Development and evaluation of antipsychotic drugs for externally triggered transdermal therapeutic systems

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Abstract---Cariprazine is a recent antipsychotic drug with an atypical neuropharmacological profile. It is promptly absorbed from the GI tract and suddenly metabolized by liver. Cariprazine has 6 h of plasma elimination half life, 9% of oral and 83% of protein binding. Administration of cariprazine in transdermal dosage form would be a lot of fascinating to regulate the mood for extended period by achieving the plasma concentration well higher than the therapeutic levels. With psychiatrical patients, schedules of daily medication should be followed to avoid adverse reactions which are notably difficult. Therefore, delivery of such drugs by transdermal route can be done. Development and maintenance of low dose drugs therapy is desired, which will limit the consequences of significant adverse effect and direct the issues of helpless consistence in the patients. Ideal quantity of the psychotropic drug can be given by transdermal route with least adverse reactions to overcome the psychic conditions. This will result in improving the health care services for long term mental illness management. In present work, we have synthesized the novel electrically responsive graft co-polymers using natural polymers with
polyacrylamide and utilized for the preparation of cariprazine containing electrically responsive transdermal therapeutic systems (ETTS). The ETTS developed using electrically-responsive graft copolymers of PAAm-g-GaG having crosslinked with glutaraldehyde have confirmed that there was augmented drug permeation under the influence of electric stimuli, while it was reduced without electric current. The Cariprazine permeability got decreased as the concentration of crosslinking agent was increased. Further, higher drug permeability rate observed with increasing electric current strength from 2 to 8 mA. There was a pulsatile pattern of drug permeability under ‘on and ‘off’ electric stimulus mode. Hence, these graft copolymers are competent biomaterials those could be used for the development of electro-sensitive transdermal therapeutic systems for on-demand drug delivery.

**Keywords**—cariprazine, natural polymers, polyacrylamide, drug delivery.

**Introduction**

The use of antipsychotics as medication began in 1933 in France. The research around developing antihistamines evolved into the introduction of promethazine.[1] This drug produced sedative side effects, so doctors started prescribing it before surgeries as a calming agent. Eventually, a doctor studied the derivatives of promethazine, altered it, and developed chlorpromazine. It was mostly used as a pre-surgery anti-anxiety pill until psychiatrists took note of the calming effect of the drug and began prescribing it to their patients. Prior to chlorpromazine, the options for treating psychotic patients were electroconvulsive therapy, hydrotherapy, and putting patients in an insulin coma. None of those are antipsychotic in nature.[2] Clozapine had more system-wide changes than just dopamine suppression, and it had a more positive response from patients. It was more effective—40-60% of people who won’t respond to a first-generation antipsychotic, do respond to clozapine.[3]

However, in Finland in 1975, 6 people taking clozapine died due to agranulocytosis (lowered white blood cell count, leading to a severe lack of immunity). A lowered neutrophil count (called agranulocytosis) can show potential problems with fighting off normal bacteria we live with all the time.[4] As new oral atypical antipsychotics have become available over the past decade, monitoring clinical day-to-day experiences in various groups of psychotic patients is useful for clinicians. Cariprazine was approved for the acute and maintenance treatment of schizophrenia and for the acute treatment of manic or mixed episodes associated with bipolar I disorder (FDA, 2015). Recently, cariprazine also got extended FDA-approval for the treatment of major depressive episodes in adults with bipolar I disorder.[5]

Cariprazine is similar to other atypical antipsychotics in exhibiting antagonistic activity at serotonin type 2A receptors. Cariprazine also acts as a partial agonist at the dopamine D3 and D2 receptors with high binding affinity and at the
serotonin 5-HT1A receptor\(^6\). Cariprazine has a similar profile to aripiprazole, except for D3, which has tenfold greater affinity than for D2, so high that extremely small doses are sufficient to get maximal D3 occupancy.\(^7\) This particular D3 receptor blockage could theoretically have pro-cognitive effects, antidepressant effects, and reduce the negative symptoms of schizophrenia. Cariprazine has other receptor properties, including moderate histamine antagonism, low \(\alpha\)-1a antagonism, and no significant affinity for muscarinic cholinergic receptors.\(^8\)

Cariprazine (Vraylar) is an oral atypical antipsychotic originated by Gedeon Richter. It is a potent dopamine D3 and D2 receptor partial agonist, which preferentially binds to the D3 receptor. Cariprazine also has partial agonist activity at serotonin 5-HT1A receptors. In September 2015, cariprazine received its first global approval in the USA for the treatment of schizophrenia and for the acute treatment of manic or mixed episodes associated with bipolar I disorder. It is also in development in a variety of countries for the treatment of schizophrenia with predominant negative symptoms (phase III), as adjunctive therapy for major depressive disorder (phase II/III) and for the treatment of bipolar depression (phase II). This article summarizes the milestones in the development of cariprazine leading to this first approval for schizophrenia and manic or mixed episodes associated with bipolar I disorder.\(^9\)

Transdermal delivery gains benefit of bypassing gastrointestinal aggravations and hepatic metabolizing effects which are unit related to the oral route. Sustained and controlled delivery is also showed, it fulfills the patient requirement and easy in application and removal. Despite fact that percutaneous route of drug administration has several benefits, as it is limited to the low penetrability from the skin, due to stratum corneum which plays role of barrier. Hence, to enhance drug permeability through the skin, various penetration enhancers, prodrugs, superfluous vehicles, iontophoresis, phonophoresis and electroporation have been utilized to maximize the degree of skin permeation. One of the chief helpful ways for rising the volume of permeation through skin is that the utilization by permeation enhancers, which showed worthy outcomes. Iontophoresis, includes applying a direct current, utilized widely as a skin invasion technique utilizing external means. Still, due to the change in pH there’s a chance of skin burning. So, the aqueous solution medication will exclusively be applied.\(^10,11\)

At present, synthetic drugs have a considerable line of treatment into controlling inflammatory disease. Analgesics, NSAIDs and cortico-steroids are found useful in treating RA. These drugs act at various sites within the schema of pathogenic mechanisms. Less compliance with older RA patients is the significant drawback in the drug therapy. Good patient compliance is observed in transdermal delivery of drug, so it is a better route of drug delivery.\(^12,13\) Transdermal therapeutic systems (TTS) are the self-contained discrete drug delivery systems that regulate the drug delivery into blood stream via skin onto application on the skin.\(^14\) Transdermal systems are flexible pharmaceutical preparations of varying sizes, containing an active component. Development of TDDS to deliver the drug molecules beyond different layers of skin into systemic circulation to bypass the first pass metabolism. It shows benefit as drug administration by IV route and to achieve rate controlled systemic delivery of drugs.\(^15\) In the permeation of most of
the drugs, Stratum corneum acts as rate limiting barrier. Thus, a few dynamic improvement innovations have emerged as strategies to upgrade extent of transdermal medication. One of such technique is Iontophoresis, which upon application of direct current (DC) as a physical mean and that follows the principle of “like repels like”, and which helps drug molecules to drive through the skin layers[16]

Materials and Methods

Materials

Cariprazine was obtained from Gedeon Richter and marketed by Actavis, India. Natural polymer like Guar Gum and Polyacrylamide, Methanol, Sodium hydroxide (NaOH) Pallets, Potassium dihydrogen ortho phosphate (KH2PO4), Sodium dihydrogen ortho phosphate (NaH2PO4) were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai. Gum Tragacanth, obtained from Loba Chemie. Mumbai. Ammonium Persulphate (APS) were obtained from Fisher Scientific, Mumbai.

Method

Pre-formulation studies

Determination of solubility

In order to acquire saturated solution in glass vial, the correctly weighed amount of QF was dissolved in a measured volume of phosphate buffer pH 7.4 to get saturated solution. The vials were kept aside for 24 h at 37 °C to achieve equilibrium with undissolved drugs particles. After filtration it was suitably diluted and the concentration was later measured at 315 nm using a UV spectrophotometer (1700, Shimadzu, Japan)

Melting Point

Capillary tube method was used to determine the melting point of drug; little quantity of drug was taken into the capillary tube closed at one end with fusion and recorded the temperature at which the drug melts by placing it in the melting point apparatus.

Determination of pH

Laboratory pH meter (Systronics, India) was used to measure the pH of 5% drug solution in double distilled water.

Partition coefficient

This study was done with the help of oil (n-octanol) and aqueous (phosphate buffer of pH7.4) phase. These two phases were taken into equal quantities, mixed well and set them for 37 °C For 24 h on water bath shaker to get both the phases saturated. The 10 ml of each phase were added to separating funnel containing 100 mg of drug and to get complete partitioning and further shaken for 6 h
vigorously at 37 °C. Then each phase was separated and set aside to determine the drug content using spectrophotometer (Shimadzu UV-1700, Japan).

**In-vitro permeation studies through rat abdominal skin**

As a preliminary step, skin permeability of the drug was evaluated through drug permeation study by using rat skin. We have performed skin permeation studies for plain drug (without enhancer) and with four different classes of permeation enhancers (10 % w/w) namely, 1, 8 cineole, span 20, oleic acid and dimethyl formamide. Abdominal hair of the healthy Wistar rats which were weighing between 150 to 200 gm were removed carefully without any skin damage and then it was excised. Dermal side of excised skin was cleaned properly to which tissues and blood vessels are attached. For the permeation study, vertically assembled Keshary-Chien diffusion cells were used. The stirrer speed was set at 100rpm, collection of samples was done from receptor compartment containing phosphate buffer of pH 7.4 and 32 °C ± 5 °C temperature was maintained during the experiment. The excised skin of rat was fixed with adhesive on donor compartment in such a way that the stratum corneum layer facing towards donor compartment and dermal layer towards receptor compartment. In the donor compartment, a 20 ml drug solution (5 mg/ml) was placed and 5ml samples from receptor compartment were collected for 24 h at different time intervals for the determination of drug quantity. The volume of sample collected from donor compartment was replaced with same quantity phosphate buffer of 7.4 pH; UV spectrophotometer (Shimadzu 1700, Japan) was used to analyze the samples at 315 nm using H 7.4 buffer as blank.[17,18]

**Formulation Studies**

**Synthesis of electrically responsive graft copolymers**

Synthesis of polyacrylamide (PAAm) grafted natural polymers like guar gum (GaG) was done with free-radical polymerization reaction. We soaked about two grams of above said natural polymers in 100 ml double distilled water overnight. The homogenous polymeric solution was heated under atmosphere of nitrogen at 80 °C and 0.43 gm of ammonium persulphate (APS) was mixed with polymeric solution and continuously stirred for 20 min to prepare solution; 10 ml of 0.105 mol acrylamide was added and polymeric reaction was carried for 60 min in the atmospheres of nitrogen. The copolymer was cooled at room temperature after 60 min and was poured into excess methanol (400 ml); filtered and fresh methanol was used for washing it repeatedly. Thus, synthesized copolymer was stored after overnight drying at 50 °C. Further, by using below mentioned equations, the grafting parameters were estimated.151[19]

**Alkaline hydrolysis of graft copolymer**

We dissolved two grams of copolymer into 100 ml solution of NaOH (0.9 M). It was stirred continuously for 60 min at 75°C in water bath shaker. After completion of reaction, it was cooled and washed with excess amount of fresh methanol; the obtained product was dried at 50°C overnight.
Characterization of grafted copolymers

The synthesized graft copolymers were characterized by;

- FTIR spectroscopy,
- Elemental (C, H & N) analysis,
- Determining NE values and
- Thermogravimetric analysis (TGA).

Fourier Transform infrared spectroscopy

FTIR was used to analyze and confirm reactions of native, grafted and hydrolyzed copolymers. Pellets were prepared by crushing the sample with potassium bromide under 600 kg of hydraulic pressure. FTIR instrument (Shimadzu, Japan) was used to take the spectra and scanned in between 500 to 4000 cm⁻¹.

Elemental (C, H & N) analysis

Samples of natural polymer, graft copolymer and partially hydrolyzed copolymers were analyzed to estimate the percent of C, H & N.

Determination of neutralization equivalent values (NE)

Neutralization equivalent is the equivalent weight of acid which can be determined by the titration with standard alkali. Two hundred milligrams of the sample were equilibrated with 0.1 N HCl for 6 h, the excess hydrogen ion concentration was back titrated with a standard sodium hydroxide solution (0.1 N). Then the equivalent weight of the carboxylic acid functional groups was calculated.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis was done on native polymers and PAAm-g-copolymers using micro calorimeter (Diamond TG/DTA, Perkin Elmer, USA) in the temperature range of 40-750°C, at heating rate of 10°C/min within the atmosphere of argon gas flowing rate at 50 ml/min to maintain inert atmosphere.

Preparation of electrically responsive transdermal therapeutic systems (ETTS)

Hydrogel reservoir

The polyacrylamide grafted copolymers were accurately weighed and dissolved in double distilled water for preparing the hydrogel reservoirs. To maintain the uniformity of polymeric solution, continuous stirring was done using magnetic stirrer. To the above polymeric solution, weighed quantities of drug along with methyl paraben were added and then various concentrations of glutaraldehyde (GA) and 0.5 ml of 0.1 N HCl were added and stirring was carried out for 30 min. Thus, formulated hydrogel reservoirs were stored in a properly closed container for developing the ETTS.
Rate controlling membranes

To prepare rate controlling membranes (RCMs), mercury substrate method was followed. The native polymers GaG along with polyvinyl alcohol (PVA) together were dissolved in distilled water in different proportions and stirred to get homogenous polymeric solution. The polyethylene glycol 200 (PEG 200), GA and 0.1 N HCl were added to the above solution with continuous stirring. This polymeric solution was poured inside the glass bangle placed over mercury in a petridish. The water was allowed to evaporate at room temperature left for 24 h. dried films were collected and kept in desiccator for further use.[20] Further, the ETTS were developed by precisely measuring an amount of hydrogel equivalent to 10 mg of drug and putting it on the backing layer; prepared RCMs were placed upon hydrogel reservoir and to avoid leakage from the system, the edges were sealed by applying heat and were stored in a closed container. The schematic diagram is shown in Figure (1) and composition of ETTS is given in Tables 1

<table>
<thead>
<tr>
<th>Codes</th>
<th>Hydrogel reservoir</th>
<th>Rate controlling membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAAm-g-GaG (%w/v)</td>
<td>Cariprazine (%w/w polymer)</td>
</tr>
<tr>
<td>BGaG1</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>BGaG2</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>BGaG3</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>BGaG4</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>BGaG5</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>BGaG6</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>BGaG7</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>BGaG8</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Figure 1. Schematic diagram of electrically-responsive transdermal therapeutic system

Evaluation of hydrogel reservoir
Measurement of pH

The graft copolymer hydrogel reservoirs of PAAm-g-GaG were evaluated for pH using a digital pH meter.
Drug content
The 500 mg of PAAm-g-GaG hydrogel reservoirs were accurately weighed and allowed to soak in 100 ml phosphate buffer of pH 7.4 for 24 h. The solution was mildly heated, cooled to room temperature and filtered. The absorbance was recorded using UV spectrophotometer (Shimadzu 1700, Japan) at 315 nm [21].

Evaluation of rate controlling membranes
Thickness
The thickness of RCMs was determined using digital micrometer at various places on the RCM; mean thickness was calculated and recorded.[22]

Water vapor transmission studies
Glass vials were used as transmission cells, which have uniform diameters. The prepared RCM (1 sqcm) was placed and fixed on the brim of cells which is containing around 1 gm fused calcium chloride. The cells were then weighed and placed in closed desiccator containing 200 ml of saturated potassium chloride solution (Relative humidity of 84%). The cells were taken out and weighed at different time intervals and amount of water vapors transmitted was determined by using following formula [23].

\[
WVT = \frac{W}{L} \times \frac{1}{S}
\]

W = Transmission of water vapor gm, L = Thickness of film in cm, S = Surface area exposed in square cm.
It indicates grams of water transmitted/hr.sqcm

In-vitro drug permeation from ETTS through rat skin
Healthy albino rats (150 to 200 gm) were selected and the hair from abdominal region of rats was cautiously removed without damaging the skin and then it was excised. Dermal side of excised skin was cleaned properly to which tissues and blood vessels are adhered. The ETTS were placed in contact with stratum corneum and was fixed on the donor compartment of Keshary-Chien diffusion cells using an adhesive. The stirrer speed was set at 100 rpm and temperature was controlled at 32 °C ± 5 °C. Donor compartment containing carbon cathode was fixed on the receptor compartment containing carbon anode. DC power source was used to regulate the applied electric current. The collection of samples was done from receptor compartment at different time intervals and drug content was determined using UV spectrophotometer (Shimadzu 1800, Japan) at 315 nm. The experiments of drug permeation were done in the below given conditions; (a) without electrical stimulus (passive diffusion), (b) with electrical stimulus using 2, 4, and 8 mA currents, and (c) and turning electrical stimulus on–off using 4 mA current.
Results and Discussion

Pre-formulation Study

The solubility of the drug in a given vehicle determines the active concentration at which the drug could be presented on to the surface of skin. Hence, a good solubility in a chosen vehicle ensures the movement of the drug through delivery systems. We were determined the solubility of drug in double distilled water and it was found to be 0.0306 mg/ml (Table 2). Drugs without sufficient lipophilicity encounters difficulty in crossing the lipid bilayer However, when the lipophilicity becomes too prominent, the drug may form a reservoir within these layers Hence, a balance of hydrophilicity and lipophilicity is desirable in the structure of drug. The octanol-water partition coefficient is thought to be a good indicator. In our study, it was found to be 3.479 for Cariprazine (Table 2). Linear correlation is seen in between the log flux and reciprocal of melting points which indicates that the penetration is better when the melting point is lower. The melting point of Cariprazine was found to be 184 °C (Table 2). The pH of the 5% w/v drug solution was found to be 6.4, which is in the range of skin pH [4-7], indicating its compatibility with skin (Table 2). To know the skin permeability of Cariprazine, we have performed the drug permeation experiments using excised abdominal skin of rat for plain drug (without enhancer) and with four different classes of permeation enhancers namely, 1, 8 cineole, span 20, oleic acid and dimethyl formamide (Figure 2). It was noticed that the Cariprazine permeation rate (without enhancer) was very low; a 28.13 % of drug permeated upto the end of 24 hr. But when penetration enhancers were used, little enhancement in drug permeation was observed. A 31.45%, 35.48%, 45.97% and 50.18% of drug was permeated with span 20, oleic acid, DMF and cineole respectively. The rank order of permeation enhancers was cineole > DMF > oleic acid > span 20. The calculated JSS, Kp and EF (enhancement factors) are shown in Table 3. It was observed that the average flux of Cariprazine was 0.0845 mg/cm2/hour, while the presence of chemical enhancers increased the transdermal flux to little extent.

Table 2
Data obtained from pre-formulation studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Solubility (mg/ml)</th>
<th>pH</th>
<th>Partition coefficient</th>
<th>Melting point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cariprazine</td>
<td>0.0306</td>
<td>6.4</td>
<td>3.479</td>
<td>184 °C</td>
</tr>
</tbody>
</table>

Figure 2. Permeation profiles of Cariprazine with or without chemical enhancers through excised rat abdominal skin
Table 3
Steady state flux, permeability coefficients and enhancement factors of Cariprazine with or without enhancer

<table>
<thead>
<tr>
<th>Donor compartmental content</th>
<th>Jss</th>
<th>Pm</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cariprazine</td>
<td>0.0845</td>
<td>0.008484</td>
<td>--</td>
</tr>
<tr>
<td>Cariprazine + Span 20</td>
<td>0.0954</td>
<td>0.009537</td>
<td>1.147</td>
</tr>
<tr>
<td>Cariprazine + Oleic acid</td>
<td>0.999</td>
<td>0.009999</td>
<td>1.208</td>
</tr>
<tr>
<td>Cariprazine + DMF</td>
<td>0.1205</td>
<td>0.012047</td>
<td>1.489</td>
</tr>
<tr>
<td>Cariprazine + Cineole</td>
<td>0.1306</td>
<td>0.013064</td>
<td>1.618</td>
</tr>
</tbody>
</table>

*Jss*: Flux (mg/cm²/hour), *Pm*: Permeability coefficient (mg/hour.cm), *EF*: Enhancement factor

**Synthesis of electrically Sensitive PAAm-g-GaG copolymer**

In electrically sensitive polyacrylamide-grafted-guar gum (PAAm- g-GaG) copolymer was synthesized and was used to prepare ETTS. The reaction was conducted with slow purging of nitrogen gas using APS as a free radical initiator at 80° C. The synthesized PAAm-g-GaG copolymer was subjected to alkaline hydrolysis with NaOH solution. When polymer solution heated at 80 oC, sulfate anion free radicals are generated by the breakdown of APS; alkoxy macroradical was formed on GaG structure by the withdrawal of hydrogen from -OH groups of GaG, thus the grafting of AAm was initiated on the backbone of GaG. Scheme 4 shows the whole reaction pathway. The graft copolymer undergoes saponification by converting–CONH2 groups to –COONa.

**Characterization of PAAm-g-GaG copolymer**

**FTIR spectroscopy**

The results of FTIR spectroscopy are depicted in Figure 3. The GaG (A) spectrum, showed the broad peak at 3350 cm⁻¹ as a result of –OH group stretching vibrations, at 1630 cm⁻¹, deformation of carbonyl group was observed. Peak at 2930 cm⁻¹ is the result of C–H cyclic aldehyde stretching vibrations and peak at 1020 cm⁻¹ relates C–O alcoholic groups stretching. Whereas, PAAm-g-GaG (B) has shown peaks at 3400 cm⁻¹ and 3200 cm⁻¹ which interrelates by overlapping the stretching vibrations of bonded–NH and –OH groups. Peaks appeared at 1610 and 1460 cm⁻¹ are due to the presence of primary amide groups on GaG, peaks at 1630 and 1430 cm⁻¹ ascribed due to deformation of carbonyl groups within primary amide. Due to aliphatic –CH stretching, peak was appeared at 2930 cm⁻¹. Therefore, this assures completion of graft reaction. Whereas in case of hydrolyzed PAAm-g-CaG (C) copolymer, peak at 3400 cm⁻¹ is due to of –OH group stretching, peak at 3200 cm⁻¹ is because of unhydrolyzed amide groups. Peak at 3200 cm⁻¹ relates to the aliphatic –OH vibration in the grafted copolymer The sharp peaks at 1660 and 1600 cm⁻¹ are because of the primary amide groups on GaG, while the peak at 1430 cm⁻¹ relates COO⁻ groups. This assures hydrolysis of grafted copolymer[24].
Figure 3. FTIR spectra of Gaur gum (A), PAAm-g-GaG (B) and hydrolyzed PAAm-g-GaG copolymer (C)

TGA Analysis

The results TG analysis for GaG and PAAm-g-GaG are shown in Figure 4. For native GaG, the mass loss was 6.10% up to 200 oC, 85.30 % upto 700 oC and further mass loss of 98.00 % was observed at 900 oC. While, for PAAm-g-GaG, the decomposition occurred after 200 oC.A 3.10% mass loss was observed upto 200oC which may be due to loss of moisture. In between 200oC to 400oC,loss of mass of 66.50 % was observed and finally it went up to 92.50% at 900oC, this was because of polymer decomposition; this mass loss was constant and noted the higher percent of mass residual. Therefore, PAAm- g-GaG showed greater thermal stability than the native GaG this assures grafting reactions of PAAm on GT [25].
Elemental analysis

Elemental analysis results are shown in Table 4. In case of native GaG, it showed of N, 38.174% of C and 4.495% of H. While, in case of PAAm-g-GaG, it showed 14.578 % of N, 41.769% of C and 5.941 % of H. It was observed that after grafting, PAAm-g-GaG has greater amount of nitrogen, which may be due to fact that the GaG back bone has greater amount of –CONH$_2$ groups on it. In case hydrolyzed PAAm-g- GaG, it showed 3.573% of N, 28.651% of C and 4.036% of H. The reduction of nitrogen content to 3.573% was seen with hydrolyzed PAAm-g-GaG; it is due to the coversion of –CONH2 groups to –COOH groups. Hence, this confirms the grafting and alkaline hydrolysis reactions [26].

<table>
<thead>
<tr>
<th>Polymer</th>
<th>N (%)</th>
<th>C (%)</th>
<th>H (%)</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GaG</td>
<td>0.00</td>
<td>38.174</td>
<td>4.495</td>
<td>1988.00</td>
</tr>
<tr>
<td>PAAm-g-GaG</td>
<td>14.578</td>
<td>41.769</td>
<td>5.941</td>
<td>1634.80</td>
</tr>
<tr>
<td>Hydrolyzed PAAm-g-GaG</td>
<td>3.573</td>
<td>28.651</td>
<td>4.036</td>
<td>365.28</td>
</tr>
</tbody>
</table>

Determination of neutralization equivalent values

Neutralization equivalent (NE) values of GaG, PAAm-g-GaG and hydrolyzed PAAm-g-GaG are shown in Table 4. As lesser the NE, greater will be the carboxyl group fractions. A 1988.00 gm, 1634.00 gm and 365.28 gm are the NE values of GaG, PAAm-g-GaG and hydrolyzed PAAm-g-GaG respectively. Greater number of –COOH groups were gained by hydrolyzed PAAm-g-GaG than the native GaG [26].
Evaluation of ETTS Hydrogel reservoir

The pH and drug content of the hydrogel reservoirs prepared were evaluated. The gel reservoirs prepared were uniform and translucent; the reservoir gels showed pH in the acceptable skin pH range i.e., between 6.2 and 7.8. The 81.30% to 89.82% was the range of drug content in gel reservoirs. It suggests the appropriate loading of drug in gel reservoirs (Table 5) [27].

Table 5
Drug content & pH of hydrogel reservoir and thickness of RCM

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Hydrogel reservoir</th>
<th>Thickness of RCM(μm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Drug content (%)</td>
<td>pH</td>
</tr>
<tr>
<td>B GaG1</td>
<td>83.28</td>
<td>7.8</td>
</tr>
<tr>
<td>BGaG2</td>
<td>81.55</td>
<td>6.5</td>
</tr>
<tr>
<td>BGaG3</td>
<td>89.82</td>
<td>6.2</td>
</tr>
<tr>
<td>BGaG4</td>
<td>81.80</td>
<td>6.9</td>
</tr>
<tr>
<td>BGaG5</td>
<td>81.30</td>
<td>6.8</td>
</tr>
<tr>
<td>BGaG6</td>
<td>81.55</td>
<td>7.7</td>
</tr>
<tr>
<td>BGaG7</td>
<td>82.54</td>
<td>7.7</td>
</tr>
<tr>
<td>BGaG8</td>
<td>82.78</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Rate controlling membranes

RCMs prepared by using GaG and PVA alone were found brittle and were not useful for developing ETTS; while RCMs formed by combination of GaG and PVA were found thin and flexible with smooth surface and were useful for developing ETTS this combination showed better film forming properties prepared by mercury surface technique. Further, film thickness and water vapor transmission (WVT) parameters were used for evaluation of RCMs. The 105 to 135 μm thickness range was reported for RCMs; thickness increased with increasing amounts of cross-linking agent, which leads to decreased permeation rate. Water vapor transmission (WVT) was determined to assess the permeability through films. It was found that water vapors were permeated through RCMs; the WVT rate was declined with higher concentration of GA in the RCMs (Figure 5) [27].

Figure 5. Water vapor transmission profiles of GaG-PVA RCMs
In-vitro drug permeation across rat skin

In-vitro drug release data of prepared ETTS in presence or absence of DC electric current with flux values are shown in Figures 6, 7, 8, 9, and Table 6. Higher concentration of drug permeation was observed with applied DC electric current and lesser amount of drug got permeated without DC electric current. The decreased permeation of drug was seen with formulation in which higher amount of PAAm-g- GaG copolymer was used; it was due to higher viscosity of formulation. Also, the drug permeation was affected by the concentration of glutaraldehyde in ETTS; higher the amount of glutaraldehyde, lower was the drug permeation (Figure 6) [28].

![Figure 6: In vitro drug permeation across rat skin in the absence of electric stimulus](image1)

![Figure 7: In vitro drug permeation across rat skin in the absence of electric stimulus](image2)
Figure 8. Effect of electric current strength on drug permeation across rat skin

Figure 9. Flux values obtained from drug permeation with or without electric stimulus
Table 6
Flux ($J_{ss}$), permeability coefficients ($P_m$), and enhancement factors ($EF$) obtained from permeation experiments

<table>
<thead>
<tr>
<th>Formulations</th>
<th>In the absence of electric stimulus</th>
<th>In the presence of electric stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$J_{ss}$ (mg/cm$^2$/h)</td>
<td>$P_m$ (mg/hour.cm)</td>
</tr>
<tr>
<td>BGaG1</td>
<td>0.0705</td>
<td>0.1002</td>
</tr>
<tr>
<td>BGaG2</td>
<td>0.0619</td>
<td>0.1184</td>
</tr>
<tr>
<td>BGaG3</td>
<td>0.0599</td>
<td>0.1239</td>
</tr>
<tr>
<td>BGaG4</td>
<td>0.0568</td>
<td>0.1213</td>
</tr>
<tr>
<td>BGaG5</td>
<td>0.0524</td>
<td>0.1233</td>
</tr>
<tr>
<td>BGaG6</td>
<td>0.0502</td>
<td>0.1179</td>
</tr>
<tr>
<td>BGaG7</td>
<td>0.0458</td>
<td>0.1047</td>
</tr>
<tr>
<td>BGaG8</td>
<td>0.0418</td>
<td>0.0974</td>
</tr>
</tbody>
</table>

$J_{ss}$: Flux (mg/cm$^2$/h), $P_m$: Permeability coefficient (mg/h.cm), $EF$: Enhancement factor

While on application of 2mA electric current, drug permeation rate from ETTS was increased, as there was lesser drug permeation without electric stimulus. Increase in the flux values were noted by two-fold. It was due to the higher amount of PAAm-g-GaG in hydrogel which makes ETTS responsive to the applied DC electric current (Figure 6). We observed that the increase in drug permeation was relying upon the nature of formulation and application of DC electric current. The permeation rate was decreased with increased amount of glutaraldehyde and increased thickness of RCMs in the formulation. While the drug permeation rate was increased with increasing applied electric current strength from 2mA to 8mA (Figure 7) [29].

The permeability coefficient ($K_p$) was estimated by equation showed below and the linear part of the permeation profiles were used to estimate the steady state flux ($J_{ss}$).

$$K_p = \frac{J_{ss}}{C_v}$$ .............................. (2)

Whereas, $C_v$ represents amount of drug present in the donor compartment. The below equation was used to estimate the range of enhancement factors ($EF$), which were ported to be in the range of 1.422 to 2.355.

$$EF = \frac{J_{ss} \text{ with electric stimulus}}{J_{ss} \text{ without electric stimulus}}$$ .............................. (3)

An enhanced permeation of drug from ETTS was seen after applying DC electric current. Flux values received for developed ETTS in passive and active conditions (with and without DC current) were between 0.0418 to 0.0705mg/cm$^2$/h and 0.0106 to 0.0179mg/cm$^2$/h [30]. The Figure 10 represents the pulsatile release behavior of ETTS; permeation rate under “on-off” condition of DC electric current was reported. Quick permeation of drug was seen when
applied DC current was ‘on’ and permeation of drug was slowed when applied DC current was ‘off’\[30\].

Figure 10. Electrical responsiveness of ETTS with “on-off” electrical stimulus

The Figure 11 reveals the change in skin structure after applying DC current. Table 7 shows the scores of changes in skin structure. The intact stratum corneum, much woven structures, no provocative cell penetration and no change in the skin extremities were seen in the normal skin. Whereas, for the skin treated with DC electric current, the intactness of stratum corneum was altered, cell structure integrity was compromised due to infiltration in the dermis and deterioration of skin appendages was recorded. The enhanced permeation of drug might be because of changes in the skin structure. However, very light change in the skin structure was observed with electric current of 2 mA and little higher changes with electric current of 8 mA\[31, 32\].

Table 7
Histopathology of rat skin without and with electric stimulus

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Parameters</th>
<th>Skin without electric stimulus</th>
<th>Skin treated with 2 mA</th>
<th>Skin treated with 8 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Stratum corneum intactness</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>02</td>
<td>Epidermis liquification</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>03</td>
<td>Subepidermal odema</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>04</td>
<td>Collagen fiber swelling</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>05</td>
<td>Inflammatory cell infiltrate</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>06</td>
<td>Skin appendages degeneration</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Scores: 0 - No Change, 1 – Very Light Change, 2 – Slight Change, 3 – Moderate Change, 4 – Marked Change.
Conclusions

Thus, it can be concluded from the present work that the synthesized graft copolymers have shown excellent electrical-responsiveness. The ETTS developed using electrically-responsive graft copolymers of PAAm-g-GaG having crosslinked with glutaraldehyde have confirmed that there was augmented drug permeation under the influence of electric stimuli, while it was reduced without electric current. The Cariprazine permeability got decreased as the concentration of crosslinking agent was increased. Further, higher drug permeability rate observed with increasing electric current strength from 2 to 8 mA. There was a pulsatile pattern of drug permeability under ‘on and ‘off’ electric stimulus mode. Hence, these graft copolymers are competent biomaterials those could be used for the development of electro-sensitive transdermal therapeutic systems for on-demand drug delivery. These systems also perform like platform technology for incorporation other molecules, which are unable to deliver through traditional transdermal route due to their molecule size, lipophilicity, ionic state etc. and also for those molecules where chronotherapy is essential.

References


Figure 11. Histopathology of rat skin without electrical stimulus (A), with 2 mA (B) and with 8 mA (C) (Hematoxylin–Eosin, 100x)
Comparison Study to Olanzapine and Aripiprazole in Rats. Scientia Pharmaceutica. 2020 Dec;88(4):50.


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