CHAPTER V

EVALUATION AND CONTROLLED RELEASE CHARACTERISTICS OF MODIFIED XANTHAN FILMS FOR TRANSDERMAL DELIVERY OF ATENOLOL

This chapter deals with the development of transdermal delivery system based on modified xanthan gum (XG) for the controlled release (CR) of an antihypertensive drug, atenolol (ATL). The formulations have been characterized by using the Fourier transform infrared (FTIR) spectroscopy, Differential scanning calorimetry (DSC), x-ray diffraction (x-RD), scanning electron microscopy (SEM). The in-vitro drug release and rat skin permeation studies were performed in phosphate buffer saline using a Keshary-Chien diffusion cell. The developed formulations were subjected to skin irritation tests on mice. Release data have been fitted to an empirical relationship to study the transport phenomenon. Results are discussed in terms of the drug release characteristics of the modified XG films.
Abstract

This chapter describes the possibility of using modified xanthan films as a matrix system for transdermal delivery of atenolol, which is an antihypertensive drug. Acrylamide was grafted onto xanthan gum by free radical polymerization using ceric ion as an initiator. Fourier transform infrared spectroscopy and differential scanning calorimetry indicated the formation of the graft copolymer. The obtained graft copolymer was loaded with atenolol and films were fabricated by solution casting method for transdermal application. Various formulations were prepared by varying the grafting ratio, drug loading and different penetration enhancers. The formulations prepared were characterized for weight, thickness uniformity, water vapor transmission rate and uniformity in drug content of the matrix. All the thin films were slightly opaque, smooth, flexible and were permeable to water vapor, indicating their permeability characteristics suitable for transdermal studies. Fourier transform infrared spectroscopy and differential scanning calorimetry studies indicated no significant interactions between drug and polymer. Drug is distributed uniformly in the matrix but showed a slight amorphous nature. Drug-loaded films were analyzed by X-ray diffraction to understand the drug polymorphism inside the films. Scanning electron microscopic studies of the placebo and drug-loaded films demonstrated a remarkable change in their surface morphology. The skin irritation tests were carried out in mice and these results suggested both placebo and drug-loaded films produced negligible erythema and edema compared to formalin (0.8 % v/v) as the standard irritant. The in vitro drug release and rat skin permeation studies were performed in phosphate buffer saline using a Keshary-Chien diffusion cell. Variations in drug release/permeation profiles were observed with different formulations prepared. Compared to in-vitro release, skin permeation was slower during 24 h study. Release data were analyzed by using the Ritger and Peppas equation to understand the mechanism of drug release as well as the estimation of n values, which ranged between 0.45 and 0.59, suggesting a Fickian diffusion trend.

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V.1. Introduction

Transdermal drug delivery (TDD) offers several advantages over the most common routes of drug administration. One of the major advantages of the transdermal therapeutic system (TTS) is the maintenance of blood concentration of a drug at the desired therapeutic level by the controlled permeation of drug through the skin. The discipline of TDD has experienced a tremendous growth over the past decade, because of the increasing number of drugs that can be delivered to the systemic circulation in clinically effective concentrations via the skin portal. This is possible despite the inherent protective function of the stratum corneum, which is primarily one of the excluding foreign substances from entering the body. Success has been achieved in the administering several drugs transdermally using TTSs with a view to maintain a constant plasma concentration of the respective drug over the predetermined time period [1].

Formulation of TTSs involves the optimization of several factors such as release rate, stability, safety, convenience of use, etc. However, the key component in a TTS, which monitors the release of an active ingredient, is the rate controlling judicially chosen polymeric membrane. The polymer should possess good film forming properties, should be non-irritating, inert and stable. Hence, the selection of a polymer is quite difficult because of the inherent diversity of structures, which requires thorough understanding of the surface and bulk properties of the polymer that can offer desired chemical, interfacial, mechanical and biological functions. Even though several polymers are already in use, yet new polymers to develop TTS are still need of the day. However, one of the major disadvantages of TDD compared to other controlled release (CR) formulations is its high cost. Hence, the less expensive natural and semisynthetic polymers have been evaluated as TTS [2-4].
Transdermal patches are of two types: membrane controlling and matrix type systems [5]. Membrane systems are constructed of rate controlling membranes, whereas in matrix controlled systems, the drug is dissolved or suspended in a hydrophilic or lipophilic matrix. Flexibility and plasticization of the films to be developed can be achieved by modification/blending with another polymer, cross-linking, etc. Advantages of such systems are that an additional free volume space can be created to accommodate the drug without any hindrance during its slow release from the matrix in addition to being biocompatible. Polyacrylamide-based hydrogel type transdermal device has been reported [6] for the transdermal delivery of peptides and calcitonin, but it was found to be necessary to improve the permeation rate of drugs by using suitable penetration enhancers that can rapidly and reversibly promote the percutaneous penetration of drugs. However, the most frequently used penetration enhancers are alcohols, fatty acids, surfactants, azones, and terpenes. Surfactant facilitated permeation of drugs through skin membranes has been studied [7,8] giving reports of significant enhancement of drugs like chloramphenicol through hairless mouse skin by sodium lauryl sulfate and acceleration of hydrocortisone and lidocaine permeating across the hairless mouse skin by the non-ionic surfactant, Tween® 80.

Atenolol (ATL) is a beta-adrenergic receptor blocking agent without membrane stabilizing or intrinsic sympathomimetic activities. It has been used for the treatment of hypertension and stable angina either alone or with other antihypertensive drugs like thiazide diuretics. It is reported that in oral applications, it can induce side effects like diarrhea, nausea, ischemic colitis and mesenteric arterial thrombosis. Atenolol is reported to be subjected to extensive hepatic first-pass metabolism following the oral administration and has a short biological half-life. The development of TDD containing antihypertensive drug and maintaining proper blood level for a long time
without any adverse effects of frequent oral administration is very important [9,10]. Therefore, it is desirable to develop a TDD system containing ATL as a model drug. As a further contribution in this area, the present study deals with the development of transdermal films using modified XG obtained by grafting acrylamide onto XG (i.e., AAm-g-XG) with ceric ion as an initiator and fabrication of transdermal films for the CR of ATL. The grafted copolymer and fabricated placebo as well as drug-loaded films have been characterized by thermal analysis. The in-vitro release studies were performed in phosphate buffer saline (PBS, pH 7.4) at 37°C using a Keshary-Chien diffusion cell for up to 24 h. These results have been fitted to empirical equation proposed by Ritger & Peppas [11] to understand the release mechanism. Such release data are important in further development of similar systems by selecting a proper drug-polymeric matrix.

V.2. Results and Discussion

V.2.1. Synthesis of Poly(acrylamide)-grafted-Xanthan Gum

In the present study, we have attempted to synthesize the graft copolymer by using ceric ion. The reaction conditions were standardized to minimize homopolymerization as well as to give better yield. The reactive vicinal group where the grafting is initiated on the XG backbone is CH2OH. The overall reaction mechanism is that ceric ion attacks the XG macrochains and generates the formation of a XG–ceric complex. The ceric(IV) ions in the complex are then reduced to ceric(III) ions by oxidizing hydrogen atom and thereby, creating a free radical onto the XG backbone. The grafting of poly(acrylamide) (pAAM) onto XG is then initiated by the free radical reacting with the monomer. In the presence of pAAM, the XG free radical is chemically coupled to the monomer unit, there by resulting in a covalent bond between pAAM and XG to create a chain reaction for propagation. Finally, termination is achieved through a combination of two radicals. The earlier methods [12,13]
reported the synthesis of graft copolymerization of AAm onto XG initiated by the \( \text{Fe}^{2+}/\text{BrO}_3^- \) redox system and CAN as the catalyst in an aqueous medium. Formulation details used in the synthetic procedure are summarized in Table V.1. The \% grafting varied from 566 to 1051 and the monomer conversion was increased with increasing the ratio of AAm to XG. In the present study, the reaction mixture was precipitated in acetone and washed several times with methanol:water (80:20, v/v) mixture and finally, with distilled water to remove the unreacted monomer and the unwanted reagents. Hence, the presence of ceric ions and homopolymers are excluded. As far as toxicology is concerned, as per the investigation made by Jakupec et al., [14] it was observed that Ce (IV) salts are not biologically stable in the aqueous media. Therefore, cerium species that circulate in the blood as colloidal compounds or protein complexes are likely to contain Ce(III). The close similarity of the ionic radii of Ce\(^{3+}\) (1.01 Å) and Ca\(^{2+}\) (1.00 Å) allows Ce\(^{3+}\) (and other light Ln\(^{3+}\) ions) to replace Ca\(^{2+}\) ions in the biomolecules.

Several drug-loaded pAAM hydrogels have also been used for \textit{in vitro} studies. In addition, \textit{in vivo} systems have been explored for the AAm-based matrices in developing superporous hydrogels. Notices that AAm blended hydrogels were found to be biocompatible [15]. In study by Makarand & Bhonde [16], the \textit{in vitro} cytotoxicity by MTT, a mitochondrial assay and NR, a lysosomal functionality assay showed no toxic effects on NIH3T3 and HeLa cells up to 40% of hydrogel extract, thus supporting the earlier observations about the biocompatibility of starting polymers. Thus, high viability of NIH3T3 and HeLa cells upon exposure to hydrogel will leach out the extracts as an indication of good tolerance of the hydrogel.
Table V.1

Synthetic Details of Polyacrylamide-grafted-Xanthan Gum

<table>
<thead>
<tr>
<th>Code</th>
<th>Mass of XG (g)</th>
<th>Mass of AAm (g)</th>
<th>Mass of initiator (mmole)</th>
<th>% Yield</th>
<th>% Grafting</th>
<th>% Grafting efficiency</th>
<th>% Conversion</th>
</tr>
</thead>
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<tr>
<td>XG1</td>
<td>1</td>
<td>7.5</td>
<td>0.05</td>
<td>78.35</td>
<td>566</td>
<td>75.46</td>
<td>88</td>
</tr>
<tr>
<td>XG2</td>
<td>1</td>
<td>10</td>
<td>0.05</td>
<td>84.45</td>
<td>829</td>
<td>82.90</td>
<td>92</td>
</tr>
<tr>
<td>XG3</td>
<td>1</td>
<td>12</td>
<td>0.05</td>
<td>88.53</td>
<td>1051</td>
<td>87.58</td>
<td>96</td>
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</tbody>
</table>

V.2.2. Preparation of Drug-Loaded Transdermal Films

Monolithic matrix type TTS loaded with ATL were prepared by solution casting method. Totally seven formulations were prepared by varying three parameters viz., grafting ratio, drug loading and penetration enhancers as shown in Table II.3 in section II.3.C.2, of Chapter II. PEG-6000 is used in concentrations of 20 % w/w as a plasticizer; polymeric films with concentrations less than 20 % w/w plasticizer were somewhat brittle and lacked the folding endurance. However, the films with a plasticizer concentration over 30 % w/w did not further improve the film properties (compared to those prepared with 20 % w/w). Hence, 20 % w/w was fixed as the optimum concentration for the plasticizer. The surfactant Tween® 80 and Pluronic F127, were used as the penetration enhancers. Various trials were taken to optimize the formulation to get the desired thickness, flexibility, drug loading and release profile.

V.2.3. Physicochemical Characterization of the Films

Physicochemical properties of the films are summarized in Table V.2. The films formed were slightly opaque and had uniform thicknesses varying from 0.082 to 0.112 mm. Drug content of the films varied from 94.26 to 98.17 %. The process employed to prepare films in this study was capable of producing films with a uniform drug content and a minimum batch variability.
Weight of the completely dried film was recorded in the range of 1.13 to 2.39 mg. Folding endurance was varied from 21 to 32; formulations F1P3 and F3P2 exhibited maximum and minimum folding endurances, respectively. Water vapor transmission (WVT) of the films varied from $1.6 \times 10^{-3}$ to $8.0 \times 10^{-4}$ g/cm$^2$/24 h. These results indicated that films were permeable to water vapor. The WVT of cellulose acetate films prepared from 2% w/v casting solution was reported [17] to be $11.9 \times 10^{-3}$ g/cm$^2$/24 h. These films were found to be suitable for developing formulation of TDD systems. Properties of the modified xanthan films were comparable with cellulose acetate films.

Table V.2

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Thickness (mm)</th>
<th>Weight (mg)</th>
<th>WVT (g/cm$^2$/24 h)</th>
<th>Folding endurance</th>
<th>Drug content (%)</th>
</tr>
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<tr>
<td>F1P1</td>
<td>0.102</td>
<td>1.80</td>
<td>$1.60 \times 10^{-3}$</td>
<td>22</td>
<td>97.26</td>
</tr>
<tr>
<td>F1P2</td>
<td>0.112</td>
<td>2.00</td>
<td>$1.35 \times 10^{-3}$</td>
<td>24</td>
<td>98.24</td>
</tr>
<tr>
<td>F1P3</td>
<td>0.111</td>
<td>2.39</td>
<td>$1.60 \times 10^{-3}$</td>
<td>32</td>
<td>96.98</td>
</tr>
<tr>
<td>F2P2</td>
<td>0.102</td>
<td>1.70</td>
<td>$1.50 \times 10^{-3}$</td>
<td>28</td>
<td>96.54</td>
</tr>
<tr>
<td>F2P3</td>
<td>0.100</td>
<td>2.04</td>
<td>$8.00 \times 10^{-4}$</td>
<td>29</td>
<td>94.26</td>
</tr>
<tr>
<td>F3P1</td>
<td>0.096</td>
<td>1.45</td>
<td>$1.08 \times 10^{-3}$</td>
<td>23</td>
<td>98.17</td>
</tr>
<tr>
<td>F3P2</td>
<td>0.082</td>
<td>1.13</td>
<td>$1.25 \times 10^{-3}$</td>
<td>21</td>
<td>95.16</td>
</tr>
</tbody>
</table>

V.2.4. Fourier Transform Infrared Spectroscopy

Grafting of AAm onto XG was confirmed by FTIR studies. FTIR spectra of (a) AAm, (b) AAm-g-XG and (c) XG are displayed in Figure V.1. In case of pure XG, a broad absorption peak at 3450 cm$^{-1}$ indicates the presence of hydrogen-bonded OH groups. Two peaks, one at 1615 cm$^{-1}$ and the other at 1476 cm$^{-1}$, are attributed to COO$^-$ groups. Additional characteristic absorption bands of XG appear at 1417 cm$^{-1}$ and 1023 cm$^{-1}$ due to C-H bending and O-H bending vibrations, respectively. In the FTIR spectrum of AAm, a
characteristic absorption band observed at 3355 cm⁻¹ is attributed to N-H stretching, whereas the bands appearing at 1673 cm⁻¹ and 1610 cm⁻¹ are due to the amide stretching vibrations. Additional characteristic absorption bands of AAm appear at 1425 cm⁻¹ and 1350 cm⁻¹, respectively due to O-H bending and C-O stretching vibrations. In the case of graft copolymer of AAm-g-XG, the bands at 1675 and 1635 cm⁻¹ are, respectively attributed to amide-I (C-O stretching) and amide-II (N-H bending) of the amide group of AAm. The peak at 3421 cm⁻¹ in AAm-g-XG matrix is attributed to a overlap of N-H stretching band of the amide group and O-H stretching band. The C-N stretching band appears at 1450 cm⁻¹. These factors are the effective evidence for the successful grafting reaction.

FTIR spectra of the pristine ATL (curve a) and the drug-loaded film (curve b) are presented in Figure V.2. ATL showed characteristic bands due to different functional groups. However, the band appearing at 3356 cm⁻¹ is due to O–H/N–H stretching vibrations, while those observed at 2924 and 2963 cm⁻¹ are due to aliphatic C–H stretching vibrations. The band at 3175 cm⁻¹ is due to aromatic C–H stretching vibrations, whereas those appearing at 1637 and 1583 cm⁻¹ are due to primary amide bond stretching and aromatic C=O stretching vibrations, respectively. The N–H bending vibrations are seen at 1516 cm⁻¹. Bands at 1180 and 1092 cm⁻¹ are due to C–O–C stretching vibrations of the ether linkages. The C–N stretching vibrations are seen at 1037 cm⁻¹, while the one that appeared at 1243 cm⁻¹ is due to aromatic C–O stretching vibrations. The peaks appearing at 3349, 2971, 2924, 1639, 1519, 1249 and 1041 cm⁻¹ for ATL have also appeared in the ATL-loaded films, indicating the chemical stability of ATL in the chosen polymeric matrix. This also indicates that ATL is not involved in any chemical reactions with either the polymer or the excipients used.
Figure V.1. FTIR spectra of (a) AAm, (b) AAm-g-XG and (c) XG.
Figure V.2. FTIR spectra of (a) pristine drug, (b) drug loaded film.
DSC is a useful technique to explain the formation of graft copolymers. DSC curves of XG (a) and graft copolymer (b) are reproduced in Figure V.3. XG showed an endothermic transition at 65°C, whereas the graft copolymer exhibited two endothermic transitions, one at 70°C and another at ~360°C. The new endothermic transition observed at 360°C in the thermogram of the graft copolymer may be due to enhanced interaction between carbonyl groups of the grafted copolymer and hydroxyl groups of XG. These results confirm the grafting of AAm onto XG.

DSC scans of the pristine drug, placebo film and the drug-loaded film are presented in Figure V.4. The endothermic peaks of ATL appeared at its melting point, 158°C (curve a), which appears at 152°C in the DSC plots of the ATL-loaded film (curve c) with a much lesser intensity. A slight shifting of the peak indicates no recrystallization of the pristine drug within the polymer matrix due to molecular interactions between the drug and the polymer. This further confirms a uniform dispersion of ATL in the polymer matrix. The placebo film (curve b) has shown two endothermic transitions at 267° and 358°C, but no peak was observed near the endothermic peak exhibited by the pristine drug. Endothermic peaks of the placebo film after loading the drug decreased to 184°C due to the possible formation of the loose polymer network as a result of the creation of extra free space after drug loading. These results suggest the absence of any interactions between the polymer matrix and the ATL.
Figure V.3. DSC thermograms of (a) XG and (b) AAm-g-XG.

Figure V.4. DSC thermograms of (a) pristine drug, (b) placebo film and (c) drug loaded film.
V.2.6. X-Ray Diffraction

The X-ray diffraction patterns of pristine drug (curve a), drug-loaded film (curve b) and placebo film (curve c) are presented in Figure V.5. The placebo film shows two peaks, one at 14.18° and the other at 16.32°. Diffractogram of the ATL shows many characteristic sharp peaks. The highest crystalline ATL peaks occurred at 2θ of 6.33°, 6.53°, 9.56°, 20.64° and 20.75°, and a series of smaller peaks are observed at 2θ angles of 16.04°, 19.29°, 22.32°, 24.68° and 26.46°. In the case of drug-loaded film, only the peaks at 6.52°, 9.74°, 16.21° and 26.09° were observed, but the peak intensities were smaller when compared to the pristine drug, indicating that the drug-loaded inside the polymer matrix is not completely in its crystalline form. This further confirms that a portion of the drug is molecularly dispersed in the polymer matrix film.

V.2.7. Scanning Electron Microscopy

Scanning electron microscopy was used to examine the surface morphology of the films. SEM images of the placebo films and the drug-loaded films are shown in Figure V.6. The placebo films exhibited a clear and homogenous surface, whereas drug-loaded films showed a uniform distribution of ATL in the polymeric matrix.

V.2.8. Skin Irritation Studies

Skin irritation studies were carried out to investigate the potential for ATL and the polymeric matrix to cause irritant or allergic reactions, which are usually produced by a specific base component. Many test protocols are described to study the irritancy levels, both in animals and humans [18]. The results depicted in Table V.4 indicate that the polymeric film (placebo and drug-loaded) as well as the USP adhesive tape produced negligible erythema and edema. On the other hand, the standard irritant viz., formalin was found to produce severe erythema and edema effects.
Figure V.5. X-RD diffractograms of (a) pristine drug, (b) drug loaded film and (c) placebo film.
Figure V.6. SEM images of (a) placebo films and (b) drug loaded films.
Table V.3

Results of Skin Irritation Tests

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Erythema</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Adhesive tape (USP)</td>
<td>1.72</td>
<td>1.61</td>
</tr>
<tr>
<td>Drug loaded</td>
<td>1.48</td>
<td>1.31</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.08</td>
<td>1.28</td>
</tr>
<tr>
<td>Formalin (0.8 % v/v)</td>
<td>3.12</td>
<td>3.29</td>
</tr>
</tbody>
</table>

V.2.9. In-vitro Release Studies

The in-vitro drug release studies were carried out in PBS at 37°C using Keshary-Chien diffusion cell with dialysis membrane acting as the diffusion barrier. The in-vitro release profiles of formulations containing graft copolymer at various grafting ratios are displayed in Figure V.7. Notice that formulation F1P1 releases the maximum amount of drug as compared to other formulations i.e., it released about 90% of the drug in about 24 h, whereas the formulations F1P2 and F1P3 released 88% and 84%, respectively under similar conditions. It is interesting to note that with increasing grafting ratio of the copolymer, a slight decrease in release rate of the drug occurred, suggesting that the release depends upon the chain length of pAAm part of the copolymer. Thus, by varying the grafting ratio, the amount of XG in the copolymer will change and the length of the side chain of the copolymer will increase. This will greatly affect the release patterns in all the grafted copolymers. The observed initial rapid release rate may be accounted for by the direct exposure of the membrane system to the diffusion media with a quick release of the drug present at the surface [19] and such an initial rapid release is attributed to the fact that polymeric matrix may form loose channels within the network, because of its hydrophilic nature and the dissolution of hydrophilic polymers.
during the diffusion process. The formation of such loose channels leads to a decrease in the mean diffusional path length of the drug molecule to leach out into the diffusion medium, thereby resulting in the higher rates of drug release from the membrane matrix. The observed initial release may be helpful to achieve the therapeutic plasma concentration of the drug in a short time along with a constant release rate at longer time period, providing CR of the drug. However, such an initial effect is related to the initial migration of the drug particles toward the surface of the membrane matrix.

The effect of drug loading on release rate from xanthan films was studied at 37°C for 24 h with different drug loadings of 20%, 30% and 40% (w/w) into the matrix. The in vitro release profiles at various drug loadings are depicted in Figure V.7. The total amount of drug released from F2P3 formulation is about 95% at the end of 24 h, whereas formulations F1P1 and F2P2 released 89% and 94%, respectively. Atenolol is a hydrophilic drug, which will therefore, exert an interaction with the water-soluble polymers, resulting in an increase of drug release. Also, the hydrophilic XG will readily absorb water molecules and will swell, resulting in the formation of large pores. Thus, as the drug loading increased; the release rate also increased. Increased release rate and the extent of drug release are due to the increase in the rate of diffusion at higher concentrations of the drug. Similar observations were reported earlier by Kim & Shin [9].

In the present study, two different penetration enhancers were used. The release profiles of these formulations are shown in Figure V.7, which indicated a slight enhanced release for formulations F3P1 and F3P2, respectively containing Pluronic F127 and Tween® 80 than the control. From the literature, it is apparent that nonionic surfactants have only a minor enhancement effect, whereas the anionic surfactants have more a pronounced effect. The effectiveness of penetration enhancer was thus determined by comparing the
drug release rate in the presence and absence of an enhancer. This was defined as the enhancement factor (EF), which was calculated by the drug release rate from the matrix containing the enhancer divided by that without the enhancer.

The results of fractional release \( \frac{M_t}{M_\infty} \) have been analyzed using an empirical equation [11]:

\[
\frac{M_t}{M_\infty} = Kt^n
\]  
(V.1)

Here the values of \( n \) represent the diffusion anomalies and \( k \) is an empirical parameter representing the interaction between drug and the polymer. Estimated values of \( n \) along with correlation coefficient, \( r \) values are presented in Table 4. In case of drug release from the swellable matrices, if \( n = 0.5 \), it indicates that transport follows the Fickian trend. For all formulations studied in this work, the values of \( n \) ranged between 0.41 and 0.53, suggesting that diffusional transport follows a Fickian trend. In case of Fickian diffusion, the rate of water absorption increases linearly when plotted as a function of square root of time. Fickian diffusion is observed when the time scale of the macromolecular relaxation is either effectively infinite or zero as compared to the time required to establish a concentration profile in the polymer [20]. These two signify the elastic and viscous Fickian diffusion limits. Matrix systems reach an equilibrium state of relaxation extremely fast with a Fickian diffusion of the drug being the dominant drug transport mechanism. With the release of surface drug, numerous pores and channels are possibly generated in the matrix structure, which further elevates the rate and extent of ATL release. Again, due to the hydrophilic nature of the polymer, when exposed to diffusion media, free volume spaces are generated between the macromolecular chains. After solvation of the polymer chains, the dimensions of polymer chain will increase due to polymer relaxation. In non-Fickian or anomalous transport, both diffusion as well as macromolecular relaxation time scales are similar and both
will control the overall rate of penetrant absorption. Case II transport is the limit when relaxation predominates. Zero-order, time-independent Case II kinetics are characterized by a linear mass uptake with time.

The steady state flux ($J_{ss}$) was also calculated from the slope of the linear portions of the straight lines obtained after plotting the mean cumulative drug released vs time. The permeability coefficient ($K_p$) was calculated using the equation [21]:

$$K_p = \frac{J_{ss}}{C_s} \quad (V.2)$$

where $C_s$ is the concentration of drug in the transdermal membrane. These results are presented in Table V.4. The flux obtained for all the formulations ranged between 1.71 and 2.56 $\mu$g/cm$^2$, whereas $K_p$ values varied from $5.39 \times 10^{-4}$ to $9.22 \times 10^{-4}$.

![Graph showing cumulative release over time](image)

**Figure V.7.** Effect of grafting ratio, drug loading and penetration enhancers on *in-vitro* release profile.
Table V.4

Results of Flux, Permeability Coefficient ($K_p$), $n$ and $r$ Values From In Vitro Release Studies

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Flux ($\mu g/cm^2$)</th>
<th>$K_p \times 10^4$</th>
<th>$n$</th>
<th>$r^{(a)}$</th>
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<tbody>
<tr>
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<td>1.88</td>
<td>9.22</td>
<td>0.51</td>
<td>0.930</td>
</tr>
<tr>
<td>F1P2</td>
<td>1.99</td>
<td>8.26</td>
<td>0.44</td>
<td>0.971</td>
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<tr>
<td>F1P3</td>
<td>1.71</td>
<td>6.10</td>
<td>0.53</td>
<td>0.968</td>
</tr>
<tr>
<td>F2P2</td>
<td>2.31</td>
<td>8.11</td>
<td>0.42</td>
<td>0.931</td>
</tr>
<tr>
<td>F2P3</td>
<td>2.56</td>
<td>5.39</td>
<td>0.41</td>
<td>0.986</td>
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<tr>
<td>F3P1</td>
<td>2.10</td>
<td>8.94</td>
<td>0.47</td>
<td>0.891</td>
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<tr>
<td>F3P2</td>
<td>1.92</td>
<td>8.53</td>
<td>0.46</td>
<td>0.978</td>
</tr>
</tbody>
</table>

$^{(a)}$ $r$ is correlation coefficient calculated at 95% confidence limit.

V.2.10. Skin Permeation Studies

In-vitro skin permeation of xanthan films was studied at 37°C using the freshly excised hairless rat skin, which is generally more permeable compared to the human skin. Skin permeation can be measured in living humans or animals or in-vitro by using the excised (human) skin in the diffusion cells. Studies with excised skin seem feasible, since the passage through skin is a passive diffusion process and the primary barrier, the stratum corneum, is composed of nonliving tissue. Previous studies reported [10,22] the use of rat skin for in vitro skin permeation. Skin permeation profiles of the formulations containing graft copolymer at various grafting ratios are shown in Figure V.8. The total amount of drug permeated from these formulations was about 85% at the end of 24 h. It was observed that the cumulative drug permeated through the rat skin was not very different. However, the enhanced skin permeation of the drug is due to its faster release rate from the transdermal film and due to less partitioning of the drug with the skin.
polymer. The skin permeation studies at various drug loadings (20%, 30%, and 40%) have been studied and these results are depicted in Figure V.8, which suggest that as the concentration of drug increased, the rate of permeation also increased. This type of increased rate and extent of permeation may be due to an increase in the rate of diffusion at the higher concentrations of the drug. Permeation profiles of the formulations containing penetration enhancers are shown in Fig. 8. About 87% of the drug is permeated for formulations F3P1 and F3P2.

The results of fractional drug permeated ($M_t / M_\infty$) through the rat skin were analyzed by using the empirical equation proposed by Ritger and Peppas [11]. Estimated values of $n$ along with the correlation coefficient, $r$ values are presented in Table V.5. For all the systems studied in this work, the values of $n$ ranged between 0.45 and 0.59, suggesting that diffusional transport follows the Fickian model. Steady state flux ($J_{ss}$) was calculated from the slope of the linear portion of the straight lines obtained by plotting mean cumulative amount permeated vs time; the permeability coefficient was calculated from Eq. V.2. These results shown in Table V.5, suggest that flux obtained for all the formulations are in the range of 0.49–3.15 $\mu$g/cm², whereas the $K_p$ values varied from $2.34 \times 10^{-4}$ to $6.64 \times 10^{-4}$.
Figure V.8. Effect of grafting ratio, drug loading and penetration enhancers on *in-vitro* atenolol permeation.

Table V.5

Results of Flux, Permeability Coefficient, n and r Values From the Skin Permeation Studies

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Flux (μg/cm²)</th>
<th>Kp x 10⁴</th>
<th>n</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1P1</td>
<td>0.49</td>
<td>2.40</td>
<td>0.45</td>
<td>0.975</td>
</tr>
<tr>
<td>F1P2</td>
<td>0.58</td>
<td>2.40</td>
<td>0.47</td>
<td>0.971</td>
</tr>
<tr>
<td>F1P3</td>
<td>0.95</td>
<td>3.42</td>
<td>0.52</td>
<td>0.968</td>
</tr>
<tr>
<td>F2P2</td>
<td>1.85</td>
<td>6.49</td>
<td>0.59</td>
<td>0.931</td>
</tr>
<tr>
<td>F2P3</td>
<td>3.15</td>
<td>6.64</td>
<td>0.56</td>
<td>0.888</td>
</tr>
<tr>
<td>F3P1</td>
<td>0.55</td>
<td>2.34</td>
<td>0.49</td>
<td>0.972</td>
</tr>
<tr>
<td>F3P2</td>
<td>0.52</td>
<td>2.45</td>
<td>0.45</td>
<td>0.959</td>
</tr>
</tbody>
</table>
V.3. Conclusions

The present study demonstrates the use of modified xanthan films to develop transdermal patches that are biocompatible and biodegradable. AAm was successfully grafted onto XG by the free radical polymerization using ceric ion initiator. FTIR spectroscopy and DSC indicated the formation of the graft copolymer. The films casted by using the graft copolymer, plasticizer and ATL by solution casting method were slightly opaque, smooth, flexible and were permeable to water vapor, indicating their favorable permeability characteristics. FTIR and DSC studies indicated no significant interactions between ATL and the polymer matrix; drug was distributed uniformly throughout the matrix with a slight amorphous morphology. X-RD indicated that drug inside the film was not completely in the crystalline form. SEM studies of the placebo and drug-loaded films demonstrated a remarkable change in surface morphology. The skin irritation test results suggested that both the placebo and the drug-loaded films have produced negligible erythema and edema compared to formalin (0.8 % v/v) as a standard irritant. The in-vitro drug release and rat skin permeation studies showed variations in drug release/permeation profiles among the different formulations developed. Compared to in vitro release studies, skin permeation was slower during the 24 h study. The obtained $n$ values indicated the drug diffusion to follow a Fickian trend. Steady state flux for skin permeation varied in the range of $0.49-3.15 \mu g/cm^2$ while the $Kp$ values varied from $(2.34$ to $6.64) \times 10^{-4}$. 
V.4. Literature Cited


