Research in Pharmaceutical Sciences, February 2021; 16(1): 1-15
Received: 06-01-2020
Peer Reviewed: 01-05-2020
Revised: 13-06-2020
Accepted: 26-12-2020
Published: 30-12-2020

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Preparation and characterization of ethylcellulose microspheres for sustained-release of pregabalin

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Abstract

Background and purpose: Pregabalin is used in the treatment of epilepsy, chronic pain, and other psychological disorders. Preparation of pregabalin in the sustained-release formulation will enhance patient compliance and reduce the incidence of side effects. The aim of this study was to prepare sustained-release microspheres for pregabalin utilizing ethylcellulose and evaluate the processing factors that influence the fabrication and the performance of the prepared microspheres.
Experimental approach: The microspheres were prepared using the water-oil-oil double emulsion solvent evaporation method. Microspheres were characterized for particle size, encapsulation efficiency, and *in vitro* drug release. The influence of the processing variables on the characteristics of the prepared microspheres was studied. Microspheres solid-state characterization performed using differential scanning calorimetry, Fourier transform infrared spectroscopy and scanning electron microscopy.
Findings/Results: The results described in the context of the current work illustrated the suitability of the water-oil-oil system in the preparation of sustained-release microspheres for pregabalin. The optimum formulation was prepared at a drug to polymer ratio of 1:3 w/w, stirring speed of 600 rpm, surfactant concentration of 1.5%, and external phase volume of 150 mL. This formula produced microspheres particle size in the range 600-1000 µm, with 87.6% yield, and 80.14 ± 0.53% encapsulation efficiency. Drug release from the microspheres was found to be diffusion controlled, with a pH-independent behavior.
Conclusion and implication
The current work presented a successful attempt to fabricate a sustained-release microsphere comprising pregabalin. This will help overcome the frequent dosing problems with conventional pregabalin dosage forms and improve product performance.

Keywords: Double emulsion; Ethylcellulose; Microsphere; Pregabalin; Solvent evaporation.

INTRODUCTION

Neuropathic pain is pain resulting from a lesion, dysfunction, or a primary disease in the nervous system (1). Around 7-8% of the general population, in an addition to an unknown population of patients suffer from long-term neuropathic pain without responding to standard treatment (2). Antiepileptic drugs used for treating epilepsy, also employed for treating neuropathic pain (3). Pregabalin has emerged as a new more potent alternative to gabapentin for the treatment of partial-onset seizures, epilepsy, pain, psychological disorders, and neuropathic pain (4). It prevents both partial and generalized seizures in humans. The primary high affinity-binding site for pregabalin in forebrain tissues for the α2-δ type 1 auxiliary subunit of voltage-gated calcium channels causes the pharmacological actions of this medication (5). According to the biopharmaceutics classification system, pregabalin is classified as a compound with high permeability and high solubility (class I).

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Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.305184

It is commercially available as hard capsules (Lyrica®) containing 25-300 mg of pregabalin along with lactose monohydrate and cornstarch. The recommended intake dose of pregabalin is 100 mg three times daily. There are several reports that multiple dosing leads to fluctuations in drug release. Such fluctuations disrupt the blood plasma levels of pregabalin and consequently cause adverse compliance. Therefore, it was strategically essential to develop a sustained release dosage preparation that provides a stable serum concentration of pregabalin. However, it is generally difficult to formulate a highly soluble drug, pregabalin in the present case, in sustained-release drug applications. This is because such high solubility may cause initial delayed, followed by a high release rate as per the definition known as the dose-dumping phenomenon (6). Recently, once-daily sustained-release tablets for pregabalin was approved by the US FDA (Lyrica® CR extended-release tablets 82.5,165, and 330 mg, Pfizer Inc., USA). Although the successful development of the sustained-release dosage, the product must be taken after an evening meal. This will cause variability in transit time and the absorption rate (7). Microspheres play an important role in drug delivery systems. This is due to their efficient carrier characteristics and small size. Microencapsulation has gained considerable attention for sustained release purposes. Microspheres can vary in size and ranging from 1 to 1000 μm. There are a variety of methods to produce microspheres, solvent evaporation method, coacervation phase separation, interfacial polycondensation, pan coating, air-suspension coating, spray drying, polymerization, ion exchange resins, and others. The various methods of preparation allow the control of dosage forms and aspects of the administration of the microspheres. Microspheres can be further developed into tablets, capsules, suspensions, effervescent tablets, and sachets. Microspheres with multiple particle systems reduce variability in transit time and absorption rate (7). The solvent evaporation method is one of the techniques to produce such microspheres. The emulsion solvent evaporation system was the first and simplest method to produce microspheres made of biodegradable polymers. Microspheres are made of drugs surrounded by a polymeric membrane created by one or more polymers. The polymeric membrane plays an important role in the size of the microsphere, encapsulation efficiency and rate of release of the drug. Ethylcellulose was used as a non-swellable, insoluble, component in matrix or coating systems. Ethylcellulose used as a polymer produces stable, pH-independent, reproducible microcapsules with the sustained-release (8). In the present work, sustained-release microspheres are produced by water-oil-oil double emulsion using a solvent evaporation technique. Furthermore, different processing variables were investigated; drug-polymer ratio, the stirring speed during the preparation of the microspheres, the surfactant concentration in the continuous phase, and the volume of the processing medium. Those factors influence the properties of prepared particles, including particle size, encapsulation efficiency, and drug release. The prepared microspheres characterized using a scanning electron microscope (SEM), differential scanning calorimetry (DSC), particle size determination, and encapsulation efficiency. The importance of this research is to produce a sustained-release formulation with a zero-order release (9). This formulation will enable patients to use pregabalin once daily, instead of thrice, increasing patient compliance, decreasing fluctuations in drug release, and hence minimizing drug plasma concentration-related side effects.

MATERIALS AND METHODS

Materials

Pregabalin, kindly donated by the Jordanian Pharmaceutical Manufacturing Company (Naor; Jordan). Ethylcellulose (viscosity grade N22) supplied by Hercules (Wilmington, USA). Acetonitrile (analytical grade) was obtained from TEDIA, (Illinois, USA). Methanol (high-pressure liquid chromatography (HPLC) grade) and phosphoric acid (85%) supplied by Merck (NJ, USA). Sorbitan sesquioleate was supplied by Sigma (NY, USA), Sodium hydroxide pellets were supplied by Frutarom LTD (Billingham, UK), Dichloromethane,

potassium dihydrogen orthophosphate, hydrochloric acid 37% extra pure, and potassium bromide (IR spectroscopy grade) were supplied by Scharlau Chemie (Barcelona, Spain), liquid paraffin and n-hexane were supplied by Sigma (NY, USA).

Preparation of pregabalin microspheres

Pregabalin microspheres were prepared using the water-oil-oil double emulsion solvent evaporation method. One g of pregabalin and 1.5, 2, or 3 g of ethyl cellulose dissolved in 40 mL of ethanol:dichloromethane (1:1 v/v). The mixture was stirred for 5 min using a magnetic stirrer. The volume of 8 mL of distilled water was added to the drug-polymer solution; the water-in-oil emulsion formed, remained under stirring conditions for 5 min. The water-in-oil primary emulsion slowly added to a stirred light liquid paraffin with stirring. Liquid paraffin volume varied as follows: 100, 150, or 200 mL containing 1, 1.5, or 2% Span® 80 used as an emulsifying agent to stabilize the prepared emulsion. The stirring of the double emulsion was carried out at room temperature (25 °C) using an overhead propeller mixer (DLH model, VELP. SCIENTIFICA, Italy) at a stirring speed of 600, 700, or 800 rpm. Stirring continued for 6 h until the complete evaporation of the volatile solvents and the formation of solid microspheres. After microsphere formation, 50 mL of n-hexane added to the medium to harden the microspheres under continuous stirring for 30 min. After 30 min, decantation followed to separate the particles from the liquid paraffin. The obtained microspheres were washed with 50 mL n-hexane three times. The particles were left to dry at room temperature for 24 h. Table 1 shows the preparation variables of different microspheres formulations. During the study of the influence of different preparation variables on the characteristics of the prepared formulas, the F3 formula considered the reference formulation as it is common to all variables and it was found later as the optimum formula (10)

HPLC analysis of pregabalin

Chromatographic separation was carried out using the Shimadzu HPLC system (Kyoto, Japan). The system consists of an LC-10AD vp pump, SIL20A autosampler, SPD10A vp UV visible detector, and SCL-10A vp interface, Shimadzu CLASS-VP software (version 6.14 SP2) was used to analyze data. Separation accomplished using ACE 5 µ C18, 250 mm × 4.6 mm (ACE, USA) HPLC column. The mobile phase for isocratic elution consisted of 0.02 M dipotassium hydrogen orthophosphate (K2HPO4): methanol:acetonitrile at the ratio of 16:3:1 v/v/v, respectively. The final pH of the mobile phase adjusted to 7.0 using sodium hydroxide. The flow rate was set at 1 mL/min. The mobile phase was filtered through a 0.45 µm regenerated cellulose membrane filter (VIVID, USA) before use. The column oven temperature was set at 25 ± 1 °C. The detection wavelength was set at 210 nm and the injection volume was 50 µL. The HPLC method was validated for the quantification of pregabalin in respect to linearity, accuracy, precision, specificity, the limit of detection and limit of quantitation (11).

Characterization of pregabalin microspheres

Particle size analysis

Microspheres particle size was determined by passing them through a series of analytical sieves (Haan, Retsch, Germany) of the following mesh sizes; 1000, 850, 600, 300, and 212 µm. The microspheres passed through the sieves from the top sieve with the largest opening to the bottom sieve with the smallest size. The particles retained in each sieve were weighed. Microsphere amounts and their particle size distribution determined. The mean particle size calculated according to equation 1:

$$Mean\ particle\ size = \frac{\sum(Mean\ fraction\ particle\ size \times fraction\ weight}{\sum Fraction\ weight} \quad (1)$$

The microspheres fraction in the particle size range between 600-850 µm size was selected for further investigations in this work.

Drug loading and encapsulation efficiency determination

The drug content for all microspheres formulas was determined, in triplicates, at the microsphere size range of 600-850 µm. Microspheres sample of 10 mg of microspheres was accurately weighed, dissolved in 25 mL

methanol, and sonicated for 1 min using water bath sonicator (Julabo sonicator model USR 3, Germany). Then, pregabalin concentration was determined by HPLC.

Drug loading and encapsulation efficiency calculated using the equations below:

$$Theoretical\ drug\ loading = \frac{weight\ of\ initial\ drug}{weight\ of\ initial\ drug\ and\ polymer} \quad (2)$$

$$Actual\ drug\ loading = \frac{weight\ of\ encapsulated\ drug}{weight\ of\ initial\ drug\ and\ polymer} \quad (3)$$

$$\%\ Drug\ loading = \frac{weight\ of\ encapsulated\ drug}{weight\ of\ microcapsules} \times 100 \quad (4)$$

$$\%Encapsulation\ efficiency = \frac{Actual\ drug\ loading}{Theoretical\ drug\ loading} \times 100 \quad (5)$$

Scanning electron microscopy

The surface morphology of the microspheres formulation F3 with particle size in the range of 600-850 µm studied using scanning electron microscopy (SEM; model FEI Quanta 200, Netherlands). The internal structure of microspheres (F3 formulation) after the release was also examined. A cross-section of the microsphere was performed to show the internal structure. All samples were mounted on aluminum stubs of conductive carbon disks then gold-coated by sputtering method at 1200 V, 20 mA using a vacuum coater (Polaron E6100, UK) thus allowing them to be electrically conductive.

DSC analysis

DSC analysis carried out using a Shimadzu DSC-50 model (Japan). Samples tested were pregabalin. Ethylcellulose, their physical mixture (1:3 drug to polymer mass ratio) and microspheres formulation (F3). Samples of 5 ± 0.2 mg of each sample placed in sealed aluminum pans then heated under the stream of nitrogen (80 mL/min flow rate) from ambient temperature to 250 °C, at a scan rate of 10 °C/min to obtain the DSC thermograms of the aforementioned preparations. An empty aluminum pan was placed alongside the sample and used as a reference.

Fourier transform infrared analysis

Fourier transform infrared (FT-IR) spectra of pure drug, physical mixture (1:3 ratio) ethylcellulose, and the microspheres F3 formulation was acquired. A small amount of the sample mixed with potassium bromide in mortal and pestle. The mixture was compressed into a desk and mounted in an FTIR spectrophotometer (IR Affinity-1, Shimadzu). FT-IR and analysis conducted over a frequency range of 4000-400 cm^{-1} (12).

Influence of the processing conditions on the prepared microspheres

Influence of polymer to drug ratio

Pregabalin microspheres formulations preparation variables are presented in Table 1. Three microspheres formulations of the different drug: polymer ratios were prepared 1:1.5, 1:2, and 1:3. Other preparation variables were kept constant to help evaluate the influence of a drug to polymer ratio on the characteristics of the prepared microspheres. For that purpose, Span® 80 was used as a surfactant at the concentration of 1.5% w/w. The stirring speed was fixed at 700 rpm. The paraffin volume used was 150 mL. The effect of polymer-drug ratio on the particle size, the encapsulation efficiency, and the release profile of the drug from the microspheres studied and recorded (13).

Table 1. Pregabalin microspheres formulations preparation variables.

Formula	Drug:polymer	Stirring speed (rpm)	Volume of paraffin (mL)	Surfactant concentration
F1	**1:1.5**	700	150	1.5%
F2	**1:2**	700	150	1.5%
F3	**1:3**	700	150	1.5%
F4	1:3	**600**	150	1.5%
F5	1:3	**800**	150	1.5%
F6	1:3	700	**100**	1.5%
F7	1:3	700	**200**	1.5%
F8	1:3	700	150	**1%**
F9	1:3	700	150	**2%**

Influence of the stirring speed

The influence of the agitation speed on the morphology, the particle size, the encapsulation efficiency, and the drug release studied and recorded. Thee formulations were prepared at varying stirring speeds of 600, 700, and 800 rpm (Table 1). The effect of different stirring speeds on the particle size, the encapsulation efficiency, and the release profile of the drug from the microspheres were studied and recorded (14). Other preparation variables were kept constant to enable comparison.

Influence of the volume of processing medium

The influence of the volume of paraffin oil on the prepared microspheres was also investigated. Three microspheres formulations were prepared where the volume of paraffin oil varied as follows: 100, 150, or 200 Ml (Table 1). The effect of increasing the volume of processing medium on the particle size, the encapsulation efficiency, and the release profile of the drug from the microspheres studied and recorded. Other preparation variables were kept constant to enable comparison.

Influence of the surfactant concentration

Three microspheres formulations were prepared to contain a different concentration of the emulsifying agent in the external phase. The concentration of Span® 80 in paraffin oil 1.0, 1.5, or 2 (w/w) (Table 1). The effect of the medium surfactant concentration on particle size, encapsulation efficiency, and drug release from the prepared microspheres was studied and recorded (15).

Release of pregabalin from the prepared microspheres

USP dissolution apparatus II (DT 60 model, Erweka, Germany) was used to carry out the release studies operating at 37± 0.1 °C. Drug release performed under sink condition. The rotational speed of the paddle was set at 50 rpm. An accurately weighed amount of microspheres containing 200 mg pregabalin was placed in 900 mL phosphate buffer solution of pH 6.8. Five mL samples were withdrawn at specific time intervals and the volume withdrawn compensated with fresh media to maintain a constant dissolution media. The samples were filtered using a syringe filter of 0.45 µm Cameo nylon. Assay of pregabalin content carried out by automatic injection of a sample size of 25 µL into the HPLC system. Percent cumulative drug released from the microspheres plotted against time and the effect of every factor on the drug release compared and studied.

In a separate investigation, a drug release study of selected microspheres representing the optimum formula was carried out in 900 mL of 0.1 N hydrochloric acid solution, pH 1.2, at 37 ± 0.1 °C, and 50 rpm. However, the release study of such preparation was only conducted for 4 h to simulate drug release in the gastric medium and compared with that in phosphate buffer (16).

Release kinetic of pregabalin from microspheres

To help understand the exact release mechanism of the drug from the prepared microspheres, *in vitro* drug release data fitted into four different mathematical models for drug release. The models were zero-order kinetics, first-order kinetics, Higuchi kinetics, and the Korsmeyer-Peppas model.

In the zero-order model, the drug release rate is independent of its concentration in the sample and represented in the following equation (17):

$$Q_t = k_0 t \quad (6)$$

where, K_0 and Q_t are zero-order rate constant and the amount of drug released at time t, respectively.

The first-order model, the drug release rate depends on the concentration of the drug that remained in the microspheres. The following equation represents this approach:

$$\ln (Q_0 - Q_t) = \ln Q_0 - k_1 t \quad (7)$$

where, Q_0 and K_1 are the initial amounts of drug present in the microspheres and the first-order rate constant, respectively.

In the Higuchi model, the drug release from the insoluble matrix is a function of the square root of time.

$$Q_t = k_H t^{1/2} \quad (8)$$

where, K_H is the Higuchi dissolution constant.

The drug release further fitted into the Korsmeyer-Peppas model. This model was developed to understand the release of a drug molecule from a polymeric matrix. The mathematical model represented in the following equation:

$$M_t/M\infty = k_t n \quad (9)$$

where, M_t is the concentration of drug released at time t, $M\infty$ is the equilibrium concentration of a drug, K_t is the drug release rate constant, and n is the diffusional exponent and used to characterize different release mechanisms.

The Korsmeyer-Peppas model was used to analyze the mechanism of drug release and the diffusion kinetics for the fraction of drug released < 0.6 and evaluate drug release from controlled-release polymeric devices, especially when the drug release mechanism is unknown or when there is more than one release mechanism. Ritger and Peppas suggested that drug release from controlled release polymeric systems could follow Fickian diffusion, or case-II transport of drug molecules (polymeric relaxation) or a combination of diffusion and polymeric relaxation (anomalous transport). The value of (n) is related to the geometrical shape (slab, cylinder, or sphere) and the type of polymer used (swellable or non-swellable) (18).

Statistical analysis

All measurements were carried out repeatedly and the results expressed as the mean ± SD. The data from the different groups statistically analyzed using a paired t-test or analysis of Variance (ANOVA). *P* values less than 0.05 are considered statistically significant. Microsoft Office Excel 2019 software used for the calculations.

RESULTS

Characterization of pregabalin microspheres

Particle size analysis

Different parameters affecting the mean microspheres particle size and microsphere characteristics were the focus of the current work investigation. These parameters included the drug-polymer ratio (F1, 1:1.5; F2, 1:2; F3, 1:3), stirring speed (F4, 600 rpm; F3, 700 rpm; F5, 800 rpm), volume of processing medium (F6, 100 mL; F3, 150 mL, F7, 200 mL), and surfactant concentration (F8, 1.0%; F3, 1.5%; F9, 2%). The results are presented in Table 2.

Effect of polymer to drug ratio

It is clear from Table 2 that by increasing the amount of drug in the formulation (F1-F3), the mean particle size of microspheres increases from 836.5 to 874.2 µm (Table 2).

Effect of stirring speed

The microspheres particle size is inversely proportional to the rotating speed. A decrease in particle size was recorded for an increasing rotating speed from 1082.2 to 844.1 µm (Table 2, F3-F5).

Volume of the external phase (paraffin oil)

The microspheres particle size is inversely proportional to the volume of paraffin oil (Table 2). An increasing volume from 100 mL (F6) to 150 mL (F3) and 200 mL (F7) resulted in a decreased microsphere size from 884.4 to 845.3 µm.

Table 2. Percent of weight fraction of each formulation and the encapsulation efficiency. Data are presented as mean ± SD.

Microsphere formulations	% Of weight fraction			*Mean particle size (µm)	*Encapsulation efficiency	*Drug loading (%)
	225-600 (µm)	600-1000 (µm)	1000-1200 (µm)			
F1	5.6	75.0	19.4	836.5 ± 8.9	60.83±0.82	83.5 ± 0.25
F2	2.9	72.0	25.1	864.1 ± 3.4	64.76 ± 2.91	89.6 ± 1.34
F3	2.3	70.0	27.7	874.2 ± 1.2	80.14 ± 0.53	91.8 ± 0.85
F4	0.0	6.0	94	1082.2 ± 13.9	73.21 ± 1.42	75 ± 2.040
F5	5.2	73.4	21.4	844.1 ± 13.7	77.06 ± 0.86	87.6 ± 1.83
F6	1.9	67.5	30.6	884.4 ± 0.9	69.71 ± 1.23	90.1 ± 0.60
F7	4.8	73.9	21.3	845.3 ± 5.4	84.25 ± 0.80	89.5 ± 1.21
F8	1.0	34.5	64.5	989.7 ± 1.4	83.13 ± 0.81	87.5 ± 0.37
F9	4.6	74.8	20.7	844.4 ± 9.1	71.15 ± 0.81	80.1 ± 0.19

*Only microspheres size at 600-1000 µm, except for F4 in which 1000-1200 was used for encapsulation efficiency.

Surfactant concentration

An increase in surfactant concentration from 1.0% (F8) to 1.5% (F3) to 2.0% (F9) (w/v) resulted in a reduction in the mean particle size of the microspheres from 989.7 to 844.4 µm. The presence of surfactant in the external oil phase stabilizes emulsion droplets against coalescence, resulting in smaller emulsion droplets and therefore smaller microspheres (Table 2) (19).

Drug encapsulation efficiency

The same parameters affecting the particle size of microspheres were investigated for the efficiency of the water-oil-oil system towards encapsulation of pregabalin. Results are presented in Table 2.

Increasing the drug-polymer ratio (F1, 1:1.5; F2, 1:2; F3, 1:3) resulted in an increase in encapsulation efficiency (60.83 ± 0.82 to 80.14 ± 0.53). Increasing stirring speed (F4; 600 rpm; F3, 700 rpm; F5, 800 rpm) did not largely affect the encapsulation efficiency. Increasing volume of processing medium (F6, 100 mL; F3, 150 mL; F7, 200 mL), caused an increase in the entrapment efficiency (69.71 ± 1.23 to 84.25 ± 0.80). Increasing surfactant concentration (F8, 1.0%; F3, 1.5%; F9, 2%) caused a decrease in encapsulation efficiency (80.14 ± 0.53 to 71.15 ± 0.81).

SEM

SEM was performed to visualize the prepared microspheres morphology F3 microsphere selected as it contained the highest proportion of the polymer i.e. drug:polymer ratio 1:3 (F3). The microspheres are white spherical with smooth surface containing tiny precipitates (Fig. 1). Figure 1B shows a scanning electron micrograph of a cross-section of the microspheres of formulation F3 after the drug release study. These microspheres showed the microsphere is porous. The pores size range from 10-50 µm (20).

DSC

The DSC thermograms of pregabalin, ethylcellulose, physical mixture of pregabalin, and ethyl cellulose, and F3 microspheres are shown in Fig. 2. Pregabalin showed a sharp melting endotherm at 201 °C whereas ethyl cellulose showed no peaks. When the drug and ethyl cellulose were physically/geometrically mixed (1:3 polymer-drug weight ratio), the mixture showed no significant shift in the melting endotherm of pregabalin. The microspheres of formulation F3 exhibited a shallow wide endothermic peak corresponding to the melting of the remained fraction of the drug.

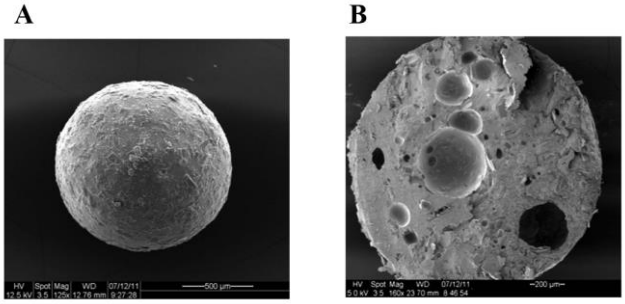

Fig. 1. Scanning electron microscope of (A) F3 microspheres and (B) cross-sectional view of F3 microsphere after drug release.

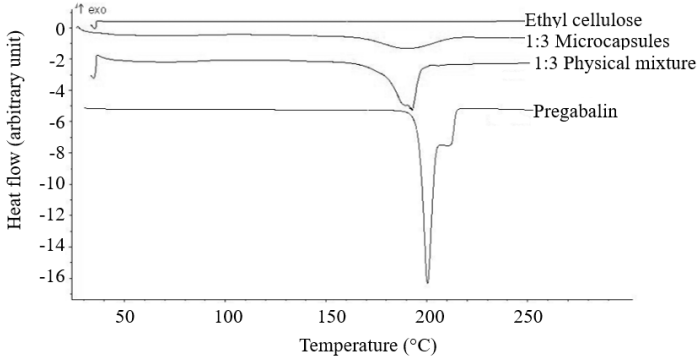

Fig. 2. Differential scanning calorimetry thermograms of ethylcellulose, F3 microspheres (drug to polymer ratio of 1:3), physical mixture of the drug to polymer ratio of 1:3, and pregabalin.

FT-IR analysis

The FT-IR spectra of pregabalin, ethylcellulose, the physical mixture (1:3 drug-polymer weight ratio), and F3 microspheres are presented in Fig. 3. The FT-IR spectrum for ethylcellulose showed a distinct band at 3482 cm^{-1} which was attributed to -OH stretching vibration. The asymmetric band was seen around 2970-2870 cm^{-1} may be due to C-H stretching vibration. The FT-IR spectrum for pregabalin showed a distinct band at 3000-2500 cm^{-1} which was attributed to -COOH and -NH stretching vibration. The bands were seen around 1760 cm^{-1} was attributed to- C=O stretching. The asymmetric band was observed around 2960-2850 cm^{-1} may be due to C-H stretching vibration. If the drug and the polymer would interact, then the functional groups in the FT-IR spectra would show band shifts and broaden compared to the spectra for the pure drug and polymer (19). The FT-IR spectra obtained from the physical mixture showed peaks, which are a summation of the characteristic peaks obtained with the pure drug and pure ethylcellulose indicating that there was no chemical interaction in the solid-state between the drug and the polymer. The FT-IR spectrum of the drug-loaded microspheres dominated by the ethylcellulose absorption, with only a minor contribution of the drug bands. This indicates that the polymer shields the drug molecules.

Drug release

in vitro release of pregabalin from microspheres prepared with different polymer-drug ratios presented in Fig. 4. *in vitro* release of pregabalin from microspheres prepared at different stirring speeds presented in Fig. 5. *in vitro* release of pregabalin from microspheres prepared using different volumes of the emulsion external phase presented in Fig. 6. *in vitro* release of pregabalin from microspheres prepared with different concentrations of the surfactant presented in Fig. 7. The influence of pH of dissolution media on the release of the drug is investigated and presented in Fig. 8.

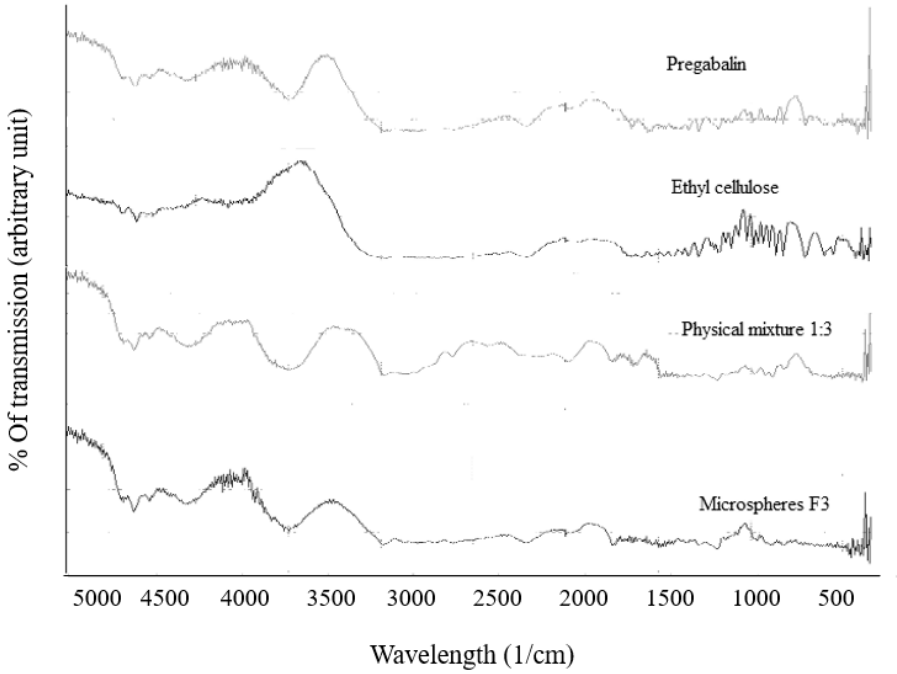

Fig. 3. The Fourier transform infrared spectra of pregabalin, ethylcellulose, the physical mixture (1:3 drug-polymer weight ratio), and microsphere formulation.

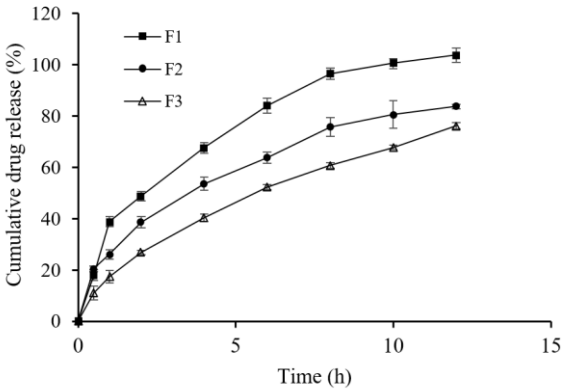

Fig. 4. Effect of polymer-drug ratio on the *in vitro* pregabalin release profile. F1, drug to polymer ratio 1:1.5; F2, drug to polymer ratio 1:2; F3, drug to polymer ratio 1:3. Data are presented as mean ± SD, n = 3.

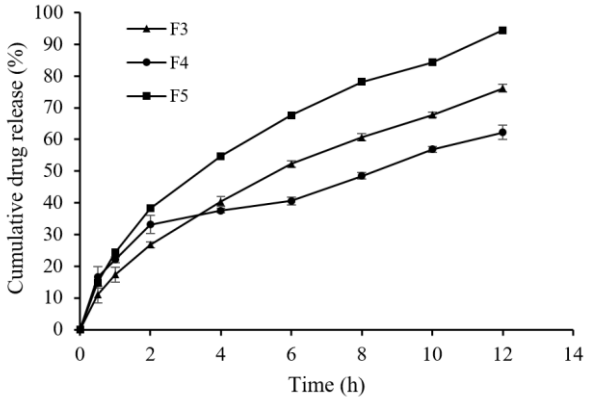

Fig. 5. Effect of the stirring speed on the *in vitro* pregabalin release. F3, F4, and F5 were stirred at 700, 600, and 800 rpm, respectively Data are presented as mean ± SD, n = 3.

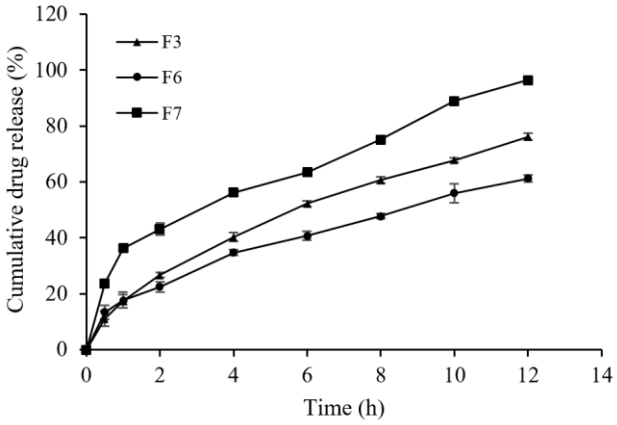

Fig. 6. Effect of the volume of processing medium (paraffin) on the *in vitro* release of pregabalin. The volume of paraffin was 150, 100, and 200 mL, respectively. Data are presented as mean ± SD, n = 3.

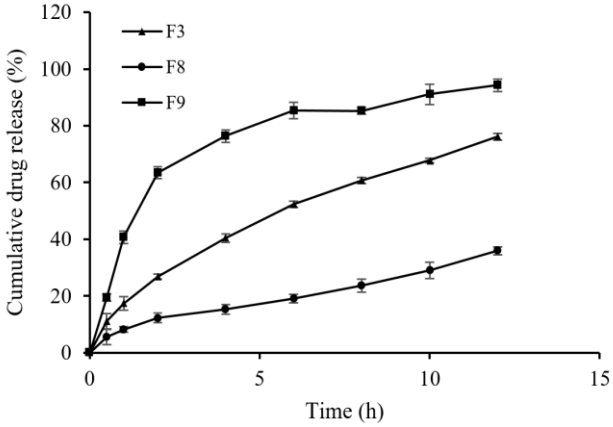

Fig. 7. Effect of the surfactant concentration on the *in vitro* pregabalin release profile. The surfactant concentrations for F3, F8, and F9 was 1.5%, 1%, and 2%, respectively. Data are presented as mean ± SD, n = 3.

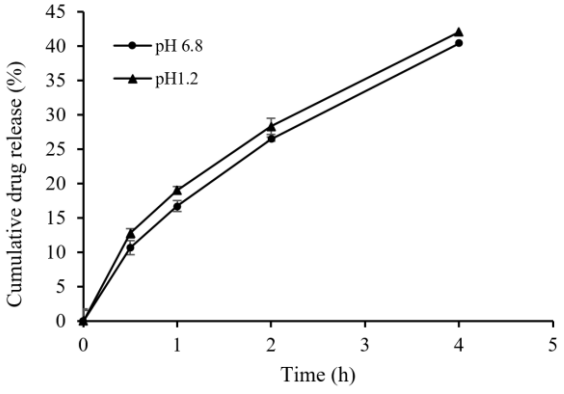

Fig. 8. Effect of the pH of the dissolution medium on the *in vitro* release of pregabalin in F3 formulation. Data are presented as mean ± SD, n = 3.

Increasing the polymer concentration in the microspheres resulted in a decrease in the magnitude of drug release F3 (26.81%) compared to F1 (48.81%, Fig. 4). This may be due to the increase in matrix thickness, and a decrease in total porosity of the microsphere, therefore producing a more sustained microsphere (21). Less polymer also caused an initial burst effect, F1 (17.9%), while initial drug release from F3 (10.63%).

Table 3. The correlation coefficients for all the release kinetics calculated using linear regression analysis. Data represent mean ± SD, n = 3.

Microspheres Formulations	Kinetic models							
	Zero-order		First-order		Higuchi model		Korsmeyer-Peppas model	
	R^2	K_0 (/h)	R^2	K_1 (/h)	R^2	K_h (/$h^{1/2}$)	R^2	N
F1	0.902	0.313	0.943	-0.465	0.983	0.327	0.919	0.669
F2	0.940	0.054	0.996	-0.134	0.998	0.234	0.992	0.501
F3	0.971	0.055	0.997	-0.110	0.999	0.238	0.999	0.638
F4	0.967	0.036	0.981	-0.063	0.980	0.154	0.982	0.380
F5	0.955	0.065	0.970	-0.202	0.997	0.283	0.998	0.622
F6	0.983	0.040	0.996	-0.067	0.997	0.171	0.993	0.518
F7	0.978	0.059	0.899	-0.229	0.985	0.249	0.969	0.396
F8	0.988	0.024	0.981	-0.030	0.956	0.100	0.983	0.549
F9	0.756	0.054	0.958	-0.208	0.880	0.245	0.980	0.853

Concerning the effects of stirring speed, there is a significant increase in the magnitude of drug release from the microspheres with the increase in stirring speed during the evaporation of the volatile solvents (Fig. 5). Drug release with F5 800 rpm was greater at 2 h (37.6%) compared to F3 600 rpm (26.47%, Fig. 5) (22). Concerning the volume of the external phase, there is an increase in the magnitude of drug release from the microspheres with increasing the volume of the external oil phase. The mean drug release from F6 (100 mL) at 2 h was 22.45%, whilst the mean drug release from F7 (200 mL) at 2 h was 43.15% (Fig. 6). F7 showed a high initial burst effect (23.83%) when compared to F3 and F6 (23). Increasing the surfactant concentration in the external oil phase increases the rate of drug release. The mean drug release form F8 (1% Span® 80) at 2 h was 12.2%, whilst the mean drug release from F9 (2% Span® 80) at 2 h was 63.51% (Fig. 7) (24). The formulation F3 that gave high encapsulation efficiency and a good release profile was selected to study the effect of changing the pH of the dissolution medium on pregabalin release from microspheres (Fig. 8). An acidic pH has a slight increase in the release rate of the drug, without a burst effect (25). This due to the increase in the solubility of the drug at lower pH. The difference in drug release at the two different pH is very small and can be considered negligible.

Release kinetic of pregabalin from microspheres

Drug release data of formulations F1-F9 were fitted to the previously described models (and the following plots were constructed: percent cumulative drug release *vs* time (zero-order kinetic model); percent cumulative drug remaining *vs* time (first-order kinetic model); percent cumulative drug release *vs* square root of time (Higuchi model), and percent cumulative drug release *vs* ln time (Korsmeyer-Peppas model). The correlation coefficients for all the release kinetics calculated using linear regression analysis. Results are presented in Table 3. As shown in Table 3, the *in vitro* release profiles of pregabalin from all the formulations is best expressed by Higuchi's equation, since the plots showed high linearity (R^2) as followed by zero-order then first order. Moreover, the release exponent *n* was within the range of 0.45-0.89, indicating a representing a non-Fickian diffusion or anomalous transport and that the drug release from microspheres was diffusion controlled through the pores and not through the swollen matrix (26).

DISCUSSION

For a proper comparison, when the effect of any parameter on drug release is considered for examination, all other parameters were, by design, remained constant. For the effect of polymer to drug ratio, and due to the increase in the amount of the polymer in the formulation (F1-F3), the mean particle size of microspheres increased from 836.5 to 874.2 µm (Table 2). This is due to an increase in the viscosity due to the increase in the amount of ethylcellulose in the microsphere. Solutions prepared with a higher concentration of the polymer will be more viscous and the viscous solution will be

harder to emulsify and will result in the production of a large droplet emulsion and larger droplets will produce larger microspheres. . Effect of stirring speed, the microspheres particle size is inversely proportional to the rotating speed. A decrease in particle size was noted for an increasing rotating speed from 1082.2 to 844.1 µm (Table 2, F3-F5). The reason is that higher rotation speed produces stronger shear force leading to the formation of smaller emulsion droplets and consequently smaller microspheres. The volume of the external phase (paraffin oil), the microspheres particle size is inversely proportional to the volume of paraffin oil (Table 2). An increase in the volume of the external phase from 100 to 200 mL resulted in a decreased microsphere size from 884.4 to 845.3µm. By increasing the volume of the external phase, the emulsion droplets can move freely in the medium, and they will have less chance to collide with each other, thereby yielding small and uniform microspheres. Authors interpret it as an indication of higher porosity of the polymer matrix. An increase in surfactant concentration from 1.0% (F8) to 1.5% (F3) to 2.0% (F9) (w/v) resulted in a reduction in the mean particle size of the microspheres from 989.7 to 844.4 µm. The presence of surfactant in the external oil phase stabilizes emulsion droplets against coalescence, resulting in smaller emulsion droplets and therefore producing smaller microspheres (Table 2) (27-28).

As with particle size of microspheres, it is most important to understand the influence of processing conditions on the microspheres encapsulation efficiency (Table 2). Encapsulation efficiency establishes the ability of the microspheres to prolong drug release and the ability to extend drug release for 24 h. Examining the effect of drug loading (F1-F3) on the encapsulation efficiency, resulted in a significant increase in the encapsulation efficiency (60.83 ± 0.82 to 80.14 ± 0.53). This can be attributed to the fact that a higher polymer ratio (in the formula F3) protects the drug molecules from leaching out toward the external phase during the microencapsulation process which leads to higher encapsulation efficiency (29). Mao *et al*. have previously reported similar findings (30). Increasing volume of processing medium [F6 (100 mL), F3 (150 mL), F7 (200 mL)], caused an increase in the entrapment efficiency (69.71 ± 1.23 to 84.25 ± 0.80). A larger volume of the continuous phase provides the higher concentration gradient, faster diffusion rate of the organic solvents, faster solidification of the microspheres, and therefore more drug entrapped within the microspheres (31). The negative effect of increasing the surfactant concentration on encapsulation efficiency is mainly related to the increase in miscibility of acetonitrile with the processing medium (liquid paraffin). The increase in miscibility resulted from reducing the interfacial tension between the two phases. As a result, accelerate the extraction of pregabalin into liquid paraffin, resulting in a decrease in encapsulation efficiency (32). When comparing our study to previous studies, previous research produced sustained release but with a maximum encapsulation efficiency of 67%, this is because one variable was only studied in the previous research. While the current research produced microspheres with an encapsulation efficiency of 80.14 %. This was achieved by studying and assessing more various process variables, until reaching the most suitable, more practical formulation as compared with other reported formulations (33).

The formation of the porous structure, clearly noticed in the cross-sectional view of the microsphere (Fig. 1B), is a result of the solvent evaporation methods carried out on a water/oil/oil system comprising dichloromethane and acetonitrile. The best fit of *in vitro* drug release profile to the Higuchi model confirms the porous nature of the microspheres and drug release takes place through the pores. Owing to its oil miscibility, dichloromethane was removed by extraction through the processing medium during stirring of the emulsion droplets. In contrast, acetonitrile, as an oil immiscible solvent, was removed by slow evaporation from the emulsion droplets. Therefore, on mixing the water-in-oil emulsion into liquid paraffin, rapid extraction of dichloromethane by the processing medium takes place leaving behind an acetonitrile-water mixture. Due to the poor

solubility of ethyl cellulose in the acetonitrile-water mixture, the latter forced out of the droplets. The migration of acetonitrile-water mixture into the outer oil phase creates a porous structure and channels through the viscous half-formed microspheres. The rate of solvent removal affects, as discussed earlier, polymer precipitation and forms the porous structure of the microspheres. The cross-section after dissolution confirms that release kinetics from the microspheres is diffusion controlled, as microspheres contained its circular shape (20). When investigating the compatibility of pregabalin with ethyl cellulose as the main component of the microspheres, DSC thermograms of the polymer-drug physical mixtures indicated no chemical interaction as the main melting endothermic peak of pregabalin encounters no significant shift at 201 °C (Fig. 2).

The drastic reduction of the melting peak of pregabalin in the thermogram in microspheres could be attributed to the incorporation of pregabalin in the ethylcellulose polymer of the prepared microspheres formulations. It is worth noting that the drug endothermic peak of the physical mixture appeared smaller and at a lower temperature than the drug alone due to the dilution/mixing effect. The FT-IR spectra obtained from the physical mixture showed peaks, which are a summation of the characteristic peaks obtained with the pure drug and pure ethylcellulose indicating that there was no chemical interaction in the solid-state between the drug and the polymer. The FT-IR spectra of the drug-loaded microspheres are dominated by the ethylcellulose absorption, with only a minor contribution of the drug bands. This indicates the drug is distributed uniformly within the polymer used in the microspheres.

The dissolution profile of pregabalin conducted in 0.05 M phosphate buffer (pH 6.8) was influenced largely by processing factors during microsphere preparation and to a very slight extent by the pH of dissolution media. Initially, (Fig. 4) increasing ethyl cellulose proportion resulted in a delayed, slower drug release. The initial burst release was also reduced. This may be due to the increase in coat thickness; therefore, it takes a longer time for the drug to diffuse into the dissolution medium. The increase of the stirring speed will increase the rate of solvent removal from the microspheres. The high vapour pressure of the evaporating solvent from the soft microspheres leads to the formation of solid microspheres with increased porosity. Therefore, drug release with F5 (prepared at 800 rpm) was greater at 2 h (37.6%) when compared with F3 (prepared at 600 rpm) (26.47%; Fig. 5). Similarly, the increase in porosity also encountered with increasing the volume of the processing medium thus leading to higher drug release, this may be due to the higher migration of drug to the surface of the microspheres during solvent evaporation from the freely moving emulsion droplets in a large volume of processing medium (Fig. 6). Concerning the effect of surfactant concentration, the high release profile obtained with increasing Span® 80 may be due to the increase in miscibility and wettability. Therefore, the amount of drug in the external phase increases due to the decrease in internal tension (Fig. 7) (34-37).

The slight increase in drug release encountered in acidic pH (Fig. 8) compared to phosphate buffer solution (pH 6.8) indicates the stable strong structure of these microspheres. This indicates the good formulation properties, which released the drug in a sustained manner even in a highly acidic medium. The solubility of the drug is much higher at lower pH.

Examining the release kinetics of pregabalin in Table 3, the plots, expressed by Higuchi's equation, showed high linearity (R^2) as compared to first-order and zero-order models. This indicates that the drug released from microspheres was diffusion controlled through pores and not through a swollen matrix. When fitted into the Korsmeyer-Peppas model, all formulations showed good linearity (R2: 0.919 to 0.999), indicating that diffusion is the dominant mechanism of drug release. The slope (n) ranges from 0.501 to 0.777 except for formulations F4 and F7. Such slope appears to indicate an anomalous transport (non-Fickian diffusion) for the formulations, which had n values < 0.5. The n value, theoretically, indicates a release from a porous material. Therefore, the n values of both formulations F4 and F7 may confirm a high initial burst effect

upon drug release. Furthermore, this agrees well with the results obtained from the dissolution of formula F4 (16.9%, 600 rpm) compared to F3 (10.6%, 700 rpm). It is suggested that the difference in the release between the two formulas (F4 and F3) is due to high drug migration when stirring speed was low resulting in a high amount of drug remaining inside the microspheres (less collision of emulsion droplets). On the other hand, formula F7 was prepared at a high external volume (200 mL) resulting, as explained above, to an increased internal porosity (38).

CONCLUSION

The current work presented a satisfactory attempt to formulate a sustained-release dosage form comprising pregabalin99.25% drug release at the end of 24 h, the beads being spherical and follows Korsmeyer-Peppas model. Microencapsulation using water-oil-oil double emulsion solvent evaporation was found to be suitable for the preparation of the drug-loaded microspheres. Ethylcellulose proved to be an efficient candidate in formulation with high encapsulation efficiency. SEM analysis revealed that the microspheres were spherical with a smooth surface and porous internal structure. DSC studies confirmed the absence of drug-polymer interaction. The *in vitro* release study revealed the ability of microspheres to prolong the drug release for a period > 12 h and the release kinetic study showed that the mechanism of drug release was diffusion controlled which means that the drug released through pores and channels present in the microsphere's matrix.

Acknowledgements

This work was financially supported by the Deanship of Research at Jordan University of Science and Technology (JUST).

Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

B. Altaani, M. Salem, and H. Yasin proposed the experiments and research design. H. Yasin performed the experiments under the supervision of B. Altaani and M. Salem. B. Altaani analyzed the results of solid-state characterizations and release studies with the contribution of other authors. M. Salem helped in the development and analysis of the drug with the contribution of other authors. H. Yasin wrote the manuscript for publication with help of other authors.

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