Research paper

An Innovative Wax-based Enteric Coating for Pharmaceutical and Nutraceutical Oral Products

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A B S T R A C T

In this work, a novel enteric coating based on natural waxes and alginate was reported. Initially, theophylline tablets were coated with emulsified ceresin wax in heated aqueous alginate solution using a fluidised bed coating technology. A coating level of 10% proved sufficient to prevent tablets from uptalking gastric medium (< 5%) and produced a delayed release profile that complies to the pharmacopeial criteria of enteric coating release. Then, a wide range of emulsions based on other natural waxes (white beeswax, yellow beeswax, cetyl palmitate, carnauba wax or rice bran wax) yielded coatings with similar disintegration times and release profiles. Interestingly, the ceresin-based coating showed a superior performance at inhibiting acid uptake and enabling highly pH-responsive drug release in comparison to different commercially available GRAS enteric coating products (Eudraguard® Control, Swanlac® ASL10, and Aquateric™ N100). The coating was stable for 6 months at 30°C and 65% RH. This innovative approach of applying hot O/W emulsion of natural waxes yielded an aesthetically attractive and stable coating with gastric protection and pH-sensitive release properties. The novel coating can be an efficient and promising alternative to overcome the shortcomings of current GRAS grade enteric coating products.

Keywords

Delayed release, nutraceutics, gastric protection, probiotics, GRAS, e/c.

1. Introduction

Nutraceuticals are herbal and food products that, in addition to their nutritional values, are used in treating or preventing diseases. The growing market of nutraceuticals exceeded $200 billion last year (Chauhan et al., 2013; World, 2017). Several nutraceutical products require often functional enteric coating to protect them against the hostile gastric environment and/or to minimise irritation and mask unpleasant taste. Nutraceutical formulations often refrain from including artificial ingredients to favour the preferences and values of nutraceuticals consumers.

Several enteric coating solutions, described as generally recognised as safe (GRAS), have been commercialised to respond to the high demand for nutraceutical product market (Barbosa et al., 2017). For instance, Sensient has developed a coating system known as Protect®, that relies on the use of naturally occurring shellac (Sensiet, 2019). However, developing shellac-based coating often involves the challenge related to ammonium and suffers stability issues and batch-to-batch variations (Farag and Leopold, 2009). Nutrateric®, Eudraguard Control® and Biotic® are other examples of semi-synthetic and synthetic products that have also been recently introduced for the GRAS enteric coating of nutraceutical products. However, these products rely on the use of ammonium and their synthetic nature limits their daily intakes (Colorcon, 2017; GmbH, 2019).

Naturally occurring waxes are esters of long chain acid and long chain alcohol (Akoh, 2017). They offer a myriad of desirable characteristics such as fat dissolving, a wide range of melting points, and malleability under different processing conditions. Due to their natural origin and favourable characteristics, natural waxes have been widely incorporated in numerous food, cosmetic as well as drug delivery systems (Akoh, 2017; Moebus et al., 2012). Based on their origin, natural waxes are often categorised into plant, animal or mineral waxes. Plant waxes, also known as vegetable waxes, are found in the surface of leaves of palms e.g. carnauba wax from *Copernicia prunifera.* Beeswax is one of the most commonly used animal waxes that was used in pharmaceutical coatings for controlled and extended release systems (Kennedy and Niebergall, 1998; Lee et al., 2019). Ceresin wax, a purified lignite-free form of ozocerite wax, is a mineral wax which is often used in combination with other excipients to provide polishing, protective and/or sustained release coating (Bagaria and Lordi, 1987). In fact, waxes have been commonly used as matrix former in cores (e.g. pellets) for extended and controlled release (Hamdani et al., 2002; Tian et al., 2008; Zou et al., 2009).

The use of waxes in pharmaceutical coating is well-established as a finishing step to achieve polished glossy tablets (Kelley, 1948). Waxes, such as stearic and palmitic acids, have also been explored for their potentials to produce functional coatings with enteric properties. However, such approach delayed drug release (up to 4 hours following pH change) and failed to produce a functional enteric coating that complied with the pharmacopeial criteria (Patil et al., 2012). Indeed, purpose-designed enteric polymers are carefully engineered to dissolve rapidly upon the pH increase in intestinal segment (Barbosa et al., 2019). In contrast, waxes and lipids coating requires digestion by enzymes via lipolysis to allow the drug release (Zechner et al., 2012). This often result in a slow drug release for wax-based coated products. For instance, it has been shown that when combined with sustained release coating polymer, waxes can results in a further delay in drug release (Tian et al., 2008). More recently, bees wax solution in petroleum ether was used to coat *Lactobacillus casei* beads via deep coating and filtration method (Mandal et al., 2014), leading to an improved survival in gastric medium. Up to the authors' knowledge, there has been no previous report of using waxes as an efficient functional enteric coating.

Sodium alginate (Alg) is a GRAS-grade water soluble polysaccharide that has been widely used for pharmaceutical applications (Dalaty et al., 2016; Layek and Mandal, 2020). It has been used as pH-dependant pore-former in delayed release systems (Barbosa et al., 2019; Czarnocka and Alhnan, 2015). However, Alg water uptake and susceptibility to acidic hydrolysis hindered its use as a functional enteric coating on its own, despite its film forming properties (Pawar and Edgar, 2012). In this context, we have previously reported that incorporating fatty glycerides in Alg-based films yielded coating systems with pH-dependent release properties (Khoder et al., 2020). However, the coated cores showed a high acid uptake (≥25%), which might not be suitable for acid-labile active ingredients.

In this work, we report for the first time an innovative approach to craft an all-natural enteric coating system by employing a hot O/W emulsion of natural waxes in Alg solution. The applicability of this approach was carefully assessed across a wide spectrum of naturally occurring waxes. The gastro-resistant properties of these novel coatings were established. Moreover, we have provided a head-to-head comparison against recently introduced GRAS-grade enteric coating products, which have not been yet evaluated independently. Long-term stability studies were also performed.

2. Materials and methods

2.1. Materials

Theophylline anhydrous, a model drug, was purchased from Acros Organics (New Jersey, USA). The excipients used for tablet compression were lactose monohydrate Lactopress (BASF SE, Germany), polyvinylpyrrolidone K90 (Sigma–Aldrich, Dorset, UK), microcrystalline cellulose (MCC) PH101 (FMC Biopolymer, Belgium), crosscarmellose sodium SD-711 (FMC Biopolymer, Belgium) and magnesium stearate (Sigma–Aldrich, Dorset, UK). The coating components were: sodium alginate (Alg) (15–20 cps, Sigma-Aldrich Dorset, UK), ceresin wax (Fisher Scientific UK), white bees (Acros, Belgium), yellow beeswax (Fischer Scientific, UK), carnauba wax (Sigma Aldrich, Dorset, UK), rice bran wax (donated by Koster Keunen, USA), and cetyl palmitate (Acros, UK). Glyceryl monostearate (GMS, Imwitor® 900K) was donated by Cremer OLEO (Germany). Methocel™ E5 Premium LV supplied by Colorcon (UK), Lycoat® RS 780 supplied by Roquette (France). Ammoniated shellac (Swanlac® ASL) was donated by AF Suter & Co Ltd. (UK). Aquateric™ N100 was supplied by FMC biopolymer (USA). Eudragaurd® Control was donated by Evonik (Darmstadt, Germany).

2.2. Model Tablet Preparation

The tablets were prepared by wet granulation as previously detailed (Czarnocka and Alhnan, 2015; Khoder et al., 2020). Briefly, powder blend (250 g of theophylline, 240 g of lactose monohydrate and 10 g of PVP K90) was granulated with 110 mL of Type II water using a mixer granulator (Erweka, Germany). Wet granules were then dried using a fan oven (Binder, Germany) to yield approximately 2-3% w/w moisture content to aid compression. Using a double-cone mixer (Erweka AR 402, Germany), the resultant granules (500 g) were blended for 10 min with 150 g of MCC, 350 g of Lactopress, 30 g of crosscarmellose sodium salt, and 10 g of magnesium stearate. The tablets were manufactured using a single-punch tablet press (Riva Minipress, Argentina). The resultant tablets demonstrated an average weight of ~600 mg and a resistant to crushing force of 120 N.

2.3. Preparation of films

a. Casted film

Alg, exemplar wax (ceresin wax) and GMS were firstly blended to produce different weight ratios (Alg: wax: GMS 12:4:1, 11:5:1, 10:6:1, 9:7:1 or 8:8:1 w/w/w). Obtained blends were then dispersed for 30 min at a concentration of 2.25% w/v in hot water (70 ^{o}C) under magnetic stirring of 150 rpm. Film casts were prepared by individually pouring 20 mL of the hot resulting

dispersions into a 100 mm Teflon-coated circular trays. Films were dried in a fan oven at 40 ^{o}C for 24 hours. Upon drying, the resultant films were peeled off and evaluated for film formation property and homogeneity.

b. Coated tablets

Initially, blends of Alg, ceresin wax and GMS at different weight ratios (12:4:1, 11:5:1, 10:6:1, 9:7:1 or 10:10:1 respectively) were prepared as mentioned above. Using magnetic stirring (150 rpm), obtained powders blends (9 g) were mixed for 45 min with 400 mL water kept at a temperature of 70 ^{o}C to yield final concentration of 2.25% w/v.

Alg: ceresin wax: GMS ratio of 10:6:1 was selected as a default condition based on film forming property study and applied and tested at three coating levels; 5, 7 and 10% WG (weight gain). In order to assess the suitability of other naturally occurring waxes, the same blend ratio (Alg: wax: GMS 10:6:1) was applied to the model core at 10% WG by replacing ceresin wax with: white beeswax, yellow beeswax, cetyl palmitate, carnauba wax or rice bran wax.

All coatings were carried out using a Strea-1 fluidised bed coater (GEA Pharma Systems AG, Bubendorf, Switzerland). The inlet air temperature was adjusted at 55 °C and outlet air temperature was 45 °C, yielding a tablet bed temperature of 42-45°C. The atomizing pressure was set at 0.35 Bar. During coating process, the coating solution was continuously stirred and maintained at 70 °C and the spray rate was ~3.4 mL/min. In order to prevent wax solidification within the tube that connects the bulk feed emulsion to the spraying nozzle, the tube was surrounded with an electric heating tube (BriskHeat, Columbus, OH, USA) and the temperature was maintained at 70 °C.

2.4. Characterization of coating liquid

The coating liquid based on Alg: wax: GMS (10:6:1) was prepared as stated in Section 2.3. To confirm the nature of emulation, 5 mg of a lipophilic dye (Nile Red) was added to the liquid at 70 ^{o}C under stirring. Aliquots of the preparation were analysed using temperature-controlled Leica light microscope equipped with QIClick camera and the images were processed using Q-Capture Pro 7 (Teledyne Imaging, Surrey, Canada).

2.5. Comparison with other commercial GRAS grade coating systems

Commercially available GRAS grade enteric products; Eudraguard® Control (neutral methacrylic polymer + Alg), Swanlac® ASL 10 (ammoniated ready solution of shellac + Alg), and Aquateric® N100 (Alg + soluble starch) were recently introduced to the market (Joao A.C.

Barbosa, 2017). In order to provide direct comparison with the novel developed coating, these coating systems were applied to the same model core following manufacturer information.

i) **Eudraguard® Control** The coating was based on 5.2% dispersion of Eudraguard® Control (available as 30% w/v dispersion), 2.8% Alg, 0.4% glycerol , and 0.16% talc in 400 mL deionized water to achieve a 10% w/v suspension (GmbH, 2015). The formulation was applied at different coating level; 2, 3, 4, 5, 7.5, and 10% WG. The coating was processed as detailed in Section 2.3 except for a flow rate of 2 mL/min.

ii) **Swanlac® ASL10** An aqueous solution of ammoniated shellac and alginate was prepared following Pencoat 770 formulation (AF Suter & Co Ltd.,UK). The coating solution was prepared by adding ammoniated shellac solution Swanlac® ASL10 (available as 25% w/v solution) to 375 mL of 2% Alg solution. The final solution has Shellac: Alg ratio of 7:3. The coating was applied at different levels: 2, 3%, 6%, 12% WG using parameters as detailed in section 2.3.

iii) **Aquateric™ N100** The coating was applied as described by Yang et al. (2016). An aqueous solution composed of 5% sodium alginate (Manucol LD, 10 cps), 3.4 % soluble starch (Lycoat RS 780) and 1.7% glycerol (plasticiser) was applied at different levels; 8%, 10%, 12% using parameters as detailed in section 2.3.

2.6. Thermal analysis

Thermogravimetric analysis (TGA) test was carried out using TA Analysis Q500 analyser (TA Instruments, Hertfordshire, UK). Samples (10 mg of Alg, GMS, ceresin wax, physical mixture and the film) were individually weighed and placed in crimp-sealed aluminium pans. The temperature was scanned from 20°C to 500°C at a heating rate of 10 °C/min under a nitrogen gas flow of 40 and 60 mL/min for furnace and samples. The thermal decomposition (or degradation) profile was analysed using TA Universal Analysis 2000 software (TA Instruments, Hertfordshire, UK).

Differential scanning analysis (DSC) was carried out using TA analysis 2000 (TA Instruments, Hertfordshire, UK). Sample of approximately 7 mg was weighed in a T0 pan and scanned from 20 °C to 180 °C for the films and to 120°C for the single ingredients including the physical mixtures as most of natural waxes melting point lie between 40°C-140°C (Endlein and Peleikis, 2011), . Samples were heated to 100 °C for 5 min to exclude the effect of humidity then cooled to -20 °C. This was followed by a heat-scan from -20 to 180 °C. Analysis was carried out under a purge of nitrogen (50 mL/min) at a heating rate of 10 °C/min. The data was analysed using TA 2000 analysis software (TA Instruments, Hertfordshire, UK).

2.7. Morphology of the coating

The colour and integrity of the coating were assessed using photographs taken by a D7100 Nikon DSLR digital camera (Nikon, Japan) integrity. Cross-sections of the coated tablets were examined using a Quanta-200 scanning electron microscope (SEM) microscope at 20 kV. Samples were placed on metallic stubs and gold scattered under vacuum for 2 min using JFC-1200 Fine Coater (Jeol, Tokyo, Japan), prior to imaging.

2.8. Disintegration test

The disintegration test was conducted in accordance with United Stated Pharmacopeia 30 standards (USP, 2007). Tablets (6 units) were placed in a ZT122 disintegration test apparatus (Erweka, Germany) that was operated for an hour in 800 mL 0.1 M HCl. Then the medium was replaced by intestinal pH 6.8 phosphate buffer, as specified in United Stated Pharmacopeia 30 (USP 30). The experiment was continued until complete disintegration of all tablets.

2.9. Acid Uptake

In order to evaluate the gastric resistance and protection capabilities of coating system, dry mass (DM) of six tablets were individually recorded and tablet were then placed for 1 hr in 800 mL 0.1 M HCl at 37°C.The tablets were drained off excess acid using filter paper and the wet mass (WM) of tablets was recorded. The acid uptake percentage was calculated according to Equation 1:

$$Acid\ uptake\ \% = \left(\frac{WM-DM}{DM}\right) * 100 \qquad \text{Equation 1}$$

2.10. In vitro drug release study

To assess the efficiency of coating systems to meet the compendial expectations drug release study was conducted in an AT-70 Smart dissolution USP II apparatus (Sotax, Switzerland) using the pH change method. Briefly, one tablet was placed in 750 mL of acidic phase (0.1 M HCl, pH 1.2) for 2 h followed by 4 h release test in intestinal phase of either pH 6.8 phosphate buffer or krebs bicarbonate buffer as simulated intestinal medium (1.18 mM KH_2PO_4, 24 mM $NaHCO_3$, 118.07 mM NaCl, 4.69 mM KCl, 2.52 mM $CaCl_2$, and 1.18 mM $MgSO_4 \cdot 7H_2O$) (Alhnan et al., 2011).

The dissolution test was performed at 37±0.5 °C with a paddle rotation at 50 rpm. , The percentage of released theophylline was determined at 5 min intervals by UV/vis

spectrophotometer (PG Instruments Ltd., UK) at the wavelength of 272 nm and path length of 1 mm. Data were analysed using Automated Lab IDISis software version 2012 (Berkshire, UK).

2.11. Stability Studies

The tablets stability was tested according to ICH guidelines at different climatic zones. The coated tablets were kept in high density polyethylene (HDPE) bottles (Fisher Scientific, UK) at room temperature, 30 °C 65% RH, or 40°C 75% RH in humidity-controlled incubator. The release profile, disintegration and acid uptake tests of the coated tablets (ceresin as an exemplar wax) were carried out as stated above directly following preparation and after 1, 3 and 6 months.

3. Results and discussion

3.1. Coating system setup

In this work, a novel enteric coating system based on emulsified waxes into aqueous Alg solution was developed. As waxes are immiscible with aqueous Alg solution, the addition of GMS, as a non-ionic emulsifying agent (HLB value= 3.8) (Remington and Beringer, 2006), was needed to stabilise the emulsion. Using ceresin as wax exemplar, a series of emulsions of different Alg: wax: GMS ratios: 12:4:1, 11:5:1, 10:6:1, 9:7:1 and 10:10:1 was initially prepared and used to create casted films to be evaluated for their coating quality. When wax ratio was increased to 10:10:1, wax and polymer separation was visually evident (data not shown). Light microscope imaging of the liquid preparation at 70 °C confirmed the formation of O/W emulsion with the presence of oily droplets coloured by lipophilic dye (Nile Red) (Fig. 1A). Following cooling sample to room temperature, the droplets readily solidified to form 3-5 μm sized wax particles (Fig. 1B).

Theophylline is a biopharmaceutical classification system (BSC) Class I drug (Qiu *et al.*, 2009). The aqueous solubility and relatively small molecular weight suggested this drug as an ideal model drug to test the enteric coating efficiency (Alhnan et al., 2010). Obtained emulsions maintained at a temperature of 70 °C were used as feed liquids to coat theophylline model core tablets. Fig. 1C displays the dissolution profiles of theophylline from tablets coated with different weight gain (WG) levels of Alg: wax: GMS (10:6:1). While a coating level of 5% and 7% released 80% and 50% of theophylline in the gastric phase respectively, increasing the coating level to 10% significantly prevented the drug release in the gastric medium (<5%). Yet it allowed a prompt release upon introducing the core to the intestinal medium (Fig. 1C). The immediate release of theophylline in the intestinal medium could be attributed to the immediate ionization of the carboxylic acid groups in Alg as the pH of the medium increases. Such ionisation can result in the electrostatic repulsion between negatively charged carboxylic groups, and hence the disintegration of the coating film and resulting dissolution (Hussan, 2012). The obtained release profile using a 10% coating level fulfills the pharmacopeial criteria of enteric coating release. Fig. 1D illustrates the dissolution of theophylline from tablets coated with different Alg: wax ratios with a coating level of 10% and shows no significant impact on the drug release profiles (Fig.1 D). It is worth mentioning that tablets coated with Alg solely without natural waxes as a blend, failed to prevent the drug release in gastric medium (Khoder

et al., 2020). On the other hand, adding GMS to Alg led to a significant control in drug release in gastric phase while inhibition of acid uptake remained limited (Khoder et al., 2020).

3.2 Morphology and characteristics of Alg- wax films

Tablets coated with Alg- wax films showed tinge of yellow-colored surface. The coated tablets were glossy and smooth in texture showing a matt finish and uniform film (Fig. 2 A). SEM images indicated that these films were approximately 80-100 µm in thickness (Figs. 2 B and C).

TGA thermographs of film components of Alg, GMS, and ceresin wax are shown in Fig. 3; no thermal degradation could be identified at the processing temperature (i.e. 70 °C). The drop in Alg weight (15%) at approximately 100 °C could be attributed to the evaporation of moisture contents (J. P. Soares, 2004). The DSC thermographs of the cast film individual ingredients illustrated an endothermic melting peak at approximately 59 and 64°C for ceresin wax and GMS respectively (O'Laughlin, 1989; Rowe, 2009) (Fig. 4A). DSC thermographs also showed a distinctive melting point (Tm) at approximately 58°C for the casted films (Figs. 4B), which corresponded to the melting point of ceresin wax. This was also observed in the thermographs of films based on different ratios of Alg: wax. The increased pattern in the endothermic melting peak might be attributed to presence of wax contents within the film. These peaks indicated that a significant portion of wax was in crystalline form within the film structure. While O/W emulsion contained molten wax droplet in aqueous Alg solution, upon evaporation of water during core coating and the sequential cooling down of the film temperature, it is possible that the wax droplet solidified into crystallized particles within the formed Alg matrix (Fig.1B).

The disintegration times of tablets coated with Alg-wax based emulsion showed resistance to open or softening in acid phase (Table1). This could be attributed to the unionization of Alg carboxylic groups and the enhanced overall lipophilic properties of coating film due to the presence of wax particles (Khoder et al., 2020; Lee and Mooney, 2012). Following pH change, the ionization of Alg polymeric chains followed by their dissolution resulted in a breakage of the coated film, leading to tablets disintegration within 11 min in simulated intestinal fluid (Table1).

3.3 Suitability of other natural waxes

In order to establish the suitability of the system to accommodate other naturally occurring waxes, the model core was coated using identical coating process by employing a range of naturally occurring wax examples i.e. white beeswax, yellow beeswax, cetyl palmitate, carnauba wax as well as rice bran wax. All the used waxes yielded coated cores that were glossy and smooth with different degrees of tinges of yellow colour depending on the nature of the employed wax (Supplementary data, Fig. S1).

Wax-based enteric coatings showed resistance to acid phase for 1 h and disintegrated in <15 min in simulated intestinal fluid pH 6.8 (Table 1). The acid uptake of the coated core following the exposure to gastric medium ranged between 4.75% and 9%. The observed variability in the acid phase uptake by coatings that are based on different waxes might be related to their chemical composition. In fact, the free fatty acid constituents and their percentages in each wax might play a significant role in maintaining local low pH (i.e. in the acid medium with non-ionized Alg), and hence decrease the acid uptake. Furthermore, the length of alkyl chain constituents can directly influence the hydrophobic nature of waxes resulting in lower permeability of the film in acid medium.

When the drug release was tested using pH-change dissolution test (Fig.5 A), all waxes-based coatings showed <10% release in the gastric phase followed by >80% released within 45 min in intestinal media (pH 6.8), that confirmed their conformity to the pharmacopeial criteria for enteric coating. In order to assess the release quality in more biologically relevant gastric medium (Fadda et al., 2009; Horter and Dressman, 1997), the coated tablets were tested in Hanks bicarbonate buffer. While all coated tablets showed resistance of drug release in the acidic media, they displayed relatively slower responses to the pH change in the bicarbonate-based buffer (Fig. 5 B). Nonetheless, the use of bicarbonate-based buffer allowed the differentiation of naturally occurring waxes. For instance, carnauba wax-based coating showed a relatively faster response to pH change with 80% release after 15 min in bicarbonate buffer pH 7.4, whilst cetyl palmitate and yellow beeswax-based coatings reached 80% release after 35 min of pH change. However, other waxes-based coatings resulted in a further delay in the response to pH change. For instance, the rice bran wax-based coating took up to 3 hours in bicarbonate buffer pH 7.4 to reach the 80% release. The inclusion of wax resulted in the embedment of ionisable hydrophobic constituents within the coating matrix such as aliphatic acid, lignoceric acid, behenic acid, and other free fatty acids such as palmitic acid (carbon atoms range from 26-30) (Tiwle, 2015). The presence of such lipophilic molecules might contribute to the reduced water imbibition into the coating matrix. Furthermore, the slower

response of the coating to the bicarbonate buffer could be attributed to its lower buffer capacity when compared with phosphate buffer (Liu et al., 2011). Indeed, the lower buffer capacity might have resulted in delayed ionization of the alginic and fatty acids, hence resulted in slow dissolution.

These findings are of a significant importance as our previous report indicated that several commercially available coatings failed to dissolve at sufficient time in bicarbonate based gastric medium (Czarnocka and Alhnan, 2015). It is also important to highlight that this unique formulation approach, while providing a sufficient protection in acid medium, it maintained a timely pH-responsive behavior.

3.4 Comparison with recently introduced GRAS-grade enteric coating

The gastric resistant properties of this novel natural coating were directly compared to some GRAS-grade coating solutions which were recently introduced to the market. The dissolution profiles of different levels of these coating products as well as their gastro-resistant properties are shown in Fig. 6 and Table 2.

Fig. 6.A shows that Eudraguard® Control (neutral methacrylic polymer + Alg), a minimal coating level of 2% was required in order to achieve a coating coverage that required that allow a sufficient inhibition for drug release in gastric medium. The addition of neutral methacrylic polymers seems to reduce the extent of Alg dissolution and maintained the integrity of the coating layer within the acidic medium. Upon pH change, a pH response was demonstrated after approximately 1 h. Furthermore, this combination showed a significant acid uptake (Table 2). While further increase in the coating thickness reduced the acid uptake, it resulted in a slower pH response of drug release beyond compendial expectations of delayed release products (Fig.6A). The slower pH response in comparison with wax-Alg based coating might be related to the slow dissolution rate of the non-ionizable methacrylic polymer chains compared to Alg.

On the other hand, shellac-Alg-based coating (Swanlac® ASL 10) demonstrated gastric resistant properties with low acid uptake (Table 3) and <10% release in acid phase at pH 1.2. However, this coating system showed a slow pH response (up to 3 h) when the pH was changed to 6.8 (Fig.6B). It has been reported that shellac has a high dissolution threshold (pH 7.3) (Farag and Leopold, 2009; Limmatvapirat et al., 2007). Therefore, the dissolution test was repeated for this product using pH change dissolution method to pH 7.4. However, theophylline release was also slow in the intestinal phase (pH 7.4) (Fig. 6C). These findings were in agreement with

our earlier report of another shellac-Alg-based coating (Protect ™) (Czarnocka and Alhnan, 2015). More recently, soluble starch (Lycoat® RS780) (Popescu et al., 2011) was included in an enteric coating design (Aquateric™ N100) in an attempt to hinder Alg dissolution and prevent film rupture from highly soluble Alg (Berger, 1953). However, the coating failed to inhibit drug release even (at WG level of up to 12%), with theophylline release percentage >10% and acid uptake of >50% (Table 4 and Fig. 6D).

4.5 Stability studies

Following storage at room temperature and at 30°C/65 RH% condition, wax-based coated tablets demonstrated a stable performance after 6 months with no significant difference in terms of release profile, disintegration time, physical appearance and acid uptake values (Figs. 7 A and B). However, while coating was relatively stable after 1 and 3 months at 40 °C 75% RH, the coating allowed the drug release at acid phase at the 6-month time point (Fig. 7C). This could be explained by the possible wax-softening upon storage for elongated period at elevated temperature. In fact, most waxes tend to congeal at a lower temperature than their melting points (<50°C) (NIIR, 2006). Although these initial stability trials indicated a suitable shelf-life for many nutraceutical products, further research on immobilizing wax molecules might improve the long-term stability of this novel coating solution.

4. Conclusion

We reported a novel enteric coating based on purely naturally occurring materials (natural waxes, Alg and fatty glycerides). Using a hot O/W emulsion of waxes in Alg aqueous solution, attractive films with gastric-resistant properties were produced. An ideal wax percentage of 25-43%w/w within Alg-based film (i.e. Alg: wax ratios of 2:4 to 9:7) deemed necessary to minimise acid uptake and meet compendial criteria for delayed release products. The described coating method proved applicable to a wide range of naturally occurring waxes. The novel coating system also demonstrated superior gastro-resistant properties when compared with other of commercially available GRAS-grade coating solutions designed for the nutraceutical market. Due to the non-synthetic nature of its constituents, this novel coating could constitute an excellent and stable alternative for nutraceuticals coating.

Credit authorship contribution statement

R Habashy: Investigation, Methodology, Writing-Original Draft**,** Methodology, conceptualization **M Khoder** Writing-review & editing, **S Zhang:** Investigation, **B C Pereira:** Investigation, **M Bohus:** Investigation, **J T Wang:** Investigation, **A Isreb:** Investigation and methodology, **M A Alhnan:** Conceptualization, Supervision, Software, Writing - review & editing, Project administration.

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Supplementary Data

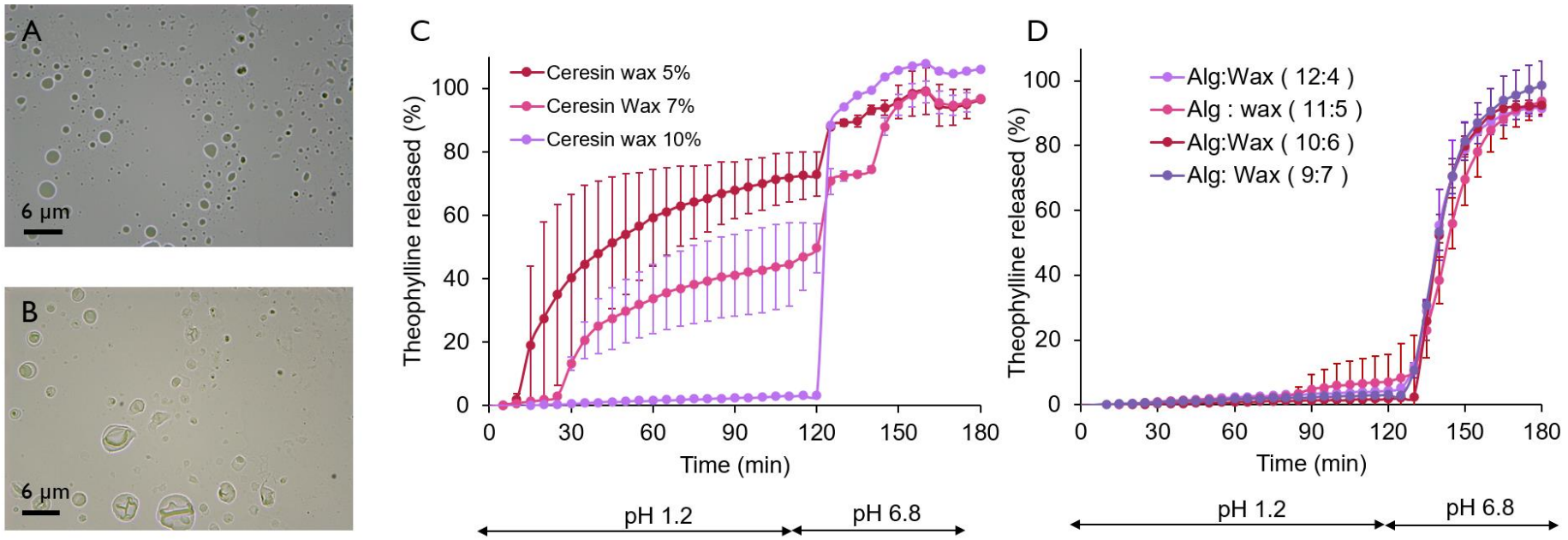


Fig. 1 Light microscopic images of the coating dispersion at (A) 70 ^{o}C and (B) 25 ^{o}C. In vitro dissolution profile of theophylline tablet coated with (C) different coating levels of Alg: wax: GMS ratio 10:6:1 and (D) different Alg: wax: GMS ratios (10% WG) using pH change UPS II dissolution test (2 hours pH 1.2 followed by pH 6.8).

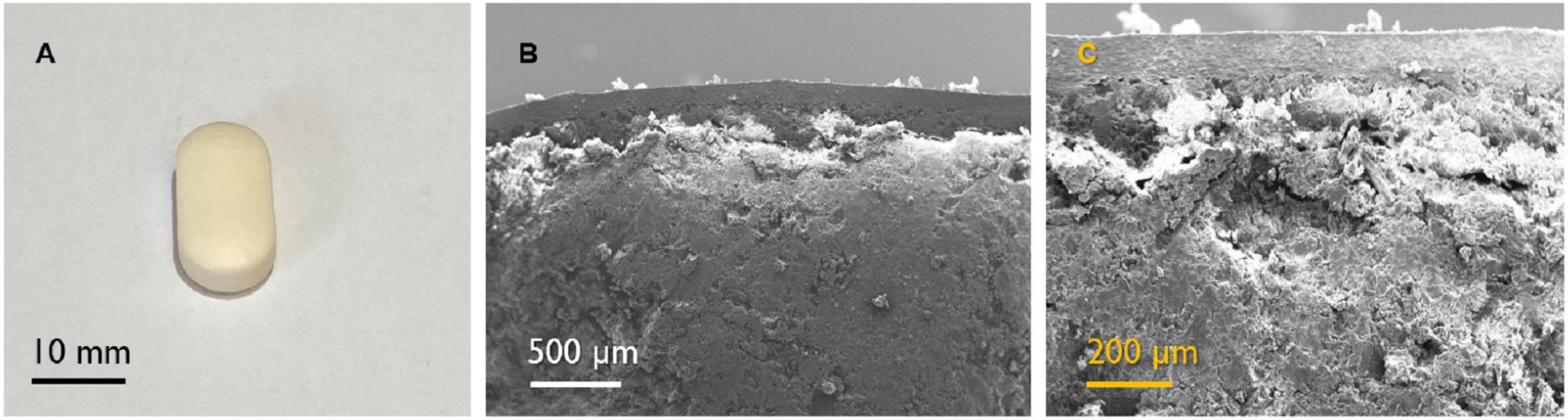


Fig. 2 Photograph of ceresin wax based coated tablet and scanning electric microscope images of the coating at (B) x 40 magnification power (C) x100 magnification power.

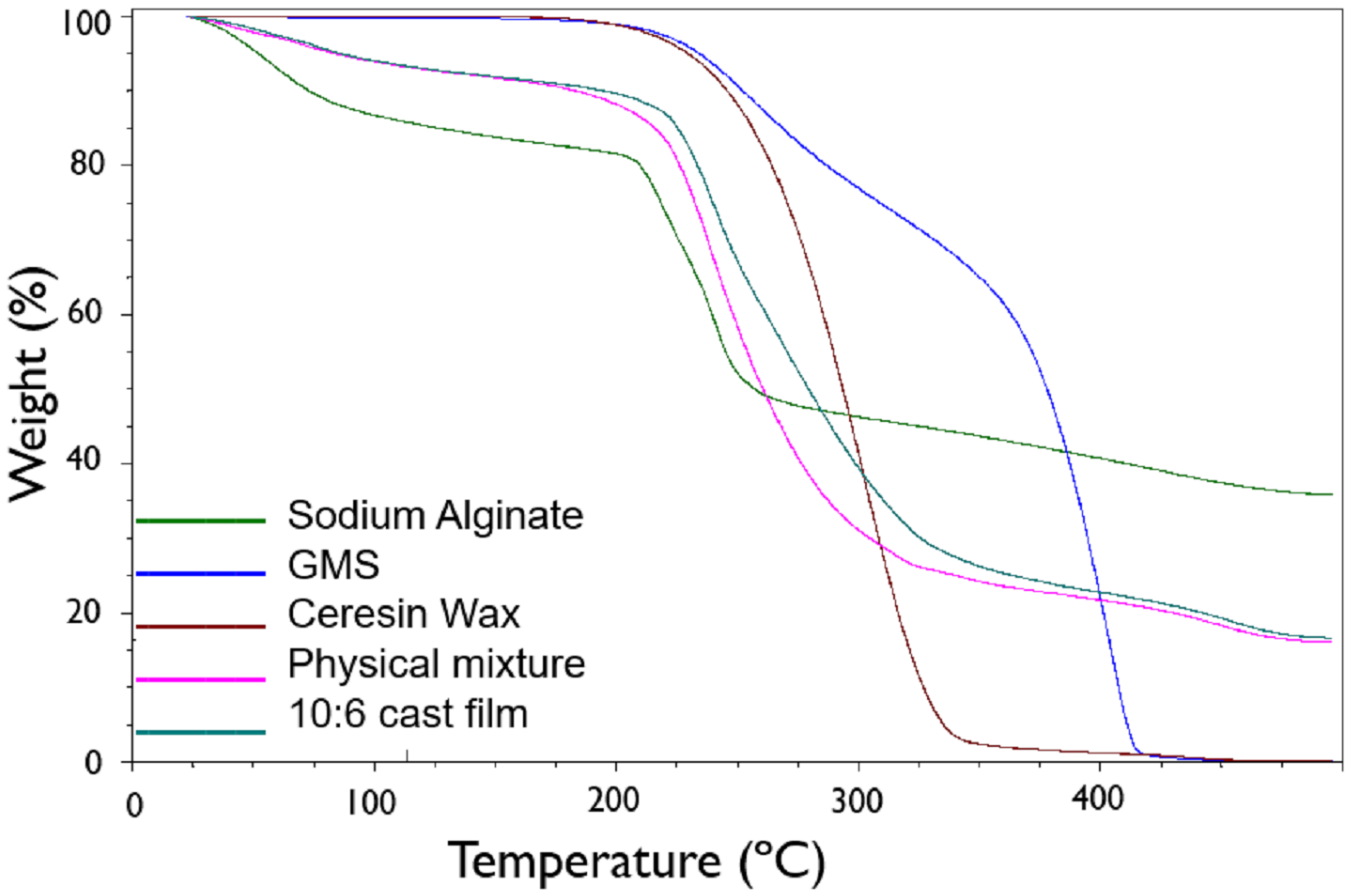


Fig. 3 TGA thermograms of Alg, GMS, ceresin wax, physical mixture and casted film

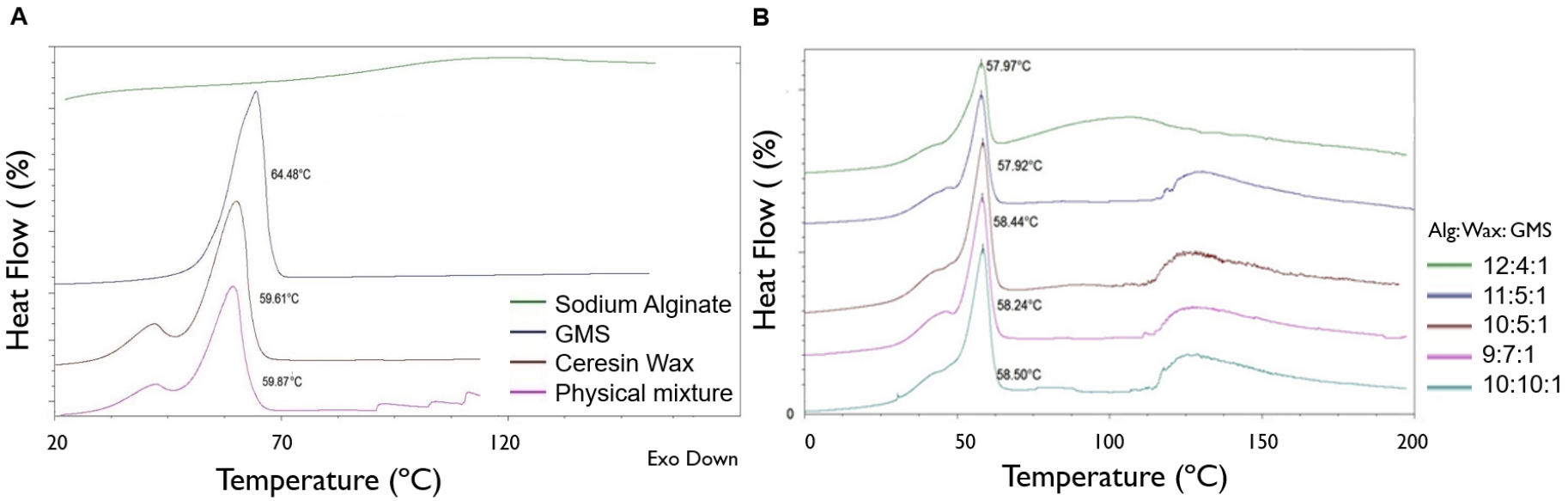
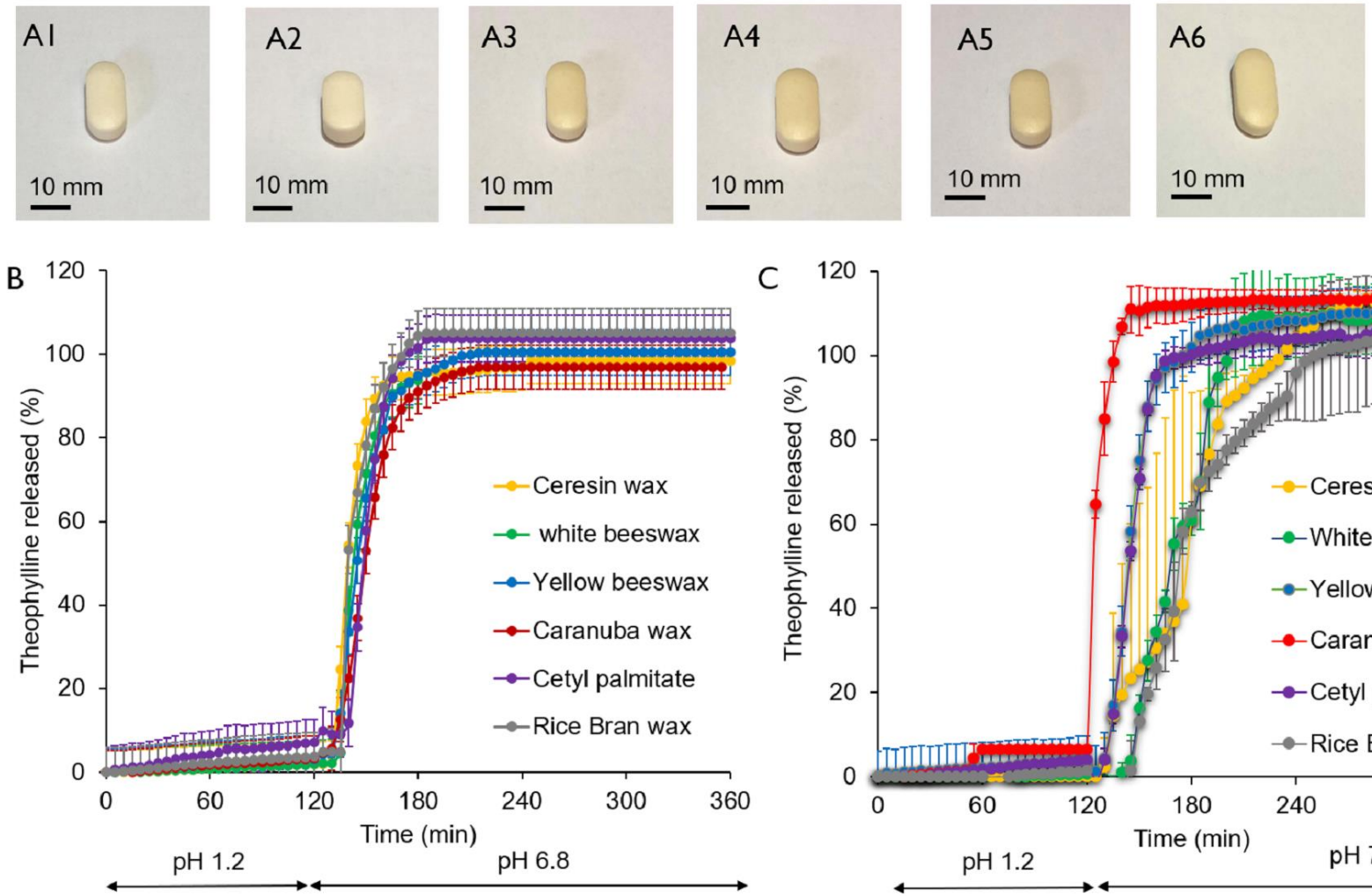


Fig. 4 DSC thermograms of (A) Alg, GMS, ceresin wax, and physical mixture, and (B) films casted of different Alg: wax ratios.

Figure 5 (A) Photograph of enteric coated tablets based on different natural waxes. In vitro dissolution studies of the enteric coated tablets using pH change UPS II dissolution test using (B) phosphate-based buffers and (C) Hanks bicarbonate buffer.



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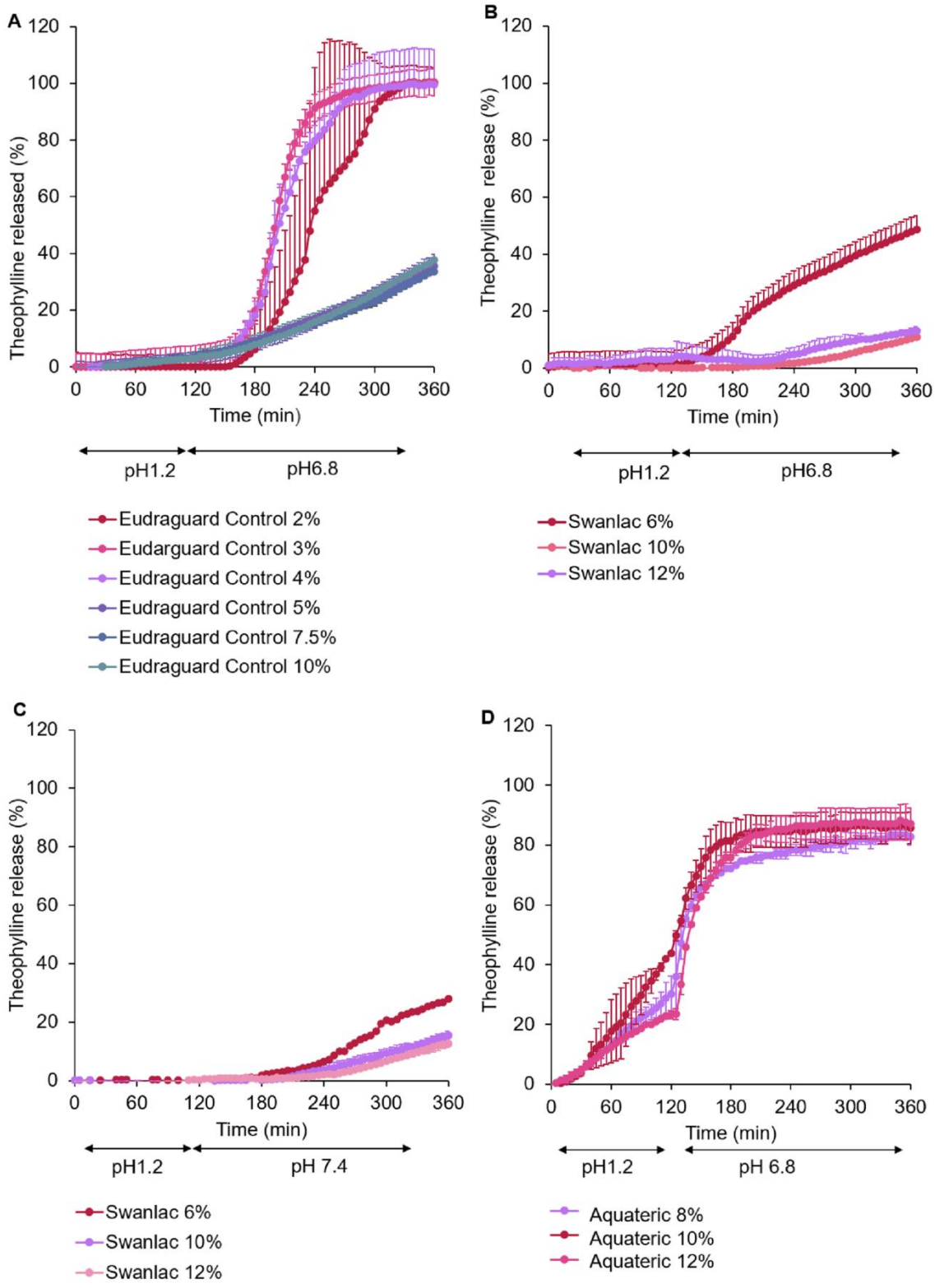

Fig. 6 In vitro dissolution profile of theophylline tablet coated with commercially available products (A) Eudraguard® Control, (B) Swanlac ®ASL 10 pH 6.8, (C) Swanlac ® pH 7.4, and (D) Aquateric ™ N100.

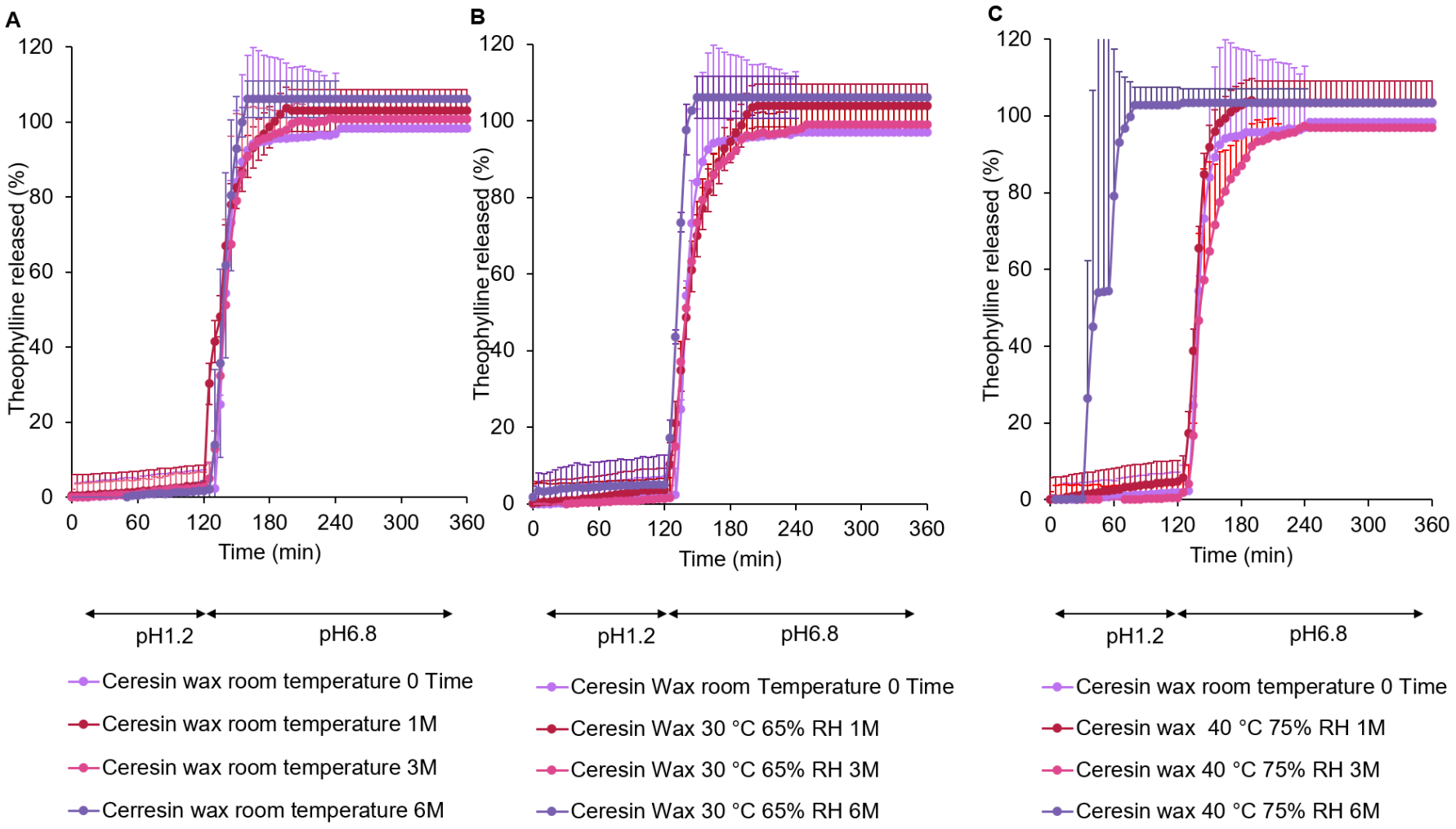

Fig. 7 In vitro dissolution profile of theophylline tablet coated with ceresin based coated tablets stored at (A) room temperature, (B) 30°C 65RH%, and (C) 40°C 75%RH.

Table 1 Gastric resistance properties of naturally occurring wax based enteric coating following USP disintegration and dissolution tests.

	Ceresin wax	White beeswax	Yellow Beeswax	Cetyl palmitate	Carnauba wax	Rice wax
In vitro drug release In phosphate buffer*	✓	✓	✓	✓	✓	✓
Lag time in buffer stage (min)	5	15	15	20	20	15
80% release time in buffer stage (min)	45	35	40	40	45	35
Disintegration test*	✓	✓	✓	✓	✓	✓
Acid medium resistance	Resisted	Resisted	Resisted	Resisted	Resisted	Resisted
Disintegration time of all tablets in SIF (min)	10.2	10.9	11.1	11.4	10.7	11.6
Acid uptake tests*						
Weight gained (%)	4.75%	5.25%	9%	8.4%	9%	6%
In vitro drug release In bicarbonate buffer*						
Lag time in buffer stage (min)	60	30	15	15	5	90
80% release time in buffer stage (min)	90	70	35	35	10	90

* Criteria for enteric coating system: ✓pass, **X** fail

Table 2 Gastric resistance properties of Eudraguard Control enteric coating following USP disintegration and dissolution tests.

	Eudraguard Control 2%	**Eudraguard Control 3%**	**Eudraguard Control 4%**	**Eudraguard Control 5%**	**Eudraguard Control 7.5%**	**Eudraguard Control 10%**
In vitro drug release In phosphate buffer*	✓	✓	✓	✓	✓	✓
Lag time in buffer stage (min)	30	35	40	?	?	?
80% release time in buffer stage (min)	110.3	47.5	62.5	>180	>180	>180
Disintegration test*	✓	✓	✓	✓	✓	✓
Acid medium resistance	Resisted	Resisted	Resisted	Resisted	Resisted	Resisted
Disintegration time of all tablets in SIF (min)	>120	>120	>120	>180	>180	>180
Acid uptake tests*						
Weight gained (%)	12.9%	11.2%	9%	8.7%	8.43%	8%

* Criteria for enteric coating system: ✓pass, **X** fail

Table 3 Gastric resistance properties of Swanlac ASL enteric coating following USP disintegration and dissolution tests.

	Swanlac ASL 6%	**Swanlac ASL 10%**	**Swanlac ASL 12%**
In vitro drug release In phosphate buffer*	✓	✓	✓
Lag time in buffer stage (min)	15	40	?
80% release time in buffer stage (min)	>180	>180	>180
Disintegration test*	✓	✓	✓
Acid medium resistance	Resisted	Resisted	Resisted
Disintegration time of all tablets in SIF (min)	>120	>120	>120
Acid uptake tests*			
Weight gained (%)	1.7%	1.8%	1.9%

* Criteria for enteric coating system: ✓pass, **X** fail

Table 4 Gastric resistance properties of Aquateric N100 (Alg + LYCOAT RS780) enteric coating following USP disintegration and dissolution tests.

	Aquateric N100 8%	**Aquateric N100 10%**	**Aquateric N100 12%**
In vitro drug release In phosphate buffer*	✓	✓	✓
Lag time in buffer stage (min)	0	0	5
80% release time in buffer stage (min)	155	45.3	69.8
Disintegration test*	**X**	**X**	**X**
Acid medium resistance	Disintegrated	Disintegrated	Disintegrated
Disintegration time of all tablets in SIF (min)	-	-	-
Acid uptake tests*			
Weight gained (%)	100%	56.7%	53.9%

* Criteria for enteric coating system: ✓pass, **X** fail