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RESEARCH ARTICLE

Preparation and Evaluation of Waxes/Fat Microspheres Loaded with Propafenone Hydrochloride for Controlled Release

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ABSTRACT

The objective of this work to prepare and evaluate beeswax microspheres loaded with Propafenone Hydrochloride. It was entrapped into gastro resistant, biodegradable, waxes and fat such as beeswax, cetostearyl alcohol, spermaceti and cetylalcohol microspheres using meltable emulsified dispersion cooling induced solidification technique utilizing a wetting agent. Solid, discrete, reproducible free flowing microspheres were obtained. The yield of the microspheres was up to 92.5%. More than 97.2% of the isolated microspheres were of particle size range 136 to 940 µm. The microspheres had smooth surfaces, with free flowing and good packing properties. Scanning electron microscope confirmed their spherical structures. The drug loaded in waxes and fat microspheres was stable and compatible, as confirmed by DSC and FTIR studies. The release of drug was controlled for more than 8 hours. Intestinal drug release from waxes/ fat microspheres was studied. The release kinetics followed different transport mechanisms. The drug release performance was greatly affected by the materials used in microsphere preparations, which allows absorption in the intestinal tract.

KEYWORDS

Propafenone Hydrochloride, Beeswax, Cetostearyl alcohol, Spermaceti, Cetylalcohol

INTRODUCTION

In recent years, various uses of wax and fat microspheres in the pharmaceutical field have come into forefront, involving the microspheres technology [1]. Over the past decades, treatment of illness has been accomplished by the administration of drugs to the human body through various conventional dosage forms. However, to achieve and maintain the drug concentrations within the therapeutics range, it is often obligatory to take the dosage form several times a day.

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This results in an undesirable see-saw pattern of drug levels in the body. The growing interest in controlled drug delivery release is because of its benefits like increased patient compliance, which is due to reduced frequency of administration and less undesirable side effects. Different waxes and fats have been used as barrier coatings due to their non-toxic and biocompatible nature.

Oral controlled release dosage forms such as micro particles, microspheres are becoming more popular than single unit dosage forms. The uniform distribution of these multiple unit dosage forms along the gastro intestinal tract could result in more reproducible drug absorption and reduced risk of local irritation.

The matrix material used in the current study has good pharmaceutical and biological properties.

In the present investigation, water is used to prepare wax/fat microspheres by meltable dispersion emulsified cooling induced solidification method. Furthermore, the process was optimized to produce microspheres to give better yield with spherical geometry and predictable dissolution pattern. Propafenone Hydrochloride (PHC) is chemically 2'-[2-Hydroxy-3-(propylamino)-propoxy]-3-Phenyl propiophenone hydrochloride[2]. PHC is a Class 1C antiarrhythmic drug[3]. PHC is used for the treatment of frequent ventricular ectopic depolarizations[4], sustained ventricular tachy arrhythmias[5] and arrhythmias related to accessory atrio-ventricular pathways[6]. Its short biological half-life (2-10 hours) necessitates that it be administered in 2 or 3 doses daily to maintain steady blood levels. The starting dose of PHC is 450 mg/day orally. It is practically insoluble in water, having only < 10% oral bioavailability[3].

Side effects attributed to propafenone include hypersensitivity reactions, lupus-like syndrome, agranulocytosis, CNS disturbances such as dizziness, lightheadedness, gastrointestinal upset, a metallic taste and bronchospasm. The side effect could be lowered by controlling the drug release and by adjusting the absorption rate. This can be achieved by employing suitable modification in the manufacturing process[7].

Previous experiment results demonstrated that the waxes/fats are biocompatible, non-immunogenic material used for the entrapment of drug and its controlled drug release in the intestinal tract[8]. Delivering the drug in the intestinal environment from waxes/fats microspheres could be manipulated by suitable coating techniques[9]. The objectives of the present study were to formulate, characterize and study the *in vitro* drug release from wax/fat microspheres loaded with Propafenone Hydrochloride.

MATERIALS AND METHOD

Propafenone Hydrochloride was purchased from Sigma-Aldrich (USA). Spermaceti was generously gifted by British Drug House, Chemical Division, Poole, England. Beeswax, cetostearyl alcohol, cetylalcohol, span20, Tween 80 and all the other chemicals and reagents were of analytical grade and were purchased from LobaChemie Pvt. Ltd., Mumbai, India.

Preparation of Waxes and Fat Microspheres

Required quantity of waxes (beeswax, spermaceti) and fats (cetostearyl alcohol, cetylalcohol) were melted separately in china dishes using water baths. To the molten wax/fat, PHC which was previously passed through sieve no. 100 was added and stirred to obtain homogenous mixture. These resultant individual mixtures were poured into 200 ml of pH 4.6 Phthalate buffer solution (to minimize the solubility of drug), which was previously heated to a temperature higher than melting point of wax/fat (>+ 5°). Tween 80 (1.9% w/w) was added to the mixtures containing beeswax, cetostearyl alcohol, cetylalcohol and span 20 (2.0% w/w) for the mixture containing spermaceti and mechanically stirred at 850 rpm using a stirrer (RQ-127A). Spherical particles are produced due to dispersion of molten wax/fat in the aqueous medium. The mixture was stirred continuously at 850 rpm at a higher temperature (>+ 5°) of the melting point of wax/fat for 3min. The temperature of the mixture in the beakers was cooled rapidly to 10° by the addition of cold water. The resultant solid spheres collected by filtration were extensively washed with water to remove any drug and surfactant residues. Air drying was carried out at room temperature for 48 h gave discrete, solid, free flowing microspheres.

Size Distribution and Size Analysis of Microspheres

Size distribution of the wax/fat microspheres was studied by sieve analysis technique. The separations of the microspheres in to various size fractions were carried out and SEM analyzed the size of microspheres.

Micromeritic Properties

Tap density of the prepared microspheres was determined using tap density tester and % Carr's index (%I) was calculated. Angle of repose was assessed to know the flowability of waxes/fat microspheres, by a fixed funnel method[10].

Scanning Electron Microscopic (SEM) Study

SEM photographs were taken using scanning electron microscope Model Joel- LV-5600, USA, at suitable magnification at room temperature. The photographs were observed for morphological characteristics and to confirm spherical nature of the microspheres.

Determination of the Sphericity

Sphericity was determined using an image analysis system. Photomicrographs were taken with a digital camera (Canon IXUS 105). The obtained images were processed by image analysis software to characterize each individual microsphere by mean Feret diameter (FD) (average of 180 calliper measurements with an angle of rotation of 1°), Aspect ratio (AR) (ratio of longest Feret diameter and its longest perpendicular diameter) and two-dimensional shape factor (*e*R)

$$eR = 2\pi r/P_m - (b/l)^2$$

Where r is the radius, P_m the perimeter, l is the length (longest Feret diameter) and b the width (longest perpendicular diameter to the longest Feret diameter) of the microsphere[10].

Differential Scanning Calorimetry (DSC)

All dynamic DSC studies were carried out on Du Pont thermal analyzer with 2010 DSC module. Calorimetric measurements were made with the help of an empty cell (high purity alpha alumina discs of DuPont Company) as the reference. The instrument was calibrated using high purity indium metal as standard. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10°/min. The runs were made in triplicate.

Fourier Transform Infrared Radiation Measurements (FTIR):

The samples (2 mg of the pure drug, drug loaded microspheres) were selected separately and dispersed in KBr powder; the pellets were made by applying 6000 kg/cm^2 and analyzed. Spectral measurements were obtained by powder diffuse reflectance on a FT-infrared spectrophotometer type Shimadzu, Model 8033, USA.

Estimation of Drug Loading

Drug incorporated wax/fat microspheres of each batch were selected and powdered in a mortar. Drug was extracted from wax/fat microspheres using methanol, filtered and analyzed for drug content after suitable dilution.

***In Vitro* Studies**

USP XXI dissolution apparatus type II was employed to study percentage of drug release from various formulations prepared. Encapsulations of the drugs-loaded microspheres were avoided, as dissolution of shell will add one more parameter to the result. Accurately weighed quantities of drug (PHC - 150 mg) loaded microspheres of each batch were taken in 900 ml dissolution medium (2 h in pH 1.2 hydrochloric acid buffer and 6 h in pH 7.4 phosphate buffer) and stirred at 100 rpm by maintaining at a temperature of 37±0.5°. The drug concentrations were determined by withdrawing the 10 ml of aliquots using guarded sample collectors periodically at an interval of 30min for first 4 h and at 60 min interval for the next 4 h. Release studies were carried out in triplicate.

RESULTS AND DISCUSSION

Evidence have[11,12] shown in the recent years that waxes and fat materials have the physical properties and behavior suitable to prepare gastro resistant, biocompatible, biodegradable microspheres to release the entrapped drug in the intestinal lumen[13]. In the present study, a modified novel meltable dispersion emulsified cooling induced solidification method was employed using inert waxes/fat (FDA approved) material and non-toxic solvents to entrap the drug. The present method is quite different from that reported by Giannola*et al*[8]. In the present

study, a modified novel meltable dispersion emulsified cooling induced solidification method was employed using inert waxes/fat (FDA Approved) and non-toxic solvents to entrap the drug. In the present study, various parameters were studied such as stirring speed and time, amount of surfactant added, volume of the aqueous phase used. Therefore the influence of the above parameters was highlighted. When the pH value of the external aqueous phase was highly alkaline, the solubility of the drug was reduced and the encapsulated amount of the drug increased. The maximum drug load was obtained at pH 4.4. Incorporation of drug into wax/fat microspheres required the addition of a surfactant at an optimum concentration to reduce the interfacial tension between the hydrophobic material and external aqueous phase. An attempt was made to incorporate drug in the waxes/fat microspheres without the addition of a surfactant. But the process was a failed, as it resulted in an aggregate cake like mass during the solidification of waxes/ fat. This may be due to repulsion resulting from high interfacial tension between the hydrophobic waxy/fat material and external aqueous phase.

To obtain an optimal surfactant concentration, various concentrations ranging from 0.5 to 2.0% w/w of the total formulation were tested. Discrete microspheres with good flow properties using an optimum concentration of surfactants 1.9% w/w (Tween-80) for beeswax, cetostearylalcohol, cetylalcohol and 2.0% w/w (Span-20) for spermaceti were obtained. Concentrations of surfactant (Tween 80) ranging from 0.5 to 1.8% w/w in case of beeswax, cetostearyl alcohol, cetylalcohol and 0.5 to 1.9% w/w (Span20) in case of spermaceti did not give reproducible microspheres. The resultant waxes/fat microspheres were composed of irregular masses, which were not possible to distinguish as individual microspheres.

In the present study, it was found that 200 ml of aqueous phase suitable for producing the spherical microspheres. Resultant microspheres did not have any surface irregularities and are non-aggregated. As the volume of external phase increased, the yield was reduced and the resultant microspheres were irregularly shaped. When the volume of the aqueous phase was less than 200 ml, the resultant microspheres were highly aggregated in nature and highly impossible to distinguish as an individual microsphere. In order to avoid the formation of irregularly shaped larger particles, in the present method, 200 ml of aqueous phase was used. Temperature of the aqueous phase was maintained at 5^0higher than the melting point of the waxes/fat in the corresponding formulations. From SEM studies it was observed that the resultant microspheres were free from surface irregularities, except some wrinkles. It was also observed that when the temperature of the aqueous phase was less than the 5^0than the melting point of the wax / fat, big flakes were produced.

Sah[14] developed microspheres using a phase ratio 1:10, but the obtained microspheres were irregular in shape and were highly aggregated. By using a phase ratio 1:3, Giannola et *al* [8] have developed spherical microspheres by using wax, but these were not hollow in nature. In the present study, to produce the spherical discrete microspheres, an optimum drug to waxes/fat phase ratio of 1:3 w/w was used. It was found that higher the amount of drug to waxes/fat ratio (2:3) produces aggregate masses during the cooling process. It may be due to reduced melting point of the waxy and fat materials.

Sieve analysis data indicated that the prepared microspheres were in the size range of 106 to 500 µm and 57.4 to 64.9% were of size fraction 250 µm shown in Table 1.

Values shown in the table mean of 3 batches (n= 3), Propafenone Hydrochloride (PHC), BWPHC (beeswax + PHC), CSPHC (cetostearylalcohol + PHC), CPHC (cetylalcohol + PHC), SPHC (spermaceti + PHC).

The sizes of the drug loaded beeswax and spermaceti microspheres were larger than ceto stearyl alcohol and cetyl alcohol microspheres Solid, discrete, free flowing microspheres were produced, after cooling.

Table 1: Size Distribution of Wax/Fat Microspheres

Formulation	Size Range (µm)					
	710	**500**	**250**	**150**	**125**	**106**
BWPHC	-	11.4	64.9	13.5	6.9	3.3
CSPHC	-	11.8	62.6	12.7	8.1	4.8
CPHC	-	13.9	60.3	14.2	8.7	2.9
SPHC	-	7.1	57.4	19.7	12.8	3.0

Values shown in the table mean percent of 3 batches (n= 3), Propafenone Hydrochloride (PHC), BWPHC (beeswax + PHC), CSPHC (cetostearylalcohol + PHC), CPHC (cetylalcohol + PHC), SPHC (spermaceti + PHC).

It was observed that the average size of the microspheres ranged between 314 to 371 µm presented in Table 2.

Table 2: Micromeritic Properties of Drug Loaded Wax/Fat Microspheres

Formulation	Size (µm)	Yield %	Angle of Repose (θ)	%I
BWPHC	371	85.10± 0.55	24.93±0.97	10.25±0.89
CSPHC	352	92.54± 0.94	27.12±1.53	13.33±0.62
CPHC	364	86.96± 0.36	26.31 ± 1.07	15.98±1.46
SPHC	314	86.13± 0.74	28.89 ± 1.15	19.67±1.39

The important factor that influences the size distribution of microspheres is the optimum stirring speed and stirring time. A stirring speed of 850 rpm and stirring time of 3min was used to obtain reproducible microspheres. It was observed that with the increase in the stirring speed from 800 to 1100 rpm there was a decrease in the average size of the spheres and recovery yield of the microspheres. It is due to small sized waxes/fat microspheres, which were lost during successive washings. When the stirring speed was lower than 850rpm, larger microsphere was a formed.

It was also found that an increase in stirring time, from 2 to 4 min (at a stirring speed of 850 rpm), there was a decrease in the recovery yield of microspheres. When the stirring time lower than 3 min, it was observed that some amount of melted material adhered to the sides of the beaker during the cooling process resulting in lower recovery of yield. Repeat batches treated at an optimized rate mentioned above proved to produce reproducible sizes, showing that stirring speed and stirring time were well controlled. Generally the micro particulate drug delivery systems are formulated as single unit dosage

forms in the form of capsule or tablet. Such micro particulate systems should possess the better and adequate micromeritic properties. The values of φ indicate reasonable good flow potential for the microspheres. The results of %I ranges from 10.25% to 19.67%, suggests good flow characteristics of the microspheres (Table 2). The better flow property indicates reasonable and good flow potential of prepared microspheres.

SEM photographs showed that the wax/fat microspheres were spherical in nature, had a smooth surface within ward dents and shrinkage, which is due to the collapse of the wall of the microspheres (fig. 1).

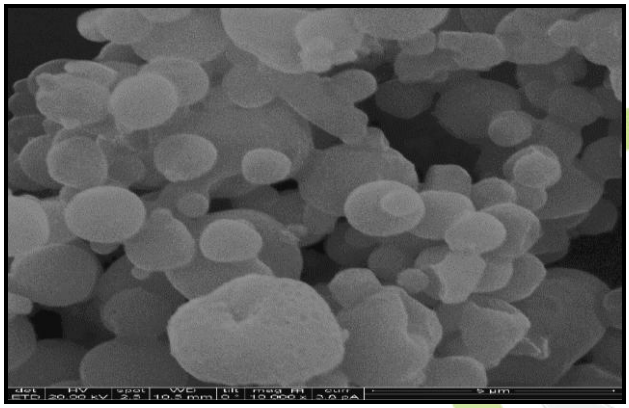

Figure 1: SEM photographs of waxes/fat microspheres loaded with Propafenone Hydrochloride showing surface dents and spherical in nature

SEM photographs reveal the absence of crystals of the drug on the surface of microsphere, indicating uniform distribution of the drug within the microspheres and further indicate that low molecular weight wax/fat produce better quality microsphere than that of high molecular weight waxes. The rate of solvent removal from the microspheres exerts an influence on the morphology of the final product [15].

From the photomicrograph image analysis, calculated Aspect ratio (AR) and two-dimensional shape factor (*e*R) was found to be 1.04-0.98 and 0.96-1.01, respectively. The obtained AR and *e*R values of the microspheres nearer to the value 1, confirming the sphericity of the microsphere.

DSC studies were performed on Pure Drug (PHC), drug-loaded microspheres. PHC exhibits a sharp endothermic peak at 173.12 °C presented in Fig. 2.

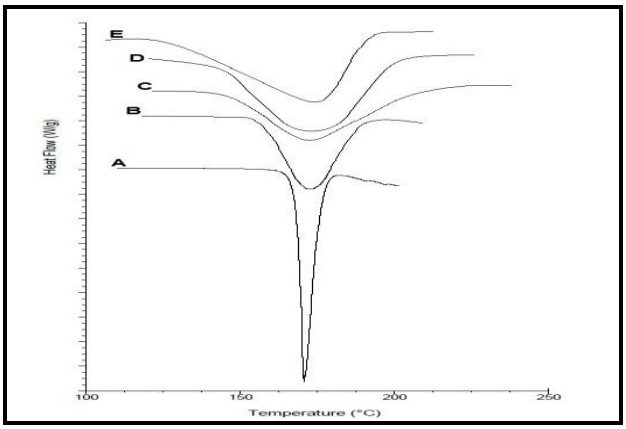

Figure 2: DSC thermograms obtained for Propafenone Hydrochloride (A), BWPHC (B), CSPHC (C), CPHC (D), SPHC (E)

The peak intensity corresponding to the melting of PHC decreased in the thermograms of drug-loaded microspheres. These results indicate that only a small fraction of the drug substance existed in the crystalline state. The DSC thermograms of drug-loaded microspheres showed peak, which corresponding to the melting of pure PHC. It indicated the absence of chemical interaction between drug and wax/fat[16]. From the FTIR studies (fig. 3), the characteristic bands for important functional group of pure drug, drug-loaded microspheres were identified.

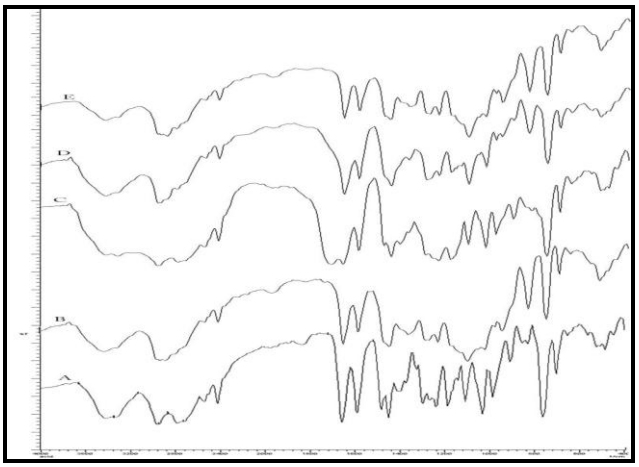

Figure 3: FTIR spectra obtained for Propafenone Hydrochloride (A), BWPHC (B), CSPHC (C), CPHC (D), SPHC (E)

FTIR spectra showed that the characteristics bands of PHC were not altered after successful encapsulation without any change in their position, indicating no chemical interactions between the drug and waxes/fat used. Compared the IR spectra at 3425 cm^{-1} due to OH stretching, 3325 cm^{-1} due to NH stretching, 2947 cm^{-1} due to aliphatic C-H stretching, 1033 cm^{-1} due to C-O stretching. FTIR spectra showed that the characteristics bands of PHC were not altered after successful encapsulation without any change in their position, indicating no chemical interactions between the drug and polymer used.

The XRD spectra recorded for the pure PHC, PHC loaded waxes/fats microspheres are presented in Fig. 4.

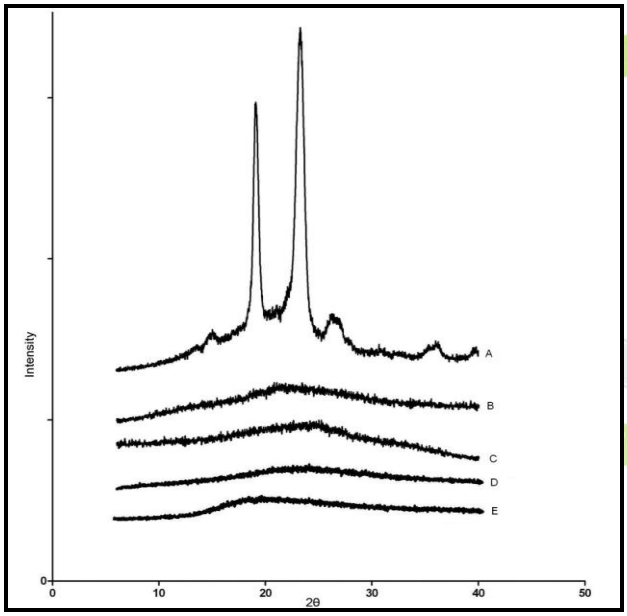

Figure 4: XRD pattern obtained for Propafenone Hydrochloride (A), BWPHC (B), CSPHC (C), CPHC (D), SPHC (E)

These studies are useful to investigate crystallinity of the drug in loaded waxes/fats microspheres. PHC has shown characteristic intense peaks between 2θº of 19.1 and 23.28 due to the presence of PHC crystals. However, these peaks were absent in PHC loaded waxes/fats microspheres. This may be attributed to the incorporation of PHC between parts of the crystal lattice of the wax, leading to a change in the crystallinity of the PHC loaded waxes/fats microspheres. These values complement the DSC data and clearly indicate the possible change in crystallinity of PHC and formulated as waxes/fats microspheres.

The percent of drug loading in the formulations were in the range of 12.33% to 15.98%. It was low in the formulations prepared by using spermaceti and high for cetostearyl alcohol.

From the release studies it was observed that, there is no significant release of drug at gastric pH from wax/fat microspheres. At the end of 8th h, *in vitro* drug release from cetostearyl alcohol (92.74%), cetyl alcohol (86.8%) microspheres was faster than beeswax (88.94%), spermaceti (80.17%) microspheres in the intestinal environment as shown in fig. 5.

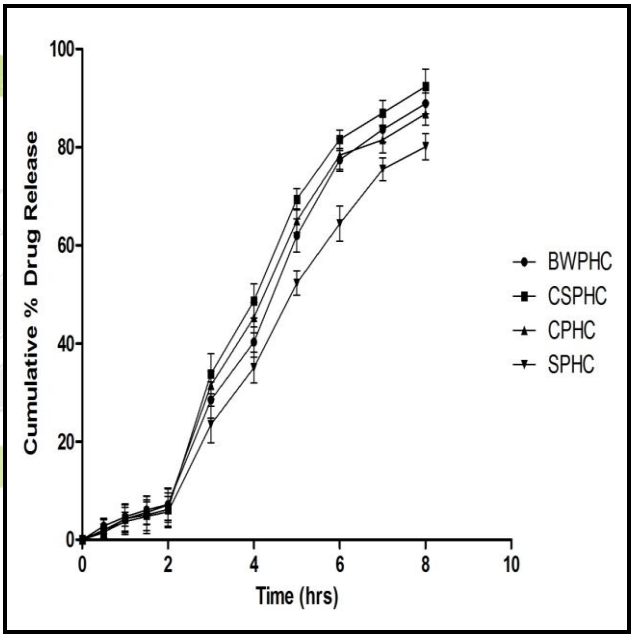

Figure 5: Cumulative % release of Propafenone Hydrochloride (PHC) from waxes/fat microspheres

The decreased *in vitro* drug release from beeswax, spermaceti microspheres was slower than that of cetostearyl alcohol, cetyl alcohol microspheres might be due to more hydrophobicity and influence of molecular weight of waxes/fat. The *in vitro* drug release was considerably retarded from the waxes/fat microspheres. The rate of drug release followed first order release kinetics and numerical data fitted into Peppa's equation showed that the

mechanism of drug release from cetostearyl alcohol, cetyl alcohol microspheres was Fickian diffusion (0.439, 0.458 respectively) and non-Fickian diffusion from beeswax, spermaceti microspheres (0.694, 0.544 respectively).

The drug release was found sufficient for oral delivery of drug. The drug release profiles were significantly affected by the properties of waxes/fatty materials used in the preparation of microspheres.

CONCLUSION

With the above results it can be concluded that Controlled Release of the carrier system can be achieved and can significantly be modified by using Waxes/Fat either alone or in combination depending on the site which further allows absorption in the intestinal tract. The future prospective of this work shall bring out an effective and economic carrier system with multiple drug delivery strategies for the developing countries.

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