DESIGN AND PHYSICOCHEMICAL CHARACTERIZATION OF LISINOPRIL–LOADED NANOCAPSULES FOR PAEDIATRIC USE

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Abstract

Lisinopril is an orally active angiotensin-converting enzyme inhibitor used for the treatment of hypertension, heart failure, and acute myocardial infarction in infants that is currently supplied as tablets. Solid dosage forms represent a problem in children with swallow difficulty and in neonates. There is no commercially available oral liquid preparation of lisinopril. Our strategy was to prepare oral liquid formulation of lisinopril based on lipid-core nanocapsules. The nanoprecipitation method was optimized for the preparation of lisinopril-loaded nanocapsules. The following characteristics of nanocapsules were determined: encapsulation efficiency, nanocapsules size and zeta potential, in vitro drug release and stability. The results revealed that after optimization of the nanoprecipitation procedure, the lisinopril-loaded nanocapsules optimized formulation size was in the nanometric range with high encapsulation efficiency. Microscopically, the prepared nanocapsules were spherical with an integral membrane. The release of lisinopril from the polymeric nanocapsules was rapid and completed in the simulated gastric fluid. Lisinopril-loaded nanocapsules may represent a useful formulation for its oral paediatric administration.

Keywords: Lisinopril, Nanocapsules, Oral liquid, Paediatric, Poly-ε-caprolacton.

1. Introduction

Lisinopril is an orally active angiotensin-converting enzyme (ACE) inhibitor. Lisinopril is the lysine analogue of enalapril that is chemically (Figure 1), (S)-1-[N2-(1-carboxy-3- phenylpropyl)-L-lysyl]-L-proline dehydrate(1). The therapeutic indications of lisinopril are; the treatment of hypertension in both adults and children, the management of heart failure in patients who are not well controlled by diuretics and digoxin, and also is used to treat hemodynamically-stable acute myocardial infarction patients. These therapeutic effects are primarily due to lisinopril ability to suppress renin-angiotensin-aldosterone system through ACE inhibition. This inhibition induces a reduction...
in both the plasma concentration of the vasopressor angiotensin II and aldosterone secretion. Lisinopril is orally absorbed with an extent of absorption of 25%. Lisinopril doesn’t bind to plasma proteins and excreted in urine unchanged. Lisinopril exhibits an effective half-life of accumulation of 12 hours after multiple dose administration. For paediatrics, the steady state plasma peak occurs within 6 h with an extent of absorption of 28%.(2).

Lisinopril is currently available as tablets with a dose range of 2.5mg to 40 mg. Oral dosage form for paediatric use of lisinopril is commercially unavailable, but two extemporaneous formulations have been developed.(3). Oral administration of liquid dosage forms is the method of choice for paediatric use as it offers several advantages specifically; the ease and convenience to use in children specially if they have problems encounter during solid dosage forms swallowing. In addition, the suitability to be used through nasogastric tubes for neonates. Moreover, oral liquid dosage forms can encourage child acceptability for medications by have attractive colours and desired flavoured taste. Liquid preparations also exert some disadvantages as require several excipients which may increase the adverse effects risk with special concern of the preservatives and solubilizing agents. Formulation of oral liquid dosage forms faces some obstacles related to drug namely, solubility, palatability and taste, and stability. Several approaches addressed these issues have been explored among them, the use of cyclodextrins complex(4), co-crystallization and amorphization of drugs(5), the use of co-solvents(6), self-emulsifying lipid systems(7), liposomes encapsulation(8) and microemulsion formation(9).

![Chemical structure of lisinopril (A), and poly-ε-caprolactone (B).](image)

**Figure 1: Chemical structure of lisinopril (A), and poly-ε-caprolactone (B).**

Nanoencapsulation of drugs is an attractive alternative for oral drug delivery. Over years, various nanocarriers were developed and applied in this route of administration viz., micelles, nanoemulsions, polyelectrolyte capsules, and the most widely studied, polymeric and lipid nanoparticles(10). Nanoparticles can be broadly categorized into nanospheres and nanocapsules; the later is characterized by the presence of a shell confining a liquid core. The nanocapsule shell may be either polymeric- or lipid-based(11). In contrast to nanospheres, that is a matrix-type
nanocarriers, nanocapsules give an opportunity for a higher lipophilic drugs encapsulation within its oil core that also protect the drug from any instability issues and reduce its direct contact with the tissues minimizing its local side effects. Moreover, nanocapsules have a lower polymeric content that made it safer and economically preferable(11). Polymeric nanocapsules can be prepared by many polymers such as polycyanoacrylate, polyD, L-lactide, polycaprolactone, poly lactic-co-glycolic acid, and Eudragit® (12–14). Among them, poly-ε-caprolactone (PCL), shown in Figure 1, is commonly used due to its biodegradability, biocompatibility and suitability for oral controlled drug delivery(15). Numerous methods have been utilized to prepare nanocapsules as nanoprecipitation, emulsion–diffusion, double emulsification, emulsion-coacervation, polymer-coating and layer-by-layer. The nanoprecipitation method is also known as solvent displacement and artificial disposition method, which involves mixing of organic solvent containing the membrane-forming polymer, the drug, and the oil (solvent phase) and a non-solvent phase that is made up with a non-solvent for the polymer (mainly water) and a surfactant(11).

The current research represents a trial aimed at designing and optimizing lisinopril-loaded polymeric nanocapsules based on poly-ε-caprolactone prepared via nanoprecipitation technique in order to formulate a liquid stable oral dosage form suitable for paediatric use. The prepared nanocapsules were physicochemically characterized in terms of encapsulation efficiency, nanocapsules size and zeta potential, microscopical characteristics, in vitro drug release and stability on storage.

2. Materials and Methods

2.1. Materials

Lisinopril was kindly obtained a gift from (RAMIDA company, Cairo, Egypt). Labrafac PG®, Labrafac®, Labrafil® M1944CS were kindly obtained from (Gattefosse, France), Campul MCM®, Captex335® were obtained as gifts from(Abitec corporation, USA). Poloxamer® 407, Sunflower oil, Tween® 80 and Span® 20 were purchased from (Sigma-Aldrich, Steinheim, Switzerland). Poly-ε-caprolactone (PCL, Mwt10.000) was supplied by (Sigma-Aldrich, Steinheim, Switzerland). All other solvents and materials used were of analytical grade. The used oils, polymer and surfactants are GRAS according to USP XXVI with no reported toxicity in children.

2.2. Preparation and optimization of lisinopril-loaded nanocapsules

2.2.1. Determination of lisinopril oil solubility

In order to select the suitable vehicle for nanocapsules core oil to trap lisinopril, solubility in different oils was determined following the method reported by Mehanna et al.(16). Specifically, 5ml of each oils was placed in a vial
where excess amount of lisinopril was added; the vials were sealed and shake at 36±1°C in thermostatically controlled water bath (Stuart, SBS40) for 24 h. The oil was filtered through a 0.45μm cellulose nitrate membrane filter. The filtrate was suitably diluted with methanol and the concentrations in the filtered samples were analyzed spectrophotometrically at λmax 260nm where no other components interfere with the drug absorbance (Perkin Elmer, Lambda, 3B, New York, U.S.A).

2.2.2. Nanoprecipitation Method

Nanoprecipitation method developed by Fessi et al.(17) was applied to prepare lisinopril-loaded poly-ε-caprolactone-based nanocapsules. The polymer and the lipophilic surfactant (Span® 20) was dissolved in acetone at 45°C, then oil solution of lisinopril was companied to form the solvent organic phase that is added to the aqueous phase containing the hydrophilic surfactant (Tween® 80 or Poloxamer® 407) under magnetic stirring at room temperature. The aqueous phase immediately turned milky with bluish opalescence due to the formation of the nanocapsule dispersion. The acetone was then removed by evaporation at 40°C under reduced pressure, on a rotary evaporator (Rotavapor, type R110; Buchi Company, Postfacn, Switzerland). The nanocapsules were collected and stored in glass vials for further characterizations.

2.2.3. Selection of surfactant and Optimization of solvent/non-solvent ratio

In order to select the suitable surfactant system that is able to form lisinopril-loaded nanocapsules, two hydrophilic surfactants (Tween® 80 and Poloxamer® 407) were tested either with or without incorporation of a lipophilic surfactant (Span®20) at 0:1 and 1:1 ratios. The total surfactant concentrations were evaluated at 100, 250 and 500 mg% w/v. The prepared nanocapsules were characterized by their mean sizes where the surfactant system and its concentration yielded the smallest size was selected for further studies. Moreover, the optimization of solvent/non-solvent volume ratio was carried out through assessing the effect of organic to aqueous phase ratio at 1:2, 1:3 and 1:5 on the mean nanocapsules size and the encapsulation efficiency of lisinopril.

2.3. Characterization of polymeric nanocapsules

2.3.1. Encapsulation efficiency estimation

The encapsulation efficiency (EE%) was determined by dialysis method, using dialysis bags of cellulose acetate (Mwt cut-off 12-14000). The prepared nanocapsules dispersion was placed into a dialysis bag that is tight up carefully. The filled bags were transferred into a magnetically stirred beaker containing reservoir medium that is distilled water/methanol (1:1). After three hours (determined during preliminary studies), samples of 5 ml were withdrawn
and spectrophotometrically analysed for lisinopril concentration. The encapsulated lisinopril was calculated indirectly and the percentage encapsulation efficiency was computed from the following equation:

\[
\text{% Encapsulation efficiency} = \frac{Q_t - Q_d}{Q_t} \times 100 \tag{1}
\]

Where; \(Q_t\) is the theoretical amount of lisinopril utilized during nanocapsules preparation and \(Q_d\) is the amount of lisinopril dialyzed. The encapsulation efficiency was determined in triplicate.

### 2.3.2. Nanocapsules size and zeta potential

The prepared polymeric nanocapsules mean size and zeta potential were measured using Zeta sizer (Nano ZS, Malvern Instruments, Westborough, MA, USA). The nanocapsules dispersion was suitably diluted with double distilled Millipore filtered water where pH was adjusted to be 7.4 during determination. The polydispersity of the nanocapsules dispersions were expressed as Span Index was calculated according to the following equation:

\[
\text{Span index} = \left[ \frac{D_{(v,90)} - D_{(v,10)}}{D_{(v,50)}} \right] \tag{2}
\]

Where \(D_{(v,10)}\), \(D_{(v,50)}\) and \(D_{(v,90)}\) are the equivalent volume diameters at 10, 50 and 90 % cumulative volumes, respectively.

### 2.3.3. Nanocapsules morphological characterization

The optimized lisinopril-loaded polymeric nanocapsules were microscopically characterized through transmission electron microscope (TEM). A drop of the nanocapsules dispersion was applied on copper-coated grids, after 2 min, the excess was drawn off with filter paper. A saturated Uranyl acetate aqueous solution was used as a negatively staining agent where its surplus was removed by distilled water. The stained air-dried samples were examined by TEM (model JEM-100S, Joel, Japan) at 50,000-magnification power at 80 KV.

### 2.3.4. In vitro release studies

The release studies of the optimized formula in comparison to the drug suspension were carried out in USP type II apparatus (Pharmatest, Germany). Simulated gastric fluid (SGF, 2 g NaCl, 80 ml HCl 1N and 1000 ml water) was used as a release medium. The release medium temperature was maintained at 37±0.5 °C and stirred at a rate of 75 rpm. The sink condition was maintained throughout the release studies. At predetermined time intervals, 5mL sample was withdrawn and analysed for its lisinopril content spectrophotometrically using SGF as a blank. In order to maintain the release medium volume constant, replacement of fresh medium was carried out after each sample withdrawal. All experiments were performed in triplicates.
2.4. Stability evaluation

Nanocapsules dispersions were stored at 4 and 25 °C with purpose of determining its stability upon storage for three months at two different temperatures mimic both the ambient and refrigerator temperatures. Determining nanocapsules encapsulation efficiency and measuring their mean size and Zeta potential comparing them with their initial values before storage governed its stability in addition to visual inspection. Statistical analysis was carried out employing the Student’s t-test where the differences are considered to be insignificant when p > 0.05.

3. Results and Discussion

Polymeric nanocapsules were prepared by nanoprecipitation method (solvent displacement method), which offers many merits viz. being simple, rapid, reproducible, one step and straightforward technique in addition, the nanocapsules formation is instantaneous and the method can be easily scaled up(11).

3.1. Selection polymeric nanocapsules core-forming oil

The goal of the study was to develop stable nanocapsules-based oral lisinopril dispersion for paediatric oral use with pharmaceutically accepted safe oil possessing a lisinopril-dissolving capacity. Sunflower oil, Captex® 335, Labrafac® CC, Labrafac® Hydro, Labrafil®, and Campul® MCM were the oils tested for their ability to dissolve lisinopril as the drug encapsulation efficiency is chiefly dependent on its solubility in the oil(18). Table I shows the determined solubility of lisinopril in various oils. Lisinopril solubility was observed to have the lowest for Campul® MCM and the highest for Captex® 335. Consequently, Captex® 335 was selected as the oil-forming nanocapsule core. Captex® 335 is a medium-chain triglyceride, is manufactured by the esterification of glycerin and fatty acids (mainly caprylic and capric) which originate from coconut and/or palm kernel vegetable sources.

Table I- Lisinopril solubility in various oils.

<table>
<thead>
<tr>
<th>Oil</th>
<th>Lisinopril saturation solubility (µg/ml)</th>
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</thead>
<tbody>
<tr>
<td>Sunflower oil</td>
<td>1370 ± 10</td>
</tr>
<tr>
<td>Captex® 335</td>
<td>2705 ± 32</td>
</tr>
<tr>
<td>Labrafac® PG</td>
<td>1540 ± 18</td>
</tr>
<tr>
<td>Labrafac®WL134!9</td>
<td>2573 ± 25</td>
</tr>
<tr>
<td>Labrafil®</td>
<td>1090 ± 15</td>
</tr>
<tr>
<td>Campul® MCM</td>
<td>800 ± 6.5</td>
</tr>
</tbody>
</table>

3.2. Optimization of lisinopril-loaded polymeric nanocapsules

The nanoprecipitation method based on solvent/non-solvent interfacial interaction, which involves Marangoni effect
that relay on complex phenomena namely, diffusion, flow and surface tension variations. Through their affinity and orientation at the interface, surfactants influence the nanocapsules properties. The presence of surfactants as an emulsifier is required for nanocapsule membrane formation and also for dispersion stability(19). Table II summarizes the effect of different surfactant combinations on nanocapsules mean size. The prepared nanocapsules showed a unimodal narrow size distribution characterized by low values of Span index. Upon close examination of table II, it could be inferred that the use of lipophilic surfactant, Span 20,(HLB=8.6) solely in the organic phase was unable to form nanocapsules in the nanometric range while the use of combination of either Tween 80 or Poloxamer 407 as hydrophilic surfactants (HLB= 15 and22,respectively) with Span 20 resulted in a reduction in the nanocapsules mean size.

**Table II- Influence of surfactants’ type and ratio on lisinopril-loaded nanocapsules**

<table>
<thead>
<tr>
<th>Surfactants</th>
<th>Ratio</th>
<th>Nanocapsules mean size (Span Index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span® 20: Tween® 80</td>
<td>1:0</td>
<td>1100±65 nm (0.83±0.3)</td>
</tr>
<tr>
<td></td>
<td>0:1</td>
<td>860±30 nm (0.71±0.09)</td>
</tr>
<tr>
<td></td>
<td>1:1</td>
<td>580±21 nm (0.85±0.1)</td>
</tr>
<tr>
<td>Span® 20: Poloxamer® 407</td>
<td>0:1</td>
<td>410±11 nm (0.40±0.05)</td>
</tr>
<tr>
<td></td>
<td>1:1</td>
<td>730±39 nm (0.68±0.12)</td>
</tr>
</tbody>
</table>

* The different formulations were realized with 100 mg PCL, 20 ml acetone, 2mg/ml lisinopril, 0.5 ml Captex 335 representing the organic phase and 20 ml of distilled water forming the aqueous phase and the total surfactant concentration was 100 mg% w/v.

Incorporation of hydrophilic surfactant in the aqueous phase merely, on the other hand, was able to produce smaller nanocapsules with Poloxamer407 being superior to Tween80 with a smaller size of 410nm. To study the effect of increasing Poloxamer concentration on nanocapsule mean size, 250 and 500 mg% w/v was tested. On increasing poloxamer concentration from 100 to 250mg% w/v, a reduction in nanocapsules mean size was observed from 410 nm to 275 nm, further increase in its concentration resulted in the formation of a larger nanocapsules (640 nm). This effect can be seen from the light of stabilization of oil/water system by Poloxamer, whichallowed forming smaller nanocapsules via inhibition of droplets coalescence by providing a mechanical layer barrier(20).Comparable results were reported for Solutol by Barras et al.(21). Hence, Poloxamer 407 at 250 mg% w/v was selected for further studies.
The choice of solvent/non-solvent volume ratio is a crucial determinant in the success of nanoprecipitation method. To address this fact, lisinopril-loaded nanocapsules were prepared utilizing 40, 120 and 200mL distilled water as non-solvent liquid corresponding to solvent: non-solvent ratios of 1:1, 1:3 and 1:5. Figure-2, illustrates that the mean size of lisinopril-loaded nanocapsules prepared by 1:1 and 1:3 solvent/non-solvent ratios were not statistically different, but upon increase the ratio up to 1:5 an increase was observed meanwhile, rise the ratio was accompanied with a significant reduction in encapsulation efficiency. The same phenomenon was observed during preparation of nanospheres by nanoprecipitation(22). This can be explained by the reduction of lisinopril available for encapsulation through partitioning out to the aqueous phase with larger volume.

3.3. Lisinopril-loaded polymeric nanocapsules characteristics

The optimized lisinopril-loaded nanocapsules composition and properties is shown in Table III. The optimized formula was a homogenous dispersion of nanocapsules with a size in the nanometric range independent on lisinopril loading. The measured nanocapsules mean size (275 nm) is within the published range for nanocapsules based on preformed polymers (23). Lisinopril-loaded nanocapsules had high encapsulation efficiency due to ability of captex® 335 to solubilize lisinopril, which enhance its encapsulation efficiency. High encapsulation efficiency of polymeric nanocapsules for benzathine penicillin was reported to be dependent on drug solubility in the oil core(24). Lisinopril-loaded nanocapsules prepared by the nanoprecipitation method, exhibited a negative zeta potential, which can be explained by the orientation of the surfactant molecules within the polymer chains. Poly-ε-caprolactone is a hydrophobic polymer where the surfactant hydrophobic tails favour to locate within its hydrophobic chains. The surfactant polar heads are protruding into the aqueous medium in order to minimize its surface free energy, which reflected on the nanocapsules surface potential. This result is in accordance with those of Joo et al. (25) who found that PCL-based nanocapsules surface potential is determined by the nature of the surfactant used during their preparation.

Table III- The composition and the physicochemical characteristics of the optimized lisinopril-loaded nanocapsules.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Optimized formula</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>20 ml</td>
<td>Macroscopical</td>
</tr>
<tr>
<td>PCL</td>
<td>100 mg</td>
<td>Fluid milky white</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>2 mg/ml</td>
<td>Bluish opalescent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captex 335</td>
<td>0.5 ml</td>
<td>Mean size</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Span Index</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Encapsulation efficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zeta potential</td>
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</tbody>
</table>

Macroscopically, Lisinopril-loaded nanocapsules appeared to be a homogenous milky white bluish opalescent fluid with no detected precipitates or aggregates. Transmission electron microscopy micrographs (Figure 3) demonstrated that the optimized lisinopril-loaded polymeric nanocapsules are closed structures, spherical in shape, and homogenous in size with no detected drug crystals. The shell structure of the nanocapsules was visible on closer examination of the photomicrographs. The capsular wall was free from holes or defects. Similar observation was outlined for Miglyol® containing nanocapsules (26). The behaviour of lisinopril-entrapped nanocapsules dispersion was found to be typical for a Newtonian system, since the increase of the shear stress was proportional to the increase of the shear rate. The optimal formulation presented a viscosity of 10.3 ± 0.15 mPa.s., Similar behaviour was observed for bixin nanocapsules (27).

Figure 2: Effect of solvent: non-solvent volume ratio (organic: aqueous phases) on lisinopril-loaded nanocapsules mean size and encapsulation efficiency.
Figure 3: Transmission electron photomicrographs of the optimized lisinopril-loaded polymeric nanocapsules prepared by nanoprecipitation method. Arrow is pointed at the capsular membrane.

The release patterns of lisinopril from the optimized nanocapsules dispersion in comparison to the free drug into simulated gastric fluid are displayed in Figure 4. The release rate of lisinopril was significantly enhanced by encapsulation into PCL nanocapsules where a rapid and complete release (100%) was observed with 20 min compared to only 40% release from its suspension under sink conditions. This rapid release of encapsulate lisinopril can be justified by considering the principles of diffusion.

The rate of lisinopril diffusion from the nanocapsules is controlled by the magnitude of lisinopril concentration gradient and its partition coefficient between the oily core of the nanocapsules and the dissolution medium. Under sink conditions and volume difference between the two compartments, rapid drug diffusion is expected. Other factors also contributed to the observed release pattern namely, the presence of surfactant and nanocapsules size(28). Surfactant molecules (Poloxamer®) can encourage drug solubility and hence its release. Tween® and Span® exhibited a similar effect on antigens release rate from poly-lactide-poly (ethylene glycol)-based microspheres(29).

The nanocapsules are very small in the nanometric range consequently they exhibited a large surface area which in turn enhance the drug release rate by providing larger area available for diffusion. Polymeric nanocapsules-loaded with griseofulvin showed an indistinguishable pattern of release (30).
Figure 4: In vitro release profiles of lisinopril-loaded nanocapsules and its suspension into simulated gastric fluid under sink conditions.

3.3. Stability evaluation

The alteration in the encapsulation efficiency and the nanocapsules size were used as physical instability tracer during the storage of lisinopril-loaded nanocapsules at 4 and 25 °C. Visually, the optimized nanocapsules formulation maintained its homogenous milky appearance. The optimized nanocapsules formulation showed a non-significant increase (280±7 nm at 4°C and 290±13 nm at 25°C) in nanocapsules means size and (-15.5± 0.44 mV at 4°C and -13.08±1.10mV at 25°C) upon storage in both temperatures, which indicated the absence of nanocapsules aggregation and this non-significant increase may be due to some polymer chains relaxation. Furthermore, the prepared nanocapsules were able to maintain its encapsulation efficiency upon storage for three months at both temperatures that denoted the stability of the nanocapsules structure and the site of lisinopril, which is confined within the nanocapsules membrane with no observed outlet diffusion. Physical stability of polymeric nanocapsules was well documented in many research papers (18,30).

Conclusion

The current research focused on the preparation and optimization of colloidal nanocapsules dispersion that could be used for oral paediatric administration of lisinopril. The nanocapsules was loaded with lisinopril by solvent displacement method utilizing poly-ε-caprolactone as a capsule-forming polymer. In order to optimize the lisinopril-loaded nanocapsules, various process variables were investigated. The optimized colloidal dispersion showed a small uniform size in the nanometric range, high encapsulation efficiency and complete drug release on contrary to drug
The prepared nanocapsules were stable at both at 4 and 25 °C. Accordingly, the lisinopril-loaded nanocapsules colloidal dispersion represents a simple stable formulation, which could be used orally for children.

References


