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Formulation of enteric coated microspheres containing pantoprazole

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Abstract:

Pantoprazole is a first-line proton pump inhibitor drug for the treatment of gastric acid secretion disorders that is known to have minimal side effects and drug interactions. To improve its stability in gastric acid, delayed-release microspheres containing pantoprazole was prepared by emulsification-solvent evaporation using a polymer-containing mixture of hydroxypropyl cellulose (HPC) and ethyl cellulose (EC), which was then coated by alginate and Eudragit® L100. The morphological characteristics of the microspheres were examined by SEM, the particle size distribution inferred by laser diffraction, and the physical state of drug substance was measured by Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and *in vitro* drug release. The three formulations of microspheres chosen for this study had an average size of 100 µm. The dissolution profile showed less than 10% of the drug was released after 120 min in 0.1-M HCl and more than 75% of drug was released after 45 min in a phosphate buffer with a pH of 6.8.

Keywords: **delayed release, enteric coated microsphere, pantoprazole.**

Classification number: **3.3**

Introduction

Gastric acid is vital for food digestion. However, excess gastric acid can cause irritation, inflammation of the oesophagus, heartburn, and stomach ulcers [1]. Many drugs have been used to treat excess gastric acid like proton pump inhibitors (PPIs), which are the first line of treatment for stomach ulcers. Pantoprazole, a third-generation PPI, has the highest efficacy and lowest side effects and drug interactions among other PPIs [2, 3]. However, pantoprazole is unstable in a gastric acid environment. There are few products containing pantoprazole for treatment of stomach ulcers in the Vietnamese market and most of them are formulated as pellets or enteric coated tablets.

In recent years, microspheres have become an effective solution to a variety of pharmaceutical challenges that include particle size, drug solubility, drug-drug interactions, drug stability, and controlled drug release [4]. Therefore, in this study, the formulation of enteric-coated microspheres containing pantoprazole by emulsification and solvent evaporation was carried out.

Materials and methods

Materials

Pantoprazole sodium sesquihydrate (USP 41) was procured from Mac-Chem Products, India. Hydroxypropyl cellulose (HPC-LMM) (EP7) and ethyl cellulose (EC 45-55 cps) were supplied by Nisso, Japan. Sodium alginate was purchased from Sigma Aldrich, Germany and Eudragit® L100 was distributed by Evonik, Germany. Ethanol, acetone, liquid paraffin, tween 20, span 80, n-hexane, and magnesium oxide (MgO) were procured from Xilong, China and were of industrial grade.

Methods

Formulation and preparation of pantoprazole microspheres: the emulsification and solvent evaporation method was used to prepare the microspheres. Briefly, polymers were weighted and dissolved in 12.5 ml of a 1:1 acetone-ethanol mixture to form a 2.5% (w/v) solution. Pantoprazole, having a 1:3 ratio with the polymers, was then dissolved in this solution. MgO was dispersed into the solution and 100 ml of paraffin containing 1% (w/v)

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Table 1. Formulations of microspheres preparation.

Formulation	Polymer concentration (%)	HPC:EC ratio	Pantoprazole (%)	MgO (%)	Tween: Span HLB=5.5 (%)
F1	2.5	0:1	0.83	0.5	1
F2	2.5	1:2	0.83	0.5	1
F3	2.5	1:1	0.83	0.5	1

emulsifier (span 80 and tween 20) was then added to this mixture and keep stirring at 500 rpm for 3 hours. The microspheres were collected by allowing the suspension to settle. Paraffin was then removed from the microspheres by centrifugation and washed three times with 30 ml of n-hexane. The microspheres were dried at 50°C for 30 min and stored in a dark bottle for further experiments. The formulation used in this study are described in Table 1.

Evaluation of microsphere: the microspheres were characterised by common methods described in Ref. [5]. Encapsulation yield of pantoprazole in the microspheres: briefly, the microspheres were dissolved in 0.1-M NaOH. The filtrate was then diluted with a phosphate buffer of pH 6.8. Pantoprazole was quantified by UV-Vis at a wavelength of 288 nm. The quantification process was validated according to ICH guidelines. The encapsulation yield of pantoprazole in the microspheres was calculated per the following formula (this experiment was repeated three times):

$$Encapsulation\ yeild\ (\%) = \frac{Amount\ of\ PAN\ in\ MS}{Amount\ of\ MS} \times 100$$

- Particle size distribution: the size distribution of the microspheres was determined by laser diffraction (Mastersizer 3000, Malvern).

- Microsphere morphological characteristics: the morphology of the prepared microspheres was analysed by scanning electron microscopy (SEM) (SEM JEOL JSM-6400, Japan). For SEM, the samples were prepared by lightly sprinkling microsphere powder on double-sided adhesive tape, which was placed on an aluminium stub. The stubs were then coated with carbon using a fine coat ion sputter.

- DSC: to determine whether there are interactions between pantoprazole and the polymer, a DSC scan was carried out for pantoprazole, a microsphere sample, and a physically mixed sample with the same proportion of microspheres. Each sample was measured over a temperature range of 30-350°C and heating speed of 10°C/min.

- FTIR: the microspheres and the physically mixed samples with the same proportion of microsphere compositions were prepared and characterised by FTIR scans over a wavenumber range of 3000 to 500 cm^{-1}.

- *In vitro* release of microspheres: a microsphere sample containing 1.6 mg pantoprazole was added to 40 ml of a 6.8-pH phosphate buffer in a 50 ml falcon tube. The falcon tube was incubated at 37°C and shaken horizontally at 125 rpm for 45 min. At an appropriate time, this mixture was then centrifuged at 5000 rpm for 5 min. Then, the supernatant was collected using a 0.45-µm filter and the released pantoprazole was quantified by UV spectrophotometry at a wavelength of 288 nm.

Formulation and preparation of delayed-release microspheres containing pantoprazole: 100 mg of microspheres (<180 µm) was dispersed in a 2% (w/v) alginate solution containing various quantities of L100 (Table 2) at 50°C. This mixture was quickly added dropwise to 50 ml of 5% $CaCl_2$ from a height of 5 cm above the solution surface. This mixture was then stirred at 150 rpm for 5 min. The microspheres were collected and washed with water and then dried at 50°C. The microspheres were stored in a dark bottle for more experiments. The *in vitro* dissolution studies were carried out in a shaking bath with uncoated microspheres in pH 1.2 for 120 min and then in pH 6.8 for 45 min.

Table 2. Formulations of delayed release pantoprazole microspheres.

Formulation	Uncoated microspheres type	Alginate (%)	L100 (%)	Microsphere (%)
F4	F3	2	0	2
F5	F3	2	5	2
F6	F2	2	5	2

Table 3. Drug encapsulation yield and *in vitro* pantoprazole released in the pH 6.8 of uncoated microspheres of pantoprazole sodium.

Formulation	Drug encapsulation yield (%)	Drug release after 45 mins (%)
F1	9.63±0.12	31.66±0.36
F2	10.61±0.20	81.74±1.46
F3	11.47±0.09	99.53±1.18

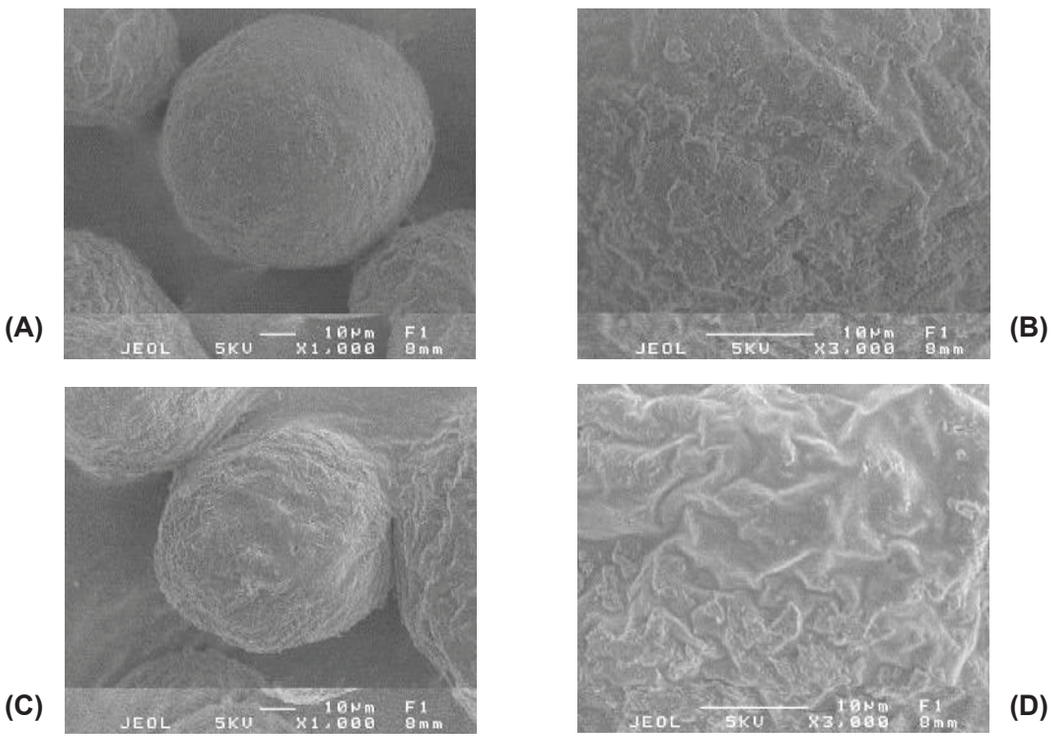


Fig. 1. SEM micrograph of microspheres F1: (A) 1000x, (B) 3000x and F3: (C) 1000x, (D) 3000x.

Results and discussion

Evaluation of microsphere formulation

The encapsulation yield and *in vitro* pantoprazole release of the prepared microspheres are described in Table 3.

The encapsulation yield increased with increasing HPC proportion. In other word, the more HPC in the formula, the greater achieved encapsulation yield. This is because adding a hydrophilic polymer maintains hydrophilic API in its dispersed phase and out of its continuous phase [6].

The drug release from the F1 formulation was relatively low, whereas the F2 and F3 formulations had a higher *in vitro* drug release profile in the pH 6.8 buffer. Therefore, increasing HPC concentration can increase the ability of pantoprazole release.

The microsphere's morphological characteristics are shown in Fig. 1. Microspheres from both formulae have a spherical shape and are an average of 100 µm in size. Although the surface of the F1 particle is smoother than F3, both formulations have a wrinkled surface.

The particle size distribution of the F3 microsphere is illustrated in Fig. 2. The average size is about 100±23 µm.

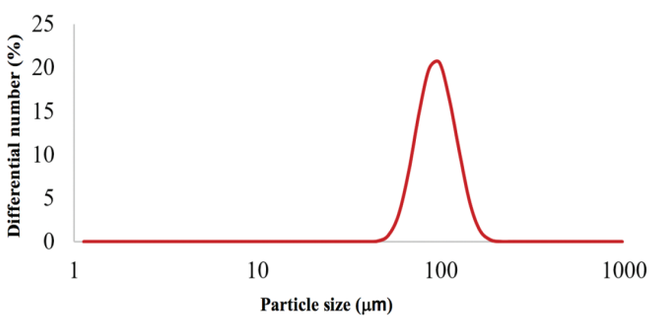


Fig. 2. Particle size distribution of the F1 microspheres.

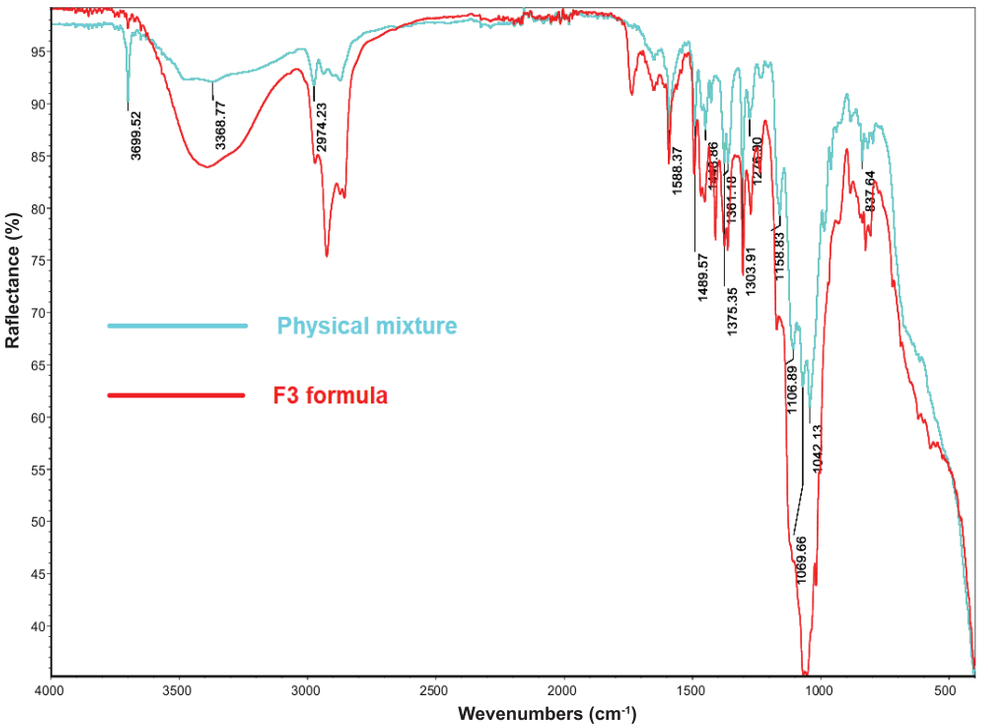


Fig. 3. The FTIR spectra of microsphere F3 (red) and physical mixture (blue).

The IR spectrum of the F3 microspheres and the physically mixed sample are illustrated in Fig. 3.

There is no significant difference between the IR spectra of the F3 formula and the physically mixed sample. Therefore, it is inferred that no chemical interaction exists between pantoprazole and the polymer-made microsphere. The absence of F3's main peak is the effect of being covered by the polymer due its large proportion in this formulation.

The DSC diagrams of the microspheres and different formulation compositions are illustrated in Fig. 4.

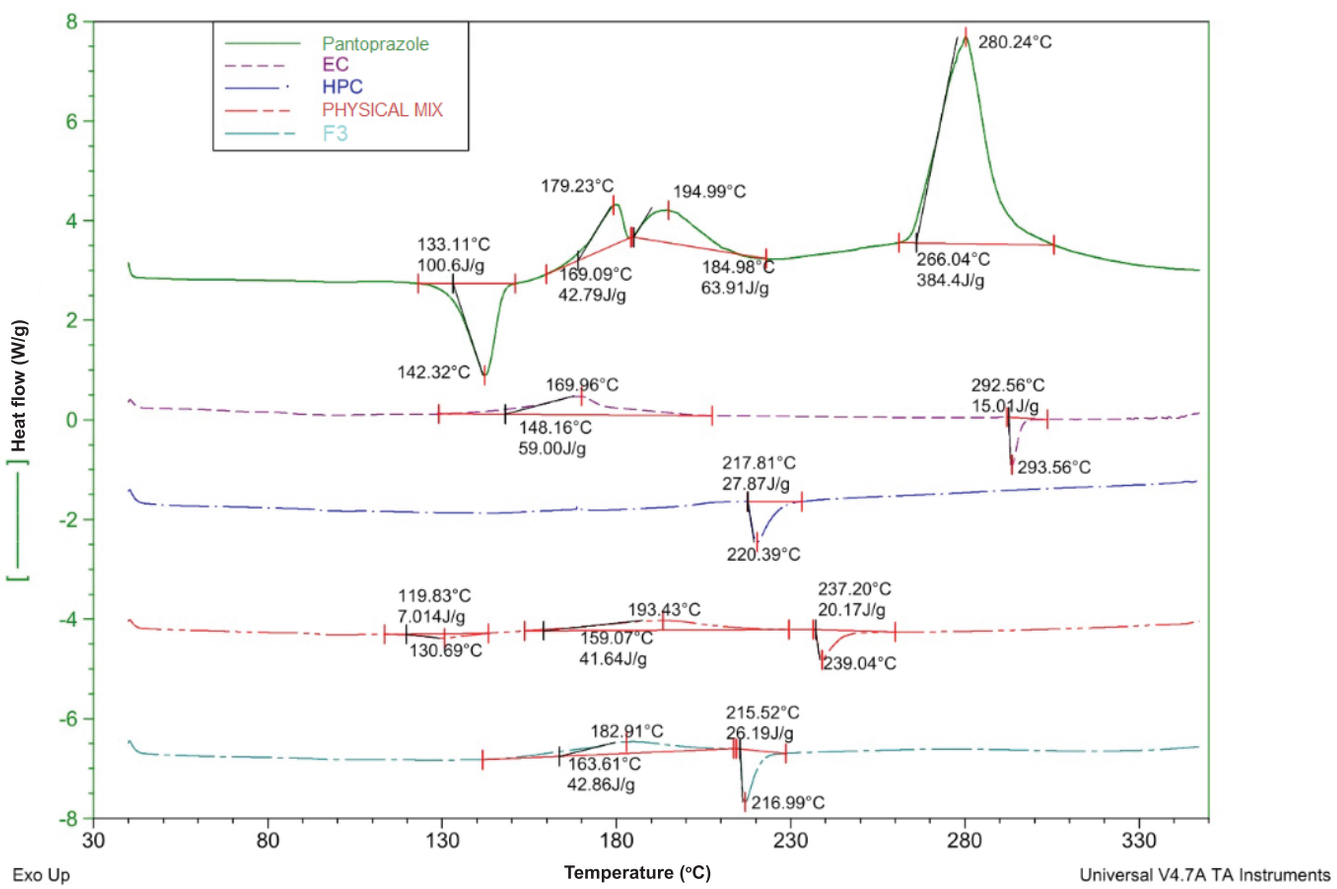


Fig. 4. The DSC thermograms of components in the formula F3.

According to diagram, F3's curve has two enthalpy transition points at 182.91°C and 217°C, which is similar to the physically mixed sample. The 130°C peak does not appear in F3's curve. This could be explained due to the cover of nearly peak of excipients (7.0 J/g difference) or Van der Waals effects of hydro connection.

Due to the high *in vitro* drug release profile in the pH 6.8 buffer, the F2 and F3 samples are used in delayed drug release formulation in the next step.

Formulation and preparation of coated microspheres

The percentage of pantoprazole content, the microparticle encapsulation efficiency, and the *in vitro* release results of the coated microspheres in a pH 1.2 buffer after 120 min are illustrated in Table 4.

The addition of 2% alginate (in formulation F5 and F6) had better API protection from leaking into the acidic medium (9.58 and 2.37%, respectively) than using Eudragit® L100 alone (F4 had 37.11% drug released in the acidic medium). The proportion of HPC and EC also affects the ability of pantoprazole release. The more EC in the formula, the more acidic protection effects (i.e., F5 vs. F6).

Table 4. Percentage content and *in vitro* drug release in the pH 1.2 of coated microsphere.

Formulation	Percentage content (%)	Encapsulation efficiency (%)	Drug release after 2 h in acidic medium (%)
F4	4.83±0.04	97.85	37.11±0.80
F5	2.31±0.03	97.79	9.58±0.08
F6	2.19±0.04	98.45	2.37±0.04

A dissolution test was carried out with the F5 and F6 microspheres and the results are shown in Table 5 and Fig. 5.

Table 5. Result of drug release in pH 6.8.

Formulation	Time of sampling		
	15 min	*30 min*	*45 min*
F5	15.09±0.74	53.62±1.96	77.23±1.02
F6	7.28±0.96	24.76±0.54	50.24±1.12

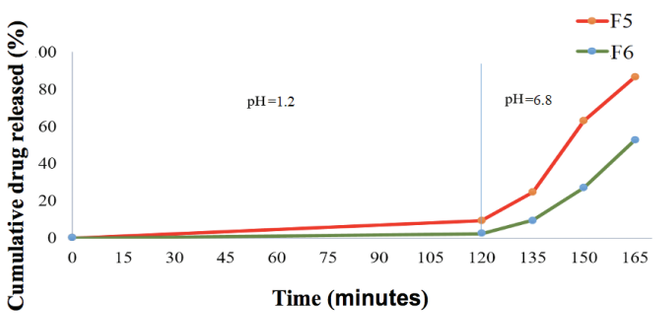
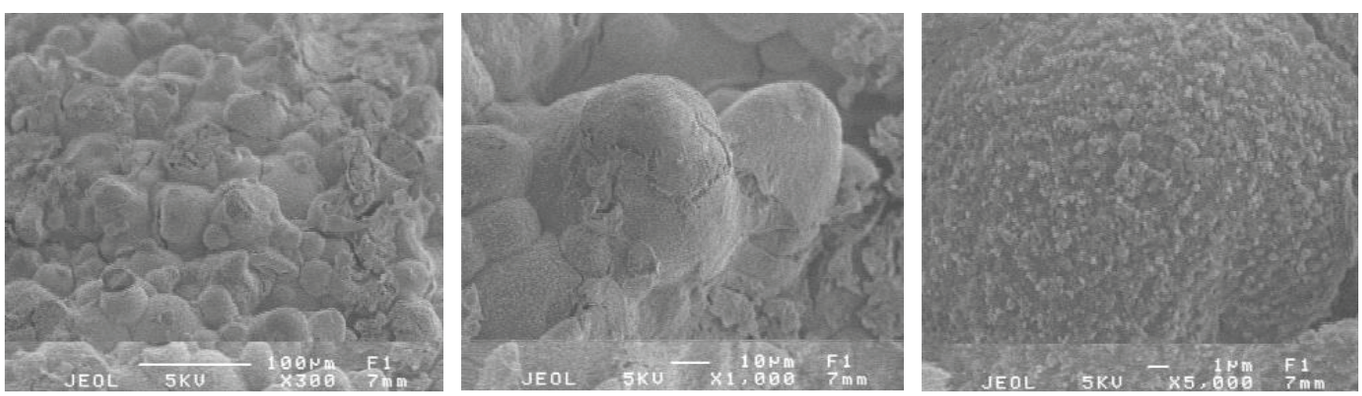


Fig. 5. Chart of *in vitro* drug release of formulations F5 and F6.

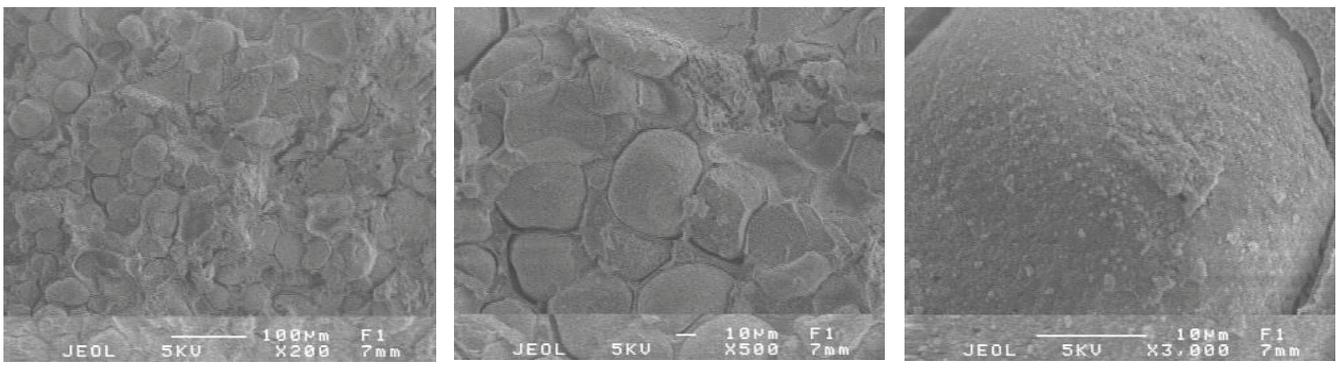
F5 showed acid tolerance after 120 min (9.58%). Furthermore, in a pH 6.8 buffer, the F5 formulation showed better API release (77.23%) than F6 (50.24%).

The surface and cross-sectional surface of the F5 formulation are shown in Figs. 6 and 7, respectively. The encapsulated microspheres were protected and intact in the alginate medium. The F3 microspheres are covered by the alginate-L100 coating layer. The cutting edge image shows that alginate gel matrix is integrated with alginate particles and filled by the Eudragit® L100 polymer.



(A) (B) (C)

Fig. 6. SEM surface photographs of formulation F5 at magnifications of (A) 300x, (B) 1000x, and (C) 5000x.



(A) (B) (C)

Fig. 7. SEM cross-section surface photographs after cutting formulation F5 at magnifications of (A) 200x, (B) 500x, and (C) 3000x.

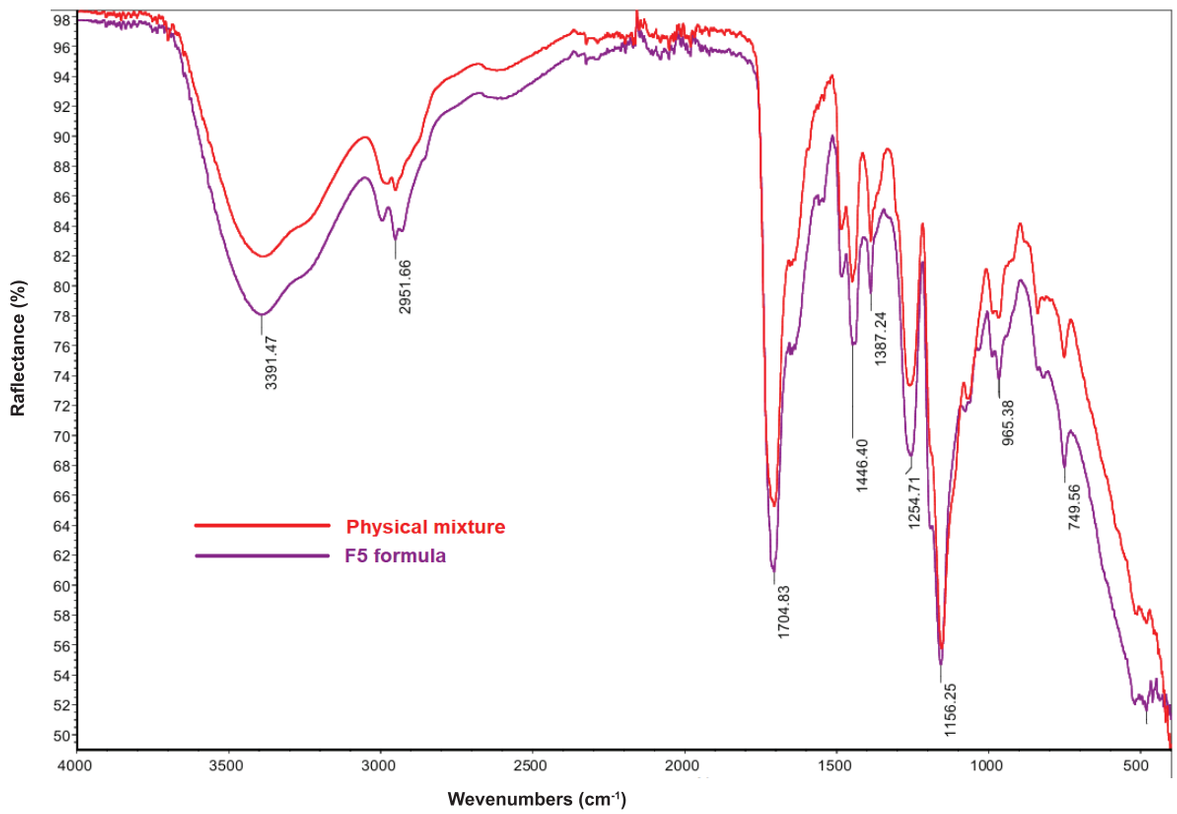


Fig. 8. The FTIR spectra of formulation F5 (purple) and the physically mixed sample (red).

The IR spectra of the F5 microspheres and the physically mixed sample of the same composition are illustrated in Fig. 8.

There are no significant differences between the spectra of the two samples, thus, there are no chemical interactions between the materials in the formulation.

Conclusions

Microspheres with 100-μm average size were successfully prepared with an encapsulation yield of 11.4%. More than 75% of the pantoprazole was released in a pH 6.8 buffer after 45 min. Moreover, the addition of alginate in Eudragite can protect the pantoprazole after

exposure to an acidic environment for 2 h (less 10%) while the release profile in the pH 6.8 buffer was still greater than 75%.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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