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pH- and electro-responsive characteristics of silk fibroin–hyaluronic acid polyelectrolyte complex membranes

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ABSTRACT

pH-responsiveness of recently developed silk fibroin (SF) and hyaluronic acid (HA) polyelectrolyte complex (PEC) membranes and their potential use in electro-responsive drug release systems were investigated. PEC membranes were prepared within a narrow pH window (3.0–3.5) for a SF–HA weight ratio of 20 and they were characterized by Atomic Force Microscopy in addition to characterization studies previously reported by our group. Swelling kinetics of the membranes was studied for a pH window of 2.5–7.4 and cyclic swelling test was performed to determine the pH-responsiveness of the membranes. It was shown that membranes swelled more in alkaline conditions and responded to variations in pH of the medium. Electric-stimuli assisted drug permeation and release studies were performed with a custom-made diffusion cell under both passive condition and electric field applied in pulsatile fashion. The instantaneous flux raised as the current was applied and then declined when the current application was terminated, and this process was repeated on subsequent applications. SF–HA complex membranes were found promising for the electric-stimuli-sensitive release of a high molecular weight and charged model drug for a membrane-permeation controlled formulation.

1. Introduction

Controlled delivery of drugs to achieve predictable and reproducible drug concentration in the blood stream is the aim of the search for the alternative routes for drug administration. Among several controlled drug delivery systems, transdermal therapeutic system has gained considerable success while generating lots of interest with the ongoing enhancements on this technique. Transdermal drug delivery systems (TDDSs) especially offer continuous administration, and thus controlled plasma levels of potent drugs with short biological half-lives. These systems allow the administration of drugs with narrow therapeutic window. Additionally, the continuous mode of administration increases patient compliance to the therapy and it brings out the ease of discontinuity of the delivery in case of toxic effects. It is generally difficult to get drugs to cross the skin at a sufficient rate to deliver at a therapeutic dose even when the drug is potent. Inherent limitations of TDDS stems from the variability of physico-chemical properties of the penetrant, low drug levels in plasma and skin irritation caused by some drugs or formulations. Passive permeability of drugs across the highly lipophilic stratum corneum is especially difficult to compounds which are hydrophilic, very lipophilic, of high molecular weight (MW > 400 g/mol) or charged (Vasil'ev et al., 2001; Wang et al., 2005). There have been many different approaches to overcome the skin barrier, including mechanical disruption (Mitragotri et al., 1995), chemical modification (Stoughton and Fritsch, 1964), electroporation and iontophoresis (Weaver et al., 1999). Iontophoresis has been reported as a promising answer for the effective transdermal drug delivery, especially for the novel biotechnologically developed drugs such as peptides and proteins that are hydrophillic, charged macromolecules and susceptible to proteolysis, chemical change and denaturation (Tabata and Ikada, 1998; Banga et al., 1999; Wang et al., 2005).

Iontophoresis is a transport phenomenon, and it stems from the movements of ions in solution due to an applied electric field across two electrodes. Iontophoretic drug delivery implies the use of small amounts of physiologically acceptable electric current across a membrane to drive charged molecules into the body. Drug is driven into the skin by electrostatic repulsion by using an electrode of the same polarity as the charge of the drug (Stamatialis et al., 2002; Eljarrat-Binstock and Domb, 2006). Electric field applied results in an electric current, which is transformed into an ionic current at the electrode/liquid or electrode/skin interface. Ionic current carries the drug ions into the skin while the counter-ions that are more mobile complete the circuit (Guy et al., 2002; Coston and Li, 2001; Wang et al., 2005).

Membrane-based systems have been used for both passive and iontophoretic TDDS (Kost and Langer, 2001; Stamatialis et

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al., 2008). Electro-responsive or electrically stimulated pH sensitive polyelectrolyte membranes and hydrogels such as chondroitin 4-sulphate hydrogels (Jensen et al., 2002) and PMMA polyelectrolyte membrane (Grimshaw et al., 1990) have been suggested to be promising for the iontophoretic delivery of large charged macromolecules such as proteins and peptides. Enhancement in iontophoretic drug delivery with the use of polyelectrolyte membranes has been attributed to increased membrane permeability due to electrically and chemically induced swelling and/or electrostatic partitioning of the charged solutes into the membrane (Grimshaw et al., 1989). Preparation of silk fibroin and hyaluronic acid PEC membranes has been reported by our group earlier (Malay et al., 2007, 2008). Fibroin provides an important set of material options with respect to its unique mechanical properties, versatility in processing, in addition to its biocompatibility, biostability and slow rates of biodegradation (Perez-Rigueiro et al., 1998; Hardy et al., 2008). Several forms of fibroin have been suggested to be used in drug preparation and in drug delivery systems. Fibroin gels were proposed for oral dosage form (Hanawa et al., 1995), membranes as controllable medicine-releasing carriers (Li et al., 2001) and fibroin/chitosan matrices for a transdermal drug delivery system (Rujiravanit et al., 2003). More recently, the protein was bioengineered for use in biomaterials as three-dimensional porous scaffolds for tissue engineering (Kim et al., 2005), coating material for sustained drug release (Wang et al., 2007a; Bayraktar et al., 2005) and microspheres for encapsulation as drug carrier systems (Wang et al., 2007b) and films (Hofmann et al., 2006) for controlled drug delivery. On the other hand, hyaluronic acid (HA), a naturally occurring lubricous biopolymer, is an attractive building block for novel biocompatible and biodegradable biomaterials with potential applications in drug delivery (Surini et al., 2003; Luo et al., 2000; Simon et al., 1997) and tissue engineering (Park et al., 2002). HA has been used in the production of artificial blood vessel and artificial skin (Choi et al., 1999; Nishida et al., 1993), whereas electrical sensitive behavior of chitosan/hyaluronic acid polyelectrolyte complexes (PEC) has been found promising for the electric current mediated drug delivery systems (Kim et al., 2003).

In the previous papers, we have reported complexation of silk fibroin and hyaluronic acid, which was followed by preparation and characterization of SF/HA PEC films (Malay et al., 2007, 2008). In this study, it was demonstrated that the membranes prepared through complex formation had a pH-responsive swelling characteristics. In addition to this, present work introduces electro-responsive controlled drug delivery of silk fibroin–hyaluronic acid membranes. In this study, Timolol maleate (TM) was chosen as a model drug since it is transdermally well tolerated in humans, it permeates through human skin (Kubota et al., 1991), and it has suitable chemical properties (weak base, $pK_a \approx 9.2$, adequate lipophilicity) for transdermal administration (Sutinen et al., 1999). TM with $pK_a = 9.21$ exists as predominantly charged ions (98.45%) at pH 7.4.

2. Materials and methods

2.1. Materials

Silk Fibroin (*Bombyx mori*) was obtained in reeled form from Bursa Institute for Silkworm Research (Bursa, Turkey). Hyaluronic acid (HA) sodium salt (MW: 1600 kDa, from *Streptococcus equi*) was provided by Fluka-BioChemica (Buchs, Switzerland) in powder form. Ethanol (absolute GR for analysis) was from Merck (Darmstadt, Germany). Calcium chloride-2-hydrate was supplied from Riedel-de Haën (Seelze, Germany), sodium carbonate (99.5%) was from Aldrich-Chemie (Steinheim, Germany). Dialysis tubing (MW Cut-off: 12–14 kDa) was obtained from Sigma (St. Louis, MO, USA). Sodium sulfide hydrate was provided by Fluka Chemie (Buchs, Switzerland). Deionized water was used during all experiments. Timolol maleate salt (MW = 432.5) was from Sigma (St. Louis, MO, USA).

Preparation steps of SF solution including degumming and dissolution processes were previously reported (Malay et al., 2007). Briefly, raw silk was kept in 50 times (v/w) of boiling aqueous 0.05% Na_2CO_3 for 30 min and this treatment was repeated three times. This was followed by washing several times with deionized water and the degummed silk was left drying at room temperature. To obtain aqueous SF solution, 1.2 g degummed silk was added to 20 times (v/w) of Ajisawa's reagent ($CaCl_2$/ethanol/water) (Yamada et al., 2001). The mixture was stirred at 78 °C to form a clear solution for 2 h. The resulting SF solution was then dialyzed against deionized water for at least 3 days at sub-ambient temperature to remove the neutral salts using cellulose tubing. Eventually, the dialysis was ended as the dialysate tested negative for chloride ion by performing silver chloride precipitation test using $AgNO_3$. The concentration of the SF solution was controlled using a rotary vacuum evaporator.

HA was provided in powder form and it was soluble in water or any buffer solutions considered. However, HA particles were prone to coagulation during dissolution; therefore HA solution was stirred overnight to ensure complete solubilization.

2.2. Preparation of SF–HA PEC membranes

SF solution, adjusted to 5 wt/v%, and HA, dissolved in water, were mixed at a weight ratio of 20 at pH of 3.2. The mixture was stirred for 4 h and casted on polyethylene Petri-dishes at room temperature. Casted SF–HA complex membranes were dried at 20 °C and 65% RH for 2 days in an environmental chamber (Angelantoni Industrie, Italy). Pure SF membranes were prepared at pH 3.2, however they were dried at 45 °C and 65% RH for 2 days to induce β-sheet structure. Dried membranes were stored in a desiccator at 10 °C until used to avoid contamination. The solubility of the membranes was controlled by immersing in deionized water and buffer solutions, whereas the thickness of the dried and swollen membranes was measured by an electronic digital micrometer.

2.3. Characterization of complex membranes

XRD, FT-IR and DSC data of the prepared membrane were reported elsewhere (Malay et al., 2008). The roughness of the membranes was determined by Contact Mode Atomic Force Microscopy (Digital Instruments MMAFM-2/1700EXL).

2.4. Swelling measurements

Swelling measurements were performed at different pH values from 2.5 to 7.4 at 37 °C and 50 °C. After immersion in citric acid buffer solution at a preset temperature and pH for 30 min, the membrane was taken from buffer solution, filter-paper dried by blotting to remove the absorbed water on the surface and then immediately weighed. The membrane was repeatedly weighed and reimmersed in solution at predetermined pH and temperature until the hydrated weight reached a constant value.

The swelling defined as, the weight of water uptake per unit weight of dried membranes (given by Eq. (1)) was calculated by measuring the weight of swollen membranes until the weight changes within 1% of previous measurement (Kost and Langer, 2001).

$$\text{Swelling } (\%) = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

where W_d is the weight of the dry membrane and W_s is the weight of the swollen membrane. Each swelling experiment was repeated three times and the average value was taken as swelling percentage. Cyclic pH-responsiveness swelling tests were performed at two pH

levels, 2.5 and 7.4, where considerable alterations in swelling were expected. Same swelling procedure was applied and the cycles were repeated for 5 times. In addition to these swelling measurements, equilibrium swelling (%) of two types of SF-based membranes at 37 °C based on pH and ionic strength of the swelling environment were determined.

2.5. Passive and electro-responsive TM delivery

Drug delivery set-up primarily consisted of a diffusion cell, Iomed iontophoretic drug delivery device used as the power supply and UV–vis spectrophotometer (Shimadzu, UV-1601) with the *in situ* flow-cell installed. Phosphate buffer saline ([NaCl] = 0.1 M) at the physiological pH 7.4 was used as the acceptor fluid. The flow of the buffer solution (approximately 2 ml/min) through the diffusion cell was provided by a peristaltic pump. A custom-made diffusion cell was used within the system. This cell includes two polytetrafluoroethylene (PTFE) parts joined end to end and an o-ring in between. The membrane is sealed with two gaskets and fixed in the middle as the parts are joined and externally squeezed. Silver/silver chloride disc electrodes (Diameter = 1 cm) were used during the experiments to prevent electrolysis of water. The silver electrode was placed in the anodal compartment and the silver chloride electrode in the cathodal compartment. The design of the cell enables the *in situ* analysis of amount of the drug delivered to the receptor solution. This was achieved by designing a small volume for the receptor chamber, which is 4.2 ml. The diameter of the opening between the compartments is 13 mm, which gives an active membrane are of 3.2 cm^2. The design of the cell ensures an undisturbed laminar flow in the receiver side and mixing was not required.

The upper chamber, having a volume of 5 ml, was filled with PBS or drug dissolved in PBS for release studies or permeability studies, respectively, via injection. As the experiment started, the PBS solution was pumped through the receptor chamber and then passed through the flow-cell installed in the UV–vis spectrophotometer, which was connected to a computer. The data was recorded and monitored by the use of software. The effluent leaving the flow-cell was collected in a beaker. Maximum current density applied was 0.5 mA/cm^2, which has been reported as the maximum acceptable density for the iontophoretic transdermal delivery producing minimal skin damage and irritation (Van der Geest et al., 1998).

3. Results and discussion

3.1. Preparation and characterization of SF/HA complex membranes

The SF–HA complex mixtures, prepared at pH 3.2, casted and dried under controlled temperature and relative humidity resulted in homogeneous transparent membranes. It was earlier shown that around this pH value, these biopolymers are oppositely charged, and capable of forming intrapolymeric complexes (Malay et al., 2007). The preliminary evaluation of the prepared membranes was their solubility in water. The membranes immersed in both water and various buffer solutions were insoluble, whereas pure SF membranes bearing β-sheet structure and pure HA membranes exhibited fast dissolution in aqueous media. At each mixing ratio within the proposed pH range, insoluble SF–HA PEC membranes were obtained regardless of 1:1 pairing of the charged groups or the weight ratios. Formation of insoluble complexes even at high excess of one of the biopolymers was shown as a proof for a greater stability of the polyelectrolyte complexes obtained in these conditions and an argument for a tight structure that could be achieved. A similar result was reported for the complex formation between chitosan and carboxymethylcellulose (Park et al., 2002).

The complex membranes underwent a color change in aqueous media; they turned to milky white as shown in Fig. 1. Such a color change was not observed for pure SF and HA membranes prepared and dried under same conditions. The color changing phenomenon in complex membranes was previously attributed to aggregative state of two biopolymers (Choi et al., 1999), that is analogous to the color change of SF–HA biopolymer mixture during the aggregation of the intrapolymer complexes with respect to induced pH changes. When pH reached a critical value (∼3), deprotonated carboxylic acid groups of HA associated with the protonated amino groups of SF, which led to the complexation of the SF and HA chains. The orientation probably caused the refraction change of the light, so the casting solution turned milky-white. As the membranes were casted and left for drying, the milky-white color gradually disappeared and dry transparent yellowish membranes were obtained. Upon exposition to aqueous environment, they regained the milky-white appearance due to associated complexes in the membrane.

AFM analyses showed that pure SF control membrane with β-sheet conformation was compact and flat with a height of 0.582 nm, whereas the formation of complexes resulted in an average height of 39.55 nm on the surface of the SF–HA complex membranes (Fig. 2(a) and (b)). FT-IR analyses of the membranes showing random coil (silk-I) structure for SF–HA complex membrane and β-sheet (silk-II) structure for pure SF membrane were reported elsewhere (Malay et al., 2008). Pure SF sample exhibited densely packed small-sized grains as shown in Fig. 2(b). It was reported that β-sheet SF membranes may exhibit several types of morphologies such as small particles, grains or nanofibrils (Putthanarat et al., 2002). It was previously reported that SF–HA complex coacervation occurs when the biopolymers are mixed within a narrow pH window of 2.5–3.5 (Malay et al., 2007). The complex coacervates were evidenced as roundish grains which also confirmed that they were liquid droplets (Fig. 2(a)). The lighter areas showed high degree of aggregation of the coacervate droplets.

Fig. 1. Appearance of dry (a) and wet (b) insoluble SF–HA complex membrane.

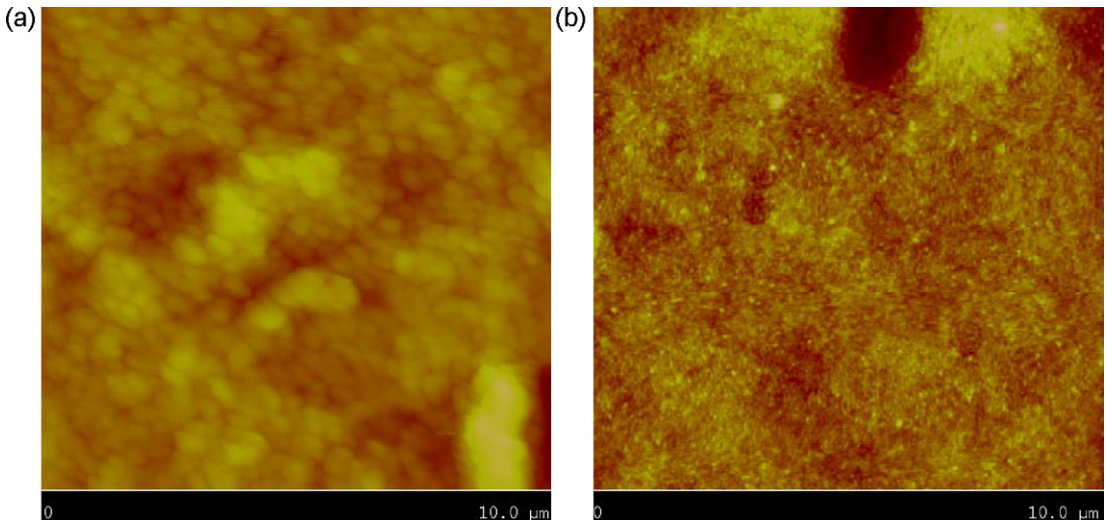

Fig. 2. AFM images of SF–HA complex membrane (a) and SF control sample with silk II conformation (b) dried under controlled conditions.

3.2. Swelling and pH-responsive drug permeation tests

The investigations on swelling ability of SF–HA membranes were carried out in the media of pH 2.5–7.4. The weight of the membranes increased rapidly and equilibrated within 3 h. The swollen membranes had the milky-white appearance during all swelling experiments. Figs. 3 and 4 show the pH-dependent swelling kinetics for the SF–HA complex membrane at 37 °C and 50 °C, respectively. The membranes swelled more in alkaline condition and shrank in acidic condition regardless of the temperature of the salt-free phosphate buffer solutions. The relatively higher swelling in alkaline condition occurred since the carboxyl groups on SF and the unreacted pendent groups on HA may have dissociated into carboxyl anion in alkali condition, which caused the ionic repulsion between anionic groups in the network resulted in the conformational stretching and the membranes swelled. On the other hand, the membranes shrank in acidic condition with respect to the coiled conformation owing to the ionic affinity.

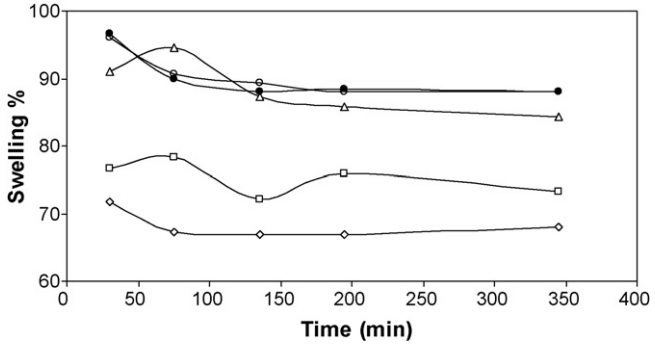

Fig. 3. pH-dependent swelling kinetics of the SF–HA complex membrane at 37 °C. pH 2.5 (◊); 3.2 (□); 5.5 (Δ); 6.5 (○); 7.4 (●).

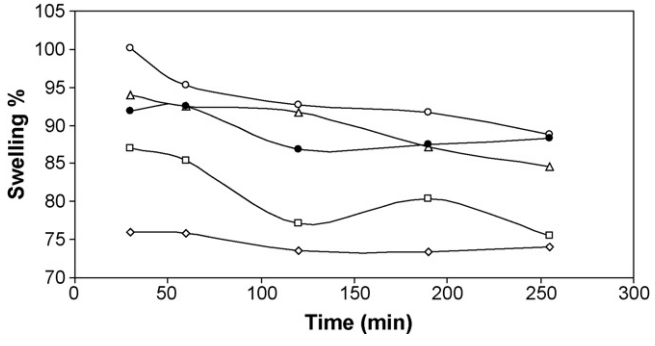

Fig. 4. pH-dependent swelling kinetics of the SF–HA complex membrane at 50 °C. pH 2.5 (◊); 3.2 (□); 5.5 (Δ); 6.5 (○); 7.4 (●).

The plots showed that the reason of swelling was mostly due to ionic repulsion of the pendent groups on SF since the swelling ratios above and below the IEP of the protein was similar. Dissociation of carboxyl groups on HA would be completed up to these pH levels since it had a pK_a value of 2.9. In the region of 2.5–4, the carboxyl groups on SF are in the form of –COOH with the excess H^+ ions present in the solution. As the pH increased, H^+ ion from carboxyl group combined with OH^- in alkali and the carboxyl groups dissociated into carboxyl anion.

The pH value and the degree of dissociation calculated according to Eq. (2) are shown in Table 1. pK_a of HA was taken as 2.5 with respect to electrophoretic mobility measurements (Sutani et al., 2002).

$$\alpha = \frac{1}{10^{(pK_a - pH)} + 1} \quad (2)$$

Degree of dissociation of the carboxyl groups on HA does not show any changes above the pH of 4–4.5, which confirmed that dissociation of carboxyl groups on HA mostly occurred within the pH window of 2.5–4.5. Thus, the alterations in swelling at alkaline pH were due to dissociation of the carboxyl groups of SF creating higher ionic repulsion with the existing negatively charged groups.

In general, for the polyampholyte gels, there occurs a u-shaped swelling profile having a minimum around the IEP of the polyampholyte showing the interaction of the anionic and cationic units. Shrinking at neutral pH was described by the presence of excess amino groups. In our case, there observed only an increase in swelling with the increase of pH approving that the cationic units

Table 1
Degree of dissociation for HA with respect to pH.

pH	α
2.5	0.50
3.2	0.83
4.5	0.99
5.5	1.00
6.5	1.00
7.4	1.00

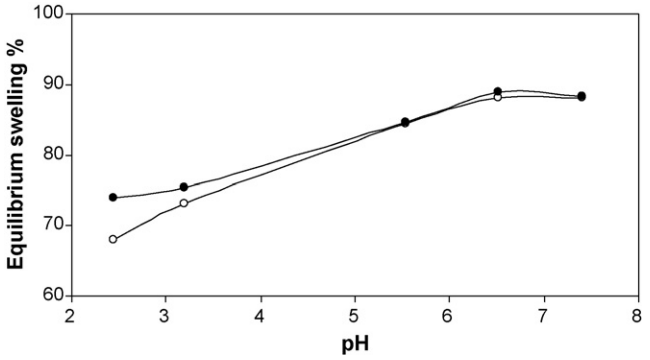

Fig. 5. Equilibrium swelling percentages of the SF–HA complex membranes with respect to pH at 50 °C (●) and 37 °C (○).

were already consumed through complexation with the dissociated units of the HA. Therefore, in all pH levels the complex membrane was negatively charged. On the other hand, higher charge density of HA caused swelling of the membranes even at highly acidic conditions. Minimum equilibrium swelling degree was recorded as 68% at pH 2.5 and 37 °C.

Equilibrium swelling of the complex membranes was found to be independent of temperature as shown in Fig. 5 below for all pH levels studied. This indicated that complex coacervates were surrounded and stabilized by the excess of HA through high yield of complexation. As a result, the water molecules were highly ordered around these groups at the temperatures examined. Temperature dependent swelling was reported for membranes mostly having hydrophobic moieties (Yoshizawa et al., 2004).

Table 2 represents equilibrium swelling (%) of two types of SF-based membranes at 37 °C based on pH and ionic strength of the swelling environment. SF–HA membrane represents polyelectrolyte complex membrane prepared at pH 3.2 whereas SF-β membrane represents pure SF membrane with silk-II conformation. Minimum swelling was obtained for SF-β membrane due to its densely packed structure evidenced by AFM analyses, and no change in swelling was observed with respect to changes in ionic strength. Maximum swelling was obtained at 0.1 M NaCl concentration and pH 7.4 which was attributed to increase in ionic osmotic pressure generated from mobile counterions in the network. Further increase of the ionic strength of the medium up to 1.0 M NaCl decreased the swelling levels, which denoted the effect of high concentration of counterions may hinder the ionic interactions between the carboxyl groups lowering the elastic-retractive force exerted on the network due to ionic repulsion within the ionic strength range studied.

In addition to all, complex membranes prepared with SF and HA were found to be highly resistant to both acidic and alkaline conditions even at elevated temperatures up to 50 °C as no disintegration or disassociation was observed during the experiments.

Table 2
Equilibrium swelling of various SF membranes with respect to pH and ionic strength.

Membrane label	pH	NaCl conc. [M]	Equilibrium swelling ratio (%)
SF–HA membrane	10.0	–	86
SF–HA membrane	7.4	–	85
SF–HA membrane	6.5	–	84
SF–HA membrane	5.5	–	81
SF–HA membrane	3.2	–	70
SF–HA membrane	2.4	–	67
SF–HA membrane	7.4	0.1	95
SF–HA membrane	7.4	1.0	75
SF–(β) membrane	7.4	0.1	53
SF–(β) membrane	7.4	–	53

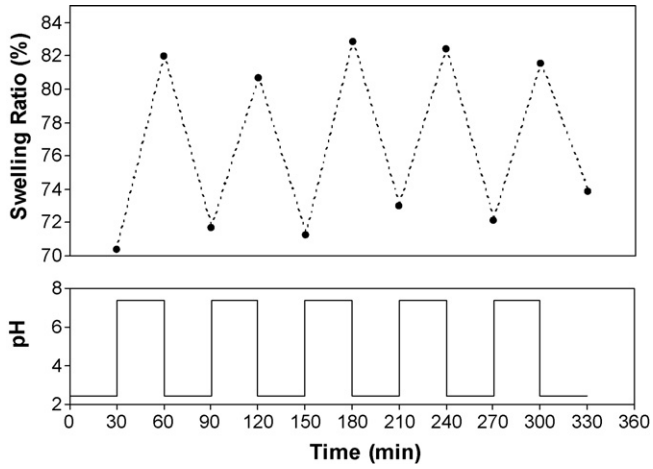

Fig. 6. pH responsive changes of water content in SF–HA complex membranes.

Fig. 6 shows pH responsive changes of the swelling degree (%) in the SF–HA membrane. The biopolymeric membrane swelled at alkaline condition (pH 7.4) and shrank at acidic condition (pH 2.5). Expansion and shrinkage was repeatedly reversible in response to change in pH.

In literature, numerous drug delivery systems based on swelling-controlled mechanism in response to variations in environmental conditions, e.g. pH, have been reported (Gupta et al., 2002). The polymeric network mesh-size of these systems showed changes with swelling, which permitted or prevented the release of the drugs. Swelling experiments employed to SF–HA complex membranes showed the membranes responded to the variations in pH regardless of the changes in temperature, which represented that SF–HA complex membranes may be utilized in pH-responsive systems for intelligent drug delivery.

3.3. Passive and electro-responsive TM delivery

The profiles for the passive (current density: $I/A = 0$) delivery and iontophoretic (current density: $I/A = 0.5\ \text{mA/cm}^2$) transport of TM through SF–HA membrane were represented in Fig. 7(a) and (b), respectively. Drug was loaded to the donor compartment with a rate of 5 mg/ml at the 15th minute. The iontophoretic delivery dose was 60 mA min (1.5 mA × 20 min) for each pulse, which was followed by 20 min of passive initial section as shown in Fig. 7(d). The higher release observed for the first pulse of the iontophoretic application could be due to a burst effect in which the drug present on the surface layer of the membrane was released instantly upon contact with the receptor solution, maintained under sink conditions. It was also observed that lag time of the membranes decreased after each pulse, which showed the response of the membrane increased upon electric field application. However, this elevation did not change the permeability of the membrane and a reversible behavior was observed since initial passive drug delivery rate was reached after each pulse.

Consequently, the instantaneous flux raised as the current was applied and then declined when the current application was terminated, and this process was repeated on subsequent applications. This showed that the release of TM by permeation through SF/HA complex membranes was enhanced by iontophoresis applied in pulsatile fashion. This represented that these membranes can be modulated for iontophoresis applications. Total mass balance calculations showed that the decline on the peaks caused by two reasons: mainly with respect to decrease in the drug concentration in the donor compartment and possibly due to fouling caused by adsorption of the drug on the membrane surface. In literature,

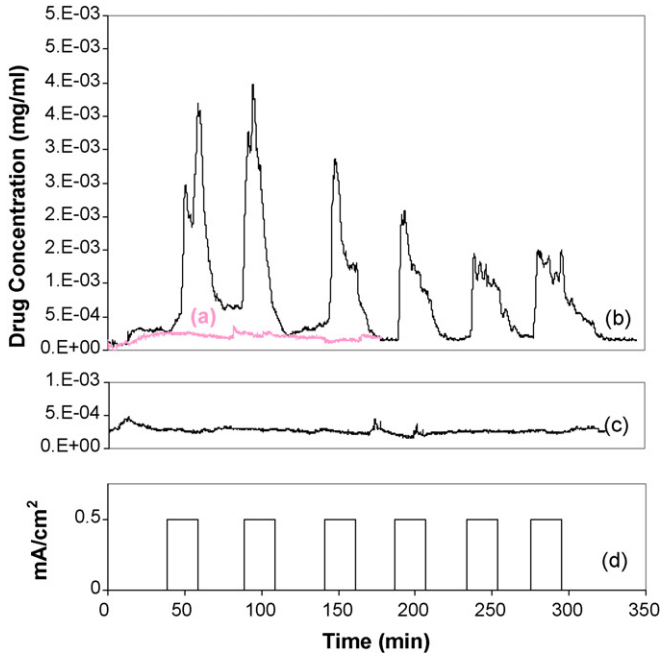

Fig. 7. TM delivery profiles through SF–HA complex membrane. TM permeation through the membrane in passive mode (a), electro-responsive TM permeation from the membrane (b), release from the drug loaded membrane (c), applied electric field profile (d).

it was reported that high adsorption of the drug to the membrane, besides the loss of valuable drug molecule, probably caused fouling of the material, which influences the membrane's permeability (Stamatialis et al., 2002). Since the membrane was shown to be negatively charged in all pH levels, and the drug molecule was positively charged, ionic interactions may have accelerated the possible adsorption mechanism.

Drug release from the drug loaded SF–HA complex membrane, Fig. 7(c), with or without current application was practically same. Formation of strong interactions between the drug and the coacervate complexes led to indistinguishable release. Denser membrane structure formed due to strong interactions did not permit the drug release from the membrane even up to 65% swelling. This showed that polymeric matrix/drug interactions as well as membrane morphology and swelling degree had an important effect on drug release.

4. Conclusions

Present study concentrated on swelling characteristics of silk fibroin (SF)–hyaluronic acid (HA) complex membranes and their potential use in controlled drug release systems. Swelling tests performed on the complex membranes exhibited that the membranes swelled more in alkaline condition and had a pH-responsive swelling behavior. No effect of temperature was observed on swelling characteristics of the membranes at various pH conditions. Swelling tests also revealed that SF–HA complexes can be good candidates for intelligent drug delivery systems, in which drug release is based on the response of the system to environmental conditions. On the other hand, drug release studies showed that the drug permeation through SF–HA complex membranes was enhanced by the application of electric field. The permeation of TM was controlled by the applied electric field in pulsatile fashion. On the other hand, drug loaded complex membrane did not give any response to the applied electric field which was attributed to strong interactions between the matrix and the drug molecules. Consequently, SF–HA complex membranes were found to be promising for electro-responsive drug delivery applications for membrane-permeation-controlled systems, rather than a matrix system in which drug was imprinted.

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