Expert Opinion

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In vitro and *in vivo* evaluation of multilayered pastilles for chronotherapeutic management of nocturnal asthma

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Objective: The present work was undertaken with an objective to design a multilayered dosage form of doxofylline, using pastillation technology, for the chronotherapeutic management of nocturnal asthma.
Research design & methods: Pastilles consisting of the drug, polyethylene glycol and colloidal silicon dioxide, were generated using an in-house laboratory-scale pastillation device. The pastilles were further coated with enteric polymers and a floating layer, using conventional coater. The pastilles were subjected to physicochemical analysis, morphological characterization, *in vitro* drug release studies and *in vivo* pharmacokinetic studies in rats.
Results: It was observed that colloidal silicon dioxide was instrumental in improving the contact angle of the pastilles. The uncoated pastilles released the drug immediately, while the enteric-coated (10% w/w) pastilles were found to have sufficient acid resistance when the coat is applied with 5% (v/v) triethyl citrate as plasticizer. The *in vivo* blood serum profile indicated that the pastilles coated with the enteric coat and the additional floating coat were effective in significantly delaying the *in vivo* drug release required for the chronotherapeutic treatment of nocturnal asthma.
Conclusion: The present work opens a new alternative to the conventional tablet or capsule dosage form for the development of both immediate-release and modified-release drug delivery systems.

Keywords: chronotherapeutic management, doxofylline, enteric coating, nocturnal asthma, pastillation

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1. Introduction

The technology 'pastillation' is widely used in chemical industries for solidification and better handling of powdered chemicals. This technology was employed in pharmaceutical field for the first time in our laboratory to fabricate a novel controlled-release dosage form called 'pastilles' (hemispherical solidified units) using solid lipids as matrix-forming agent [1]. This technology can also be extended for the design of immediate-release dosage forms using solid poly ethylene glycols (PEG) as the matrix-forming agent [2]. Furthermore, the PEG pastilles can be enteric coated to achieve pulsatile drug delivery.

'Chronotherapeutics' refers to a treatment method in which *in vivo* drug availability is timed to match rhythms of disease in order to maximize therapeutic outcomes and minimize side effects. This principle is applicable for the effective treatment of nocturnal asthma. It is a well-known fact that circadian rhythms have significant influence on the disease processes and physiological events. In case of asthmatic patients, lung function (e.g., peak expiratory flow rate or FEV1) is usually highest

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at 16.00 h and lowest at 04.00 h and it is generally in the latter time when asthma symptoms are most prevalent [3]. In a study, it has been reported that about 53% deaths due to asthma occur between midnight and 08.00 h [4]. In a large survey, involving 7729 asthmatic patients, it was observed that 74% patients awakened one night per week and 64% awakened three nights per week due to nocturnal asthmatic attack [5]. Such a condition demands considerations of diurnal progress of the disease rather than maintaining constant plasma drug level. This requirement can be addressed by the design of a pulsatile/time-programmed drug delivery system, which on administration at bedtime (night) would release the drug rapidly only in the early morning hours to get maximum bronchodilatory effect.

Pulsed or pulsatile-release dosage forms play a significant role in achieving the goal of chronotherapeutics as they are designed to manifest the rapid and transient release of a certain amount of molecules within a short time period immediately after a predetermined no-drug release period or lag time [6]. These novel drug delivery systems have been attempted not only for the chronopharmacotherapy of diseases that show circadian rhythms in their pathophysiology [7]; but also for avoiding degradation of acid labile and sensitive active ingredients in upper gastrointestinal (GI) tract (proteins and peptides) [8] and avoiding pharmacokinetic drug–drug interactions between concomitantly administered drugs [9]. These dosage forms are also useful in avoiding the development of resistance to certain drugs such as isosorbide nitrate [10,11]. Pulsatile multiparticulate system has also been developed to add the advantage of uniform GI transit in comparison with single-unit dosage form apart from chronotherapeutic treatment [12,13].

Methylxanthines, an important class of bronchodilators, are a group of structurally related compounds that are widely used in the treatment of asthma and chronic obstructive pulmonary disease. Theophylline is a widely prescribed drug of this class. This drug has been used in the past for the development of pulsatile-release formulations aiming to treat nocturnal asthma [14,15]. Doxofylline [16], a new bronchodilator xanthine drug used for the treatment of asthma and chronic obstructive pulmonary disorder, has been used for the present study. Doxofylline (7-(1,3-dioxolan-2-ylmethyl)theophylline) differs from theophylline by the presence of a dioxolane group at 7th position and has shown good bronchodilator activity. Unlike theophylline, doxofylline has less affinity for $\alpha 1$ and $\alpha 2$ receptors, does not antagonize calcium channel blocker receptors and does not interfere with the influx of calcium into the cells, which together account for the absence of cardiac side effects in its administration, thus improving safety profile of the drug [16].

As no modified-release formulation of doxofylline is available in market, therefore, the objective of the present work was to explore multilayered pastilles to design as a modified-release formulation of doxofylline for the chronotherapeutic management of nocturnal asthma.

2. Materials and methods

2.1 Materials

Doxofylline was a kind gift from Euro Drugs (India). PEG 4000 and triethyl citrate were procured from Rankem (India). Colloidal silicon dioxide (Aerosil 200) and Eudragit L100-55 were generous gifts from Evonik Degussa India Pvt. Ltd (India). HPMC K15 M was obtained from Colorcon (India). All other chemicals and solvents were of analytical grade and were used as received.

2.2 Formulation method of pastilles

The method of pastille fabrication using a laboratory-scale device developed in-house was similar to that of our previous report as shown in **Figure 1** [1]. Briefly, the device 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 melt alone or its mixture with colloidal silicon dioxide along with the drug was then drawn into the pre-heated syringe and allowed to fall drop-wise (with pressure regulation managed manually with plunger of the syringe) on to 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.

2.3 Coating of pastilles

2.3.1 Preparation of coating solution

The coating solution for enteric coat layer was prepared by dissolving Eudragit L100-55 (5.0 g) and triethyl citrate (plasticizer) (0.25 g in enteric coat 1 and 0.5 g in enteric coat 2) 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 no. 100 mesh (ASTM), was dispersed along with 2% w/w talc in the above solution.

2.3.2 Coating process

The coating of the 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

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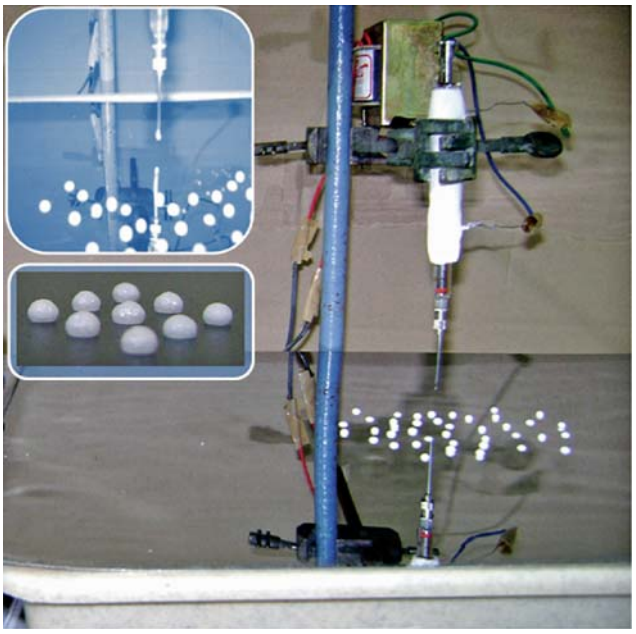

Figure 1. In-house laboratory design of pastillation device.
Reproduced with permission from Informa Healthcare [2].

Technology, India) at temperature and pressure conditions of 35°C and 400 mmHg for 2 h. All the three coatings were done using same procedure. The pastilles were then manually filled into size '0' capsules. The formulation composition of the prepared batches is shown in **Table 1**. Batch IV pastilles were coated with enteric coat 1, batch V pastilles were coated with enteric coat 2 and batch VI pastilles were prepared by coating with enteric coat 2 followed by floating coat.

2.4 Contact angle measurement

Contact angle of the solidified hemispherical drops, that is, pastilles, was measured against solid stainless steel plate by photographic method [17]. The photographs were proportionally magnified and processed using Adobe Photoshop® software. The angle of contact was determined manually and confirmed mathematically using the following equation:

$$\theta = 2 \tan^{-1} 2h/d$$

where h is the height of the drop from the plate and d is the diameter of the drop. Both of these dimensions can be measured directly from the photograph for calculating the contact angle.

2.5 Analytical method

The analytical methods used were as follows:

2.5.1 *Assay*

Uncoated pastilles equivalent to 100 mg of doxofylline were transferred into a dry 25 ml volumetric flask. Twenty milliliters of distilled water was added and sonicated in an ultrasonic water bath for 10 min with occasional swirling. Volume was made up to the mark with distilled water. Ten milliliters of the above solution was filtered through 0.45 µm nylon filter, diluted appropriately with distilled water and was analyzed spectrophotometrically at 273 nm.

2.5.2 *Drug content uniformity*

For drug content uniformity, uncoated pastilles equivalent to 10 mg of doxofylline were transferred individually into six dry 25 ml volumetric flasks and further processed as per the assay method.

2.6 Scanning electron microscopy

The surface morphology of the multilayered pastilles was observed using scanning electron microscope (FEI Quantum 200E instrument).

2.7 Friability study

A sample of 1 g pastilles was accurately weighed and placed in the drum of the Roche friabilator (Campbell Electronics, Mumbai, India). The drum was rotated at 25 rpm for 4 min, and the pastilles were removed, dedusted and accurately weighed. Friability was calculated by using following relationship:

$$\% F = (W_0 - W_f / W_o) \times 100$$

where W_0 is the initial weight of pastilles and W_f is the weight after friability test.

2.8 Drug release study

Drug release studies of coated pastilles were carried out on six units using USP Dissolution Apparatus II (Electrolab TDT-06P, Electrolab, India) with 500 ml of 0.1N HCl as dissolution medium maintained at 37 ± 0.5°C at 50 rpm paddle speed for 2 h followed by pH 6.8 phosphate buffer at similar condition for next 6 h (drug release study was withheld if complete drug release took place at earlier time point). Drug release studies of uncoated pastilles were carried out only in 0.1N HCl for 1 h. Five milliliters of aliquots were withdrawn at predetermined intervals with replacement. The samples were filtered using 0.45 µ nylon syringe filter and analyzed spectrophotometrically at 273 nm.

2.9 *In vivo* 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.

2.9.1 *Animal experimental protocol*

Male albino Wistar rats of 300 ± 25 g were divided into three groups comprising six animals each. They were fasted for 12 h before the experiment but had free access to water. After light anesthetization with ether, the formulation (coated pastilles of mean diameter 1.5 ± 0.2 mm freshly dispersed in 5.0 ml of 1.0% aqueous polyvinyl alcohol solution) containing drug

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Table 1. Formulation design and evaluation parameters of prepared batches.

Ingredients	P-I	P-II	P-III	P-IV	P-V	P-VI
DOX (mg)	500	500	500	500	500	500
PEG 4000 (mg)	2000	2000	2000	2000	2000	2000
Colloidal silicon dioxide (mg)	-	75	150	75	75	75
Enteric coat 1 (%)	-	-	-	10 ± 5*	-	-
Enteric coat 2 (%)					10 ± 5*	10 ± 5*
Floating layer (%)	-	-	-	-	-	20 ± 5‡
Assay (%)	100.12 ± 1.11	100.09 ± 1.91	99.61 ± 2.17	98.12 ± 1.21	98.08 ± 2.19	99.06 ± 1.98
Drug content uniformity	100.19 ± 2.13	99.98 ± 2.21	98.89 ± 1.81	99.01 ± 0.91	98.21 ± 1.27	99.12 ± 2.12

*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. Assay, drug content uniformity and coating layer weight gain values are represented as mean ± SD.

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equivalent to 5.70 mg/kg body weight was orally administered. Formulation batches P-II, P-IV and P-V were administered to group I, II and III, 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. Food was withheld but free access to drinking water was provided during the entire study period [18]. 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.

2.9.2 *Quantification of serum drug concentration*

A liquid–liquid extraction method was employed for extraction of drug from the serum and was analyzed using a validated high-performance liquid chromatography (HPLC) method [19]. Serum sample (500 µl) was poured into microtubes and vortex mixed for 2 min. Methanol (400 µl) was added to precipitate serum proteins, and the sample was again 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 (Sheldon Manufacturing, USA) at 40°C. The residue was reconstituted in 200 µl mobile phase and 20 µl of the reconstituted sample was injected for HPLC analysis. The HPLC system consisted of two delivery pumps (Waters Corp, USA), a diodearray detector (Waters 2998) and software (Empower Node 2054). A rheodyne manual injector (USA) attached with 20 µl sample loop was used for loading the sample. C_{18} reverse-phase 250 × 4.6 mm 5 µm ODS2 column (Waters, Ireland) and a C_{18} guard column were used. The mobile phase was 18:82 acetonitrile–12.5 mM potassium dihydrogen orthophosphate buffer (pH adjusted to 3.0 with orthophosphoric acid), which was passed at a flow rate of 1 $mlmin^{-1}$. The injection volume was 20 µL, elute was monitored at 275 nm and the sensitivity was 0.0007 AUFS. The retention time of the drug was approximately 9.5 – 10 min. Standard calibration curve constructed over the concentration range of 25 – 1000 ng/ml was used to determine serum drug concentrations.

Pharmacokinetic parameters such as 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-t}) and the time span during which the plasma concentrations were at least 50% of the C_{max} value ($HVD_{t50\%\ Cmax}$) for doxofylline were obtained for each subject by non-compartmental 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 R△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 [20].

2.9.3 *Statistical analysis*

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

2.10 Stability study

Pastilles of finalized batch were packed in 30 cc HDPE bottles, sealed and kept at 40°C/75% relative humidity in stability chamber (Narang Scientific Works Pvt. Ltd, New Delhi, India) for a period of 3 months as per ICH guidelines. Samples withdrawn after 3 months were analyzed for physical appearance, drug content (assay) and drug release test.

3. Results and discussion

Pastillation technology is a well-established technique in chemical, agrochemical and petrochemical industries for conversion of dusty hazardous chemical powders into hemispherical solidified units (pastilles) by dropping their melt on a cooled stainless steel surface. Unfortunately, the potential of this technology for the development of oral multiparticulate dosage forms has not been recognized by the pharmaceutical industry. If implemented, this dosage form can be an alternative for the line extension of the existing product baskets and also for designing patent non-infringing formulations. The first attempt in this direction was made in our laboratory and controlled-release pastilles were successfully designed [1].

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In continuation, the present study was designed to prepare pastilles for immediate release. These were then further coated with hydrophilic and enteric polymers in order to delay the drug release so as to match its release with the onset of asthma in the early morning hours of the day. The batches were then subjected to *in vitro* and *in vivo* evaluation.

3.1 Contact angle of pastilles and analytical testing

As described in our previous report [1], contact angle above 85° describes a proper hemisphere shaped pastille (**Figure 2A**) due to which these multiparticulate dosage forms have the ability to exhibit sufficient flowability during large-scale handling. In the present study, immediate-release pastilles were fabricated using PEG as the matrix former. Unlike lipid-based pastilles, the desired hemispherical shape of pastilles using PEG could not be achieved (**Figure 2B**). The pastilles formed using PEG alone were found to have extremely low contact angle (< 45°). Such pastilles were more or less flat whose sharp edges were found to be highly susceptible to damage during handling. The reason for this may be described on the basis of viscosity of the PEG melt. As compared with our previous study where lipid was used as the matrix former, the viscosity of the lipid (stearic acid) melt was comparatively higher (observed visually) than that of PEG melt. It may be attributed that being of lower viscosity, the rate of spreading of the PEG melt is faster than its rate of freezing on the cold plate, which results in flattened pastilles with extremely low contact angle. Therefore, an attempt was made to improve the viscosity of the PEG melt by addition of colloidal silicon dioxide. Addition of colloidal silicon dioxide (3.75% w/w of PEG – batch P-II) improved the viscosity of PEG, which in turn improved the contact angle (> 70°) significantly (**Figure 2C**). In addition, presence of colloidal silicon dioxide in the matrix also improved the friability of the pastilles compared with batch P-I, making it more robust to withstand the shear during shipping and handling (friability of batches P-I and P-II were 0.596 and 0.104%, respectively). However, the maximum contact angle achieved was less than that of the lipid-based pastilles. Furthermore, increase in colloidal silicon dioxide quantity (batch P-III) resulted in a highly viscous melt, which was difficult to pass through the needle. As these pastilles have lower contact angle, they may require additional aid to help them flow from the hopper during capsule filling.

Another reason due to which good contact angle (> 85°) could not be achieved despite addition of colloidal silicon dioxide is probably due to significant increase in the solidification time (by approximately 1 min) of PEG in presence of colloidal silicon dioxide. The later phenomena may be described using a diagrammatic model wherein a single pastille has been divided into two horizontal plates as shown in **Figure 3A**. As soon as the melt is dropped, the lower plate in contact with the cold surface starts getting solidified. On the other hand, the dissipation of heat from the upper plate to the cold surface is extremely low due to higher viscosity. In addition, the increased solidification time of the melt delays the overall freezing process and the lag time thus available is sufficient for the upper layer to spread on the edges of lower plate, resulting in almost disk-shaped pastilles. Therefore, any provision to increase the rate of freezing may prove to be useful in minimizing the extent of spreading. This is possible either by further reduction in the temperature of the cold plate (below 0°C) or by additional provision to blow chilled air gently on the surface of the melt as soon as it is dropped when fabricated at a large scale (**Figure 3B**). The drug content uniformity and assay values are shown in **Table 1** and were found to be satisfactory. The size of coated pastilles of batch B-VI was measured by using vernier calipers and was found to be 2.0 ± 0.5 mm.

3.2 *In vitro* drug release and morphological study

Figure 4 shows doxofylline release profiles from the uncoated batches (P-I and P-II) in acidic medium for 1 h and coated pastilles (batches P-IV, P-V and P-VI) for 2 h in acidic dissolution medium (0.1 N HCl) followed by 2-8 h in alkaline dissolution medium (pH 6.8 USP phosphate buffer). Though batch P-I did not form pastilles of proper dimension, it was subjected to drug release study to understand the effect of colloidal silicon dioxide on the release of the drug. Both uncoated pastilles were found to release the drug within 1 h in the acidic medium, which is due to the high solubility of polyethylene glycols in aqueous medium. However, batch P-II showed slightly retarded drug release profile as compared with batch P-I, which may be attributed to two different factors. The first factor is the hydrophobic nature of colloidal silicon dioxide, which reduces the exposure of PEG to the aqueous environment. The second factor is the compactness that the pastilles have attained due to addition of colloidal silicon dioxide, which retards the solubilization rate of PEG. Pastilles coated with 10% of enteric polymer (batch P-IV) was found to release about 40% of the drug in acidic medium indicating insufficient gastric protection of the applied coat. As can be observed from the SEM micrographs (**Figure 5A**), the coated surface shows severe cracks and pores, which indicate lack of effective film formation. The micrographs suggest for increase in concentration of plasticizer, which is required for imparting flexibility to the polymer film. Therefore, the plasticizer concentration was increased from 5 to 10% with similar coating weight build-up (batch P-V). The attempt was successful in preventing drug release in acid for 2 h (< 10% drug release after 2 h in acidic release medium (acceptable as per USP 27 specification limit)), which is due to a smooth enteric film formation as can be seen in SEM micrograph (**Figure 5B**). Moreover, the same coat immediately released the drug in the alkaline condition within 1 h. Furthermore, the formulation of batch P-V was coated with 20% floating layer (batch P-VI) consisting of $NaHCO_3$ with an intention to further delay the gastric transit time, which is required for the drug release in the early morning hours to manifest its chronotherapeutic effect. The floating layer helped the pastilles to float all throughout the study in

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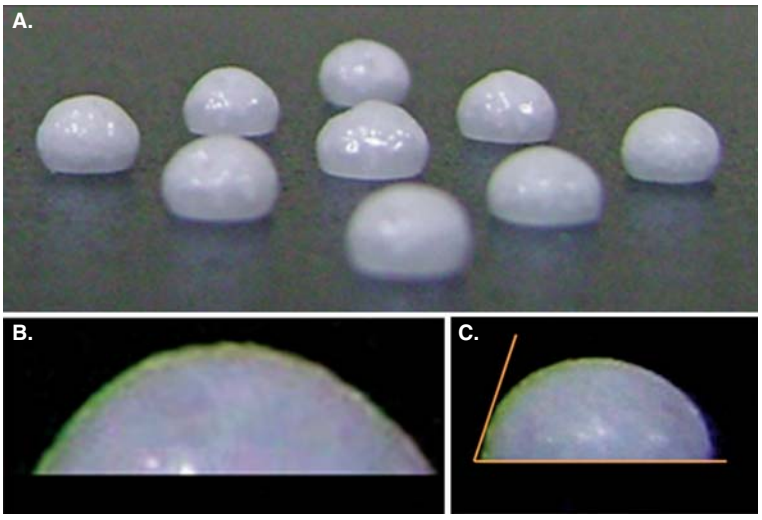

Figure 2. Pastilles with **A)** desired contact angle (above 85°), **B)** with contact angle ≤ 45° and **C)** with contact angle above 70°.

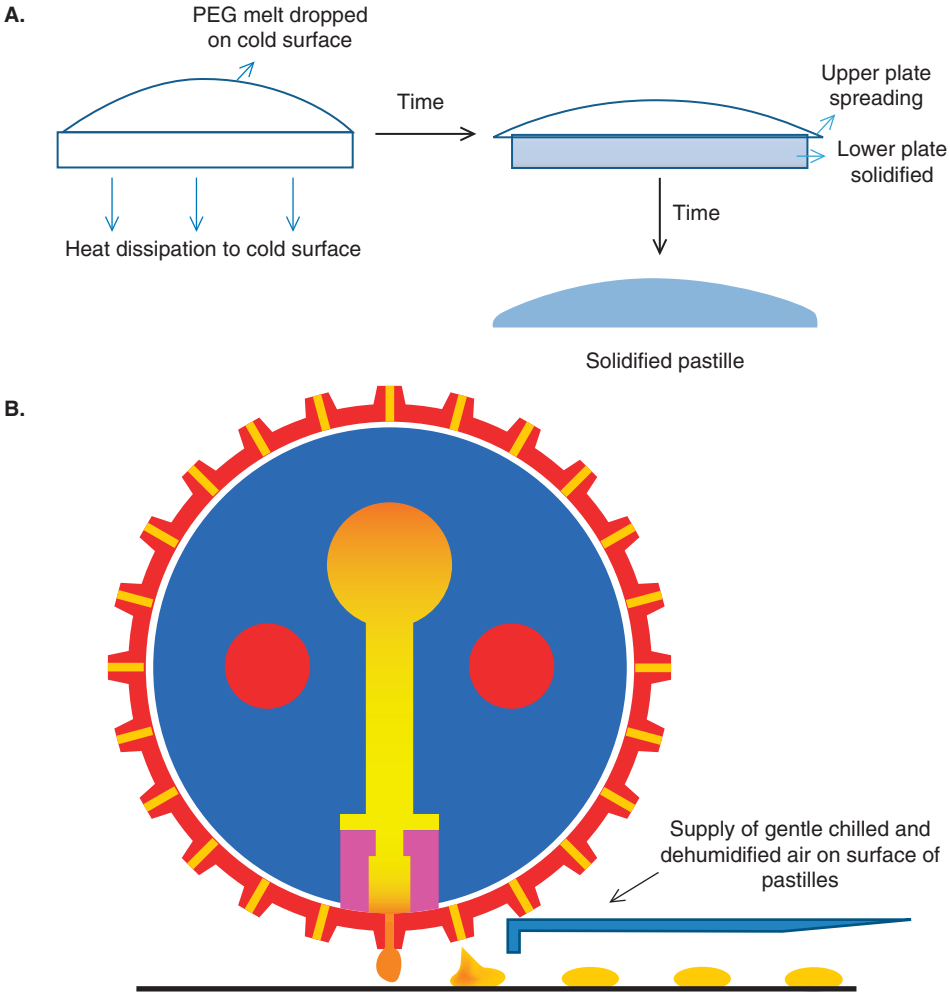

Figure 3. Diagrammatic representation of A) the phenomenon that results in significant increase in the solidification temperature, **B)** additional arrangement to improve contact angle of PEG pastilles in large-scale production.

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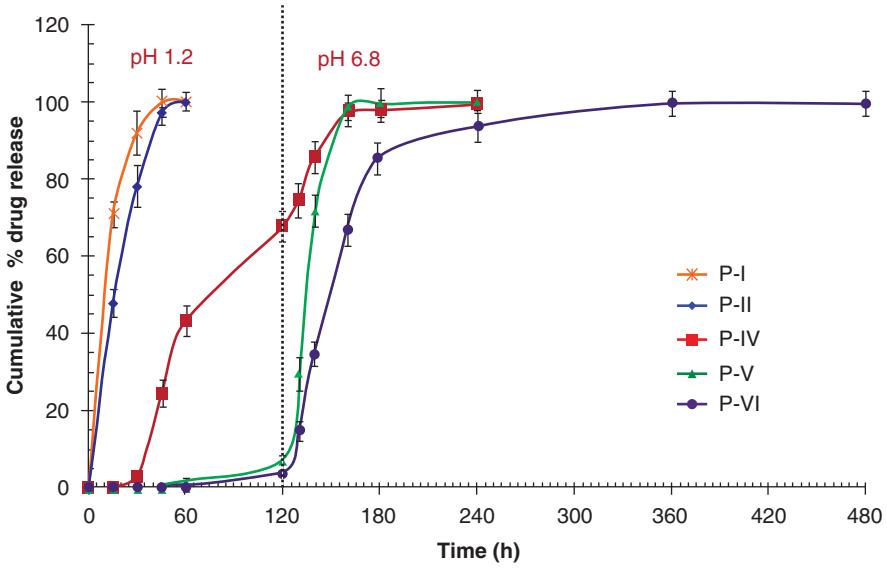

Figure 4. Drug release profiles of prepared batches (n = 6, bars represent SD).

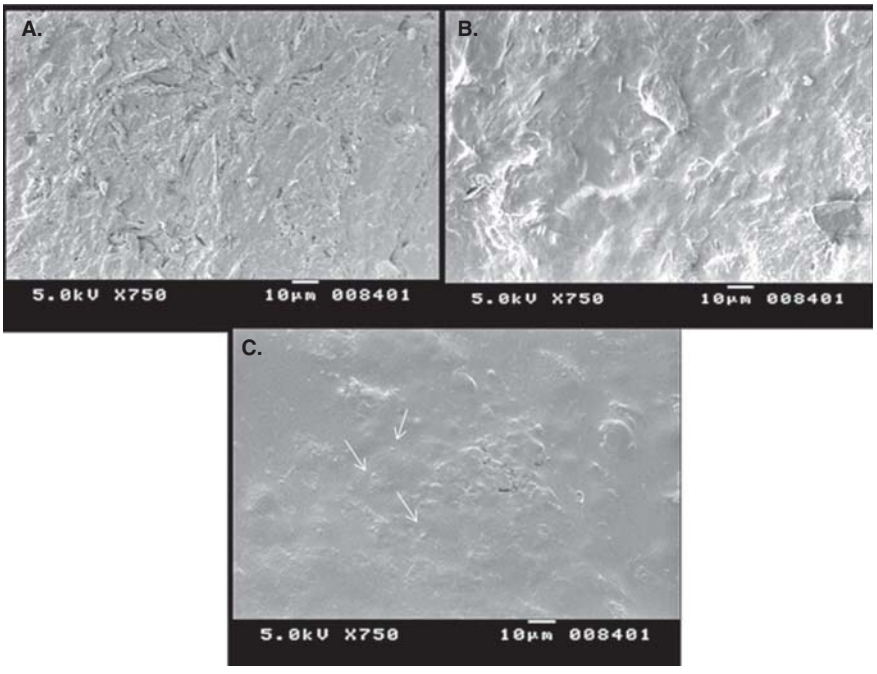

Figure 5. Surface morphology of coated pastilles using SEM A) batch P-IV, **B)** batch P-V and **C)** batch P-VI.

both the dissolution media (**Figure** 4) and also in retarding the drug release as can be clearly observed in **Figure** 6. **Figure** 5C is the SEM micrograph of floating coat wherein the spots marked with arrows are probably the particles of sodium bicarbonate embedded in the polymer coat. Furthermore, it was decided to subject both batches P-V and P-VI for animal studies in order to find out the *in vivo* effect of the coating.

3.3 *In vivo* study

Three doxofylline pastille formulations (uncoated, enteric coated and enteric coated with additional floating coat) were evaluated in *in vivo* study on male albino Wistar rats to study the effect of the coating on the pharmacokinetic behavior of the drug. Like theophylline, doxofylline is also an effective methylxanthine with better safety profile, which can be used

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Figure 6. PEG pastilles floating in dissolution jar containing 0.1 N HCl.

in the treatment of nocturnal asthma. Formulating a multiparticulate solid dosage form with sufficiently delayed drug release would be an advantage as after late night administration the drug would be available in the early morning hours when it is actually required. **Figure 7** presents the mean (n = 6) doxofylline serum concentration versus time profiles of the three formulations, while **Table 2** summarizes the pharmacokinetic parameters. **Table 2** shows that there are no statistically significant differences in the AUC_{last} and C_{max} 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. However in **Figure 7**, 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 B-VI in comparison with B-V. 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 HPMC layer. This phenomenon causes floating of the pastilles, which causes delay in gastric transit time and finally results in increasing the lag phase. Furthermore, in case of B-VI, the drug release was much slower as compared with 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, which probably 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 batch P-VI 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 B-VI also showed a significant increase ($p < 0.001$) as compared with B-I and B-V, which is indicative of longer duration of action and increased efficacy of formulation. The characterization of *in vivo* drug release profile using R△ indicates intermediate sustained-release effect.

It is important to discuss here that the *in vitro* drug release study does not reflect the effect of floating layer on the exact time of drug release in the GI tract, except that it indicates the ability of the formulation to float in the dissolution media. However, in *in vivo* study of the formulation with floating layer, the T_{max} was achieved at 6 h, which is a result of the lag phase generated due to the floating capacity of the pastilles in the stomach. Therefore, if the formulation is administered at 10.00 h, the T_{max} would be achieved at 04.00 h, which matches with the peak time of low lung function and thus would show maximum therapeutic effect.

3.4 Stability studies

On comparing the stability data of the stored samples with that of the initial samples, it was observed that neither the physical appearance nor the drug release profiles of the stored samples were influenced by the storage condition (**Figure 8**). No significant change in drug content (assay) of the formulation was found after 3 months (99.87 ± 2.13 %) of storage. This indicates that the formulation is physically stable and capable of withstanding the environmental fluctuations during storage and handling.

4. Discussion

The development of new solid oral formulation is primarily important as there is limited number of dosage forms available, such as tablets or capsules, for any particular drug. This issue intensifies further when the design of a formulation is restricted by intellectual property rights. This usually increases the cost and restricts the scope of formulation development of certain drugs. Therefore, there is a need to design new simple dosage forms and also to upgrade existing manufacturing technology, which can open alternatives and new avenues in the field of drug delivery for preparing patent non-infringing formulations of existing drug products and help patients to receive the treatment at an affordable price. The present development of pulsatile-release pastilles opens a potential alternative to the conventional tablet or capsule dosage form for line extension or preparing patent non-infringing products of existing drugs at commercial scale. The developed formulation approach in the present study may be used as platform technology for drugs that cure diseases that require chronotherapeutic treatment such as asthma, hypertension, osteoarthritis, ulcers, allergic rhinitis, rheumatoid arthritis, myocardial infarction.

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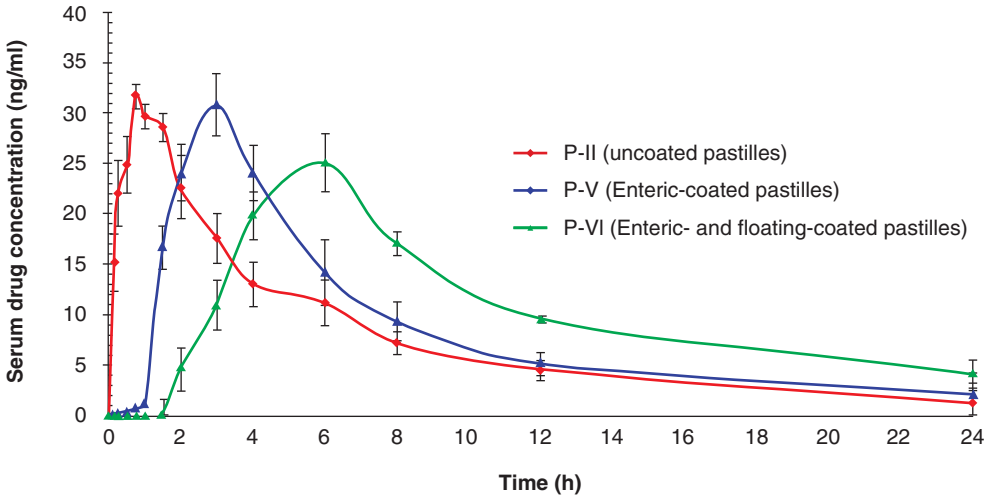

Figure 7. Serum drug concentration–time profile of prepared batches in rats (bars represent SD).

Table 2. Pharmacokinetic parameters of immediate-release and two final pulsatile formulations in rats.

Pharmacokinetic parameters	P-II (Uncoated pastilles)	P-V (Enteric-coated pastilles)	P-VI (Enteric and floating-coated pastilles)
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
R△	-	1.33	2.11

Values are represented as mean ± SD.

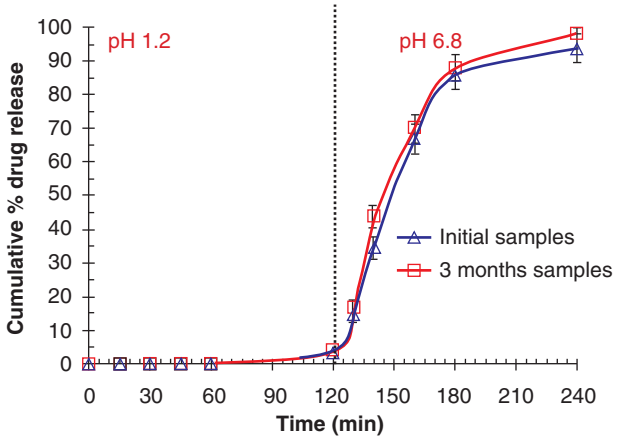

Figure 8. Comparison of drug release profiles of initial and stability samples of batch P-VI (n = 6, bars represent SD).

5. Conclusion

The present study involving a novel technology 'pastillation' was successfully employed for the development of immediate-release pastilles. This dosage form after coating with enteric and floating coat was also found to be effective in achieving the desired delayed *in vivo* drug release profile required for chronotherapeutic treatment of nocturnal asthma. The prepared formulation showed a lag time of approximately 5 h in drug release, which would be instrumental in achieving effective plasma drug concentration in early morning hours (03.00 – 06.00 h) if the dosage form is administered at 22.00 h last night. Thus, the treatment is expected to prevent the onset of asthmatic attack in the morning and reduce the chances of awakening and discomfort to the patient. In this way, the present work opens a new alternative to the conventional tablet dosage form for the development of both immediate-release and pulsatile drug delivery systems.

Declaration of interest

The authors declare no conflict of interest. Dali Shukla gratefully acknowledges the financial assistance provided by Indian Council of Medical Research, New Delhi, India, in the form of Senior Research Fellowship to support this research work.

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