PREPARATION AND IN VITRO EVALUATION OF DICLOFENAC SODIUM LOADED WAX/LIPID MICROCAPSULES BY EXPERIMENTAL DESIGN

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ABSTRACT

The primary goal of the present investigation was to study the main and interaction effects of selected process variables on the response variable(s) in the congealable disperse-phase encapsulation method using experimental design and thereby identifying the global solution to optimize the response variable(s) for sustaining the drug release using wax/lipids as release rate modifiers. For this model a drug Diclofenac Sodium (DFS) was microencapsulated with stearyl alcohol (SA), cetostearyl alcohol (CA), carnauba wax (CW), white bees wax (BW), and microcrystalline wax (MCW). The microcapsules were prepared at 1:1 coat: core ratio. A 2-level-3-factor (2^3) full factorial experimental design with 3 replications was used to prepare the microcapsules (SAMC, CAMC, CWMC, BWMC, and MCWMC) selecting surfactant (Tween 80) concentration, stirring speed, and cooling temperature of the molten dispersion as independent variables (predictors) and percentage drug released in 60 minutes from microcapsules-Y_{60} as dependent variable (response). The results revealed that, within the gamut of the levels of predictors studied, the global solution to minimize this response variable is: Stirring speed 200 rpm, Tween 80 concentration 1%, and Cooling temperature 10°C.

KEY WORDS: Congealable disperse-phase encapsulation, Full Factorial Experimental Design, Replications, Variable.

1.INTRODUCTION

Microencapsulation by congealable disperse-phase method has been proposed as a simple and useful technique to produce microspheres for achieving sustained release, without using any harmful organic solvents (Varshosaz and Keihanfar,2001). Waxy materials have major applications in sustained release systems as they have physical properties and behavior suitable to prepare gastro resistant, biocompatible, biodegradable microspheres to release the entrapped drug in the intestinal lumen (Gifani,2009; Bennet,1975).

The methodology of Design of Experiments (DoE) provides proven strategies and methods of experimental design for performing and analyzing test series in a systematic and efficient way. All experimental parameters are varied in an intelligent and balanced fashion so that a maximum of information is gained from the analysis of the experimental results. In most cases, the time and money spent on the experimental investigation will be greatly reduced (Montgomery,1976).

Knowledge of the main and interaction effects of the process and formulation variables helps in the optimization of any process. Hence, in the present investigation a model drug Diclofenac Sodium (Bennet,1975) (DFS) was microencapsulated with wax/lipid materials by congealable disperse-phase encapsulation method by selecting three variables, surfactant (Tween80) concentration, stirring speed, and cooling temperature of the molten dispersion as independent variables (predictors) each at 2 levels- low and high; using full factorial experimental design. The objective was to study the magnitude of main and interaction effects of the predictors and to rank their contribution on response variable-percentage drug released in 60 minutes from microcapsules (Y_{60}).

2.MATERIALS AND METHODS

Materials

Diclofenac Sodium (gift sample from Amoli Organics, Ahmadabad), Stearyl alcohol (MERCK), Microcrystalline wax (Sarabhai M Chemicals), Cetostearyl alcohol (Loba-Chemie), White Bees wax (Loba-Chemie), Carnauba wax (Loba-Chemie), and Tween 80 (Loba-Chemie) were used. All other chemicals and reagents used were of analytical grade.

Preparation of Microcapsules

DFS was microencapsulated with various wax / lipid materials like stearyl alcohol (SA), cetostearyl alcohol (CA), carnauba wax (CW), white beeswax

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(BW), and microcrystalline wax (MCW) by a congealable disperse-phase encapsulation method reported earlier (Vilivalam and Adeyeye,1994), with minor modifications. Microcapsules (SAMC, CAMC, CWMC, CWMC, and MCWMC) were prepared at a coat: core ratio of 1:1 with each of the wax/lipid material. A 2^3 full factorial experimental design with 3 replications was used to prepare the microcapsules with each of the wax/lipid coat material. In this experimental design, stirring speed, surfactant (Tween 80) concentration, and cooling temperature of the molten dispersion were selected as independent variables (predictors) and percentage drug released in 60 minutes (Y_{60}) was chosen as dependent variable (response). The coded and actual values of the independent variables are shown in the Table 1.

Table1. Coded and Actual Values of the Independent Variables

Variable	Low level (-1)*	High level (+1)*
Tween 80 concentration	0.5%	1.0%
Stirring speed	200 rpm	300 rpm
Cooling Temperature	10°C	20°C

* Coded values are indicated in the parentheses

DFS (1.0 gm) was dispersed in molten wax/ lipid material (1.0 gm). The dispersion was then added slowly in a thin stream to 100 ml of 0.1N Hydrochloric acid, containing Tween 80, maintained at 80-90° C, while stirring. Stirring was continued for 5 minutes to emulsify the wax dispersion and to form spherical microspheres. The mixture was then cooled to room temperature with ice, while stirring. The encapsulated product was then collected by vacuum filtration, washed and air dried to obtain discrete microcapsules. This procedure was followed with each of the wax/lipid material, varying the combinations of the independent variables, as per the experimental design matrix shown in Table 2.

Characterization of Microcapsules

Drug Content: An accurately weighed thoroughly ground DFS loaded microcapsules was pulverized and digested in pH 6.8 buffer. The drug was extracted with the solvent overnight; filtered using 0.45 ìm filter and the amount of medicament in the filtrate was assayed after appropriate dilution by measuring the absorbance at 276 nm in Shimadzu UV- 2550 UV/ Visible Spectrophotometer. The drug content was estimated in triplicate.

Microencapsulation Efficiency: Microencapsulation efficiency was calculated using the following formula:

Microencapsulation efficiency = (estimated percentage drug content/theoretical percentage drug content) × 100. (1)

Yield: The percentage yield of microcapsules was calculated using the following formula:

% yield= weight of microcapsules (g)/initial weight of DFS (g) + initial weight of wax (g) × 100 (2)

Morphology and Size Distribution: The morphology of microcapsules was evaluated by the scanning electron microscopy (SEM) (JSM-6510LV).

Particle size range and distribution of microcapsules were determined by sieve analysis.

Measurement of Micromeritic and Packing Properties of Microcapsules

The angle of repose of microcapsules was determined by the fixed- funnel and free-standing cone method. Hausner ratio and Carr's Index were determined from bulk density measurements (Amareshwar,2010).

Sphericity: Ten microcapsules were observed by optical microscopy, fitted with a camera lucida connected to a grabbing board. The size and shape descriptors characterizing each particle as in its silhouette were calculated. Two parameters, the silhouette breadth (*B*, smallest dimension) and length (*L*, largest dimension), were noted, and the degree of sphericity (Xinde,2007) ø is defined as: $\text{ø} = \frac{L}{B}$

Porosity: The porosity of the microcapsules was calculated from the density values using the following equation (Ozyazici,1996):

$$\text{å} = 1 - \{(C_{EC} \times \tilde{n}_{DFS} + C_{DFS} \times \tilde{n}_{EC})\, \tilde{n}_{M}\} / \tilde{n}_{DFS} \times \tilde{n}_{EC}$$

where: å, porosity of the microcapsules; C_{EC}, weight per cent of EC; C_{DFS} , weight per cent of DFS; $\tilde{n}_{DFS}$, density of DFS particles; $\tilde{n}_{EC}$, density of the EC particles; $\tilde{n}_{M}$, density of the microcapsule particles.

Thickness: The thickness of the microcapsule wall was calculated using the following equation (Luu and Cartier,1973).

$$h = r/3 \times \{(1-P) \times d_1 / (P \times d_2) + (1-P) \times d_1\}$$

where h is the thickness of the envelope (in μ); r is the value of the average radius (in μ); P is the ratio of the mass of the nucleus to the total mass of the microcapsules; d_1, is the density of the material of the nucleus (in g/cm^3); d_2 is the density of the material of the envelope (in g/cm^3).

Release Rate Studies

Microcapsules equivalent to 100 mg of DFS were filled in hard gelatin capsules and were evaluated for in-vitro release studies. The study was carried out in USP XXII basket apparatus at a rotational speed of 50 rpm at 37 ± 0.5°C in 900 ml phosphate buffer (pH 6.8). Samples (10 ml) were withdrawn at regular time intervals and filtered through 0.45 μm membrane filter. The drug content was determined in the filtrate at 276nm either directly or after appropriate dilution with the dissolution medium.

To find out the kinetics and mechanism of drug release, data obtained from release rate studies was fitted to both linear and non-linear kinetic models (Zhang,2010) (Table 3).

Table 3. Fitting of Data from Release Rate Studies to Linear and Non-Linear Models

Linear Models		Non- linear Models	
Zero-order	$F = k_0.t$	Gompertz	$F = F_{max}.e^{-e^{k.(t-y)}}$
First-order	$F = 100.(1-e^{-k_1.t})$	Logistic	$F = F_{max} \cdot \frac{1}{1+e^{-k.(t-y)}}$
Higuchi	$F = k_H.t^{0.5}$	Quadratic	$F = 100.(k_1.t^2 + k_2.t)$
Korsmeyer-Peppas	$F = k_{KP}.t^n$	Weibull	$F = F_{max}.(1-e^{-\frac{t^\beta}{\alpha}})$

F denotes fraction of the drug released at time t; k stands for release rate constant in respective models; n indicates the exponent- If the n value is 0.5 or less, the release mechanism follows Fickian diffusion, and the values $0.5 < n < 1$ indicate a non-Fickian release (anomalous/zero order release). The drug release follows zero-order and case-II transport if the n value is 1. For the values of n higher than 1, the mechanism of drug release is regarded as super case-II transport. F_{max} is the maximum fraction of the drug released at infinite time; k stands for release rate constant in respective models; á, â, and ã denote scale factors in respective models. The criteria considered for selecting the best fit model were: the adjusted coefficient of determination ($R^2_{adjusted}$) and standard error of mean (SEM) (Costa and Sousa,2001).

To characterizing drug release curve, area under the release curve (AUC), mean residence time of the drug substance molecules in the dosage form (MRT), mean dissolution time (MDT), and dissolution efficiency up to 30 minutes (DE_{30}) were also calculated (Zhang,2010).

Further, data obtained from release rate studies was fitted for the following probability distributions using Anderson-Darling test statistic (De,2006) as the criterion: normal, log-normal, exponential, Weibull, logistic, and log logistic.

Shelf Life Prediction

For the purpose of shelf life prediction, the selected formulation (MCWMC) was tested for potency (drug content) by testing at 0, 6, 12, and 18 months. The resultant data was subjected to linear regression and linear regression line was drawn with the potency data. The lower and upper 95% confidence lines were also graphed. The accepted definition of shelf life time is the x-axis coordinate for the intersection of the lower 95% confidence line with 90% drug activity (Chemtob,1986).

3.RESULTS AND DISCUSSION

For microencapsulation with wax/lipid materials a congealable disperse-phase encapsulation was employed. The microcapsules were prepared at 1:1 coat: core ratio using 2^3 full factorial experimental design with 3 replications with each of the wax/lipid material.

To illustrate the main and interaction effects of the predictors on the response, the results of DoE in the preparation of MCWMC are shown in Figs.1-3.

In addition to these, surface plots were also shown, which indicate the combined effect of toluene: petroleum ether ratio and stirring speed on the T_{50} values holding stirring time both at low (Fig.4) and high (Fig.5) levels. These results indicate that:

- Increasing the stirring speed increases Y_{60}
- Increasing the Tween 80 concentration decreases Y_{60}
- Increasing the cooling temperature decreases Y_{60}

Increasing the stirring speed facilitates the emulsification process. At higher stirring speeds aggregation of microcapsules was minimum, which resulted in finer microcapsule formation. At low stirring speeds large aggregates of microcapsules were observed resulting in small surface area of microcapsules. Hence, Y_{60} values increased at higher stirring speed.

This is in agreement with the earlier report (Varshosaz and Keihanfar,2001).

Tween 80 was used to stabilize the oil-in-water emulsion. Tween 80 in its high levels up to 1.0 % increased the HLB, so the interfacial surface tension between water and wax was decreased and consequently the drug solubility (or loss) in the aqueous phase was increased, which might have resulted in lower drug entrapment in the microcapsules (Varshosaz and Keihanfar,2001). This could be the reason for decreased Y_{60} values.

An important process variable was the rate of cooling of the external aqueous phase after emulsification (Bodmeier,1992). The hardening of the oily internal phase (containing wax and drug) and formation of the microspheres are dependent on cooling. At high cooling a rigid wall of microcapsule forms, which reduces the dissolution rate of the drug. Hence, Y_{60} values decreased at high cooling temperature.

Results of ANOVA indicated that: With all the wax/lipid materials, stirring speed showed greater positive effect on response variable than cooling temperature and Tween 80 concentration showed negative effect except in the case of MCWMC. In this case although the effects of Tween 80 concentration and cooling temperature were opposite to those with other coat materials, they may be considered insignificant as $p>0.05$. Stirring speed* Tween 80 concentration (interaction) exerted a negative influence on Y_{60}, except in the case of MCWMC. In this case although this interaction was positive, it may be considered insignificant as $p>0.05$. Stirring speed* cooling temperature (interaction) exerted a positive influence on Y_{60} in the case of SAMC and CAMC. In other cases, although this interaction showed opposite (negative) effect, it may be considered insignificant as $p>0.05$. Tween 80 concentration* cooling temperature (interaction) exerted a negative influence on Y_{60} in the case of SAMC, CAMC, and CWMC. In other cases, although this interaction showed opposite (positive) effect, it may be considered insignificant as $p>0.05$. The three way interaction Stirring speed* Tween 80 concentration* cooling temperature showed a negative effect on Y_{60} with all microcapsules.

Since the target is to minimize Y_{60} values for sustained release, it may be concluded that the global solution to maximize this response variable is: Stirring speed 500 rpm, Tween 80 concentration 1%, and Cooling temperature 10°C.

Since 24 formulations were prepared as per the experimental design matrix shown in Table 2, a formulation as per the global solution to minimize the response variable is further studied. The results are presented in the following paragraphs.

To characterize the microcapsules, three parameters were calculated: the drug content, the microencapsulation efficiency, and the weight yield. Low s.d values in the mean percent drug content ensured uniformity of drug content in each batch of pellets. Also, loading efficiency and weight yield are satisfactory (Table 4). SEM picture of the selected formulation MCWMC is shown in Fig.6 which indicates spherical nature and uniform coating of the microcapsules.

Angle of repose, Hausner ratio and Carr's Index values indicate good flow characteristics of microcapsules (Vilivalam and Adeyeye,1994) (Table 5). Sphericity values (Table 5) indicate that pellets are of uniform coat thickness and have good flow properties. Porosity values ranged from 12.3-16.3%. Thickness ranged from 0.25-0.90ìm (Table 5).

The release profile of drug from microcapsules is shown in Fig.7. The differences in DFS release rate from microcapsules may be attributed to the physical and chemical properties of the different wax/lipid materials.

Stearyl alcohol being polar facilitates higher release rates of entrapped drug from microcapsules. Cetostearyl alcohol usually consists of about 50–70% stearyl alcohol and 20–35% cetyl alcohol. The combined stearyl alcohol and cetyl alcohol comprise at least 90% of the material. Cetyl alcohol belongs to polar (class I) lipids. It allows more rapid penetration of water into the matrix and/or more matrix erosion (Gren and Nystro,1999). Bees wax has hydroxyl and hydroxyl acid groups, which make it more susceptible to hydration in the dissolution medium (Adeyeye and Price,1994). Microcrystalline wax is composed of a mixture of straight-chain and randomly branched saturated alkanes obtained from petroleum (Adeyeye and Price,1994). Hence, the entrapped drug had little affinity for the dissolution medium. The drug might be entrapped in a compact dense wax matrix that posed a significant hindrance to fluid penetration and passive drug diffusion. Carnauba wax is extremely hydrophobic in nature with lower wettability. Carnauba wax contains lower

percentage of free fatty acids and hydroxyl number but contains higher percentage of fatty esters (ester value of 75–85).In addition; Carnauba wax contains 5% of resins (Reza,2003; Cusimano and Becker,1968; Evrard and Delattre,1996; Shellhammer,1997). These factors may account for the observed low dissolution behavior of Carnauba wax. Since the microcapsule formulations prepared in the present study contained no wax modifiers, formation of pores and cracks did not occur to facilitate drug release and the impervious hydrophobic matrix of Carnauba wax decreased drug release.

The calculated values of Dissolution Efficiency upto 30 minutes (DE_{30}), Mean Dissolution Time (MDT), Mean Residence Time (MRT), Area under the Release Curve (AUC), and T_{50} values were calculated and shown in Table 6. It is evident that, dissolution efficiency decreased from SAMC to CWMC, mean dissolution time increased from SAMC to CWMC, mean residence time increased from SAMC to CWMC, AUC values increased from SAMC to CWMC, and T_{50} values increased from SAMC to CWMC. The reasons for these observations may be attributed to the differences in the physical and chemical properties of the different wax/lipid materials as discussed in the above paragraph.

When the data from release rate studies was fitted to linear models, it was observed that the drug release may be described by first order kinetics. 'n' values in Peppas equation ranged from 0.233 to 0.367suggesting that Fickian diffusion is involved in drug release. When the data was fitted to non-linear models (Table 7), it was observed that they could also be used to fit the data. But, however, convergence problem may occur. This is in agreement with the literature report (Adams,2002). Among the probability distributions verified, it was observed that normal distribution fitted well (based on high value of Anderson-Darling test statistic for normal distribution).

For predicting shelf-life the graph between time (months) and potency (%) was plotted (Fig.8). It was found that the shelf life of MCWMC was 27.5253 months.

4.CONCLUSIONS

DFS could be microencapsulated by various lipid materials used in the present study. $A2^3$ full factorial experimental design with 3 replications was used to prepare the microcapsules selecting surfactant (Tween 80) concentration, stirring speed, and cooling temperature of the molten dispersion as independent variables (predictors) and percentage drug released in 60 minutes from microcapsules-Y_{60} as dependent variable (response). The results revealed that, within the gamut of the levels of predictors studied, the global solution to maximize this response variable is: Stirring speed 500 rpm, Tween 80 concentration 1%, and Cooling temperature 10°C.Carnauba wax has shown the maximum retardant effect on DFS release, while stearyl alcohol has shown the least among the coat materials employed. Further extension of the drug release from microspheres prepared with stearyl alcohol, cetostearyl alcohol, and white beeswax may require tableting. Whilst, drug release from microcrystalline wax microcapsules is inherently sustained, poor drug release from those prepared with carnauba wax may cause severe bioavailability problems, unless a wax modifier is included in the particular formulation. Both linear and non-linear models are suitable to fit the data obtained from release rate studies. The calculated shelf life of selected formulation MCWMC was 27.5253 months.

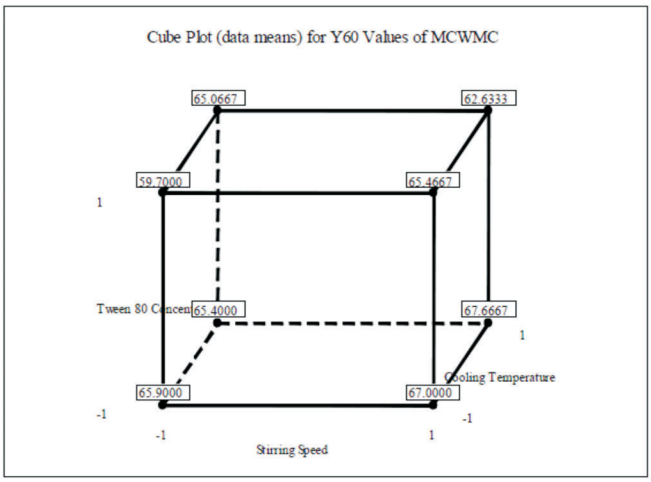

Fig.1. Cube Plot for Y_{60} Values of MCWMC

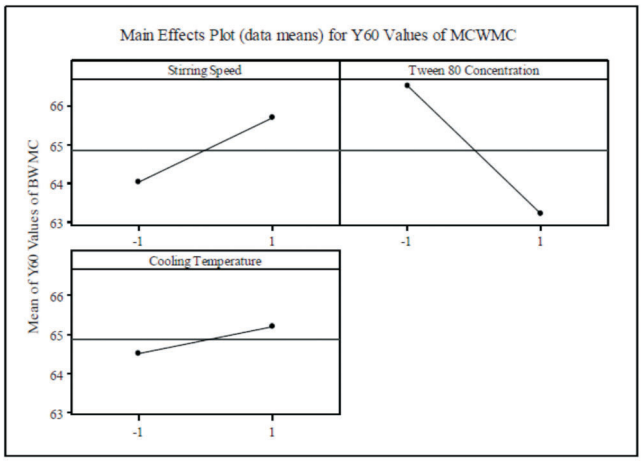

Fig.2. Main Effects Plot for Y_{60} Values of MCWMC

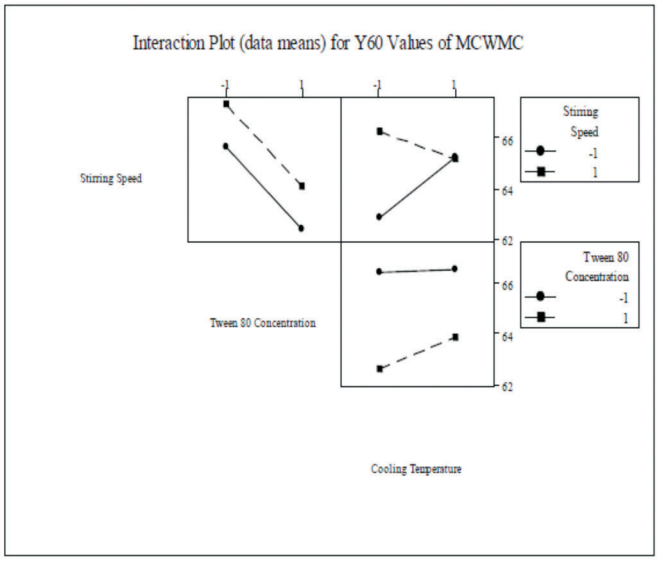

Fig.3. Interaction Plot for Y_{60} Values of MCWMC

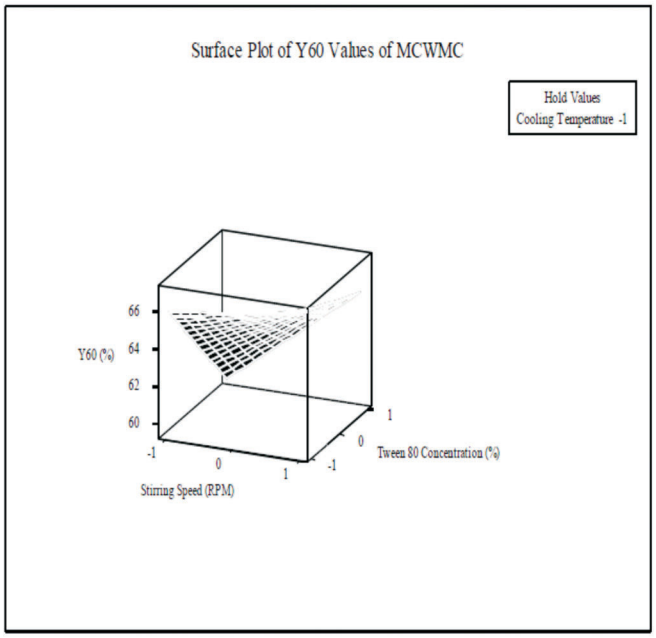

Fig.4. Surface Plot for Y_{60} Values of MCWMC

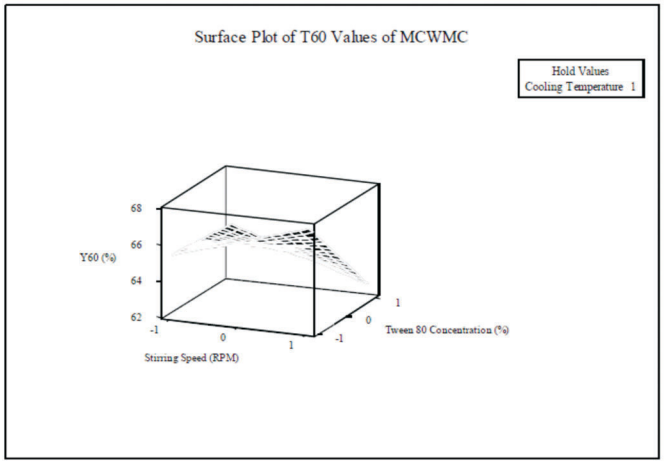

Fig.5. Surface Plot for Y_{60} Values of MCWMC

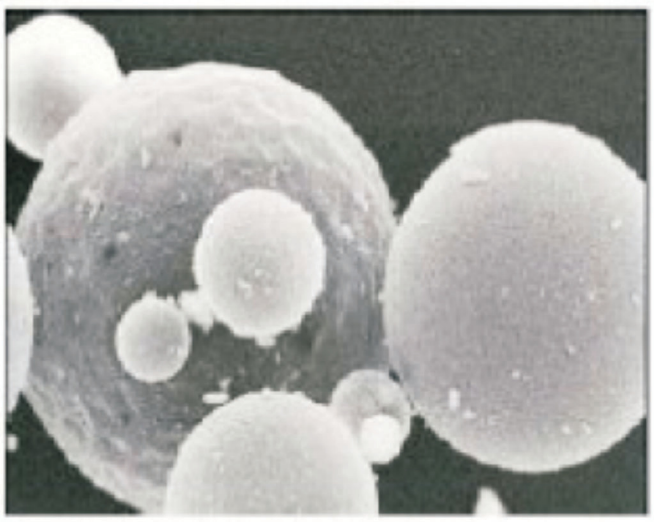

Fig.6. SEM picture of MCWMC

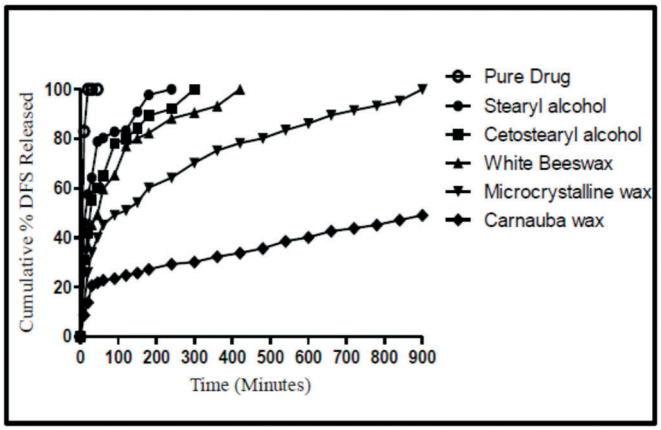

Fig.7. Release Profile of DFS from wax/lipid Microcapsules

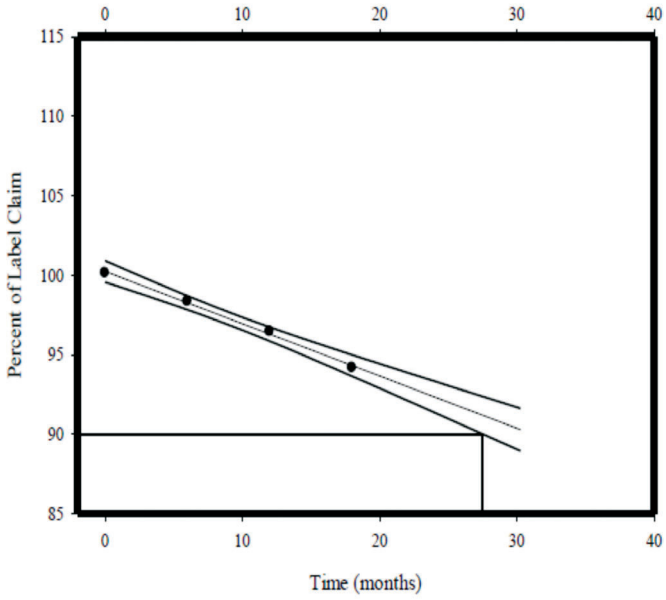

Fig.8. Prediction of Shelf Life of MCWMC

Table 2. 2^3 Full Factorial Experimental Design Matrix with 3 Replications

Run Order	Stirring Speed	Tween 80 Concentration	Cooling Temperature
1	-1	1	1
2	-1	-1	-1
3	1	1	1
4	-1	1	-1
5	-1	-1	-1
6	1	1	-1
7	-1	-1	1
8	-1	1	-1
9	1	-1	1
10	1	1	-1
11	1	1	1
12	1	-1	-1
13	-1	1	1
14	-1	-1	1
15	-1	1	1
16	1	-1	-1
17	1	1	1
18	-1	1	-1
19	1	-1	1
20	-1	-1	-1
21	-1	-1	1
22	1	1	-1
23	1	-1	-1
24	1	-1	1

Table 4. Drug Content, Microencapsulation Efficiency, and Yield of Wax/Lipid Microcapsules

Formulation	Drug Content (mg/100 mg MC) Theoretical	Estimated	Microencapsulation Efficiency	Yield %
SAMC	50.00	46.10 (0.13)	92.20 (0.24)	92.70 (1.21)
CAMC	50.00	45.95 (0.29)	91.90 (0.37)	82.59 (1.23)
BWMC	50.00	40.25 (0.87)	80.50 (0.35)	81.20 (1.17)
MCWMC	50.00	40.15 (0.39)	80.30 (0.21)	92.70 (1.08)
CWMC	50.00	40.12 (0.40)	80.20 (0.45)	95.31 (1.34)

Table 5. Sphericity, Thickness, Flow, and Packing Properties of EC Microcapsules

Formulation	Angle of Repose (°)	Hausner Ratio	Carr's Index	Sphericity	Thickness µm	Porosity %
SAMC	25.15 (0.45)	1.18 (0.28)	15.0 (0.13)	0.59 (0.12)	0.25 (0.99)	15.3 (0.70)
CAMC	26.23 (0.49)	1.23 (0.26)	18.8 (0.50)	0.67 (0.07)	0.57 (1.13)	14.7 (0.79)
BWMC	32.45 (0.53)	1.13 (0.53)	13.9 (0.90)	0.70 (0.85)	0.69 (0.99)	13.3 (0.89)
MCWMC	23.23 (0.48)	1.14 (0.14)	11.8 (0.42)	0.90 (0.10)	0.80 (2.23)	12.7 (0.87)
CWMC	21.24 (0.59)	1.13 (0.21)	11.1 (0.35)	0.95 (0.05)	0.90 (0.99)	10.3 (0.76)

*** Values in parentheses are standard deviations**

Table 6. Dissolution Parameters of Wax/Lipid Microcapsules

Formulation	Coat: Core Ratio	DE_{120} (%)	MDT (min)	MRT (min)	AUC (%.min)	T_{50} (min)
SAMC	1:1	71.4 (0.18)	40.52 (0.42)	57.43 (0.57)	19948 (0.76)	12.2 (0.86)
CAMC	1:1	61.1 (0.85)	63.91 (0.93)	79.85 (0.44)	23609 (0.63)	45.9 (0.39)
BWMC	1:1	53.4 (0.71)	90.03 (0.27)	113.35 (0.76)	32998 (0.18)	68.1 (0.71)
MCWMC	1:1	38.9 (0.97)	227.39 (0.87)	266.38 (0.15)	67261 (0.52)	191.3 (0.37)
CWMC*	1:1	20.1 (0.97)	260.06 (0.32)	414.79 (0.84)	31421 (0.51)	859.0 (0.58)

* shown for comparison

Table 7. Fitting of Data from Release Rate Studies to Non-linear Models

Formulation	Gompertz	Logistic	Quadratic	Weibull
SAMC	R^2 - 0.9204 R^2_{adj} - 0.9005 SEM- 9.0851	R^2 - 0.9889 R^2_{adj} - 0.9861 SEM- 3.3916	R^2 - 0.7702 R^2_{adj} - 0.7128 SEM- 15.4348	R^2 - 0.9674 R^2_{adj} - 0.9511 SEM- 3.8548
CAMC	R^2 - 0.9364 R^2_{adj} - 0.9223 SEM- 8.1311	R^2 - 0.9961 R^2_{adj} - 0.9952 SEM- 2.0257	R^2 - 0.8783 R^2_{adj} - 0.8512 SEM- 11.2492	R^2 - 0.9925 R^2_{adj} - 0.9893 SEM- 2.2568
BWMC	R^2 - 0.9466 R^2_{adj} - 0.9369 SEM- 7.3261	R^2 - 0.9962 R^2_{adj} - 0.9956 SEM- 1.9423	R^2 - 0.9027 R^2_{adj} - 0.8850 SEM- 9.8873	R^2 - 0.9939 R^2_{adj} - 0.9918 SEM- 2.1286
MCWMC	R^2 - 0.9314 R^2_{adj} - 0.9242 SEM- 7.7076	R^2 - 0.9932 R^2_{adj} - 0.9925 SEM- 2.4290	R^2 - 0.9202 R^2_{adj} - 0.9117 SEM- 8.3171	R^2 - 0.0000 R^2_{adj} - 0.0000 SEM- 26.9208
CWMC	R^2 - 0.8864 R^2_{adj} - 0.8744 SEM- 4.4971	R^2 - ** R^2_{adj} - ** SEM- **	R^2 - 0.8905 R^2_{adj} - 0.8790 SEM- 4.4147	R^2 - 0.9572 R^2_{adj} - 0.9465 SEM- 2.5564

** Data did not converge

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