In Vitro/In Vivo Evaluation and Bioavailability Study of Amitriptyline Hydrochloride from the Optimized Oral Fast Dissolving Films

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Abstract

The present investigation was undertaken with the objective of developing fast dissolving film(s) of a tricyclic antidepressant drug amitriptyline hydrochloride in order to improve its bioavailability, optimize its therapeutic action especially when used to treat major depression and to enhance the compliance for the developmentally disable, mentally ill, elderly and pediatric patients. Fast dissolving dosage forms have acquired great importance in pharmaceutical industry because of their unique properties. Ten formulations were prepared by solvent casting method using different polymer types, plasticizer types, surfactant concentrations and different ratio of hydroxypropyl methyl cellulose and maltodextrin. The prepared films were evaluated for folding endurance, thickness, drug content, in vitro/in vivo disintegration time, drug release and tensile test. The optimized formula F8 containing HPMC 15cp and maltodextrin in 1:1 ratio showed minimum in vitro/in vivo disintegration time 16.8, 13.2 seconds respectively, highest dissolution rate i.e. T80% 1.1 minutes, D2 min (%) 89.77% and satisfactory mechanical properties. The optimized film was further evaluated for bioavailability compared with a marketed solution (Amitriptyline Hydrochloride®), the study based on cross over design using experimental animals (rabbits). The pharmacokinetic results revealed that the fast dissolving films has higher peak blood concentration (Cmax, 0.927µg/ml) within shorter time (Tmax, 2 hours), indicating rapid absorption and faster onset of action with acceptable bioavailability value. These findings suggest that the fast dissolving film containing amitriptyline hydrochloride is expected to become one of choices for the treatment of acute depression.

Keywords: Amitriptyline Hydrochloride, Fast dissolving films, Bioavailability

1 Introduction

Despite the tremendous advancement in the drug delivery system, oral route is the most acceptable drug delivery route for patient compliance aspects. There are many drug delivery systems accessible for the oral administration of the drugs. All these systems are related with their own merits and demerits. The change in lifestyle and changes in patient population in the emerging and developing countries, painless, simple cost and effective delivery system is predictable to have a bright future. The industries have diverted their research focuses from conventional dosage forms to new drug delivery technologies such as fast dissolving drug delivery system. Rapid dissolving oral dosage forms is also known as fast dissolve, quick disintegrating and rapid melt drug delivery systems were first developed in the late 1970s as an alternative to capsules, tablets and syrups for people experiencing difficulty in swallowing traditional solid dosage forms. Fast dissolving films (FDF) are the most advanced form of fast dissolving oral dosage forms. It is developed on the base of the technology of transdermal patch. The delivery system consists of thin elegant films of edible hydrophilic polymers of various sizes and shapes like square, rectangle or disc. The strips may be flexible or brittle, transparent or opaque which is simply placed on the tongue or any oral mucosal tissue and due to saliva, it instantly wet, hydrates and adheres onto the site of application. It rapidly disintegrates and dissolves to release the drug for oromucosal absorption or will maintain the quick-dissolving aspects which allow for gastrointestinal absorption to be achieved.
when swallowed. Fast dissolving films provide rapid, precise dosing in a safe, efficacious format that is convenient, portable and can be used anywhere anytime. Amitriptyline hydrochloride (AMT HCl) is a tricyclic antidepressant, which is widely prescribed for the treatment of major depression. It has low bioavailability (30-60%) due to extensive first-pass effect. The aim of this study was to formulate Amitriptyline Hydrochloride as fast dissolving films, to improve the pharmacokinetic properties of the drug through enhancing the rate of drug absorption, avoiding hepatic first-pass metabolism and to provide effective and satisfactory dosage form for patients suffering from dysphagia, nausea or vomiting and unconscious patients.

2 Materials and Methods

2.1 Materials

Amitriptyline HCl (Samara Drug industry-Iraq), Hydroxy propyl methyl cellulose (HPMC15cp) (Provizerpharma-India), Poly vinyl alcohol (PVA) (Provizerpharma-India), Sodium carboxy methyl cellulose (NaCMC) (Provizerpharma-India), Maltodextrin (MDX) (Sigma-Aldrich-USA), Tween 80 (Riedel-De-Haen-Germany), Glycerine (GCC-UK), Citric acid (Panreac-Espana), Sodium saccharine (Avonchem limit-UK), Amitriptyline HCl Solution (Wockhardt-UK), Diltiazem HCl (Asia pharmaceutical industries-Iraq), Methanol HPLC grade (Biosolve B France), Acetonitrile HPLC grade (Biosolve B France), n-Hexane HPLC grade (Biosolve B France), Acetonitrile HPLC grade (Biosolve B France).

2.2 Methods

2.2.1 Preparation of oral film

Ten formulations were prepared (F1-F10), using solvent casting method with different types of polymers and composition as shown in table 1. The plasticizer, sodium saccharine, citric acid and mannitol were dissolved in suitable volume of water with heating and continuous stirring to form a clear viscous solution. After cooling, a suitable amount of polymers (an aqueous dispersion of film forming polymer was separately prepared) were added to the previous solution. Pre-dissolved AMT HCl in water was added with stirring for 4 hours, until uniformly viscous solution achieved, which was kept undisturbed condition at least for 24 hours to remove the entrapped air. The resulting solution was poured into a 9 cm petri dish and allowed to dry in hot air oven at 40 °C for 24 hours, the dried batch carefully removed, cut into desired size.

2.2.3 Evaluation of oral films

2.2.3.1 Visual inspection

Properties such as homogeneity, color, transparency and surface of the oral films were inspected for all the prepared oral films.

2.2.3.2 Thickness measurements

The thickness of the film was measured by micrometer screw gauge at different strategic points. Each film was measured at 5 positions (center and four corners), and the mean thickness was calculated.

2.2.3.3 Drug Content Uniformity

The film was allowed to dissolve in 100 ml phosphate buffer pH 6.8 contained in 100 ml volumetric flask, with stirrer maintained at 37°C for 3 hours and left for 24 hr at room temperature. The filtered solution was diluted and analyzed by UV-spectrophotometer at λmax 239 nm in triplicate, the average drug content was calculated.

2.2.3.4 Surface pH study

The surface pH of the oral dissolving film is calculated in order to investigate the risk of any side effects in vivo, since acidic or alkaline pH may cause irritation to the oral mucosa and it is measured to maintain the surface pH as close to neutral as possible. A combined pH electrode is used for this purpose. The film was slightly wet with the help of 1 ml of distilled water and kept for 30 seconds. The pH was measured by bringing the electrode in contact with the surface of the formulation and allowing it to equilibrate for 1 minute. The average of three determinations for each film was determined.

2.2.3.5 Folding endurance

The folding endurance is expressed as the number of folds (number of times of folding the film at the same plain) required to break the specimen or developing visible cracks or folded up to 300 times manually, which was considered satisfactory to reveal good patch properties and gives an indication of brittleness of the film.

2.2.3.6 Tensile test

The mechanical properties of the films were determined using Tinius Olsen testing instrument (Model H50KT, UK). The sample was cut into a dumbbell shape, then held between two clamps and the strip was pulled by the top clamp at a rate of 10 cm/min. The force and elongation were determined as the film broke. The mechanical properties include tensile strength, percent elongation, elastic modulus and strain were computed for the characterization of the film. Tensile strength is the maximum stress applied to a point at which the strip specimen breaks, as described from the following equation:

\[
\text{Tensile strength (TS)} = \frac{\text{Force at break (N)}}{\text{Initial cross – Sectional area of the sample}}
\]

Percent elongation at break (E %) was calculated using the flowing equation

\[
\%E = \frac{D_1 - D_2/D_0} \times 100
\]
Where $D_0$ is the distance between the tensile grips before the fracture of the film, and $D_f$ is the distance between the tensile grips after the fracture of the film.

Young’s modulus or elastic modulus (EM) is the measure of stiffness of the strip was calculated from the following equation, 
\[
\text{F/A} = \text{EM} \times \left(\frac{D_f - D_0}{D_0}\right)
\]

Where $F$= breaking load (N) and $A$ = cross-sectional area of the sample.

Strain value was calculated from the flowing equation:
\[
\text{Strain} = \frac{\text{tensile strength}}{\text{elastic modulus}}
\]

Fast dissolving film should possess moderate TS, high %E, low EM, high strain.

Table 1: Composition of Different Fast Dissolving Films Containing AMT HCl (Quantities are expressed in terms of %w/w)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
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<tr>
<td>AMT HCl</td>
<td>11.31</td>
<td>11.31</td>
<td>11.31</td>
<td>11.31</td>
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<td>11.31</td>
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<tr>
<td>HPMC15cp</td>
<td>45.35</td>
<td>-</td>
<td>-</td>
<td>45.35</td>
<td>45.35</td>
<td>45.35</td>
<td>45.35</td>
<td>22.67</td>
<td>30.23</td>
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<td>PVA</td>
<td>-</td>
<td>45.35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>NaCMC</td>
<td>-</td>
<td>-</td>
<td>45.35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22.67</td>
<td>15.12</td>
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<tr>
<td>Glycerin</td>
<td>21.54</td>
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<td>Tween 80</td>
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<td>Citric acid</td>
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<td>2.23</td>
<td>2.23</td>
<td>2.23</td>
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</tr>
<tr>
<td>Na saccharin</td>
<td>3.43</td>
<td>3.43</td>
<td>3.43</td>
<td>3.43</td>
<td>3.43</td>
<td>3.43</td>
<td>3.43</td>
<td>3.43</td>
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</tr>
</tbody>
</table>

2.2.3.6 Disintegration test

2.2.3.6.1 In-vitro disintegration test

The test was performed using USP disintegration test apparatus, using 250 ml phosphate buffer pH 6.8 at 37±0.5°C as medium, 2×2 cm² film was placed in the tube of the basket and the disks were placed over it.

2.2.3.6.2 In-vivo disintegration test

The time required for complete disintegration in the oral cavity was calculated from three healthy volunteers. The mouth cavity was rinsed with a cup of water, the film was placed on the tongue and subsequently the tongue was gently moved. The time required for disintegration in the mouth was determined. The data were represented as a mean of three replicate determinations.

2.2.3.7 In-vitro dissolution study

The dissolution study was carried out using USP dissolution apparatus type II paddle apparatus in 250 ml phosphate buffer (pH 6.8) kept at 37 ± 0.5°C with rotation speed 50 rpm. Film of 4 cm² size.
was immersed and fixed on a jar glass, five milliliters samples were withdrawn at the time interval from 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40 and 45 min and an equal volume of the fresh dissolution media at the same temperature was replenished. The collected samples were filtered and analyzed spectrophotometrically at measured λmax. The % release of AMT HCl from film was measured; results were expressed as mean of three determinations6.

2.2.3.8 Characterization of selected formula using scanning electron microscopy (SEM)

SEM (SEM Inspect S50-Netherlands) is utilized to assess the surface morphological characteristics of the optimized formulation. The sample for SEM was mounted on round brass stub using double backed adhesive tape and then sputter coated for 30 seconds with gold palladium under an argon atmosphere. The stub containing the coated sample was placed in the scanning electron microscope chamber. Microphotographs were taken on different magnification17.

2.2.3.9 Bioavailability test

2.2.3.9.1 Study design

The protocol for the study was approved by the animal care committee in National Center for Drugs Control and Researches in Iraq. Twenty four male rabbits (weighed 1850-2250 g) were selected for this study and only 6 rabbits continued in this study because of several difficulties such as (disease, death of animals) during the study and difficulty to continue sampling as decided schedule times). All six rabbits were healthy during the period of the experiment. The study was a simple crossover design with three-week washout period, in order to reduce variability arising from physiological variables (e.g. gastric emptying, pH), in addition to the variability arising from residual effects. The rabbits were fasted overnight (to minimize the effects of food on pharmacokinetic profile) with free access of water and sine. The rabbits were randomly divided into two groups (A and B) each group consist of three rabbits. Group A were anesthetized by intravenous injection of pentobarbital in a dose of 25 mg/kg, the rabbits were then placed on a table with the lower jaw supported in a horizontal position and the selected formula F8 was placed carefully on the rabbit's tongue. The rabbits were anaesthetized to ensure the maintenance of the film in the oral cavity without escaping down the gastrointestinal tract. A suitable volume of the commercial AMT HCl solution equivalent to the applied dose was administered orally to group B via gastric gavage18, 19. Blood samples (2ml) were drawn from the marginal ear vein at zero time and 1, 2, 3, 4, 6, 8, 10 and 24 hours. Blood samples were collected in heparinized tubes and immediately frozen at -20°C until analysis 20.

2.2.3.9.2 Chromatographic conditions

A Knauer HPLC system (Germany) was employed in the bioavailability test and consists of a Wellchrom K-1001 pump, a Rheodyne 7125 injector and a K 2501 UV detector with a thermostatic column compartment connected to a Eurochrom 2000 injector. An isocratic high performance liquid chromatography was performed on an analytical C18 column (Knaure;150 ×4.6 mm :5 μm particle size; 25cm length ) supported by guard column C18- 4 mm diameter (Germany). The wave length was set at 254 nm. The mobile phase was a mixture of acetonitrile and buffer solution of (0.02 M) KH2PO4, (35:65 v/v) and adjusted to pH 3.0 by orthophosphoric acid. Chromatographies were carried out using flow rate of 1.5 ml min⁻¹ and at 40°C 21.

2.2.3.9.3 Preparation of stock solutions

The primary stock solutions of AMT HCl standard and internal standard (I.S diltiazem HCl) were prepared by dissolving separately 10 mg of each compound in 10 ml of methanol using volumetric flasks, to produce concentration of 1 mg/ml. These were stored at -20°C. The working standard solution of the strength 10μg /ml was prepared by diluted the stock solution 100 fold via using the mobile phase 22.

2.2.3.9.4 Sample extraction procedure

To one milliliter of blood, 50 μl of I.S (10µg/ml freshly prepared) and 200 μl of 0.5 M NaOH were added together with 4 ml n-hexane. The resulted mixture was vortexed for 5 min., followed by centrifugation at 2000 rpm for 10 min, the organic layer was isolated carefully, transferred to another tube and evaporated at 40°C under stream of nitrogen. The dry residue was reconstituted with 500 µl mobile phase and vortexed for 30 seconds. The solution was injected into autosampler vials of HPLC 23.

2.2.3.9.5 Pharmacokinetic and statistical calculations

The peak concentration (Cmax) and peak times (Tmax) were derived directly from the experimental points. The other pharmacokinetic parameters of two formulations were computed by non-compartmental analysis using (Kinetica® software-version 5, Thermo Fisher scientific, USA), mean value (±SD) were calculated for each parameter. The pharmacokinetic parameters of the two products (the prepared film and commercial solution of AMT HCl) were compared by one way analysis of variance (ANOVA).

3 Results and Discussions

3.1 Visual inspection

The appearance of all prepared AMT HCl fast dissolving films which contain different film forming polymers showed homogenous, transparent, flexible, non sticky, smooth in the texture properties with elegant appearance.
3.2 Thickness measurement

The thickness of the films is essential to be uniform as it is directly associated to the precision of dose. Thickness of the films was found to vary between (0.05-0.229 mm) (table 2). The low ±SD values in the film thickness measurements ensured uniformity of thickness in each formulation and the method used for the formulation of the film is reproducible with dose accuracy23.

3.3 Drug content uniformity

All the prepared films were found to be within uniform quantity of the drug, and the preparations met the criteria of United State Pharmacopeia (USP), as shown in table 2. This indicates that the drug was uniformly dispersed throughout the films24.

3.4 Surface pH study

The surface pH of films was found to be in the range of (6.1 - 6.89), which is within the range of salivary pH. It indicates that the fast dissolving film not create any types of oromucosal irritation.

3.5 Folding endurance

The results indicated that most of the formulas showed satisfactory folding endurance, the low folding endurance of the films below the acceptable level (usually <100 times) were found in the formulations (F10), this is related to weak bonds between polymer chains that cannot maintain their integrity upon folding25, as shown in table 2.

3.6 Effect of film forming polymer types

Formulations (F1-F3) that were prepared using different primary film forming polymers (HPMC 15cp, PVA, NaCMC) to study the effect of different polymer types on the disintegration, mechanical properties and release profile of prepared films. The rank order of AMT HCl films in vitro/in vivo disintegration time is as follows: PVA(F2) > NaCMC (F3) > HPMC (F1), as shown in table3. Formula F1 film gave shorter DT compared to other formulations. This may be due to the high solubility of HPMC film in polar solvents and therefore undergoes disintegration rapidly without forming a gel residue, ensuring rapid matrix disintegration26.

The results display the effect of film forming polymer types on the mechanical properties of the prepared films (F1-F3), formula F1 gave satisfactory mechanical property. Formula F2 showed higher strain, higher TS, higher E% than F1, this may be due to formation of strong hydrogen bonds between polymer (PVA) and plasticizer there by imparting the polymer flexibility to withstand rupture27. While F3 showed lower strain value, as shown in table 3.

The effect of changing types of the polymer on the drug release profile from AMT HCl fast dissolving films was conducted by comparing the release profile of formulations (F1-F3) as shown in figure 1. The time required for 80% of drug to be released (T80%) and percent drug dissolved in 2 minutes (D2 min) are scheduled in table 4. Formulation F2 showed higher dissolution rate than other formulas, this is attributed to the high solubility of PVA in water, and the loosely bounded PVA molecules were easily eroded, allowing the rapid release of the drug 28. while Formula F4 showed lower dissolution rate since Na CMC dissolved rapidly leading to the formation of channels inside the matrix of the film which enhanced the diffusion of the drug across such channels followed by the formation of a stable gel layer which in turn will control the release of drug from the film29.

Table 2: The Physical Parameters of The Prepared Oral Films of AMT HCl

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Film Thickness (mm) ± SD</th>
<th>Drug Content Uniformity (%) ± SD</th>
<th>Surface pH ± SD</th>
<th>Folding endurance ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.081±0.034</td>
<td>98.10±0.45</td>
<td>6.89±0.1</td>
<td>&gt;300</td>
</tr>
<tr>
<td>F2</td>
<td>0.229±0.04</td>
<td>98.8±0.12</td>
<td>6.83±0.09</td>
<td>&gt;300</td>
</tr>
<tr>
<td>F3</td>
<td>0.130±0.06</td>
<td>98±0.12</td>
<td>6.68±0.08</td>
<td>&gt;300</td>
</tr>
<tr>
<td>F4</td>
<td>0.105±0.07</td>
<td>96.32±0.225</td>
<td>6.81±0.07</td>
<td>&gt;300</td>
</tr>
<tr>
<td>F5</td>
<td>0.09±0.078</td>
<td>95.8±0.33</td>
<td>6.83±0.032</td>
<td>&gt;300</td>
</tr>
<tr>
<td>F6</td>
<td>0.20±0.087</td>
<td>96±0.09</td>
<td>6.10±0.03</td>
<td>&gt;300</td>
</tr>
<tr>
<td>F7</td>
<td>0.08±0.034</td>
<td>98±0.14</td>
<td>6.74±0.07</td>
<td>&gt;300</td>
</tr>
<tr>
<td>F8</td>
<td>0.061±0.078</td>
<td>98.3±0.34</td>
<td>6.76±0.03</td>
<td>&gt;300</td>
</tr>
<tr>
<td>F9</td>
<td>0.076±0.054</td>
<td>97.0±0.17</td>
<td>6.09±0.02</td>
<td>&gt;300</td>
</tr>
<tr>
<td>F10</td>
<td>0.05±0.034</td>
<td>91.3±0.22</td>
<td>6.72±0.01</td>
<td>15.3±0.76</td>
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</table>

3.7 Effect of different plasticizer types

Three types of plasticizers; glycerin, propylene glycol (PG) and poly ethylene glycol 400 (PEG400) in concentration of 21.54% w/w (represented in formulas F1, F4, F5) were prepared to study the...
effect of plasticizer types on DT, mechanical properties and in-vitro drug release profile of AMT HCl oral film.

Table 3 indicates that changing plasticizer type caused non significant effect (p>0.05) in the in vitro/in vivo disintegration time of oral films; this may be due to the fact that three types of plasticizer enhanced the disintegration time by facilitated the diffusion of fluid into the film since plasticizer alter the densely packed chains of HPMC texture by forming a more porous and less dense polymer structure that breaks at lesser force, resulting in faster disintegration of the film.

On other hand the results of tensile test revealed that, formula F1 causes significant improvement (p<0.05) in mechanical properties of the films in comparison to F4 and F5, this may be due to the small size and arrangement of glycerin which makes it easily inserted between the chains of the polymer lead to provide more influence on the mechanical properties than the larger molecule.

Table 4 and figure 2 showed that F1 significant increase (p˂0.05) in the release profile of AMT HCl film in comparison to F4, F5. Since the films plasticized with glycerin adsorbed more humidity leading to increase in the hydrophilic character of films, reducing the internal hydrogen bonds between the polymer chains and enlarged the internal space in the molecular structure of polymer.

3.8 Effect of incorporation of different concentrations of surfactant (Tween 80)

The effect of different concentrations of surfactant, 2.5% (F6) and 5% (F7) in comparison to F1 (not contain surfactant ) on the disintegration time, mechanical properties and in-vitro drug release profile of AMT HCl oral films were studied.

Table 3 revealed that, the increase in concentration of tween 80 caused significant reduction (p<0.05) in disintegration time, since tween 80 is emulsifying agent, so it facilitates the diffusion of fluid into the film resulting in faster disintegration of the film. Therefore formulation F7 disintegrated faster than F6 and F1.

Table 4 and Figure 2 showed that F7 gave fastest dissolution rate, this may be due to the ability of the surfactant to improve wettability of the film and reduced the surface tension, making the drug easily accessible by dissolution medium, immediately dissolves and diffuses from the interface between the film surface and the surrounding medium.

3.9 Effect of polymeric blend and polymeric blend ratio

Formula F7 (not contain MDX), F8 (contain HPMC:MDX in the ratio 1:1 , F9(contain HPMC:MDX in the ratio 2:1) in addition to F10 ( contain HPMC:MDX in the ratio 1:2) were used to study the effect of polymeric blend and the polymeric blend ratio on the in vitro/in vivo disintegration time, mechanical properties and in-vitro drug release profile of AMT HCl oral films.

Table 3 shows an enhancement in the disintegration time with the addition of MDX compared with formula without MDX, this could be attributed to the presence of MDX in the formulation that facilitates water penetration into the film structure due to its high water-solubility, leading to decrease the thickness of the films and reduced the DT.

The results in table 4 showed that as the amount of MDX increased, there was significant increase (p<0.05) in the TS in addition significant increase (p<0.05) in the E% and strain of the films. This indicates that formulation F7 disintegrated faster than F6 and F1.
may be due to the ability of MDX to impart greater ductility to the films, which supports the mechanical properties. Further increase in the amount of MDX (F31) caused significant suppression in the mechanical properties of the film leading to brittle, weak and sticky films, since there will be further reduction in the amount of the 1° polymer (HPMC) that affects the mechanical properties of the films dramatically.

Table 3: The Disintegration and Mechanical Properties of The Prepared Oral Films of AMT HCl

<table>
<thead>
<tr>
<th>Formula code</th>
<th>In vivo DT(sec) ± SD</th>
<th>In vitro DT(sec) ± SD</th>
<th>Tensile Strength (MPa)</th>
<th>Elongation%</th>
<th>Elastic Modulus</th>
<th>Strain</th>
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<tbody>
<tr>
<td>F1</td>
<td>27±3.3</td>
<td>30.2± 2</td>
<td>17.78</td>
<td>41.40</td>
<td>43.36</td>
<td>0.41</td>
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<td>F2</td>
<td>114±3.3</td>
<td>117±2.1</td>
<td>19.17</td>
<td>150.0</td>
<td>5.5</td>
<td>1.5</td>
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<tr>
<td>F3</td>
<td>40.74±1</td>
<td>42.2±1</td>
<td>5.29</td>
<td>14.503</td>
<td>37.78</td>
<td>0.14</td>
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<tr>
<td>F4</td>
<td>27.9±1</td>
<td>30.8±1.3</td>
<td>19.53</td>
<td>28.4</td>
<td>69.75</td>
<td>0.28</td>
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<tr>
<td>F5</td>
<td>28.1±1.4</td>
<td>32.1±2.2</td>
<td>22.4</td>
<td>19.1</td>
<td>117.69</td>
<td>0.19</td>
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<tr>
<td>F6</td>
<td>24.5±1.7</td>
<td>26.3±1.9</td>
<td>15.42</td>
<td>43.24</td>
<td>35.86</td>
<td>0.43</td>
</tr>
<tr>
<td>F7</td>
<td>21.4±0.8</td>
<td>23.8±1.1</td>
<td>11.76</td>
<td>45.98</td>
<td>25.57</td>
<td>0.46</td>
</tr>
<tr>
<td>F8</td>
<td>13.2±2</td>
<td>16.8±0.8</td>
<td>5.961</td>
<td>53.0</td>
<td>11.25</td>
<td>0.53</td>
</tr>
<tr>
<td>F9</td>
<td>18.7±1</td>
<td>22.2±1.3</td>
<td>8.111</td>
<td>47.80</td>
<td>16.9</td>
<td>0.48</td>
</tr>
<tr>
<td>F10</td>
<td>7.34±0.5</td>
<td>11.8±2.6</td>
<td>1.435</td>
<td>5.33</td>
<td>2.66</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 4: In-vitro Dissolution Parameters in Phosphate Buffer (pH6.8)

<table>
<thead>
<tr>
<th>Formula</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
</tr>
</thead>
<tbody>
<tr>
<td>D_{2min}(%)</td>
<td>41.3</td>
<td>44.98</td>
<td>36.31</td>
<td>31.03</td>
<td>23.1</td>
<td>60.34</td>
<td>73.68</td>
<td>89.77</td>
<td>79.67</td>
<td>95.0</td>
</tr>
<tr>
<td>T_{80%(min)}</td>
<td>5.20</td>
<td>4.88</td>
<td>5.41</td>
<td>9.67</td>
<td>15.3</td>
<td>3.1</td>
<td>2.6</td>
<td>1.1</td>
<td>2.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

On the other hand, it was found that as the amount of MDX increased there was significant increase (p< 0.05) in the drug release, as shown in table 4 and figure 4, this may be due to the fact that the presence of HPMC alone (F7) lead to the formation of viscous gel layer that decreases the mobility of drug particles in swollen matrices leading to decrease the release rate. But upon the addition of MDX (F8-F10) resulted in reducing the viscosity of the 1° polymer with immediate solubilization of the drug in water thus guide to faster drug release.

Based on the above physical and mechanical evaluations of all 10 formulations (F1-F10), indicated that formula F8 having fastest in vivo disintegration time and satisfactory release rate and mechanical properties, so selected as the optimum formula for formulation of amitriptyline hydrochloride fast dissolving films and
chosen for further investigation, including: scanning electron microscopy, bioavailability test.

Figure 4: Effect of polymer blend (HPMC:MDX) in different ratios on the release of AMT HCl from the prepared films in phosphate buffer (pH 6.8) at 37°C

3.10 Characterization of selected formula using scanning electron microscopy (SEM)

The SEM image in figure 5 for the selected AMT HCl film revealed that the surface morphology of the formulation (F8) had a regular texture with little pores without any scratches on the film and uniform distribution of active pharmaceutical ingredients in the film matrix.

Figure 5: Surface SEM Photograph of AMT HCl fast dissolving film (F8)

3.11 Bioavailability test

The concentration of AMT HCl in rabbits blood was determined using a reproducible, sensitive and rapid HPLC method, which showed two separated peaks chromatogram for AMT and internal standard (diltiazem HCl), the chromatographic conditions and extraction procedure yielded a clear chromatogram for analyte as shown in figure 6.

The retention times of drug (AMT HCl) and internal standard (diltiazem HCl) were detected in the mobile phase and blood and were recorded to be 6.1± 0.05 min for AMT HCl and 4.7± 0.07 min for internal standard. A straight line with high regression coefficient ($R^2 = 0.996$) was obtained by plotting the peak height ratio versus the concentration.

Figure 6: Chromatogram of rabbits blood contains 10μg/ml of IS (A) and blood containing 10μg/ml of AMT HCl and IS (B)

The mean blood concentration of AMT HCl versus time profile revealed that first order pharmacokinetic model without lag time and first order elimination rate was considered to be the best fit to explain the generated data (figure 7). The average pharmacokinetic parameters (table 5) revealed that $C_{\text{max}}$ of AMT HCl from the film formula (F8) (0.927μg/ml) was significantly higher than $C_{\text{max}}$ of Amitriptyline Hydrochloride® solution (0.630μg/ml). The result also showed that the time required to reach peak concentration ($T_{\text{max}}$) from the prepared film (2hr) is significantly less than that required by the drug from Amitriptyline Hydrochloride® solution (6 hr). $T_{\text{max}}$ reflects the rate of absorption from the formulation, therefore the drug from prepared film showed the higher rate of entrance into the blood stream and gave faster onset of action. The mean residence time (MRT) which describes the average time for the drug molecules to reside in the body. From the results; the MRT of the drug from the prepared film was significantly less than that from Amitriptyline Hydrochloride® solution indicated that the drug persist for shorter time in the blood after film administration, and this agreed with the higher elimination rate constant (ke) (0.367 hr$^{-1}$) and lower half life $t_{1/2}$ (1.886 hr) in comparison to that from commercial solution where Ke (0.260 hr$^{-1}$) and $t_{1/2}$ (2.664 hr). The area under the curve (AUC) of the drug from the prepared film was found to be non significantly different ($p> 0.05$) from the AUC of the commercial solution. Therefore, the relative bioavailability of drug from the prepared film in comparison to solution was found to be 81.447% which is within the bioequivalence acceptable range of 80-125%.

4 Conclusions

In vitro and in vivo evaluation of the AMT HCl fast dissolving films confirmed their potential as an innovative dosage form to improve delivery of amitriptyline hydrochloride. Therefore, the oral fast dissolving film is considered to be potentially useful for the treatment of disease where the quick onset of action is desired, also improved patient compliance.
Table 5: Statistical Comparison of Mean Pharmacokinetic Parameters Following Administration of 40mg/kg AMT HCl in Orodispersible Film (F8) and Commercial Solution to Six Rabbits (n = 6)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Selected formula (F8)(±SD)</th>
<th>Commercial solution (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (μg/ml)</td>
<td>0.927 ±0.122</td>
<td>0.630 ±0.233</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr.)</td>
<td>2 ±0.11</td>
<td>6 ±0.09</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (μg.h/mL)</td>
<td>3.832±0.243</td>
<td>4.705±0.214</td>
</tr>
<tr>
<td>$K_e$ (hr$^{-1}$)</td>
<td>0.367±0.04</td>
<td>0.260 ±0.06</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr.)</td>
<td>1.886±0.045</td>
<td>2.664 ±0.047</td>
</tr>
<tr>
<td>MRT</td>
<td>4.065±0.224</td>
<td>7.399 ±0.232</td>
</tr>
</tbody>
</table>

*MRT=Mean residence time (hr)

Figure 7: Logarithmic mean blood concentration versus time curve for AMT HCl after single oral dose of the selected formula and commercial solution

5 Competing interests

Formulation AMT HCl FDF that provide rapid rate of absorption and rapid onset of action and improved patient compliance.

6 Author’s contributions

NKM and ZDS carried out literature review of the formulation and in vitro/in vivo evaluations.

NKM, MGA, MMG and ZDS participate in bioavailability study.

ZDS carried out the formulation of FDF of AMT HCl, in vitro evaluation and the bioavailability study.

7 References

