DEVELOPMENT OF TRADITIONAL CHINESE DRUG LOADED HYDROGEL SYSTEM WITH DUAL RESPONSIVENESS (PH AND TEMPERATURE) FOR TEXTILE BASED TRANSDERMAL THERAPY

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Abstract

Thermoresponsive polymer pluronic F-127 was combined with pH-responsive polymers *N,N,N*-trimethyl chitosan and polyethylene glycolated hyaluronic acid to develop dual responsive (pH/temperature) hydrogel. Gallic acid, which is the principal component of traditional Chinese drug Cortex Moutan, was loaded into this hydrogel, and various morphology and physico-chemical characterizations along with drug release property of the hydrogels were studied to validate its applicability and efficiency in textile based transdermal therapy for the treatment of atopic dermatitis. Rheology and tube inversion method were performed to confirm thermoresponsive property of the hydrogel and pH -responsiveness of hydrogel was observed by changing pH of external media.

Keywords: Hydrogel; Dual responsive; Thermoresponsive; pH-responsive; Transdermal therapy

1. INTRODCUCTION

Textile fabrics coated with drug-loaded hydrogel systems have been applied to fight against atopic dermatitis (AD) which is a common skin disease caused due to *Staphylococcus aureus* infection [1,2]. The textile based transdermal therapy can effectively provide both moistures and drugs to the AD infected areas on skin [3]. The hydrogels made of thermoresponsive polymers such as poly(*N*-isopropylacrylamide), pluronic F-127 (PF127) can form gel near body temperature and find suitable and effective drug delivery applications [4]. pH-responsive hydrogels are useful for drug delivery applications as polymers containing pendant acidic (hyaluronic acid) or basic groups (chitosan) are capable of showing changes in their properties in response to changes in pH of external environment [5]. For last few years, dual responsive hydrogels made of both pH-responsive polymers and thermoresponsive polymers have been gaining attention as drug delivery systems because these can be effectively applied for cancer therapy, textile based skin therapy, and these drug delivery systems are reported to be capable of showing better drug loading capacity and site-specific drug delivery [6,7].

Thermoresponsive polymer PF127 and chemically synthesized pH responsive polymers *N,N,N*-trimethyl chitosan (TMC) from chitosan and polyethylene glycolated hyaluronic acid (PEG-HA) from hyaluronic acid (HA) were used to develop dual responsive (pH/temperature) hydrogel in this study and gallic acid which is the principal component of traditional Chinese drug, Cortex Moutan, was loaded into it. The physico-chemical characterizations and morphological properties of the hydrogels along with release property of the hydrogel for drug (gallic acid) were done to elucidate its applicability and efficiency in textile based transdermal therapy.

2. MATERIALS AND METHODS

2.1. Materials

PF127, chitosan (low molecular weight), methoxy-polyethylene glycol amine (OMe-PEG_{2000}-NH_2), HA (8-15 KDa), gallic acid, *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (NHS), *N*-hydroxysuccinimide, methoxypolyethylene glycol amine (EDC), sodium iodide, 1-methyl-2-pyrrolidinone, iodomethane solution (methyl iodide), phosphotungstic acid hydrate, sodium hydroxide, and deuterium oxide were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Synthesis of PEG-HA and TMC, and their chemical characterizations

PEG-HA was prepared in deionized water from HA and OMe-PEG-NH_2 using NHS and EDC as amide coupling agents at room temperature [8], followed by dialysis (3.5-5.0 KDa cut dialysis membrane) against de-ionized water, and finally freeze-dried to get solid sample. TMC was prepared from chitosan, methyl iodide, and sodium iodide using one-step methylation process [9]. Chitosan (0.25 g) and sodium iodide (0.6 g) were first reacted in 10 ml of 1-methyl-2-pyrrolidinone with stirring at 60 °C for 24 h, followed by reaction with 2 ml of methyl iodide and 2 ml of 15% (w/v) aqueous sodium hydroxide solution at room temperature for 2 h under stirring. The product was precipitated using ethanol, followed by its isolation through centrifugation and the same process was repeated for 2 times. The product in 5 ml of water was exchanged with chloride by adding 30 ml of HCl (1 M) in ethanol (96%) and the water soluble TMC was obtained after centrifugation, followed by washing with ethanol and ether, and finally drying in vacuums at 40 °C. ^{1}H-NMR (500 MHz NMR spectrometer, Varian Unity Inova) and FT-IR (Nicolet iS50 FT-IR, Thermo Scientific) analyses were done for the compounds along with pristine chemicals.

2.3. Preparation of hydrogel

The hydrogel (PF127/TMC/PEG-HA) was formed from PF127, TMC, and PEG-HA in deionized water-ethanol mixture along with gallic acid used as a drug in the system. PF127 (1.35 g), TMC (0.008 g), PEG-HA (0.008 g), and gallic acid (0.15) g were added in a mixture of water (7.74 g) and ethanol (0.55 g), followed by incubation at 4 °C to obtain a clear solution. The formation of hydrogel was carried out applying heat to observe sol-gel transition using rheology study and tube inversion method [1]. Rheological measurement and tube inversion experiments were done in the temperature range of 5 to 50 °C. Rheological measurements of formulation PF127/TMC/PEG-HA with drug (gallic acid) was carried out with DVE Brookfield viscometer using spindle 5 (50 rpm) and the temperature was increased manually at a rate of 4 ºC/min over the range of 5 to 50 °C. The gelation temperature of the formulation was determined from the inflection points of observed data of dynamic viscosity. In tube inversion method, the flowability of 10 ml formulation (PF127/TMC/PEG-HA with drug) in 20 ml capped glass vial was checked within the temperature range of 5 to 50 °C.

The formation of hydrogel using heat as an external stimulator is schematically presented in Figure 1 along with its future progress and applications in textile based transdermal therapy.

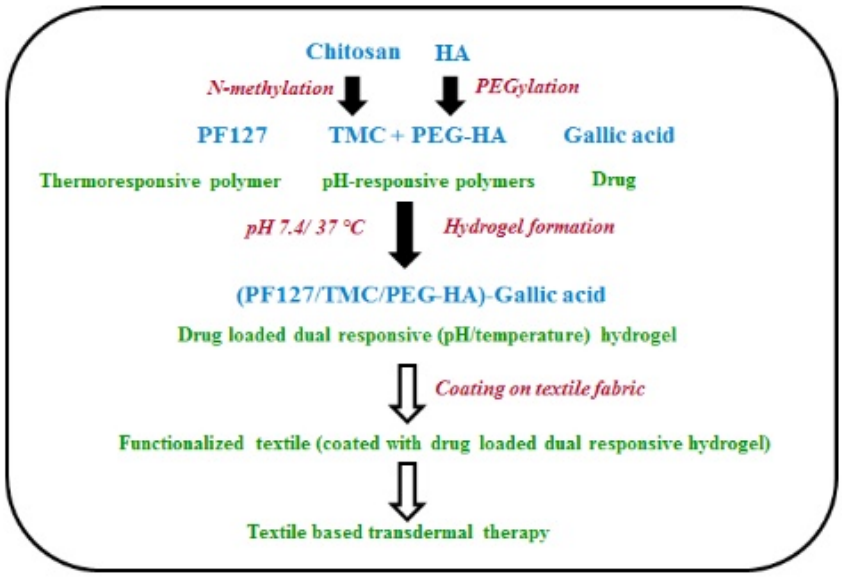

Figure 1. The formation and application of drug loaded dual responsive (pH/temperature) hydrogel in textile based transdermal therapy; schematic presentation

2.4. pH-responsiveness of hydrogel materials by swelling ratio

0.2 g of freeze-dried hydrogel (PF127/TMC/PEG-HA) was swelled separately in 2 ml of pH 5.4 and 7.4 buffers for 4 h at 30 ºC. At different time intervals, sample was collected and dry weight was taken after removing adhered liquid to determine the swelling ratio of freeze dried sample under different pHs. The swelling ratio of hydrogel was determined from equation (1) [10].

$$\text{Swelling ratio} = \left(\frac{W_t - W_0}{W_0} \right) \quad (1)$$

where W_t is the final weight of hydrogel after swelling and W_0 is the initial weight of the freeze dried hydrogel sample.

2.5. Characterization of hydrogels

The SEM of freeze-dried PF127/TMC/PEG-HA was performed using JSM-6490 (Jeol) and TEM of the same formulation in sol phase was done using JEM-2011 (Jeol). PF127/TMC/PEG-HA in sol phase was used during storage of 14 days at 30 °C for zeta potential measurements (ZetaPlus zeta potential analyzer, BIC). The degradation of hydrogel (PF127/TMC/PEG-HA) at pH 7.4 under mechanical stirring (70 rpm) was monitored using PBS at 37 ºC [11].

2.6. The drug release of hydrogel formulations in vitro

The cumulative release of gallic acid from PF127/TMC/PEG-HA was monitored at pH 7.4 using dialysis bag method for 5 days [1]. At designated time intervals, 5 ml of the release media was collected for analysis of gallic acid and replaced with equal volume of fresh PBS

to maintain sink conditions and the drug [gallic acid] released in the buffer (pH 7.4) from hydrogel was measured spectrophotometrically at 265 nm (UH5300, Hitachi).

3. RESULTS AND DISCUSSION

3.1. ^{1}H NMR and FTIR analyses of TMC and PEG-HA

^{1}H-NMR spectra of TMC showed (figure not shown) characteristic peak at 3.36 ppm for *N*-trimethyl group along with the peaks in the range of 3.85-3.55 ppm for H-3 to H-6 and peak for H-2 at 2.68 ppm. ^{1}H-NMR of PEG-HA (figure not shown) showed (-NH-CH_2-CH_2-O-) and ethylene H peak of OMe-PEG_{2000}-NH_2 at 2.76 and 3.60 ppm, respectively, along with glucosidic H at 3.21 ppm and aeetyl H peak at 1.89 ppm of HA.

The FT-IR spectra of TMC (figure not shown) showed characteristic peak of O-H stretching (3431 cm^{-1}), C-H stretching of pyranose ring (2919 cm^{-1}); C-O stretching of amide (1654 cm^{-1}); C-H stretching of methyl-TMC (1503 cm^{-1}); C-H bending of CH_3CO (1390 cm^{-1}); C-O-C stretching (1158, 1066 cm^{-1}). The FTIR spectra of PEG-HA (figure not shown) showed characteristic peaks of O-H stretching (3428 cm^{-1}), C-H stretching of pyranose ring (2892 cm^{-1}), C-O stretching of acetylated NH_2 (1644 cm^{-1}), and C-N stretching (1473 cm^{-1}).

3.2. Thermoresponsive property of hydrogel by rheological analysis and tube inversion method

The dynamic viscosity (Pa.s) changes of system PF127/TMC/PEG-HA against temperature in the range of 5-50 °C (figure not shown) showed gradual increase in dynamic viscosity values with increase in temperature above 25 °C and the formulation exhibited sol-gel transition at 37 °C. The flowability of PF127/TMC/PEG-HA formulation with temperature change was determined by tube inversion method (figure not shown) and it was found that the formulation showed reversible sol-gel transition at 37 °C. PF127chains in the formulation PF127/TMC/PEG-HA use temperature and show gel formation with other two compounds (TMC and PEG-HA) by reversible physical linking of the polymer chains [2].

3.3. Characterizations of hydrogel formulations

PF127/TMC/PEG-HA under mechanical stirring (70 rpm) for 14 days in 0.1 (M) PBS (pH 7.4) at 37 °C showed remaining weight of 45.4% after 14 days (figure not shown). Thereby, PF127 polymer chains along with TMC and PEG-HA formed interconnected network. PF127/TMC/PEG-HA formulation in sol phase exhibited negative zeta potential (-16.3 mV ± 5.9) and the value did not change significantly over storage for 14 days at 30 °C (-14.6 mV ± 7.7).

The characteristic FTIR peaks of drug (gallic acid) loaded freeze dried PF127/TMC/PEG-HA (figure not shown) are O-H stretching and C-H stretching at 3445 cm^{-1} and 2891 cm^{-1}, respectively, of PF127, TMC, PEG-HA, and gallic acid in hydrogel, and CH_2-O-R stretching, R-C-O symmetric stretching, and R-C-O asymmetric stretching at 1282, 1110, and 964 cm^{-1}, respectively, of PF127 in hydrogel.

Figure 2A showed SEM image of freeze-dried hydrogel PF127/TMC/PEG-HA where hydrogel after freeze-drying formed agglomerated porous structure with irregular pore size. The interconnected porous structure accumulated drugs within their pores. Figure 2B showed TEM image of PF127/TMC/PEG-HA formulation in sol state where micellar aggregates in the range of 100 to 500 nm were formed by PF127 chains along with TMC and PEG-HA.

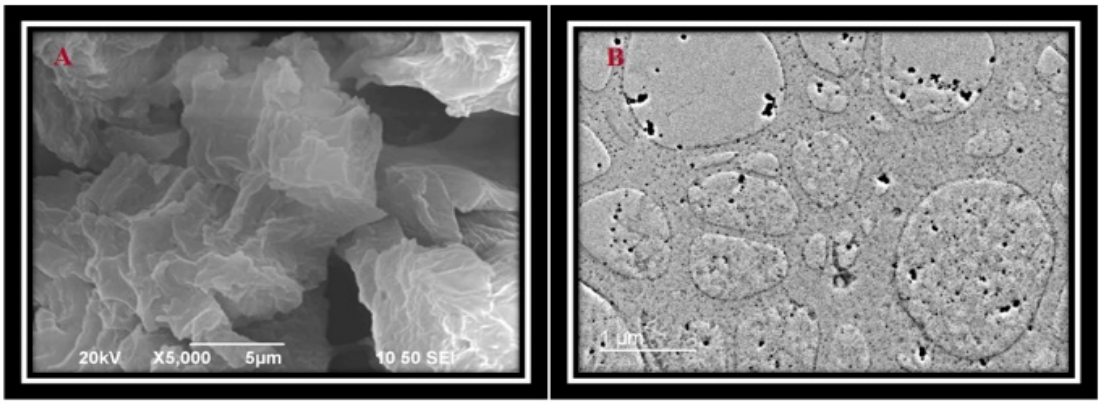

Figure 2. SEM (A) and TEM (B) images of PF127/TMC/PEG-HA) hydrogel formulation

3.4. pH-responsive property of hydrogel by swelling study

The swelling study results of freeze-dried hydrogel (PF127/TMC/PEG-HA) in 0.1 (M) acetate buffer of pH 5.4 at 30 °C (figure not shown) indicated that it completely dissolved in the buffer within 2 h. The rapid dissolution of gel structure under acidic condition occurred due to increased hydrophilicity of PF127 by polymer water interaction [2,4]. Freeze dried PF127/TMC/PEG-HA in 0.1 (M) PBS of pH 7.4 at 30 °C (figure not shown) showed the swelling ratio of 5.01 after 4h suggesting that the interconnected porous network of PF127/TMC/PEG-HA accumulated high amount of water in the swelled state.

3.5. Cumulative release study

The cumulative release of gallic acid from PF127/TMC/PEG-HA was performed at 37 °C using 0.1 M PBS of pH 7.4 for 5 days (figure not shown). It showed release of 64.5% drug (gallic acid) within 5h and the maximum release of 88.0% drug was observed after 5 days. The cumulative release results of PF127/TMC/PEG-HA for gallic acid indicated that this hydrogel system could be used as an effective drug delivery system for transdermal therapy. Moreover, the sustained drug delivery pattern indicated that after being coated on textile fabric, it would be capable of giving protection against skin pathogenesis related to AD.

4. CONCLUSIONS

Gallic acid was loaded into dual responsive (pH/temperature) hydrogel (PF127/TMC/PEG-HA) for application in textile based transdermal therapy. TMC and PEG-HA made form chitosan and HA, respectively, were confirmed by ^{1}H-NMR and FTIR, and combined as pH responsive polymers with thermoresponsive polymer PF127 to develop this dual responsive hydrogel. Various physico-chemical and morphological characterizations like SEM, TEM, zeta potential, FTIR, pH responsiveness, thermoresponsiveness, and gel degradation under mechanical agitation were done along with drug release study to assess its efficiency and applicability as a drug delivery system for treatment of AD through textile based transdermal therapy.

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