Hybrid Materials based on Dendritic Polymer and Halloysite

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This study was focused on the synthesis of some new hybrid materials based on poly(amidoamine) dendrimer and halloysite designed for 5-Fluorouracil encapsulation. Different techniques like Spectroscopic methods (FTIR, XPS and UV-Vis) and thermogravimetric analysis were used to characterize the hybrid materials. The morphology of the hybrid materials was pointed out using Scanning Electron Microscopy (SEM) analysis. The obtained results showed that the drug encapsulation efficiency of the hybrid materials was strongly influenced by the interactions between dendrimer-halloysite and drug molecules.

Keywords: dendrimer, halloysite, 5-fluorouracil, UV-Vis, X-Ray Photoelectron Spectroscopy

The hybrid materials based on polymers and layered silicates represent a new concept of drug delivery system. Depending on the polymer-silicate ratio, polymer structure, drug structure and silicates properties, these systems can be classified as follows:

(1)- *hybrid systems* in which the drug entrapment occurred within the layered silicates and the polymer acts as a polyelectrolyte in order to adjust the drug release rate [1].

(2)-*polymer-clay nanocomposites* in which the polymer matrix is the main host for drug encapsulation. In this case the layered silicates are used in low concentrations to increase the mechanical properties or other properties like swelling degree [2-7].

(3)- *hybrid systems* in which both polymeric and layered silicates act like hosts for drug molecules [8,9]. An example of such of system was developed by our research group [8]. These hybrid systems involve two type of hosts: sodium montmorillonite used as inorganic host and two type of poly(amidoamine) dendrimers with different generations as a polymeric host. The using of dendrimers for drug encapsulation was motivated by the special hyperbranched structure with a high concentration of terminal functional groups which ensure their ability to encapsulate drug molecules by covalent or non-covalent interactions [10-12].

The montmorillonite is the most used classical clay involved in the development of drug release systems due to its high cation exchange capacity [13].

Halloysite is another natural clay which exhibits a similar chemical structure with that of kaolin but with a different morphology that depends on several factors like crystal structure, chemical composition, dehydration effects, content and type of impurities [14]. The Halloysite with a tubular morphology, characterized by an external diameter of 50-80 nm, lumen of 10-15 nm and length of about 1000 nm, shows attractive properties that recommend it as a nanocontainer which can be loaded with corrosion inhibitor agents, drug molecules and other active agents [15, 16].

Due to the hydrophilic nature, the halloysite can be introduced into the polar polymers such as polyamides, polyacrylates, chitosan, pectin, starch, polyvinyl alcohol in order to enhance the thermal or/and mechanical properties [17]. In addition this layered silicate can be used as rheological additive, flame retardant agent and as a special additive in packaging industry. The new class of hybrid catalysts used for atom transfer radical polymerization (ATRP) technique is another example of halloysite application. In this case transition metals are immobilized within the halloysite structure [18]. The acid treated halloysite was found to be an effective catalyst in the thermal recycling of polymeric wastes [19, 20].

The main goal of our study consists in the development of new organic-inorganic materials based on dendrimer and halloysite. Third generation Poly(amidoamine) dendrimer (PAMAM G3) with ethylene core and terminal amino groups was used as an organic host, while halloysite clay with a tubular morphology represents the inorganic component involved in the hybrid material synthesis. The synthesis of hybrid materials was performed using aqueous solutions with different *p*H value (*p*H = 1.2; *p*H=7.4 and *p*H =11). The synthesized hybrid materials were characterized using different techniques like FTIR Spectroscopy, X-Ray Photoelectron Spectroscopy (XPS), UV-Vis Spectroscopy and thermogravimetrical analysis. The morphology of hybrid materials was pointed out using Scanning Electron Microscopy (SEM).

Experimental part

Raw materials

5-Fluorouracil (5FU) and third generation poly (amidoamine) dendrimer (PAMAM G3) with ethylene-diamine core and amino terminal groups were supplied from Sigma-Aldrich.

Halloysite clay (HNT) was also supplied from Sigma-Aldrich. The HNT exhibits a diameter between 30-70 nm, a length of 1.3μm, a pore size volume of 1.26-1.34 mL/g and a surface area of 64 m^2/g.

Synthesis of PAMAM-G3-HNT-5FU hybrid materials

The synthesis of hybrid materials based on PAMAM G3, halloysite and 5FU were performed using the following strategy. In the first step 0.1 mL of PAMAM-G3 was concentrated using a rotary evaporator and then 10 mL of solution with different *p*H (1.2; 7.4; 11) were added. In the second step 0.075 g of 5FU were added to the solutions and the resulted solutions were maintained for 1 h at room

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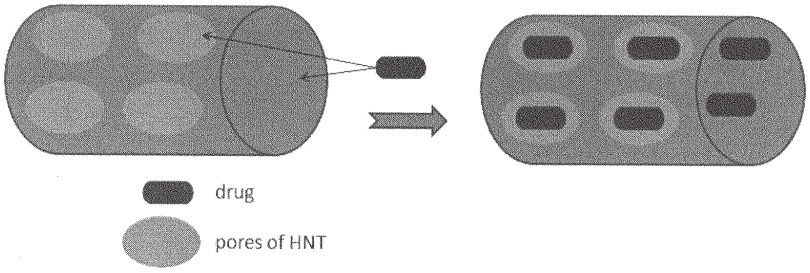

Fig. 1. Drug encapsulation within the HNT pores and lumen

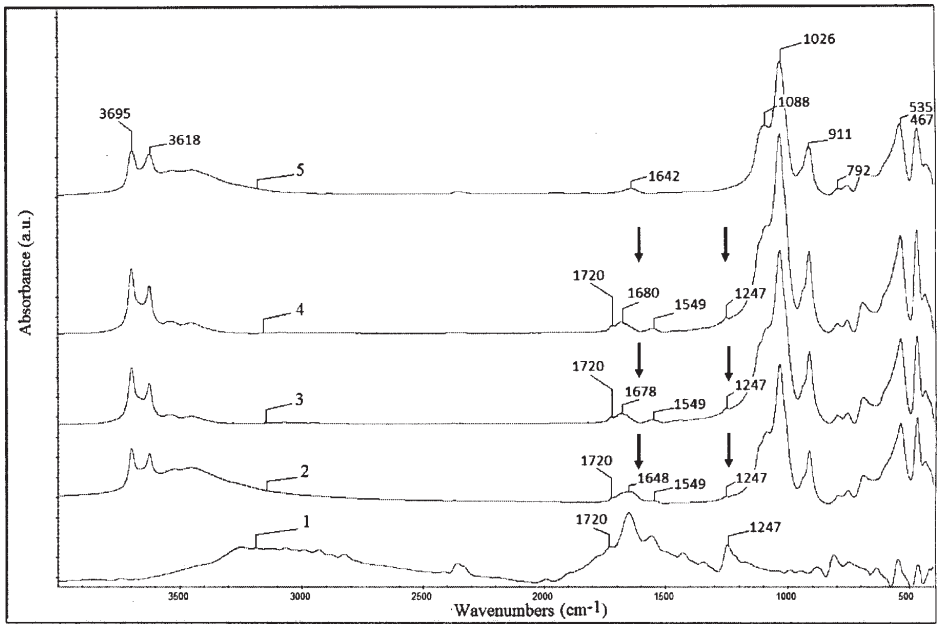

Fig.2. FTIR spectra of 1-5FU; 2-PAMAM-G3-HNT-5FU-1.2; 3-PAMAM-G3-HNT-5FU-7.4; 4-PAMAM-G3-HNT-5FU-11; 5-HNT

temperature, under stirring until the drug was dissolved. In the last step 0.4 g of HNT were added. The obtained suspensions were stirred for 24 h at room temperature and then was centrifuged at 4000 rpm for 10 min. The hybrid materials were freeze dried at -60°C for 3.5 h. The synthesized hybrid materials using solutions with different pH value were abbreviated as PAMAM-G3-HNT-5FU-1.2, PAMAM-G3-HNT-5FU-7.4 and PAMAM-G3-HNT-5FU-11.

Characterization techniques

FTIR spectra were recorded on a Bruker VERTEX 70 spectrometer using 32 scans with a 4 cm^{-1} resolution.

Thermogravimetrical analysis (TGA) was done on a Q 500 TA equipment. The samples of 3 mg were heated from 20 to 800 °C at a scanning rate of 10°C/min under a constant nitrogen flow rate (40 mL/min).

UV-Vis spectra were recorded using a UV-3600 Shimadzu equipment provided with a quartz cell having a light path of 10 mm.

X-Ray Photoelectron Spectroscopy (XPS) spectra were recorded on Thermo Scientific K-Alpha equipment, fully integrated, with an aluminum anode monochromatic source.

The morphology of the final hybrid materials was studied using a Scanning electron microscope SEM Quanta Inspect F. The samples were sputtered with a thin gold layer.

In vitro drug release

The release of 5FU from organic-inorganic hybrid materials was performed in a shaking bath by suspending dialysis tube membrane (MWCO =3500 Da) containing a certain quantity of hybrid materials and 5 mL buffer solution of *p*H 7.4 in 200 mL of the same buffer solution. Rotation speed was 100 rpm, and the temperature was kept constantly at 37°C. At precise interval times, 3 mL of the dissolution medium were manually taken out and replaced with fresh dissolution medium. The concentration of 5FU was determined by UV adsorption at 266 nm.

Results and discussions

Due to the dendrimer properties, HNT morphology (tubular morphology) and porosity, the drug retention (encapsulation) within the hybrid host can take place via multiple interactions which include: (1) drug adsorption on the HNT pores, (2) drug entrapment within the HNT lumen, (3) drug adsorption on the dendrimer by supramolecular complexes formation [21] (fig. 1).

Characterization of hybrid materials

FTIR analysis

The presence of organic fractions (PAMAM-G3 and 5FU drug) within the HNT was firstly checked by FTIR Spectroscopy for all the samples prepared at different *p*H values (fig. 2).

As one may observe from figure 2 the FTIR spectrum of unmodified HNT shows several characteristics peaks as follows: 3695 and 3618 cm^{-1} (assigned to stretching vibration of inner-surface hydroxyl groups), 1642 cm^{-1} (assigned to interlayer water), 1088-1026 cm^{-1} corresponding to Si-O stretching vibration, 911 cm^{-1} assigned for O-H bending vibrations of inner hydroxyl group, 792 cm^{-1} for Si-O symmetric stretching, 535 cm^{-1} assigned for Al-O-Si bending vibrations, 467 cm^{-1} assigned for Si-O-Si bending vibrations [22- 24].

The presence of dendritic polymer (PAMAM-G3) within HNT was confirmed by FTIR analysis through the presence of new peak at 1549 cm^{-1} assigned to the amide II vibration involved in the dendrimer structure. This peak appeared at the same value of wavenumbers for all hybrid systems. Regarding the peak corresponding to the amide I vibration from the dendrimer structure, this was also identified in the FTIR spectra of hybrid materials at different

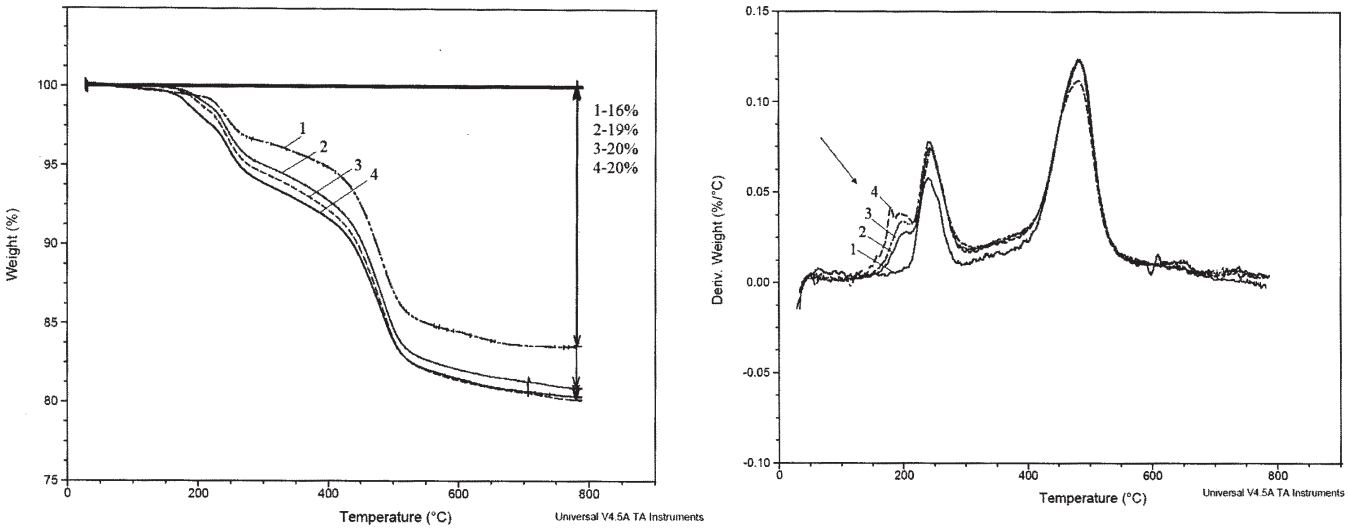

Fig. 3. TG and DTG curves of 1-HNT; 2-PAMAM-G3-HNT-5FU-1.2; 3- PAMAM -G3-HNT-5FU-11; 4- PAMAM -G3-HNT-5FU-7.4

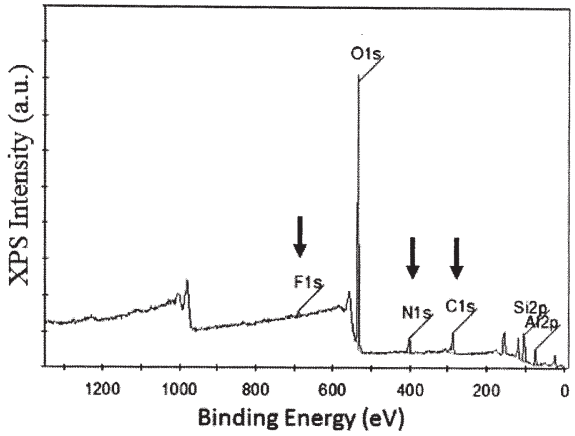

Fig.4. XPS survey spectrum of PAMAM-G3-HNT-5FU-7.4 hybrid material

wavenumbers (1648, 1678 and 1680 cm^{-1}) values which increased with the increases of pH value.

The presence of drug molecules within the hybrid host was also confirmed by FTIR analysis. The peaks at 1720 and 1247 cm^{-1} corresponding to carbonyl and ring stretching vibrations from the 5FU structure were detected.

TGA characterization

The presence of organic fraction within HNT was also confirmed by thermogravimetrical analysis (fig. 3). The thermogravimetrical results showed that the hybrid materials exhibit a different degradation behaviour in comparison with the HNT. The increase of the weight loss for hybrid materials was assigned to the thermal degradation of organic fractions (PAMAM G3 and 5FU). The presence of organic fractions was also identified in DTG curves of hybrid materials through the appearance of new peak with a T_{max} around 178-194 °C. The PAMAM-G3 dendrimer exhibits a similar thermal stability like 5FU and therefore it was difficult to assign that peak for drug or dendrimer degradation.

XPS characterization

The XPS analysis was used to confirm the presence of organic fractions (PAMAM-G3 and 5FU) on the clay surface (fig. 4). Figure 4 shows an example of XPS spectrum of hybrid material based on PAMAM-G3, HNT and 5FU synthesized at *p*H 7.4.

The presence of signals assigned to C1s, N1s and a small signal at a binding energy of 687eV corresponding to F1s demonstrated the presence of dendrimer and drug molecules within the HNT surface. In addition the XPS results highlighted a dependence of drug quantity retained on the PAMAM G3-HNT hosts surface on *p*H value (table 1). For instance in case of hybrid material synthesized at *p*H higher than 7 the presence of 5FU on the material surface was detected, while for hybrid material synthesized using an acid solution the signal assigned to the F1s was not detected.

These results lead to the conclusion that the interactions between the hybrid materials components (dendrimer-drug-clay) are strongly influenced by the solution *p*H used for materials synthesis.

SEM analysis

The morphology of hybrid materials based on PAMAM-G3, HNT and 5FU was studied using SEM analysis (fig. 5). The SEM images of hybrid materials showed that the tubular morphology of HNT was maintained regardless the pH value of the reaction medium.

UV-VIS analysis

The study of drug encapsulation within the hybrid hosts based on HNT and PAMAM G3 was performed using UV-VIS analysis. The drug encapsulation efficiency (EE (%)) was calculated with the following equation (1) [25]:

$$EE\,(\%) = \frac{\text{Total amount of 5FU - Total amount of free 5FU}}{\text{Total amount of 5FU}} \times 100 \quad (1)$$

The changing of reaction medium *p*H value used for the hybrid material synthesis has a strongly influence on the dendrimer charging and also on to the HNT charge.

Thus at a *p*H value higher than 6 the HNT surface can be considered positive which allows negative species loading. At *p*H values lower than 6, the HNT exhibits a negative zeta-potential [26].

Regarding the dendrimers charging, the experimental results reported in the literature showed that the dendrimers with amino terminal groups are macromolecular compounds which exhibits a weak polyelectrolytes behaviour. A decrease of *p*H value leads to an increase of protonation degree of amino groups from dendritic structure and thus the charge increases [27-29].

At lower *p*H the drug was mainly encapsulated within dendrimers because the access of drug to the HNT was hindered by the dendrimer which covers the surface due

Sample	Si2p %	Al2p %	C1s %	O1s %	N1s %	F1s %
PAMAM-G3-HNT-5FU-1.2	16.2	14.3	12.6	52.4	4.5	-
PAMAM-G3-HNT-5FU-7.4	13.2	15.1	14.7	50.8	4.8	**1.4**
PAMAM-G3-HNT-5FU-11	16.3	13.3	13.6	49.9	5.2	**1.7**

Table 1
XPS ANALYSIS RESULTS FOR HYBRID MATERIALS SYNTHESIZED AT DIFFERENT *p*H VALUES

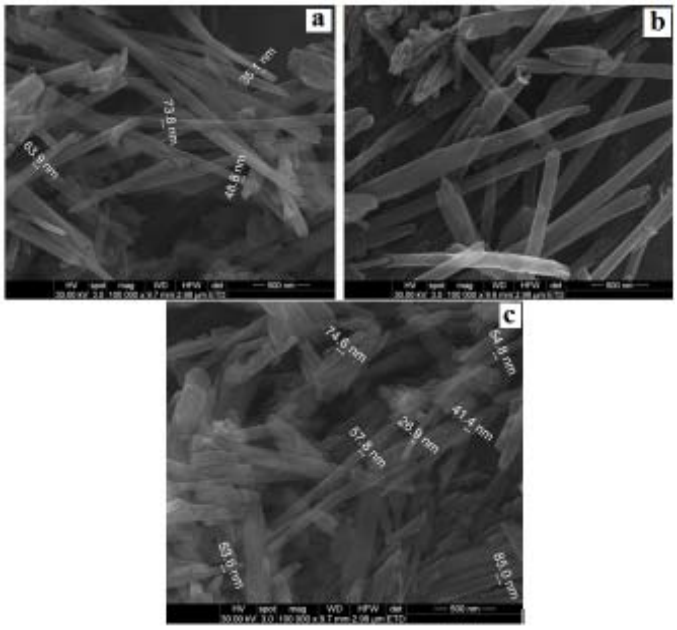

Fig.5. SEM images of hybrid materials synthesized at different pH values: a-1.2, b-7.4, c-11

to the electrostatic interactions occured between the negative HNT surface and positive charged dendrimer. In this case the HNT loses the host role. Therefore this hybrid material exhibits the lowest EE value (21 %).

In case of hybrid system obtained at pH 7.4 the dendrimer charge density decreases (only the primary terminal amino groups were protonated) and thus the HNT surface was covered by a lower dendrimer concentration. The HNT partially regains the host role for drug entrapment. Due to the presence of two hosts (dendrimer and HNT) the drug encapsulation efficiency increased (EE=28 %).

The highest drug encapsulation efficiency (EE=49 %) was achieved for hybrid material synthesized at *p*H 11. At this *p*H value the electrostatic interactions between dendrimer and HNT significantly decreased. In addition at this *p*H value the HNT activation could occured [30].

Conclusions

Some new hybrid materials based on polyamidoamine and halloysite were developed in order to be used in biomedical applications. These hybrid systems were designed for 5-Fluorouracil drug encapsulation. The presence of organic fractions (PAMAM-G3 and drug molecules) within HNT was investigated using different characterization methods (FTIR Spectroscopy, thermo-gravimetrical analysis, X-Ray Photoelectron Spectroscopy and UV-Vis spectroscopy).

The drug encapsulation efficiency of the hybrid hosts (PAMAM-G3-HNT) was strongly influenced by the *p*H value of the reaction medium in which the hybrid materials were synthesized. The highest drug encapsulation efficiency was achieved at *p*H 11.

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: 27.11.2013