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In vitro and *ex vivo* assessments of surfactant-free topical curcumin emulgel

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ABSTRACT: Curcumin has been used in many diseases due to its high therapeutical potential in recent years. Although curcumin is a frequently used natural polyphenolic compound products, its low solubility and poor permeability limits the dermal efficacy of curcumin. Emulgels are a new generation of semi-solid formulations that combine the advantages of both emulsions and gels. The biggest limitation of emulsions is surfactant related irritation problems caused by the use of high amount of surfactants. In this study, it was aimed to increase the skin permeability of curcumin by developing surfactant-free emulgel formulations. In the study, emulgels were developed by Carbopol 940 gels as the aqueous phase, olive oil and curcumin methanol solution as the oil phase. No surfactant was used additionally to show the stabilizing effect of Carbopol in emulgels.

The emulgel formulations were subjected to physicochemical characterization by means of organoleptic properties, pH, rheological and mechanical properties. Mechanical properties were carried out by texture profile analyzes to determine the structure related properties such as hardness, compressibility, adhesiveness, cohesiveness and elasticity. The spreadability of the formulations was also determined with Texture Analyzer. Obtained emulgels showed good rheological, mechanical and spreadability properties. The transdermal permeation of the chosen emulgel was studied ex vivo against hydrogel prepared with the same amount of Carbopol. The emulgel formulation significantly increased transdermal permeation compared to the hydrogel. This emulgel formulation successfully passed from stress tests.

As a conclusion, the novel surfactant-free emulgel formulation was successfully developed to increase curcumin permeation through the rat skin.

KEYWORDS: Emulgel, surfactant-free, curcumin, texture analysis, spreadability, 2^3 factorial design

1. INTRODUCTION

Curcumin is a polyphenolic compound isolated from the rhizomes of Curcuma longa l., popularly known as "turmeric, Indian saffron, saffron root" (C. Longa). Studies have shown that curcumin has a wide range of biological and pharmacological effects with its antioxidant, anticarcinogenic, antimutagenic, antidiabetic, antibacterial, antiviral, anti-inflammatory, and antinociceptive effects. It is suggested that curcumin is effective and safe for the prevention and treatment of many diseases, including cancer. Curcumin has also been reported to have significant potential for the topical treatment of diseases such as wounds and skin cancer [1-4].

Chemical formula of the curcumin is $C_{21}H_{20}O_6$ with molecular weight of 368.39 Daltons, it is poorly soluble in water (0.00575 mg/mL) and soluble in organic solvents. It exhibits oral bioavailability of about 1% due to its low solubility, high first pass effect and rapid clearance. For this reason, approaches for solubility enhancement is important in the bioavailability of curcumin [5,6]. Various studies have been carried out by combining curcumin with permeability enhancers, encapsulating curcumin into carrier systems such as liposomes, micelles and nanoparticles [2,5,7,8].

Gels are highly preferred topical dosage forms because of the attractive appearance and giving pleasant cooling sensation. They are divided into two types: The first is hydrophobic gel (organogels), which contains polyethylene or fatty oils gelled with colloidal silica, aluminum or zinc soaps, and the second is hydrophilic

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gel (hydrogel) that consists water and hydrophilic solvents such as glycerol or propylene glycol [9,10]. These gels, which have many advantages, are still widely used.

Emulgels (or gelled emulsions) are a new-generation of topical formulations that consist of an emulsion and a gel and have the advantages of both. It can be prepared by adding an oil-in-water emulsion or a water-in-oil emulsion to the gel base [10,11]. Emulgel is an emerging field in topical drug delivery and is less commercially available. Since the distribution of hydrophobic drugs inside the gels is not homogeneous, emulgels have superiority over emulsions; therefore, hydrophobic drugs can be produced in an emulsion and then combined with a gel. They appear to be a good choice to achieve a dual controlled release effect for BCS Class II drugs with poor solubility and high permeability [11]. Emulgels show properties such as thixotropy, non-oily, easy application, easy removal, softening, non-staining, long shelf life, being biologically inert and acceptable to the patient [11,12]. In emulgels the use of different vegetable oils with emollient properties in the oil phase in the emulsion can alleviate significant skin dryness and irritation caused by the drug.

An emulsion is a thermodynamically unstable system consisting of at least two immiscible liquid phases. In order to keep two immiscible liquids together, the adhesive forces between the liquids must be greater than the cohesive forces. For this reason, surfactants are generally used to prevent the separation of the two liquid phases. The biggest limitation of emulsions is the problems caused by the use of high amount of surfactants and surfactant related irritation. Academic and industrial researchers have been working on surfactant-free emulsions for many years to prevent the disadvantages of traditional surfactants. The Society for Dermopharmacy, which is formed by pharmacists, dermatologists and other related professionals, uses the term "emulsifier-free" to stabilize emulsions macromolecules (molecular weight in more than 5000 g/mol) instead of classical surfactant [13]. There are various approaches that have been developed to reduce or eliminate surfactant use. These approaches are biopolymer conjugates and complexes [14-17], pickering stabilization with colloidal particles [18-20] and solidification of the oil phase [21-23].

In our study, it was aimed to develop surfactant-free emulgel formulations in order to increase the permeability of curcumin. The emulgel stabilization is provided by Carbopol 940, which is used as a gelling agent. The pH, viscosity, texture profile analysis (TPA) and spreadability properties of the obtained emulgels were investigated. The stress tests of surfactant-free emulgel was shown with stress tests by means of freeze-taw and centrifugation. The effect of surfactant-free emulgel on the curcumin permeability was investigated by examining the ex-vivo permeation of the optimum formulation versus the hydrogel formulation.

2. RESULTS AND DISCUSSION

In this study it was aimed to increase the skin penetration of curcumin by producing surfactant-free emulgel formulations. Emulgels are effective drug delivery systems in terms of dermatologic and cosmetic applications. They are promising drug delivery systems for hydrophobic drugs, consisting of gel phase and water-in-oil (w/o) or oil-in-water (o/w) emulsions, and are frequently used in the delivery of analgesic, anti-inflammatory, anti-fungal, anti-acne drugs and for the preparation of cosmetic formulations. Emulgels have many other features such as non-oily, easy to apply, easy to remove, emollient and transparency [24].

The emulgels in this study were prepared by cold-homogenization using homogenizer at 7000 rpm. The oil phase of the formulations contained a constant amount of olive oil and the aqueous phase was produced with high or low concentrations of C940 and TEA (1 and 0.5 w/v, respectively). Olive oil was chosen as oil phase because of its fatty acid components. It is reported that oleic acid, which is mainly found in olive oil, protects curcumin from autoxidation. It also affects the penetration of drugs as a penetration enhancer. The oil/water weight ratio of 0.3-0.4 (w/w) was found to be optimum in terms of viscosity and dermal application [21]. In this study, the oil phase was fixed at 0.28 according to the preliminary studies. C940 has been used to increase the viscosity of formulations to provide a thickening effect. It also affects the texture properties and stability of the emulgel system by surrounding the oil droplets as a protective layer [11,25,26].

3.1. Physicochemical Properties of Emulgels

The emulgels (E1-E8) were evaluated for their physical appearance, pH and viscosity. In terms of the appearance of emulgels, the dominant yellow color originated from curcumin and olive oil was obtained (Figure 1). The color difference in emulgel formulations varied according to the amounts of curcumin. All emulgels were odorless and smooth. No phase separation/visual difference was observed after 8 weeks of storage (Table 1).

Figure 1. Appearance of emulgels after preparation

The pH values for emulgels were measured between 4.9 and 7.8 and the obtained viscosity values were between 26.30 and 78.70 P (Table 1). Formulation parameters did not significantly affect pH and viscosity according to ANOVA statistics ($p>0.05$) given in Table 4. All emulgels, except E5 formula with a pH of 7.8, were found suitable for skin application due to their pH value.

Table 1. Physicochemical properties of formulations

Formulation	Color	Grittiness	Homogenity	Phase separation	pH ± SD	*Viscosity ± SD (cPx10³)
E1	Yellow	None	Yes	None	6.3 ± 0.07	51.23 ± 0.20
E2	Yellow	None	Yes	None	6.6 ± 0.06	67.23± 0.49
E3	Yellow	None	Yes	None	6.0 ± 0.03	63.56 ± 0.05
E4	Yellow	None	Yes	None	5.6 ± 0.02	78.70 ± 0.65
E5	Yellow	None	Yes	None	7.8 ± 0.03	42.26 ± 0.31
E6	Yellow	None	Yes	None	6.4 ± 0.50	40.03 ± 0.06
E7	Yellow	None	Yes	None	5.3 ± 0.50	26.30 ± 0.06
E8	Yellow	None	Yes	None	4.9 ± 0.02	35.30 ± 0.06

*Results were obtained using vibro viscometer (AND, SV-10)

Since curcumin emulgels do not contain surfactant, the presence of carbopol gains importance in terms of increasing the viscosity and modifying the rheological properties. C940 provides a shear-thinning or thixotropic property, which affects the product removal or skin application and resistance to incompatibility such as creaming, flocculation or coalescence [27]. Kim et al, (2003) reported a Herschel-Bulkley type of shear-thinning flow property by the use of C934 [24,28].

Therefore, the rheological properties of C940 hydrogels alone was also studied by a rotational viscometer and the direct viscosity values obtained was used against the rpm values of the viscometer to show the shear-thinning property of the gels (Figure 2). The logaritmic scale provided inside the graph represents a bingham type of flow.

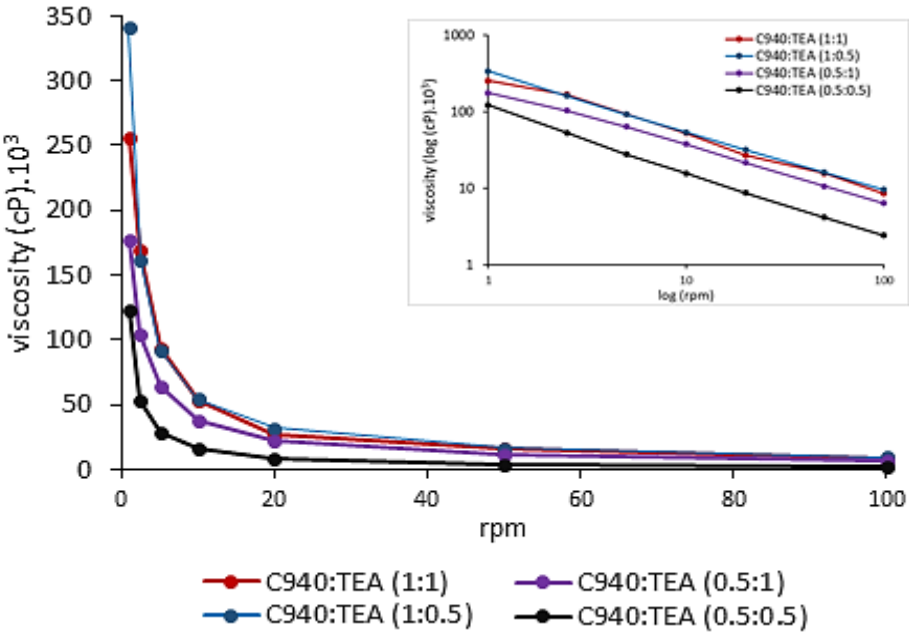

Figure 2. Rheological properties of different C940:TEA combinations used in emulgel preparation.

3.2. Evaluation of the Mechanical Properties of Emulgels

The choice of suitable formulation highly depends on mechanical, adhesive and rheological properties. Studies on mechanical features of semi-solid and disperse systems are important to mimic the real situations such as ease of removal from the container, spreading onto the skin; therefore, patient acceptability and ease of use are highly influenced by the mechanical parameters of the formulations. Texture profile analysis (TPA) is a suitable method for determining the mechanical properties such as hardness, compressibility, cohesiveness, elasticity and adhesiveness of emulgels [29,30]. It is also useful tool in providing information about batch-to-batch uniformity, quality and stability of formulations [31].

In TPA analysis, hardness and compressibility two parameters directly obtained from force-time curves and are used for interpreting the ease of removing the product from the container or skin application of the product. Hardness defines as the maximum peak force during the first compression cycle whereas compressibility defines the work required to deform the product during the first compression of probe [30,32].

Cohesiveness is the ratio between compression areas of two different cycles and in TPA analysis this parameter is generally used for predicting the product performance on application area by evaluating the the extent of structural reformation. Adhesiveness is the negative area of the first compression cycle, which represents the work required to overcome attractive forces between the product surface and the probe surface under physiological conditions. The lower cohesiveness values indicate the ease of spreading of the formulation, while higher values of adhesiveness are advantageous due to the increased contact retention time on the application surface

Elasticity, can be defined as the ratio between the time rates of two compression cycles. It is related with the reconstruction of the product after deformation in time. The decrease in the numerical value of elasticity shows the increase in the elasticity of gel, which indicates greater product elasticity and therefore better skin retention [30,32,33]. The results of TPA analysis are given in Table 2.

Table 2. TPA results for emulgels (n=4) ±SD

Batch	Hardness (N)	Compressibility (N.sec)	Adhesiveness (N.sec)	Cohesiveness	Elasticity
E-1	0.279±0.022	1.003±0.079	0.257±0.019	0.863±0,029	0.935±0.009
E-2	0.291±0.026	1.110±0.110	0.334±0.128	0.835±0.058	0.936±0.027
E-3	0.306±0.022	1.056±0.042	0.317±0.045	0.820±0,048	0.942±0.021
E-4	0.326±0.033	1.181±0.127	0.315±0.075	0.821±0,053	0.983±0.017
E-5	0.195±0.024	0.758±0.096	0.245±0.060	0.836±0,045	0.931±0.028
E-6	0.175±0.027	0.700±0.130	0.158±0.097	0.871±0,054	0.961±0.037
E-7	0.214±0.015	0.819±0.052	0.218±0.038	0.836±0,036	0.952±0.032
E-8	0.190±0.010	0.753±0.023	0.195±0.018	0.867±0,030	0.953±0.002

Carbopols are reported to increase the hardness, compressibility and adhesiveness in emulgel formulations whereas causes a decrease in cohesiveness and elasticity values [31].

3.3. Spreadability Properties of Emulgels

An emulgel must show good spreadability property for suitability of skin application. Spreadability is an interrelated feature with the durability/resistance of a product against externally applied force. While durability is measured by the applied force to deform the surface of the product, spreadability is the deformation of the entire structure due to an external force. In this case, although the firmness of the two products is similar, their application to the skin surface may be different. Therefore, firmness and work of shear are often evaluated together.

When the spreadability data is examined, the required work and firmness of the gels increase as the carbopol concentration increases in the emulgels (Table 3). This situation is directly related to the increase in the hardness and viscosity of the gel as the amount of carbopol increases in emulgels. As this component increases, the spreadability of the gel decreases.

In spreadability studies the peak force which represents firmness shows the ability of product flow. The higher value of firmness means the formulation has a higher consistency which negatively affects the spreadability [34].

Table 3. Spreadability results for emulgels (n=4)

Batch	Work ± SD (N)	Firmness ± SD (N.sec)
E-1	10.819 ± 1.238	12.768 ± 2.209
E-2	12.089 ± 2.982	14.736 ± 6.033
E-3	15.393 ± 2.074	20.169 ± 4.108
E-4	12.720 ± 3.073	12.930 ± 6.142
E-5	9.758 ± 1.631	11.815 ± 3.404
E-6	7.239 ± 1.066	7.600 ± 2.311
E-7	7.642 ± 2.050	8.234 ± 2.992
E-8	7.806 ± 0.933	8.792 ± 2.286

Table 4. MLR results and variance analysis p values obtained by ANOVA.

Responses		Carbopol Concentration	TEA Concentration	Curcumin Concentration	Intercept	R
Viscosity	**Coefficient**	16.595	40.265	-0.5018	3.7250	0.8283
	p	0.3340	0.0561	0.5431	0.8613	
pH	**Coefficient**	3.350	-0.650	-0.0775	5.225	0,8622
	p	**0.0383**	0.5867	0.2320	**0.0230**	
Hardness (N)	**Coefficient**	0.2144	-0.0476	0.0003	0.1173	0.9831
	p	**0.0005**	0.0802	0.7751	**0.0122**	
Compressibility (N.sec)	**Coefficient**	0.6598	-0.1188	-0.0027	0.5575	0.9665
	p	**0.0018**	0.2543	0.5766	**0.0092**	
Adhesiveness	**Coefficient**	-0.2032	0.0256	-0.0009	-0.1086	0.8662
	p	**0.0266**	0.6882	0.7814	0.2386	
Cohesiveness	**Coefficient**	-0.0366	0.0296	-0.0010	0.8640	0.6589
	p	0.2783	0.3679	0.5306	**0.0000**	
Elasticity	**Coefficient**	-0.0007	-0.0334	-0.0018	1.0022	0.7728
	p	0.9743	0.1769	0.1456	**0.0000**	
Spreadability/Work	**Coefficient**	9.2880	-1.8280	0.0934	3.4290	0.8955
	p	**0.0179**	0.4884	0.4771	0.3406	
Spreadability/Firmness	**Coefficient**	12.081	-1.603	0.2232	0.9240	0.8399
	p	**0.0450**	0.7215	0.3473	0.8758	

Significance was shown in bold ($p<0.05$)

3.4. Stress test results

Carbomer gels are formed by the interchain entanglement of the carboxyl groups in the acrylic acid chain and electrostatic repulsion occured by balancing the pH. They are reported to produce microgel particles by neutralization of -COOH with amines such as TEA. This polyelectrolyte microgel particles strengthen the o/w interface through partial solubilization into the oil droplets [24]. They lower the interfacial pressure between two immiscible phases and set up an interfacial layer surrounding the droplets through adsorption. Therefore, C940 can be utilized as a emulsifier in stabilizing oil and water mixtures. Carbopol based stabilization is mainly results from steric stabilization [35,36].

To demonstrate the effect of C940 on emulgel stability, centrifugation was performed as a stress test on E1, E3, and E5 formulations. Samples taken from different parts of the surface of the products were investigated optically by microscope (Leica DM 4000B- DFC 280) at different magnifications before and after centrifugation (Figure 3).

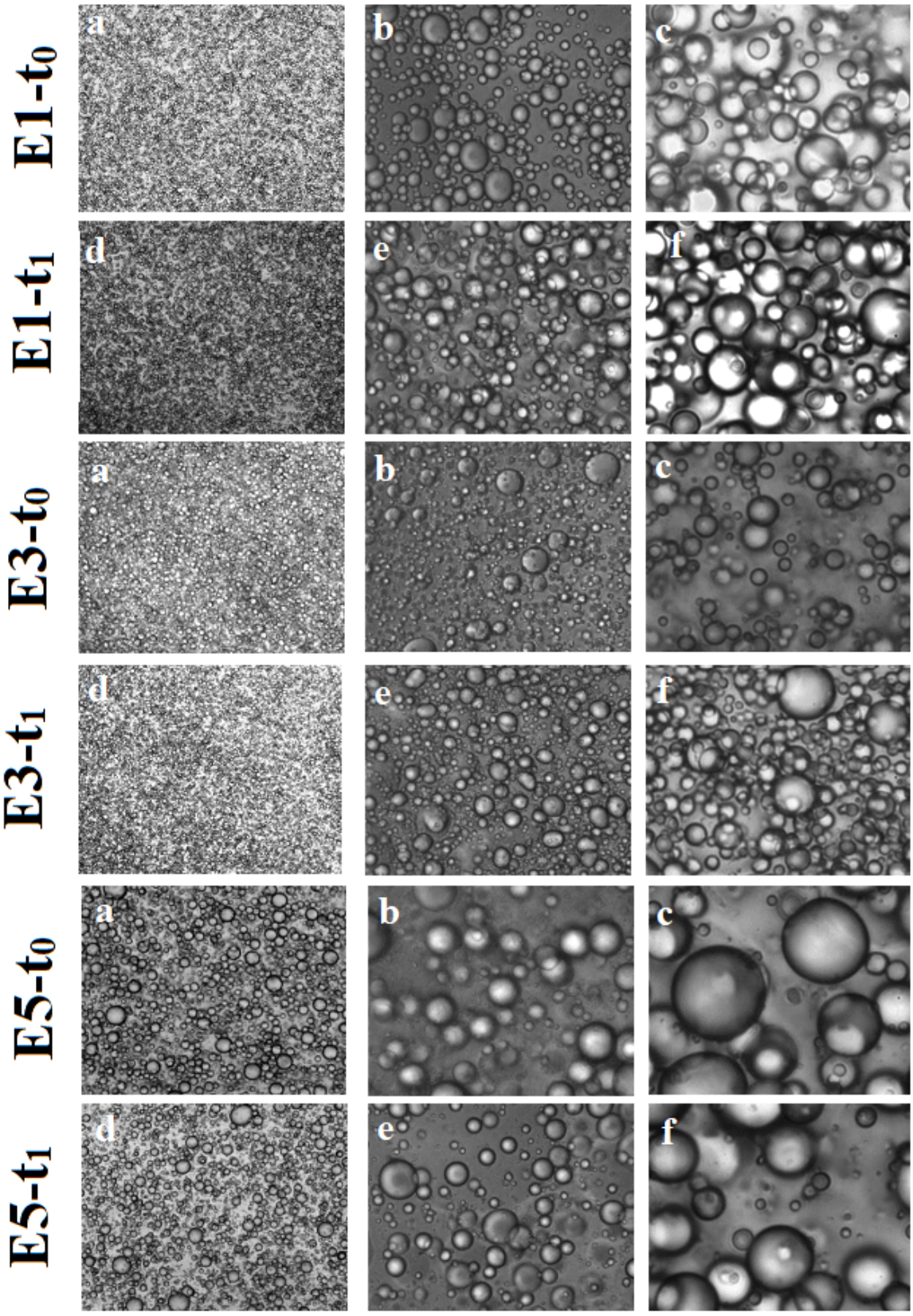

Figure 3. Centrifuge test results for E1, E3 and E5. Micrographs from a to c represents 10x, 40x, 100x magnifications for t_0 and from d to f represents 10x, 40x, 100x magnifications for t_1.

Masmoudi et al (2006) reported that the increase in droplet size while a decrease in the droplet number indicates the coalescence in emulgels [7]. In our study, no difference was observed in the number of droplets

and the droplet size per unit area of the image at applied magnifications. Independent from its amount, both 1% and 0.5% C940 with 1% and 0.5% TEA provided an emulgel that are resistant to stress condition. When the droplets were evaluated with optical microscopy at the same conditions, it was visually seen that, E1 and E3 emulgels prepared with 1% C940 had smaller droplets than E5 prepared with 0.5 % C940.
Freeze-thaw method was additionaly performed on E3 formulation, which was chosen for ex-vivo study. No visual difference was observed between the before and after samples. The oil droplets in microscope at 10x, 40x and 100x magnifications did not show significant difference in droplet size or number (evaluated visually) which is interpreted as no coalescence was occured. The viscosity value at 20 rpm was not significantly changed (p= 0.2169) with ANOVA test (Figure 4).

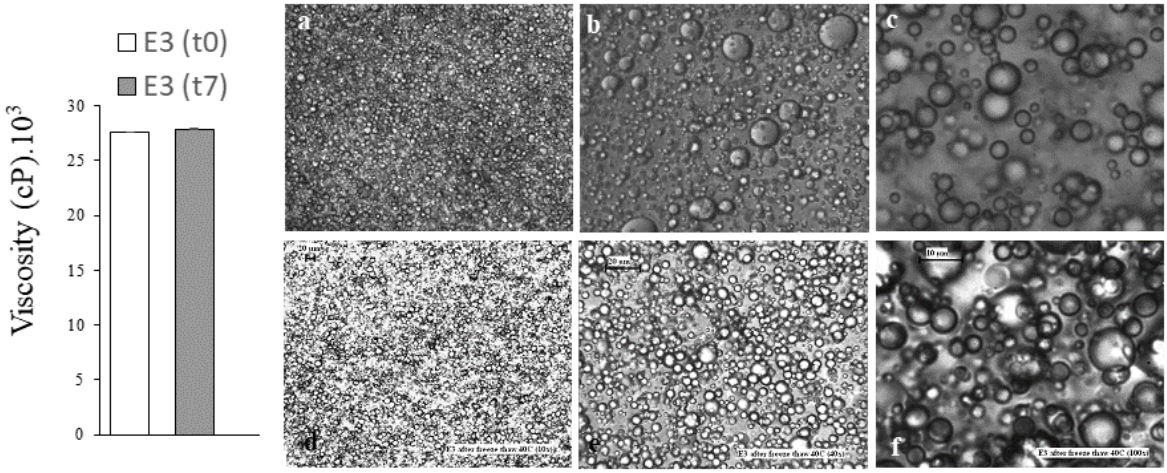

Figure 4. Freeze-thaw results for E3: Viscosity measurements were done at 20 rpm with TC spindle at room temperature. Micrographs from a to c represents 10x, 40x, 100x magnifications for t_0 and from d to f represents 10x, 40x, 100x magnifications for t_7.

All these results indicated that C940 can enhance the stability in emulgels and thus emulgels can be can be prepared as surfactant-free by the use of carbopol.

3.5. Ex-vivo permeability study results

When the rat skin permeation profile was examined, it was observed that emulgels significantly increased permeability compared to hydrogels (Figure 5). In our study, curcumin was mixed with olive oil homogenously, then these oil droplets were covered by Carbopol. This strategy prevents the auto-oxidation of curcumin and provides drug stability during the skin penetration. Besides, olive oil acts as a penetration enhancer as shown in literature [25,38,39]. The suggested mechanism for increased permeation of curcumin can be either disintegrating of the intracellular highly ordered lipid structure by olive oil and co-solvent (methanol) or changing the conformational structure in the intracellular proteins or might be due to increasing the partitioning of curcumin into stratum corneum [38-40].

In hydrogels, curcumin was homogeneously dispersed in the gel, but its penetration through the skin was found to be very low. This shows that emulgels are more suitable for transdermal use while hydrogels are suitable for topical use. Because in topical applications, it is desired that the product stays on the skin surface without passing through the layers of the skin.

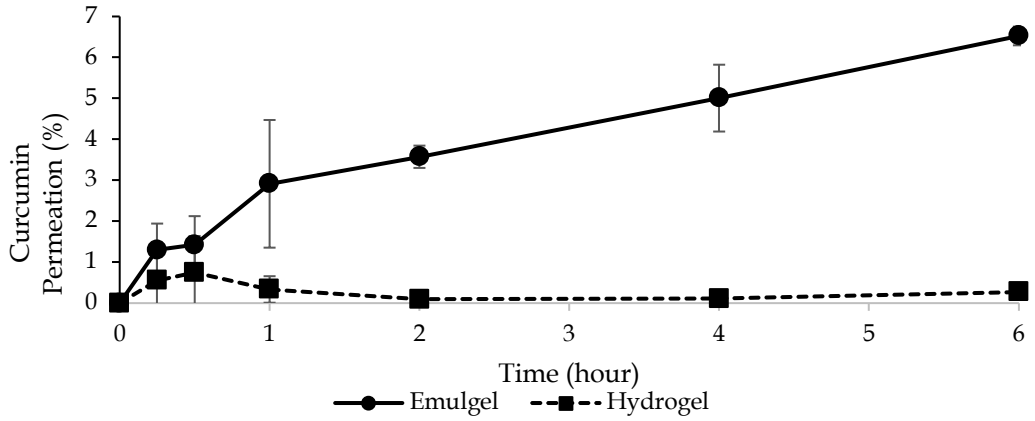

Figure 5. Ex-vivo permeability result for E3 in comparison with hydrogel

3. CONCLUSION

Curcumin has been used for years topically due to its wide range of biological and pharmacological effects such as antioxidant, anticarcinogenic, antimutagenic, antidiabetic, antibacterial, antiviral, anti-inflammatory, and antinociceptive. However, its low solubility and poor permeability limits the dermal efficacy of curcumin. Emulgels, combining the advantages of both emulsions and hydrocolloids can be a good strategy to increase the penetration of curcumin. Therefore, an emulgel formulation developed with carbopol 940 and olive oil was studied.

Olive oil is a highly used oil in both food and cosmetics, as a carrier system and C940 has stabilizing effect on emulgel formulations. In our study the physical stability of the formulation was successfully provided by C940 without using a surfactant in the emulsion phase of the formulation. The obtained gel formulations were subjected to physicochemical characterizations such as appearance, pH and viscosity. Then, texture analyzes were carried out to determine the spreadable and structural properties of the emulgel. According to the results, the emulgels showed good rheological, mechanical and spreadability properties.

The transdermal permeability studies of the emulgels were studied using a whole piece of skin taken from the rats' abdominal region. The emulgel formulation significantly increased curcumin skin penetration compared to the hydrogel formulation containing the same amount of gel agent and curcumin. As a result, surfactant-free emulgel formulations were successfully developed and can be good option for permeation of poorly soluble drugs through the skin.

4. MATERIALS AND METHODS

4.1. Materials

Curcumin (95% purity) was purchased from BulkSupplements, USA. Carbopol 940 were purchased from Düzey Lab. Triethanolamine (TEA), methanol and Tween 80 were purchased from Merck, Germany. PBS (Phosphate Buffered Saline) were purchased from Amrecso, USA. All chemicals and solvents were analytical reagent grade.

4.2. Preparation Emulgels

Carbopol 940 (C940) was dispersed in distilled water at certain concentrations (1-0.5% w/v) by stirring with a heating magnetic stirrer at 400 rpm for 1 hour. Curcumin (0.01-0.02 w/v %) was dissolved in 4 mL of methanol in an ultrasonic bath. Then, the oily phase was formed by mixing the olive oil and curcumin solution, and C940 was included in the dispersion by mixing with the aid of a homogenizer at 7000 rpm (WiseTis, Japan) for 5 minutes. TEA was mixed with emulgels until the gelation occurred. While olive oil was kept constant in the formulations, C940 and the neutralizing agent TEA (1-0.5 w/v %) were used at different concentrations to obtain gels (Table 5).

Table 5. Composition of Emulgel Formulations

Contents	E1	E2	E3	E4	E5	E6	E7	E8
Olive oil (%)	20	20	20	20	20	20	20	20
Carbopol 940 (%)	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5
Curcumin (%)	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01
TEA (%)	1.0	1.0	0.5	0.5	1.0	1.0	0.5	0.5

In preparation, 2^3 factorial designs comprising of 2 level and 3 factors were connected in arrange to decide the impact of C940, TEA and curcumin concentrations on the physicochemical properites of emulgel formulations. Therefore, independent variable chosen for structuring micromatrices were the concentrations of C940 (X_1), TEA (X_2), Curcumin (X_3). Viscosity, pH, texture analysis and spreadability properties were dependent factors. Thus, a 2^3 factorial design was utilized with 3 components (X_1, X_2, X_3) at 2 levels to explain the impacts of independent variables on measured responses. The factorial design is summarized in Table 2. The measured responses were subjected to to multiple linear regression analysis to evaluate the impacts of independent components. The multiple regression model is given as Equation (1) where Y is the measured response, X_i is the level of the independent factor, B_i is coefficients and B_0 is the intercept. The linear regression module of Statistica 8.0 for Windows (Statsoft Inc., OK, USA) was utilized to calculate B coefficients with their standard errors and descriptive statistics of regression for the model.

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 \quad \text{Eq.1.}$$

Table 6. Variable level of 2^3 factorial design for emulgel formulation

Variable level	-1 (low)	+1 (high)
C940 Concentration (%) (w/v) (X_1)	0.5	1.0
TEA Concentration (%) (w/v) (X_2)	0.5	1.0
Curcumin Concentration (w/v) (X_3)	0.01	0.02

4.3. Evaluation of emulgels

Emulgels were evaluated by means of their organoleptic, pH, rheological, mechanical and spreadability properties. The effect of C940 on emulgel stability was shown by evaluating the changes in viscosity and optical properties after stress conditions were applied to the optimum emulgel formulation. Ex-vivo permeability study was carried out on the E3 formula.

4.3.1. Organoleptic Properties

The emulgel formulations were visually evaluated as homogeneity, color, phase separation and grittiness during the 8 weeks.

4.3.2. pH

The pH measurements of the prepared formulations were carried out with a pH-meter (Mettler Toledo S 220, Switzerland). The measurements were repeated 3 times at room temperature, and the arithmetic mean and standard deviation (SD) values were calculated.

4.3.3. Rheological properties

The viscosity of emulgels were studied by a sine-wave vibro viscometer (AND, SV-10, Japan). The mechanism of this viscometer depends on vibration of the sensory plates driven by the electromagnetic force of the same frequency, which is measured as resonance (amplitude) at constant shear rate. Measurements with vibro viscometer was carried out at 50 Hz at 30 ± 0.20 °C.

The effect of C940 on rheological properties were evaluated using a rotational viscometer (Brookfield DV II, Scintech Ins., VA, USA), TC spindle with increasing rpms of 1, 2.5, 5, 10, 20, 50 and 100 at room temperature.

4.3.4. Mechanical properties

The mechanical properties of the emulgels were determined by texture profile analysis (TPA) method using TAXT Plus Texture Analyzer (Stable Micro Systems, UK) attached with a 5 kg load cell. A height calibration was ensured before the experiments.

Briefly products were placed in a 50 mL beaker. The height of the product is arranged as a 5 cm. Two-Cycle Compression Test was applied for TPA. In the method pre-test speed of 1.5 mm/s; test speed of 1.0 mm/s and post-test speed of 1.5 mm/s; target mode distance of 10 mm, and trigger force of 0.05 N was applied. Cylindrical perspex probe of 10 mm diameter was immersed twice into each formulation and 15 s of delay period was allowed between two compressions. The smeasurements were made for each formulation at 25 ± 1 °C.

Hardness, compressibility, cohesiveness, elasticity and adhesiveness were calculated with the values obtained from the force-time graph. Cohesiveness and elasticity were calculated by Equations 2 and 3:

$$Cohesiveness = \frac{2nd\ Compression\ Area}{1st\ Compression\ Area} \quad \text{Eq. 2}$$

$$Elasticity = \frac{Time\ for\ 2nd\ Compression}{Time\ for\ 1st\ Compression} \quad \text{Eq. 3}$$

4.3.5. Spreadability properties

The spreadability of the emulgels was measured with the TAXT Plus Texture Analyzer using two conical probes, fixed and movable (TTC Spreadability Rig-HDP/SR) are used in drivability tests. Calibration of the system was provided by placing the movable probe in the fixed cone. After the movable cone is calibrated at a distance of 18 mm from the fixed cone, the movable cone touches the fixed cone and is positioned 2 mm above the fixed cone. In our study, the product was transferred to the fixed cone without creating air bubbles, and the mechanism was operated and the strength (also called as firmness, N) and work of shear (N.sec) values required for spreadability were taken from the force-time graph.

4.3.6. Stress test

The stability of surfactant-free emulgels was investigated by applying stress conditions on selected emulgels (E1, E3, and E5). The formulations were chosen because of their increased amount of C940 and TEA. For this purpose, centrifugation (Sigma, 30-300 KS) was applied on 4 g of emulsion at 3000 rpm at 32°C for 1 hour and the samples were evaluated optically.

The E3 formulation was investigated additionally by freeze-thaw (aging and temperature approximation) method by cycling 6 times at 5 ± 3°C and 38°C ± 2°C. The changes in the viscosity values and microscopic appearance of the formulation were evaluated before and after the freeze-thaw study.

4.4. Ex-Vivo Permeability

The skin permeation of emulgel (E3) was compared with an hydrogel formulation consisting the same amounts of C940, TEA and curcumin without addition of methanol and olive oil by using fullthickness skin from the abdominal region of male Wistar rats was used. Before use, the skins were thoroughly shaved, washed with pH 7.4 phosphate buffer, dried and wrapped in parafilm, and stored in a deep freezer at -80°C until use. For ex-vivo skin permeation studies, approval was obtained for the use of donated tissue with the approval of the local ethics committee, dated 08.12.2016 and numbered 148677, obtained from the Trakya University Animal Experiments Local Ethics Committee.

Modified Franz diffusion cells were used for determining the skin penetration of curcumin [41]. Briefly, emulgel or hydrogel consisting of 1.5 mg curcumin was applied onto the stratum corneum layer, which was placed in donor compartment (3.41 cm^2 surface area). The dermal layer was in contact with the receptor compartment, which 50 mL of medium was placed. The diffusion cell was stirred at 37 ± 0.5°C with a magnetic stirrer at 300 rpm. 1 mL samples were taken at regular intervals (0.25., 0.50., 1., 2., 4., and 6. hours) replaced by 1 mL of dissolution medium. All the samples taken were analyzed in UV spectrophotometer (Optizen POP) with a UV spectrophotometric method in dissolution medium between 1.25-50 µg/mL at λ_{max} of 425 nm. The assay method was validated according to ICH Q2(R1) guideline by analytical validation parameters of accuracy, precision, limit of detection, limit of quantification. The relative standard deviations of precision and accuracy results were within the acceptable limits.

Phosphate buffered saline pH 7.4 was used as dissolution medium with the addition of 0.5 % Tween 80 as a surfactant which provide the sink condition for poorly soluble drugs.

4.8. Statistical Analysis

SPSS (Version 20.0, Chicago, IL) was used for all statistical analyses. Mean value and standard deviation were calculated using descriptive statistics. Significant differences were considered when $p<0.05$.

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