lack of fit	11.99	6	2.00	3.14	0.1439	Insignificant
	Pure error	2.55	4	0.64			
	Core total	129.75	12				
R²	Encapsulation efficiency	0.9725				Release rate	0.8880
Adjusted R²		0.9528					0.8656
Predicted R²		0.8621					0.7749
Adequate precision		26.459					18.043

Simultaneous optimization of encapsulation efficiency and release rate

After generating the model polynomial equations that relate the dependent and independent variables, encapsulation efficiency and release rate were optimized simultaneously. Hence, final optimal experimental parameters were obtained using both numerical and graphical optimization techniques of Design-Expert 8.0.4 software, which allows the compromise among various responses and searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the set. The optimization was done with constraints for encapsulation

efficiency, in the range of 25–56.98 % and release rate of 27 h^{-1/2} in 12 h as the goals to locate the optimum setting of independent variables in the new formulation as shown in Table 6.

Numerical optimization technique based on the desirability function approach is one of the most widely used methods for optimization of multiple response processes. This function searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the design. Figure 6 shows the predicted optimum values and the corresponding levels of parameters according to the set goals. The dot indicates the best solution found by the Design-Expert solver.

Table 6: Constraints of factors and responses for optimization of diclofenac sodium microcapsules

Factor constraints				
Factor	Low	High		
Polymer: drug (w/w)	2:1	5:1		
Stirring rate (rpm)	500	1200		
Response constraints				
Response	Goal	Lower limit	Upper limit	Importance
Encapsulation efficiency (%)	Maximize	25	56.98	+++
Release rate (hr ^{-1/2})	Target = 27	26	30.05	++++

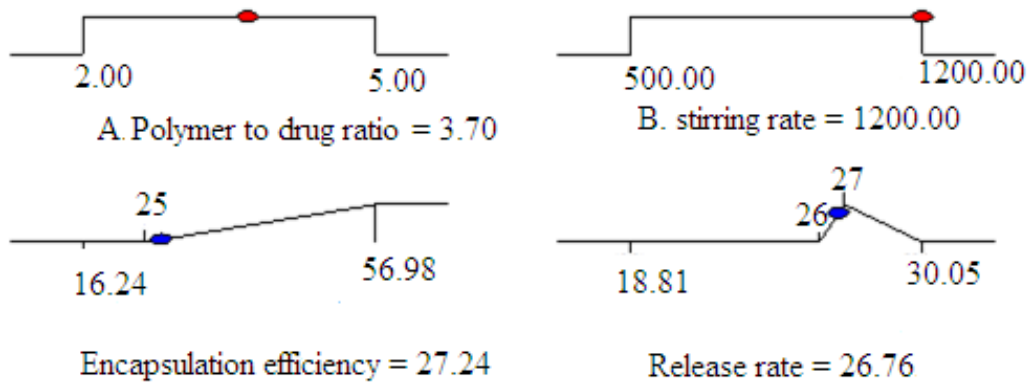


Fig. 6: Desirability ramp for numerical optimization of four goals, namely the polymer to drug ratio, stirring rate, encapsulation efficiency and release rate.

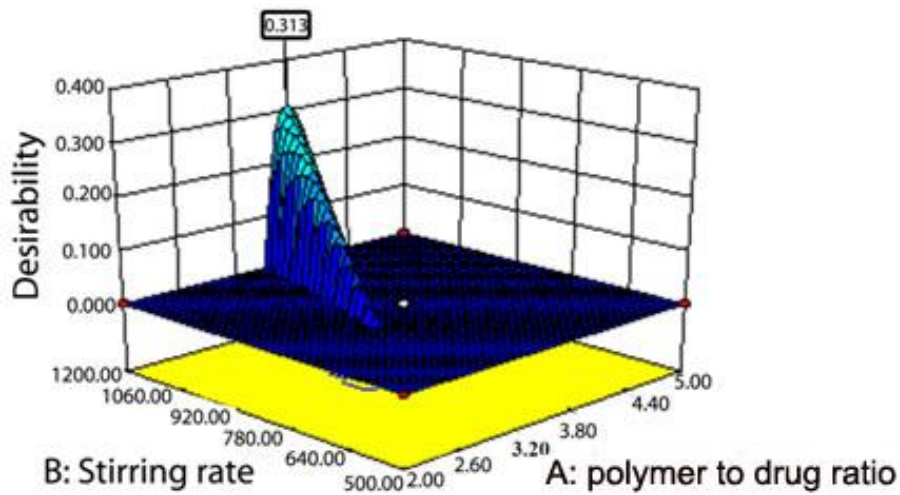


Fig. 7: 3D view of most desirable operating conditions.

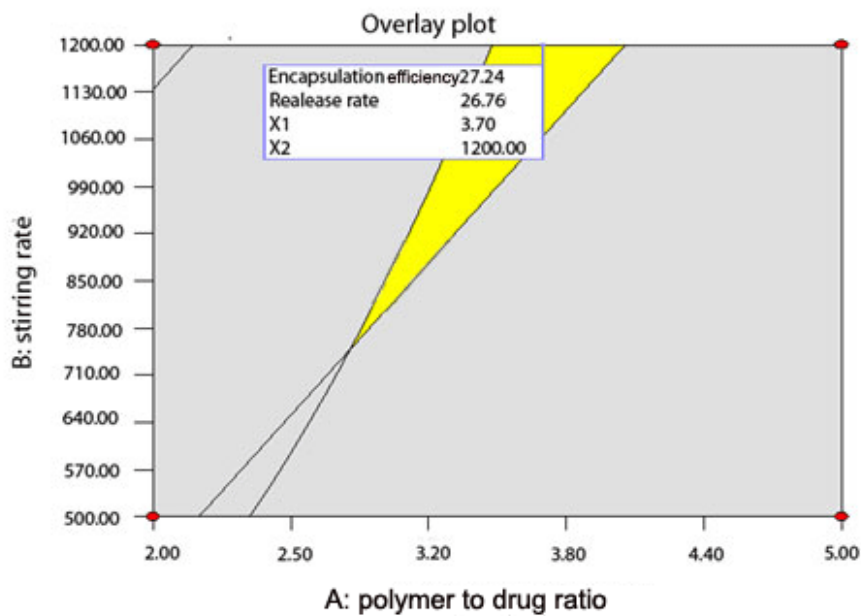


Fig. 8: Optimum region identified by overlaying plots of the two responses (encapsulation efficiency and release rate) as functions of polymer to drug ratio and stirring rate.

The optimization is accomplished by converting each response Y_i ($i = 1, 2, \dots, m$) into a dimensionless desirability scale that defines a partial desirability function (d_i), combining the individual desirability to obtain the composite or global desirability function (d), and finally maximizing the global desirability function (d) and identifying the optimal factor settings. The scale of the desirability function ranges between $d = 0$, for a completely undesirable response, and $d = 1$ for a fully desired response above which further improvements would have no importance [30].

A partial desirability (d_i) for release rate is 0.769 and 0.070 for encapsulation efficiency indicating better desirability is obtained for release rate. The overall desirability was obtained by combining individual desirability. In this case, the overall desirability of 0.313 was obtained as shown in Figure 7.

The graphical optimization allows a visual selection of the optimum conditions according to certain criteria. The same criteria proposed in the numerical optimization were introduced in the graphical optimization. Figure 8 shows the overlay plot in which the yellow area represents the area satisfying the imposed criteria. The point identified by the flag was chosen in the graph as representative of

the optimized area corresponding to polymer to drug ratio of 3.7:1 and stirring rate of 1200 rpm. Under these conditions the model predicts encapsulation efficiency of 27.24 % and release rate of $26.76 \text{ h}^{-1/2}$.

Validation of optimum formulation

To check the suitability of the model equation for predicting the optimum response, values were tested using the recommended optimum conditions. Three batches of microcapsules were prepared according to the optimized formulation. Then, encapsulation efficiency and release rate of each batch were determined. Table 7 shows the test conditions of the optimum and their experimental and predicted values for both response variables, along with the calculated percentage prediction errors. The good correlation between predicted and experimental values justified the validity of the response model and the existence of an optimum point. As shown in Table 7, the predicted errors were below 5%, indicating that the response surface methodology (RSM) optimization technique was quite useful for optimizing diclofenac sodium controlled release microcapsules.

Table 7: Experimentally prepared formulations based on the predicted values and the evaluation of encapsulation efficiency and release rate (n = 3)

Response	Predicted value	Experimental value	% error
Encapsulation efficiency (%)	27.24	26.45 ± 0.0216	2.99
Release rate ($\text{h}^{-1/2}$)	26.76	27.87 ± 0.114	3.98

Evaluation of the optimized formulation of diclofenac sodium microcapsules

The optimized formulation of diclofenac sodium microcapsules was further evaluated for different characteristic properties as shown in Table 8. The bulk density and tapped density values

were very close (the difference was 0.03). The angle of repose and Carr's index values were 29° and 5.56, respectively, indicating free flowing property. For free flowing powder, bulk and tapped densities are closer in value [31]. The microcapsules obtained were observed under microscope and revealed spherical shape.

Table 8: Characteristic properties of optimized diclofenac sodium microcapsules (n = 3, mean \pm SD)

Parameters	Experimental values
Bulk density (g/cm^3)	0.56 ± 0.01
Tapped density (g/cm^3)	0.59 ± 0.011
Angle of repose ($^\circ$)	29.00 ± 0.46
Carr's Index (%)	5.56 ± 0.85
Particle size (μm)	306 ± 2.58
Yield (%)	58.45 ± 1.7

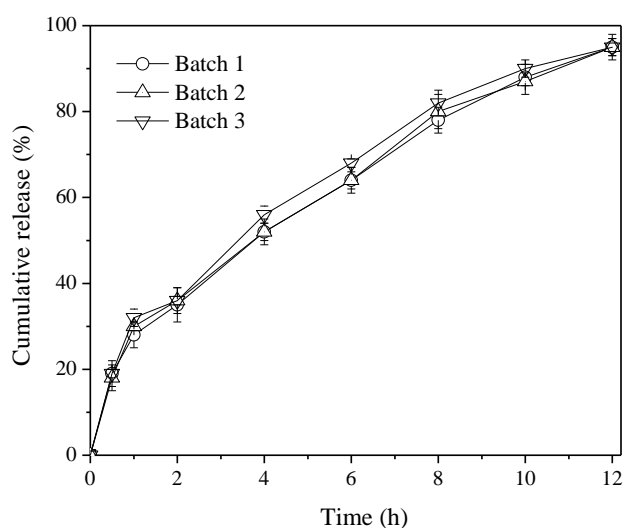


Fig. 9: The release profiles of the three batches of the optimized diclofenac sodium microcapsule formulations.

The release profiles of the three batches were almost similar with minimum burst effect (Figure 9). The ANOVA results of the release profiles based on dissolution efficiency values of the three batches, $61.71 \pm 0.75\%$, $61.83 \pm 0.63\%$ and $62.65 \pm 0.47\%$, revealed that there was no statistically significant difference ($P > 0.05$) in release profiles of the formulations.

The release kinetics for the optimized formulation indicated the best fit for Higuchi equation with $R^2 \geq 0.992$ and n values for the Korsmeyer-Peppas model in the range between 0.494 and 0.505, which indicates drug release by diffusion and erosion mechanisms. For spherical particles, the release exponent (n) between 0.43 and 0.85 indicates non-Fickian release mechanism [32].

CONCLUSIONS

The results of this study demonstrate that diclofenac sodium microcapsules can be prepared using the resin of *B. papyrifera* as a wall material by emulsion solvent evaporation method. The preliminary studies indicated both formulation variables (polymer to drug ratio, volume of dispersed phase) and process variables (stirring rate and temperature) influenced the characteristics of the prepared microcapsules. The polymer to drug ratio and stirring rate were, however, found to be critical factors for encapsulation efficiency and drug release from the microcapsules. The RSM based on CCD was successfully used to optimize the polymer to drug ratio and stirring rate with respect to encapsulation efficiency and release rate. The optimal conditions were obtained at 3.7:1 polymer to drug ratio and 1200rpm stirring rate. Under these conditions, the encapsulation efficiency and release rate were 27.24% and 26.76 $\text{h}^{-1/2}$, respectively. The experimental values of the diclofenac sodium loaded microcapsules prepared under the optimum conditions were within 5% of the predicted values. The optimum formulation provided discrete, spherical and freely flowing microcapsules. The *in vitro* drug release exhibited minimum burst release with sustained release for 12 h. The kinetic study showed the optimized formulation followed Higuchi square root kinetic model with non-Fickian diffusion release mechanism. Therefore, the results of the present study indicated that the resin of *B. papyrifera* can be used as alternative wall material for microencapsulation.

Declaration of Interest

The authors report no conflicts of interest

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