Formulation, *in-vitro* and *in vivo* evaluation of ketorolac tromethamine controlled release drug delivery system

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ABSTRACT

Ketorolac tromethamine is a potent non-steroidal drug with potent analgesic and anti-inflammatory activity. Its oral administration is associated with a high risk of adverse effects such as irritation, ulceration and bleeding of gastrointestinal tract. The present study focuses on the development of controlled release drug delivery system of ketorolac microcapsules as one of the multi-particulate formulations and are prepared to obtain prolonged drug delivery in order to decrease ulcerogenicity, improve bioavailability and stability and also target a drug at specific sites. Microcapsules were prepared by air suspension technique using Eudragit RS100, Eudragit RL100 and ethyl cellulose in ratios of 1:1,1:2 and 1:3 drug to polymer. The effect of various formulation and process variables on the flowability, drug loading and *in-vitro* drug release was studied. IR, DTA and X-ray investigations revealed that there was no interaction between the drug and the polymers used. The flow characteristic showed Hausner's ratios of <1.25 and Carr's index of 5-16 % of the systems prepared while those of the drug alone were >1.25 and > 40% indicating good and excellent flow of the systems and extremely poor flowability of the drug. Ketorolac tromethamine content in different microcapsules formulations was not affected by neither the polymer type nor drug to polymer ratio and ranged between 98 -99.5 %. The *in-vitro* drug studies showed that amounts of drugs released from Eudragit RS100 based microcapsules at pH 1.2 were 38 , 22, and 14 % from drug to polymer ratios of 1:1, 1:2 and 1:3 respectively. Amounts of drug released at pH 7.4 were 72, 70, and 51% respectively. The amount of drug released from Eudragit RL100 based microcapsules at pH 1.2 were 35, 27and 19% drug to polymer ratios of 1:1, 1:2 and1:3 respectively. Amounts of drug released at pH 7.4 were 63, 61 and 52 %. The amount of drug released from ethyl cellulose based microcapsules at pH 1.2 were 22, 16 and 13% drug to polymer ratios of 1:1, 1:2 and 1:3 respectively. Amounts of drug released at pH 7.4 were 75, 68 and 58% respectively. The *in-vitro* release studies showed that the release rate of the drug has been modified. This study presents a new approach based on microencapsulation technique for obtaining modified release drug delivery system. The enteric-coated formulations which showed best release profiles were tested *in vivo*. The enteric-coated formulations using Eudragit RS100, Eudragit RL100 and ethyl cellulose at a ratio of (1:3) significantly suppressed ulceration by avoiding the exposure of ketorolac to ulcer prone area of gastro-intestinal tract.

Key words: Ketorolac microcapsules, ketorolac controlled release system, Enteric coated ketorolac tromethamine, Air suspension technique, Ketorolac delivery system

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs worldwide and their analgesic, anti-inflammatory and anti-pyretic therapeutic properties are thoroughly accepted. More than 30 million people use NSAIDs every day, and they account for 60% of the US over-the-counter analgesic market. Like many other drugs, however, NSAIDs are associated with a broad spectrum of side effects, including gastrointestinal (GI) and cardiovascular (CV) events, renal toxicity, increased blood pressure, and deterioration of congestive heart failure among others. In this review, we will focus on upper and lower GI tract injury [1].

The damage of gastric and duodenal mucosa caused by NSAIDs has been widely studied. These upper GI side effects include troublesome

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symptoms with or without mucosal injury, asymptomatic mucosal lesions, and serious complications, even death. About 30 to 50% of NSAID users have endoscopic lesions (such as subepithelial hemorrhages, erosions, and ulcerations), mainly located in gastric antrum, and often without clinical manifestations. Generally, these lesions have no clinical significance and tend to reduce or even disappear with chronic use, probably because the mucosa is adapted to aggression (2, 3).

On the contrary, up 40% of NSAIDs users have upper GI symptoms, the most frequent being gastro-esophageal reflux (regurgitation and/or heartburn) and dyspeptic symptoms (including belching, epi-gastric discomfort, bloating, early satiety and postprandial nausea) (2). Ketorolac tromethamine is a potent non-steroidal analgesic drug, frequently used for treatment of rheumatoid arthritis, osteoarthritis, a variety of other acute and chronic musculoskeletal disorders and mild to moderate pain (4-6). The drug acts as anti-inflammatory by inhibiting prostaglandin synthetase cyclooxygenase. It is 36 times more potent than phenyl butazone, and twice as that of indomethacin (7).

Ketorolac tromethamine is used clinically for the management of post-operative and cancer pain. Previous studies have suggested that ketorolac·s analgesic efficacy may be greater than other non-steroidal anti-inflammatory drug sand comparable with that of morphine in models of acute pain (8-12). Regardless of the route of administration, the biological half-life of the drug ranges from 4 to 6h in human (13), its oral bioavailability is estimated to be 80 %. The oral administration of ketorolac tromethamine is associated with high risk of adverse effects such as irritation, ulceration, bleeding of gastrointestinal tract, edema as well as peptic ulceration (14). These attributes make ketorolac tromethamine a good candidate for controlled release dosage form , so as to ensure slow release of the drug eliminating the disturbance of the gastrointestinal tract.

Drug delivery system can control the release of the drug and targets drug moiety to a specific site in the body and has a great impact on the human health care system. The use of solid dispersion of a drug and certain polymers is an exclusive technology that has been used for the controlled release of drugs for increasing patient compliance. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivery of a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microcapsules as carriers for drugs. Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsule. In a relatively simplistic form, a microcapsule is a small sphere with a uniform wall around it. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell coating or membrane. Most microcapsules have diameters ranging from less than one micron to several hundred microns in size (15-17).

Considerable evidences exist in the literature to demonstrate the effect of formulation on the efficacy of orally administered pharmaceuticals (18-20).Various physicochemical properties of the formulation contribute directly to the release and subsequent absorption of the drug to affect its onset, intensity, residence and duration of action (21-23). Various polymeric coatings have been investigated to control the release of drugs in the GIT (17, 18, 23, and 24). Acrylic resin and celluloses have been used to encapsulate drug particles and to obtain microcapsules with certain physicochemical properties and/or to alleviate certain side effects of the parent drug (26-30).

Several types of acrylic resin polymers with distinct structures and properties were developed for coating. These polymers, commercially available under the trade name Eudragits, are physiologically inert, resist enzymatic attack and are not absorbed by digestive tract. The cationic types Eudragit-E is soluble under weak acidic conditions and can be used for film coating or to isolate incompatible ingredient. The anionic types Eudragit-L and Eudragit-S are used as enteric coating agents and can be used to influence the dissolution rate of drugs in intestine. The permeable polymers, Eudragit retard-L and Eudragit retard-S are insoluble in the pH range of the digestive tract but swell in an aqueous medium and exhibit a distinct permeability for water as well as water soluble drugs. These permeable polymers are used in the formulation of controlled release medication. Ethyl cellulose is the ethyl ether of cellulose; it is mainly used either alone or with other polymers in formulations required for extended or controlled release. Microencapsulation technology involves a basic understanding of the general properties of microcapsules, including the nature of the core and coating material, the release characteristics of the coated particles and the

method chosen for microencapsulation and also on the properties of the obtained microcapsules **(14, 16, 31-33)**.

The main objective of this work was to obtain controlled release ketorolac tromethamine formulation by microencapsulation of the drug by the fluidized bed technique **(25, 26)** using Eudragit RS100, Eudragit RL100 and ethyl cellulose as coating materials in different proportions (1:1, 1:2 and 1:3) drug-polymer ratios. Investigation of the effect of various processing and formulation factors such as drug to polymer ratio, nature of polymer on the yield production, flowability of the product , *in-vitro* release rate of the drug from the obtained microcapsules were performed. The possibility of the presence of interaction between the drugs and polymers was determined by infrared spectral analysis (IR), differential thermal analysis (DTA) and X- ray as well as the ulcerogenic activity of the drug were evaluated and compared with those of the uncoated drug.

MATERIALS AND METHODS

Materials: Ketorolac tromethamine (Sigma-Aldrich, St. Louis, Mo, USA) was a gift sample kindly supplied by Amriya pharmaceuticals industries, Alexandria, Egypt, Eudragit RL100 and Eudragit RS 100 were purchased from RÖhm Pharma GMBH, Darmstadt (Germany), Ethyl cellulose was obtained from Sigma- Aldrich Chemi (Germany). All other reagents and chemicals were analytical grades and were used as received.

Coating of Ketorolac tromethamine with Eudragit RS100, Eudragit RL 100 and Ethyl cellulose:

Preparation of the coating solution: Coating solutions with concentration of 5% w/v Eudragit RS100, Eudragit RL100 or Ethyl cellulose in acetone-isopropyl alcohol mixture (1:1) were prepared by dissolving 30gm of each Eudragit RS100, Eudragit RL100 or Ethyl cellulose separately in 200ml solvent mixture **(25,26)**.

Coating technology: Reviewing the literature about air suspension technique revealed that microencapsulation by this technique reduces processing time and improves the product properties. It was also proven to be more convenient method especially in case of thermolabile materials. The process consists simply of supporting 30gm drug in the vertical container simply fluidized from below by a stream of air. The exhaust filter was shaken from time to time to keep the entire drug inside the container. After adjusting the atomized compressed air, the solution of 5%

w/v of either Eudragit or Ethyl cellulose in acetone-isopropyl alcohol mixture (1:1) was sprayed over the bed. The spraying pump was adjusted to be 10 rpm to give a suitable droplet size from the sprayed solution. The temperature was maintained at 35-40º C during the coating process.

The volume of the solution needed to produce the desirable microcapsules was 200ml. When the microcapsules have been formed, the spray was turned off and the product was left to fluidize inside the apparatus for about 60 minutes for complete drying at the same temperature. The same procedure was followed to obtain 1:2 and 1:3 drug to polymer ratios. The encapsulated particles were stored in a desiccator over anhydrous calcium chloride for 48hrs before any further study. Table (1) shows the operating condition in coating ketorolac tromethamine powder

Infrared spectral analysis: The IR spectrum was used to determine the interaction of the drug with the polymers used. The infrared spectra of samples were obtained using a spectrophotometer (FTIR, Jusco, Japan). Samples were mixed with potassium bromide (spectroscopic grade) and compressed into discs using hydraulic press before scanning from 4000 to 400 cm^{-1}

Differential Thermal Analysis (DTA): The physical state of drug in the microspheres was analyzed by Differential Thermal Analyzer (Mettler-Toledo star 822e system, Switzerland). The thermo grams of the samples were obtained at a scanning rate of 10°C/min conducted over a temperature range of 25-220°C, respectively.

X-ray Diffractometry (XRD): X-ray Diffractometry of ketorolac microspheres were performed by a diffractometer using model (Joel JDX-8030, Japan) equipped with a graphite crystal monochromator (Cu-Kα) radiations to observe the physical state of drug in the microspheres.

Scanning Electron Microscopy (SEM): The shape and surface morphology of ketorolac loaded microspheres were studied using (Jeol, JSM-840A scanning electron microscope, Japan). The gold coated (thickness 200Å; Jeol, JFC-1100E sputter coater, Japan) microspheres were subjected to secondary imaging technique at 150 tilt,15mm working distance and 25 Kv accelerating voltage.

Flowability testing: The flowability of the prepared powders was tested by measuring their angle of repose as well as the calculation of Carr's compressibility index.
Measuring the angle of repose: The fixed height cone method was adopted **(34)** where the diameter

of the formed cone (d) was determined according to the following equation:

$$\text{Tan}\,\theta = \frac{2h}{d}$$

Where (h) and (d) are the height and the diameter of the cone respectively.

Determination of Carr's compressibility index: A fixed weight of the powder either drug or microcapsules was poured in a 25 ml graduated cylinder , the powder was allowed to settle with no outer force and the volume occupied was measured as V_I (initial bulk volume).The cylindrical graduate was then tapped on a plan surface at a one inch distance till a constant volume was obtained . The tapped volume of the powder was then recorded as (V_T). The initial and tapped bulk densities were then calculated according to the following equation:

Initial Bulk Density $\quad \rho_I = \dfrac{M}{VI}$

Tapped Bulk Density $\quad \rho_T = \dfrac{M}{VT}$

Where (M) is the mass of the powder. The percentage compressibility (Carr's index) was then determined from the following equation:

Carr's index = $\dfrac{\rho T - \rho I}{\rho T}$

Drug content determination: Percentage yield can be determined by calculating the initial weight of raw materials and the finally obtained weight of microcapsules. Percentage yield can be calculated by using the formula:

Percentage yield = $\dfrac{\text{Percentage yield}}{\text{Theoritical yield}} \times 100$

Accurately weighed microcapsules were taken in a stoppered test tube and extracted with 5×10 ml quantities of phosphate buffer pH 7.4.The extracts were filtered and collected into 100 ml of volumetric flask and made up to the volume with phosphate buffer (pH 7.4). The solutions were subsequently diluted suitably with pre warmed phosphate buffer (pH 7.4) and spectrophotometric absorbance was recorded at 323 nm **(35)** (UV-Visible recording spectrophotometer, SHIMADZU (UV-160A) (Japan). Percentage drug entrapment and the percentage entrapment efficiency (PEE) were calculated by the formula given below **(36-38)**.

PEE = $\dfrac{\text{Drug loading microcapsules}}{\text{Theoritical drug loading}} \times 100$

***In-vitro* drug release studies:** The release rate of ketorolac tromethamine microcapsules was studied using USP dissolution test apparatus employing paddle type (Paddle type , Copley , England). Accurately weighed samples of microcapsules were used which were calculated to contain 10 mg of the tested drug. They were placed in 900 ml of dissolution media (two types pH 1.2, 0.1 N HCL and pH 7.4 phosphate buffers). Paddle speed of 100 rpm and temperature of 37.5°C ± 0.2 was employed. Aliquots (5ml) were withdrawn , filtered through 0.45 membrane filter at 5, 10, 15, 20, 30, 45, 60, 90,120,150 and180 min. and replaced with equal volume of pre warmed fresh medium to maintain constant volume and keep sink condition **(39)**. The drug concentration and the percentage drug released were determined with respect to time spectrophotometrically at 323 nm **(39)**. The *in-vitro* dissolution studies were performed in triplicate for each sample and the results were reported as mean ± SD.

***In-vivo* Ulcerogenicity Studies:**

Experimental Animals: Male Wister rats, weighing 180-200gm, were obtained from National researches center (Cairo, Egypt). Rats were maintained at 22±1°C on a 12h light-dark cycle allowed rat chow and water ad libitum. Five groups of rats (n=6 animals per groups) were used. The allocation of animals to all groups was randomized. *In-vivo* experimental protocols had the approval of the institutional animal ethics committee (IAEC) (IAEC/PROPOSAL/DB-4/2010). Before the start of the experiments, rats were housed individually in wire mesh cages to avoid coprophagy under controlled environmental conditions. Food was withdrawn for 36h **(40)** but water was allowed ad libitum. The absence of ulcers in some of the treated groups has revealed that the pre-fasting conditions alone doesn't induce ulcer. As described in the studies **(41-43)**, on the morning of the experiments each fasted rat was orally administered 1 ml suspension of the assigned drug by oral gavage in a dose equivalent to 5 mg per kg of ketorolac tromethamine or different ketorolac microcapsules systems. Magnetic stirring was utilized to obtain a well-dispersed of each drug and microcapsules suspension. Six hours later **(44)**, each animal was removed, anaesthetized with ether, and the abdomen was opened. Each stomach was excised, dissected along the greater curvature and contents were empties by gently rinsing with isotonic saline solution. Each stomach was pinned out on a flat surface with the mucosal surface uppermost.

Macroscopic examination of gastric ulcers: The ulcer incidence represented as hemorrhagic lesions and gastric ulcers were examined and assessed macroscopically with the help of a 10x

binocular magnifier immediately after the animals were sacrificed. To quantify the induced ulcers in each stomach, the scoring systems described in the literature **(44)** was employed. The induced ulcers in these experiments were in the form of small spots punctiform lesions and thus each was given a score between 1 and 4.Ulcers of 0.5 mm diameter were given a score of 1 whereas ulcers of diameters 1 and 2mm were given scores of 2 and 4, respectively. Stomach with no pathology was assigned a score of zero. For each stomach, an ulcer index was calculated as the sum of the total score of ulcers. Six determinations were made for each drug suspension administered. The average ulcer index is presented as the mean (n=6) ± standard error.

Histopathological examination of stomach sections: For histological examination, the stomach was surgically extirpated from each group and opened through vertical incision along the greater curvature and photographs were taken of the inside surface of the stomach. The stomach tissues were then washed in 0.9% salaine and a portion of it was kept in 10% buffered formalin for histopathological studies. The sections were then stained with hematoxylin and eosin. The tissue sections were examined under an Olympus BX51 (Olympus Corporation, Tokyo, Japan) microscope and images were captured with a digital camera attached to it.

Statistical analysis: All data are presented as the mean ± the standard error (S.E.).Significant differences between the percentages released of the drug measured during *in-vitro* dissolution studies also between the ulcer indexes of the different treatment groups following *in-vivo* study were determined by one-way analysis of variance (ANOVA) using the SPSS® statistical package (version 10, 1999, SPSS Inc., Chicago, IL). Statistical differences yielding P ≤ 0.5 were considered significant.

RESULTS AND DISCUSSION

Infrared spectral analysis: Infrared studies (Figures 1a, 1b, 1c and 1d) reveal that there is no appearance of new peaks and disappearance of existing peaks, which indicated that there is no interaction between the drug and the polymers used. The IR spectrum of ketorolac tromethamine exhibited peaks at $3350.01 cm^{-1}$ due to N-H and NH2 stretching and peaks at $1469.43 cm^{-1}$ and $1430.88 cm^{-1}$ due to C=C $1469.43 cm^{-1}$ and $1430.88 cm^{-1}$ due to C=C aromatic and aliphatic stretching, on the other hand, peak at$1383.19 cm^{-1}$ is due to-C-N vibrations, peak at $1047.59 cm^{-1}$ is due to -OH bending which confirms the presence of alcoholic group. Peaks at $702.09 cm^{-1}$, $725.54 cm^{-}$

^{1},$771.71 cm^{-1}$ and $798.11 cm^{-1}$ confirm C-H bending (Aromatic), thus confirms the structure of ketorolac tromethamine. IR studies show no interaction between drug and excipients. Addition peaks were absorbed in microcapsules which could be due to the presence of polymers and indicated that there was no chemical interaction between ketorolac tromethamine and other excipients. The spectra showed no incompatibility between the polymers and ketorolac tromethamine. The spectra of the polymers and the pure drug are given in the figures (1-a, 1-b, 1-c and 1-d).

Differential Thermal Analysis (DTA): In order to confirm the physical state of the drug in the microspheres, DTA of the drug alone, and drug loaded microspheres were carried out (Fig 2-a, 2-b, 2-c, 2-d and 2-e). The DTA trace of drug showed a sharp endothermic peak at 168.88°C, its melting point. The physical mixture of drug and blank microspheres showed the same thermal behavior 168.76°C as the individual component, indicating that there was no interaction between the drug and the polymer in the solid state. The absence of endothermic peak of the drug at 168.88°C in the DTA of the drug loaded microspheres suggests that the drug existed in an amorphous or disordered crystalline phase as a molecular dispersion in polymeric matrix **(45, 46)**.

X-ray diffractometry (XRD): In order to confirm the physical state of the drug in the microspheres, powder X-ray diffraction studies **(47)** of the drug alone, physical mixture of drug, and drug loaded microspheres were carried out. X-ray diffractograms (Fig. 3-a, 3-b, 3-c, 3d and 3-e) of the samples showed that the drug is still present in its lattice structure in the physical mixture where as it is completely amorphous inside the microspheres. This may be due to the conditions used to prepare the microspheres lead to cause complete drug amorphization.

Scanning Electron Microscopy (SEM): The surface morphology of the ketorolac tromethamine and ketorolac tromethamine loaded microspheres were studied by scanning electron microscopy (Fig. 4). SEM photograph of the drug indicated that the drug exists in crystal form. Surface smoothness of MS was increased by increasing the polymer concentration, which was confirmed by SEM.

Flowablity testing: The effect of coating of ketorolac tromethamine with different polymers on the flow properties of the drug is illustrated in Table (2). Table (2) shows the results of the flowablity testing of ketorolac tromethamine as well as the drug coated with Eudragit RS100, Eudragit RL100 and Ethyl cellulose it is clear that;

Carr's index for the drug alone was found to be 52.38 ± 0.18 %indicating extremely poor flow. The microcapsules based on Eudragit RS 100 showed an index between11.12 ± 0.92and 14.82± 1.73 %, while those based on Eudragit RL100 showed an index between 11.19 ± 0.86 and 14.66 ± 1.22%. The microcapsules based on Ethyl cellulose showed an index between 13.22± 1.86 and 15.16± 1.19% indicating excellent and good flow **(48)**. In all formulations, the ratio of 1: 3 drug to polymer showed the best flowability results. It is evident from Table (2) that the angle of repose decreases by inclusion of the drug in microcapsules. Also by increasing the ratio of the polymers, the angle of repose decreases i.e. flowability increases.

Drug content determination: Table (3) shows the results of ketorolac tromethamine content in different microcapsules formulation , it is clear that the percentage yield of different microcapsules formulations varied from 98.10 ±2.30% to 99.67 ±1.81%. From the results in the Table it is evident that drug to polymer ratio did not play any rule in the entrapment efficiency of the drug. This is in contrast to the results obtained by Trivedi *et al* **(48)** who reported that by increasing the polymer ratio in certain formulations from 1:1 to 1:5 was followed by increasing the drug entrapment efficiency. Swetha *et al* **(49)** prepared micro sponges containing etodolac with different types of polymers including Eudragit and ethyl cellulose. The authors proved that the ratio of the polymer in the delivery system has no effect on the percentage entrapment efficiency.

In-vitro **drug release studies:** The release profile of ketorolac tromethamine microcapsules prepared from different drug to polymer ratios at different values are shown in Figures (5-7). Figure (5-a) shows the release of the drug as well as the release of the drug from EudragitRS100 microcapsules (pH 1.2). It is clear that the drug percentages released all over the experimental time period (180 min) were 40.22 ± 0.66, 25.28 ± 1.90and 18.33 ± 1.36% from the ratios 1:1 ,1:2 and 1:3 drug to polymer respectively i.e. by increasing the polymer to drug ratio there is a significant decrease in the drug release (P < 0.05). Figure (5-b) shows the release of the drug as well as the release of the drug from Eudragit RS 100 microcapsules (pH 7.4). It is clear that the drug percentages released after 30min were 67.18 ± 1.23, 63.22 ± 1.82and 45.75 ± 0.62. The amount of the drug released was inversely proportional to the polymer ratio in the microcapsules.Figure (6-a) shows the release of the drug as well as the release of the drug from Eudragit RL100 microcapsules at (pH 1.2). It is obvious that all over the experimental time period, the percentages drug released were 37.63 ± 1.02,

28.22 ± 2.09 and 20.62 ± 2.60 with drug - polymer ratios of 1:1, 1:2 and 1:3 respectively. Figure (6-b) shows the release of the drug as well as the release of the drug from Eudragit RL 100 microcapsules at (pH 7.4). It is clear that the drug percentages released after 30 min were 58.33 ± 1.63, 54.82 ± 1.83and 35.13 ± 1.76.The reduction of the drug released was significantly affected by the polymer ratio in the microcapsules formulation (P < 0.05). Figure (7-a) shows the release of the drug as well as the release of the drug from Ethyl cellulose microcapsules (pH 1.2). It is obvious that drug percentages released all over the experimental time period (180 min) were 26.53 ± 2.09, 20.92 ± 2.03 and 15.98 ±1.02% in case of drug-polymer ratios of 1:1, 1:2 and 1:3 respectively. Figure (7-b) shows the release of the drug at (pH 7.4) from Ethyl cellulose microcapsules systems. It is clear that the percentages of drug released after 30 min were 69.16±1.34, 40.18 ±1.76 and 32.18 ± 1.23 in case of drug - polymer ratios of 1:1, 1:2 and 1:3 respectively. From the above figures (5-7) , it is clear that microcapsules based on Eudragit RS 100, Eudragit RL 100 and ethyl cellulose can be prepared successfully by using simple techniques and could be used as controlled release formulations on account of their safety , stability, hydrophobicity as well as compact film-forming nature among water-insoluble polymers. The alteration obtained in the release rate of the drug may be due to the entrapment which modifies the release of the drug which accompanied by reduction in the severity of the side effects compared with the control .

The big difference in the release profile between the controlled-release drug contained in the microcapsules and the drug itself will be reflected on the bioavailability. There will be an increase in the relative bioavailability due to the slow release of the drug from the microcapsules formulations **(51-53)** which is accompanied with a longer absorption and distribution period for the drug loaded microcapsules than that of the free form.

In-vivo **Ulcerogenicity Studies:**
Macroscopic examination of gastric ulcers: Figure (8) shows gastric mucosal injury of the stomach of untreated rats and others treated with pure drugs and different microcapsule systems. Gross study of gastric Lumina of control group figure (8-a) showed completely apparent normal gastric mucosa regarding normal rauga and mucus covering layer. Single doses of ketorolac (8-b) evoked focal area of congestion, spots of hemorrhagic area (pin pointed hemorrhagic area covered with mucous) and wide spread hemorrhaging as indicating by the red spots which are blood clots. Single doses of microcapsules

systems using Eudragit RS100, Eudragit RL100 as well as ethyl cellulose (1:3) drug to polymers (8-c), (8-d), and (8-e) respectively showed apparently normal gastric mucosa with little pin pointed hemorrhagic area covered with mucous. Also, there were a significant reduction in both number of ulcer per rat and cumulative ulcer diameter for microcapsules systems than free drug.

Results in table (4) revealed that untreated rats showed ulcer incidence of zero, however, group II rats showed ulcer incidence of 100% which confirmed that gastric ulceration of ketorolac is reported after single dose administration of (5mg/kg) of the free drug which is consistent with the dose used clinically **(51)**. As shown in table (4), microcapsules of ketorolac using Eudragit RS100, Eudragit RL100 as well as ethyl cellulose (1:3) drug to polymers significantly suppressed the induced ulcers. The enteric coated formulations of ketorolac with Eudragit RS100, Eudragit RL100 as well as Ethyl cellulose (1:3) resulted in a mean percentage reductions of the induced ulcer of 16.66%, 16.66% and 0% respectively compared to the free drug which showed ulcer incidence of 100%. Table (4) also showed the ulcer index of ketorolac as a free drug and microcapsules of ketorolac using Eudragit RS100, Eudragit RL100 as well as ethyl cellulose (1:3) drug to polymers. Microcapsules showed reductions of the ulcer index of 0.33± 0.41, 0.52±0.61 and 0 respectively compared to the free drug which showed ulcer index of 5.34± 0.32.

Histological Observations: The histological pattern of the mucosal specimens was studied by examining the histology of the treated and control samples. Histopathological examination of Hx&E stained stomach sections of control rats showed completely normal gastric mucosa with excess mucous layer (Fig.9-a). In group II dose of (5mg/kg) of ketorolac tromethamine, showed pronounced necrotic gastric mucosa with sever dilated congested blood vessels in the lamina properia with sever edema infiltrated by inflammatory cells (neutrophil infiltration) (Fig.9-b).

The histological pattern of group III experienced completely normal mucosal with a thin rim of mucous similar to control group (Fig. 9-c) .However, group IV showed intact gastric epithelial lining cells, while two rats showed dilated basal crypt with mild mononuclear cellular infiltration (Fig.9-d). Group V showed intact gastric epithelial lining cells and parietal cells analogous to those observed for control fed plain water (Fig.9-e).

CONCLUSION

Encapsulating of drug in a carrier and slow diffusion of the drug into the mucosal media could alleviate the problem of gastric ulceration. Microencapsulation of ketorolac using Eudragit RS100, Eudragit RL100 as well as Ethyl cellulose at drug to polymer ratio of (1:3) significantly reduced gastric irritations and gastric ulcers compared to the free drug. The previous results, it is clear that the major contribution of the local ulcerogenic effects of ketorolac can be appreciated from the decreased incidence and magnitude of ulcers following the use of enteric coated formulations. It possible to overcome the problem of gastric damage during the use of ketorolac, by avoiding the exposure of the drug to the ulcer-prone area of the GI tract.

Table (1): Operating Condition in Coating Ketorolac tromethamine powder:

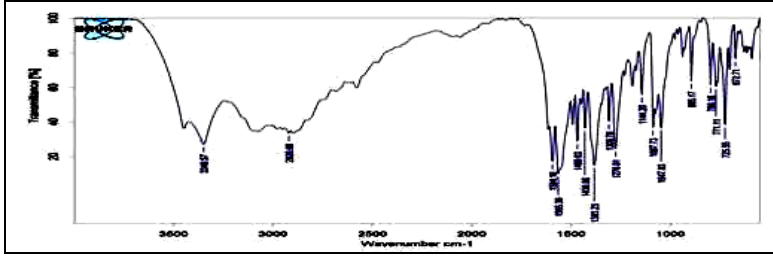| Operating Condition in Coating Ketorolac tromethamine powder	
Core material	Ketorolac tromethamine
Inlet air temperature (°C)	(60)
Material temperature (°C)	(35-40)
Out let air temperature (°C)	(33-36)
Air flow rate (m^3 / min.)	(0.75-0.9)
Spray rate (ml / min.)	(6.9)
Spray pressure (atm.)	(1.5-2.0)
Diameter of spray nozzle (mm)	(0.8)
Drying conditions	(40°C,60min)
Mesh size	(80-250)
Charged weight (gm.)	(30)

Figure (1-a): FTIR Spectrum of ketorolac tromethamine.
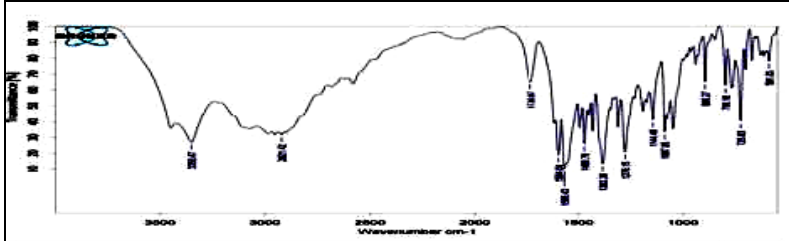

Figure (1-b): FTIR Spectrum of ketorolac tromethamine coated with Eudragit RS 100.
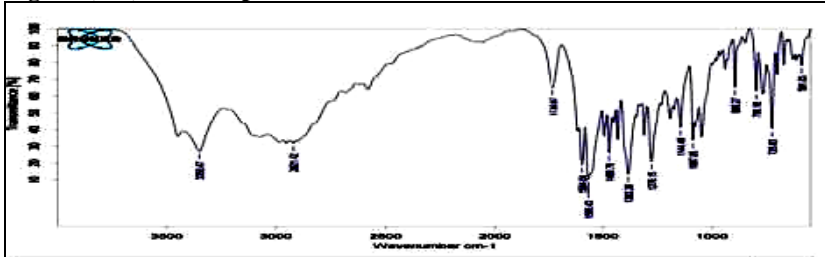

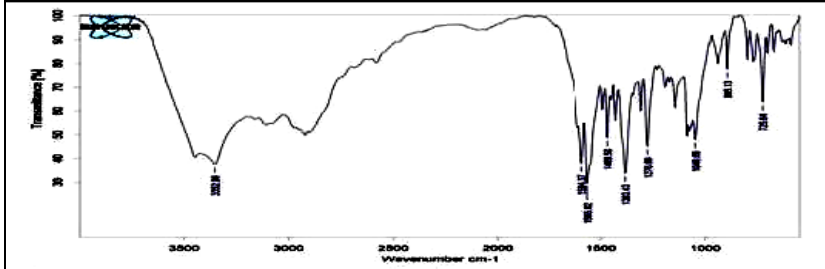**Figure (1-c): FTIR Spectrum of ketorolac tromethamine coated with Eudragit RL 100.**

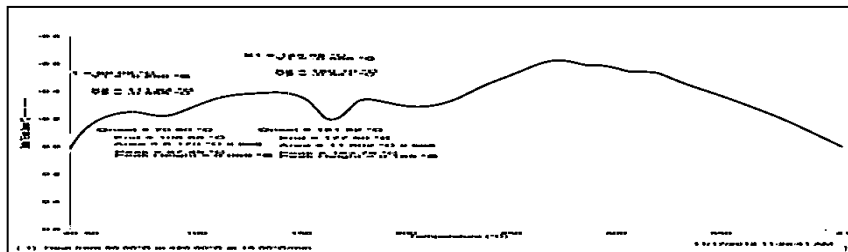**Figure (1-d): FTIR Spectrum of ketorolac tromethamine coated with Ethyl cellulose.**

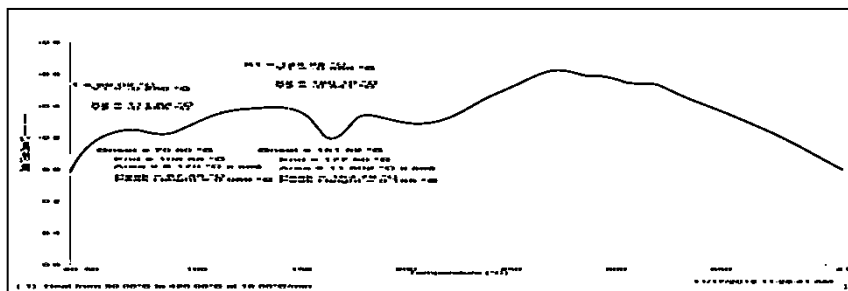**Figure (2-a): DTA Spectrum of ketorolac tromethamine.**

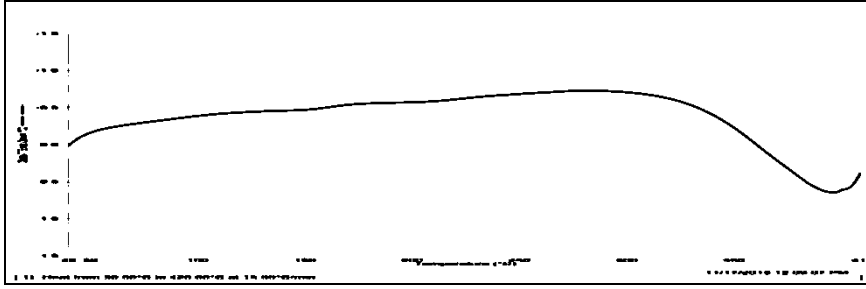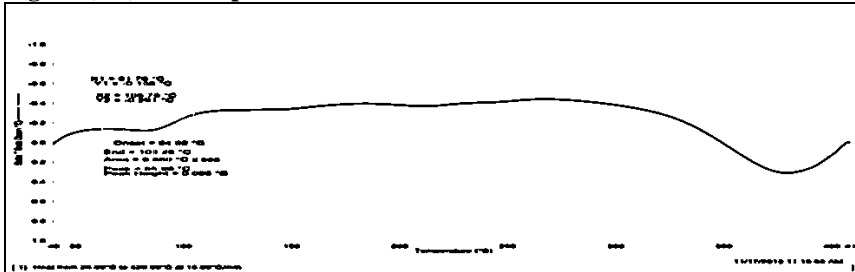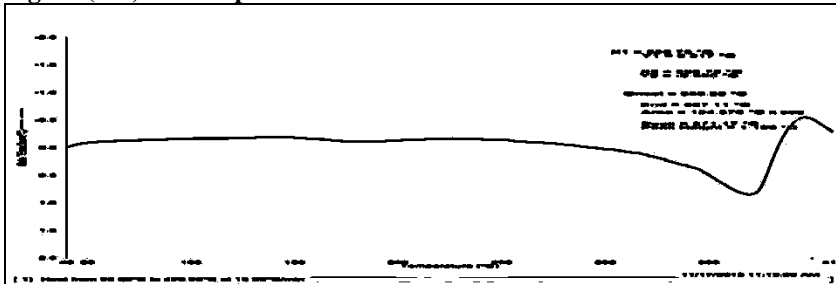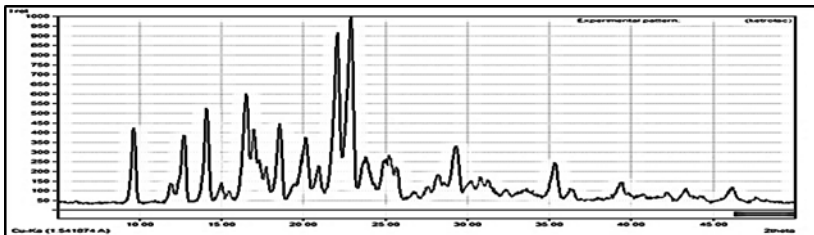**Figure (2-b): DTA Spectrum of ketorolac tromethamine physical mixture.**

Figure (2-c): DTA Spectrum of ketorolac tromethamine coated with Eudragit RS 100.

Figure (2-d): DTA Spectrum of ketorolac tromethamine coated with Eudragit RL100.

Figure (2-e): DTA Spectrum of ketorolac tromethamine coated with Ethyl cellulose.

Figure (3a): X -ray of ketorolac tromethamine.
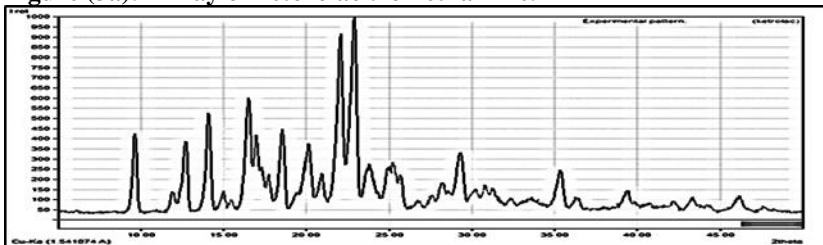
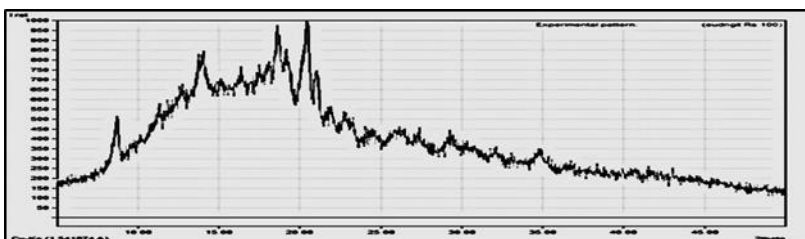**Figure (3b): X -ray of ketorolac tromethamine physical mixture.**

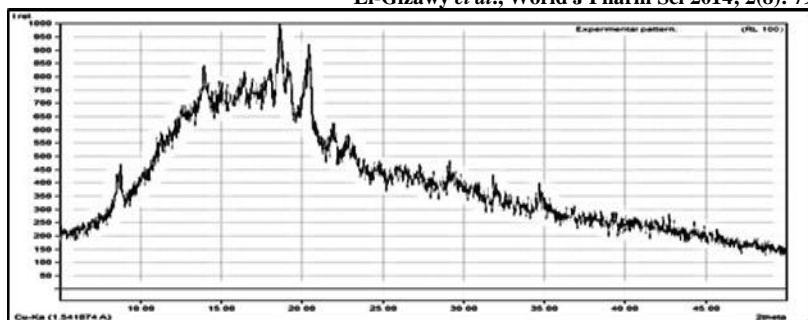**Figure (3c): X -ray of ketorolac tromethamine coated with Eudragit RS 100.**

Figure (3d): X -ray of ketorolac tromethamine coated with Eudragit RL 100.
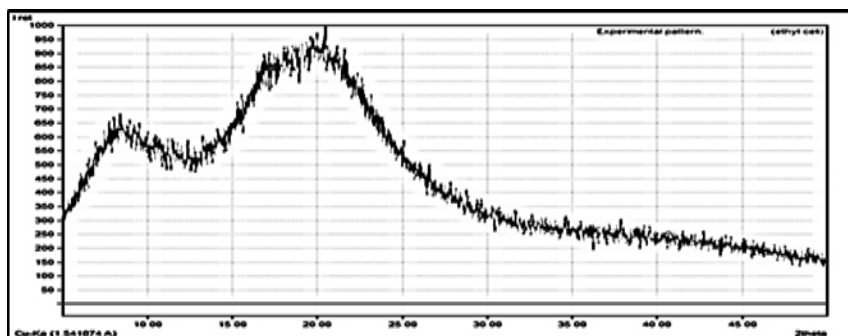

Figure (3e): X -ray of ketorolac tromethamine coated with Ethyl cellulose.
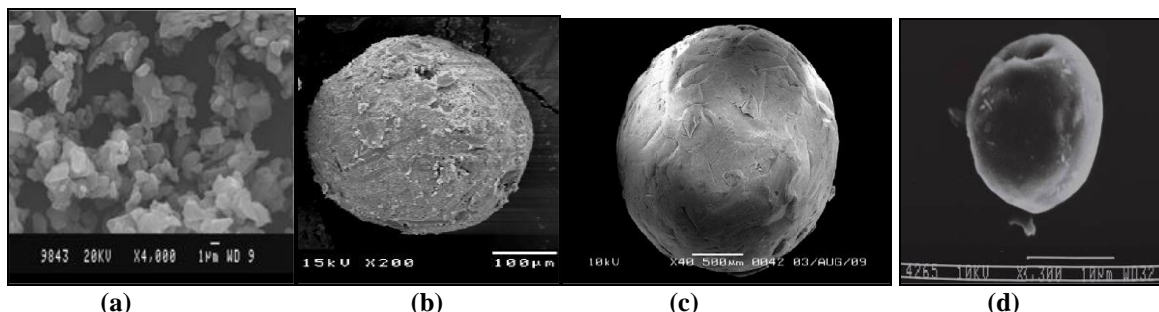

| (a) | (b) | (c) | (d) |

Figure (4) Scanning electron micrograph of Ketorolac Tromethamine Microparticles
(a) Ketorolac tromethamine (b) ketorolac coated with Eudragit RS100 (c) ketorolac coated with Eudragit RL100   (d) ketorolac coated with Ethyl cellulose.

Table (2) Flowability test of ketorolac tromethamine and its microcapsules form.

| Parameter	Drug alone	Eudragit RS100 based microcapsules			Eudragit RL100 based microcapsules			Ethyl cellulose based microcapsules	
		Core / Coat ratio							
	-	1:1	1:2	1:3	1:1	1:2	1:3	1:1	1:2
Carr's index (%)	52.38	14.82	13.9	11.12	14.66	13.66	11.19	15.16 13.22	14.82
Angle of repose	58.00	30.11	26.13	20.09	30.19	25.19	16.22	30.29 19.66	25.17

Table (3): Ketorolac tromethamine content in different microcapsules formulations in phosphate buffer (pH7.4).

Microcapsule	Drug: polymer ratio	Ketorolac tromethamine Content (%)
Eudragit RS 100	1:1	98.10 ± 2.30
	1:2	98.63 ± 1.72
	1:3	± 1.16 98.58
Eudragit RL 100	1:1	99.10 ± 1.36
	1:2	99.67 ± 1.81
	1:3	98.73 ± 1.36
Ethyl cellulose	1:1	± 1.23 99.32
	1:2	99.67 ± 1.96
	1:3	± 1.21 98.83

(Mean ± SD, n=3).
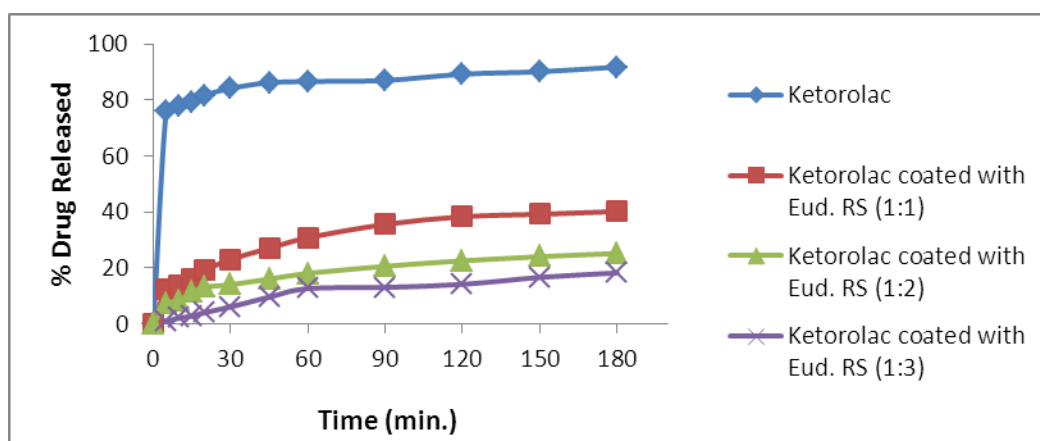
Figure (5-a): Release of ketorolac tromethamine from ketorolac-Eudragit RS 100 microcapsules in 0.1 N HCl   (pH 1.2).
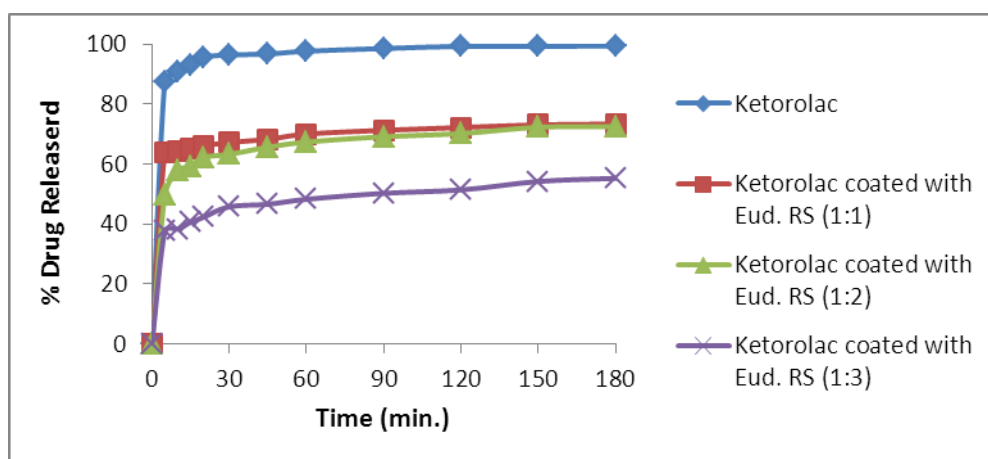
Figure (5-b): Release of ketorolac tromethamine from ketorolac -Eudragit RS 100 microcapsules in phosphate buffer (pH 7.4).
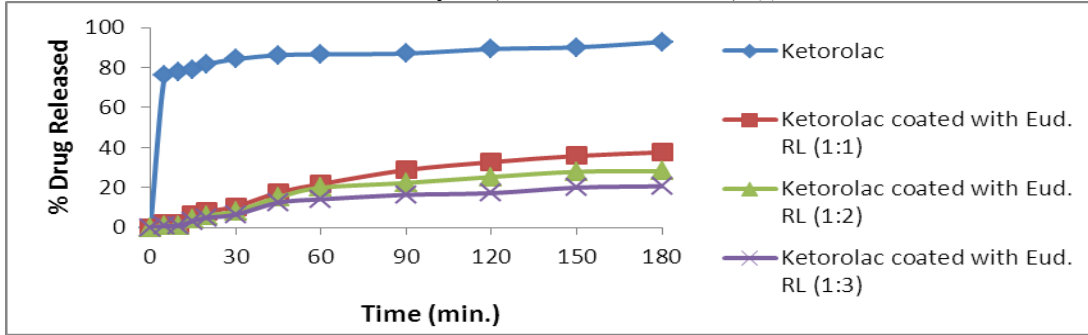
Figure (6-a): Release of ketorolac tromethamine from ketorolac-Eudragit RL 100 microcapsules in 0.1 N HCl (pH 1.2).
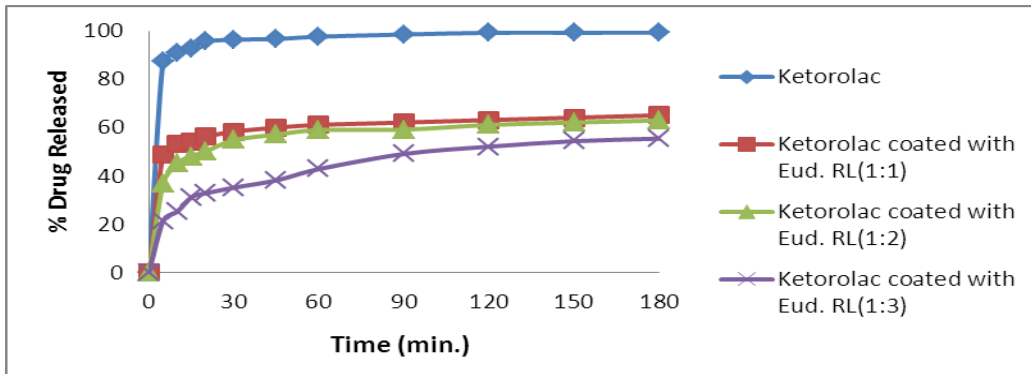

Figure (6-b): Release of ketorolac tromethamine from ketorolac -Eudragit RL100 microcapsules in phosphate buffer (pH 7.4).
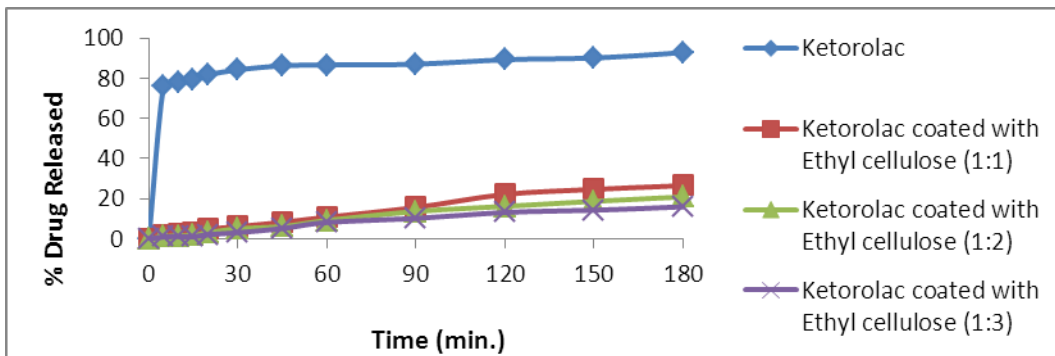

Figure (7-a): Release of ketorolac tromethamine from ketorolac-Ethyl Cellulose microcapsules in 0.1 N HCl (pH 1.2).
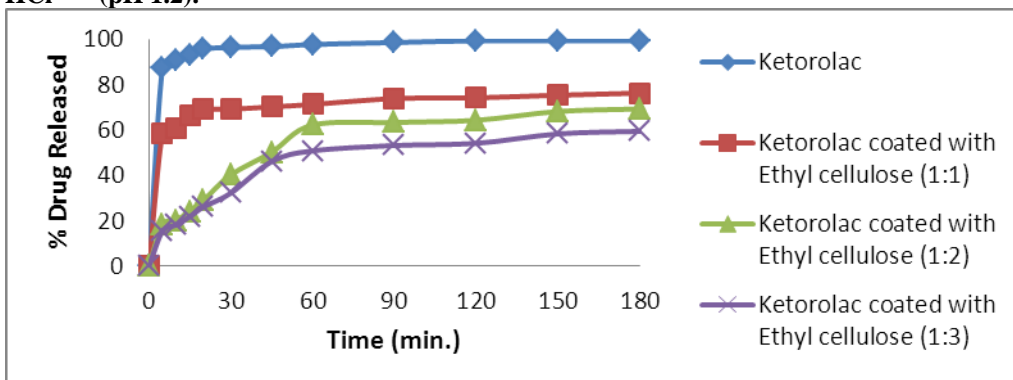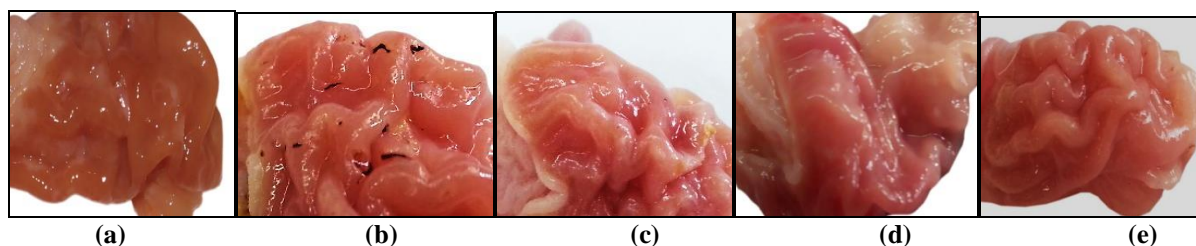

Figure (7-b): Release of ketorolac tromethamine from ketorolac-Ethyl Cellulose microcapsules in phosphate buffer (pH 7.4).
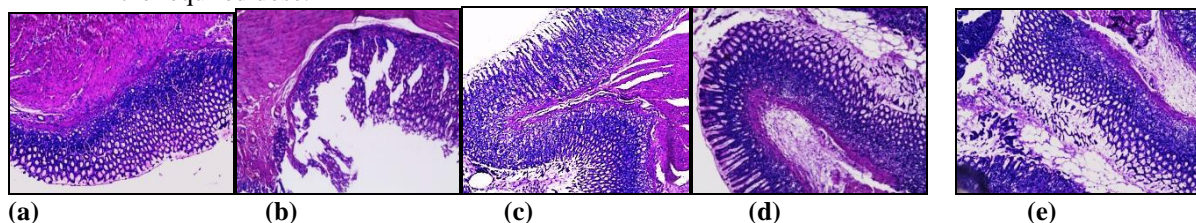
(a) (b) (c) (d) (e)

Figure (8): Representative image showing morphological changes in rat gastric tissues after administration of ketorolac as free drug and ketorolac coated with different polymers, (a) Control (b) Ketorolac free drug (c) ketorolac coated with Eudragit RS100 (d) ketorolac coated with Eudragit RL100 (e) ketorolac coated with Ethyl cellulose.

Table (4): Effect of ketorolac and ketorolac microcapsules on ulcer incidence

Group number	Treatment·	Ulcer incidence	Ulcer index
I	(Control group)	0% (0/6)	0
II	Ketorolac (free drug)	100% (6/6)	5.34± 0.32
III	Ketorolac: Eudragit RS 100 (1:3)	16.66% (1/6)	0.33± 0.41
IV	Ketorolac: Eudragit RL 100 (1:3)	16.66% (1/6)	0.52±0.61
V	Ketorolac: ethyl cellulose (1:3)	0% (0/6)	0

- Six rats were randomly allocated for each group.
- Control group received orally 1ml distilled water and each treatment group received 1ml solution of ketorolac tromethamine or suspension of ketorolac tromethamine microcapsules systems containing the required dose.

(a) (b) (c) (d) (e)

Figure (9): Representative image showing histological observations in rat gastric tissues after administration of ketorolac as free drug and ketorolac coated with different polymers, (a) Control (b) Ketorolac free drug(c) ketorolac coated with Eudragit RS100 (d) ketorolac coated with Eudragit RL100 (e) ketorolac coated with Ethyl cellulose.

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