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Interactions between PAMAM dendrimers and gallic acid molecules studied by spectrofluorimetric methods

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Abstract

Interactions between gallic acid molecules and different types of polyamidoamine (PAMAM) dendrimers with modified surfaces were studied by spectrofluorimetric methods. Changes in fluorescence intensity of gallic acid and in a position of spectrum were monitored. It was found that the extent of gallic acid incorporation into dendrimers depends on a type of a dendrimer.

Keywords: PAMAM dendrimers; Gallic acid; Host–guest systems; Encapsulation; Fluorescence

1. Introduction

Dendrimers are a relatively novel and the most intensively investigated class of polymers. In contrast to traditional polymers, dendrimers are monodisperse macromolecules characterised by a well defined structure. All dendrimers consist of a core molecule and radically attached layers of branched monomers. The outer layer of monomers creates a shell built from many terminal functional groups. Dendrimers possess not only a high concentration of end groups on the surface but also empty internal cavities [1]. These two features make them very promising materials as capture and delivery systems for small molecules. It was possible to trap inside dendrimers such small molecules as *p*-nitrobenzoic acid, rose bengal, or eosin Y [2–4]. This specific property can find a utilization in biomedical applications. Dendrimers can serve as both drug carriers and entrapping agents for toxins [5,6]. By entrapping drugs inside a dendrimer, slow release can be achieved. It is especially important for cytostatic drugs because it reduces side-effects [7]. Besides medical-oriented studies, basic studies on host–guest systems were also performed to reveal relationships between a dendrimer structure and its capacity to serve as a host macromolecule [8–10].

In our studies the binding of gallic acid by polyamidoamine (PAMAM) dendrimers was examined. PAMAM dendrimers are based on an ethylenediamine core and branched units are built from methyl acrylate and ethylenediamine. Dendrimers are characterised by the generation number which indicates how many layers of monomers were attached. Different types of the fourth generation

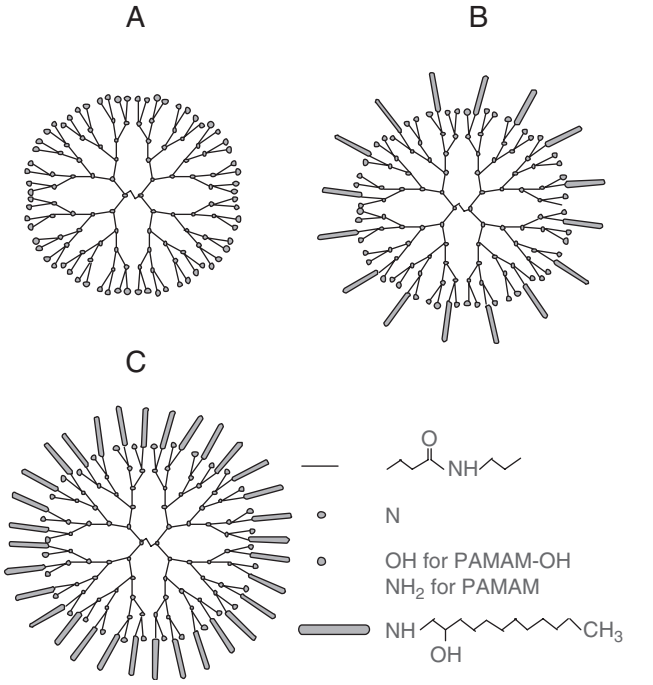

Fig. 1. The structures of PAMAM G4 and PAMAM–OH G4 (A), PAMAM-C_{12}-25% (B) and PAMAM-C_{12}-50% (C) dendrimers.

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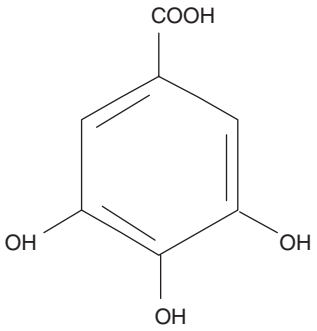

Fig. 2. Chemical structure of gallic acid.

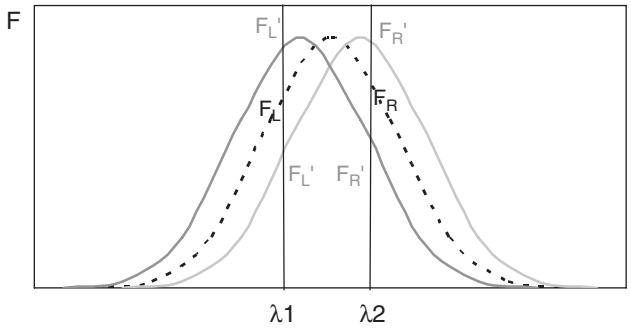

Fig. 4. The scheme of double-wavelength method to evaluate the position of emission maximum.

(G4) of PAMAM dendrimers were used. All of them possess 64 terminal groups. The difference between them was based on a kind of end groups. PAMAM dendrimers have amino groups on the surface. The surface of PAMAM-C_{12}-25% and PAMAM-C_{12}-50% dendrimers were modified in 25% and 50% by attaching *N*-(2-hydroxydodecyl) groups. PAMAM–OH dendrimers, instead of possessing amino groups, are hydroxy-terminated. The schematic structures of all dendrimers are presented in Fig. 1.

Gallic acid is a naturally occurring plant phenol. Its chemical name is 3,4,5-Trihydroxybenzoic acid (Fig. 2). Gallic acid is known by its antioxidant properties. It possesses a scavenging activity against several types of free radicals and protects cells from damage induced by UV-B or ionizing irradiation [11]. However, for higher concentrations gallic acid acts as a prooxidant [12].

In our studies gallic acid has been chosen as a model compound to investigate relationships between the dendrimer structure and the ability to encapsulate small molecules. Gallic acid is a fluorescent molecule and its fluorescence is sensitive to changes in its environment [13].

2. Experimental

All types of dendrimers (10% wt solutions in methanol) were obtained from Aldrich (UK). Gallic acid was purchased from Sigma (USA) and it was dissolved in methanol at a concentration of 50 μmol/l. Then increasing concentrations of dendrimers were added from a stock solution in methanol. Fluorescence spectra were taken with a Perkin-Elmer LS-50B spectrofluorometer using excitation wavelength of 270 nm. The emission spectra were recorded from 290 to 500 nm. The excitation and emission slit widths were set to 5 and 3 nm, respectively. Samples were contained in 1-cm path length quartz cuvettes and were continuously stirred. It was checked that dendrimers were not excited by 270-nm wavelength and did not emit fluorescence.

3. Results and discussion

The decrease in the fluorescence intensity was observed upon addition of dendrimers. The changes were the least pronounced for PAMAM–OH dendrimers. For all types of dendrimers, their increasing concentration caused a linear reduction in the fluorescence of gallic acid. Thus data were analysed by the Stern–Volmer equation:

$$\frac{F^0_{375}}{F_{375}} = 1 + K_{SV} \cdot c$$

where F^0_{375} and F_{375} are, respectively, fluorescence intensities for excitation wavelength 375 nm in the absence and in the

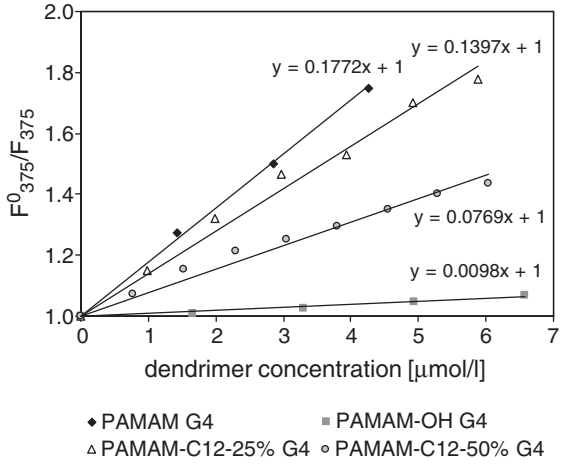

Fig. 3. The Stern–Volmer plots for gallic acid fluorescence quenched by dendrimers.

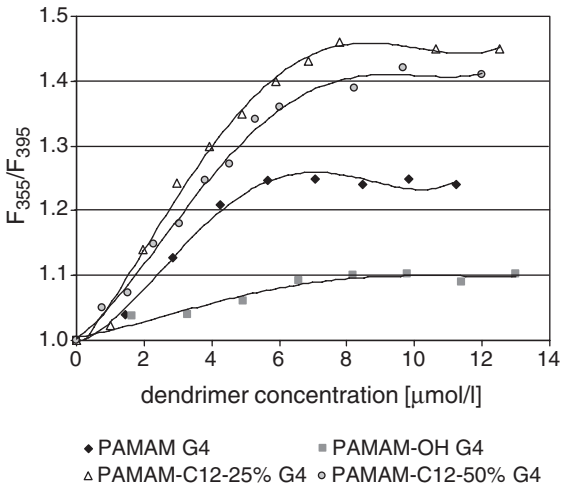

Fig. 5. The effect of dendrimers on the position of emission maximum.

Table 1
The Stern–Volmer constants of the quenching of gallic acid fluorescence by dendrimers and the number of gallic acid molecules interacting with one molecule of dendrimer

Name, generation	K_{SV} [l/mmol]	n
PAMAM	177.2	10
PAMAM-C_{12}-25%	139.7	7
PAMAM-C_{12}-50%	76.9	6
PAMAM–OH	9.8	8

presence of a dendrimer, K_{SV} is the Stern–Volmer dynamic quenching constant, and c is the dendrimer concentration. Stern–Volmer plots are shown in Fig. 3.

Changes in the fluorescence intensity were accompanied by a blue-shift in the position of emission maximum. The blue-shift of gallic acid emission maximum indicates that gallic acid was transferred into more hydrophobic environment inside dendrimers. To be more accurate we decided to register the changes in the position of a spectrum as a ratio of fluorescence intensities at two wavelengths: on the left and on the right slopes of the spectrum [14] (Fig. 4). In our case F_L and F_R equalled to 355 and 395 nm, respectively. For each type of dendrimers the position of emission maximum changed linearly with an increasing dendrimer concentration and for higher concentrations achieved a plateau (Fig. 5). This feature was very useful to calculate the number of gallic acid molecules interacting with one molecule of dendrimer:

$$n = \frac{c_{GA}}{c'_{den}}$$

where c_{GA} is the gallic acid concentration and c'_{den} is the concentration of dendrimer which corresponds to the saturation point.

The calculated Stern–Volmer constants and n values are given in Table 1.

The aim of the studies was to compare the behaviour of gallic acid in the presence of different types of dendrimers. The weakest interactions were observed for hydroxy-terminated dendrimers. The gallic acid fluorescence was not quenched and the blue-shift was only slight. It seems that quenching occurs through the contact between dendrimer amino end groups and a carboxyl group of gallic acid. The more amino groups exist on the surface, the bigger K_{SV} value is. Moreover, the more amino groups is substituted by *N*-(2-hydroxydodecyl) chains, the less molecules of gallic acid can interact with one molecule of dendrimer. On the other hand, the presence of long chains on the surface allows gallic acid to enter deeper into the dendrimer structure, which was observed by a significant blue-shift. The biggest blue-shift occurred for PAMAM-C_{12}-25% dendrimers. Further modification of the dendrimer surface and replacing 50% of amino groups by *N*-(2-hydroxydodecyl) groups did not increase the effect. On a contrary, the shift towards shorter wavelengths was slightly smaller. It could happen due to sterical restrictions which are a consequence of too densely packed surface.

4. Conclusions

Fluorescence parameters of gallic acid are strongly related to its microenvironment. That is why we were able to conclude where gallic acid molecules were located in the presence of dendrimers. Especially the final ratio F_{355}/F_{395}, that corresponds to a saturation point, showed how deep gallic acid molecules were incorporated into the dendrimer. The bigger value, the deeper incorporation.

It was shown that the efficiency and depth of incorporation of small molecules into dendrimers depend on the dendrimer's structure and a kind of end groups. In case of acidic compounds the presence of amino groups on the surface provides attractions that facilitate the incorporation. Modifying surface by attaching long chains allows small molecules to enter deeper.

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