Polyphenols have antioxidant character due the presence of several phenol functional groups. In human health, they are thought to be useful in combating oxidative stress, a syndrome causative of neurodegenerative and cardiovascular diseases. The main source of polyphenol antioxidants is nutritional, since they are found in most vegetables and fruits. We used tannic acid as polyphenol model. The polyphenols are encapsulated in chitosan matrices because chitosan is a natural bioactive material and it is pursued to release the polyphenols only in a certain medium. In this paper, the obtaining strategy of chitosan nanostructure membranes with tannic acid, as Controlled Delivery System, is presented.

Keywords: chitosan, cationic surfactant, tannic acid, morphological analysis

The material properties and the microstructure of the scaffold are important parameters that determine the suitability of a material for tissue growth and controlled drug release. In recent years, there has been a significant increase of interest in using chitosan for such applications.

Chitosan, a copolymer of glucosamine and N-acetylg glucosamine units linked by 1-4 glucosidic bonds, can be obtained by N-deacetylation of chitin (the primary structural polymer in arthropod exoskeletons), which is the second most abundant natural polymer [1-3]. The degree of deacetylation is a structural parameter, which influences physicochemical properties such as the molecular weight, the elongation at break and the tensile strength. It also influences biological properties, namely the biodegradation by lysozyme, the wound-healing properties and the osteogenesis enhancement.

Chitosan was selected as matrix for immobilization of the polyphenols because of an unusual combination of its properties, which includes an excellent membrane-forming ability, high permeability toward water, good adhesion, biocompatibility, non-toxicity and high mechanical strength.

Tannins are water-soluble polyphenol compounds of varying molecular masses that have the ability to react with proteins, polysaccharides and other macromolecules.

Tannic acid is a hydrolysable polyphenol formed from the secondary metabolism of plants. It is most commonly found in the bark and fruits of many plants and has a structure consisting of a central carbohydrate (glucose) and 10 galloyl groups [4, 5]. Several authors have demonstrated that tannic acid and other polyphenols have antimutagenic and anticarcinogenic activities [6-11]. Moreover, the consumption of polyphenol-rich fruits, vegetables, and beverages, such as tea and red wine, has been linked with inhibitory and preventive effects in various human cancers and cardiovascular diseases [5, 8, 10-21], which may be related - at least in part - with the antioxidant activity of polyphenols [11-16]. Dietary tannins are thought to reduce the digestibility and metabolisable energy of feeds through direct interaction with proteins and carbohydrates from both exogenous and endogenous sources.

Tannic acid should not be used continuously or in high quantities because it slows down the absorption of iron and possibly other trace minerals [22]. Also, large amounts can cause liver and kidney damage. From this point of view, the dosage in a very important prophylactic parameter and new strategy of delivery are in progress [5, 6, 17-19].

As polymer, we used chitosan that acts like an additional transport barrier which enables a slower and more extended rate of release for the active substance entrapped in this matrix.

In nanostructures development, the nanocontainers for drug controlled release represent one of the new strategies [23, 24].

In this paper are presented studies regarding the obtaining of nanostructure films and membranes which contain nanocontainers with tannic acid in different concentrations. These films and membranes systems were morphologically investigated by Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) methods.

Materials and methods
Previous studies [3] were performed on chitosan membranes in combination with a cationic and anionic surfactant, in different concentrations. As cationic surfactant was used cetyl trimethylammonium bromide (CTAB) and as anionic surfactant, sodium dodecyl sulfate (SDS). It was observed that the CTAB - chitosan membranes are more hydrophilic than the SDS - chitosan membranes, which mean that they are better aspirants for drug release. The optimum results were obtained for chitosan in concentration of 2% and 3% and for CTAB in concentration of 6mm.

The chitosan was given by Sherbrooke University (Quebec, Canada). The N-deacetylation degree was 88%, average molecular weight number was $M_n = 150000$, average molecular weight was $M_w = 350000$ and the polydispersity index was 2.33.

The tannic acid, with a molecular weight of 1701.20 was acquired from Sigma Aldrich.

The cationic surfactant, cetyl trimethylammonium bromide ($C_{16}H_{33}NBr$) (CTAB), was purchased from Chemapol.
All solutions were made using a 1% (wt/wt) acetic acid (with a purity of 99.5%, from Chemical Company) in distilled water solution.

For films preparation we used microscope slide glass supports with a 25 · 25 mm² area. The glass supports were cleaned first with a dish detergent, followed by a thorough rinsing with deionized water and ethyl alcohol (with a purity of 94%, from Chemical Company). After that, they were sonicated (Sonopuls, Bandelin) for 10 minutes while submerged in a 2% RBS 35 concentrate solution obtained from Fluka, followed by a second rinse with ethanol and deionized water.

Two matrices of 2% and 3% chitosan solutions were prepared by mixing the 6m m cationic surfactant (CTAB) with different concentrations of tannic acid (1, 2, 3, 5, 7 and 10mm). These solutions were stirred at 2000 rpm for 3 h at room temperature, followed by centrifugation at 4000 rpm for 60 min.

The films were obtained by depositing on supports by spin-coating method using a WS-400B-6NPP/LITE spin-coater.

The membranes were obtained by dry phase inversion [2]: the prepared solutions were cast in a Teflon mold and left to dry in a thermostat chamber at 50°C for 24 h.

The films morphology was analyzed by AFM using a home-made apparatus, with a standard silicone nitride tip (NSC21) and tips radius 10-20 nm. The analysis was made in tapping mode.

The membranes morphology was investigated by SEM on a VEGA TESCAN microscope.

**Results and discussions**

In figure 1 are presented the AFM images of pure 2% chitosan, 2% chitosan with 6mm CTAB and 2% chitosan with 2mm tannic acid as blank tests.

It can be observed that the chitosan and tannic acid - chitosan system films are homogeneous while the CTAB - chitosan system films have a heterogeneous morphology (the two, chitosan and CTAB, have cationic behaviour).

In figures 2 and 3 are presented the AFM images of 2% and 3% chitosan based films with 6 mm CTAB and different concentration of tannic acid. From de AFM images obtained it can be observed that there is a tendency for the tannic acid - CTAB nanocontainers to agglomerate in clusters both in the case of 2% and 3% chitosan based films. Also, it doesn’t appear net structures of those type that can be seen from the bottom of Fig. 1 for CTAB and the fact that these structures have a geometrical form approximately of the same type and increase in ponderosity with the increase of the tannic acid concentration entitle us to believe that they contain a tannic acid – CTAB mixture (they are containers).

These clusters are well defined by the chitosan matrix and for that reason we suppose that they will have a different solubility behaviour unlike the chitosan matrices. Analysis of the films AFM images reveals that the formed nanocontainers have a dimension in the range of 50 – 500 nm.

From the SEM images (figs. 4 and 5) it can be observed that the membranes are dense, homogenous, without pore structures, except for a few accidental craters that don’t have any spatial regularity.

**Fig. 1.** The 2D (a) and 3D (b) topographies, and phase contrast (c) of 2% chitosan film (top), 2% chitosan with 2mm tannic acid film (middle), 2% chitosan with 6mm CTAB film (bottom); the scale of square area is 3 x 3 µm²
Fig. 2. The 2D (a) and 3D (b) topographies, and phase contrast (c) of 2% chitosan and 6mm CTAB films in combination with: 3mm tannic acid (top), 7mm tannic acid (middle), 10mm tannic acid (bottom); the scale of square area is $3 \times 3 \mu m^2$.

Fig. 3. The 2D (a) and 3D (b) topographies, and phase contrast (c) of 3% chitosan and 6mm CTAB films in combination with: 3mm tannic acid (top), 7mm tannic acid (middle), 10mm tannic acid (bottom); the scale of square area is $3 \times 3 \mu m^2$. 
Fig. 4. The SEM micrographs of transversal section (a), transversal section details (b) and air-facing surface (c) of 2% chitosan and 6 mCTAB membranes in combination with: 3 m tannic acid (top), 7 m tannic acid (middle), 10 m tannic acid (bottom)
In figure 4 are shown the SEM images of 2% chitosan based membranes. It is obvious that at low concentration the tannic acid is structured in small spheroids.

In the case of 3% chitosan based membranes (fig. 5), the SEM images demonstrate that at high concentration, the tannic acid presents an octahedral configuration. The SEM images demonstrate an ordering of the tannic acid – CTAB containers in horizontal planes, which means that these nanocontainers will control release of the active substance (tannic acid, in this case) as the chitosan matrix is subject of the swelling process.

The air-facing surface SEM images indicate that while at low tannic acid concentration the nanocontainers are distributed as islands, with the concentration increasing these planes become better distributed.

Moreover, it seems that with the tannic acid concentration increasing there is a tendency of the containers to homogenize (dissolute) in chitosan matrix and this is in good concordance with the AFM images from figure 1 of tannic acid – chitosan system film. This fact means that over a concentration of 10 mm tannic acid the containers formation is diminished.

Conclusions

The fact that the nanocontainers are orderly structured in planes, entitle us to affirm that as the chitosan is swelled the tannic acid - CTAB containers are dissolved and will release the active substance (tannic acid). In the case of 2% chitosan membranes the containers show a spheroid structure while in the case of 3% chitosan membranes it can be observed that they have a crystallite structure with octahedral geometry. Conclusively, we obtained matrices with tannic acid – CTAB containers at an optimum concentration of 5 – 9 mm tannic acid.

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