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Synthesis and characterization of a polyurethane transdermal carrier for lupeol

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Abstract

A few polyurethane (PUR) copolymers were recently considered as possible material in the drug delivery systems domain. This study intends to obtain a transdermal carrier for lupeol. PUR nanocapsules were synthesized by interfacial polycondensation combined with spontaneous emulsification. An organic phase containing lysine diisocyanate ester in water-miscible solvent (acetone) and a homogeneous aqueous phase formed by water, diols, polyether and a surfactant (Tween®20) were used in the synthesis. After the optimization of the work procedure, was obtained around 600 nm size nanocapsules which were characterized by pH, size and zeta potential measurements, SEM and DSC and no significant modifications compared with an ethalon were reported

Keywords: lupeol, drug carrier, polyurethane, hollow nanocapsule, lysine diisocyanate ester

1. Introduction

Lupeol (3β-hydroxylup-20(29)-ene) is a pharmacologically bioactive triterpenoid found in a variety of plants, including mango, acacia visco and others [1-3].

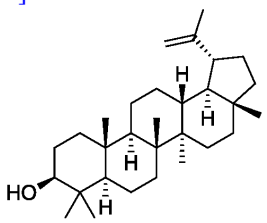

Figure 1. Lupeol structure

Many bioactivities and bioassays of this compound were reviewed [4], which suggest its useful medicinal properties with diversity of action against different diseases. In literature, it was reported to be antiangiogenic [5], antioxidative and anti-inflammatory in nature [6-8].

A 1998 study found lupeol to decrease paw swelling in rats by 39%, compared to 35% for the standardized control compound indomethacin [9]. It also plays very important role in normalization of lipid profile [10], protective effect in hypercholesterolemia associated with renal damage [11] and suppression of immune factors [12-14]. Lupeol inhibits cancer growth and ameliorates inefficiency of cancer cells to undergo apoptosis [15].

It has been found to induce differentiation and inhibit the cell growth of mouse melanoma and human leukemia cells [16, 17]. In another study, lupeol, betulin, and methyl betulinate, glycosides (b-D-glucosides, a-L-rhamnosides, and a-D-arabinosides) were synthesized and tested in vitro for cytotoxicity against three cancerous cell lines: human lung carcinoma (A-549), human colon adenocarcinoma (DLD-1) and mice melanoma (B16-F1) [18].

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Pentacyclic triterpenes are important pharmacological active compounds and a modern techniques can improve their solubility and biological activity [19].

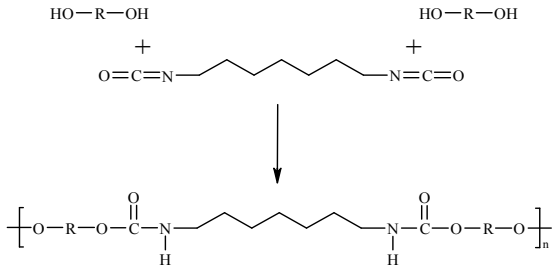
The major classes of nanoparticles used for nanotech medical applications include: liposomes, nanoshells, carbon-based particles (carbon nanotubes and fullerenes), nanoemulsions, nanocrystals, and polymer-based nanomaterials [20].

The aim of this study was the obtaining of nanocapsules with around 600 nm diameter and we studied the differences between nanocapsules with lupeol and empty nanocapsules [20].

2. Materials and Method

Lysine diisocyanate ester (LDI) was furnished by Hangzhou Imaginechem Co., Ltd (China). Mono-ethylene glycol (MEG) is from Lach-Ner s.r.o. (Czech Rep.), 1,4-butanediol (1,4-BD) from Carl Roth GmbH (Germany) and lupeol from Aldrich (Germany); all the other raw materials, polyethylene glycol (PEG 200), the solvent (acetone) and surfactant (Tween®20) were obtained from Merck (Germany).

The polyaddition reaction between diols and diisocyanates for the polyurethane products synthesis is:



It was used a hydroxyl component / isocyanate component ratio = 1.1 : 1 because the hydroxyl excess is easier to be removed and the quantity of products from secondary reactions (amines) will be decreased.

Based on other previous results, the protocol for obtaining PUR nanocapsules by interfacial polycondensation combined with spontaneous emulsification presents the following steps:

1. Preparation of the organic phase: 20 mL solution 5% diisocyanate in acetone was preheated at 40 ^{o}C.
2. Preparation of the homogeneous aqueous phase: 40 mL mixture of MEG, 1,4-BD, PEG 200 and Tween®20 in distilled water (1:1:2:1 ratio) was preheated at 40 ^{o}C. 30 mg of lupeol was inserted into this phase in one batch.
3. The organic phase was rapidly poured into the aqueous phase under magnetic stirring (5000 rpm) and heating (40 ^{o}C). This is the moment when the nanocapsules precipitate instantaneously.
4. The stirring is still maintained for four hours at 40 ^{o}C in order to ensure the maturation of the nanocapsules walls.
5. The solvent (acetone) and a part of water is removed by slow evaporation by keeping the suspension as thin layers (approx. 3 mm) in Petri dishes at 60 ^{o}C in oven for 12 hours.
6. The products were purified by three times cycle of centrifugation and redispersion in a mixture (water-acetone 1:1 v/v) in order to eliminate secondary products (amines).

After the samples were well dried, we studied their solubility and we measured the pH of nanocapsules solutions at the same concentration. The nanocapsules aspect and physico-chemical properties were finally characterized by pH, size and charge measurements, SEM, DSC and FT-Raman spectroscopy.

Electronic scanning microscopy (SEM). PU nanoparticles shape and morphology were examined using a scanning electron microscope Hitachi 2400S (Hitachi Scientific Ltd., Japan) using a voltage of 10 kV. A thin-layer covering device (Bio-Rad SC 502, VG Microtech, England) was used to obtain an electric conductivity to the surface of the samples. The air pressure was between 1.3-13.0 mPa.

Thermal behavior. The thermal decomposition examinations were carried out with a Mettler-Toledo 821e instrument between 30-300 ^{o}C because it is well-known that the urethane group (-NH-COO-) is stable in this temperature range.

Size and zeta potential. The particles size and charge were measured using a Zetasizer Nano series equipment Nano-Zs, Malvern Instruments (Table I). The samples were diluted at a ratio of 1:5000 (v / v).

The nanocapsules present a very low solubility in water even after the bath sonication. The measurements were carried out two times for each sample.

3. Results and Discussion

The PUR nanocapsules present a low solubility and did not disperse well in the water even after the bath sonication at room temperature. The pH values of nanocapsules solutions were measured with a Schott TitroLine by simply plunging the electrode into the aqueous solutions (1:5000 v / v). The empty nanocapsules samples present an average value of 6.44 for pH and in the case of nanocapsules with lupeol samples it was 6.62. The slightly acid pH is due to the characteristics of its components and it demonstrates the absence of secondary products (amines). Besides, these pH values (close to 5) are appropriate for products intended for cutaneous administration.

The particles size and charge were measured using a Zetasizer Nano series equipment (Table I). The measurements were carried out three times for each sample.

Table 1. The Zetasizer characterization for the PUR nanocapsules

Batch No.	Particle size (nm)		Zeta Potential (mV)
	Mean ± SD	Polydispersity index	Mean ± SD
1	643 ± 201	0.6	23 ± 1.0
2	632 ± 192	0.5	27.5 ± 0.4

The large values for the diameter of each bench can be attributed to particle aggregation. The zeta potential values are important because if all the particles have a zeta potential which is more negative than - 30 mV or more positive than + 30 mV the dispersion should remain stable. The second batch, with lupeol, is more stable than the empty nanocapsules batch.

The shape and morphology of the nanocapsules were investigated by a scanning electron microscope Hitachi 2400S (Hitachi Scientific Ltd., Japan) using a voltage of 10 kV and a thin layer coating device (Bio-Rad SC 502, VG Microtech, England) in order to induce electrical conductivity of the sample surface; air pressure was 1.3-13.0 mPa.

It was detected the existence of nanocapsules with irregular shapes (Figure 2; the shape and the sizes does not depend on the introduction of lupeol.

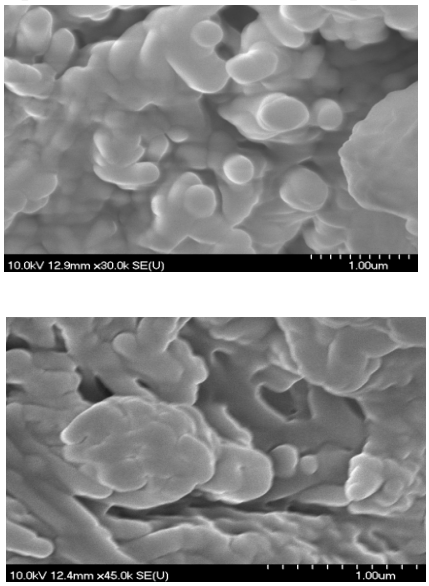


Figure. 2. Scanning electron microscopy of empty PUR nanocapsules (1) and nanocapsules with lupeol (2)

The thermal decomposition examinations were carried out with a Mettler-Toledo 821e instrument between 30-350 ^{o}C because it is well-known that the urethane group (-NH-COO-) is stable in this temperature range. Both samples present a good stability in the studied temperature range with a small peak around 70 ^{o}C due to the volatile raw materials (Figure 3).

All these measurements indicated that such type of nanocapsules can be a drug deliverys system for a water insoluble compound. They are stable forms and can be applied in future applications even on dermal site [1, 20]. Future in vivo evaluations of such formulations will establish the efficacy but the maintaining of stability for the presented formulations lead to the idea of possible new lupeol-delivery systems.

It was obtained hollow PUR nanocapsules for lupeol transdermal delivery by using the interfacial polycondensation technique combined with spontaneous emulsification. The nanocapsules suspensions present pH values which are appropriate for products intended for cutaneous administration.

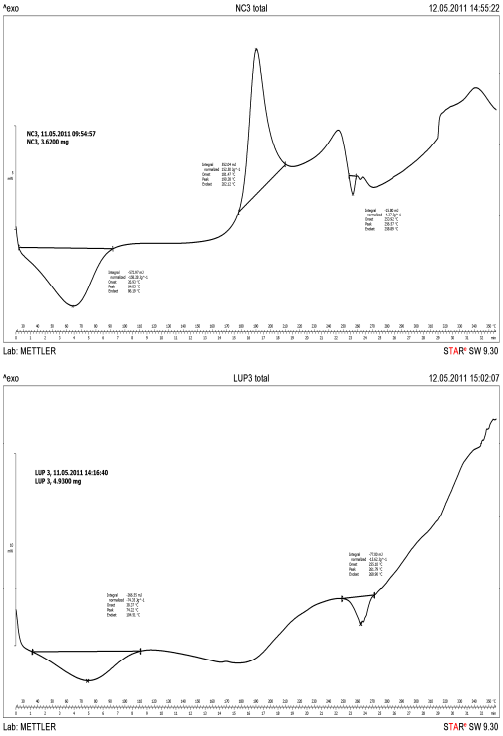


Figure 3. The thermal decomposition curves of empty polyurethane nanocapsules (1) and nanocapsules with lupeol (2)

4. Conclusion

PUR nanocapsules are proper delivery systems for pentacyclic triterpenes like lupeol. The PUR nanocapsules with lupeol present a better stability and do not tend to cluster as empty nanocapsules. The drug used not influence the thermal stability of the polymer chains. The comparative analysis of the samples performance revealed that the PUR nanocapsules are suitable as transdermal drug delivery systems.

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