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Preparation and evaluation of Venlafaxine hydrochloride Microspheres by Ionotropic Gelation method

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ABSTRACT

Introduction: Venlafaxine hydrochloride (VHL) an antidepressant drug shows burst effect with fluctuating drug levels in systemic circulation due to its higher solubility and shorter elimination half life of 4 hrs. **Background:** In order to achieve the patient compliance the dose frequency and dose size of VHL can be reduced by entrapping it in gastro-resistant hydrophilic natural polymers. **Method:** Ionotropic gelation method. **Results and Discussion:** SEM confirmed cracks on the surface of calcium-alginate microspheres and due to these cracks low drug content and entrapment efficiency was observed. With the increase in the concentration of pectin, a higher number of free carboxylic groups of pectin were able to interact with Ca++ counter-ions and thus increases the drug entrapment . The characteristic peak of VHL was not more detectable in its physical mixture with pectin and sodium alginate due to the strong exothermic effect associated to the pectin decomposition that overlaps and masks the drug fusion peak indicating decrease of crystallinity and uniformity of dispersion at molecular level. The best fit model was Korsmeyer peppas for the formulations (F_2-F_4) with higher correlation coefficient value, *n* value, however, appears to indicate a coupling of diffusion and erosion mechanisms so called anomalous diffusion, whereas formulation F_1 was best fitted to Higuchi model, indicates diffusion mechanism. **Conclusion:** Conclusively the optimal formulation comprising of 0.5%w/v VHL, 1.75%w/v sodium alginate and 0.9%w/v pectin was identified to provide maximum % CDR and a controlled drug release profile without burst release extending up to 12 hrs.

Keywords: Anti-depressant activity, calcium-alginate-pectinate microspheres, calcium-alginate microsphere, Non-fickian diffusion.

INTRODUCTION:

VHL commercially known as “Effixor” with the therapeutic dose of 75-375 mg and is a bi cyclic phenyl ethyl amine. Due to its ready solubility in water (572mg/ml) and shorter half life (4hr) the VHL is an ideal choice for controlled release formulations. It is selective reuptake inhibitor of serotonin and nor epinephrine and to a lesser extent of dopamine[12].

In order to get controlled release of VHL various research papers had already published such as triple layer tablets containing hypromellose, Chitosan nanoparticles, glyceryl behenate matrix tablet, resinates using ion exchange resins, complexation with Indion 244, sustained release tablets, extended release tablets and micro beads etc.

Literature review suggested that different methods were used for preparation of microspheres. Ionotropic Gelation method was selected as it can be carried out under very mild condition in aqueous and organic solvent free environment and does not require any special equipment and can be performed at room temperature. In this method physical cross linking occurs via ionic interaction at room temperature and at physiological pH. Moreover drugs with higher solubility and shorter half life such as verapamil HCl, furosemide, diltiazem HCl, theophylline and tetracycline HCl etc were reported by above method [10,15].

In this study natural polymers sodium alginate and pectin were selected for microspheres preparation. Both polymers were frequently used for microsphere preparations intended for oral drug delivery; because of nontoxic, biocompatible and biodegradable nature. But due to their solubility and biodegradation in the upper GIT they were unable to sustain drug release for more than 4-5 hrs. This drawback of sodium alginate and pectin can be adjusted by preparing complexes with divalent cations (like Ca^{++}). These complexes are insoluble and stable in acidic pH and also used as a matrix material to achieve a controlled release of drug due to its hydro gel forming property [5,16].

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MATERIALS AND METHODS:

Materials

Venlafaxine hydrochloride (VHL) was obtained as a generous gift

sample from Akum drugs, Haridwar, UK, India. Sodium alginate and Pectin was purchased from s.d fine-chemicals, Mumbai. Calcium chloride was purchased from Qualigens chemicals, Mumbai. All other chemicals used were of analytical grade.

Preparation of drug loaded calcium-alginate and calcium-alginate-pectinate microspheres

Calcium-alginate microspheres were prepared by Ionotropic gelation method. 0.5%w/v of drug was added to 1.75 %w/v aqueous solution of sodium alginate in F_1 (Table 1.). This solution was dropped manually through a needle size no. 26 G from a hypodermic syringe in to 2%w/v solution of calcium chloride. The gelled microspheres formed were allowed to harden in gelling bath for at least 30 min. After washing with distilled water, they were dried at room temperature until constant weight were achieved and then transferred to desiccators under vacuum [17].

Calcium-alginate-pectinate microspheres (F_2) were prepared by adding 0.5 %w/v of drug to an aqueous solution comprising 1.75%w/v sodium alginate and 0.3%w/v aqueous solution of pectin. Similarly F_3 and F_4 were prepared by adding 0.5 %w/v of drug to an aqueous solution comprising 1.75 %w/v sodium alginate and 0.6%w/v and 0.9%w/v aqueous solution of pectin respectively. Further procedure was followed as explained above in the preparation of Calcium-alginate microspheres.

Characterization of calcium-alginate and calcium-alginate-pectinate microspheres

Morphology of microspheres

The external morphology of microspheres was studied by FEI Quanta™ 200 scanning electron microscope, USA at 15 kv. The microspheres were coated with gold palladium under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then observed with a scanning electron microscope.

Micromeritic studies

The micromeritic properties of pure drug and prepared microspheres were characterized such as: determination of bulk density, dynamic angle of repose and average particle size.

Determination of bulk density

1g of pure drug and prepared microspheres were subjected in to 10 ml graduated measuring cylinder separately and the initial volume and poured density were noted down. The graduated cylinder was dropped on to a hard surface until no further changes in volume was noted. The tapped density was then obtained by dividing the weight of sample in gram by the final volume in cm^3 of the material contained in the cylinder. Percentage compressibility index and Hausner ratio were also calculated, using the formula [1].

Determination of Dynamic angle of repose

Dynamic angle of repose of pure drug and prepared microspheres were determined by placing 1 g of pure drug and prepared microspheres separately in rotating cylinder in lab fabricated equipment and allowed to rotate at 25 rpm for 5 min. The angle made by the bulk of the pure drug and prepared microspheres against the horizontal tangent, was recorded and dynamic angle of repose was calculated [1].

Determination of Average particle size

Average particle size of prepared microspheres was also studied by sieve method. 1 gm of microspheres was placed on the top of series of six standard sieves ranging from sieve no: 16 to 100, arranged from bottom to top in ascending order of aperture size. The sieves were mounted on mechanical sieve shaker and shaken for 10 min. The weight of microspheres on each sieve was measured and the average particle size was determined.

Determination of drug content and % entrapment efficiency

100 mg of microspheres were crushed in glass mortar and triplicate samples of 10 mg of the crushed microspheres were dissolved in 10 ml of phosphate buffer pH 7.4, vortexed for 5 min and filtered through watt man filter paper. The filtered samples were diluted 50 times with phosphate buffer pH 7.4 and drug content was assayed by UV spectrophotometer at 225 nm.

In vitro drug release study

The dissolution medium was 0.1 N HCl as simulated gastric fluid (SGF) (900 ml, pH 1.2) for 2 hr, followed by phosphate buffer as simulated intestinal fluid (SIF) (900 ml, pH 7.4) for the rest of 10 hr. 1 ml samples were withdrawn at specified time intervals (0-12 hr) and equal volume of fresh medium was replaced immediately. After a suitable dilution (10 times), samples were analyzed by UV spectrophotometer.

Kinetics of drug release

To find out the mechanism of drug release from microspheres, the dissolution data of each batch was fitted to various kinetic equations, namely Zero order, First order, Higuchi square root of time and Peppas-Korsmeyer [3,19].

Differential Scanning Calorimetry (DSC) studies

DSC thermo grams were obtained using an automatic thermal analyzer system. Thermal behavior was studied under normal conditions with perforated and sealed quartz pans and with a nitrogen gas flow of 200 ml/min. The samples were heated at 10 ? C/min over temperature range of 50-250 ? C. The reference sample used in all determination was alumina. The spectra obtained were analyzed for incompatibility.

Effect of agitation intensity and different concentrations of pectin on drug release from microspheres

To find out the effect of agitation intensity and different concentrations of pectin on drug release from microspheres, three rotational speed (50,100 and 150 rpm) and pectin concentrations (0.3, 0.6 and 0.9 %w/v) were selected. Dissolution was first carried out in SGF for 2 hr followed by dissolution in SIF for 10 hr.

Comparison of the best formulation with the marketed formulation

In vitro release study of the selected formulation and marketed preparation Venlift-OD 75 mg capsule (Torrent pharmaceuticals) was compared.

Stability studies

The prepared microspheres (20 mg) were placed in sealed, clear glass vials and stored at ambient humidity conditions at room temperature (30 °C ± 2 °C), oven temperature (45 °C ± 2 °C) and in refrigerator (5-8 °C) for a period of 60 days. The samples were assayed for drug content and evaluated for physical stability at regular intervals of two months.

RESULTS:

Sem study of calcium-alginate microspheres revealed spherical shape with rough, irregular, porous, depression and cracks on the surface **Fig.1 (a)**, whereas calcium-alginate-pectinate microspheres were of uniform size, spherical, smooth surface which was free from cracks were observed **Fig.1 (b)**.

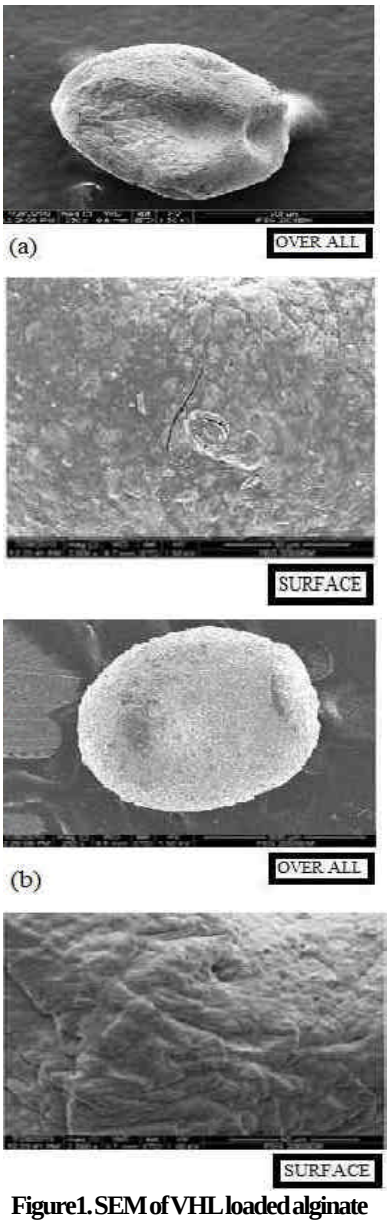

Figure1. SEM of VHL loaded alginate microspheres (a) Calcium-alginate microsphere and (b) Calcium-alginate- pectinate microspheres

Table1. Composition, comparative micromeritic and physicochemical evaluation of pure drug and drug loaded microspheres

Batch code	Drug (%w/v)	Composition Sodium alginate (%w/v)	Composition Pectin (%w/v)	Micromeritic evaluation Poured density	Micromeritic evaluation Tapped density	Micromeritic evaluation Comp-ressibility	Micromeritic evaluation Hausner's ratio	Dynamic angle repose (degree)	Average size (mm)	Physicochemical evaluation Actual Drug Content (mg/ml)	Physicochemical evaluation %EE	Physicochemical evaluation % CDR after12 hr
Pure drug				0.31	0.59	47.61	1.90	75	19.50	-	-	99.28 ± 0.03 (within 2 hr)
F1	0.5	1.75	-	0.83	0.90	8.32	1.09	22(Good)	610	34.62 ± 0.07	66.30 ± 0.67	84.54 ± 0.36 (within 6 hr)
F2	0.5	1.75	0.3	0.9	1.0	9.1	1.10	25(Passable)	635	39.33 ± 0.09	72.57 ± 0.53	74.71 ± 0.24
F3	0.5	1.75	0.6	0.98	1.10	10.90	1.12	24(Passable)	650	42.47 ± 0.04	74.81 ± 0.40	78.12 ± 0.26
F4	0.5	1.75	0.9	1.0	1.11	9.9	1.11	20(Good)	660	46.92 ± 0.04	79.33 ± 0.41	83.66 ± 0.32

The value of poured density and tapped density was highest with F_4 formulation and lowest with the pure drug. All formulations showed lower values for compressibility index, Hausner 's ratio and dynamic angle of repose than the pure drug **(Table 1)**. Average particle sizes of microspheres were ranged between 610 µm to 660 µm and highest drug loading and percentage entrapment efficiency was exhibited by F_4 microspheres.

Pure drug was completely dissolved (99.28 ± 0.03%) within 2hr. Formulations F_1, F_2, F_3 and F_4 released 31.34 ± 0.43% , 26.22 ± 0.26 %, 22.62 ± 0.16% and 17.44 ± 0.25% of VHL respectively at pH 1.2 (SGF) within 2hr and 84.54 ± 0.36% (within 6 hr) , 74.71 ± 0.24%,78.12 ± 0.26% and 83.66 ± 0.325% within 12hrs at pH 7.4 (SIF) shown in **Fig.2**. Formulation F_4 follows a slower gradually declining drug release phase extending up to 12 hrs.

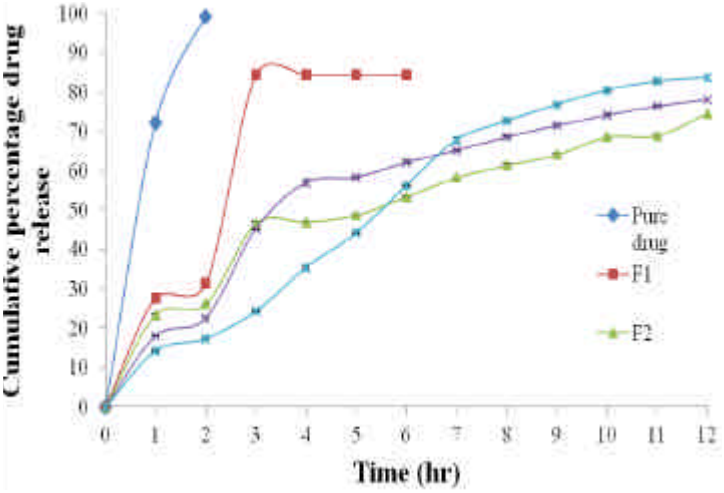

Figure2. Comparative percentage drug release profile of pure drug and prepared microspheres (F_1-F_4)

From the various release kinetic models applied to determine the mechanism of drug release from microspheres, the highest correlation coefficient (r^2) was found for Korsmeyer peppas model in calcium-alginate-pectinate microspheres (F_2-F_4) and for Higuchi model in calcium-alginate microspheres. When the *in-vitro* release profiles of formulations (F_2-F_4) were plotted according to Korsmeyer's eqn, they showed high linearity (R^2= 0.9969) with a comparatively high slope (*n*) value ranging from 0.08 to 0.1.

The DSC thermo grams of pure VHL showed a sharp endothermic peak at 217 ^{0}C (Fig.3.). Similarly, the melting peaks of sodium alginate were observed at 62 ^{0}C as endothermic and at 231 ^{0}C as exothermic peaks. The additional component pectin showed an endothermic peak at 153 ^{0}C. Sharp endothermic peaks of VHL, sodium alginate, pectin and the exothermic peaks of sodium alginate were reduced in case of physical mixtures and microsphere.

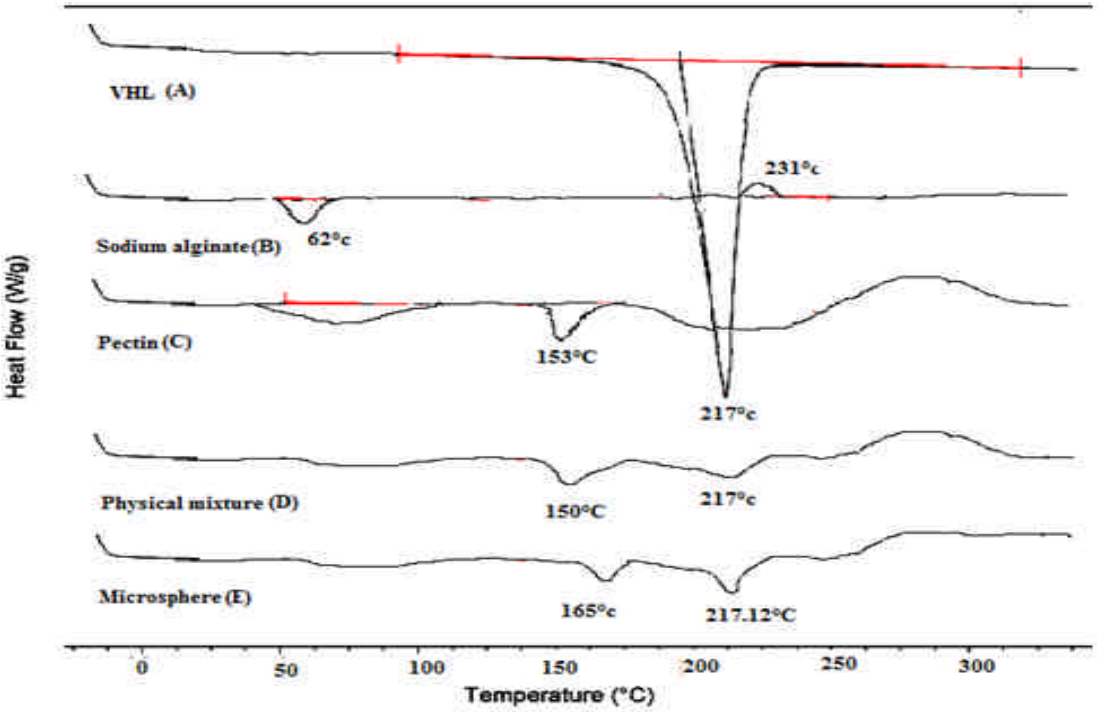

Figure3. DSC thermo grams of VHL, sodium alginate, pectin, physical mixture and microsphere

The variation in agitation intensity does not have any significant effect on release profile of the optimized formulation (Fig.4.) and the higher concentration of pectin retarded the drug release in SGF, while enhanced it in SIF (Fig.5.). Comparative profile showed higher percentage of drug released from optimised formulation than the marketed preparation (Fig.6.). It is evident from the data in Table 2.that the microspheres were physically and chemically stable for the test period of 60 days.

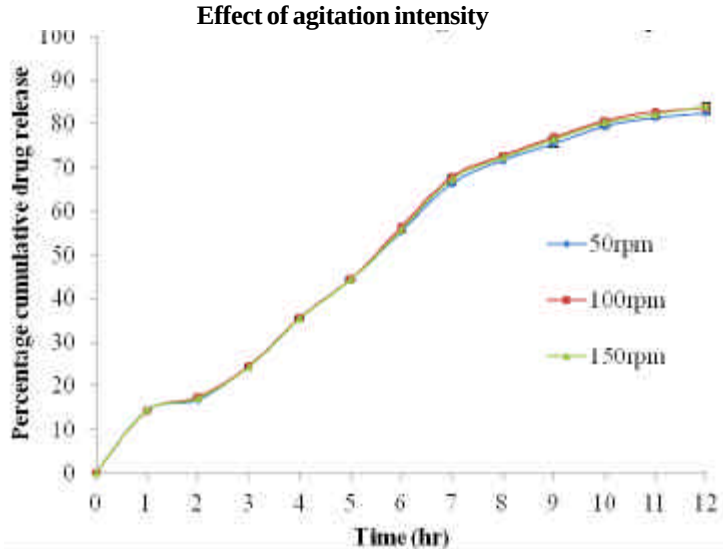

Figure4. Effect of agitation intensity on drug release

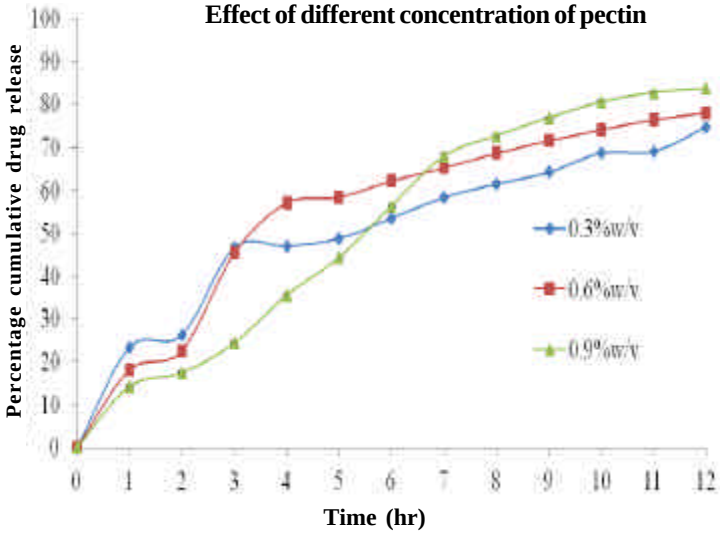

Figure5. Effect of different concentration of pectin on drug release

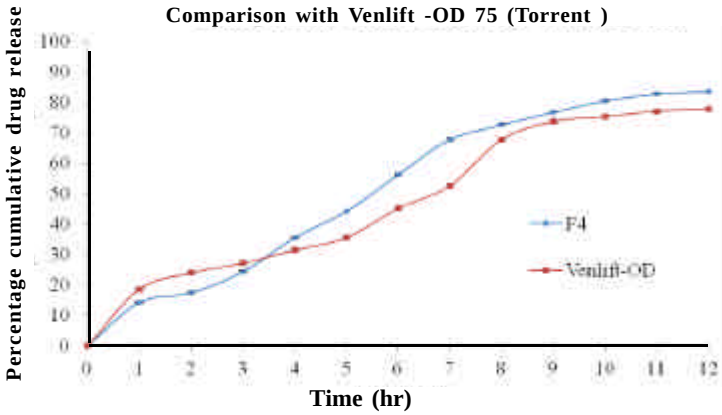

Figure6. Comparison of the optimized formulation (F_4) with the marketed formulation (venlift-OD 75 cap)

Table2. Kinetic data of VHL microspheres stored under variable storage conditions

Time (months)	Drug content under variable storage conditions (mg/ml) Room temperature	Oven temperature	Refrigerator
	(30 ± 2^{O}C)	(45 ± 2^{O}C)	(5-8^{O}C)
0	46.541	46.543	46.671
1	46.673	46.813	46.639
2	46.789	46.849	46.578

DISCUSSION:

SEM confirmed cracks on the surface of calcium-alginate microspheres and due to these cracks low drug content and entrapment efficiency was observed. And these results are supported by low *in vitro* release profile of drug, whereas the addition of pectin results in crack-free surface in case of calcium-alginate-pectinate microspheres. The drug content and % entrapment efficiency increased progressively with increasing sodium alginate and pectin concentrations resulted in the formation of larger microspheres entrapping greater amounts of the drug. This may be attributed to the greater availability of active calcium-binding sites in the polymeric chains thus greater degree of cross-linking among polymers.

With the increase in the concentration of pectin, a higher number of free carboxylic groups of pectin were able to interact with Ca++ counterions and thus increases the drug entrapment [13].

The best fit model was Korsmeyer peppas for the formulations (F_2-F_4) with higher correlation coefficient value, *n* value, however, appears to indicate a coupling of diffusion and erosion mechanisms so called anomalous diffusion[14], whereas formulation F_1 was best fitted to Higuchi model, indicates diffusion mechanism.

The thermal curve of VHL exhibited a profile typical of a pure, crystalline, anhydrous, drug with a sharp endothermic peak due to its melting process. Sodium alginate showed an endothermic peak corresponds to its melting and an exothermic peak due to its decomposition. The thermal profile of pectin attributes to a melting phenomenon, followed by an exothermic event ascribed to a decomposition process. The phenomenon of enthalpic relaxation, appearing as a broad flex, is well visible, with the thermal data for pectin. The characteristic peak of VHL was not more detectable in its physical mixture with pectin and sodium alginate due to the strong exothermic effect associated to the pectin decomposition that overlaps and masks the drug fusion peak indicating decrease of crystallinity and uniformity of dispersion at molecular level. Thermal curve of VHL charged microspheres showed broad flex, which appears increased in intensity and shifted at higher temperature due to growing number of intermolecular crosslink's formed between carboxylic groups of sodium alginate and pectin with the counter ions Ca^{++}. The VHL peak was strongly reduced in intensity, indicating a marked decrease of crystallinity but not complete drug amorphization in the microsphere. The drug was uniformly dispersed at the molecular level and completely goes in to polymer system. These findings indicate that there was no appreciable change in thermal properties of the drug and the polymers in the formulations prepared [6].

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