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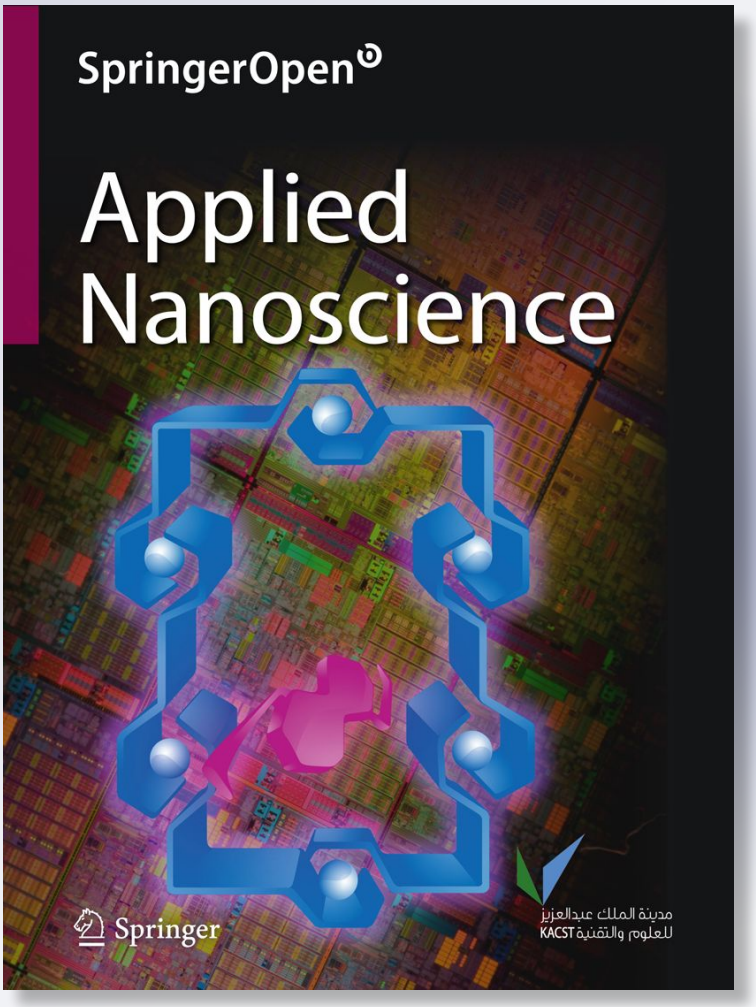

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ORIGINAL ARTICLE

Enhanced encapsulation of metoprolol tartrate with carbon nanotubes as adsorbent

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Abstract A highly water-soluble antihypertensive drug, metoprolol tartrate (MT), was selected as a model drug for preparation of multi-walled carbon nanotubes (MWCNTs)-impregnated ethyl cellulose (EC) microspheres. The present investigation was aimed to increase encapsulation efficiency of MT with excellent adsorbent properties of MWCNTs. The unique surface area, stiffness, strength and resilience of MWCNTs have drawn much anticipation as carrier for highly water-soluble drugs. Carbon nanotubes drug adsorbate (MWCNTs:MT)-loaded EC microspheres were further optimized by the central composite design of the experiment. The effects of independent variables (MWCNTs:MT and EC:adsorbate) were evaluated on responses like entrapment efficiency (EE) and t_{50} (time required for 50% drug release). The optimized batch was compared with drug alone EC microspheres. The results revealed high degree of improvement in encapsulation efficiency for MWCNTs:MT-loaded EC microspheres. In vitro drug release study exhibited complete release form drug alone microspheres within 15 h, while by the same time only 50–60% drug was released for MWCNTs-impregnated EC microspheres. The optimized batch was further characterized by various instrumental analyses such as scanning electron microscopy, powder X-ray diffraction and differential scanning calorimetry. The results endorse encapsulation of MWCNTs:MT adsorbate inside the matrix of EC microspheres, which might have resulted in enhanced encapsulation and sustained effect of MT. Hence, MWCNTs can be utilized as novel carriers for extended drug release and enhanced encapsulation of highly water-soluble drug, MT.

Keywords Multi-walled carbon nanotubes · Central composite design · Ethyl cellulose · In vitro drug release · Microspheres

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Introduction

High water solubility of the active pharmaceuticals necessitates a controlled and sustained release medication (Choudhury and Kar 2009). There are several drug delivery systems available to sustain the release rate of highly water-soluble drugs. Among them, microencapsulation technique has been a prime choice by most researchers (Yang et al. 2001; Luzzi and Palmier 1984). Microencapsulation being a multi-unit particulate system bypasses the variations associated with gastric emptying and different transit time (Ghebre-Sellassie 1994). The uniform distribution of multiparticulate system along the gastrointestinal tract (GIT) could lead to more reproducible drug absorption and minimize risk of local irritation as compared to single unit dosage form (Galeone et al. 1981). Numerous methods have been used to prepare polymeric microparticulate drug delivery systems of highly water-soluble drugs (Benita et al. 1984; Bodmeier and McGinity 1987a, b; Jeffery et al. 1993; Amperiadou and Georgarakis 1995a, b; Genta et al. 1997; Schlicher et al. 1997; Ghorab et al. 1990; Cheu et al. 2001). Different polymeric materials have been enveloped to formulate microparticulate systems (microspheres), which include chitosan, gelation, alginate, ethyl cellulose, D-L-lactide-co-glycolide and their copolymers (Jeyanthi and

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Panduranga Rao 1988; Hazrati and DeLuca 1989) Ethyl cellulose being a biocompatible and water-insoluble polymer is one of the extensively studied encapsulating materials for sustained release (Benita and Donbrow 1982; Amperiadou and Georgarakis 1995a, b; Yang et al. 2000; Wade and Weller 1994; Chowdary et al. 2004). This is attributed to its high safety, stability, easy fabrication and low cost. Acetone was selected as a solvent for emulsification processes based on human safety concern over the disadvantageous impact of residue solvent (Sah 1997; Allemann et al. 1993). Unfortunately, the number of shortcomings associated with this carrier includes high drug leakage, low mechanical strength and serious swelling due to their open structure and large pore size (Choi and Han 2005; Ito et al. 2008; O'Donnell and McGinity 1997; Blandino et al. 2000; Blandino et al. 2001; Bajpai and Saxena 2004; Boonsongrit et al. 2008). These drawbacks restrict the use of EC as a polymer for microencapsulation. Various attempts have been done to overcome the problems associated with EC, which includes cross linking (Anal and Stevens 2006), solvent evaporation (Yang et al. 2005), ion exchange (Abdekhodaie and Wu 2008) and surface coating (Zhang et al. 2008), but to date none of them have shown distinguished effectiveness.

Nanotechnology offers lots of openings and benefits for new drug molecules with extensively enhanced characteristics. Pharmaceutical innovations have chiefly concentrated on the design of modified release drug delivery systems using diverse newly discovered excipients. In the last few years, several carriers have been developed for effective drug delivery of highly water-soluble drugs. Carbon nanotubes (CNTs), being one of such carriers, represent allotropes of carbon with cylindrical tubules-shaped concentric graphitic (a hexagonal lattice of carbon) sheets and capped by fullerene-like hemispheres with a length to diameter ratio >1,000,000 (Iijima 1991). Their inert nature (Bonard et al. 1998; Niyogi et al. 2002), large surface area (Wu and Liao 2007), strong hydrophobic property (Tagmatarchis and Prato 2005) and available flexibility for physiochemical manipulation (Tasis et al. 2006) have sparked much interest in the pharmaceutical scientist. Wide amount of research is dedicated to their innovative applications in the design of the modified release dosage form. CNTs are mechanically stable substances with special ability to incorporate material into their inner hollow spaces (Ajima et al. 2005) or adsorption over the surface (Davydov et al. 2005). CNTs-doped microspheres of nicotinamide adenine dinucleotide with increased loading efficiency were developed by Lu et al. (2005). Jiang et al. (2006) have also developed microspheres containing bovine serum albumin (BSA) using CNTs as a carrier to overcome the drawbacks of polymeric microspheres. They observed considerable reduction of BSA leakage with dramatic increase in the strength of microspheres. In light of these, CNTs-impregnated microspheres may effectively overcome the drawbacks associated with microspheres containing water-soluble drug.

Metoprolol tartrate (MT) is a highly water-soluble antihypertensive drug with a half-life of 3–4 h. The short biological half-life provides suitability of the drug for sustained drug delivery. The basic nature attributes to high p*K*a value (9.5) of the drug. It is prescribed alone or in combination with other antihypertensive agents for the long-term management of angina pectoris and myocardial infarction. Moreover, it is readily and completely absorbed from the GIT with a bioavailability of about 50% (Martindale 2009; Chrysant 1998). Since MT requires multiple daily dosage to maintain adequate plasma concentrations, it was selected as a model drug for sustained release polymeric microparticulate formulation using MWCNTs as adsorbent (Dadashzadeh et al. 2003; Gambhire et al. 2007).

Hence, the present investigation aimed at preparing MWCNTs-impregnated EC microspheres of a highly water-soluble drug, MT, to achieve sustained release with elimination of multiple daily dosing.

Materials and methods

Materials

MT was obtained as a gift sample from Sun Pharmaceutical (Mumbai, India). Multi-walled carbon nanotubes (MWCNTs) were procured from Nanoshel LLI (Wilmington, USA). Ethyl cellulose (EC) and Tween 80 were obtained from Hi-Media (Mumbai, India). Other chemicals were of analytical grade and used as received without further modification. Deionized water was used throughout the study.

Adsorption isotherm

Several reports demonstrated that CNTs had good adsorption capacities for different materials due to their hollow and layered nanosized structures that have a large specific surface area (Long and Yang 2001; Li et al. 2002; Peng et al. 2003). MT solutions were prepared by dissolving solid in deionized water. Batch adsorption studies were performed in glass bottles with MT solution (100 mL) of the prescribed concentration, ranging from 5 to 50 mg dm^{-3} (5 mg dm^{-3} interval), and adsorbent material (MWCNTs, 0.1 g) was added to each bottle. The bottles were capped with glass stoppers followed by sonication for 5 min and subsequently subjected to isothermal shaking incubator (Nova, Mumbai, India) for 24 h at 30°C. After equilibration, the suspension was filtered through a

0.22-μm filter and the filtrate was analyzed for the presence of any unadsorbed MT content by double beam UV/visible spectrophotometer (Shimadzu 1700, Tokyo, Japan) at 276 nm (Shinde et al. 2009). The amount of MT absorbed by the adsorbent material (MWCNTs) was calculated based on a mass balance equation as given below:

$$q = \frac{(C_0 - C_{eq}) \times V}{W}$$

where q is the amount of MT adsorbed by MWCNTs, mg g^{-1}; C_0 and C_{eq} are the initial and equilibrium concentration of MT in the solution, mg dm^{-3}; V is the solution volume, dm^3; and W is the dry weight of adsorbent, g.

Adsorption isotherm models

Literature survey has cited various examples of drug adsorption on the various carriers. So far, kaolin (Afolabi 2006), chitosan (Alkhamis 2001) and antacids (Aideloje et al. 1998) have been studied for drug adsorption, and it has been observed that depending on the surface area and nature of the adsorbent, the extent of adsorption varies. Langmuir, Freundlich and Sips adsorption isotherm has been used to study the same. In the case of adsorption of drug on MWCNTs, the same method has been employed to study drug adsorption by MWCNTs.

The representation of the Langmuir model is given as:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$$

where q_e is the equilibrium MT concentration on the adsorbent, mg g^{-1}; C_e is the equilibrium concentration of MT in the solution, mg l^{-1}; q_m is related to the maximum adsorption capacity of adsorbent mg g^{-1}; and K_L is the constant term related to energy of adsorption, dm^3 mg^{-1}.

The representation of the Freundlich model is:

$$q_e = K_F C_e^{1/n}$$

where K_F is the Freundlich constant related to the adsorbent capacity, dm^3 g^{-1}, and $1/n$ measures the surface heterogeneity.

Sips model is a combination of Langmuir and Freundlich models and is stated as:

$$q_e = \frac{q_{max} K_{eq} C_e^n}{1 + K_{eq} C_e^n}$$

where K_{eq} represents the equilibrium constant of Sips equation, dm^3 mg^{-1}; and q_{max} is the maximum adsorption capacity, mg g^{-1}. Sips isotherm model is distinguished by the heterogeneity factor, n. Data obtained from adsorption isotherm method were fitted to the Langmuir, Freundlich and Sips isotherm models using a method of least square. From the nonlinear regression coefficient (R^2) and nonlinear Chi-square test (χ^2) value, the best fit model was predicted.

Preparation of drug-nanotubes adsorbate

Drug-nanotubes adsorbate (MT:MWCNTs) was prepared by the nanoprecipitation method (Ajima et al. 2004). Accurately weighed amount of MT was dissolved in 25 mL of deionized water followed by addition of MWCNTs in the required quantity. The mixture was further subjected to sonication (Frontline FS-4, Mumbai, India) for 15 min followed by incubation in hot air oven (Nova, Mumbai, India) at 40°C for 48 h. The residue left after evaporation (MWCNTs:MT) was analyzed for drug content by the UV method at 276 nm.

Drug content estimation of adsorbate

As much as 10 mg of adsorbate was added to 50 mL of deionized water with subsequent sonication for 30 min. The resultant solution was kept at ambient temperature for 24 h for complete extraction of MT from the adsorbate. The dispersion was filtered through Whatman filter paper (Sartorius Stedim, Bangalore, India) and analyzed by UV spectroscopy.

Preparation of microspheres

MWCNTs-impregnated EC microspheres of MT were prepared by the solvent evaporation technique (Cheu et al. 2001). Briefly, ethyl cellulose was dissolved in 25 mL of acetone along with the prepared adsorbate. The dispersion was further subjected to sonication for 15 min with intermediate shaking. The resultant dispersion was added dropwise to 250 mL of light liquid paraffin (containing 0.2% w/v Tween 80) with continuous stirring by magnetic stirrer (Remi Labs, Mumbai, India). After 2 h, microspheres were collected and washed with petroleum ether with subsequent air drying at room temperature. A similar method was utilized for preparing EC microspheres of MT without incorporation of MWCNTs.

Experimental design

The MWCNTs:MT ratio and polymer concentration play a crucial role in the preparation of MWCNTs-impregnated EC microspheres of MT. Center composite design (CCD) was employed for systemic study of joint influence of the effect of independent variables [MWCNTs:MT (X_1) and EC:drug adsorbate (X_2)] on responses such as drug entrapment efficiency (EE) and time for 50% drug release (t_{50}). The selections of dependent variables were done on the basis of the aim of the present investigation (enhanced

encapsulation and sustained drug release). Based on preliminary trials, two factors were determined as follows: MWCNTs:MT ratio (X_1): 0.25–2.75 and EC:drug adsorbate ratio (X_2): 0.10–0.30. In this design, two factors with five levels were probed to investigate the main effects and interaction of the two factors on two responses (Table 1). The design consists of nine runs (4 factorial points, 4 star points and 1 center point) and four replicated runs (center points) yielding 13 experiments in total. The main purpose of the replication runs was to increase the precision and to minimize experimental error. A third-order quadratic model incorporating interactive and polynomial terms was used to evaluate the response.

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2 + b_6X_1^2X_2 + b_7X_1X_2^2$$

where, Y_i was the dependent variable, b_0 was arithmetic mean response of the 13 runs and b_i was the estimated coefficient for factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) were included to investigate nonlinearity (Liu et al. 2009).

Data were further analyzed by Microsoft Excel® for regression analysis. Analysis of variance (ANOVA) was implemented to assure that there was no significant difference between the developed full model and the reduced model. Response surface plots were plotted to study response variations against two independent variables using Design Expert® Version 8 software.

Table 1 Central composite design batches

Batch	MWCNTs:MT (X_1)	EC:adsorbate (X_2)
1	1	−1
2	−1	1
3	$-\alpha$[a]	0
4	−1	−1
5	0	$-\alpha$
6	α	0
7	1	1
8	0	α
9–13	0	0

Factor	Level				
	$-\alpha$	−1	0	1	α
X_1 (MWCNTs:MT)	0.25	0.62	1.50	2.38	2.75
X_2 (EC:adsorbate)	0.10	0.13	0.20	0.27	0.30

[a] $\alpha = 1.414$

Characterization of microspheres

Yield of microspheres

Fresh microspheres impregnated with MWCNTs and of drug alone were collected and weighed separately. The measured weight of microspheres was divided by the total amount of all nonvolatile components that were used for the preparation (Patel et al. 2006).

$$\%\,\text{Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of excipients and drug}} \times 100$$

Entrapment efficiency (EE)

Intact microspheres (equivalent to 100 mg of drug) were crushed and extracted with methanol. The extract was further filtered by Whatman filter paper (Sartorius Stedim, Bangalore, India) and diluted to 100 mL with methanol. The drug content of filtrate was determined spectrophotometrically at 276 nm (Patel et al. 2006). The amount of drug entrapped in the microspheres was calculated as:

$$\text{EE} = \frac{\text{Practical drug content}}{\text{Theoretical drug load expected}} \times 100$$

Micromeritics

Sphericity of microspheres Photomicrographs of randomly selected microspheres were utilized to calculate the area (A) and perimeter (P) of the microspheres. The shape of the microspheres was estimated by computing the shape factor and circularity factor (Jadhav et al. 2007; Singh et al. 2007). It was calculated by the following equation.

$$\text{Shape factor} = P'/P, \quad \text{where } P' = 2\pi(A/\pi)^{1/2}$$

$$\text{Circularity factor} = P^2/12.56 \times A$$

Determination of flow properties The flow behavior of microspheres was quantified by the angle of repose and Carr's Index (Lee et al. 2000; Parul et al. 2008).

Instrumental analysis

Scanning electron microscopy (SEM) Surface topography of MWCNTs, drug adsorbate and optimized batch of microspheres were observed under a scanning electron microscope (Model JSM 5610LV, Jeol, Japan). The samples were attached to the slab surfaces with double-sided adhesive tapes and the scanning electron photomicrograph was taken at 1,000–15,000× magnification.

Powder X-ray diffraction (PXRD) Samples of MT, MWCNTs, adsorbate, EC microspheres (without MWCNTs) and EC microspheres impregnated with MWCNTs were subjected to X-ray diffraction (PANalytical X'pert PRO-

6340, India) to investigate their X-ray diffraction patterns. The data were recorded over a range of 2°–100° at a scanning rate of 5 × 10^3 cps using a chart speed of 5 mm per 2°.

Differential scanning calorimetry (DSC) 5–10 mg of fresh samples as mentioned for PXRD were studied by differential scanning calorimeter (Linseis STA PT-1600, Germany). The samples were hermetically sealed in an aluminum crucible before analysis. The system was purged with nitrogen gas at a flow rate of 60 mL min^{-1}. Heating was done between 30 and 300°C at a rate of 10°C min^{-1}.

In vitro drug release

Accurately weighed amount of microspheres (equivalent to 100 mg of drug) from each batch were subjected to dissolution studies using USP dissolution test apparatus type I in 900 mL of pH 6.8 phosphate buffer maintained at 37 ± 0.5°C; 5 mL aliquots were withdrawn at 10-min time intervals for the first hour while the remaining samples were withdrawn at 60-min time intervals for 24 h. Each sampling was followed by the simultaneous addition of fresh medium. The samples were filtered through Whatman filter paper (Sartorius Stedim, Bangalore, India) before being analyzed for the amount of drug released.

Results and discussion

Adsorption isotherm of MWCNTs for MT

Adsorption isotherm of MWCNTs for MT was studied using Langmuir, Freundlich and Sips adsorption isotherm models. The results of nonlinear R^2 and χ^2 for the three adsorption isotherms are shown in Table 2. The maximum adsorption capacity (q_m) of the MWCNTs for MT was 443.04 mg g^{-1} (Table 2). The R_L value of the MWCNTs (0.028) obtained from the Langmuir isotherm model for the initial MT concentration of 50 mg dm^{-3} indicated favorable adsorption of MT onto MWCNTs. The adsorption isotherm data for MT exhibited good fit with Langmuir model ($R^2 = 0.9971$) and Sips model ($R^2 = 0.9969$) than the Freundlich model ($R^2 = 0.8542$). The results of the nonlinear χ^2 for the three adsorption isotherms indicated that the Langmuir isotherm model appeared to be the best fitting model for the adsorption isotherm data of the MWCNTs, because it displayed the lowest Chi-square, χ^2 (5.43) with the highest R^2 (0.9971) values. The value of n for MT adsorption onto MWCNTs was close to unity ($n = 1.005$), indicating homogeneous adsorption. Also, there was close similarity between the maximum adsorption capacity values obtained from Langmuir (443.04 mg g^{-1}) and Sips (444.67 mg g^{-1}) isotherm models (Table 2). From the adsorption isotherm data, it is concluded that the MWCNTs is a good adsorbent for MT with homogeneous adsorption to reduce batch to batch variability.

Table 2 Constant for equilibrium isotherm models with error analysis values for MT:MWCNTs adsorbate

Isotherm model	Parameter value (at constant temperature, 30°C)				
	K_L	q_m	R_L	R^2	χ^2
Langmuir	0.0572	443.04	0.028	0.9971	5.43
Isotherm model	Parameter value (at constant temperature, 30°C)				
	K_F	$1/n$	R^2	χ^2	
Freundlich	15.7575	0.3547	0.8542	78.45	
Isotherm model	Parameter value (at constant temperature, 30°C)				
	q_{max}	K_{eq}	n	R^2	χ^2
Sips	444.67	0.042	1.005	0.9969	14.31

K_L constant term related to energy of adsorption, dm^3 mg^{-1}; q_m maximum adsorption capacity of adsorbent, mg g^{-1}; R_L adsorption coefficient; R^2 regression coefficient; χ^2 Chi-square value; K_F Freundlich constant, dm^3 g^{-1}; q_{max} maximum adsorption capacity, mg g^{-1}; K_{eq} equilibrium constant, dm^3 mg^{-1}; n heterogeneity factor

Experimental design (CCD)

Preliminary investigations of the process parameters revealed that factors such as ratio of MWCNTs:drug (X_1) and EC:drug adsorbate (X_2) exhibited significant influence on rate of entrapment efficiency (EE) and in vitro drug release; hence, they were utilized for further systematic studies. Both selected dependent variables (EE and t_{50}) for all 13 batches showed a wide variation of 84.17–97.78% and 5.88–15.05 h, respectively (Table 3). The data clearly

Table 3 Results of experimental design batches

Batch	X_1	X_2	EE (%)	t_{50} (h)
1	2.38	0.13	87.42 ± 0.26	10.89 ± 0.97
2	0.62	0.27	92.94 ± 0.47	09.71 ± 0.52
3	0.25	0.20	86.08 ± 0.18	08.93 ± 0.65
4	0.62	0.13	88.04 ± 0.33	08.42 ± 0.48
5	1.50	0.10	84.17 ± 0.46	05.88 ± 0.42
6	2.75	0.20	96.01 ± 0.81	11.95 ± 0.78
7	2.38	0.27	93.84 ± 0.57	12.83 ± 0.11
8	1.50	0.30	97.78 ± 0.34	15.05 ± 0.61
9	1.50	0.20	92.04 ± 0.42	09.63 ± 0.45
10	1.50	0.20	89.97 ± 0.84	09.61 ± 0.81
11	1.50	0.20	90.52 ± 0.25	09.74 ± 0.47
12	1.50	0.20	91.85 ± 0.55	09.72 ± 0.48
13	1.50	0.20	89.47 ± 1.01	09.91 ± 0.77

X_1 MWCNTs:MT, X_2 EC:adsorbate, EE entrapment efficiency, t_{50} time required for 50% drug release

indicate the strong influence of X_1 and X_2 on selected responses (EE and t_{50}). The polynomial equations can be used to draw conclusions after considering the magnitude of coefficients and the mathematical sign carried: positive or negative. For EE, coefficients b_3, b_4 and b_5 were found to be insignificant, as p values were more than 0.05 and hence removed from the full model. Similarly, for t_{50}, values of b_5 and b_7 were insignificant and hence removed from the full model (Table 4). Table 5 shows the results of analysis of variance (ANOVA) performed to justify the removal of insignificant factors. The high values of correlation coefficients for EE and t_{50} indicate a good fit. The critical values of F for EE and t_{50} were found to be 5.41 ($df = 3, 5$) and 5.79 ($df = 2, 5$), respectively, at $\alpha = 0.05$. Moreover, calculated F value [0.1742 (EE), 0.8475 (t_{50})] was found to be less than critical value, which suggests no significant difference between the full and reduced model. The data of all the 13 batches of factorial design were used to generate interpolated values using Design Expert® Version 8 software. High levels of both X_1 and X_2 were found to be favorable for sustained release and high entrapment efficiency. Multiple linear regression analysis (Table 4) also revealed positive values of coefficient b_1 and b_2 for both responses. This indicated that as MWCNTs:drug ratio (X_1) and EC:drug adsorbate ratio (X_2) were increased, there was a significant improvement in EE and t_{50}.

Influence of formulation composition factor on entrapment efficiency (EE)

The major aim of the present investigation was to increase the entrapment efficiency of a highly water-soluble drug, MT. Response surface plot for EE (Fig. 1) illustrated strong influence of two factors (MWCNTs:MT ratio and EC:adsorbate ratio). An EE of 97.78% was observed with MWCNTs:drug ratio 1.5 and EC:adsorbate ratio 0.30 (Batch 8). This might be attributed to the ability of MWCNTs to prevent leaching of drug after encapsulation inside the matrix of EC microspheres and also due to increase in the amount of EC in the formulation that ultimately increase the polymeric matrix from which MT has to release.

Influence of formulation composition factor on in vitro drug release (t_{50})

A strong influence of both independence variables (MWCNTs: drug ratio and EC: adsorbate ratio) was observed on in vitro drug release (t_{50}) (Fig. 2). The highest t_{50} value (15.05 ± 0.6114) was observed with Batch 8 (MWCNTs:MT ratio 1.5 and EC:adsorbate ratio 0.30). It indicates retardation of drug release, which again endorsed the resilience and stiffness of MWCNTs along with increased amount of EC with respect to drug.

Table 5 Calculation of testing the model in portions

	DF	SS	MS	R^2
EE				
Regression				
FM	7	174.5569	24.9367	0.9690
RM	4	173.9737	43.4934	0.9658
Error				
FM	5	6.4767	1.2953	
RM	8	7.2479	0.9059	
t_{50}				
Regression				
FM	7	59.1275	8.4467	0.9996
RM	5	58.8046	11.7609	0.9941
Error				
FM	5	0.0232	0.0046	
RM	7	0.3465	0.0494	

DF degree of freedom, *SS* sum of squares, *MS* mean of squares, *R* regression coefficient

Table 4 Summary of regression analysis

Coefficients	b_0	b_1	b_2	b_{11} [a]	b_{22} [a]	b_{12} [a]	b_{112}	b_{122} [a]
EE								
FM	90.7700	3.5113	4.8125	0.0249	−0.0100	0.3801	−1.9825	−3.4413
RM	90.7792	3.5113	4.8125	–	–	–	−1.9825	−3.4413
t_{50}								
FM	9.5651	1.0678	3.2425	0.3965	0.4090	0.1625	−2.4350	0.3296
RM	9.5651	1.1132	3.2425	0.3965	0.4090	–	−2.4350	–

FM full model, *RM* reduced model, *EE* entrapment efficiency, t_{50} time required for 50% drug release

[a] Response is insignificant at $p = 0.05$

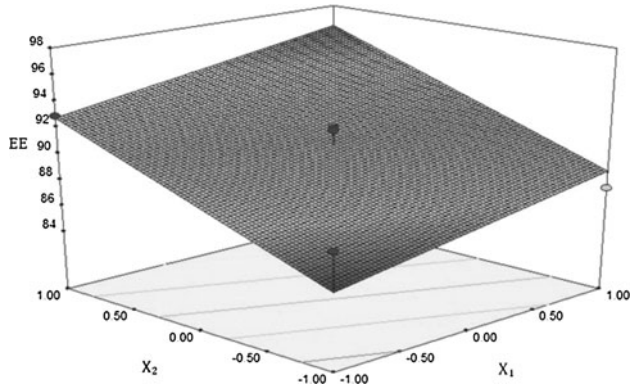

Fig. 1 Response surface plot showing effect of entrapment efficiency of variables [MWCNTs:MT (X_1) and EC:adsorbate (X_2)]

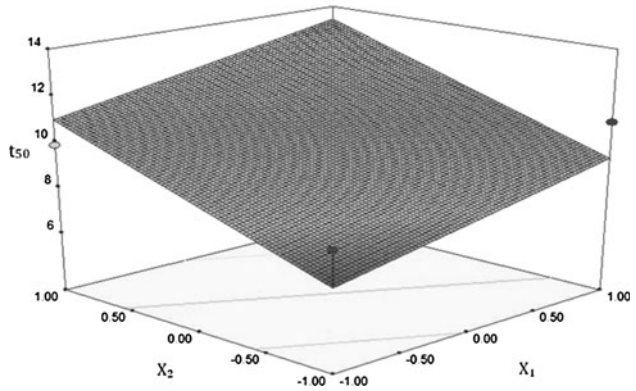

Fig. 2 Response surface plot showing effect of t_{50} (time required for 50% drug release) of variables [MWCNTs:MT (X_1) and EC:adsorbate (X_2)]

Characterization

Yield of microspheres

The total yield of microspheres was determined by dividing the measured weight with the weight of the total amount of nonvolatile compound. The percentage yield of microspheres of different batches was in the range of 92–97 wt% (Table 6). Statistically insignificant difference was observed for the total yield of microspheres for microspheres prepared in the presence or absence of MWCNTs, which suggests no major effect of MWCNTs on the yield of microspheres. These results were far better than others previously reported (Shabaraya and Narayanacharyulu 2003).

Entrapment efficiency

EC microspheres of highly water-soluble drug without incorporation of MWCNTs had very less entrapment efficiency, because most drugs escaped from the polymeric matrix of microspheres (data not shown). The effect of CNTs on the entrapment efficiency of the prepared microspheres was very pronounced (Table 3). The entrapment efficiency of the prepared microspheres varied from 84.17 to 97.78%. The entrapment efficiency was increased significantly with increasing MWCNTs concentration, which might be attributed to the interaction between the drug and MWCNTs. It will diminish the overall drug leaching from the formulation with entrapping more amount of drug. The entrapment efficiency was found to be highest for Batch 8. These results were far better than those previously reported 38.9–72.1% (Shabaraya and Narayanacharyulu 2003).

Table 6 Characterization of experimental design batches

Batch	Yield (%)	Angle of repose	Carr's Index	Shape factor	Circularity factor
1	95.58 ± 0.41	21.34 ± 0.17	21.56 ± 0.65	1.142 ± 0.048	0.948 ± 0.004
2	96.08 ± 0.45	19.45 ± 0.21	27.64 ± 0.55	1.265 ± 0.063	0.952 ± 0.065
3	94.72 ± 0.61	18.51 ± 0.66	29.65 ± 0.41	1.366 ± 0.016	0.964 ± 0.065
4	94.14 ± 1.04	22.21 ± 1.21	19.14 ± 0.58	1.165 ± 0.016	1.065 ± 0.015
5	96.45 ± 1.48	26.14 ± 0.54	31.15 ± 1.66	1.054 ± 0.061	1.056 ± 0.098
6	93.56 ± 1.03	25.42 ± 0.45	28.15 ± 1.54	0.954 ± 0.121	1.118 ± 0.051
7	95.21 ± 1.15	26.41 ± 0.14	26.54 ± 0.19	0.969 ± 0.015	1.061 ± 0.05
8	97.22 ± 0.34	18.15 ± 0.30	15.04 ± 0.14	1.012 ± 0.004	1.001 ± 0.004
9	96.18 ± 0.47	29.16 ± 0.66	32.65 ± 0.48	1.018 ± 0.015	0.965 ± 0.061
10	95.05 ± 1.42	29.07 ± 1.31	31.45 ± 0.21	1.041 ± 0.016	0.951 ± 0.065
11	92.77 ± 0.45	29.97 ± 1.15	27.00 ± 0.67	0.984 ± 0.026	0.916 ± 0.065
12	94.13 ± 1.14	31.08 ± 0.15	29.15 ± 0.64	0.949 ± 0.014	1.042 ± 0.056
13	95.45 ± 1.11	27.45 ± 0.16	28.58 ± 0.34	0.985 ± 0.055	1.052 ± 0.067

Results are mean of triplicate observations ± SD

Micromeritics

The formed microspheres of all batches were very good in shape and circularity. A statistically insignificant difference was observed for all batches of microspheres, indicating that the shape and circularity of the microspheres (Table 6) were not significantly influenced by MWCNTs. According to the literature, microspheres with CI values between 5 and 15% have very good flowability. A statistically insignificant difference was observed in values of Carr's Index for different batches of microspheres (Table 6) suggesting that microspheres of all batches have an excellent flowability irrespective of the presence of MWCNTs. Moreover, the angle of repose of most of the batches indicated a very good flow, which suggested no significant effect of MWCNTs on the flow properties of microspheres. Furthermore, an excellent flow property of microspheres indicates an absence of aggregation and ease of handling. This will help in producing a uniform batch of microspheres for oral delivery.

Instrumental analysis

Scanning electron microscopy (SEM) The surface morphology of pure MWCNTs, drug adsorbate and MWCNTs-impregnated EC microspheres was determined by scanning electron microscopy (SEM) (Figs. 3, 4, 5). SEM confirmed the fiber-like structure of pure MWCNTs, drug absorbance on the surface of MWCNTs and spherical structure of MWCNTs-impregnated EC microspheres. From the photograph of microspheres, it was quite clear that drug adsorbate was trapped inside the microspheres, which might prevent drug diffusion easily.

Powder X-Ray diffraction X-ray diffraction pattern of MT, MWCNTs, drug adsorbate and EC microspheres with and without MWCNTs impregnation are illustrated in Fig. 6. The X-ray diffractogram of pure MT had sharp peaks at diffraction angles (2θ) of around 12°, 19°, 21° and 26°, indicating a typical crystalline pattern. The spectrum of MWCNTs showed three reflections of higher intensity at 2θ of around 26°, 43° and 72°. The X-ray pattern of drug adsorbate indicated characteristics peaks of both MT and MWCNTs with slight poor reflection in the range of 5°–75°. All major characteristic crystalline peaks of MT appear in the diffractogram of adsorbates, indicating the absence of any significant conversion of crystalline state. The lower intensity of the drug peaks in the diffractogram of adsorbate was attributed to the physical interaction of the drug and MWCNTs. MWCNTs-impregnated EC microspheres exhibited complete absence of sharp peaks of pure MT, which suggest decreased crystallinity and may be responsible for enhanced encapsulation of drug inside the matrix of EC.

Differential scanning calorimetry (DSC) Differential scanning calorimetry gives reliable information on the physicochemical state of the ingredients of microspheres

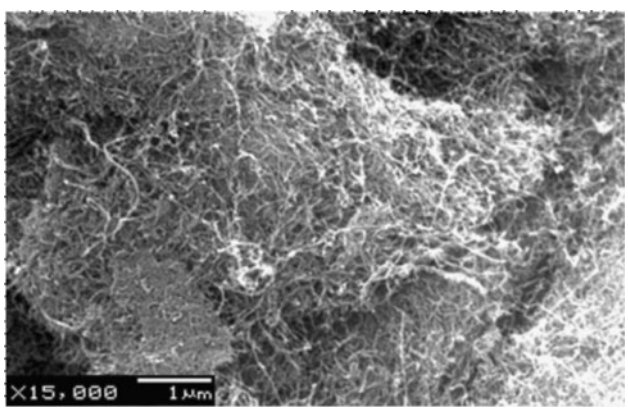

Fig. 3 SEM of MWCNTs

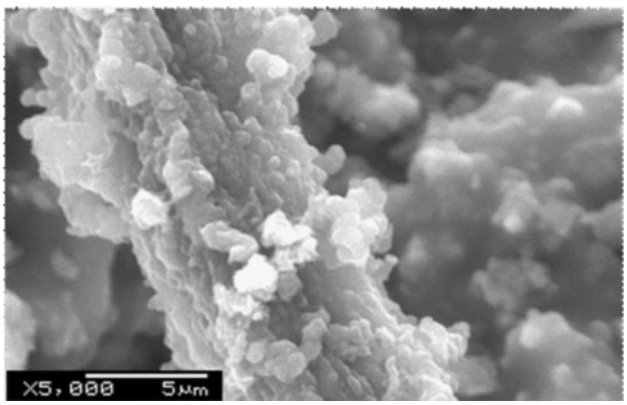

Fig. 4 SEM of MWCNTs:MT adsorbate showing drug (MT) adsorption on surface of MWCNTs

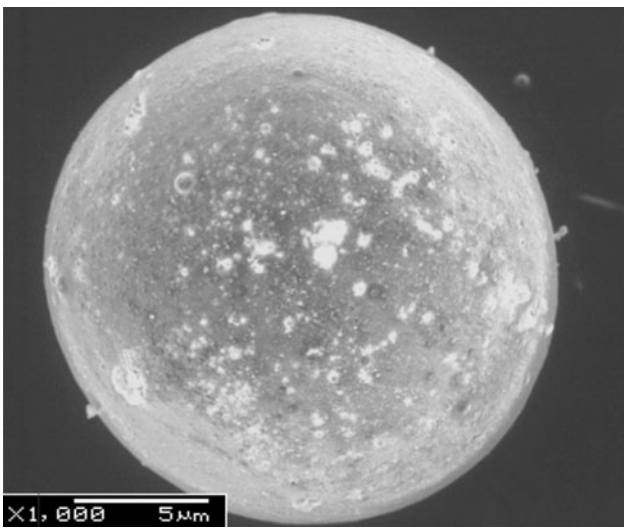

Fig. 5 SEM of MWCNTs-impregnated EC microsphere

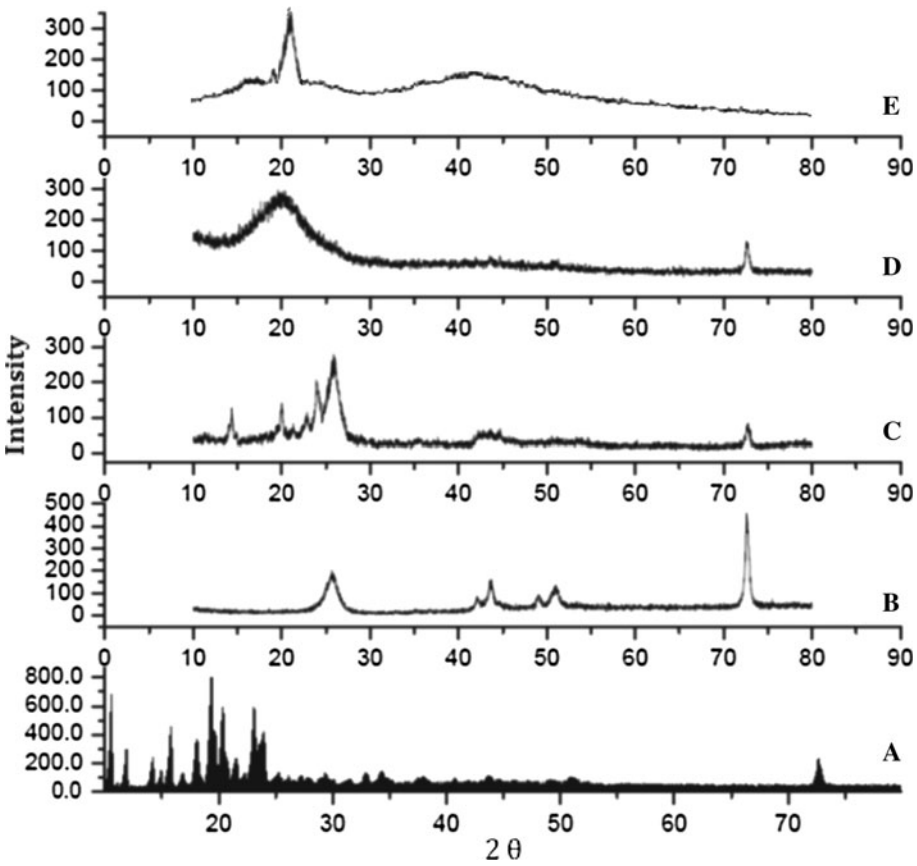

Fig. 6 PXRD pattern of *A* MT, *B* MWCNTs, *C* MWCNTs:MT, *D* EC microspheres impregnated with MWCNTs and *E* EC microspheres without MWCNTs

and the possible interaction between drug and other components of microspheres. The DSC curves are shown in Fig. 7. MT showed an endothermic peak at 125.9°C corresponding to its melting point and one exothermic peak at 238°C, which is still under further evaluation. Drug adsorbate also showed a similar characteristic peak with decreased intensity showing its stability during the nanoprecipitation process. The DSC curve of adsorbate had very little shift toward the lower side, which attributes physical interaction MT over the surface of MWCNTs. There was a total disappearance of endothermic peaks of the drug in drug-loaded microspheres, which supports complete entrapment of drug inside matrix of microspheres.

Dissolution studies

The release of any drug molecule from the reservoir-type formulation predominantly depends upon pore size of the reservoir, molecular weight and hydrophobicity of the drug molecules. Devices with a larger pore size cannot control the release of drugs effectively from the reservoir. Drug release studies of all batches of microspheres were performed to evaluate the potential ability of CNTs in sustaining the release rate of drug from the microspheres. The dissolution studies of MWCNTs-impregnated microspheres revealed sustained drug release up to 24 h, while microspheres without MWCNTs exhibited 90% drug release within 8 h (Fig. 8). Batch 8 was considered as an optimized formulation for sustained release microspheres of MT on the basis of its ability to sustain drug release up to 24 h with almost 15% drug release in the first hour. Moreover, it also exhibited the highest value for t_{50} (15.05 hours) and EE (97.78%) among all batches formulated.

This finding was in close agreement with the results obtained by Zhang et al. (2010) for theophylline. As per literature review, hydrophobic interaction and pore size of the nanometer scale of MWCNTs make it difficult for the dissolution medium to penetrate inside the matrix of microspheres. For highly water-soluble drugs (MT), simple entrapment inside the nanocavity was likely to be effective for producing adequate sustained release property. From the dissolution study, it was considered that nanometer-scale diameter of MWCNTs and hydrophobic interaction were the prime factors governing the release of MT. The data obtained for in vitro release were fitted into various release kinetic model equations (zero-order, first-order, Hixson-Crowell, Weibull, Kosemeyer-Peppas and Higuchi release models). The in vitro drug release of optimized batch displayed highest regression coefficient for Higuchi's model indicating diffusion to be the predominant mechanism of drug release (Costa and Manuel 2001; Reza et al. 2003; Gohel et al. 2000).

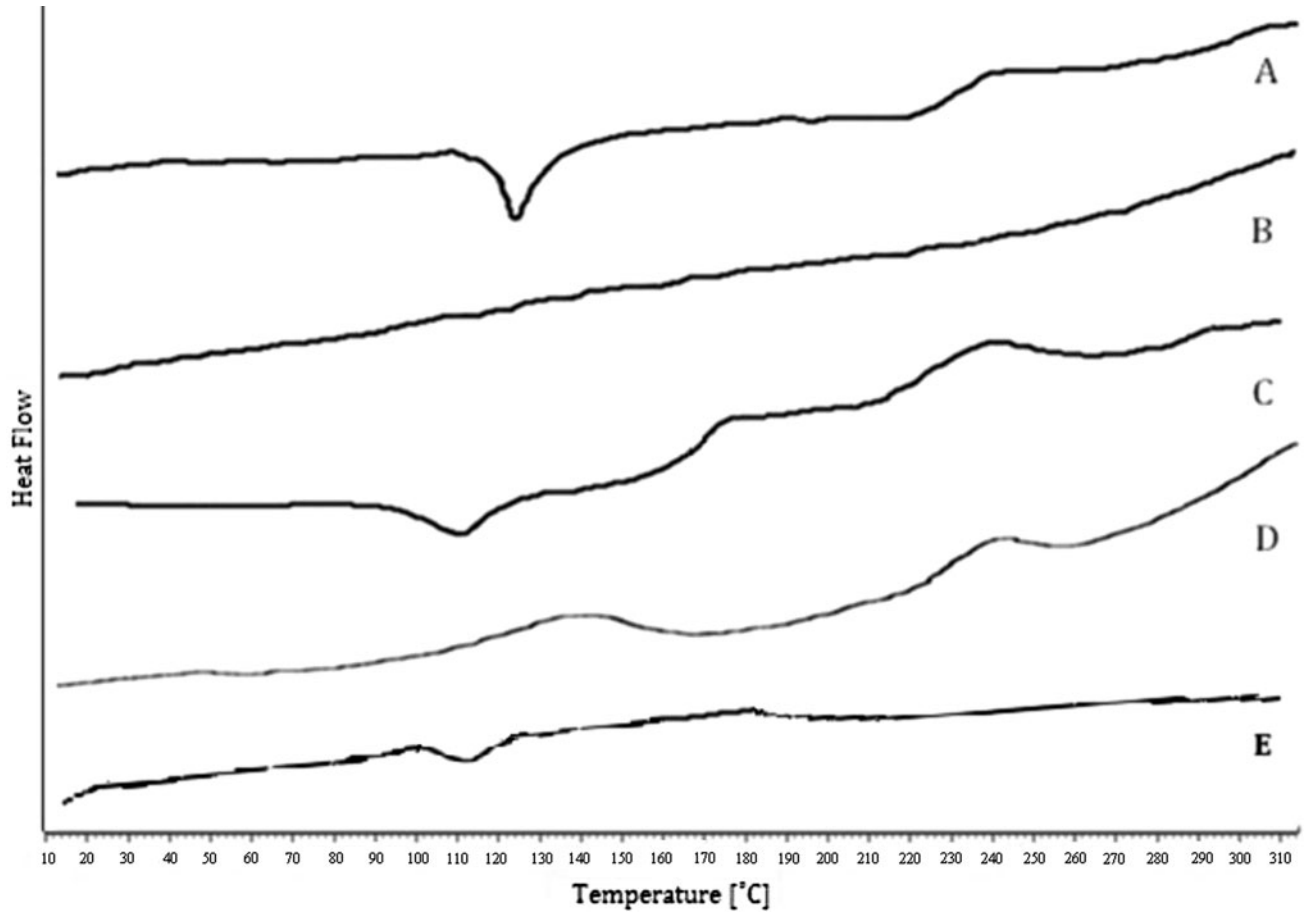

Fig. 7 DSC thermogram of *A* MT, *B* MWCNTs, *C* MWCNTs:MT, *D* EC microspheres impregnated with MWCNTs and *E* EC microspheres without MWCNTs

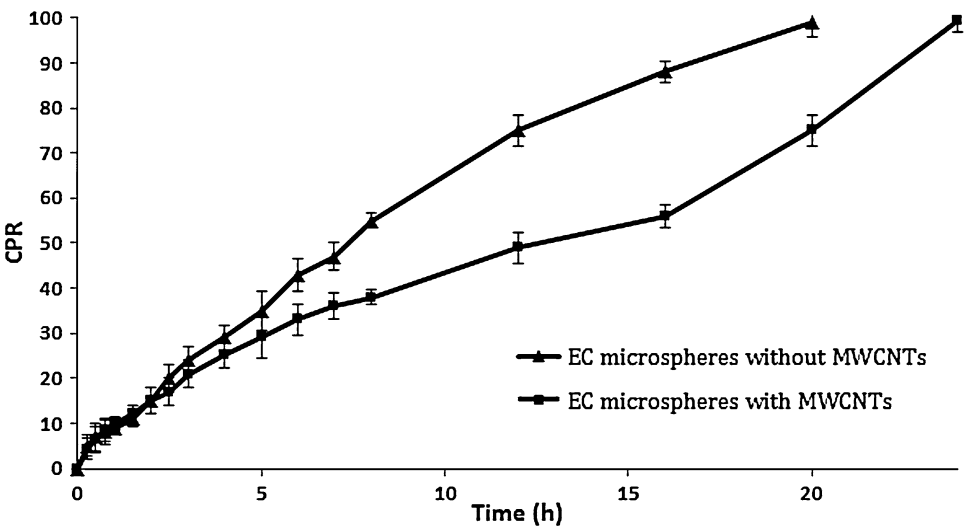

Fig. 8 Comparison of in vitro drug release profiles of EC microspheres with and without impregnation of MWCNTs

Conclusions

The present study has been a satisfactory attempt to formulate a sustained release microparticulate system of a highly water-soluble drug, MT, with an objective of minimizing frequency of daily dosing. It can be concluded from experimental results that CNTs-doped EC microspheres revealed high total yield, and the excellent flow

behavior for all batches was independent of the amount of MWCNTs incorporated. Drug entrapment efficiency of EC microspheres prepared with drug adsorbate was very high for all batches, which favors its use as an excellent carrier for highly water-soluble drugs, like MT. Moreover, the central composite design of the experiment had been successfully employed for optimizing the formulation. The optimized formulation (Batch 8) revealed sustained release up to 24 h with only 15% drug release at the end of the first hour. it was best fitted to Higuchi model out of all kinetic models applied. Further, the retention characteristics, as well as entrapment efficiency, are far better than all other previous reports. However, the formulation still required an extensive in vivo study before utilizing MWCNTs as a carrier for the products used by human beings.

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