PREPARATION AND EVALUATION OF LIPID MATRIX MICROENCAPSULATION FOR DRUG DELIVERY OF AZILSARTAN KAMEDOXOMIL

Firas Aziz Rahi, Muath Sheet Mohammed Ameen, Krar Kadhim Mohammed Jawad

The aim of the work is to consolidate azilsartan-kamedoxomil (AZM) into lipid matrix controlled-release microparticles to enhance its permeability because AZM belongs to Biopharmaceutical classification (BCS) IV, which is characterized by poor permeability and to protect AZM from light and humidity and execute a prolonged release profile. Materials and methods. A reversed-phase HPLC method was created and validated to estimate the drug. AZM microparticles formulations were invented using melt dispersion technique and waxy materials such as carnuba wax, beeswax, and stearic acid in the ratio of waxy-substance: drug ranging from 0.25:1 to 1:1 and stabilizer, namely; tween 80 and Poloxamer 407 in the ratio of stabilizer: drug ranging from 0.5:1 to 1:1. Azilsartan formulations were assessed for azilsartan-medoxyomil content, loading, entrapment efficiency, the zeta potential, the particle size, the morphology by scanning electronic microscopy (SEM), and in-vitro release profile.

Results. Zeta potential results for microparticle formulations using beeswax and carnuba range from −21.1 mV to −26.6 mV and −20.6 mV to −26.7 mV, respectively. This difference indicates that the azilsartan microparticles containing stearic acid are better stabilized with a 25.3−29.7 mV zeta potential. Furthermore, the release from azilsartan microparticle formulations containing stearic acid exceeded 80 % after 8 h. It remained for 24 h, while release from beeswax did not exceed 65 % after the same period and less than 60 % in the case of carnuba formulations.

Conclusions. The formulation (AZSP4) exhibited the highest zeta potential and released exceeding 80 % of AZM over 8 hours and remained over a day. AZSP4 microparticles formulation containing poloxamer 407, in a 0.8:0.8:1 drug: stearic acid: poloxamer ratio proved the ability of stearic acid microencapsulation employing poloxamer as a stabilizer in a certain ratio could prolong the release of AZM.

Keywords: azilsartan kamedoxomil, controlled release, microparticles, antihypertensive drug, HPLC method


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surface charge, in vitro drug release, and scanning electron microscopy

![Fig. 1. Chemical structure of AZM](image)

2. Research planning (methodology)

The principal steps of the research include:

1. Creating the HPLC method for AZM and its analytical validation per international guidelines.
2. The planning of formulations of azilsartan kamedoxomil microparticles. Waxy materials were used to prepare extended-release microparticles capable of permeability into biological fluids to increase bioavailability.
3. Assessing the formulations prepared.
4. The selection of the best formulation and further characterized morphologically by scanning electron microscopy (SEM) to investigate the smoothness of the surface.

3. Materials and methods

Azilsartan kamedoxomil (99.8, HPLC) was purchased from MSN Labs, India. Carnuba wax and bees-wax were gifted from Wadi Elrafideen Company for pharmaceuticals, Baghdad, Iraq. Poloxamer 407 was purchased from BASF pharma, Germany. Ammonium acetate and acetonitrile, HPLC grade; E. Merck, Darmstadt, Germany. Sodium hydroxide and hydrochloric acid; Schuarlo, Spain. Tween-80, acetone, was purchased from Sigma-Aldrich chemicals. Purified water was obtained from a Milli-Q purification unit.

3. 1. HPLC method development

The system components were as follows; an X-bridge C18 (5 μm, 25 cm×4.6 mm) was the stationary phase and 0.05 M ammonium acetate (pH 5): acetonitrile in the ratio 20:80 was the mobile phase with 2 ml/min flow. A photodiode array detector was used to measure AZM at 254 nm while injecting 20 μl. The assay was performed at 25 °C employing Waters HPLC apparatus 2695 with a binary pump.

3. 2. Microparticles preparation

The microparticles of AZM were produced as follows; the stabilizer (Tween 80 or /and Poloxamer 407) was weighed precisely and put in a beaker containing water (100 ml), and stirred on a thermostated mechanical shaker at 90 °C. Then, wax was added while shaking to melt. AZM 0.8g was dissolved in 20 ml of acetone and added portion-wise to the dispersion of melted wax by stirring at 20,000 rpm for 20 min on a magnetic stirrer with a hotplate. Next, cold water was added to convert the loose microparticles to solid microparticles. Eventually, the dispersion was filtered to collect microparticles using a vacuum pump, washed with water, and dried. To assess the volumetric size distributions, a Malvern Mstersizer 2000 (Malvern UK) was employed. Table 1 revealed the azilsartan microparticle formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Bees-wax (g)</th>
<th>Carnuba wax (g)</th>
<th>Stearic acid (g)</th>
<th>Poloxamer 407 (g)</th>
<th>Tween 80 (g)</th>
</tr>
</thead>
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<tr>
<td>AZBP1</td>
<td>0.2</td>
<td>----</td>
<td>----</td>
<td>1</td>
<td>----</td>
</tr>
<tr>
<td>AZBP2</td>
<td>0.4</td>
<td>----</td>
<td>----</td>
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<td>----</td>
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<tr>
<td>AZBP3</td>
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<tr>
<td>AZBP4</td>
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<td>----</td>
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<tr>
<td>AZBP5</td>
<td>0.8</td>
<td>----</td>
<td>----</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>AZBP6</td>
<td>0.4</td>
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<tr>
<td>AZBP7</td>
<td>0.6</td>
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<td>AZBP8</td>
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<tr>
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<td>----</td>
<td>----</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
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<td>AZBP11</td>
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<tr>
<td>AZBP14</td>
<td>0.8</td>
<td>----</td>
<td>----</td>
<td>0.5</td>
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</table>

3. 3. Characterization of loaded microparticles

Encapsulation efficiency determination.

The centrifugation method was utilized to calculate the entrapment efficiency of AZM. First, Microparticle powder (including azilsartan medoxomil equivalent to 40 mg) was dispersed and centrifuged at a speed of 20000 rpm for 30 min in a centrifuge of refrigeration characteristics to concentrate the resulting supernatant liquid. Then, a filtration process was carried out for the concentrated liquid aiming to determine the free drug concentration, and this was performed after dilution with a fresh phosphate buffer saline of pH 7.4, and the results were obtained. The following equation was used to measure the entrapment efficiency [12, 13].

\[
\% \text{Entrapment efficiency} = \frac{\text{weight of drug incorporated} \times 100}{\text{weight of drug initially taken}}.
\]

Drug loading capacity.

The drug loading capacity of AZM was obtained by the following equation [14].
%Drug loading capacity = \frac{weight\ of\ drug\ encapsulated}{weight\ of\ microparticles} \times 100.

**Particle size and surface charge investigation.**

Testing of the stability (as zeta potential) and size uniformity of AZM microparticles were estimated using photon correlation spectroscopy with a Zetasizer (Malvern Instruments, Malvern, UK). Every sample was determined three times after being amply diluted with filtered deionized water, and the results were indicated as mean±SD.

**Scanning electron microscopy.**

The surface morphology of azilsartan microparticles was examined by scanning electron microscopy (SEM). The process involves coating the azilsartan microparticles with gold was achieved by a Spater coater device Baltec SCD 005. The microparticles were imaged employing an SEM-Jeol JSM 6460LV instrument.

**In vitro drug release studies.**

Powder of the microparticles corresponding to 40 mg of azilsartan medoxomil underwent dissolution using a specific dialysis membrane (mass cut-off of 12 KDa) [15]. Consequently, the bags were supported and placed in the apparatus type I of the USP dissolution tester, rotating at 50 rpm in 250 ml of deaerated phosphate buffer with pH 7.8 [16]. We withdrew 5 ml aliquots at intervals of 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours and replaced them with a fresh medium to conserve sink condition. Then the released azilsartan medoxomil per cent from microparticles was computed.

**Statistical analysis.**

The linearity residual involves standard deviation, the limit of detection (LOD), and the limit of quantitation (LOQ) were calculated utilizing the GraphPad Prism® program.

**4. Results**

An HPLC method was invented to estimate AZM, and validation parameters per ICH Q2 and USP 43 guidelines were performed [17, 18]. Before analyzing the actual samples, the system suitability was executed, including tailing factor (T), capacity factor (k’), and plate count (Bayoumi, 2018) [19]. The estimations were within the criteria illustrated in Fig. 2 and Tables 2, 3. For example, in Fig. 2, the peak with a retention time of 5 min refers to AZM showed low tailing and good symmetry, while the first peak refers to the mobile phase used, and there is no interference between the two peaks.

The calibration curve was built where Y-axis is the peak area (µV), and X-axis represents the concentration (µg/ml). The correlation coefficient (r2) obtained 0.9998 within limits (not less than 0.99). The recovery ranged from 99.6 % to 100.3 %. Moreover, the method was precise as the estimations of the same sample were close, and the RSD was less than 1 % or within the limit of ±2 %. The method sensitivity was emphasized by calculating LOD and LOQ [20] for AZM using GraphPad Prism® to be 0.83 µg/ml and 2.52 µg/ml, respectively. The results are represented in Table 3.

The AZM content ranged from 91.6 % to 94.6 %, and the entrapment efficiency of microparticles prepared by beeswax, carnauba wax and stearic acid ranged from 73.5–81.2 %, 69–78 % and 85.69–89.27 %, respectively as shown in Table 4.

**Table 2**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Number of theoretical plates</th>
<th>Tailing factor</th>
<th>Capacity factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item name</td>
<td>Not less than 2000</td>
<td>Not more than 2</td>
<td>Not less than 2</td>
</tr>
<tr>
<td>Azilsartan medoxomil</td>
<td>5649±1.29</td>
<td>0.75±0.05</td>
<td>5.26±0.02</td>
</tr>
</tbody>
</table>

Fig. 2. HPLC spectrum of azilsartan medoxomil
was –29.7 mV which indicates stability and lack of agglomeration among microparticles on time. The polydispersity index (PDI) of the microparticles prepared with carnauba wax and stearic acid matrices had the highest zeta potential, ranging from 25.3–29.7 mV, close to ideal stabilization.

Furthermore, the azilsartan microparticles prepared by beeswax, ranging from 0.37 to 0.97, were uniform in particle size distribution.

The mean size of azilsartan microparticles ranged from 3.41 to 8.13 μm, and the zeta potential of the azilsartan loading microparticles, including beeswax, was in the range of –21.1 to –26.6 mV while microparticles embedded in carnauba wax was from –20.6 to –26.7 mV. Furthermore, the azilsartan microparticles prepared by stearic acid matrices had the highest zeta potential, ranging from 25.3–29.7 mV, close to ideal stabilization. Moreover, the zeta potential of the formulation (AZSP4) was –29.7 mV which indicates stability and lack of agglomeration among microparticles on time. The polydispersity index (PDI) was not uniform in microparticles prepared with beeswax ranging from 0.37 to 0.97. However, the PDI of the microparticles prepared with carnauba wax and stearic acid ranged 0.37–0.59 and 0.41–0.58, authenticating uniformity of the particle size distributions.

The mean size of azilsartan microparticles was 65 % after 8 h showing the highest release of azilsartan from beeswax matrices. The last one was (AZBP4) which released 35 % of the drug after 8h while the release of pure azilsartan medoxomil was 80 % after 4 h. There-
fore, the release of azilsartan kamedoxomil from beeswax was slower than desired.

In microparticles containing carnuba wax in Fig. 5, the best formula released less than 60% after 8 h, while the other formulae containing carnuba wax had a much lesser release. Therefore, the release of azilsartan kamedoxomil from beeswax and carnuba wax in the used percent was slower than desired. However, the azilsartan microparticles consisting of stearic acid in incorporation with poloxamer 407 had the most desired release, especially AZSP4, whose release exceeded 80% after 8 h and extended for 24 h, revealed in Fig. 6.

Fig. 4. Dissolution rate of AZM microparticles prepared from beeswax (values represent mean±SD, n=6)

Fig. 5. Dissolution rate of AZM microparticles prepared from carnuba wax (values represent mean±SD, n=6)

Fig. 6. Dissolution rate of azilsartan microparticles prepared from stearic acid (values represent mean±SD, n=6)
5. Discussion

Depending on previous results concerning entrapment efficiency, mean size, zeta potential and release, the formulation AZP4 was chosen for further investigation. The size of the microparticles of formulation AZP4 is illustrated in Fig. 7, where the mean size was 5.87 µm. Furthermore, an SEM study was performed on the optimized formula; Fig. 8 showed the SEM photograph of azilsartan microparticles AZP4. The release of AZM from AZSP4 was more than 80 % after 8 h and prolonged to 24. Therefore, the best formulation was AZSP4 which showed acceptable release. The formulation AZSP4 showed the best characteristics containing stearic acid and poloxamer 407 since poloxamer 407 is a copolymer which consists of a hydrophobic portion of polyoxypolyene located between the two hydrophilic polyoxyethylene portions that may convert to gel at body temperature [21] where the azilsartan microparticles did not agglomerate showing a smooth surface with some cracks and a spherical shape.

To achieve controlled release, the literature lacks trials to microencapsulate azilsartan kamedoxomil in lipid waxy matrices. In our work, we successfully prepared loaded azilsartan kamedoxomil microparticle formulations using waxes which is the first trial to load azilsartan in lipid waxy matrices. However, some researchers tried to prepare azilsartan self-microemulsifying particles [22], such as Madan JR et al. formulated AZM in self-microemulsifying particles using soya lecithin to enhance solubility. Therefore, complexes of the drug with soya lecithin were prepared, characterized and assessed. The particles produced were filled in a hard gelatin capsule and introduced for in vitro drug release. The particles consisting of Syloid® XDP 31509 [mesoporous silica gel] had drug loading, particle size, polydispersibility index and zeta potential of 99.2, 201 nm, 0.544 and –19.7 mV, respectively, enhanced in vitro dissolution of AZM when compared to pure AZM. At the same time, in our research the AZM were incorporated in waxy matrices to achieve controlled release extending for 24 hours, the best microparticles formula showed results 86.7, 5.87 µm, 0.58 and –29.7 mV for drug loading, particle size, polydispersibility index and zeta potential, respectively [22]. Therefore, the microparticles in our research showed comparable PDI value and higher zeta potential compared to self-emulsifying particles, while the dissolution profile was different due to the required purpose.

Furthermore, Jassem & Ayash, 2017 developed AZM nanosuspension to improve its solubility and bioavailability using povidone k 30 and sodium lauryl sulphate. The particle size of the optimized formula F3 was 157 nm entrapment efficiency 95.8, PDI of 0.005 and improved release by more than 80 % after 30 min [23]. Moreover, Ma et al. prepared nanocrystals of AZM to improve its solubility by freeze-drying, decreasing the agglomeration of particles while solidifying. They prepared AZM nanocrystals using a stabilizer (sodium deoxycholate) to aid Poloxamer 188, and the best nanocrystals had mean particle sizes ranging from 300 nm and released more than 90 % after 20 min (pH 6) [24].
**Limitations of the study:** The proposed analytical method cannot be used to determine other drugs combined with AZM without investigating the resolution between peaks and confirming no interference. Furthermore, the waxes used to make microencapsulation in this research may not be suitable for another drug.

**Prospects for further research:** The presented paper describes the HPLC method, which can be used as a stability-indicating assay to assess AZM after storage at a higher temperature. Also, the formulation of AZM may be used to formulate microencapsules of AZM in combination with another antihypertensive drug.

6. Conclusion

The best microparticle formulation for azilsartan (AZSP4) released more than 80 % of AZM after 8 hours and lasted up to 24 hours. The formula contained stearic acid and poloxamer 407 (0.8:0.8:1 drug: stearic acid: poloxamer 407). Stearic acid combined with Poloxamer 407 in a certain ratio can produce lipid-loaded azilsartan microparticles with high zeta potential values, good stability, and good shape, extending AZM release for 24 hours. To distribute the produced microparticles orally with well controlled release behavior, capsules or tablets may be used.

**Conflict of interests**

The authors confirm that they have no conflict of interest correlated to this research, whether financial, personal, authorship or otherwise, that may impact the research and its results revealed in this paper.

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