Evaluation of the effect of polymer composition on the rheological, mechanical properties and drug released behavior of novel Eudragit L100-55/ gelatin gastro-resistance uncoated capsule

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Abstract

Capsules have been investigated as a popular oral dosage form among communities due to their simplicity and ease of production. Capsules that are considered in the gastro-resistance category can be very beneficial due to enhanced drug absorption, improved stability, targeted release, etc. This study investigated the effect of enteric polymers on uncoated hard capsule fabrication and dissolution properties. The polymers used in this study included HPMCPh and Eudragit L100-55. Eight different formulations based on HPMCPh, Eudragit, and gelatin were examined to identify the ideal formulation for the product of uncoated enteric hard capsules with preferred physicochemical and gastro-resistance properties. The results reveal that the capsules containing Eudragit (F1), HPMCPh (F2), Eudragit/HPMCPh/gelatin (F3), and Eudragit/gelatin (F4) are steady within the simulated stomach environment, and drug release does not occur for 120 minutes. The outcomes demonstrate that, among the proposed formulas, the F4 formula is suitable both in terms of capsulation form and delayed release properties and shows no microbial growth. The properties of the optimized sample were studied by FTIR, FESEM, tensile strength, humidity, and rheology. The results illustrated that gelatin-based hydrogels with Eudragit (F4) are potential candidates for manufacturing uncoated enteric hard capsules that inhibit drug release in a gastric pH medium and act as a pH-sensitive drug release system.

1. Introduction

Hard capsules are one of the most common oral forms of solid dosage that are often used for delivering medicinal compounds [1]. Hard capsules are easier to use compared to tablets. These are typically easier to swallow, fast released, and absorbed in the stomach. They can be easily opened and the contents can be mixed with food or liquids for individuals who have difficulty swallowing whole capsules or tablets. This flexibility is particularly beneficial for children, elderly individuals, or those with swallowing difficulties. Some medications have an unpleasant taste, which can be masked more effectively in hard capsules compared to tablets. The capsule shell helps to prevent direct contact between the medication and taste buds, making it more palatable. Hard capsules can be customized with different colors, shapes, and markings to aid in identification. Also, the production and quality control of tablets require more time, formulation development, and quality control [2–4].

Capsules provide an easy delivery system for nutrients (especially powders) without the need for creating complex formulas. They have been used in developing a series of drugs that are tested in animal or clinical trials due to their simplicity and rapid formulation. Hard capsules can be produced in diverse sizes and with different materials depending on their main purpose and content. Most of the hard capsules are made of gelatin. Gelatin capsules are vital capsules in the capsule industry [5–7].

Gelatin is one of the most popular gelling agents and a natural polypeptide polymer that can be used for hydrogel formulations based on gelatin and in drug delivery [8, 9]. Gelatin exhibits the thermoreversible sol–gel transition, which is the furthermost popularly thermoreversible gelling agent for gel formulations at the typical temperature of the human body and thus enables the rapid release of the drug [10, 11]. In

recent studies, some physical properties of gelatin, such as modifications within the structure of the gel during the gelation procedure, have been considered [12, 13].

Reviews show that the gelatin capsules themselves cannot control the drug release process at the pH level of stomach acid, and also, the gel networks produced by gelation do not show strong mechanical properties. Although the combination of some compounds such as alginate and pectin with gelatin creates stronger mechanical networks compared to gelatin, their gels do not demonstrate useful pH responsivity for drug-release profiles [14, 15].

Several studies have indicated that achieving controlled drug release within the gastrointestinal tract of the human body through the use of a single gelling agent, consisting mainly of biocompatible and biodegradable polymers, is not a straightforward process. Therefore, the application of additional polymers, in combination with gelatin, can ensure the control of drug release conforming to thermal and pH responsiveness [16, 17]. Nevertheless, limited studies have been done about what happens when gelatin and other polymers react to alterations in pH or temperature [18].

In the technology of enteric hard capsules, one way is to add an additional coating step to the capsules with acid-sensitive polymers through a delayed-release mechanism. These methods do not show a pH-generated release but are a replacement for relying on a time delay in the expectation of timely emptying from the stomach. For the preparation of coat enteric capsules, there are two processes. Firstly, the drug particles or pellets are coated with enteric-coated materials, and in a second way, the enteric coating solution is sprayed on the outer layer of the capsule and then filled with drugs. These methods have some problems such as coated drug capsules must be made by uniformly spraying the gastric solution through the coating process on the out of drug particles or capsules, the preparation process is complex, and there are a lot of organic solvents in the enteric coating solution which has great safety risks to operators and manufactures workspaces with possible effects on the manufacturing prices [19–21].

Consequently, altering of initial composition is one of the most recent techniques implemented in the development of enteric empty capsules. The present approach entails the inclusion of a polymer that exhibits resistance to stomach acid through changes in the formulation of the capsule. As a consequence, the enteric characteristics are developed in the capsules through the modification of additive ratios [19].

Eudragit (polymethacrylates) and HPMCPh (hydroxypropyl methylcellulose phthalate) are synthetic polymers that display pH sensitivity and act as enteric polymers owing to the presence of phthalyl and acrylic groups covalently linked to the hydrophobic polymer chains. The pH-dependent solubility property makes these polymers suitable for drug delivery applications in the context of targeted intestinal release [22].

The pH-sensitive polymers are polymers that contain functional groups that changes in the pH of the environment leading to acceptance or donation of these functional groups and changes in the polymer's structure, solubility, or other properties. The polymers containing carboxylic groups display pH-dependent

solubility whereby the carboxylic groups become ionized under high pH (may deprotonate) thus their conformation has been changing and expanding due to the repulsion between the negative charges of the carboxylates, so facilitate polymer dissolution. while at low pH, the carboxylic groups are not ionized (may protonate) and their conformations are close causing insolubility and leading to shrinkage. Combinations of Eudragit with HPMC or talc stabilized loaded drugs provided a controlled release of them [23–25].

The present study aimed to develop a formulation for hard gelatin gastro-resistant capsules utilizing a widely used polymer in the pharmaceutical industry, specifically for acid-resistant pharmaceutical capsules. Uncoated enteric capsules based on Eudragit (an acrylic polymer) and gelatin have not been reported in the literature so far. Accordingly, the objective of this study is to improve the formulation of hard gastro-resistant capsules using one of the most common polymers used in the pharmaceutical industry for the product of acid-resistant pharmaceutical capsules. The effect of polymer and solvent was studied on capsule shells based on the capsule formation and enteric properties.

2. Materials and Methods

2.1. Raw materials and chemicals

All raw materials used in this work were utilized in an as-received form without any additional process and purification. Gelatin type B was acquired from Rousselot (France), (HPMCPh) (hydroxypropyl methylcellulose phthalate) and Eudragit L100-55 (poly (methacrylic acid-co-ethyl acrylate) (1:1)) were provided in-kind as samples from LOTTE Fine Chemical (South Korea) and Rahavard Tamin pharmaceutical co., respectively. Polyethylene Glycol 400 (PEG-400; $H(OCH_2CH_2)_nOH$) was used as a plasticizer, Trisodium phosphate ($Na_3PO_4.12H_2O$), tryptic soy broth (TSB) and Hydrochloric acid 35% (HCl), were acquired from Merck. Aqueous solution of ammonia (NH_3, 1N) and sodium hydroxide (NaOH, 0.2N) were used as the solvents for HPMCPh and Eudragit. Sodium lauryl sulfate (SLS; $NaC_{12}H_{25}SO_4$) was purchased from Godrej Industry (India). Colloidal Nano silicon dioxide (SiO_2) was obtained from Evonik (Germany), Propylene glycol (PG) and Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) were purchased from Kimyagaran Emrooz Chemical Industries Co.(Iran) and Behansar (Iran), respectively. Further additives, including Propylparaben ($C_{10}H_{12}O_3$) and Methylparaben ($C_8H_8O_3$) were provided by UENO Fine Chemical Industry (Japan). Pantoprazole sodium sesquihydrate (PSS; $C_{16}H_{17}F_2N_3NaO_5S$) was purchased from Sigma-Aldrich. The material for determination of microbial levels was tryptic soy agar (TSA), sabouraud dextrose agar (SDA), eosin methylene blue agar (EMB), rappaport-vassiliadis soya peptone (RVS), cystine tryptic agar (CTA), macConkey broth (MACB) and mannitol salt agar (MSA) that acquired from Merck.

2.2. Preparation of enteric solution

This study has explored the potential of ammonia and sodium hydroxide solutions as suitable solvents for Eudragit and HPMCPh since these polymers exhibit insolubility in deionized water (DI-water) and can

be dissolved in organic solvents. For the preparation of the eight formulations in Table 1 on a laboratory scale, Eudragit, HPMCPh, or a combination of them were carefully weighed and then dissolved in 160 mL ammonia (IN) or 320 mL NaOH (0.2N).

10 g Na_3PO_4 was added to the solution to help dissolve better the solution. While the solution was vigorously stirred, gelatin was added. Then, 2 g PEG–400 was mixed until the solution attained a state of homogeneity. PEG–400 was added to the mixture as a plasticizer to reduce the stiffness and make the polymer more flexible. Furthermore, a combination of 0.14 g SLS, 0.1 g colloidal SiO_2 and 0.2 gr $ZnSO_4$ were amalgamated and added to the mixture. Then, 0.45 gr methylparaben and 0.1 gr propylparaben mixed with 1 gr PG were added to the prepared mixture and stirred for 30 minutes. Subsequently, the final solution was transferred to a bain-marie at 55°C for 5 hours to expel any gas bubbles that may have formed. After which, the viscosity of the solution was assessed. In the instance of the ammonia-containing solution, the solutions were subjected to the external environment for a duration of 12 h to remove the excess ammonia [19]. Scheme 1 shows the preparation methods of all samples.

Table 1 Improved formulations for fabricated capsules.

Formulation / Substance	Formule1 (F1)	Formule2 (F2)	Formule3 (F3)	Formule4 (F4)	Formule5 (F5)	Formule6 (F6)	Formule7 (F7)	Formule8 (F8)
HPMCPh	-	60	60	-	60	-	-	60
Eudragit	50	-	50	50	50	50	50	-
Gelatin	-	-	30	30	30	-	30	-
NH_3(1N)	-	-	-	-	160	160	160	160
NaOH(0.2N)	320	320	320	320	-	-	-	-
Methylparaben	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Propylparaben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PG	1	1	1	1	1	1	1	1
$ZnSO_4$	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
PEG-400	2	2	2	2	2	2	2	2
Na_3PO_4	10	10	10	10	10	10	10	10
SLS	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
SiO_2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Viscosity (cp)	900	1092	1410	1200	870	574	745	630
Capsulation form	defect	defect	defect	✓	Pouring	Pouring	Pouring	Pouring
Enteric property	✓	✓	✓	✓	-	-	-	-

✓: The test was successful, and the capsule has enteric properties and shape as capsule.

-: The enteric properties of capsule were not studied

Defect: The capsule formed on the pin had problem in many cases

Pouring: The capsule did not form on the pins

Eight formulae were chosen for additional research after different amounts of gelatin, Eudragit, and HPMCPh were tested to determine the material ratio used in the enteric capsule formulation. According to the characteristics of sodium hydroxide and reducing its health risk for human consumption, it was chosen as a solvent for its better industrial scale and pleasant smell compared to ammonia. Also, the studies showed that the solution made with ammonia as a solvent (F5, F6, F7, and F8) had a relatively low viscosity, which caused the capsule did not have a proper form on the pins. Low viscosity also leads to pouring on the pins during the capsule manufacturing process. Table 2 lists the dissolution results of the final formulations, and this result was performed in the laboratory.

Table 2
Final formulation tests

Formulation	Temperature ℃	Defect	stomach environment test	intestine environment test	Release
F1	37	✓	Intact	Opening begin	Release begins almost at the 120th minute and continues until after 10 min
F2	37	✓	Intact	Opening begin	Release begins almost at the 120th minute and continues until after 10 min
F3	37	✓	Intact	Opening begin	Release begins almost at the 120th minute and continues until after 10 min
F4	37	×	Intact	Opening begin	Release begins almost at the 120th minute and continues until after 8 min

Multiple polymeric formulations were evaluated to attain the most favorable gastro-resistant outcomes for every enteric polymer. Various polymeric formulations were evaluated in order to attain the most favorable gastro-resistance from each enteric polymer. Decreasing the polymer concentration leads to the production of capsules with less thickness. This subsequently renders them more susceptible to breakage, whether during the stripping or subsequent handling procedures.

2.3. fabrication of capsule

The prepared solution was transferred into a steel dish at 45–48°C and 38–40% humidity conditions. A dish at the capsule manufacturing machine refers to a vessel that holds the melting solution during the manufacturing capsule process. It is typically a flat surface where the dipping step is done inside. To simulate the method in laboratory conditions, a container made of steel with a dimension of 10×5×7 cm^3, and the dipping of the capsules is done manually inside this container (Fig. 1a). The dip-coating method is used for capsule fabrication. In this method, after immersing the pin bars in the enteric glue solution, a thin coating was applied to the pin bars. It was shaped by turning it up and down. Figures 1b–e depicts the pin bars of various formulations. The capsules were stripped off the pins after complete drying at

room temperature under blowing air. After cutting, the cap and body were joined to the pre-locked orientation, and the empty enteric capsules were obtained. Figure 1 shows the prepared enteric hard capsules (F1, F2, F3, and F4) on a laboratory scale.

2.4. Physical instruments

The quality control process for capsules was defined through the implementation of a series of physical evaluations including diameter and size measurements, humidity, and disintegration tests. In the present investigation, FTIR spectra were recorded between scan range 400 and 4000 cm^{-1} in KBr pellets on Shimadzu Varian 4300 FTIR spectrophotometer. Surface and cross-section field emission scanning electron microscopy (FESEM) was achieved using a ZEISS GeminiSEM 560 device. The FESEM cross-sections of samples were prepared by immersion them in liquid nitrogen and cutting samples. Then, in order to higher contrast and prevent the accumulation of electrons on the surface, the samples were coated with a thin gold film. Tensile strength (TS) and elongation at break were measured using the GOTECH universal testing machine and ASTM standard method D882 was used for the determination of strength properties. The films were tested by placing samples (130mm ×20mm) between two tensile grips at a distance of 100mm. The tests were performed at a speed of 5mm/min, at 20 °C, using a 200N load cell.

2.5. Investigation of gelling ability using rheology

The rheological properties of the F1 and F4 samples were investigated via a rheometer (Physica MCR 300, Anton Paar Ltd., Austria) utilizing a circular disk parallel plate with a diameter of 25 mm and a gap of 0.5 mm. An amplitude sweep was conducted at a consistent angular frequency of 1 Hz to define the limit of linear viscoelasticity. The strain amplitude was kept at 0.4% during the test. The contributions of the liquid-like form (viscous modulus (G'')) and solid-like form (elastic modulus (G')) were noted through temperature sweeps from 90 to 10°C at a speed of -2°C min^{-1} to assess the thermogelling attributes (angular frequency = 1 Hz). The oscillatory rheological determination as a function of time was conducted at a consistent frequency of 1 Hz to evaluate the time of gelation. The gel point or gelation time was specified as the time that the loss modulus and shear storage modulus were identical [26].

2.6. Drug release from gastro-resistant uncoated capsules filled with pantoprazole

The investigation of drug release from capsules filled with 10 mg of Pantoprazole was performed using the USP-711 dissolution apparatus. Each formulation was tested three times. The profile release of Pantoprazole was carried out using the paddle approach.

The capsules were first tested in 0.1M HCl (900 mL, pH 1.2) for 120 min at 37 ± 0.5°C as a simulated gastric media, while the rotational speed of the paddle was maintained at a constant rate of 100 rpm. After that, the tests were performed in phosphate buffer (pH = 6.8) as a simulated intestinal medium for 10 min at 37.0 ± 0.5 ℃. The dissolution testing included two media that were prepared according to the

USP. The release of Pantoprazole from F1, F2, F3, and F4 capsules was quantified over specific intervals in both the stomach-like and intestinal-like media. At regular intervals, 10 mL samples were taken from the media. Following sampling, fresh medium was placed in the reservoir. A 0.45 µm nylon filter is used to filter. Then, the drug release was studied using a UV-visible spectrophotometer (Shimadzu UV-1800) at the wavelength of maximum absorption (λ_{max}) of 289 nm [27, 28].

2.6.1. Pantoprazole Calibration

To prepare the stock solutions of pantoprazole, a quantity of 10 mg of pantoprazole was dissolved in a 100 mL solution composed of 0.1 N hydrochloric acid with a pH of 1.2, and the next solution with the same concentration was made in phosphate buffer with a pH of 6.8. Dilute solutions with specific concentrations (5–30 µg/mL) were made with the same solvent from the stock solutions [29]. After preparing the stock solution, each calibration curve was drawn separately.

2.7. Determination of Microbial Levels

The investigation of microbial levels was performed using the USP-61, 10 gr of capsules were dissolved in a 90 mL phosphate buffer solution. The next solution was made by solving 10 gr of capsules in 90 mL tryptic soy broth. 1 mL of each solution was taken and spread into a special medium. The medium that contained the capsule solution was incubated at 35–37°C for 72–120 h depending on the type of microorganism. After the incubation stage, the number of living colonies was calculated.

3. Results and Discussion

Eight different formulations of uncoated hard gastro-resistant capsules were investigated in this study. The formula with ammonia as a solvent had a relatively low viscosity and the capsules' form was not good on the pins. Dimensional and dissolution tests were performed on F1, F2, F3, and F4. The experimental findings demonstrate that F1, F2, F3, and F4 formulations exhibit superior properties in terms of enteric development and acid resistance, as presented in Table 2. Observations show that the gelatin was homogeneously mixed with either HPMCPh or Eudragit. After preparing the F3 solution containing gelatin, HPMCPh, and Eudragit and placing it in a Bain-Marie for degassing, it was observed that the solution was not uniform and had become two phases. Therefore, it was necessary to stir the solution before dipping. As a result, the F3 formulation found it challenging to produce capsules with smooth walls. On an industrial scale, phase separation can pose issues in the feed tanks for manufacturing hard capsules. The prepared capsules from F1, F2, and F3 formulations showed defects (Fig. 1(b-d)) and did not have suitable walls and domes in many cases. Among these capsules, which were of poor quality, some capsules seemed to have suitable appearance, Therefore, this number of capsules was selected from the others and their drug release was studied.

3.1. Physical parameters

According to Table 3, four different formulations were subjected to experimentation with the aim of achieving the suitable wall, dome, smoothness, shoulder, and gastro-resistance. In the absence of gelatin (as a gelling agent), the prepared capsules are very breakable and can be damaged when stripping off the pin. The capsules were prepared in "1" size. The wall, domes, shoulders, and dimensions were measured using quality control gauges of the capsules. The physical parameters of the capsules are within the standard ranges [30–32]. The results show that all formulations are suitable for the production of capsules on a laboratory scale [30].

Table 3
dimensions of produced capsules under stable conditions which were measured using quality control gauges of the capsule.

	F1		*F2*		*F3*		*F4*	
Items	*Cap*	*body*	*Cap*	*body*	*Cap*	*body*	*Cap*	*body*
Size (mm)	9.81 ± 0.02	16.62 ± 0.02	9.81 ± 0.01	16.63 ± 0.01	9.81 ± 0.01	16.62 ± 0.02	9.80 ± 0.02	16.61 ± 0.02
Wall (mm)	0.086 ± 0.004	0.088 ± 0.004	0.086 ± 0.003	0.087 ± 0.004	0.088 ± 0.003	0.089 ± 0.003	0.087 ± 0.004	0.088 ± 0.004
Dome (mm)	0.13 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Shoulder (mm)	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
Capsule (mm)	19.8 ± 0.1		19.8 ± 0.1		19.8 ± 0.1		19.8 ± 0.1	

3.2. Tensile studies of produced capsules

The tensile strength (TS) of the enteric uncoated capsule film used in the production of the enteric capsule (F4) was measured using the universal testing machine. Besides, a gelatin solution was prepared to compare the tensile strength of the enteric uncoated film with it. To prepare films of these polymers that have the same formulation as capsules, the solution of each polymer was cast on a smooth surface with the same thickness as capsules and then allowed the films to dry in the same conditions as the capsules were dried (38–40% relative humidity and 23 ± 2°C temperature). The film specimens of the polymer were produced according to ASTM standard method D882 with some modifications and can be seen in Figs. 2a and 2c [18, 33].

The result demonstrates that the strength of the F4 is similar to the documented strengths of gelatin that are utilized in the formulation of capsules [18, 34]. The measured strength of the F4 formulation illustrated suitable resilience elastic deformation compared to other enteric capsules, while it was lower than gelatin capsules (the tensile strength of gelatin was ~ 80MPa) [18]. The tensile strength was acceptable for F4 formulation film (~ 43MPa), which can result in the mechanical stability of the capsule throughout its distinct stages of packaging, storage, or transportation[18, 35, 36]. Moreover, the F4

capsules successfully passed the acid challenge test and the following drug release in buffer met the acceptable criterion. These results suggested that the compatibility of gelatin with the F4 formula resulted in the improved brittleness of the films compared to other capsule formulations that have been reported (Figs. 2b and 2d). The Young's moduli measured from the F4 and gelatin film were 11.20 MPa and 23.14 MPa, respectively.

3.3. Fourier transforms infrared spectroscopy (FTIR)

Fourier transforms infrared spectroscopy (FTIR) spectrums of F1, and its combination with gelatin at enteric capsule (F4) was analyzed by Thermo Scientific Nicolet iS 10 spectrometer and shown in Fig. 3. F1 showed the characteristic C-Hx vibrations at 1389cm^{-1}, 1430cm^{-1}, 1482, and 2925cm^{-1}. The vibration bending mode of O—H is detected at 1262 cm^{-1}. The FTIR spectrum of F1 showed characteristic bands of the ester groups at 1155 and 1733 cm^{-1}. The FTIR spectrum of the F1 exhibits similarities with Eudragit, which suggests that the presence or absence of PEG-400 did not cause a change in the spectrum [37, 38].

The FTIR spectrum of F4 showed the characteristic band of N—H stretching vibration modes belonging to gelatin at 3446 cm^{-1}. Due to the wideness of the peak in this area, the characteristic bond of O-H stretching vibration modes belonging to gelatin and primary alcohol at PEG-400 can overlap with N-H stretching vibration modes. The bending vibration of the N—H bond is appropriate to gelatin and is detected at wavenumber 1269 cm^{-1}. The stretching and bending modes of C = O and C-H are observed at 1711, 1106, 2922, and 1400 cm^{-1} respectively. In addition, C-Hx vibrations can be discerned at 1400, 1451, and 2922cm^{-1}[39]. The v(C = O) in IR spectra of the F4 is red-shifted by 22 cm^{-1} with a characteristic shoulder band at lower energy than F1. These can be attributed to the interaction between gelatin and Eudragit.

3.4. Humidity of uncoated enteric capsules

Loss on drying (LOD) is a thermogravimetric method that is commonly used to determine the moisture content of uncoated enteric capsules. In this method, to measure the percentage of moisture within the capsules, a hygrometer is utilized at a specific temperature. The vacant gelatin capsules have a moisture content of 13–16%. If the humidity is low, the capsule becomes brittle, and if the humidity is high, the active pharmaceutical ingredient (API) will stick to the capsule wall [40].

Figure 4 illustrates that HPMCPh present in the F2 and F3 formulations causes the primary humidity of the capsules to be higher than other products at the moment of production, and then over time, the humidity decreases with a mild slope. After 48 h, the LOD rate within the delayed-release capsules remained almost constant with no observable variations. It can be concluded that the moisture content of the prepared capsules is almost similar to gelatin capsules. The environmental conditions and measured values were the same in all four types of samples (F1, F2, F3, and F4). It should be noted that F1 capsules demonstrate lower relative humidity than other products among prepared capsules (F1, F2, F3, and F4),

while the F2 capsules have the highest relative humidity. The F4 formulation as the selected formulation is acceptable and appropriate in terms of moisture content.

3.5. Surface and cross-section morphology analysis using field emission scanning electron microscopy (FESEM)

The study of surface and cross-sectional features of F1, F2, F3, and F4 samples were analyzed to explore the morphology and porosity of the prepared capsules (Fig. 5). The FESEM images of the F1 capsule reveal a relatively rough surface and cross-section (Figs. 5a and 5b). The use of only Eudragit caused the unevenness of the surface and cross-section. Figures 5c and 5d show the FESEM images of F2 in terms of cross-section and surface. As it can be seen, the presence of HPMCPh results in a relatively rough cross-section and smooth surface. The addition of gelatin to the formulation leads to a smooth surface and cross-section (Fig. 5(c-f)). In other words, the roughness in the surface and cross-section has been eliminated by adding gelatin to the F4 (Figs. 4g and 4h).

3.6. Rheological behaviors

In order to examine the effect of gelatin on the rheological properties of F4 and evaluate the synergistic interaction between gelatin and Eudragit, the solutions of F1 and F4 were prepared. Figures 6a and 6b show the temperature dependence of the storage modulus (G'), which characterizes the elasticity of the capsule, and the loss modulus (G"), which represents the capsules' F1 and F4 viscosity. Gelatin molecules have random coli conformation at high temperatures [41]. During the process of cooling the samples below the temperature at which gelling occurs, the gelatin gradually forms a tri-helical conformation. This process leads to the construction of a weak network [42]. Therefore, the gelatin compounds can be considered as a soft solid, such as a gel [43].

The enteric solution achieves similar fluidity, and the value of G' is relatively low. As the temperature decreases, the gelation occurs, causing the value of the storage modulus (G') to suddenly rise. The temperature was reduced from 90 to 10°C through a cooling process with a rate of − 2°C/min. As shown in Fig. 6b, the point at which the liquid-like hydrogel turns into a solid-like scaffold is considered as the gelation temperature and is described as the temperature at which G" equates to G' and examined for F1 and F4 [44].
The elasticity behavior of the uncoated enteric capsule capsules as a function of temperature was followed by G" and G' (Fig. 6). The gelation temperature shows the thermal sensitivity of the scaffolds. At temperatures above the gelation point, the G' is less than G". However, at the gelation temperature or even near this temperature, G' exhibits a rapid increase. Figure 6a exhibits that the gelation temperature for F4 is 40.1°C, while no gelation was observed for F1.

The gelation time is the time that liquid forms to transform into the gel. In the time sweep rheology analysis results, setting time that prevents capsules from defecting is considered when the storage modulus (G') of the scaffolds is equal to the loss modulus (G") at a specified temperature. As illustrated

in Fig. 6c, G' is always less than G" and they did not cross over at any time, in other words, gelation does not occur. In F4, G' exhibits lower values than G" at the start of gelation, which represents effective viscous properties (Fig. 6d). After a while, the G' improves quicker than the G", which indicates that the solution phase has changed into a soft-solid gel with the best elastic behavior [45, 44]. The setting time was obtained at 21 s for the modulus diagram against time at 40.1°C (temperature gelation). A long gelation time is considered as a defect in the capsule and results in a capsule with an unsuitable wall, dome, and shoulder which falls outside of the predetermined range.

3.7. Study of drug release

The dissolution test was performed according to USP specifications for F1, F2, F3, and F4 products. The absorption of the drug and the availability of its physiologic effects depend on whether the drug substance is in a state of absorption at the site of absorption. The rate and extent of drug dissolution in simulated test conditions over a specific time are tested by dissolution testing. This test provides a tool for quality control to ensure that different batches of pharmaceutical products have similar drug characteristics. Figures 7a and 7b indicate the calibration diagrams of pantoprazole in 0.1 N HCl (pH = 1.2) and phosphate buffer (pH = 6.8) media, respectively. As observed in these diagrams the calibration curve for pantoprazole had excellent linearity in the specified concentration range. The correlation coefficients (R^2 values) for HCl (0.1 N, pH = 1.2) and phosphate buffer (pH = 6.8) are 0.9849 and 0.9892, respectively. The temperature setting of the dissolution apparatus was established at 37 ± 0.5°C with a rotation speed of 100 rpm. Subsequently to the performed stability test, the uncoated enteric capsules were introduced into the dissolution apparatus [27, 28]. 8 mL of samples (F1, F2, F3, and F4) were manually withdrawn from HCl solution every 10 min (10 to 120 min), then the collected media were substituted with phosphate buffer at pH = 6.8. The experiment persisted for an additional duration of 20 minutes. Due to the present results, all capsules exhibit stability in the acidic environment of the stomach (pH = 1.2) for 120 min. Upon transferring the capsules to the phosphate buffer medium as a small intestinal media simulator (pH greater than 6), pantoprazole was progressively released. The complete release for F4 and other capsules (F1, F2, and F3) occurred within 10 and 14 minutes, respectively (Fig. 7c).

3.8. Microbial Limit Test

Hard-shell capsules as a drug-delivery system, are very important that it does not contain harmful microorganisms. The investigation of microbiological content in the gastro-resistance uncoated capsule F4 was performed using the USP-61 microbiological examination of nonsterile products. The microorganisms that were investigated using this method are Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella species, and Escherichia coli. To perform the microbial growth test, the F4 capsules were placed into a cultivation environment and incubated for 48–72 h. After incubation, no microbial growth was seen. For the fungal growth test, the special medium was incubated for 72–120 h. Results recognized that F4 does not exhibit any fungi activity. These results indicate that a combination

of methylparaben and propylparaben as preservative was effective in inhibiting microbial growth in the F4 capsules. The microbial content of F4 capsules is presented in Table 4.

Table 4
Microbial measurement of F4 capsules

Microbial Attribute	Specification	Result
Total Aerobic Bacterial Count	*N.M.T 1000 CFU/gr*	*< 40*
Total Molds & Yeasts Count	*N.M.T 100 CFU/gr*	*Negative*
Escherichia coli	*Should be Absent in 1 gr*	*Negative*
Salmonella species	*Should be Absent in 10 gr*	*Negative*
Pseudomonas aeruginosa	*Should be Absent in 10 gr*	*Negative*
Staphylococcus aureus	*Should be Absent in 10 gr*	*Negative*

4. Conclusions

We have successfully designed and developed new uncoated gastro-resistant capsule shells by improving and in some cases changing the chemical composition of hard empty gelatin capsules, employing compositing with Eudragit. The combination of gelatin and Eudragit (F4) resulted in a higher crossover strain (G' = G") compared to Eudragit alone (F1), accordingly suggesting a strong gel network that is suitable for capsule formation. The temperature at which gelation occurs and the time taken to reach it for the final uncoated capsule formulation (F4) were determined to be 40.1°C and 21 seconds, respectively. The present investigation on drug release demonstrated that F4 capsules are potential candidates for manufacturing delayed-release intestinal uncoated capsules in the simulated stomach environment. These capsules did not release pantoprazole as the drug model for 120 min and then was progressively released to the duodenum. Microbial studies show that F4 formulation fulfilled the requirements of medicinal capsules in terms of microbial content and it can be said that it is free of harmful microorganisms. The optimization of the formulation of hard capsules for the development of uncoated gastro-resistance hard capsules can lead to a reduction in the production time and also the total cost of these capsules. Therefore, the F4 formulations, which consist of a gelatin hydrogel blended with Eudragit can be an excellent candidate to delayed-release intestinal uncoated capsules.

Declarations

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Author Contributions

CRediT authorship contribution statement: **Ramin Ramezani Kalmer**: conceptualization, supervision, project administration, and visualization, **Afzal Karimi**: methodology. **Samira Gholizadeh Dogaheh**: writing-review and editing, validation, resources, and data curation. **Mojgan Ghanbari**: investigation, formal analysis, editing, and software. **Dariush Samandarian**: methodology. **Atefeh Sadjadinia**: investigation and formal analysis. **Hamed Ramezanalizadeh**: data curation, validation, and resources. **Seyedehmaryam Moosavi**: investigation and methodology. Notes The authors declare no competing financial interest.

Conflicts of interest or competing interests

The authors have no conflicts of interest to declare

Data and code availability

NotApplicable

Supplementary information

NotApplicable

Ethical approval

NotApplicable

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Scheme 1

Scheme 1 is available in the Supplementary Files section.

Figures

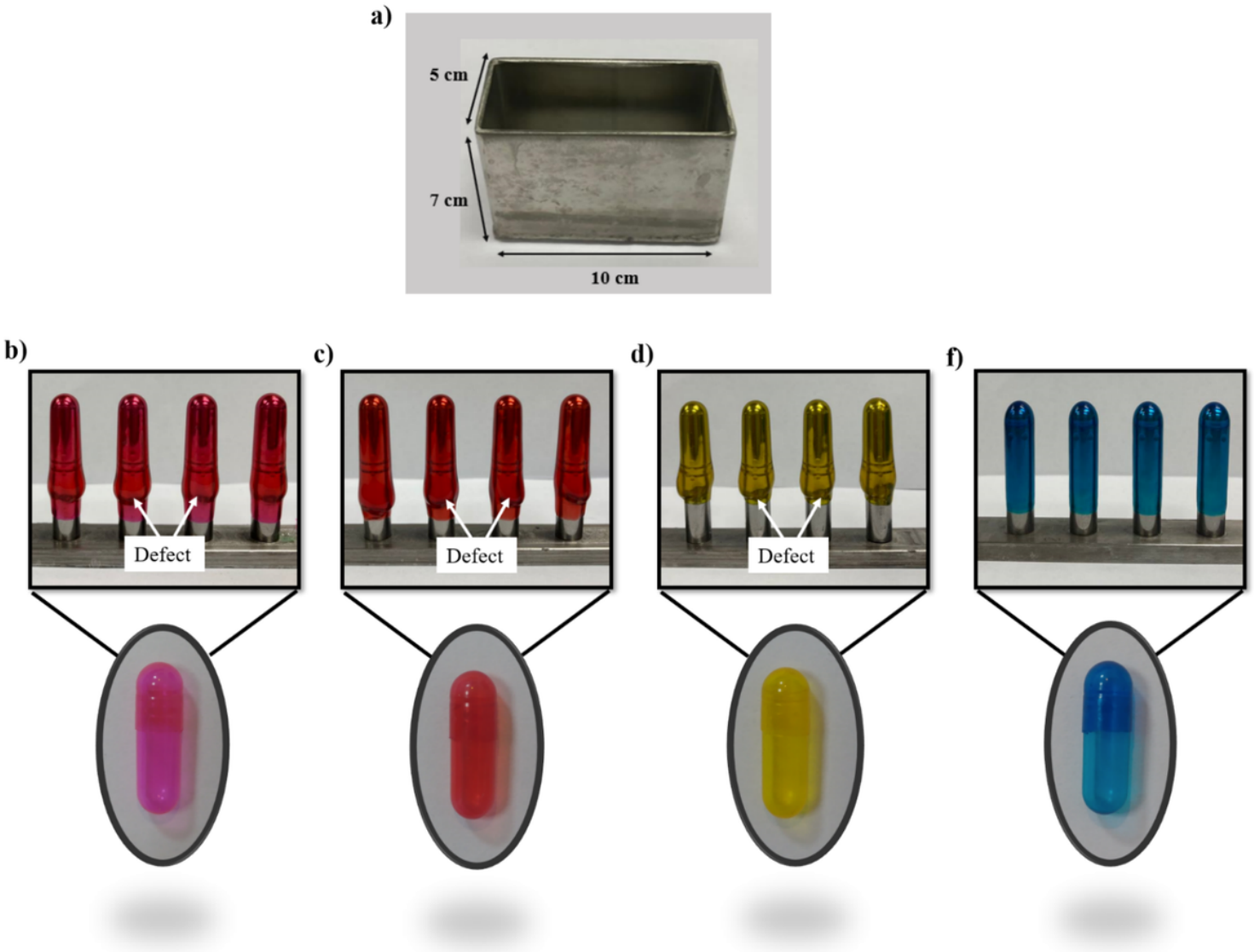

Figure 1

(a) Showing and dimension and shape of the steal dish. Photograph of the uncoated enteric layer on the pin bars for the different formulations and as-prepared enteric hard capsules produced on a laboratory scale (38-40% relative humidity and 23±2 °C temperature) of (b) F1, (c) F2, (d) F3, and (e) F4.

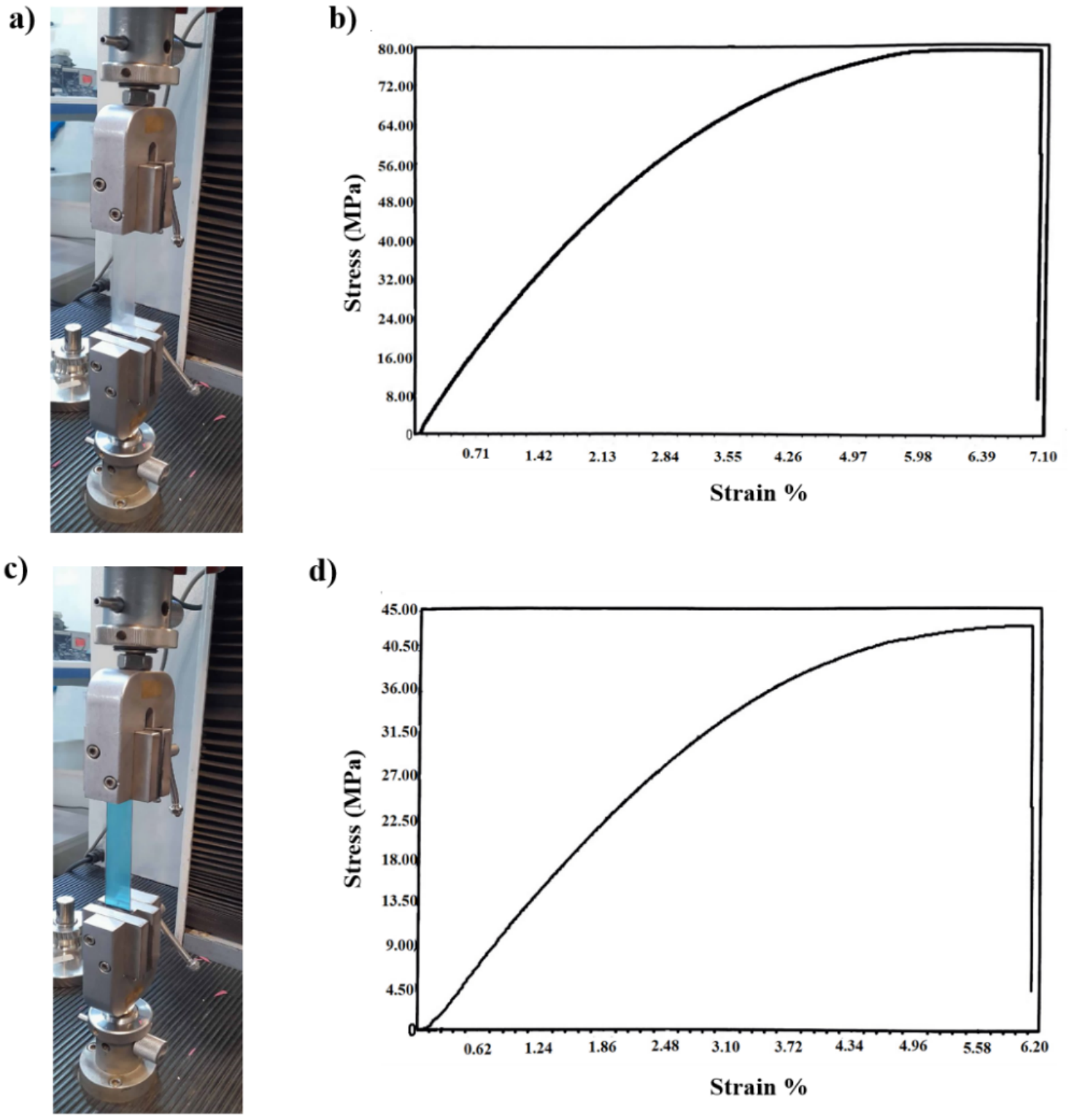

Figure 2

Test setup of tensile strength (a) gelatin film (c) F4 enteric capsule film. Stress vs strain% plots polymeric films of (b) gelatin and (d) F4.

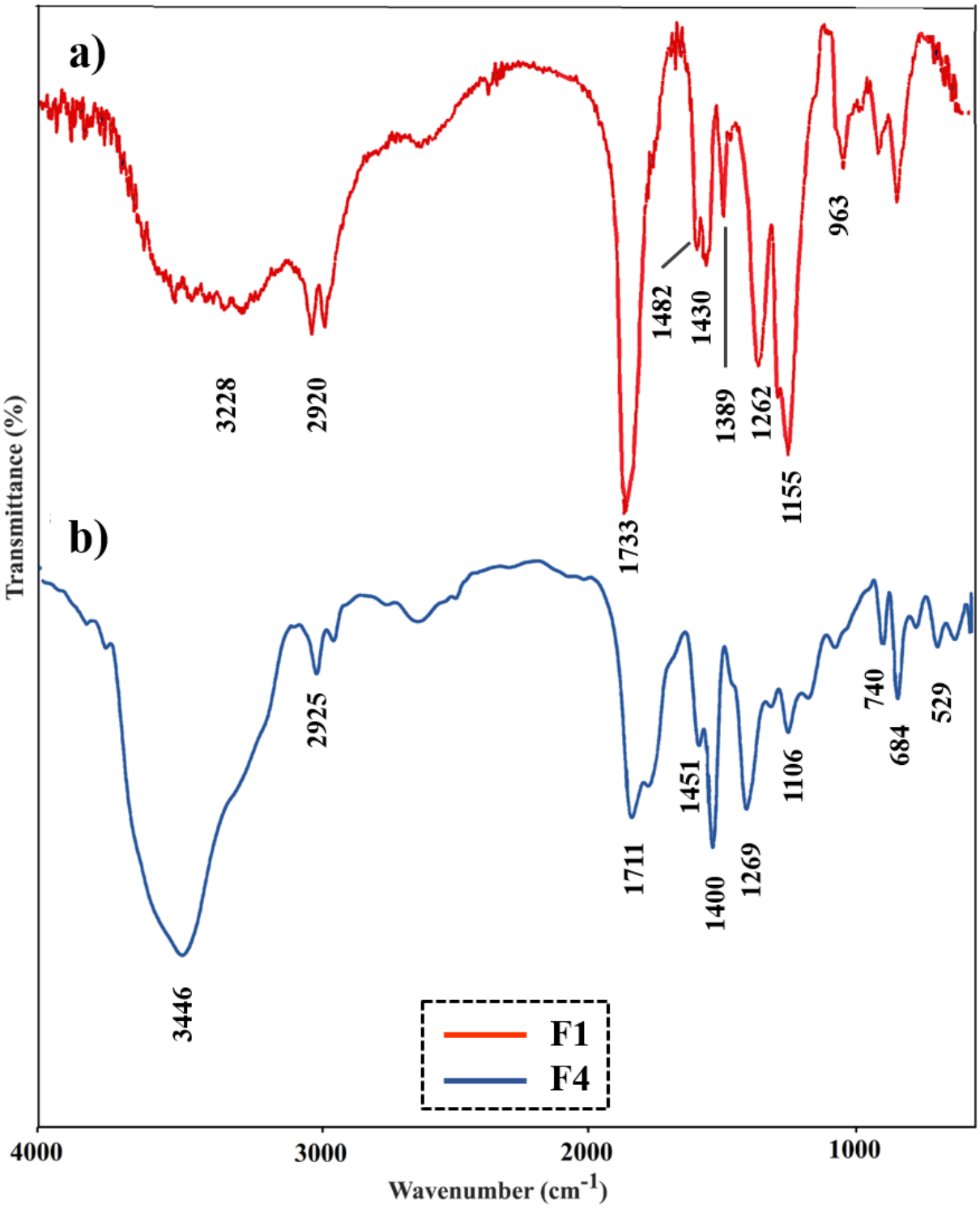

Figure 3

FTIR spectra of (a) F1(Eudragit) and (b) F4 (Eudragit / gelatin) enteric capsules.

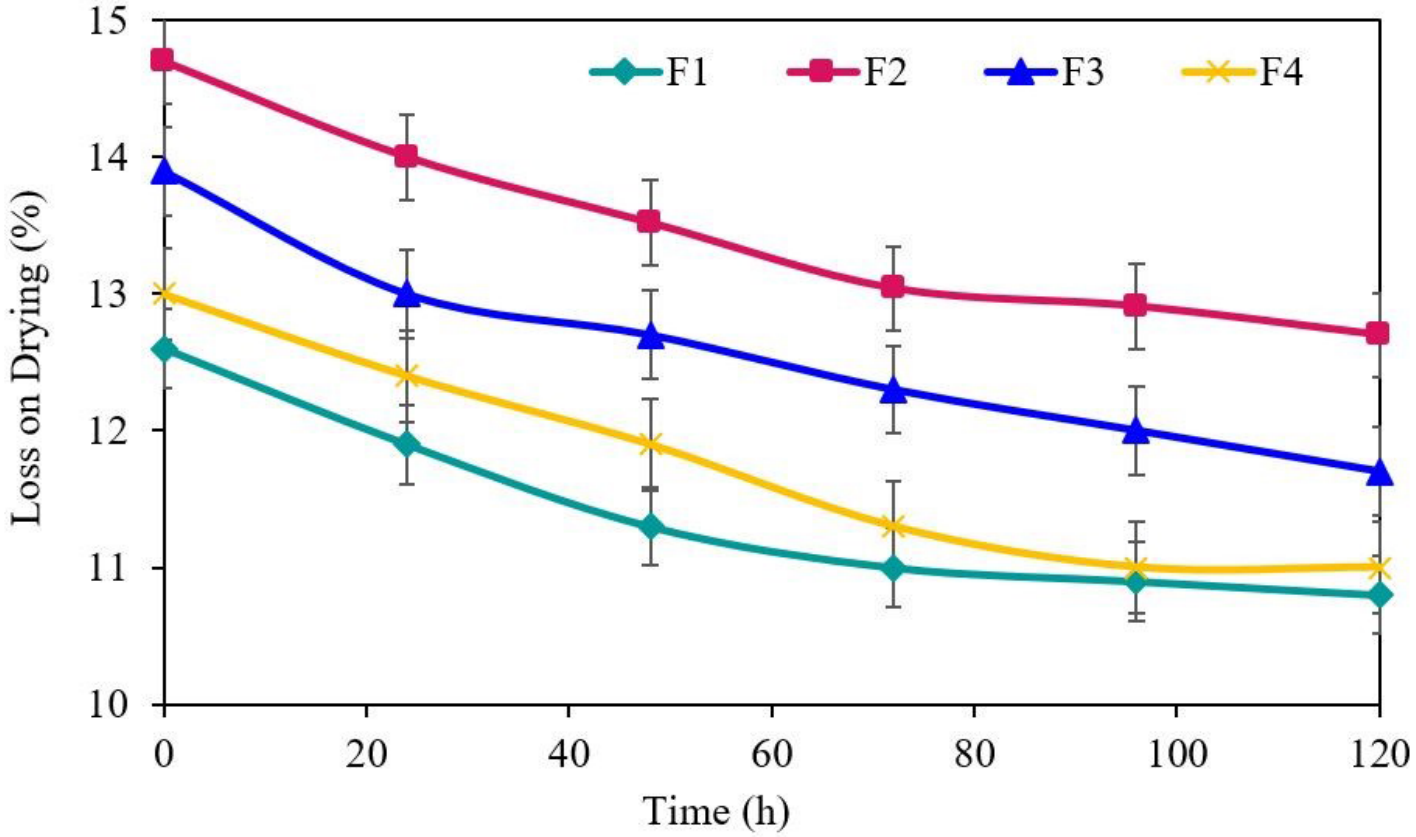

Figure 4

Loss on drying (%) of F1, F2, F3, and F4.

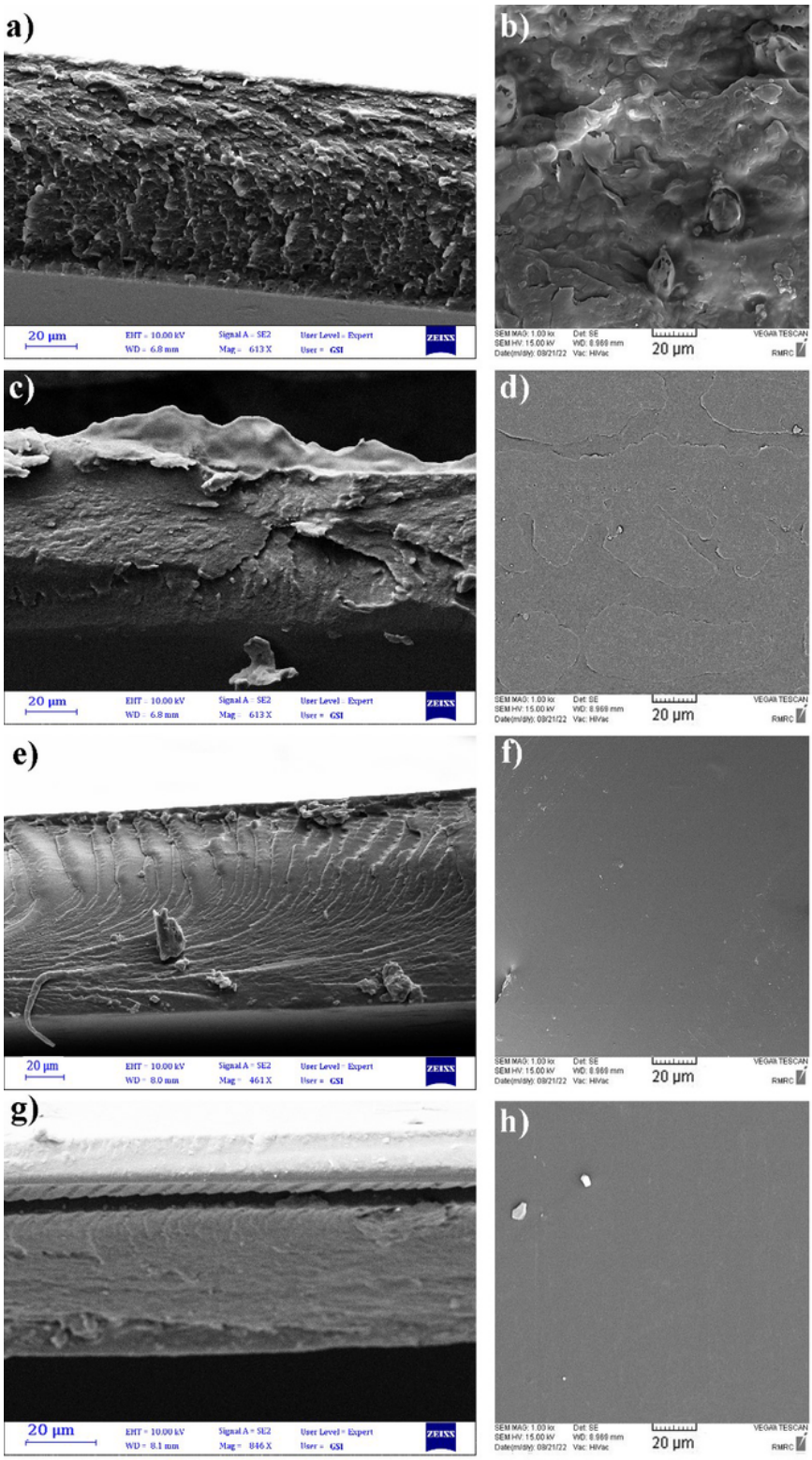

Figure 5

FESEM images of the cross-section and surface of (a, b) F1, (c, d) F2, (e, f) F3 and (g, h) F4 capsules respectively.

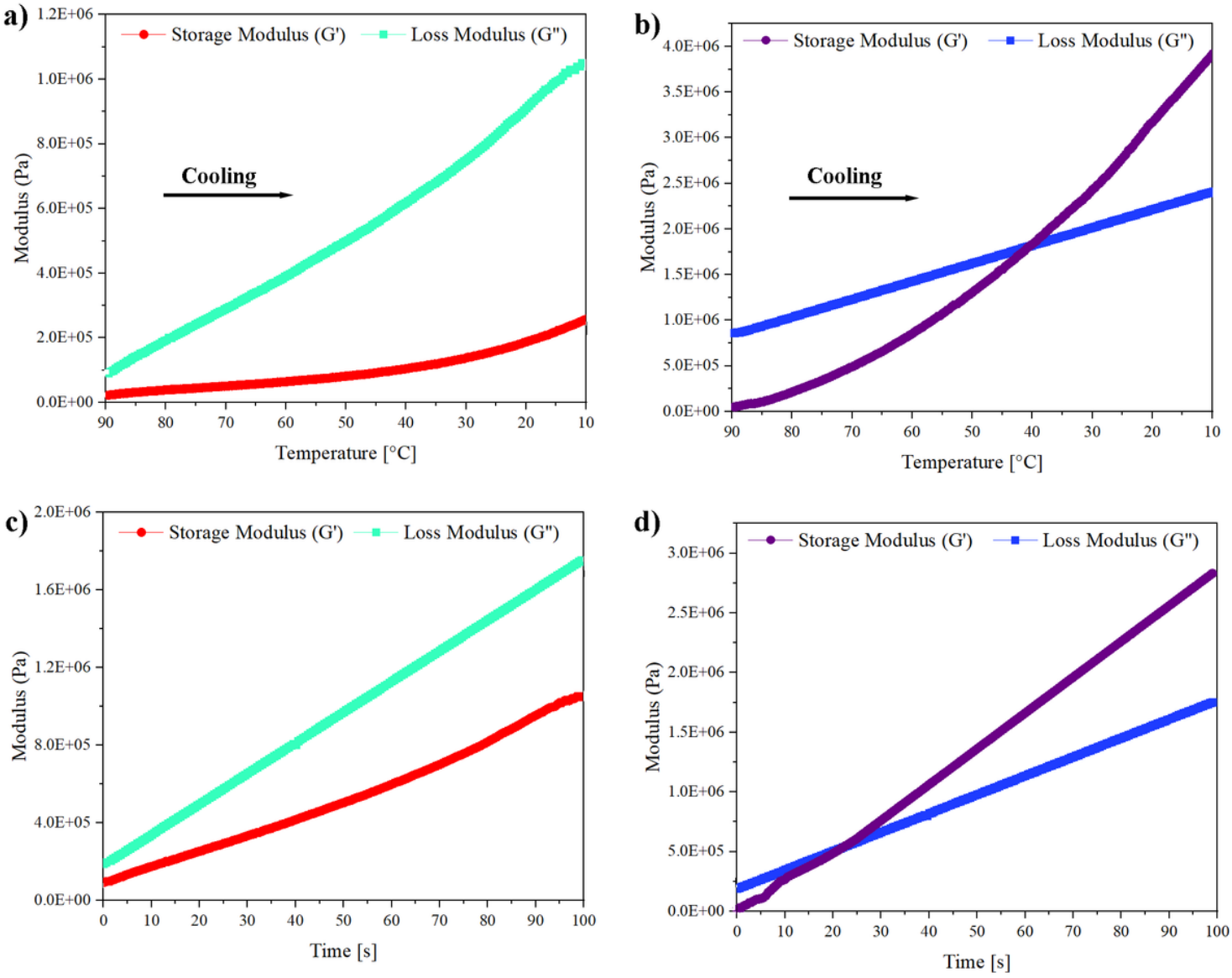

Figure 6

Temperature sweeps of (a) F1 and (b) F4 uncoated enteric capsules from 90 to 10 °C, time sweeps of (c) F1 and (d) F4.

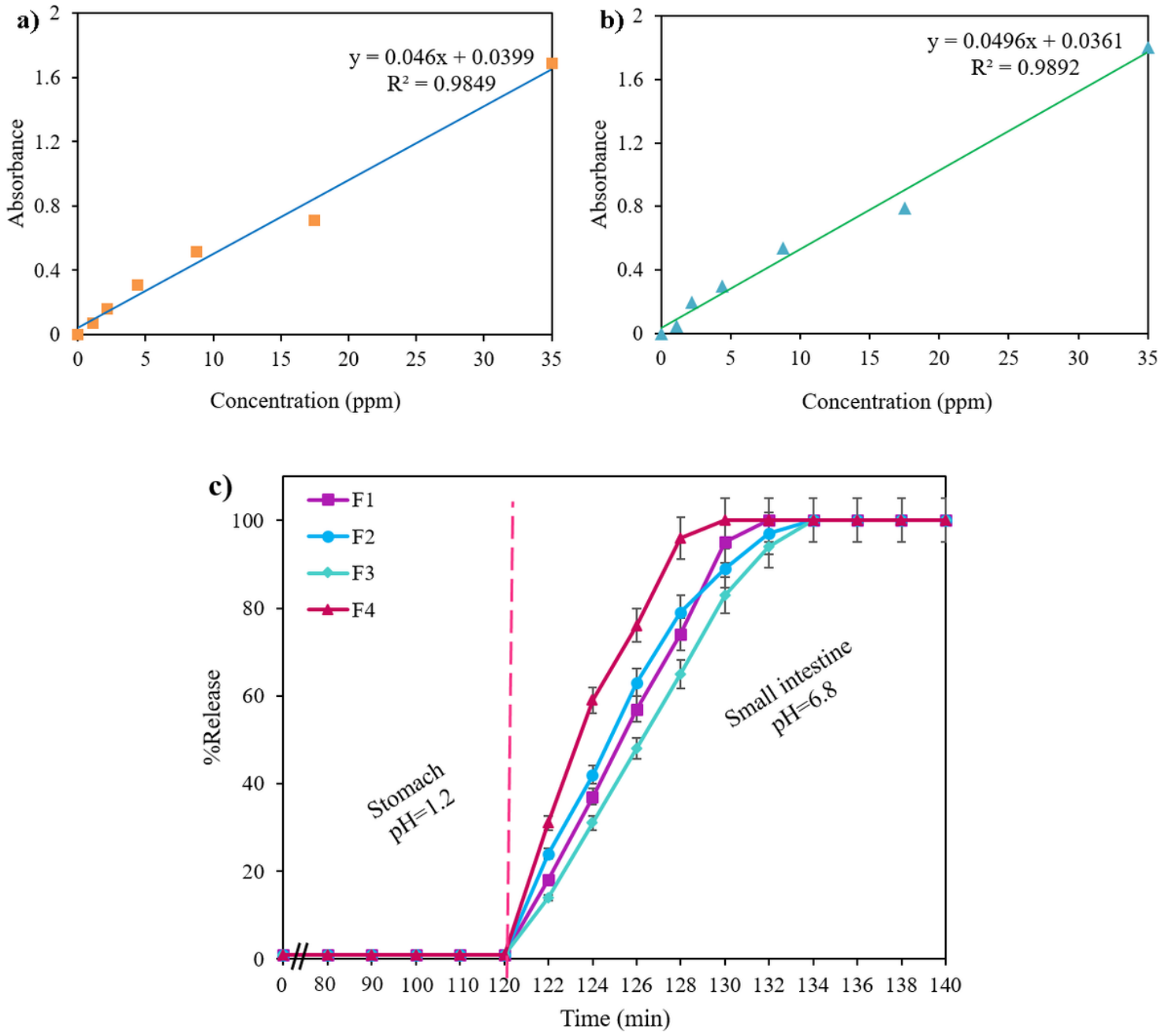

Figure 7

Pantoprazole calibration in (a) HCl 0.1 N (pH = 1.2), (b) phosphate buffer (pH = 6.8), and (c) pantoprazole release diagram.

Supplementary Files

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- Scheme1.png