STUDIES OF MUCOADHEZIVE MATRIXES BASED ON CHITOSAN AND *LYTHRUM SALICARIA* L. PLANT EXTRACT

Ph. D. Student Irina Mihaela Iancu[1]
Assoc. Prof. Dr. Laura Adriana Bucur[2]
Assoc. Prof. Dr. Verginica Schröder[3]
Dr. Eng. Manuela Rossemary Apetroaei[4]
Assist. Dr. Valeriu Iancu[5]
Prof. Dr. Victoria Badea[6]
[1, 6] Ovidius University of Constanta, Faculty of Dental Medicine, Department of Microbiology, Constanta, Romania
[2, 3, 5] Ovidius University of Constanta, Faculty of Pharmacy, Constanta, Romania
[4] Mircea cel Batran Naval Academy, Faculty of Navigation and Naval Management, Constanta, Romania

ABSTRACT

The *Lythrum salicaria* L. plant, from the *Lythraceae* family, has multiple beneficial effects on the human body, through pharmacological properties imprinted by its secondary metabolites, namely tannins.

Chitosan-based biomedical materials are of increasing interest precisely due to the uniqueness of their properties, namely biocompatibility, nontoxicity, biodegradability, antimicrobial and antioxidant nature. The combination of chitosan with the plant extract aimed at obtaining new matrices, with clearly superior characteristics, compared to each material (chitosan and plant extract). This could be due to the presence of amino groups in the structure of chitosan, known to be active at a pH slightly acidic and which could be chemically bound to the phenolic groups of tannic acids (the predominant components of the plant extract).

The study aimed to obtain for the first-time mixtures of different concentrations of aqueous solutions of *Lythri herba* plant extract with standard chitosan 1 % in lactic acid (1 %) solution, which allowed achieving compatible and stable membranes. Microscopic evaluation of the membranes were made, following the uniformity of the surfaces, the homogeneity, the distribution of chitosan relative to the extract, and their stability in PBS saline buffer. The behavior of these membranes gives us a perspective on their use in dentistry and pharmaceuticals.

In addition, the current paper has shown the existence of chitosan in the composition of the obtained membranes and their ability to maintain constant hydration and flexibility over a certain period.

Keywords: *chitosan, Lythri herba, membranes, epifluorescence microscopy*

INTRODUCTION

Lythrum salicaria L. is a well-known medicinal plant since ancient times, used for conditions such as diarrhea and dysentery. Its name comes from the Greek word *lithron*, which means coagulated blood and probably refers to its hemostatic properties or the color of its flower and *salicaria* due to the shape of the leaves similar to that of willow species (Salix sp.) [1].

Lythrum salicaria L. is a rich source of polyphenols, including ellagitannins, flavonoids, flavan-3-oils, phenolic acids, and anthocyanosides [1], which adds value to the extracts of this plant species for medical applications.

At present, the scientific interest in the development of biodegradable films through elementary and easy methods has increased. Natural polymers, such as chitin, chitosan, cellulose, or gelatin have become an acceptable choice; due to the different advantages they have [2]. Chitosan is the most used biopolymer in medical fields due to its non-toxic, biodegradable, biocompatible, antimicrobial, antioxidant nature [3], as well as for its abundant availability and its low-cost [4].

Recent studies evaluate chitosan-based membranes in which plant extracts, or their secondary metabolites have been incorporated [5], precisely due to the increased ability of chitosan to form membranes [6] and its mucoadhesive property [7]. The ease of processing into gels, nanoparticles, microparticles, membranes, nanofibers, and even in the form of sponges is another advantage of chitosan [8].

Given the properties of the biopolymer and the reactivity of its amino groups, membranes made for the first time from weakly acidic solutions of standard chitosan (lactic acid 1%) in which were incorporated the aqueous extract with various concentrations of *Lythri herba*, are to be evaluated in terms of surface characteristics, stability, and hydration.

MATERIALS AND METHODS

The materials used in this study were dry *Lythri herba* extract, obtained by the concentration method with rotavapor and lyophilization of the aqueous extract from the floral tips of *Lythrum salicaria* L. (harvested in August 2019, from Năvodari area, Dobrogea, Romania), and standard chitosan powder (from Sigma Aldrich) with medium molar mass (300-400 kDa) and deacetylation degree (DDA) between 75% - 85%.

Obtaining membranes with 1% standard chitosan concentration in diluted lactic acid solutions and aqueous Lythri herba extract

The mixture obtained from 1% standard chitosan (CS) in 1% lactic acid solution was poured on a Teflon support and placed in the oven at a temperature of 50 °C, for 3 hours. The standard chitosan membrane (1 %) obtained has a yellowish, uniform appearance and was chosen as a reference in this study. Similarly, by slightly mixing the acidic chitosan solutions with aqueous plant extract (v / v = 1: 1), standard chitosan membranes (1%) were obtained mixed with *Lythri herba* extract, of different concentrations (0.5 g/L, 1 g/L, and 2 g/L) and their colors were ranged from light brownish to the dark brown.

Microscopic evaluation of new membranes obtained

In order to perform this evaluation, fragments of a few mm from each membrane were sectioned, making preparations, which were subsequently observed with the Optika Microscopes Italy epifluorescence microscope, Series B-350, model B-353LD2 at magnitude X200, used in the field of fluorescence B (ex 450-480 nm.).

The percentage hydration (%) determination of new membranes

The hydration properties of the membranes were measured by evaluating their hydration degree using the improved method of Al-Dhubiab *et all*, (2016) [9]. The surfaces of 1 cm^2 with initial weight (m_i) were cut from membranes, immersed into a small volume of saline PBS phosphate-buffered and kept in an incubator at 37 °C temperature for 10, 20, and 30 minutes. After each time interval, the small pieces of membranes were removed from the solution, dried slightly, and weighed again (m_f). The percentage of membrane hydration was determined using the equation of Nair *et all*, (2013) [10]:

$$\text{Hydration (\%)} = \frac{m_f - m_i}{m_f} \cdot 100, \text{ where:}$$

m_i = initial mass of the membrane, m_f = final mass of the membrane after being kept in the buffer solution.

RESULTS AND DISCUSSIONS

Obtaining membranes with 1% standard chitosan concentration in diluted lactic acid solutions and aqueous Lythri herba extract

The obtained membranes are mixtures of diluted acidic solutions with standard chitosan (1%) and aqueous *Lythri herba* extracts of different concentrations (0.5 g/L, 1 g/L and 2 g/L). Known for its many benefits, including regeneration [11], the plant offers potential in terms of developing products with mucoadhesive applications and gives protective, antibacterial and healing action in case of lesions of the oral mucosa.

Solubilized chitosan in the diluted lactic acid solutions (through the amino groups from its structure) has combined with extract components and the membranes obtained have a uniform appearance, slightly brownish color, due to the presence of tannins from the extract composition (Figure 1).

Suitable for the working protocol, the membranes obtained are according to Figure 1.

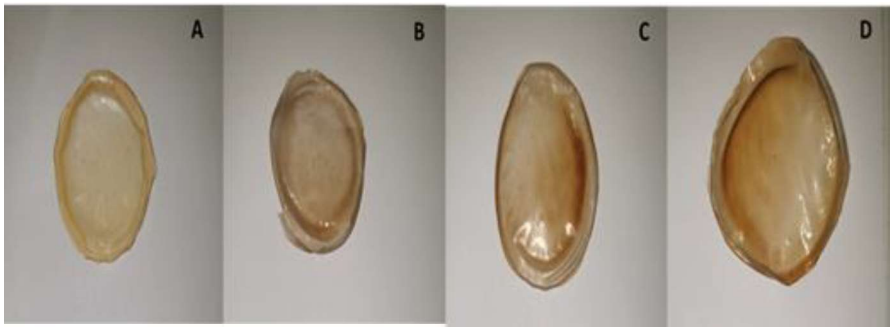

Fig. 1. *1 % standard chitosan membranes (CS) in lactic acid and aqueous Lythri herba extract.* ***A****. 1 % CS membrane in lactic acid,* ***B.*** *1 % CS membrane in lactic acid with 0.5 g/L Lythri herba,* ***C.*** *1 % CS membrane in lactic acid with 1 g/L Lythri herba,* ***D.*** *1 % CS membrane in lactic acid with 2 g/L Lythri herba*

Microscopic evaluation of new membranes obtained

The microscopic analysis confirms the presence of chitosan in membranes, due to its ability to emit autofluorescence at the wavelengths specified above, stating that for this analysis no specific dyes are used. Furthermore, the epifluorescence microscopy clearly highlights the details of the multilamellar arrangement in parallel layers and the oblique arrangement of filaments specific to chitosan structures (Figure 2). In addition, the microstructure of these membranes has numerous porosities and the colored components of *Lythri herba* are visibly included in the membranes.

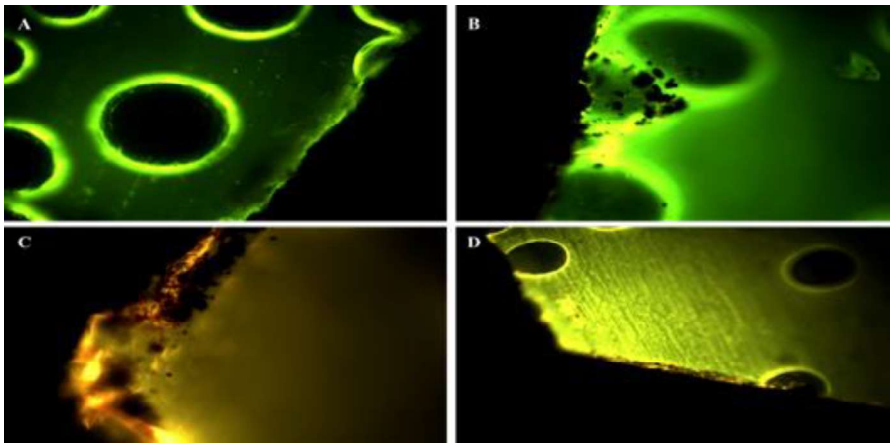

Fig. 2. *Chitosan membranes in 1 % lactic acid solution under the epifluorescence microscope (x200),* ***A*** *–CS (1 %) membrane,* ***B*** *–CS (1 %) membrane with Lythri herba extract (0.5 g/L),* ***C*** *– CS (1 %) membrane with Lythri herba extract (1 g/L),* ***D*** *–CS (1 %) membrane with Lythri herba extract (2 g/L)*

The percentage hydration (%) determination of new membranes

In mucoadhesive applications, knowing the hydration degree for membranes is especially useful, as it could be an important preliminary aspect for other tests, such as biocompatibility. Our study highlights the structural stability and the possibility to establish a contact time between chitosan-extract mixtures and mucoadhesive surfaces.

Following exposure of the membranes in PBS buffer, an increase in their hydration was observed in the first 10 minutes after exposure, followed by stagnation in terms of weight in the following time intervals (Figure 3) compared to CS (1 %) membrane, chosen as a reference sample.

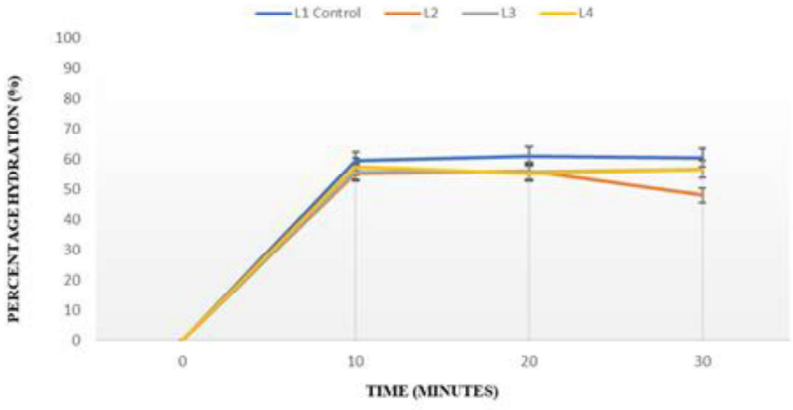

Fig. 3. *Degree of hydration of CS (1 %) membranes in lactic acid solution tested as a function of time (minutes) (± SD).* ***L1*** *(CS in 1 % lactic acid),* ***L2*** *(a mixture of CS 1 % in lactic acid + Lythri herba extract 0.5 g / L),* ***L3*** *(mixture of CS 1 % in lactic acid + Lythri herba extract 1 g/L),* ***L4*** *(mixture of CS 1 % in lactic acid + Lythri herba extract 2 g/L)*

At the time interval of 10 minutes, the membranes in lactic acid solution (1 %) appeared to be stable, without deformations, and showing a tendency to roll. The colors of the tested membranes, during hydration, did not have any noticeable change, which denotes the stability of the bonds (of chemical nature) between chitosan and the components of the analyzed extract.

At the time interval of 20 minutes all tested membranes in diluted lactic acid solution (1 %) exhibit stability, without deformations, but visibly they are much more flexible. Membrane L1, the reference sample (standard chitosan in 1 % lactic acid) is softer and more flexible than membranes with aqueous *Lythri herba* extracts (L2, L3, L4) incorporated. Compared to the membranes that remained in the alkaline buffer PBS for 10 minutes, it is no longer observed the same tendency of rolling of the edges; except for this observation of the L4 membrane is making (Figure 4).

At the hydration time interval of 30 minutes, the membranes in 1% lactic acid solution are visibly softer, without deformations, and no longer show the same

tendency to roll as the membranes after the time interval of 10 minutes, except for this observation making the L3 membrane, whose edges have rolled more.

Low mechanical resistance in water of standard chitosan (1 %) membranes were common points of the various studies performed [12], [13], a statement also proven in the current paper on the L1 membrane of standard chitosan (1 %) in 1 % lactic acid solutions.

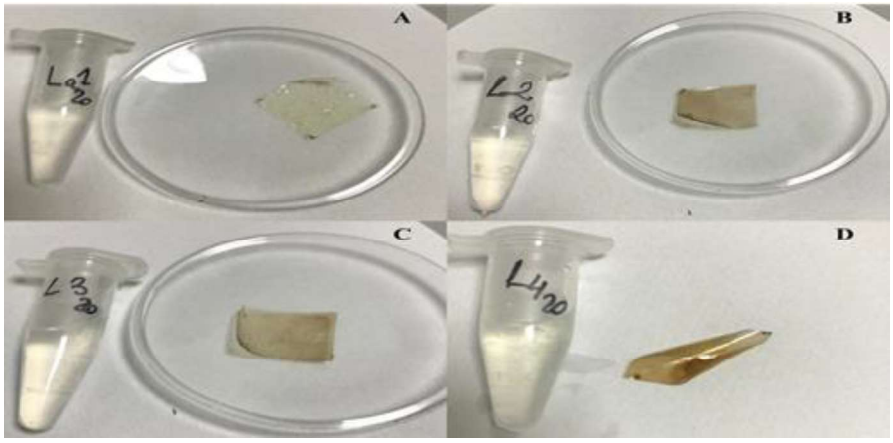

Fig. 4. *CS membranes in 1% lactic acid tested.* ***A*** *- L1 (CS in 1 % lactic acid),* ***B*** *- L2 (mixture of CS 1 % in lactic acid + Lythri herba extract 0.5 g/L),* ***C*** *- L3 (mixture of CS 1 % in lactic acid + Lythri herba extract 1 g/L),* ***D*** *- L4 (mixture of 1 % CS in lactic acid + Lythri herba extract 2 g/L)*

This study presents strong stability and flexibility of the membranes made from the mixture of chitosan with *Lythri herba* extract. The results obtained confirm the hypothesis of the study to create from chitosan a favourable matrix for the compounds of the analyzed plant, to promote obtaining a resistant, and flexible system, but also with therapeutic potential induced by the components of Lythri herba extract.

The correlation between extract concentration and hydration stability of membranes reveals an interaction with chitosan, which is even higher as the extract quantity increases. This information is supported by data from the literature according to which chitosan interacts, through its amino groups, with some groups of polyphenols contained in plant extracts [14].

Our studies on the cytotoxic activity of the extract [15] showed low toxicity of *Lythri herba* solutions, which further strengthens the potential support that the extract could induce.

As a result, our study focused to obtain stable chitosan-extract membranes with promising applications for the therapy of mucosal surfaces or those interacting with aqueous solutions.

CONCLUSION

To the best of our knowledge, this is the first time when membranes with potential in therapeutic applications are obtained by mixing in a compatible and stable ratio of solutions of standard chitosan (1%) in lactic acid (1%) with the aqueous solutions of *Lythri herba* plant extract.

Epifluorescence microscopy confirmed the presence of fluorescent chitosan in membranes and clearly showed its multilamellar, oblique, and superficial layer arrangement.

The membranes obtained for the first time showed constant hydration over time and had a flexible, elastic, and deformation-free behavior, these features are necessary for mucoadhesive biomaterials, to their use in the medical and pharmaceutical field.

Following the analyses carried-out, our study showed the therapeutic potential of *Lythrum salicaria* L., which could be improved by combining with chitosan, at appropriate concentrations in the form of membranes (mucoadhesive films), in order to increase the number of pharmaceutical or biomedical applications.

REFERENCES

[1] Piwowarski, J. P.; Granica, S.; Kiss, A. K. Lythrum salicaria L. Underestimated medicinal plant from European traditional medicine. A review. *Journal of ethnopharmacology*, 2015, 170: 226-250.

[2] Sakthiguru, N., & Sithique, M. A. (2020). Fabrication of bioinspired chitosan/gelatin/allantoin biocomposite film for wound dressing application. *International journal of biological macromolecules*, 152, 873-883.

[3] İlk, S., Ramanauskaitė, A., Bilican, B. K., Mulerčikas, P., Çam, D., Onses, M. S., ... & Zang, L. S. (2020). Usage of natural chitosan membrane obtained from insect corneal lenses as a drug carrier and its potential for point of care tests. *Materials Science and Engineering*: C, 110897.

[4] Hu, Q., & Luo, Y. (2016). Polyphenol-chitosan conjugates: Synthesis, characterization, and applications. *Carbohydrate polymers*, 151, 624-639.

[5] Kaya, M., Khadem, S., Cakmak, Y. S., Mujtaba, M., Ilk, S., Akyuz, L., ... & Deligöz, E. (2018). Antioxidative and antimicrobial edible chitosan films blended with stem, leaf and seed extracts of Pistacia terebinthus for active food packaging. *RSC advances*, 8(8), 3941-3950.

[6] Bajić, M., Ročnik, T., Oberlintner, A., Scognamiglio, F., Novak, U., & Likozar, B. (2019). Natural plant extracts as active components in chitosan-based films: A comparative study. *Food Packaging and Shelf Life*, 21, 100365.

[7] Khutoryanskiy, V. V. Advances in mucoadhesion and mucoadhesive polymers. *Macromolecular bioscience*, 2011, 11.6: 748-764.

[8] Ahmed, S., & Ikram, S. (2016). Chitosan based scaffolds and their applications in wound healing. *Achievements in the life sciences*, 10(1), 27-37.

[9] Al-Dhubiab, B. E., Nair, A. B., Kumria, R., Attimarad, M., & Harsha, S. (2016). Development and evaluation of buccal films impregnated with selegiline-loaded nanospheres. *Drug Delivery*, 23(7), 2154-2162.

[10] Nair, A. B., Kumria, R., Harsha, S., Attimarad, M., Al-Dhubiab, B. E., & Alhaider, I. A. (2013). In vitro techniques to evaluate buccal films. *Journal of Controlled Release*, 166(1), 10-21.

[11] Jouravel, G., Guénin, S., Bernard, F. X., Elfakir, C., Bernard, P., & Himbert, F., New biological activities of *Lythrum salicaria* L.: Effects on keratinocytes, reconstructed epidermis and reconstructed skins, Applications in dermo-cosmetic sciences. *Cosmetics*, 2017, 4(4), p. 52

[12] De Masi, A., Tonazzini, I., Masciullo, C., Mezzena, R., Chiellini, F., Puppi, D., & Cecchini, M. (2019). Chitosan films for regenerative medicine: fabrication methods and mechanical characterization of nanostructured chitosan films. *Biophysical Reviews*, 1-9.

[13] Hps, A. K., Saurabh, C. K., Adnan, A. S., Fazita, M. N., Syakir, M. I., Davoudpour, Y., ... & Dungani, R. (2016). A review on chitosan-cellulose blends and nanocellulose reinforced chitosan biocomposites: Properties and their applications. *Carbohydrate polymers*, 150, 216-226.

[14] Jing, Y., Diao, Y., & Yu, X. (2019). Free radical-mediated conjugation of chitosan with tannic acid: Characterization and antioxidant capacity. *Reactive and Functional Polymers*, 135, 16-22

[15] Iancu, I., M., Bucur, L., A., Schroder, V., Mireşan, H., Mihai, S., Iancu, V., Badea, V., Phytochemical evaluation and cytotoxicity assay of Lythri herba extracts, *Farmacia*, 2021, 69(1), 51-58