Preparation and in-vitro evaluation of floating microspheres of gabapentin

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Abstract:

Gabapentin dosage form could be designed to release the drug in the stomach at a rate providing the maximum amount of drug absorbable by the upper intestinal segment using the multiple- unit floating system to increase the gastric residence time (GRT) of the dosage forms. The floating microspheres were prepared by the solvent evaporation method using polymers ethyl cellulose and cellulose acetate. The microspheres were characterized for their particle size, shape, percentage yield, drug entrapment, buoyancy ratio, drug- polymer interaction and in-vitro drug release kinetics. In addition, effects of the polymer type, polymer: drug ratio, solvent system compositions and temperature on the microspheres characteristics were studied. Investigations using optical microscope revealed spherical shape microspheres, with a size range at (45-1050µm), as well as high yield (99%), high drug entrapment (98%), high buoyancy ratio (≥95) and no drug-polymer interaction. A modified release rate (reach ten hours) was obtained.

Key words: Floating microspheres, Gabapentin, Ethyl cellulose and cellulose acetate.

التحضير والتقييم المختبري للحويصلات الدقيقة الطافية لعقار الكابابنتين

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الخلاصة:

يمكن تصميم شكل دوائي للكابابينتين لتحرر العقار في المعدة بمعدل يزود المقدار الأعلى من العقار قابل للامتصاص بواسطة الجزء المعوي العلوي باستخدام نظام الطفو المتعدد الوحدة صمم لزيادة وقت بقاء العقار في المعدة. تم تحضير الكريات المجهرية الطافية بطريقة تبخير المذيب باستخدام بوليمرات مثل اثيل سيليلوز وخلات السيليلوز.وقد تم تقييم الكريات المجهرية مختبريا من حيث الحجم، الشكل، الكمية، تحميل العقار، نسبة الطفو، تداخل العقار والبوليمر وتحرر العقار.

بالإضافة إلى دراسة تأثير نوع البوليمر، نسبة العقار للبوليمر، مكونات المذيب، معدل وزمن الخلط ودرجة الحرارة على مواصفات الكريات المجهرية. اظهرت النتائج باستخدام جهاز المجهر الضوئي إن

شكل الكريات دائرية و مدى الحجم كان (45- 1050مايكروميتير) ، ناتج عالي (99%)، نسبة تحميل العقار(98%)، نسبة طفو عالية (≤95%)، ولا يوجد تداخل كيمياوي بين العقار والبوليمر.وقد تم الحصول على معدل تحرر للعقار (يصل عشر ساعات).

Introduction:

Gabapentin [1-(aminomethyl) cyclohexaneacetic acid] is a white to off–white crystalline solid. M.p. 162° to 166°. It is freely soluble in water (4491 mg/L at 25°), basic and acidic aqueous solutions, with dissociation constants pK_a of 3.68 and 10.7(1). Gabapentin is an antiepileptic drug, related structurally to γ-aminobutyric acid (GABA). An absolute bioavailability of approximately 50% makes gabapentin a good candidate for improvement of oral bioavailability (2). It has a relatively short half-life (5-7 hours), which leads to substantial fluctuation in the plasma concentration of the drug. Frequent dosing is necessary to maintain reasonably stable plasma concentrations. It is typically absorbed from the upper intestine i.e. it has a narrow absorption window and is absorbed by active transport through a large neutral amino acid (LNAA) transporter. This transporter is located in the upper small intestine, has limited transport capacity, and becomes saturated at high drug concentrations. Consequently, the plasma levels of gabapentin are not dose proportional and, therefore, higher doses do not give proportionately higher plasma levels. Since the LNAA transporter responsible for gabapentin absorption is present only in the upper region of the intestine, the dosage form used to provide gabapentin should be designed to release gabapentin in the stomach at a rate such that the maximum amount of the drug is available in the intestinal segment(3).

A modified release drug delivery system with prolonged residence time in the stomach is of particular interest for drugs (i) which are locally active in the stomach; (ii) with an absorption window in the stomach or in the small intestine; (iii) which are unstable in the intestinal or colonic environment; and (iv) with low solubility at high pH values (4).

Floating drug delivery systems (FDDS) or hydrodynamically balanced systems (HBS) are among the several approaches that have been developed in order to increase the gastric residence time (GRT) of dosage forms. Both single and multiple unit systems have been developed(5). Oral multiple unit dosage form such as microspheres have received much attention as modified/controlled drug delivery systems. These systems distribute more uniformly in the gastrointestinal tract, thus resulting in a more uniform drug absorption and reducing patient-to-patient variability (6).

2. Experimental work:

2.1. Materials:

Gabapentin supplied by medico laboratories (Syria), ethyl celloulose (ethoxy content 48%, 7cps) and cellulose acetate were obtained from ACROS organic/USA. Span80, acetone, conc. HCl, liquid paraffin, n- hexane, potassium hydrogen phosphate, orthophosphoric acid from BDH chemicals, Ltd., Liverpool, England. All other reagents were of analytical grade.

2.2. Equipments:

Electronic melting point apparatus (Stuart TM.) SMP 10, Bibby Scietific (limited stone Staffordshire, ST15, UK), KNAUER HPLC system (Germany), Erweka® dissolution apparatus, Heidolph® R2R 2041 Mechanical Stirrer (Germany), Titerteck® ultrasonic cleaner, Labtech® Daihan lab. water bath, vacuum, DENVER® sensitive balance (Germany), NOVEL microscopy (China), FTIR Spectroscopy, Shimadzu (Model No. 8400S) and pH meter, Hanna instruments (Romania).

2.3. Methods:

Preparation of microspheres

The microspheres were prepared by the oil/oil (O/O) emulsion-solvent evaporation technique [6]and[7]. The polymer or polymer mixture was dissolved in 30 ml of organic solvent or solvent mixture by ultrasonication. Accurately weighed amount (0.5gm) of gabapentin was dispersed in this solution with stirring. This dispersion was rapidly poured into liquid paraffin (100ml). The resultant (O/O) emulsion was continuously agitated at certain temperature using a mechanical stirrer at stirring speed (400 rpm) and the organic solvent was removed completely by evaporation. The solidified microspheres were filtered, washed with 200 ml n-hexane, then dried under vacuum at room temperature over night.

Different gabapentin microspheres were prepared using different drug:polymer ratios (which maintaining a constant amount of drug), different temperatures, organic dispersed phase type and polymer types. The composition of each microspheres formula prepared is given in (table -1). Span 80 (1ml) was used as an emulsifier to prevent coalescence during the formation of gabapentin microspheres and was added to the dispersion phase (liquid paraffin) because of its oily nature.

(Table-1) : The Composition and Conditions of the Microspheres Formulas coded (FET1- FC3).

Formula Code	Polymer Type	Polymer Amount (gm)	Drug Amount (gm)	Drug:polymer ratio	Solvent System	Temp. (°C)
FET1	EC	0.5	0.5	1:1	Acetone	30
FET2	=	=	=	=	=	35
FET3	=	=	=	=	=	45
FEP1	=	=	=	=	acetone	=
FEP2	=	=	=	=	Acetone+ methanol	=
FE1	=	1	=	2:1	Acetone	=
FE2	=	1.5	=	3:1	=	=
FE3	=	2	=	4:1	=	=
FE4	=	3	=	6:1	=	=
FE5	=	4	=	8:1	=	=
FEC1	EC+CA	0.5+0.5	0.5	2:1	=	=
FEC2	=	0.75+0.75	=	3:1	=	=
FEC3	=	1+1	=	4:1	=	=
FEC4	=	1.5+0.5	=	=	=	=
FEC5	=	0.5+1.5	=	=	=	=
EC1	CA	1	=	2:1	=	=
FC2	=	1.5	=	3:1	=	=
FC3	=	2	=	4:1	=	=

Hint: EC= ethyl cellulose, CA=cellulose acetate, FET= effect of change of temperature, FEP= effect of change of solvent system, FE= effect of change of drug: polymer (EC) ratios, FEC = effect of change of drug: polymer (EC+CA) ratios and FC= effect of change of drug: polymer (CA) ratios.

Characterization of microspheres:

Percent yield value of microspheres:

The percent yield value of the microspheres was determined by accurately calculating the ratio of the solidified total microspheres amount of each formulation to the total solid materials weight used in the dispersed inner phase, which was then multiplied by 100 and expressed as a percentage (8).

Determination of encapsulation efficiency of microspheres:

An adequate quantity of microspheres was weighed, milled

and then added to a certain volume of 0.1N HCl. and the resulting dispersion was ultrasonicated for 30min. The solution was filtered with a 0.45 µm pore size filter and the drug concentration was determined using HPLC instrument consist of degasser, pump, UV detector and column heater. Data collection and analysis were performed using ChromoGate Client/series software. Separation was achieved on a column C18 (250mm×4.6mm in diameter). The elution was under low pressure gradient(LPG) at 1ml/min. with a mobile phase of methanol- KH_2PO_4 (pH2.5)(27:73 v/v). UV detection was at 210nm(9). The retention time of gabapentin was found to be about 7.2min. Detection of unknown samples was occurred by the standard curve prepared by injection of different known concentrations of gabapentin (0 -3000 µgm/ml). the percentage of encapsulation efficiency was calculated by dividing the percent actual drug loading on the percent theoretical drug loading and then multiplied by 100%.

Determination of The buoyancy percentage:

Microspheres (100mg) were spread over the surface of 300ml of 0.1N HCl, the mixture was stirred at 100rpm with a magnetic stirrer. After 8 hours, the layer of buoyant microspheres was separated by filtration. Particles in the sinking layer were separated by filtration. Particles of both types were dried, then weighed. The buoyancy percentage was determined by dividing the weight of buoyant microspheres on the total weight of particles multiplied by 100% (10).

Determination of size and shape of microspheres:

The size and shape of microspheres were determined using a microscope fitted with an ocular micrometer and stage micrometer.

Fourier-transform infrared Spectroscopy (FTIR:)

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug, pure polymer, drug-loaded microspheres and physical mixtures of drug and polymer using FTIR. Samples were prepared in KBr disks. The scanning range was (500- 4000 cm^{-1}).

In-vitro drug release studies:

The in vitro release studies of drug loaded microspheres were carried out at 37°C and 100 rpm using 0.1N HCl pH 1.2 (500 ml) USP dissolution apparatus under sink conditions. Accurately weighed samples of microspheres (containing approximately 300 mg drug) were added to the dissolution media and at different time intervals, 5 ml was withdrawn and replaced by an equal volume of fresh dissolution medium. The samples were analyzed using HPLC system. The concentrations of gabapentin in test samples were calculated using a regression equation of the calibration curve.

Release kinetics:

To find out the mechanism of drug release from microspheres, the data obtained from in vitro release studies were fitted to the following kinetic equation (Korsmeyer-Peppas):

$\text{Log } Q_t/Q_\circ = \text{Log } K_p + n \text{ Log } t$

Where Qt is the amount of drug released in time t and $Q_\circ$ is the initial amount of drug in the microspheres. Kp is the release rate constant (Peppas constant), n is the release exponent indicative of mechanism of release. In spherical matrices, if n approximates to (0.5), a Fickian diffusion mediated drug release occurs; if ($0.5 < n < 1$), non-Fickian transport occurs; and erosion mediated release occurs if n approaches one (11).

3. Results and discussion:

Preparation of microspheres:

The (O/O) emulsion-solvent evaporation method was used to prepare floating microspheres of gabapentin. Liquid paraffin was selected as an outer phase, since gabapentin and ethyl cellulose (EC) were very slightly soluble in liquid paraffin. Acetone with a dielectric constant of 20.7 was chosen as a disperse (inner) phase because solvents with dielectric constants between 10-40 show poor compatibility with liquid paraffin and the system of these solvent/liquid paraffin was reported to be applicable to the microencapsulation process [6]. As alternative component, methanol was added within the dispersed phase to act as co-solvent for gabapentin and give solution form-dispersed phase. This form gave relatively less uniform microspheres which may be due to the difference in the evaporation property between methanol and acetone. The emulsifying agent span 80 was used to reduce the interfacial tension and prevent coalescence and agglomeration of drops and stabilizes the emulsion. The stirring speed was 400 rpm, which represents (by trials) the best speed to get more uniform gabapentin microspheres with highest encapsulation efficiency. The temperature was varied to study the effect of temperature on the formation and properties of microspheres, where increasing the temperature of the continuous phase can accelerate the solvent evaporation, however the temperature should not be too high so as not to disnature the drug and not to reach the boiling point of solvent (12). It was found that by changing the temperature, there is a change in the properties of the resulted microspheres as shown in table (2).

The type and amount of polymer used are important factors in preparation of microspheres. EC and cellulose acetate (CA) were used as drug carriers due to their availability ,hydrophobicity and good biocompatibility (12 and 13).

Determination of production yield and encapsulation efficiency:

The production yield percents and encapsulation efficiency of gabapentin floating microspheres are shown in (table-2). All microsphere formulations were produced with high production yield and encapsulation efficiency (except FEP2). The yield of production ranged from (80-100%). The encapsulation efficiency of floating microspheres using EA and CA was between (78-100%). The results have shown that EC and CA are suitable polymers for the encapsulation of a hydrophilic drug (6).

(Table-2): Properties of the Floating Microspheres Formulas of Gabapentin (formulas FET1-FC3)

Formula Code	(%) yield	(%) Encapsulation efficiency	Buoyancy Ratio(%)	Particle Size range (µm)	Particle shape
FET1	99	78	75	45- 215	Irregular mostly, less uniform
FET2	=	85	81	65-210	Irregular, less uniform
FET3	=	89	94	125-210	More regular and uniform
FEP1	=	90	97	120- 210	less uniform
FEP2	=	54	74	250-500	less uniform, larger
FE1	100	99	93	370-500	Less uniform with clumps
FE2	80	=	89	440- 574	Larger particles
FE3	100	100	82	480 – 645	Irregular
FE4	99	99	29	585- 749	More irregular
FE5	86	80	23	590- 790	=
FEC1	100	96	39	650-800	Large regular
FEC2	=	100	18	625-850	=
FEC3	96	90	50	740-860	=
FEC4	98	94	25	875-1050	Irregular
FEC5	97	97	10	835-1081	Large, regular
EC1	100	100	47	570-790	Large, uniform
FC2	97	81	42	580-850	Large, uniform
FC3	=	87	88	585- 960	Large, less uniform

The production yield and encapsulation efficiency of the microspheres were investigated based on variation of temperature, polymer type and its amount, it appears that the production yield and encapsulation efficiency were not affected by these variations ($p > 0.05$). However, the encapsulation efficiency was found to be affected by the solvent system state (solution or dispersion) ($p< 0.05$). This is due to presence of methanol which has less evaporation rate than acetone and less compatibility with liquid paraffin. The effect of temperature on the production yield and encapsulation efficiency is related to the effect of temperature used for evaporation on the uniformity or shape of microspheres which is optimum at 45°C. From table-2, one can notice that as the polymer amount increased , the encapsulation efficiency of EC microspheres decreased relatively. This phenomenon was due to the fact that higher polymer amount produced smaller size droplets and, since smaller droplets have a larger total surface area, the diffusion of the drug from them is faster and more of the drug is lost, resulting in the formation of microspheres with a lower drug content. A similar finding was reported previously by Sengel et al. (6).

Determination of the buoyancy percentage:

Good in vitro floating behavior was observed in most of prepared microspheres formulas. Table-2 shows the buoyancy percentages of floating microspheres containing EC and/or CA. There is significant effect of the temperature and the dispersed phase state on the buoyancy ratio ($p < 0.05$) due to change of density of microspheres. For the floating microspheres prepared using ethyl cellulose alone, a significant effect for the amount of EC on the buoyancy ratio was observed ($p < 0.05$), i.e. as the amount of EC increase, the buoyancy ratio decrease, this is due to the effect of polymer on the size and uniformity of microspheres. While for the microspheres prepared with CA, there is a significant effect for the polymer amount on the buoyancy ratio ($p<0.05$), but due to the different properties of CA, it appears that as the polymer amount increase, there is relative increase in buoyancy ratio, this is due to the changes in the density of microspheres. As a combination of both polymers EC and CA is used, a variation in buoyancy ratio was obtained depending on the ratio of one polymer to another.

Particle size measurement:

The shape of microparticles is mostly spherical with some irregularities (Figures 1,2,3 and 4). Table-2, indicated that there is significant effect for the temperature, the type of polymer and amount on the size of microspheres ($p<0.05$) by extension of the particle size range. For the temperature, as the temperature increased, the size distribution shifts toward larger size

[12]. While for the effect of polymer, difference in the shape and size of microspheres was noticed due to the difference in the physical properties of polymers used .

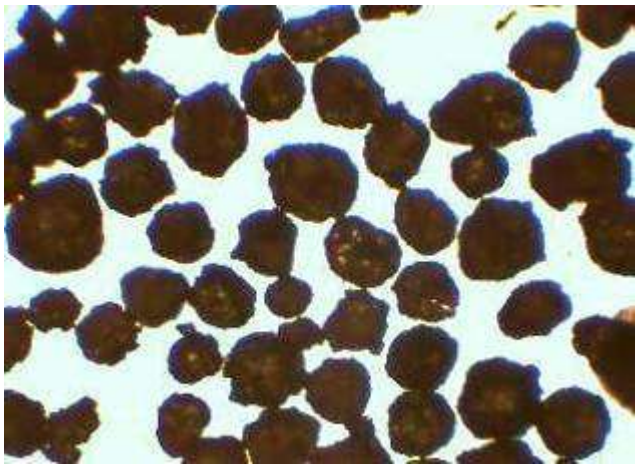

Figure-1: EC microspheres (FE1) as seen by optical microscopy under (4x SF)

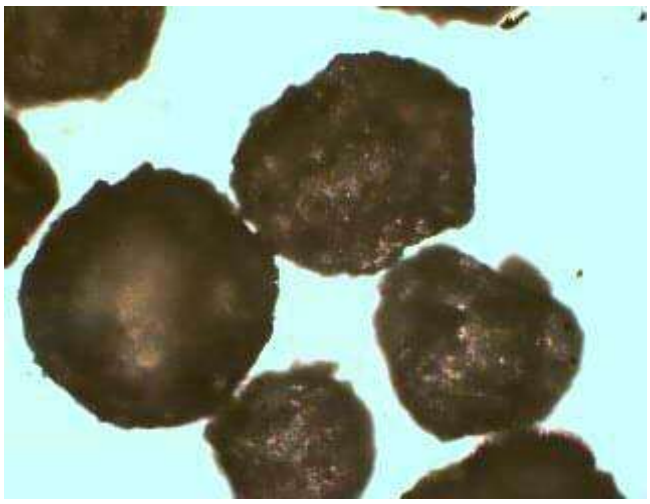

Figure-2: EC microspheres (FE1) as seen by optical microscopy under (10x SF)

Figure-3: CA microspheres (FC1) as seen by optical microscopy under (10x SF)

Figure-4: CA microspheres (FC1) as seen by optical microscopy under (10x SF)

FTIR Studies:

The FTIR spectra obtained for gabapentin, EC, CA, physical mixtures of gabapentin-EC and gabapentin-CA, gabapentin-loaded EC microspheres and gabapentin-loaded EC/CA microspheres are illustrated in (Figures 5,6,7,8,9, 10 and 11) respectively. The results showed that the characteristic peaks due to pure gabapentin (N-H bending, N-H and O-H stretching), pure EC and CA(O-H ,C-H stretching and C-C bending) have appeared in microspheres, without any change in their position after successful encapsulation, indicating no chemical interaction between gabapentin and EC and/or CA which is important for the release. Also by comparison between the physical mixtures and the prepared microspheres, the same result was obtained.

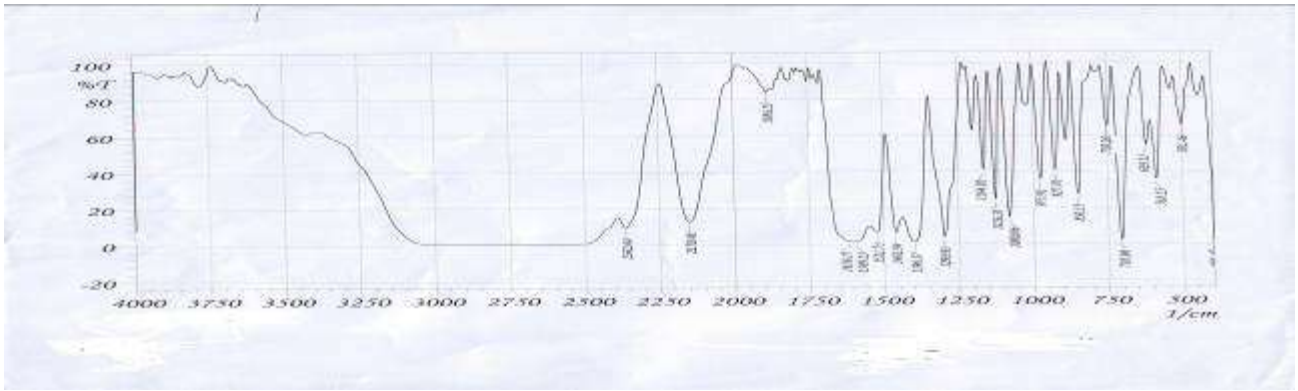

Figure-5: The FTIR spectrum of Gabapentin

Figure-6: The FTIR spectrum of EC

Figure-7: The FTIR spectrum of CA

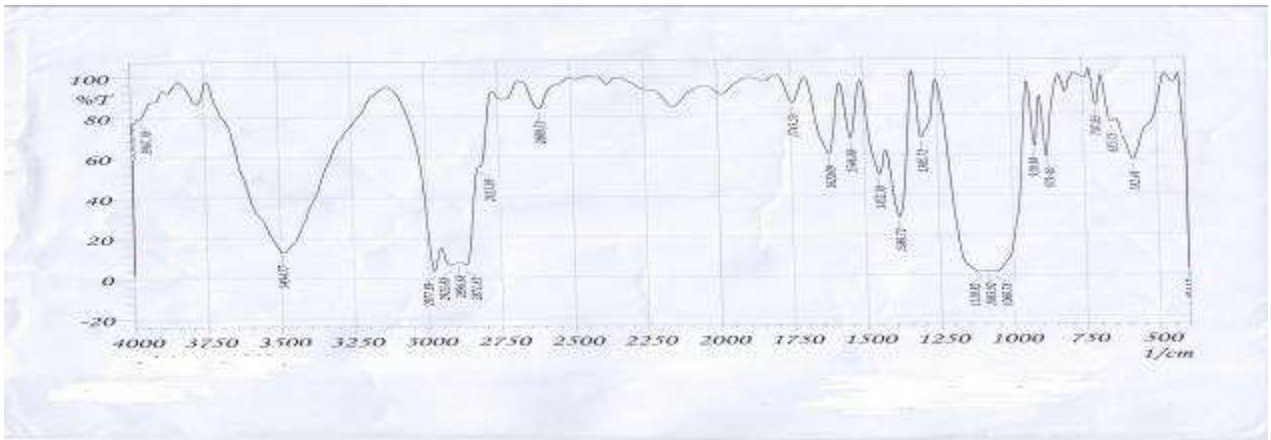

Figure-8: The FTIR spectrum of the physical mixture of gabapentin and EC

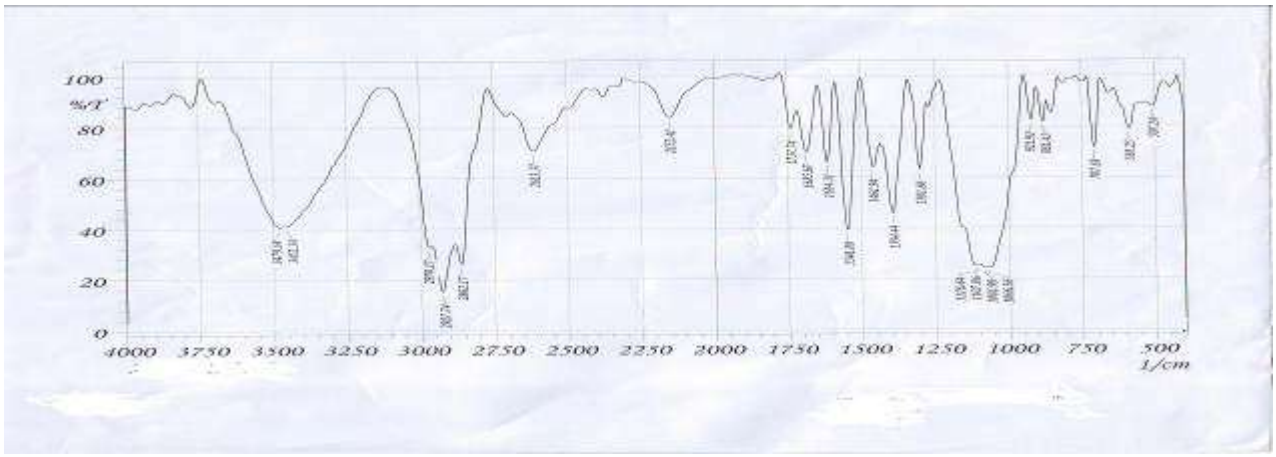

Figure-9: The FTIR spectrum of gabapentin-loaded EC microsphers

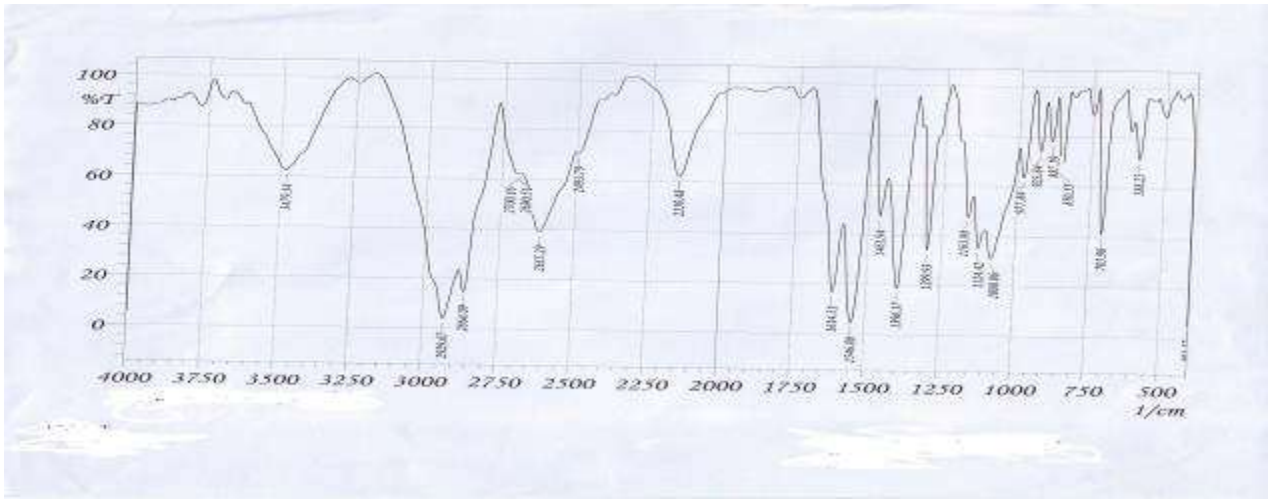

Figure-10: The FTIR spectrum of the physical mixture of gabapentin, EC and CA

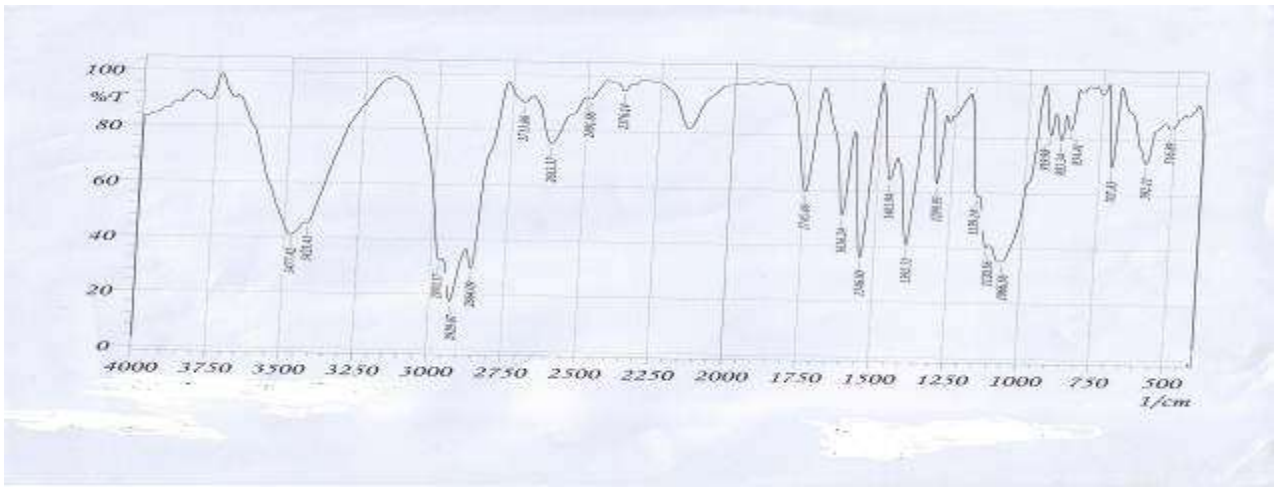

Figure-11: The FTIR spectrum of gabapentin-loaded EC/CA microspheres

In-vitro drug release study:

Formulas with good properties (FET3, FEP2, FE1, FE2, FE3, FEC3 and FC3) were taken for the in vitro release study using 0.1N HCl pH 1.2 dissolution medium, the times required to reach 50% and 100% (complete release) of the drug amount loaded into microspheres (T50% and T100%) for each one were determined as shown in table-3.

Table -3: The release date for gabapentin formulas

Formula code	T50% (hr.)	T100%(hr.)
FET3	0.5	4
FEP2	0.3	1.3
FE1	**3.5**	**8**
FE2	**4**	**9**
FE3	**5**	**10**
FEC3	0.25	5
FC3	0.25	0.5

FE1, FE2 and FE3 prepared formulas gave good release pattern in comparison with others as seen in figures (12, 13 and 14) respectively. We see that times required to reach 50% of the gabapentin amount loaded into microspheres formulas (FE1, FE2 and FE3) are (3.5, 4 and 5 hours respectively) while the times required to get complete release of gabapentin are (8, 9 and 10 hours respectively). The release of gabapentin from microspheres consisted of two phases. The first phase was an initial rapid release of the drug at the first hour, followed by a slower rate of release. This result is consistent with the release of other drugs encapsulated within the same polymer[14]. The release of drug is more retarded by use of ethyl cellulose as main polymeric material because it can form hydrophobic matrix including the drug, the type of polymer has significant effect ($p<0.05$) on the release of gabapentin specially at the first initial phase. For EC, the drug-polymer ratio has no significant effect ($p> 0.05$) on the release of drug at the first initial phase.

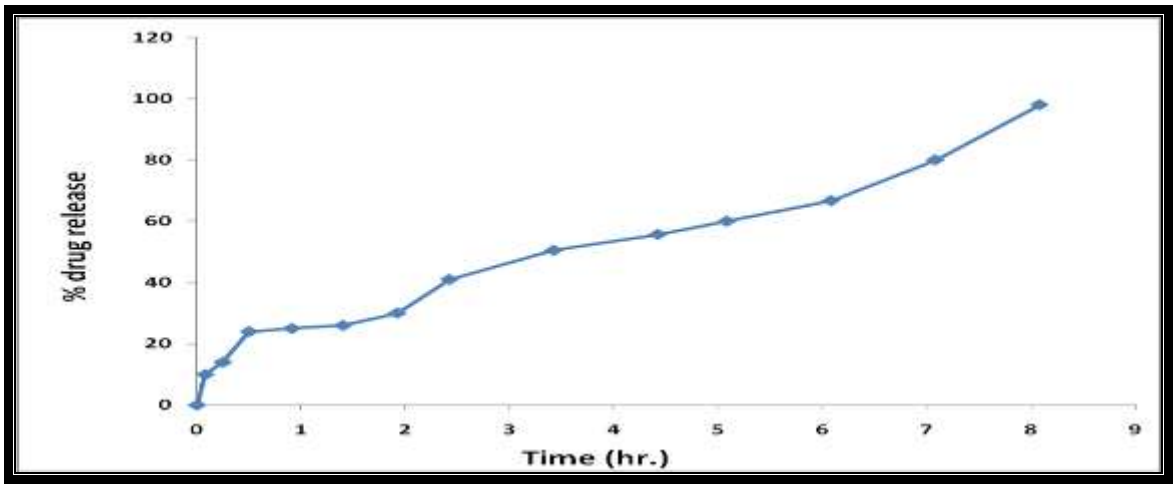

Figure-12: The release profile of gabapentin (FE1)

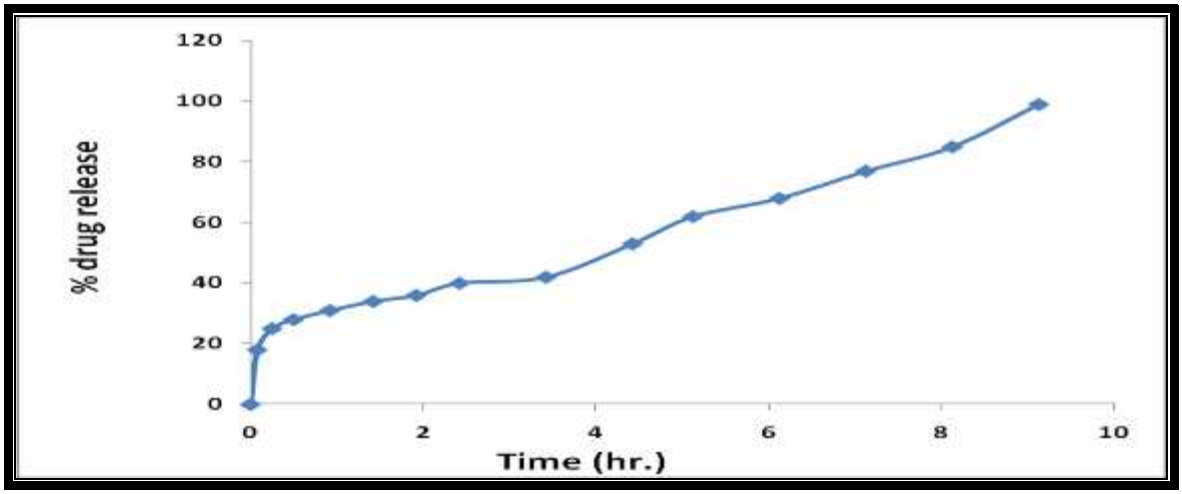

Figure-13: The release profile of gabapentin (FE2)

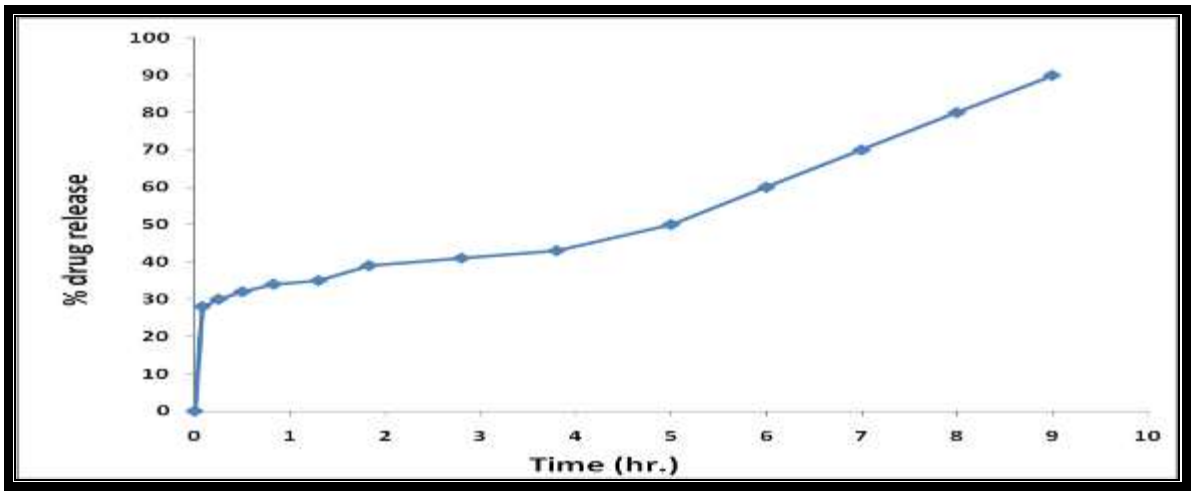

Figure-14: The release profile of gabapentin (FE3)

The release kinetics:

Depending on values of (n), one can conclude the mechanism of release of gabapentin from the prepared microspheres (FE1, FE2 and FE3) as shown in table-4. The value of (n) is below (0.5), which indicates that a fickian diffusion dominates the drug release through the swelling matrix and hydrophilic pores. While depending on values of R^2, the Korsmeyer- Peppas model is best fitted the formula FE1.

Table- 4:the release kinetics of the formulas (FE1, FE3 and FEC3)

Formula code	Kp	n value	R^2	The release mechanism
FE1	0.29	0.47	0.95	a fickian diffusion mediated drug release
FE2	0.35	0.32	0.91	=
FE3	0.4	0.23	0.74	=

Conclusions:

Based on the data obtained, one can prepare floating microspheres of gabapentin by use of solvent evaporation- (O/O) emulsion method using ethyl cellulose as polymeric coating material. The type of polymer and its ratio is important factor in design of modified release formulation. As well as, the conditions used in preparation of microspheres are important for determining the size and shape of microspheres. And finally the mechanism of release of drug from the most successful formula is mainly a fickian mechanism (mediated diffusion).

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