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A thermo-sensitive release system based on polymeric membrane for transdermal delivery of doxycycline HCl

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ABSTRACT

A class of intelligent thermo-sensitive membranes was synthesized by immobilizing the thermo-sensitive polymeric gel, poly(*N*-isopropylacrylamide) (PNIPAAM) or PNIPAAM-*co*-2 mol% acrylic acid (AA) on the surface and inside the pores of a hydrophilized polyvinylidene fluoride (PVDF) membrane, hereafter referred to as PNIPAAM-PVDF membrane or 2%AA-PVDF membrane, respectively. Such gel-modified membranes have drastically different permeation properties upon the volume-phase transition of the immobilized gel at its lower critical solution temperature (LCST). The permeability and flux of 2%AA-PVDF membrane at its LCST (33 °C) almost doubled compared to those at 32 °C for the agent doxycycline HCl partitioning from its solution/dispersion in light mineral oil in the donor reservoir bounded by the membrane. Further *in vitro* studies with a mouse skin mounted beneath the 2%AA-PVDF membrane demonstrated that its release can be switched on and off at the LCST of the gel. At 32 °C, there was no doxycycline HCl release through skin for 24 h, while at 33 °C, the ailing condition, 30 μg/cm^2 of doxycycline HCl was accumulated in the receptor through the skin after 24 h with the permeability and flux being very close to those from a regular PVDF/mouse skin composite. Since human skin temperature changes from 32 °C to 33 °C under certain feverish conditions, the gel-modified membranes can be exploited as a transdermal controlled-release system for treating fever symptoms. However, for smaller molecules such as caffeine, different configurations yielded almost the same permeation profiles.

1. Introduction

A transdermal drug delivery (TDD) system based on a membrane-based aqueous–organic partitioning system [1–4] for treating fever (which is the symptom of many diseases [5] like malaria) involves the coating and filling the pores of the membrane with a thermo-sensitive polymer gel. Ideally, the gel collapses and opens up the membrane pores for TDD at feverish temperature of the skin. The choice of a thermo-sensitive polymer gel and its collapse mechanism is crucial for the design of such TDD systems.

Since 1940s many papers have investigated comb-like polymers with long side-chains which can crystallize independent of the main chain [6]. Such polymers may vary in their chemistry details but have one common characteristic: they always possess long alkyl groups extending from the main-chain. The specific property of these polymers is that as their melting point is a critical structure transforming juncture, their permeability can be changed dramatically because of a different morphology around this temperature. That is, below the melting point of the side-chain crystals, the rigid but brittle nature of those semi-crystalline materials becomes effective permeation obstacle with the branch chain-based crystallinity; above such a point they appear softer like a fluid and have much higher permeability owing to their amorphous structure. This unique thermo-sensitive property has found polymers of this category applied to a wide range of areas such as medical, agrochemical and industrial areas in which temperature-controlled release is required. Examples of such applications are seed coating linking germination to soil temperature, pesticide optimum-timing release related to the certain temperature, and nitroglycerin transdermal release in a pulsatile way [7].

Volume-phase transition property of certain polymers has also been applied for thermo-controlled release widely [8–13]. In aqueous solution, such a polymer has a lower critical solution temperature (LCST), below which its linear or branch form dissolves in water and precipitates above it. If it is crosslinked, it forms a thermally responsive hydrogel, which swells below and de-swells above the LCST. Such crosslinked gels in water shrink abruptly above LCST [14].

Among such "intelligent" polymers, poly-*N*-isopropylacrylamide (PNIPAAM) has been intensely studied. In an aqueous solution it has an LCST of about 32 °C [10–12], which is around the normal skin temperature [15]. Its linear or branch form dissolves in water below the LCST and precipitates above it. If it is crosslinked, it

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forms a temperature-responsive hydrogel, which swells below and de-swells above the LCST. Such reversible thermo-sensitive polymer or its gels have been applied in many biomedical/biochemical fields: biosensing, immobilization of enzymes or bioactive materials, controlled drug release, protein purification and affinity separation, etc.

In the case of a TDD system, regulation of the drug delivery is achieved by turning "on" and "off" the rate controlling membrane in the transdermal device. This membrane is fabricated from a thermo-sensitive polymer that undergoes a reversible phase transition at a certain temperature. When the device is in contact with warm skin, the membrane is below its LCST and is "crystalline", therefore, impermeable. But with an ailing body, in most cases the skin temperature rises a little so that the polymer membrane can be warmed above the LCST. Then it becomes amorphous and very permeable to deliver the drug into the blood through skin steadily and continuously until the disease is cured and the skin temperature is back to normal.

In order to make the LCST of PNIPAAM match the changes of physical temperature, several strategies of achieving chemical modifications by tailoring it may be followed: change its hydrophilic/hydrophobic balance [11], i.e., adding extra hydrophilic comonomer like acrylic acid (AA) [10]; change the block length of PNIPAAM [16]; adjustment of pH of polymer solution [17], etc. Among those studied, AA comonomer has been investigated most intensively and presented satisfactory results [10,11]. This approach has been adopted here with transport/release experiments carried out at two temperatures, 32 and 33 °C, to simulate no fever and onset of fever conditions. TDD of doxycycline HCl from a reservoir of the agent in light mineral oil with/without enhancers ethanol/linoleic acid [4] has been studied here using the principle of aqueous–organic partitioning through a porous PVDF membrane appropriately modified. The TDD of caffeine from a reservoir of the agent in 1-octanol through a similar configuration has also been investigated. In a number of experiments, a composite of this membrane and a mouse skin was employed.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Doxycycline HCl (molecular weight (MW): 480.1), Sigma brand, was donated by Integrated Pharmaceuticals, Boston, MA. Caffeine (MW: 194.2) was purchased from Fisher Scientific, Fair Lawn, NJ.

N-isopropylacrylamide (NIPAAM), acrylic acid (99%), N,N-methylene bisacrylamide (99%) (BIS), N,N,N′,N′-tetramethylene diamine (TEMED) and ammonium persulfate (APS) were purchased from Sigma–Aldrich, Milwaukee, WI.

Potassium phosphate monobasic anhydrous and linoleic acid were obtained from Sigma (St. Louis, MO). Light mineral oil, 1-octanol, ethanol, methanol (HPLC-grade) and acetonitrile (HPLC-grade) were purchased from Fisher Scientific, Fair Lawn, NJ. Phosphate buffered saline (PBS) was supplied by Fluka, Milwaukee, WI.

2.1.2. Membranes

Polyvinylidene fluoride (PVDF) Durapore® hydrophilized films were purchased from Millipore Corp. (Bedford, MA). The polymer PVDF is naturally hydrophobic; an additional polymerized layer on the membrane surface and pores makes it hydrophilic. The geometrical characteristics of the membrane are listed in Table 1 [4,18].

Thermo-sensitive gels, PNIPAAM and PNIPAAM-*co*-AA were synthesized by radical polymerization.

Skin membranes: Male hairless mice, strain SkH1, 8 weeks old, were supplied by Charles River Laboratories (Wilmington, MA). Mice were euthanized by carbon dioxide asphyxiation. Their skins were excised and kept at −30 °C until used.

2.2. Experimental procedure

2.2.1. Drug solution

When doxycycline HCl is the agent, and light mineral oil is the solvent, the agent has a very low solubility [4]. The agent amount added was 500 mg into 50 ml of light mineral oil so that there was enough agent present in a suspension. In another case, the agent sample (500 mg) was suspended into 50 ml of 10% (v/v) ethanol in light mineral oil and agitated over a magnetic stirrer for 12 h. Fifteen minutes before applying the agent solution into the donor part of the diffusion cells, 10% (v/v) of enhancer (linoleic acid) was sometimes added into the solution as a suspension and thoroughly mixed.

For caffeine as the agent, an amount of 20 mg of the agent was transferred into 20 ml 1-octanol. Then the suspension was stirred for 12 h before use.

2.2.2. Preparation of thermo-sensitive PVDF membrane

Thermo-sensitive gels based on PNIPAAM and PNIPAAM-*co*-2%AA, were synthesized by radical crosslinking copolymerization in aqueous solution. The synthesis route is shown in Fig. 1 [13]. First an amount of 10 g NIPAAM, 0.5 mol% BIS, 1 mol% APS were transferred into 190 ml water with or without 2 mol% AA. After bubbling with nitrogen for 10 min in a water/ice bath [9], 1 mol% TEMED was added into the solution along with 20 small pieces of PVDF membranes. Then the system was left to polymerize in a water bath at room temperature for 6 h [9]. Before the diffusion experiments, the coated/soaked PVDF membranes were carefully pulled out from the gel solutions and the gel attached on both surfaces of membrane was removed by a tweezer.

2.2.3. Preparation of mouse skin membrane

To prepare skin membranes, the mouse skins were first taken out from the freezer and put into a beaker filled with room temperature water until they were defrosted. The dorsal sites of mouse skins were removed from the adhering fat deposits and then were cut into small pieces of appropriate size and carefully mounted on top of the diffusion cells and left to hydrate for 1 h [4,19].

2.2.4. LCST study of thermo-sensitive gel

The LCSTs of thermo-sensitive gels were determined by differential scanning calorimetry (DSC) using TA Instruments Q100 (TA Instruments, New Castle, DE). Scanning temperatures were from 0 °C to 50 °C at a scanning rate of 5 °C/min.

2.2.5. SEM imaging

Scanning electron micrographs of membranes were obtained using LEO 1530 VP FE-SEM (Carl Zeiss, New York, US).

Table 1
Characteristic properties of polymeric PVDF membrane.

Microporous membrane	Material	Pore size (μm)	Porosity[a]	Tortuosity	Membrane thickness (μm)
PVDF film	Polyvinylidene fluoride	0.1	0.7	2.58[b]	100

[a] Supplied by manufacturer.
[b] Chen et al. [18].

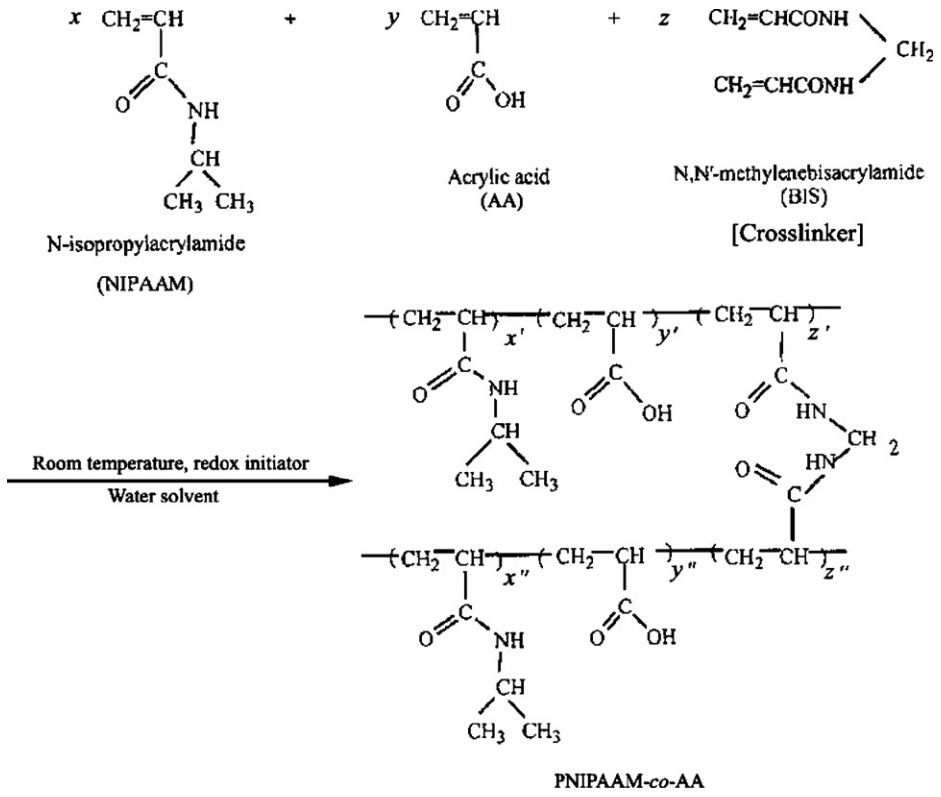


Fig. 1. Schematic of PNIPAAM-*co*-AA synthesis (modified from ref. [13]).

2.2.6. In vitro drug-release studies

Gel-modified PVDF membrane was cut to the shape fitting the Franz diffusion cell. The setup of Franz diffusion cell and sampling has been discussed elsewhere [4,19]. The water temperature of the circulating bath was adjusted so that the surface temperatures of the mouse skin would be either 32 °C or 33 °C, simulating the conditions of normal and fever skin temperatures, respectively. All results are based on an average of three parallel measurements using three different Franz cells.

2.2.7. HPLC analysis

HPLC analysis of caffeine was performed using a Hewlett Packard 1100 LC with a reverse-phase C18 column (Microsorb-MV^{TM}, 15 cm, 5 μm, Agilent Technologies) at a flow rate of 1 ml/min. Caffeine was detected at 270 nm with a mobile phase composition of acetonitrile:methanol:water (10:20:70, by volume ratio) and injection volume of 20 μl. HPLC method for doxycycline HCl has been described elsewhere [4].

2.3. Data processing

The permeation parameters of the agents (i.e., caffeine and doxycycline HCl) were obtained by the following procedure. First, the cumulative corrected amount (μg/cm^2) of an agent permeated through the skin is plotted versus time (h). The slope of the linear portion of the graph is the average flux value (J) at steady state (μg/cm^2 h). The quantities Q_1 and Q_{24} are the accumulations, respectively, for 1 and 24 h in the receptor chamber.

Permeability (P) was evaluated using the following equation

$$P = \frac{\Delta C_{\text{receptor}}}{\Delta t \times A_{\text{receptor}}} \times \frac{V_{\text{receptor}}}{C_{\text{donor}}} \text{ (cm/h)} \quad (1)$$

where Δt (h) is diffusion time, $\Delta C_{\text{receptor}}$ (μg/ml) is the change in agent concentration in the receptor reservoir in Δt (h), C_{donor} (μg/ml) is the agent concentration in the donor reservoir; and A (cm^2) is the area of receptor. Statistical analysis was performed using one-way analysis of variance.

3. Results and discussion

Two types of gel-modified membranes were synthesized and characterized in this study, i.e., the PNIPAAM-PVDF membrane and the 2%AA-PVDF membrane. The LCSTs of the corresponding gels are presented first. Then the release profiles of doxycycline HCl and caffeine through these membranes will be presented. In the end, the release profiles of doxycycline HCl and caffeine through the composite of 2%AA-PVDF membrane and mouse skin are discussed.

3.1. LCSTs of thermo-sensitive polymeric gels

The LCSTs of PNIPAAM and PNIPAAM-*co*-2%AA were found to be 32.6 °C and 33.0 °C, respectively, by DSC. The normal skin temperature is usually 32 °C [15], and in certain situations, skin temperature is 33 °C during a fever. These LCSTs, therefore, match the change of variation in skin temperature when a fever takes place.

3.2. Release profiles from porous thermo-sensitive membranes

Based on LCSTs of the gels, the permeation experiments using doxycycline HCl suspension in light mineral oil as a donor solution were carried out at 32 °C and 33 °C through PNIPAAM-PVDF and PNIPAAM-*co*-2%AA-PVDF membranes, respectively.

Figs. 2 and 3 illustrate the release profiles at 32 °C and 33 °C through PNIPAAM-PVDF membrane and PNIPAAM-*co*-2%AA-PVDF membrane, respectively. The corresponding permeation data are listed in Table 2.

From these data, it is obvious that both gel-modified membranes exhibit a higher permeability, flux and accumulation at 33 °C than at 32 °C. This is because the gels undergo a volume-phase

Table 2
Permeation data of doxycycline HCl released through different thermo-sensitive membranes at different temperatures.

Temp. (°C)	PNIPAAM-PVDF			2%AA-PVDF		
	P (cm/h)	J (μg/cm² h)	Q_1^* (μg/cm²)	P (cm/h)	J (μg/cm² h)	Q_1^* (μg/cm²)
32	0.14 ± 0.06	1440 ± 630	1210 ± 590	0.11 ± 0.09	1050 ± 840	880 ± 700
33	0.16 ± 0.03	1560 ± 280	1770 ± 510	0.19 ± 0.06	1900 ± 570	1390 ± 380

$^*Q_1$: receptor concentration after 1 h.

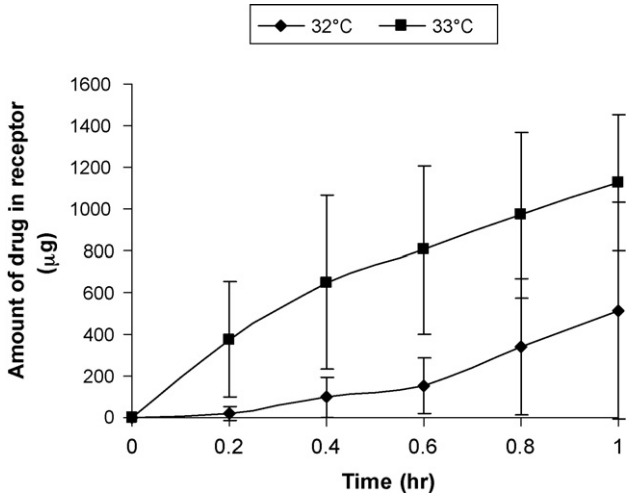


Fig. 2. *In vitro* release of doxycycline HCl using light mineral oil as vehicle through PNIPAAM-PVDF membrane at two temperatures over 1 h.

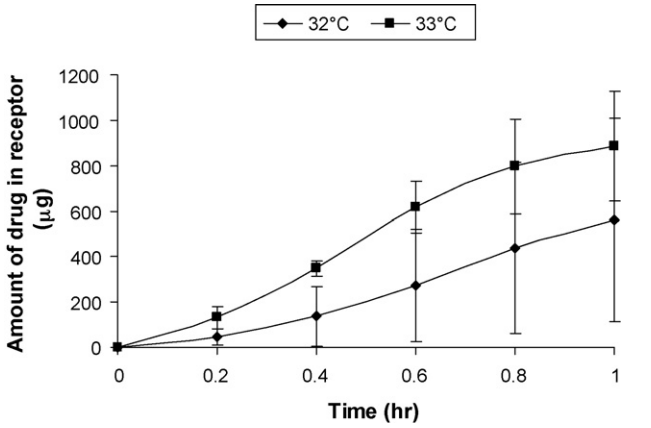
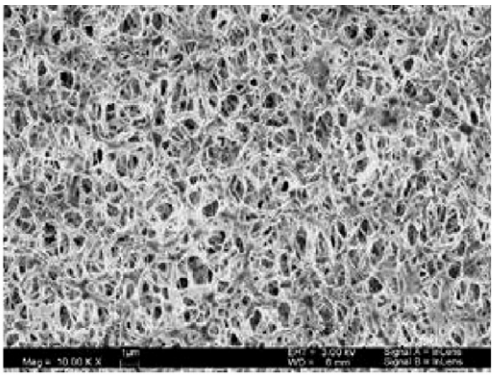
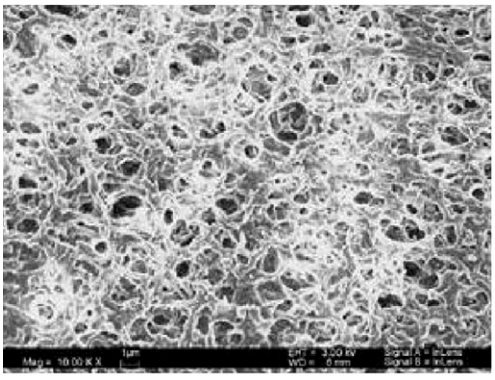


Fig. 3. *In vitro* release of doxycycline HCl using light mineral oil as vehicle through 2%AA-PVDF membrane at two temperatures over 1 h.

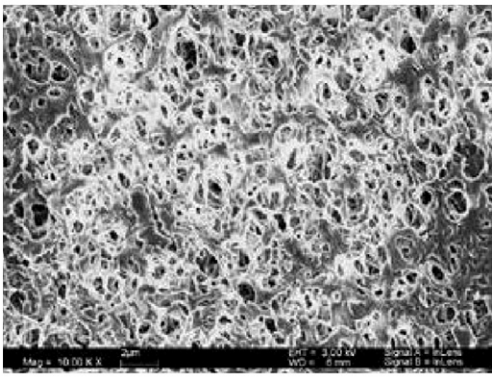
transition and collapse at 33 °C. Membrane pores are therefore opened up to allow enhanced diffusion. The SEM micrographs in Figs. 4 and 5 clearly show that the thermo-sensitive gel, based on either PNIPAAM or PNIPAAM-*co*-2%AA, was both partially coated on the surface of PVDF membrane and invaded into PVDF membrane pores during synthesis. As described in Section 1, it is believed that at a temperature lower than the LCST, e.g., 32 °C, the polymer chains are expanded and occupy membrane pores, which partially block the transport path of doxycycline HCl; when, however, the temperature is raised to 33 °C, the polymer underwent volume-phase



(a) Surface image of PVDF membrane without modification

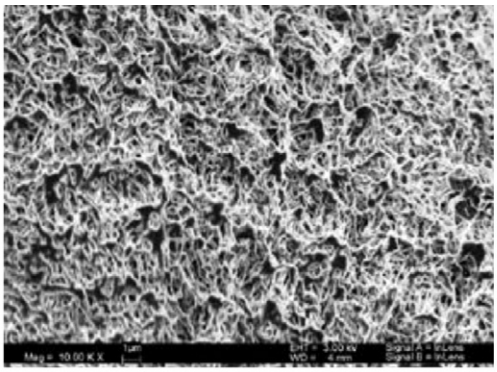


(b) Surface image of PNIPAAM-PVDF membrane

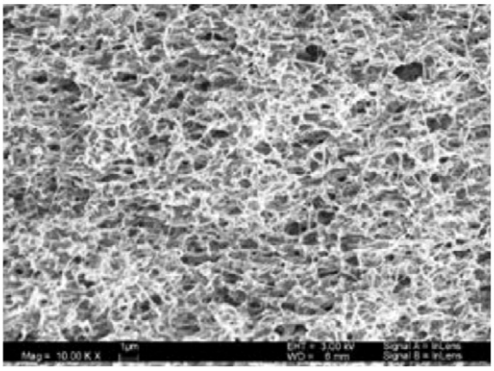


(c) Surface image of 2% AA-PVDF membrane

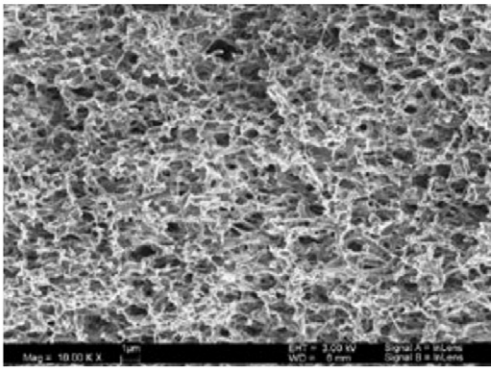
Fig. 4. SEM images of PVDF membrane surfaces with and without modification by thermo-sensitive gels.



(a) Cross section image of PVDP membrane without modification



(b) Cross section image of PNIPAAM-PVDP membrane



(c) Cross section image of 2%AA-PVDP membrane

Fig. 5. SEM images of PVDF membrane cross-section with and without modification by thermo-sensitive gels.

Table 3
Permeation data of DoxyHCl through 2%AA-PVDF and mouse skin membranes over 24 h[a].

Configuration	Temperature (°C)	P (cm/h)	J (μg/cm² h)	Q_{24} (μg/cm²)
2%AA-PVDF + mouse skin	32	0	0	0
	33	2.6E−4 ± 3E−5	2.6 ± 0.3	30 ± 6
Regular PVDF membrane + mouse skin	25 (room temperature)	2.7E−4 ± 5E−5	2.7 ± 0.5	63 ± 9

[a] At the 47th hour, DoxyHCl release was detected in the receptor for the 32 °C system.

transition; the chains collapsed and opened up membrane pores resulting in an increased permeability, flux and accumulation. This situation is depicted in Fig. 6 [8].

The high values of observed standard deviations in Fig. 2 and Table 2 especially at 32 °C are likely to be a result of the variations of the coating on different membrane samples. The coating process wherein the membrane samples were immersed in the polymerization bath and their surfaces were cleared of the gel afterwards was very nonuniform. Such a non-homogeneous coating of the PVDF membranes will lead to considerable transport variability especially for larger molecules like doxycycline HCl.

From Table 2, one can find that the permeation through PNIPAAM-*co*-2%AA-PVDF membrane exhibits a much stronger dependence on temperature than those through PNIPAAM-PVDF. Therefore, 2%AA-PVDF membrane was selected to form a composite with the mouse skin to simulate a TDD environment. The release properties through the composite are discussed next.

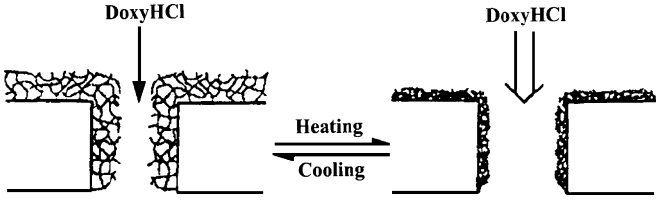


Fig. 6. Thermo-sensitive permeation of doxycycline HCl through polymeric gel-immobilized PVDF membrane (adapted from [8]).

3.3. Release profiles through a composite of porous thermo-sensitive PVDF and mouse skin membranes

Table 3 shows the permeation data of doxycycline HCl release through the composite of 2%AA-PVDF and the mouse skin membranes. For the sake of comparison, the permeation data through

Table 4
Permeation data of caffeine through 2%AA-PVDF and mouse skin membranes.

Configuration	Temperature (°C)	P (cm/h)	J (μg/cm² h)	Q_{24} (μg/cm²)
2%AA-PVDF + mouse skin	32	0.03 ± 0.001	303 ± 12	4120 ± 120
	33	0.03 ± 0.002	315 ± 19	4120 ± 530
Regular PVDF + mouse skin	25 (room temp.)	0.09 ± 0.006	892 ± 62	4820 ± 50

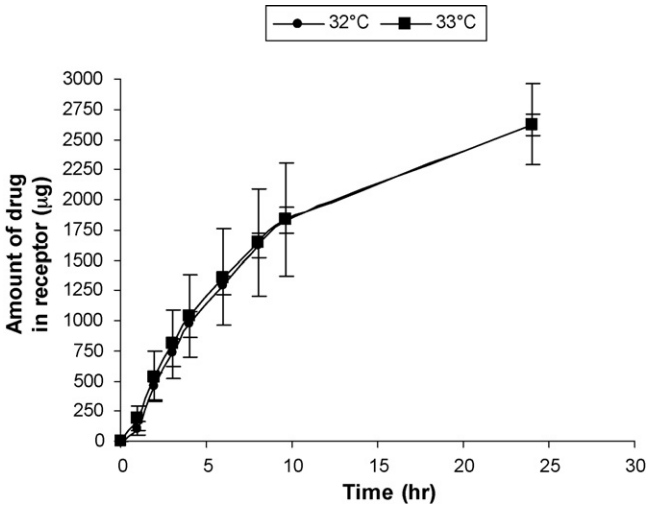


Fig. 7. *In vitro* release of caffeine using 1-octanol as vehicle through 2%AA-PVDF membrane at two temperatures over 24 h.

a composite of a regular PVDF membrane and a mouse skin under the same experimental configuration are also shown in Table 3. By inspection, it is obvious that there is no release of doxycycline HCl from this system for the first 24 h at 32 °C, which is the normal skin temperature, while at 33 °C, which is the skin temperature during a fever, the release data are very close to those from the regular PVDF system except for the 24 h accumulation. This deviation is believed to originate from the increased lag time of agent penetration due to the immobilized thermo-sensitive gel on the PVDF membrane and in its pores. This results in a reduced time period for more agents to permeate. Nevertheless, this system achieves the so called "on-off" switch function expected from an intelligent TDD system. It is to be noted that a small amount of doxycycline was detected in the receptor reservoir at 32 °C after 47 h.

In addition to the permeation experiments using doxycycline HCl, a relatively large MW agent (MW 480.1), a low MW agent, namely, caffeine (MW 194.2) was also studied using the same gel-modified PVDF membranes at 32 °C and 33 °C. The purpose was to explore whether the "on/off" switching capability of the TDD system is still functional when low MW agents are involved. From Fig. 7, one observes little difference in permeation data at these two temperatures. Therefore, for low MW agents such as caffeine, their diffusional rate barely changes in the particular gel-modified PVDF membranes used here regardless of the gel states (i.e., the collapsed state or swollen state). Fig. 7 shows the release profiles of caffeine at two different temperatures, and Table 4 illustrates the permeation data as well as the data of release through a regular PVDF membrane and mouse skin for comparison.

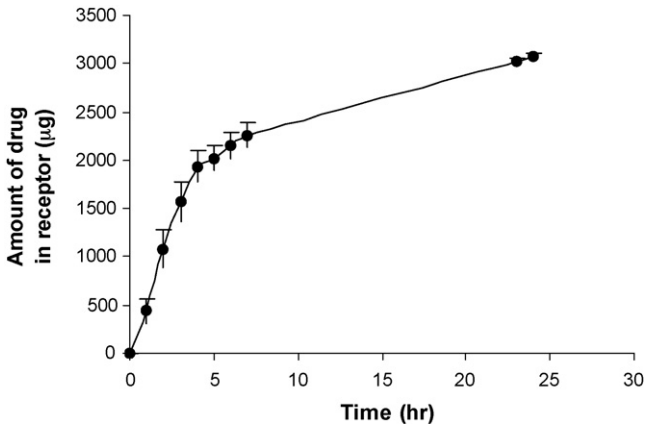


Fig. 8. *In vitro* release profile of caffeine with 1-octanol as vehicle through PVDF membrane and mouse skin over 24 h.

In terms of sustained release, the gel-modified PVDF membranes outperformed the regular PVDF membrane. This is seen in the release profile in Fig. 8. After 24 h, the thermo-sensitive system continued to exhibit a linear release profile, while that of the regular PVDF membrane levels off. As shown in Fig. 7, there is little difference between the release profiles at 32 °C and 33 °C through the 2%AA-PVDF membrane. The release profiles almost overlapped with each other, and the permeation of 33 °C was just slightly higher than that at 32 °C.

4. Concluding remarks

Based on the transport results for two agents, doxycycline HCl (MW: 480) and caffeine (MW: 194), it is indicated that the gel-modified PNIPAAM-PVDF and PNIPAAM-*co*-2%AA-PVDF membranes exhibit the on/off switching capability for high MW agents such as doxycycline HCl. For low MW agents, such as caffeine, the switching capability is no longer observed. Therefore, other thermo-sensitive polymeric gels are needed to make the PVDF membrane more "dense" at 32 °C to minimize the release of smaller MW agents, or employ membranes of smaller pore size/porosity immobilized by PNIPAAM gel to achieve the same goal.

This research assumed that the skin temperatures under normal condition and during a fever are 32 °C and 33 °C, respectively. This is valid for certain situations. However, it is important to note that the variations of both body and skin temperatures are more complicated. Skin temperatures of different body parts, adults and children vary [20,21]. When fever occurs, with less circulation in the capillaries of skin tissue, the skin temperature could even go lower than normal [22]. Therefore, a number of other aspects have to be considered in future to design such a thermo-sensitive TDD system to be more useful.

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