



Development and physicochemical characterization of alginate composite film loaded with simvastatin as a potential wound dressing



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ABSTRACT

Previously, studies have demonstrated that topical application of simvastatin can promote wound healing in diabetic mice via augmentation of angiogenesis and lymphangiogenesis. This study aimed to formulate and characterize simvastatin in alginate-based composite film wound dressings. Biopolymers used for composite films were sodium alginate blended with pectin or gelatin. The films were prepared and characterized based on their physical properties, surface morphology, mechanical strength and rheology. Then, in vitro drug releases from the films were investigated and, finally, the cell viability assay was performed to assess the cytotoxicity profile. From the pre-formulation studies, alginate/pectin composite film showed to possess desirable wound dressing properties and superior mechanical properties. The in vitro drug release profile revealed that alginate/pectin film produced a controlled release drug profile, and cell viability assay showed that the film was non-toxic. In summary, alginate/pectin composite film is suitable to be formulated with simvastatin as a potential wound dressing.

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1. Introduction

Wound dressing has a predominant function in wound management. Chronic wounds, such as diabetic leg ulcers, are long lasting wounds that do not undergo the gradual process of healing and instead remain in the inflammatory phase of management. Medicated dressing by impregnating a drug of different pharmacological actions is an effective approach for achieving a quick and optimum healing response (Pawar, Tetteh & Boateng, 2013).

Simvastatin, an inhibitor of HMG-CoA reductase, is conventionally used for lipid lowering effects in cardiovascular diseases. Recently, its therapeutic effects beyond plasma cholesterol lowering in cell regeneration, such as the induction of bone tissue regeneration (Bae et al., 2011; Tanigo, Takaoka, & Tabata, 2010), anti-inflammatory activity on skin (Adami et al., 2012) and fracture healing (Fukui et al., 2012), have been demonstrated. Asai et al. (2012) successfully demonstrated that the topical application of simvastatin promotes wound healing in diabetic mice by augmenting angiogenesis and lymphangiogenesis. The statins appear to protect against ischemic injury and stimulate angiogenesis. This angiogenic effect is partially mediated

by direct regulation of the proliferation of endothelial cells and capillary morphogenesis via the PI3-kinase/Akt pathway (Kureishi et al., 2000). Simvastatin also promotes capillary morphogenesis in vitro and exerts an anti-apoptotic effect on lymphatic endothelial cells, which suggests the lymphangiogenic effects of simvastatin in wound healing (Asai et al., 2012). Currently, there is a lack of study in the literature with respect to developing a wound delivery system containing simvastatin for the chronic wound healing application.

In the wound care market, a plethora of wound dressings, ranging from conventional gauze to modern dressings, are available. Moisture-retentive dressings have been preferred in chronic wound management, owing to their ability to provide a moist environment, which is essential for effective chronic wound healing. Film dressings are known to be one of the most popular choices. Film dressings are simple, thin and semipermeable for effective water vapor and oxygen exchange. When in contact with wound exudates, the film transforms into a gel, creating a moist environment around the wound area. Moreover, the pliable feature of a film dressing is useful for application on flexible body areas such as joints (Moura, Dias, Carvalho, & de Sousa, 2013).

Natural biopolymers such as alginate, pectin and gelatin have been widely investigated as materials for wound dressings (Mogoşanu & Grumezescu, 2014). Among these biopolymers, alginate dressings are the most commonly used in different stages

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of wound healing due to alginate being a biocompatible and biodegradable polymer (Lee & Mooney, 2012) that possesses good film forming characteristics (Wang, Liu, Holmes, Kerry, & Kerry, 2007). Moreover, it can trigger macrophage activity and increases cytokine levels in wounds (Yang & Jones, 2009). Pectin is a complex polysaccharide consisting mainly of esterified D-galacturonic acid. It is found in the most primary cell walls of terrestrial plants and is also one of the major constituents in citrus fruit by-products. Pectin offers several advantages in wound healing, such as (1) its hydrophilicity which permits the removal of exudates by forming a soft gel over the wound bed, (2) the retention of an acid environment during pectin solubilization which may act as bacterial or viral barrier and (3) its ability as binding agent for protecting growth factors, which responsible for new cell generation, from degradation (Munarin, Tanzi, & Petrini, 2012).

Gelatin is a peptide produced by the hydrolysis of collagen extracted mainly from animal sources and is widely used in food and pharmaceutical applications due to its biocompatibility, safety and biodegradability (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011). It also gives prompt hemostasis, prevents wound contracture and aids in cell adhesion and proliferation during wound healing process (Boateng, Burgos-Amador, Okeke, & Pawar, 2015).

Previous research has suggested that composite film is more superior to single polymeric film and is a simple approach for enhancing the mechanical properties of films (Han, Yan, Chen, & Li, 2011; Thu, Zulfakar, & Ng, 2012). The scope of the present work was to develop simvastatin-loaded film wound dressings composed of alginate, pectin and gelatin. The films were prepared using the solvent cast technique and characterized based on their physical properties, surface morphology, mechanical strength and rheology. Then, in vitro drug releases from the films were investigated and, finally, the cell viability on human fibroblast cells of the films was carried out to assess their cytotoxicity profiles.

2. Materials and methods

2.1. Materials

Sodium alginate (CAS No. 9005-38-3, $M_w \sim 20\text{--}40$ kDa) and gelatin (CAS No. 9000-70-8, Ph. Eur grade, type B) were obtained from Sigma-Aldrich (UK). Simvastatin (CAS No. 79902-63-9, pharmaceutical secondary standard) was supplied by Sigma-Aldrich (USA). High methoxy pectin pure (CAS No. 9000-69-5, $M_w \sim 30,000\text{--}100,000$) was purchased from R&M Chemicals (UK). Glycerol ($\geq 99.5\%$) was procured from Sigma-Aldrich (Germany). Calcium chloride dehydrate and ethanol were obtained from Merck (Germany). All other chemicals were of analytical grade, and distilled water was used throughout.

2.2. Preparation of gels and films

Aqueous gel (blank) and drug-loaded gels were prepared using the solvent casting method. Five percent (5% w/w) sodium alginate (SA) and SA composite films blended with pectin (SA-PC) or gelatin (SA-GL) of equal mass fractions (1:1) were prepared. The compositions of gels for film casting are presented in Table 1. The gels for casting films were prepared by dissolving the polymers in distilled water containing glycerol with a constant stirring at 1000 rpm for 2–3 h at 40 °C. These homogenized gels were sonicated for at least 1 h and were then left to stand at room temperature until all remaining air bubbles were eliminated. The blank films were dried cast by pouring the gel (20 g) into a plastic Petri dish ($d = 90$ mm) and then drying in oven at 45 °C for 48 h. The drug-loaded polymeric gels were prepared by incorporating 5 mL of ethanolic solution

Table 1

Composition of the alginate and composite film dressings containing simvastatin.

Film composition ^a	SA	SA-SIM	SA-PC	SA-PC-SIM	SA-GL	SA-GL-SIM
Sodium alginate (g)	5	5	2.5	2.5	2.5	2.5
Pectin (g)	–	–	2.5	2.5	–	–
Gelatin (g)	–	–	–	–	2.5	2.5
Glycerin (g)	2.5	2.5	2.5	2.5	2.5	2.5
Simvastatin (g)	–	0.1	–	0.1	–	0.1
Ethanol (mL)	–	5	–	5	–	5
Distilled water (mL)	92.5	87.4	92.5	87.4	92.5	87.4

^a Abbreviation: SA—sodium alginate, PC—pectin, GL—gelatin, SIM—simvastatin.

of SIM (20 mg/mL) into the gels to attain a drug concentration of 2% w/w in gels. These drug-loaded gels were dried in an oven as described earlier for blank gels and eventually stored in a desiccator.

2.3. Film thickness and mechanical properties

The thickness of films was measured by using a digital caliper (Digimatic Micrometer MDC-S, Mitutoyo Co., Japan, sensitivity = 0.001 mm) at five positions (one at the center, four near the edges). This test was carried out in triplicate for each film and mean values were recorded. Tensile strength (TS) and percentage elongation at break (%E) of the films were determined using the Instron Universal Testing Machine (Model 5567, USA) according to the ASTM D 882-02 standard (ASTM, 2002). The sample specimens were conditioned at 25 °C and 50 ± 5% RH for 48 h prior to analysis. Films were cut to dumbbell-shaped strips that were 30 mm long and 5 mm wide using an ASTM standard dumbbell shape template and a cutter press (GT-7016-A Gotech Testing Machine, Malaysia). The mechanical properties of specimens were measured by stretching the films at a crosshead speed of 5 mm/min to their breaking point. At least five replicates from each type of film were used for this analysis.

The tensile strength (N/mm²) and elongation at break (%E) were calculated based on the following equations:

$$TS = \frac{\text{Maximum load at break}}{\text{Transverse section area}} \quad (1)$$

$$\%E = \frac{\text{Extension of length at rupture}}{\text{Initial length}} \times 100 \quad (2)$$

2.4. Water vapor transmission

A procedure for water vapor transmission (WVT) of the film over 24 h was adopted based on the modification of the previously described method (Thu et al., 2012). Films were cut into a disc-like shape. Five grams of calcium chloride as a desiccant was added to a dry glass vial, and films of defined diameter (exposed diameter = 1.1 cm) were sealed to the cap using a rubber ring and then mounted onto the brim of a vial. The vials were weighed and put into a humidity chamber (Terchy HRM 80 FA, Taiwan) at constant relative humidity (84%) and temperature (25 °C). The presence of a fan in the chamber ensured a uniform RH throughout the experiment. Gain in the weight of the vial was recorded every 1 h for a period of 8 h and then at 24 and 48 h. Triplicated measurements was run, and mean values were obtained. WVT over 24 h was determined using the following equation:

$$WVT = \frac{W}{S} \quad (3)$$

where W is the gain in weight of the desiccant over 24 h and S is the exposed surface area of the film (m²), WVT (g/m²/day).

2.5. Rheology

The flow properties of gels (before drying) and rehydrated films were assessed by using a Bohlin Gemini II rheometer (Malvern Instruments, UK) equipped with cone and plate geometry. For rehydrated samples, films were transformed into gel by introducing the amount of water lost after drying the gel in an oven. The water loss was calculated using by the following equation:

$$\text{Water loss (g)} = \text{weight of gel before drying} - \text{weight of dried film} \quad (4)$$

Next, approximately 1 mL of gel was placed over the Peltier plate and an experiment was performed using a cone with a diameter of 40 mm and an angle of 4° at a constant shear rate of 250 s⁻¹ for 3 min. Three replicates of each sample were taken and the mean value was calculated. Apparent viscosities at a shear rate of 250 s⁻¹ were also calculated.

2.6. FTIR analysis

Fourier Transform Infrared (FTIR) analysis was performed by using a spectrophotometer (Perkin Elmer Spectrum 100 FTIR Spectrometer, USA) fitted with an Attenuated Total Reflection assembly. Small pieces of dry films were placed on an ATR crystal and the maximum force was applied by using an ATR pressure clamp to allow for optimum contact with samples. The FTIR spectra of the pure drug, blank and drug-loaded films were recorded within the range of 4000 to 550 cm⁻¹ and a uniform resolution of 2 cm⁻¹.

2.7. X-ray diffraction

X-ray diffraction patterns of the pure drug, polymers, physical mixtures and film formulations were obtained by using the D8 Advance X-ray diffractometer (Bruker AXS, Mannheim, Germany). The system was operated during the experiment by using Lynx-Eye as a detector at a voltage of 40 kV and at a constant current of 50 mA. The scanning was conducted over the 2θ range from 10° to 40° using Nickel-filtered CuKα radiation (λ = 0.15406 nm) with a scan speed of 4.2°/min. The pure powder samples of the drug and polymers were poured into the cavity of the sample tray, and film specimens of approximately 0.5 mm thick were cut to fit the square tiles of the sample holder before running the test.

2.8. Microscopic examination

The morphology of films was determined by using a polarized microscope (Olympus BX41, Japan) attached to a camera (UC30, Germany). The films were trimmed to approximately 2 cm² and placed onto a glass slide covered with a slip. The images were recorded at 10× magnification under bright and polarized light.

2.9. Films expansion profile

A clear solution of gelatin was obtained by adding 4 g of gelatin powder into 100 mL of distilled water at 70 °C with constant stirring at 700 rpm. Then, 20 g of this 4% w/v gelatin solution was poured into a Petri dish and stored at 25 °C overnight to form a gel. Next, each drug-loaded film was trimmed into a circular shape of defined size (22 mm in diameter) then placed at the center of the gelatin gel (in the Petri dish). The change in the diameter of the films was recorded at predetermined time intervals. The test was performed

in triplicate for each formulation, and the mean value was used to calculate the expansion behavior using the following equation:

$$E = \frac{D_t - D_0}{D_0} \times 100 \quad (5)$$

where E is the expansion ratio, D_t is the diameter of the film after expansion, and D_0 is the diameter of the film before expansion.

2.10. In vitro drug release study

The Franz diffusion cell (PermeGear Inc., USA) was used to determine the in vitro SIM release profile of the drug-loaded films. PBS–ethanol (9:1) was used as a dissolution media. The pH of the dissolution media was adjusted to 7.4 to simulate the natural chronic wound environment (pH = 7.15–8.90). The receptor cell was filled with the dissolution medium and the temperature was maintained at 37 ± 1 °C during the experiment, with constant agitation (50 rpm). The films (SA–SIM, SA–PC–SIM, SA–GL–SIM), with a diameter of 2.1 cm, were placed on top of the receptor compartment over the cellulose acetate membrane (0.45 μm). Aliquots of the sample (0.5 mL) were withdrawn at fixed intervals, and an equal amount of fresh medium was added to maintain a constant volume. To prevent evaporation of the medium, the cell cap and sampling arm were wrapped with parafilms during analysis. The concentration of the SIM in the samples was determined spectrophotometrically (UV-1601, Shimadzu, Kyoto, Japan) at 238 nm. The cumulative release of SIM from the films was calculated and the results were plotted over time, finally the drug release data was fitted to Korsmeyer–Peppas equation (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983).

2.11. Cell viability study

Primary human dermal fibroblast cells (HDF; ATCC PCS-201-012) were used for cell viability assay. Cells were cultured in a fibroblast basal medium (ATCC, PCS-201-030) plus a serum-free fibroblast growth kit (ATCC PC-201-040), supplemented with 0.1% gentamicin penicillin/streptomycin (Thermo-Fisher Scientific, USA). Cells were incubated at 37 °C under 5% CO₂, and the culture medium was refreshed once every 48 h. For cell viability assay, cells were seeded in 96-well plates at a density of 1 × 10⁴ cells per well and incubated for 24 h. The drug released from the SA–PC–SIM film was obtained by placing the film on the Franz diffusion cell as described in Section 2.10. The receptor solution was withdrawn after 24 h and the SIM content was quantified with a UV spectrophotometer. The drug solution was sterilized via filtration through 0.22 μm syringe filters. Later, cells were treated separately with 100 μL of the drug solution (containing 84 μg/mL of SIM) or standard SIM solution of the same concentration, and then 100 μL of culture media was added and incubated for 24 h. Cells treated with PBS only were used as a control. After 24 h, 10% (v/v) Alamar Blue® (Invitrogen, USA) was added and the plates were incubated for 5 h. Thereafter, optical densities of each sample were measured at 570 nm using a microplate reader (Tecan infinite M200 Pro, Switzerland). Cell viability was calculated using the following formula:

$$\text{Cell viability(\%)} = \frac{A_{\text{film}}}{A_{\text{control}}} \times 100 \quad (6)$$

where A_{film} is the absorbance of film extract with cells at 570 nm and A_{control} is the absorbance of the control with cells at 570 nm.

2.12. Statistical analysis

Statistical analysis was performed using one-way ANOVA, followed by Tukey's multiple comparisons test, by using GraphPad

Prism version 6.00 for Mac (GraphPad Software, La Jolla, California, USA). The statistical significance was accepted at $p < 0.05$.

3. Results and discussion

3.1. Pre-formulation

Gels at a polymer concentration of 5% (w/w) were found to be easy to handle and free of undissolved lumps. Thus, they are suitable to be formulated into films. All of the blank and drug-loaded formulations were smooth in texture and pliable. They were easy to remove from the container. The presence of glycerin as a plasticizer was crucial because glycerin increases the space between polymer chains and helps increase the flexibility of films. Therefore, a flexible, non-brittle and conformable dressing for application to various wound sites is obtained. The addition of ethanol as a co-solvent was to enhance the solubility of simvastatin, a hydrophobic drug (Log $P = 4.68$) in the aqueous gel. However, the addition of ethanolic solution to the drug gave a slight translucence appearance to the gel. In this study, 20 g of gel was ideal to be used to cast the film dressing in a Petri dish with desirable properties. Films obtained from casting with less than 20 g were too thin, curled easily and were difficult to remove from the Petri dish as it could torn easily. On the other hand, thick films (cast from more than 20 g) were not ideal as they possessed less flexibility and appeared less translucent. Oxygen and water vapor exchange could also be reduced with thick film dressings.

The SA-PC films appeared translucent, while SA and SA-GL films appeared opaque yellowish-brown. Translucency is an important feature of film dressing, as it allows for a wound's inspection without having to remove the dressing.

3.2. Thickness and mechanical properties

The thickness of the film mainly depends on the method of preparation, amount of gel poured into the dish and flatness of the surface during drying. Therefore, to obtain films of uniform thicknesses, fixed amounts of gels were poured into the Petri dishes and films were dried in a flat tray. The average thicknesses of films are shown in Table 2. These results showed that thicknesses of films were slightly increased by incorporating SIM, which might be due to the greater amount of solids present in these films. Moreover, the thicknesses of PC-containing films were also slightly higher than those of other film formulations. The thickness uniformity within the formulations was evident from the low S.D. values, which indicated the reproducibility of film casting.

The mechanical properties of the films are shown in Table 2. Films were evaluated based on their tensile strength and elongation at break, brittleness and elasticity, respectively. The results suggested that the composite films showed enhancements in both tensile strength and percent elongation at break compared with single polymeric film, which might be attributed to the formation of intermolecular bonding between polymers. Previously, it has been shown that films made of single polymer possessed poorer

mechanical properties compared to composite films (Boateng et al., 2015; Mishra, Majeed, & Banthia, 2011). This was likely due to the weak intermolecular forces, molecular symmetry and chain stiffness of the polymer chains. Hence, a composite or blended film, i.e., combining two or more polymers to make a film is one of the approaches in improving the physical and mechanical properties of a film (Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011; Wang, Hu, Du, & Kennedy, 2010).

Moreover, the drug-loaded films exhibited a higher tensile strength and slightly lower elongation at break than the blank films. This could be due to the difference in thickness of films in the presence of the drug, as an increase in thickness enhanced the tensile strength and subsequently reduced the elongation of films at break. Furthermore, plasticizer had a significant impact on the films by reducing their rigidity, which in turn lowered their tensile strength and enhanced elongation. This effect could be explained due to the increased free volume between the polymeric chains and reduced polymeric interactions in the presence of the plasticizer. Flexible dressing is desirable, as its pliable nature does not act like rough sheets over a wound and therefore enhances patient compliance. Previous literature suggests that the values of tensile strength and elongation at break for skin are in the range of 2.5 to 16 MPa and 70%, respectively (Pereira et al., 2013). The mechanical profiles of films prepared in this study were comparable to these values, suggesting that the flexible films were prepared by blending alginate and pectin with mechanical properties acceptable for skin applications.

3.3. Water vapor transmission

Generally, an ideal wound dressing should provide an optimum rate of moisture transmission to inhibit both the accumulation of exudates and excessive dehydration at the wound site. It is reported that the WVT rate for normal skin is 204 g/m²/day, whereas for injured skin, it can range from 279 to 5138 g/m²/day for a first degree burn and granulating wounds, respectively (Lamke, Nilsson, & Reithner, 1977). The WVT rates for SA-SIM, SA-PC-SIM and SA-GL-SIM were calculated to be 1455, 1385 and 1394 g/m²/day, respectively. These indicated that all of the films were permeable to water vapor and could maintain a moist environment on the wound site for low to medium level exudative wounds without excessive dehydration. Moreover, the results showed that there was only a significant difference in the WVT between the SA-SIM and SA-PC-SIM formulations ($p < 0.05$). Maintaining high humidity is necessary for healing a wound, accelerating cell migration and facilitating epithelialization at the wound site (Mohamad, Mohd Amin, Pandey, Ahmad, & Rajab, 2014). This study assessed the WVT over a 24-h period, assuming the films were applied as a daily dressing.

3.4. Rheological studies

The rheological profiles of the rehydrated films and gels before drying are shown in Fig. 1. Both gels and rehydrated films presented shear thinning with pseudoplastic flow behavior. No syneresis was

Table 2
The thickness, mechanical (data expressed as mean \pm S.D., $n = 3$) and kinetic data of SA and composite film formulations.

Formulation	Thickness (mm)	Tensile strength (N/mm ²)	Elongation at break (%)	Crystallinity (%)	Diffusion exponent ^a (n)	Constant ^a (k)	Regression coefficient ^a (r ²)
SA	0.177 \pm 0.001	1.28 \pm 0.18	26.83 \pm 2.93	29.84 \pm 1.60	–	–	–
SA-SIM	0.193 \pm 0.005	1.43 \pm 0.21	26.38 \pm 0.34	31.64 \pm 0.71	0.841	1.969	0.981
SA-PC	0.197 \pm 0.002	1.23 \pm 0.23	75.19 \pm 3.69	36.09 \pm 0.03	–	–	–
SA-PC-SIM	0.213 \pm 0.004	3.32 \pm 0.58	61.49 \pm 2.06	37.07 \pm 1.57	0.771	2.209	0.982
SA-GL	0.179 \pm 0.004	0.89 \pm 0.05	114.56 \pm 10.11	19.69 \pm 0.95	–	–	–
SA-GL-SIM	0.190 \pm 0.002	1.78 \pm 0.02	97.66 \pm 1.20	29.79 \pm 0.04	1.076	1.166	0.927

^a Data of drug release fitted to the kinetic model using Korsmeyer–Peppas equation.

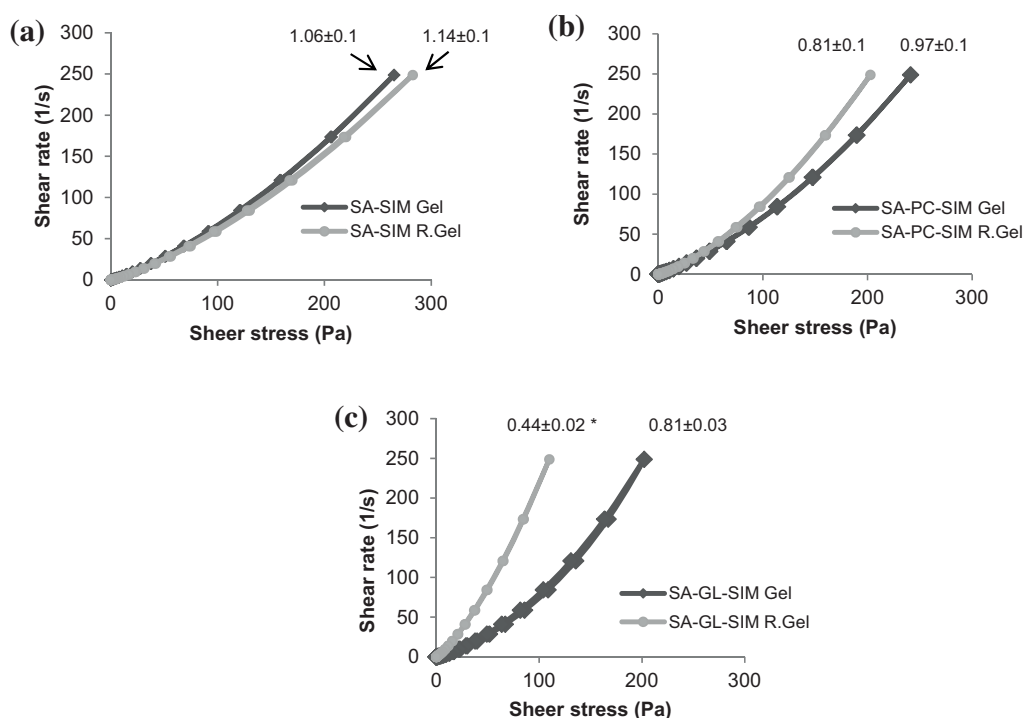


Fig. 1. Rheology profile of gel and rehydrated gel of (a) SA-SIM, (b) SA-PC-SIM, and (c) SA-GL-SIM. The values shown at the apex of the curve are the average apparent viscosities (Pa s) at 250 s^{-1} ($n=3$). The asterisks (*) represent significant difference ($p < 0.05$) compared to gel.

observed. This observation was similar to those of previous studies and is an indication that formulations were able to maintain flow properties during the reversion from film to gel (Thu et al., 2012). The apparent viscosity at 250 s^{-1} of SA-GL-SIM gels was significantly reduced ($p < 0.05$) compared with gels, while no significant differences were observed for SA alone and SA-PC-SIM. Although the previous study showed that the addition of gelatin could enhance the dispersion of a drug in an alginate film (Thu & Ng, 2013), the gelatin polymer here could not regain its coil-helices conformation after rehydration, a structure which is crucial in the gelation process, thereby presenting a drop in the overall viscosity.

A desirable wound dressing on a wound site should be capable of maintaining sufficient viscosity to prevent flow for a prolonged time (Matthews, Stevens, Auffret, Humphrey, & Eccleston, 2006). The rehydration of film in this study was done to simulate the situation where the film reabsorbs wound exudates and becomes a gel. It is crucial that the rheological properties do not change drastically after rehydration, as this will also affect the drug release profile of the film formulation. Because the properties of gelatin gels are very sensitive to temperature variations, the previous thermal history of the gel (Djabourov & Papon, 1983; Young, Wong, Tabata, & Mikos, 2005), i.e., of SA-GL films containing simvastatin, was not preferable because the variable in film formulation may become one of the manufacturing concerns.

3.5. FTIR studies

FTIR was performed in order identify any chemical interactions between the matrix and drug within the film. The FTIR spectra of pure drug, polymers and physical mixtures in powder form are shown in Fig. 2A, whilst Fig. 2B represents FTIR spectra of blank and drug-loaded films. The FTIR spectra (Fig. 2A) of SIM shows characteristic peaks at 3548 cm^{-1} (O–H stretching vibration), 2955 cm^{-1} and 2877 cm^{-1} (C–H stretching vibration) and 1699 cm^{-1} (stretching vibration of C–O and C=O carbonyl groups). Pure SA powder revealed a broad peak at 3297 cm^{-1} representing stretching of OH group, peaks at 1596 cm^{-1} and 1404 cm^{-1}

corresponding to COO through asymmetric and symmetric stretching vibration, respectively. The band from the C–O–C stretching vibration appeared at 1025 cm^{-1} , which was credited to the saccharide structure of the pure SA (Pereira et al., 2013; Thu & Ng, 2013). PC powder showed its characteristic bands at 3403 cm^{-1} and 2938 cm^{-1} which is attributed to –OH and –CH stretching vibration peaks; 1737 cm^{-1} representing the carbonyl of the esterified pendant group; 1377 cm^{-1} belonging to the –OH bending vibration peak and 1017 cm^{-1} suggested to be the glycosidic bonds linking two galacturonic sugar units (Mishra et al., 2011). On the other hand, pure GL at 3304 cm^{-1} revealed a wide absorption band due to the stretching vibration of O–H bound to N–H group. Peaks at 1741 cm^{-1} and 1631 cm^{-1} proposed to be the primary amide due to C=O stretching and secondary amide owing to NH bending vibration, respectively. In addition, C–O–C stretching at 1219 cm^{-1} was observed.

All the physical mixtures of SA-SIM, SA-PC-SIM, SA-GL-SIM in dry form showed the presence of the prominent peak of SIM at 1697 cm^{-1} , 1698 cm^{-1} , 1697 cm^{-1} , respectively. In contrary, the IR spectra of blank and drug-loaded films did not show major differences from each other in terms of the presence of SIM peaks. In other words, there was no characteristic peak of the corresponding drug (SIM) observed in the drug-loaded films that indicated a uniform inclusion or dispersion of the drug within the film matrix and lack of obvious chemical reaction between the drug and the matrix which proves the homogenous mixture of the SIM-matrix (Boateng, Pawar, & Tetteh, 2013; Dong, Wang, & Du, 2006). However, in case of the films, they showed a strong broad peak at $3274\text{--}3278\text{ cm}^{-1}$ indicated the O–H groups, which can be attributed to water molecules due to the presence of bound-water in the films.

3.6. X-ray diffraction (XRD)

XRD diffractograms of the pure drug, polymer, blank and drug-loaded films are presented in Fig. 3. From the XRD pattern in Fig. 3A, pure SIM and PC exhibited crystalline patterns, whereas SA and GL

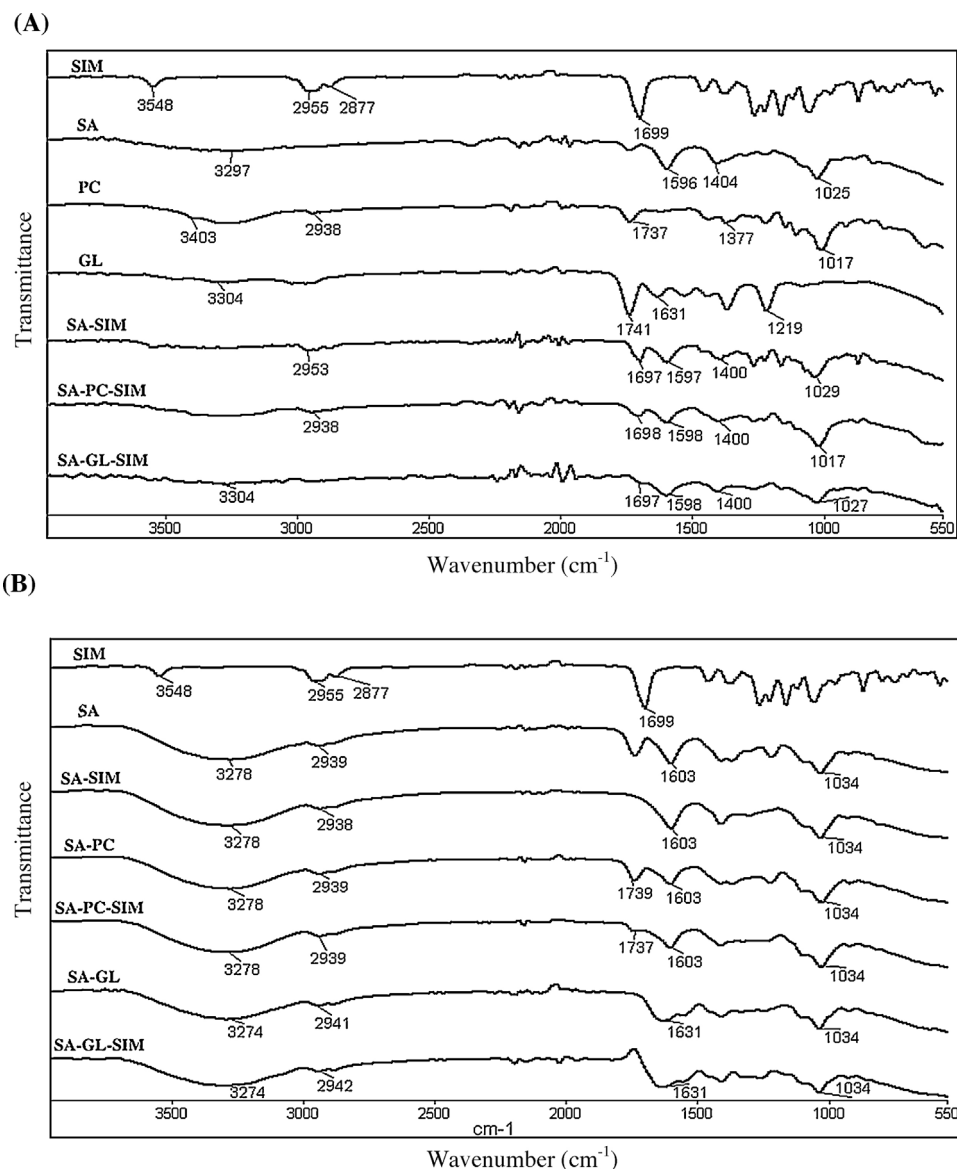


Fig. 2. FTIR spectra of (A) pure compounds, (B) pure drug, blank and drug-loaded films.

showed no crystalline peaks, indicating the amorphous nature of these polymers. SIM showed various distinct sharp peaks at 9.4° , 10.96° , 15.64° , 16.58° , 17.26° , 18.84° , 19.42° and 22.6° , which was due to its regular crystallization and was comparable with previous reports (Singh, Philip, & Pathak, 2012). Moreover, pectin showed its crystalline peaks at 14.64° , 18.68° , 20.56° and 28.36° . The diffractogram of SA powder showed two crystalline peaks at 13.6° , 21.7° and 23.7° , whereas gelatin powder showed a peak at 7.6° and a typical broad crystalline peak at 21.4° ; our reported positions of crystalline peaks were similar to those previously reported by Thu and Ng (2013).

In Fig. 3B and Table 2, SA-PC film showed more crystallinity (36.1%) than SA (29.8%) and SA-GL (29.8%) blank films. This may be due to the presence of pectin in the formulation. On the other hand, drug-loaded films also showed a higher crystallinity index compared with blank films due to the addition of SIM in the matrix. Addition of the drug showed a slight increase in the crystallinity percentage, which could be the result of the formation of crystalline microaggregates of the drug and polymers (Thu & Ng, 2013). Overall, the diffraction patterns of films suggested that there is good compatibility between the matrix film and SIM.

The crystallinity index calculated for each of the formulations is given in Table 2.

3.7. Microscopic examination

Fig. 4 shows the microscopic surface morphology of the film formulations, in both bright and polarized (dark) fields. Generally, the blank films (Fig. 4a) displayed uniform structures under bright light, with SA film being coarse and composite films appearing smooth. However, when viewed under polarized light, crystalline formation (indicated by arrow X) is detected in the SA-PC composite film. In Fig. 4b, pure SIM appeared as rod-like crystalline particles under both bright and polarized fields. In drug-loaded films, the presence of SIM was indicated with the appearance of dark solids in the bright fields. Their structures can also be observed as bright solids under cross-polar fields. Maltese crosses were observed in the polarized field of SA-PC-SIM. Maltese cross patterns are characteristic of polymer semi-crystalline structure when viewed with polarized light. The Maltese cross is observed because polymers are birefringent. Birefringence is the optical property of a material having a refractive index that depends on

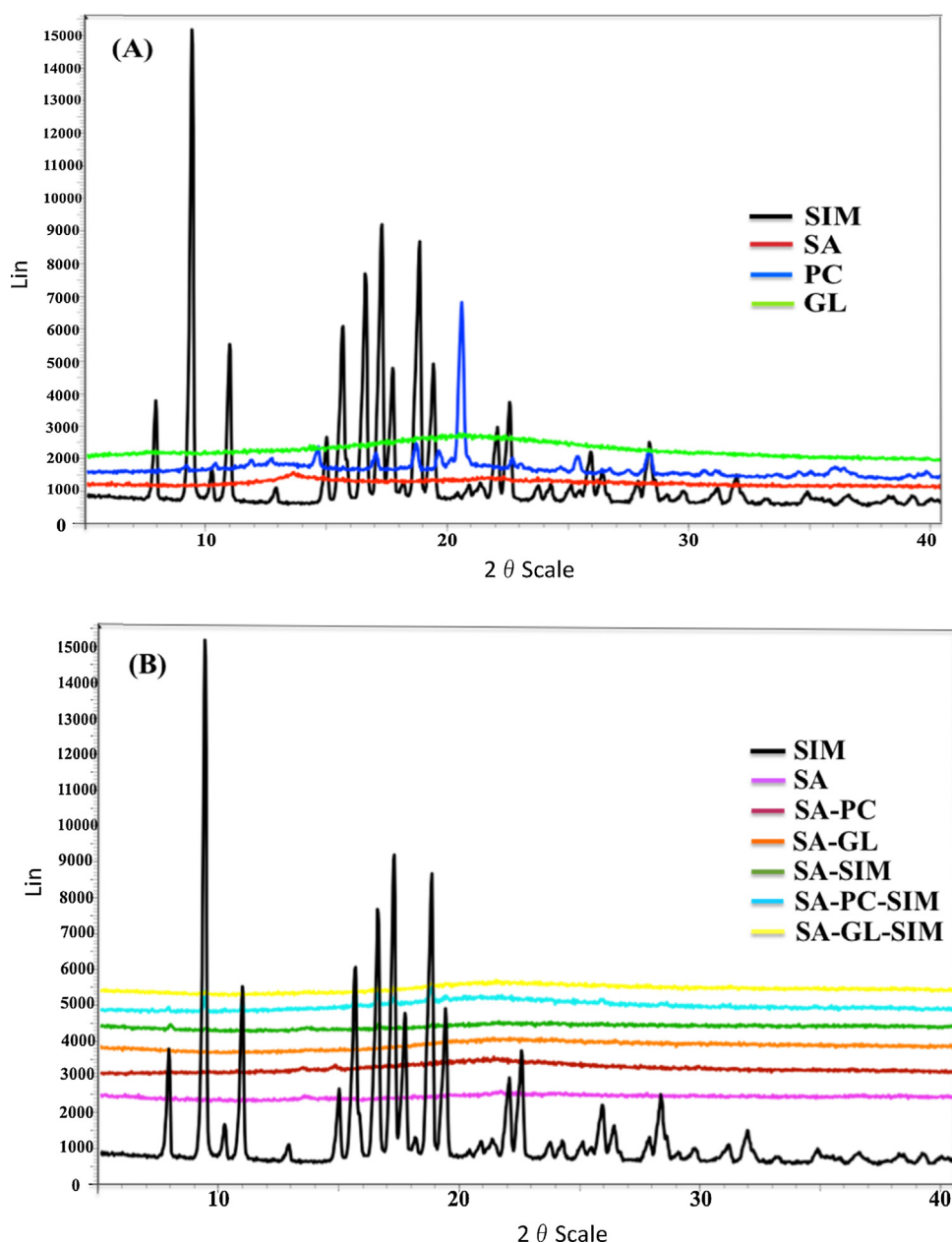


Fig. 3. XRD patterns of (A) pure compounds, (B) pure drug, blank and drug-loaded films.

the polarization and propagation direction of light. These optically anisotropic materials, such as biopolymers, are said to be birefringent. However, the Maltese crosses are not apparent in other films. This may imply that the alginate-pectin composite produced higher crystallinity (which was previously confirmed in the X-ray diffraction studies) and could be a reason for the higher tensile strength of the SA-PC film. These films also demonstrated a good compatibility within the film matrix and SIM, observed by their uniform appearance, without any phase separation and noticeable cracks.

3.8. Film expansion study

When a dried film comes in contact with a moist wound surface, wound exudates will be absorbed into the film matrix. This causes the film to hydrate and expands on the wound surface and eventually turns into a gel. This film expansion study was carried out in order to measure the rate of expansion (or hydration) of the

film dressings. Gelatin model was employed here as it simulates the wound moist surface environment (Matthews, Stevens, Auffret, Humphrey, & Eccleston, 2005).

The percentage increase in expansion of the drug-loaded films as a function of time is presented in Fig. 5a. The disk-shaped films were hydrated slowly on the gelatin surface, expanded gradually in all directions and eventually transformed into gels. The hydration rate was observed to be slower in the composite film containing PC, followed by SA alone film, after the first 3 h. The SA films containing GL exhibited the highest expansion and it was most likely attributed to its poor mechanical strength. SA-SIM and SA-GL-SIM expanded gradually up to $46.97 \pm 5.24\%$ and $90.91 \pm 0.0\%$ of their initial sizes, respectively, in 4 h. Moreover, these films were unable to retain their circular shape after 4 h. On the contrary, SA-PC-SIM retained its disc-like shape for 5 h and expanded by 50% of its initial size. This could be correlated to the mechanical strength of the films, as SA-PC-SIM also exhibited higher tensile strength than SA-SIM and SA-GL-SIM, as already shown in Table 2.

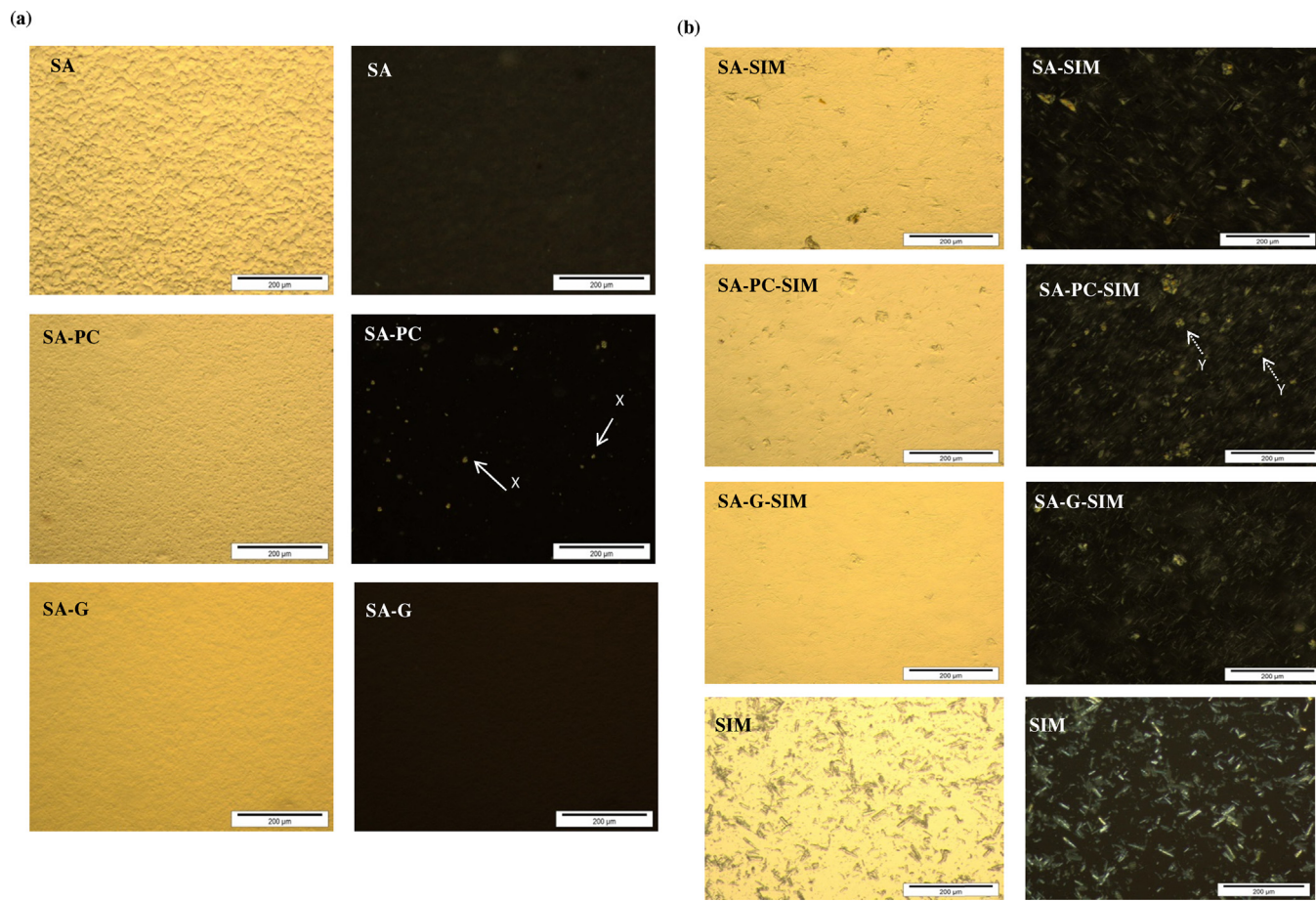


Fig. 4. Microscopic images of (a) blank films and (b) drug-loaded films and simvastatin viewed under bright (left column) and polarized (right column) light. X indicates possible formation of microcrystalline structures. Y indicates formation of maltose-cross structures.

The rehydrated SA–GL–SIM exhibited the least apparent viscosity (Fig. 1c) and therefore expanded quicker over the gelatin surface; thus, a higher expansion ratio value was observed. SA–GL–SIM may be useful for low-suppurating wounds. Comparing the expansion values of SA–SIM and SA–PC–SIM did not reveal any significant differences, whereas SA–SIM vs. SA–GL–SIM and SA–PC–SIM vs. SA–GL–SIM revealed significant differences ($p < 0.05$) after 24 h. A wound dressing which maintains its structure and shape for a longer period of time is desirable for heavy exudative wounds. And this feature was more apparent with films containing pectin.

3.9. *In vitro* drug release

Franz diffusion cell drug release studies were employed to compare the SIM release behavior of drug-loaded films. Drug release profiles in terms of cumulative amount as a function of time are shown in Fig. 5b. Because SIM is a poor water-soluble drug, a small amount of ethanol (10%) was used in release media while maintaining the pH of the chronic wound environment of 7.15–8.19 (Pawar, Boateng, Ayensu, & Tetteh, 2014). The results indicated that SIM-loaded SA and SA–PC displayed similar drug release behavior. In contrast, higher drug release was achieved from the formulation containing GL. SA–PC–SIM films exhibited the lowest cumulative drug release over 28 h (0.826 ± 0.069 mg/cm²), followed by SA–SIM films (0.929 ± 0.101 mg/cm²), while SA–GL–SIM showed the highest cumulative drug release (1.736 ± 0.134 mg/cm²) at this time. Moreover, the fluxes of SA–SIM, SA–PC–SIM and SA–GL–SIM were 0.0311 ± 0.003 , 0.0282 ± 0.003 and 0.0562 ± 0.0007 mg/cm²/h,

respectively. The release profiles could be correlated to the expansion ratio of formulations because they can act as a rate-limiting step in drug release. SA–SIM and SA–PC–SIM exhibited slower drug release, which may be correlated to their lower expansion ratio (~46% and ~50%, respectively). On the other hand, because of a higher expansion ratio of the film containing GL (~90%), the drug release from these films was higher and faster.

The release profile of formulations showed immediate release without lag time. Moreover, the SA plain film showed insignificant difference in the release of the drug compared with the blend film SA–PC. For the first 18 h, the drug release and fluxes for SA and SA–PC were not significantly different, but later drug release dropped slightly for the film formulation containing PC. Drug release from SA–PC was slower than that from SA–GL and SA alone, which may be due to the formation of microaggregates between the drug and polymers. The presence of pectin may delay chain relaxation in the presence of wound exudates as the drug begins to diffuse from the swollen films. It is believed that slower drug release from the medicated dressing is desirable because it helps in lengthening the action and release of the active drug. Moreover, it helps in enhancing patient compliance as applying a slow release medicated dressing reduces the frequency of replacing a wound dressing.

In order to investigate the SIM release mechanism from the films, Korsmeyer–Peppas equation was fitted on release data based on following equation:

$$\frac{M_t}{M_\infty} = kt^n \quad (7)$$

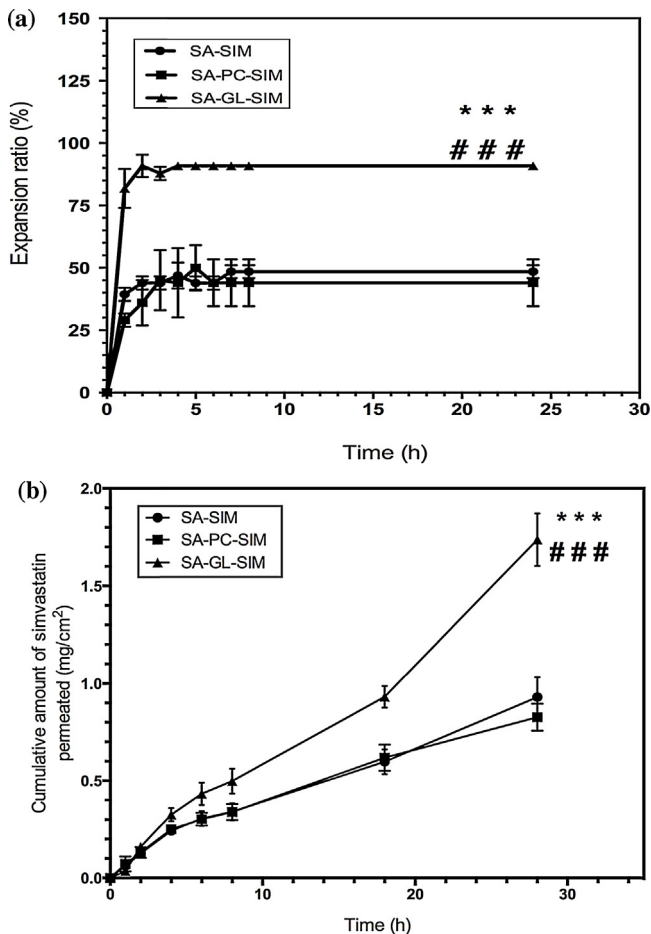


Fig. 5. Expansion (a) and in vitro drug release profile (b) of drug loaded film formulations (mean \pm S.D., $n = 3$). The asterisks (*) represent significant difference ($p < 0.05$) of SA-GL-SIM from SA-SIM and hash (#) represents significant difference ($p < 0.05$) SA-GL-SIM from SA-PC.

where Mt/M_∞ represents fraction of drug releases at time t while ' n ' and ' k ' represent diffusional exponent and the release rate constant, respectively. The values of ' n ' and ' k ' were calculated from the slope and intercept of graph between $\ln(Mt/M_\infty < 0.6)$ and $\ln(t)$ (Korsmeyer et al., 1983).

The values of n , k and r^2 of SA-SIM, SA-PC-SIM and SA-GL-SIM are summarized in Table 2. The value of ' n ' specifies the drug release mechanism characteristic of release system. For thin films, $0.5 \leq n$ corresponds to a Fickian diffusion mechanism, $0.5 < n < 1.00$ to non-Fickian (Anomalous) transport, and $n > 1.0$ to super case II transport (Ritger & Peppas, 1987). The kinetic data (Table 2) showed values of ' n ' for SA-SIM and SA-PC-SIM were between 0.5 and 1.0 which inferred that drug release mechanism from these films was governed by non-Fickian (Anomalous) transport. On the other hand, value of ' n ' for SA-GL-SIM films was higher than 1.0 that suggested case II transport from these films.

3.10. In vitro cell viability assay

A wound dressing should not cause any toxicity to the wound tissue (Zhang, Yang, & Nie, 2008). A cytotoxicity assay was performed to explore the in vitro and, subsequently, to predict the in vivo biocompatibility of these materials for their respective applications.

The cell viability assay of SA-PC-SIM film was assessed by measuring its cytotoxicity upon normal human dermal fibroblast cells. After 24 h of incubation with the drug-loaded film's receptor

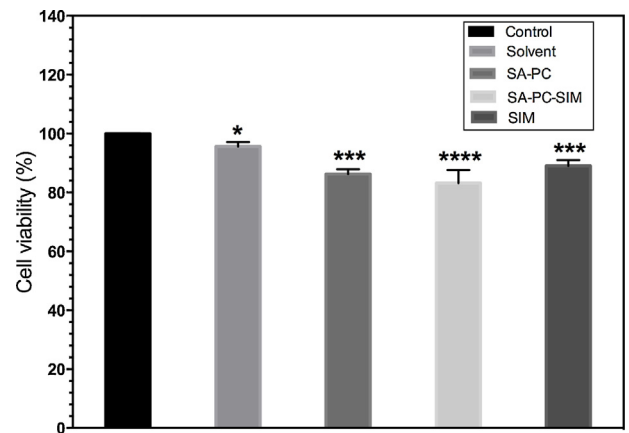


Fig. 6. Cell viability assay using human dermal fibroblast cells (mean \pm S.D., $n = 3$). The asterisks (*) represent significant difference ($p < 0.05$) from control (culture media).

solution, cell viability was 83.20% with respect to untreated cells (100%). As indicated in Fig. 6, the viability of the cells was evaluated in the presence of solvent (PBS:ethanol at a 9:1 ratio) and the SA-PC film's solution, and they were found to be 95.67% and 86.28%, respectively. Moreover, viability assay was also conducted with the pure drug solution, and the cell viability was 89.10%. As reported by ISO 10993-5 (2009), percentages of cell viability above 80% are considered as non-cytotoxic; cell viability for the formulations was 83.20%, which indicates that the developed wound dressing can be considered as a safe and non-toxic dressing for skin cells.

4. Conclusion

In the present study, three alginate-based dressings (SA, SA-PC and SA-GL) loaded with SIM were prepared using the solvent casting method. The composite film of SA-PC-SIM showed to be more superior compared to SA and SA-GL in terms of mechanical properties, expansion profile and drug release. The FTIR data revealed a good homogeneity and the lack of chemical reaction between polymers and the drug while XRD showed that the developed films were in an amorphous state. The preliminary cell viability assay suggested that the SA-PC dressing loaded with SIM is non-toxic to human dermal fibroblasts cells. Further investigations in vivo are required to establish the film formulation toxicity and efficacy profiles.

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References

- Adami, M., Prudente, A. S., Mendes, D. A., Horinouchi, C. D., Cabrini, D. A., & Otuki, M. F. (2012). Simvastatin ointment, a new treatment for skin inflammatory conditions. *Journal of Dermatological Science*, 66(2), 127–135.
- Asai, J., Takenaka, H., Hirakawa, S., Sakabe, J., Hagura, A., Kishimoto, S., et al. (2012). Topical simvastatin accelerates wound healing in diabetes by enhancing angiogenesis and lymphangiogenesis. *American Journal of Pathology*, 181(6), 2217–2224.
- ASTM. (2002). *ASTM D 882-02: Standard test method for tensile properties of thin plastic sheeting*. American Society for Testing Materials.
- Bae, M. S., Yang, D. H., Lee, J. B., Heo, D. N., Kwon, Y. D., Youn, I. C., et al. (2011). Photo-cured hyaluronic acid-based hydrogels containing simvastatin as a bone tissue regeneration scaffold. *Biomaterials*, 32(32), 8161–8171.
- Boateng, J., Burgos-Amador, R., Okeke, O., & Pawar, H. (2015). Composite alginate and gelatin based bio-polymeric wafers containing silver sulfadiazine for wound healing. *International Journal of Biological Macromolecules*, 79, 63–71.

- Boateng, J. S., Pawar, H. V., & Tetteh, J. (2013). Polyox and carrageenan based composite film dressing containing anti-microbial and anti-inflammatory drugs for effective wound healing. *International Journal of Pharmaceutics*, 441(1–2), 181–191.
- Djabourov, M., & Papon, P. (1983). Influence of thermal treatments on the structure and stability of gelatin gels. *Polymer*, 24(5), 537–542.
- Dong, Z., Wang, Q., & Du, Y. (2006). Alginate/gelatin blend films and their properties for drug controlled release. *Journal of Membrane Science*, 280(1–2), 37–44.
- Fukui, T., Ii, M., Shoji, T., Matsumoto, T., Mifune, Y., Kawakami, Y., et al. (2012). Therapeutic effect of local administration of low-dose simvastatin-conjugated gelatin hydrogel for fracture healing. *Journal of Bone and Mineral Research*, 27(5), 1118–1131.
- Gómez-Guillén, M. C., Giménez, B., López-Caballero, M. E., & Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocolloids*, 25(8), 1813–1827.
- Han, D., Yan, L., Chen, W., & Li, W. (2011). Preparation of chitosan/graphene oxide composite film with enhanced mechanical strength in the wet state. *Carbohydrate Polymers*, 83(2), 653–658.
- ISO. (2009). Biological evaluation of medical devices, Part 5: Tests for in vitro cytotoxicity. In *ISO 10993-5*. Geneva, Switzerland: ISO.
- Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., & Peppas, N. A. (1983). Mechanisms of solute release from porous hydrophilic polymers. *International Journal of Pharmaceutics*, 15(1), 25–35.
- Kureishi, Y., Luo, Z., Shiojima, I., Bialik, A., Fulton, D., Lefer, D., et al. (2000). The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nature Medicine*, 6, 1004–1010.
- Lamke, L., Nilsson, G., & Reithner, H. (1977). The evaporative water loss from burns and the water–vapour permeability of grafts and artificial membranes used in the treatment of burns. *Burns*, 3(3), 159–165.
- Lee, K. Y., & Mooney, D. J. (2012). Alginate: Properties and biomedical applications. *Progress in Polymer Science*, 37, 106–126.
- Matthews, K., Stevens, H., Auffret, A., Humphrey, M., & Eccleston, G. (2005). Lyophilised wafers as a drug delivery system for wound healing containing methylcellulose as a viscosity modifier. *International Journal of Pharmaceutics*, 289(1–2), 51–62.
- Matthews, K., Stevens, H., Auffret, A., Humphrey, M., & Eccleston, G. (2006). Gamma-irradiation of lyophilised wound healing wafers. *International Journal of Pharmaceutics*, 313(1–2), 78–86.
- Mishra, R. K., Majeed, A. B. A., & Banthia, A. K. (2011). Development and characterization of pectin/gelatin hydrogel membranes for wound dressing. *International Journal of Plastics Technology*, 15(1), 82–95.
- Mohamad, N., Mohd Amin, M. C., Pandey, M., Ahmad, N., & Rajab, N. F. (2014). Bacterial cellulose/acrylic acid hydrogel synthesized via electron beam irradiation: accelerated burn wound healing in an animal model. *Carbohydrate Polymers*, 114, 312–320.
- Mogoşanu, G. D., & Grumezescu, A. M. (2014). Natural and synthetic polymers for wounds and burns dressing. *International Journal of Pharmaceutics*, 463(2), 127–136.
- Moura, L. I. F., Dias, A. M. A., Carvalho, E., & de Sousa, H. (2013). Recent advances on the development of wound dressings for diabetic foot ulcer treatment—A review. *Acta Biomaterialia*, 9(7), 7093–7114.
- Munarin, F., Tanzi, M. C., & Petrini, P. (2012). Advances in biomedical applications of pectin gels: A Review. *International Journal of Biological Macromolecules*, 51, 681–689.
- Pawar, H. V., Boateng, J. S., Ayensu, I., & Tetteh, J. (2014). Multifunctional medicated lyophilised wafer dressing for effective chronic wound healing. *Journal of Pharmaceutical Sciences*, 103(6), 1720–1733.
- Pawar, H. V., Tetteh, J., & Boateng, J. S. (2013). Preparation, optimisation and characterisation of novel wound healing film dressings loaded with streptomycin and diclofenac. *Colloids Surf B*, 102, 102–110.
- Pereda, M., Ponce, A. G., Marcovich, N. E., Ruseckaite, R. A., & Martucci, J. F. (2011). Chitosan–gelatin composites and bi-layer films with potential antimicrobial activity. *Food Hydrocolloids*, 25, 1372–1381.
- Pereira, R., Carvalho, A., Vaz, D. C., Gil, M. H., Mendes, A., & Bártolo, P. (2013). Development of novel alginate based hydrogel films for wound healing applications. *International Journal of Biological Macromolecules*, 52, 221–230.
- Ritger, P. L., & Peppas, N. A. (1987). A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *Journal of Controlled Release*, 5(1), 37–42.
- Singh, H., Philip, B., & Pathak, K. (2012). Preparation characterization and pharmacodynamic evaluation of fused dispersions of simvastatin using PEO–PPO block copolymer. *Iranian Journal of Pharmaceutical Research*, 11(2), 433–445.
- Tanigo, T., Takaoka, R., & Tabata, Y. (2010). Sustained release of water-insoluble simvastatin from biodegradable hydrogel augments bone regeneration. *Journal of Controlled Release*, 143(2), 201–206.
- Thu, H.-E., & Ng, S.-F. (2013). Gelatine enhances drug dispersion in alginate bilayer film via the formation of crystalline microaggregates. *International Journal of Pharmaceutics*, 454(1), 99–106.
- Thu, H.-E., Zulfakar, M. H., & Ng, S.-F. (2012). Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing. *International Journal of Pharmaceutics*, 434(1–2), 375–383.
- Wang, L. Z., Liu, L., Holmes, J., Kerry, J. F., & Kerry, J. P. (2007). Assessment of film-forming potential and properties of protein and polysaccharide-based biopolymer films. *International Journal of Food Science & Technology*, 42, 1128–1138.
- Wang, Q., Hu, X., Du, Y., & Kennedy, J. F. (2010). Alginate/starch blend fibers and their properties for drug controlled release. *Carbohydrate Polymers*, 82, 842–847.
- Yang, D., & Jones, K. S. (2009). Effect of alginate on innate immune activation of macrophages. *Journal of Biomedical Materials Research, A*, 90, 411–418.
- Young, S., Wong, M., Tabata, Y., & Mikos, A. G. (2005). Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *Journal of Controlled Release*, 109(1–3), 256–274.
- Zhang, X., Yang, D., & Nie, J. (2008). Chitosan/polyethylene glycol diacrylate films as potential wound dressing material. *International Journal of Biological Macromolecules*, 43(5), 456–462.