Lupulone Delivered in Nanoparticles and the Promise to Defy Aging

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Abstract: This study reports synthesis and characterization of nanocomposite matrices (NP) designed to fight aging/photoaging of the skin. All materials used in the preparations were nontoxic, sustainable and beneficial to the skin. Zein (Z), from corn, is a moisture attractor. Lupulone (L) from hops is anti-inflammatory, antioxidant, antibacterial, stimulates circulation and collagen production, and has calming effect. Gum arabic (GA), from acacia trees, is used in foods as stabilizer/emulsifier. Chitosan (C), from chitin, is recommended by its properties: antioxidant, antibacterial, and filmogenic. A green procedure was used in the synthesis, replacing ethanol (typically used to dissolve Z/L) with 80:20 propylene glycol in water. The NP were characterized by FTIR, encapsulation efficiency of L, SEM, size and zeta potential. Antioxidant assays showed activity in all NP. They were assayed against MMP-1 (collagenase) as well. NP containing only Z and GA stimulated MMP-1 activity, which recommends them for use in wound healing. Those containing L too displayed inhibitory activity against MMP-1, stronger as L content increases. This anti-collagenase effect recommends them for use as anti-aging agents. Kinetic studies of L release from NP displayed levels too low for practical applications. To address this problem, in another set of preparations, C was added in the synthesis. The intent was to mitigate the strong lipophilic Z-L interactions. L release was positively impacted by C presence in the matrices. Increase due to C presence was afforded in antioxidant activity as well, although more modest than expected, since C is a known antioxidant. The anticollagenase activity was present and displayed the same variation as in the NP without C. The nanomatrices described are antioxidants, moisture attractors, and collagen synthesis stimulators which makes their promise to defy ageing very clear.

Keywords: chemistry, nanoparticles, zein, lupulone, antioxidant, collagen, MMP-1

(Title and abstract in Romanian)

Lupulona Încapsulată in Nanoparticule Promite Să Încetinească Îmbătrânirea

Rezumat: Acest studiu raportează sinteza şi caracterizarea unor nanomatrice (NP) concepute să combată îmbătrânirea/fotoîmbătrânirea pielii. Toate materialele folosite în preparare sunt netoxice, naturale şi folositoare pielii. Zeina (Z), din porumb, este hidratantă. Lupulona (L), din hamei, este antiinflamatoare, antioxidantă, antibacteriană, stimulează circulaţia şi producţia de colagen şi are efect calmant. Guma arabică, din răşina pomilor acacia, este folosită în alimente ca stabilizator/emulsificator. Chitosanul (C), obţinut din chitină, este antioxidant, antibacterian şi filmogenic. Sinteza, eco-friendly, a înlocuit etanolul (folosit pentru dizolvarea Z/L) cu 80% propilenglicol în apă. NP au fost caracterizate prin FTIR, eficiența încapsulării pentru L, SEM, mărimea particulelor şi potenţial zeta. Toate NP au demonstrat efect antioxidant. Testate împotriva MMP-1 (colagenază), NP conţinând numai Z and GA au stimulat activitatea enzimei, ceea ce le recomandă pentru tratarea rănilor. NP conţinând şi L au avut efect inhibitor, mai puternic la conţinut ridicat de L. Efectul anticolagenază recomandă aceste NP pentru aplicaţii ca agenți anti-îmbătrânire. Fiindca viteza eliberarii L din NP nu a atins valori practice, un alt set de NP a fost preparat în care s-a adăugat C pentru a atenua interacţiunile lipofile Z-L. C a fost benefic pentru cinetica eliberarii L şi a contribuit la creşterea activitătii antioxidante a NP, dar nu la nivelul anticipat. Activitatea anti-colagenază a fost prezentă cu aceleaşi variaţii ca în setul de NP fără C. Proprietăţile matricilor prezentate (efect hidratant, antioxidant, stimulator al sintezei colagenului) le recomandă ca agenţi ce combat îmbătrânirea pielii.

Cuvinte cheie: chimie, nanoparticule, zeină, lupulonă, antioxidant, collagen, MMP-1

Skin care products which fight chronological aging and photoaging are of high interest in a time when the number of seniors is on the rise. Adding to this the effect prolonged exposure to UV radiation has (sunburn, inflammation, oxidative stress, photocarcinogenesis), such products have become of general interest. Ultraviolet radiation (all ranges) is particularly dangerous due to the potential to damage collagen in the extracellular matrix (ECM), which in some cases can lead to fully stopping collagen synthesis (Pandel et al., 2013). Topical applications used as protection are not very effective due to the barrier represented by the *stratum corneum* in the epidermis (Leonida & Kumar, 2016). Due to their size, nanomaterials have a strong penetration power into living tissues and, due to their large surface to volume ratio, allow use of lower doses for efficient treatment.

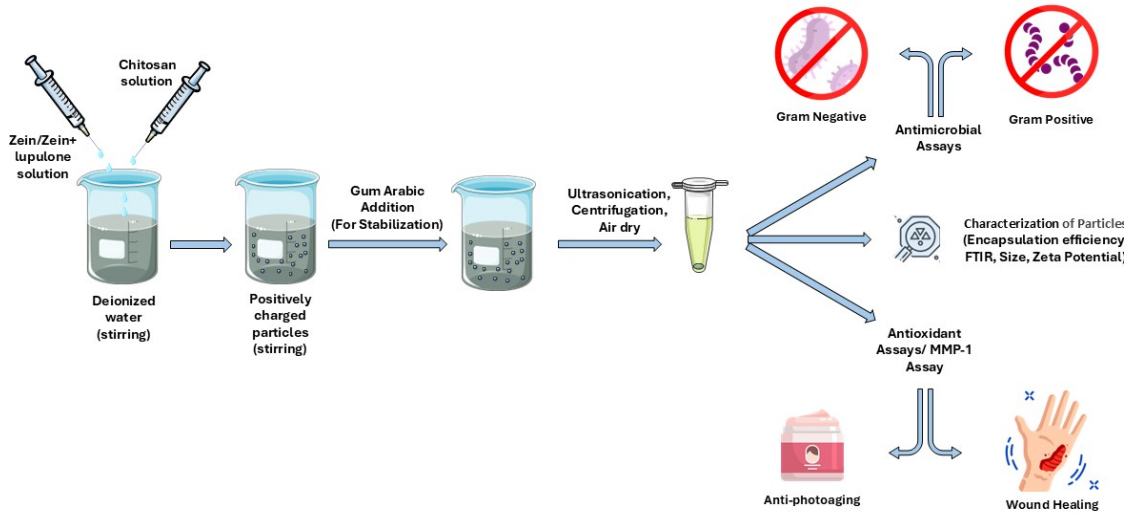

Figure 1. Preparation of composite matrices containing Z/L in different ratios (2:3 Z:GA in all preparations)

The present study reports the synthesis and characterization of nanocomposite matrices designed to address these concerns: chronological aging of skin, oxidation due to oxygen in the air, environmental agents, and bioavailability of bioactive agents. All materials used in the preparations were GRAS (generally regarded as safe) compounds obtained from natural sources. Zein (Z), a protein from corn rich in prolamin, is a powerful moisture attractor (Leonida et al., 2023). Lupulone (β-acid, L) from hops helps reduce inflammation, is antioxidant, antibacterial, stimulates circulation in the skin and collagen production, and has a calming effect (Natarajan et al., 2008). Gum arabic (GA), obtained from the sap of acacia trees, a mixture glycoproteins/ polysaccharide, is used in the food industry as stabilizer/emulsifier (Leonida et al., 2023). Chitosan (C) is an inexpensive biopolymer

obtained from chitin by deacetylation and is recommended by its filmogenic, antioxidant and antibacterial properties (Leonida et al., 2011). An environment-friendly, antisolvent procedure was used in the synthesis, which replaced the ethanol typically used to dissolve zein and lupulone with 80:20 propylene glycol in water. (Figure 1). Different Z:L ratios were used in the preparations.

FTIR spectra of the composites displayed the presence of all components and efficient L encapsulation. They evidenced the same type of complex interactions like those between zein and another lipophilic agent a-tocopherol in a matrix where GA was replaced by C (Luo et al., 2011) , although the particles were larger in the present study (Table 1, adapted from (Leonida et al., 2023), with permission). The encapsulation efficiency (EE) increased as the ratio Z:L increased. The same trend was displayed when peppermint oil was encapsulated in zein-based nanomatrices (Luo et al., 2011) while higher EE values were reported for L encapsulated in a hydrophilic matrix (Leonida et al., 2018).

Table 1. Characterization of the composites containing different Z_a:L_b ratios. Z:GA was 2:3 in all samples. Size and zeta potential values for nanoparticles suspended at 1 g/L in propylene glycol:water (80:20)

Sample	Encapsulation Efficiency (%)	Size (nm)	Zeta Potential (mV)
Z-GA	N/A	306	24.1
Z_1-GA-L_1	29.2	467	11.2
Z_2-GA-L_1	33.7	548	13.8
Z_4-GA-L_1	60.3	909	12.8

When L content was low, it is likely that L molecules were deposited on the surface of zein nanoparticles, in addition of the migration into the lipophilic core, affording large size complexes. As L content increased, rearrangements were likely (for example outward migration) upon GA addition, which led to decrease in size. Luo *et al* (Luo et al., 2011) and Nagibzadeh *et al* (Naghibzadeh et al., 2010) reported the same trend for composites encapsulating lipophilic agents in polysaccharide-based nanomatrices. GA, negatively charged at pH 5, was used as stabilizer for the positively charged zein-based particles adding its emulsifying effect as additional stabilizer for the complexes. A sharp decrease in zeta

potential was recorded when neutral L was added, before complexation with GA. We relate this to conformational changes in Z (evidenced by FTIR spectra) due to hydrophobic interactions with L leading to decrease in the number of exposed positive charges. The positive zeta potential values are a good predictor of antibacterial activity of the discussed particles. Scanning electron microscopy (SEM) studies did not show morphological differences between nanocomposites with and without L.

Antioxidant assays showed activity in all composites (Figure 2). A remarkable finding was the antioxidant effect of the Z:GA nanoparticles, while none of the components is a known antioxidant. This observation is ascribed to the benefits of delivery at the nanolevel.

Collagenases, a type of metal metalloproteinases (MMP-1, MMP-8, MMP-13) degrade triple-helical collagen. In healthy tissues, endogenous inhibitors like tissue inhibitors of metalloproteinases (TIMPs) maintain homeostatic balance in their activities (Kumar et al., 2024). Under various pathological conditions, that balance is disrupted by either the over- or underexpression of MMPs or TIMPs. Skin exposure to UV radiation results in overexpression of MMPs (Gimeno et al., 2021) leading to degradation of the collagen and elastic fibers, including microfibrils in the skin. The result is skin sagging and wrinkle formation. MMPs play an essential role in the wound healing/tissue remodeling process (Leonida & Kumar, 2016). MMP-1 is found to be important in the migration of cells like keratinocytes resting on fibrillar collagen, and also in angiogenesis (Caley et al., 2015).

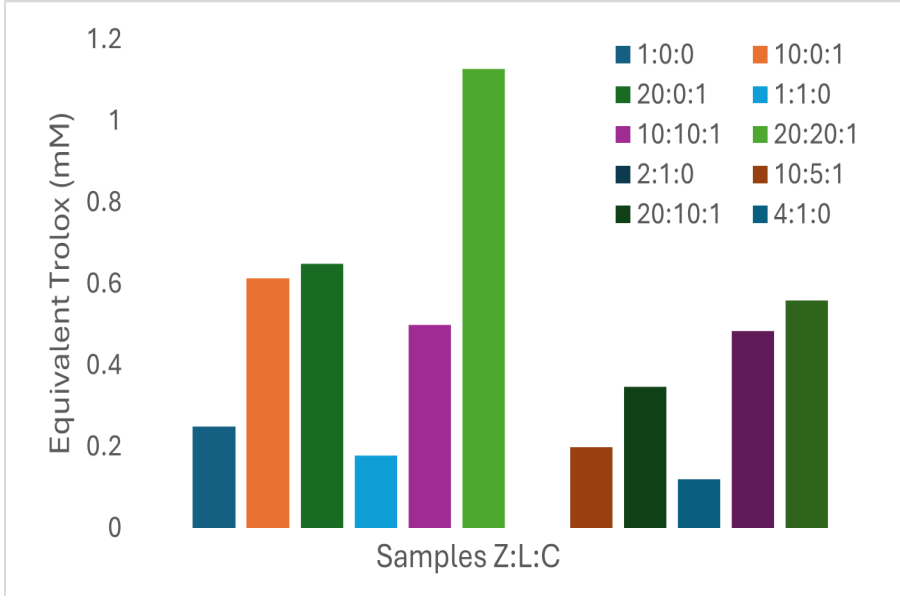

Figure 2. Antioxidant activities of the Z:L and Z:L:C samples, all at 2:3 Z:GA ratio

Kinetic studies conducted for the release of L from composites displayed sustained profiles over 7 days (Figure 4, adapted with permission from (Leonida et al., 2023)). All particles showed initial "burst effect" due, presumably, to the release of L adsorbed on the surface of the lipophilic particles. This is longer (one day) than the same effect recorded for the release of L from hydrophilic matrices (chitosan-tripolyphosphate, ~ 4 h) (Leonida et al., 2018). However, the levels released were too low for practical applications. To address this problem, in another set of preparations, C was added in the synthesis, at two different Z:C ratios (10:1 and 20:1) and using the same Z:L ratios as for the first set. The intent was to modulate the strong lipophilic interactions between Z and L. The release of L was positively impacted by C presence in the matrices and increased with increase in C content in the preparation.

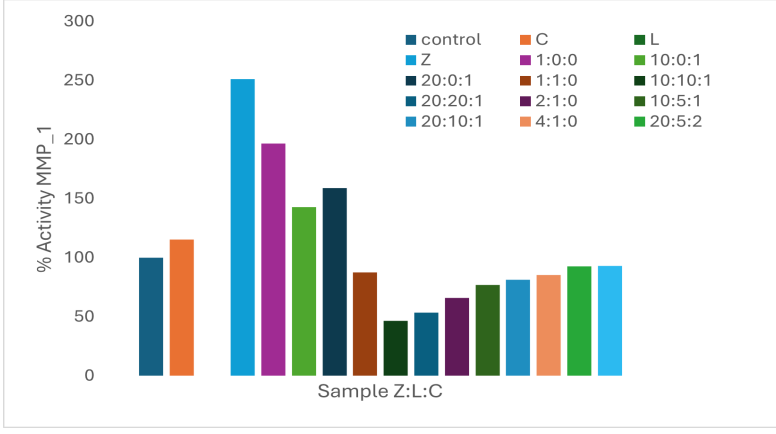

Figure 3. Influence of the Z:L and Z:L:C samples, all at 2:3 Z:GA ratio, on MMP-1 activity

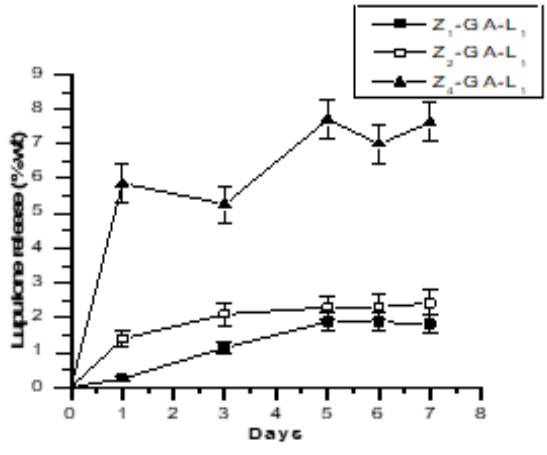

Figure 4. Release of L from Z:L:GA matrices

C presence afforded an increase in antioxidant activity of the composites as well (Figure 3), although more modest than anticipated, since chitosan is a known antioxidant. The lower-than-expected antioxidant activity in both types of particles may be correlated to the difference in time scale between the L release (slow kinetics) and the time required by the antioxidant assay (minutes).

The anticollagenase activity was present and displayed the same variation like in the nanoparticles without chitosan (Figure 4). It is remarkable that the composites displayed anticollagenase activity at all if we take into account the slow kinetics of the release and the difference (several orders of magnitude) between the concentration of L in the assay of the control sample compared to the one in the MMP-1 assay of the particles containing L. The effect of lupulone was powerful enough to mitigate the potent MMP-1 activating effect of zein in the particles. It is more difficult to assess the impact of C presence in the matrix on enzyme activity probably due to the masking effect of the comparatively high Z content.

The nanocomposites reported herein have potential for use in formulations for skin treatment and skin care. The particles containing only Z-GA, powerful activators of MMP-1, have promise for wound healing and tissue remodeling. Addition of filmogenic C may open the possibility of delivery in self-sticking bandages. The presence of L in some of the composites is beneficial for topical applications in which it may mitigate the occurrence of contact dermatitis and pruritus reported in hop pickers (Cookson & Lawton, 1953). The nanomatrices described herein are antioxidant agents, moisture attractors, and collagen synthesis stimulators which makes their promise to defy ageing very clear.

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