Systems Based on Dendrimers and Antitumoral Drug Synthesized by Non-covalent Method
The influence of dendrimers generation

SORINA ALEXANDRA GAREA*, **ADI GHEBAUR**, **CORINA ANDRONESCU**
[1]University Politehnica of Bucharest, 149 Calea Victoriei, 010072, Bucharest, Romania

The main goal of this study was to investigate the capacity of dendritic polymers to encapsulate the antitumoral drug (5-fluorouracil) using non-covalent methods. As nanoscale containers four polyamidoamine dendrimers (PAMAM) types of different generations were used. The antitumoral drug encapsulation efficiency of these dendrimers was pointed out using different methods like FTIR Spectroscopy, X-Ray Photoelectron Spectroscopy (XPS) and Thermogravimetric analysis (TGA). The obtained results showed that the dendrimers generation exhibits a strong influence on the 5-fluorouracil encapsulation.

Keywords: dendrimer, antitumoral drug, FTIR analysis, X-Ray Photoelectron Spectroscopy

Dendrimers are considered a relatively new class of polymers which exhibit special features like a high degree of molecular uniformity and monodispersity, three dimensional structures, variable reactive surface induced by the presence of functional groups on the surface, typical architecture [1]. Dendrimers present similar size with some biological structures. For instance fifth generation polyamidoamine dendrimers exhibit the same size and shape as hemoglobin (5.5 nm diameter) [2].

These polymers are used in some special applications in the biomaterials fields (drug delivery systems, imaging agents) due to their unique properties [3]. Thus dendrimers have found some special applications in pharmaceutical field [4-10]. Due to the special architecture and reactive surface these dendritic polymers may be considered as a nanoscale container which is able to interact with the drugs in different ways like association with surface groups (by electrostatic attraction, hydrogen bonding and van der Waals interactions) or/and encapsulation of drugs into the void spaces [11].

The interaction between drug and dendrimer is favoured as the generation increases because the functional groups from the dendrimer surface doubles from a generation to another. Not all the functional groups from dendrimer structure are involved in drug interaction due to the steric hindrance which may appear. The interaction between the dendrimer and drug could be achieved through covalently conjugation or by non-covalent encapsulation.

The synthesis of some dendrimer-drug complex may enhance the drug solubility, stability, bioavailability and controll its release . The most antitumoral drugs like 5-fluorouracil, cisplatin, doxorubicin, paclitaxel are known for the poor water solubility. By conjugation of these drugs with dendritic molecules it is possible to change this drawback. The synthesis of dendrimer-drug is influenced by some factors like the number of surface groups, molecular weight of dendrimers, steric hindrance factor, biocompatibility and toxicity [4, 11, 12].

The aim of the present work was to synthesize different systems based on dendritic polymers and an antitumoral drug using non-covalent method. Another goal of this work was to study the influence of PAMAM generation on the drug encapsulation.These complexes were characterized using different methods like FTIR Spectroscopy, X-Ray Photoelectron Spectroscopy (XPS) and Thermogravimetric analysis (TGA).

Experimental part
Materials
The poly(amidoamine) dendrimers (PAMAM) with ethylenediamine core, aminic terminated groups and different generations (generation 1 (**PAMAM G1**), generation 3 (**PAMAM G 3**), generation 4 (**PAMAM G4**) and generation 5 (**PAMAM G5**)) were supplied from Sigma-Aldrich Company. In scheme 1 the chemical structure of PAMAM G1 is shown and the main characteristics of dendritic polymers with various generatios are summarized in table 1.

PAMAM G1

Scheme 1. The chemical structure of the PAMAM G1

* email :garea_alexandra@yahoo.co.uk; Tel: 0214022710

Table 1
THE MAIN CHARACTERISTICS OF THE DENDRITIC POLYMERS USED FOR
ANTITUMORAL DRUG ENCAPSULATION

PAMAM type	Molecular weight (g/mole)	Number of surface primary aminic groups
PAMAM G1	1429.85	8
PAMAM G3	6908.84	32
PAMAM G4	14214.17	64
PAMAM G5	28824.81	128

Fluorouracil (5-FU) and chloroform were supplied by Sigma-Aldrich Company and used as received.

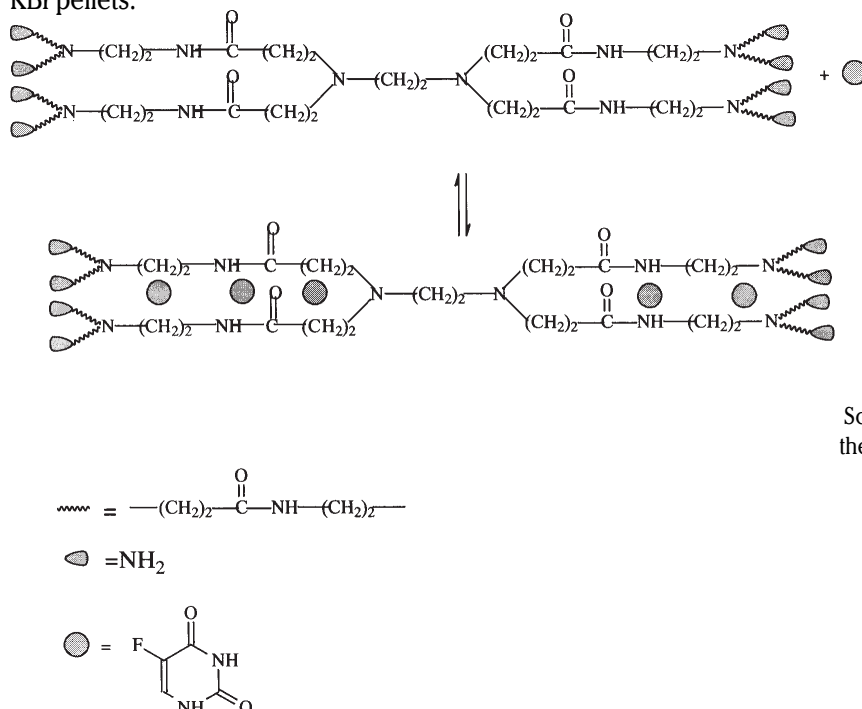*Encapsulation of antitumoral drug into the dendritic polymers by non covalent method*

The synthesis of dendrimer-drug systems was done according to the method described by [14] who synthesized some biconjugated systems based on PAMAM and doxorubicin as antitumoral drug. In our study we used other antitumoral drug and dendritic polymers with different generations.

A precise amount of 5-FU (6:1 drug: dendrimer molar ratio) was dissolved in 8 mL deionized water and then was mixed with dendritic polymers which exhibit various generations (PAMAM G1, PAMAM G3, PAMAM G4, PAMAM G5). The solution was stirred for 12 h at room temperature and then the solvent was evaporated using a rotaevaporator. The obtained transparent gel was treated with 5 ml chlorofom for 24 h and finally after the evaporation of the solvent a yellow solid was obtained. The solid was grounded to obtain a yellow fine powder and dried at 50°C for 24 h.

Characterization methods
FTIR analysis
FTIR spectra of drug, dendrimers and drug-dendrimer systems were recorded on a Bruker VERTEX 70 spectrometer using 32 scans with a resolution of 4 cm⁻¹ in 4000-500 cm⁻¹ region. The samples were analyzed from KBr pellets.

X-Ray Photoelectron Spectroscopy (XPS)
The XPS spectra were recorded on a Thermo Scientific K-Alpha equipment using a monochromated AlK$_\alpha$ source (1486.6 eV) at a pressure of 2 x 10⁻⁹ mbar. Charging effects were compensated by a flood gun and binding energy was calibrated by placing the C1s peak at 284.8 eV as internal standard.

Thermogravimetric analysis (TGA)
TGA tests were done on a Q 500 TA equipment using nitrogen and a heating rate of 10 °C/min, from 30 to 800°C.

Results and discussions
Incorporation of antitumoral drug (5-FU) in different dendritic polymers

The drug can give some interactions with dendritic polymers due to the reactive surface which includes aminic functional groups. Also the dendrimer architecture allows the encapsulation of drugs into the void spaces. Depending on the PAMAM generation, the drug can be entrapped within the branched structure of dendrimer as in scheme 2.

Characterization of dendrimer-antitumoral drug systems
FTIR analysis
The dendrimer-antitumoral drug complexes were first characterized by FTIR Spectroscopy.

In the first step it was necessary to record the FTIR spectra of the neat dendrimers (PAMAM G1, PAMAM G3, PAMAM G4 and PAMAM G5) and antitumoral drug in order

Scheme 2. The encapsulation of drug into the dendritic polymer (PAMAM G1) by non covalent method
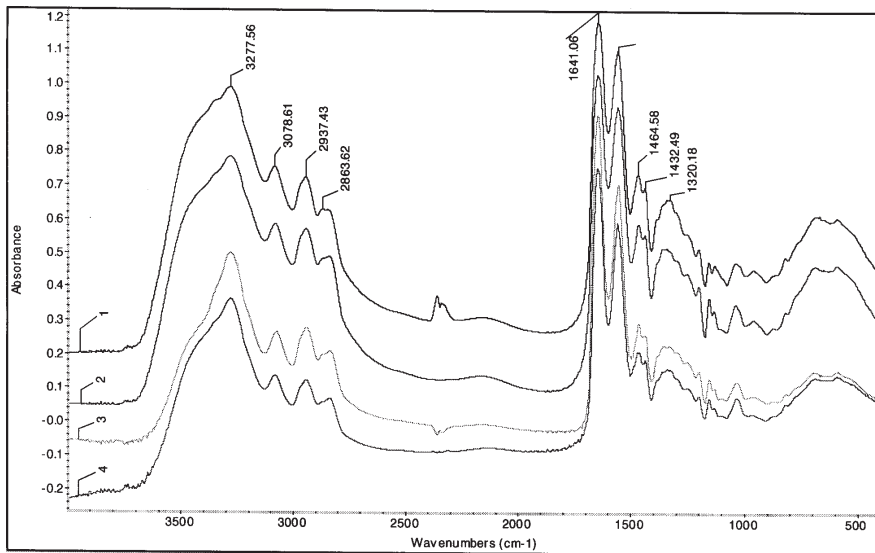
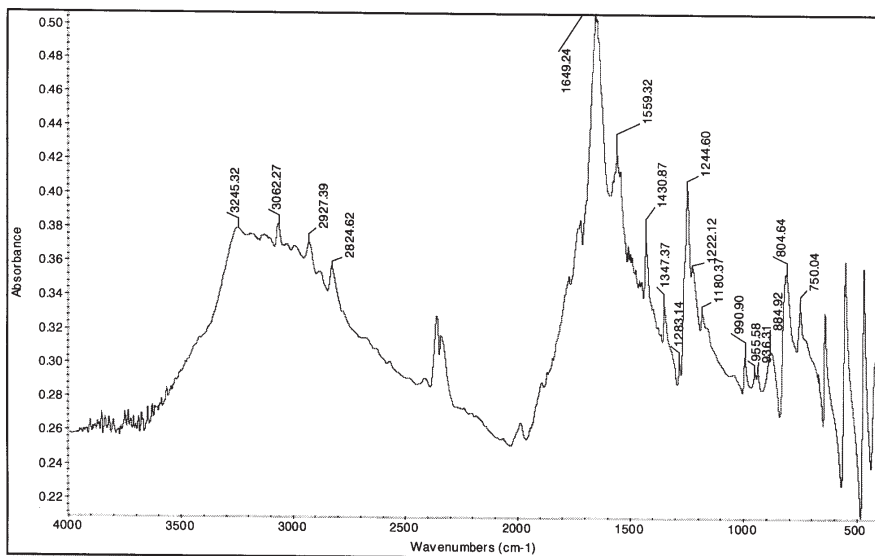Fig. 1. FTIR spectra of dendrimers: 1-PAMAM G1, 2-PAMAM G3, 3-PAMAM G4, 4-PAMAM G5

Fig. 2. FTIR spectrum of 5-FU

to be used for comparison with the FTIR spectra of drug-dendrimer complexes.

The FTIR spectra of dendrimers and 5-FU are shown in figure 1 and figure 2 and the spectral assignments are listed in table 2 and 3.

Figure 3 presents the FTIR spectra of complexes based on antitumoral drug and dendritic polymers (PAMAM G1-5-FU, PAMAM G3-5-FU, PAMAM G4-5-FU and PAMAM G5-5-FU). This characterization method was usefull to detect if some interactions between drug and dendrimers may occured. The FTIR results showed that the presence of

Table 2
THE MAIN SPECTRAL ASSIGNMENTS FOR DENDRITIC POLYMERS

Wave number (cm^{-1})	Spectral assignment
3277	N-H stretching vibration
2937, 2863	-CH$_2$- asymmetric and symmetric stretching vibration
1641	Amide I mode
1559	Amide II mode
1464, 1432	-CH$_2$- scissoring vibration
1320	-CH$_2$- twisting vibration

Table 3
SPECTRAL ASSIGNMENTS FOR 5-FU

Wave number (cm^{-1})	Spectral assignment
3245	N-H stretching vibration
3062	C-H stretching vibration
2927, 2824	-CH$_2$- asymmetric and symmetric stretching vibration
1649	-C=C- stretching vibration carbonyl stretching vibration
1430	N-H in plane bending vibration
1244	Ring stretching vibrations
1222	C-F stretching vibration
990	N-H and C-H wagging vibration
804	C-F stretching vibration
750	Pyrimidine ring vibration

5-FU was not detected in all dendritic polymers. The generation of dendrimers exhibits an influence on drug incorporation.

Fig. 3. FTIR spectra of: 1-PAMAM G1-5-FU, 2-PAMAM G3-5-FU, 3-PAMAM G 4-5-FU, 4-PAMAM G5-5-FU
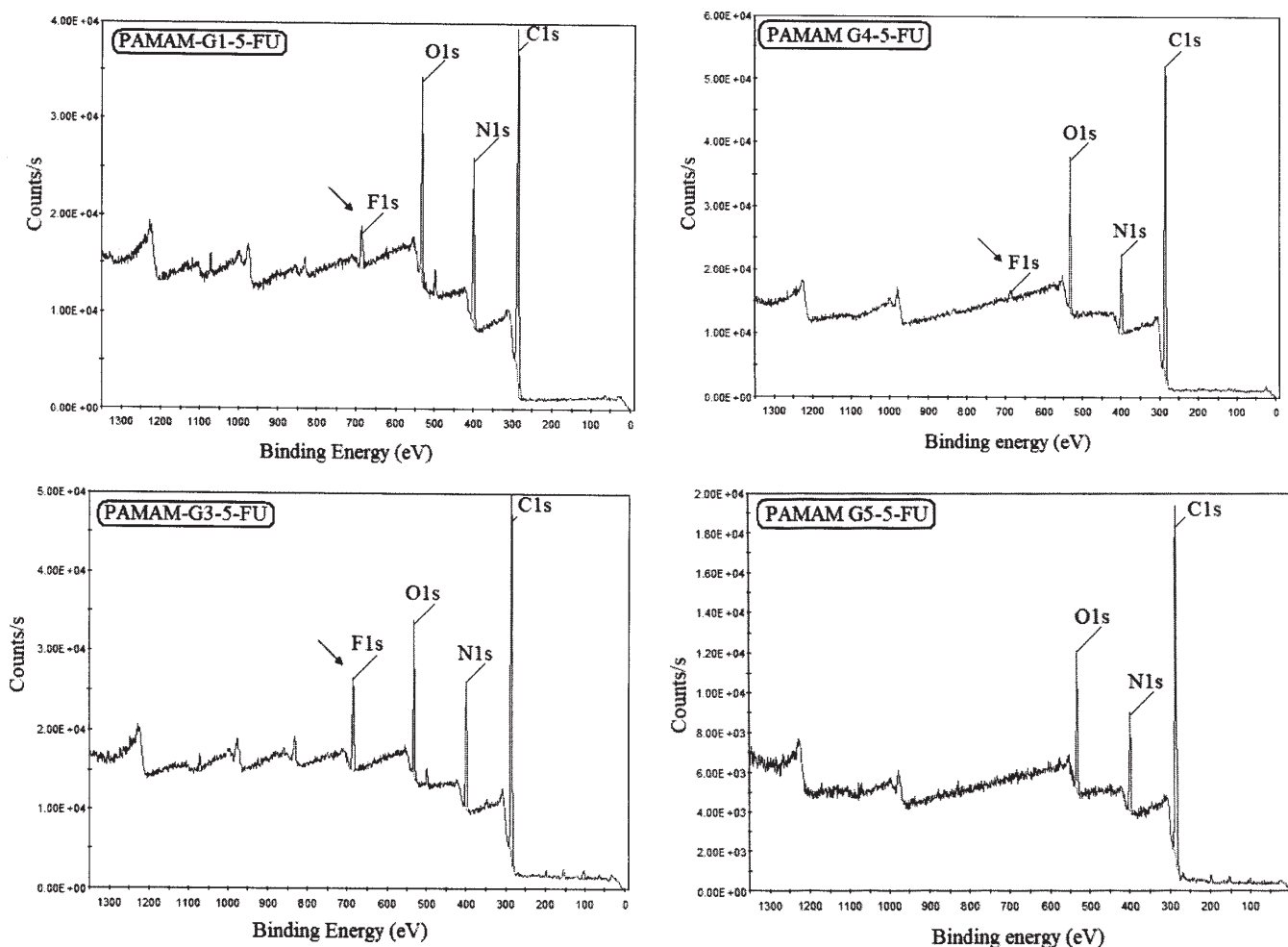
Fig. 4. XPS survey spectra of some different PAMAM polymers treated with 5-FU

In almost all the cases (PAMAM G1, PAMAM G3, PAMAM G4) the presence of 5-FU was proved by the presence of the peaks at 813 and 775 cm^{-1} which are also detected in the FTIR spectrum of 5-FU. These peaks are observed in the complexes at frequency slightly higher than in drug FTIR spectrum, this fact suggesting the presence of some interactions between the 5-FU and dendritic molecules. In the case of PAMAM G5 used as polymeric host the FTIR results proved that the drug encapsulation does not occured. This fact may be due to the presence of steric hindrance which frequently occured in case of dendritic polymers which exhibit high generations.

X-Ray Photoelectron Spectroscopy (XPS)

The XPS analysis was very useful to check the presence of 5-FU into the dendritic host. The XPS spectra of dendrimers with different generations treated with 5-FU are shown in figure 4. The presence of the peak assigned to F1s is an important prove that the 5-FU was encapsulated into the dendrimer host. This peak was detected only for the systems which exhibit dendritic polymers with lower generation (G1, G3 and G4). Also the intensity of this peak depends on the dendrimer generation. For the dendrimers with higher generation than 4 the encapsulation of 5-FU was hindered probably due to the steric factors. In case of

a

b
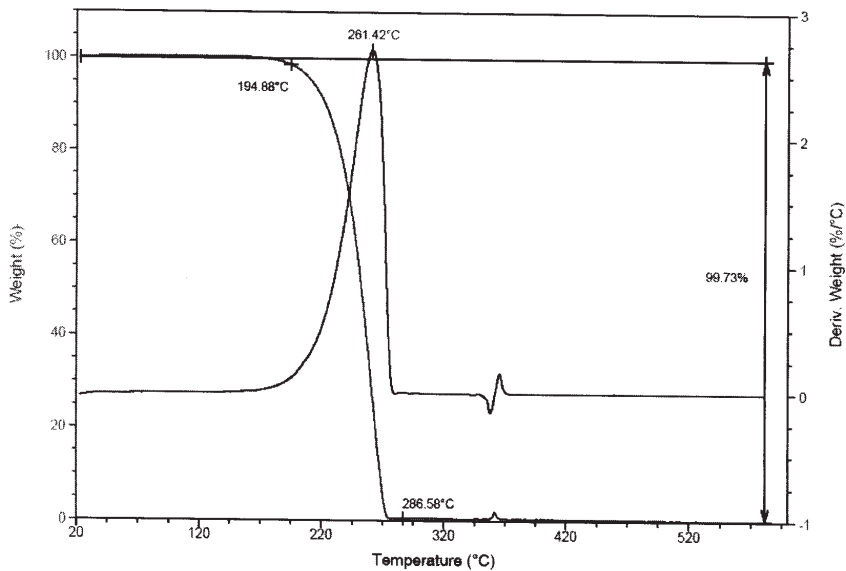
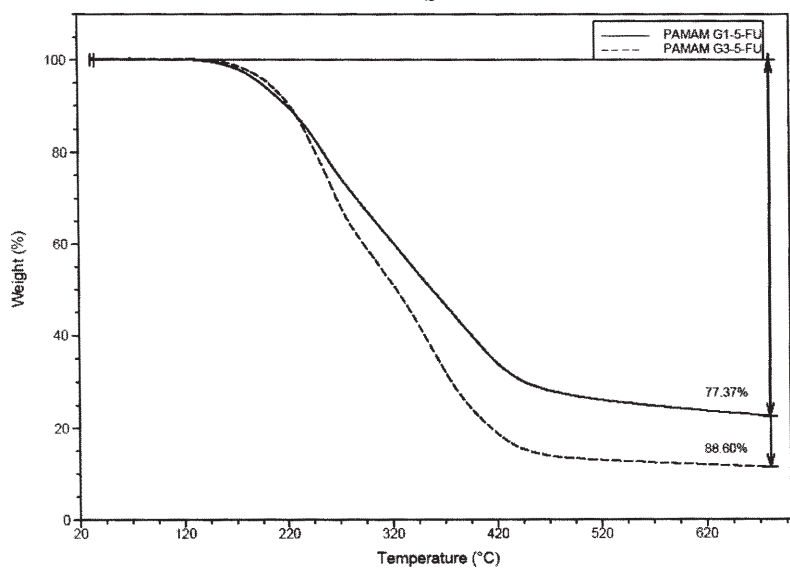Fig. 5. TG and DTG of 5-FU (a) and PAMAM G1 (b)
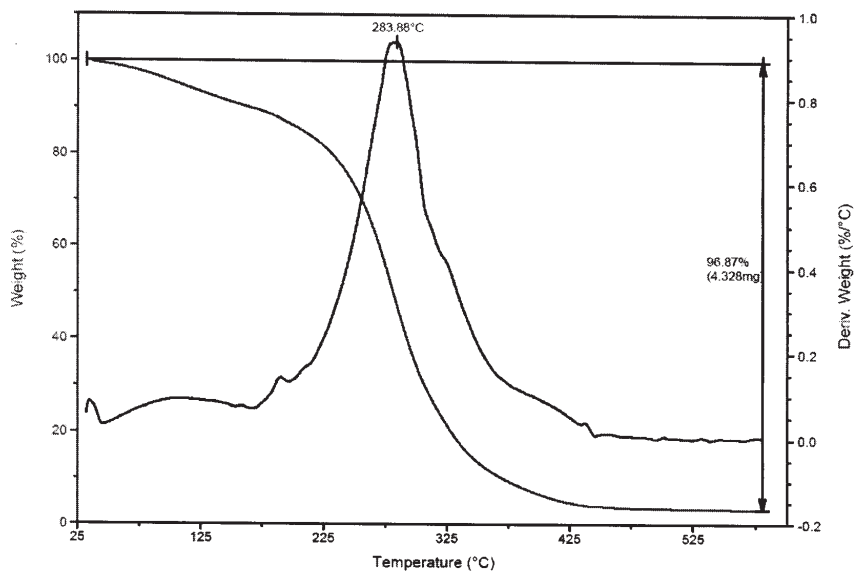
Fig. 6. TGA curves of different drug-dendrimer complex synthesized by non-covalent method: 1-PAMAM G1-5-FU, 2-PAMAM G3-5 FU

PAMAM G5 the absence of the peaks assigned for F1s confirms the FTIR data.

The best result regarding the drug encapsulation was achieved by PAMAM G3.

Therefore it may be concluded that the generation of dendrimers represents an important factor which may influence the encapsulation of a drug using non-covalent methods.

Thermogravimetric analysis

The thermal stability of 5-FU and PAMAM dendrimer are shown in figure 5. The drug shows an initial degradation at 194°C and a complete thermal breakdown at about 287°C. For the PAMAM dendrimer a total degradation was achieved at 500°C.

The thermal stability characterization was done only for the systems based on PAMAM G1 and PAMAM G3 which proved a high capacity to encapsulate the antitumoral drug (5-FU) in comparison with PAMAM G4 and PAMAM G5. As one may observe the both systems exhibit similar initial degradation temperature but the PAMAM G3-5-FU complex shows a higher weight loss at 700 °C than PAMAM G1-5-FU.

Conclusions

Some complexes based on antitumoral drug and various dendritic polymers of different generations were synthesized. The incorporation of the drug into the dendritic polymers strongly depends on the dendrimer generation. The FTIR analysis showed the presence of some peaks which exist in the drug FTIR spectrum only in the spectra of dendritic polymers with low and medium generations (PAMAM G1, PAMAM G3, PAMAM G4). In case of dendritic polymer of high generation (PAMAM G5) the incorporation of drug was hindered by the steric factors.

The XPS analysis confirms the order of dendrimer encapsulation efficiency proved by FTIR analysis.

References

1. PISANI, M.J., WHEATE, N.J., KEENE, F. R., ALDRICH-WRIGHT, J. R., GRANT COLLINS, J., Journal of Inorganic Biochemistry,103, 2009, p. 373
2. ESFAND, R., TOMALIA, D.A., Drug Discov. Today, 6, 2001, p. 427.
3. SVENSON, S., TOMALIA, D.A., Advanced Drug Delivery Reviews, 57, 2005, p. 2106.
4. D'EMANUELLE, A., ATTWOOD, D., Advanced Drug Delivery Reviews, 57, 2005, p. 2147
5. DEVARAKONDA B., OTTO, D. P., JUDEFEIND, A., HILL, R. A., VILLIERS, M. M, International Journal of Pharmaceutics, 345, 2007, p. 142.
6. CHENG, Y., XU, T., European Journal of Medicinal Chemistry, 43, 2008, p. 2291
7. MARKATOU, E., GIONIS, V., CHRYSSIKOS, G. D., HATZIANTONIOU, S., GEORGOPOULOS, A., DEMETZOS, C., International Journal of Pharmaceutics, 339, 2007, p. 231
8. MA, M., CHENG, Y., XU, Z., XU, P., QU, H., FANG, Y., XU, T., WEN, L., European Journal of Medicinal Chemistry, 42, 2007, p. 93
9. CHENG, Y., QU, H., MA, M., XU, Z., XU, P., FANG, Y., XU, T., European Journal of Medicinal Chemistry, 42, 2007, p. 1032.
10. CUI, D., XU, Q., GU, S., SHI, J., CHE, X., Journal of Pharmacy and Pharmacology, 3, 2009, p. 227.
11. DUNCAN, R., IZZO, L., Advanced Drug Delivery Reviews, 57, 2005, p. 2215.
12. PATRI, A,K, KUKOWSKA-LATALLO, J.F., BAKER Jr, J.R., Advanced Drug Delivery Reviews, 57, 2005, p.2203.
13. WOLINSKYA, J.B, GRINSTAFF, M.W., Advanced Drug Delivery Reviews, 60, 2008, p. 1037.
14. PAPAGIANNAROS, A., DIMAS, K., PAPAIOANNOU, G.T., DEMETZOS, C., International Journal of Pharmaceutics, 302, 2005, p. 29