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# ATR-FTIR Characterization of *Janus* Nanoparticles. Part I: Implementation of Spectroscopic Descriptors

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ABSTRACT

The present work deals with original bicompartmental lipid *Janus* nanoparticles (JNPs), which are characterized by the presence of an oily compartment associated with an aqueous compartment delimited by a phospholipid-based bilayer. The size of JNP varies between 150 and 300 nm. As JNP are promising candidates for cutaneous application, the purpose of this study was to implement reliable infrared descriptors over time of JNP, to follow the physical stability of JNP in open air and over time. Therefore, a comparative study with the nanoemulsion and the physical mixture formulations was conducted by attenuated total reflection by FTIR spectroscopy. We defined herein spectroscopic descriptor reflecting the integrity of the JNP. Principal component analysis and orthogonal partial least square–discriminant analysis were used to validate the relevant descriptor and permitted to extract relevant and useful information from the spectral data. Dynamic light scattering measurements were also carried and gave supporting data for our conclusion on the fate of JNP over time.



## Introduction

*Janus* is a Roman god with 2 faces, one turned toward the past and the other toward the future. The term *Janus* particles is used to describe particles which are the combination of 2 distinct sides. At a macroscopic level, mainly 2 kinds of *Janus* particles can be described: patchy and compartmented particles. Patchy particles are defined as particles with precisely controlled patches of varying surface, whereas compartmented particles are composed of multiple phase–separated domains in the core. In the field of drug delivery, mainly 4 types of applications have been addressed with Janus nanoparticles (JNPs): co-encapsulation of active pharmaceutical ingredients which have disparate solubility or charge, dual-phase release kinetics, drug targeting and theranostic.[1] In the present work, nanoparticles combine 2 separate compartments of opposite chemical polarity, a lipid compartment bonded to an aqueous compartment which is encased in a phospholipid-based bilayer.[2] Cryogenic transmission electron microscopy (cryo-TEM) can be used to visualize the coexistence of these 2 compartments by direct observation of the sample in a frozen hydrated state (Fig. 1).[3] Owing to this morphology, JNPs are very promising tools, able to incorporate hydrophilic and lipophilic molecules with distinct activities in the same nanoformulation and are very interesting in the drug delivery and cosmetic domains. Moreover, stored in closed container, the aqueous dispersion of nanoparticles formulated with this method is stable for 20 months at room temperature. For this study, the investigated formulation did not contain active ingredients to visualize the fate of the pure vehicles.

As JNP seems to be candidates for cutaneous application, a rapid and noninvasive technique is required to study the behavior of JNP before and after cutaneous application. The technique of dynamic light scattering (DLS) measures the size distribution of a dispersion of nanoparticles, and cryo-TEM enables nanoparticles to be visualized, but these 2 methods cannot be used for *in vivo* studies. The attenuated total reflection by FTIR spectroscopy (ATR-FTIR) method is thus a very informative analytical tool, enabling the direct analysis of the surface of a sample contacting the ATR crystal, and it has been shown that it is suitable for the study of cutaneous tissue.[4-6] For all these reasons, ATR-FTIR was selected to develop spectroscopic descriptors of JNPs in the present article.

*Abbreviations used:* JNP, *Janus* nanoparticles; NE, nanoemulsion; PM, physical mixture; ATR-FTIR, attenuated total reflection by FTIR spectroscopy; PCA, principal component analysis; OPLS–DA, orthogonal partial least square–discriminant analysis; DLS, dynamic light scattering; Cryo-TEM, cryogenic transmission electron microscopy; PEG, polyethylene glycol; $D_H$, hydrodynamic diameter; Pdi, polydispersity index; ν, stretching; δ, bending; s, symmetric; as, asymmetric.

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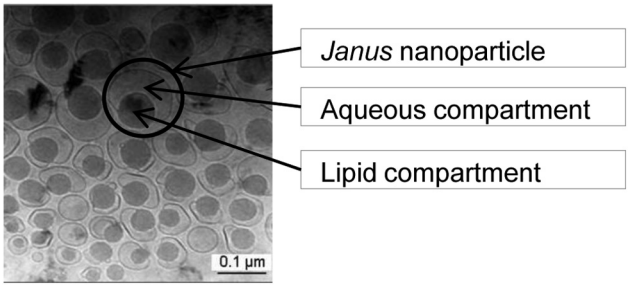


**Figure 1.** Cryo-EM image of the dispersion of JNP.

The first step of the study involved formulating JNP, nanoemulsion (NE), and physical mixture (PM) followed by measuring the size distribution of the 3 formulations. ATR-FTIR was used to select characteristic descriptors of JNP, a comparative study with the NE, and the PM formulations was conducted. In the second step, principal component analysis (PCA) and orthogonal partial least square–discriminant analysis (OPLS-DA) were carried out for the 3 formulations JNP, NE, and PM and validated the previously selected characteristic descriptors. Chemometrics highlighted the organization into 3 groups (PCA). The difference between these groups was explained by identifying discriminant variables (OPLS-DA). DLS was used to obtain supporting data to conclude on the fate of JNP.

## Materials and Methods

### *Materials*

Gelucire 50/13® (a mixture of mono-, di-, and triacylglycerols of palmitic and stearic acid [20%], mono and diesters of polyethylene glycol [PEG] 1500 [~70% with a majority of diesters], and free PEG 1500 [~8%]) and Labrafil M2125CS® (a mixture of mono-, di-, and triglycerides of linoleic acid and mono and diesters of PEG 300 [55% glycerides, 45% PEG ester]) were obtained from Gattefossé (Saint-Priest, France). Phospholipon 90G® (purified phospholipid mixture obtained from soybean lecithin consisting primarily of phosphatidylcholine (94%-102%), lysophosphatidylcholine (≤4%), and tocopherol) was synthesized by Lipoid (Köln, Germany). Water was purified with the Milli-Q water system (Millipore, Martillac, France).

### *Methods*

#### *Preparation of JNP, NE, and PM*

For JNPs preparation, the aqueous phase consisted of 2% (w/w) Gelucire 50/13®, 1% (w/w) Phospholipon 90G®, and 77% (w/w) Milli-Q water, and the lipophilic phase was 20% (w/w) Labrafil M2125CS®. The mixture of Gelucire 50/13®/Phospholipon 90G® was dispersed in Milli-Q water and heated in a 70°C water bath for 5 min with mechanical stirring. The oil phase consisting of Labrafil M2125CS® was also heated at the same temperature. The oil phase was added dropwise in the aqueous phase, and the mixture was stirred with an Ultra-Turrax T18 (Janke & Kunkel GMBH & Co. KG, IKA®-Labortechnik, Staufen, Germany) at 7000 rpm (rotations per minute). Rotor speed was then increased to 11,000 rpm for 5 min. To reduce and standardize the size of particles, the dispersion was passed through a high-pressure homogenizer (APV-2000) at pressures of 600 and 200 bar for the first and second stages, for 5 min at 70°C. The final product was a whitish dispersion of nanoparticles in water.

The same JNP formulation method was used for the formulation of NE but without phospholipon 1% (w/w) 90G®. The PM has the same qualitative and quantitative composition as JNP. The only difference is that PM was obtained by simple magnetic stirring of all the compounds.

#### *DLS: Measuring the Size Distribution of JNP, NE, and PM*

The hydrodynamic diameter ($D_H$) and polydispersity index (Pdi) were measured with a Zetasizer Nano ZS90 (Malvern Instruments, Orsay, France), piloted by Zetasizer Software. The Zetasizer Nano ZS90 detector was positioned at 90° to the sample, and the attenuation was determined automatically by the Nanosizer during the measurement sequence. The measurement cell contained 1.5 mL of water to which was added 5 µL of the dispersion of the nanoparticles (a 1/300 dilution), and cell contents were homogenized. Each sample was analyzed in triplicate at controlled temperature (25°C).

#### *Infrared Vibrational Spectroscopy: ATR-FTIR Spectra Recording*

The infrared spectrophotometer used for the study was ATR-FTIR Spectrum 2 from PerkinElmer (Waltham, MA) piloted by PerkinElmer Spectrum software (version 10.4.2.279). The spectral range used was 450-4000 $cm^{-1}$ with resolution of 4 $cm^{-1}$, and 16 scans were acquired by run. The background of the ATR-FTIR spectrophotometer was air.

In an air-conditioned chamber at 22°C, 3 kinetics were carried out for JNP, NE, and PM, using 25 $CaF_2$ windows as it does not absorb in the mid-infrared. One drop of 25 µL was deposited on the $CaF_2$ window. Kinetics was held over 4 h with spectral measurements every 10 min (from 0 to 240 min). Experiments were performed in triplicate for the 3 formulations mentioned previously (JNP, NE, and PM). Each window was deposited on the crystal ATR, and a spectrum was recorded on the surface of the

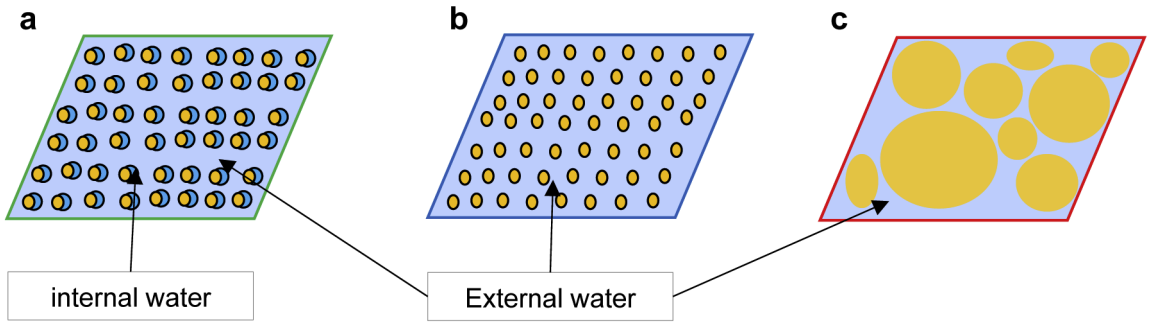


**Figure 2.** Diagrammatic representations of JNP (a), NE (b), and PM (c). (a) JNP is a stable dispersion with bicompartmental objects, $D_H$ and Pdi were 222 ± 66 nm 0.069 ± 0.025 and respectively. (b) NE: is a stable dispersion with monocompartmental objects, $D_H$ and Pdi were 154 ± 50 nm and 0.107 ± 0.041 respectively. (c) PM: is an unstable dispersion with very heterogeneous objects in size, Pdi > 0.2. External water: constituted by the continuous phase of the dispersion of JNP, NE and PM. Internal water: constituted by the hydrophilic compartment of each nano object for JNP.