CHITOSAN-GELATINMICRO/NANOPARTICLES AS A CONTROLLED DELIVERY OF DEXKETOPROFEN TROMETAMOL FOR TOPICAL APPLICATIONS IN WOUND CARE

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CHITOSAN-GELATINMICRO/NANOPARTICLES AS A CONTROLLED DELIVERY OF DEX-KETOPROFEN TROMETAMOL FOR TOPICAL APPLICATIONS IN WOUND CARE (Abstract): Progress in the nanotechnology field has led to the discovery of various drug delivery systems for biomedical applications in the last few decades. Materials and methods: Micro/nanoparticles (MPs) based on chitosan (CS) and gelatin (GEL), designed as a drug delivery system for biomedical applications, were prepared using a double crosslinking process within an emulsion-phase separation system. Results: The obtained MPs were characterized using scanning electron microscopy (SEM), which revealed diameters ranging from 0.467 to 4.596 µm. The swelling properties of the MPs were investigated in a neutral environment with a pH of 5.5. Conclusions: The applicability of the prepared materials was further evaluated for drug loading and in vitro delivery of Dexketoprofen trometamol (DEX), demonstrating excellent capacity, which recommends them for effective wound care, including postoperative wounds. Keywords: CHITOSAN, GELATIN, MICROPARTICLES, NANOPARTICLES, DOUBLE CROSSLINKING, REVERSE EMULSION, DEX-KETOPROFEN TROMETAMOL, DRUG DELIVERY.

INTRODUCTION
Nanotechnology is utilized to design various particulate systems that serve as effective drug delivery supports for a range of biomedical applications due to their unique properties. Natural polymers, especially polysaccharides, are particularly advantageous for biomedical use because of their chemical structure, which impart essential qualities such as biocompatibility, non-toxicity, biodegradability, abundance, relatively low cost, and renewable nature (1, 2).
Among natural polysaccharides, CS is the most frequently used support material for polymeric particles. It is highly regarded for its exceptional properties, including biocompatibility, biodegradability, non-toxicity, mucoadhesion, and the presence of highly reactive amine groups. CS can be chemically modified to improve specific characteristics, such as solubility, mucoadhesion, and stability, thus expanding its potential applications in drug delivery (3).

Similar to CS, GEL is a biodegradable polymer that has attracted growing interest in recent years due to its diverse applications and favorable properties, such as biodegradability, biocompatibility, non-toxicity, and ease of cross linking and chemical modification (4, 5). As a natural and renewable substance, GEL is utilized in various fields, including bioengineering, pharmaceuticals, and the food industry (6, 7). It is derived from the partial hydrolysis of collagen, which is found in animal tissues such as bones, hides, and pigskin (8).

The diversity and effectiveness of CS, respectively gelatin-based particle systems for drug targeting and controlled drug release applications have been well-documented in the literature. Particles based on CS and/or GEL prepared via emulsification and crosslinking method, containing different bioactive molecules have been extensively recorded in the literature (9-13). Thus, GEL and CS particles represent a promising carrier system for controlled drug delivery technology.

The current study involves the preparation of MPs based on CS and GEL through a double crosslinking process using a water-in-oil emulsion. The MPs were characterized in terms of morphology, swelling behavior, and drug loading/release properties, which were controlled by adjusting synthesis parameters such as polymer concentration and the GEL/CS ratio. *In vitro* tests were conducted to demonstrate the MPs' capacity for drug loading and release of DEX. We can state that the prepared MPs can be used as support for post-surgical drug administration. Usually, acute wounds are associated with inflammation and in uncontrolled inflammation, proinflammatory macrophages and inflammatory mediators such as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) become overexpressed. As a result, acute wounds become chronic and difficult to heal, incurring additional costs to healthcare systems and especially decreasing the quality of life of affected patients (14). Therefore, a non-steroidal anti-inflammatory drug such as DEX can effectively contribute to the wound healing process and thus benefit both patients and healthcare system management.

**MATERIALS AND METHODS**

**Materials**

The following materials were used: low molecular weight Chitosan (CS, deacetylation degree 75-80%, Sigma Aldrich, Saint Louis, MO, USA), Gelatin (GEL, Sigma Aldrich, Saint Louis, MO, USA), Glutaraldehyde (GA, Sigma Aldrich, Saint Louis, MO, USA) –25% aqueous solution, sodium sulfate (Sigma Aldrich, Saint Louis, MO, USA), Acetone (Sigma Aldrich, Saint Louis, MO, USA), Hexane (Sigma Aldrich, Saint Louis, MO, USA), Dexketoprofen trometamol (DEX, Sigma Aldrich, Saint Louis, MO, USA), Tween 80 (Sigma Aldrich, Saint Louis, MO, USA), Span 80 (Sigma Aldrich, Saint Louis, MO, USA), Milli-Q ultrapure distilled water (DW, Merck).

**Methods**

**GEL-CS Microparticles synthesis**

The chosen method for MPs preparation involved a double crosslinking (ionic and covalent) technique using a water-in-oil emulsion (10, 11, 12). First, a CS solution was prepared by dissolving the polymer in
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a 2% (w/w) aqueous acetic acid solution. Second, GEL was dissolved in double distilled water at 40°C. According to specific polymer ratios, the CS and GEL solutions were mixed, and Tween 80 was added to the solution (1%, w/w) and stirred vigorously for 10 minutes. Next, separately, the organic phase was prepared by mixing a 1% (w/w) Span 80 solution in toluene. The MPs were obtained by gradually adding the polymer solution to the organic phase (water/oil ratio 1/4) while homogenizing with an UltraTurrax at 20,000 rpm for 10 minutes, leading to emulsion formation. Subsequently, freshly prepared crosslinker aqueous solutions, namely sodium sulfate (10%), were added at a rate of 1 mL/min with continuous stirring for another 10 minutes. Afterward, the emulsion was transferred to a round-bottom flask and mechanically stirred at 1000 rpm. Subsequently, 10 mL of a saturated GA solution in toluene was added, and the mixture was stirred for another 1 hour. The MPs were separated by centrifugation at 10,000 rpm and repeatedly washed with acetone and water to remove unreacted compounds. After washing with hexane, the obtained MPs were dried at room temperature.

Techniques used for characterization

MPs morphology was investigated by SEM technique. SEM micrographs were recorded with a HITACHI SU 1510 (Hitachi SU-1510, Hitachi Company, Japan) Scanning Electron Microscope, MPs were fixed on an Aluminum stub and coated with a 7 nm thick gold layer using a Cressington 108 device before observation.

MPs swelling behavior in aqueous media

MPs swelling characteristics in the aqueous environment were evaluated by the gravimetric method using a buffer solution (pH 5.5) (Phosphate Buffer Solution, PBS). 0.030g of MPs were immersed in a 2 mL Eppendorf tube containing 1 mL of PBS. The suspensions were monitored and maintained at room temperature under mechanical stirring (100 rpm) for 24 hours when equilibrium swelling was attained. MPs suspensions were ultra-centrifuged (12,000 rpm), withdrawn from the buffer solutions, and weighed at specified time intervals, respectively immediately replaced with an equivalent volume of fresh buffer. The maximum swelling degree was determined with equation (Eq.) (1):

\[ Q\% = \frac{w_s - w_0}{w_0} \times 100 \]  

where:

- \( w_s \) - the weight of swollen CPH;
- \( w_0 \) - the weight of dry CPH.

MPs drug loading capacity

DEX is the tromethamine salt of S-(+)-2-(3-benzyolphenyl) propionic acid, an analgesic, antipyretic, and anti-inflammatory drug that belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs)(15). DEX was utilized as a model drug for loading into the microparticle (MPs) drug delivery system. The loading process of DEX was conducted via a diffusional mechanism. Specifically, MPs (0.030g) were suspended in 1 mL of an aqueous DEX solution (c = 20 mg/mL, dissolved in PBS pH=5.5) and dispersed by sonication for 10 minutes. These suspensions were maintained at room temperature with gentle mechanical stirring for 72 hours. After, MPs samples were centrifuged (15,000 rpm, 5 min). The amount of DEX encapsulated in the MPs was quantified by determining the drug content in the supernatant, calculated using Eq. (2) derived from a previously prepared calibration curve.

\[ y = 3.9395x + 0.0092; \quad R^2 = 0.9917 \]
Drug encapsulation efficiency (DEE) represents the quantity of added drug (%) that is encapsulated in the formulation. The DEE of LEV from CPH was calculated with Eq. (3). The DEX entrapment efficiency of MPs was determined with Eq. (4).

\[
\text{DEE} = \frac{\text{Actual drug (LEV) content loaded in CPH}}{\text{Theoretical drug content (LEV)}} \times 100 \tag{3}
\]

Entrapment efficiency = \(\frac{\text{The total amount of LEV} - \text{Amount of LEV from supernatant}}{\text{Total amount of LEV}}\) \tag{4}

All tests were accomplished with a spectrophotometer UV-VIS Nano Drop ND-1000. DEX-loaded MPs were freeze-dried.

**In vitro DEX release from MPs**

To assess the *in vitro* drug release properties, the release of DEX-loaded MPs was conducted in PBS with a pH of 5.5, ensuring a slower release rate of the drug. The DEX release process involved immersing the DEX-loaded MPs in a 2 mL Eppendorf tube containing 1 mL of PBS. The suspensions were maintained at 37°C with continuous gentle stirring at 50 rpm. At predetermined time intervals, the suspensions were ultracentrifuged at 12,000 rpm, and the amount of released DEX was quantified using UV-VIS spectrometry at 258 nm. Each determination was performed in triplicate, and the results were averaged.

**RESULTS**

The selected method for the MPs preparation was a double crosslinking (ionic and covalent) technique within a water-in-oil emulsion. The method advantage is due to a reduced amount of covalent crosslinker required, which subsequently decreases the final product's toxicity. The fundamental principle of this process involves forming an interconnected/interpenetrated network initially through predominantly ionic crosslinking, followed by a covalent crosslinking step. The experimental parameters used for the preparation of the MPs are detailed in first table.

<table>
<thead>
<tr>
<th>TABLE I. MPs Preparation parameters</th>
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<tbody>
<tr>
<td>Sample code</td>
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</tr>
<tr>
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<td>M7</td>
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<td>M8</td>
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The parameters taken into consideration for the preparation process were total initial concentration and the GEL/CS ratio. The initial water/oil phase ratio, surfactant concentration, and ionic and GA content in the aqueous/organic phase were kept constant.
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throughout the study.

The morphology of the prepared micro-particulate systems was examined using SEM (fig. 1). The results indicate that the MPs exhibit a spherical shape, are well-individualized, generally possess a micron diameter, and demonstrate a dimensional polydispersity. Moreover, two primary particle populations were observed: the first consisted of particles in the micron range, characterized by rough surfaces and an approximately spherical shape, while the second population comprised submicron particles with a distinct spherical shape and smooth surfaces. Also, the images confirm the obtaining of MPs with variable diameters which ranged between 0.550 – 1.36 µm for M1-M4, respectively 0.467 – 4.596 µm for M4-M8. The adapted emulsion double crosslinking procedure effectively produces clear particulate systems with relatively low dimensional dispersity, as evidenced by the SEM micrographs.

To further understand the interaction between the MPs and the aqueous environment, swelling studies were conducted in a neutral medium (pH = 5.5) using a gravimetric method. The water swelling behavior provides insights into the interactions between double-crosslinked MPs CS-GEL and the selected environment, as well as its suitability for the drug loading and release processes. Figure 2 displays the kinetic data for all samples, while table II presents the maximum swelling degree values obtained. The results revealed that the maximum swelling degree for the M1-M4 samples ranged from 706% to 787%, and for the M5-M8 samples, it ranged from 681% to 788%. The swelling kinetics, as shown in Figure 2, indicate that an equilibrium phase is established, demonstrating the good stability of the particulate formulations. Also, was observed that when the ratio between GEL and CS is in favor of GEL the water uptake increased, demonstrating good water-CS interactions, most likely favored by the hydrophilic GEL.

DISCUSSION

Considering the swelling results which demonstrate that MPs presented a high ability to swell, DEX was loaded in neutral conditions ensuring the encapsulation of a higher amount of drug. The ability of MPs to encapsulate drugs was analyzed using DEX as a model drug. The DEX content in the supernatant was analyzed using UV-VIS spectrophotometry (at 258 nm wavelength) based on an established calibration curve. Table III presents the DEX loaded MPs amounts and loading efficiency. MPs were able to entrap DEX between 0.46 – 0.48 mg drug/mg particles. Therefore, the DEX loading efficiencies values calculated with Eq. 4 were between 98.0 – 99.0%, showing a high capacity of the MPs as support for the biologically active compound.

The DEX release capacity of MPs was analyzed by the diffusion method in PBS pH = 5.5 at 37°C. The released DEX for all samples is presented in Table IV and the kinetic data are displayed in Figure 3 a) and b). The results showed a fast release phase which is reached within the initial 6 hours followed by a slower phase (characterized by a slow release) until 72 hours. The sustained release of DEX can be explained by the fact that the released drug was adsorbed in the MPs network due to excellent hydrophilicity of the polymer network which it seems that it is maintained also after the crosslinking procedures. The maximum released amount of DEX varied between 0.146 and 0.301 DEX mg/mg MPs (Table IV, Figure 3a). Also, the release efficiency of DEX for all MPs samples, values between 30 and 65% were obtained (tab. IV, fig. 3b), the highest efficiency being noticed for the sample M4 and M8 which has been shown also a high-swelling degree.
Fig. 1. Micro/Nanoparticles SEM images.
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TABLE II.
Microparticle swelling degree in PBS pH=5.5

<table>
<thead>
<tr>
<th>Sample code</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>M7</th>
<th>M8</th>
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<tr>
<td>Swelling degree, %</td>
<td>706</td>
<td>750</td>
<td>768</td>
<td>787</td>
<td>681</td>
<td>740</td>
<td>753</td>
<td>788</td>
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</table>

![Fig. 2. Microparticles Swelling degree](image)

TABLE III.
Microparticles Loading DEX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>M7</th>
<th>M8</th>
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<tbody>
<tr>
<td>DEX mg / mg MPs</td>
<td>0.479</td>
<td>0.477</td>
<td>0.511</td>
<td>0.478</td>
<td>0.48</td>
<td>0.481</td>
<td>0.481</td>
<td>0.464</td>
</tr>
<tr>
<td>DEX loading Efficiency, %</td>
<td>98.93</td>
<td>98.67</td>
<td>98.8</td>
<td>98.73</td>
<td>99.27</td>
<td>99.33</td>
<td>99.33</td>
<td>99.07</td>
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TABLE IV.
Microparticles Releasing DEX

<table>
<thead>
<tr>
<th>Sample code</th>
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<th>M2</th>
<th>M3</th>
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<th>M6</th>
<th>M7</th>
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</thead>
<tbody>
<tr>
<td>DEX mg / mg MPs</td>
<td>0.146</td>
<td>0.256</td>
<td>0.30</td>
<td>0.299</td>
<td>0.175</td>
<td>0.238</td>
<td>0.259</td>
<td>0.301</td>
</tr>
<tr>
<td>DEX releasing Efficiency, %</td>
<td>30.42</td>
<td>53.57</td>
<td>58.87</td>
<td>62.50</td>
<td>36.41</td>
<td>49.49</td>
<td>53.95</td>
<td>64.88</td>
</tr>
</tbody>
</table>
Fig. 3. MPs ability to *in vitro* release DEX: a) DEX mg/mg MPs; b) DEX releasing Efficiency, %
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The results of release kinetics were analyzed also based on the Korsmeyer-Peppas mathematical model. From the linear plotting of DEX release data (figs. 4a, 4b), it can be observed that \( n \) is smaller than 1, indicating that the drug release mechanism follows a Non-Fickian transport (16, 17).

**Fig. 4.** Korsmeyer-Peppas model for a) M1-M4 samples; b) M5-M8 samples
The diffusional exponent \((n)\) values, determined using the Korsmeyer-Peppas model, ranged from 0.37 to 0.48. In the case of M1 - M4 samples the diffusional exponent \((n)\) values were between 0.43 and 0.48. It can be observed that the values of the exponent \(n\), which characterize the release mechanism, are notably close to 0.5 \((0.5 < n < 1.0)\). The results suggest that the transport/release mechanism is predominantly governed by diffusion. Moreover, in the case of samples M5 – M8 was observed that the values of the exponent \(n\) are lower than 0.5. This indicates an anomalous transport, which combines both diffusion and swelling mechanisms of the analyzed samples (18).

CONCLUSIONS
The present work demonstrates the amenability of CS-GEL for the preparation of particulate systems through a double crosslinking process into a reverse emulsion. SEM micrographs revealed two primary particle populations: the first consisted of micron-sized particles with rough surfaces and an approximately spherical shape, while the second population comprised submicron particles with a distinct spherical shape and smooth surfaces. Additionally, the images confirmed the formation of MPs with variable diameters ranging from 0.550 to 1.36 \(\mu\)m for M1-M4, and from 0.467 to 4.596 \(\mu\)m for M4-M8. MPs behavior in aqueous media with pH = 5.5 was studied, demonstrating a higher water retention capacity.

The MPs water uptake properties are significantly influenced by polymer concentration and CS-GEL ratio employed in the preparation stage, and they are correlated with the particle size. The drug loading and release capacity of MPs, tested using DEX, showed a good diffusional loading capacity, up to 0.5 mg of DEX per mg of MPs in a neutral environment. A rapid drug release was observed within the first 6 hours, followed by a slower phase, characterized by a constant release, up to 72 hours. Drug release kinetics analysis demonstrated that the drug transport/release mechanism is predominantly diffusional.

The obtained result confirms that CS-GEL-based MPs are a promising carrier system for controlled drug delivery for biomedical applications, including topical application of DEX to support the normal wound healing process, with all the advantages of prolonging the time of action and minimizing the specific side effects of NSAIDs, which will significantly improve patient compliance with this type of treatment.

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CONFLICT OF INTEREST
The authors declare no conflicts of interests.

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