UNIVERSITY OF MEDICINE AND PHARMACY

"CAROL DAVILA", BUCHAREST

DOCTORAL SCHOOL

PHARMACY FIELD

Implications of the properties of chitosan in the development of some intranasal insulin release systems targeting diseases at the CNS level

ABSTRACT OF THE DOCTORAL THESIS

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Introduction

The increase in the number of people diagnosed with diseases of the central nervous system motivated us to pay attention to the nasal administration of active pharmaceutical ingredients [1, 2]. This type of administration is less used, but has multiple advantages and needs to be explored in order to increase the bioavailability of active pharmaceutical ingredients. Up to date, a number of studies have been carried out for the intranasal administration of drugs with local and systemic action, but a greater interest is being allocated to those with action in the central nervous system.

The well-known characteristics of chitosan and carboxymethyl chitosan polymers were the basis for the development of hydrocolloidal systems with nasal administration, because the impact of the mucoadhesive properties and of enhancing the permeability of the active pharmaceutical ingredient through the nasal mucosa is beneficial, thus favoring their bioavailability at the level of the central nervous system. The studies carried out so far have shown that the polymer and its derivatives are biodegradable, biocompatible and do not present toxicity, which makes them compatible with mucosal administration.

Insulin is a hormone, currently used to treat diabetes, but what is less known is that it also has actions at the level of the central nervous system. Insulin is considered a neuroprotein because it has been shown to have potentially beneficial therapeutic effects in conditions associated with the central nervous system, such as Alzheimer's disease, Parkinson's disease, and cognitive impairment. It also has the ability to modulate nicotine cravings and limit the symptoms associated with abstinence syndrome and favoring smoking cessation.

1. Chitosan. General aspects of the application of chitosan in the formulation of medicinal products

Chitosan is a natural polysaccharide, generally obtained from a marine source [3]. Chitosan and its derivatives have mucoadhesive properties [4, 5], they enhance the permeability through the nasal mucosa due to the positive charge given by the amino groups, thus stimulating the bioavailability of the substances [6].

Regarding the surface tension of the polymer, it decreases with the increase in chitosan concentration [7, 8].

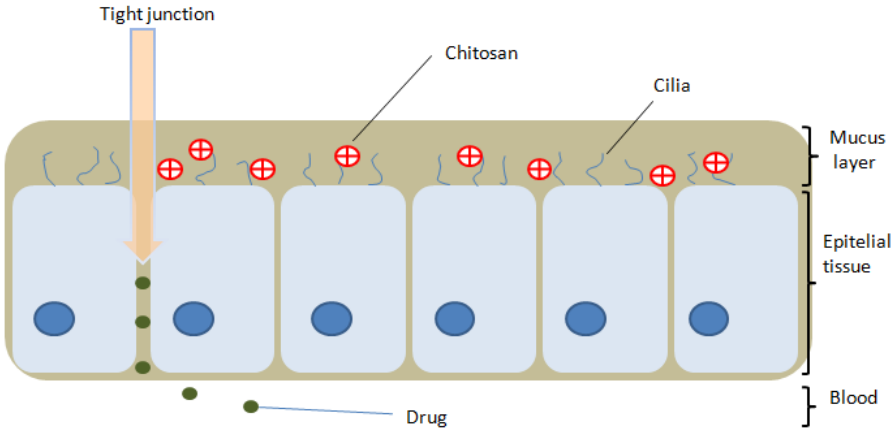

Figure 1.1. Mucoadhesive property of chitosan on the nasal mucosa and opening of the epithelial tight junction effect (Adapted after Popescu, R., M.V. Ghica, C.E. Dinu-Pirvu, V. Anuta, D. Lupuliasa, and L. Popa, New Opportunity to Formulate Intranasal Vaccines and Drug Delivery Systems Based on Chitosan. Int J Mol Sci 2020, 21 [6])

The interactions between chitosan and proteins [9], lipids [10] or other molecules [11] lead to compounds that have their own applications or to the modification of the characteristics of the substances involved in the connection (conformational changes [12], increased solubility [13], etc.).

Chitosan and its derivatives have their own actions (antibacterial [14, 15], antifungal [16], antitumor [17], antioxidant [18], hemostatic [19], immunostimulatory [20], neuroprotective [21] and others) and can be used as such or in combination with other molecules of the same class to achieve a synergistic effect [22-24].

Studying the properties of chitosan and its derivatives is necessary to evaluate their influence in the development of nasal formulations. Studies to date have shown that chitosan is biodegradable, biocompatible and non-toxic making it suitable for mucosal delivery, leading to the formulation of nasal drug delivery systems [25-27].

2. Chitosan-Based Formulations for Intranasal Administration

The nasal route shows higher bioavailability compared to the oral route, avoiding hepatic [28] and intestinal metabolism [29]. Biologically active drugs reach the brain directly, from the nasal cavity, along the trigeminal nerve and through the olfactory system, bypassing the blood-brain barrier in a non-invasive way [28, 30], or are absorbed through the nasal epithelium into the systemic circulation and then they pass through the BBB into the CNS [31]. Systems that transport the biologically active molecules in the body without being invasive and without penetrating the BBB are considered third generation [32].

Nasal vaccine can be used to overcome the disadvantages of the conventional and injectable ones [30]. These can prevent outbreaks or pandemics, leading to a more effective management of transmissible diseases. The use of nasal vaccines can increase the percentage of people immunized [33]. As the nasal route is non-invasive and rapid [34], the vaccine can be easily administered at home without the need for qualified medical personnel [28]. The use of chitosan as an adjuvant in the formulation of nasal vaccines helps to obtain a superior immune response [35].

According to the literature, numerous researches include drug delivery systems containing chitosan or its derivatives for intranasal administration and incorporating different active substances, such as: hydrogels with ropinirole or insulin [36], nanoparticles with methylprednisone or insulin [37], microspheres with carvedilol or diltiazem [38], emulsions with quetiapine or zolmitriptan [39] and so on.

Besides the well-known property of insulin to regulate glucose metabolism in the treatment of diabetes [39, 40], intranasal administration also shows favorable effects in CNS-related diseases [41, 42] through its neuroprotective action [43]. Different clinical studies aim to investigate the potential therapeutic effects of insulin in diseases such as: memory impairment, Alzheimer's disease, Parkinson's disease, smoking cessation and others [44].

3. Working hypothesis and general objectives

The research presented in the present thesis was based on the biodegradable, biocompatible and non-toxic characteristics of chitosan, but most importantly, on the mucoadhesive properties and its ability to improve the penetration through the nasal mucosa of active pharmaceutical ingredient in general, but especially of insulin.

Studies carried out to date have shown the benefits of intranasal insulin administration, and in this work, we aim to evaluate the properties of hydrocolloidal systems based on chitosan and its derivatives containing insulin.

The main objective of the doctoral work is represented by the formulation and evaluation of hydrocolloidal systems based on chitosan and, respectively, using the derivative - carboxymethyl chitosan, which incorporates insulin, with the aim of transporting the active substance from the nose to the brain.

4. General research methodology

The general research methodology, which was developed within the collective of the Physico-Chemical and Colloidal Discipline of the Faculty of Pharmacy, within the "Carol Davila" University of Medicine and Pharmacy in Bucharest.

The pH was evaluated using the METTLER TOLEDO Seven Compact pH-meter. The calibration of the device was carried out with a standard solution with pH 4, then with pH 7 before making the measurements.

The evaluation of the surface properties consisted in the determination of the contact angle by the sessile drop method and the surface tension by the falling drop method using the goniometer CAM 101 (KSV Instruments, Finland) based on the Young-Laplace equation. The results obtained from the two determinations were included in the calculation of the work of adhesion, the work of cohesion and the spreading coefficient of the hidrocolloidal systems.

Determination of the rheological profile of the systems was performed with the Multi Visc viscometer (Fungilab SA, Barcelona, Spain), using the adapter for reduced viscosities for chitosan and PVA-based systems. The experiments were carried out at the conditioning temperature (4 – 8 ^{0}C), and the mathematical modeling of the rheological profiles was used to determine the viscosity. For the second study, the Lamy Rheology RM100 Plus viscometer (Lamy Rheology Instruments, Champagne au Mont d'Or, France) was used, at a temperature of 35 ^{0}C. The Power Law Model was applied to analyze the flow behavior.

The *in vitro* release studies, corresponding to the second experiment, were performed on Franz diffusion cells (Hanson apparatus), using synthetic cellulose acetate membrane, using phosphate buffer, at 35^{0}C. At predefined time intervals, samples were taken, which were analyzed spectrophotometric at 271 nm.

5. Development and preliminary evaluation of chitosan-based intranasal hydrocolloidal systems with insulin

The first study involved the development, formulation and preliminary evaluation of intranasal hydrocolloid systems based on chitosan and polyvinyl alcohol with insulin. Research was focused on determining the surface properties of the hydrocolloid systems, especially mucoadhesiveness; as well as rheological profiles, as key factors to overcome mucociliary clearance and improve therapeutic efficacy after intranasal drug administration.

To design the experimental matrix in this study, the Modde 13 program was used, in which there are 3 independent variables X1: chitosan concentration (1%, 1.5%, 2%); X2: ratio between chitosan and PVA 1% (1:2, 1:1, 2:1) and X3: amount of insulin (5 IU/g, 7.5 IU/g, 10 IU/g) [45]. In addition, the Minitab statistical program was used to generate the regression equations for each response.

The contact angle values for the hydrocolloidal systems analyzed in this study were between 54.84 ± 1.52 ^{0} and 66.74 ± 0.59 ^{0}; which indicates an increased wetting capacity and a good spreading capacity of the systems. The surface tension values obtained from the determinations are lower than that of the nasal mucosa [4].

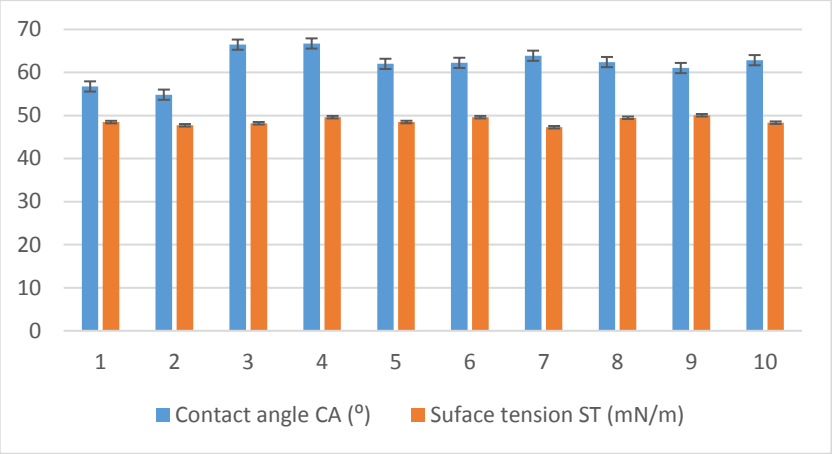

Figure 5.1. Comparative graph of contact angle and surface tension

According to the graphical representation of the regression coefficients, the increase in chitosan concentration has an inversely proportional influence on the adhesion forces and proportional to the viscosity.

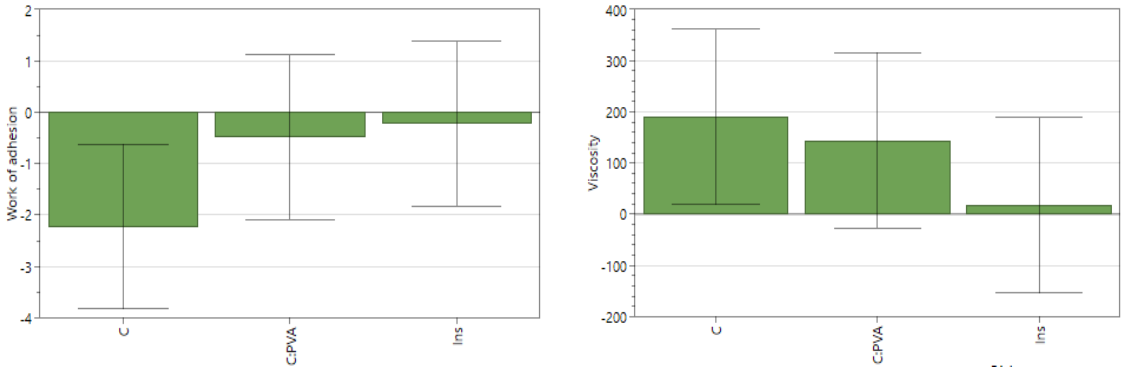

Figure 5.2. Regression coefficient plots for to the work of adhesion and viscosity

Putting together the results of the study, regarding the superficial properties and rheological profiles, the hydrocolloidal system 1 (chitosan 1%, chitosan:PVA = 2:1, insulin = 5 IU/g) can be considered the optimal sample and can be used for further research.

6. Physico-chemical characterization and initial evaluation of intranasal hydrocolloid insulin systems based on carboxymethyl chitosan – hyaluronan

The aim of this study was to develop and characterize nasal systems based on carboxymethyl chitosan (the soluble derivative of chitosan [46]) and sodium hyaluronate

with insulin, which would combine the mucoadhesive properties of the polymers and stimulate insulin absorption [47-49]. We evaluated the influence of formulation factors on the superficial properties, rheology and *in vitro* release of insulin using complementary Design of Experiments strategies.

A factorial design of 2^3 was developed, where there are three independent variables at two levels of variation, where X1 is the concentration of CMC (1% or 2%), X2 is the ratio of CMC to NaHA (1/1 or 1/2) and X3 us the amount of insulin (20 IU/ml or 30 IU/ml).

All studied samples recorded the contact angle values lower than 90 ^{0}. The determinations made in this experiment showed that formulation S1 had the closest value to the physiological surface tension of the nasal mucosa. The work of adhesion calculated based on contact angle and surface tension was over 80 mN/m for all systems. The flow behavior of the eight systems was pseudoplastic.

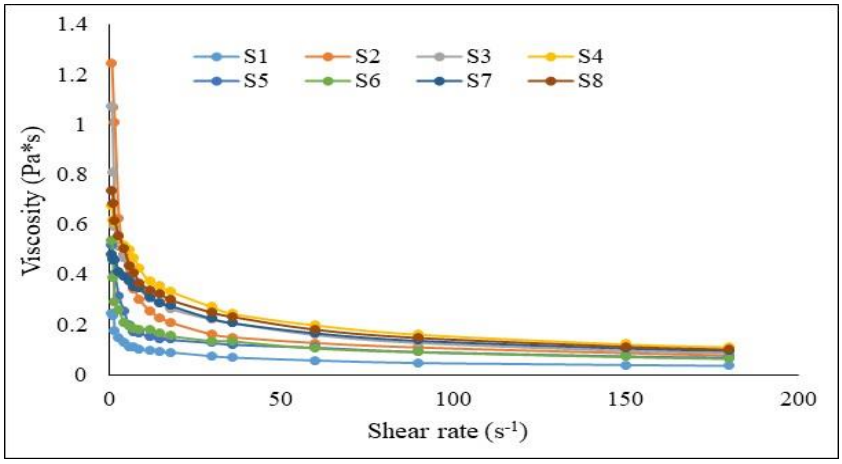

Figure 6.1. The influence of share rate on the viscosity

The release kinetics followed the zero-order model for S1, S4, S7 and S8 with R^2 greater than 0,9934 and the rest of the systems the Power-law model with R2 > 0,9958; and all samples had cumulative drug release higher than 60%.

Modde software was used to design the experimental plan and provide 3D representation of the response surface and response contour, to observe the trend of the response variables based on the changes in the formulation parameter [50]. Minitab statistical software was used to analyze the data and complement the screening provided by the Modde program by generating regression equations and Pareto charts for each response factor.

The work of adhesion was influenced by the amount of insulin (X3); and by the CMC/NaHA ratio (X2). The CMC/NaHA ratio had pronounced significance in terms of the consistency index and the release of the amount of active substance.

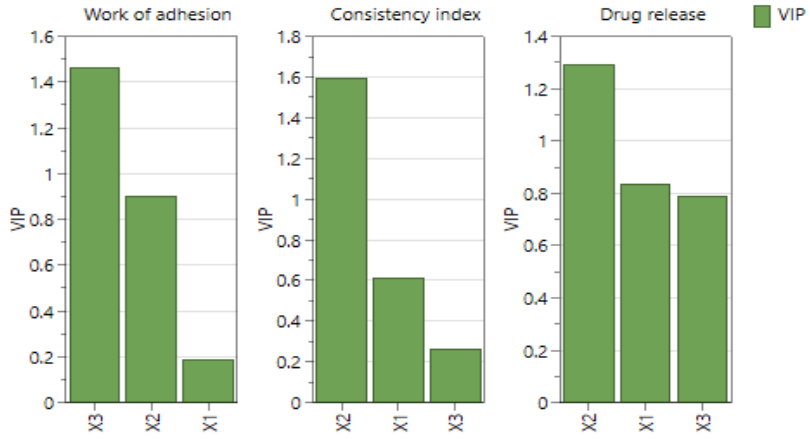

Figure 6.2. Variable importance in the projection (VIP) of the independent parameters

The S7 system is the most eloquent system compared to the predictive point of reference generated with the Modde program.

7. Conclusions and personal contributions

The doctoral thesis, entitled "*Implications of the properties of chitosan in the development of some intranasal insulin release systems targeting diseases at the CNS level*" aims to overcome the limitations given by the blood-brain barrier on active pharmaceutic ingredients acting on the central nervous system, by outlining the characteristics of chitosan and its derivatives in the formulation of medicinal systems for nasal administration.

The objectives proposed in this doctoral thesis were met, and that are: the selection of a medicinal substance with new applications discovered within the diseases associated with the central nervous system; the formulation and development of two hydrocolloid systems based on chitosan or carboxymethyl chitosan associated with other polymers, to enhance the properties of the polymers; evaluating the impact of the formulation parameters on the response variables with the help of two statistical programs. These interpretations can be used as a starting point for further studies.

These hydrocolloid systems can be included in other researches, for administration on the nasal mucosa or with applicability on other mucous membranes and in which to incorporate other active substances from different drug classes, offering new opportunities for treatment.

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List of scientific papers developed in relation to the research topic

ARTICLES PUBLISHED IN ISI INDEXED JOURNALS WITH IMPACT FACTOR

1. **Popescu R**, Dinu-Pîrvu C-E, Ghica MV, Anuța V, Popa L. Physico-Chemical Characterization and Initial Evaluation of Carboxymethyl Chitosan–Hyaluronan Hydrocolloid Systems with Insulin Intended for Intranasal Administration. International Journal of Molecular Sciences, 2024, 25(19), 10452.

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