SUPERCRITICAL CARBON DIOXIDE IMPREGNATION/DEPOSITION OF NAPHTHOQUINONES IN MESOPOROUS SILICA PARTICLES

Nicolás A. Gañán[a], Ana M. A. Dias[b], Julio A. Zygadlo[c], Esteban A. Brignole[a], Mara E. M. Braga[b], Hermínio C. de Sousa[b]*

[a] Planta Piloto de Ingenieria Química (PLAPIQUI – CONICET)
Universidad Nacional del Sur
CC 717 – 8000, Bahia Blanca, Argentina

[b] CIEPQPF, Chemical Engineering Department, FCTUC
University of Coimbra
Rua Sílvio Lima
Pólo II-Pinhal de Marrocos, 3030-790 Coimbra, Portugal

[c] Instituto Multidisciplinario de Biología Vegetal (IMBiV – CONICET), FCEFyN
Universidad Nacional de Córdoba
Avenida Vélez Sarsfield 1611, X5016GCA Córdoba, Argentina

E-mail: hsousa@eq.uc.pt

Abstract. Juglone, 1,4-naphthoquinone and plumbagin are three related naphthoquinones known for their toxicity, allelopathic activity and skin peeling effects. They naturally occur in several vegetable species, especially in walnut trees (*Juglans spp*.). In this work, these naphthoquinones are separately impregnated/deposited in mesoporous silica particles (SBA-15) using carbon dioxide as solvent in a static high pressure cell operating at 45ºC for 14 hs. Two pressure conditions (120 and 250 bar) and two depressurization rates (5 and 10 bar/min) were studied in order to determine their influence on impregnation/deposition yield. The total amount of impregnated naphthoquinones was determined spectrophotometrically by dissolution with ethanol, showing that best results were achieved at 120 bar and fast depressurization rate (10 bar/min). Controlled release kinetics in a mixture of physiological serum and ethanol (10% v/v) was also studied for a 48 h period at 32ºC and the corresponding release profiles were determined and compared. Preliminary permeation/diffusion profiles through a dialysis membrane (100 Da) were also determined in a Franz cell, using impregnated particles in suspension.

Keywords: juglone, 1,4-naphthoquinone, plumbagin, supercritical fluid impregnation, mesoporous silica particles

1. Introduction

Naphthoquinones are a widespread family of phenolic compounds naturally occurring in various species of plants and microorganisms as secondary metabolites. These compounds present different types of biological activity, including antimicrobial, anti-inflammatory, cytotoxic and allelopathic effects, among others which justify the use of plant extracts rich in naphthoquinones for centuries in traditional medicines [1].

Juglone (5-hydroxy-1,4-naphthoquinone) is one of the most studied compounds within this family. It naturally occurs in fresh walnut leaves, roots, husks and wood (*Juglans spp*) [2]. Besides its biocidal properties [3,4], it has been used for hair dying and skin coloring, and has a potential cosmetic application as

peeling agent. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) has gained great interest in the last decade due to its antioxidant, anti-inflammatory, anticancer, antibacterial, and antifungal effects [5]. It has

been pointed out that naphthoquinones biological activity, as well as their physicochemical properties, depends on the chemical structure and can be strongly modified by the presence and position of functional groups such as hydroxyl or methyl [6,7].

These properties make them suitable for topical applications on skin, both for pharmacological and cosmetological purposes. To ensure their efficient and sustained release onto and through the skin, as well as to preserve their bioactivity, these natural based drugs should be incorporated into a carrier matrix that can be a gel or any other organic, inorganic or composite matrix. Mesoporous silica nanoparticles are being used in medicine as drug delivery systems since the 1990´s [8] and several applications have been reported for instance in dental, hair and skin care formulations [9].

Impregnation/deposition using supercritical fluids as solvents, and especially supercritical carbon dioxide, constitutes an attractive technology for particles loading due to several advantages over other solvents and methods [10]. Carbon dioxide is a non-expensive, non-flammable and non-toxic solvent, and during depressurization it evaporates completely, leaving a solvent-free product. Its solvent power can be tuned by small changes in temperature and/or pressure and the use of small amounts of cosolvents can enhance its affinity for polar and water-soluble drugs.

Based on these facts, this work aims to study the impregnation/deposition of 1,4-naphthoquinone, juglone and plumbagin on mesoporous silica nanoparticles using supercritical carbon dioxide as solvent, to evaluate the effects of different operating conditions on the loading yield. The controlled release of the drugs in a physiological-type medium is also studied and kinetic profiles are determined, in order to get information for further formulation design. A preliminary study of the permeation/diffusion of naphthoquinones through a polymeric membrane as a proxy of skin is also shown. Results are discussed in terms of the drugs chemical structures and their interactions with the supercritical solvent, the aqueous medium and the silica particles surface.

2. Materials and methods

2.1 Samples and chemicals

1,4-naphthoquinone (≥ 97%), juglone (≥ 95%) and plumbagin (≥ 95%) were obtained from Sigma-Aldrich and were used without further purification. Carbon dioxide (≥ 99.5%) from Praxair, Spain, was used for the impregnation/deposition experiments. Ethanol (≥ 99.5%, p.a., Panreac Quimica SA, Spain), physiological serum (sodium chloride 0.9%, Aga, Portugal) and Milli-Q water were used for the spectrophotometric analysis and for the release and permeation experiments. SBA-15 silica nanoparticles were provided by ClaytecInc (average BJH Framework Pore Size 8.5 nm, total pore volume: 0.93 cm^3/g, surface area: 718 m^2/g). A dialysis membrane (6/Spectra/Por, MWCO: 8000 Da) was used in the impregnation/deposition as well as in the controlled release experiments. A 100 Da dialysis membrane (cellulose ester, Spectra/Por, Biotech) was used in the permeation/diffusion experiments.

2.2 Supercritical fluid impregnation/deposition

The impregnation/deposition experiments were performed in a 100 cm^3 volume high-pressure stirred reactor schematically shown in Figure 1.

In each experiment 90 mg of silica particles were loaded into three dialysis membrane packings (of 30 mg each) and placed into a stainless-steel basket fixed to the agitator axis. The same amount of drug was placed into the reactor in order to maintain a high concentration in the solvent-phase. Then CO_2 is delivered using an ISCO Pump until reaching the desired pressure. At this point, all valves are closed and stirring is turned on. Two pressure levels (120 and 250 bar) and two depressurization rates (5 and 10 bar/min) were tested. All experiments were carried out at the same temperature (318 K) and stirring (150 rpm) for 14 hs. After this period, the expansion valve is open and the system is depressurized at constant rate.

After each experiment, the membrane packings were carefully removed from the reactor, opened and the loaded silica particles were stored in eppendorfs in a freezer (at 263 K), protected from light, until further analysis.

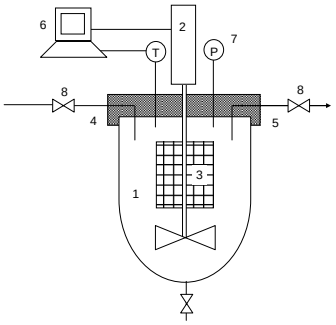

Figure 1. High-pressure stirred reactor. 1. Vessel, 2. Agitator, 3. Sample basket, 4. Inlet line, 5. Outlet line, 6. Computer (temperature and agitation control), 7. Pressure gauge, 8. Valves

2.3 Quantification of the total impregnated/deposited amount of each naphthoquinone

The amount of impregnated/deposited drug was quantified spectrophotometrically. An aliquot of 5-6 mg of impregnated particles was washed with 5 ml of ethanol, vigorously mixed and centrifuged at 6000 rpm for 15 min. The liquid supernatant was then carefully removed. This procedure was repeated 4 times in total until complete redissolution of the drug. The supernatant was analyzed using a UV-vis spectrophotometer (Model 650, Jasco, Japan) and previously determined calibration curves. The absorbance of the samples was measured at 332, 423 and 420 nm for 1,4-naphthoquinone, juglone and plumbagin, respectively. Analyses were made in duplicate.

2.4 Controlled release experiments

In order to obtain further information about the efficiency of the impregnation/deposition process to modulate the release of the drugs in a biological-type medium, controlled release experiments were performed using a mixture of ethanol (10 % v/v) and physiological serum as receptor solution. This percentage of ethanol is suitable for cosmetic applications and enhances naphthoquinone's solubility in aqueous media.

For each sample, 10 mg of particles impregnated at the best yield condition were loaded into a dialysis membrane packing and placed into a flask with 20 ml of receptor solution. The flasks were closed and placed into a shaker at constant temperature (305 K) and stirring (100 rpm) for 48 hs. Samples were taken from the solution at regular intervals and analyzed spectrophotometrically using a UV-vis spectrophotometer (Model 550, Jasco, Japan) and previously determined calibration curves measured at the above referred wavelengths. After each analysis, the solution sample was returned to the flask. Samples were analyzed each 20 min during the first hour, then each 60 min up to 480 min and finally at 24 hs and 48 hs. In this way, an accumulative release profile could be obtained for each drug. Experiments were performed in triplicate.

2.5 Permeation/diffusion experiments

A vertical Franz diffusion-cell apparatus (12 ml) from Analysesysteme (Bechenheim, Germany) was used to study the permeability of naphthoquinones through a preconditioned membrane (100 Da) that is clamped between donor and receptor compartments. The contact area between compartments is 1.77 cm^2. Measurements were performed at 32 ºC using a thermostatized air bath. The receptor compartment contained a mixture of ethanol 10 % (v/v) and physiological serum while the donor compartment contained a suspension of juglone-loaded SBA-15 particles impregnated in the best yield condition and dispersed in the same solution. The amount of loaded particles was calculated in order to keep a concentration of juglone in the donor compartment much higher than in the receptor compartment during the whole experiment (sink conditions). Receptor solution was stirred at 500 rpm. Aliquots (0.5 ml) were withdrawn at constant time intervals and immediately analyzed for drug concentration spectrophotometrically (at 423 nm). The removed aliquot was replenished with equal amount of receptor solution. More details about the Franz diffusion-cell and the use of polymeric membranes for transdermal delivery studies can be found in Hwang et al. [11].

3. Results and discussion

3.1 Impregnation/deposition yield

A macroscopic visualization of silica nanoparticles before and after impregnation/deposition with 1,4-naphthoquinone is shown in Figure 2 together with the chemical structures of the different molecules studied in this work.

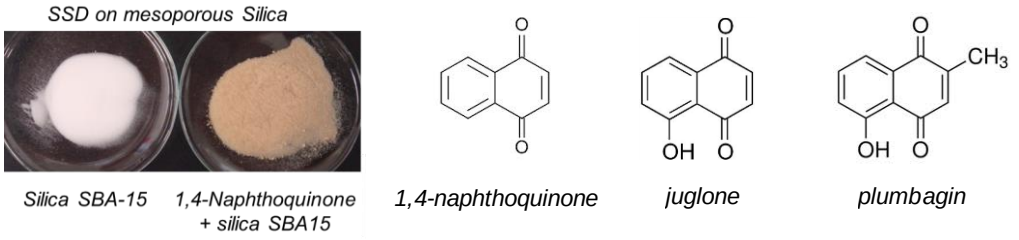

Figure 2. SBA-15 silica nanoparticles before and after SSI of 1,4-naphthoquinone using $scCO_2$ as solvent and chemical structures of the studied naphthoquinones.

Total impregnation/deposition yields for each naphthoquinone are shown in Figure 3 for each pressure and depressurization rate condition. These values were determined by redissolution of the compounds in ethanol and spectrophotometric quantification. The effect of pressure is easily identified: yields are higher at lower pressure for 1,4-naphthoquinone and plumbagin. In the case of 1,4-naphthoquinone, yields at 120 bar are 4-6 times higher than when operating at 250 bar and for plumbagin, this difference is lower. In the case of juglone at 5 bar/min this difference is not verified.

On the other hand, the effect of the depressurization rate is more complex. At lower pressure, yield is higher when depressurization is faster for juglone and 1,4-naphthoquinone, and slightly lower for plumbagin. At higher pressure, yield is only slightly higher for 1,4-naphthoquinone and plumbagin and lower for juglone.

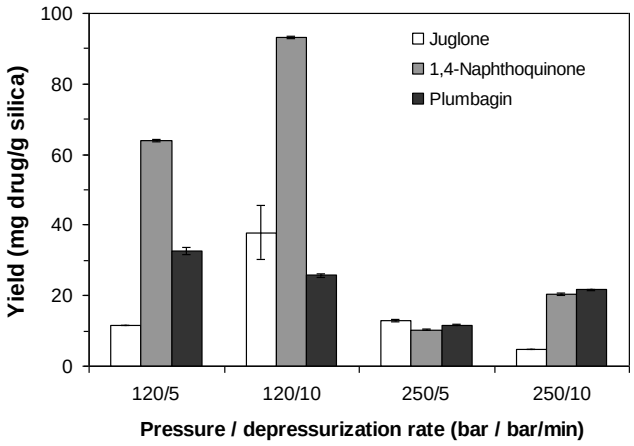

Figure 3. Naphthoquinones total impregnation/deposition yields obtained at different pressure and depressurization rate conditions (determined by redissolution in ethanol).

According to solubility data of naphthoquinones in supercritical CO_2 previously reported in literature [12,13], the initial drug loaded into the cell is completely dissolved at both pressure levels (i.e., the solvent phase is homogeneous) and is the same in all experiments. Therefore the observed effects cannot be explained in terms of solubility limitations, but instead in terms of CO_2–drug–silica interactions, mainly the CO_2–drug and drug–silica interactions. The first is represented by the solubility at a given temperature and pressure, and can be described in terms of solvent density and drug volatility. The second is more specific, and depends on the drug chemical structure and the presence of functional groups capable to interact with the hydroxyl groups of the silica surface. These factors will determine the partition coefficient of the drug between the CO_2 (solvent phase) and the silica particles surface which ultimately will determine the total impregnation/deposition yield.

When the experiment is performed at higher pressure, the CO_2 density and its solvent power are higher than at lower pressure (at constant temperature). Therefore, when equilibrium is reached, CO_2–drug interactions are favored and a lower amount of drug is expected to be adsorbed on the particles surface.

On the other hand, the depressurization step decreases solvent density and drug solubility, leading to drug precipitation or deposition. In this way, the drug is partly impregnated or adsorbed onto the particles surface and partly deposited, with no specific interaction with the surface. When depressurization starts at a higher pressure level and/or when is performed at a slow rate, more drug is removed (re-dissolved in the solvent phase) before it begins to precipitate on the silica particles. This explains that the best impregnation/deposition yields were generally obtained at conditions of lower pressure (120 bar) and faster depressurization rate (10 bar/min), and the lowest yields at the opposite conditions (250 bar and 5 bar/min).

3.2 Controlled release kinetics

The kinetic curves obtained in the controlled release experiments for particles impregnated at 120 bar and 10 bar/min depressurization rate (corresponding to the best yield conditions) showed that more than 90% of mass of each drug is released after 24 hs and completely released after 48 hs.

In this case, the receptor solution–drug–silica interactions have to be analyzed in order to explain the results. Similarly to the case of the impregnation/deposition, the release process is governed by the partition coefficient of the drugs between the solvent phase and the silica surface, which in turn depends on the drugs solubility and the specific chemical interactions between hydroxyl and carbonyl groups. Due to the fact that the volume of receptor solution and the amount of drug sample are the same during the whole experiment, it can be assumed that results obtained after 48 hs correspond to an equilibrium state, and therefore the distribution coefficient is high: naphthoquinones tend to be dissolved rather than adsorbed onto the particles.

However, the kinetic profiles are different, especially within the first 10 hs of experiment. Plumbagin is more easily released, followed by 1,4-naphthoquinone and juglone. Naphthoquinones solubility in aqueous solutions is very low, but it is greatly enhanced by the presence of ethanol, so that solubility is not a limiting factor. The differences must be then explained in terms of naphthoquinone's interactions with the silica particles which depend on their different chemical structures. These results can also give information about the impregnation/deposition process efficiency. As mentioned above, the drugs are partly adsorbed onto the particles surface and partly just deposited or precipitated. This last part is more easily released and dissolves during the first minutes. The initial slope of the kinetic curves was observed to be almost the same for the three naphthoquinones. After dissolution of the deposited/precipitated fraction, the adsorbed material starts to be released in a much slower process. In this region juglone presents the slowest release ratio indicating that is may be strongly adsorbed to the silica particles.

3.3 Permeation/diffusion experiments

Figure 4 shows the diffusion/permeation profile of juglone from impregnated silica nanoparticles, represented as the accumulated mass of drug in the receptor solution along time.

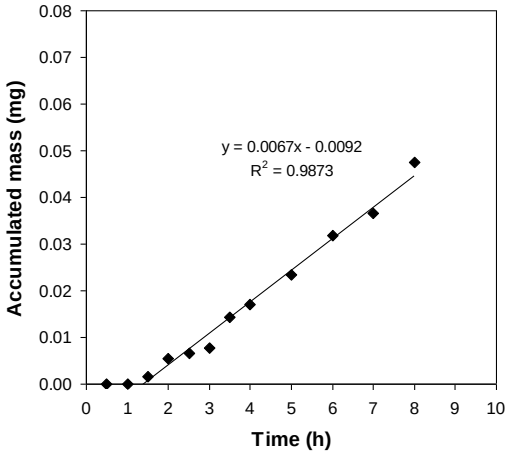

Figure 4. Diffusion/permeation profile of juglone in 10% (v/v) ethanol-physiological serum through a 100 Da dialysis membrane (T=32ºC, 500 rpm).

During the first hour, juglone was not detected in the receptor solution. This initial period is likely to correspond to the swelling of the membrane and its saturation with juglone. After this period, the transfer of juglone to the receptor solution begins at an approximately constant rate. The diffusion/permeation rate was calculated by linear regression of the measured data and it was found to be 6.7 μg/h which corresponds to a flux (specific rate per unit area) of 3.8 μg/h.cm^2. Note that these values should be considered as preliminary and have to be confirmed with further experiments.

4 Conclusions

The results obtained in this work demonstrate the feasibility of using SSI to impregnate/deposit natural based drugs, namely 1,4-naphthoquinone, juglone and plumbagin into mesoporous silica nanoparticles using supercritical carbon dioxide as solvent. The high solubility of these drugs into $scCO_2$ justifies the use of the technique to prepare materials to be used for pharmaceutical and/or cosmetic applications due to greener character of the solvent and also because it can be applied at mild conditions originating solvent free products.

The obtained results indicate that lower pressures and higher depressurization rates lead to higher total impregnated/deposited drug amounts for all the studied molecules being this effect more pronounced for 1,4-naphthoquinone. These tendencies were explained by the different partition coefficients that were established at each condition and that depend on specific $scCO_2$–drug–silica interactions. Release and permeation preliminary results permitted to infer that juglone is strongly adsorbed on silica surface and that it permeates a model membrane (100 Da) at 3.8 μg/h.cm^2.

Acknowledgements

A.M.A. Dias acknowledges FCT-MCTES for fellowship (SFRH/BPD/40409/2007). N.A. Gañan acknowledges Erasmus Mundus Project (EADIC II) for financial support.

References

[1] P. Babula, V. Adam, L. Havel, R. Kizek, Noteworthy Secondary Metabolites: Naphthoquinones – Their Occurrence, Pharmacological Properties and Analysis, Curr. Pharm. Anal. 5 (2009) 47–68.
[2] J.S. Amaral, R.M. Seabra, P.B. Andrade, P. Valentão, J.A. Pereira, F. Ferreres, Phenolic profile in the quality control of walnut (*Juglans regia* L.), Food Chemistry 88 (2004) 373–379.
[3] A.M. Clark, T.M. Jurgens, C.D. Hufford, Antimicrobial activity of juglone, Phytotherapy Research 4 (1990) 11–14.
[4] P. Babula, V. Adam, R. Kizek, Z. Sladky, L. Havel, Naphthoquinones as allelochemical triggers of programmed cell death, Environmental and Experimental Botany 65 (2009) 330–337
[5] S. Padhye, P. Dandawate, M. Yusufi, A. Ahmad, F.H. Sarkar, Perspectives on medicinal properties of plumbagin and its analogs, Medicinal Research Reviews 9 (2010) 1–28.
[6] L.M. Hughes, C.A. Lanteri, M.T. O'Neil, J.D. Johnson, G.W. Gribble, B.L. Trumpower, Design of anti-parasitic and anti-fungal hydroxy-naphthoquinones that are less susceptible to drug resistance, Molecular and Biochemical Parasitology 177 (2011) 12–19.
[7] K. Ollinger, A. Brunmark, Effect of hydroxy substituent position on 1,4-naphthoquinone toxicity to rat hepatocytes, J. of Biological Chemistry 266 (1991) 21496–21503.
[8] J. Andersson, J. Rosenholm, M. Lindén, Mesoporous silica: An alternative diffusion controlled drug delivery system, in Topics in Multifunctional Biomaterials and Devices, Ed. N. Ashammakhi (2008).
[9] A. Mihranyan, N. Ferraz, M. Strømme, Current status and future prospects of nanotechnology in cosmetics, Progress in Materials Science 57 (2012) 875–910.
[10] J.M. Cocero, A. Martín, F. Mattea, S. Varona, Encapsulation and co-precipitation processes with supercritical fluids: Fundamentals and applications (Review), J. of Supercritical Fluids 47 (2009) 546–555.
[11] Y.K. Hwang, J.W. Choi, C.H. Hwang, W. Park, K.-D. Park, K.-D. Suh, J.W. Kim, Synthetic polymer membranes as a proxy of skins in permeation studies of biologically active compounds, Macromolecular Research, 20 (2012) 379–384.
[12] S. Marceneiro, M.E.M. Braga, A.M.A. Dias, H.C. de Sousa, Measurement and correlation of 1,4-naphthoquinone and of plumbagin solubilities in supercritical carbon dioxide, J. of Chemical & Engineering Data, 56 (2011) 4173–4182.
[13] S. Marceneiro, P. Coimbra, M.E.M. Braga, A.M.A. Dias, H.C. de Sousa, Measurement and correlation of the solubility of juglone in supercritical carbon dioxide, Fluid Phase Equilibria, 311 (2011) 1–8.