Physicochemical Characterization of Orally-Active Meglumine Antimoniate/Beta-Cyclodextrin Nanoassemblies: Non-Inclusion Interactions and Sustained Drug Release Properties

P. S. Martins, R. R. Ribeiro, A. P. C Bahia, R. L. M. Neto, F. Frézard*
Departamento Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Pampulha, 31270-901 Belo Horizonte, MG, Brazil

A. M. C. Pimenta
Departamento Bioquímica-Imunologia-ICB, Universidade Federal de Minas Gerais, Brazil

A. L. Melo
Departamento Parasitologia-ICB, Universidade Federal de Minas Gerais, Brazil

L. Le Moyec
CNRS 7033, Université Paris XIII, France

C. Demicheli
Departamento Química-ICEX, Universidade Federal de Minas Gerais, Brazil
(Received on 1 July, 2008)

β-cyclodextrin (β-CD) is widely used as a component of pharmaceutical formulations, classically to improve the solubility and oral bioavailability of poorly water-soluble drugs through formation of drug/β-CD inclusion complexes. Unexpectedly, the association of the highly water-soluble drug meglumine antimoniate (MA) with β-CD turned this antimonial compound orally-active in a murine model of leishmaniasis. To get insight into the mechanisms responsible for the enhanced oral efficacy of MA, the MA/β-CD composition was characterized physicochemically, using thermogravimetry, circular dichroism, mass spectrometry (ESI-MS), osmometry and photon correlation spectroscopy. The freeze-dried MA/β-CD was found to form nanoassemblies in water, as a result of multiple non-inclusion interactions between MA and β-CD, which behave as a sustained release system of the MA drug.

Keywords: cyclodextrin, antimony, meglumine antimoniate, sustained release, nanobiotechnology

I. INTRODUCTION

Even though the pentavalent antimonials meglumine antimoniate (MA) and sodium stibogluconate are still the first-line drugs against all forms of leishmaniasis, their use in the clinical setting shows several limitations. These compounds have to be given parenterally, daily, for at least 4 weeks. Antimony therapy is often accompanied by local pain during intramuscular injections and by severe systemic side effects, requiring very careful medical supervision. The appearance of drug resistance is another important problem in the treatment of this disease. All these factors contribute to compliance difficulties and treatment failures.

The search for an orally-active formulation of MA led us to prepare and evaluate a composition comprising MA and β-cyclodextrin (β-CD). Even though hydrophilic β-CD is not expected to enhance the absorption of water-soluble (BCS class III) drugs by the oral route, the MA/β-CD composition rendered MA orally active in a murine model of cutaneous leishmaniasis [1]. This unexpected result was attributed, in part, to the fact that the heating of equimolar mixture of MA and β-CD (first step of preparation of MA/β-CD composition) induced the depolymerization of MA from high molecular weight Sb complexes into 1:1 Sb-meglumine complex, resulting in an enhanced oral bioavailability of Sb [2]. Since the pre-heated MA+β-CD mixture still produced significantly lower serum Sb levels when compared to the MA/β-CD composition [2], we inferred that the freeze-drying process (second step of preparation of MA/β-CD composition) was required for achieving a high absorption of Sb by oral route.

The aim of the present work was to get insight into the physicochemical alterations induced by the freeze-drying step, through characterization of MA/β-CD composition by circular dichroism, mass spectrometry (ESI-MS), osmometry, thermogravimetry and photon correlation spectroscopy.

II. EXPERIMENTAL METHODS

II.1. Samples preparation

The MA/β-CD composition was prepared by mixing β-CD and the 1:1 Sb^V-megumine complex in water at a 1:1 β-CD/Sb molar ratio, heating the mixture for 48 h at 55^{o}C under stirring and finally freeze-drying the resulting solution.

II.2. Physicochemical analyses

In circular dichroism (Jobin Yvon-Spex Mark CD6 dichrograph) and mass spectrometry (ESI-Q-ToF mass spectrometer) analyses, solutions containing the antimonial compounds were prepared at 0.01 mol/L of Sb and kept at 25^{o}C; spectra were registered at different time intervals. The following solutions of antimonials were used as controls. MA: solution of MA in water obtained from the dilution of a concentrated (0.7

*Electronic address: frezard@icb.ufmg.br

mol/L of Sb) MA solution. MA+CDh: pre-heated MA+β-CD obtained from the dilution in water of an equimolar MA+β-CD mixture at 0.05 mol/L Sb previously heated for 48 h at 55^{o}C under stirring.

In the photon correlation spectroscopy analysis (ZEN3500, Malvern Instruments), lyophilized samples were prepared in deionized water at the final β-CD concentration of 2.5 mmol/L and kept at 25^{o}C. The mean hydrodynamic diameter and polydispersity index were determined different times after the preparation of the solution.

In the osmometry experiment, MA and MA/β-CD were prepared at 0.04 mol/L Sb in water from the lyophilized samples; solutions were kept at 25^{o}C and osmolarity was measured immediately and at different time intervals after dissolution (freezing point osmometer, μOSMETTE Model 5004, Precision Systems Inc.).

The circular dichroism signal is given as $\Delta\varepsilon$, which is the differential molar dichroic absorption coefficient ($\Delta\varepsilon = \varepsilon_L - \varepsilon_R$ in $Lcm^{-1}\ mol^{-1}$) and is expressed in terms of the molar concentration of Sb.

Sb-meglumine complex ions were easily characterized by ESI-MS as containing Sb by the distinctive isotope pattern of Sb (ratio of ^{121}Sb:^{123}Sb, 57:43). Each species is indicated in the following with the *m*/*z*value of the first peak of its isotopic cluster.

III. RESULTS AND DISCUSSION

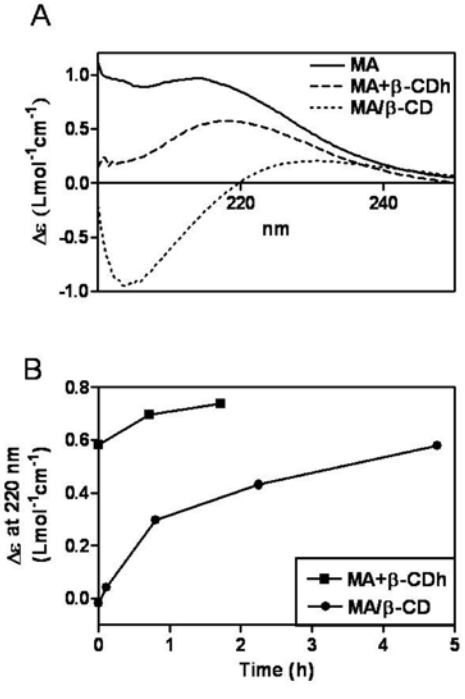

FIG. 1: A: circular dichroism spectra of different compositions of MA, just after preparation in water at 0.01 mol/L Sb at 25 ° C. B: kinetics of change of circular dichroism signal at 220 nm.

Figure 1A shows the circular dichroism spectra obtained for MA, MA+β-CDh (pre-heated MA+ β-CD) and MA/β-CD (lyophilized MA+β-CD), immediately after their preparation in water at 0.01 mol/L Sb and 25^{o}C. As reported previously [2], upon heating of MA in the presence β-CD, the circular dichroism spectrum of MA shows a decrease of its intensity and a red-shift, as a result of the formation of a ternary meglumine-Sb-β-CD complex. Strikingly, following submission of the heated MA+β-CD mixture to freeze-drying, MA spectrum suffered marked changes, with the appearance of a negative Cotton effect centered at 205 nm, indicating the occurrence of additional interactions between MA and β-CD.

When the circular dichroism spectrum of MA/β-CD composition was registered as a function of time, it was found to return slowly to a spectrum characteristic of MA (Fig. 1B), indicating that the MA/β-CD composition released MA. The kinetic was found to be biphasic with a first rapid phase and a slow late phase.

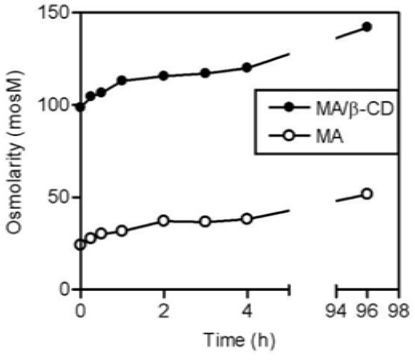

FIG. 2: Time-course of variation of the osmolarity of MA and MA/β-CD solutions at 25 ° C, just after the dissolution of lyophilized compounds in water at 0.04 mol/L of Sb.

When the osmolarity of MA and MA/β-CD solutions was measured as a function of time, just after the dissolution of these compounds in water at 0.04 mol/L of Sb, a slow increase of the osmolarity was observed in both cases (Fig. 2), indicating the occurrence of a slow dissociation of high molecular weight species into low molecular weight ones. The increase of osmolarity of MA solution as a function of time was reported previously and attributed to the hydrolysis of the more polymerized complexes into 1:1 Sb-meglumine complex [3]. Strikingly, the increase of osmolarity of MA/β-CD solution during 96 h was greater than that of MA solution (44 mosM vs 28 mosM), supporting the hypothesis that MA/β-CD complexes also slowly dissociate.

Thermogravimetry analysis of MA/β-CD composition indicated the presence of 9 water molecules per Sb atom, suggesting that β-CD did not release originally included water molecules [4] into the bulk water upon association with MA and that non-inclusion interactions are taking place.

TABLE I: Sb-β-CD complexes identified by ESI(-)-MS in the MA/β-CD composition

Sb-β-CD complexes[a]	*m*/*z*
$[\beta\text{-CD(NMG)(Sb)}_2\text{(OH)}_4 - 8H]^{2-}$	816
$[\beta\text{-CD(NMG)}_2\text{(Sb)}_2\text{(OH)}_3 - 9H]^{2-}$	904
$[\beta\text{-CD(NMG)(Sb)(OH)}_2 - 4H]_2^{2-}$	1479

Table 1 displays the main anionic species identified by ESI(-)-MS in MA/β-CD and their proposed structural formula. The formation of 1:2:1, 2:2:1 and 2:2:2 meglumine-Sb-β-CD complexes is proposed. When compared to the ternary 1:1:1 meglumine-Sb-β-CD complex previously identified [2], these new species exhibit higher molecular weight and ionization state, presumably as a result of multiple associations. Comparing the ESI(-)-MS spectra of MA/β-CD, immediately and 24 h after its dissolution in water, indicated a decrease of the relative abundance of these species, suggesting that some of these species may contribute to the enhanced absorption of Sb by oral route.

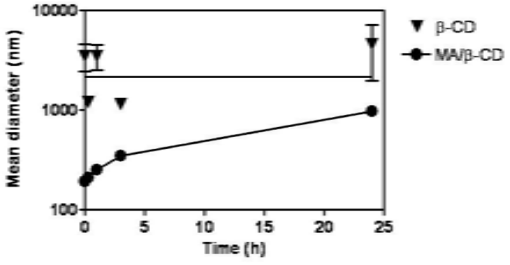

FIG. 3: Time-course of variation of the mean hydrodynamic diameter of particles after reconstitution of MA/β-CD or β-CD in water, as determined by photon correlation spectroscopy.

Figure 3 shows the time-course of variation of the mean hydrodynamic diameter of particles, as determined by photon correlation spectroscopy, after reconstitution of MA/β-CD or β-CD in deionized water at 2.5 mmol/L of β-CD.

Initially, a mean hydrodynamic diameter of 190 nm with a polydispersity index of 0.37 was measured. The mean hydrodynamic diameter was found to increase as a function of time. From 0 to 3 h, the increase in particle size was accompanied by a decrease of the particle count. At 24 h, a mean diameter of 971 nm was registered with a polydispersity index of 0.75. When β-CD alone was studied, lower particle counts were observed, mean hydrodynamic diameters higher than 1000 nm with polydispersity indexes of 1 were determined.

IV. CONCLUSIONS

The orally-active freeze-dried MA/β-CD forms nanoassemblies in water, as a result of multiple non-inclusion interactions between MA and β-CD, which behave as a sustained release system of the MA drug. These nanoassemblies most probably contribute to the enhanced antileishmanial efficacy of MA by oral route following its association with β-CD.

Acknowledgements

Research supported by the Brazilian agencies: CNPq (472032/2004-6; 307726/2006-1; 477003/2004-4), FAPEMIG (EDT1806/02; REDE 2825/05; CEX549/04; CBB1014/05; CBB 165/07).

[1] C. Demicheli, R. Ochoa, J. B. B. da Silva, A. L. de Melo, C. A. M. Falcão, B. Rossi-Bergmann, R. D. Sinisterra, and F. Frézard, Antimicrob. Agents Chemother, **48,** 100 (2004).

[2] P. S. Martins, R. Ochoa, A. M. C. Pimenta, L. A. M. Ferreira, A. L. Melo, J. B. B. Silva, R. D. Sinisterra, C. Demicheli, and F. Frézard, Int. J. Pharm. **325,** 39 (2006).

[3] F. Frézard, P. S. Martins, M. C. M. Barbosa, A. M. C. Pimenta, W. A. Ferreira, J. E. de Melo, J. B. Mangrum, and C. Demicheli, J. Inorg. Biochem. **102**, 656 (2008).

[4] K. Lindner and W. Saenger, Angew. Chem. Int. Ed. Engl. **17,** 694 (1978).