Preparation of Nanoparticulate Polymeric Matrices Highly Loaded with Gentamicin by PCA

Ventosa N[1a]*, Elizondo E[1a], Sala S[1b],Imbuluzqueta E[2], González D[2], Gamazo C[3], Blanco-Príeto M.J[2],Veciana J[1a]

[1] Molecular Nanoscience and Organic Materials Department. [a]ICMAB-CSIC/CIBER-BBN, [b]CIBER-BBN/ICMAB-CSIC. Campus UAB s/n 08193-Bellaterra ,SPAIN.
[2] Department of Pharmacy and Pharmaceutical Technology, University of Navarra, Irunlarrea s/n, Pamplona 31080, SPAIN.
[3] Department of Microbiology, University of Navarra, Irunlarrea s/n, Pamplona 31080, SPAIN.
Contact e-mail: ventosa@icmab.es; Fax: (+34) 935805729

INTRODUCTION

From an aesthetic perspective, it is attractive to build all desirable pharmacological features of a drug- such as solubility, stability, permeability to biological membranes, and targeting to particular tissues, cells and intracellular compartments- into the drug molecule itself. But it would be simpler and perhaps more powerful to obtain these features by decoupling the biological action of the drug from the other biochemical and physicochemical characteristics that determine these key features of its pharmacology [1]. Thus, the need of meliorating the pharmacological properties of the existing compounds more than the synthesis of new ones, has aroused an increasing interest in the development of new systems for the controlled delivery of these drugs *(Drug Delivery Systems, DDS)*. Micro and nanoparticles composed by a biologically active compound and a biodegradable polymer, acting as a carrier, represent one of the most studied systems in the field of nanomedicine [2]. In such formulations, the polymer confers stability to the drug, enhances its intracellular penetration and permits a more controlled release reducing, as a result, the number of required dosis.

The intracellular infections constitute an important group of diseases where the use of these devices is of vital importance. In the particular case of brucellosis, the intracellular location of the bacteria *Brucella*, renders treatment difficult since most antibiotics, such as gentamicin, known to be efficient in vitro, do not actively pass through cellular membranes [3]. This low *in vivo* efficiency causes chronic infections both in animals and humans, who become infected by contact or ingestion of infected animal products, such as milk or derivatives [4]. The enhancement of the intracellular penetration of gentamicin, a really hydrophilic aminoglicoside, by using biodegradable polymers as drug carriers has already been studied [5]. Despite the promising results observed for the micro and nanoparticulated formulations, there are still problems like the low loading of the polymer or the poor robustness of their preparation methodologies.

As an alternative, the development of new processes that use compressed fluids, mainly CO_2, for the preparation of micro or nanoparticulate materials has extended considerably since the early 90s, being of increasing interest in the drug delivery field, as it has been recently reviewed [6]. As the compressed CO_2 has already been used for the production of drug/polymer composites [7], we have developed a PCA based method in order to check if the compressed fluid based processes constitute a good alternative for the production of nanoparticulate polymeric matrices loaded with high quantities of gentamicin, with the goal of enhancing its intracellular penetration. In this work, we have chosen a copolymer with

maleic anhydride (MAcop) as Gm carrier, due to its excellent properties for the oral administration of active compounds. This polymer, not only protects the drug from degradation in the gastrointestinal tract, but also has bioadhesive properties that favour a major contact with the mucosa.
Gentamicin sulphate, commercial form used in the present work, is a highly water-soluble drug with negligible solubility in organic solvents. Therefore, a previous chemical modification was necessary for processing it by PCA.

MATERIALS AND METHODS

Materials:

Maleic anhydride copolymer was kindly given by ISP International Corp. (Sant Joan Despí, Spain). Gentamicin sulphate was purchased from Molekula (Dorset, UK). Bis(2-ethylhexyl) sulfosuccinate sodium salt (AOT) was supplied by Sigma (Tres Cantos, Spain). Carbon dioxide (high purity SCF grade) was obtained from Carburos Metálicos S.A (Barcelona, Spain). The organic solvents, HPLC grade, were supplied by Teknokroma (Sant Cugat del Vallès, Spain).

Hydrophobic ion pairing of gentamicin sulphate:

The ionic complex of gentamicin (Gm) and the anionic surfactant Bis(2-ethylhexyl) sulfosuccinate sodium salt (Aerosol OT ™, AOT), GmAOT, was prepared by the HIP method as described elsewhere [7]. Briefly, a solution of 5 mol of AOT in chloroform was added to an equal volume of an aqueous solution of Gm for the stoichiometric complexation of its 5 ionizable amino groups and therefore the replacement of the sulphate counter ions, dealing to a 1:5 Gm:AOT ionic complex (Figure 1).

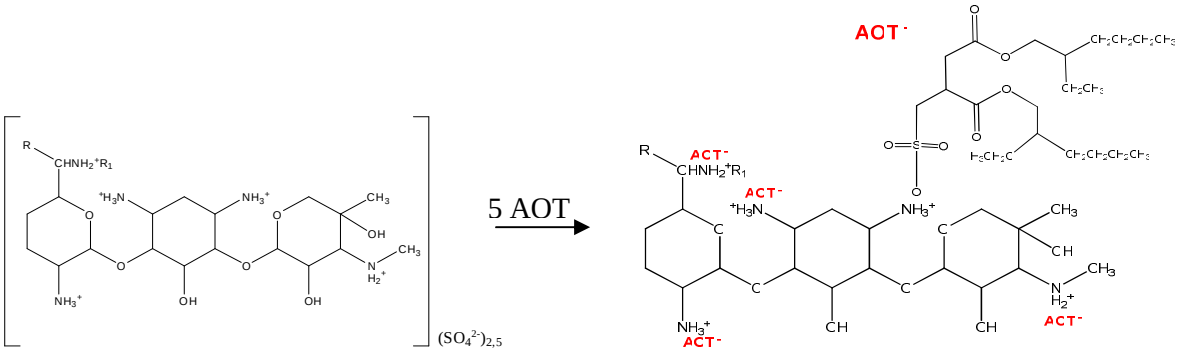


Figure 1: Hydrophobic Ion Pairing of gentamicin

Antisolvent precipitation apparatus and procedure:

PCA experiments of both compounds, GmAOT and polymer, either alone or together, were performed using the experimental set-up showed in Figure 2. As schematized, a constant flow of CO_2 was continuously supplied inside a 300 ml high-pressure autoclave. The pressure inside the autoclave was kept constant using a back pressure regulator valve. When constant values of temperature and pressure were achieved, pure solvent was fed into the autoclave through an atomization nozzle (100 µm diameter), at a constant flow, for several minutes after

a constant X_{CO2} was reached inside the vessel. The solvent flow was then stopped and a liquid solution of the compound was sprayed inside the autoclave at the same flow rate as the pure solvent. The micronized solid, produced through the PCA process during the spraying of the solution, was collected at high pressure on a filter placed inside the autoclave and the mother liquid was depressurized after the back pressure valve. When all solution volume had been delivered, the CO_2 flow continued for an hour in order to remove the solvent retained in the precipitate, and the autoclave was depressurized to atmospheric pressure in order to withdrawn the micronized materials.

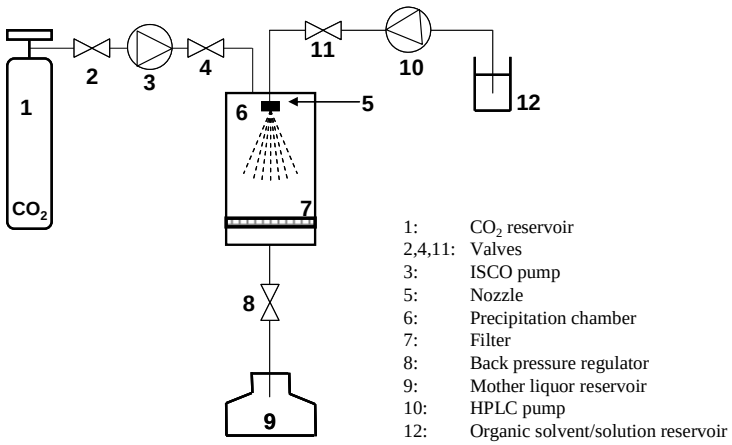


Figure 2: PCA apparatus

Precipitates characterization:

Fourier Transformation Infrared Spectroscopy (FTIR):

The qualitative chemical composition of the GmAOT complex and the GmAOT:MAcop composites was studied by FTIR (Spectrum One, PERKIN ELMER, USA) using the Universal Attenuated Total Reflectance (UATR) accessory.

Elemental analysis:

The elemental analyzer CARLO ERBA, EA1108 model, was used for determining the exact CHNS composition of the GmAOT complex. N and S, only present in the drug and surfactant respectively, were used to confirm the 1:5 proportion between both compounds in the HIP complex.

Nuclear Magnetic Resonance (NMR):

The study of the drug content in the different GmAOT:MAcop matrices prepared was performed by nuclear magnetic resonance (Bruker-DPX250). The ^{1}H NMR spectra of the composites were performed at ambient temperature using acetone-d_6 as solvent. The spectra analysis for drug quantification was carried out with the Top Spin software (Bruker). The proportions between GmAOT:MAcop were calculated by integrating non-overlapped signals of the polymer and the drug.

Scanning Electron Microscopy (SEM):

The morphology and particle size of the precipitates were examined by scanning electron microscopy using HITACHI S-570 and JEOL JSM-6300 (Japan) microscopes. The powders were deposited onto carbon tape previously stuck to an aluminium stub and coated with gold using a sputter coater (K550, Emitech, UK) for 4 minutes. Several images of different parts of the samples were analyzed, in order to perform a representative analysis of the precipitates.

RESULTS

GmAOT complex

A hydrophobic complex between gentamicin and the anionic surfactant AOT was prepared by the Hydopobic Ion Pairing method. The elemental analysis confirmed a 1:5 drug:surfactant proportion in the complex. The solubility of GmAOT was studied in various organic solvents, exceeding 0,5 g/ml in the case of acetone, methanol, ethanol, isopropanol, butan-1-ol, ethyl acetate, tetrahydrofurane, pentane and heptane.

Once ion paired, the Gm was then micronized by PCA following the above described procedure. It was observed that for the preparation of powdered GmAOT, high initial concentrations of the antibiotic in acetone were necessary, since bellow 0,3 g/ml instead of a powder, a waxy material was obtained. The morphology of the powder was examined by scanning electron microscopy, observing that though the microparticles suffered from coalescence, the roughness of the surface was much higher than in the non-processed GmAOT, resulting in a major accessible surface area (Figure 3).

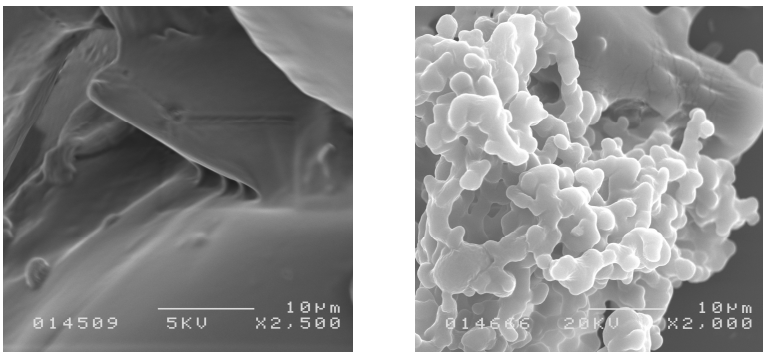
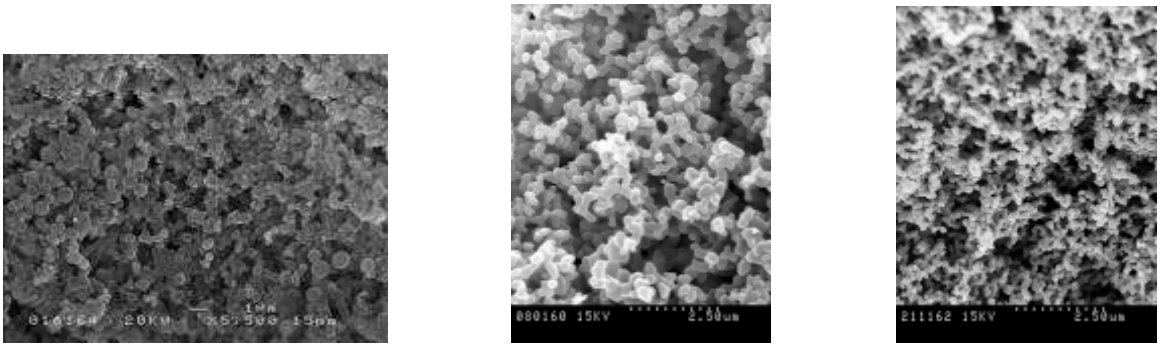


Figure 3: SEM images of non-processed (left) and processed GmAOT (right)

GmAOT:MAcop composites

Various GmAOT:MAcop composites were prepared by spraying solutions with different proportions of drug and polymer into the precipitation chamber. In order to check the GmAOT loading in the polymeric matrices, the ^{1}H NMR spectrum of the different composites was analyzed. The integration of non-overlapped signals from the drug and the polymer provided their real proportions in the precipitates. It was confirmed that the proportions were maintained after spraying, proving that composites of desired proportions could be obtained by PCA. It is important to underline that by this methodology much higher quantities of Gm could be loaded into the polymeric matrices compared to conventional methodologies. The morphology and particle size of these composites was analyzed by SEM, observing that in all cases nanoparticulate and homogenous polymeric matrices were obtained. It was also detected that whereas the particle size diminished upon increasing the GmAOT proportion in the composite, the coalescence between the primary particles was more evident in that direction (Figure 4).



0,09:1 0,37:1 1:1

Figure 4: SEM images of GmAOT:MAcop nanoparticulate composites with different w:w proportions

CONCLUSION

The antisolvent precipitation of a hydrophobic complex of gentamicin, GmAOT, resulted in a microparticulate material with a much higher surface area than the non-processed complex. By the PCA method, nanoparticulate polymeric matrices loaded with different proportions of the ion paired gentamicin were obtained. The use of this methodology allowed much higher loadings than the ones obtained by conventional technologies, therefore, reduced doses would be needed and an easier and faster treatment could be provided to the patients

AKNOWLEDGMENTS

This work was supported by the CIDEM from the *Catalan Government*, the CIBER-BBN and the DGI from the Spanish Government under projects CONSOLIDER-C EMOCIONA (CTQ2006-06333/BQU), and NANOFAR (NAN2004-09159-C04-01) . The authors thank ISP International Corp. for supplying the biodegradable polymer and Raul Solanas and Emili De la Serna (MATGAS) for the experimental assistance. Elisa Elizondo thanks CSIC for her PhD bursary.

REFERENCES

[1] HUBELL, J. A., Science, Vol. 300, **2003**, p. 595
[2] JAIN, R. A., Biomaterials, Vol. 21, **2000**, p. 2475
[3] (a) ROLAIN, J. M.., MAURIN M., RAOULT D., Journal of Antimicrobial Chemotherapy, Vol. 46, **2000**, p. 811; (b) Nature News in Brief: "Brucellosis fears hamper Yellowstone bison plans", Nature, Vol. 452, **2008**, p.677
[4] BLASCO, J. M., GAMAZO, C., Investigación y Ciencia, Vol. 218, **1994**, p. 56
[5] (a) LECAROZ, C., BLANCO-PRÍETO, M. J., BURRIEL, M.A., Journal of Antimicrobial Chemotherapy, Vol. 58, **2006**, p. 549; (b) LECAROZ, C., GAMAZO, C., BLANCO-PRIETO, M. J., Journal of Nanoscience and Nanotechnolgy, Vol. 6, **2006**, p. 3296; (c) LECAROZ, C., BLANCO-PRÍETO, M. J., et al., Antimicrobial Agents and Chemotherapy, Vol. 51, **2007**, p. 1185
[6] Special issue: "Drug Delivery Applications of Supercritical Fluid Technology", Advanced Drug Delivery Reviews, Vol. 60, **2006**, issue 3
[7] (a) REVERCHON, E., ANTONACCI, A., Biotechnology and Bioengineering, Vol. 97, **2007**, p. 1626; (b) GHARDERI, R., ARTURSSON, P., CARLFORS, J., European Journal of Pharmaceutical Sciences, Vol 10, **2000**, p. 1; (c) FALK, R., RANDOLPH, T. W., Pharmaceutical Sciences, Vol. 15, **1998**, p. 1233
[8] FALK, R., RANDOLPH, T. W., MEYER J. D., NELLY, R. M., MANNING, M. C., Journal of Controlled Release, Vol. 44, **1997**, p. 77