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Preparation and *In Vivo* Evaluation of Ulcerogenic Activity of Piroxicam Microspheres

Esmat E Zein[1*], Ahmed A Donia[2], Sania E El-Kayad[2] and Karima El-Dosoky[3]

*[*1]Department of Pharmaceutics, Faculty of Pharmacy, Egyptian Russian University, Cairo*

[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, Tanta University, Tanta, Egypt

[3]Department of Clinical Pathology, Faculty of Medicine, Tanta University, Tanta, Egypt.

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ABSTRACT

The aim of the present study is to reduce the ulcerogenic effect of piroxicam by controlling its dissolution rate. Piroxicam is a class II drug according to BCS, thus dissolution is the rate limiting step for its bioavailability. To control the bioavailability of piroxicam and reduce its side effects dissolution rate was attempted to be controlled by preparing microspheres having a solid dispersion structure. Two different polymers were used one is solid dispersing polymer to enhance dissolution rate of piroxicam and the other is a retarding polymer in order to control its release. Depending on the ratio of the two polymer combinations, drug release can be controlled. Percentage yield and entrapment efficiency of prepared formulations ranges from 45.21% ± 0.01 to 87.79% ± 0.01 and 23.87% ± 0.89 to 56.13% ± 7.06, respectively depending on polymer concentration. Characterization of piroxicam and other formulations using DSC and FTIR analysis reflects possibility of transformation of the drug from crystalline to amorphous state. Release of piroxicam was faster from microspheres having solid dispersion structure (about 74.98% ± 1.5 after 15 min). In order to control the release of the drug, ethyl cellulose as well as eudragit Rs100 was added. The release pattern of piroxicam from different prepared formulations followed Higuchi matrix kinetic model. In vivo ulcerogenic studies revealed that piroxicam containing eudragit S100 was the formula of the least ulcer incidence (50%) showing gastric mucosa with (mild mucosal edema as well as minimal sloughed area and also minimal vascular congestion) than those showed by other animals.

Keywords: Ulcerogenic, Piroxicam, Polymers, Microspheres

INTRODUCTION

The permeability besides the solubility behavior of a drug is a key determinant of its oral bioavailability [1]. Formulation of poorly soluble compounds for oral delivery now presents one of the interesting challenges to formulation scientists in the pharmaceutical industry where more than 40% new chemical entities are practically insoluble [2].

Piroxicam is a member of the oxicam group of non-steroidal anti-inflammatory drugs (NSAIDs) that is indicated for acute or long-term use in the relief of signs and symptoms of osteoarthritis and rheumatoid arthritis and also has gastrotoxic as well as duodenotoxic effects [3,4].

According to the Biopharmaceutical Drug Classification System (BCS) piroxicam is a class II drug, characterized by low solubility-high permeability, where drug dissolution is the rate limiting step in drug absorption and bioavailability [5].

Solid dispersion systems in which the drug is dispersed in solid water-soluble matrices either molecularly or as fine particles have shown promising results in increasing bioavailability of poorly water-soluble drugs [6,7]. Solid dispersion techniques including dissolution method, fusion method and fusion-dissolution method were commonly used.

Microencapsulation is one of the most interesting fields in

Corresponding author: Esmat E Zein, Department of Pharmaceutics, Faculty of Pharmacy, Egyptian Russian University, Cairo, E-mail: dr.esmat.zein@gmail.com

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the area of pharmaceutical technology by which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of polymeric material [8,9].

Microencapsulation for oral use has been employed to sustain the drug release and to reduce or eliminate gastrointestinal tract irritation. In addition, multiparticulate delivery systems spread out more uniformly in the gastrointestinal tract. Small particle size, are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa [10,11].

Emulsion solvent evaporation method was used for the preparation of microspheres due to its ease of fabrication without compromising the activity of drug and it requires only mild conditions such as ambient temperature and constant stirring [12,13].

Piroxicam may cause serious GI side effects, including ulceration as well as intestine and stomach perforation, which can also be fatal [14,15]. Piroxicam side effects are similar to other NSAIDs, its GI damage is the most serious one (GI adverse effects is 3.7-10 fold) [16].

Attempts to overcome the undesired effects of piroxicam include modifications in the manner of administration [17-19]; in pharmaceutical forms [20], in the preparation of pro-drugs [21]; and in the synthesis of complexes [22].

The aim of the present study is preparation of piroxicam microspheres with different polymers to obtain different dissolution patterns and comparing it's *in vivo* gastro ulcerogenic activity with free piroxicam and piroxicam microspheres containing eudragit S100 which was previously prepared by El-Kayad et al. [23].

MATERIALS AND METHODS

Materials

Piroxicam was obtained as a gift sample from Medical Union Pharmaceuticals, Ismailia, Egypt. Ethyl cellulose, Eudragit Rs100 and Eudragit L100-55 were obtained as gift samples from Sigma for Pharmaceutical Industries, Quesna, Egypt. Aerosil (ISO-CHEM, China). Ethanol, methanol, dichloromethane, sodium lauryl sulphate (pharmaceutical grade) were obtained from El Nasr pharmaceutical chemicals company, Cairo, Egypt. All other chemicals used were of analytical grade.

Equipment

Mechanical paddle stirrer (Heidolph RZR-2000), U.V. visible spectrophotometer (Shimadzu UV-visible UV-160 A, Japan), USP II dissolution apparatus (paddle type, Copley Scientific Dis 6000, Nottingham, UK).

Determination of piroxicam by UV-visible spectrophotometric method

A stock solution of piroxicam in methanol (1000 µg/ml) was prepared. The standard stock solution was further diluted to the required concentration for method development and validation. Calibration curve was constructed at different pH values (1.2, 6.8 and 7.4) using 0.1 N HCl and phosphate buffer, respectively. Ultraviolet absorbance of the solutions was determined spectrophotometrically (Thermo, Evo300pc, USA) at the wavelength of maximum absorbance at 334, 354 and 353 nm for pH values 1.2, 6.8 and 7.4, respectively [24].

Preparation of piroxicam microspheres

The microspheres were prepared using emulsion solvent evaporation technique with certain modifications [25]. **Table 1** represents the composition of piroxicam microsphere formulations. Eudragit L100-55 is used as a solid dispersing carrier; eudragit Rs100 and ethyl cellulose were selected as controlled release polymers. The drug and polymers were dissolved in a mixture of methylene chloride and ethanol (1:1 v/v) to give a clear solution. Aerosil was suspended uniformly in the drug polymer solution under vigorous agitation. The resultant drug-polymer-aerosil suspension was poured slowly into 200 ml distilled water containing 0.08% SLS with agitation using mechanical stirrer (700 rpm) at room temperature. The suspension was finely dispersed into translucent emulsion droplets immediately under agitation where the drug and polymer co-precipitated in the emulsion droplets. With agitation translucent emulsion droplets turned into opaque microspheres. Agitation was continued for complete evaporation of organic solvent [26]. Microspheres were filtered, washed several times with distilled water and then allowed to dry at ambient temperature to be used for further analysis.

Table 1. The composition of the proposed piroxicam microspheres.

Formula	Piroxicam (g)	Aerosil (g)	Eudragit L_{100-55} (g)	Eudragit Rs_{100} (g)	Ethyl cellulose (g)	Surfactant conc. (%)	Internal phase (ml)	External phase (ml)
F1	0.5	1	0.5	-	-	0.08	26	200
F2	0.5	1	1	-	-	0,08	30	200
F3	0.5	2	0.5	-	-	0.08	32	200
F4	0.5	2	1	-	-	0.08	36	200
F5	0.5	2	1	0.5	-	0.08	44	200
F6	0.5	2	1	1	-	0.08	44	200
F7	0.5	2	1	1.5	-	0.08	44	200
F8	0.5	2	1	-	0.5	0.08	48	200
F9	0.5	2	1	-	1	0.08	44	200
F10	0.5	2	1	-	1.5	0.08	44	200

Characterization of the prepared microspheres

Surface morphology (SEM): The surface morphology and texture of the prepared microspheres were determined using scanning electron microscope (SEM). A small amount of each sample was spread on aluminum stub and coated with gold then placed in SEM chamber using SEM (JEOL-JSM-5200 LV, Japan). SEM photomicrograph was taken at acceleration voltage of 25 KV.

Percentage-yield: The prepared microspheres were collected after drying and weighed [27]. Percentage yield of the microspheres was calculated as follow:

% yield of prepared microspheres = (actual weight of the product/total weight of excipients and drug) × 100

Fourier transformed infrared spectroscopy (FT-IR): Interaction between drug and polymers was investigated using IR spectrophotometer. IR spectroscopy was performed using Fourier- transform infrared spectrophotometer, (Jasco, Japan). Eudragit L100-55, eudragit Rs100, ethyl cellulose, aerosil, piroxicam, prepared formulations and physical mixture between drug and different polymers spectrum were recorded using FTIR spectrophotometer. Samples were mixed with potassium bromide (spectroscopic grade) and compressed into disks using hydraulic press before scanning between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} [28].

Entrapment efficiency: The entrapment efficiency (%) of the prepared microspheres was evaluated using the method of Gangadhar et al. [29]. with certain modification. 25 mg of each prepared formula were crushed into powder and were completely dissolved in 100 ml of phosphate buffer solution (pH 7.4) using magnetic stirrer. 5 ml of the obtained solution was filtered using syringe filter (0.45 µm) and the concentration of the drug was determined spectrophotometrically at 353 nm after appropriate dilution [30,31]. The actual drug loading and encapsulation efficiency (EE %) were calculated using the following equations:

Encapsulation efficiency (%) = (Actual drug loading/Theoretical drug loading) × 100

Differential scanning calorimetry (DSC): DSC studies were performed using a DSC Perkin Elmer with thermal analyzer. A known weight of the test sample was loaded in aluminum pans which were crimped and mounted on the DSC before heating under nitrogen flow (20 ml/min). Thermal results were recorded while heating from 30 to 400°C at a heating rate of 10°C/min. An empty aluminum pan was used as a reference. DSC thermograms of pure substances, their physical mixture and drug loaded microspheres were recorded.

***In vitro* drug release study**

In vitro drug release from the prepared microspheres was performed at different pH values (1.2 and 6.8) at 37 ± 0.5°C. The release of piroxicam from microspheres was determined using type II dissolution apparatus (Copley, NG 42JY, Nottingham, UK). Microspheres equivalent to 20 mg were weighed and added to 900 ml of dissolution medium with a stirring rate of 100 rpm. For microspheres having solid dispersion structure release was measured at pH 1.2 for 1 h. The pH of the dissolution medium was kept at 1.2 for 2 h then adjusted to 6.8 for 4 h to evaluate release of piroxicam from microspheres containing eudragit Rs100 and ethyl cellulose. Samples (5 ml) were withdrawn from the dissolution medium at various time intervals and replaced with 5 ml fresh media to keep sink conditions. The amount

of drug released at each time interval was calculated and the cumulative amount of drug released was calculated as a function of time to construct the drug release profile.

Release kinetics studies

To determine the possible release mechanism of different prepared formulations the release data was fitted to different kinetic models. Thus, the release data was fitted to zero order, first order and Higuchi kinetic models [32].

***In vivo* ulcerogenicity studies**

Animals, treatment and collection of tissue samples: Male Wistar-strain rats weighing (160-180) g were obtained from National researches center (Cairo, Egypt). In vivo ulcerogenicity studies were conducted according to the procedure reported by previous study with some modifications [33].

Animals were maintained at 22 ± 1°C with 12 h light/dark cycle using galvanized wire cages and allowed rat chow and water *ad libitum* for 14 days to get adapted to laboratory conditions. *In vivo* experimental protocols were approved by the Animal Care and Use Committee and were in accordance with all recommendations in the University Guide for the Care and Use of Experimental Animals.

The animals were divided into four groups each containing 6 animals (n=6). Animals were fasted 40 h with free access to water [34]. The first group of animals is the control group, the second group of animals was treated with free piroxicam (30 mg/kg), the third group of animals was treated with piroxicam microspheres containing eudragit S100 in the ratio (1:3) in a dose equivalent to (30 mg/kg) of piroxicam while the fourth group of animals was treated with piroxicam microspheres containing aerosil and eudragit L100-55 in the ratio (1:4:2) in a dose equivalent to (30 mg/kg) of piroxicam.

Piroxicam and prepared formulae were administrated orally to each corresponding group as 1 ml suspension by oral gavage using an intubation needle fitted onto a syringe of appropriate size in a dose equivalent to 30 mg/kg of piroxicam or its equivalent in different formulations [35,36].

6 h later, each animal was removed from its cage, anaesthetized with ether and the abdomen was opened. Each stomach was excised, dissected along the greater curvature and contents were emptied by gently rinsing with isotonic saline solution [37].

Macroscopic examination of gastric ulcers: After the animals were sacrificed, each stomach was pinned out on a flat surface with the mucosal surface uppermost. Then a 10x binocular magnifier was used to examine and assess presence of hemorrhagic lesions and/or gastric ulcers expressed as the ulcer incidence.

The number of erosions per stomach was assessed for severity according to the scoring system described [38]. The grade of lesions was scored according to the following scale- 0: no pathology; 1: small (1-2 mm ulcers); 2: medium (3-4 mm ulcers); 4: large (5-6 mm ulcers); 8: ulcers (greater than 6 mm). The sum of the total ulcer scores in each group of rats was divided by the number of animals in the group to give the mean ulcer index for that group.

Histopathological examination of stomach sections: The collected stomachs samples were fixed overnight in 10% w/v buffered formalin. Each specimen was sectioned, processed overnight and then embedded in paraffin. The paraffin blocks were sectioned and the slides were stained with a standard haematoxylin and eosin stain then photographed under 20x magnifications using a Nikon Eclipse 80i light microscope (Nikon Corporation, Japan) [39].

RESULTS AND DISCUSSION

Surface morphology

SEM was used to investigate surface morphology and texture of the obtained microspheres. The prepared microspheres showed dense texture. Presence of pores in the internal matrix of the microspheres may be attributed to evaporation of organic solvent from firstly formed emulsion droplets which later form the microspheres. Some traces of aerosil particles may be found on the surface of the microspheres as illustrated in **Figure 1**.

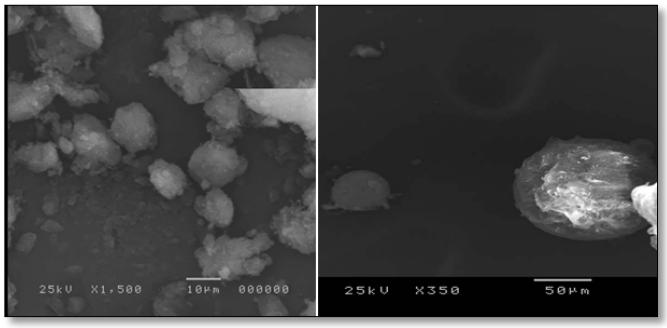

Figure 1. Scanning electron microphotograph of microspheres.

Percentage yield

The percentage yield of different formulations was represented in **Table 2** ranging from 45.21 ± 0.01 to 87.79 ± 0.01. The percentage yield of microspheres having solid dispersion structure is less than those containing controlling release polymers. Formula F3 and formula F4 having high percentage of aerosil has been found to have the least yield. This can be attributed to that aerosil have high porosity and specific surface area to act as dispersing agent may cause loss of the drug. By increasing polymer amount, percentage yield of the obtained formulations is increased. This was approved by work of other researchers who study the effect of the polymer concentration on the percentage yield of the resulting microspheres [40].

Table 2. The characteristics of the prepared microspheres.

Formula	Yield percent (%)	Actual drug loading (%)	Theortical drug loading (%)	Entrapment efficiency (%)
F1	61.25 ± 0.19	17.44 ± 1.11	50	34.87 ± 2.21
F2	62.13 ± 0.07	18.70 ± 2.35	33.33	56.13 ± 7.06
F3	45.21 ± 0.01	15.49 ± 1.32	50	30.99 ± 2.65
F4	57.73 ± 0.18	7.95 ± 0.30	33.33	23.87 ± 0.89
F5	73.69 ± 0.01	6.72 ± 0.26	25	26.88 ± 1.05
F6	59.42 ± 0.05	8.65 ± 1.27	20	43.26 ± 6.36
F7	87.79 ± 0.01	5.81 ± 0.33	16.67	34.88 ± 1.98
F8	81.25 ± 0.00	8.33 ± 0.42	25	33.30 ± 1.66
F9	85.52 ± 0.04	6.82 ± 0.89	20	34.09 ± 4.49
F10	78.47 ± 0.04	6.74 ± 0.61	16.67	40.45 ± 3.65

Each result is the mean of 3 determinations ± SD

Entrapment efficiency

Entrapment efficiency varies according to polymer type, drug to polymer ratio and aerosil percentage as shown in **Table 2**. Effect of aerosil on entrapment efficiency is due to the fact that aerosil particles have high porosity and large specific surface area leading to drug loss during evaporation of organic solvent within the preparation process. Thus increasing amount of aerosil decreases entrapment efficiency as shown for formula F2 which has the highest entrapment efficiency (about 56%) having the least amount of aerosil and the highest amount of eudragit L100-55. Increasing polymer percentage increases the entrapment efficiency due to better coating of drug resulting from precipitation of polymer on the surface of the dispersed phase which leads to preventing of drug diffusion across the phase boundary [41]. Similar results were obtained by Mehta et al. [42] and Sharma et al. [43].

Fourier transformed infrared spectroscopy (FTIR)

The FTIR spectra of piroxicam, polymers and the different formulae **(Figure 2)** shows the drug characteristic peaks, including carbonyl and second amide group at 1632 cm^{-1} and 1529 cm^{-1}, respectively which indicate presence of intramolecular hydrogen bond within piroxicam structure which come in accordance with published data of the same compound [44,45].

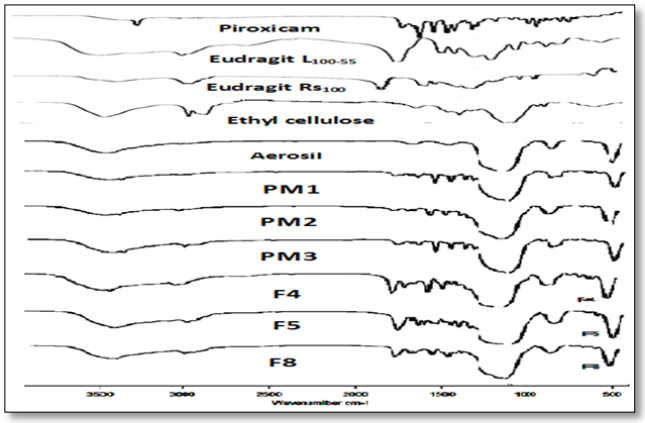

Figure 2. FTIR of piroxicam, eudragit L100-55, eudragit Rs100, ethyl cellulose, aerosil, PM1, PM2, PM3 (physical mixture of formula F4, F5 and F8 components) and prepared microspheres (formula F4, F5 and F8).

For eudragit L100-55 characteristic IR peaks are 1736 cm^{-1} due to esterified carboxyl group vibration, 1182 cm^{-1} and 1268 cm^{-1} peak are due to ester vibration [46]. Aerosil show characteristic peaks at 1110 cm^{-1} and 3426 cm^{-1}.

Accordingly, the results ruled out the possibility of disappearance of intramolecular hydrogen bonding as 1632 cm^{-1} stretching peak which is involved in the formation of this intramolecular hydrogen bond shifted to higher value 1642 cm^{-1}. For IR spectrum of microspheres containing eudragit Rs100 presence of 1527 cm^{-1}, 1601 cm^{-1}, 1329 cm^{-1} peak may be due to intermolecular interaction between drug and polymer. The same results were obtained by other investigators studying interaction between piroxicam and eudragit polymers [47]. Physical mixture spectrum indicates

only the summation of different components of microspheres.

Differential scanning calorimetry (DSC)

Piroxicam shows a sharp endothermic melting peak at 200.09°C that indicates its crystalline nature that correlated with published data [48,49]. Preparation of piroxicam as solid dispersion structure microspheres with eudragit L100-55 reduced the melting temperature (T_m) of the drug and lowered the enthalpy of the endothermic peak so that the melting transition of the drug almost disappeared for formula F4. This effect can indicate possible transformation of the drug from crystalline to amorphous form. Other formulations containing eudragit Rs100 and ethyl cellulose polymers show disappearance of melting transition peak of piroxicam indicating presence of drug in amorphous form. Melting of piroxicam could be observed in physical mixtures of drug and other polymers. This indicated drug: polymer solid state interaction induced by heating which is similar to DSC results published by other researchers for solid dispersion of piroxicam with polyvinylpyrrolidone [50,51] **(Figure 3)**.

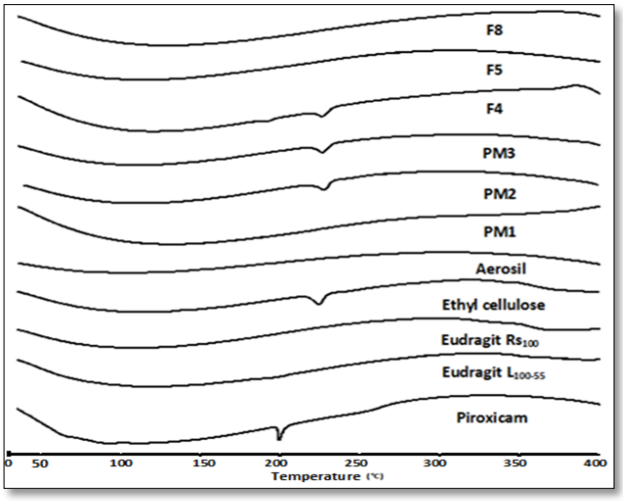

Figure 3. DSC thermogram of piroxicam, eudragit L_{100-55}, eudragit Rs_{100}, ethyl cellulose, aerosil, PM1, PM2, PM3 (physical mixture of formula F4, F5 and F8 components) and prepared microspheres (formula F4, F5 and F8).

***In vitro* release results**

According to biopharmaceutical classification system piroxicam is a class II drug having low solubility and high permeability so drug release is a crucial and a limiting step for oral drug bioavailability particularly for drugs with low gastrointestinal solubility and high permeability. By improving the drug release profile of these drugs it is possible to enhance their bioavailability and reduce side effects [52].

As piroxicam has both acidic and basic groups, its solubility is pH dependent so the difference in the degree of the dissolution of the drug is dependent on the ionization of the drug at different pH values [53]. Release of the drug at different pH values is illustrated in **Figure 4**.

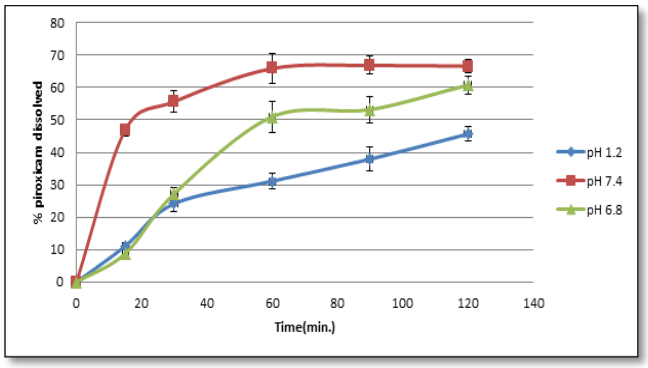

Figure 4. Dissolution profiles of piroxicam in a free form at different pH values.

Microspheres have in their structure eudragit L100-55 as solid dispersing carrier and aerosil as drug dispersing and anti-adhesion agent. **Figure 5** represents dissolution of microspheres having solid dispersion structure. It was found that increasing amount of aerosil or eudragit L100-55 increases release rate of the drug from microspheres. The function of eudragit L100-55 as a dispersion agent for dug is more significant than that of aerosil. Aerosil has large specific surface area for the drug dispersion which decreases hydrophobicity of the drug and also increase porosity of microspheres contributing to improving release rate of drug from microspheres while its property of insolubility might not contribute to form the drug dispersion so much as eudragit L100-55 polymer. The formula showing solid dispersion properties was the formula containing drug, aerosil and eudragit L100-55 in the ratio of 1:4:2 where the drug release was greatly enhanced.

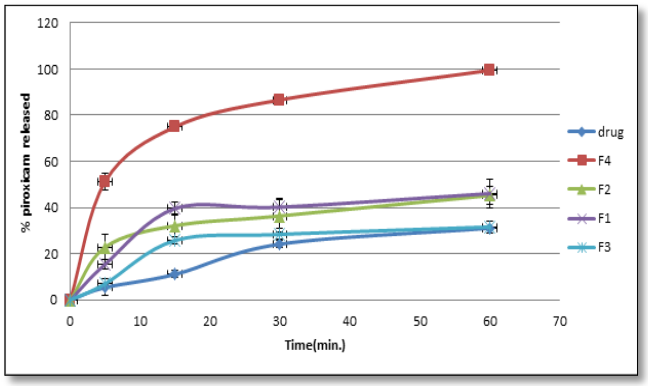

Figure 5. The release profile of piroxicam from various microspheres at pH 1.2.

For controlling drug release from the previously prepared microspheres, eudragit Rs100 and ethyl cellulose was added as retarding agent for solid dispersion structure formula in different ratios. According to the ratio between eudragit

L100-55 and retarding polymers (eudragit Rs100 and ethyl cellulose) the drug release rate from microspheres could be controlled. **Figure 6** indicates dissolution of those microspheres having retarding polymer at pH 1.2. It was found that formula F6 show the least release at pH 1.2 about (38.2%) and F9 shows the highest release about (66.4%) then the release of different formulations was continued for another 4 h at pH 6.8. **Figure 7** shows dissolution of microspheres having retarding polymer at pH 6.8. The release decreased from formula F5 to F7 for formulations containing eudragit Rs_{100} with formula F7 showing release about (88.4%) for 4 h at pH 6.8. formula F8 is the formula showing the least release and the most retarding effect about (83.3%) of drug released at pH 6.8 for 4 h indicating that the retarding ability of ethyl cellulose was higher than that of eudragit Rs_{100}. These release patterns were similar to that investigated by Cui et al. [26].

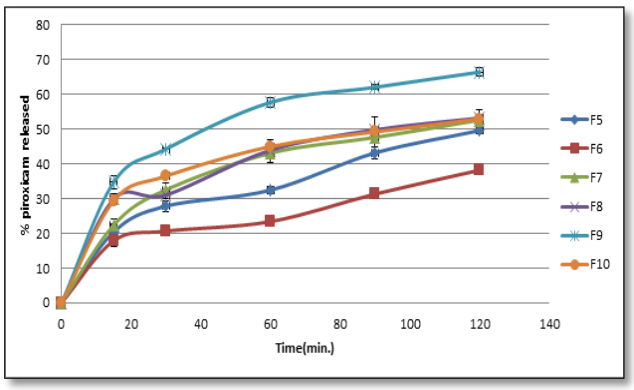

Figure 6. The release profile of piroxicam from microspheres containing eudragit Rs_{100} and ethyl cellulose polymers at pH 1.2.

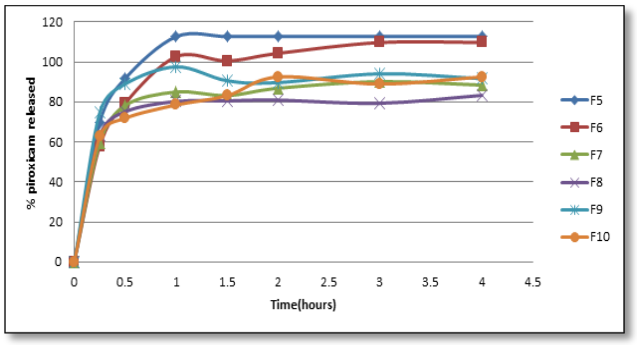

Figure 7. The release profile of piroxicam from microspheres containing eudragit $Rs_{10}0$ and ethyl cellulose polymers at pH 6.8.

The results of the kinetics of drug release are presented in **Table 3** which is obtained by fitting the release data to different kinetic models.

Table 3. The recorded correlation coefficient after fitting the release data to different kinetic models.

Formula	Release kinetics		
	Zero order	1st order	Higuchi
F1	0.6505	0.4328	0.8743
F2	0.7008	0.3940	0.9229
F3	0.6861	0.5145	0.8789
F4	0.6615	0.3616	0.9041
F5	0.8784	0.5160	0.9869
F6	0.8549	0.5099	0.9635
F7	0.8220	0.4910	0.9797
F8	0.7771	0.4550	0.9544
F9	0.7525	0.4416	0.9507
F10	0.7307	0.4365	0.9389

From **Table 3** it was revealed that the drug release follows Higuchi kinetic model in all of the formulations. This result was concluded from the higher correlation coefficient of Higuchi kinetic model compared with that of other models. As the release data fitted to Higuchi matrix kinetic model then the drug release is diffusion controlled. Similar results were obtained by Liu et al. [54] and Babay et al. [55].

***In vivo* ulcerogenicity studies**

Macroscopic analysis: Experimental design, animal groups as well as ulcer incidence and ulcer index of different formulations are illustrated in **Table 4**.

Table 4. Macroscopic results of gastric ulcers of different formulations.

Group No.	Treatment	Ulcer incidence	Ulcer index
I	Control group	0% (0/6)	0.0 ± 0.0
II	Piroxicam 30 mg/kg	100% (6/6)	2.66 ± 0.35
III	Piroxicam:eudragit S_{100} (1:3) 30 mg/kg	50% (3/6)	0.83 ± 0.23
IV	Piroxicam:aerosil:eudragit L_{100-55} (1:4:2) 30 mg/kg	83% (5/6)	1.33 ± 0.30

Figure 8 shows macroscopic observations of stomach mucosa of the animals of different groups which differ according to presence or absence of hemorrhagic lesions.

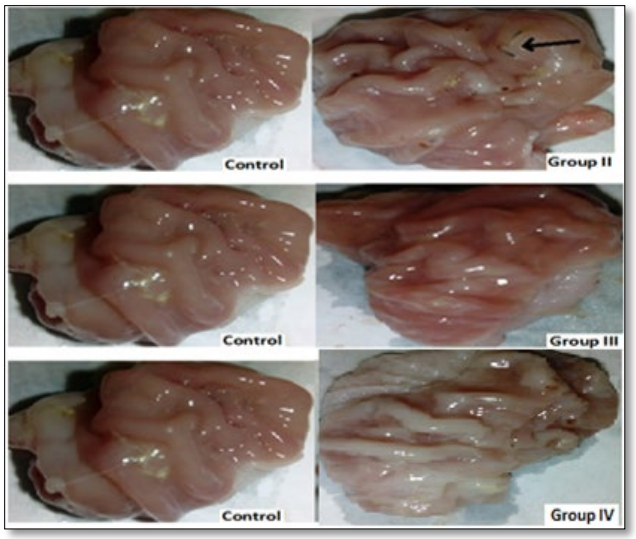

Figure 8. Macroscopic observations in stomach mucosa of rats of different groups.

As shown in **Figure 8** the ulcer incidence is represented by presence of hemorrhagic lesions. The control group shows normal mucosa without any hemorrhagic lesions on its surface while mucosa of the group treated with piroxicam free drug showed appearance of wide spread of hemorrhagic area indicated by dark red spots which are blood clots. The ulcer incidence for piroxicam treated group (group II) was 100% compared with control group (group I) which has ulcer incidence 0%. On the other hand the group treated with eudragit L_{100-55} containing piroxicam microspheres (group IV) has ulcer incidence of 83% with spots of hemorrhagic area beside lesions. Eudragit S100 containing piroxicam microspheres (group III) was the group of the least ulcer incidence (50%) as it showed suppression of gastric ulcer more than group II and group IV as illustrated in **Table 4**.

Histopathological analysis: The results of the histopathological analysis of different stomach specimens of the animals of different groups after investigation under microscope are illustrated in **Figure 9**.

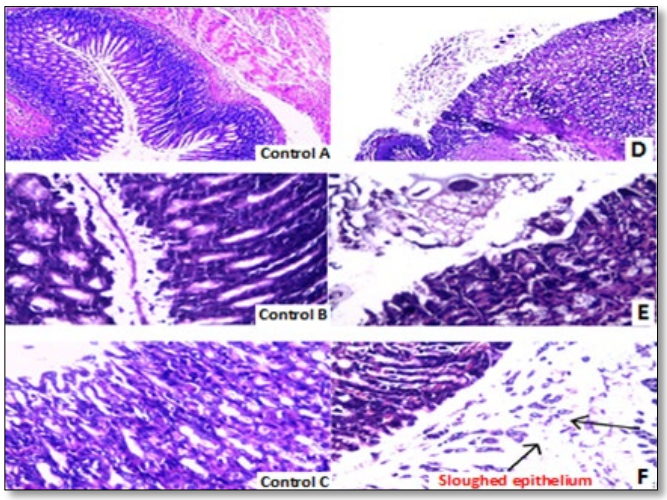

Figure 9. Histopathological photographs of stomach specimens stained with hematoxylin and eosin: A (H&E*250), B and C (H&E*400) from control rats. D (H&E*250) from rats treated with piroxicam (30 mg/kg). E from rats treated with eudragit S_{100} containing piroxicam microspheres. F from rats treated with eudragit L_{100-55} containing piroxicam microspheres.

The histological pattern of the tested groups was studied to assay its ulcerogenic effect. Gastric mucosa of control group represented by **Figure 9A** which displays normal control group gastric mucosa (covering epithelium gastric glands and intact musculature) while **Figure 9B** shows higher magnification of **Figure 9A** having normal epithelium and

Figure 9C was that of gastric mucosa showing normal covering epithelium and normal parietal cells.

Piroxicam treated rat (group II) represented by **Figure 9D** show stomach mucosa of gastro-esophageal junction of the treated group with piroxicam (30 mg/kg) after 6 h having focal superficial degeneration, congestion and sloughing of gastric mucosa with wide inflammatory cellular infiltration and dense mononuclear cell infiltration, respectively.

Figure 9E (group III) show stomach mucosa of animals treated with microspheres containing piroxicam and eudragit S_{100} (equivalent to 30 mg piroxicam/kg) after 6 h. It shows gastric mucosa having (mild mucosal edema with minimal sloughed area and vascular congestion).

Figure 9F (group IV) show stomach mucosa of animals treated with microspheres containing piroxicam and eudragit $L_{100\text{-}55}$ (equivalent to 30 mg piroxicam/kg) after 6 h. It shows superficial diffuse, sloughing of the covering epithelium, severe congestion and inflammatory cellular infiltration of (group IV). So according to the obtained results it was found that group III of microspheres containing eudragit S_{100} and piroxicam was the group of decreased in vivo gastric ulcerogenic activity compared to other groups.

CONCLUSION

Microspheres of piroxicam with different polymers of eudragit $L_{100\text{-}55}$, eudragit Rs_{100} and ethyl cellulose were successfully prepared. It was found that eudragit $L_{100\text{-}55}$ was effective as solid dispersing carrier for preparation of piroxicam microspheres having solid dispersion structure. Eudragit Rs_{100} and ethyl cellulose were added to control dissolution rate of piroxicam microspheres. So it was concluded that increasing or decreasing the ratio of eudragit $L_{100\text{-}55}$ to that of eudragit Rs_{100} and ethyl cellulose resulted in preparation of piroxicam microspheres with desired dissolution results. Comparing the *in vivo* gastric ulcerogenic effect of eudragit S_{100} containing piroxicam microspheres and eudragit $L_{100\text{-}55}$ containing piroxicam microspheres to that of free piroxicam, it was found that eudragit S_{100} containing formula was the formula of the least ulcer incidence and minimal sloughed mucosal and vascular congestion.

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