# Physicochemical/structural investigation of lipid nanoparticles with high lecithin amounts loaded with patent protected pyrazoloquinolinone ligand DK-I-60-3

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## Introduction

Lipid nanoparticles are being intensively investigated for the formulation of the drugs with poor solubility substances (1). They represent colloid dispersions of the particles with lipid matrix that is solid at room and body temperature. Because of the low capacity of triglycerides for the drug substances incorporation, alternatively, high amounts of lecithin could be added to increase the solubilization (2). This was used for the incorporation of DK-I-60-3 (7-methoxy-d3-2-(4-methoxyd3-phenyl)-2,5-dihydro-3Hpyrazolo[4,3-c]quinolin-3-one), novel deuterated pyrazoloquinolinone ligand, with very low solubility in water as well as in oils (3,4). However, because of amphiphilic nature of lecithin, its localization within nanoparticles should be analyzed, especially with respect to stability, drug loading capacity and drug localization, because it may additionally influence the drug release mechanism (2).

## Methods

Lipid nanoparticle dispersions (Table 1) were prepared by hot high pressure homogenization method (65 ºC, 800 bar, 20 cycles) on EmulsiFlex-C3 (Avestin Inc., Canada). The particle size expressed as hydrodynamic diameter (z-ave), polydispersity index (PDI) and zeta potential were determined on Zetasizer Nano ZS90 (Malvern Instruments Ltd.,Worcestershire, U.K.). The morphology of the nanoparticles was analyzed by atomic force microscopy (AFM) using NTEGRA Prima Atomic Force Microscope (NT-MDT). The structure of the nanoparticles was determined by nuclear magnetic resonance (NMR) on Bruker Ascend 400 spectrometer (Bruker, Rheinstatten, Germany).

Table 1: Composition of developed formulations

| | LNP10-PL | LNP10-DK | LNP15-PL | LNP15-DK |
|---|---|---|---|---|
| **Softisan® 154** | 7,00% | 7,00% | 10,50% | 10,50% |
| **Lecithin (Lipoid® S75)** | 3,00% | 3,00% | 4,50% | 4,50% |
| **Butylated hydroxytoluene** | 0,05% | 0,05% | 0,05% | 0,05% |
| **DK-I-60-3** | - | 0,10% | - | 0,15% |
| **Polysorbate 80** | 2,00% | 2,00% | 3,00% | 3,00% |
| **Ultra-purified water** | to 100,00% | to 100,00% | to 100,00% | to 100,00% |

## Results

### 1. Physicochemical characterization

Table 2: Mean hydrodynamic diameter (z-ave), polydispersity index (PDI) and zeta potential(ZP) of developed formulations.

| Formulation | z-ave (nm) | PDI | ZP (mV) |
|---|---|---|---|
| **LNP10-PL** | 74.09 ± 0.80 | 0.300 ± 0.006 | -50.5 ± 0.7 |
| **LNP15-PL** | 74.58 ± 1.12 | 0.264 ± 0.007 | -39.2 ± 1.8 |
| **LNP10-DK** | 70.37 ± 0.15 | 0.233 ± 0.008 | -41.3 ± 0.9 |
| **LNP15-DK** | 91.87 ± 1.50 | 0.271 ± 0.006 | -34.0 ± 2.0 |

### 2. Microscopic analysis

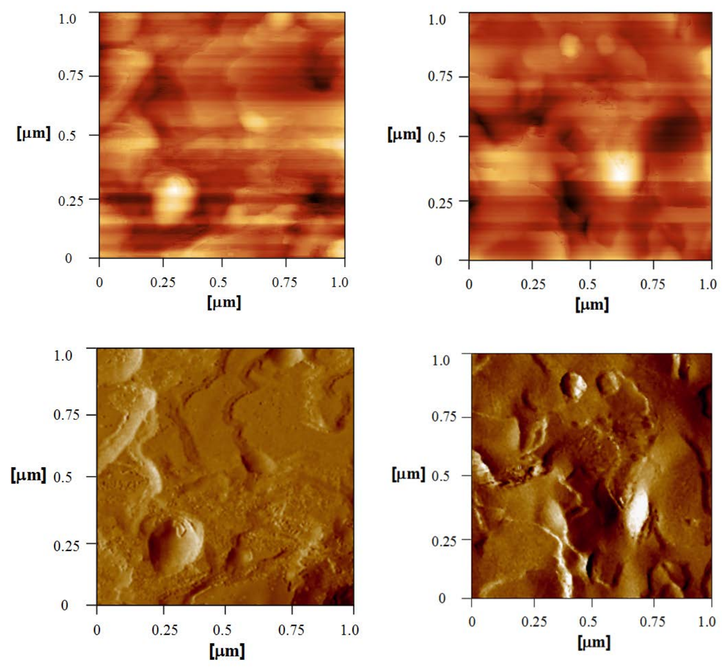
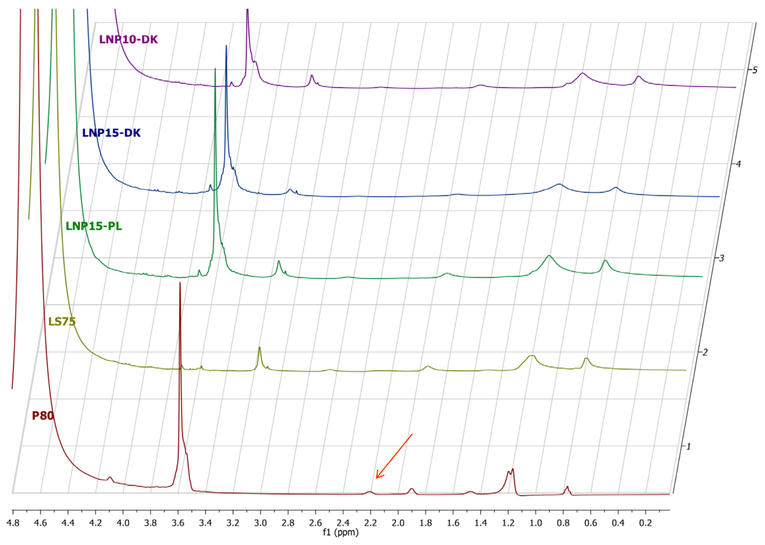



Figure 1: AFM micrographs of LNP10-DK (left) and LNP10-DK (right): 2D topography (up) and signal-error (down) of the sample at the 1 x 1 µm area.

✓ Confirmation of the submicron size of particles.
✓ Stabilizers layer visible on micrographs.

### 3. Structural characterization

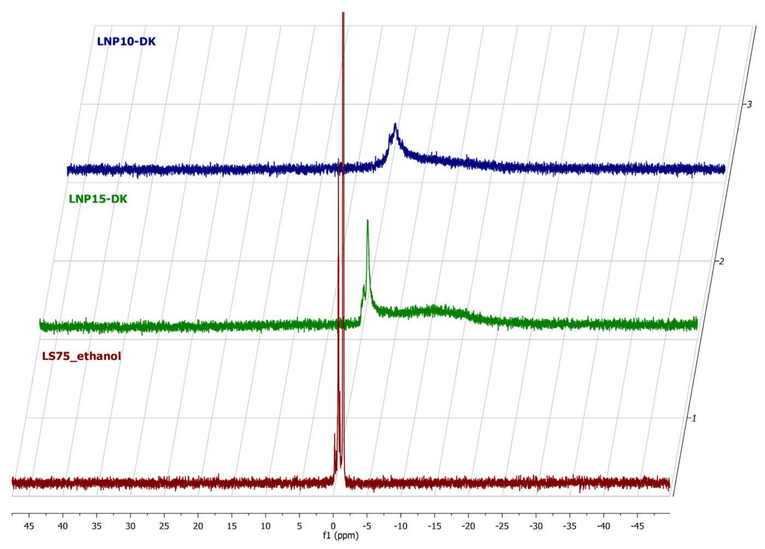



✓ Loss of the signal marked by arrow → immobilization of the hydrophobic part of P80

✓ Broadening of signals from LS75 → partial immobilization of lecithin

✓ Additional broadening of LS75 signals in DK-loaded samples → interaction between DK-I-60-3 and lecithin

Figure 2: $^{1}$H NMR spectra of the reference samples (aqueous dispersion of polysorbate 80 (P80) and lecithin (LS75)) and developed dispersions.




✓ Decreased signal intensity → confirmation of the partial lecithin immobilization

Figure 3: $^{31}$P NMR spectra of the reference sample (lecithin ethanol solution (LS75_ethanol)) and developed dispersions.

## Conclusion

We propose the layered structure of the nanoparticles with lipid core composed of crystalized triglycerides and the shell composed of stabilizers. The ligand DK-I-60-3 was probably localized close to the lecithin molecules in the outer shell, while polar groups of polysorbate 80 were in the aqueous phase contributing to the stabilization of the particles as they were mainly stabilized by the electrostatic effects of the lecithin.

## References

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