Preparation and Characterization of Pharmaceutical Grade Chitosan And Hydrated Chitosan Gums for Topical Preparations

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ABSTRACT
The main goal of this work is the preparation and characterization of hydrated gums using chitosan for further formulation and topical applications. To achieve this goal several gums from pharmaceutical quality chitosan of low (Mw= 72,180 g/mol) and medium. (Mw= 103,000 g/mol) molecular weight with a 4% w/w were employed. The prepared hydrated gums were characterized by infrared spectroscopy, scanning electron microscopy and rheological analyses. The spectroscopic study shows no great difference between both gums and most of chitosan bands are exhibited. The rheological studies shows that both gums exhibit a non Newtonian flow independent of the time, with pseudoplastic for low molecular weight chitosan and plastic behavior for the medium molecular weight.

Key Words: Hydrated gums, Chitosan, Chitin, Pseudoplastic, Rheologic.

INTRODUCTION
The chitosan is a copolymer of ß-(1->4) linked to 2-acetamide-2-deoxy-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. There are several chitosan degrees which difference depends on the Deacetylation degree and molecular weight¹.

Chitosan is currently receiving a great deal of interest for medical and pharmaceutical applications. Chitosan is known for its biocompatibility allowing its use in various medical applications such as topical ocular applications, implantation or injection. Furthermore, chitosan is metabolized by certain human enzymes², e.g. lysozyme, and can be considered as biodegradable³⁴. Besides, Chitosan acts as a penetration enhancer by opening epithelial tight-junctions⁵⁶. Due to its positive charges at physiological pH⁷, chitosan is also bio-adhesive, which increases retention⁸ at the site of application. Chitosan also promotes wound healing and has bacteriostatic effects. Chitosan is of low cost and is ecologically interesting. The chitosan is a biopolymer, biocompatible and biodegradable with antifungal and antibacterizide, properties (Candida albicans, Trichophyton mentagrophytes y Microsporum canis)⁹ and antibacterial (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa y Salmonella paratyphi B)¹⁰ besides this improves the effect in the wound dressing injuries. In medical and pharmaceutical applications, chitosan can be used as a component in hydro gum. There are several possible definitions of a hydro gum; hydro gums can be defined as macromolecular networks in water or biological fluids. Based on the definition, hydro gums are often divided into two classes depending on the nature of their network, namely entangled networks and networks formed by physical interactions.

There are several alternative to covalently cross linked hydro gums. On the other hand, chitosan shows the ability to form biocompatible gel that can be applied directly on the injuries and for that reason this polymer becomes with enormous potential in the biomedical field.

In this study chitosan gums of low molecular weight (Mw=72,180 g/mol) with 92% degree of Deacetylation and medium molecular weight (Mw=103,200 g/mol) with 97% degree of Deacetylation, both with a 4% w/w concentration and characterized by several methods. The information obtained from the gums characterization will be fundamental for the formulation of these semisolids of topical use in the treatment of cutaneous injuries of different etiologies.

EXPERIMENTAL

Synthesis of chitosan pharmaceutical grade
The chitosan pharmaceutical grade was synthesized from chitin at Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak.¹³

The Process

Raw material
Dried/wet shells of prawns, squilla, crabs, lobsters etc., could be
utilised. The shells thus used should be thoroughly free from sand and extraneous matter, so as to reduce the ash content of the final product to less than 2%.

**Clarification of raw material**
The shells are boiled with water (ground water) for 30 minutes in a mild steel vessel to remove protein stuck to head and shell. The boiled raw material is allowed to cool and it is washed with forced water stream to remove all traces of proteins and washed with chemically neutral solution to neutralize all the excess acid-base ions. (Could be tested with a pH paper).

**Depigmentation and Deproteinisation**
The Deproteinisation and the Depigmentation of the shells is done by adding 6% Caporite solution and thoroughly mixed, transferred to a mild steel vessel lined with fiber glass and kept for 24 hours in the room temperature for the complete removal of pigments present in the shell and 94% of the proteins all precipitated out which is later separated out through centrifugation or surface capturing mechanism and the mass was dried at room temperature. The obtained mass is grinded and filtered. Then 50 g of mass was reacted with 500ml 3.5% NaOH (w/v) for 2 hours at 65ºC. The mixture was cooled and washed with ion free water until the mixture was neutral (Tested with pH paper indicator).

**Demineralisation**
For Demineralisation 30g of residue was mixed with 1.0M HCl and stirred for 30 minutes at the room temperature. The mixture obtained was solid and it was washed and dried at 60ºC to yield chitin powder.

**Removal of water** (If necessary).

Excess water is removed using a screw press till the moisture is below 60%. The product thus obtained is called chitin.

**Deacetylation of Chitin**
It is the process of conversion of chitin to chitosan. Chitin was heated at 95ºC for about one and a half hour with 42% caustic soda in a mild steel vessel. Excess alkali was drained off and the mixture was washed with water several times till it is free from alkali. Eighty percent of the alkali, thus removed could be reused in subsequent cycles.

**Removal of water**
Excess water was removed in a screw press and the product thus obtained is wet chitosan.

**Drying**
The above product was sun dried for 8 hours or in drier till the moisture content is less than 5%. Care should be taken not to exceed the drier temperature beyond 60º C. Chitosan thus obtained is in the form of flakes which were further milled and finely powdered.

**Preparation of Chitosan hydrated gums**
Chitosan hydrated gums at 4% w/w of low molecular weight (Mw=72.180 g/mol) with a 92% degree of Deacetylation and medium molecular weight (Mw=103.200 g/mol) with a 97% degree of Deacetylation. The chitosan powder was dispersed in lactic acid solution at 3.2%, 2% Hydro aerated acacia and heated at 85ºC with stirring for 24 h until complete solution. The gums was neutralized with NaOH 1M up to pH= 5 and later on filtered.

**Infrared Spectroscopy**
The hydrated gum chitosan composition was obtained by FT-IR NicoletMagna550. The gels were spread between two KBr windows. The spectra were recorded with 64 accumulations.

**Scanning Electron Microscopy**
The morphology of the gels was studied by SEM using a JEOL Model JSM 6380. The samples were treated with gold sputtering to obtain a thickness of 150 Å.

**Rheological Analysis**
The rheological behavior of the hydrated chitosan gums was measured in a rotation viscometer Haake Rotovisco a 500 head and the rotors MV-I and MV-II for both gels of different molecular weight, respectively. The temperature was kept constant at 25±1ºC.

**RESULTS AND DISCUSSION**
In the FT-IR, some characteristic bands of hydrated chitosan gums are shown below. The IR spectrum of both gums is quite similar. A β-C-C band at 850-853 cm⁻¹ is shown. The β-C-H band at 2790-2881 cm⁻¹ and the β-C-H(CH₂) at 2931-2933 cm⁻¹. The amide band at 1316-1410 cm⁻¹ is shown due to the low amount of chitin present. On the other hand, the bands corresponding to lactic acid are present in both formulations. The β-C-H of the acid appears at 3564-3514 cm⁻¹. The β-C-O exhibit different absorptions at 1043 and 1021 cm⁻¹ for low and medium molecular weight. The β-C=O of the lactic acid at 1654 cm⁻¹ with medium molecular weight gums is more intense. The βCH₂ of the lactic acid appears at 1453 and 1449 cm⁻¹, respectively.

Figure 1.a & b showing bands
1. \( \beta\text{C-C} \) band at 3290 cm\(^{-1}\)
2. \( \beta\text{C-H} \) band at 2790-2881 cm\(^{-1}\)
3. \( \beta\text{C-H(CH}_2\text{)} \) at 1246 cm\(^{-1}\)
4. The amide band at 1649 cm\(^{-1}\) is shown due to the low amount of chitin present.
5. The \( \beta\text{C-H} \) of the acid appears at 3278 cm\(^{-1}\)
6. The \( \beta\text{C-O} \) exhibit different absorptions at 1060 and 1000 cm\(^{-1}\) for low and medium molecular weight.
7. The \( \beta\text{C}=\text{O} \) of the lactic acid at 1321 cm\(^{-1}\) with medium molecular weight gums is more intense.
8. The \( \beta\text{CH}_2 \) of the lactic acid appears at 1453 and 1449 cm\(^{-1}\), respectively.

The morphological study of the due shows the formation of a homogeneous chitosan films (fig. 2 and 3) once the water has been evaporated from the gums at 30°C. The surface is smooth and they look like a film on the surface, no difference between both. This characteristics is favored if the further application is for the cutaneous injuries protecting from contaminant agent and avoiding the loss of body fluids.

![Figure 2: SEM of hydrated chitosan gum of low molecular weight at 4%](image2)

![Figure 3: SEM of hydrated chitosan gum of medium molecular weight at 4%](image3)

As we can see in figures 4 and 5, both gums show a non Newtonian behaviour, independent of the time, in fact, their viscosity thus not remain constant but changes with the speed velocity applied. The chitosan gum of low MW show a pseudoplastic flow which is characteristic of dispersed system of polymeric type. When a force is applied over the system, which is characteristic of dispersed polymeric systems. When a force is applied over the system, the chitosan chains are forming an structure in the aqueous media can separate and aligned in the flow direction, decreasing their viscosity with the increase of displacement. On the other hand, the chitosan gum of medium MW exhibit a plastic flow with a yield value of 20 equivalents to 710 dynes/cm\(^2\).

![Figure 4: Rheogram of the hydrated chitosan gum of low molecular weight at 4%](image4)

![Figure 5: Rheogram of the hydrated chitosan gum of medium molecular weight at 4%](image5)

The gum formulation of semisolid of topic application will be necessary to incorporate in the formulation some additive to increase the viscosity and produces some degree of tixotropy\(^{11}\)to the mixture. In practical terms that means to obtain an easy extraction of the flask when decreases the viscosity of the preparation, besides remains in the same place of application without flow because increases their viscosity when the product remains quite constant\(^{12}\) and stable.
CONCLUSIONS
1. The gels of medium and low molecular weight hydrated chitosan gums do not exhibit a great difference in their IR spectrum, only the intensity and shift of some bands was observed. This indicates no difference in their composition.
2. The capacity of the gums to form adhesive and flexible films after their application at 37°C will be very useful for topical preparations.
3. In order to formulate these gums as semisolid systems for topical application would be recommendable to incorporate some agent that increase the viscosity in the formulation and give tixotropy to the preparation. When the viscosity of the preparation decreases by stirring and in that way facilitates the extraction of the pot and besides remains stable with the increase of the viscosity when remains quite.

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