pH- and Temperature-Sensitive *In Vitro* Release of Salicylic Acid through Poly(vinyl alcohol-*g*-acrylamide) Membranes

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ABSTRACT: In this study, acrylamide (AAm) was grafted onto poly(vinyl alcohol) (PVA) in solution with UV radiation, and membranes were prepared from the graft copolymer (PVA-*g*-AAm) for transdermal release of salicylic acid (SA) at *in vitro* conditions. Permeation studies were carried out using a Franz-type diffusion cell. Release characteristics of SA through PVA and PVA-*g*-AAm membranes were studied using 2.0 mg/mL SA solutions. Effects of the presence of AAm in the copolymer, pH of donor and acceptor solution, and concentration of SA and temperature on the release of SA were investigated. Permeation of SA through the membranes was found to be pH-dependent, and increase in pH generally increased the release percentage of SA, and the presence of AAm in the membrane positively affected the permeation. The effect of concentrations of SA on the permeation was also searched using saturated solution of SA, and permeated amount of SA was found to be less than in the case of unsaturated SA solution. Studies showed that the release of SA from PVA-*g*-AAm membranes was temperature-sensitive and increase in temperature increased the permeation rate. 82.76% (w/w) SA was released at the end of 24 h at (39 ± 1)°C, and the overall activation energy for the permeation of SA through PVA-*g*-AAm membranes was found to be 19.65 kJ/mol. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 3005–3012, 2008

Key words: graft copolymers; membranes; drug delivery systems; pH–temperature sensitive; salicylic acid

INTRODUCTION

The skin as a route for systemic drug administration has become very attractive, and skin-penetration studies play an essential role in the optimization of drug and formulation design in dermal and transdermal delivery. The most applied polymers onto skin belong to various classes, as cellulose derivatives, chitosan, carageenan, polyacrylates, poly(vinyl-alcohol) (PVA), poly(vinylpyrrolidone), and silicones.[1] PVA is a biocompatible, chemically stable, nontoxic, and noncarcinogenic polymer, and it is desirable for both bioseparations and cell encapsulation.[2–9] However, crystallinity limits the performances of PVA membranes. To reduce the crystallinity and thus to increase the membrane permeability of PVA, it is generally modified with hydrophilic compounds.[10,11] Polyacrylamide is also a biocompatible polymer[12] that can be easily synthesized either chemically or by using radiation[13] and is used to increase the hydrophilicity of PVA. Aminabhavi and Naik[14,15] have increased the hydrophilicity of PVA membranes by grafting PVA with acrylamide (AAm) and tried to separate aqueous organic solutions by pervaporation, which is one of the membrane-separation techniques.

SA is an active component of aspirin, and the regular use of aspirin by adults appears to reduce the risk of many diseases such as colon cancer, lung cancer, breast cancer, Alzheimer and heart diseases, etc. However, it has the drawback of dyspepsia and gastrointestinal problems. One way to overcome these drawbacks is to use transdermal route. There is limited number of studies related with the transdermal release of SA using synthetic polymeric membranes.[16–22]

In this study, AAm was grafted on PVA by using UV radiation, and membranes were prepared from the copolymer for the transdermal release of SA. The effects of pH of donor and acceptor solutions, the concentration of SA, and the temperature were investigated.

EXPERIMENTAL

Materials

PVA ($\overline{M}_w$ = 72,000 g/mol, degree of saponification >98%), SA ($C_7H_6O_3$; 138.12 g/mol), acetone (C_3H_6O; 58.08 g/mol; 0.79 kg/L), acrylamide (C_3H_5ON; 71.08 g/mol), dimethyl sulfoxide (DMSO; $(CH_3)_2SO$), and hydroquinone ($C_6H_6O_2$) were all from Merck (Hohenbrunn, Germany). Disodium hydrogen phosphate ($Na_2HPO_4{\cdot}2H_2O$), sodium dihydrogen phos-

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TABLE I
The Elemental Analysis of PVA-*g*-AAm and the Percentage of Acrylamide in the Copolymer

N%	8.9
C%	48.8
H%	8.0
AAm % of PVA-*g*-AAm	45.0

phate ($NaH_2PO_4 \cdot 2H_2O$), and benzophenone ($C_{13}H_{10}O$) were purchased from Fluka (Steinheim, Germany). Sodium acetate (CH_3COONa) and acetic acid (CH_3COOH) were all Reidel products (Steinheim, Germany).

Synthesis of PVA-*g*-AAm copolymer

PVA (10% w/v) and AAm (6*M*) were put into a three-necked UV-cell (Helios GR.E.125W, Helios Italquartz) equipped with a magnetic stirrer and N_2 inlet. After a mixing period of 30 min in N_2 atmosphere, 0.1% (w/w) benzophenone was added as a photosynthesizer. Polymerization reaction was carried out for 6 h with UV light. At the end of the polymerization period, a saturated solution of hydroquinone was supplemented to the polymerization mixture, and the synthesized copolymer was precipitated in excess amount of acetone, filtered, and dried at 60°C in a drying oven. Then the precipitate was dissolved in DMSO, undissolved polyacrylamide was removed, copolymer was reprecipitated in acetone, filtered, and vacuum-dried. The percentage of AAm in the copolymer was calculated from the elemental analysis of PVA-*g*-AAm as 45.0% (w/w) by using eq. (1) (Table I).

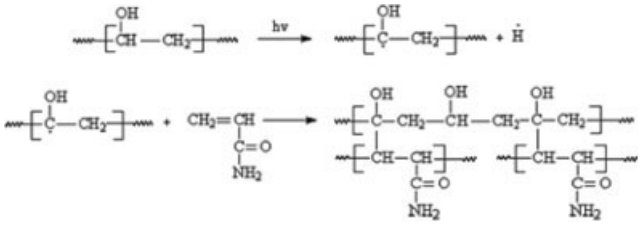
$$\%A = (a/M_e)M_m \quad (1)$$

where $\%A$ is the mass % of the monomer in the copolymer, a the mass % of an element (N%) in the copolymer other than carbon and hydrogen, M_m the molar mass of the monomer, and M_e is the atomic weight of the element for which the elemental analysis was performed.

The proposed mechanism for the grafting process was given in Scheme 1.

Preparation of PVA membranes

PVA membranes were prepared by using aqueous solution of PVA at a concentration of 5.0% (w/v). Predetermined amount of polymer solution was cast onto the petri dishes. After complete dryness, they were heat-treated at 150°C for 1 h for crosslinking, and they were preserved in the studied buffer solutions till to use. The thickness of the PVA membranes was determined as 20 ± 5 μm by using precision micrometer (Aldrich) (Milwaukae, USA).



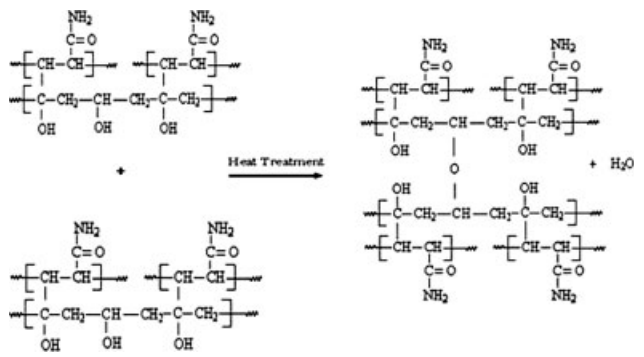
Scheme 1 Proposed mechanism for grafting AAm on PVA.

Preparation of PVA-*g*-AAm membranes

PVA-*g*-AAm membranes were prepared from the aqueous solutions of the copolymers at a concentration of 5% (w/v). Similar to the PVA membranes, PVA-*g*-AAm membranes were also heat-treated at 150°C for crosslinking and preserved in buffer solutions. The thickness of the PVA membranes was determined as 20 ± 5 μm. The proposed crosslinking reaction can be given as Scheme 2.

Infrared analysis

Infrared spectra of PVA and PVA-*g*-AAm membranes were measured with Fourier transform infrared (FTIR) Spectrometer (Unicam Co., Mattson 1000) (UK) and were presented in Figure 1. The spectrum of PVA shows a characteristic broad band at around 3440 cm^{-1} corresponding to O—H stretching vibrations of the hydroxyl group of PVA. These O—H stretchings are also observed in the spectra of PVA-*g*-AAm, indicating that all the hydroxyl groups in PVA are not involved in grafting. The peak due to N—H vibrations of the primary amide groups overlaps with the O—H stretching vibrations. The absorption bands at 3000–2850 cm^{-1} belong to aliphatic C—H bond. The C=O stretching vibrations of PVA appear at around 1720 cm^{-1} because of the unhydrolyzed vinyl acetate groups present in PVA. The increased band intensity at 1720 cm^{-1} can be attributed to the additional C=O groups introduced



Scheme 2 Proposed crosslinking reaction mechanism of PVA-*g*-AAm membranes.

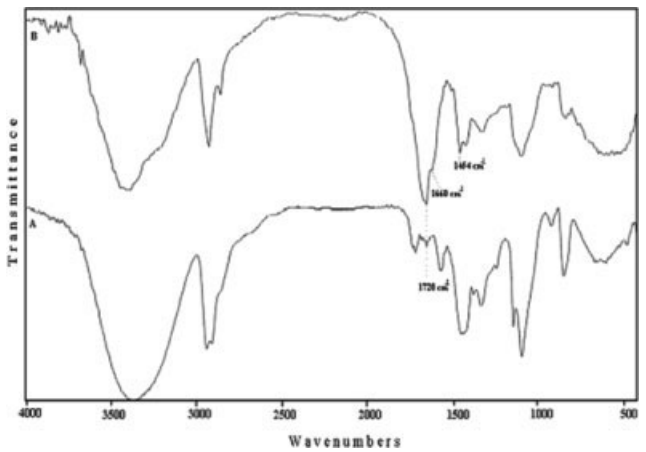


Figure 1 FTIR spectrum of (A) PVA and (B) PVA-*g*-AAm membranes.

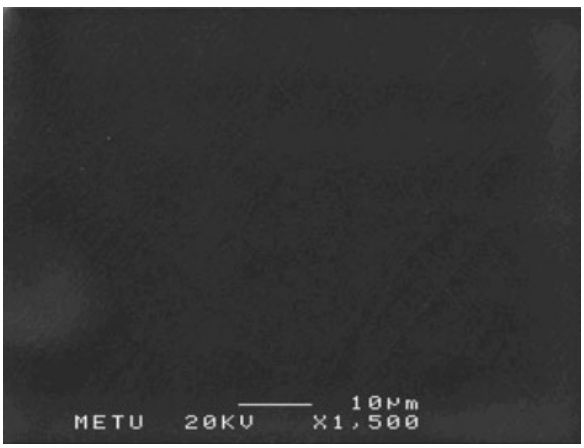


Figure 2 SEM micrograph of PVA membrane (×1500).

due to the grafting of AAm on to PVA. Grafting was also confirmed by the appearance of absorption at 1660 cm^{-1} corresponding to antisymmetric N—H bending and the C—N strechings at 1454 cm^{-1}. The stretching vibrations at around 1150–1000 cm^{-1} belong to C—O bonds.

Scanning electron microscope studies

For scanning electron microscope (SEM) analysis, the dried membranes were sputtered with gold in vacuum before viewing under the microscope (Model JEOL, JEM-100CXII). It was determined that both PVA and PVA-*g*-AAm membranes were homogenous–nonporous membranes as reflected by SEM micrograph in Figures 2 and 3 respectively.

Swelling studies

Swelling degree percentage, SD%, values of the membranes were computed at a temperature of (32 ± 1)°C at a pH range of 2.10–7.4 by using eq. (2).

$$\mathrm{SD\%} = \frac{W - W_0}{W_0} \times 100 \qquad (2)$$

where W and W_0 are the wet and dry masses of the membranes, respectively.

Permeation experiments

Permeation experiments were carried out at (32 ± 1)°C, (37 ± 1)°C and (39 ± 1)°C by using Franz diffusion cell, which consists of two parts (donor and acceptor) that is separated by a membrane. The donor compartment holds the drug preparation and the acceptor compartment the receiver medium. Three milliliters of 2 mg/mL salicylic acid (SA) solution at different pH values (pH, 2.10–7.40) was used as donor solution in the upper compartment, and phosphate (pH 7.4) or acetate (pH, 2.1–5.0) buffer solution was used as acceptor solutions in the lower compartment of the cell. Silicone grease was used to produce leak-proof seal between the membrane and two compartments of the cell. The sampling arms and the donor compartment of the cell were occluded to prevent the evaporation of the solvent. The lower compartment of the cell (acceptor solution) was stirred by using a magnetic stirrer operating at constant rotation rate to obtain uniform composition during the permeation. The receiver solution was sampled periodically, and samples were replaced with equal volume of fresh receiver solution. The analysis of the samples was carried out spectrophotometrically at 298 nm by using Unicam UV2-100 UV–Vis Spectrophotometer. All of the data points are the average of at least three experimental results. The experiments are fairly reproducible.

Permeability coefficient (P) is a measure of the permeation ability of a membrane and it can be determined from the eq. (3).[22]

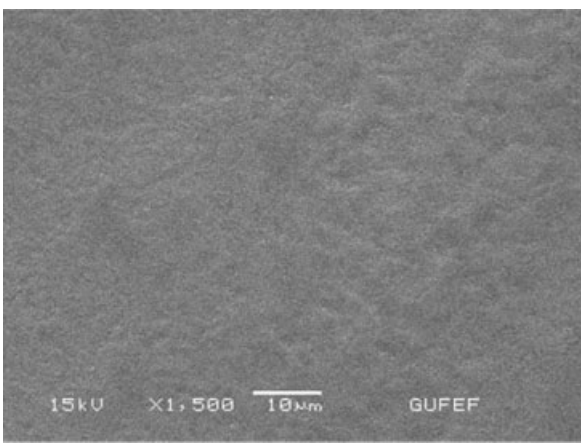


Figure 3 SEM micrograph of PVA-*g*-AAm membrane (×1500).

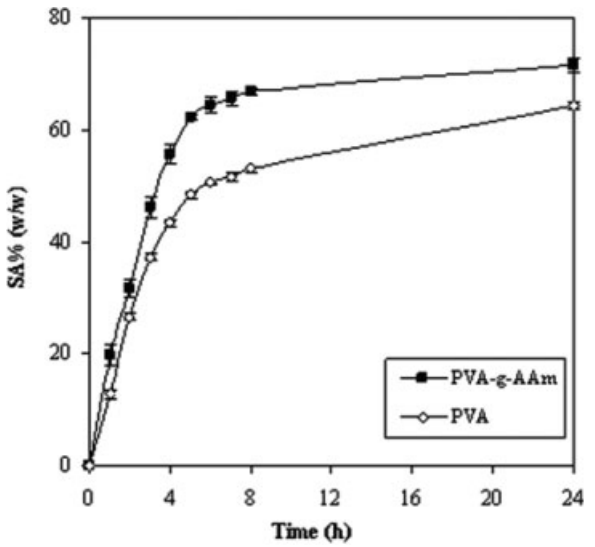


Figure 4 Effect of the presence of AAm on the performance of PVA-*g*-AAm membranes [SA = 2.0 mg/mL; T = (32 ± 1)°C; pH (donor) = 2.1; pH (acceptor) = 7.4].

$$Q_t = \frac{PC_0^D}{L}\left(t - \frac{L^2}{6D}\right) \quad (3)$$

where C_0^D is the concentration of the donor side of the cell, D the diffusion coefficient, L the thickness of the membrane, t the time, and Q_t is the amount of drug diffused through the membrane unit area at time t.

RESULTS AND DISCUSSION

Effect of the presence of AAm on the permeation behavior of PVA membranes

To determine the effect of the presence of AAm on the release of SA through the PVA-*g*-AAm membranes, permeation studies were carried out using 2.0 mg/mL of SA as donor solution (pH 2.1) at (32 ± 1)°C, and the pH of the acceptor solution was kept constant at pH 7.4. The results were represented in Figure 4.

As it is seen from the figure, the release percentage of SA through the PVA-*g*-AAm membranes is greater than the PVA membranes, especially after 4 h of permeation time. The transfer of the molecules through a membrane is mainly affected from the swelling behavior of the membrane. As the SD% value of a membrane increases, the amount of the free volume which is suitable for the permeation and diffusion of the molecules increases. SD% values of PVA and PVA-*g*-AAm membranes were determined at (32 ± 1)°C at a pH of 2.1 by using eq. (2) and given in Table II. As it is seen from the table, SD% value for PVA-*g*-AAm membrane is much greater than PVA membranes, and it can be stated that the presence of AAm increases the hydrophilic character of PVA membranes. This could be the reason for getting the high release % of SA from the PVA-*g*-AAm membranes.

The permeability coefficients of PVA and PVA-*g*-AAm membrane were calculated by using eq. (3) and given in Table II. From the P values of the membranes, it is clearly seen that introduction of the AAm group to the PVA membranes increases the permeability for SA. Although there is a very big difference between the SD% values of PVA and PVA-*g*-AAm membranes, the difference between the P values is not so great. This may be the result of the interaction of —COOH groups of SA with the amide groups of PVA-*g*-AAm by hydrogen bonding. Diffusion coefficients for the permeation of SA through PVA and PVA-*g*-AAm membranes were also calculated by using Fick's first law and presented in Table II.

Effect of pH of donor solution on the release of SA through PVA-*g*-AAm membranes

The effect of the pH of the donor solution on the release characteristics of SA through the PVA-*g*-AAm membranes was studied at (32 ± 1)°C by using 2.0 mg/mL of SA solution at pH values of 2.1–7.4. The pH of the acceptor compartment was kept constant at 7.4 by using phosphate buffer solution. The result of the permeation studies was given in Figure 5.

As it is reflected from the figure, although the released amount of SA increased with the increase of pH in the range of pH of 3.5–7.4, SA% value at pH 2.1 is close to that obtained at pH 7.4. This could be explained by the acidic hydrolysis of amide groups of PVA-*g*-AAm membrane at pH 2.1.

The mechanism of the hydrolysis of N-substituted or *N,N*-disubstitude amides in aqueous acid solutions is suggested to be as follows[23] (Scheme 3).

The hydrolysis of the amide groups of PVA-*g*-AAm makes the membrane more hydrophilic by C=O and —OH groups at pH 2.1 than pH 3.5, 4.3, and 5.0, resulting in high SD% value [T = (32 ± 1) C] as given in Table III. As it is seen clearly from the table, SD% of the PVA-*g*-AAm membrane is high at

TABLE II
SD% Values Permeability and Diffusion Coefficients of PVA and PVA-*g*-AAm Membranes

Membrane	SD% (w/w) at pH = 2.1	P (10^4 cm^2/h)	D (10^4 cm^2/h)
PVA	60.60	2.01	3.81
PVA-*g*-AAm	462.00	2.64	6.96

T = (32 ± 1)°C; pH (donor) = 2.1; pH (acceptor) = 7.4.

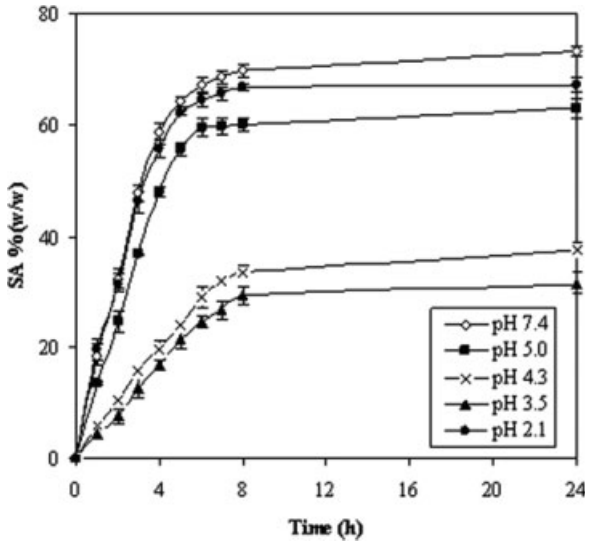


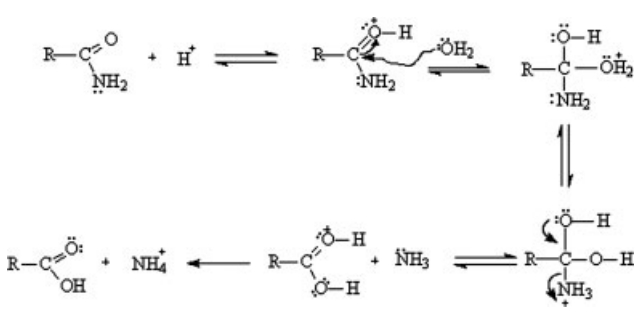
Figure 5 Effect of pH of the donor solution on the release of SA through PVA-*g*-AAm membrane [SA = 2.0 mg/mL; $T = (32 \pm 1)^\circ C$; pH (acceptor) = 7.4].

pH 2.1 compared to pH 3.5, 4.3, and 5.0, leading to high amount of SA release.

Effect of pH of acceptor solution on the release of SA

To determine the effect of ionization upon the transport of SA across the PVA-*g*-AAm membranes, the permeation process was carried out using an acceptor solution that has the same pH with the donor solution. The concentration of the SA solutions and the temperature were kept constant at 2.0 mg/mL and $(32 \pm 1)^\circ C$, respectively. The permeation profile of the membranes was given in Figure 6.

As it is seen from the figure, percentage release values of SA at pH 3.5, 4.3, and 5.0 are all above 60% (w/w) release. Furthermore, from the comparison of Figure 6 with the Figure 5, it can also be said that permeated amount of SA increased for pH (3.5–5.0) when the acceptor and donor parts have the same pH.



Scheme 3 The mechanism for the hydrolysis of N-substituted or *N,N*-disubstituded amschides in aqueous acid solutions.

TABLE III
SD% Values of PVA-*g*-AAm Membranes in 2.0 mg/mL SA Solution and Ionization % of SA at Different pH Values [$T = (32 \pm 1)^\circ C$]

pH	SD% of PVA-*g*-AAm membrane (w/w)	Ionization % for 2.0 mg/mL SA
2.10	462	9.00
3.50	344	72.00
4.30	357	94.00
5.00	415	99.00
7.40	424	99.99

It is suggested in early studies that only the unionized forms of drugs are able to pass through the membranes.[16] However, there has been increasing evidence that ionized species can also contribute to the transdermal permeation of ionizable drugs.[24] The *in vitro* diffusion of compounds can not only be significantly influenced by the physicochemical properties of the vehicle and the drug but also affected from the type of the membrane and the receiver phase.[21] Increase in pH generally increases the SD% of PVA-*g*-AAm membranes and percent ionization of SA as given in Table III. Increase in degree of swelling of the membranes allows the SA to pass through the membrane, both in ionized and unionized forms because of the increase in the free volumes of the membrane material. In the case of adjusting the donor solution pH in the range of 2.1–7.4 while setting the acceptor solution pH to 7.4, the permeated unionized SA molecules tend to be ionizable at this pH with a very high percentage (99.99%), and salicylate ions probably interact with

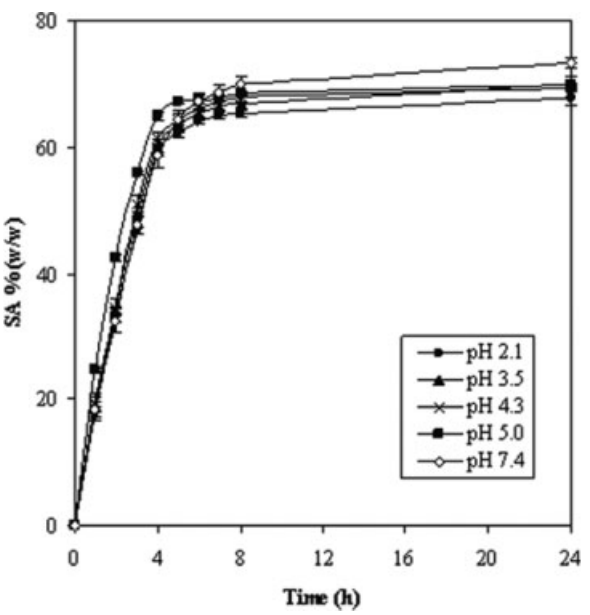


Figure 6 Effect of pH of the acceptor solution on the release of SA through PVA-*g*-AAm membrane [SA = 2.0 mg/mL; $T = (32 \pm 1)^\circ C$].

TABLE IV
Permeability Coefficients of PVA and PVA-*g*-AAm Membranes for the Saturated and Unsaturated SA Solutions

Membrane	SA (mg/mL)	P (10^4 cm^2/h)
PVA-*g*-AAm		
Unsaturated	2.00	2.64
Saturated	3.61	1.56
PVA		
Unsaturated	2.00	2.01
Saturated	3.61	1.03

T = (32 ± 1)°C; pH (donor) = 2.1; pH (acceptor) = 7.4.

the membrane material via hydrogen bonding at the acceptor side, and so this causes the observed flux of SA to decrease (Fig. 5). However, the lower pH values than 7.4 in the acceptor compartment prevents such interaction because of low ionization of SA, and so high swelling and low ionization causes an increase in release percentage of SA through PVA-*g*-AAm membranes. The reason of getting close release percentages of SA at pH 2.1 and 7.4 could be attributed to the possible hydrolysis of amide groups of PVA-*g*-AAm at pH 2.1 as stated previously, which leads to high SD% of PVA-*g*-AAm membranes at this pH value (Table III).

Effect of SA concentration on the release of SA

To investigate the effect of the concentration on the permeation behavior of PVA-*g*-AAm membranes, saturated solutions of SA at pH 2.1 were prepared and permeation studies were performed by keeping the pH of the acceptor solution at pH 7.4. The temperature was kept at (32 ± 1)°C. The permeability coefficients of the membranes were calculated from the slope of the linear portion of Q_t versus t graphs according to the eq. (3), and they were presented in Table IV together with the P values of PVA membranes for comparison purposes. As it is seen from the table, the permeability of PVA-*g*-AAm membrane for the saturated solution of SA is lower than the unsaturated SA, and the presence of AAm increased the permeability of PVA-*g*-AAm membranes for both saturated and unsaturated SA solutions. The decrease in permeability for saturated solutions supports the idea that the transport of SA through the PVA-based membranes occurred mainly in dissociated form.

If the linear plot of Q_t versus t is extrapolated to Q_t axis, the resulting intercept includes ($L^2/6D$) term called as lag time [eq. (3)]. Figure 7 shows this plot, and as it is seen from the figure, the resistance of the PVA-*g*-AAm membrane to the permeation of SA is negligible.

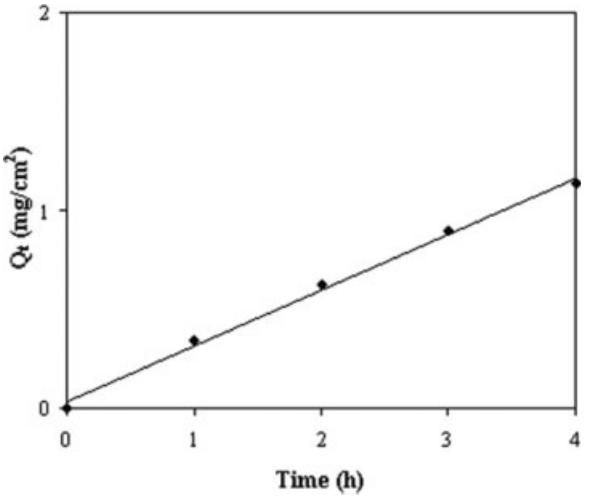


Figure 7 Q_t versus t diagram for the permeation of saturated SA through PVA-*g*-AAm membrane [T = (32 ± 1)°C; pH (donor) = 2.1].

Effect of temperature on the release of SA

To investigate the dependence of the permeation of SA on the temperature for the PVA-*g*-AAm membranes, the permeation studies were done at (32 ± 1)°C, (37 ± 1)°C, and (39 ± 1)°C. The pH of 2.00 mg/mL SA solution was kept at 2.1 and the pH of the acceptor compartment was adjusted to 7.4. The results of the permeation were presented in Figure 8.

As it is reflected from the figure that the release of SA is temperature-dependent and increase in temperature increased the released amount of SA. The increase in the permeated amount of the SA with

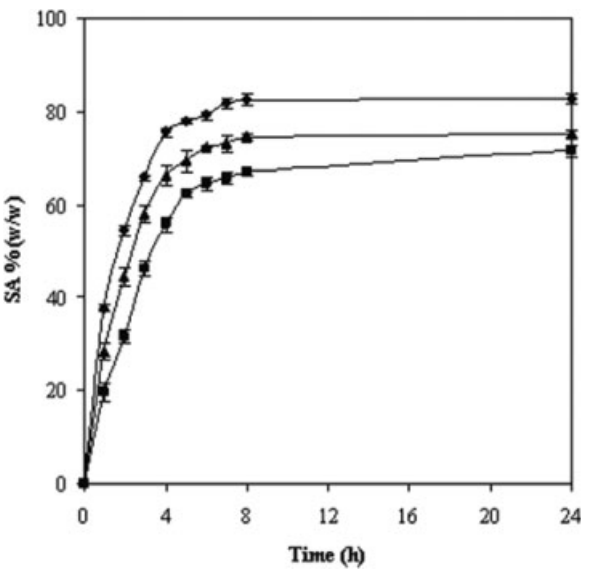


Figure 8 The effect of the temperature on the permeation of SA through PVA-*g*-AAm membranes [■ (32 ± 1)°C; ▲(37 ± 1)°C; ◆ (39 ± 1)°C; SA = 2.0 mg/mL; pH (donor) = 2.1; pH (acceptor) = 7.4].

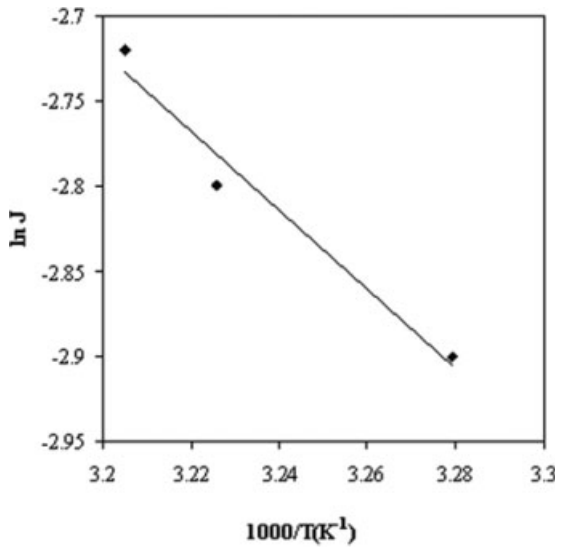


Figure 9 ln J versus $1/T$ diagram for the permeation of SA through PVA-*g*-AAm membranes.

temperature can be explained by free volume theory.[25] According to this theory, the thermal motion of polymer chains in the amorphous regions randomly produces free volume. As the temperature increases, the frequency and the amplitude of the chain jumping increase and the resulting free volumes become larger for the diffusion of SA molecules, leading to high percentage release. Şanlı et al.[22] studied the release of SA through PVA/PVP membranes and found that released amount of SA increased with the increase in the temperature. Neuhoff et al.[26] also studied the effect of the temperature on the permeation of SA solutions through caco-2-cell monolayers. They have stated that the transport of SA was temperature-dependent, and the activation energy (which were estimated from the Arrhenius plots) for the transport of SA through caco-2-cell monolayers changed between 50 and 70 kJ/mol depending on the pH of donor compartment (pH, 5.0–7.4) when the pH of acceptor compartment was kept constant at 7.4.

Additionally increase in temperature increases the mobility of the permeants.[27] Therefore, interaction between the permeating particles and also with the membrane material decreases. This also accelerates the permeation positively and results in an increase in the permeated amount of SA. Similar trends were observed in the studies given in the literature. Huang and Yeom[28] stated that the interaction between the permeated molecules and also with the membrane material decreases with an increase in the temperature. The same relation was also stated by Asman and Şanlı.[29]

The transfer of the molecules through a polymeric membrane follows an Arrhenius type of relation.[25,28,30]

$$J = A \exp\left(\frac{E_a}{RT}\right) \quad (4)$$

where J is the mass transferred per unit area in unit time [mg/(cm^2 h)], A is the preexponential factor, and E_a is the overall activation energy in kJ/mol. E_a was determined from the slope of linear regression line of logarithmic permeation rate versus the reciprocal of the absolute temperature within PVA-*g*-AAm membranes (Fig. 9), and the overall activation energy of permeation was found to be 19.65 kJ/mol.

CONCLUSIONS

Following results can be withdrawn from the study:

1. AAm was grafted on PVA by using UV radiation and the percentage of AAm in the copolymer was calculated as 45.0% (w/w).
2. Grafting AAm onto PVA increased the hydrophilic character of the PVA membranes.
3. Grafting of AAm onto PVA affected the permeation behavior of PVA membranes and the released amount of SA from PVA-*g*-AAm membranes was found to be higher than that of PVA membranes.
4. pH affected the permeation of SA through PVA-*g*-AAm membranes and increase in pH of the donor solution generally increased the release percentage of SA when the pH of the acceptor solution was kept at 7.4.
5. It was determined that at high SD% values of PVA-*g*-AAm membranes and at low ionization percentages of SA, the released percentage of SA was found to be high.
6. The released % of SA for saturated SA solution was found to be lower than the unsaturated SA.
7. Temperature affected the permeation rate of SA through PVA-*g*-AAm membranes. Increase in temperature increased the permeated amount of SA.
8. Overall activation energy for the permeation of SA through PVA-*g*-AAm membranes was found to be 19.65 kJ/mol.
9. Highest permeation of 82.76% (w/w) of SA for PVA-*g*-AAm membranes was obtained at (39 ± 1)°C at the end of a period of 24 h.

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