FT-IR Spectroscopy and Thermogravimetrical Characterization of Prodrugs Based on Different Dendritic Polymers and Antitumoral Drug

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The aim of the present study was to synthesize and characterize some bioconjugated systems based on dendritic polymers and paclitaxel. Three types of polyamidoamine dendrimers (PAMAM) with amine terminated groups and different generations (PAMAM-G1-NH_2, PAMAM-G4- NH_2, PAMAM-G6-NH_2) were used as polymeric hosts. The bioconjugated systems were characterized using FT-IR Spectroscopy and thermogravimetrical analysis.

Keywords: dendrimers, paclitaxel, FTIR spectroscopy

Paclitaxel is a diterpenoid extracted from the bark of yew (Taxus brevifolia). In 1992 this compound was approved as chemoterapeutic agent for a wide range of tumor for brest cancer, ovarian cancer, non–small cell lung cancer (NSCLC), head and neck cancer and cervical cancer [1-7]. Paclitaxel is called a mitotic inhibitor [8]. Like other antitumoral agents (cisplatin, doxorubicin), Paclitaxel is insoluble in water and in his natural state is toxic for human body. Another disadvantage of Paclitaxel is the high price because it is found in extremely low amount in the barks and roots of taxus [9-13].

In order to increase the Paclitaxel water solubility different methods like microparticles, complexation with cyclodextrines and drug dispersion in carriers, nano-suspension, inclusion complex formation technique, floating granules, modification of the crystal habit, solubilization by surfactants were developed [14, 15]. A recent method used to enhance the water solubility of Paclitaxel involves the prodrug conjugation using small molecules or polymers as solubilization entites [16].

Dendrimers can be used in this case due to the high number of functional groups which implies a high number of cavities [17].

Dendritic polymers like polyamidoamine (**PAMAM**) are highly hyperbranched synthetic polymers with some special structures which involves a well defined spherical structure, nanometer scale size, reactive functional groups and a hydrophilic behaviour. The surface functional groups can be used for covalently attach diferent compounds like drugs, targeting agent and imaging agent.

Through the covalent binding of drug functional groups of dendrimers it is obtained a complex called prodrug [18]. Different linkers like succinic acid, succinic anhydride, glutaric acid, gallic acid [19], Gly-Leu-Phe-Gly tetra-peptide are considered good candidates for PAMAM dendrimer conjugates. Recently aminoacids were proposed as versatile linkers for dendrimers [20].

The objective of the current study is to characterize the conjugated systems based on Paclitaxel and different dendritic molecules using FTIR Spectroscopy and thermogravimetrical analysis.

Experimental part

Materials

The antitumoral drug (Paclitaxel), dimethylsulfoxide (DMSO), N,N2 -Dicyclohexylcarbodiimide (DCC) and poly(amido amine) dendrimers (PAMAM) with ethylene-diamine core (EDA), aminic functional terminated groups and different generations (PAMAM-G1-NH_2, PAMAM-G4-NH_2, PAMAM-G6-NH_2) were supplied by Aldrich Chemical Company.

Synthesis of bioconjugated systems based on various dendritic polymers with terminal aminic groups and Paclitaxel by covalent method

The linking of the drug to the dendritic chain was done using a covalent method which consists in several steps according to figure 1. For the bioconjugated synthesis it was used a modified method reported in literature [21].

In the first step 20 mg of Paclitaxel was dissolved in 5 mL anhydrous DMSO and then 4.4 mg succinic acid was added. The reaction mixture was stirred for 48 h at room temperature in the presence of 4.6 mg DCC. This step was performed in order to introduce carboxylic acid groups into the drug molecule structure. The final product of this first step was purified by filtration and then the product was liophilized for 24 h.

In the *second step* the purified carboxylated modified drug was dissolved in 2 mL DMSO and 20 mg of DCC was added as activating agent and was stirred for 2 h. 1 mL of dendrimer methanolic solution was evaporated in order to remove methanol and then was added to the reaction mixture and stirred at room temperature for 3 days. The final product was purified by dialysis method for 3 days using DMSO as buffer. The product was liophilized for 24 h.

Characterization techniques

FTIR analysis

FTIR spectra of drug, dendrimers and complexes 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.

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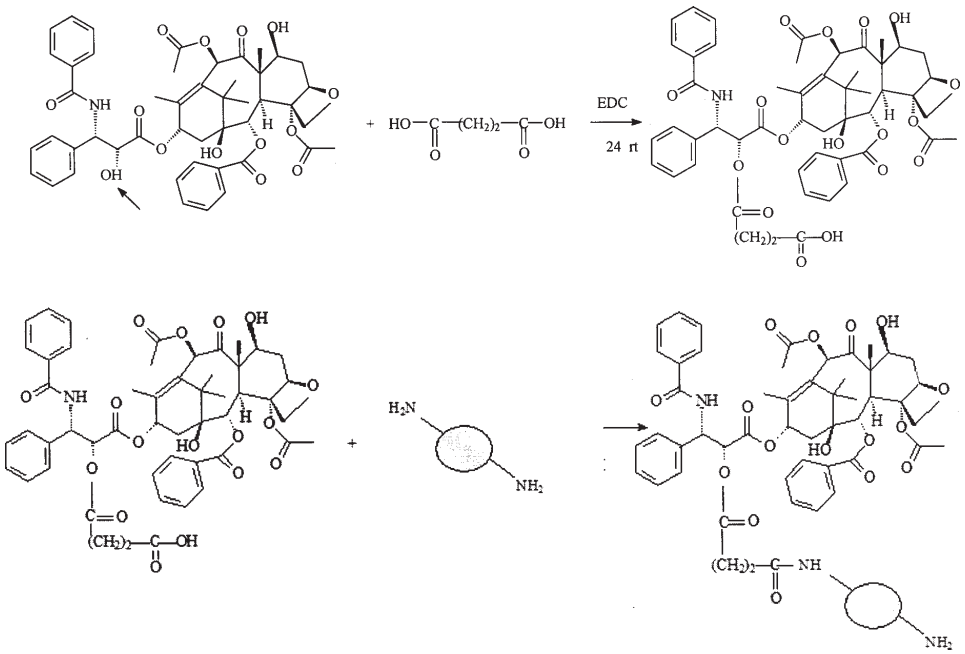

Fig. 1. Synthesis of PAMAM -NH_2-drug bioconjugates

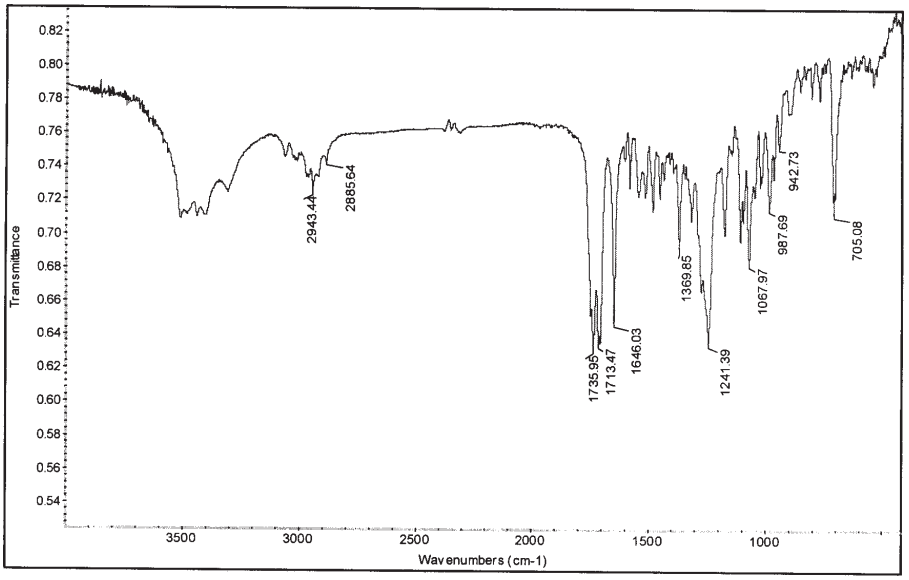

Fig. 2. FT-IR spectrum of Paclitaxel

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

FT-IR Spectroscopy may be considered a suitable techniques for studying the interactions of drug with polymers. In the present study we report the use of FT-IR Spectroscopy to characterize the bioconjugated systems based on Paclitaxel and different PAMAM dendritic polymers. In the literature a relatively few published studies regarding FT-IR Spectroscopic characterization of dendrimer-antitumoral drug complexes were reported [22-25].

In the first step it was necessary to record the FTIR spectrum of the antitumoral agent (Paclitaxel) in order to be used for comparison with those of drug-dendrimer bioconjugated systems.

The FTIR spectrum of Paclitaxel is shown in figure 2.

The main infrared peaks of the Paclitaxel are as follows: N-H stretching vibrations at 3479-3300 cm^{-1}, CH_2 asymmetric and symmetric stretching vibrations at 2976-2885 cm^{-1}. The peaks situated at 1735 and 1713 cm^{-1} are assigned to C=O stretching vibration from the ester groups. The amide bound was located around 1646 cm^{-1}. Ester bond stretching vibrations and C-N stretching vibrations are situated at 1241 cm^{-1}, and 1273 cm^{-1} respectively.

Absorption at 1604, 1068, 943 and 705 were assigned to the aromatic bonds. Thus, the C=C stretching vibrations are located at 1604 cm^{-1} and C-H out of plane deformation bands at 1068, 943 and 705 cm^{-1}.

In the next figures (figs. 3-4) are shown the FTIR spectra of bioconjugated systems based on different dendritic polymers (PAMAM-G1-NH_2, PAMAM-G4-NH_2) and Paclitaxel.

The presence of Paclitaxel within dendritic molecules (PAMAM-G1-NH_2, PAMAM-G4-NH_2, PAMAM-G6-NH_2) was proved by FTIR Spectroscopic analysis.

Thus, in all FTIR spectra of bioconjugated systems based on PAMAM polymers and Paclitaxel some characteristic peaks of Paclitaxel drug are detected. The presence of new peaks at 954 and 706 cm^{-1} in the FTIR spectra of bioconjugated systems proved the PAMAM-drug interactions. The peak at 954 cm^{-1} appears at higher frequency than in Paclitaxel spectrum and this fact suggests the presence of some interactions between the Paclitaxel and dendrimers.

The other peaks detected in the FTIR spectra of bioconjugated compounds were assigned to the

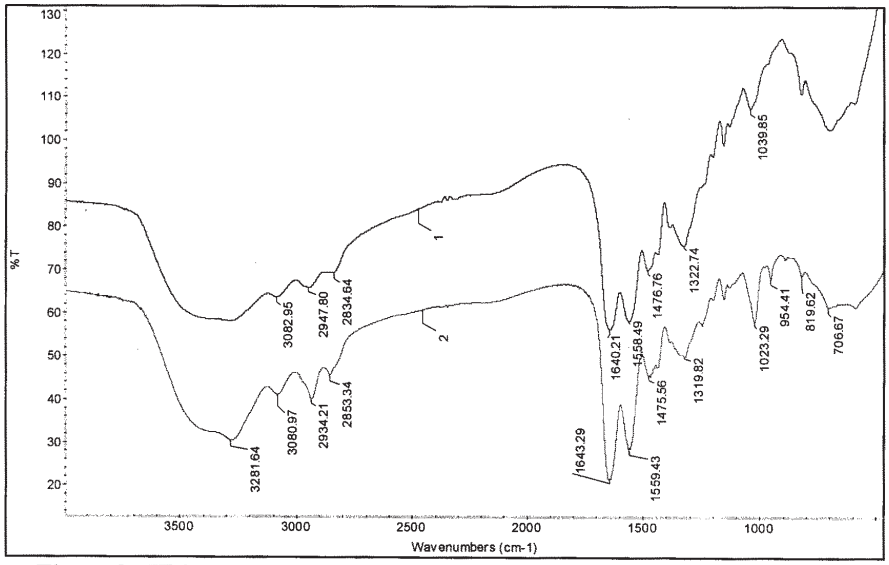

Fig. 3. FT-IR spectrum of 1- PAMAM-G1-NH_2, 2- PAMAM-G1- NH_2-Paclitaxel bioconjugated system

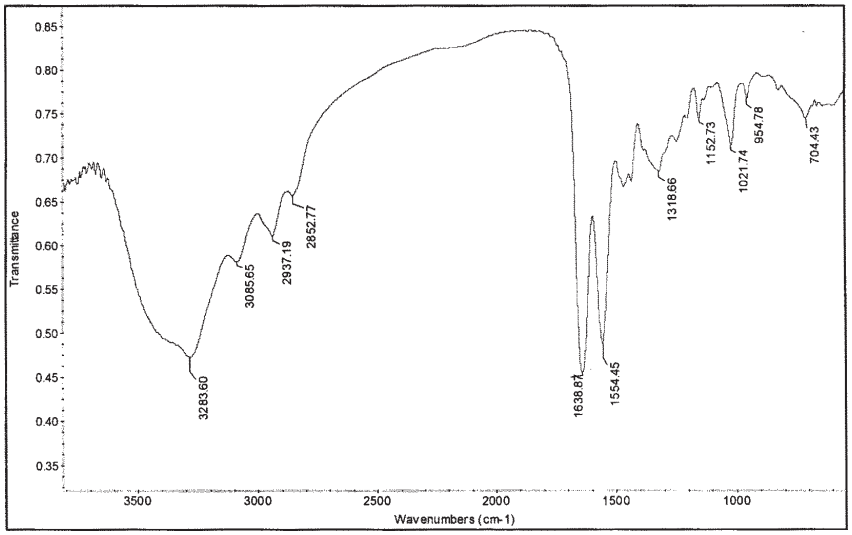

Fig. 4. FT-IR spectrum of PAMAM-G4-NH_2-Paclitaxel bioconjugated system

Table 1
SPECTRAL ASSIGNMENTS FOR BIOCONJUGATED SYSTEMS

Wave numbers (cm^{-1})			Spectral assignments
PAMAM G1-Paclitaxel	**PAMAM G4-Paclitaxel**	**PAMAM G6-Paclitaxel**	
3395-3310			NH stretching vibrations
2934, 2853	2937, 2853	2933, 2853	C-H stretching vibrations
1643	1639	1640	Amide I mode vibrations
1554	1554	1557	Amide II mode vibrations
1475	1471	1467	CH_2 scissoring vibrations
1319	1319	1321	CH_2 twisting vibrations

characteristic bonds presented in PAMAM molecules. Assignments of these peaks are summarized in table 1.

Thermogravimetric analysis

The TGA and DTG curves of Paclitaxel and bioconjugated systems are shown in figure 5 and the thermogravimetrical data are summarized in table 2.

The Paclitaxel shows an initial degradation temperature at 231.6°C, while the PAMAM-Paclitaxel bioconjugates exhibit a lower thermal stability in comparison with drug molecules. For the bioconjugated systems a total degradation was achieved at 500°C.

The DTG curve of Paclitaxel exhibits one peak (240.4 °C) assigned to the degradation of drug molecules. For all the bioconjugated systems the DTG curves present two peaks assigned to the polymer-drug complexes degradation.

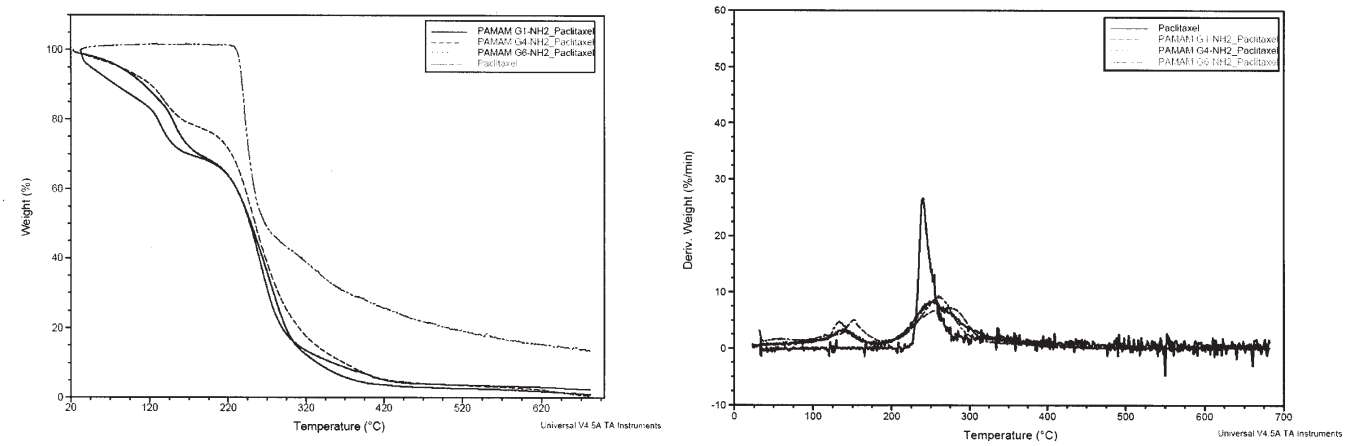

Fig. 5. TGA and DTG curves of Paclitaxel and bioconjugated systems based on different dendritic polymers and Paclitaxel

Sample	Weight Loss %	T_{max} (°C)
Paclitaxel	86.5	240.4
PAMAM-G 1-NH_2-Paclitaxel	98.9	151.8; 273.9
PAMAM-G 4-NH_2-Paclitaxel	99.6	136.9; 251.8
PAMAM-G 6-NH_2-Paclitaxel	97.6	133.7; 260.3

Table 2
THERMOGRAVIMETRIC DATA FOR PACLITAXEL AND BIOCONJUGATED SYSTEMS

Conclusions

Some bioconjugated systems based on Paclitaxel and various dendritic polymers (PAMAM-G1-NH_2, PAMAM-G4-NH_2 and PAMAM-G6-NH_2) which exhibit different generations were synthesized. The incorporation of the Paclitaxel within the dendritic polymers is shown by the presence of some new peaks which exist in the drug molecules. Thermogravimetric analysis indicates a different thermal degradation mechanism of bioconjugated systems than for drug molecules.

Acknowledgements: Executive Agency for Higher Education, Research, Development and Innovation Funding (UEFISCDI) and National Research Council (CNCS) are gratefully acknowledged for the financial support through the research project for the stimulation of the forming of young independent research teams "New controlled drug released systems based on polymer-layered silicate nanocomposites for cancer therapy", TE-142, No. 5/4.08.2010

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Manuscript received: 23.11.2011