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Short communication

Preformulation and development of preliminary nanoemulsion carrier for patent protected compound GL-II-73

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Introduction

Nanopharmaceuticals offer a good option to avoid some of the difficulties that novel drug candidates confront. They can be tailored to adjust their water solubility, half-life, biodistribution, and govern the release of the integrated medication. Because of the excipients utilized, lipid nanocarriers (liposomes, nanoemulsions (NEs), nanoparticles) have been used to increase brain targeting (Bisso et al., 2020; Ilić et al., 2023).

The investigated compound (GL-II-73) - (4R)-8-ethynyl-6-(2-fluorophenyl)-N,N,4-trimethyl-4H-benzo[f]imidazo[1,5-a] [1,4]diazepine-3-carboxamide is imidazobenzodiazepine (IBZD) ligand that acts as positive allosteric modulator on α-GABAA receptors and was shown to possess combined antidepressant and pro-cognitive effects, making it a promising candidate for further research (Prevot et al., 2019).

This work aims to investigate the physicochemical features of GL-II-73 to pick the best parenteral nanodelivery system for prospective research to assess its parameters.

Materials and methods

The saturation solubility of GL-II-73 was determined by adding it in excess to various oils (medium chain triglycerides, soybean, castor, and fish oil) to assess the oil solubility for the substance and select the optimal oil phase composition capable of incorporating the highest concentration of the GL-II-73. It was necessary to test the substance's solubility in buffers of various pH values to determine whether it has pH-dependent solubility. This investigation was carried out by incubating GL-II-73 and studied mediums on vortex for 24 hours and centrifuging to isolate supernatants from which the GL-II-73concentration was evaluated using the LC-MS/MS method. The measurements were taken three times. In addition, after a 24-hour equilibration interval and determination of the GL-II-73 concentration, the log P value was obtained in an octanol/water system.

Based on these findings, preliminary GL-II-73 (NE) was prepared using the high pressure homogenization method. In brief, the oil and aqueous phases were prepared separately and heated to 50 ˚C. They were then pre-mixed at the rotor stator homogenizer before being homogenized for 10 discontinuous cycles at 800 bar on the high pressure homogenizer. After diluting the sample in 1:500, v/v, ultra-purified water, the resulting formulations were characterized in terms of droplet size using the dynamic light scattering (DLS) technique on a Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, U.K.). On the same equipment, the NE's zeta potential (ZP) was measured. In addition, the pH and conductivity of the samples were examined. The ultrafiltration technique was used to evaluate the encapsulation efficacy (EE) by depositing 2 ml of the material in Amicon Ultra-4; NMWL 10 kDa filter units and centrifuging at 4500 rcf for 90

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minutes. The EE was computed as %EE = ((A $_{formulation}$ A $_{filtrate}$)/A $_{formulation}$) 100, where A $_{formulation}$ represents the compound content in the formulation and A filtrate represents the filtrate, which was diluted in methanol and analyzed for Gl-II-73 content using the LC-MS/MS technique. During the one-month storage period, these conditions were monitored.

Results and discussion

Table 1 shows the solubility of GL-II-73 in the oils and buffers tested. The relatively good oil solubility, together with the log P value of 2.09, suggested that NEs could be promising carriers for GL-II-73. The highest oil solubility was detected in medium chain triglycerides, making them the oil phase of choice for future formulation development. Based on the solubility in 0.1 M HCl and phosphate buffer pH 7.4, it is possible to deduce that GL-II-73 has pH dependent solubility, with increased solubility observed as pH decreases, most likely due to the presence of ionizable functional groups and multiple H-bond acceptors. This suggested that the best EE would most likely be obtained by increasing the pH of the aqueous phase and keeping the chemical entrapped in the NE droplets. Solubility in organic solvents revealed that methanol is the best solvent for GL-II-73, as expected given its greater polarity index compared to isopropanol, which is why it was chosen for future characterization.

Table 1. GL-II-73 solubility in selected solvents

Solvent	Solubility (µg/ml)
Water (pH 5.2)	1,001.10 ± 39.94
0.1 M HCl (pH 1.2)	5,370.70 ± 195.26
Phosphate buffer (pH 7.4)	951.37 ± 41.38
Medium-chain triglycerides	4,489.70 ±148.32
Soybean oil	3,055.05 ± 137.42
Castor oil	2,820.65 ± 183.68
Fish oil	2,395.07 ± 331.00
Isopropanol	131,047.81 ± 6,902.35
Methanol	> 1,469,735.25 ± 93,891.20

*The values are presented as means ± SD.

Based on the solubility study, NE of the following composition was prepared: oil phase - medium chain tryglicerides (20%, w/w), soybean lecithin (2%, w/w), buthylhidroxytoluen (0.05%, w/w) and aqueous phase polysorbate 80 (2%, w/w), glycerol (2.25%, w/w), sodium oleate (0.03%, w/w), GL-II-73 (0.2%, w/w) and highly purified water to 100. The values of physicochemical parameters (Z-ave, PDI, ZP, pH, conductivity, drug content and encapsulation efficacy), measured both initially and after one month of storage (Fig. 1), indicate suitability for parenteral administration.

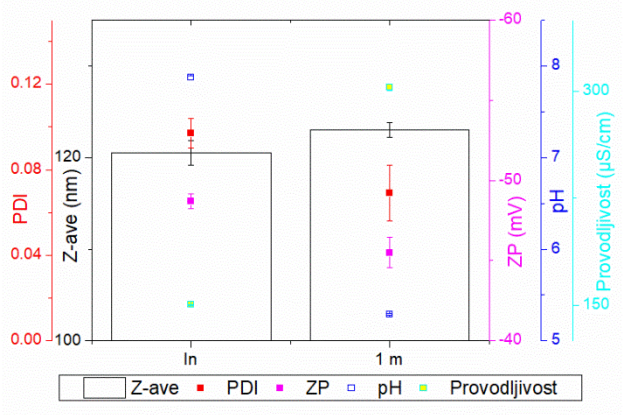

Fig. 1. Stability of GL-II-73 nanoemulsions after one month of storage at room temperature**)**

Conclusion

Preliminary studies suggested that NEs are good prospective carriers for GL-II-73, but further research is needed for stability optimization.

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