Digest Journal of Nanomaterials and Biostructures Vol. 5, No 4, October-December 2010, p. 919-925

LIPOSOMES CONTAINING UNDOPED AND Au^+ / Ag^+ DOPED TITANIUM DIOXIDE NANOPARTICLES

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The paper presents the obtaining and characterization of liposomes containing undoped and metallic ions (Au^+ and Ag^+) doped titanium dioxide nanoparticles by using the ultrasonication method. Commercial "empty" liposomes and undoped and doped (with Au^+ and Ag^+ ions) titanium dioxide (obtained by sol-gel method) were used for obtaining the liposome nanocapsules and these were analyzed by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDS). Generally, the liposomes are unilamellar and have a higher uniformity, being formed by separated capsules with diameters up to 300 nm, as is revealed by SEM and TEM analyses. These liposomes contain titanium dioxide nanoparticles as is demonstrated by EDS analysis, but the concentration of metallic ions cannot be evaluated. The approximate concentration (on the basis of EDS analysis) of titanium dioxide in the liposomes was 7% and 1-2% TiO_2 (undoped or doped, respectively).

(Received September 25, 2010; accepted October 21, 2010)

Keywords: liposomes, metallic ions doped titanium dioxide, nanoparticles, nanocapsules, SEM, TEM, EDS

1. Introduction

Liposomes are some of the most used matrices for protection and controlled release of bioactive compounds and they are microspheres or nanospheres containing empty cavities resulted by phospholipids assembling in aqueous systems. Generally, the membrane is formed by two or more double lipidic layers, which contain aqueous phase where they are suspended [1-5]. These special properties of liposomes generate various applications such as: models for biological membranes and carriers for drugs [1, 2]. Bioactive compounds can interact with liposomes according to their solubility and polarity; these can be inserted in the lipid bilayer region, intercalated in the polar groups region, adsorbed on the membrane surface, anchored by a hydrophobic chain, or encapsulated in the aqueous inner cavity of liposomes. The first type of liposomes was multilamellars and was obtained by lipid-water interaction in different ratios. Modern liposomes are unilamellars, with well defined characteristics. Obtaining methods belong to the inverse phase evaporation (large unilamellars vesicles – LUV, with diameters of 100-1000

nm) and ultrasonication (small unilamellar vesicles – SUV, with diameters of 25-100 nm) [1]. Some liposomes are formed as polymer/liposome composites, in order to enhance the bioavailability and stability [5].

Liposomes are widely used in many fields such as in the pharmaceutical field (applications in the encapsulation of some anticancer – anthracyclins, antifungal, antiviral, or antibiotic drugs) [4,6-10], in the food field (for controlled release of proteinases in order to enhance the developing of aromas from some special types of cheese, for the encapsulation of food aqueous phases for reducing the vapor pressure, for encapsulation of some food enzymes, antioxidants, non-volatile flavor compounds, food dyes, and vitamins [3]).

Titanium and titanium dioxide are widely used in biomedical applications. In order to enhance the bioavailability of these compounds some liposome nanocapsules containing titanium dioxide were obtained by using lecithin [12-14] and these liposomes can be decomposed upon illumination with near-UV light [12].

In this research we obtain liposomes containing nanoparticles of titanium dioxide, undoped or doped with 1% Au^+ or Ag^+ ions with biomedical applications and evaluate the type and dimensions of liposomes by electronic microscopy and the composition of these liposomes by energy-dispersive X-ray spectroscopy.

2. Materials and method

Materials. Titanium dioxide undoped or doped with metallic ions (1% Au^+ or Ag^+ ions) was obtained previously by sol-gel route and the obtained nanocrystals have dimensions between 10 and 20 nm according to [15]. Commercial lipid mixture (Sigma-Aldrich) containing ~58 mg lyophilized powder/vial (with a composition of 63 μmoles L-α-phosphatidylcholine, 9 μmoles cholesterol, and 18 μmoles stearylamine) was used for obtaining the liposomes.

Obtaining the liposomes containing titanium dioxide nanoparticles. The ultrasonication method was used for obtaining the liposomes containing undoped or metallic ions doped (Au^+ or Ag^+) titanium dioxide nanoparticles. Thus, 25 mg undoped or doped TiO_2 was suspended in 10 ml distilled water and the suspension was ultrasonicated in a flask (under cooling on ice) by using an Ultrasonic Liquid Processor Vibra Cell VC 505, 500 W, with the following conditions: amplitude 80%, ultrasonication time 15 minutes, pulse on 30 s, pulse off 15 s. Liposomes contain titanium dioxide were obtained from 14.6 mg commercial lipid mixture which are ultrasonicated in the same conditions with 4 ml distilled water and 1 ml TiO_2 suspension. After decantation, the liposome suspension was separated and analyzed by SEM, TEM, and EDS analyses.

Scanning electron microscopy (SEM) analysis / Energy-dispersive X-ray spectroscopy (EDS). A JEOL JSM 5510-LV apparatus coupled with EDS system was used for morphological and dimensional analysis of the liposomes containing titanium dioxide nanoparticles. The following conditions were used for SEM analysis: voltage of 15 kV, 300-150 000× magnitude level; analysis was performed on the non-covered and carbon-coated liposomes for EDS analysis. Carbon deposition was performed by using a JEOL JEE 4B vacuum evaporator, at a vacuum of 10^{-5} torr.

Transmission electron microscopy (TEM) analysis. A JEOL JEM 1010 apparatus, with a Mega View III CCD camera for acquisition of images and an acceleration voltage of 100 kV, were used for TEM analysis.

3. Results and discussion

Liposomes containing even undoped titanium dioxide or Au^+/Ag^+ ions doped titanium dioxide were obtained as translucent suspensions (after the gravimetric decantation of the resulted suspension, the non-encapsulated titanium dioxide nanocrystals being separed on the bottom of the ultrasonication flask).

Scanning electron microscopy analysis of the uncoated and carbon-coated liposomes revealed that the most of liposome particles are unilamellar with spherical shapes and a relatively

higher dimensional uniformity. Thus, for the undoped TiO_2 containing liposomes most of liposomes have lower (50-100 nm) and medium (100-300 nm) diameters, and only few are larger (multilamellar) with dimensions of 2-3 μm (Figures 1a and 1b). In the case of Au^+ doped TiO_2 containing liposomes a higher percent of larger liposomes exists (a majority with diameters of 2-2.5 μm, even higher – 5 μm), but also a relatively higher number of liposomes with medium dimensions (Figures 1c and 1d). In both cases some relatively larger (approximately 50-60 nm) crystals having nail-like shapes are adherent to the liposomes. More dimensional uniformity have been observed in the case of Ag^+ doped TiO_2 containing liposomes: most of the liposomes are unilamellar and have diameters between 200 and 300 nm, few of them are larger (up to 1 μm) and also some liposomes have diameters lower than 200 nm (Figures 1e and 1f).

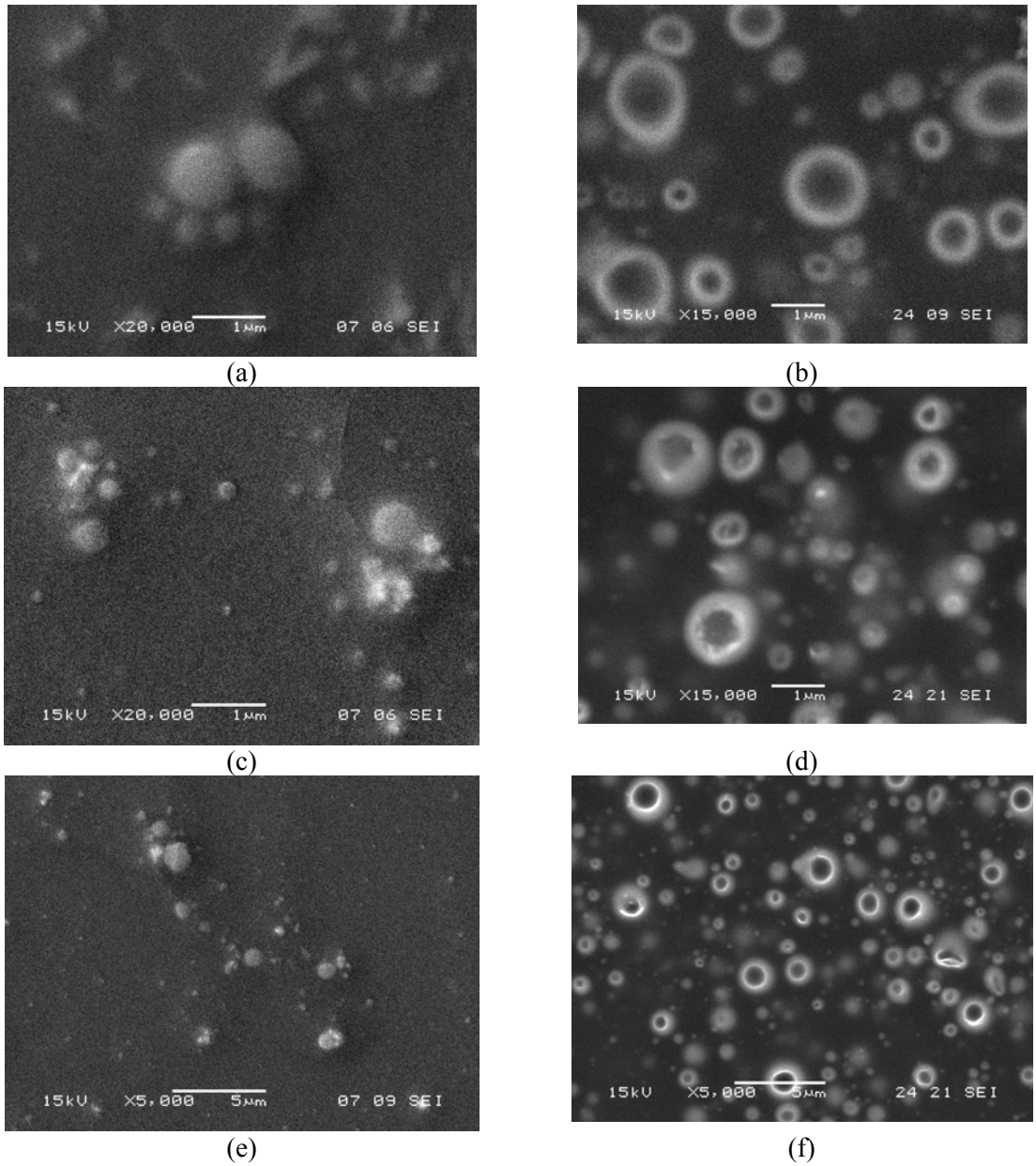

Fig. 1. SEM (normal and carbon-coated) images (left and right, respectively) of liposomes containing undoped (a and b) and Au^+ (c and d)/Ag^+ (e and f) doped titanium dioxide

Transmission electron microscopy of TiO_2 containing liposomes indicate that some of unilamellar particles contain TiO_2 nanocrystals, but some of larger crystals only adhere to the liposomes. Thus, the undoped TiO_2 containing liposomes are well formed, unilamellar, with

dimensions up to 1µm in most cases, and with titanium dioxide crystals inside or adherent to the liposomes (Figure 2a and 2b). Au^+ doped TiO_2 containing liposomes are more larger and contain also TiO_2 nanoparticles (with 20-40 mm lenght) inside or attached to the liposome surface (Figure 2c and 2d). Ag^+ doped TiO_2 containing liposomes are smaller (as is revealed by TEM analysis), densely, and have especially TiO_2 nanocrystals inside the liposomes (Figures 2e and 2f).

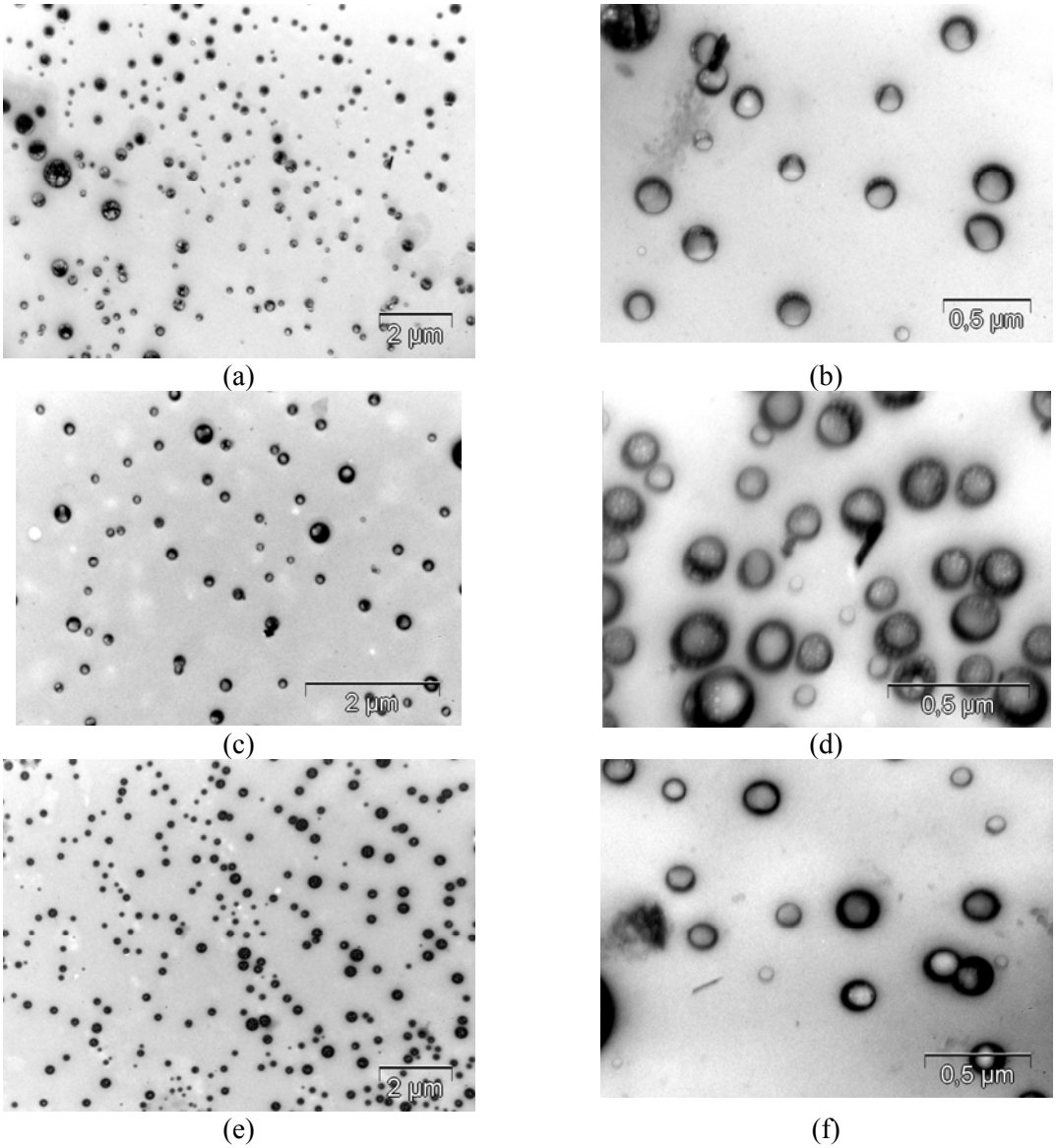

Fig. 2. TEM images for liposomes containing undoped (a and b) and Au^+ (c and d) or Ag^+ (e and f) doped titanium dioxide

The EDS analysis of carbon-coated liposomes containing undoped or Au^+/Ag^+ doped TiO_2 nanoparticles allows to evaluate the approximate content of titanium dioxide in liposomes. Thus, the L-α-phosphatidylcholine/cholesterol/stearylamine mixture content (on the basis of compound concentrations furnished by provider), the undoped or doped titanium dioxide nanoparticles, as well as the water concentration in the final liposomes could be calculated by knowing the relative concentration of the main elements: C, Ti, and O from EDS analysis. Ti is present only in titanium dioxide nanoparticles, O is present in all components, but C is present in organic mixture used for obtaining these liposomes (phosphatidylcholine, cholesterol, and sterylamine, in known concentrations) as well as on the surface of liposomes (EDS was performed on the carbon-coated samples). The concentration of lipid mixture is 85.7% phosphatidylcholine, 6.0% cholesterol, and

8.3% stearylamine, and the elemental relative concentration is 78.07% C, 2.22% N, 15.93%O, and 3.79% P (hydrogen is neglected), but the concentration of C from the surface of liposomes could not be established. Titanim dioxide have 60% Ti and 40% O (metallic ions are neglected). If C from the liposome surface is neglected, the lipid mixture content could be evaluated by using the percentage of C from EDS analysis. By knowing the Ti percentage the approximate TiO_2 concentration of the resulted liposomes can be established. Finally, the concentration of water results from the percentage of O, after the excluding of the O percentage corresponding to the already known lipid mixture and titanium dioxide concentrations. The concentration of Au^+ and Ag^+ ions in the final liposomes cannot be calculated on the basis of EDS due to the very low concentration in the final liposomes, but they can be evaluated by knowing the initial composition of doped titanium dioxide nanoparticles (we pressume that the metallic ions concentration are not modified by nanoencapsulation process).

Three EDS spectra were obtained for every sample and the results are the averages of these determinations. The undoped TiO_2 containing liposomes are 4-10 fold more concentrated in TiO_2 nanocrystals than in the case of doped samples. Thus, the concentration of undoped TiO_2 in the final liposomes was 7.4±2.00% (Figure 3), while the Au^+ and Ag^+ doped TiO_2 containing liposomes have only 2.2% and 0.8% (standard deviation is poor in these cases) of such nanocrystals, respectively (Figures 4 and 5).

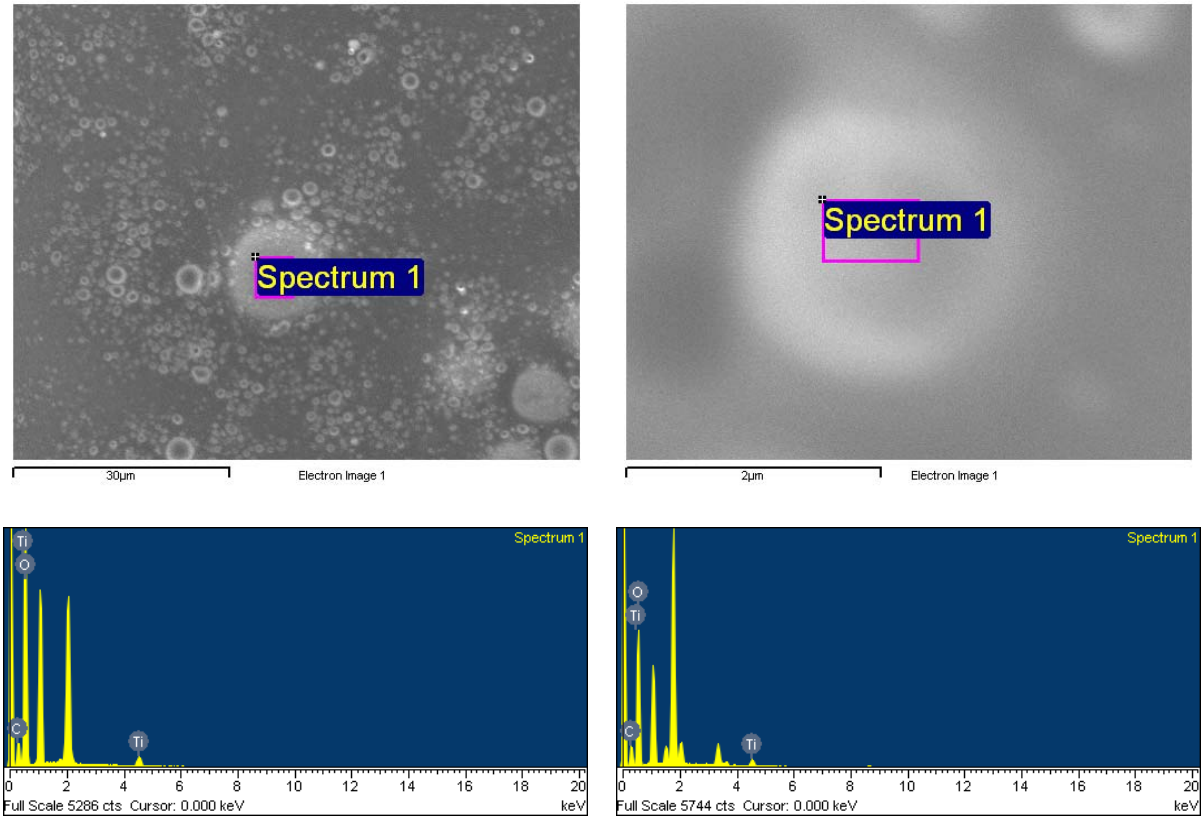

Fig. 3. EDS analyses of liposomes containing undoped titanium dioxide for two relevant nanocapsules (8.3% and 8.7% TiO_2, left and right images, respectively).

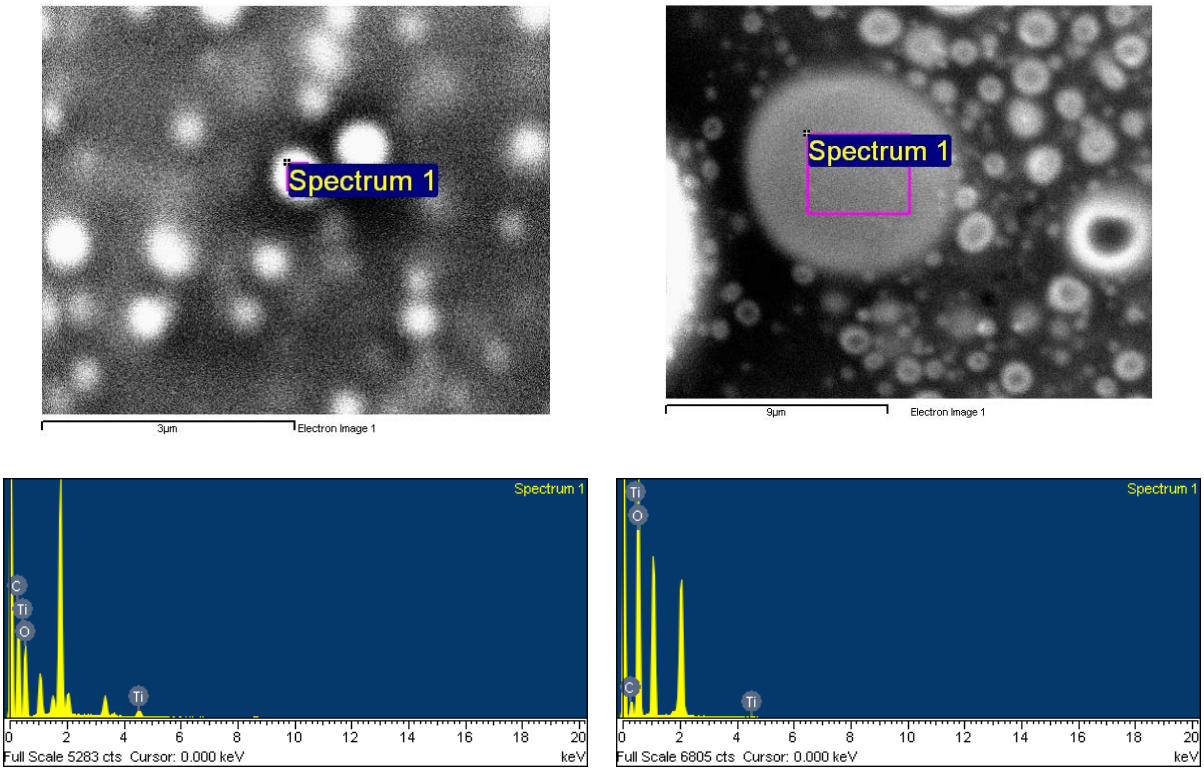

Fig. 4. EDS analyses of liposomes containing Au^{+} doped titanium dioxide for two relevant nanocapsules (6.0% and 0.3% TiO_2, left and right images, respectively)

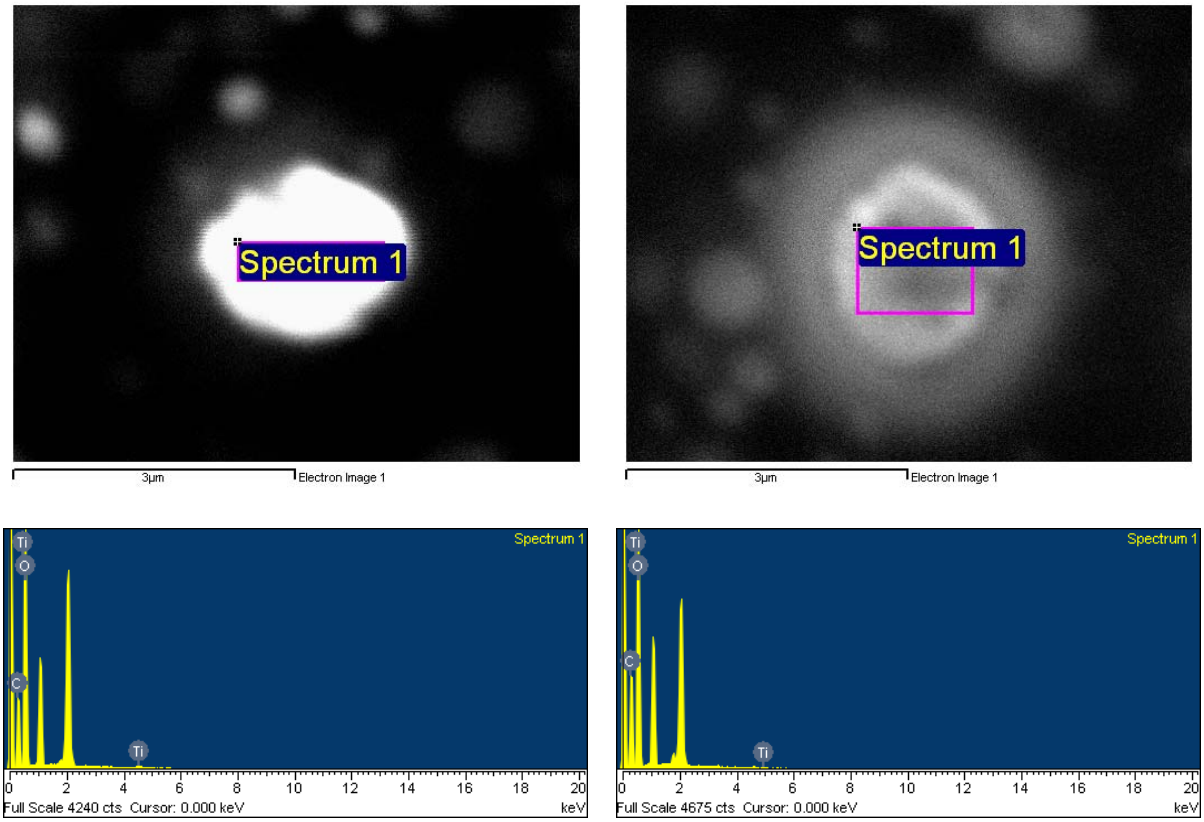

Fig. 5. EDS analyses of liposomes containing Ag^{+} doped titanium dioxide for two relevant nanocapsules (1.5% and 0.8% TiO_2, left and right images, respectively)

4. Conclusions

Unilamellar liposomes with higher uniformity, stability, and diameters up to 300 nm, having an approximate TiO_2 concentration of 7% in the case of undoped titanium dioxide nanocrystals and up to 2% in the case of Au^+ / Ag^+ doped titanium dioxide were obtained by using the ultrasonication method.

These undoped or metallic ions doped titanium dioxide containing liposomes could be appropriate to be used in the treatment of various diseases due to the posibility to furnish the titanium dioxide nanocrystals at cellular and molecular levels, but furthermore studies on bioactivity and toxicology of these nanocapsules will be needed.

Acknowledgements

This work was supported by Ministry of Education and Research from Romania, PN2 41070/2007 IMUNONANOMAT.

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