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The effect of lavandula essential oils on release of niflumic acid from collagen hydrolysates

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Abstract—The aim of this paper is to design and characterize some drug delivery systems (DDS) based on collagen hydrolysates (H), niflumic acid as a non-steroidal anti-inflammatory model drug and two essential oils of *Lavandula officinalis L.* (LO) and *Lavandula stoechas L.* subsp. *Stoechas* (LS), for use in healing cutaneous wounds with post-lesion inflammatory response. The essential oils are characterized by GC-MS. The wettability capacity of collagen hydrolysate powders was assessed by contact angle measurement. Niflumic acid release was investigated using a modified Franz diffusion cell, and the diffusion coefficient, time-lag and drug flux were determined. The kinetic parameters were found to be influenced by different concentrations and types of essential oils. These therapeutical products, based on collagen hydrolysates and synergistic drug associations, could have potential biomedical application in wound healing treatment.

Keywords: Collagen Hydrolysate, Niflumic Acid, Lavandula Essential Oils, Release Kinetics

INTRODUCTION

An important issue that has to be considered in cutaneous wound healing is the management of the post-lesion inflammatory response. Non-steroidal anti-inflammatory drugs (NSAIDs) represent a reliable strategy for treating wound site inflammation and consequently pain. In many cases, wound healing is delayed by bacterial infections that can be fatal in cases of severe wounds [1]. Natural products of plant or animal origin and their derivatives are important sources of novel therapeutic molecules [2,3]. Collagen, in different forms (powders, hydrogels, solutions, films/membranes, matrices) has been reported to be one of the first materials used as a carrier of bioactive components [4-8]. Collagen hydrolysate was proved to be a bioavailable source of peptides and amino acids with therapeutic effects [9]. Type I collagen in form of sponges or membranes is suitable for skin replacement and burn wounds due to its mechanical strength and biocompatibility [10,11]. Collagen-based materials have been either used in practice as implants or tested as implants in clinical studies, without adverse effects [12].

Medicinal plants are an important source of natural antimicrobial biomolecules and alternative anticancer agents. [13-15]. Essential oils are complex mixtures of different compounds, such as sesquiterpenoids, benzoids, polypropanoids, and fatty acid derivatives, [16] with various pharmaceutical and biological activities, like antibacterial, antifungal, antimutagenic, antidiabetic, antiviral, anti-inflammatory, and antiprotozoal properties [17]. Yang et al. [18] reported that flavonoids may potentially inhibit the viability of the HeLa human cervical carcinoma cell line and P-388 mouse leukemia cell line. Other authors suggest that sesquiterpenes show anticancer activity [19]. Lavandula oil is the most commonly used and the most versatile of all the essential oils [20]. As our previous works showed, *L. stoechas* is rich in ketones while *L. officinalis* has a high amount of esters and alcohols. The most important ether found in both oils was 1.8 cineol (eucalyptol), one of the most efficient therapeutic compounds used in many diseases [21]. Eucalyptol was used in preclinical tests as a novel permeation enhancer on Wistar albino rats, proving to be effective in transdermal delivery [22].

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Our aim was to design and characterize some drug delivery systems based on natural therapeutic compounds such as collagen hydrolysates and antimicrobial essential oils of *Lavandula officinalis L.* (LO) and *Lavandula stoechas L.* subsp. *stoechas* (LS) in association with niflumic acid as a non-steroidal anti-inflammatory drug, for potential medical use.

1. Materials

Plant material: Aerial parts of *Lavandula officinalis L.* and *Lavandula stoechas L.* subsp. *stoechas* were harvested at full-flowering stage in July 2014 from the botanical gardens of the Field Crops Department of Mustafa Kemal University. The isolation of essential oils was performed by drying the aerial parts at 35°C in an oven, to a constant weight, followed by their hydrodistillation with 1 L distilled water, for 3 h, using a Neo-Clevenger apparatus. The oils were dried over anhydrous sodium sulfate and then stored in dark color (amber) glass bottles, at −4 °C ready for GC-MS analysis. The collagen hydrolysate (CH) with an average molecular weight of about 6,000 Da was obtained by acid hydrolysis of calf pelt at 125 °C for 8 h with the previously described technology [21]. Niflumic acid (NA) was purchased from ICN Biomedicals Inc. (USA) and glutaraldehyde (GA) from Merck (Germany). Sodium hydroxide, ethanol (Et) and phosphate buffer solution (PBS), pH 7.4 were of analytical grade.

2. Drug Delivery Systems (DDS) Preparation

The collagen hydrolysate : ethanol solutions with a 9 : 1 ratio (w/w) and 0.0, 0.25; 0.50; 1.0 and 2.0% (w/v) *Lavandula officinalis L.* and *Lavandula stoechas L.* subsp. *stoechas* essentials oils respectively were prepared, and then lyophilized using the previously reported method [1,21]. The resulting powders were later dissolved in distilled water in the presence of niflumic acid (0.25%) as sodium salt (w/v), and thus the drug delivery systems were obtained. The samples were coded as shown in Table 1 and divided in two series, HLO and HLS, depending on their content in essential oils, respectively *Lavandula officinalis L.* and *Lavandula stoechas L.* subsp. *stoechas* oil.

3. Methods

3-1. Gas Chromatography-mass Spectrometry (GC-MS) Analysis

The analysis of the essential oils was carried out using a Thermo Scientific Focus Gas Chromatograph equipped with MS, autosampler and TR-WAXMS-A (5% phenyl polysilphenylene-siloxane, 0.25 mm×60 m.I.D., film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV, mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 °C, MS Ionization source 319 temperature was 220 °C, the injection port temperature was 220 °C. The samples were injected with 250 split ratio. The injection volume was 1 µl. Oven temperature was programmed in the range of 50 °C to 220 °C at 3 °C/min. The structure of each compound was identified by comparison with its mass spectrum (Wiley). The data were handled using *Xcalibur* software.

Table 1. Codification and compositions of the designed DDS based on collagen-lavandula oil-niflumic acid

DDS	DDS compositions	
	Essential oil* (%)	Niflumic acid** (%)
H 0.00	0	0.25
HLO 0.25	0.25	0.25
HLS 0.25	0.25	0.25
HLO 0.50	0.50	0.25
HLS 0.50	0.50	0.25
HLO 1.00	1.00	0.25
HLS 1.00	1.00	0.25
HLO 2.00	2.00	0.25
HLS 2.00	2.00	0.25

*Reported to volume of hydrolysate solution (v/v)

**Reported to volume of hydrolysate solution (w/v)

4. Evaluation of Collagen Hydrolysate Powders Wettability

The wettability capacity of collagen hydrolysate (reference sample) and collagen hydrolysate - essential oil powders was assessed by contact angle measurement at room temperature, using a KSV Cam 101 Scientific Instrument. The contact angle evaluation was performed using the following procedure, according to Nowak et al. [23]: on a glass microscope slide the powder was placed on a double sided adhesive tape. The pendant drop dynamic method was applied, using distilled water dispensed with a Hamilton syringe [7]. The drop shape was monitored with a digital camera and mathematically described by the Young-Laplace equation [8,23]. At least six independent measurements on different powder surface locations were averaged.

5. Niflumic Acid Release Kinetics

Niflumic acid release kinetics was investigated using a modified Franz diffusion cell fitted with a cellophane membrane (Autogen Bioclear Ltd.) and a peristaltic pump (Masterflex, Cole-Parmer) as previously reported [25]. A phosphate buffer solution of pH 7.4 at 37 °C±0.5 °C (ThermoHaake P5 Ultrathermostat) was used as receiving medium and continuously stirred by a rotating Teflon-coated magnet placed inside the receptor compartment. The amount of niflumic acid released at different time intervals was spectrophotometrically assessed at 287 nm (Perkin-Elmer spectrophotometer) using the previously determined calibration curve ($A_{1\%}^{1\,cm}$=777, R=0.9986) [26].

RESULTS AND DISCUSSION

According to GC-MS results, 31 and 32 different components were identified in *Lavandula officinalis L.* and *Lavandula stoechas L.* subsp. *stoechas* essential oils, respectively. The main components are presented in Table 2.

Fenchone (42.00%), eucalyptol (17.56%), camphor (18.11%) and borneol acetate (1.77%) were the main components for *L. stoechas* oil, while for *L. officinalis*, linalool (24.99%), linalyl acetate (45.04%), camphor (6.28%) and eucalyptol (4.54%) were found in high concentrations. The wettability capacity for the collagen hydrolysate powders, quantified by contact angle measurement, was further determined. The dynamic contact angle determinations led to the description of the drop shape time variation. The images of the drop shape recorded at different time intervals are exemplified in Figs. 1-3(a)-(f) for collagen hydrolysate powder with no essential oil, with 0.50% *Lavandula officinalis L.* essential oil, and with 1.00% *Lavandula stoechas L.* subsp. *Stoechas* essential oil respectively.

Table 2. *Lavandula officinalis L.* and *Lavandula stoechas L.* subsp. *stoechas* essential oils components

Retention time	Components	*Lavandula officinalis L.* oil, %	*Lavandula stoechas L.* subsp. *Stoechas* oil, %
3.64	α-Pinene	0.26	0.82
4.35	Camphene	0.45	1.34
5.14	β-Pinene	0.12	0.28
5.45	Sabinene	0.82	0.13
6.50	Myrcene	0.22	-
6.89	α-Terpinene	-	0.23
7.41	Limonene	0.76	0.64
7.61	**Eucalyptol**	**4.54**	**17.56**
8.84	γ-Terpinene	-	0.44
9.08	Ocimene	0.43	-
9.66	Cymene	0.14	0.87
10.03	α-Terpinolene	-	0.48
13.97	**Fenchone**	0.19	**42.00**
15.68	Linalool oxide	1.22	0.23
16.57	Trans-sabinene hydrate	0.31	0.09
16.78	Trans-Linalool oxide	0.44	0.44
17.47	α-Cubebene	0.21	0.39
18.33	**Camphor**	**6.28**	**18.11**
19.90	**Linalool**	**24.99**	0.75
20.25	**Linalyl acetate**	**45.04**	-
20.88	**Borneol acetate**	0.11	**1.77**
21.08	Fenchyl alcohol	-	0.45
21.39	Trans-Caryophyllene	0.93	-
21.77	4-Terpineol	0.27	0.32
22.17	Lavandulyl acetate	1.99	-
22.61	Myrtenal	0.43	1.21
23.66	Trans-Pinocarveol	0.21	0.43
23.77	β-Cadinene	0.46	-
24.13	Cryptone	-	0.32
24.55	Cis-Verbenol	-	0.41
24.69	Lavandulol	0.37	-
24.93	Myrtenyl acetate	-	0.78
25.26	α-Terpinenyl acetate	-	0.94
25.36	2-Methylisoborneol	4.01	-
27.37	α-Amorphene	-	0.53
29.56	Epoxylinalool	0.79	-
30.26	Carveol	-	0.40
30.81	p-Cymen-8-ol	0.25	-
34.66	Limonene oxide	0.34	-
34.83	Caryophyllene oxide	0.63	-
36.42	Viridifloral	-	0.65
37.62	α-Copaene	-	0.12
38.23	Viridiflorol	-	0.99
41.16	Globulol	-	0.32
42.49	α-Bisabolol	0.68	-

As shown in Figs. 1-3, the contact angle decreases with time due to progressive imbibition of the liquid into the powder particles.

The dynamic drop method also gave rise to advancing and receding contact angles as a function of time. The difference between these extreme values is called contact angle hysteresis [8], exemplified in Fig. 4 for collagen hydrolysate powders.

According to Fig. 4, similar trends of variation of contact angles and their hysteresis are obtained for powders from both series, as well as for H powder. The average contact angles for the tested powders were lower than 90°, with values between 49.06±2.55° and

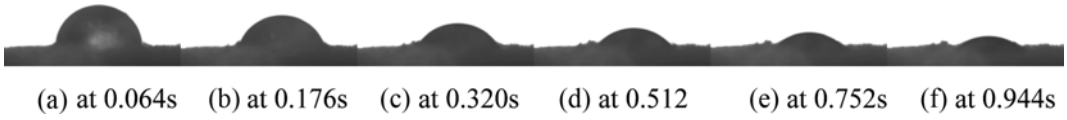

Fig. 1. Images of the drop shape at different time points for the collagen hydrolysate powder without essential oil.

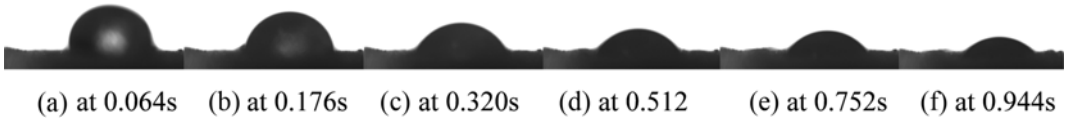

Fig. 2. Images of the drop shape at different time points for the collagen hydrolysate powder with 0.50% *Lavandula officinalis L.* essential oil.

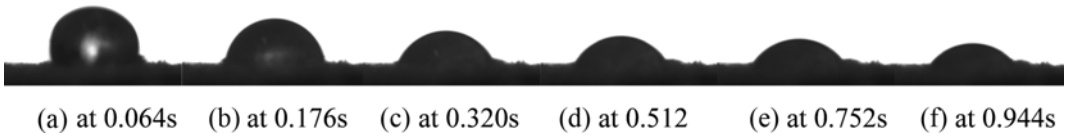

Fig. 3. Images of the drop shape at different time points for the collagen hydrolysate powder with 1.00% *Lavandula stoechas L.* subsp. *stoechas* essential oil.

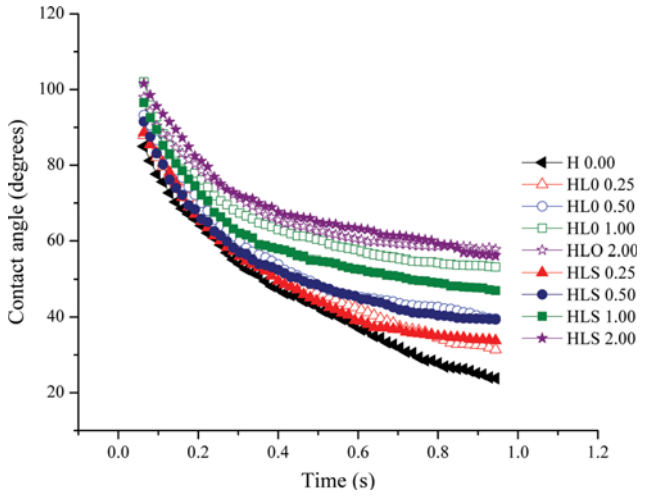

Fig. 4. Variation of contact angle for collagen hydrolysate powders.

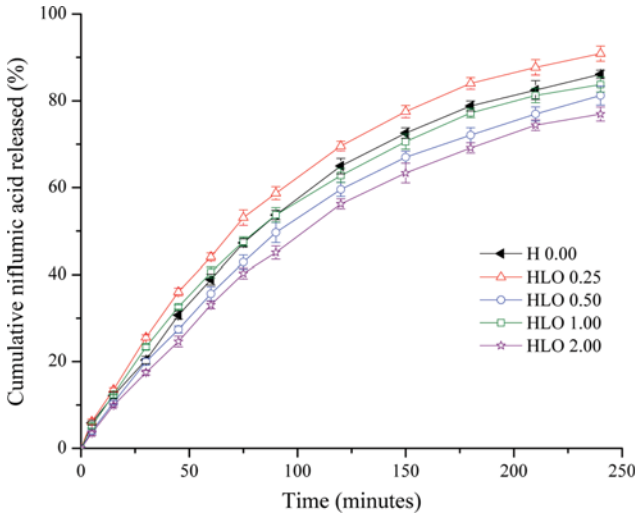

Fig. 5. Plots of cumulative percentage of niflumic acid released versus time for DDS with essential oil of *Lavandula officinalis L.* and with no essential oil.

69.07±3.53° for the HLS series, between 50.07±3.05° and 67.20±3.48° for the HLO series, and 45.16±2.96° for the H sample, respectively. The contact angle hysteresis ranged between 45.41±2.25° and 54.77±3.23° for the HLS series, between 40.26±3.12° and 56.45±3.09° for the HLO series, and 61.20±2.96° for the H sample. Note that both wetting parameters are influenced by the essential oil concentration for each series, as well as by the source of the essential oil. The smallest average contact angle value and the highest hysteresis are recorded for the hydrolysate powder with no essential oil, while a decrease in hydrophilicity and hysteresis is noticed as the concentration in essential oil increases. The wetting characteristics indicate a good hydrophilicity and a suitable wettability of the collagen hydrolysate powder surface by the liquid. The powder wettability is an indicator for the affinity between liquid and solid phases and is important for the dissolution process involved in the DDS preparation [23,24,27]. The kinetic data recorded for the prepared DDS conducted to release profiles illustrated in Figs. 5 and 6, and are expressed as the percentage of drug release versus time.

For the assessment of the niflumic acid release mechanism from the tested DDS the kinetic data were fitted according to the general equation suggested by Peppas [28]:

$$\frac{m_t}{m_\infty} = k \cdot t^n \quad (1)$$

where, m_t is the amount of drug released vs. time t, m_∞ - the total drug contents in the tested collagen hydrolysates, m_t/m_∞ - the fractional release of the drug at the time t, k - the kinetic constant, and n - the release exponent, indicating the mechanism of drug release. Various kinetic models were tested (first order, zero order, Higuchi) and the highest values for the correlation coefficients, ranging

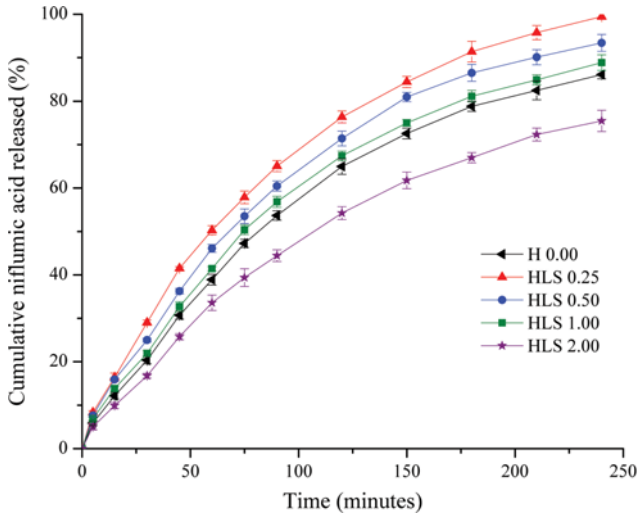

Fig. 6. Plots of cumulative percentage of niflumic acid released versus time for DDS with essential oil of *Lavandula stoechas L.* subsp. *stoechas* and with no essential oil.

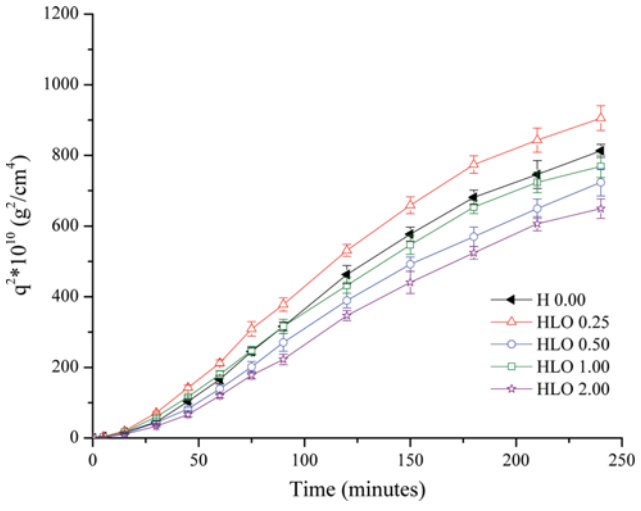

Fig. 7. Linear plots of q^2 versus time for DDS with essential oil of *Lavandula officinalis L.* and with no essential oil.

between 0.9927 and 0.9963 (Table 4), were obtained for the Higuchi model. This highlighted the fact that the cumulative niflumic acid release depends linearly on the square root of time (n=0.5), indicating a Fickian diffusion drug release mechanism.

The *in vitro* niflumic acid release kinetics was evaluated by three parameters: diffusion coefficient, time-lag and drug flux. The first two parameters are specific to the Higuchi model and were determined from the following equation [25]:

$$D = \frac{q^2 \cdot \pi}{4 \cdot C_0^2 \cdot t} \quad (2)$$

where, D is the drug diffusion coefficient (cm^2/min) q - the amount of drug released per unit of membrane surface (g/cm^2), C_0 - the initial drug concentration in the hydrolysate (g/mL), t - the drug release time (min). The time-lag was assessed from the regression line q^2 as a function of time (Figs. 7 and 8).

The drug flux was evaluated by the following equation [25]:

$$J = \frac{q}{t} \quad (3)$$

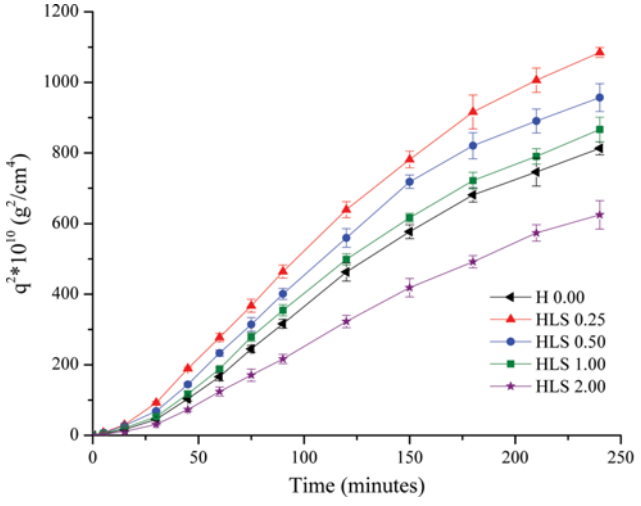

Fig. 8. Linear plots of q^2 versus time for DDS with essential oil of *Lavandula stoechas L.* subsp. *stoechas* and with no essential oil.

where, the significance of the q and t parameters was previously specified.

Table 3. Results of the release studies for the designed DDS

DDS	System responses			
	Diffusion coefficient $D \cdot 10^5$ (cm^2/s)	Time-lag T_{lag} (min)	Flux $J \cdot 10^6$ ($g/cm^2 \cdot s$)	Correlation coefficient for Higuchi model
H 0.00	4.69±0.13	7.86±0.73	1.20±0.014	0.9938±0.0009
HLO 0.25	5.21±0.18	4.04±0.39	1.25±0.019	0.9937±0.0006
HLS 0.25	6.17±0.15	2.27±0.59	1.34±0.014	0.9944±0.0014
HLO 0.50	4.08±0.18	9.35±0.74	1.15±0.021	0.9958±0.0005
HLS 0.50	5.54±0.21	4.15±0.11	1.28±0.025	0.9927±0.0005
HLO 1.00	4.43±0.17	5.21±0.32	1.13±0.021	0.9951±0.0003
HLS 1.00	4.96±0.16	5.98±0.27	1.22±0.015	0.9939±0.0008
HLO 2.00	3.74±0.14	11.33±0.60	1.09±0.019	0.9948±0.0007
HLS 2.00	3.54±0.17	10.49±0.64	1.05±0.022	0.9963±0.0008

Table 3 lists the values obtained for the kinetic parameters.

Table 3 shows that for the HLS series the diffusion coefficient and flux were higher, respectively, the time-lag was smaller for an essential oil concentration between 0.25-1.00% than for collagen hydrolysate with no essential oil (H). In the case of the HLO series, for an essential oil concentration of 0.25% and 1.00%, the time-lag was smaller than the one obtained for H, while the diffusion coefficient and flux were higher only for the HLO 0.25.

CONCLUSIONS

The niflumic acid release from all designed DDS followed a diffusional transport mechanism. The kinetic parameters were influenced by different concentrations and types of essential oils. For the HLS series, the combination of niflumic acid and essential oil in a concentration ranging from 0.25 to 1.00% leads to optimum values for the kinetic parameters compared to niflumic acid-collagen hydrolysate (H). In the case of the HLO series, it was only for the 0.25% concentration of the essential oil that all the kinetic parameters were optimum compared to the niflumic acid-collagen hydrolysate. These DDS, based on a synergistic therapeutical association of a drug and an essential oil, can have a potential biomedical application in wound healing, and further studies are recommended to test their antimicrobial efficiency.

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