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Optimization of long circulating mixed polymeric micelles containing vinpocetine using simple lattice mixture design, *in vitro* and *in vivo* characterization

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**A B S T R A C T**

The aim of this study was to increase the *in vivo* mean residence time of vinpocetine after IV injection utilizing long circulating mixed micellar systems. Mixed micelles were prepared using Pluronics L121, P123 and F127. The systems were characterized by testing their entrapment efficiency, particle size, polydispersity index, zeta potential, transmission electron microscopy and *in vitro* drug release. Simple lattice mixture design was planned for the optimization using Design-Expert® software. The optimized formula was lyophilized, sterilized and imaged by scanning electron microscope. Moreover, the *in vivo* behavior of the optimized formula was evaluated after *IV* injection in rabbits. The optimized formula, containing 68% w/w Pluronic L121 and 32% w/w Pluronic F127, had the highest desirability value (0.621). Entrapment efficiency, particle size, polydispersity index and zeta potential of the optimized formula were 50.74 ± 3.26%, 161.50 ± 7.39 nm, 0.21 ± 0.03 and −22.42 ± 1.72 mV, respectively. Lyophilization and sterilization did not affect the characteristics of the optimized formula. Upon *in vivo* investigation in rabbits, the optimized formula showed a significantly higher elimination half-life and mean residence time than the market product. Finally, mixed micelles could be considered as a promising long circulating nanocarrier for lipophilic drugs.

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1. Introduction

Vinpocetine, as a vincamine derivative, is used for the treatment of cerebrovascular circulatory disorders as cerebral infarction and cerebral arteries cirrhosis (Hasa et al., 2011). It has limited oral use due to its low water solubility (≈5 μg/ml), slow dissolution rate, poor absorption and significant first-pass metabolism during first pass that lead to very low bioavailability (≈7%) (El-Laithy et al., 2011). Additionally, it has very rapid elimination rate with short half-life (2 h) that results in frequent drug dosing (three times daily) (Zhuang et al., 2010).

Vinpocetine was previously investigated after intravenous injection for the control and treatment of both acute and chronic cerebral ischemia (Gulyás et al., 1998; Szakall et al., 1998). Intravenous administration could overcome some of vinpocetine drawbacks as poor absorption and first pass, but its very low water solubility and short elimination half-life still persist. Aqueous solubility could be enhanced by decreasing the medium pH due to the basic nature of vinpocetine (pKa = 7.1) (Hasa et al., 2011). Citric acid was previously utilized as an enhancer for the solubility and dissolution of vinpocetine (Ning et al., 2011). After vinpocetine solubilization by pH modification, the only remaining difficulty that affects the reliability of intravenous administration is the
rapid elimination rate. To overcome this problem, long circulating nanocarriers could be utilized for formulation of intravenous sustained release vinpocetine.

Inclusion of hydrophobic drugs into polymeric micelles has been found to be very attractive concept for solubilization and bioavailability enhancement (Zhao et al., 2012). Micelles containing single polymer have been used in drug delivery for long time, but lately binary micelles replaced them. The disadvantages of mono micellar systems include low drug loading, large particle size and low stability (Kulthe et al., 2011). On the other hand, mixed micelles exhibit synergistic properties, such as increased micelle stability, drug loading and entrapment efficiency (Gao et al., 2005, 2008).

Pluronics are triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) with the structure PEO–PPO–PEO. Previous studies demonstrated that pluronic copolymers can form spherical micelle by self-assembly (Parmar et al., 2011). Pluronics show low cytotoxicity and weak immunogenicity after topical and systemic administration although they are not biodegradable polymers. This may be due to having molecular weight less than 15 kDa which could be easily filtered and cleared by the kidney (Batrakova et al., 2004; Huang et al., 2009).

Pluronic micelles have a core–shell structure with hydrophobic core acting as solubilization depot for the non polar drugs and hydrophilic corona preventing aggregation, protein adsorption, and recognition by the reticulo-endothelial system (RES), which leads to longer blood circulation time (Zhang et al., 2011). Also, the small size of polymeric micelles (<100 nm) could increase blood circulation time due to escaping from capturing by the mononuclear phagocytic system in the liver and bypassing the filtration of inter-endothelial cells in the spleen (Lu and Park, 2013). Presence of pluronics with different hydrophilic–lipophilic balance (HLB) could help in achieving the optimum thermodynamic and kinetic stabilities for the formed micelles. It was assumed that low HLB pluronics, would increase the thermodynamic stability of the micelles due to the tight hydrophobic interactions with hydrophobic propylene oxide blocks. On the other hand, the high HLB pluronics would increase the kinetic stability of the micelles due to the steric hindrance that minimize micelle aggregation (Abdelbary and Tadros, 2013; Lee et al., 2011).

The aim of this work was to formulate mixed micelles using the lipophilic Pluronic L121 (HLB: 1–7), intermediate Pluronic P123 (HLB: 7–12) and hydrophilic Pluronic F127 (HLB: 22) (Alexandridis and Lindman, 2000). Statistical design was utilized for optimization of the formulated micellar dispersion. The optimized formula was lyophilized, sterilized and investigated for its in vivo behavior after intravenous injection in rabbits.

2. Materials and methods

2.1. Materials

Vinpocetine was kindly supplied by Nile Pharma, Cairo, Egypt. Pluronic L121 (PL121), Pluronic P123 (PP123), Pluronic F127 (PF127), mannitol and sodium lauryl sulphate were purchased from Sigma–Aldrich, St. Louis, USA. Potassium dihydrogen phosphate and disodium hydrogen phosphate were supplied by SISCO Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals and solvents were of analytical grade and used without further purification.

2.2. Preparation of mixed micelles

Micelles were prepared using the thin film hydration technique (Wei et al., 2009). Briefly, vinpocetine (25 mg) and pluronic(s) (total weight: 1000 mg) were accurately weighed and dissolved in 10 mL chloroform:methanol mixture (in ratio, 2:1 v/v) in round-bottom flask (capacity, 250 mL). The organic solvent was evaporated under reduced pressure using rotary evaporator (Rotavapor, Heidolph VV 2000, Burladingen, Germany) revolving at 150 rpm for 1 h at 60 °C. Hydration of the formed thin film was performed using 20 mL distilled water. During hydration, the flask was rotated at 150 rpm for 1 h at 60 °C under normal pressure. Finally, the micellar dispersion was sonicated using bath sonicator (Ultrasonic bath sonicator, Model SH 150–41, PCI Analytics Pvt. Ltd., Mumbai, India) for 2 min to reduce particle size (Oh et al., 2004).

2.3. Statistical design of the study

Simple lattice mixture experimental design was utilized to investigate the effects of the different variables on the characteristics of mixed micelles using Design-Expert® software (Version 7, Stat-Ease Inc., MN, USA). The independent variables were the percentages of PL121 (X1), PP123 (X2) and PF127 (X3). Monitored responses were entrapment efficiency (Y1): EE, particle size (Y2): PS, polydispersity index (Y3: PDI), as dependent variables. Table 1 illustrates the composition of the prepared mixed micelles formulae. Ten formulae had been generated with 14 runs as formulae M1, M3, M8 and M10 were prepared twice. Formulae were having the same total surfactant(s) to drug ratio with different percentages of the three pluronics to investigate the effect of hydrophilicity/lipophilicity on the micellar characteristics. Moreover, desirability values were calculated for the optimization of variables to attain the superior formula.

2.4. Characterization of the mixed micelles formulae

2.4.1. Determination of entrapment efficiency (EE)

Formulae were centrifugated at 14,000 rpm and temperature 4 °C for 1 h using cooling centrifuge (Beckman, Fullerton, Canada) to separate un-entrapped precipitated drug from the encapsulated nano-dispersed. Samples aliquots from supernatant were diluted with methanol to disrupt the micellar structure (Jin et al., 2011). Drug was spectrophotometrically analyzed in the diluted supernatant at λmax 268 nm. EE was calculated using the following equation (Mu et al., 2010):

\[
EE\% = \frac{\text{weight of drug in micelles}}{\text{weight of added drug during preparation}} \times 100
\]

2.4.2. Analysis of particle size, polydispersity index and zeta potential

The particle size (PS) analysis of micelles was performed using dynamic light scattering (Zetasizer Nano ZS-90, Malvern instruments, Worcsershire, UK). Before measurement, samples were diluted with distilled water, if required, until being translucent. Additionally, polydispersity index (PDI) was measured to assess the particle size distribution. Finally, zeta potential (ZP) of the diluted formulae samples, having pH ranged between 5.5 and 6.5, was analyzed for evaluation of their physical stability. Measurements were done in triplicates for three independent samples of each formula and the average values ±SD were calculated.

2.4.3. Morphological evaluation of the dispersed micelles by transmission electron microscopy (TEM)

Morphology of the optimized formula was examined using transmission electron microscope (JEM-1230, Jeol, Tokyo, Japan). The negative staining technique was utilized as one drop of diluted sample was placed on carbon coated copper grid and stained by 2% w/v phosphotungstic acid. The samples were investigated using TEM at 100 kV, after drying at room temperature.
2.5. Lyophilization of the optimized micellar formula

The optimized formula was frozen at -20°C in presence and absence of 5% w/v mannitol as a cryoprotectant. Then, samples were lyophilized at -45°C and pressure of 7 x 10^-3 mbar for 24 h (Novalyphie-NL 500; Savant Instruments Corp., USA). EE, PS, PDI and ZP were analyzed for the lyophilized samples after reconstitution and compared to the original values recorded before lyophilization. Data was statistically analyzed applying one way ANOVA using SPSS 19 software (IBM Corporation, Armonk, NY, USA). Post-hoc multiple comparisons were performed using Fisher's least significant difference test and the results were considered significantly different when p-values were less than 0.05.

2.6. In vitro vinpocetine release from the optimized formula

In vitro vinpocetine release from micellar dispersion, before and after lyophilization was carried out in USP I dissolution apparatus (Pharm Test, Hainburg, Germany) using the membrane diffusion technique. Dialysis tubing membranes (Spectra Por®, molecular weight cut off 12–14 kDa, Sigma–Aldrich, St. Louis, USA) were immersed in the dissolution medium overnight prior to experiment. The lyophilized formula was reconstituted to its original volume using distilled water before conducting the dissolution testing. Samples from each formula (equivalent to 5 mg of vinpocetine) were added to glass cylinders (6 cm length and 2.5 cm internal diameter) tightly covered with the dialysis membrane tubing from one end. The loaded cylinders were fixed at the shafts of the USP dissolution tester apparatus (Abdelhary and Tadros, 2013; Aburahma and Abdelbary, 2012). The used dissolution medium was 500 mL phosphate buffer saline (PBS, pH 7.4) containing 0.5% sodium sulphate to achieve sink conditions (Nie et al., 2011). Rotation speed was set at 50 rpm and the temperature was adjusted to ±0.5 °C throughout the study. Dissolution of vinpocetine market product (Vinporal®, Amriya Pharmaceuticals, Alexandria, Egypt) was carried out under the same conditions for comparison. Samples (3 mL each) were withdrawn at time intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 8, 24 and 48 h and spectrophotometrically analyzed at Amax 268 nm. The release study was repeated three times and the average percentages dissolved (±SD) were illustrated against time. Similarity factor (f2) was calculated for comparison of release profiles utilizing the following equation:

\[ f_2 = 50 \times \log \left( \left[ 1 + \frac{1}{n^2} \sum_{j=1}^{n} \left( R_j - T_j \right)^2 \right]^{-0.5} \times 100 \right) \]  

where n is the sampling number, T and R are the percentages of drug released from the test and reference micellar formulae, respectively, at each time interval j (Costa and Sousa Lobo, 2001). Moreover, half-lives of the drug release were calculated and statistically compared to detect presence or absence of any significant difference between the calculated values.

2.7. Effect of sterilization by gamma radiation

Samples of the lyophilized formula were packed in dry ice inside a polyurethane container to avoid aggregation or melting due to the probable temperature elevation induced by T-radiation (T-irradiator, Gamma cell 1000; BEST theratronics. Ontario, Canada) (Yehia et al., 2012). Radiation dose was 2.5 Mrad as stated in the united states pharmacopoeia (Convention, 2006). After reconstitution of the sterilized formulæ with sterile water for injection, EE, PS, PDI and ZP were re-measured and statistically compared with the equivalent results before sterilization. Moreover, testing of the in vitro drug release profile was re-conducted and similarity factor (f2) was calculated to compare the drug release profiles before and after sterilization.

2.8. Morphological evaluation of the lyophilized matrices by scanning electron microscopy (SEM)

The surface properties of the lyophilized optimized formula, before and after sterilization were evaluated using scanning electron microscope (JXA-840; JEOL Japan). Samples were gold coated under vacuum using an ion sputter and then, examined.

2.9. In vivo quantification of vinpocetine concentrations in plasma

2.9.1. Study design and animals

Six healthy New Zealand male rabbits weighing 3–4 kg were used throughout the study. The animals were randomly divided into two groups, each consisted of three: group I, administered the optimized formula and group II, administered the marketed product (Vinporal®, Amriya Pharmaceuticals, Alexandria, Egypt), with volume equivalent to 3.2 mg vinpocetine. The equivalent rabbit dose was calculated according to the following equation, based on the body surface area (Reagan-Shaw et al., 2008):

\[ \text{Human equivalent dose (mg/kg)} = \frac{\text{Animal dose (mg/kg)} \times \text{Animal Km}}{\text{Human Km}} \]  

Briefly, rabbits were fasted overnight with free access to water before experimentation. The protocol of the study was reviewed and approved by the institutional review board of Genuine Research Center, Cairo, Egypt. Blood samples (approximately 3 mL) were withdrawn from the ear vein into heparinized glass tubes at the following time intervals; 0.25, 0.5, 1, 2, 3, 4, 5, 8, 24 and 48 h following the IV dosing. The withdrawn blood samples were centrifuged at 4000 rpm for 15 min at 4°C. Then, plasma was transferred directly into 5 mL plastic tubes and stored at -70°C till drug analysis. The study was repeated after washing period of seven days to fulfill the crossover design.

2.9.2. Sample preparation

A plasma sample of 500 µL was transferred to a 10 mL glass test tube, then 50 µL of internal standard (IS; Torsemide) working solution (1000 ng/mL) was added. After vortex mixing for 10 s, 4 mL aliquot of ether was added using Dispensette Organic (Brand GmbH, Postfach, Germany) (Lin et al., 2014). The sample was vortex-mixed for 3 min using vortex. The organic layer was transferred to clean glass tubes and evaporated to dryness using centrifugal vacuum concentrator Vacufuge 5301 (Eppendorf, Germany) at 40°C. Dry residues were then dissolved in 200 µL of mobile phase and vortexed for 1 min for reconstitution, and 20 µL was injected using the autosampler.

A sensitive, selective and accurate LC–MS/MS method was developed and validated before the study for determination of vinpocetine. A shimadzu prominence (Shimadzu, Japan) series LC system equipped with degasser (DGU-20A3), solvent delivery unit (LC-20AB) with an auto-sampler (SIL-20 AC) was used to inject 20 µL aliquots of the processed samples on a Luna C18 (phenomenex, USA) (50 x 4.6) mm, 5 µm particle size. The guard column was phenex C18 (5 x 4.0) mm, 5 µm particle size. All analysis was carried out at room temperature.

The isocratic mobile phase consisted of a mixture of methanol–2 mM ammonium acetate–formic acid (40:60:0.1, v/v/v); after filtration and degassing of the mobile phase, it was delivered at a flow rate of 0.3 mL/min into the mass spectrometer's electrospray ionization chamber (Xia et al., 2010). MS/MS detection in negative ion mode using a MDS Sciex (Foster City, CA, USA) API-4000 mass
spectrum was used for quantitation. The mass spectrometer was equipped with a turbo ionspray interface at 450 °C. The ion spray voltage was set at 5500 V. The common parameters, viz. curtain gas, nebulizer gas, collision gas and auxiliary gas were set at 25 psi, 20 psi, 5 psi and 40 psi, respectively. The compound parameters: collision energy, declustering potential, entrance potential and collision exit potential were 100 V, 40 V, 8 V, 7 V for vinpocetine and 20 V, 30 V, 9 V, 12 V for Torsemide (IS), respectively. Multiple reaction monitoring (MRM) mode detection of the ions was performed in the, monitoring the transition of the m/z 351.17 precursor ion to the m/z 280.20 for vinpocetine and m/z 348.99 precursor ion to the m/z 263.90 for IS. The analytical data were processed by Analyst software version 1.6 (Applied Biosystems Inc., Foster city, CA).

2.9.3. Pharmacokinetic and statistical analysis

The pharmacokinetic parameters of vinpocetine after IV injection of the investigated formulae were analyzed by non-compartmental pharmacokinetic models using Kineticra® software version 5 (Thermo Fisher Scientific Inc., Waltham, MA). The peak plasma concentration (Cmax) was directly obtained from the concentration–time data. In addition, the values of the area under the plasma concentration–time curve from time zero to the last time point and to infinity (AUC0–t and AUC0–∞) were calculated in accordance with the linear trapezoidal rule. Also, the terminal elimination rate constant (Ke), elimination half-life (t1/2) and mean residence time (MRT) were calculated using the linear regression of the terminal portion of the ln (concentration)–time curve.

3. Results and discussion

3.1. Characterization of the prepared micellar formulae

3.1.1. Determination of entrapment efficiency (EE)

EE percentages for the prepared vinpocetine mixed micelles ranged between 6.97% and 94.08% as shown in Table 1 and Fig. 1a. The calculated EE values were best fitted to polynomial analysis with special cubic model. Adequate precision was calculated by the Design-Expert software to demonstrate the signal to noise ratio to ensure that the model could be applied to navigate the design space, whereas a ratio greater than 4 is desirable (de Lima et al., 2011). On the other hand, predicted R2 was calculated as a measure of how good the model could predict a response value by comparing the calculated value with the adjusted R2 (Annadurai et al., 2008). Adequate precision was 17.85 with reasonable difference between the predicted R2 (0.8619) and the adjusted R2 (0.9457). The calculated equation for the EE% analysis was:

\[
\text{EE\%} = 92.55X_1 + 20.23X_2 + 14.27X_3 - 140.02X_4X_5 - 11.77X_1X_3 - 36.50X_2X_5 + 864.35X_1X_2X_3 
\]

(4)

Factorial statistical analysis revealed that changing surfactants percentages had a significant effect on the EE% (p < 0.001). It was observed that increasing PL121 led to EE% increase. This might be attributed to the high lipophilicity of PL121 (HLB: 1–7) that provide a favorable medium for the entrapment of a water insoluble drug like vinpocetine. Similar results were obtained by Xu et al. who observed the increase in folate loading after incorporation of PL121 in the formulated mixed micelles (Xu et al., 2012).

3.1.2. Analysis of particle size, polydispersity index and zeta potential

The mean PS of micellar formulae were in the nano range between 10.70 and 315.39 nm as shown in Table 1. PS values were subjected to polynomial analysis using quadratic model. Adequate precision was 9.36 with reasonable difference between the predicted R2 (0.7981) and the adjusted R2 (0.8243). The calculated equation for the PS analysis was:

\[
\text{PS} = 218.83X_1 + 16.79X_2 + 30.56X_3 + 767.94X_4X_2 + 8.79X_1X_3 + 32.74X_2X_3
\]

(5)

Fig. 1b illustrates the effect of independent variables on the mean PS. From the presented figure, it is clear that the incorporation of different pluronic percentages showed significant effects on the mean PS (p-value = 0.011). It could be noticed that the formulae M1 (containing 100% w/w PF127), M2 (containing PF127 and PF123, in ratio 1:1 w/w) and M3 (containing 100% w/w PF123) were having the lowest particle size values. This might be attributed to the polar and semi-polar nature of PF127 and PF123, respectively, which could enable them to form relatively small micelles in water. Similar results were obtained in previous studies utilizing PF123 alone or in a mixture with PF127 for the micellar solubilization of paclitaxel, whereas the particle size was nearly 20 nm (Han et al., 2006; Wei et al., 2009). On the other hand, incorporation of PL121 in the remaining formulae (M4–M10) led to significant increase in the particle size. These results were in accordance with the previously noticed findings by Abdelbary et al. who prepared mixed micelles containing different concentrations of PL121 and PF123 for brain targeting of olanzapine (Abdelbary and Tadros, 2013).

Additionally, PDI values were also investigated and the obtained results were displayed in Table 1 and statistically illustrated in Fig. 1c. It was found that all the prepared micellar formulae had values ranged between 0.21 and 0.69 which could be considered as an acceptable mid range (Cho et al., 2014). Polynomial analysis

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Experimental runs, independent variables, and measured responses of the simple lattice mixture design for vinpocetine micellar formulae.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulan</td>
<td>PL121 (% w/w)</td>
</tr>
<tr>
<td>M1</td>
<td>0.00</td>
</tr>
<tr>
<td>M2</td>
<td>0.00</td>
</tr>
<tr>
<td>M3</td>
<td>0.00</td>
</tr>
<tr>
<td>M4</td>
<td>16.67</td>
</tr>
<tr>
<td>M5</td>
<td>16.67</td>
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<tr>
<td>M6</td>
<td>33.33</td>
</tr>
<tr>
<td>M7</td>
<td>50.00</td>
</tr>
<tr>
<td>M8</td>
<td>50.00</td>
</tr>
<tr>
<td>M9</td>
<td>66.67</td>
</tr>
<tr>
<td>M10</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Fig. 1b illustrates the effect of independent variables on the mean PS. From the presented figure, it is clear that the incorporation of different pluronic percentages showed significant effects on the mean PS (p-value = 0.011). It could be noticed that the formulae M1 (containing 100% w/w PF127), M2 (containing PF127 and PF123, in ratio 1:1 w/w) and M3 (containing 100% w/w PF123) were having the lowest particle size values. This might be attributed to the polar and semi-polar nature of PF127 and PF123, respectively, which could enable them to form relatively small micelles in water. Similar results were obtained in previous studies utilizing PF123 alone or in a mixture with PF127 for the micellar solubilization of paclitaxel, whereas the particle size was nearly 20 nm (Han et al., 2006; Wei et al., 2009). On the other hand, incorporation of PL121 in the remaining formulae (M4–M10) led to significant increase in the particle size. These results were in accordance with the previously noticed findings by Abdelbary et al. who prepared mixed micelles containing different concentrations of PL121 and PF123 for brain targeting of olanzapine (Abdelbary and Tadros, 2013). Additionally, PDI values were also investigated and the obtained results were displayed in Table 1 and statistically illustrated in Fig. 1c. It was found that all the prepared micellar formulae had values ranged between 0.21 and 0.69 which could be considered as an acceptable mid range (Cho et al., 2014). Polynomial analysis
with quadratic model was utilized for the statistical evaluation of the PDI values. Adequate precision was 5.13 with reasonable difference between the predicted $R^2$ (0.2544) and the adjusted $R^2$ (0.4286). The calculated equation for the PS analysis was:

$$PDI = 0.46X_1 + 0.62X_2 + 0.60X_3 - 0.24X_1X_2 - 1.05X_1X_3 - 0.75X_2X_3$$  (6)

It could be concluded from the statistical analysis that changing pluronics percentages had no significant effect on the PDI values with p-value of 0.084.

Finally, the zeta potential of the prepared formulae was investigated and negative charges were observed on their surfaces with values ranging between -18.3 and -35.40 mV. As stated in literature, the zeta potential is an indicator for the dispersion stability. High zeta potential could provide high repulsion forces protecting the dispersed nanoparticles from aggregation (Wang et al., 2013). From the obtained findings, it is evident that the obtained zeta potential range would present a reasonable degree of stability for the prepared micellar formulae.

3.1.3. Morphological evaluation of the dispersed micelles by transmission electron microscopy (TEM)

The morphological characteristics of the optimized micellar formula were investigated using TEM, as presented in Fig. 2. The TEM micrographs showed vesicles with uniform spherical shape.

![Fig. 2. Transmission electron micrographs of the optimized formula, at different magnifications.](image-url)
These vesicles appeared to be well dispersed, non-aggregated with smooth surface. The mean particle size demonstrated by the TEM micrograph was in good agreement with that measured by the Zetasizer. As previously mentioned, the observed PS was less than 100 nm which could achieve long circulation through avoidance of capture and digestion by the RES (Cabral et al., 2011; Wei et al., 2009).

3.2. Selection of the optimized micellar formula

Optimization of all independent variables at the same time is nearly unachievable as the optimum condition obtained with one response might have antagonizing effect on another one (Singh et al., 2012). The desirability function approach is widely used in researches for the optimization of multiple response processes. It was calculated to choose the optimum composition with the maximal EE, minimal PS and PDI. The highest desirability value was 0.621, as shown in Fig. 1d. This desirability value was for the optimized formula containing 68% w/w PL121 and 32% w/w PF127. Such findings are in harmony with that obtained by Oh et al. who found that PL121/PF127 mixtures (in ratio, 1:1 w/w) formed stable dispersions with small particle size. Furthermore, Oh et al. found that mixed PL121/PF127 micelles showed nearly 10-fold higher solubilization capacity compared to PF127 micelles (Oh et al., 2004). After preparation and investigation of the optimized formula, EE, PS, PDI and ZP were 50.74 ± 3.26%, 161.50 ± 7.39 nm, 0.21 ± 0.03 and −22.42 ± 1.72 mV, respectively. Consequently, this formula was selected for further investigations.

3.3. Characterization of the lyophilized optimized micellar formula before and after sterilization

Lyophilized samples of the optimized formula in presence and absence of mannitol showed no significant differences in the measured parameters (EE, PS, PDI and ZP), either to each other or to the original formula before lyophilization \((p \geq 0.05)\). So, it could be concluded that the micellar dispersion do not need cryoprotectant. This might be due to the presence of PF127 which had long hydrophilic polyethylene oxide chain that might prevent sticking of the adjacent tubular aggregates formed by the lipophilic pluronic (Oh et al., 2004; Wei et al., 2009). These findings were consistent with the previous observations of Szleifer et al. who demonstrated that lipid vesicles could be sterically stabilized by incorporation of PF127 (Szleifer et al., 1998). The mean EE of the reconstituted lyophilized formula, without mannitol, was found to be 49.37 ± 4.10%. PS, PDI and ZP were 163.03 ± 6.22 nm, 0.19 ± 0.01 and −23.55 ± 2.16 mV. After sterilization, all the re-measured parameters had no significant differences to the original formula before sterilization with p-value ≥ 0.05.

3.4. In vitro vinpocetine release from the optimized formula

The release profiles of vinpocetine from the optimized micellar formula before, after lyophilization and after sterilization in comparison to the market product in PBS (pH 7.4) containing sodium lauryl sulphate are illustrated in Fig. 3. The optimized formula showed significant sustainment of the drug release when compared to the market product with \(f_2\) of 25. Moreover, the drug release half-life significantly increased from 2.38 h, in case of the市场 product to 6.53 h, in case of the optimized formula \((p < 0.001)\). Dissolution profile could be considered similar if \(f_2\) value lies between 51 and 100 (Costa and Sousa Lobo, 2001). From these data, it could be deduced that the prepared micellar dispersion achieved the targeted sustained release profile for vinpocetine. On the other hand, drug release from the optimized formula after lyophilization and sterilization were similar to the original formula with \(f_2\) values of 55 and 52, with preserved release half-life values of 6.41 and 6.76 h, respectively. These results could indicate the capability of the used techniques during lyophilization and sterilization in the preservation of the optimized formula characteristics.

![Figure 3](image-url)  
**Fig. 3.** Release profiles of vinpocetine from the optimized formulae before, after lyophilization and after sterilization, in comparison with the market product in PBS at 37°C.

![Figure 4](image-url)  
**Fig. 4.** Scanning electron micrographs of the lyophilized optimized formula, before (a) and after (b) sterilization.
On the other hand, significantly higher elimination half-life ($t_{1/2}$) and mean residence time (MRT) have been reported in case of the optimized formula ($p < 0.001$). These results could indicate the ability of the optimized nanomicellar dispersion to achieve intravenous sustained profile when compared to the market product containing the drug in solubilized form. These results were in accordance with that previously reported by Wei et al. who studied the in vivo behavior of pluronics mixed micelles after IV injection in rats (Wei et al., 2009).

4. Conclusion

Mixed micelles achieved significant in vitro and in vivo sustainment behavior and could be considered as a promising nanocarrier for the intravenous delivery of the hydrophobic drug: vinpocetine, having rapid elimination rate with low elimination half-life. Additionally, it could be deduced that the presence of three surfactants with different HLB in the same mixed micellar formula did not offer any extra advantage over those containing two surfactants only. This might be due to that the controlling factor is the characteristics of the hydrophilic shell and the lipophilic core, nevertheless the number of surfactants incorporated.

References


4.2.3. Morphological evaluation of the lyophilized matrices by scanning electron microscopy (SEM)

The scanning electron micrographs of the lyophilized optimized formula are shown in Fig. 4, before (a) and after (b) sterilization. Both micrographs demonstrate the porous nature of the investigated matrices without any noticeable difference. These results could support the observed rapid reconstitution due to the rapid water penetration through the lyophilized porous matrices.

3.6. In vivo quantification of vinpocetine concentrations in plasma

The LC–MS/MS assay has a good linearity from 0.5 ng/mL to 500 ng/mL with acceptable within and between day reproducibility. The lower limit of vinpocetine quantification in plasma was 0.5 ng/mL. The intra-day accuracy of the method ranged from 95.6% to 103.9% (data not shown), while the intra-day precision calculated as CV% ranged from 4.1% to 9.5%. The inter-day accuracy ranged from 96.2% to 106.5%, while the inter-day precision ranged from 4.7% to 8.2%. The accuracy of freeze and thaw stability ranged from 89.0% to 93.4%, while its precision ranged from 4.4% to 7.9%.

The vinpocetine mean plasma concentration–time profiles following single IV dose administration of the optimized formula and the market product are shown in Fig. 5. Corresponding pharmacokinetic parameters are summarized in Table 2. It could be observed that the optimized formula had significantly lower maximum plasma concentration (C_{max}) and elimination rate constant (K_e), when compared to the market product ($p < 0.001$).

Table 2

<table>
<thead>
<tr>
<th>Pharmacokinetics parameters</th>
<th>Treatment (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimized formula</td>
</tr>
<tr>
<td>C_{max} (ng/ml)</td>
<td>150 ± 6.07</td>
</tr>
<tr>
<td>AUC_{0-24} (ng h/ml)</td>
<td>507.06 ± 41.40</td>
</tr>
<tr>
<td>AUC_{24-∞} (ng h/ml)</td>
<td>550.05 ± 39.15</td>
</tr>
<tr>
<td>K_e (h^{-1})</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>14.19 ± 2.72</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>9.94 ± 1.96</td>
</tr>
</tbody>
</table>

* Data are the mean values (n = 6) ± SD.


