

Preparation and gamma scintigraphic evaluation of colon specific pellets of ketoprofen prepared by powder layering technology

*Subhabrota M, Souvik R, Subhadeep C

Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences, Banitabla, Uluberia, Howrah, West Bengal, 711316, India.

Received 27 July 2010; Revised 28 Feb 2011; Accepted 1 March 2011

ABSTRACT

Background and the purpose of the study: Multiparticulates by powder layering process have advantages of the uniform distribution of the binder solution, easy-to-clean pan and the possibility of applying the successive functional film coating using the same equipment. This study relates to a multiparticulate formulation comprising pellets with a multilayer of pectin-ethyl cellulose on non pareil seeds by powder layering technology. The pellets were prepared to target ketoprofen in colon based on the microbial enzyme dependent drug release mechanism.

Methods: Multiparticulate formulation by powder layering technology was prepared by conventional pan coating process to evaluate the effect of 59% methoxylated pectin and 45 cps ethyl cellulose on coating label. The formulations were tagged with ^{99m}Tc-DTPA, a tracer in gamma scintigraphy study to evaluate the transit behavior of drug loaded pellets and compared with uncoated pellets to evaluate its specific release.

Results: The transit behavior and scintigraphy image clearly indicates that the formulation can delay the drug release prior to colon. In albino rabbit, the coated pellets released drug in the colon indicating that site specificity has been achieved with pectin/ethyl cellulose coating at 1:2 ratio with 20% coating label.

Major conclusion: Formulation containing pectin and ethyl cellulose with suitable coating label may be suitable as a coating formulation for colon delivery of ketoprofen and can be successfully evaluated by gamma scintigraphy method.

Keywords: Colon specific delivery, Powder layering, Non-pareil seed.

INTRODUCTION

Various approaches have been designed for oral colonic drug delivery including a) taking advantages of the apparent consistency of small intestine transit time b) utilization of the pH changes within the Gastrointestinal (GI) tract and c) exploitation of bacterial enzyme in the colonic region. Because of the poor site specificity of pH dependent systems due to the variations of the pH in the GI tract (1) and poor site specificity of the timed release dosage forms (2) exploitation of the bacterial enzyme localized in the GI tract has been one of the best approaches for colon targeting. Activity of colonic bacteria on polysaccharide based carrier systems includes pectin and its salts have been extensively investigated (3, 4). The problem encountered with pectin is its solubility and swelling property in aqueous media. As a consequence, film-coating consisting of pectin alone is unable to prevent the release of drugs during stomach and the small intestinal transit (5). It has been depicted that combination of ethyl cellulose and pectin could

provide protection to a drug against enzymatic breakdown in the upper G.I tract to produce more universal 'colonic' coating (6, 7).

Formulation of Multiparticulates by powder layering process comprises the deposition of successive layers of drug entities on non pareil seeds (8). The most attractive features of this system are the uniform distribution of the binder solution, easy-to-clean pan and the possibility of applying the successive functional film coating using the same equipment (9).

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic effects (10) by inhibition of prostaglandin biosynthesis. It undergoes metabolism in the liver *via* conjugation with glucuronic acid, hydroxylation of the benzoyl ring, and reduction of its keto group (11).

Gamma scintigraphy, a non-invasive imaging technique, has been used to determine the in-vivo behavior of various colon delivery systems. By incorporating small amounts of gamma-emitting radionuclides into the dosage forms, GI transit

pattern within the body and site of disintegration and release can be determined (12).

In this study, ketoprofen loaded pellets were coated with different ratio of pectin and ethyl cellulose by powder layering technology using conventional pan coating equipment. The coated drug loaded pellets were evaluated in vitro and gamma scintigraphy was done in rabbit to study the in vivo transit pattern.

MATERIAL AND METHODS

Materials

Ethyl Cellulose (45cps) was kindly provided by Dow Chemical Company Ltd (UK). Pectin (esterification degree of 59%) was a generous gift sample of Herbstreith & Fox (Germany). Pectinex Ultra SP-L (Pectinolytic Enzyme) was obtained from Novo Ferment (Switzerland). One milliliter of enzyme has an activity of 9500 PG/ml. The plasticizer triethyl citrate (TEC) kindly provided by Polynt SPA (Italy) and Ketoprofen by Aarti Drugs Limited (Mumbai, India). All other chemicals were of analytical grade.

Preparation of Ketoprofen pellets by Powder Layering Technology

Drug containing pellets were prepared by accurately weighing the non-pareil seed of 30 mesh size and dried at 35°C. These dried non-pareils were charged into the coating pan and 5% PVP (polyvinyl pyrrolidone) as a binder solution was sprayed with the help of spray gun (attached with compressor) till the bed become wet. Immediately the required amount of powder drug was layered on to the wet bed of pellets. Pan rotation continued till the dry powder adheres onto the wetted pellets properly. Drying bed temperature and blowing air temperature were maintained properly to avoid overheating of drug loaded pellets, which may cause separation of the drug from the pellets after several pan rotation.

Formation of coating layer

Pectin in acetone: isopropyl alcohol of 40:60 was prepared in different ratios and Talc (anti-sticking agent) was added to the solution based on the solid dry weight (5% w/w) of pectin present and mixed for 30 min. The weighted quantity of ethyl cellulose was dissolved in ethyl alcohol containing 10% w/w (solid dry weight of ethyl cellulose) of TEC as a plasticizer. The ethyl cellulose solution was then added to non-aqueous pectin solution to produce coating formulation and sprayed onto the loaded pellets until the pellets achieved desire coating level shown in table 1.

Statistical optimization by RSM (Response Surface Methodology)

The composition of controlled release multiparticulate formulation of Ketoprofen was carried out according to the optimization procedure by using Design

Expert Software (design expert 8.0.3 trial version). Based on the Pre-formulation study the Ratio of EC (X_1) and Coating Level (X_2) were selected as the independent variables and studied at 3 levels each. The central point (0,0) was studied in triplicate. All other formulations and processing variables were kept invariant throughout the study. Percent of the drug release at 6 hrs (Y_1), Time at which 50% of drug was released (T_{50}) (Y_2) and time at which 80% of the drug was released (T_{80}) (Y_3) were taken as the response variables. The optimized formulation was subjected for Gamma Scintigraphy study.

Fourier transformed infrared spectroscopy (FT-IR)

IR spectroscopy of ketoprofen loaded pectin/ethyl cellulose pellets were taken on fourier transformed infrared spectrophotometer (FT-IR 840, Shimadzu, Japan). The pellets of drug and KBr were prepared by compressing the powders at 20 psi for 10 min on KBr press. The mixture was grounded into a fine powder using an agate mortar before compressing into a disc and the spectra were scanned in the wave number range of 2000-500 cm^{-1} and stored in a personal computer which was coupled to FT-IR. The characteristic peaks of IR transmission spectra of the pure and formulation of ketoprofen were recorded.

Differential scanning calorimetry (DSC)

Thermograms of ketoprofen- pectin-EC pellets were obtained using a Perkin Elmer-Jeda DSC instrument equipped with an intra-cooler. Powder samples were hermetically sealed in perforated aluminum pans and heated at a constant rate. Purge gas-nitrogen at a flow rate 20 ml/min and heating temperature of 100 °C was used to maintain inert atmosphere. In this technique the difference in energy input into a substance and reference material is measured as a function of temperature as the specimens are subjected to controlled temperature program (13).

Morphology of pellets by scanning electron microscopy (SEM)

The surface morphology of pellets of optimized formulation was examined before and after dissolution using scanning electron microscope. The samples were fixed on a brass stub using double-sided tape and then gold coated in vacuum by a sputter coater. The pictures were taken at excitation voltage of 10 KV and at 400X magnification by using JSM-840A scanning Microscope; Jeol-Japan (14).

Gamma Scintigraphic study

Preparation of DTPA-Ketoprofen mixed pellets

Ketoprofen was first mixed with DTPA before layering onto the non pareil. Immediately the required amount of drug powder i.e Ketoprofen-DTPA mixture was layered on to the wet bed of pellets. Pan rotation continued till the dry powder

Table 1. Processing conditions for the preparation of Ketoprofen pellets.

Formulations	Non pareil (mg) Sugar pellets	Ketoprofen (mg)	Ratio of pectin: ethyl-cellulose	Coating label in %	Talc (% w/w) Anti-sticking agent	TEC (% w/w) Plasticizer
F1	600	100	1:1	10	5	10
F2	600	100	1:1	15	5	10
F3	600	100	1:1	20	5	10
F4	600	100	1:2	10	5	10
F5	600	100	1:2	15	5	10
F6	600	100	1:2	20	5	10
F7	600	100	1:4	10	5	10
F8	600	100	1:4	15	5	10
F9	600	100	1:4	20	5	10

adheres onto the wetted pellets properly. Drying bed temperature and blowing air temperature was maintained properly to avoid overheating of drug-DTPA loaded pellets, which may cause separation of drug and DTPA from the pellets after several pan rotation. After layering the drug loaded multiparticulate formulation were kept in an oven for 2 hrs at 35°C (15, 16).

Labeling of pellets

Tagging with the radioactive substance with the ketoprofen pellets was carried out in Regional Radiation Medicine Centre & Variable Energy Cyclotron Centre, under the Department of Atomic Energy, Government of India. Gamma Scintigraphy study on healthy albino rabbit was performed in Cancer Centre & Welfare Home (Thakurpukur, Kolkata, India). DTPA-ketoprofen pellets were prepared by using powder layering technology and an amount of the pellets (10 mg/kg) weight of drug-DTPA were soaked with ^{99m}Tc which was previously reduced with stannous chloride from 10MBq to 4MBq/dose by direct labeling technique (17). MBq strength of radioactive label was determined by CAPINTEC CRC-15R detector (Pittsburgh, U.S.A) with ionization chamber of a Colimeter. Then the labeled pellets were coated with pectin/ethyl cellulose to achieve 20% coating level (optimized formulation) and dried for 15 min in a fume cupboard. The Specification of gamma camera was: INFINIA (GE Company), Resolution: 256x256 with zoom 2, Counter time: 5 min, Acquisition time: 5 min, Photo peak: 140 keV \pm 10 (10% window).

Quality control of ^{99m}Tc -DTPA Ketoprofen mixture

Radiochemical purity and labeling efficiency of the ^{99m}Tc -DTPA-labeled Ketoprofen preparation was determined by Instant Thin-Layer Chromatography (ITLC) on 2, 3 and 20 cm chromatography strips (18, 19). ^{99m}Tc -DTPA- Ketoprofen preparation (5 μ l), was applied to the strip at the starting point, which was 2 cm from one end of the chromatographic strip. Two successive analyses were performed

on the same strip. Between two analyses, an air-drying period of 2 minutes was allowed. After development in the solvent, the strips were cut into two portions (top: bottom; 1:3), and activity in each portion was measured in the form of counts using a gamma scintillation counter for determination of the mobility of the radioactive samples and the corresponding spots. The activity of each segment was then expressed as percentage of the total activity on the strip.

Stability of radiolabelled pellets coated with pectin-ethyl cellulose

Stability of radioactivity (C.P.S) of ^{99m}Tc -DTPA labelled coated pellets was determined using a CRC-15R detector with ionization chamber. Dissolution tests for the release of the radioactive material as a test and without radioactive material as a standard were carried out in 900 ml of 0.1M HCl for 2 hrs at pH of 6.8 for 3 hrs and pH of 6.0 for one hour at $37 \pm 0.5^\circ\text{C}$ using the USP XXIII basket type dissolution apparatus. The rotation speed was 100 rpm. At 30 min intervals, 5 ml of samples were taken and replaced with fresh solution. At the end of the dissolution testing, the pellets were recovered from the dissolution medium and blotted dry with tissue paper. The activity (counts per 20 s) of the test solutions was determined. The percentage of radioactivity in the pellets after 6 hrs of dissolution testing was calculated from the initial and remaining final activity (20).

In vivo imaging study on rabbit

The recommendations of "Institutional Animal Ethical Committee" according to the rules of "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCEA, Registration No. 1126/bc/07) India] for the care and use of laboratory animals were strictly followed throughout the experiment. Twelve male albino rabbits which were one year old and weighted 2.5-3.6 kg were used to monitor the in vivo transit behavior of the ketoprofen pellets

coated with pectin/ethyl cellulose. Rabbits were divided into 2 groups and were fasted for 12 hrs prior to the gamma scintigraphy study. Drug loaded radiolabelled pellets with polymer coating were orally administered in suspension form to animals of group I and those without coating to group II followed by sufficient volume of drinking water. All 4 legs of the rabbit were tied over a piece of board, and the location of the pellets in the stomach was monitored by keeping the subjects under gamma camera. The gamma camera had a field view of 40 cm and was fitted with a medium-energy collimator. The 140 keV gamma rays emitted by ^{99m}Tc were imaged. The gamma images were recorded using an online computer system, stored on magnetic disk, and analyzed to determine the distribution of activity in the stomach, and intestine and colonic region. At the time intervals of the gamma scanning, the animals were freed and allowed to move and carry out normal activities but were not allowed to take any food or water until the stomach was completely free from the formulation (21). Ketoprofen loaded radiolabelled pellet distribution was measured by the number of radioactive count recorded within the stomach and colon region in the scintigraphy image. The mean of the counts was then calculated for radioactive decay and expressed as a percentage of the dose (22). The transit profile for coated pellets was characterized by the half-lives ($T_{50\%}$) for gastric emptying, for colon arrival and for intestinal transit (the time interval between $T_{50\%}$ values for gastric emptying and colon arrival).

RESULT AND DISCUSSION

FT-IR analysis

In the FT-IR spectrum the characteristics peaks corresponding to $-\text{COOH}$ (1597.73 cm^{-1} for the symmetric stretch), an aromatic $-\text{CH}=\text{CH}-$ (1024.98 cm^{-1} for symmetric bend), a CH_2 (2975 cm^{-1} for scissors stretch) and $-\text{C}=\text{O}-$ group (1693 cm^{-1} for symmetric stretch) were present. A peak at 2350 cm^{-1} was due to $\text{O}=\text{C}=\text{O}$ of CO_2 present in air. The spectrum of ketoprofen formulation and ketoprofen pure drug showed that there was no significant interaction between drug and other polymers and plasticizer as shown in figure 1.

Differential scanning calorimetry (DSC)

DSC scan was recorded for ketoprofen powder and pellets are depicted in figure 2. The pure ketoprofen displayed a single sharp endothermic peak at 96.47°C corresponding to the melting point of the drug and an identical peak was also observed in the pellet formulations. The thermographic result showed that the drug retains its identity in the coated pellet formulations. Additional peaks in the formulation were due to other components present in the pellets.

Scanning electron microscopy (SEM)

Large amount of particles with spherical, smooth and discrete surfaced with small amounts of crystals of the drug were visible on surface indicating that the concentration of polymeric solution was sufficient for complete coating. Scanning electron photomicrographs of the optimized formulation are shown in figures 3A and 3B.

In vitro drug release

Figure 4 shows the drug release profiles of the labelled and the unlabelled coated pellets after 24 hrs dissolution testing in simulated gastrointestinal conditions. The cumulative amount of ketoprofen released from the pellets after 12 hrs dissolution testing were $64.21\pm 0.79\%$ (labelled), and $58.19\pm 0.30\%$ (unlabelled) and after 24 hrs were $93.59\pm 0.87\%$ and $89.12\pm 0.86\%$ respectively. The similarities in the drug release profiles indicate that the labeling process had no adverse effect on the kinetics of drug release.

Gamma Scintigraphy study

Quality control of ^{99m}Tc -DTPA Ketoprofen mixture

The activity of each segment of chromatographic strip was analyzed and the percentage of the activity at the origin and the solvent front was determined. Typical autoradiogram of ITLC performed for the evaluation of the labeling efficiency is shown in figure 5. ^{99m}Tc -DTPA-Ketoprofen formulation remained at the origin and free technetium travelled with the solvent front ($\text{RF}=0.9885-0.9924$). ITLC indicated a labeling efficiency of 91%. The main labeled species migrated with the solvent in front of the second solvent, whereas approximate reduction of 8% of the radioactivity indicates the radiochemical purity and labeling efficiency of the ^{99m}Tc -DTPA-labeled Ketoprofen preparation (18, 19)

Stability of radiolabelled pellets

The stability of the ^{99m}Tc -DTPA labelled drug pellets coated with pectin-ethyl cellulose was evaluated in simulated gastrointestinal fluids. The amount of radioactivity released from the pellets after dissolution testing in 0.1M HCl (pH of 1.2) for 2 hrs, pH of 6.8 for 3 hrs and phosphate buffer solutions of pH 6.0 at 6th hour were $01.874\pm 0.11\%$, $14.947\pm 0.7\%$, and $24.032\pm 2.6\%$, respectively is shown in figure 6. Thus, almost 76% of the estimated radioactivity in the pellets at the beginning of the tests remained bound to the pellets core after 6 hrs of dissolution testing in each medium. Thus, the labeling procedure was satisfactory because there was no sudden release of radioactivity into the dissolution media.

In vivo imaging study on rabbit

The pectin/EC coated ketoprofen pellets with 1:2 ratios with 20% coating level showed optimum

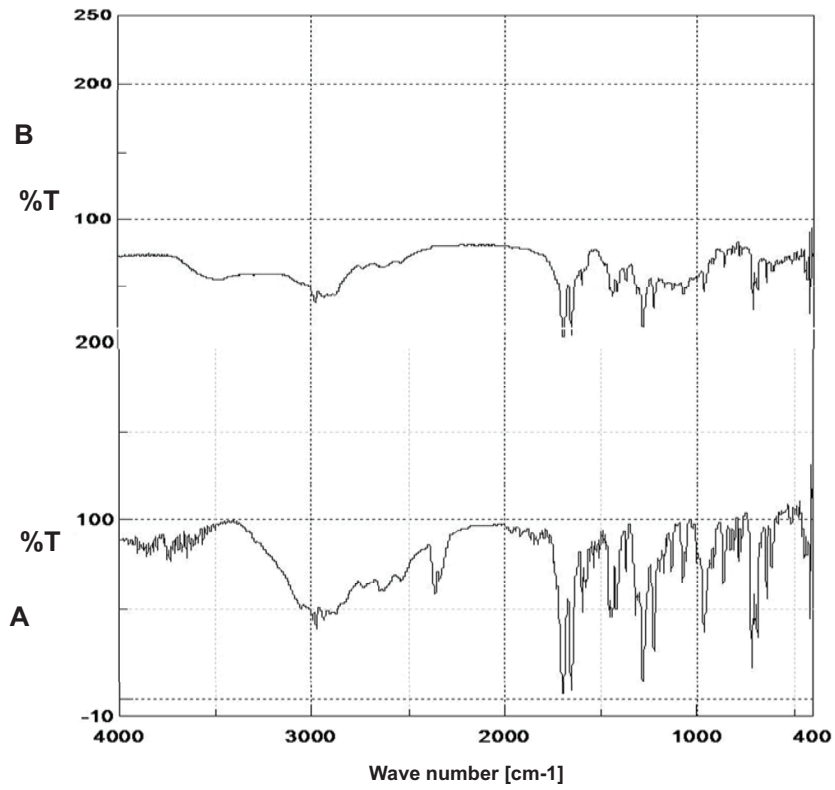


Figure 1. Characteristic peaks of IR transmission of pure Ketoprofen (A) & Ketoprofen formulation (B).

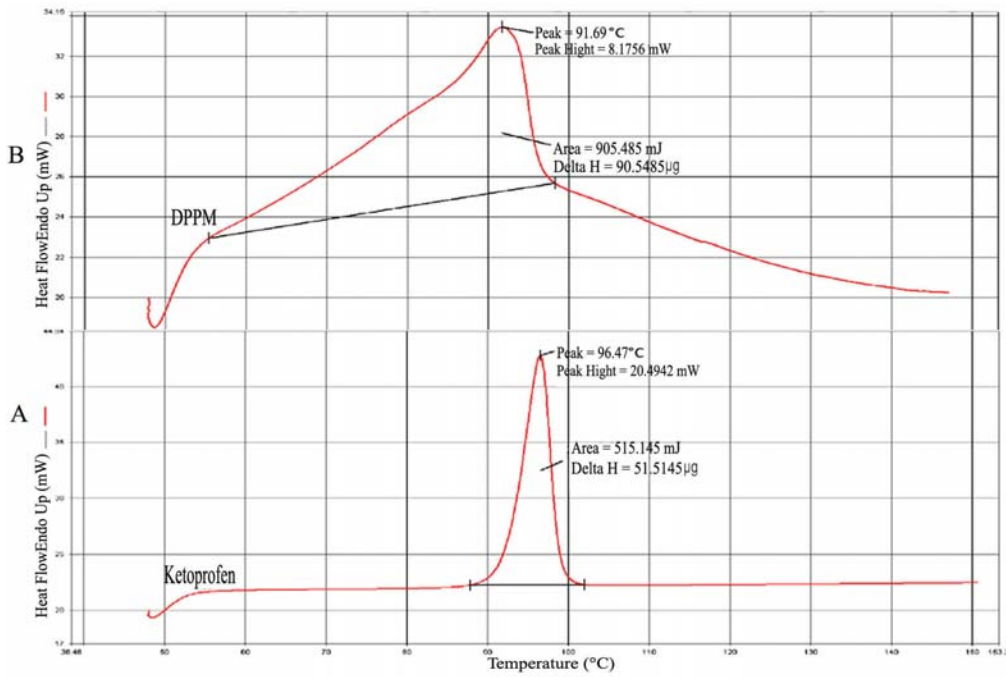


Figure 2. DSC Thermogram of pure Ketoprofen (A) & drug polymer physical mixture (B).

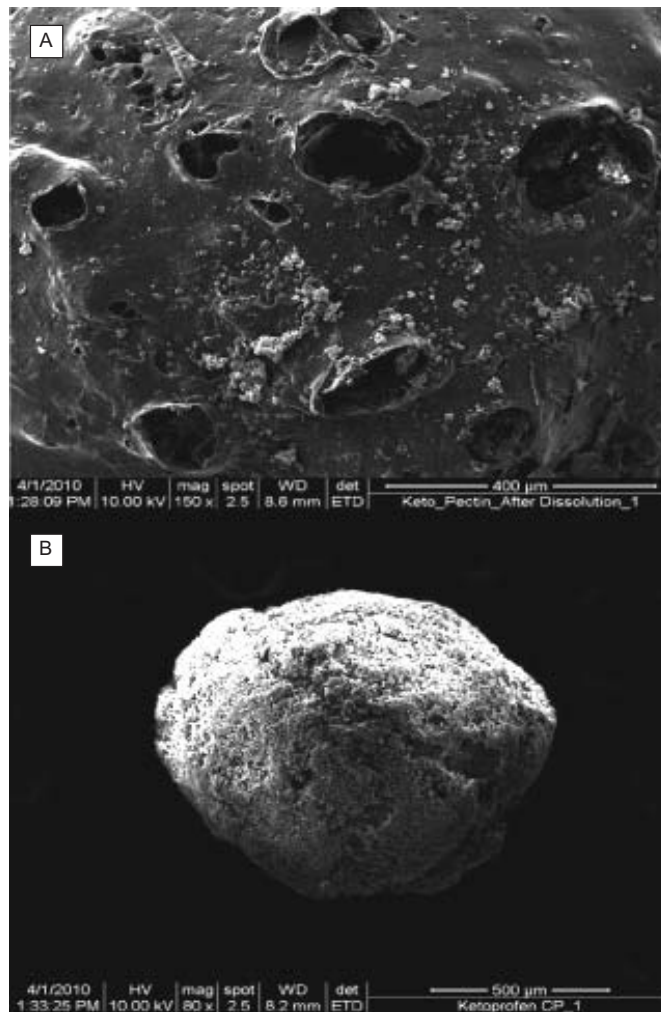


Figure 3. SEM of Ketoprofen-Polymer loaded multiparticulate formulation (A) and Surface morphology of Ketoprofen-Polymer loaded multiparticulate formulation (B).

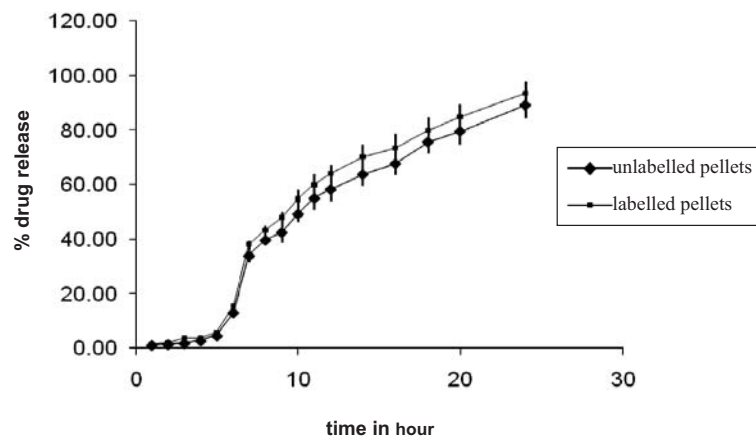


Figure 4. % drug release from labelled and unlabelled pellets.
 * All values are expressed as Mean \pm SD, n=3.

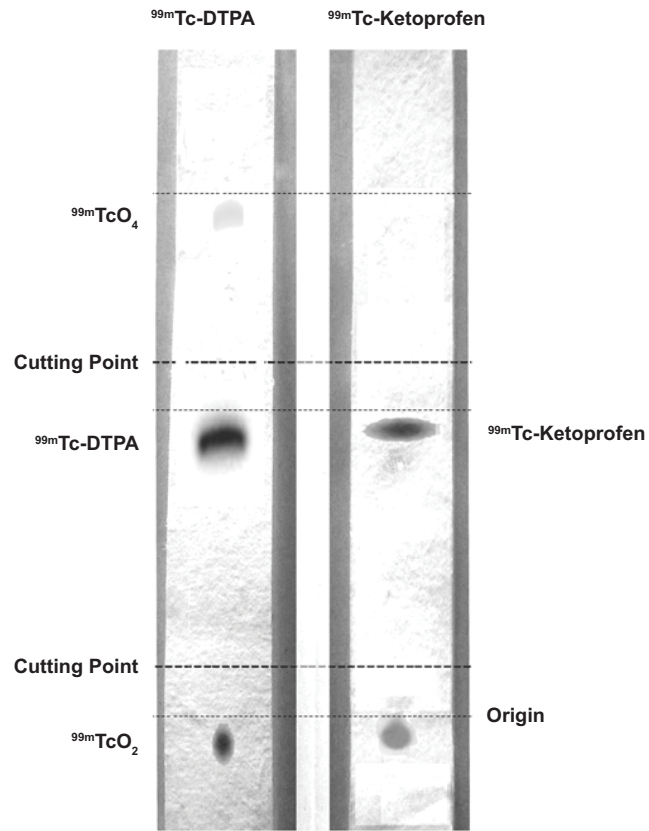


Figure 5. Autoradiogram of ITLC performed for the evaluation of the labeling efficiency of Ketoprofen with ^{99m}Tc-DTPA.

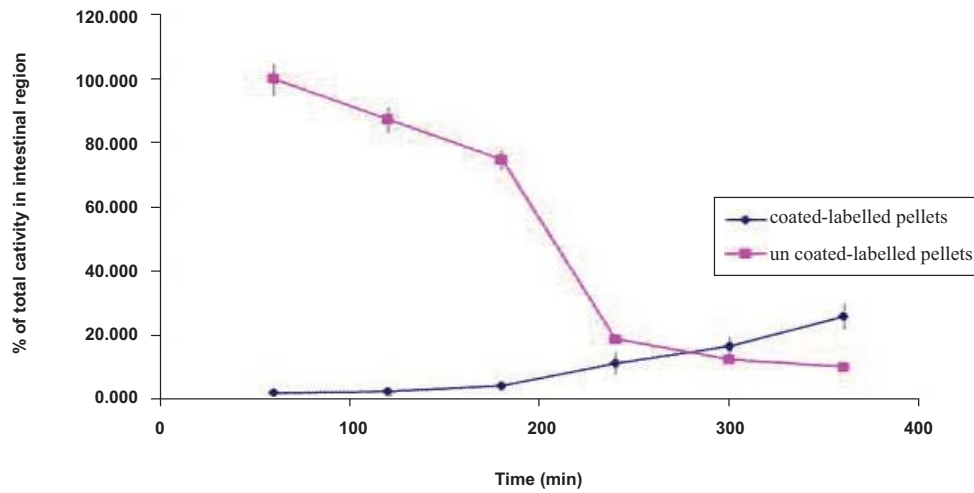


Figure 6. Percentage of the activity of the coated-radio labeled pellets.

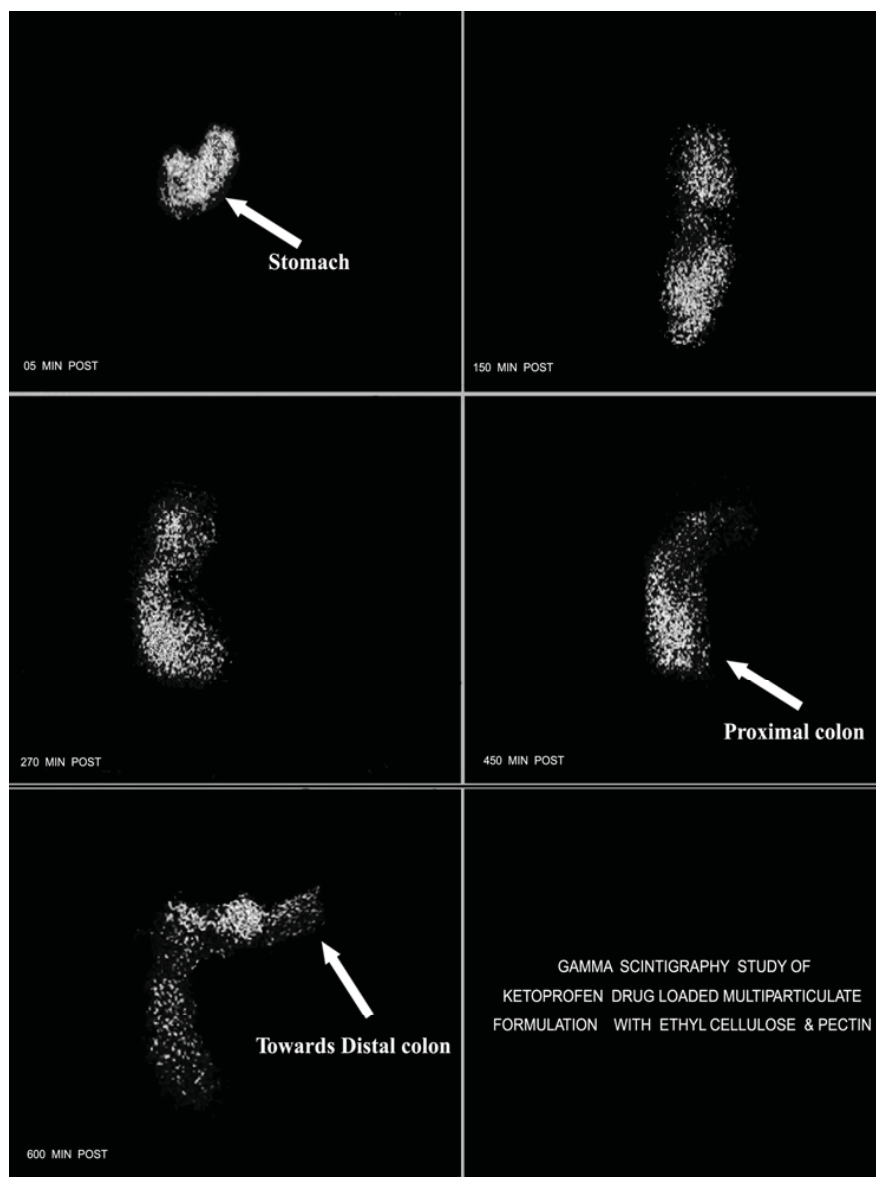


Figure 7. Gamma scintigraphy images of Ketoprofen multiparticulate formulation in rabbit.

in vitro and controlled release behavior and as a result was finally selected for in vivo gamma scintigraphy study and the results were compared with uncoated pellets. A gamma image of the ^{99m}Tc -labeled coated pellets is shown in figure 7. Gastric retention time of almost 6 hrs was achieved in all rabbits. A sufficient number of counts of ^{99m}Tc -DTPA labeled ketoprofen pellets for the 10th hour of the study period showed very good colon arrival and retention time. Gamma scintigraphy images during the study clearly indicated that the coated formulation with Pectin/EC remained intact and uniformly distributed in the colon for the 10 hrs study period. Coated drug loaded pellets remained

intact until it reached to the colon, where as in the presence of pectinolytic enzyme, the pellets disintegrated and released the drug.

Transit of drug-loaded radiolabelled pellets

Transit of pellets in different region was expressed as the time for 50% ($T_{50\%}$) to leave the GIT or to arrive into the colon. Six rabbits were subjected to study the transit time of drug loaded radiolabelled pellets. Emptying of pellets was rapid in some subject but slow in others. (Table 2). The mean of gastric emptying, small intestinal transit and colon arrival time was found to be 0.58, 5.57 hrs and 6.05 hrs respectively.

Table 2. Transit of drug-loaded radiolabelled pellets in different parts of GIT.

Subject	Gastric emptying T _{50%} (hrs)	Small intestinal transit T _{50%} (hrs)	Colon arrival T _{50%} (hrs)
1	0.59	4.29	5.05
2	1.02	6.19	6.29
3	1.09	6.04	6.27
4	0.52	5.46	6.11
5	1.01	5.28	6.21
6	0.54	6.14	6.39
Mean	0.58	5.57	6.05
SD	0.22	0.73	0.50
Median	0.52	5.75	6.24
n	6	6	6

CONCLUSIONS

From the results of the study it appears that Gamma Scintigraphy based modeling of multiparticulate formulation of ketoprofen coated with pectin/ethyl cellulose appears suitable for localizing targeted drug delivery to the colon. Scintigraphy-based modeling of the drug delivery to the colon provides a valuable perspective towards the transit behavior of oral controlled release multiparticulate formulation as the colon arrival time (T_{50%}) ranges

between 5.05 to 6.39 with average of 6.05. So the prepared pellets can successfully deliver the drug ketoprofen to the target site-colon at desirable time.

ACKNOWLEDGEMENT

The authors are indebted to Dr. Santanu Ganguly, Head of section, Regional Radiation medicine centre, Department of Atomic energy, Govt. of India for his technical advice and kind support.

REFERENCES

- Ashford M, Fell JT, Attwood D, Sharma HL, Wood Head PJ. An in vivo investigation into the suitability of pH dependent polymers for colonic targeting. *Int J Pharm*, 1993; 95: 193-199.
- Davis S, Hardy JH, Taylor MJ, Stockwel A, Whalley DR, Wilson CG. A comparative study of the gastrointestinal transit of a pellet and tablet formulation. *J Pharm Pharmacol*, 1984; 36: 740-742.
- Raghavan CV, Chithambaram M, Joseph AJ, Thengungal KR. An in Vitro and in Vivo Investigation into the Suitability of Bacterially Triggered Delivery System for Colon Targeting. *Chem Pharm Bull*, 2002; 50: 892-895.
- Ugurlu T, Turkoglu M, Soyogul Gurer U, Gurbuz Akarsu B. Colonic delivery of compression coated nisin tablets using pectin/HPMC polymer mixture. *Eur J Pharm Biopharm*, 2007; 67: 202-210.
- Semde R, Amighi K, Devleeschouwer JM, Moes JA. Effect of Pectinolytic enzyme on the theophylline release from pellets coated with water insoluble polymers containing pectin HM or calcium pectinate. *Int J Pharm*, 2000; 197: 169-179.
- Wei H, Qing D, Ying CD, Bai X, Fang FL. Study on colon-specific pectin/ethyl cellulose film-coated 5-fluorouracil pellets in rats. *Int J Pharm*, 2008; 348: 35-45.
- Wakerly Z, Fell JT, Attwood D, Parkins D. Studies on drug release from pectin/ethyl cellulose film coated tablets: a potential colonic delivery system. *Int J Pharm*, 1997; 153: 219-224.
- Jackson IM, Roberts S, Timmins P, Sen H. Comparison of laboratory scale processing in the production of coated pellets. *Pharm Technol*, 1989; 1: 29-32.
- Nastruzzi C, Cortesi R, Esposito E, Genovesi A, Spadoni A, Vecchio C, Menegatti E. Influence of Formulation and Process Parameters on Pellet Production by Powder Layering Technique. *AAPS Pharm Sci Tech*, 2000; 1: 9.
- Kantor TG. Ketoprofen: a review of its pharmacologic and clinical properties. *Pharmacotherapy*, 1986; 6: 93-103.
- Lemke TL, Williams DA, Roche VF, Zito SW. Foyes Principles of Medical Chemistry. 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2009. 979.
- Hodges LA, Connolly SM, Band J, Mahony BÒ, Ugurlu T, Turkoglu M, Wilson CG, Stevens HNE. Scintigraphic evaluation of colon targeting pectin-HPMC tablets in healthy volunteers. *Int J Pharm*, 2009; 370: 144-150.
- Devi SKU, Thiruganesh R, Suresh S. Preparation and characterization of pectin pellets of aceclofenac for

- colon targeted drug delivery. *J Chem Pharm Res*, 2010; 2: 361-374.
14. Ghaffari A, Oskoui M, Helali K, Bayati K, Tehrani MR. Pectin/chitosan/eudragitRS mixed-film coating for bimodal drug delivery from theophylline pellets: preparation and evaluation. *Acta Pharm*, 2006; 56: 299-310.
 15. Arulsudar N, Subramanian N, Mishrap, Chuttani K, Sharma RK, Murthy RSR. Preparation, characterization, and biodistribution study of technetium-99m -labeled leuprolide acetate-loaded liposomes in ehrlich ascites tumor-bearing mice. *AAPS Pharm Sci*, 2004; 6: 5.
 16. Abrahamsson B, Alpsten BM, Jonsson AE, Lundberg PJ, Sandberg A, Sundgren M, Svenheden A. Gastrointestinal transit of a multiple-unit formulation (metoprolol CR/ZOK) and a non-disintegrating tablet with the emphasis on colon. *Int J Pharm*, 1996; 140: 229-235.
 17. Basit WA, Newton JM, Short MD, Waddington WA, Ell PJ, Larry LF. The effect of polyethylene glycol 400 on gastrointestinal transit: implications for the formulation of poorly water soluble drugs. *Pharm Res*, 2001; 18: 1146-1150.
 18. Koukourakis MI, Koukouraki S, A. Giatromanolaki A, Archimandritis SC, Skarlatos J, Beroukas K, Bizakis JG, Retalis G, Karkavitsas N, Helidonis ES. Liposomal doxorubicin and conventionally fractionated radiotherapy in the treatment of locally advanced non-small-cell lung cancer and head and neck cancer. *J Clin Oncol*, 1999; 17: 3512-3521.
 19. Sanada S, Ando A, Ando I, Hiraki T, Hisada K. A single-strip mini-paper chromatographic method for rapid purity-control of ^{99m}Tc-labeled radiopharmaceuticals. *Eur J Nucl Med*, 1986; 12: 390-393.
 20. Kwakye KO, Fell JT, Sharma HL, Smith AM. Gamma scintigraphic evaluation of film-coated tablets intended for colonic or biphasic release. *Int J Pharm*, 2004; 270: 307-313.
 21. Jain SK, Agrawal GP, Jain NK. Evaluation of porous carrier-based floating orlistat microspheres for gastric delivery. *AAPS Pharm Sci Tech*, 2006; 7: 90.
 22. Sinha A, Ball DJ, Connor AL, Nightingale J, Wilding IR. Intestinal performance of two mesalamine formulations in patients with active ulcerative colitis as assessed by gamma scintigraphy. *Pract gastroenterol*, 2003; 27: 56-69.