PREPARATION AND EVALUATION OF MICROSPHERES WITH DUAL POLYMERIC FILM TO MINIMIZE BURST EFFECT OF HIGHLY WATER SOLUBLE DRUG

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ABSTRACT

Double-layered controlled release microspheres of venlafaxine were prepared using ethyl cellulose (EC) as a wall forming hydrophobic polymer. Usually, it is difficult to control initial sudden release of highly water soluble drugs. In this regard, double-layered microparticles were prepared not only for controlling initial burst effect but also to sustain the release of venlafaxine for an extended period of time. Solvent evaporation technique based on O/O emulsion was applied to prepare modified release microparticles. To minimize initial burst effect, developed microparticles were again coated with same hydrophobic polymer. Percent recovery, drug loading efficiency and particle size were measured. FTIR, SEM and kinetic models were applied to characterize venlafaxine microspheres. Packing and flow properties of microparticles were satisfactory. FTIR spectra of EC, venlafaxine and venlafaxine loaded microspheres were recorded. SEM analysis showed the formation of spherical microspheres with slightly rough surface. Burst effect of venlafaxine was significantly retarded in double-layered microparticles. Drug release databased on highest values of correlation coefficient, was suitably explained by Higuchi kinetics and mechanism of release was Fickian diffusion. This study strongly suggests that double-layered microspheres can successfully be applied for controlling the dose dumping and sustaining the release of highly water soluble drug for an extended period of time.

Keywords: Burst-effect, Double-layered, Venlafaxine, Characterization, Ethyl cellulose.

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INTRODUCTION

Both natural and synthetic polymers can be used to prepare microspheres. Cellulose and cellulose derivatives such as ethyl cellulose (EC), hydroxypropylmethyl cellulose (HPMC), cellulose acetate butyrate, cellulose acetate, cellulose acetate trimellitate, cellulose acetate phthalate, cellulose acetate propionate, hydroxypropylmethyl cellulose phthalate, hydroxypropylmethyl cellulose acetate succinate, carboxymethyl cellulose, methyl cellulose, sodium cellulose sulfate and sodium carboxymethyl cellulose are commonly applied polymers in development of pharmaceutical products [**1**].

Double-layer or multi-layer microparticles offer an attractive and robust approach in drug delivery.

Manipulation of particle parameters, such as polymer type, structural configurations and layer thickness offer a greater variety for controlling rate and kinetics of drug release. Better control over drug release can be achieved by double-layered or multilayered microparticles containing drug core surrounded by a second layer. The second layer over microparticles acts as barrier membrane and slows down drug release. The outer shell allows drug to be released from core through diffusion. Appropriate selection of polymers helps to achieve the desired release profile of drug and eliminates undesired effects associated with single layered microparticle. Matsumoto and coworkers prepared microparticles of PLGA containing cisplatin. The second coating

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of microparticles with poly(D,L-lactide) showed a better control over initial burst release [**2–6**]. Similarly, Shi and coworkers prepared double-layered microparticles and observed a sustained release profile for hydrophilic bovine serum albumin and hydrophobic cyclosporine A [**7**].

Kinetics of drug release from polymeric devices is influenced and controlled based on physical and chemical attributes of polymers. Diffusion is involved in all these systems. In non-biodegradable polymeric devices, drug release is based on concentration gradient by either matrix swelling or diffusion. In biodegradable polymeric devices, drug release is controlled by the rate of hydrolytic degradation of polymer chains that lead to erosion. Mathematical modeling of drug release data furnish important information regarding mass transport and chemical processes involved in release of drug. Mathematical models further explain the effect of design parameters, such as device geometry (shape, size) and drug loading on drug release mechanism. Mathematical modeling also facilitates optimized device design for desired predictable release profile with a minimum number of experiments performed [**8**].

The solubility of venlafaxine in water is >500 mg/ml. Further, immediate release formulations of venlafaxine are causing higher incidences of nausea, dizziness, non-adherence, cessation and failure of antidepressant therapy. Therefore, it is desirable to reduce burst effect and develop sustained release formulations of venlafaxine [**9, 10**].

We have successfully prepared and evaluated different microparticle formulations of venlafaxine. Among various formulations, double-layered microspheres (E4, E5) showed promising effect to control burst release and maintain sustained release up to 10 hours.

MATERIALS AND METHODS
Materials
Ethyl cellulose 400 cps, methyl acetate, paraffin oil, methanol and n-heptane were purchased from Sigma Aldrich, Germany. Venlafaxine hydrochloride was provided by Mass Pharma, Lahore Pvt. Ltd. as a gift sample. All other ingredients used in this study were of analytical grade and purchased from local chemical market.

Preparation of Microspheres
Microspheres of EC were prepared using solvent evaporation method. **Table 1** contains formulation variables. Drug and polymer were dissolved in predetermined ratio in 20 ml of methyl acetate. 10 mg magnesium stearate was added to paraffin oil and continuously stirred at 650 rpm with help of magnetic stirrer (Velp Scienifca, Germany). The polymer-drug mixture was slowly dropped in beaker containing 100 ml paraffin oil over a period of six hours till complete evaporation of organic solvent. As organic solvent is evaporated, microparticles grow and harden. Adhered paraffin oil was removed by washing microspheres with n-heptane. To prepare double-layered microspheres, prepared microspheres were again suspended in fresh paraffin oil and rest of procedure was same as used for single layered microspheres. The prepared microspheres were dried in oven at 45°C and stored in air-tight glass jars for further study.

Table 1: Composition of EC microspheres.

Formulation	EC (g)	Venlafaxine (g)	Magnesium stearate (mg)
E1	1.0	1.0	10
E2	2.0	1.0	10
E3	3.0	1.0	10
E4	2:1	1.0	10
E5	2:2	1.0	10

Percent Recovery
Percent product recovery of EC microspheres was calculated to determine the efficiency and suitability of preparation method. Following mathematical relation was adapted to measure % yield:

$$\text{Product recovery} = \frac{\text{Mass of microspheres}}{\text{Theoretical mass of polymer+drug}} \times 100 \qquad (1)$$

Drug Incorporation Efficiency
Encapsulation efficiency of EC microspheres was measured by dissolving accurately measured weight (10 mg) of E1, E2, E3, E4 and E5 microspheres in 20 mL water-methanol v/v. Methanol dissolves EC layer. The solution was filtered through Millipore and diluted suitably with distilled water. The filtrate was stirred for one h at 40°C to remove organic solvent. After evaporation of methanol, 1 ml of solution was diluted to 100 mL and absorbance of venlafaxine was measured at 226 nm using a UV-VIS spectrophotometer (UV-1100, Schimadzu). Encapsulation efficiency of EC microspheres was calculated as follows:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Calculated venlafaxine concentration}}{\text{Theoretical venlafaxine concentration}} \times 100 \qquad (2)$$

Rheological Studies
Tapped Density of Microspheres
Tapped density is used to analyze packing properties of microparticles into capsules. Tapped density also influence flow and mixing properties of microspheres in preparation of tablets. The tapped

density of microspheres was measured by using a conventional taping method. For this purpose, 10 mL measuring cylinder was used and total number of tapings was fixed to 100. 100 tapings are sufficient to achieve plateau condition. Following formula was used to calculate tapped density [11].

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after 100 tapings}} \quad (3)$$

Angle of Repose

Angle of repose was calculated by passing EC microparticles through a funnel on a flat surface. The radius (r) and height (h) of microparticle heap achieved after passing through funnel on flat surface was also measured. Following mathematical relation was applied to calculate angle of repose.

$$\text{Tan } \theta A = \frac{h}{r} \quad (4)$$

Hausner's Ratio

Hausner's ratio is an important tool to assess flowability of microparticles. It can be calculated by following relation. A value of Hausner's ratio less than 1.2 indicates good flow.

$$\text{Hausner's ratio} = \frac{\text{Volume of microspheres before tapping}}{\text{Volume after tapping}} \quad (5)$$

Carr's Index

It is also called compressibility index. Carr's index is an indirect measure of bulk density, surface area, size, shape, and cohesiveness of microparticles. Following formula was used to measure Carr's index.

$$\text{Carr's index} = \frac{\text{Initial volume} - \text{Final volume}}{\text{Initial volume}} \times 100 \quad (6)$$

FTIR

FTIR analysis was carried out to study the interactions between EC and venlafaxine hydrochloride. The spectra were obtained for pure venlafaxine, EC and drug-loaded microspheres. Midac USA 2000 FTIR instrument was used to record spectra. Samples were prepared using KBr discs. The spectra were recorded at a scanning range of 500-4000 cm^{-1} at resolution of 2 cm^{-1}.

In-Vitro Dissolution and Mathematical Modeling

Measured quantity of microparticles was placed in dialysis bag and tied to paddle of USP dissolution apparatus II. Buffer of pH 7.4 was used as release media. The volume of release media was 900 ml and paddle speed was 50 rpm. Temperature of release media was maintained at $37 \pm 1°C$ throughout the study. An aliquot of 2 ml was collected at various time intervals and replaced with fresh equal amount of buffer. The aliquot was suitably diluted with distilled water and analyzed at 226 nm spectrophotometrically. The amount of drug released at each time interval was measured by using standard calibration curve. The cumulative release was calculated by following relation.

$$\text{Cumulative release} = \frac{F_t}{F_\infty} \times 100 \quad (7)$$

Where, F_t and F_∞ represent amount of drug released at time t and total amount of drug released from microspheres, respectively.

Drug release from various drug delivery devices is assumed to follow different mechanism and patterns. Mathematical modeling of release data is commonly performed to assess the mechanism of drug release from different carriers. Zero order release equation describes that drug release is at constant rate and is independent of its concentration [12]:

$$F_t = K_0 t \quad (8)$$

First order equation explains systems where drug release is dependent on its concentration from a device [13]:

$$\log F_t = \log F_0 - K_1 t \quad (9)$$

Hixon-Crowell equation is given below [14]:

$$F_0^{1/3} - F_t^{1/3} = K_{HC} t \quad (10)$$

Higuchi's equation is based on drug release from insoluble matrix from insoluble polymeric system. It describes the time independent process based on Fick's law [15]:

$$F_t = K_H t^{1/2} \quad (11)$$

Korsmeyer-Peppas mathematical equation is used to assess the mechanism of release [16]:

$$\frac{F_t}{F_0} = K_{kp} t^n \quad (12)$$

Particle Size and Morphological Analysis

Particle size of microspheres was measured using optical microscope (Nikon, Japan). Surface morphology of EC microparticles was examined using scanning electron microscopy (Hitachi, S 3000H, Japan). EC microparticles were sprinkled on a double sided adhesive tape attached to an aluminium stub. Excess EC microparticles were removed and stub sputter was coated with gold using vacuum evaporator to render microparticles electrically conductive. Surface properties were analyzed at 25 KV under the scanning electron microscope [17].

RESULTS

FTIR

FTIR spectrum of venlafaxine as shown in **Fig. 1** (B) illustrates characteristic peaks at 3330 cm^{-1} due to stretching of O-H. The peak at 2825 cm^{-1} is due to C-H stretch. Peak at 1208 cm^{-1} indicates C-O stretching vibration. Peak of C-O-C stretch shoulders at 1028 cm^{-1}. Fig. 1 (C) shows only a slight shift in some of group's characteristic of venlafaxine. No new band was detected in spectra of venlafaxine loaded EC microparticles that

excludes potential interaction between venlafaxine and EC. Therefore, venlafaxine was compatible and stable in EC microsphere formulations.

Particle Size and Morphology

E1 microparticles were spherical in shape and with smooth surface. Smooth surface of microparticles own to high plasticizing properties of EC. However, surface morphology of E5 is slightly rough. Rough surface may be attributed to double layering which may impart some uneven morphology due to difference in thickness of second layer over the surface of microparticles. Mean particle size of microparticles is provided in **Table 2**. Size of EC microparticles increases parallel to EC ratio and highest particle size was found for double-layered E5 microparticles. As ratio of EC is increased, the coating layer becomes more and more thick producing particles with larger size.

Percent Recovery and Drug Loading Efficiency

Table 2 contains percent yield, % drug loading efficiency and mean particle size of different microsphere formulations. Only a slight increase in percent yield was observed on increase of EC ratio. However, % drug incorporation gradually increased on increasing ratio of EC. Apparent decrease in drug incorporation efficiency in E4 and E5 is not real. Drug loading efficiency of E4 and E5 actually represents venlafaxine entrapped in first layer. The second coating applied was devoid of drug.

Flow Characteristics of Microspheres

Flow properties of formed microspheres have been displayed in **Table 3**. The values of Carr's index, Hausner's ratio, angle of repose and tapped density help to understand flow and packing properties of microparticles. These properties are used to predict behaviour of microparticles for filling into capsules and flow through hopper and compressibility. Carr's index exhibited excellent flow properties as per the scale given by Carr. Values of Hausner's ratio and angle of repose also proved excellent flowability of EC microparticles. Hausner's ratio less than 1.25 indicates free flow property. The values of angle of repose were close to 30° indicating good free flowing nature of EC microparticles. Excellent flow properties of microspheres may be attributed to spherical shape and low size distribution.
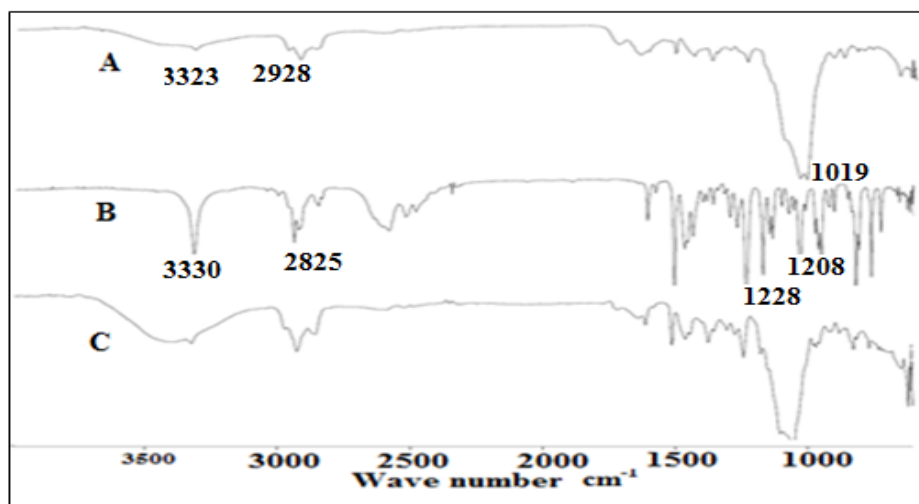

Figure 1: FTIR spectra of (A) EC (b) venlafaxine HCl (C) EC microspheres.

Table 2: Mean values of product yield, drug entrapment efficiency and particle size.

Formulation	EC:drug ratio	Percent yield	Percent loading efficiency	Particle size (µm)
E1	1:1	87.89	59.55	149 ± 13.39
E2	2:1	92.27	67.67	167 ± 11.67
E3	3:1	92.69	72.36	171 ± 15.50
E4	(2:1):1	85.45	66.86	261 ± 12.45
E5	(2:2):1	83.67	66.39	274 ± 14.07

Table 3: Micromeritic features of EC microspheres.

Sample	Angle of repose	Hausner's ratio	Carr's index	Tapped density (g/mL)
E1	27.56±1.79	1.13±0.03	9±1.45	0.25
E2	27.29±2.11	1.12±0.03	10±1.24	0.28
E3	29.55±1.29	1.14±0.02	10±2.19	0.29
E4	25.57±1.41	1.15±0.01	9±1.49	0.33
E5	27.07±1.07	1.11±0.02	9±1.89	0.32
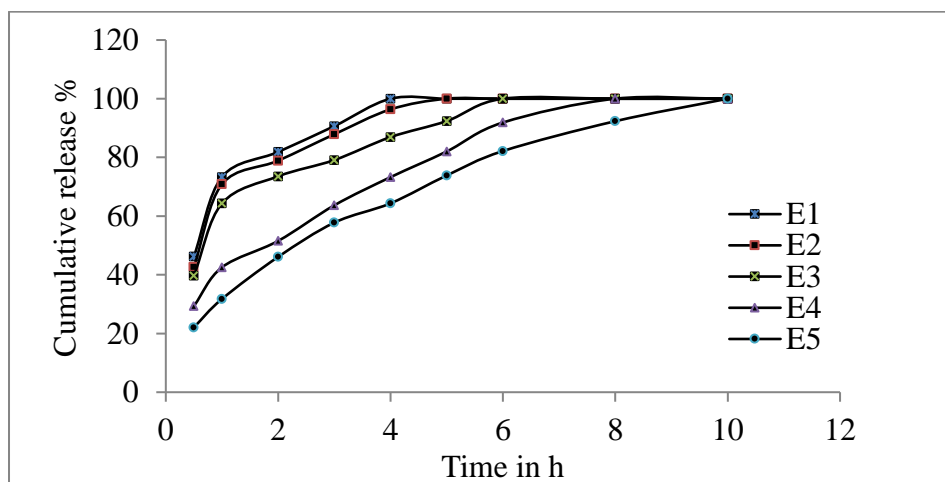
Figure 2: Release profile of EC microspheres.

Kinetics and Mechanism of Release

Dissolution kinetics of EC microspheres is shown in **Fig.2**. Release rate and burst effect of venlafaxine was reduced on increasing ratio of EC. Cumulative release of drug after completion of 1st hour dissolution study was 73.34 %, 70.85 %, 64.34 %, 42.56 % and 31.78 % from E1, E2, E3, E4 and E5, respectively. Rate and extent of drug release was dependent on EC ratio and decreased slightly on increasing ratio of EC. Burst effect was significantly reduced in double-layered microparticles. High burst effect in E1 and E2 is due to sudden dissolution of highly water soluble venlafaxine on contact with water. Double-layered microparticles were able to extend and maintain sustained release up to 10 hours. On increasing ratio of EC, particle size is increased and due to hydrophobic nature, water penetration is restricted. When water penetration into microsphere is restricted, diffusion of drug from core is also reduced. Burst effect in E5 is significantly less than that of E1. This phenomenon can be explained on the fact that outer coating of microsphere is devoid of drug and water has to pass the outer barrier layer first to dissolve and diffuse venlafaxine out in dissolution media.

Drug release kinetics of polymeric microparticles can be categorized into three main processes: (a) drug diffusion from non-biodegradable polymer is termed as diffusion-controlled system, (b) enhanced drug diffusion due to polymer swelling is termed as swelling-controlled system and (c) drug release due to polymer degradation or erosion is called erosion-controlled system.

Drug release data fitted to various kinetic models showed highest linearity for Higuchi model followed by First order kinetics. Release of venlafaxine was controlled by multiple mechanisms. Multiple mechanisms may include diffusion coupled with polymer chain relaxation or glassy-rubbery transition of polymer on contact with dissolution media.

CONCLUSION

Modified release microspheres can be prepared by O/O solvent evaporation using EC as wall forming material. Drug release was slowed down on increasing ratio of EC. Double-layer formulation (E5) efficiently reduces burst effect to 31.78 % compared to that of 73.34 % for E1 after completion of 1st hour of dissolution study. All EC microparticles showed excellent packing and free

flowing properties. FTIR spectra proved stability and compatibility between EC and venlafaxine. SEM micrographs indicate the development of spherical particles with smooth surface and even surface. Particle size increased with increasing ratio of EC. Single-layered microparticles achieved 100 % cumulative drug release within 6 hours. However, double-layered microspheres were apparently superior in controlling burst effect as well as extending sustained release of venlafaxine up to 10 hours.

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