

# SOLUBILITY IMPROVEMENT OF AN ANTHELMINTIC BENZIMIDAZOLE CARBAMATE BY ASSOCIATION WITH DENDRIMERS

L. Fernández<sup>1</sup>, E. Sigal<sup>1</sup>, L. Otero<sup>2</sup>, J. J. Silber<sup>2</sup> and M. Santo<sup>1\*</sup>

<sup>1</sup>Departamento de Física, Facultad de Ciencias Exactas Físicoquímicas y Naturales, Universidad Nacional de Río Cuarto, Phone: + 54 0358 4676146, Fax: +54 0358 4676530, Río Cuarto, (X5804BYA), Argentina.  
E-mail: msanto@exa.unrc.edu.ar

<sup>2</sup>Departamento de Química, Facultad de Ciencias Exactas Físicoquímicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, (X5804BYA), Argentina.

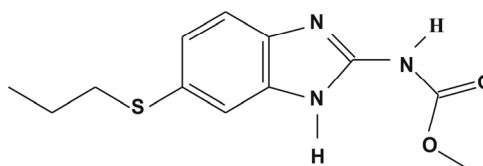
(Submitted: November 3, 2010 ; Revised: June 16, 2011 ; Accepted: June 29, 2011)

**Abstract** - The improvement of aqueous solubility of methyl (5-[propylthio]-1H-benzimidazol-2-yl) carbamate, albendazole (ABZ) using polyamidoamine (PAMAM) dendrimers as solubility enhancers was investigated. Full generation PAMAM dendrimers with amine terminal groups, (G3), with hydroxyl terminal groups (G3OH) and half generation PAMAM dendrimers with carboxylate terminal groups (G2.5 and G3.5), were chosen for this study. The nature of dendrimer-ABZ association was investigated by UV absorption, fluorescence emission measurements and by <sup>1</sup>H-NMR spectroscopy. The results obtained show that these polymeric structures have the capacity to enhance the solubility of ABZ, both lipophilic and specific hydrogen bond interactions contributing to the guest-host association. Although all studied dendrimers have hydrophobic internal nanoenvironments with similar dimensions, their surfaces differ significantly and the nature and the localization of the interactions involved in ABZ-dendrimer association depend on the type of terminal groups.

**Keywords:** Dendrimer; Solubility; Albendazole; Anthelmintic; Host-guest interaction.

## INTRODUCTION

Albendazole (ABZ), methyl (5-[propylthio]-1H-benzimidazol-2-yl) carbamate, (Scheme 1), is a broad spectrum anthelmintic agent (Casulli *et al.*, 2006), that has been successfully used in clinics for the treatment of cerebral cysticercosis, a serious public health problem in underdeveloped countries (Zongde *et al.*, 2005). Furthermore, ABZ is a potential anticancer agent that is currently under development for the treatment of cancer (Zhao *et al.*, 2008), (Mohammad *et al.* 2010) A major problem associated with the formulation and effectiveness of ABZ is its poor aqueous solubility, 0.61 µg/ml, which may account for its low and irregular bioavailability in humans (Wu *et al.*, 2005).



**Scheme 1:** Methyl (5-[propylthio]-1H-benzimidazol-2-yl) carbamate, Albendazole (ABZ)

The absorption in the intestinal tract of most orally administered drugs depends on their solubility and permeability properties (Daniel-Mwambete *et al.*, 2004). The solubility can be modified by drug complexation with different excipients and, through this process, the oral bioavailability of drugs with a low aqueous solubility can be greatly improved.

\*To whom correspondence should be addressed

Previous studies showed that complexation with povidone and cyclodextrins increases the solubility of ABZ, improving its bioavailability (Moriwaki *et al.*, 2008; Pacioni *et al.*, 2008).

In the last two decades, a novel polymeric nanoarchitecture for solubility enhancement has attracted the attention of many scientists. These compounds, named dendrimers, have been tried successfully for enhancing the solubility of hydrophobic compounds. Dendrimers with a hydrophobic core and hydrophilic periphery have been shown to exhibit micelle-like behavior and to have container properties in solution (Svenson, 2009).

Dendrimers are highly branched and mono-disperse macromolecules with symmetrical architecture (Meredith and Grinstaff, 2011). They consist of a central core, branching units and terminal functional groups. This type of architecture induces the formation of a nano-environment, which determines its solubilizing or encapsulating properties; while the external groups primarily characterize the solubility and chemical behavior (Jang *et al.*, 2009; Astruc *et al.*, 2010). Neutral and negatively charged dendrimers do not interact with biological environments and hence are compatible for clinical applications, as elucidated by various studies reviewed by Jain *et al.* (2010).

The objective of this work is to examine the possible improvement of the aqueous solubility of ABZ using ethylenediamine core polyamidoamine (PAMAM) dendrimers as solubility enhancers. The use of dendrimers for solubility enhancement has been studied extensively (Wang *et al.*, 2008). Namazi and Adeli (2005) used a new type of dendrimer with poly(ethylene glycol) as core and citric acid as the branching unit for the solubility enhancement of mefenamic acid and Diclofenac. Also, Generation 4 PAMAM dendrimers have been studied to enhance risperidone solubility. Temperature, pH and ionic strength conditions are evaluated in order to improve drug-dendrimer complexation (Prieto *et al.*, 2011).

Over the last several years, substantial progress has been made towards the therapeutic applications of dendrimers for cancer treatment (Wolinsky and Grinstaff, 2008); diverse dendrimers were used to increase the solubility of anticancer drugs, as well as to reduce drug toxicity. Recently, Hai *et al.* (2011) have reported a PEGylated fourth generation PAMAM dendrimer as nanocarrier of the anticancer drug doxorubicin, encapsulated in the interior of PAMAM molecules; this study provides a potential application of this dendrimeric system for current brain cancer therapy.

Our previous studies using functionalized polyamide amine dendrimers were focused on the analysis of the interactions between dendrimers and several biologically important guests (Santo and Fox, 1999). We analyzed the solubilization and release of different hydrophobic compounds by interaction with polyamidoamine and polypropylenimine dendrimers (Fernández *et al.*, 2006). Moreover, we investigated a first generation of a new dendrimer as candidate for intravenous administration of an antichagasic compound. The results obtained show that guest-host specific interactions result in good drug solubilization and that they can be controlled by varying the solution pH, allowing drug delivery (Fernández *et al.*, 2008).

In the present work, the effect of several PAMAM dendrimers on the aqueous solubility of the hydrophobic drug ABZ was studied. Full generation PAMAM dendrimers with amine terminal groups, G3, with hydroxyl terminal groups, G3OH, and half generation PAMAM dendrimers with carboxylate terminal groups, generation G2.5 and generation G3.5, were chosen for this study (Scheme 2). The influence of the different terminal groups on ABZ-dendrimer association was analyzed.

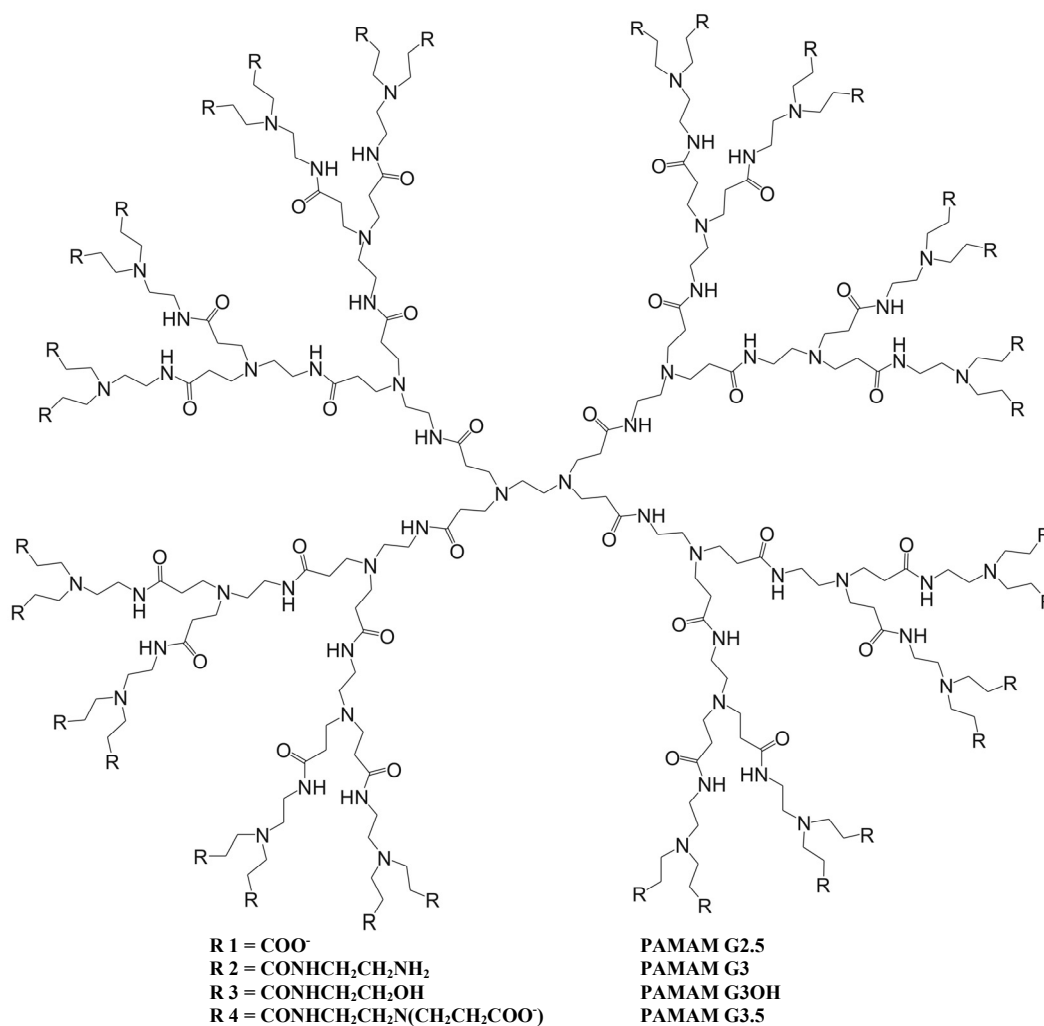
## MATERIALS AND METHODS

### Materials

PAMAM G3, G3OH, G2.5 and G3.5 dendrimers in methanol solution and ABZ were obtained from Sigma-Aldrich. The organic solvents (methanol, dimethylformamide, toluene, benzene, dimethyl sulfoxide) and water of HPLC quality were purchased from SINTORGAN and were used without further purification. The UV cut-off point of the solvents in a UV cell of 10 mm against air was used as the purity criterion.

### Methods

UV visible spectroscopic measurements were performed using a Shimadzu U.V.2401PC spectrophotometer at  $20.0 \pm 0.2^\circ\text{C}$ . A Spex Fluoromax apparatus was employed for the fluorescence measurements at the same temperature. Dendrimer/ ABZ fluorescence spectra were taken using an excitation wavelength of  $\lambda=295\text{nm}$ , and the emission registered in the 300-500 nm range. NMR data were recorded using a Bruker 400 MHz Advance II. Semiempirical calculations were carried out using the HyperChem software, version 5.0, running on a Pentium III personal computer.



Scheme 2: PAMAM dendrimer structures

## EXPERIMENTAL

### UV Absorption Spectroscopy

The electronic absorption spectra of ABZ were recorded in solvents of different polarity (methanol, dimethylformamide, toluene, benzene, dimethyl sulfoxide and water). The stock solution of ABZ was prepared by dissolving the solute in methanol at  $1.0 \times 10^{-3}$  M. Appropriate aliquots of the solute stock solution were transferred into 5 mL volumetric flasks and the methanol evaporated off under a nitrogen atmosphere. The samples were then diluted to the final volume with the corresponding solvent to roughly  $5.0 \times 10^{-5}$  M, except for water (less than  $1.0 \times 10^{-6}$  M), sonicated for 10 minutes, and stored at room temperature in darkness until further use.

### Solubilization Enhancement of ABZ

Stock solutions of ABZ were prepared by dissolving the guest in methanol at  $1.0 \times 10^{-3}$  M and stored in darkness. Appropriate aliquots of the solute stock solutions were transferred into 5 mL volumetric flasks and the solvent evaporated off under a nitrogen atmosphere. The samples were diluted to the appropriate volume with HPLC-grade water, sonicated for 10 minutes, and stored at room temperature in darkness. To perform a calibration graph, the absorbance corresponding to the  $\lambda_{\max}$ , for solutions with different concentrations, was determined. Subsequently, a saturated solution of ABZ in HPLC-grade water was prepared and the absorbance corresponding to the  $\lambda_{\max}$  for the

saturated solution was determined to calculate the water solubility of ABZ.

Stock solutions of ABZ were prepared by dissolving the guest in methanol at  $1.0 \times 10^{-3}$  M and stored in darkness. Then appropriate amounts of PAMAM dendrimers in methanol and ABZ stock solution were transferred into 5 mL volumetric flasks, the samples were diluted with 1 mL of methanol, sonicated for 20 min and allowed to equilibrate in darkness overnight. Afterwards, the solvent was evaporated off under nitrogen atmosphere and the samples were diluted to the appropriate volume with HPLC-grade water. A small amount of drug precipitated from solution and was removed via filtration through a  $0.46 \mu\text{m}$  Millipore membrane. Samples for NMR analysis were prepared using deuterated solvent ( $\text{D}_2\text{O}$ ).

## RESULTS AND DISCUSSION

### Solubility Enhancement of ABZ

The capacity of PAMAM dendrimers to enhance the solubility of ABZ was studied by spectroscopic methods for different ABZ-dendrimer systems in aqueous solution using full G3 and G3OH and half generation G2.5 and G3.5 PAMAM dendrimers.

### UV Absorption Spectroscopy

The spectra of ABZ at different concentrations of aqueous G3 dendrimer are shown in Figure 1. The spectrum of ABZ in water is also included for reference. The G3 dendrimer concentration was varied from  $5.2 \times 10^{-6}$  M to  $1.1 \times 10^{-4}$  M. ABZ was added in excess of its aqueous solubility limit,  $2.4 \times 10^{-6}$  M, so that the solubility enhancement could be observed. The analytical concentration of ABZ was  $1.0 \times 10^{-4}$  M, the real ABZ concentrations may be calculated depending on the extent of solubility enhancement in the presence of different dendrimer concentrations.

The observed increase in the absorbance, due to ABZ solubilization enhancement, is attributed to its association with the dendrimer. As expected,

upon increasing the dendrimer concentration the solubilization increases (Figure 1). In addition, the ABZ absorption band, observed in pure water at 298.9 nm, shifts to lower wavelength with the increase in dendrimer concentration. To analyze the observed spectral shift, the electronic absorption spectra of ABZ were recorded in solvents of different polarity. The experimental  $\lambda_{\text{max}}$  shifted from 298.5 nm and 297.5 nm in polar solvents such as dimethyl sulfoxide and dimethylformamide, respectively, to 295.2 nm in nonpolar solvents like benzene and toluene. Thus, there is a blue shift with decreasing solvent polarity. Consequently the shift observed in the absorption band of ABZ with the increase of dendrimer concentration indicates a decrease in microenvironment polarity around ABZ. These results could indicate that ABZ solubility increases due to its association with lipophilic microenvironments defined within the dendrimeric structure. Similar results have been obtained using hydroxyl terminal dendrimers, G3OH.

The absorption spectra of ABZ in aqueous solutions of the PAMAM carboxylate terminal dendrimers G2.5 and G3.5 and the PAMAM hydroxyl terminal dendrimer G3OH also show that dendrimeric solutions improve drug solubility with respect to aqueous medium. The maximum solubility enhancement obtained for each dendrimer is listed in Table 1.

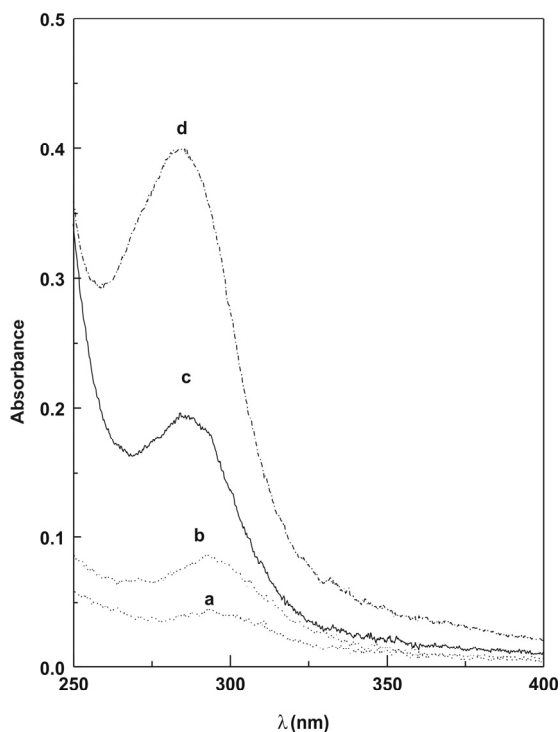
The study of different ABZ-dendrimer systems at equal concentration of the dendrimers indicated that the increase in solubility depends on both the size of the dendrimers and the type of end groups (Figure 2). In cationic (amine terminated) and neutral (hydroxyl terminated) dendrimers, the ABZ absorption band shifted to a wavelength lower than that observed in water, indicating that ABZ is surrounded by a lipophilic environment. The association may be due to the incorporation of ABZ in the hydrophobic microenvironment of dendrimers. In aqueous solutions of anionic half generation dendrimers, the ABZ band shifted to higher wavelength, indicating that ABZ is surrounded by a more polar environment. In these host-guest systems, ABZ may be preferentially located close to the terminal groups of the dendrimeric structures.

**Table 1: Characterization of the PAMAM dendrimers used and their solubility enhancement capacity.**

PAMAM Dendrimer	Terminal group	Surface charge <sup>b</sup>	Molecular weight (g/mol)	Solubility enhancement
G3	32 NH <sub>2</sub>	Cationic	6909	10 S <sub>w</sub> <sup>a</sup>
G3 OH	32 OH	Neutral	6941	5 S <sub>w</sub> <sup>a</sup>
G2.5	32COOH	Anionic	6267	7 S <sub>w</sub> <sup>a</sup>
G3.5	64COOH	Anionic	12931	4 S <sub>w</sub> <sup>a</sup>

<sup>a</sup> S<sub>w</sub> =  $2 \times 10^{-6}$  M Water solubility.

<sup>b</sup> from (Klajnert, 2003)

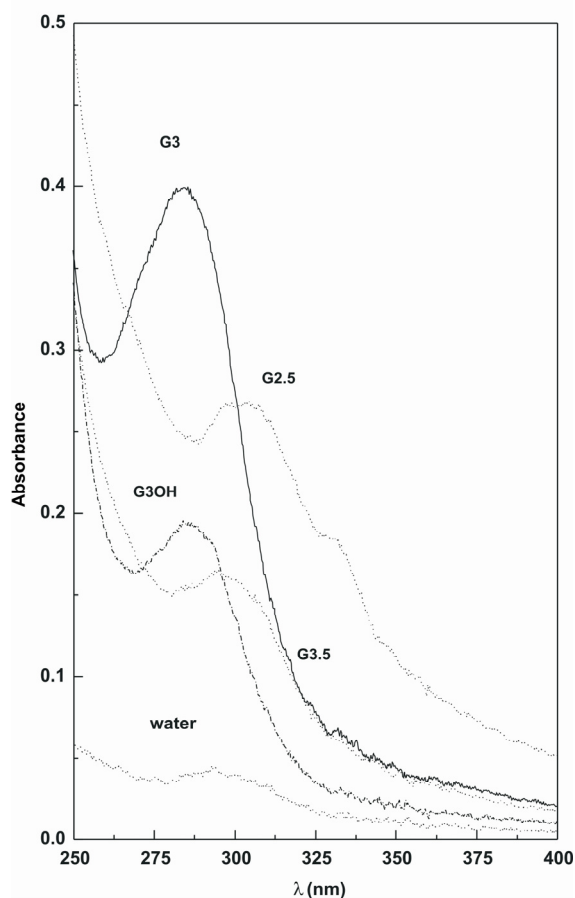


**Figure 1:** Absorption spectra of ABZ in water (a) and in aqueous solutions with different concentrations of G3 dendrimer: (b)  $1.0 \times 10^{-5}$  M, (c)  $5.2 \times 10^{-5}$  M and (d)  $1.1 \times 10^{-4}$  M.

The increase in solubility was higher in G3 than in G2.5 and G3.5 PAMAM dendrimers. Dendrimer-mediated solubility enhancement depends mainly on factors such as generation size, dendrimer concentration, core, and terminal functionality (Gupta *et al.*, 2006). In this system, we assume that the low encapsulation efficiency of half generation PAMAM dendrimers is a consequence of their anionic character.

### Fluorescence Emission Spectroscopy

ABZ has a single broad fluorescence emission band in aqueous solution. Representative fluorescence spectra are shown in Figure 3. The fluorescence intensity with respect to pure water increases significantly with an augment in dendrimer



**Figure 2:** Absorption spectra of ABZ in water and in aqueous solutions with all of the PAMAM dendrimers studied at  $[\text{dendrimer}] = 1.0 \times 10^{-4}$  M.

concentration, indicating an enhancement in ABZ solubility.

As can be observed in Figure 3, the emission intensity of ABZ in G3 is lower than that observed for G2.5 and G3.5, although the solubility enhancement of ABZ is higher using G3 dendrimers. The low intensity observed for the emission of ABZ in G3 dendrimers can be explained by considering the quenching capacity of tertiary amines present at the branching points in PAMAM dendrimers where the guest should be localized (Wade *et al.*, 1999).

This result reinforces the assumption that the association between ABZ and full generation dendrimers G3 and G3OH occurs preferably in the interior of the dendrimeric structure, where the emission intensity could be quenched by the tertiary amines. Moreover, the association of ABZ with half

generation dendrimers (G2.5 and G3.5) occurs preferably in the proximity of the carboxylate surface groups. The terminal groups of G2.5 and G3.5 dendrimers are unable to quench ABZ emission, so the contribution to the emission intensity is greater.

### <sup>1</sup>H NMR Studies

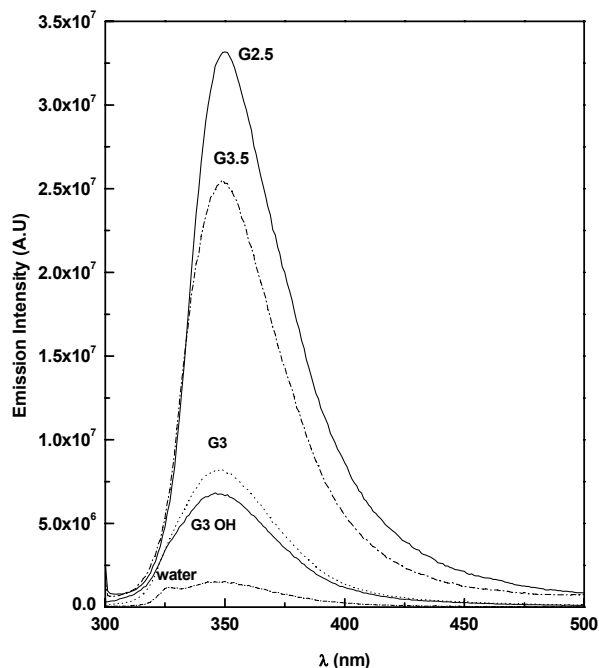
NMR spectroscopy is a very useful technique to investigate intermolecular interactions in solutions because it gives information on the formation of aggregates, ion pairing, encapsulation, and size variations (Biosselier et al., 2008). The <sup>1</sup>H-NMR technique was employed here in order to analyze further the proposed molecular interaction between ABZ and dendrimers. Among the different dendrimers studied, G3 was chosen for the NMR study because of its high solubilization effect on ABZ.

Five broad peaks are observed in the <sup>1</sup>H-NMR spectrum of a G3 dendrimer in D<sub>2</sub>O, corresponding to: CONHCH<sub>2</sub> indicated as H<sub>a</sub>, CONHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, H<sub>b</sub>, NCH<sub>2</sub>CH<sub>2</sub>CONH, H<sub>c</sub>, CH<sub>2</sub>CH<sub>2</sub>NR<sub>2</sub>, H<sub>d</sub>, CH<sub>2</sub>CONH, H<sub>e</sub>. Scheme 3 (δ ppm) H<sub>a</sub>=3.11; H<sub>b</sub>=2.65; H<sub>c</sub>=2.59; H<sub>d</sub>=2.48; H<sub>e</sub>= 2.29. The <sup>1</sup>H-NMR spectrum of ABZ in D<sub>2</sub>O shows seven kinds of protons (δ ppm) at 1.19 (t,3H), 1.86 (sext, 2H), 3.30 (m,2H), 4.1 (s,3H),

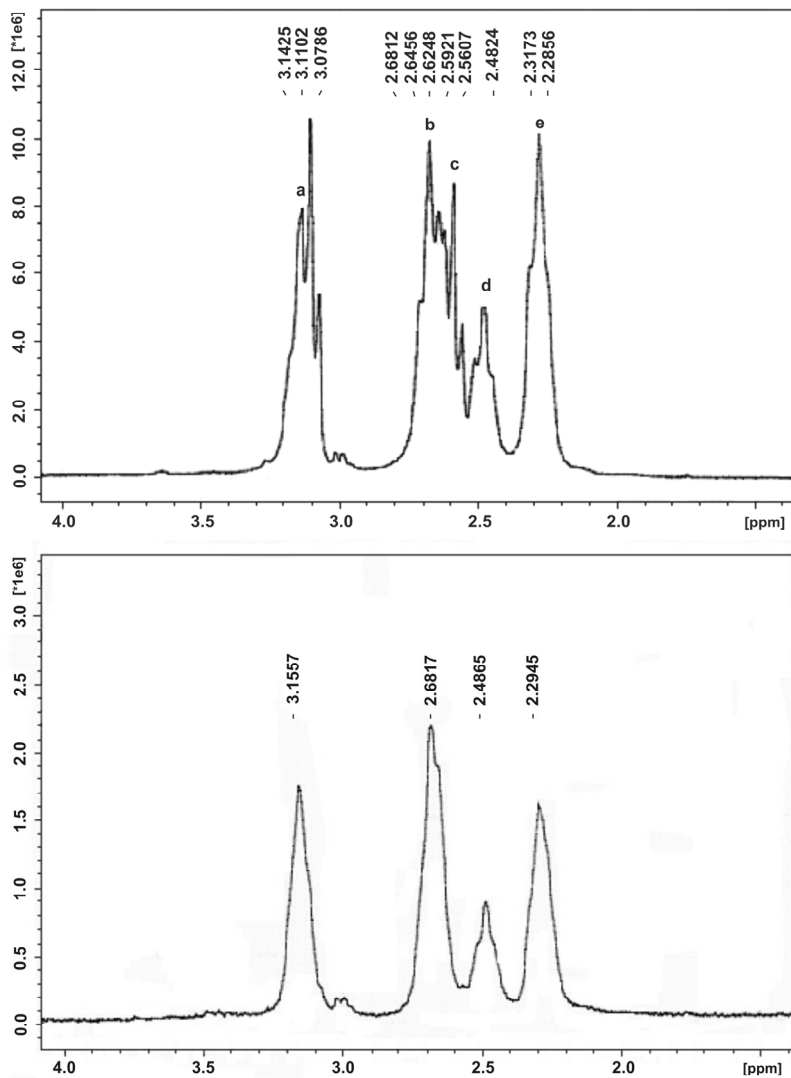
7.92 (dd,1H), 8.01(d,1H), 8.32(d,1H).

The NMR study of the host-guest system was performed using similar drug and dendrimer concentration. However, the signal strength of the protons of ABZ is much lower than that observed for the different protons of dendrimers, as the contribution of the number of protons per signal is higher in the latter. Therefore, the ABZ signals do not interfere in the analysis of the signal shifts in the dendrimer NMR spectrum.

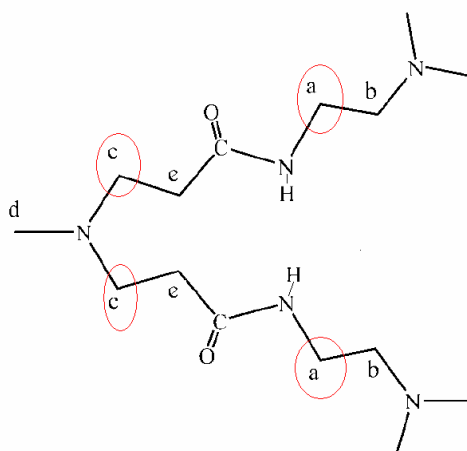
The <sup>1</sup>H-NMR spectra obtained are shown in Figure 4. Changes in the proton peaks of the dendrimer induced by the incorporation of ABZ in the aqueous dendrimeric solutions were observed. Band broadening and chemical shifts are good indicators of ABZ-dendrimer complex formation. When the guest is added to the dendrimer solution, the H<sub>c</sub> signal shifts downfield from 2.59 to 2.68 ppm. This is probably due to ring current effects produced by the aromatic ring of ABZ when this compound associates with the internal groups of the dendrimer (Scheme 3). No shift is observed for protons near the end groups (H<sub>b</sub>). These results indicate that there are interactions with the internal nanoenvironment of the dendrimers, but that the ABZ-G3 interactions with surface groups, if any, are very weak.



**Figure 3:** Fluorescence emission spectra of ABZ in water and in aqueous solutions of all of the PAMAM dendrimers studied ( $\lambda_{exc} = 295$  nm)



**Figure 4:** Expanded region of  $^1\text{H-NMR}$  spectra of G3 dendrimer/ $\text{D}_2\text{O}$  solution (top) and G3 dendrimer/ABZ/ $\text{D}_2\text{O}$  solution (bottom). For assignments see text.



**Scheme 3:** Chemical Structure and Atom Labeling in the G3 PAMAM Dendrimer interior repetitive unit.

### Possible Mechanism of Interaction Between PAMAM Dendrimers and ABZ

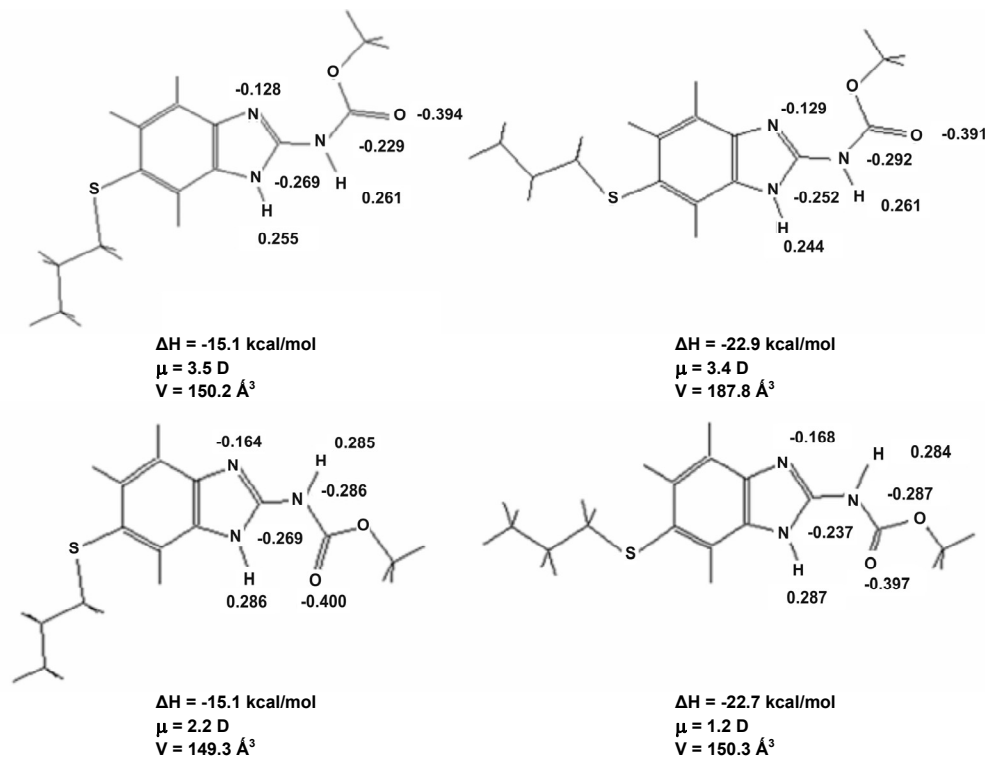
The structure of dendrimers depends on their generation. Lower generations of dendrimers have an asymmetric shape and open structure, whereas the higher generations possess a nearly globular shape and densely packed end groups. In our studies, we chose to investigate third generation dendrimers which are almost spherical but have substantial flexibility. Molecular simulation of the structures of the PAMAM dendrimers showed that lower generation ones ( $G < 4$ ) possess an open structure and are well below De Gennes dense-packing transitions and, consequently, are accessible for guest inclusion (Meredith and Grinstaff, 2011; Fréchet and Tomalia, 2001).

Several studies suggest that ionic interactions, hydrogen bonding and hydrophobic drug-dendrimer interactions are the mechanisms by which dendrimers exert their solubilizing effect (Gupta *et al.*, 2006). PAMAM dendrimers have primary amines on the surface and amide and tertiary amines in their interior, which could act as hydrogen bond donors and acceptors. It is clear from its structure that ABZ also has hydrogen bond donor and acceptor groups. So the formation of an intramolecular hydrogen bond between the carbonyl of the carbamoyl moiety and the proton of the amine group

on the aromatic ring of ABZ is possible.

The predominant conformers and charge density distributions were predicted from theoretical calculations performed by semi-empirical molecular orbital methods AM1 (Dewar *et al.*, 1985). Calculations were performed starting from standard bond lengths and bond angles. All geometries were fully optimized by minimizing the energy with respect to the geometrical variables without symmetry constraint, using a gradient of 0.01 kcal/mol and the Polak–Ribierie algorithm as convergence criteria. This semi-empirical method does not describe the solvating processes, but it may provide some information about the interaction sites and the bond distances and changes in the charge distribution of the different atoms present in the main structure.

To suggest a possible localization for the hydrogen-bond interactions, the calculated charge density distributions for different atoms of the active molecule were analyzed. (Scheme 4). A negative charge distribution is localized on the nitrogen of the aromatic ring, the carbonyl group of the carbamoyl moiety and the ester oxygen. All these groups are characterized as good hydrogen bond acceptors (Etter, 1990). Furthermore, a positive charge distribution localized on the proton of the amide group and the proton of the amine group on the aromatic ring shows that both protons could participate in hydrogen bonding interactions.



**Scheme 4:** Charge distribution, calculated enthalpy of formation,  $\Delta H$ , dipole moment,  $\mu$ , and molecular volume,  $V$ , of the predominant ABZ conformers, calculated using semiempirical AM1 method.



From the analysis of the possible conformers obtained, Scheme 4, we can conclude that both structures, with and without intramolecular hydrogen bonding, are possible and the contribution of the different structures will depend on the interactions of ABZ with its environment. It is possible that the intramolecular hydrogen bonding prevents stronger interaction with the environment. Moreover, the amide proton is a hydrogen bond donor site available in both conformations.

In summary, from these results it can be inferred that ABZ could participate in both inter- and intramolecular hydrogen bonding. Therefore, hydrogen bonding between ABZ and PAMAM dendrimers could contribute to facilitate the association in this host-guest system and be a possible mechanism contributing to the solubility enhancement.

In addition to hydrogen bonding, the solubilization properties of dendrimers could also be due to the hydrophobicity of their microenvironment. Previous studies have shown that the internal microenvironment in the dendrimeric structure is less polar than the bulk aqueous phase (Fernandez *et al.*, 2006). The hydrophobic guest isolates itself from the outer interface of the host to afford minimum contact with polar and aqueous domains. The storage space of the dendrimers is controlled by geometrical parameters of the branch cell (branching angles, rotation angles and repeat-unit segment length) and by the shape and size of the available internal dendrimer microenvironment that influences the host-guest interactions (Tomalia *et al.*, 2003). The maximum amount of entrapped guest molecules is proportional to the shape and size of the guest molecules. The molecular volume of ABZ (Scheme 4) allows accessibility into the dendrimer. Hence, in aqueous medium, highly hydrophobic ABZ could be solubilized within low polarity environments of the dendrimer. As observed in the spectroscopic studies, a decrease in the polarity of the environment causes a bathochromic shift in the absorption maximum of ABZ. The gradual blue shift of the ABZ band with the increase of dendrimer concentration with respect to pure water indicated a decrease of the microenvironment polarity around ABZ. Therefore, hydrophobic interactions help the incorporation of guests into the lipophilic interior of dendrimers and ABZ-dendrimer hydrogen bonding stabilizes the association of this host-guest system.

## CONCLUSION

In the present work, we investigated different PAMAM dendrimers as solubility enhancers of

ABZ. The results obtained show that these polymeric structures have the capacity to increase the solubility of poorly water soluble drugs such as ABZ. The association of the hydrophobic compound and the aliphatic dendrimer can be rationalized by the poor water solubility of the guest and by the affinity between ABZ and the lipophilic microenvironments of the dendrimeric structures. From our results, we can conclude that both lipophilic and specific hydrogen bond interactions contribute to the ABZ-dendrimer association, enhancing its solubility. The difference in solubility enhancement found with the different dendrimers studied could be attributed to the nature of the ABZ-dendrimer interactions, which depend on the identity of the surface functional groups.

## ACKNOWLEDGEMENTS

Financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT-Argentina), and the Secretaría de Ciencia y Técnica de la Universidad Nacional de Río Cuarto (SECYT-UNRC) is gratefully acknowledged.

## NOMENCLATURE

ABZ	Albendazole
PAMAM	Polyamidoamine dendrimer
G2.5	Half generation 2.5 polyamidoamine dendrimer with carboxylate terminal groups
G3	Full generation 3 polyamidoamine dendrimer with amine terminal groups
G3.5	Half generation 3.5 polyamidoamine dendrimer with carboxylate terminal groups
G3OH	Full generation 3 polyamidoamine dendrimer with hydroxyl terminal groups
<sup>1</sup> H-NMR	Nuclear Magnetic Resonance of protons
UV	Ultraviolet-visible

## REFERENCES

Astruc, D., Biosselier, E. Ornelas, C., Dendrimers designed for functions: from physical, photophysical and supramolecular properties to

- applications in sensing, catalysis, molecular electronic, photonics and nanomedicine. *Chemical Reviews*, 110, 1857-1959 (2010).
- Biosselier, E., Ornelas, C., Pianet, I., Aranzaes, J. R., Astruc, D., Four generations of water-soluble dendrimers with 9 to 243 benzoate tethers: Synthesis and dendritic effects on their ion pairing with acetylcholine, benzyltriethylammonium, and dopamine in water. *Chemistry-a European Journal*, 14, (18), 5577-5587 (2008).
- Casulli, A., Gomez Morales, M. A., Gallinella, B., Turchetto, L. and Pozio, E., 2-Hydroxypropyl- $\beta$ -Cyclodextrin improves the effectiveness of albendazole against encapsulated larvae of *Trichinella spiralis* in a murine model. *Journal of Antimicrobial Chemotherapy*, 58, 886-890 (2006).
- Daniel-Mwambete, K., Torrado, S., Cuesta-Bandera, C., Ponce-Gordo, F., Torrado, J. J., The effect of solubilization on the oral bioavailability of three benzimidazole carbamate drugs. *International Journal of Pharmaceutics*, 272, 29-36 (2004).
- Devarakonda, B., Hill, R., Villiers, M., The effect of PAMAM dendrimer generation size and surface functional group on the aqueous solubility of nifedipine. *International Journal of Pharmaceutics*, 284, 133-140 (2004).
- Dewar, M. J. S., Zoebisch, E. G., Healvy, A. F., Stewart, J. J. P., AM1: A new general purpose quantum mechanical molecular model. *Journal of the American Chemical Society*, 107, 3902-3909 (1985).
- Etter, M., Encoding and decoding hydrogen-bond patterns of organic compounds. *Accounts of Chemical Research*, 23, 120-126 (1990).
- Fernández, L., Calderón, M., Marinelli, M., Strumia, M., Cerecetto, H., Gonzalez, M., Silber, J. J., Santo, M., Evaluation of a new dendrimeric structure as prospective drugs carrier for intravenous administration of antichagasic active compounds. *Journal of Physical Organic Chemistry*, 21, 1079-1085 (2008).
- Fernández, L., González, M., Cerecetto, H., Santo, M., Silber, J. J., Solubilization and release properties of dendrimers. evaluation as prospective drug delivery systems. *Supramolecular Chemistry*, 18, (8), 633-643 (2006).
- Fréchet, J. M. J., Tomalia, D. A., *Dendrimers and other dendritic polymers*, First Eds., Wiley Series in Polymer Science, Wiley, Chichester, England, (2001).
- Gupta, U., Agashe, H., Jain, N., Dendrimers: Novel polymeric nanoarchitectures for solubility enhancement. *Biomacromolecules*, 7, 649-658 (2006).
- Hai He, Yan Li, Xin-Ru Jia, Ju Du, Xue Ying, Wan-Liang Lu, Jin-Ning Lou, Yan Wei PEGylated poly(amidoamine) dendrimer-based dual-targeting carrier for treating brain tumors. *Biomaterials*, 32, 478-487 (2011)
- Jain, K., Kesharwani, P., Gupta, U., Jain, N. K., Dendrimer toxicity: Let's meet the challenge. *International Journal of Pharmaceutics*, 399, 122-142 (2010).
- Jang, W., Selim, K. M., Lee, C., Kang, I. K., Bioinspired application of dendrimers: From biomimicry to biomedical applications. *Progress in Polymer Science*, 34, 1-23 (2009).
- Klajnert, B., Stanislawska, L., Bryszewska, M., Palecz, B., Interactions between PAMAM dendrimers and bovine serum albumin. *Biochimica et Biophysica Acta - Proteins and Proteomics*, 1648, 115-126 (2003).
- Mintzer, M. A. and Grinstaff, M. W., Biomedical applications of dendrimers: a tutorial, *Chem. Soc. Rev.*, 40, 173-190 (2011).
- Pourgholami, M. H., Szwajcer, M., Chin, M., Liauw, W., Seef, J., Galettis, P., Morris, D. L., Links, M., Phase I clinical trial to determine maximum tolerated dose of oral albendazole in patients with advanced cancer. *Cancer Chemother. Pharmacol.*, 65, 597-605 (2010).
- Moriwaki, C., Costa, G. L., Ferracini, C., Moraes, F., Zanin, G., Pineda, E., Matioli, G., Enhancement of solubility of albendazole by complexation with  $\beta$ -cyclodextrin. *Brazilian Journal of Chemical Engineering*, 25, 255-267 (2008).
- Namazi, H., Adeli, M., Dendrimers of citric acid and poly (ethylene glycol) as a new drug-delivery agents. *Biomaterials*, 26, 1175-1183 (2005).
- Neerman, M., Chen, H., Parrish, A., Simanek, E., Reduction of drug toxicity using dendrimers based on melamine. *Molecular Pharmaceutics*, 1, 390-393 (2004).
- Pacioni, N., Sueldo Ocello, V., Lazzarotto, M., Veglia, A., Spectrofluorimetric determination of benzoimidazolic pesticides: Effect of p-sulfonatocalix[6]arene and cyclodextrins. *Analytica Chimica Acta*, 624, 133-140 (2008).
- Prieto, M. J., Temprana, C. F., Del Río Zabala, N. E., Marotta, C. H., Del Valle Alonso S., Optimization and in vitro toxicity evaluation of G4 PAMAM dendrimer-risperidone complexes. *European Journal of Medicinal Chemistry*, 46, 845-850 (2011).
- Santo, M., Fox, M. A., Hydrogen bonding interactions between Starburst dendrimers and several molecules of biological interest. *Journal of Physical Organic Chemistry*, 12, 293-307 (1999).

- Svenson, S., Dendrimers as versatile platform in drug delivery applications. *European Journal of Pharmaceutics and Biopharmaceutics*, 71, 445-462 (2009).
- Tomalia, D. A., Huang, B., Swanson, D. R., Brothers, II, H. M., Klimash, J. W., Structure control within poly(amidoamine) dendrimers: Size, shape and regio-chemical mimicry of globular proteins. *Tetrahedron*, 59, 3799-3813 (2003).
- Wade, D. A., Torres, P. A., Tucker, S. A., Spectrochemical investigations in dendritic media: evaluation of nitromethane as selective fluorescence quenching agent in aqueous carboxylate-terminated polyamido amine (PAMAM) dendrimers. *Analytica Chimica Acta*, 397, 17-31 (1999).
- Wang, F., Bronich, T. K., Kabanov, A. V., Rauh, R. D., Roovers, J., Synthesis and characterization of star poly( $\epsilon$ -caprolactone)-b-poly(ethylene glycol) and poly(L-lactide)-b-poly(ethylene glycol) copolymers: Evaluation as drug delivery carriers. *Bioconjugate Chemistry*, 19, 1423-1429 (2008).
- Wolinsky, J. B., Grinstaff, M. W., Therapeutic and diagnostic applications of dendrimers for cancer treatment. *Advanced Drug Delivery Reviews*, 60, 1037-1055 (2008).
- Wu, Z., Medlicott, N., Razzak, M., Tucker, I., Development and optimization of a rapid HPLC method for analysis of ricobendazole and albendazole sulfone in sheep plasma. *Journal of Pharmaceutical and Biomedical Analysis*, 39, 225-232 (2005).
- Zhao, Y., Buck, D. P., Morris, D. L., Pourgholami, M. H., Day, A. I., Collins, J. G., Solubilization and cytotoxicity of albendazole encapsulated in cucurbit[n]uril. *Organic & Biomolecular Chemistry*, 6, 4509-4515 (2008).
- Zongde, Z., Xingping, L., Xiaomei, W., Hong, Z., Yamping, S., Liren, Ch., Yongmin, L., Analytical and preparative resolution of enantiomers of albendazole sulfoxide by HPLC on amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phases. *Journal of Biochemical and Biophysical Methods*, 62, 69-79 (2005).