Hybrid Drug Release Systems based on Dendrimers and Montmorillonite

SORINA ALEXANDRA GAREA*, ADI GHEBAUR, EUGENIU VASILE
University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, 149 Calea Victoriei, 010072, Bucharest, Romania

The objective of this study was to develop some new hybrid hosts based on dendrimers and montmorillonite designed for 5-Fluorouracil drug encapsulation. The presence of drug within the hosts was pointed out using different methods like FTIR Spectroscopy, X-Ray Photoelectron Spectroscopy (XPS), Thermogravimetric analysis (TGA), UV-Vis Spectroscopy and X-Ray Diffraction. The UV-Vis results showed that the dendrimer type involved in the hybrid hosts influenced the drug release.

Keywords: antitumoral drug, dendrimer, montmorillonite, drug release system

The drug release can be adjusted using different strategy like synthesis of some pH-sensitive hydrogels, encapsulation of drug molecules within various hosts like inorganic hosts, polymeric microspheres, nanoparticles and biodegradable nanospheres and attachment to fluorescent magnetic graphene oxide hybrid materials [1-6].

Different polymers types were used in combination with inorganic hosts in order to develop new hybrid systems which can be used as host for drug encapsulation [7-11].

These organic-inorganic hybrid systems combine the advantages of polymers with the features of inorganic component.

Dendrimers can be considered a suitable class of polymer which can be used for the synthesis of new drug delivery systems due to their excellent properties like biocompatibility, high water solubility, encapsulation capacity of large amount of drugs due to their hyper-branched structure and conjugation capacity with targeting molecules [12,13]. The presence of terminal functional groups provide the ability to attach different substances which can act as imaging contrast agents or targeted agents for cancer tumor [14-16].

Montmorillonite is a natural clay and due to his high cationic exchange capacity a relatively high drugs quantity intercalates through the silicate layers. The drug-montmorillonite interaction can be influenced by several factors like *p*H value [17], reaction temperature [18], drug concentration [19] and reaction time [20].

This study was focused on the development of new hybrid hosts based on different dendritic polymers and montmorillonite for 5-Fluorouracil encapsulation. The encapsulation and drug release was studied using different methods like Spectroscopic methods (X-Ray Photoelectron Spectroscopy (XPS), UV-Vis and FT-IR Spectroscopy), X-Ray Diffraction (XRD) and Thermogravimetric analysis (TGA).

Experimental Part

Materials and methods

The antitumoral drug (5-Fluorouracil)-**5FU** and poly(amido amine) dendrimers (PAMAM) with ethylene-diamine core, aminic terminated groups and different generations (generation 1 (**PAMAM G1**), generation 3 (**PAMAM G3**)) were supplied by Aldrich Chemical Company.

Na-Montmorillonite (MMT-Na) (used as inorganic component in the hybrid hosts) with a Cationic Exchange Capacity (CEC) value of 92.6 meq/100g clay was purchased from Southern Clay Products.

Synthesis of hybrid materials based on montmorillonite, 5FU and dendritic polymers

The synthesis of hybrid materials based on montmorillonite, dendritic polymers (PAMAM G1, PAMAM G3) and 5FU were performed using the following procedure. 75 mg of 5FU were dissolved in 10 mL of solution with 11 pH value and then 0.4 g of montmorillonite and 100 μL of PAMAM G1/PAMAM G3 were added.

The obtained suspensions were stirred for 24 h at room temperature and then centrifuged at 4000 rpm for 10 min. The hybrid materials abbreviated MMT-PAMAM-G1-5FU, MMT-PAMAM-G3-5FU were liophylized at -60°C for 3.5 h.

A dendrimer free sample was also synthesized using a modified methodology reported [21]. 75 mg of 5FU were dissolved in 10 mL of solution with 11 *p*H value and then 0.4 g of MMT-Na were added. The obtained suspension was stirred for 24 h at room temperature and then centrifuged at 4000 rpm for 10 min.

The sample abbreviated MMT-Na-5FU was liophylized at -60°C for 3.5 h.

Characterization techniques

FTIR spectra were registered on a BRUCKER VERTEX 70 equipment using 32 scans and 4 cm^{-1} resolution. The samples were analyzed from KBr pellets.

X-Ray Diffraction (XRD) analysis was performed on a XRD 6000 SHIMADZU diffractometer.

The XPS spectra were recorded on Thermo Scientific K-Alpha equipment, fully integrated, with an aluminum anode monochromatic source.

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

UV-Vis absorbance of 5-FU was measured at λ = 266 nm on a UV-3600 Shimadzu equipment provided with a quartz cell having a light path of 10 mm and equipped with a Syringe Sipper Type N.

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

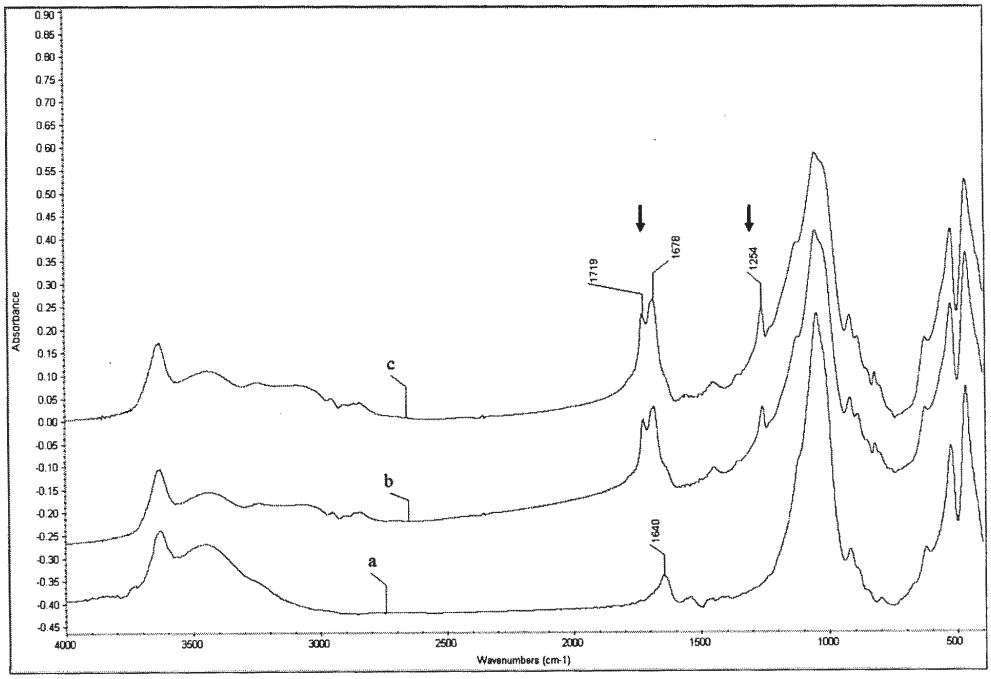

Fig. 1. FTIR spectra of a. MMT-Na; b. MMT-PAMAM G1-5FU; c. MMT-PAMAM G3-5FU

In vitro drug release

The release of 5 FU from hybrid hosts was performed in a thermostated 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 200mL of the same buffer solution. Rotation speed was 100 rpm, and the temperature was kept constantly at 37°C. At intervals of 30 min, the dissolution medium was taken out and the 5 FU concentration was determined by UV absorption at 266 nm and then the analyzed solution was put back to maintain a constant volume.

Results and discussions

Characterization of hybrid materials

FTIR analysis

The hybrid materials based on montmorillonite, 5FU and dendritic polymers were initially characterized by FTIR Spectroscopy.

The presence of 5FU within hybrid hosts was proved by the appearance of new peaks at 1719, 1678 and 1254 cm^{-1} which are also detected in the FTIR spectrum of drug. These peaks were assigned to the carbonyl stretching vibrations, -C=C- of pyrimidine stretching vibration and ring stretching mode vibration.

XRD analysis

The 5FU can be retained in hybrid hosts (MMT-PAMAM-G1/MMT-PAMAM-G3) via two possible routes:

1. incorporation of the drug molecules within the dendritic polymers and 2.encapsulation of the drug within the silicate layers.

In our previous article we proved that the dendritic polymers (PAMAM G1 and PAMAM G3) were mainly accumulated on the surface of montmorillonite due to the interactions with the hydroxyl groups from the surface of inorganic host [22]. The XRD results confirm a small increase of basal distance which leads to the conclusion that the PAMAM molecules are difficult to be intercalated within the layered silicates so that the PAMAM molecules are mainly attached to the surface of layered silicate regardless of PAMAM generation.

The capacity of dendritic polymers to encapsulate the 5-Fluorouracil using non-covalent method was tested in our previous study and we may concluded that the incorporation of the 5FU molecules into the dendrimers can occurred and it strongly depends on the dendrimer generation [23].

The XRD results showed some differences between the hybrid hosts treated with 5FU (table 1).

The MMT-PAMAM-G1-5FU system exhibits the highest value of interlamelar distance (d_{001}) which leads to the conclusion that some interactions between drug, dendrimer and layered silicates occurred. This increase of interlamelar distance is difficult to be assigned to the intercalation of drug between the silicate layers or to the partial intercalation of dendrimer within the silicates because both lead to a small increase of interlamelar distance.

TGA tests

The presence of 5FU within the hybrid hosts was also investigated using thermogravimetrical analysis (table 2, fig.2).

As one may observe the hybrid hosts exhibit higher weight loss than unmodified MMT (MMT-Na) assigned to the thermal degradation of dendritic molecules and 5FU fraction retained within these hosts.

XPS analysis

The XPS analysis was used to confirm the presence of 5FU within hybrid hosts (MMT-PAMAM-G_1, MMT-PAMAM-

System	2θ (°)	d_{001} (Å)
MMT-Na	7.34	12.02
MMT-Na-5FU	7.09	12.46
MMT-PAMAM-G1-5FU	6.69	13.19
MMT-PAMAM-G3-5FU	7.05	12.52

Table 1
XRD RESULTS FOR DIFFERENT HOSTS TREATED WITH 5FU

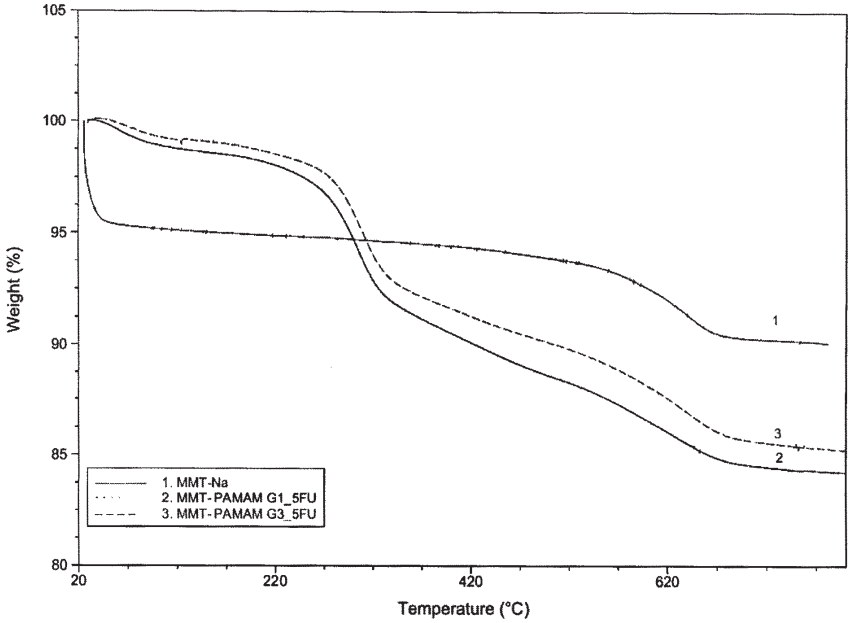

Fig.2. TGA curves of different hybrid host treated with 5FU

Table 2
TGA RESULTS FOR DIFFERENT HOSTS TREATED WITH 5FU

System	Weight Loss (%)
MMT-Na	10
MMT-PAMAM-G1-5FU	16
MMT-PAMAM-G3-5FU	15

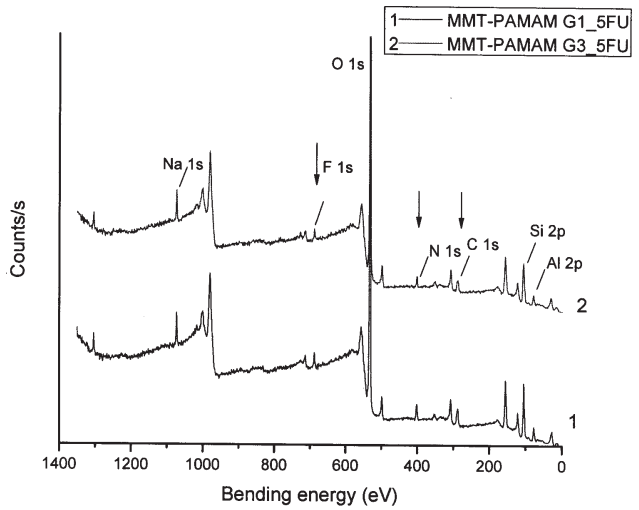

Fig. 3. XPS survey spectra of hybrid hosts treated with 5FU

Table 3
XPS RESULTS FOR INORGANIC AND HYBRID HOSTS TREATED WITH 5FU

System	C1s	N1s	F1s
MMT-Na	-	-	-
MMT-Na-5FU	5.6	2.07	1.63
MMT-PAMAM G1-5FU	8.42	3.51	2.17
MMT-PAMAM G3-5FU	7.08	2.63	2.22

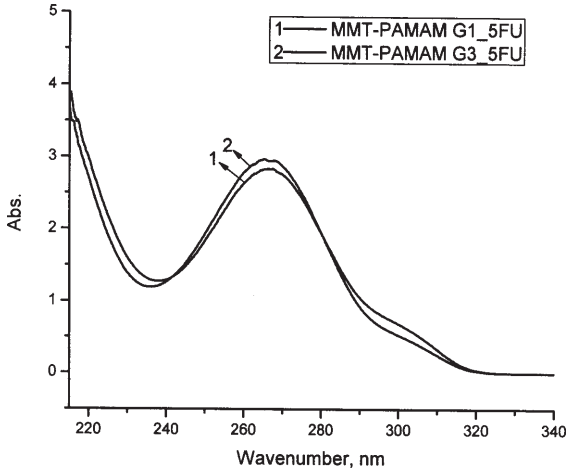

Fig. 4. UV-Vis spectra of water resulted after centrifugation

G_3) (fig.3). The presence of the drug molecule within hybrid hosts was proved by the appearance of new peak assigned to F1s. This peak was detected for all systems but in different percentages (table 3).

UV-Vis analysis

The UV-Vis analysis was useful to establish the 5FU encapsulation efficiency within designed hosts and also to represent the curve of drug release against time.

The encapsulation efficiency (EE (%)) was calculated with the following equation [24]:

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

As one may observe from figure 4 the drug was not fully encapsulated in the hosts. A high quantity of free drug was detected in the water resulted after centrifugation (fig. 4). The UV-Vis data showed that the highest EE of 5FU was achieved for MMT-PAMAM-G_1 (22 %) while MMT-PAMAM-G_3 exhibits 18 % EE.

Regarding on the drug release from hybrid hosts we observed that the 5FU release occurred with a lower rate in case of hybrid systems which contain a dendrimer with a low generation value (MMT-PAMAM-G_1) (fig. 5). The hybrid system which contains PAMAM-G1 dendrimer exhibits a more space available for drug intercalation between silicate layers and thus the drug release occurred with a lower rate.

In case of hybrid system based on PAMAM-G_3 which exhibits a hyperbranched structure than PAMAM-G1 an accumulation of dendritic chains onto the surface of montmorillonite may hinder the access of 5-FU to the gallery of layered silicates and thus the montmorillonite loses the host properties. The XRD results indicated a very small increase of basal distance for this system and thus we may concluded that in this case the drug molecules

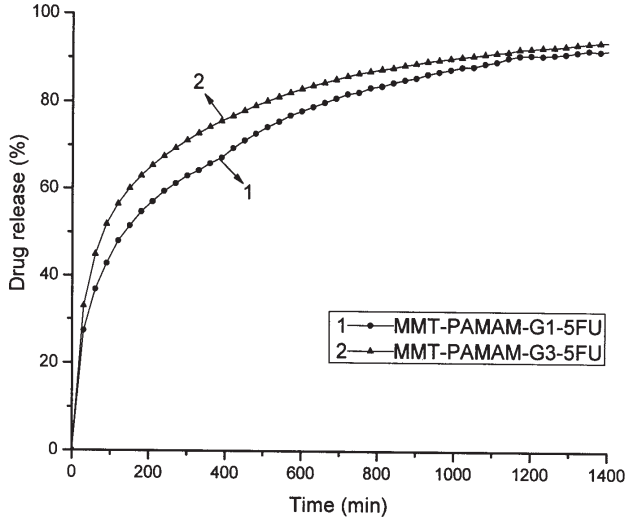

Fig 5. 5 FU release from different hybride hosts

are mainly entrapped within the polymeric host (PAMAM-G_3) and were released with a higher rate than the drug intercalated between the silicate layers.

Conclusions

The 5-Fluorouracil encapsulation within some new hybrid hosts based on different dendrimers and montmorillonite was investigated using different spectroscopic methods (FTIR, XPS, UV-Vis), XRD and thermogravimetrical analysis. It was shown that the drug encapsulation and release is influenced by the dendimer type. In case of hybrid system which contains dendrimer characterized by a higher generation (PAMAM-G_3) the drug encapsulation occured especially within the organic host, the access to the inorganic host beeing hindered.

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

References

1. KEVADIYA, B.D., THUMBAR, R.P., RAJPUT, M.M., RAJKUMAR, S. BRAMBHATT, H., JOSHI, G.V., DANGI, G.P. MODY, H.M., GADHIA, P.K. BAJAJ H.C., Eur. J. Pharm. Sci., 47, nr. 1, 2012, p. 265
2. BHAT, S.K., KESHAVAYYA, J., KULKARNI, V.H., REDDY, V.K.R., KULKARNI, P.V., KULKARNI, A.R., J. Appl. Polym. Sci., 125, nr. 3, 2012, p. 1736
3. CHANG, L., CHEN, S., JIN, P., LI, X., J. Colloid. Interf. Sci., 388, nr. 1, 2012, p. 9
4. WILSON, B., AMBIKA, T.V., DHARMESH KUMAR PATEL, R., JENITA, J.L., PRIYADARSHINI, S.R., Int. J. Biol. Macromol., 51, nr. 5, 2012, p. 874
5. CALDERINI, A., PESSINE, F.B.T., FRANCHI, G.C., NOWILL, A.E., Int. J. Nanotechnol., 9, nr.. 10-12, 2012, p. 851.
6. MISHRA, R.K., RAMASAMY, K., MAJEED, A.B.A., , J. Appl. Polym. Sci., 126, nr. S2, 2012, p. E98.
7. SALCEDO, I., AGUZZI, C., SANDRI, G., BONFERONI, M.C., MORI, M., CEREZO, P., SÁNCHEZ, R., VISERAS, C., CARAMELLA, C., Appl. Clay. Sci., 55, 2012, p. 131.
8. AGUZZI, C., CAPRA, P., BONFERONI, C., CEREZO, P., SALCEDO, I., SÁNCHEZ, R., CARAMELLA, C., VISERAS, C., Appl. Clay. Sci., 50, 2010, p. 106.
9. DARDER, M., COLILLA, M., RUIZ-HITZKY, E., Chem. Mater., 15, nr. 20, 2003, p. 3774.
10. PINTO, F.C.H., SILVA-CUNHA, A., PIANETTI, G.A., AYRES, E., ORÉFICE, R.L., DA SILVA, G.R., J. Nanomater., 2011, 2011, p.1
11. NANDA, R., SASMAL, A., NAYAK, P.L., Carbohyd. Polym, 83, nr. 2, 2011, p. 988.
12. YANG, W., CHENG Y., XU, T., WANG, X., WEN, L., Eur. J. Med. Chem., 44, nr. 2, 2009, p.862
13. DUNCAN, R., IZZO, L., Adv. Drug. Deliver. Rev., 57, nr. 15, 2005, p. 2215
14. KONDA, S.D., AREF, M., WANG, S., BRECHBIEL, M., WIENER, E.C., Magn. Reson. Mater. Phy., 12, nr. 2-3, 2001, p. 104.
15. ZHANG, Y.H., THOMAS, T.P., LEE, K.H., LI, M.H., ZONG, H., DESAI, A.M., KOTLYAR, A., HUANG, B.H., HOLL, M.M.B., BAKER J.R., Bioorgan. Med. Chem., 19, nr. 8, 2011, p. 2557
16. TEKADE, R.K., KUMAR, P.V., JAIN, N.K., Chem. Rev., 109, nr. 1, 2009, p. 49.
17. SCIASCIA, L., LIVERI, M.L.T., MERLI, M., Appl. Clay. Sci., 53, nr. 4, 2011, p. 657
18.FENG, S.S., MEI, L. , ANITHA, P., GAN, C.W., ZHOU, W.Y., Biomaterials, 30, nr. 19, 2009, p. 3297
19. MENG, N., ZHOU, N.L., Appl. Clay. Sci., 70, 2012, p. 22.
20. SANCHEZ, R.M.T., GENET, M.J., GAIGNEAUX, E.M., AFONSO, M.D., YUNES, S., Appl. Clay. Sci., 53, nr. 3, 2011, p.366.
21. LIN, F.H., LEE, Y.H., JIAN, C.H., WONG, J.M., SHIEH, M.J., WANG, C.Y., Biomaterials, 23, nr. 9, 2002, p. 1981
22. GÂREA, S.A., IOVU, H., GHEBAUR, A., Nanotechnology In Polymers, Editura Studium Press LLC, Vijay Kumar Thakur şi A. S. Singha, U.S.A, 2012, p. 11, ISBN: 1-933699-90-6.
23. GÂREA, S.A., GHEBAUR, A., ANDRONESCU, C., Mat. Plast., 2011, **48**, no. 1, p. 17
24. ELDEEN, A., YASSIN, B., ANWER, M.K., MOWAFY, H.A., EL-BAGOY, I.M., EL-BAGORI, MOHSEN, A., ALSARRA, I.A.,. Int. J. Med. Sci., 7, nr. 6, 2010, p. 398

Manuscript received: 4.12.2012