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IMPACT OF MORPHOLOGY ON D-LIMONENE PAYLOAD AND DIFFUSION IN BIODEGRADABLE PROLONGED RELEASE FILMS

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In this study, D-limonene is integrated into films made with different ratios of gelatin to β-CD. The FTIR, vacuum liquid absorption method and gravimetric analysis were used to assess porosity and swelling behavior. Inclusion and retention were detected by FTIR analysis in distinctive peaks at 870–890 cm^{-1}. Films with a 3:1 gelatin to β-CD ratio had a higher D-limonene payload. Porosity and swelling are enhanced with increasing β-CD content. These analyses highlight the potential of gelatin-β-CD matrices for developing prolonged-release systems for volatile organic compounds.

Keywords: β-cyclodextrin, gelatin, prolonged release, D-limonene, carriers, FTIR-spectroscopy, porosity, inclusion complex.

Introduction

New biodegradable materials are gaining increasing interest nowadays as a key driver forward to a sustainable and circular economy. It is trendy: new packaging materials, deep customization, and personalization [1]. One of such possible implementations is functional films, which can create a range of possibilities for customization and digitalization. Prolonged release, as a property, aims to describe controlled release parameters of the material and give us a tool for information entropy reduction in different applications.

Biodegradable film morphology refers to the structural characteristics and surface properties of films made from organic, decomposable materials. Main morphological properties include surface characteristics, such as porous surface textures that increase surface area and support adsorption processes. For example, eggshell powder/chitosan films exhibit non-adhesive surfaces with advanced structural properties. Structural properties may vary – some of them may have fibrous structures, other uniform matrices like gelatin-based films. Several factors impact film morphology, including material composition, production process, incorporation of nanoparticles, and treatment methods like UV radiation [2, 3].

The way gelatin and β-cyclodextrin(β-CD) interact to create porosities within the matrix remains not very deeply explored, specifically when d-limonene and additional crosslinkers like transglutaminase are introduced.

Protein-based biomaterial gelatin is derived from collagen and has gelling, thickening, and stabilizing properties, widely used in food, pharmaceutical, photographic, and biomedical applications. Molecular weight usually ranges between 30–100 kDa and density is 1.3–1.4 g/cm³. The main functional properties of gelatin films are high hydrophilicity due to polar groups, and hydrogen bond formation. They contain hydroxyl, carbonyl, and amino acid groups. As key functions mentioned are good barrier properties for oxygen, carbon dioxide, and aromatic compounds [4]. B-cyclodextrin is usually white powder with a density of 1.44–1.46 g/cm³ moderately soluble in water. Adding β-CD into gelatin's protein network usually disrupts it, creates additional free volume in the film matrix, increases intermolecular spaces, and enables the formation of microcavities and channels. The main interaction mechanisms are the formation of hydrogen bonds within gelatin protein chains, generating electrostatic interactions, and increasing chain mobility. Some studies report an increase in swelling and porosity in films containing β-CD [5].

D-limonene is commonly used in many applications and demonstrates impacts in pest prevention, herbicidal activities, and antioxidant functions, and is

used as an aroma additive. It is unstable and quickly evaporates, so forming inclusion complexes with β-CD helps stabilize it [6].

This study aims to evaluate the morphology of gelatin and β-cyclodextrin films, formed with d-limonene inclusion complexes, analyze the impact on the payload of d-limonene in these films, and assess diffusion rate, porosity, and swelling parameters.

Materials and research methods

The main objects of this study were films formed with β-cyclodextrin (β-CD), gelatin, transglutaminase as crosslinker, and D-limonene with different weight ratios. An inclusion complex has been formed between D-limonene and β-cyclodextrin and further added to the gelatin matrix to form a film. Other film samples have been prepared without D-limonene and with the addition of transglutaminase as a crosslinker for comparison.

β-CD was sourced from LogicGroup (Ukraine) and used without further purification, D-limonene was obtained from UkrAroma (Ukraine). High molecular gelatin 240 bloom was sourced from Trobas (Netherlands).

Methods for film creation. Gelatin/β-cyclodextrin films with D-limonene were prepared by creating inclusion complexes following the protocol of Petrovic et al. [7] with a few modifications. Films without D-limonene were produced by stirring a 10 % gelatin solution with β-CD in ratios of 3:1, 3:2, and 1:1 for about 30 min at room temperature and then casting the mixture onto a flat surface. Approximately 2 g of β-CD was dissolved in 7 ml of an alcohol/distilled water (1:6) mixture, and then D-limonene was added with a weight ratio of 1:1 (D-limonene:β-CD). The solution was stirred for 1 hour at 50 °C (±5 °C), and after the precipitated material was mixed with 10 % gelatin solution with different ratios of 3:1, 3:2, 2:1, and 1:2 and cast on a flat surface. A sample with transglutaminase as a crosslinker has been produced by adding it to the gelatin solution with a weight ratio of 10:1 (gelatin: transglutaminase). The experiment was conducted at a temperature of 17–19 °C.

Methods of analysis. Several methods for measuring morphology in terms of porosity are based on optical microscopic analysis, quantitative methods like vacuum liquid absorption/displacement, swelling measurements, gravimetric methods, and others. In this study, we use FTIR, gravimetric analysis, vacuum liquid absorption method, and optical images [8, 9].

The liquid absorption method in a vacuum for measuring the porosity of materials is widely used to analyze biodegradable films. This method involves immersing the material in a liquid and measuring the volume of liquid absorbed, which correlates to the volume of the pores within the material. Ethanol (96 %) has been used as the liquid due to the possibility of gelatin water sorption [8, 9].

The porosity range has been calculated by weighting samples before vacuum absorption by ethanol 96 % and after using the following formula adopting the method proposed by Kavya et al. [10] and modified to analyze films:

$$P = \frac{V_{etanol}}{V_{scaf\ fold} + V_{etanol}}; \quad (1)$$

$$V_{etanol} = \frac{W_{dry} - W_{wet}}{d_{etanol}}; \quad (2)$$

$$V_{scaf\ fold} = \frac{W_{dry}}{d_{polymer}}, \quad (3)$$

where $P - porosity$; $d_{etanol} = 0.789\ \text{g/cm}^3$;
$d_{polymer} = 1.36\ \text{g/cm}^3$

The gravimetric method has been used to evaluate the release rate by weighing the samples daily using an analytical grade weigher (RADWAG AS220 R2).

Optical properties were evaluated with microscopy images of film surfaces using a microscope MICROmed XS-5520 LED.

Created gelatin/β-cyclodextrin films were characterized using Fourier-transform infrared spectroscopy (Spectrum Two, PerkinElmer). FTIR is an analytical technique for studying the interactions within inclusion complexes, particularly between host molecules like cyclodextrins and guest molecules like essential oils and other hydrophobic compounds [11]. The data were processed by One-Way ANOVA with groupings by Tukey's HSD test.

Results and discussion

Films, after casting, were dried for three days in order to achieve a stable state and mechanical properties. We conducted an experiment on D-limonene stability, 1 ml of D-limonene evaporated for 24 hours with 97 % evaporated from the Petri dish, so we assume that D-limonene, which was not complexed within the film matrix, evaporated during this time.

Porosity and FTIR analysis were performed on the third day of casting. On the same day, we measured the weight of each sample and took it as a base for porosity and swelling analysis using the vacuum absorption method.

The overall size of the films is 25×60×2 mm with some fluctuations because of different viscosity during casting, which is impacted by different proportions between gelatin/β-CD.

In order to analyze how much D-limonene is integrated into each film, FTIR peaks were evaluated. D-limonene is a monocyclic monoterpene composed mainly of C-H bonds, particularly in its methyl and methylene groups, which exhibit IR absorption around 870–890 cm^{-1}. It contains a double bond, which can participate in reactions, usually absorbing IR radiation near 1660 cm^{-1} if it remains unsaturated.

Spectra were taken in the range 4000–400 cm^{-1}. However, only the spectral region of the greatest interest is presented in this paper. In Figure 1, the most diagnostic region for D-limonene in this gelatin–β–CD film lies around 880 cm^{-1}, where the ring-related C–H bending modes of the monoterpene typically appear. This peak is strong and distinct in the original D-limonene spectrum (dLim), showing the relatively high concentration of the monocyclic terpene there.

By comparing the various gelatin–β-CD formulations, we can see the approximate amount of D-limonene that has been taken up or retained in each film. Gelatin: β-CD ratio 3:1 shows a higher load of D-limonene based on a peak around 870–890 cm^{-1} compared to other ratios 3:2, 2:1. Notably, W43 and W43d (which have the B33 ratio but have been swollen in D-limonene) show higher absorption compared to their un-swollen counterparts, which indicates significant initial evaporation of D-limonene from the film [12, 13].

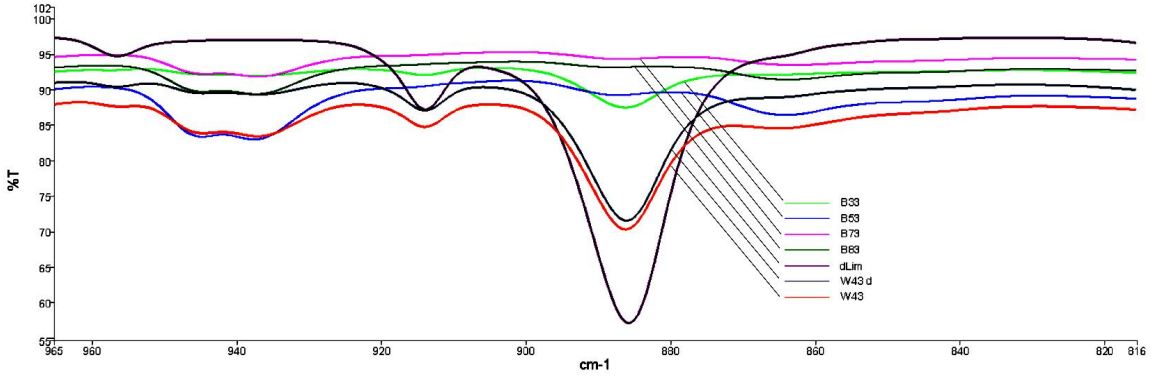

Fig. 1. FTIR on films with D-limonene and gelatin/β-CD ratios (B33 – 3:1, B53 – 3:2, B73 – 2:1, B83 – 1:2, W43 & W43d – 3:1 swelled in D-limonene with 2 days difference measurement, dLim original D-limonene)

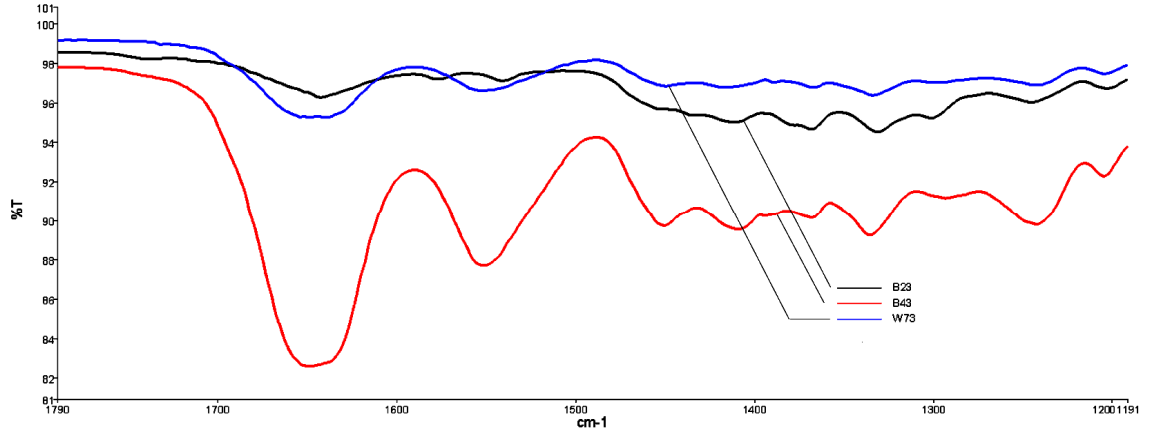

Fig. 2. FTIR on films B23 – gelatin/β-CD(3:1)/transglutaminase, B43 – gelatin/β-CD(3:2), W73 – gelatin/β-CD(1:1)

FTIR spectra provide indirect insight into porosity by revealing the strength of interaction between gelatin and β-CD (and crosslinker) within the film matrix. When there is more extensive hydrogen bonding or covalent crosslinking among the components, the polymer network tends to become denser, often resulting in smaller pore sizes. In Fig. 2, the amide-I (~1650 cm^{-1}) and amide-II (~1550 cm^{-1}) regions can show shifts or intensity changes when the gelatin is crosslinked or when β-CD interacts strongly with the protein backbone.

Data in Table 1 show some trend in which higher βCD content in the gelatin matrix generally corresponds to higher porosity and higher swelling ratios, particularly in the samples containing D-limonene (e.g., B83 with a 1:2 gelatin/β-CD ratio shows the highest porosity at 25.57 % and swelling ratio at 20.45 %). This aligns with previous reports indicating that β-CD, when present in larger amounts, can create a more open or microporous network as it disperses among the protein chains, sometimes forming microcavities or channels [9, 14]. These voids facilitate solvent uptake (leading to higher swelling) and increase free volume. Table 1 does not present the data on porosity and swelling for films without D-limonene due to their high density and the difficulties of determining these parameters.

Films with a lower proportion of β-CD tend to exhibit lower porosity and less swelling, showing a denser protein network. The gelatin alone can form a relatively compact matrix via hydrogen bonding and partial triple-helix formation; when β-CD is introduced at moderate levels, it may integrate smoothly into the gelatin network without introducing extensive channels and act as a crosslinker as well. With the β-CD fraction increase, as well as adding potential inclusion sites for D-limonene, it also disrupts the gelatin structure to form additional -voids, increasing both porosity and solvent uptake capacity [14].

Also, D-limonene can amplify the effects of additional porosity in films. As D-limonene diffuses into the β–CD–gelatin film, it may plasticize or slightly swell the matrix, loosening intermolecular interactions and creating additional free volume. This relation can be seen in the higher porosity and swelling ratios for the films with the availability of D-limonene compared to similar films in gelatin ratio, but without essential oil. These observations align with previous studies showing that cyclodextrins, especially when present at higher loadings, enhance porosity and solvent absorption in protein-based films [9, 14].

Next, films were evaluated using the gravimetric method by weighing samples daily and calculating the approximate release rate of non-stable D-limonene; the data have been added in Table 2.

$$\%Loss(n\rightarrow n+1) = \\ = [Mass\ (n) - Mass\ (n+1)]/Mass\ (n) \times 100\% / \quad (4)$$

Table 1

Porosity(%) and swelling (%) of films

	with D-limonene			
Sample	B33	B53	B73	B83
Gelatin/β-CD ratio	3:1	3:2	2:1	1:2
Porosity	9.07±1.22 [b]	11.73±0.46 [b,c]	15.13±2.01 [c]	25.57±1.51 [d]
Swelling	5.91±0.69 [b]	7.71±0.36 [b,c]	10.43±1.70 [c]	20.45±1.76 [d]

Note. The results are presented as mean ± SD with groupings by Tukey's HSD test; means sharing any letter are not significantly different (p > 0.05).

Table 2

Films' weight drop rate by days

Film	Day 1→2, %	Day 2→3, %	Day 3→4, %	Day 4→5, %	Day 5→6, %	Total, %
B33 (3:1)	2.57	2.06	2.00	1.86	1.22	≈10
B53 (3:2)	1.76	1.29	1.19	1.04	0.54	≈ 6
B73 (2:1)	1.45	0.96	0.84	0.78	0.21	≈5
B83 (1:2)	0.59	0.21	0.10	0.28	2.05	≈3

The film with the largest proportion of gelatin shows a fairly steady (~2 %) daily loss until day 5→6, dropping to ~1.22 %, while the film with the largest amount of β-CD has the smallest drop around 3 %. It is not correlated with porosity rates, as porosity typically facilitates diffusion, but taking into account the FTIR analysis, there is a high probability that most of the d-limonene was released during the film drying process, and shows a low presence of D-limonene in these films. Few studies highlight similar trends of β-CD impact into films. For example, a study by Ibáñez et al. (2020) describes challenges associated with volatility and the potential volatile organic compounds loss during drying as well as the role of porosity into diffusion [6].

Conclusions

Biodegradable gelatin-based films with different ratios of β-CD and D-limonene were produced using the casting method. Psychochemical properties of films have been evaluated by measuring porosity, swelling, and gravimetric weight loss as diffusion metrics together with FTIR analysis for payload.

A larger gelatin ratio shows some reduction in film porosity, as well as adding transglutaminase as a crosslinker, making the film structure more dense. Initially, D-limonene is better retained in the film with a gelatin to β-CD ratio of 3:1; however, the approximate release rate during the first week is around 10 % of the overall film weight, based on the gravimetric method.

Morphology analysis provides insights into film structure and helps understand diffusion behavior as well as its impacts on the amount of retained essential oil.

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Impact of morphology on D-limonene payload and diffusion in biodegradable prolonged release films

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ВПЛИВ МОРФОЛОГІЇ НА ЗАВАНТАЖЕННЯ ТА ДИФУЗІЮ D-ЛІМОНЕНУ В БІОРОЗКЛАДНИХ ПЛІВКАХ ПРОЛОНГОВАНОГО ВИВІЛЬНЕННЯ

Досліджено плівки, виготовлені з різним співвідношенням желатину до β-CD, інтегровані D-лімоненом. Пористість і набухання отриманих плівок оцінювали за допомогою ІЧ-Фур'є, гравіметричного аналізу та методу вакуумної абсорбції рідини. FTIR аналіз підтвердив включення та утримання D-лімонену у вигляді характерних піків при 870–890 см$^{-1}$, причому більший вміст D-лімонену був виявлений у плівках із співвідношенням желатину до β-CD 3:1. Із збільшенням вмісту β-CD пористість і набухання плівки підвищувалися, що ґрунтується на можливому утворенні мікропорожнин, індукованих β-CD. Цей аналіз підкреслює потенціал матриць на основі желатину і β-CD для розроблення систем пролонгованого вивільнення летких органічних сполук.

Ключові слова: β-циклодекстрин, желатин, пролонговане вивільнення, D-лімонен, носії, FTIR-спектроскопія, пористість, комплекс включення.