EXPERT OPINION

Evaluation of *in vivo* behavior of controlled and pulsatile release pastilles using pharmacokinetic and γ-scintigraphic techniques

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Objective: To evaluate the *in vivo* behavior of controlled and pulsatile release pastilles for chronic treatment of asthma and chronic obstructive pulmonary disease (COPD) and for the chronotherapeutic management of nocturnal asthma, respectively.

Research design & methods: The prepared immediate release and controlled release pastilles were subjected to *in vivo* pharmacokinetic studies in rats. Whereas, pulsatile release formulation was subjected to γ-scintigraphic study in rats to study the gastrointestinal transit of the formulations and its results were correlated with the previous pharmacokinetic data.

Results: The *in vivo* pharmacokinetic study of controlled release pastille formulation showed significant decrease in C_{max} with increase in t_{max}, which indicates that the effect of dosage form would last for longer duration. Thus, the prepared formulation can be useful for the chronic treatment of asthma and COPD. The γ-scintigraphic study and pharmacokinetic data indicated that the pastilles coated with the enteric coat and the additional floating coat were effective in significantly delaying the *in vivo* drug release (by 4–5 h) required for the chronotherapeutic treatment of nocturnal asthma.

Conclusion: This study opens a new alternative to the conventional tablet or capsule dosage form for the development of both immediate and modified release drug delivery systems.

Keywords: controlled release, doxofylline, *in vivo* study, pastilles, pharmacokinetic evaluation, pulsatile release, γ-scintigraphy

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1. Introduction

Pastillation, a technology for fabrication of novel dosage form "pastilles," has been developed and has been discussed in detail in our previous review and research communications [1-3]. Controlled release formulation was developed for prolonged release of doxofylline [4] to improve patient compliance by reducing dosing frequency for chronic treatment of asthma and COPD, whereas the pulsatile release dosage form was developed for treatment of nocturnal asthma. The *in vitro* studies carried out have shown the effectiveness of the developed dosage forms [2,3].

In vitro drug release study in bio-relevant media is an important test during formulation development to predict the *in vivo* performance of dosage forms. Controlled release drug delivery systems are supposed to maintain their integrity in the entire gastrointestinal tract while it continuously releases the drug during its transit. On the other hand, pulsatile release dosage forms are expected to disintegrate after sufficient lag period during which it reaches the intestinal area. Several factors, especially the peristaltic movements, enzymatic actions, presence of bile salts, rate of gastric transit time, and the pH variations significantly influence the behavior

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Table 1. Formulation composition of prepared batches for *in vivo* studies.

Composition of ingredients	mg/batch			
	Immediate release (PEG based) pastilles Batch I	Controlled release (lipid-based) pastilles Batch II	Pulsatile release with enteric coat Batch III	Pulsatile release with enteric + floating coats Batch IV
DOX	500	500	500	500
Stearic acid		1700		
Benefat		75		
PEG 4000	2000		2000	2000
Colloidal silicon dioxide	75		75	75
Enteric coat (%) (5 g Eudragit L100 55 and 0.5 g triethyl citrate (plasticizer) & 2% talc in 100 ml methanol)			10 ± 5*	10 ± 5*
Floating coat (%) (1 g HPMC K15M, 0.1 g triethyl citrate (plasticizer) in 100 ml IPA DCM mixture (60:40 v/v). 10% $NaHCO_3$ & 2% talc was dispersed in the above solution				20 ± 5‡

*Amount of enteric coat applied was calculated in terms of percentage weight gain with respect to the weight of uncoated pastilles.
‡Amount of floating coat applied was calculated in terms of percentage weight gain with respect to the weight of enteric-coated pastilles. Coating layer weight gain values are represented as mean ± SD.

of the dosage forms in the gastrointestinal tract (GIT). In particular, the GI transit rate is frequently affected by various factors like inter-subject difference [5], meals [6], disease states [7], etc., resulting in remarkable alteration of the absorption profile of orally administered drugs. The variations in the above-mentioned factors are responsible for the variability in the inter-subject bio-analytical results usually observed. The primary disadvantage of *in vitro* experimental studies is that it can sometimes be very challenging to extrapolate their results to understand the performance of the dosage form in an intact organism and over-interpretation of their results can sometimes lead to erroneous conclusions. Therefore, the study of *in vivo* pharmacokinetic performance of the developed dosage form becomes very important.

The prepared controlled release formulation is a matrix system whereas the pulsatile release formulation is a reservoir type system. In the pulsatile system, the release is dependent on the functional coating layers (enteric and floating) applied on the immediate release pastilles. The factors influencing the drug release from an enteric-coated formulation include the type of polymer used for coating and the thickness of the coat applied [8]. The bioavailability of drugs from enteric-coated formulations can be variable, primarily because of differences in the rate at which the dosage form passes through the gastrointestinal tract and the subsequent site of drug release. On the one hand, if the formulation is retained in the stomach because of delayed gastric emptying, and the pH of the stomach contents increases above a certain threshold, the coating may begin to dissolve, leaving an acid-labile drug vulnerable to degradation. On the other hand, the formulation may move rapidly through the intestine, which may also decrease its bioavailability if the erosion of the enteric coat and the subsequent drug release occurs in a segment of the gastrointestinal tract where absorption is impaired [9]. Gamma scintigraphy is an ideal noninvasive technique for obtaining information on the gastrointestinal transit of a dosage form [10-12].

Gamma scintigraphy has proven to be of great value in assisting product development as well as in the testing of marketed products in pharmaceutical field [8,13-19]. In this technique, the gamma rays emitted by the radiolabeled dosage form or an anatomical site are focused on a sodium-iodide crystal of a gamma camera after passing through a collimator and the resultant flash of light is subsequently detected by photomultiplier tubes. The analog signal is then digitized and this permits quantitative image processing, which helps to monitor the location of the dosage form in the GIT. Most scintigraphic systems are able to separate emissions on the basis of the peak photon energies so that two isotopes can be monitored simultaneously and independently. This permits a concurrent, noninvasive behavioral study of two different dosage forms or two discrete components within the same delivery device [20]. The use of gamma scintigraphy to assess GI transit in experimental and clinical practice has been well established and utilized [20-24] to evaluate the gastric emptying profile even in rats [25,26] and cats [27].

Therefore, the aims of this study were to evaluate the bioavailability of doxofylline from the controlled release formulation relative to the immediate release pastille using pharmacokinetic study; to evaluate the rate of gastrointestinal

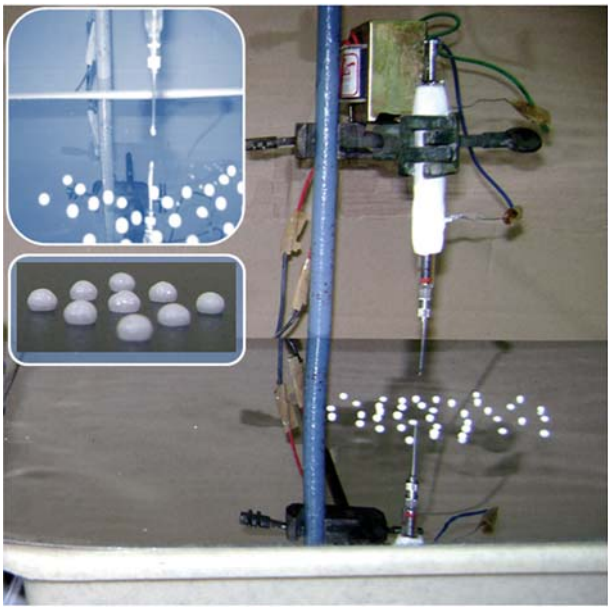

Figure 1. In-house laboratory design of pastillation device.
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transit of pulsatile release formulations using gamma scintigraphy; and to correlate the pharmacokinetic and scintigraphic data of pulsatile release formulation.

2. Materials and methods

2.1 Materials

Pure sample of doxofylline was a gift from Euro Drugs, Hyderabad, India. Acetonitrile (HPLC grade), methanol (HPLC grade), and potassium dihydrogen orthophosphate (AR grade) were obtained from Rankem, India. Double distilled water was used throughout the HPLC procedure.

2.2 Preparation of immediate, controlled, and pulsatile release formulations

The method of pastille fabrication using a laboratory scale device developed in-house was similar to that of our previous report [1] and the optimized compositions of the formulations based on the results of *in vitro* drug release study are shown in **Table 1**.

2.2.1 Formulation method of pastilles (lipid/PEG-based)

Briefly, the device (**Figure 1**) consisted of a glass syringe with stainless steel plunger, hypodermic needles (metallic), a metallic plate, heating coil and a 1.5 Amp transformer. The heating coil was wrapped on the external surface of an open-ended ceramic tube and coated with a thick layer of ceramic clay for insulation. The coil was then connected with the transformer before being connected to electricity. The syringe with hypodermic needle (needle size-20G) attached was inserted into the ceramic tube. This assembly was arranged over the metallic plate with the help of a burette holder. The metallic plate was cooled with the help of ice cubes in the ice tray placed below it. The PEG/lipid melt (with colloidal silicon dioxide in case of PEG) along with the drug was then drawn into the preheated syringe and allowed to fall drop-wise (with pressure regulation managed manually with plunger of the syringe) onto the cold plate at a dropping height of 1 mm to generate pastilles (**Figure 1** insets). The pastilles were then allowed to solidify and were finally scrapped with the help of a sharp metallic scrapper. Lipid-based controlled release, PEG-based immediate release, and cores of pulsatile release pastilles were prepared by this method.

2.2.2 Enteric coating of PEG-based pastilles for pulsatile release

2.2.2.1 Preparation of coating solution

The coating solution for enteric coat layer was prepared by dissolving Eudragit L100 55 (5.0 g) and triethyl citrate as plasticizer (0.5 g) in 100 ml methanol followed by dispersion of 2% w/w Talc. The coating solution for floating coat layering was prepared by dissolving HPMC K15 M (1.0 g) and triethyl citrate (plasticizer) (0.1 g) in 100 ml mixture of isopropyl alcohol and dichloromethane (60:40 v/v). Sodium bicarbonate (10% w/w), crushed and passed through #100 mesh (ASTM) was dispersed along with 2% w/w talc in the above solution.

2.2.2.2 Coating process

The coating of the PEG-based pastilles was carried out by spray coating technique using conventional coating pan (Macro Scientific Works, India). Coating process was carried out at bed temperature below 32 °C and the weight gain was periodically checked till it reached the desired weight. After coating, the pastilles were air-dried overnight and finally vacuum-dried (Decibel Digital Technology, India) at temperature and pressure conditions of 35 °C and 400 mm Hg for 2 h. Both the coatings were done using same procedure. Batch III pastilles were coated with enteric coat and Batch IV pastilles were prepared by coating with enteric coat followed by floating coat.

All the batches were evaluated for their *in vivo* behavior.

2.3 Bio-analytical method development

2.3.1 Chromatographic conditions

The HPLC system consisted of two delivery pumps (Waters Corp., USA), a diode array detector (Waters 2998) and a system controller (Empower Node 2054). A rheodyne manual injector (USA) attached with 20 µl sample loop was used for loading the sample. A C_{18} reverse-phase 250 × 4.6 mm 5 µm ODS2 column (Waters, Ireland) and a C_{18} guard column were utilized for drug separation. The mobile phase was 18:82 acetonitrile–12.5 mM potassium dihydrogen orthophosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) and the flow rate was maintained at 1 ml/min. The injection volume was 20 µl, elute was monitored at 275 nm and the sensitivity was 0.0007 AUFS.

2.3.2 Preparation of samples for calibration curve and for analysis

Stock solution (1 mg/ml) of doxofylline was prepared in methanol. Working solution (100 µg/ml) was prepared by appropriate dilution of the stock solution which was used for preparing spiking stock solutions for construction of eight-point calibration plots (25 – 2500 ng/mL). All stock solutions were kept refrigerated (4°C) when not in use. Calibration standard samples were prepared in bulk by spiking 500 µl control serum with 100 µl respective spiking stock solution; they were then divided into smaller volumes and stored at -20°C until analysis.

2.3.2.1 Drug extraction procedure from serum

A liquid–liquid extraction method was employed for extraction of drug from the serum [28]. Serum containing doxofylline (500 µl) was pipetted into microtubes and methanol (400 µl) was added to precipitate serum proteins. The sample was then vortex mixed for 8 min and centrifuged at 3500 rpm for 10 min. Supernatant (400 µl) was transferred to a glass tube and evaporated in a vacuum oven at 40°C. The residue was reconstituted in 200 µl mobile phase and 20 µl of the reconstituted sample was injected for HPLC analysis.

2.3.3 Extraction efficiency

Recovery of doxofylline was determined from samples at concentrations of 25, 250, and 2500 ng/ml. Five replicates of each sample were extracted as described above and analyzed by HPLC. The extraction recovery at each concentration was calculated by using the following equation:

$$\text{Recovery (\%)} = \frac{(\text{Peak area after extraction})}{(\text{Peak area after direct injection})} \times 100$$

2.3.4 Assay method validation

For determining the intra-day and inter-day precision of the assay method, samples spiked with 25, 250, and 2500 ng/ml of doxofylline were analyzed and both the precisions were evaluated. Five replicates at each concentration were processed as described above, on days 1, 2, 3, 4 and 5, to determine inter-day precision. Intra-day precision was measured on same day at initial time and after 6, 12, 18 and 24 h and percentage error was calculated by use of the equation:

$$\text{Error (\%)} = \frac{(\text{calculated concentration - Added concentration})}{(\text{Added concentration})} \times 100$$

2.4 *In vivo* pharmacokinetic study

In vivo studies were carried out as per the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. The study protocol was approved by the Animal Ethical Committee of Banaras Hindu University.

2.4.1 Animal experimental protocol for pharmacokinetic study

Male albino Wister rats of 300 ± 25 g were divided into groups comprising of six animals each. They were fasted for 12 h before the experiment with free access to water. After light anaesthetization with ether, the formulation (pastilles of mean diameter 1.5 ± 0.2 mm freshly dispersed in 5.0 ml of 1.0% aqueous polyvinyl alcohol solution) containing drug equivalent to 5.70 mg/kg body weight was orally administered.

For *in vivo* (pharmacokinetic) study of controlled release pastilles vs. immediate release pastilles, rats were divided in two groups of six each. One group was administered with immediate release pastilles and other one with controlled release pastilles. In case of *in vivo* (pharmacokinetic) study of two differently coated pulsatile release pastilles with uncoated immediate release pastilles, the rats were divided in three groups, each of six rats. Group I, II, and III were administered with uncoated pastilles, enteric-coated pastilles and enteric-coated pastilles with additional floating coat pastilles, respectively.

Blood (0.5 mL) was collected from the retro-orbital vein at 0, 0.25, 0.50, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h during the study. All blood samples were allowed to clot and then centrifuged for 10 min at 3000 rpm and finally the serum obtained was transferred to clean tube for storage at -20 °C until analysis. Sampling was done each time from alternate eye and the sampling was done at time point 1, from three rats out of six of a group and then at time point 2 from the other three rats of same group and likewise alternately, to reduce the number of blood withdrawals and vein punctures from each rat and each eye.

Pharmacokinetic parameters like peak plasma concentration (C_{max}), time to reach peak concentration (T_{max}) and area under the curve from time zero to last measured concentration ($AUC_{0\text{-last}}$) and the time span during which the plasma concentrations were at least 50% of the C_{max} value ($HVD_{t50\%\ Cmax}$) [HVD-half value duration] for doxofylline were obtained for each subject by noncompartmental pharmacokinetic models using Kinetica® software (version 5). The ratio between the $HVD_{t50\%\ Cmax}$ values of the test formulation and the immediate release formulation expressed as RΔ was also calculated to check any possible sustained release effect. A ratio of 1.5, 2 and > 3 indicates low, intermediate and strong sustained release effect, respectively [29].

All values are expressed as mean ± standard deviation (SD). Statistical analysis of the data was undertaken using one-way analysis of variance test followed by Tukey's multiple comparison test with Graphpad Prism statistical software program (version 5.03).

2.5 Gamma scintigraphy

2.5.1 Radiolabeling of pastilles

Gamma-scintigraphic studies were carried out to determine the location of pastilles after oral administration and their extent of transit through the gastrointestinal tract. The study was conducted in the Spec labs, Pune, India. Technetium (^{99m}Tc) was chosen for radiolabeling of the pastilles because

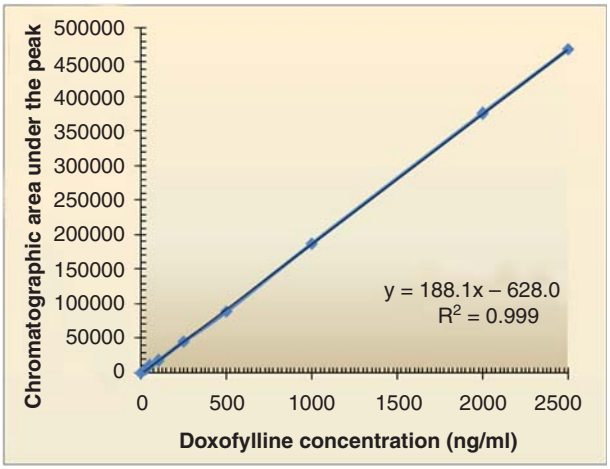

Figure 2. Standard curve of doxofylline extracted from serum.

of its short half-life of 6 h and very less amount of electron emission [30]. (S)-4-{2,3-bis[bis(carboxymethyl)-aminoprolyl] isothyocynate} of DTPA (CITC-DTPA) was used to provide the necessary ^{99m}Tc species for radiolabeling the pastilles. Pastilles labeled with ^{99m}Tc were administered to adult Wister rats. For radiolabeling, 5 ml of distilled water was added to approximately 1 g of pastilles (uncoated/coated) in a sterile glass vial. In another similar vial, 100 ml of ^{99m}Tc was taken and 5 mg of stannous chloride dihydrate (1 mg/ml in 10% acetic acid) was added. The pH was adjusted to 7.5 with 0.5 M sodium bicarbonate. The reduction of ^{99m}Tc was assured by Thin Layer Chromatography (Silica Gel) (TLC-SG). When the reduction was found to be 99%, the suspended pastilles were added to it and the mixture was left for 2 min to complete the reaction. The radiolabeling was done at the surface of the uncoated/coated pastilles as the aim was only to locate the formulation during its gastrointestinal transit and its disintegration. Therefore, specific radiolabeling of drug molecules was not carried out. The radiolabeling efficiency was evaluated with TLC-SG strips as stationary phase and 100% acetone as mobile phase.

$$\%\,\text{Radiolabelling} = \frac{\textit{Radioactivity (count) retained in the lower half of the strip}}{\textit{Initial radioactivity associated (total count present) with the strip}} \times 100$$

Radiochemical impurity in the form of unconjugated technetium was determined by ascending TLC-SG [12].

$$\%\,\frac{\text{R}}{\text{H}}\,\text{Technetium} = \frac{\text{Counts present in the lower part of the strip}}{\text{Total count present in the strip}} \times 100$$

2.5.2 *Stability of radiolabeled pastilles*

Stability tests of ^{99m}Tc-labeled pastilles were carried out to confirm the binding of sodium pertechnetate to the pastilles throughout the duration of the study [31,32]. One gram of radiolabeled pastilles was added to three tubes containing different standard buffers solutions of pH 1.2, 6.8 and 7.4, respectively, which were kept under stirring in a water bath maintained at 37°C. At predetermined time intervals, 0.2 ml of sample was taken using a pipette with a glass wool filter tip and at the end of the experiment the pastilles were recovered, washed and dried. The radioactivities of the samples, pastilles and the filtrate were counted in an auto gamma counter (Siemens). The sum of radioactivity of pastilles, the filtrate and the extreme samples was expressed as the total radioactivity.

2.5.3 *Animal study protocol for gamma scintigraphy*

In order to determine the gastrointestinal transit of pastilles in the GIT, imaging studies were performed using high specific activity of ^{99m}Tc-labeled pastilles. Three adult male Wistar rats (300 ± 25 g) were used in each group. The pastilles were administered at a dose of 10 mg/kg body weight by oral gavage, after overnight fasting for 8–10 h. Animals were given free access to water, but food was restored 1–2 h after dosing. The animals were anaesthetized with diazepam and serial scintigraphic examination was done at 0.5, 1, 1.5, 2, 3, 4 and 5 h to assess the mobilization of the pastilles in the GIT, using a large field view gamma camera (Siemens) equipped with a high-resolution, parallel-hole collimator and interfaced to a dedicated computer. Images were recorded for a preset time of 1 min/view with a 15% window centered to include the 140 keV photopeak of ^{99m}Tc.

3. Results and discussion

3.1 Bio-analytical method

The chromatogram containing doxofylline has shown a retention time of 9.75 min. The calibration plot of doxofylline in the concentration range 25 – 2500 ng/ml are shown in **Figure 2**. The calibration/regression equation of doxofylline obtained from its eight-point calibration plot was y = 188.1x - 628 (r^2 = 0.999), where y represents the doxofylline-to-standard peak-area ratio, x represents the concentration of doxofylline, m is the slope of the plot, and c is the intercept. The calibration curve is linear throughout the concentration range studied.

The recovery (mean ± SD) of doxofylline at 25, 250 and 2500 ng/ml was 95.06 ± 2.98, 98.91 ± 2.12 and 99.11 ± 1.65% (n = 6), respectively. Thus, the recovery of doxofylline with this method was found to be consistent and efficient.

Further, the intra-day and inter-day coefficients of variation (CV, %) and error (%) values are shown in **Table 2**. These values were within specified limits of less than 15% for inter-day and intra-day precision [33,34]. Data showed good consistency of results and the method was validated for its precision.

3.2 *In vivo* study

3.2.1 *Pharmacokinetic study of controlled release pastilles*

The pharmacokinetic study was carried out by oral administration of the PEG-based (immediate release) and the lipid-based (controlled release) pastilles in two different groups

Table 2. Inter-day and Intra-day precision data of assay of doxofylline.

Intra-day precision (n = 5)				
Added conc. (ng/ml)	**Measured conc. (ng/ml)**	**Standard deviation**	**Coefficient of variation (%)**	**Error (%)**
25	25.08	1.67	6.66	0.32
250	251.29	9.23	3.67	0.52
2500	2516.21	82.51	3.28	0.65
Inter-day precision (n = 5)				
Added conc. (ng/ml)	**Measured conc. (ng/ml)**	**Standard deviation**	**Coefficient of variation (%)**	**Error (%)**
25	26.13	2.71	10.37	4.52
250	249.61	7.37	2.95	-0.16
2500	2496.15	49.26	1.97	-0.15

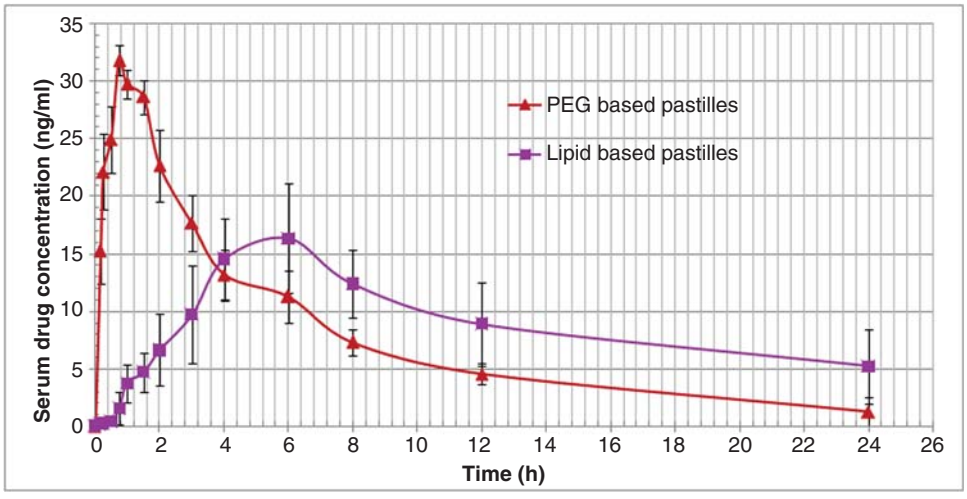

Figure 3. Serum drug concentration time or pharmacokinetic profile of PEG- and lipid-based pastilles (n = 6) in rats (Vertical bars represent mean ± sd).

of rats (n = 6). The mean serum drug concentration–time profiles obtained after oral administration of the two different types of pastilles are shown in **Figure 3**. The pharmacokinetic parameters are listed in **Table 3**. From statistical analysis of the AUC_{last} values of both the groups, it was observed that there was a slight increase of AUC_{last} in controlled release group but the increment was insignificant ($p > 0.05$). The differences of C_{max} and T_{max} values between the two groups were found to be statistically significant ($p < 0.05$ for C_{max} and $p < 0.01$ for T_{max}). The C_{max} was found to decrease to half its value while the T_{max} shifted by about 5 h in controlled release pastilles. The increase in the $HVD_{t50\%\ Cmax}$ values of controlled release group than immediate release group was significant in terms of sustained release behavior as indicated by its high Δ value of 3.59 which indicates a strong sustained release or drug release retardation.

The T_{max} (0.75 ± 0.06 hrs) values of the PEG-based pastilles clearly indicate the ability of the formulation to release the drug immediately upon reaching the GIT which is due to the hydrophilic nature and high solubility of PEG. Therefore, PEG-based pastilles can be a suitable design and also a potential alternative for the conventional immediate release dosage forms. The dramatic shift in T_{max} (6.0 ± 1.58 h) of the lipid based pastilles with respect to PEG pastilles is indicative of the lipid carrier to control the release of the drug even under the gastrointestinal environment. The above explanation is in agreement with the high Δ value (> 3.0) obtained in the calculation of the pharmacokinetic parameters and thus can be suitable for once-a-day administration. In addition, the immediate and controlled release behavior observed in *in vivo* pharmacokinetic studies is in agreement with the observations in *in vitro* drug release studies [2]. The reduced C_{max} of the lipid pastilles with respect to the PEG pastilles is due to slow and controlled release of the drug from the dosage forms.

Table 3. Pharmacokinetic parameters of PEG- and lipid-based pastilles in rats.

Pharmacokinetic parameters	PEG-based pastilles Batch I	Lipid-based pastilles Batch II
C_{max} (ng/ml)	31.83 ± 1.28	16.32 ± 3.69
T_{max} (h)	0.75 ± 0.06	6.0 ± 1.58
AUC_{last} (ng/ml*h)	182.56 ± 19.98	210.39 ± 59.6
HVD (h)	3.18 ± 0.21	11.43 ± 1.52
RΔ		3.59

*Values are represented as mean ± SD.

3.2.2 *Pharmacokinetic study of pulsatile release pastilles*

Three doxofylline pastille formulations (uncoated, enteric-coated and enteric-coated with additional floating coat) were evaluated in *in vivo* study on male albino Wister rats to study the effect of the coating on the pharmacokinetic behavior of the drug. Although this study has been reported earlier [3], it has been briefly discussed here to correlate the obtained results with γ-scintigraphic data of the present study. **Figure 4** presents the mean (n = 6) doxofylline serum concentration vs. time profile of the three formulations, while **Table 4** summarizes the pharmacokinetic parameters.

Table 4 shows that there are no statistically significant differences in the AUC_{last} values of the uncoated pastilles and coated formulations ($p < 0.001$). This indicates similar drug availability of all the compared formulations and that application of an enteric coat did not influence the bioavailability of doxofylline, significantly. Suppressed C_{max} and high value of t_{max} for pastilles with enteric and floating coat indicate sustained and controlled release property of the formulation as compared to uncoated and enteric-coated pastilles. However, no significant difference was observed between C_{max} and AUC_{last} values of uncoated and enteric-coated pastilles ($p < 0.001$), indicating the inability of only enteric coating to significantly increase bioavailability.

However in **Figure 4**, an initial lag time was observed before the drug release initiated from both coated pastilles. This is due to acid resistance of the coating polymer (Eudragit L100 55), which dissolves only at pH > 5.5 (i.e., after gastric transit of the coated pastilles). The lag time was longer in case of pastilles with additional floating coat in comparison to enteric-coated pastilles. This could be attributed to the presence of additional coat containing sodium bicarbonate, which generates carbon dioxide gas in presence of acidic medium and gets entrapped in the hydroxypropyl methyl cellulose (HPMC) layer. This phenomenon causes floating of the pastilles, which causes longer gastric transit time and finally results in increase in the lag phase. Further, their drug release was much slower as compared to the enteric-coated pastilles. Apart from the floating property, the other reason for such retarded *in-vivo* profile may be attributed to the presence of the viscous HPMC coat. Probably this coat takes time to dissolve completely during which the alkaline medium penetrates and dissolves the enteric coat to allow slow diffusion of the drug through the HPMC layer. It may be noted here that the difference in the *in vitro* drug release profile of enteric-coated pastilles with additional floating coat with respect to enteric-coated pastilles was also observed in *in-vivo* conditions with significant shift ($p < 0.01$) in T_{max} by about 3 h. Therefore, application of an additional floating coat on the enteric coat showed a beneficial effect in further delaying and reducing the rate of drug release. The $HVD_{t50\%\ Cmax}$ value of pastilles with floating layer also showed a significant increase ($p < 0.001$) as compared to uncoated and enteric-coated pastilles which is indicative of longer duration of action and increased efficacy of formulation. The characterization of *in-vivo* drug release profile using RΔ indicates intermediate sustained release effect. Thus, if the dosage form is administered at time of sleep in the night it would release the drug early morning and help to prevent the onset of nocturnal asthma attack during the period. Further the effect would last longer in comparison to immediate release or enteric-coated formulation.

3.2.3 *Gamma scintigraphy studies of pulsatile release pastilles*

The gamma scintigraphic study was conducted in order to assess gastro-retentive behavior and overall gastrointestinal transit of the two optimized enteric-coated formulations in rats in comparison with uncoated PEG-based pastilles. The pharmacokinetic study confirmed the presence of lag time in drug release of both pulsatile release formulations and moderate sustained release behavior in formulation with additional floating layer coating [3].

Amount of reduced/hydrolyzed (R/H) ^{99m}Tc was determined using pyridine: acetic acid: water (PAW) in the volume ratio of 3:5:1.5 as mobile phase and TLC-SG strip as the stationary phase [12]. The reduced/hydrolyzed technetium remained at the point of application whereas free pertechnetate and labeled complex moved with the solvent front.

The stability of ^{99m}Tc-labeled pastilles was tested in standard buffer solutions of pH 1.2, 6.8 and 7.4 in order to confirm that the activity does not leach out from the pastilles during transit time of the formulation through GI tract. The activity released from ^{99m}Tc-labeled pulsatile formulations and immediate release formulations at different pH is shown in **Table 5** in the study period of 6 h. The sufficient amount of stability ensures successful gamma imaging for the duration of the study.

The instruments and the laboratory set up during gamma scintigraphy experimentation are presented in **Figure 5(A-D)**. **Figures 6-8** show typical gamma scintigraphic images of radio-active labeled pastilles (uncoated pastilles, enteric-coated pastilles and enteric-coated with additional floating layer coated pastilles, respectively) passing through the GI tract post-dose. It is difficult to discriminate jejunum from ileum in the gamma scintigraphy images due to small size

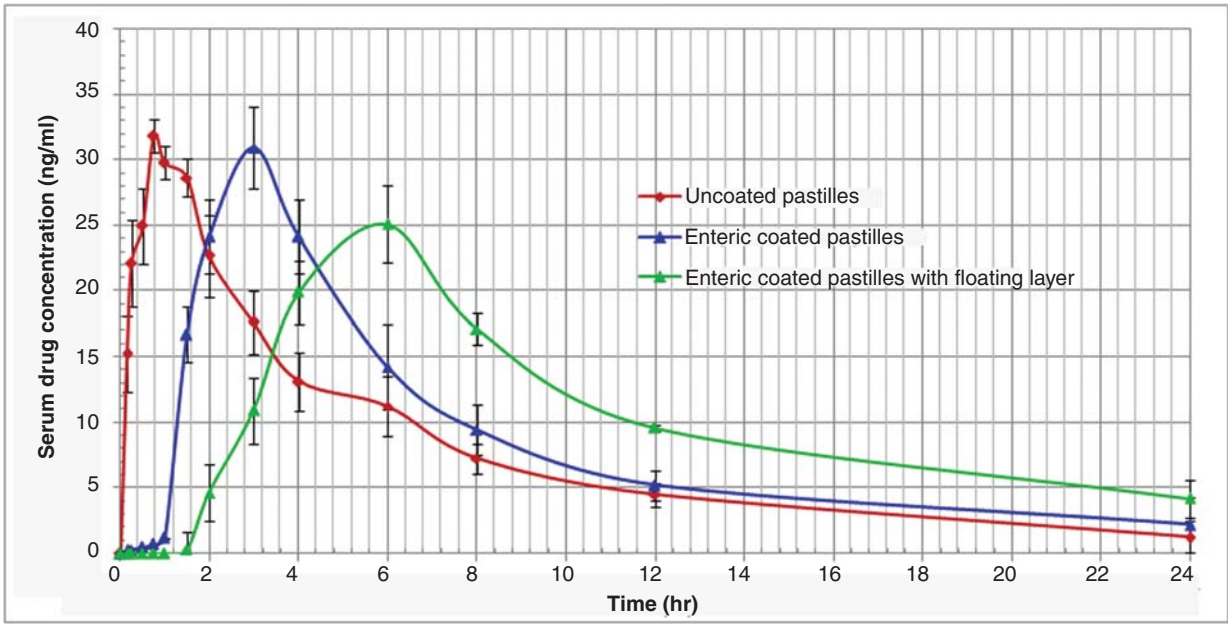

Figure 4. Pharmacokinetic profile of prepared pulsatile release pastilles and uncoated immediate release pastilles (n = 6) in rats (Vertical bars represent mean ± sd).

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Table 4. Pharmacokinetic parameters of immediate release and two optimized pulsatile formulations in rats (3).

Pharmacokinetic parameters	(Uncoated pastilles) Batch I	(Enteric-coated pastilles) Batch III	(Enteric & floating-coated pastilles) Batch IV
C_{max} (ng/ml)	31.83 ± 1.28	30.92 ± 2.12	25.12± 2.41
T_{max} (h)	0.75 ± 0.06	3.0 ± 0.27	6.0 ± 0.82
AUC $_{last}$ (ng/ml*h)	182.56 ± 19.98	201.47 ± 29.7	241.68 ± 42.7
$HVD_{t50\%\ Cmax}$ (h)	3.18 ± 0.21	4.23 ± 0.15	6.70 ± 0.13
RΔ		1.33	2.11

	Statistical analysis		
	I vs. III	I vs. IV	III vs. IV
C_{max} (ng/ml)	Not significant $p > 0.05$	Significant $p < 0.05$	Significant $p < 0.05$
T_{max} (h)	Significant $p < 0.01$	Significant $p < 0.001$	Significant $p < 0.001$
AUC $_{last}$ (ng/ml*h)	Not significant $p > 0.05$	Not significant $p > 0.05$	Not significant $p > 0.05$
$HVD_{t50\%\ Cmax}$ (h)	Significant $p < 0.001$	Significant $p < 0.001$	Significant $p < 0.001$

*values are represented as mean ± SD.

of the rat intestine. Therefore, the intestinal region was monitored closely by examining the radioactivity relative to its position in the stomach.

The gamma scintigraphy images (**Figure 6A and B**) of uncoated pastilles tested showed that the radioactivity was attenuated within 0.5 h. This indicates that in the presence of gastric fluid, PEG matrix dissolved completely and behaved as an immediate release dosage form. The enteric-coated pastilles were found to maintain their matrix integrity till 1.5 h in the gastric region, indicating the absence of gastric fluid influence on the coating applied as can be seen in **Figure 7(A,B & C)**. At 2 h the pastille was located in the jejunum area where it started to dissociate as observed by the distorted radioactive image in **Figure 7D & E**. This is in

Table 5. Stability data of radiolabeled pastilles in the form of ^{99m}Tc activity released in buffer solutions of different pH.

pH	Uncoated pastilles Batch I	Enteric-coated pastilles Batch III	Enteric & floating-coated pastilles Batch IV
1.2	0.13	0.21	0.16
6.8	0.25	0.32	0.27
7.2	0.36	0.34	0.31

Data represents % radioactivity released in solution.

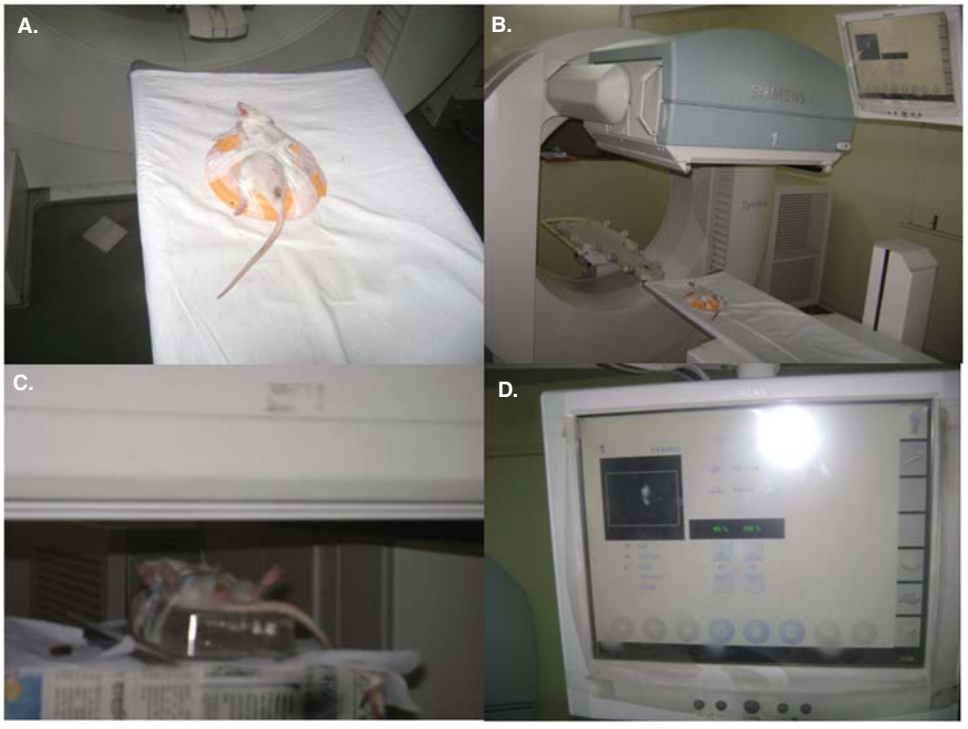

Figure 5. Images of Gamma Scintigraphy study on rats.

agreement with the pharmacokinetic data which also indicate that the t_{max} was achieved in 3 h for enteric-coated pastilles and 0.75 h for immediate release pastilles. So, there is a lag time of 2 h which has been due to its enteric coating (which dissolves at pH above 5.5) in the gastric region.

On the other hand, gamma scintigraphy study of enteric-coated pastilles with additional floating coat showed the retention of the formulation within the stomach for 2 h (**Figure 8A, B,C and D**). In the next hour, the pastille can be observed to have migrated into the jejunum area where the integrity of the formulation is still maintained (**Figure 8E**). This indicates that the floating coat is not only valuable in retaining the dosage form in the stomach for more than 2 h but also in protecting the enteric coat from alkali environment for an hour. Further, in the 4th hour the dosage form was found to reach the ileum region where it can be seen to start dispersing with attenuated radioactivity which is probably because the $t_{1/2}$ of ^{99m}Tc is around 4 – 5 h (**Figure 8F**). The image of 6th hour shows complete disintegration of the dosage form (**Figure 8G**). The results related to the gastric transit and disintegration of the pastilles in the intestinal lumen is in good agreement with the data observed in pharmacokinetic and *in-vitro* drug release study. The t_{max} for the floating pastilles can be observed to be about 1 h after the pastilles were found to start dissociating in the intestine which is the time required for the drug to get released, absorbed and finally attain C_{max}.

Thus, from the pharmacokinetic and γ-scintigraphic study, it was found that the enteric-coated pastilles with additional floating coat was having a lag time of 4 – 5 h in drug release and after that also it releases drug slowly to show prolonged effect. Therefore, if the formulation is administered at 22.00 h, the t_{max} would be achieved at 04.00 h, which

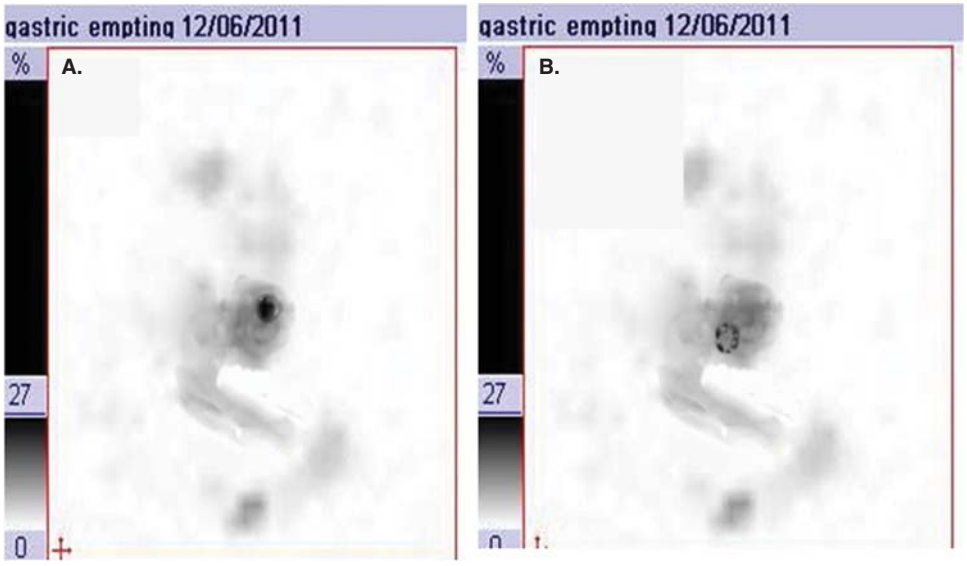

Figure 6. Gamma Scintigraphy study of uncoated PEG pastilles on rats at time point (A) 0.5 h and (B) 1 h.

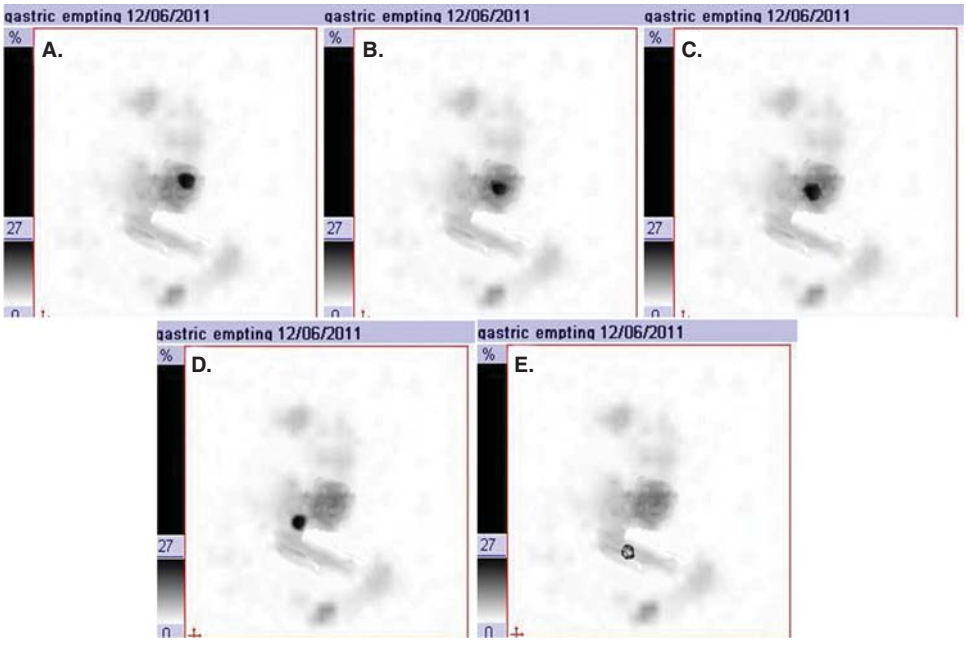

Figure 7. Gamma Scintigraphy study of enteric-coated PEG pastilles on rats at time point (A) 0.5 h, (B) 1 h, (C) 1.5 h, (D) 2 h and (E) 3 h.

matches with the peak time of low lung function and thus would show maximum therapeutic effect.

4. Conclusion

The *in vivo* pharmacokinetic study of controlled release pastille formulation showed successful retardation of drug release behavior which is in confirmation with the *in vitro* drug release data. The C_{max} was decreased significantly with increase in t_{max}, which indicates that the effect of dosage form would last for longer duration. Thus, the prepared formulation can be useful for the chronic treatment of asthma and COPD.

The *in vivo* study including pharmacokinetic and gamma scintigraphic imaging confirm the ability of the pulsatile release formulations to release the drug only after a desired period of time as specifically required for the treatment of

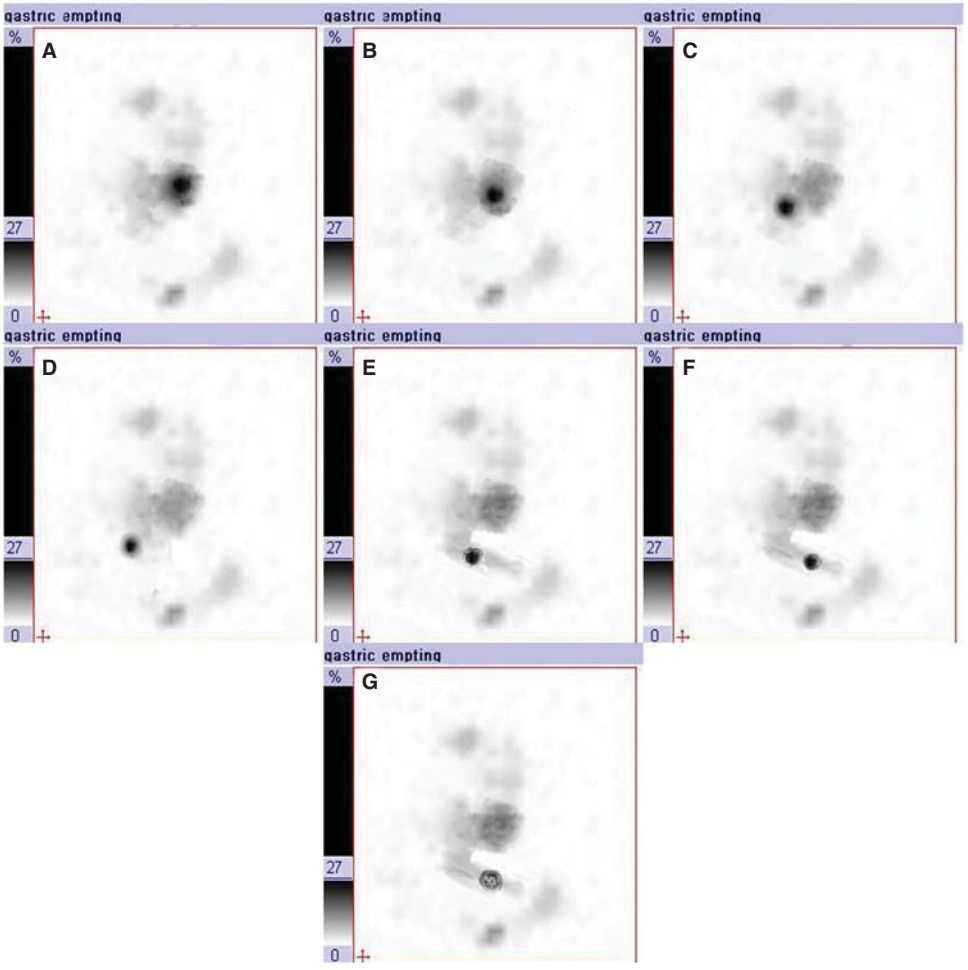

Figure 8. Gamma Scintigraphy study of enteric-coated PEG pastilles (with additional floating coat) on rats at time point (A) 0.5 h, (B) 1 h, (C) 2 h, (D) 3 h, (E) 4 h, (F) 5 h and (G) 6 h.

nocturnal asthma. These results are also in agreement with the *in vitro* drug release study which indicates the efficiency of the coating system. This study also confirms the ability of PEG-based pastilles to act as an immediate release dosage forms and thus opens a new alternative for the conventional tablets and capsules. Further coating of these dosage forms with appropriate polymers can significantly impart functional properties to control the release of the drug in a predetermined fashion. As an extension of this study, the immediate release pastilles can also be coated with extended release polymers to design a reservoir-based controlled release formulation.

Declaration of interest

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