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The effect of prolonged incubation time on the interaction between PAMAM dendrimers and iminodiacetic acid derivatives

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ABSTRACT

Dendrimers, highly branched spherical polymers, are known for their ability to act as solubility enhancers of various types of drugs. In the present study, we investigated the potential of poly(amidoamine) (PAMAM) dendrimers G1 – G4 in concentration from 0 to 10 mg/mL for solubility of two iminodiacetic acid derivatives after prolonged (72 hour) incubation time. The experiments were conducted by means of the equilibrium solubility method which is based on the spectrophotometric measurements of compounds' absorbance solubilized in the presence of PAMAM dendrimers. We reported that PAMAM dendrimers contribute to significant solubility enhancement of tested compounds. This effect is concentration- and generation- dependent. The results of solubility studies after 72 h incubation time suggest that the interactions between iminodiacetic acid derivatives and PAMAM dendrimers, mainly electrostatic and hydrophobic, are stable in aqueous environment.

Keywords: PAMAM dendrimers, solubility studies, iminodiacetic acid, electrostatic interactions

INTRODUCTION

Dendrimers, a relatively new class of chemical compounds, are complex, multi-branched, three-dimensional polymers, which possess, in comparison to traditional linear polymers, well-defined chemical structure [8]. In a structure of typical dendrimer we distinguish three elements: an initiator core, interior layers known as generations which are built of repeating units, attached to the initiator core and multiple peripheral functional groups [8,13]. PAMAM dendrimers are the first dendrimers which were synthesized [10]. This dendrimer family receives widespread attention throughout the world and has been under the most active investigation.

Over the past decade interest in utilization of host-guest properties of dendritic polymers in the field of drug delivery has increased. Interactions between dendrimers and biologically active molecules might be based on three various mechanisms. Firstly, encapsulation of drugs molecules into dendrimers might be based on hydrogen bonding interaction. Fox and co-workers showed that lower generation PAMAM dendrimers incorporate guest molecules at external (surface amino groups) and internal (interior amido groups) coordination sites [15,16]. Interaction between biologically active compounds and dendrimers may also be a consequence of electrostatic interactions which may occur between peripheral amine groups of PAMAM dendrimers and acidic, water insoluble molecules such as benzoic acid and salicylic acid [13]. The last way of utilization of dendrimers as drug delivery vehicles is covalent conjugation of drugs molecules to their surface groups [16].

Apart from utilization of dendritic polymers in the field of drug delivery systems these multi-branched molecules attract the attention of scientists as solubility enhancers of various types of drugs. There are numerous studies confirming that dendrimers are capable of binding and solubilizing small acidic molecules with low water solubility [7,12]. PAMAM dendrimers with amine-terminated surface groups might be potential carriers for NSAIDs (non-steroidal anti-inflammatory drugs) which possess carboxyl groups. There are several NSAIDs which have been successfully encapsulated into or complexed with PAMAM dendrimers (e.g. aspirin, indomethacin, flurbiprofen, ketoprofen, ibuprofen, diclofenac and naproxen). These interactions have led to the increased solubility of NSAIDs [3,6]. There have been many researchers who describe the possibility to enclose within the dendrimers structure not only drug molecules, but also genetic materials, targeting agents, dyes either by encapsulation, complexation, or conjugation.

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The aim of this study was to determine effect of PAMAM dendrimers generation 1 - 4 on solubility of two iminodiacetic acid derivatives: N-(4-methylacetanilide) iminodiacetic acid (1) and N-(2,4-dimethylacetanilide)iminodiacetic acid (2) after prolonged incubation time (72 h). Analogues of iminodiacetic acid complexed with gadolinium are contrasting compounds characterized by high affinity to liver cells and enable performing high-resolution imaging of this organ applying the magnetic resonance imaging (MRI). MRI, a widely used, non-invasive diagnostic tool, allows visualizing detailed internal structure (three-dimensional images) and limited function of the human body. Commercially available MRI contrast agents such as Magnevist (Gd(III)-diethylenetriaminepentaacetic acid) and Dotaren (Gd(III)- N,N', N'',N'''– tetracarboxymethyl-1,4,7,10-tetraazacyclododecane), are characterized by short circulation times, inefficient differentiation between normal and diseased tissues, and do not target specific organs or regions of the body [14]. Therefore, it is of vital importance to synthesize novel MRI contrast agents with improved contrast enhancement. It might be achieved by conjugation of gadolinium-based contrast agents with polymers such as poly(amino acids), polysaccharides, proteins and dendrimers [9]. Thus, within this study, we present the development of the theme concerning interactions between iminodiacetic acid derivatives and PAMAM dendrimers and their stability.

MATERIALS AND METHODS

Materials and synthesis of derivatives of N-(2-phenylamine- 2- oxoethyl)- iminodiacetic acid

PAMAM dendrimers generation 1-4 and substrates for synthesis of iminodiacetic acid derivatives such as nitrilotriacetic acid, 4-methylaniline, 2,4-dimethylaniline were purchased from Sigma Aldrich. All the chemicals and solvents were used without further purification.

Synthesis of iminodiacetic acid derivatives was described earlier [11]. Briefly, the syntheses of N-(4-methylacetanilide)iminodiacetic acid and N-(2,4-dimethylacetanilide)iminodiacetic acid involve the reaction between *in situ* obtained nitrilotriacetic acid anhydride and 4-methylaniline or 2,4-dimethylaniline, respectively (Fig. 1).

Aqueous solubility studies.

The influence of PAMAM dendrimers (generation 1 – 4) in concentration from 0 to 10 mg/mL on aqueous solubility of two derivatives of iminodiacetic acid was determined with the equilibrium solubility method described in detail previously [11].

In brief, to the diluted solutions of PAMAM dendrimers (generation 1–4) in appropriate concentrations the excess of compounds were added and obtained suspensions were subjected to ultrasonic effects. The difference

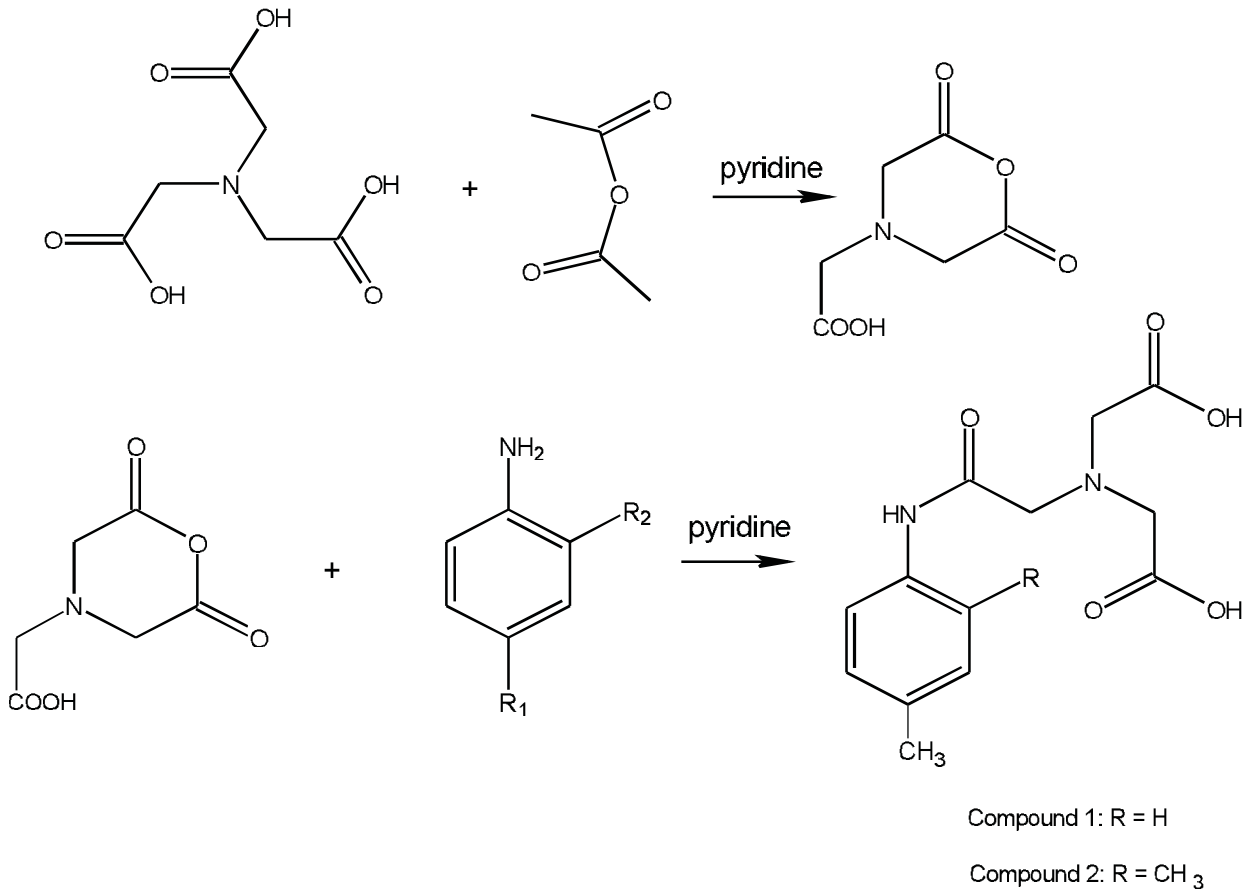

Fig. 1. Synthesis of derivatives of N-(2-phenylamine-2-oxoethyl)iminodiacetic acid

is that in the current study solutions were mechanically incubated for 72 hours, whereas in the previous one for 24 hours. After centrifugation the saturated solutions were diluted to a proper concentration with bidistilled water, followed by spectrophotometric measurements of absorbance by Perkin-Elmer UV-VIS spectrophotometer. All the measurements were carried out between 190–400 nm. The solubility of iminodiacetic acid derivatives in the presence of PAMAM dendrimers was calculated according to the measured absorbance and the calibration curve. Double distilled water was also used as a blank. We conducted three repeats of each sample.

RESULTS AND DISCUSSION

Solubility enhancement of newly developed drugs has always been a challenge to the scientists because the hydrophobicity of these compounds contributes to difficulties during product development and unsatisfactory bioavailability.

During the last decade numerous scientific teams evaluated the effect of PAMAM dendrimers on solubility of various types of drugs. Among them are NSAIDs such as: salicylic acid, indomethacin, flurbiprofen, diclofenac, mefenamic acid, piroxicam, naproxen, ibuprofen, ketoprofen, diflunisal, phenylbutazone, and anticancer drugs: paclitaxel, and methotrexate [16]. It was found that concentration, generation number, pH of the solvent, temperature, and dendritic architecture influence the efficiency of dendrimers as solubilizing agents [11,16].

It has been stated that the drugs solubility enhancement in the presence of PAMAM dendrimers is assigned to: the nonpolar cavities in the interior of dendrimers that can entrap hydrophobic drugs; cationic functional groups on the surface of dendrimers that can interact by electrostatic interactions; nitrogen and oxygen atoms in the structure of dendrimers that can interact with guests by hydrogen bond formation [1,4,16].

In the current study, the effect of G1–G4 PAMAM dendrimers on the process of solubilization of two iminodiacetic acid derivatives was investigated by means of UV-VIS spectroscopy after prolonged incubation time. The obtained results are presented in Figures 2 and 3, from which it is clear that the solubility of iminodiacetic acid analogues was affected by concentration and generation of PAMAM dendrimers.

The apparent solubility of both derivatives of iminodiacetic acid increased in an approximately linear manner as a function of PAMAM dendrimer concentration over the whole concentration range. It is the result of the increase in the number of amines on the surface of PAMAM dendrimers which may interact electrostatically with the carboxyl groups of the tested compounds.

Furthermore, the solubility of compounds **1** and **2** was the highest in the presence of G4 PAMAM dendrimers because the number of primary and tertiary amines within the dendrimer structure increases with generation number. Therefore, dendrimer of higher generation has a tendency to interact with more particles of hydrophobic compound than lower one.

Results of our study are consistent with most of foreign researchers and our previous one [11]. Generally, the solubility of tested drugs increased with the concentration and higher generation number of the dendrimer. Such observations were confirmed for most of examined drugs, for example solubility of NSAIDs (naproxen, ketoprofen, ibuprofen and diflunisal) was higher in the presence of G4 PAMAM dendrimers than in the presence of G2 and G3 PAMAM [2].

To the best of our knowledge, there are not available scientific publications concerning the effect of time on the solubility of drugs in the presence of PAMAM dendrimers. Therefore, we decided to compare the effec- tiveness of PAMAM dendrimers as solubility enhancers of iminodiacetic acid analogues after 24 and 72 hours incubation time. The results are presented in Figure 4 and 5. The data concer-

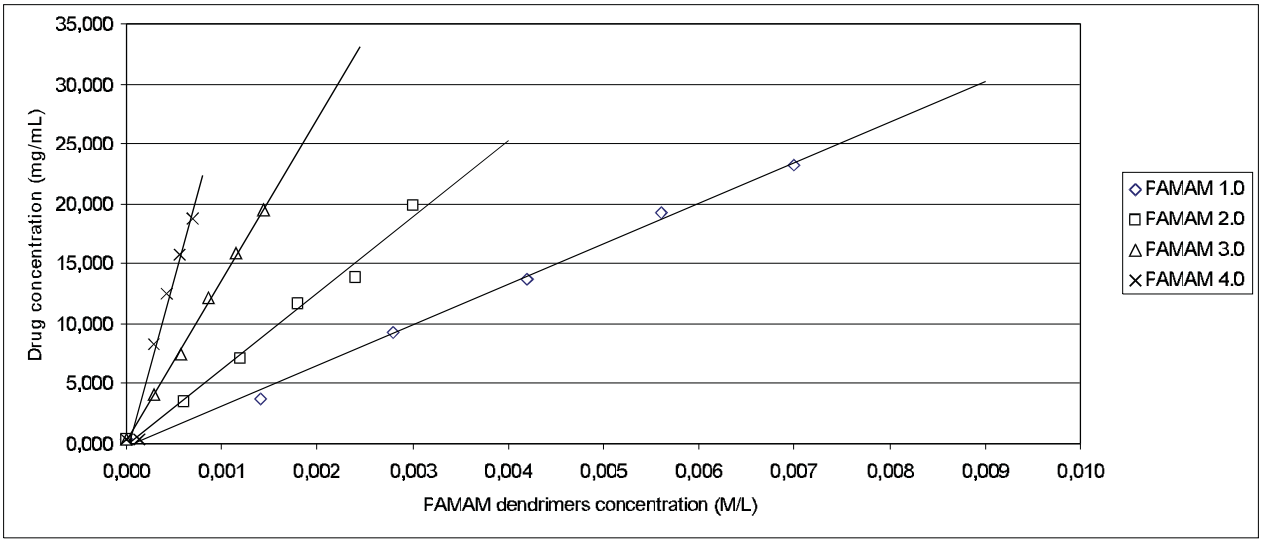

Fig. 2. Solubility of compound 1 at different concentrations and generations of PAMAM dendrimers after 72 h incubation time

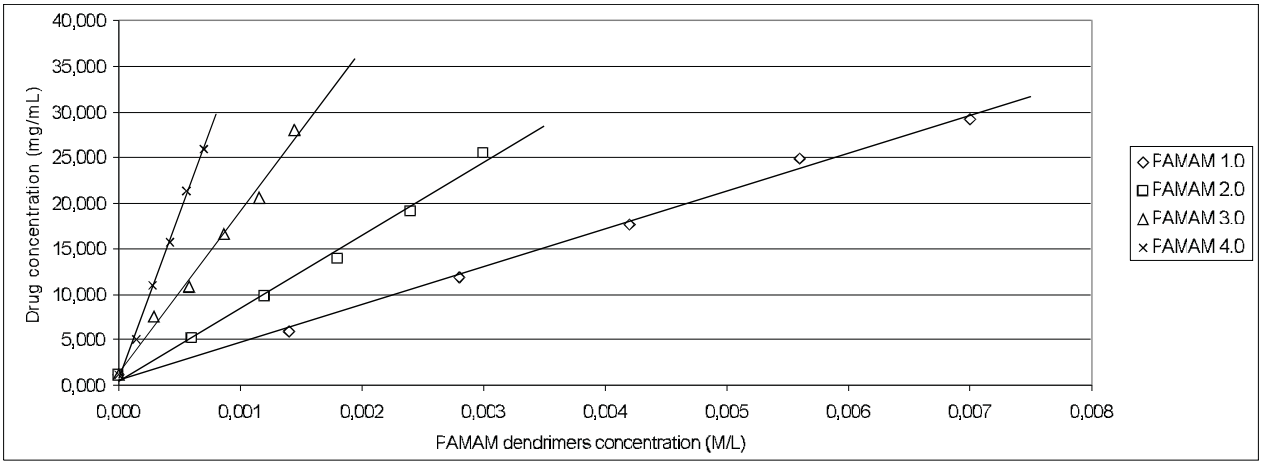

Fig. 3. Solubility of compound 2 at different concentrations and generations of PAMAM dendrimers after 72 h incubation time.

ning solubility of compounds 1 and 2 after 24 hours incubation were published in our previous paper [11].

Figures 3 and 4 present the solubility of compounds 1 and 2 in the presence of the highest dendrimer concentration used in our studies (10 mg/ml). Solubility enhancement of both compounds in the presence of the same mass concentration of G1-G4 PAMAM dendrimers is on the same level due to the fact that PAMAM dendrimers of various generations in the same mass concentration possess similar number of primary amine groups, which are responsible for solubility enhancement of anionic compounds.

The results of our previous work and those described within this study reveal that the prolonged incubation time

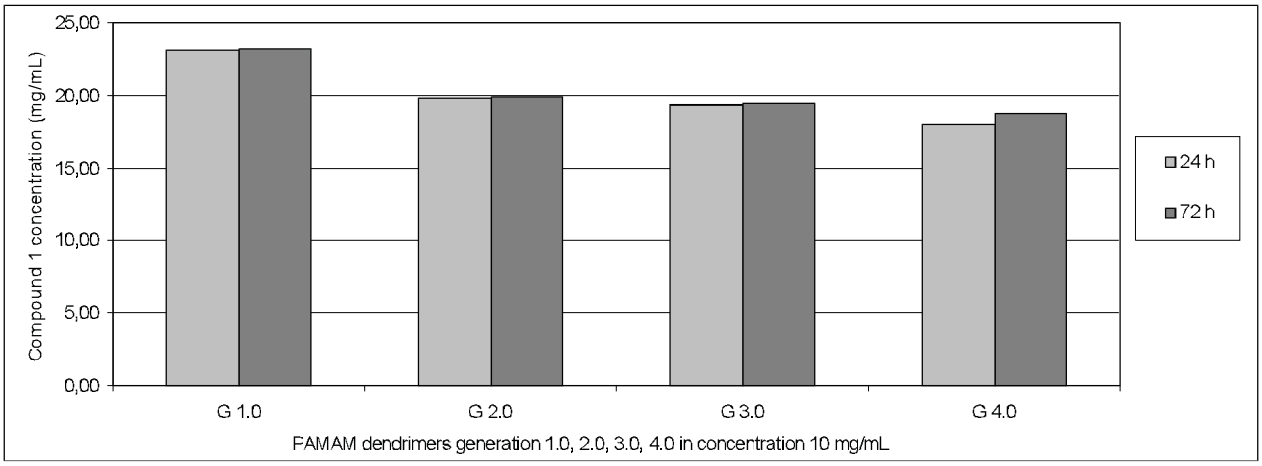

Fig. 4. Comparison of the influence of incubation time on solubility of compound 1

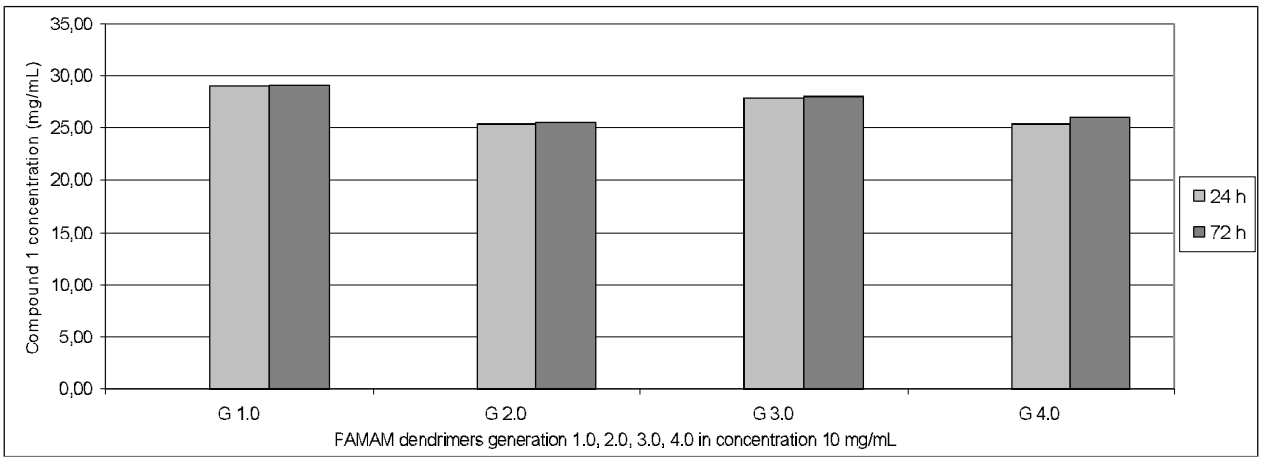

Fig. 5. Comparison of the influence of incubation time on solubility of compound 2

does not influence significantly the solubility of iminodiacetic acid derivatives. Figures 3 and 4 show that for all generation of PAMAM dendrimers solubility enhancement remain on the same level. The highest differences were noted for generation 4; for compound 1 the solubility after 24 h was 17.97 mg/mL [11] and after 72 h it was 18.71 mg/mL. In case of compound 2 the solubility after 24 h was 25.42 mg/mL [11] and after 72 h – 25.99 mg/mL.

These results suggest that interactions created between iminodiacetic acid analogues and PAMAM dendrimers are stable over 72 hours. Our previous ^{1}H NMR and 2D-NOESY studies revealed [11] that interactions between tested compounds and G1-G4 PAMAM dendrimers are based mainly on electrostatic interaction between the surface amine groups of PAMAM dendrimers and the carboxyl groups of compounds. Furthermore, hydrophobic interactions are involved in the mechanism of complex formation. Thus, it was of vital importance to determine the stability of these interactions.

Little scientific information concerning interactions between PAMAM dendrimers and double charged drugs is available. To the best of our knowledge, the only example of drug whose interactions with dendrimers were investigated is methotrexate. According to the results of the study solubility of methotrexate in the presence of PAMAM dendrimers was not increased because two carboxylic acid groups present in the structure of methotrexate form cross-linking structures and form large aggregates with dendrimers which precipitate in aqueous solutions [17]. Also in case of Congo Red and indocyanine green, drugs bearing multiple charges, precipitates were observed [5].

Results of the current study confirm that interactions between derivatives of iminodiacetic acid and PAMAM dendrimers are strong and stable over 72 hours in contrast to other multiple charged drugs.

CONCLUSION

Within the study the effect of PAMAM dendrimers G1-G4 on solubility of two analogues of iminodiacetic acid after prolonged incubation time was described. Amine terminated dendrimers contributed significantly to the solubility enhancement of tested compounds. We reported that the solubility of compounds was connected with concentration and generation of dendrimer. The results of solubility studies after 72 h incubation are almost identical with those recorded after 24 h incubation (reported previously [11]) which suggest that the interactions between iminodiacetic acid derivatives and PAMAM dendrimers, mainly electrostatic and hydrophobic, are strong and stable in aqueous environment.

The results of our study provide new insight into the interaction mechanism of complexes formed by PAMAM dendrimers and drugs containing two carboxylic groups, which might be useful in the development of novel MRI contrast agents.

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