SINTEZA ȘI CARACTERIZAREA MATERIALELOR HIBRIDE MEDICAMENT –ARGILE PENTRU APLICAȚII BIOMEDICALE UTILIZATE CA SISTEME CU ELIBERARE CONTROLATĂ- PARTEA I

SYNTHESIS AND CHARACTERIZATION OF DRUG - MINERAL CLAY HYBRID MATERIALS FOR BIOMEDICAL APPLICATIONS AS DRUG DELIVERY SYSTEMS – PART I

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In order to obtain a drug delivery system used in tumoral treatment, this study presents the synthesis of mineral clay /antitumor drug hybrid materials. There were used three types of mineral phases with different structural characteristics as matrix and epirubicin, fludarabine, gemcitabine as active substances. The obtaining process was performed by mixing an aqueous solution of the drugs with the swelled clay. For the active substances incorporation, drug-solutions were heated and added slowly in clays suspensions (at 60°C, using magnetic stirring - 350 rpm). The dried hybrid materials were obtained after the separation was performed by centrifugation of cooled suspension.

The synthesized hybrid materials were characterized using different experimental techniques as X-ray diffraction, thermal analysis, scanning electron microscopy etc.. The characterization gave information about the proper interlayer intercalation of cytostatic, the good adsorption of drug into the matrix and the microstructure.

În vederea obținerii unui sistem cu eliberare controlată folosit în tratamentul antitumoral, acest studiu prezintă sinteza unor materiale hibride medicament- argile. Au fost utilizate trei tipuri de faze minerale cu diferite caracteristici structurale ca matrice suport și epirubicină, fludarabină și gemcitabină ca substanțe active. Procesul de obținere a fost realizat prin amestecarea unei soluții apoase cu argilele. Pentru incorporarea substanțelor active, soluțiile medicamentoase au fost încălzite și adăugate încet în suspensiile argilelor (la 60°, sub agitare magnetică- 350 rpm). Materialele hibride uscate au fost obținute după separarea prin centrifugare a suspensiilor răcite.

Materialele hibride sintetizate au fost caracterizate folosind diferite tehnici precum difracția de raze X, analize termice, microscopie electronică de baleiaj, etc. Caracterizarea oferă informații despre intercalarea interstrat adecvată a citostaticelor, o adsorbție bună în matrice și microstructură.

Keywords: *epirubicin, fludarabine, gemcitabine, kaolinite, halloysite, montmorillonite, hybrid materials, drug delivery systems*

1. Introduction

Mineral clays are natural materials often used in drugs systems. Most important mineral clays are: kaolinite ($Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$), halloysite ($Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O + 2H_2O$), montmorillonite ($Al_2O_3 \cdot 4SiO_2 \cdot H_2O + H_2O$) and ilite.

Kaolinite and halloysite though the similar type of structure, less water is bound in halloysite readily removed by heating at 50 ° - 100 ° C, to form metahalloysite, halloysite like structure [1].

Characteristic of montmorillonite(MMT) is that between basic structural layers is fixed water adsorption, chemically unbound that is easily eliminated by heating. Water networked in MMT is harder to eliminate than kaolinite. As a result of their structure, montmorillonite clays, are characterized by a high plasticity. This is provided by placing the water in the package between the basic layers which for a certain thickness, acts as a lubricant [2].

Montmorillonite also has a high specific surface area, very good adsorption property, high cationic exchange capacity, outstanding adhesion capability and very good drug carriers.

Thus, MMT is a common ingredient in pharmaceutical products, such as excipient and active substance. The interplay of organic compounds between the layered anorganic material provides a very favorable route for preparing the organic-inorganic hybrid materials that combine the properties of both the anorganic host and the foreign organic compound [3].

Montmorillonite is considered a medical clay. In a strong detoxification process, MMT can adsorb toxin from food, bacterial toxins associated with gastrointestinal disorders, hydrogen ions and metabolic acidosis toxins, these results may lead to

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a variety of symptoms such as nausea, vomiting and diarrhea. These are typical symptoms of side effects caused by anticancer drugs [4].

Iillite are a group of minerals derived from muscovite with similar structure to montmorillonite. However, illite are mineral clays with high plasticity.

Among the approaches suggested to achieve a controlled release formulation, the ion exchange can be achieved by mixing the solid substrate (ion exchange) with the ionic medicinal solution. In biological fluids, "counter-ions" can displace the drug from the substrate and can wear in the body. The ion exchanger can then be removed or biodegraded.

MMT has a much higher capacity compared for the cation exchange with other pharmaceutical silicates (such as talc, kaolin and clay mineral fibers) [5,6].

Currently, most antitumor drugs are administered intravenously. Intravenous route is the most direct and variable adsorption methods beyond the gastrointestinal tract. Lead to an immediate and complete bioavailability and thus a precise dosage. However, this approach may be hazardous, because they are administered to normal tissue very high concentrations of the drug. Chemotherapy IV is designed to provide the maximum tolerated dose of the cytotoxic agent to kill cancer cells in a short period of treatment followed by a period of several weeks without any further use. In addition to possible side effects, this route requires visits to hospital care and palliative treatment [7-13].

Active substances used for preparation of the mineral clay /antitumor drug hybrids were epirubicin, fludarabine, gemcitabine, cytostatic with a large usage spectra in cancer treatment (breast cancer, stomach cancer, lung cancer, ovarian cancer, etc.).

Montmorillonite, in addition to the property of the drug carrier, a muco-adhesive provides a pharmaceutical formulation, making it possible to pass through the gastrointestinal barrier. MMT can improve the adsorption properties of gastrointestinal disorders, which are one of the most common side effect of chemotherapy. For these reasons, controlled release systems that support the use of MMT is an interesting concept for the development of drug release using as the carrier material that also has therapeutic effect, capable of reducing the side effects of the drug incorporated [3] .

The purpose of this study is to synthesize a drug delivery system based on three different types of mineral clays- as matrix and three different antitumoral drugs– as active substances. In order to establish its antitumoral activity and posibilty to be used as drug delivery systems, the materials are characterised.

2. Experimental

2.1. Materials

There are used for this purpose three types of clay minerals and three types of chemotherapy: epirubicin, fludarabine, gemcitabine with the following propeties:

• Epirubicin ($C_{27}H_{29}NO_{11}$), is a red powder with 1.18 mg/mL theoretical water solubility, often used in breast cancer, stomach cancer, lung cancer, colorectal cancer, ovarian cancer.

• Fludarabine ($C_{10}H_{13}FN_5O_7P$) is a white powder with 2.97 mg/mL theoretical water solubility, used in chronic lymphocytic leukemia.

• Gemcitabine ($C_9H_{11}F_2N_3O_4$) is a white powder, with 22.3 mg/mL theoretical water solubility, used in lung cancer, pancreatic cancer breast cancer, ovarian cancer, bladder cancer.

The materials used and their preparations have been assigned the following codes shown in Table 1.

2.2. Preparation of the clay hybrid materials

The Clay – drug hybrid materials were obtained starting from clay materials and cytostatic as can see in Figure 1.

The clay -drug hybrid materials have been obtained by mixing an aqueous solution of the active substance with the swelled clay.Swelling clays was obtained by gradually introducing 1 g of the clay in 50 mL distilled water pre-heated to a temperature of 60° C. The suspension was maintained at this temperature and under magnetic stirring (350 rm) for 4 hours.For

Table 1

Materials code/ *Codurile materialelor*

Drug / Clay	epirubicin	fludarabine	gemcitabine
Clay 1 Hydratated sodium aluminosilicate	1E	1F	1G
Clay 2 Muscovite, Kaolinite	2E	2F	2G
Clay 3 Magnesium and Iron Hidrosilicate, Calcium Aluminosilicate Hydrate	3E	3F	3G

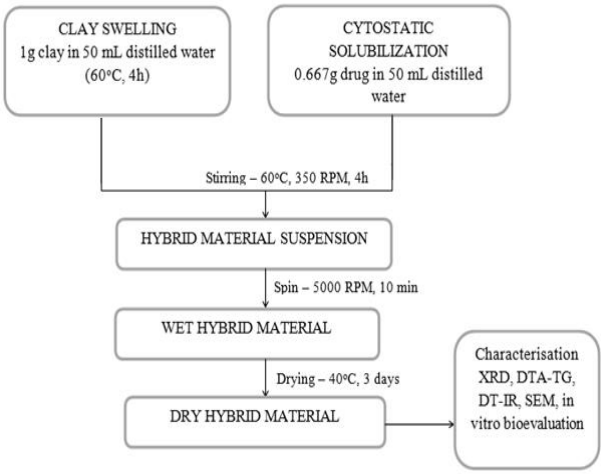


Fig. 1 - Preparation of hybrid materials- diagram / *Schema de obţinere a materialelor hibride.*

incorporation of the drug was prepared solutions (50 mL, each) using 0.667 g of each type of chemotherapy (the mass ratio of clay: cytostatic = 60: 40). After solubilisation and heating the solution to 60° C, they were added slowly over a suspension of the clay (which is also at 60° C under magnetic stirring - 350 rpm) for a period of 4 hours.After cooling the suspension, the hybrid material was separated by centrifugation at 5000 rpm for 10 min. This was followed by drying them for 3 days at 40° C. Separately, the same amount of swelling clay minerals are subjected to the same conditions and after drying were used as reference samples.

2.3. Characterization

The materials used in the synthesis and the obtained hybrids were characterized from both chemical and microstructural points of view using different experimental techniques: X-ray diffraction (XRD), thermal analysis (DTA-TG), scanning electron microscopy (SEM).

For the identification of crystalline phases of materials and their cristalinity degree, X –ray Schimatzu was carried out on a Schimatzu diffractometer XRD 6000-Ni-filtered CuKa(χ=1.5406Å) radiation, scanning speed of 2°/min in 2θ range of 3-40°.

Additional information regarding the structure are presented in the thermal analysis (DTA-TG), using a a differential thermal analyzer Shimadzu DTG-TA 51H (30–1000° C temperature range and 10° C/min heating rate).

Morpho-structural study of the materials was carried out using a scanning electron microscopy Hitachi S 2600 N.

3. Results and discussion

3.1. X-ray diffraction

In the Figure 2 is shown the specific diffractogram of clays used in Table 2. X-ray diffraction data indicated the presence of clay mineralogical phase with a certain degree of crystallinity, as follows:

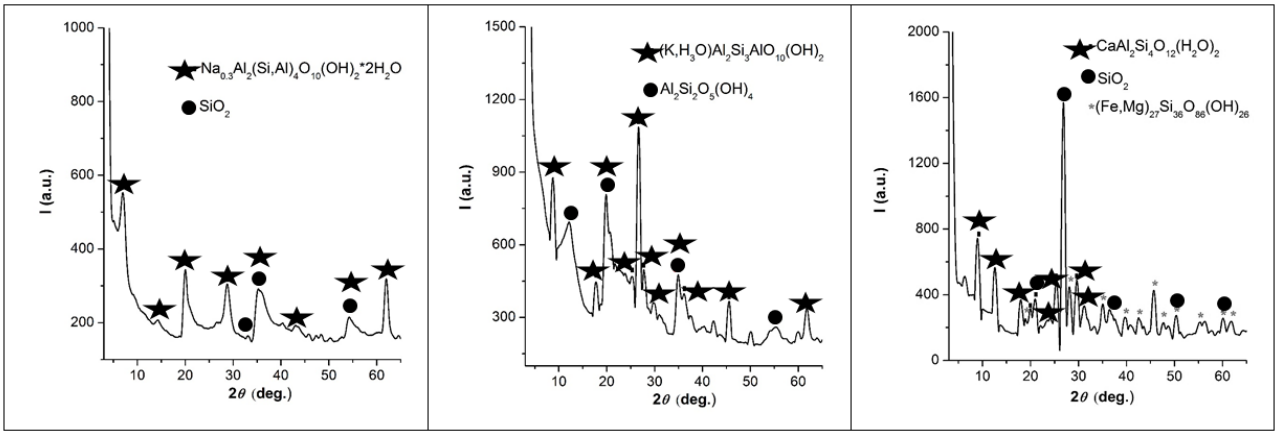
• to highlight clay 1 is mainly characteristic lines of a hydrated sodium aluminosilicate (ASTM [43-0688]) and iron oxide (ASTM [84-0307]);

• to highlight 2 clay mainly illite interference characteristics (ASTM [26-0911]) and halloysite (ASTM [29-1487]);

• to emphasize the 3 clay-specific interference hidroxisilicat magnesium and iron (ASTM [41-0594]) and a hydrated calcium aluminosilicate (ASTM [72-2194]); also identifies lines of silicon oxide-quartz (ASTM [78-1252]), this can be explained by the fact that the clay is a natural clay.

In the Figure 2 is shown the results obtained by X-ray diffraction clays used in accordance with Table 1.

In Figure 3 is the X-ray diffraction pattern of the clays and hybrid materials used based on them as shown in Table 1. Is noted for all types of clay,



a b c

Fig. 2 - XRD patterns of: a)clay 1; b) clay 2; c) clay 3/ *Difractograme de raze X ale mineralelor argiloase: a) argila 1; b) argila 2; c) argila 3.*

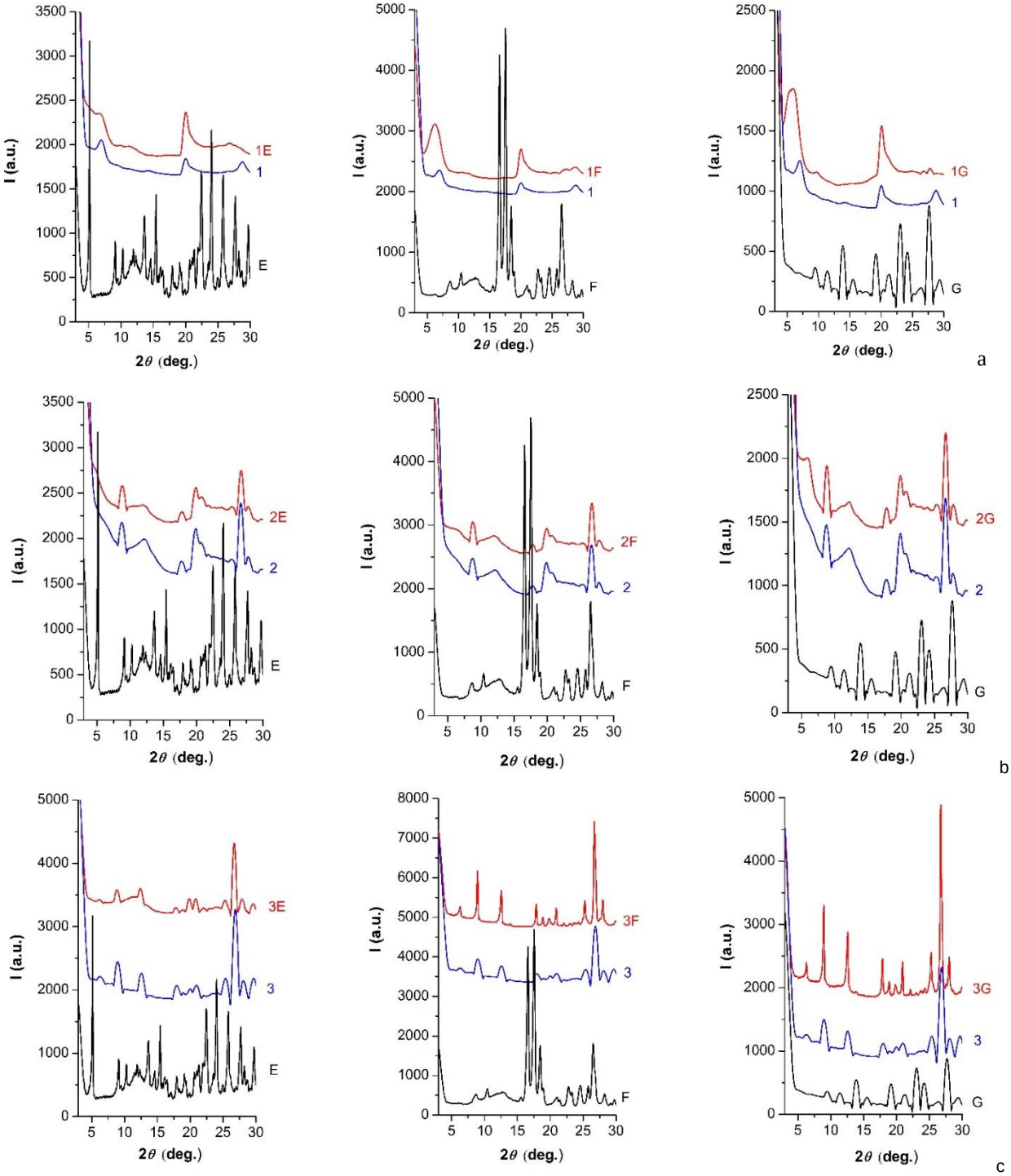


Fig.3 - XRD patterns of inflated clay (a.- clay 1, b. – clay 2, c. – clay 3) and correspondent hybrid materials/ / *Difractograme ale argilei (a.- argila 1, b. –argila 2, c. argila 3) gonflate și ale materialelor hibrid realizare cu aceasta.*

the introduction of epirubicin results in a good interlayer intercalation, it was made out by reducing interference characteristic clay mineralogical phases of their movement or their disappearance. In the case of hybrid materials based on the other two drugs, this is less evident, although the data in Table 1 shows that the molecular weight of epirubicin is the largest, but has groups favoring ion exchange (hydroxyl groups in higher concentration).

3.2. Thermal analysis

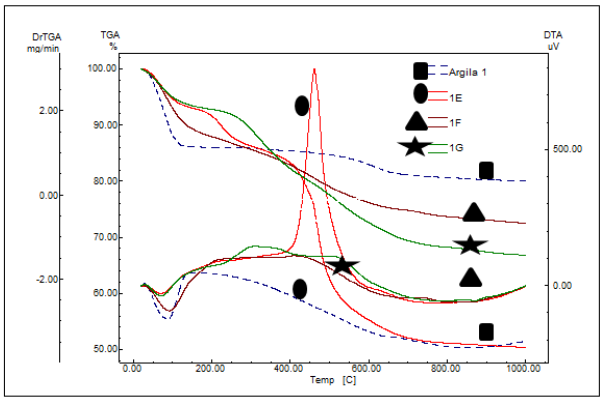
X-ray diffraction data are supplemented by data from complex thermal analysis, shown in Table 2 and Figure 4. It is noted that the total mass loss for swollen clays vary as followed: $P.T._{clay\ 1} \approx P.T._{clay\ 2} > P.T._{clay\ 3.}$ Clay 1 shows the highest weight loss, probably due to the interlayer intercalation of large quantities of water. In addition, the hybrid material containing epirubicin has the highest loss of weight in all cases. This can be explained

Table 2

Total weight loss – TG/ *Pierederea de masă totală - TG*

Probe code	Teperature range				Total weight loss (%)
	30° - 150°	150° - 500°	500° - 650°	650° - 1000°	
Clay 1	10.85	1.1	3.62	0.86	**16.43**
1E	6.87	30.42	9.26	2.92	**49.46**
1F	10.32	10.32	3.72	2.77	**27.12**
1G	6.20	15.91	6.77	3.92	**32.80**
Clay 2	11.96	1.67	1.39	1.65	**16.66**
2E	4.38	15.48	6.39	2.86	**29.11**
2F	4.96	8.43	3.18	2.02	**18.58**
2G	3.77	7.71	2.87	2.50	**16.85**

	30° - 150°	150° - 500°	500° - 650°	650°-750°	750°-1000°	
Clay 3	1.84	2.81	2.85	4.38	1.09	**12.98**
3E	3.92	32.90	2.76	1.68	1.27	**41.89**
3F	1.95	3.60	2.62	0.97	1.33	**10.48**
3G	1.64	3.38	2.38	0.73	1.39	**9.35**



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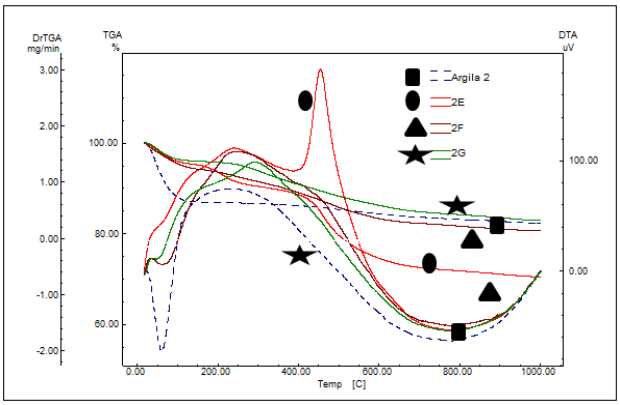

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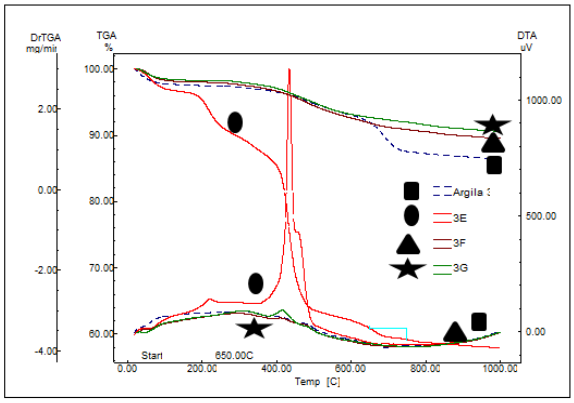

c

Fig. 4 -TDA-TG patterns of inflated clay (a. – clay 1, b. – clay 2, c. – clay 3) and correspondent hybrid materials/
DTA- TG ale argilelor (a.- argila 1, b. – argila 2, c.- argila 3) gonflate și ale materialelor hibrid realizare cu aceasta.

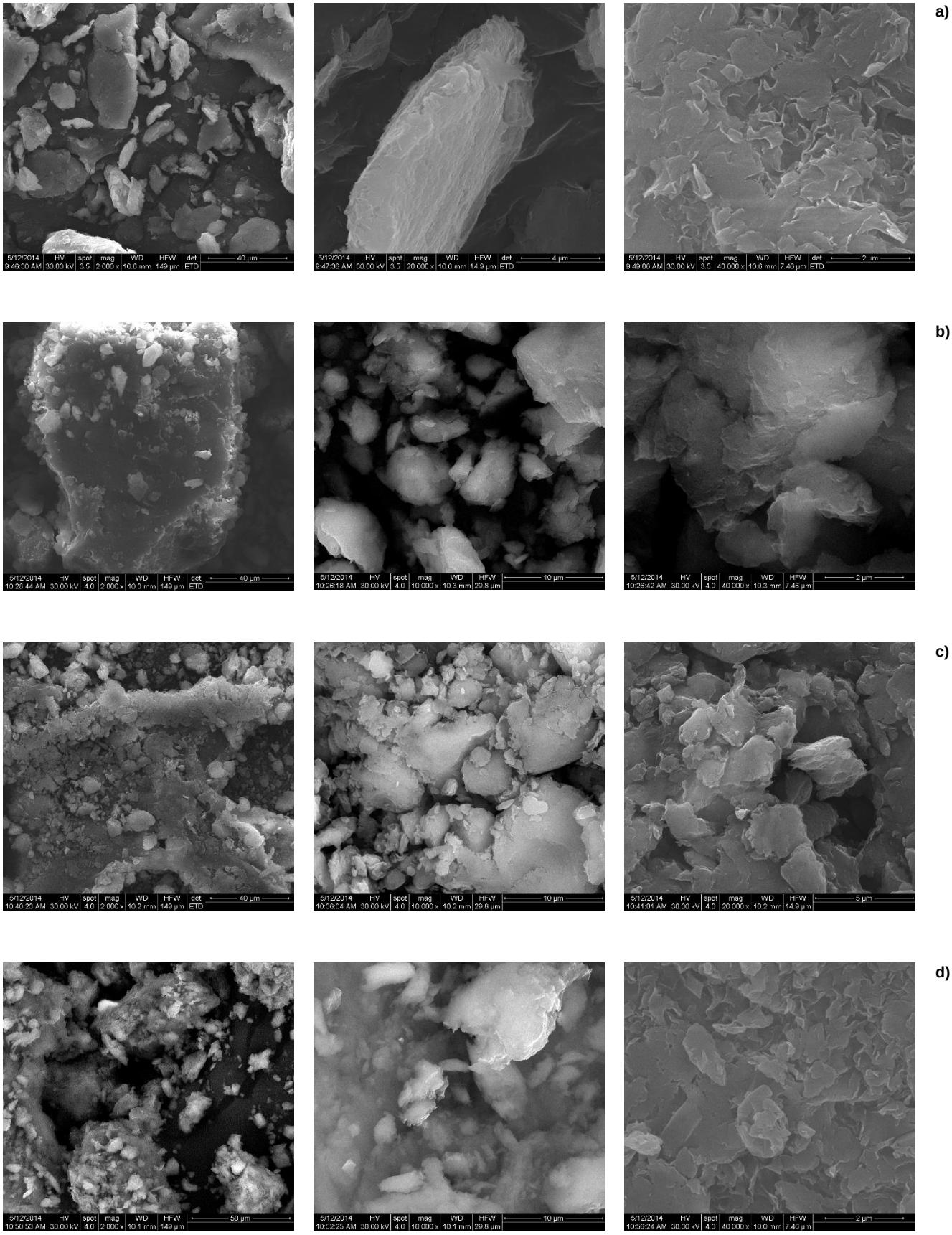

Fig. 5 - SEM images of a) clay 1 ,b) 1E, c) 1F, d) 1G/ *Imagini SEM: a) argila 1; b) 1E ; c) 1F, d) 1G.*

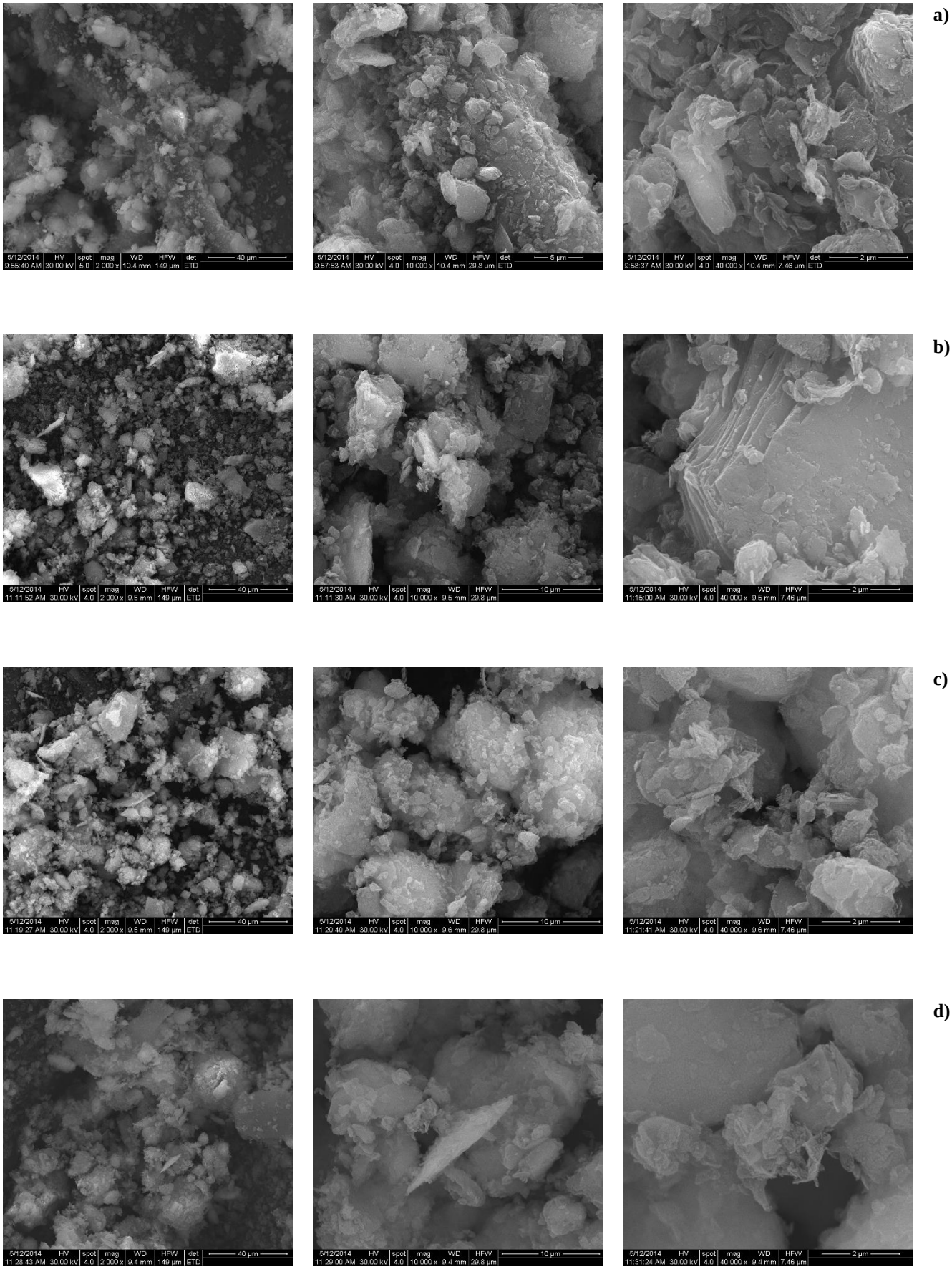

Fig. 6 - SEM images of : a) clay 2 ,b) 2E, c) 2F, d) 2G/ *Imagini SEM ale: a) argila 2, b) 2E, c) 2F, d) 2G.*

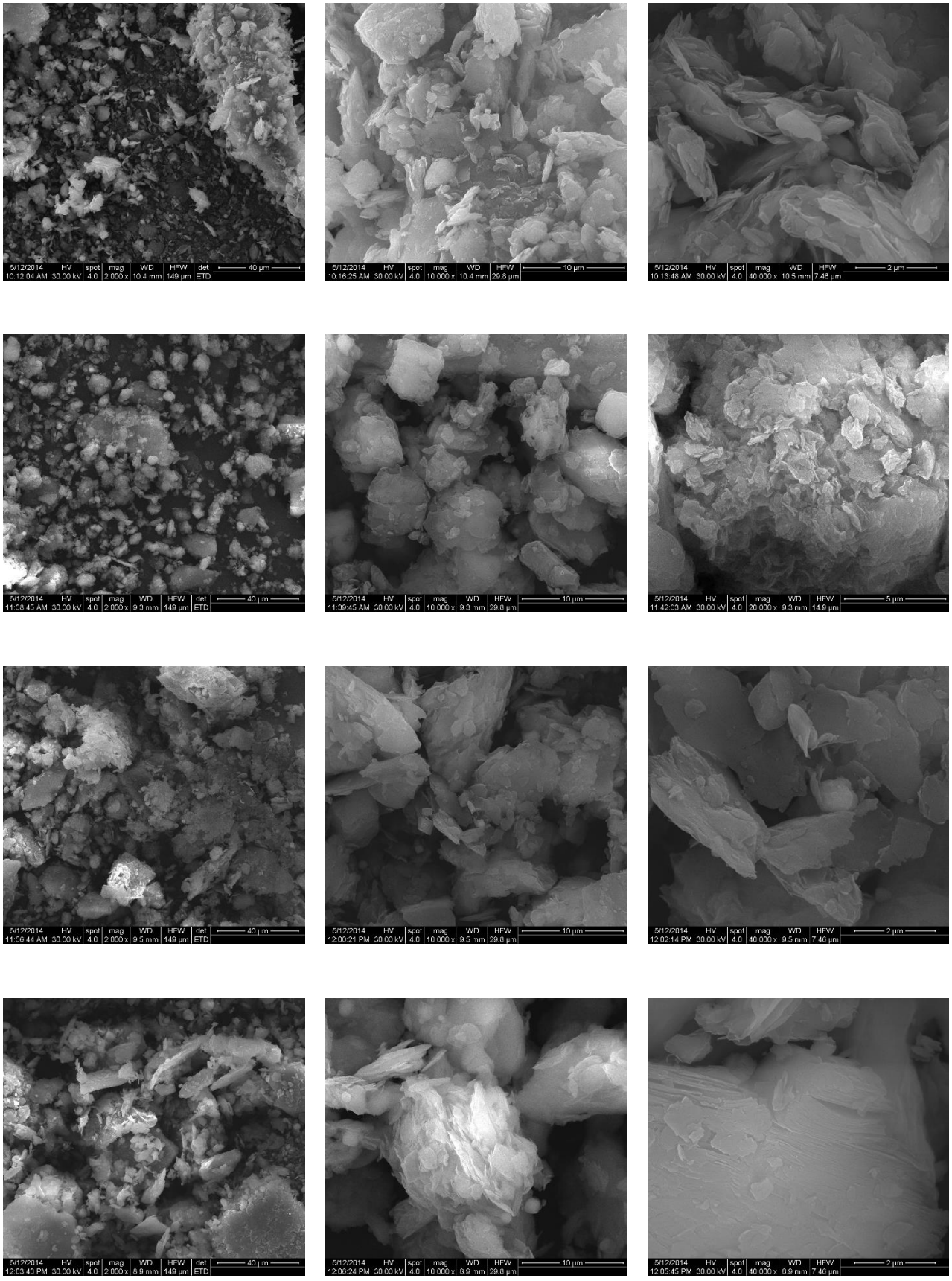

Fig. 7- SEM images of : a) clay 3, b) 3E, c) 3F, d) 3G/ *Imagini SEM ale: a) argila 3, b) 3E, c) 3F, d) 3G.*

by a good interlayer intercalation, but also by adsorption on the clay surface, showing the loss in the range of 150° - 500° C, which is attributed to the combustion of the product.

3.3. Scanning electron microscopy (SEM)

Both the clays and the hybrid material synthesized were characterized by scanning electron microscopy.

For electron microscopy images shown in Figures 5, 6, 7 it is noted that the clays have a platelet microstructure (see Figures 5, 6, 7), and decreases the particle size of the clay 1 to 3 in that clay 3 is much finer. The introduction of drugs clays have the effect of network congestion and the formation thereof on the surface of clay particles of film, indicating that the drug is absorbed.

4. Conclusions

Recent studies show the possibility of using mineral clay as drug delivery system [1-3]. Thus, the main focus of the present study is the preparation and characterization of clay/antitumor drug hybrid materials. With potential applications in drug delivery systems, the hybrid materials were obtained starting from three types of mineral phases with different structural characteristics as matrix and three types of cytostatic (epirubicin, fludarabin, gemcitabine) as active substances [2].

The materials used in the synthesis and the obtained hybrids were characterized from both chemical and microstructural points of view using different experimental techniques: X-ray diffraction (XRD), thermal analysis (DTA-TG), scanning electron microscopy (SEM). From the XRD patterns and DTA-TG analysis it is noted that the cytostatic has breached the gallery of the clays (diffraction peaks from plane family [0 0 1] shift left on hybrid, incrementing the interplanar c-spacing by ~0,4Å) creating a good interaction with it (major weight loss shifts from 30°C-150°C – physical water loss – to 150°C-500°C – cytostatic loss). The influence of epirubicine upon mineral morphology was highlighted by the appearance of conglomerates on the clay surface in SEM.

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