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In vitro Drug Release from Acetylated High Amylose Starch-Zein Films for Oral Colon-Specific Drug Delivery

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Abstract

This study describes the preparation of free films of zein with and without acetylated high amylose maize starch (HAS) and their corresponding coated tablets as a novel approach to colonic drug delivery. We hypothesise that the embedding of a digestible starch component within the inert zein would allow the film to remain intact until the large intestine is reached. Free films of zein alone and starch/zein were prepared and characterised. SEM and AFM images of film surface showed that films were morphologically inhomogeneous, particularly at lower HAS/Zein ratios; however, nanothermal analysis data suggested that these differences in appearance within the same film are not compositional differences, i.e. the systems are not phase-separated. Moreover, FT-IR could detect no molecular interaction between the two polymers. Paracetamol tablets were coated with HAS/Zein aqueous based coatings of different compositions to a TWG of 20%. Drug release from zein alone and 1:5 HAS/Zein coated tablets under upper gastrointestinal conditions (pH 1.2, pH 6.8 with pepsin and pancreatin included) was very similar (for example approximately 12% and 14% of the drug was released, respectively, after 6 hours in a sequential *in vitro* test), suggesting that release in this region is limited and is not influenced by the presence of HAS in the ratio to zein under study. Studies using an *in vitro* colon model showed that under simulated colonic conditions, the drug release was significantly ($p < 0.05$) more rapid from 1:5 HAS/Zein, compared to the zein alone coating formulation. These data therefore support the potential use of zein-starch mixed films for colonic targeting purposes.

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1. Introduction

Zein is the major storage protein of corn (Shukla and Cheryan, 2001), and constitutes 44-79% of the endospermic protein content (Lawton, 2002). It is composed of a mixture of different peptides that can be classified on the basis of their solubility and molecular weight into α (19 and 22 kDa), β (17-18 kDa), γ (16 and 27 kDa) and δ (10 kDa) zeins (Esen, 1986). Among these, α-Zein accounts for 75-85% of the total zein and γ-zein is the next most abundant fraction and accounts for approximately 20% of the total. Zein is particularly rich in hydrophobic amino acids, but deficient in polar or ionisable amino acids (Gianazza et al., 1977; Righetti et al., 1977; Shukla and Cheryan, 2001); it is biodegradable and biocompatible (Dong et al., 2004) and has generally recognized as safe (GRAS) status. Based on its amino acid constitution, zein is recognized for being soluble in aqueous alcohol solutions but not in water (Swallen, 1941). Indeed, its ability to form tough, glossy and hydrophobic coatings has been widely explored in food industry (Bai et al., 2003; Gennadios and Weller, 1990; Weller et al., 1998), while it has also been investigated for its potential use as a pharmaceutical excipient in encapsulation (Katayama and Kanke, 1992; Wang et al., 2005) and coating processes (Beck et al., 1996; O'Donnell et al., 1997; Winters and Deardorff, 1958). For an overview on the uses of zein as an excipient for oral solid dosage forms, readers are referred to Berardi et al. (2018).

Here we investigate the potential use of zein as a colonic delivery excipient. There has been considerable interest in colon targeting as this region of the gastrointestinal tract can be liable to many pathological conditions such as Crohn's disease, ulcerative colitis and carcinomas. Colon-specific drug delivery may not only treat local disorders but may also reduce the administered doses and associated systemic side effects (Leopold, 2001). Colonic delivery of bioactives is also valuable for manipulation of the gut microbiota which is now known to play a critical role in our health and disease. Such delivery can be achieved by using polymeric coatings that are resistant to digestion in the upper gastrointestinal tract (GIT) yet are selectively degraded by the colonic microbiota (Basit, 2005). The use of naturally available polysaccharides, such as glassy amylose (McConnell et al., 2007; Siew et al., 2004), high amylose maize starch (Freire et al., 2010, 2009b), starch derivatives (Karrout et al., 2011, 2010, 2009), chitosan (Fan et al., 2009; He et al., 2009) and pectin (He et al., 2008; Wakerly et al., 1997) has been reported for this application. However, these materials are hydrophilic

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in nature, which makes them either soluble or swellable in aqueous gastrointestinal environments, potentially resulting in drug release prior to the target region being reached by the dosage form. To limit this problem, these excipients have been mixed with water-insoluble polymers, such as ethyl cellulose, as reported by Liu et al. (2003) and Freire et al. (2009a, 2009b). Thus, it has been suggested that the water-insoluble zein could represent a valuable alternative to ethyl cellulose as a film-forming polymer for colonic delivery (Liu et al, 2006; Tang et al, 2015).

This study aims to demonstrate the feasibility of using zein and high amylose starch/zein mixed coatings in colon-targetted drug delivery systems via assessment of their resistance to release in the upper GI tract. It has been found that heat treatment of high amylose starch generates a retrograded form that is more resistant to pancreatic α-amylases, yet susceptible to those present in the colon (Freire et al., 2009a). In this study, free films of heat treated acetylated high amylose maize starch (HAS) and zein were prepared and characterized using a range of techniques including scanning electron microscopy (SEM), atomic force microscopy (AFM), nano-thermal analysis (n-TA), and Fourier-transform infrared (FT-IR). In particular, the miscibility of the films is assessed as this is integral to the release properties. Paracetamol-loaded tablets were film coated with zein alone and HAS/zein aqueous dispersions and the drug release was assessed in simulated gastric and intestinal fluids with pepsin and pancreatin enzymes, respectively. In this manner it is intended that the ability of zein films to prevent or reduce drug release within the upper intestinal tract will be evaluated. Finally, drug release was assessed under simulated colonic conditions using a batch culture fermentation system, inoculated with human faecal bacteria.

2. Materials and Methods

2.1 Materials

Zein from maize (Z3625) was obtained from Sigma-Aldrich (Germany) and used as received. This product was plasticized with polyethylene glycol 400 (Sigma Aldrich, UK). Acetylated high amylose maize starch (HAS) with a minimum degree of substitution (DS) of 0.06 was obtained from Roquette (Italy) and used as received. The amylose content of this starch is 51%.

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The model drug, paracetamol was obtained from Alfa Aesar (UK) and was used as received. Avicel PH 301 was obtained from FMC BioPolymer (UK). Anhydrous calcium phosphate dibasic of 97% purity was purchased from Acros Organics (USA). Polyvinylpyrrolidone K30 (PVP K30) and magnesium stearate were purchased from Aldrich (UK). Partially Gelatinized Starch (Pre-gelatinised starch) was purchased from Colorcon (UK) as the grade Starch 1500. All excipients were used as received. Pepsin from porcine gastric mucosa and pancreatin from porcine pancreas were supplied by Sigma (UK).

2.2 Preparation of Zein and Starch–Zein Aqueous Dispersions

Aqueous zein dispersions (10% w/w) were prepared by a precipitation method described by Li et al. (Li et al., 2010). The dispersion was plasticized with 30% (w/w) polyethylene glycol 400 (PEG 400) corresponding to the dry weight of zein. In order to prepare starch-zein dispersions, an aqueous dispersion of acetylated high amylose maize starch (HAS) was prepared by dispersing 3 g of HAS in 20 mL water, and heating to 80 ± 5 ˚C for 30 minutes, based on the method proposed by Freire et al. (Freire et al., 2009a). The HAS dispersion was allowed to cool down and then mixed with the pre-plasticized aqueous zein dispersion. Mixing using a magnetic stirrer was continued overnight to ensure complete dispersion of the two polymers. HAS–Zein dispersions were prepared in 1:3, 1:5 and 1:7 ratios, corresponding to the dry weight of both polymers. Zein (alone) dispersions were prepared by admixing the pre-plasticized aqueous zein dispersion with distilled water (same volume as used for the preparation of the HAS-Zein dispersions) overnight using a magnetic stirrer.

2.3 Preparation of Free Films

Zein alone, HAS alone and HAS/Zein dispersions were prepared as described in Section 2.2. Free films were prepared by a casting/solvent evaporation technique, as described in the work of Gillgren et al. (Gillgren et al., 2009). The aqueous dispersions (9 g each) were poured onto 90 mm plastic petri dishes and dried in an oven at 45 ± 5 °C for 24 hours. After drying, the films were stored in a desiccator over P_2O_5 (0 % RH) at room temperature for subsequent studies.

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2.4 Characterization of Free Films

2.4.1 Scanning Electron Microscopy (SEM)

The surface morphology of zein alone and HAS/Zein free films as well as morphology of coated tablets were studied using SEM. Samples were mounted onto stubs using double-sided tape and were gold coated by a Polaron SC7640 sputter gold coater manufactured by Quorum Technologies (UK). The thickness of the gold coating was about 15 nm. The imaging process was performed in a high vacuum environment with a JEOL JSM5900 LV SEM (Japan), mounted with a tungsten filament with an acceleration voltage of 5-20 kV.

2.4.2 Nano-Localized Thermal Analysis (n-LTA)

Topographic images were acquired with a Caliber atomic force microscope (AFM) from Bruker AFM (UK) equipped with an AN-200 Thermalever probe from Anasys instruments (USA). The scan area for all AFM images was 50 µm x 50 µm with a resolution of 512 pixels and a scan rate of 0.5 Hz. Localized thermal analysis was carried out using the same system as described above but equipped with a nanoTA2 controller (Anasys Instruments, USA). A typical measurement is carried out by generating a topographical image of the film surface using the AFM and then selecting a point on the surface for interrogation. A voltage is then applied to the probe by the nanoTA2 controller and the deflection of the probe monitored. As the probe heats the material directly beneath the probe, the material expands, forcing an upward deflection of the probe. As the temperature reaches a thermal transition such as a melting point, the material softens and the probe penetrates into the surface. At this point the temperature profile is stopped and the probe retracted. Measurements were carried over a range of room temperature to 200 °C at a heating rate of 10 °C/s.

2.4.3 Attenuated Total Reflectance Fourier Transform Infra-Red (ATR-FTIR)

Fourier-transform infrared (FTIR) measurements were carried out in attenuated total reflectance (ATR) mode. The infrared spectra of the samples were collected using a Bruker IFS 66/S spectrophotometer (Bruker Optics Ltd., UK), fitted with a Golden Gate

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ATR accessory from Specac Ltd. (UK). Measurements were carried out over a spectral range of 4,000 to 550 cm^{-1} with a total of 16 scans acquired at a resolution of 4 cm^{-1}.

2.5 Preparation and Film Coating of Paracetamol-Loaded Tablets

The formulation was produced via wet granulation as described in Table 1. The granules were loaded onto a Manesty E-2 single station tablet press (Manesty Machines Ltd., UK) and were compressed using round 7 mm diameter plain, normal concave punches. Tablet specifications tests including weight uniformity, content uniformity and tablet thickness were determined (Table 1). Hardness was measured using an Erweka TBH 28 hardness tester (Germany) and friability was determined using Erweka TAR friabilator (Germany) at 100 rotations. The disintegration time was measured in distilled water and in 0.1 M HCl at 37 ± 0.5 ºC in a Copley DTG 2000 apparatus using disks (UK).

Coating was performed on ten tablets batches using a top-spray fluidized-bed coater (Mini Coater/Drier 2, Caleva Process Solutions Ltd, UK). The coating was carried out in a bed temperature of 45 °C, atomization pressure of 0.7 bar, pump rate of 1.09 rpm and fan speed of 13 to 13.5 m/s. A drying stage was incorporated into the process by turning off the spray and keeping the coated tablets at the same bed temperature for 15 minutes. The tablets were then cured in an oven for 10 hours at 45 °C. The film thickness is expressed in terms of the percentage total weight gain (TWG), and tablets with a TWG of 20% were obtained.

2.6 In Vitro Drug Release Studies

Dissolution studies were carried out on a Copley CIS 8000 dissolution bath (Copley Scientific, UK) with a rotation speed of 50 rpm according to the BP Apparatus I basket method. The tests were performed in 900 mL of freshly prepared simulated gastric fluid (SGF, 2 g L^{-1} NaCl, 3.2 g L^{-1} pepsin, 80 mL L^{-1} 1M HCl (BP 2011)) pH 1.2 for 2 hours at 37 ± 0.5 °C, followed by 6 hours in freshly prepared simulated intestinal fluid (SIF, 6.8 g L^{-1} KH_2HPO_4, 10 g L^{-1} pancreatin, 77 mL L^{-1} 0.2 M NaOH (BP 2011)) pH 6.8. Samples of 10 mL were withdrawn at predetermined times, filtered through a 0.2 µm membrane filter (Sartorius Stedim Biotech GmbH, Germany) and replaced with an equivalent volume of fresh medium. The paracetamol concentration was determined spectrophotometrically (Lambda XLS UV/VIS, Perkin-Elmer, USA) at a wavelength of 243 nm and with reference to an appropriate standard curve. Samples that contained

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the pancreatic digestive enzymes were centrifuged at 13,000 rpm for 30 minutes prior to filtration and UV measurements. The dissolution studies were carried out in triplicate and the average drug release ± SD was calculated.

2.7 Batch Culture Fermentation studies

Drug release from the tablets was also assessed under simulated colonic conditions using a batch fermentation system. Fermenters consisted of glass bottles, fitted with the standard dissolution baskets and a magnetic stirrer. The vessels were filled with prereduced basal culture medium, prepared based on work of Hughes et al. (Hughes et al., 2008). This medium contained per litre: 2 g peptone water (Oxoid Ltd., UK), 2 g yeast extract (Oxoid), 0.1 g NaCl, 0.04 g K_2HPO_4, 0.01 g $MgSO_4.7H_2O$, 0.01 g $CaCl_2.6H_2O$, 2 g $NaHCO_3$, 0.005 g haemin (Sigma), 0.5 g L-cysteine HCl (Sigma), 0.5 g bile salts (Oxoid), 2 mL Tween 80, 10 µL vitamin K (Sigma), and 4 mL of 0.025% (w/v) resazurin solution. The fermenters were then inoculated with a 10% (w/w) faecal slurry which was prepared by homogenizing fresh human faeces and prereduced phosphate-buffered saline (PBS 8 g L^{-1} NaCl, 0.2 g L^{-1} KCl, 1.15 g L^{-1} Na_2HPO_4, and 0.2 g L^{-1} KH_2HPO_4) pH 7.3, in a Stomacher 400 (Seward, UK) at 230 rpm for 45 s. Unhomogenized fibrous material was removed by filtration through filter bags (Stomacher® lab system, BA6141/STR bag filters made for Seward LTd.). The final pH was adjusted to 6.8. The tablets were placed in the dissolution baskets, then introduced into 100-mL batch culture fermenters and stirred using a magnetic flea at 100 rpm for 24 hours. All additions, inoculations and incubations were carried out inside an anaerobic cabinet (10% H_2, 10% CO_2, and 80% N_2) at 37 °C. Control experiments using autoclaved faecal sample (i.e. dead bacterial cells and no enzymatic activity) were run on a subsequent day. Experiments were conducted in triplicate.

One-mL samples were removed at predetermined times over a 24-hour period, centrifuged at 14,000 rpm for 10 minutes, and filtered through 0.2 µ m filters prior to analysis for paracetamol by high-performance liquid chromatography (HPLC; Dionex, UK). The mobile phase consisted of acetonitrile (MeCN)-water (25:75 v/v) adjusted to pH 2.5 with phosphoric acid and pumped at 1.7 $mL.min^{-1}$ through a C18, 5 µm, 250 x 4.6 mm column (Phenomenex, UK). UV detection was carried out at 245 nm.

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3. Results and Discussion

3.1 Characterization of Free Films

SEM and AFM images of the surfaces of the free films were obtained to gain information regarding the miscibility of the two polymers and overall quality of the films. SEM images of the surfaces obtained using different HAS and zein ratios (w/w) are presented in Figure 1. The acetylated high amylose maize starch (HAS) alone film (Figure 1a) showed granular structures of non-uniform architecture. The swelling of starch granules upon heat treatment at 80 °C, as reported by Freire et al. (Freire et al., 2009a) could possibly explain the formation of these granular structures; the same group (Freire et al., 2009b) reported similar results using a starch type with approximately the same amylose content to the one used in this study. The authors stated that despite the intense swelling of starch granules, most of the granules did not burst and formed aggregates at the surface of the free films. The zein alone film (Figure 1b) had a compact surface made up of smaller spherical particles. Similar structures were observed by Li et al. (Li et al., 2010). The 1:7 and 1:5 HAS/Zein films (Figure 1c and d) showed a smooth surface along with some spherical features similar to those observed with zein alone films. The 1:3 HAS/Zein film had a smooth and homogenous surface (Figure 1e), in contrast to that observed with films of lower HAS/Zein ratios. These images therefore suggest that a more homogenous dispersion of the two phases occurred at higher HAS/Zein ratios. This may be associated with the adhesion between zein and starch as proposed by Habeych et al. (Habeych et al., 2008), who suggested that increasing zein concentration promoted poorer adhesion between the components, which is in keeping with the observations made here.

AFM topographic images were also acquired to identify surface features of the films (Figure 2_A), and were in alignment with the SEM images (Figure 1). HAS alone free film exhibited large circular features, similar to those seen in SEM images, while the surface of zein alone film was made up of smaller spherical particles. This is in agreement with Li et al. (Li et al., 2010) who reported that the plasticized zein films prepared from aqueous dispersion were more compact, smoother and composed of smaller colloidal particles compared to the plasticized films prepared form organic solutions. HAS/Zein films showed different structural patterns which were intermediate between the features displayed by the single components and were dependent on the

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HAS/Zein ratio of the film. Films having the lowest HAS/Zein ratio, i.e. 1:7 showed mainly spherical particles. Films with the highest ratio, i.e. 1:3 had the smoothest surface (height difference = 400 nm) and those of 1:5 ratio were made up of spherical particles along with smoother areas. Again, these results might suggest a poor adhesion between the two polymers at lower HAS/Zein ratios compared to the higher ratios.

To determine the nature of the different structures observed in the topographic images of the film surface, single point LTA measurements were carried out at various locations on the film surface. This technique consists of the application of a heated nanoprobe onto a surface and the measurement of the position of the probe as a function of temperature; more details can be found in (Craig et al., 2002). Each individual measurement has a scale of scrutiny of circa 500nm by 500nm. Figure 2_B shows the corresponding LTA responses. Whilst HAS alone films showed no measurable transitions up to degradation (and hence is not included in Figure 2B), zein alone films were relatively consistent with softening occurring at 75.7 ± 5.3 °C; this softening temperature is related to the glass transition of zein. HAS/Zein films of 1:7, 1:5 and 1:3 ratios showed a trend whereby one softening point, in most cases, was detected at circa 115.7 ± 3.8, 135.9 ± 6.6 and 142.2 ± 10.4 °C, respectively. It is worth noting that although the topographic images of film surface were inhomogeneous, particularly at lower HAS/Zein ratios, only one softening transition was detected in the corresponding LTA measurements. Chanvrier et al. (Chanvrier et al., 2005) reported that although starch and zein are immiscible, only one thermal transition was detected, possibly because the peaks due to the main relaxation of starch and zein were superimposed. Indeed, Chanvrier et al. (Chanvrier et al., 2006) found that starch and zein have similar Tg values and thus deduced that the transitions measured on the starch-zein blends corresponds to the superimposition of the glass transition of each component. However, it should be also noted that the softening temperature detected by LTA increased with increasing the HAS/Zein ratio, i.e. with the addition of starch. This type of behaviour might indicate that some physical interactions between zein particles and starch granules occurred at the interaction surface between the components. This result is not in keeping with the findings of (Chanvrier et al., 2006; Corradini et al., 2007) , where the transition temperatures of immiscible blends remained nearly constant for all starch-zein ratios. This may reflect

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the differing nature of the measurements; nanothermal analysis measures the temperature of physical softening rather than the associated thermodynamic event, hence the differences may reflect the manner in which the starch is altering the thermomechanical properties of the films.

Moreover, FT-IR spectra of the mixed films with various HAS/Zein ratios were obtained in the spectral region of 4000 to 500 cm^{-1} to investigate any possible interactions between the two polymers (Figure 3); HAS alone and zein alone films were scanned for comparison. The FTIR spectrum of zein protein consists of the typical protein bands including amide I from 1750 to 1600 cm^{-1} and amide II from 1500 to 1400 cm^{-1} (Forato et al., 2003). HAS alone film showed distinct peaks at 1005 cm^{-1}, 1080 cm^{-1} and 1153 cm^{-1} in addition to a small peak at 1240 cm^{-1} which is typical of acetylated starches. No spectra of the mixed HAS/Zein films, regardless of the ratio, exhibited any significant shifts in the amide I and II peaks and no new peaks could be detected even at the highest HAS content, suggesting a lack of chemical interaction between the two polymers.

Overall, SEM and AFM images indicate that starch/zein films were morphologically inhomogeneous, particularly at lower starch/zein ratios. However, the NTA measurements did not indicate distinct transition populations, a characteristic of systems that are homogeneous at the scale of scrutiny (circa 500nm by 500nm) hence at least some of the topographic inhomogeneity noted may reflect architectural rather than compositional distribution. It is also interesting to note the increase in the measured softening temperature as the HAS/Zein ratio increased, reflecting the changes in thermoresponsive behaviour as the composition is altered. This again is a typical characteristic of blended systems.

3.2 Paracetamol Release Profile from Tablets Film Coated with Starch-Zein Dispersions

3.2.1 Effect of Starch to Zein Ratio on Paracetamol Release

Paracetamol tablets were coated with HAS/Zein aqueous based coatings of different compositions to a TWG of 20%. The effect of starch to zein ratio on paracetamol

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release is shown in Figure 4. The test was carried out under acidic conditions in order to maximally challenge the formulation, as the dibasic calcium phosphate within the tablet core is soluble in dilute acids, potentially exerting an osmotic pressure against the coating after dissolution of (at least part of) the tablet core. Drug release from zein alone and 1:5 HAS/Zein coatings was comparable. Increasing the starch content in film coatings increased the drug release rate and extent in comparison to coatings with no or less starch. We suggest that the drug release through zein-based coatings occurs mainly via diffusion and increasing the hydrophilic content in the water insoluble film coat will influence the drug release. The swelling of starch in aqueous environments will disrupt the structure of the film and create aqueous channels through which more drug release can occur (Siew et al., 2000). The results of drug release also suggest that the water insoluble zein domains could control the swelling of starch only at lower starch concentration, whereas rupture of the film coating occurred at the higher ratio, i.e. 1:3. Similarly, reducing the coating thickness of the 1:5 HAS/Zein ratio to a TWG of 10% resulted in film rupture and premature drug release in the upper GIT (Figure S1). Thus, formulations with 1:5 HAS/Zein ratio coated to a TWG of 20% were used for further investigations.

3.2.2 Paracetamol Release in Simulated Gastric and Intestinal Fluids

A key requirement of a colonic-targetted drug delivery system is to minimize early drug release in the upper gastrointestinal tract (Basit, 2005). To investigate this, zein alone and 1:5 HAS/Zein coated tablets were tested in conditions simulating the stomach and the small intestine. The drug release profiles from zein alone and 1:5 HAS/Zein coated formulations in simulated gastric fluid with pepsin (SGF with pepsin) for 2 hours and subsequently in simulated intestinal fluid with pancreatin (SIF with pancreatin) for a further 6 hours are shown in Figure 5. Approximately, 12% and 14% of the drug was released after 6 hours, respectively. The release profile of HAS/Zein based coatings was very similar to that of zein alone, suggesting that the starch present in the films is resistant to digestion by the enzymes present in SGF and SIF, at least over this test duration chosen to reflect the average transit time of solid dosage forms in the stomach (2 hours) and small intestine (4 hours) (Yang, 2008).

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The values of % drug release obtained here were comparable to those obtained with pectin/zein complex hydrogel beads in environments similar to the stomach and small intestines (Liu et al., 2006). However, these values were higher in comparison to those obtained for ethyl cellulose-based films (Freire et al., 2009b; Siew et al., 2000). This difference could be explained by the fact that although zein, like ethyl cellulose, is water insoluble, it swells in the presence of an aqueous medium (Berardi et al., 2017a, 2017b), thus increasing the porosity and permeability of the coating. The difference in release can also be attributed to differences in the formulation of the core substrate and drug used. Nevertheless, the drug release under conditions simulating the upper GIT suggests that high amylose maize starch-based films can be used potentially for colon-specific delivery.

3.2.3 Paracetamol Release in Simulated Colonic Fluids Using a Batch Culture Fermentation System

The dissolution testing explained above provided information about the resistance of the coating to drug release under conditions resembling those of the upper gastrointestinal tract. In this section, the susceptibility of pseudolatex-based zein alone and HAS/Zein coatings to digestion by the colonic microbiota was investigated using a batch culture fermentation system inoculated with human faecal bacteria. Figure 6 shows the paracetamol release from zein alone and 1:5 HAS/zein coated tablets under colonic (with faecal content) and control (with autoclaved faecal content) conditions.

For both formulations, the drug release in simulated colonic conditions (solid-lines) was greater than in the control conditions (dashed-lines), indicating that both systems are being subjected to digestion by the gut microbiota. It is interesting to note the following. Firstly, the difference in profiles between the zein alone and the HSA/zein coated systems was greater for the batch culture fermentation system than for the *in vitro* study shown in Figure 5. Secondly, in both cases release under the colonic conditions was more rapid than the control, the difference being greater for the HSA/zein systems. Taken together, the results indicate that there are multiple influences in operation within the simulated colonic environment. The composition of the fermentation study media, irrespective of autoclaving, compared to the *in vitro* study shown in Figure 5, does appear to influence the release, possibly by solubilising

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or by modifying the swelling behaviour of the film components which may promote release from zein-based systems. Thirdly, there is indeed evidence (i.e. the greater release compared to the control) that the presence of the HSA increases the release via digestion by the microbiota (the difference is statistically significant ($p<0.05$)), hence the hypothesis on which the study has been based is supported. Further optimisation of the formulation may increase the difference between the HSA loaded and unloaded coats, particularly given that the level of starch present was relatively low. Perhaps surprisingly, there is some evidence of the zein also being digested by the microbiota, probably reflecting the presence of bacterial proteases in the colon model system. Nevertheless, overall the fermentation study has demonstrated that the presence of HSA may render the coats susceptible to digestion by colonic bacteria.

The question arises as to the likely relationship to the in vivo situation, particularly amongst the ulcerative colitis patient population. It has been reported that the total colonic transit time in individuals with ulcerative colitis is on average 24.3 hours as opposed to 51.7 hours in healthy individuals (Hatton et al., 2018; Hebden et al., 2001); This makes the formulation developed here potentially suitable to orally deliver the entire drug payload to the diseased colon despite the reduced residence time.

SEM images of the surface of tablets coated with zein alone and 1:5 HAS/Zein after 24 hours in control and colonic conditions are shown in Figure 7. The zein coated tablet had a fairly smooth surface before the release study (a), but showed signs of extensive digestion under colonic conditions (c), compared to the control (b) conditions. More significantly, tablets coated with HAS/Zein (Figure 7, lower row) showed a complete loss of the coating structure and integrity under colonic conditions (c), whereas the coating was highly porous under control conditions (b).

The release of paracetamol from zein alone or HAS/Zein coated tablets is postulated to occur mainly via drug diffusion through the aqueous channels formed either by the spaces between the polymeric chains or by the dissolution of some film ingredients along with some erosion and/or degradation of the polymeric membrane in the presence of digestive enzymes.. Pre-existing microcracks are also a possibility but the thickness of the coat and the absence of evidence of such structures from SEM may render this mechanism less likely; however crack generation on swelling is also

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feasible.

Although macroscopically zein alone coatings remained intact after 24 hours exposure to the colonic media, SEM images indicated a degree of surface erosion (Figure 7, upper row_ c). The fact that these polymeric coatings retained their structural integrity further suggests that these coated systems are primarily diffusion-controlled, with the exception of HAS/Zein tablets that showed a complete loss of the coating structure under colonic conditions (Figure 7, lower row_ c). While further optimisation in terms of, for example, coating thickness and HSA content is necessary to bring this approach to a pre-clinical stage, the investigation here nevertheless indicates that the principle of using HSA-loaded zein coatings is a promising approach for selective delivery to the colon.

4. Conclusions

The present work has examined the use of high amylose maize starch (HAS) and zein film coatings as potential vehicles for colon-specific drug delivery. HAS/Zein free films showed no evidence for phase separation or chemical interaction between the two polymers. Both zein alone and 1:5 HAS/Zein coated tablets showed limited drug release under upper gastrointestinal tract conditions, indicating that the starch resisted digestion by pepsin and pancreatin enzymes and that zein effectively suppressed the swelling of starch and the consequent drug release. Under colonic conditions, the drug release from 1:5 HAS/Zein was complete and significantly ($p < 0.05$) higher than zein coated tablets, suggesting that the starch in this film was susceptible to digestion by the colonic microbiota. The drug delivery device for colonic-specific targeting described here is thus promising and provides a robust drug delivery platform that could be further engineered to tailor drug release profiles to specific needs.

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Figure captions

Figure 1 SEM images of free films surface of (a) HAS alone, (b) zein alone, (c) 1:7 HAS/Zein, (d) 1:5 HAS/Zein and (e) 1:3 HAS/Zein films.

Figure 2 (A) AFM topographic images and (B) corresponding LTA measurements of (a) HAS alone (b) zein alone (c) 1:7 HAS/Zein (d) 1:5 HAS/Zein and (e)1:3 HAS/Zein free films.

Figure 3 FT-IR spectra of zein alone film, HAS alone film, and 1:7, 1:5 and 1:3 HAS/Zein films in the spectral region of 500 to 4000 cm^{-1}.

Figure 4 Dissolution profiles in 0.1 M HCl of paracetamol tablets coated (TWG = 20%) with zein alone, 1:5 HAS/Zein and 1:3 HAS/Zein pseudolatexes. Each point represents the mean value ± SD (n = 3).

Figure 5 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with zein pseudolatex alone and 1:5 HAS/Zein pseudolatex in SGF (with pepsin) pH 1.2 (2 hours) then SIF (with pancreatin) pH 6.8 (6 hours). Each point represents the mean value ± SD (n = 3). (Error bars within data points).

Figure 6 Release profile of paracetamol from tablets coated (TWG = 20%) with zein alone (black lines) and 1:5 HAS/Zein dispersions (red lines) in colonic (solid lines) and control (dashed lines) conditions. Each point represents the mean ± SD (n = 3).

Figure 7 SEM images of paracetamol tablets coated with zein alone (upper row) and tablets coated with 1:5 HAS/Zein (lower row) aqueous dispersions: prior to any treatment (a); and after 24 hours immersion in: control (b); and colonic (c) conditions.

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Table 1 Formulation and specification tests data for paracetamol-loaded tablets.

Chemical	**Content (g)/batch**	**Specification test**	**Mean (SD)**
Calcium phosphate dibasic	423.75	Weight uniformity (mg, n = 20)	212.7 (2.5)
Paracetamol	150.00	Content uniformity (mg, n = 10)	43.3 (0.9)
MCC	75.00	Thickness (mm, n= 20)	3.9 (0.03)
PVP *	18.75	Hardness (kp, n = 20)	12.0 (1.0)
Partially gelatinized starch	75.00	Disintegration time (minutes, n = 6):	
Water	As required	In water	1.78 (0.4)
Magnesium stearate	7.50	In 0.1 M HCl	0.8 (0.3)
Total	**750.00 g**		

*Added as 100 mL of 18.75% aqueous solution during granulation.

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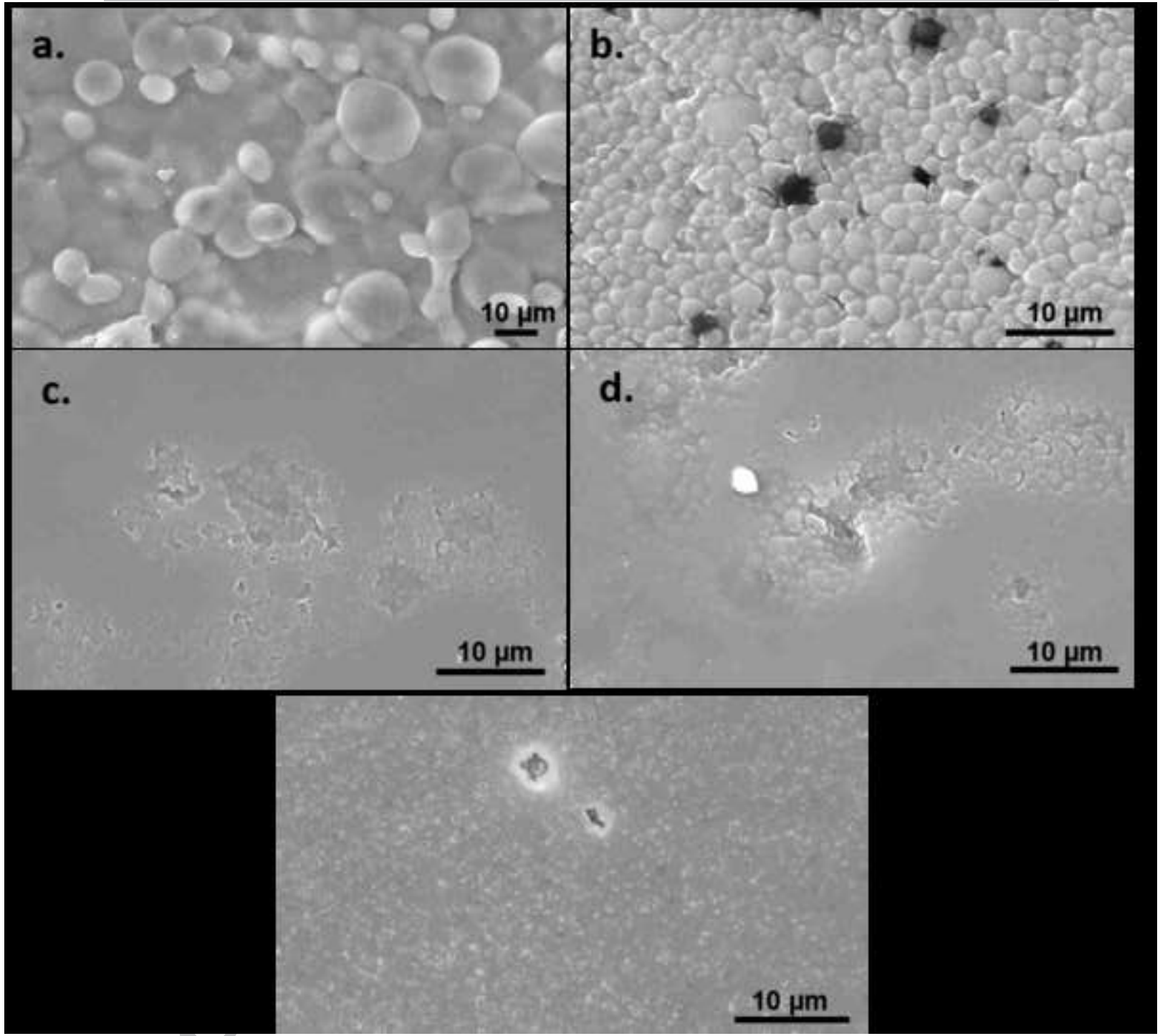

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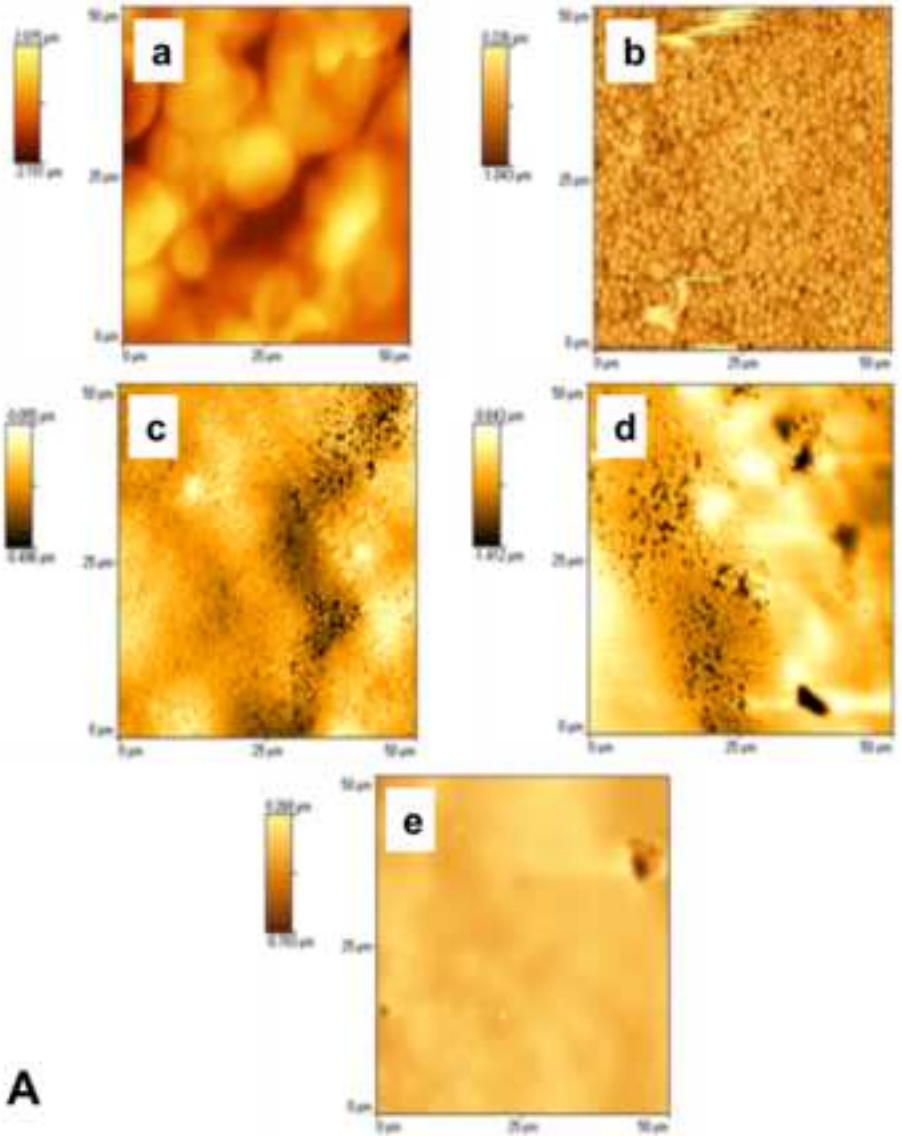

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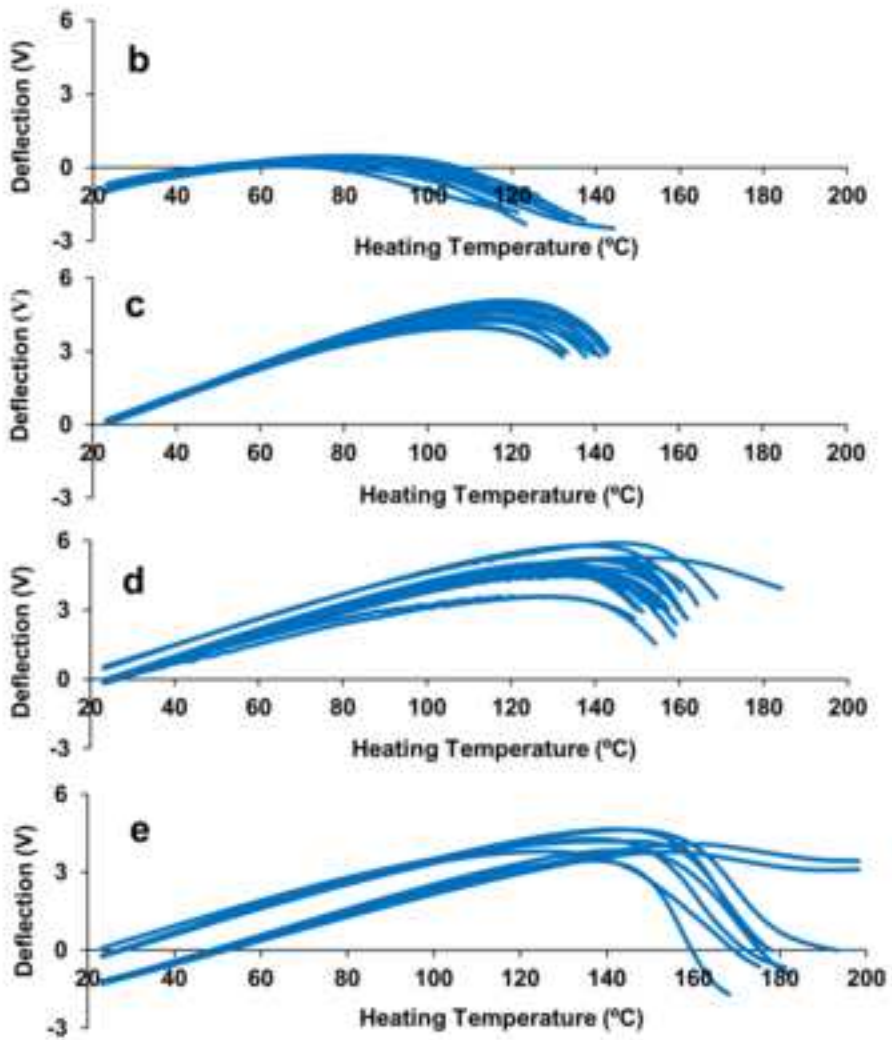

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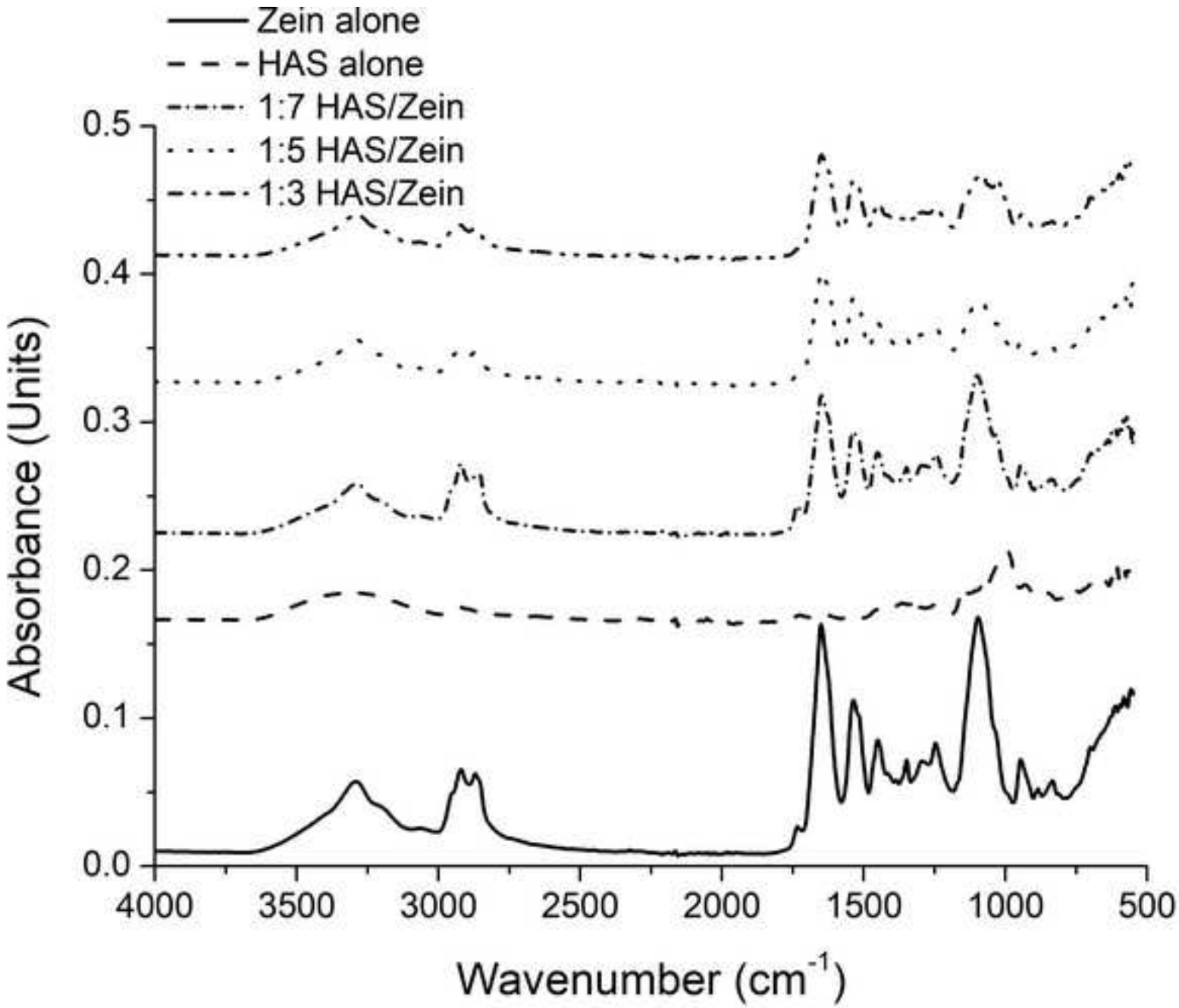

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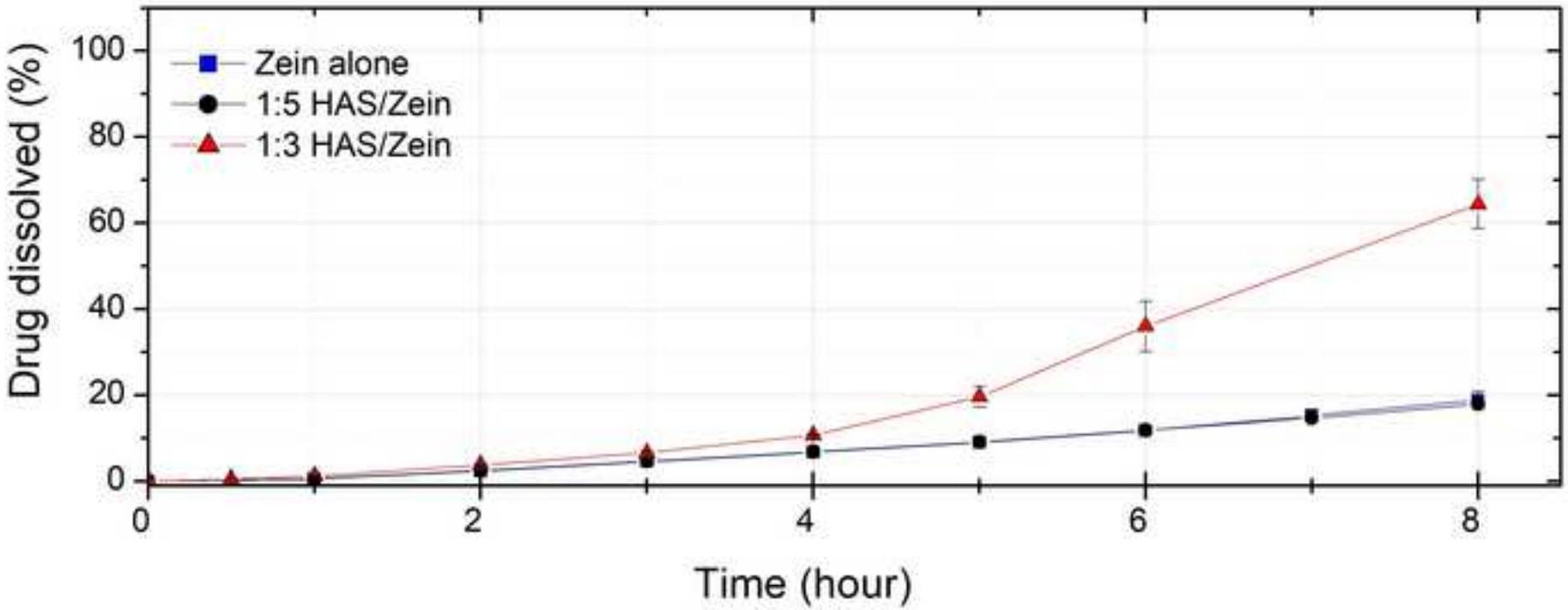

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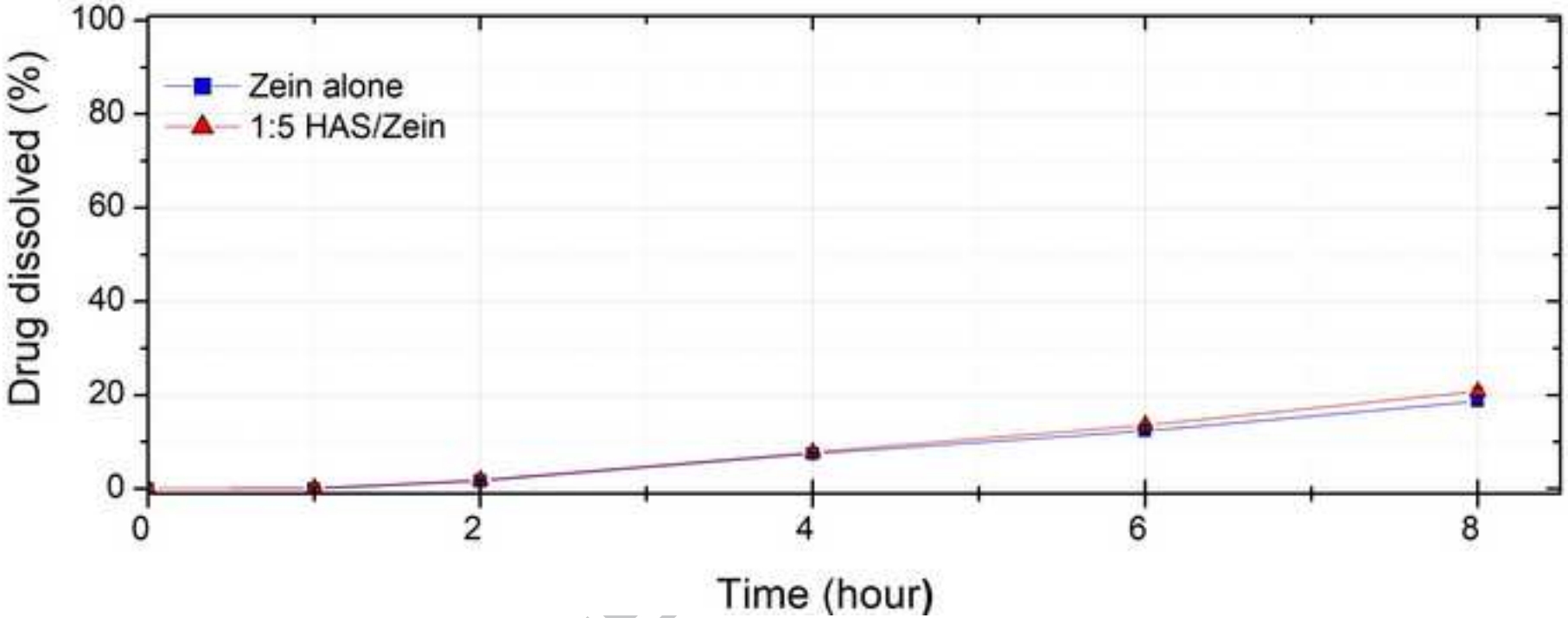

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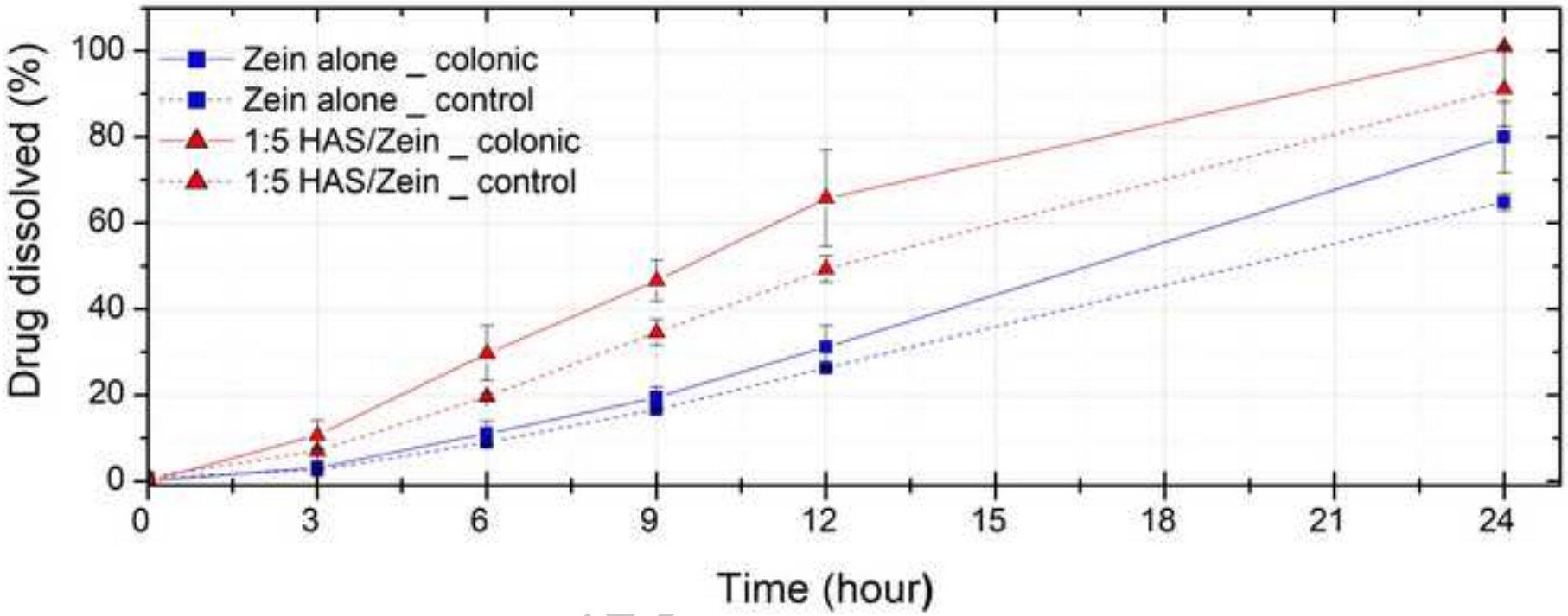

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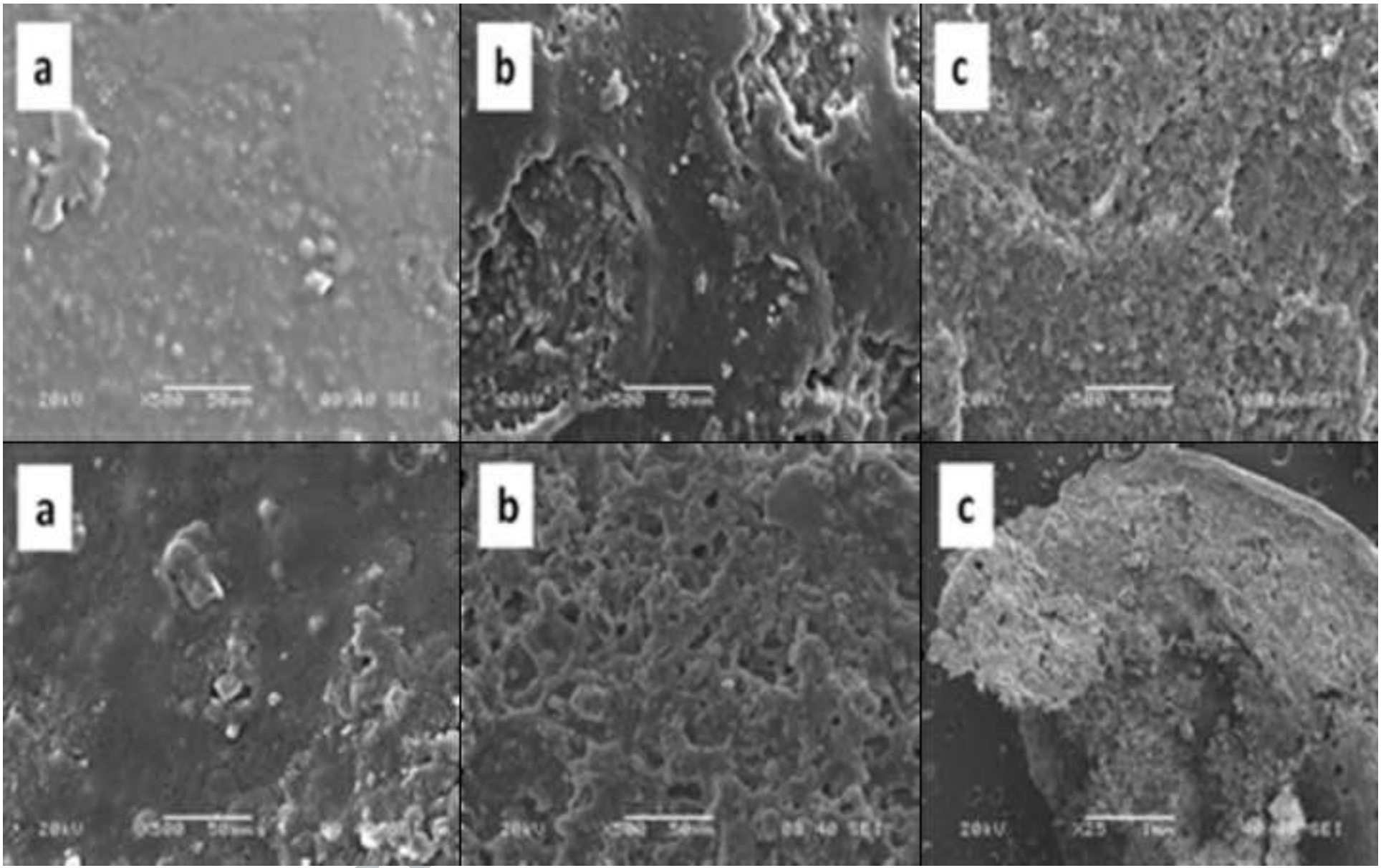

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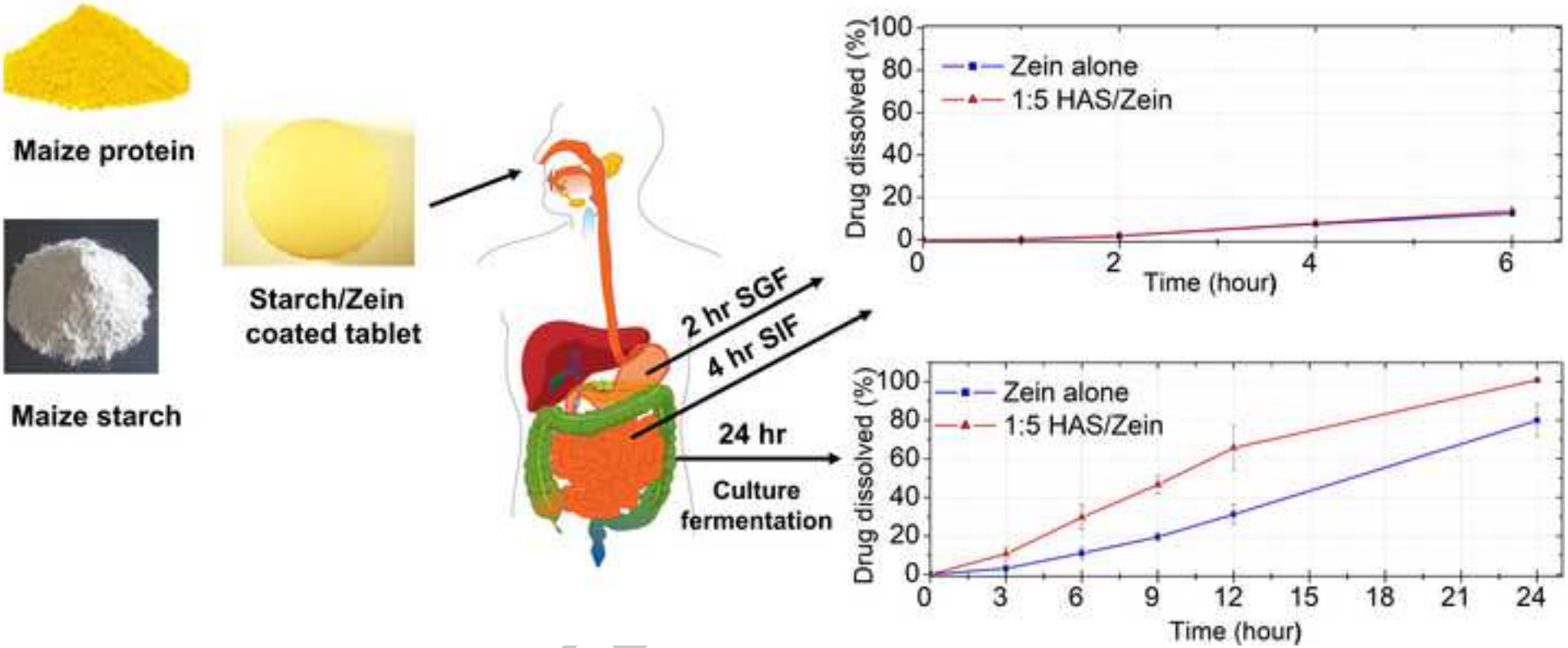

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