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Encapsulation effects on the structure-dynamics on drug carriers revealed by neutron scattering

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In this work, we present an overview of a new approach to look into encapsulated systems by means of Elastic and Inelastic Neutron Scattering. The results report on the influence of complexation of the anticancer drug paclitaxel encapsulated into a bio-nanocomposite and of the local anesthetics Bupivacaine (BVC·HCl, $C_{18}H_{28}N_{20}$·HCl·H_2O), Ropivacaine (RVC·HCl, $C_{17}H_{26}N_{20}$·HCl·H_2O) complexed into the cyclic β-cyclodextrin (β-CD) oligosaccharide. We show how neutrons, with wavelength of 1 Å and energy close to 1 kcal/mol, might be a new method to discern if encapsulation of a drug alters or not its conformation in a way that affects its functionality. In addition, our approach offers a method for revealing the structural parameters of magnetic nanoparticles designed for drug delivery.

Introduction

Magnetic micro- and nanoparticles are often used in biomedical applications as contrast agents in magnetic resonance imaging (MRI) as well as for magnetic cell sorting and immunoassay in pathology laboratories [1]. Considering the lower doses needed by magnetic carriers in comparison to the treatment of cancer through tumor targeting, great interest exists on experimental work focusing on their development. Many factors, however, must be considered when designing such magnetic nanoparticle-based targeting systems, including physical parameters, such as magnetic properties and size of the carrier particles, field strength as well as drug/gene binding capacity. Recently, we have shown that we can verify the structural properties of a newly developed bio-nanocomposite (bio-NCP) targeting system by means of neutron-diffraction studies, namely the synthesis reproducibility, particle size and modification [2]. This approach can result in valuable advances to its experimental design as described below.

The second example for the application of neutron scattering is in pharmacology and it is related to the invaluable information that can be obtained about the influence of complexation on the physical properties of a pharmaceutical molecule [3]. Thermal analysis is considered to be one of the most suitable approaches for the determination of an efficient and successful encapsulation process [4], while further information can also be obtained by infrared spectroscopy (IR), Raman scattering (RS) and Nuclear Magnetic Resonance (NMR) [5]. However, (i) optical spectroscopy is a surface technique, which regularly cannot penetrate the sample, (ii) the mass of the encapsulated pharmaceutical molecules is very small when compared to the complex molecule mass, thus the signal of the encapsulated molecule is weak, and vibrational changes might not be detectable. Moreover, solid-state NMR spectroscopy has provided a great deal of useful information on such systems, but

great care has to be taken in such an analysis since the sample preparation process can induce amorphization of the sample or a pronounced structural rearrangement of the guest molecule inside the encapsulation cavity [6]. Consequently, inelastic neutron scattering (INS) is an excellent alternative since neutrons penetrate easily into matter and are sensitive to hydrogen. With INS no special sample preparation is needed and lattice modes and molecular vibrations can be recorded in one measurement. Here, we discuss the dynamical behavior of a new delivery system for long-acting anesthetics that will avoid readministration. Such an approach helps reducing the risk of systemic toxicity associated with individual peripheral nerve blocks.

Results

Development of a bio-nanocomposite (bio-NCP) for the treatment of breast cancer

Breast cancer is the second most common cancer in women. It tends to spread to different parts of the body; in particular to the bones. Nowadays, Paclitaxel (PTX) ($C_{47}H_{51}NO_{14}$) is considered the most effective anticancer drug against this disease; however, low water solubility and the fact that the drug damages healthy cells still limit its clinical application [7]. These issues can be minimized by developing a bio-nanocomposite (bio-NCP) formed firstly by encapsulating magnetic nanoparticles in a polymeric shell and then impregnating the surface with apatite nano-crystals—a main component of the bone tissue with great affinity to breast cancer cells [2]. Antitumor drugs can be further incorporated into the carrier, Figure 1, and by means of magnetic delivery technique the drug carrier can be guided directly to the breast cancer site by external magnetic fields. Furthermore, due to the apatite crystals on its surface, the (bio-NCP) can bind to tumor cells, leading to increased uptake of the drug at the target site, with reduced side effect.

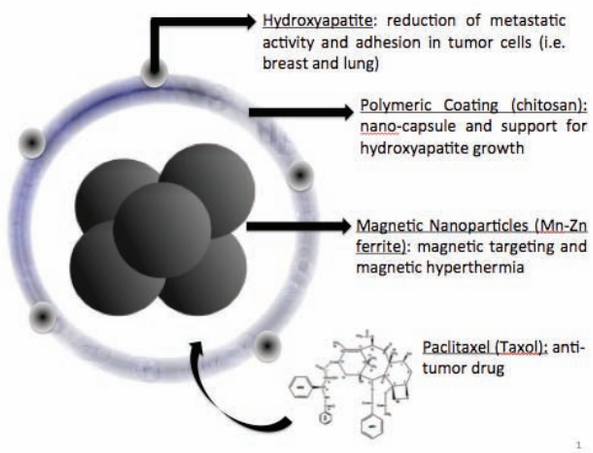

Figure 1. Schematic view of the newly developed bio-NCP. The PTX molecule is also shown.

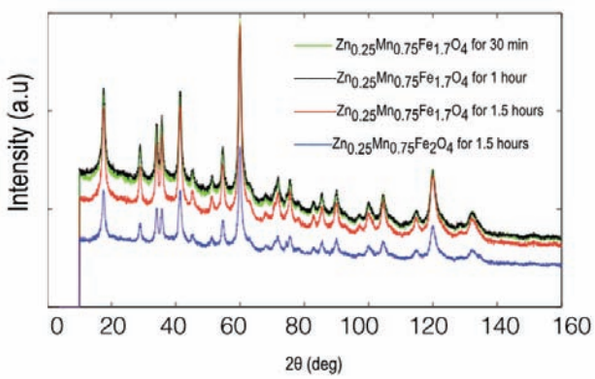

Figure 2. NPD data measured at HRPT with λ = 1.494 Å for a sample synthesized with the nominal composition $Zn_{0.25}Mn_{0.75}Fe_2O_4$ and reaction time of 1.5 h and for samples synthesized under iron deficiency with the nominal composition $Zn_{0.25}Mn_{0.75}Fe_{1.7}O_4$ and reaction times of 30 min, 1 h and 1.5 h.

The first step towards the development of the bio-NCP was to map the ionic distribution in the structure of the manganese-zinc ferrite nanoparticles ($Zn_{0.25}Mn_{0.75}Fe_yO_4$) with y = 1.70 or 2 obtained from different synthesis times. This particular compound was chosen because Mn and Zn ferrites provide the opportunity to obtain a wider range of magnetic properties, by tuning the molar ratio between Mn, Zn and Fe, as well as their distribution within the spinel structure providing for different application requirements. Moreover, these metals, in low quantities, are non-toxic to humans. By means of neutron powder diffraction (NPD), using the thermal diffractometer HRPT at the Paul Scherer Institute (PSI, Switzerland) and the time-of-flight neutron diffractometer POLARIS (ISIS, United Kingdom), this first step was achieved and is discussed below.

The change in the background observed in the NPD data measured at HRPT with λ = 1.494 Å, shown in Figure 2, mainly originates from the presence of H-atoms as amorphous species in the sample that develops over time. The background increase seen in the raw data between 30 and 60 min is most likely attributed to the formation of the oxide-hydroxide shell, which is characteristic of the synthesis method—Ostvald ripening. On the other hand, after 90 min synthetization time the reduced background

indicates that the oxides are replacing the hydroxide species by dehydration. Although the NPD data analysis showed difficulties, because of the magnetic contribution at low angles (after appropriate corrections and using the GSAS program [2, 8]), we were able to demonstrate that no drastic variations in the composition occur when the synthesis condition changes. These results allow us to conclude that the structural properties of the magnetic nanoparticle itself are easily controlled through synthesis parameters and that, therefore, the Mn/Zn ratio as well as the magnetic properties can be tuned. In our work, the bio-NCP achieves high magnetic response even at weak magnetic fields and is a promising material for early diagnosis and treatment of breast cancer.

Characterization of Local Anesthetics (LAs)

Local anesthetics (LAs) are pharmaceutical compounds used to attenuate or eliminate local pain in medical and dental procedures through various routes of administration, such as injective, topical, dermal and mucosal. Leaves of a South American indigenous plant (*Erythroxylon coca*) were among the first LAs ever used. Bupivacaine (BVC, $C_{18}H_{28}N_2O$) was the first made available as a longer acting amide derivate LA in 1963. However, its toxic effects on brain and heart [9], provided stimulus to develop new amide-like local anesthetics. The bupivacaine analog that has come into clinical practice in the United States in the early 1990s, was ropivacaine (RVC, $C_{17}H_{26}N_2O$). Unlike formulations of other local anesthetics in clinical use, RVC is prepared as the single levorotatory isomer rather than as a racemic mixture of the levo- and dextro-forms. Yet the relationship between the anesthetic potency of RVC and its margin of safety has also become a source of controversy [10]. Thus, it is a challenge to develop new ways to deliver long-acting anesthetics to reduce the risk of systemic toxicity associated with individual peripheral nerve blocks. To this aim the oligosaccharides of cyclodextrin (CD) are regarded as excellent encapsulation host agents due to their pre-organized macrocyclic structure. The cyclic β-cyclodextrin (β-CD) is one of the most widely used because of its favorable cavity dimensions and complex formation stability with a wide range of small to medium sized guest molecules [11]. Considering that the outcome of the process of complexation is a unique molecule with different physical properties relative to the free LAs or of the CDs, changes in the thermal analysis results, such as the loss of the endothermal melting peak of the crystalline drug as well as of the cyclodextrin, are expected [4]. INS can provide highly needed complementary information in addition to the results obtained by IR and Raman scattering. In particular, quasi-elastic neutron scattering (QENS) can be used to probe the dynamics and associated geometry of proton motions under confinement on a broad time scale of ns to ps. [3].

The dynamical behavior of BVC hydrochloride monohydrate in the form of racemate (BVC·HCl, $C_{18}H_{28}N_2O{\cdot}HCl{\cdot}H_2O$), RVC hydrochloride monohydrate (RVC, HCl, $C_{17}H_{26}N_2O{\cdot}HCl{\cdot}H_2O$), cyclic β-cyclodextrin (β-CD) oligosaccharide and the respective inclusion complexes in the form of powder samples were studied using the IN6 spectrometer (ILL, Grenoble, France). The measurements were carried out at 300 K using wavelength λ_i = 5.1 Å and energy resolution ΔE = 75 μeV. The dynamical susceptibility, χ(ω), presented in Figure

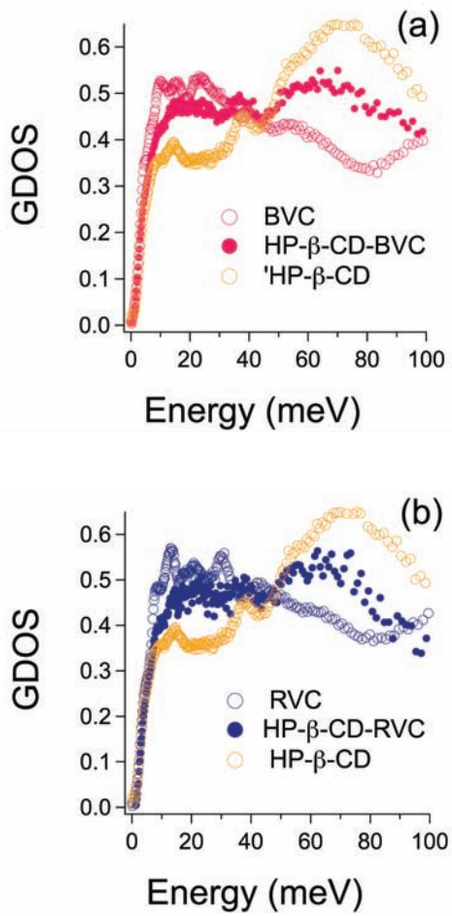

Figure 3. Experimentally determined dynamical susceptibility, χ(w), of BVC, RVC and respective completed systems at 300 K.

3 was calculated after elimination of detectors with intensity from diffraction peaks using the program LAMP. To minimize the effects of multiple scattering during the measurements, the sample transmission was kept at 0.9.

The differences in the low-frequency vibrational spectra of the BVC and RVC molecules are related to the distinct symmetry properties exhibited by the compounds, according to previous X-ray diffraction studies. Concerning the complexes, even if well-defined vibrational modes cannot be distinguished, the observation of the overall shape of the vibrational spectra of the drugs as well as of the β-CD molecules, confirms that the former are confined into a preserved sugar cage [3]. Moreover, it seems that new hydrogen bonds are formed between the drug compounds and the sugar molecules. This observation is in full agreement with previous NMR measurements in similar systems [12]. To confirm this idea, DFT calculations are now underway.

Conclusion and further perspectives

Hydrogen bonds are ubiquitous to our bodies and the world around us. Although most hydrogen bonds exhibit weak attractive forces, with a binding strength of about one-tenth of a normal covalent bond, they are very important, for without them our daily lives would be impossible. If we could see inside ourselves at the molecular level we would observe a marvelous display of chemical reactions taking place to keep the body healthy. When a foreign drug enters our inner world, it can interfere with these reactions via mechanisms common to solution chemistry—including hydrogen bonding, dipole-dipole interactions, charge-transfer and covalent bonding—with (unpredictably) beneficial, benign or catastrophic consequences. Clearly, understanding the structure of a drug in terms of its hydrogen bonds and their interaction with our body chemistry is vital to the challenge of designing new and improved therapeutic drugs. Since many drugs are toxic, their side effects can be often reduced by encapsulating them into some sort of controlled delivery systems before being delivered to the targeted areas inside the human body. However, it is of great importance to determine if, and which, changes take place when such an approach is followed. To this end we are trying to develop a challenging research program by combining neutron scattering techniques to commonly used experimental techniques and with pharmaceutical studies to tackle this problem. Here, we give a glimpse of our research, however, this idea still is at its infancy and further validation is needed.

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References

1. J. Dobson, *Drug Development Research* **67**, 55 (2006).
2. M. L. Martins, M. J. Saeki, M. T. F. Telling, J. P. R. L. L. Parra, S. Landsgesell, R. I. Smith, H. N. Bordallo, *J Alloys Compd*. **584,** 514 (2013).
3. J. Fischer, N. Tsapatsaris, E. de Paula, H. N. Bordallo, *Eur. Phys. J. Special Topics*, **223,** 1831 (2014).
4. L. M. A. Pinto, M. B. de Jesus, E. de Paula, A. C. S. Lino, J. B. Alderete, H. A. Duarte, Y. Takahata, *Journal of Molecular Structure (Theochem)*, **678**, 63 (2004).
5. V. Crupi, A. Fontana, M. Giarola, G. Guella, D. Majolino, I. Mancini, G. Mariotto, A. Paciaroni, B. Rossi, V. Venuti, *The Journal of Physical Chemistry B*, **117**, 3917 (2013).
6. F. G. Vogt and M. Strohmeier, *Molecular Pharmaceutics* **9**, 3357 (2012).
7. P. Lv, W. Wei, H. Yue, T. Yang, L. Wang, G. Ma, *Biomacromol*. **12**, 4230 (2011).
8. R. Ignazzi, BSc Thesis, Niels Bohr Institute, University of Copenhagen, Copenhagen, Denmark (2014).
9. G. A. Albright, *Anesthesiology* **51**, 285 (1979).
10. O. Huet, L. J. Eyrolle, J. X. Mazoit, Y. M. Ozier, *Anesthesiology* 99:1451 (2003).
11. H. M. C. Marques, *Flavour Fragr. J.* **25**, 313 (2010).
12. D. R. de Araujo, S. S. Tsuneda, C. M.S. Cereda, F. D. G.F. Carvalho, P. S.C. Preté, S. A. Fernandes, F. Yokaichiya, M. K.K.D. Franco, I. Mazzaro, L. F. Fraceto, A. F.A. Braga, E. de Paula, *European Journal of Pharmaceutical Sciences* **33**, 60 (2008).