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Design and gamma scintigraphic evaluation of colon specific pectin-EC pellets of secnidazole prepared by powder layering technology

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The aim of the present study was to prepare a colon targeted pellet formulation of secnidazole and to evaluate the formulation *in vitro* and *in vivo* by a gamma scintigraphy method. Pectin/ethyl cellulose in different ratios and in different coating labels with plasticizer was used to prepare secnidazole pellets by a powder layering technique. The formulations were tagged with ^{99m}TC-DTPA, a tracer in gamma scintigraphy to evaluate its transit behavior in rabbits. Morphology and compatibility were studied using Scanning Electron Microscopy, IR spectroscopy and Differential Scanning Calorimetry were used for the characterization of prepared pellets. The *in-vitro* study suggested that pectin (59%) esterification and ethyl cellulose 45cps at 20% coating label led to an optimum bacterial enzyme dependent released behavior. The optimized formulation was subjected to an *in-vivo* transit study. Scintigraphy images clearly indicated that the formulation can delay the drug release prior to the colon. The average time of gastric emptying and colon arrival was 57 min and 6.08 h, respectively. The coated pellets prepared by powder layering technology successfully released drug in the colon indicating that site specificity has been achieved with pectin 59% esterification and ethyl cellulose 45 cps at 1:2 ratio with 20% coating label.

1. Introduction

The delivery of drugs to colon for systemic action or a local effect is valuable in a variety of circumstances. These include the topical treatment of diseases such as ulcerative colitis, Crohn's disease and colon carcinoma and the potential for the oral delivery of peptides and other labile drugs which are unstable in the upper part of gastrointestinal (GI) tract (Ashford et al. 1993; Jain et al. 2007). The requirement for an oral colonic drug delivery system is to reduce the drug release to a minimum prior to the cecum. If systemic absorption from the colon is desired, the drug should be released rapidly thereafter. Different types of pectin have been studied as components for oral formulations intended to release their active substance in the colon. Their mode of action is based on their sensitivity towards the polysaccharides by the bacteria in colon (Ahrabi et al. 2000). The major problem encountered with pectin containing targeted drug delivery systems is their solubility and swelling properties in aqueous media. As a consequence, film-coating consisting of pectin alone is unable to prevent the release of drugs during the transit through the stomach and the small intestine (Semde et al. 2000).

To overcome the problem of dissolution of pectin in the upper GI tract, many approaches have been evaluated to create an effective pectin-based drug delivery system. Among these approaches, the combination of pectin with water insoluble polymers as film-coating materials appears especially promising. It was reposted that a combination of ethyl cellulose and pectin could protect a drug in the upper GI tract while allowing enzymatic breakdown and drug release in the colon (Wei et al. 2008). Furthermore, the combination of pectin and ethyl cellulose applied as a film coating may offer a potential approach to a more universal 'colonic' coating (Wakerly et al. 1997).

Pelletization techniques widely used in pharmaceutical industries are direct pelletization, extrusion-spheronization and layering. The layering process is the process in which drug in powder, solution or suspension form is layered onto seed materials. Powder layered pellets possess higher pellet density and smoother surface than do the suspension layered pellets due to the greater consolidation resulted from tumbling and colliding of pellets (Sinchaipanid et al. 2004). The most attractive features of this powder layering system are the uniform distribution of the binder solution, as well as the easy-to-clean pan and the possibility of applying the successive functional film coating using the same equipment (Nastruzzi et al. 2009).

Secnidazole (α, 2-dimethyl-5-nitro-1*H*-imidazole-1-ethanol) is structurally related to the commonly used 5-nitroimidazoles, metronidazole and tinidazole. These drugs share a common spectrum of activity against anaerobic microorganism and they appear particularly effective in the treatment of amebiasis, giardiasis, trichomoniasis and bacterial vaginosis. In these cases, the treatment with secnidazole is shorter and significantly more effective than the treatment with other imidazole drugs (Rivera et al. 2000).

Gamma scintigraphy, a non-invasive imaging technique, has been shown to be successful in determining the *in-vivo* behavior of various colon delivery systems. By incorporating small amounts of gamma-emitting radionuclides into the dosage

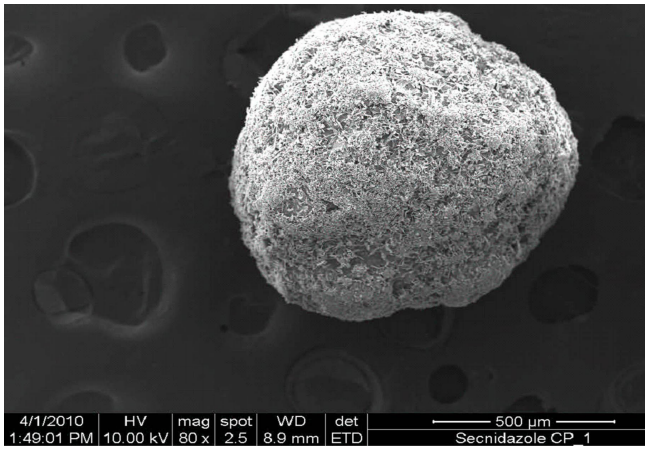

Fig. 1: SEM of Secnidazole drug loaded pellet at 80X magnification coated with pectin HM 59% and EC 45 cps with 20% TWG

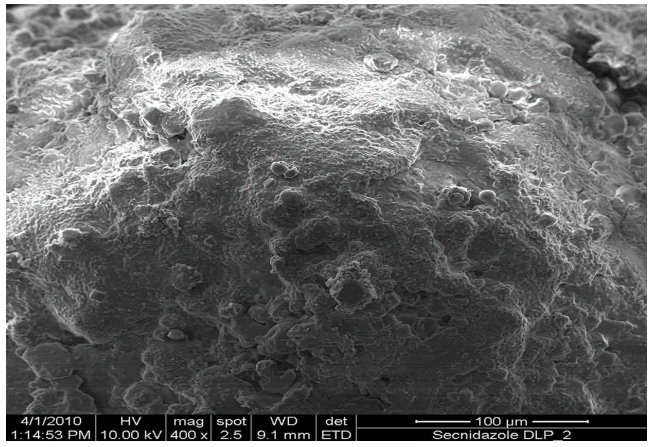

Fig. 2: SEM of Secnidazole drug loaded pellet's closed view at 400X magnification coated with pectin HM 59% and EC 45 cps with 20% TWG

forms, it is possible to establish the GI transit patterns of these systems within the body and determine site of disintegration and release (Hodges et al. 2009).

In this study, secnidazole drug loaded pellets were coated with different ratios of pectin and ethyl cellulose (45 CPS) by powder layering technology using conventional pan coating equipment. The coated drug loaded pellets were evaluated *in vitro* in presence of pectinolytic enzyme and *in vivo* by gamma scintigraphy in rabbits to study the transit pattern.

2. Introduction, results and discussion

2.1. Powder layering process

The aim of the work described here was to design a colonic drug delivery formulation for a better treatment of colorectal diseases. The pellets made of pectin/ethyl cellulose achieve microbial enzyme dependent drug release kinetics. Pellets containing secnidazole (model drug) were prepared using the conventional coating system which is able to process pellets of desire size and shape. This process led to the formation of multiple layers of drug particles that adhere to one another due to capillary pressure and interfacial forces originating from the liquid phase, allowing the enlargement of the initial cores. Intraparticellar solid bridges were formed after each wetting-powder cycle by the complete removal of water through a stream of warm air.

2.2. Scanning electron microscopy (SEM)

Scanning electron microscopy was performed to characterize the surface of the formed pellets. Pallets with spherical, rough and discrete surfaced with less crystals of the drug are visible on surface indicated that the concentration of polymeric solution is sufficient for complete coating. Scanning electron photomicrographs of the optimized formulation are shown in Figs. 1–2.

2.3. FT-IR analysis

In the FT-IR spectrum the characteristic peaks correspondend to a NO_2 group (1527.6 and 1358.3 cm^{-1} for the asymmetric and symmetric bends), a CH_3 group (1466.4 cm^{-1} for asymmetric bend), a CH_2 group (1489.0 cm^{-1} for scissors bend) and C-N groups (1271.8 cm^{-1}). The spectra of secnidazole and secnidazole formulation show that there was no significant interaction between drug and other polymers and plasticizes.

2.4. Differential scanning calorimetry (DSC)

DSC scan was recorded for secnidazole and secnidazole pellets as depicted in Figs. 3–4. Pure secnidazole displayed a single sharp endothermic peak at 82.76 °C corresponding to the melting point of the drug and an identical peak was also observed in the pellet formulations. The thermographic result shows that the drug retains its identity in the coated pellet formulations. The additional peak in the formulation is due to other components present in the pellets.

2.5. In vitro drug release

The *in vitro* release of secnidazole from the ^{99m}Tc-DTPA labelled and the unlabelled coated pellets was investigated to determine possible effect of the labeling process on the kinetics of drug release from the tablets. Tests were conducted in 900 ml of dissolution medium maintained at 37 ± 0.5 °C with a paddle rotation speed of 100 rpm. The pH of the medium was varied over the course of the experiment: 0.1N hydrochloric acid (pH 1.2) was used for the first 2 h followed by phosphate buffer pH 6.8 for the next 3 h and finally pH 6.0 for rest of the time. Samples 5 ml were withdrawn at predetermined times using an automated sampler. The secnidazole concentration in each sample was determined with an UV-visible spectrophotometer.

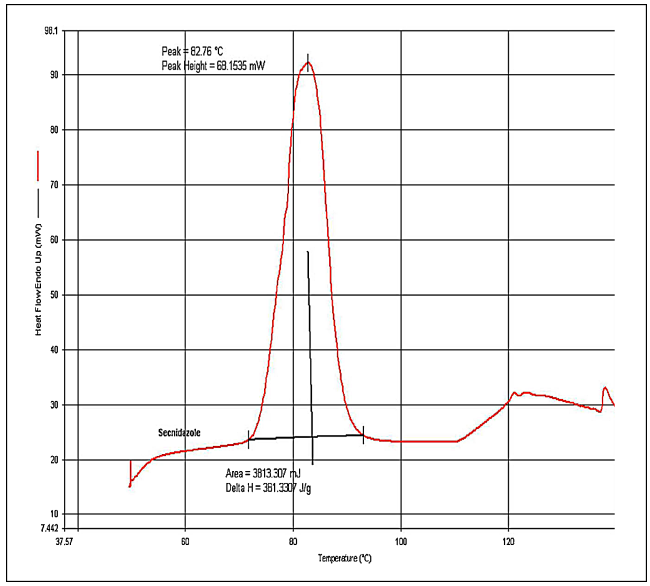

Fig. 3: DSC thermogram of secnidazole pure drug

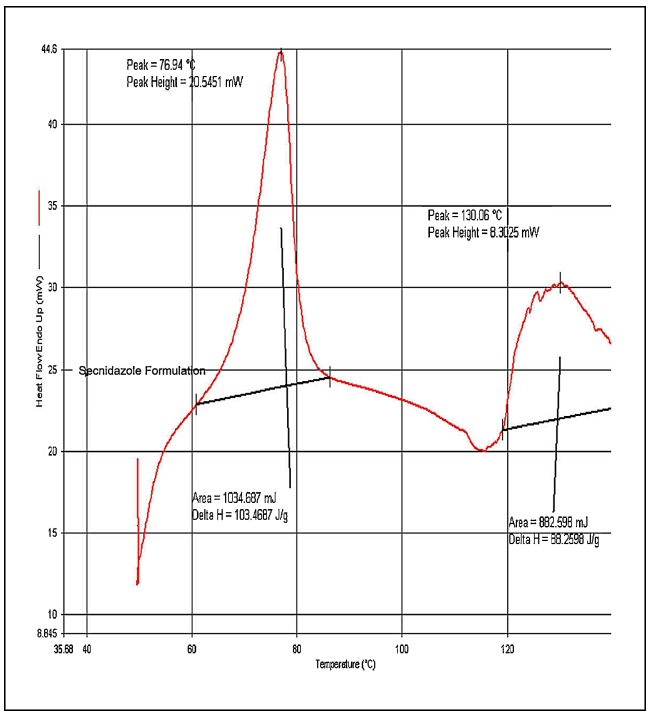

Fig. 4: DSC thermogram of secnidazole formulation

Fig. 5 shows the drug release profiles of the labelled and the unlabelled coated pellets after 24 h dissolution testing in simulated gastrointestinal conditions. The cumulative amount of secnidazole released from the pellets after 12 h dissolution testing were $64.21 \pm 0.79\%$ (labelled), and $58.19 \pm 0.30\%$ (unlabelled) and after 24 h was $93.59 \pm 0.87\%$ and $89.12 \pm 0.86\%$ respectively. The similarity in the drug release profiles indicates that the labeling process had no adverse effect on the kinetics of drug release.

2.6. *Gamma scintigraphy study*

2.6.1. *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 0.1 M HCl (pH 1.2) for 2 h, pH 6.8 for 3 h and pH 6.0 phosphate buffer solutions at 6th h was $01.87 \pm 0.11\%$, $14.95 \pm 0.7\%$, and $24.03 \pm 2.6\%$, respectively, shown in Fig. 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 h of dissolution testing in each medium. Thus,

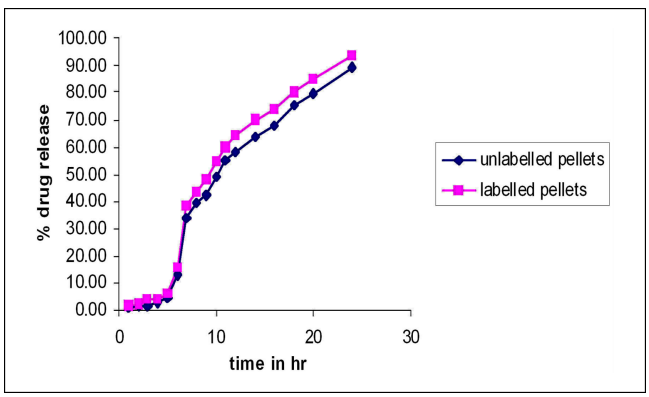

Fig. 5: . Drug release vs time graph of unlabelled and labelled pellets of secnidazole

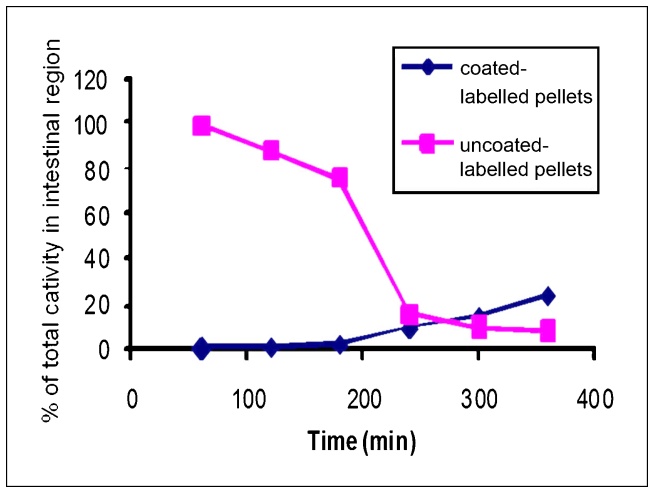

Fig. 6: Radioactivity release from coated and uncoated pellets in intestine region

the labeling procedure is satisfactory in that there is no sudden release of radioactivity into the dissolution media (Kwakye et al. 2004)

2.6.2. *In-vivo imaging study on rabbit*

The pectin/EC coated secnidazole pellets with 1:2 ratio with 20% coating level showed optimum *in-vitro* and controlled release behavior so was finally selected for an *in vivo* gamma scintigraphy study and the results were compared with uncoated pellets. Gamma images of the ^{99m}Tc-labeled coated pellets are shown in Fig. 7. Gastric retention time of almost 6 h was achieved in all the rabbits. A sufficient number of counts of ^{99m}Tc-DTPA labeled secnidazole pellets for the 10 h 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 study period of 10 h. Coated drug loaded pellets remain intact until they reach the colon. After reaching the colon, in presence of pectinolytic enzyme, the pellets start disintegrating and releasing the drug.

2.6.3. *Transit of drug-loaded radiolabelled pellets*

Transit of pellets in different regions is expressed as the time for 50% ($T_{50\%}$) to leave the GIT or to arrive at the colon. Six subjects (rabbits) were subjected to a transit study of drug loaded radiolabelled pellets. It was observed that in some subjects the empting of pellets was rapid but in another subject a slow empting was observed (Table 1). The average time of gastric empting

Table 1: Transit of drug-loaded radiolabelled pellets in different parts of GIT

Subject	Gastric emptying $T_{50\%}$ (h)	Small intestinal transit $T_{50\%}$ (h)	Colon arrival $T_{50\%}$ (h)
1	0.51	4.34	5.03
2	1.03	6.17	6.34
3	1.09	6.01	6.38
4	0.42	5.51	6.14
5	0.49	5.55	6.19
6	0.56	6.21	6.42
Mean	0.57	5.63	6.08
SD	0.23	0.70	0.53
Median	0.52	5.78	6.27
n	6	6	6

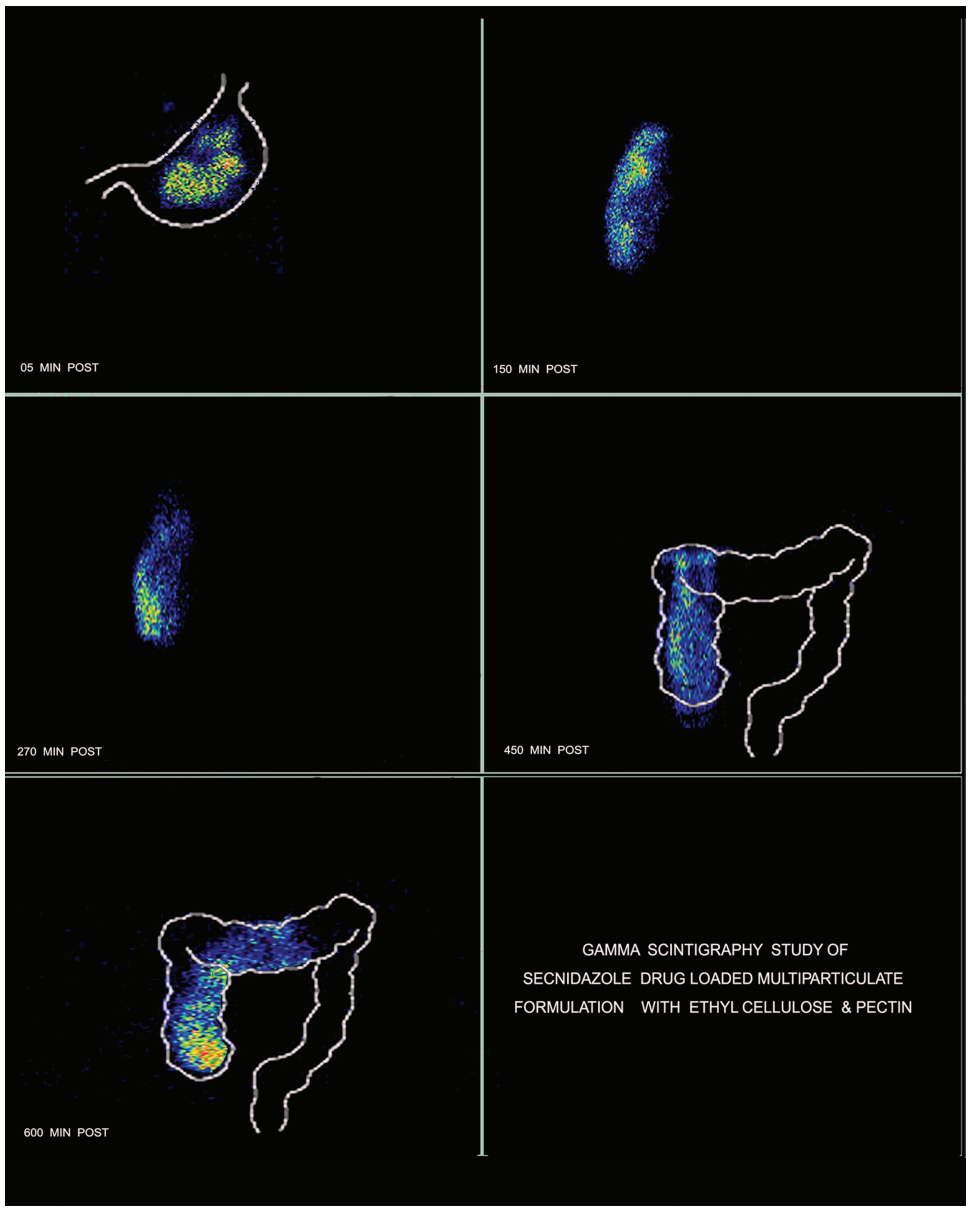

Fig. 7: Gamma scintigraphy image of ^{99m}Tc-DTPA labelled coated pellets of secnidazole at periodic interval

was 57 min with 5.63 h of small intestinal transit and 6.08 h to colon arrival.

3. Experimental

3.1. Materials

Ethyl Cellulose (45cps) was kindly provided by Dow Chemical Company Ltd, Middlesex, England. Pectin (59%) esterification was a generous gift sample of Herbstreith & Fox, Germany. Pectinex Ultra SP-L (Pectinolytic Enzyme) obtained from Novo Ferment, Switzerland. 1 ml of enzyme has an activity of 9500 PG/ml with a PG being the milliequivalence of reducing (carbonyl)groups liberated from pectin per minutes per unit of enzyme. The plasticizer TEC (triethyl citrate)-K PH EPs was kindly provided by Polynt SPA, Italy and secnidazole by Aarti Drugs Limited, Mumbai, India. All other chemicals were of analytical grade.

3.2. Preparation of secnidazole 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 for removal of moisture present. 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) until the bed became wet. Immediately the required amount of powder drug was layered on to the wet bed of pellets. Pan rotation was continued until the dry powder adhered onto the wetted pellets properly. Drying bed temperature and blowing air temperature were maintained properly to avoid over heating of drug loaded pellets, which may cause the separation of drug from the pellets after several pan rotations.

3.3. Formation of coating layer

Pectin in 40:60 of acetone:isopropyl alcohol 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 2.

3.4. Morphology of pellets

The surface morphology of pellets of optimized formulation was examined before and after dissolution using a 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.

Table 2: Processing conditions used for the study

Formulations	Non pareil (mg)	Secnidazole (mg)	Ratio of pectin: ethyl-cellulose	Coating label in %	Talc (% w/w) Anti-sticking agent	TEC (% w/w) Plasticizer
F1	500	500	1:1	10%	5% w/w	10% w/w
F2	500	500	1:1	15%	5% w/w	10% w/w
F3	500	500	1:1	20%	5% w/w	10% w/w
F4	500	500	1:2	10%	5% w/w	10% w/w
F5	500	500	1:2	15%	5% w/w	10% w/w
F6	500	500	1:2	20%	5% w/w	10% w/w
F7	500	500	1:4	10%	5% w/w	10% w/w
F8	500	500	1:4	15%	5% w/w	10% w/w
F9	500	500	1:4	20%	5% w/w	10% w/w

3.5. Fourier transformed infrared spectroscopy (FT-IR)

IR spectroscopy of secnidazole loaded pectin/ethyl cellulose pellets was performed on a Fourier Transformed Infrared Spectrophotometer (840, Shimadzu, Japan). The pellets of drug and KBr (potassium bromide) were prepared by compressing the powders at 20 psi for 10 min on a KBr press. The mixture was ground 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} coupled to a personal computer. The characteristic peaks of IR transmission spectra of secnidazole pure drug and secnidazole formulation were recorded.

3.6. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is valuable in studying the beginning of melting of a compound. The temperature at which the suspected melting endothermic peak begins is considered to be the beginning of melting. Thermograms of secnidazole-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.

3.7. Gamma scintigraphic study

3.7.1. Labeling of pellets

Tagging the radioactive substance with the secnidazole pellets was done in the Regional Radiation Medicine Centre & Variable Energy Cyclotron Centre, under the Department of Atomic Energy, Government of India. Gamma scintigraphy study on healthy albino rabbits was done in Cancer Centre & Welfare Home, Thakurpukur, Kolkata, India. DTPA-secnidazole pellets were prepared by using powder layering technology. 10 mg/kg body weight of drug-DTPA pellets were socked with ^{99m}TC which was previously reduced with stanus chloride from 10MBQ to 4MBQ/dose. MBQ strength of radioactive label was determined by CAPINTEC CRC-15R detector with ionization chamber of a Colimeter. Then the labeled pellets were coated with pectin/ethyl cellulose to achieve a 20% coating level (optimized formulation) and dried for 15 min in a fume cupboard. The specification of gamma camera was as follows-
Model: INFINIA made by GE Company, Resolution: 256 × 256 with zoom 2, Counter time: 5 min, Acquisition time: 5 min, Photo peak: 140 keV ± 10 (10% window).

3.7.2. Stability of radiolabelled pellets coated with pectin-ethyl cellulose

The stability of radioactivity (counts per 20 s) of ^{99m}Tc-DTPA labelled coated pellets was determined using a CRC-15R detector with a 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 0.1 M HCl for 2 h, 6.8 pH for 3 h & 6.0 pH for 1 h at 37 ± 0.5 °C using the USP XXIII basket type dissolution apparatus. The rotation speed was 100 rpm. At 30 min intervals, 5 ml 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 counted. The percentage of radioactivity in the pellets after 6 h of dissolution testing was calculated from the initial and final activity remaining (Kwakye et al. 2004).

3.7.3. In-vivo imaging study on rabbit

The recommendations of the "Institutional Animal Ethical Committee" ["Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA Regn. No. 1126/bc/07CPCSEA) India] for the care and use of laboratory animals were strictly followed throughout the experiment. Twelve 1-year-old male albino rabbits with 2.5 to 3.6 kg weight were used to monitor the *in vivo* transit behavior of the secnidazole pellets coated with pectin/ethyl cellulose. Rabbits were divided into 2 groups (group I and group II). The animals were fasted for 12 h 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 non-coating to group II followed by a 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 a 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 a magnetic disk, and analyzed to determine the distribution of activity in the stomach, intestine and colonic region. In between 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 formulation had emptied the stomach completely (Jain et al. 2006). Secnidazole loaded radiolabelled pellet distribution was measured by the number of radioactive counts 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 (Sinha et al. 2003). The transit profile for coated pellets was characterized by the half-lives ($T_{50\%}$) for gastric empting, for colon arrival and for intestinal transit (the time interval between $T_{50\%}$ values for gastric emptying and colon arrival).

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