EUCALYPTUS-COLLAGEN COMPOSITE GELS FOR DENTISTRY APPLICATIONS

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Composite gels based on collagen and eucalyptus oil were prepared in order to be used in dentistry. The main components such as β-Cymene (32.1%), Eucalyptol (36.59%), Cryptone (4.35%) and Spathulenol (3.82%) of *Eucalyptus camadulensis* Dehnh. essential oil were determined by GC-MS. The eucalyptus oil dissolved in different concentration of ethanol (4 and 8% v/v) was embedded in collagen gels (0.4 and 0.8% w/w) and cross-linked with tannic acid (0.2 and 0.4% w/w). The stability of gels was depending on collagen concentration, only the gels with 0.8% of collagen being stable. The rheological analysis of composite gels showed that formulation containing a combination of ethanol and tannic acid at the ratio of 8% v/v: 0.4%w/w resulted in the highest consistency index and yield stress. All the 0.8% collagen gels exhibited a suitable pseudoplastic behaviour and could be recommended for an adequate administration in the oral cavity.

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1. Introduction

Selection of biomaterials used for oral cavity is of prime importance and these materials need to be as natural as possible. Currently, collagen-based biomaterials have found many dental applications because of their biological properties as biocompatibility and biodegradability, where the degradation can be controlled by cross-linking [1]. Tannic acid showed the ability to bind proteins being used as cross-linker for collagen [2, 3]. Covalent bonds between phenolic compounds and proteins are more rigid and thermally stable than other interactions [4]. Moreover, tannic acid is an astringent that helps to stop oral bleeding [5].

The oral environment is different from the rest of the body due to the presence of saliva and oral microbiota [1]. Other major dental problem is the infected tissue which became more and more resistant to antibiotic. One of the best solutions for this problem could be the use of natural antimicrobial agents such as essential oils. Eucalyptus oils are widely used in medicinal and pharmaceutical products [6] being placed under GRAS (Generally Regarded as Safe) category by

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Food and Drug Authority of USA and classified as non-toxic [7]. Some researches showed that eucalyptus essential oils are effective even against resistant strains of microbes and they show not only toxicity against a wide range of fungi and bacteria but also possess antiviral activity. Because of its properties Eucalyptus oil is used as analgesic, anti-inflammatory, and antipyretic remedies [8, 9].

Among the biomaterial forms used in dentistry the most suitable one is the gel. It is easy to be handled, applied and gains cavity shape. Hence, for the design of novel biomaterials, rheological properties of collagen become significant [10]. The rheology is a powerful tool for physico-chemical characterization of gels providing useful information for their administration. Thus, it is crucial for a suitable application and drug controlled release to evaluate the influence of the formulation factors on the semisolid flow properties [11-14].

In the present paper we prepared composite gels based on collagen, eucalyptus essential oil and tannic acid and we determined their stability and reological performance by flow rheometry in view of their use in dentistry.

2. Experimental

Materials

Plant material: Leaves of *Eucalyptus camadulensis* L Dehnh. were harvested in July 2012 from the botanical gardens, Field Crops Department of Mustafa Kemal University.

Isolation of the essential oils: dried at 35^{o}C in oven, to constant weight, leaves of *Eucalyptus camadulensis* Dehnh. 100 g were extracted by hydrodistillation with 1 L distilled water for 3 h using Neo-Clevenger apparatus. Yield of obtained *Eucalyptus camadulensis* Dehnh. oils was 1.2%. The oils were dried over anhydrous sodium sulfate and then stored in dark color (amber) glass bottles, at -4^{o}C ready for GC-MS analysis.

Collagen gel with initial concentration of 2.11% (w/w) and with 2.5 pH was prepared as we described in our previous papers [15, 16]. Tannic acid (TA) was supplied by Sigma-Aldrich, Germany. Sodium hydroxyde and ethanol (Et-OH) were of analytical grade.

Composite gel preparation

Collagen gels of different concentrations were adjusted at 7.4 pH with 1M sodium hydroxyde (under stirring). *Eucalyptus camadulensis* Dehnh. oil (0.5% v/v) was dissolved in different concentration of Et-OH and added to collagen gels and than cross-linked with tannic acid. The composition of the obtained gels is given in Table 1.

Table 1. Compositions of the designed collagen gels

Gel	Collagen gel*, %	Et-OH**	TA**
G1	0.4	4	0.2
G2	0.8	4	0.2
G3	0.4	8	0.2
G4	0.8	8	0.2
G5	0.4	4	0.4
G6	0.8	4	0.4
G7	0.4	8	0.4
G8	0.8	8	0.4

* reported to dry substance of collagen, ** reported to volume of gel

Methods

GC-MS analysis: Analysis of the essential oils was carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, auto sampler and TR-WAXMS-A (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 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^{o}C, MS Ionization source

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 their mass spectrum (Wiley). The data were handled using Xcalibur software program.

Stability of obtained gels was determined by centrifugation. 5 g of the gels were put into tubes and centrifugated at 4000 - 4500 rot / min during 30 minutes, for 3 times using a Centrifuga Sigma Tip 3-15 centrifuge with speed of max. 14500 rot/min and 0.8 L capacity. The samples are considered stable if after the centrifugation cycle they are not separated into phases.

Rheological behaviour of collagen gels was analyzed using a rotational viscometer MultiVasc-Rheometer Fungilab equipped with a standard spindle TR 8. The operational conditions of rheological experiments were detailed in our previuos studies [13]. The up curves for shear rate-shear stress rheograms were recorded at two temperatures: 23°C and 37°C. The designed systems flow properties were determined using Table Curve 2D v5.01 software. Each experiment was carried out in triplicate.

3. Results and discussion

According to GC-MS results, 34 different components were identified in the eucalyptus essential oil. The main components are presented in Table 2.

Table 2. Eucalyptus camadulensis L. essential oil components

Retention Time	COMPONENTS	% peak area
12.96	α-Pinene	1.60
13.39	δ-3 CARENE	1.50
13.86	2,4(10)-thujadien	0.12
15.16	Sabinene	0.92
15.75	β-Pinene	0.55
16.77	Phellandrene	4.21
17.24	α-Terpinene	0.70
17.71	β-Cymene	32.10
18.10	Eucalyptol	36.59
19.26	γ-Terpinene	2.50
19.97	Sabinene hydrate	0.08
20.58	α-Terpinolene	0.41
21.21	Linalool	0.64
22.68	Cis-p-2-Mentene-1-ol	0.49
23.60	1-Terpineol	0.43
24.05	3-Cyclohexen-1-one, 3,5,5-trimethyl	0.21
25.47	Terpinen-4-ol	2.71
26.06	Cryptone	4.35
27.39	cis-Z-α-Bisabolene epoxide	0.02
28.69	Cuminal	1.52
29.16	Piperitone	0.31
30.36	Phellandral	1.15
31.95	Bicyclo[4.4.0]dec-5-ene-1-acetic acid	0.16
32.62	3-oxo-7,8-dihydro-α-ionol	0.04

Retention Time	COMPONENTS	% peak area
34.29	β-Guaiene	0.02
34.82	Ledene	0.02
35.72	Cadinene	0.04
36.35	trans-Caryophyllene	0.12
37.18	γ-Gurjunene	0.35
38.12	Neoalloocimene	0.62
39.59	Patchoulene	0.84
39.91	Dehydroaromadendrene	0.12
40.34	δ-Cadinene	0.06
43.05	Spathulenol	3.82

The main components are β-Cymene (32.1%), Eucalyptol (36.59%), Cryptone (4.35%) and Spathulenol (3.82%).

The obtained gels were subjected to centrifugation. The gels with 0.8% collagen (G2, G4, G6 and G8) were stable during stability tests meanwhile, the ones with 0.4% collagen (G1, G3, G5 and G7) were separated into two phases.

The rheological studies were performed only on the stable gels.

The rheological profiles corresponding to the ascending rheogram shear stress as a function of shear rate, obtained at 23°C and 37°C for G2, G4, G6 and G8 gels are given in Figures 1 and 2.

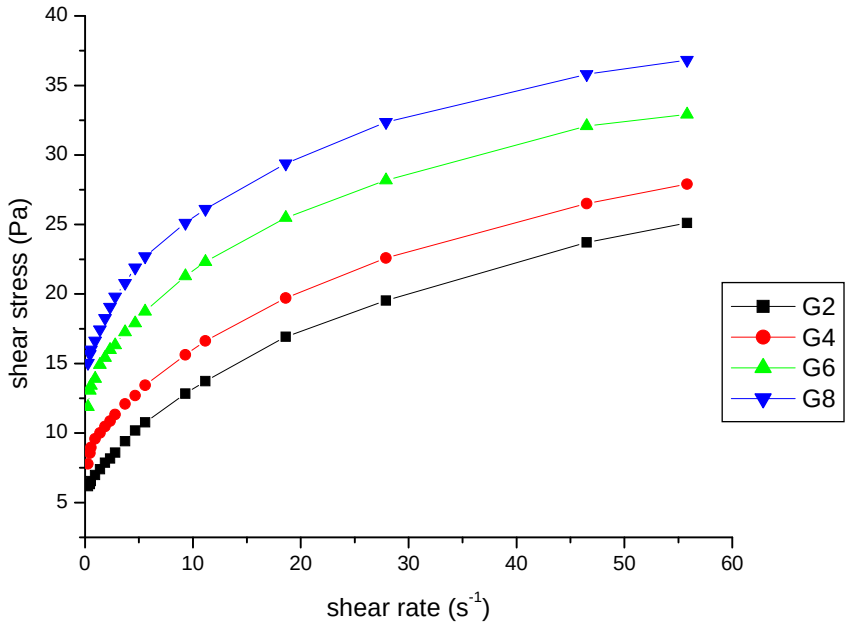

Fig. 1 – The flow profiles for collagen gels tested at 23°C

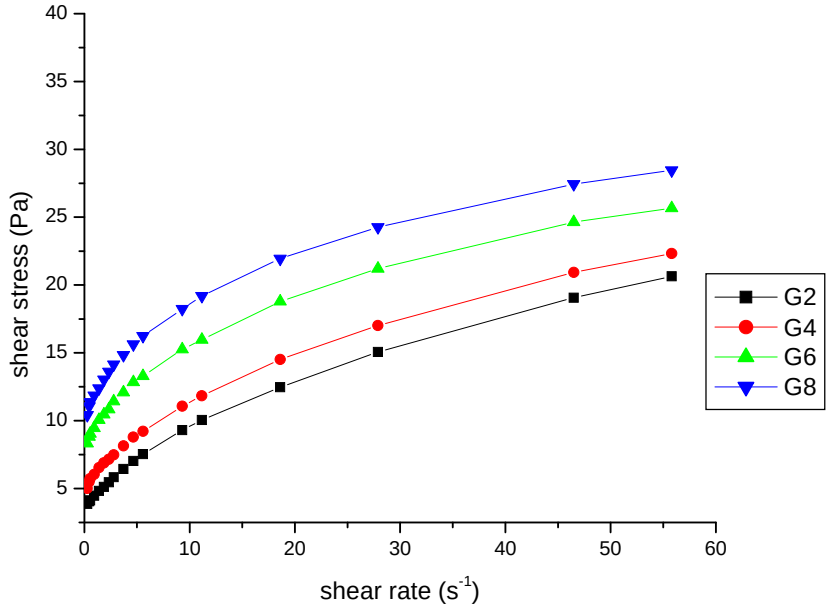

Fig. 2 – The flow profiles for collagen gels tested at 37°C

The flow curves presented in Figures 1 and 2 showed that the gels present a non-newtonian behaviour at both temperatures, the shear stress increasing with shear rate increase.

In order to quantify the flow behaviour of the designed gels, the experimental data were subjected to different rheological models testing: Ostwald-de Waele and Herschel-Bulkley specific to pseudoplastic systems, Bingham and Casson characteristic to plastic systems, respectively (Table 3) [12, 13, 16]. Determination coefficients (R^2) values obtained for these rheological models and their corresponding equations are listed in Table 3.

The meaning of the terms included in the above mentioned models is as follows: τ - shear stress (Pa), $\dot{\gamma}$ - shear rate (s^{-1}), η - dynamic viscosity (Pa·s), τ_o - yield stress (Pa), K - consistency index ($Pa \cdot s^n$), n - flow index.

Table 3. Determination coefficients (R^2) values of different rheological models for the designed gels analyzed at 23°C and 37°C

Gel	Determination coefficient R^2 for different rheological models							
	Ostwald-de Waelle $\tau = K \cdot \dot{\gamma}^n$		Herschel-Bulkley $\tau = \tau_0 + K \cdot \dot{\gamma}^n$		Bingham $\tau = \tau_0 + \eta \cdot \dot{\gamma}$		Casson $\tau^{0.5} = \tau_0^{0.5} + \eta^{0.5} \cdot \dot{\gamma}^{0.5}$	
	23°C	**37°C**	**23°C**	**37°C**	**23°C**	**37°C**	**23°C**	**37°C**
G2	0.9815	0.9853	0.9979	0.9992	0.9405	0.9587	0.9895	0.9942
G4	0.9803	0.9811	0.9983	0.9992	0.9280	0.9491	0.9874	0.9930
G6	0.9818	0.9791	0.9976	0.9985	0.8976	0.9198	0.9779	0.9857
G8	0.9857	0.9832	0.9974	0.9982	0.8744	0.8959	0.9685	0.9832

Analyzing the values obtained for the determination coefficients of the above rheological models, it is noticed that the shear stress dependence of shear rate can be described with Herschel-Bulkley model. The R^2 values for this model range from 0.9974 to 0.9983 for the gels tested at 23°C and from 0.9982 to 0.9992 for the same formulations tested at 37°C, respectively.

The values of the rheological parameters specific to Herschel-Bulkley model for the semisolid formulations tested at both working temperatures are summarized in Table 4.

Table 4. Values of rheological parameters specific to Herschel-Bulkley model for the collagen gels assessed at 23°C and 37°C

Gel	Yield stress – τ_o (Pa)		Consistency index - K (Pa·s^n)		Flow index - n	
	23°C	37°C	23°C	37°C	23°C	37°C
G2	4.410	2.780	2.654	1.768	0.515	0.577
G4	6.062	3.941	3.376	2.147	0.468	0.537
G6	8.886	6.304	5.207	3.304	0.386	0.444
G8	9.970	7.441	7.093	4.601	0.336	0.382

As it can be seen in Table 4, all the gels exhibit at both temperatures a flow index smaller than 1. This aspect is associated with a pseudoplastic behaviour. Regarding the gels biomedical utilization, the shear-thinning behaviour with yield stress offers the advantages of a suitable syringeability for administration in the oral cavity and an easy spreadability at the application site [13, 17].

According to Table 3, the values of flow parameters specific to Herschel-Bulkley model show that ethanol and tannic acid induce changes in the rheological behaviour of collagen-eucalyptus oil gels. Thus, both ingredients affect the yield stress and the consistency index in a added volume/amount-dependent manner.

Thus, doubling the volume of ethanol from 4% to 8% keeping the tannic acid amount at the same level (0.2%), the yield stress increase similarly at both temperatures (37.46% at 23°C and 41.76% at 37°C), while the consistency index has a more important increase at 23°C (27.20%) compared to 37°C (21.43%). In turn, maintaining the ethanol concentration at the same level (lower) and varying the tannic acid concentration from 0.2% to 0.4%, the same flow parameters values increase more markedly: the yield stress rises 2.01 times and consistency index - 1.96 times at lower temperature, while for the superior temperature the increase is about 2.27 times and 1.87 times, respectively.

For the highest level of tannic acid the increase of ethanol determines a small increase of the yield stress (12.19% - 18.03%) and a bigger rise of the consistency index (36.22%-39.28%) at both temperatures. A significantly higher increase of the above rheological characteristics is recorded for the variation of tannic acid from 0.2% to 0.4% when the ethanol is kept at the 8% concentration. Thus, the yield stress values are 1.65 and 1.89 times higher and the consistency index rises 2.10 and 2.14 times for 23°C and 37°C respectively,

This rheological analysis indicates that the influence of ethanol is more important for the gels prepared with the lower level of tannic acid. It could be also concluded that, beside the own pharmacological action, the tannic acid significantly modifies the flow parameters of collagen gels.

The incorporation of tannic acid and ethanol at the maximum levels (0.4% w/w and 8% v/v) significantly modifies the rheological descriptors specific to Herschel-Bulkley equation compared to the gels prepared with the lower levels of these formulation factors: yield stress increases 2.26 times at 23°C and 2.68 times at 37°C, consistency index rises 2.67 times at 23°C and 2.60 times at 37°C.

Besides the ethanol and tannic acid added in different ratios, another factor that influences the rheological properties is the temperature. Thus, for all formulations the temperature increase is resulting in a decrease of the yield stress between 25.36% and 36.96% and of the consistency index between 33.38% and 36.54%.

4. Conclusions

Composite gels with the same concentrations of Eucalyptus essential oils and different concentration of collagen, tannic acid and ethanol were prepared. Their stability was determined by centrifugation and the results showed that only the gels with 0.8% of collagen were stable. The rheological properties of the collagen-eucalyptus essential oil gels were also investigated. The rheological measurements reflected the effect of the formulation factors combination in different ratios on the flow descriptors. Thus, the formulation containing a combination of ethanol and

tannic acid at the ratio of 8% v/v: 0.4%w/w resulted in the highest consistency index and yield stress. Moreover, the monitoring of flow parameters showed that the designed gels possess suitable rheological characteristics (pseudoplastic behaviour with shear thinning) recommending them for an adequate administration in the oral cavity.

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