FABAD J. Pharm. Sci., 35, 35-44, 2010

RESEARCH ARTICLE

In Vitro Comparison of O/W Type Emulsion Gel and Liposome Gel for Cosmetic Application of Kinetin

Şeyda AKKUŞ ARSLAN*°, Tuba İNCEÇAYIR*, Figen TIRNAKSIZ*

In Vitro Comparison of O/W Type Emulsion Gel and Liposome Gel for Cosmetic Application of Kinetin

Summary

Kinetin is a highly potent growth factor that is capable of delaying or preventing age-related changes of human skin fibroblasts. The purpose of this study was to develop and characterize topical kinetin-loaded emulsion gel and liposome gel formulations. Emulsion formulation was prepared using the spontaneous emulsification method. Pseudo-ternary phase diagram for oil/water (o/w) type emulsion was constructed using caprylic/capric/linoleic triglyceride (Miglyol®818), surfactant [egg lecithin (phosphatidyl choline content 80%)], cosurfactant (absolute ethanol), poloxamer 188 (Lutrol®F68) and water. Poloxamer 188 and water were used as the aqueous phase. Liposome was prepared by conventional film hydration method using 9:1 w/w phosphatidylcholine:cholesterol as lipophilic phase. The encapsulation efficiency of liposome was found to be 84.5%. The droplet/vesicle sizes were 3.88 µm and 5.30 µm for o/w type emulsion and liposome systems, respectively. Emulsion and liposome formulations were incorporated in hydroxypropyl methylcellulose (HPMC®K15M) and carbomer (Carbopol®974) gels for improving the viscosity for topical administration, respectively. The reograms exhibited a non-Newtonian behavior. Kinetin release from emulsion and liposome gels were evaluated through artificial membrane (cellulose acetate membrane with molecular cut-off 12000 Dalton, D-0405). The results of the study indicated that the emulsion and liposome gels may be promising vehicles for topical delivery of kinetin.

Key Words: *Emulsion, liposome, kinetin.*

Kinetinin Kozmetik Uygulaması için Emülsiyon ve Lipozom Jellerinin Kıyaslanması

Özet

Kinetin, insan derisi fibroblastlarında olan yaşa bağlı değişiklikleri geciktiren veya önleyen son derece güçlü bir büyüme faktörüdür. Bu çalışmanın amacı, topikal kinetin yüklü emülsiyon jel ve lipozom jel formülasyonları geliştirmek ve karakterize etmektir. Emülsiyon formülasyonu spontan emülsifikasyon yöntemi ile hazırlanmıştır. Yağ/su tipi emülsiyon için üçgen faz diyagramı kaprilik/kaprik/linoleik trigliserit (Miglyol®818), sürfaktan [yumurta lesitini (fosfatidilkolin içeriği %80)], kosürfactan (absolü etanol), poloxamer 188 (Lutrol®F68) ve su kullanılarak oluşturulmuştur. Su fazı olarak poloxamer 188 ve su kullanılmıştır. Lipozom, lipofilik faz olarak 9:1 a/a fosfatidilkolin:kolesterol kullanılarak konvansiyonel film hidrasyon yöntemi ile hazırlanmıştır. Lipozomun enkapsülasyon etkinliği %84.5 olarak bulunmuştur. Yağ/su tipi emülsiyon ve lipozom sistemleri için damlacık/vezikül büyüklüğü sırasıyla 3.88 µm ve 5.30 µm'dir. Topikal uygulama için viskoziteyi iyileştirmek amacıyla emülsiyon ve lipozom formülasyonları sırasıyla hidroksipropilmetilselüloz (HPMC®K15M) ve karbomer (Carbopol®974) jelleriyle birleştirilmiştir. Reogramlar non-Newtonian davranış göstermektedir. Emülsiyon ve lipozom jellerinde yapay membrandan (selüloz asetat membran, moleküler cut-off değeri: 12000 Dalton, D-0405) kinetin geçişleri değerlendirilmiştir. Çalışmanın sonuçları, kinetinin topikal verilişi için emülsiyon ve lipozom jellerinin uygun taşıyıcılar olabileceğini göstermektedir.

Anahtar Kelimeler: *Emülsiyon, lipozom, kinetin.*

Received: 10.01.2012
Revised: 10.03.2012
Accepted: 22.03.2012

* Gazi University, Faculty of Pharmacy, Dept. Pharmaceutical Technology, 06330, Etiler-Ankara, TURKEY
° Corresponding Author E-mail: seydaakkus@gmail.com

INTRODUCTION

Cytokinins are plant hormones with many essential signaling roles in plant growth and development (1, 2). Kinetin, the first cytokinin isolated and identified in 1955, is a highly potent growth factor that promotes cell division and ensures orderly growth and development of plants (3, 4). More importantly, kinetin has been shown capable of delaying or preventing age-related changes of human skin fibroblasts grown in laboratory culture (5, 6). The new data on the biological properties of kinetin have yielded the commercial applications of kinetin in cosmeceutics as a powerful antioxidant and antiageing molecule (5, 7). One open-label study about the clinical safety and efficacy of kinetin 0.1% lotion on human skin demonstrated that topical kinetin (0.01%-0.1%) can improve some of the clinical signs of mild to moderately photodamaged skin, such as skin texture, fine wrinkles, skin color and blotchiness and can help to restore normal skin barrier function (8). Another clinical study investigated the anti-aging effects of topical kinetin 0.03% in combination with niacinamide 4% versus niacinamide 4% alone in Asians. It was found that kinetin combined with niacinamide might be used as an adjunctive therapy for anti-aging purposes of the skin (9). This synthetic cytokinin plant hormone is available in conventional creams and lotions (0.01%, 0.05%, and 0.1%) (10).

Emulsions and liposomes have been investigating as delivery systems to cosmetic active substances (11-14).

Emulsions are the most common type of formulations used for cosmetic products. The most typical emulsion is one which oil is dispersed in water (oil-in-water emulsion, o/w). Coalescence represents a more serious stability problem because it is irreversible for o/w type emulsions. This process can lead to phase separation. The uses of gelling polymer agents can ensure the long term stability of o/w emulsions. Different characteristics can be investigated for emulsions and liposomes. The simplest of these include observation of pH, viscosity and droplet size of internal phase. There are different methods to prepare emulsion systems. These are high pressure homogenization (15), ultrasonication (16), microfluidization (17), emulsion inversion point method (18) and spontaneous emulsification (19). In this study o/w type, lecithin and poloxamer based emulsion, prepared using spontaneous emulsification method, forms when an organic phase and aqueous phase are mixed. The organic phase is a homogeneous solution of oil, lipophilic surfactant and water-miscible solvent (or cosurfactants). The aqueous phase consists on hydrophilic surfactant and water (20). Emulsion forms spontaneously when organic phase and aqueous phase are mixed. In this method water-miscible solvent, such as ethanol is later evaporated by vacuum.

Liposomes are microscopic vesicles composed of one or more liquid lipid bilayers arranged in concentric fashion enclosing an equal number of compartments (21). Liposomes are excellent formulations as cosmetic carriers, owing to their biodegradability, biocompatibility, low toxicity, and ability to entrap lipophilic and hydrophilic active molecules (22).Topical application of liposomes has many advantages over the conventional dosage forms (23). In general liposomes are more effective than conventional formulations due to bilayer composition, structure and their interaction with stratum corneum (24-26).

Liposome vesicles and emulsion droplets embedded into a suitable gel matrix could be attractive candidates for the use as delivery vehicles for topical application of kinetin. The aim of this study was to formulate and characterize liposome gel and emulsion gel formulations of kinetin for topical application. This study was conducted to evaluate the release behaviors of kinetin from these formulations. Up to now, as far we know, there is no report in the literature proposes the entrapment of kinetin in liposome and emulsion, which are dispersed in gel formulations.

EXPERIMENTAL

MATERIALS

Egg lecithin (phosphatidyl choline (PC) content 80%), cholesterol (CH), caprylic/capric/linoleic triglyceride (Miglyol®818) and poloxamer 188

(Lutrol®F68) were supplied from Lipoid GmbH (Germany), Merck (Germany), Hanseler (Germany) and BASF (Germany), respectively. Kinetin and absolute ethanol were purchased from AppliChem (Germany). Hydroxypropyl methylcellulose (HPMC®K15M) by Dow (USA) and Carbopol®974 by Noveon (USA) were used as gelling agents for emulsion and liposome formulations, respectively. Cellulose acetate membrane with molecular cut-off 12000 Dalton (D-0405) was supplied from Sigma (USA). Chloroform, sodium hydroxide and potassium dihydrogen orthophosphate were of analytical grade.

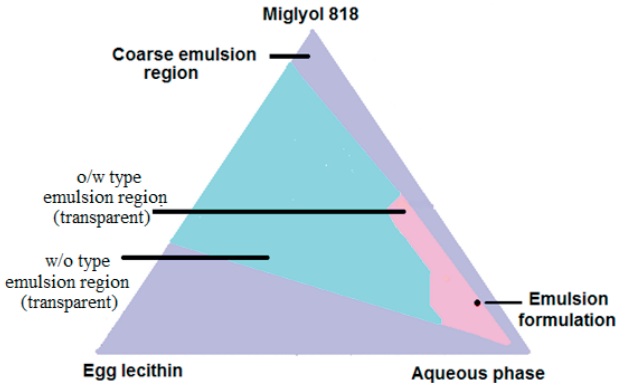


Figure 2. Pseudo-ternary phase diagram of the oil, surfactant and aqueous phase at ambient temperature.

METHODS

Preparation of emulsion

Emulsion formulation was prepared using the spontaneous emulsification and solvent evaporation method (Figure 1) (27). Pseudo-ternary phase diagram was constructed using water titration method at ambient temperature (25°C) to find out the concentration range of components for the existence range of transparent emulsion region. Samples were prepared with 1:100 weight ratio of surfactant (egg lecithin) to cosurfactant (ethanol) (K_m=1:100) and ethanol was then evaporated. Pseudo-ternary phase diagram is presented in Figure 2. The specific surfactant:cosurfactant weight ratio was chosen for the optimized formulation based on the pre-study conducted by the authors (28), (data not shown). The mixture of surfactant and cosurfactant was mixed with oil phase. Aqueous phase, which was formed of poloxamer 188 (8%) and water, was added drop by drop and stirred using magnetic stirrer until homogeneous dispersion was obtained. The end point of the titration was the point in which the solution becomes cloudy or turbid.

O/W type emulsion region was determined according to the HLB value of the surfactant pair (egg lecithin and poloxamer 188). The appropriate ratio of components was chosen for the optimized emulsion formulation (Table 1). The hydrophilic-lipophilic balance (HLB) value of the system was 16.9. The appropriate amount of kinetin was dissolved in the oil phase consisted of Miglyol 818, lecithin and ethanol. Oil phase was dispersed into the aqueous phase. The mixture was stirred for 30 minutes. Thus,

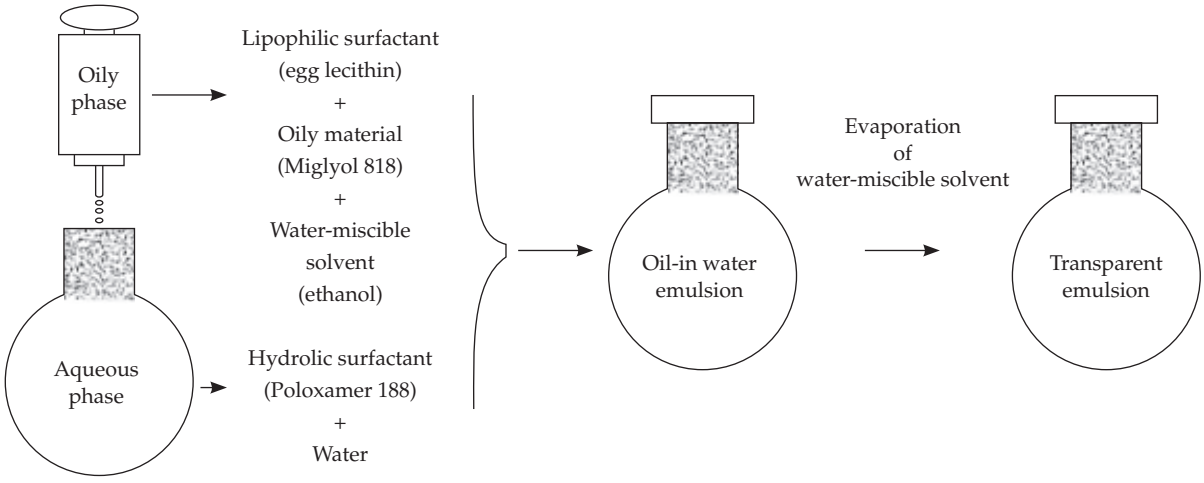


Figure 1. Schematic representation of emulsion solvent evaporation method (27).

a clear and transparent system was obtained. Ethanol in the formulation was later evaporated by vacuum evaporation at 40°C. This system was transparent, but unfortunately it became translucent due to coalescence after a few days. At the end of 7 days, a complete separation of the oil and aqueous phases was observed.

HPMC K15M gel (5% w/w) was used as the gel matrix to provide the stability of emulsion. Appropriate amount of HPMC K15M was dispersed in bidistilled water at 90°C. The dispersion was stirred 30 minutes at ambient temperature. The resulting gel was stored at 4°C in the dark. Emulsion formulation was mixed with equal amount of HPMC K15M gel (1:1, w/w) until a homogeneous emulsion gel was obtained.

Table 1. Composition of emulsion formulation and emulsion gel.

Kinetin	0.2 g
Miglyol 818	13 g
Lutrol F68	6.64 g
Lecithin	4 g
Distilled water	up to 100 mL
Absolute ethanol (evaporated later)	400g

Emulsion formulation containing 0.2% kinetin	10 g
HPMC gel (5%)	10 g

The final concentration of kinetin in emulsion gel was 0.1% (Table 1).

Preparation of liposome

Liposome dispersion containing kinetin (0.2%, w/w) were prepared by conventional film hydration method. PC:CH in mass ratio of 9:1 and kinetin were dissolved in chloroform in a round bottomed flask. The solvent was then removed by vacuum evaporation using rotary evaporator (Heidolph, Germany) at 40°C such that a thin lipid film was deposited on the wall of the flask. The lipid was resuspended in appropriate amount of phosphate buffer pH 7.4 solution by vigorous vortexing for 5 minutes and allowed to swell for 1 hour at room temperature (Table 2). Small bilamellar vesicles were obtained following the sonification of liposome dispersion for 15 minutes. pH value of obtained liposome dispersion was measured by a pH meter (CG 840, Germany). The liposome dispersion was kept at 4°C and protected from light, prior to use.

As a vehicle for liposome dispersion, Carbopol gel base (2%, w/w) was prepared by suspending appropriate amount of Carbopol 974 in bidistilled water, stirring for 4 h at room temperature. Then 10% (w/v) aqueous solution of sodium hydroxide was added up to pH 7.0 for gelification. The resulting gel was stored at 4°C in the dark. Liposome dispersion

Table 2. Composition of liposome formulation and liposome gel.

Kinetin	100 mg
Phosphatidylcholine	450 mg
Cholesterol	50 mg
Phosphate buffer (pH 7.4)	up to 50 mL
Chloroform (evaporated later)	50 mL

Liposome formulation containing 0.2% kinetin	10 mg
Carbopol gel (2%)	10 mg

was mixed with equal amount of Carbopol gel until a homogeneous liposome gel was obtained. The final concentration of kinetin in liposome gel was 0.1% (Table 2).

Droplet or vesicle sizes of emulsion and liposome

The average diameters of droplets for emulsion system and the average diameters of vesicles for liposome system were determined using laser diffraction particle sizer (Sympatec GmbH Laser Particle Sizer, Germany) at a temperature of 25±0.1°C. Samples were analyzed within the day of their preparation.

Images of emulsion and liposome

The existence of liquid crystalline phase for emulsion and the lamellarity of liposome vesicles were investigated by Leica DMEP Polarized Microscope system (USA) under 10x100 magnifications.

Rheological evaluation and pH values of emulsion and liposome gels

Rheological properties of emulsion gel and

liposome gel were measured at 25°C using a Brookfield RVIII + Rheometer (USA) with spindle no 52 with a shear rate interval from 1 to 18. pH values of systems were measured by a pH meter (CG 840, Germany).

Encapsulation of kinetin in liposome

The encapsulation efficiency of kinetin in liposome was determined on the basis of "free" no encapsulated portion of kinetin spectrophotometrically (λ=266 nm, Shimadzu UV 1700, USA) Liposome dispersion was centrifuged at 160.000 x g (4°C) for 120 minutes in a ultracentrifuge (HITACHI, Micro Ultracentrifuge GX series CS 150GXL, Japan). The supernatant was removed and analyzed for the determination of the amount of the unentrapped kinetin.

Drug release studies

Gel systems (emulsion gel, liposome gel, 2.5% HPMC K15M gel and 1% Carbopol gel) including 0.1% kinetin were used as samples for the donor compartment in the release study. Franz type diffusion cells with an active surface area of 3.8 cm^2 and a receptor phase volume of 15 mL were set at 32°C with magnetic stirring of the receptor compartment. The receptor compartment was filled with pH 5.2 phosphate buffer. The cellulose acetate membrane with molecular cut-off 12000 Dalton (D-0405, Sigma) was fixed in the diffusion cell. The membrane was soaked prior to use overnight in pH 5.2 phosphate buffer solution. The buffer solution in the receptor compartment was withdrawn at predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6 hours) and replenished with fresh buffer solution. The study was performed in triplicate replicates. The samples were analyzed spectrophotometrically (λ=266 nm, Shimadzu UV 1700, USA) for kinetin content.

The calibration graph of kinetin in pH 5.2 buffer was demonstrated to be linear (r^2=0.999) over the concentration range 0.5 – 10 µg/mL. The regression equation was as follows: y=11.5x-0.0378, where y is the concentration of kinetin (µg/mL) and x is the absorbance of kinetin. The limit of quantification of the method is 0.5 µg/mL. The recoveries of kinetin using spectrophotometric assay varied between 92.8% and 100%. The other components of liposome and emulsion formulations exhibit no significant absorbance at 266 nm.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). The models (zero order, first order and Higuchi models) were tested by fitting the release experimental data to the appropriate equations. The correlation was used as an indicator of goodness-of-fit of the equation to the experimental data. Release rates of kinetin from formulations were estimated. The significance of the differences between different formulations was tested using one-way analysis of variance (ANOVA). The differences were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Preparation of systems

The prepared emulsion was transparent and appeared like a homogenous single-phase liquid. No traces of undissolved kinetin or other solid ingredients were found in the formulation. The spontaneous emulsification process that was used to prepare kinetin loaded emulsion system is a simple method because it requires no energy input or heat application. System also does not contain organic solvent (29). Thus, the emulsion obtained by spontaneous emulsification process is very suitable for the preparation of cosmetic formulations. A study showed that the ratio of surfactant to cosurfactant as well as the amount and the type of oil were found to have an impact on the release and loading capacity of kinetin in the emulsion system (30). To produce a stable o/w type emulsion system, it is usually necessary to ensure the high viscosity of aqueous phase. The hydrophilic gelling polymer such as HPMC is a way of improving the long-term stability of the system. This offers the possibility of improving oil droplet resistance to coalescence during storage time. In general, the emulsion systems are known to enhance cutaneous absorption of both hydrophilic and hydrophobic active substances (31-33), which makes the emulsion gel as a promising vehicle for the topical application of kinetin.

Small unilamellar vesicles can be prepared by sonication of multilamellar vesicles (34). The modalities of performing the sonification process

can influence the vesicle particle size and homogeneity (35). Kinetin loaded small bilamellar vesicles were obtained following sonification of liposome dispersion composed of PC/CH at molar ratios of 9:1 at room temperature. The liposomes generally presented in gel system in order to ease for application onto the skin and to ensure physical stability. Thus, kinetin would be advantageous when entrapped in liposomes; since these drug delivery systems potentially improve moisturizing effect.

Characteristics of emulsion gel and liposome gel

The pH value of the emulsion gel and the liposome gel were 5.0±0.0 (n=3) and 7.0±0.0 (n=3), respectively. The encapsulation efficiency of kinetin for liposome was found to be 84.5±0.02%. The physicochemical properties of the drug, especially solubility and partition coefficient can be important determinants for the extent of its liposome incorporation. Therefore the high encapsulation efficiency of kinetin in liposome is due to the lipophilic nature of kinetin (logP=2.19±0.02) (n=3) (28). The result can be explained by considering that when kinetin is dissolved in the mixture of PC-CH, it will be located within the liposome bilayer, where the acyl chains of phospholipids provide a favorable environment for the lipophilic kinetin molecules. Theoretically, it is understandable that kinetin can achieve high entrapment efficiency because of its lipophilic character.

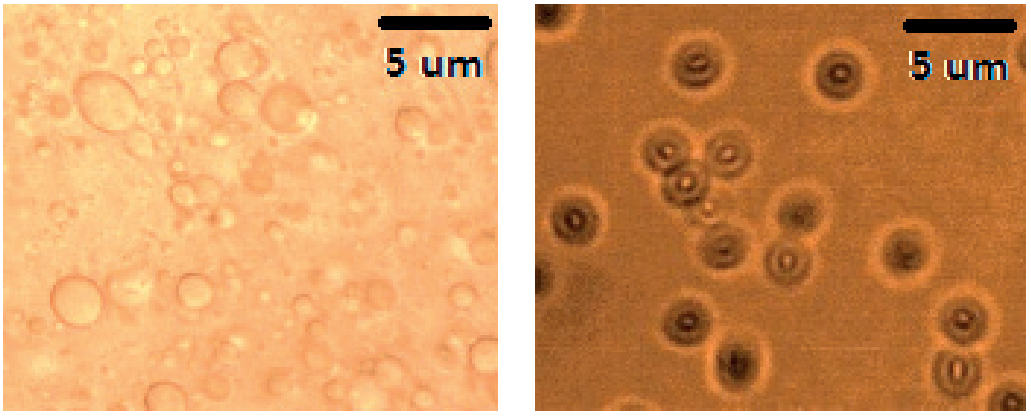
Droplet size of emulsion gel and vesicular size of liposome gel

Microscopic observations of the emulsion gel and liposome gel confirmed the formation of droplets and spherical vesicles respectively. The liquid crystalline structure for the emulsion gel did not exist in o/w transparent region (Figure 3). The type of liposome was determined as bilamellar using polarized microscope.

The droplet/vesicle size of emulsion and liposome gel formulations measured by laser diffraction particle sizer was 3.88±0.10 µm (mean±SD, n=3), 5.30±0.02 µm (mean±SD, n=3), respectively. There were no significant changes in droplet/vesicle size of the emulsion and the liposome by incorporation into gels ($p<0.05$).

Rheological evaluation of emulsion gel and liposome gel

Rheological behavior of topical formulations has a profound effect on the spreadability of the formulation, its retention and contact time on the skin surface (36-38). The appropriate viscosity is required for the application of topical formulations in humans. This can be achieved by their incorporation in a vehicle suitable for topical application. One of the limitations of conventional topical dosage forms on the skin is relatively short residence time of the drug at the site of application. As a controlled release and prolonged retention on the skin is often required for the desired effect, research efforts have been directed to using hydrophilic polymers to improve active substance delivery. The prepared liposome and emulsion formulations were incorporated in Carbopol and HPMC gels for improving the viscosity, respectively. The viscosities of emulsion gel, liposome gel, HPMC



(a) (b)

Figure 3. Images of emulsion gel (a) and liposome gel (b) (magnification 10x100)

K15M (2.5%) and Carbopol (1%) gels were shown in Table 3. Figure 4 represents the reograms of the emulsion gel and the liposome gel. The flow curves of both formulations revealed a Non-Newtonian flow behavior. The prepared gel formulations were exhibited shear-thinning property. As viscosity values of the emulsion gel and liposome gel formulations were similar at 4 s^{-1} of shear rate. Viscosity did not seem to have an effect on the difference between kinetin releases from the formulations.

Table 3. The viscosity values of the gel systems.

Formulation	Viscosity (mPa.s) (n=3) (Mean value±SD) (Shear rate= 4 s-1)
Emulsion gel	21569.30±0.42
Liposome gel	20227.90±0.00
HPMC gel (2.5%)	22563.80±0.00
Carbopol gel (1%)	54670.00±0.00

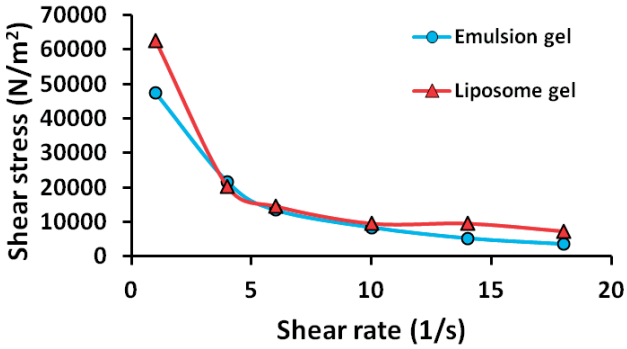


Figure 4. Reograms of emulsion gel and liposome gel.

Drug release studies using artificial membrane

The cumulative amount of kinetin release from gel systems during 6 hour are presented in Figure 5. The cumulative percentages of kinetin release from liposome gel were significantly lower than emulsion gel. The total amount of kinetin release from emulsion gel, liposome gel, Carbopol gel (1%) and HPMC gel (2.5%) were 10.7, 1.53, 13.2, and 85.8 %, respectively. The obtained experimental data were analyzed using Higuchi matrix kinetic model (Table 4).

It can be observed that liposome gel formulation

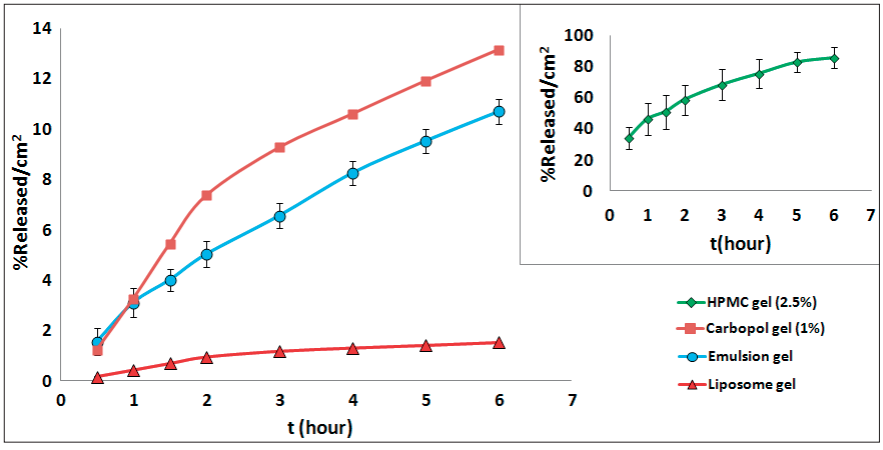


Figure 5. Comparative release of kinetin from gel systems.

Table 4. Release parameters of kinetin from the gel systems.

		Emulsion gel	Liposome gel	1% Carbopol gel	2.5% HPMC gel
Higuchi model	Slope (released %1/cm²/h)	5.26	1.68	6.83	29.8
	Intersection (released %/cm²)	–2.29	–0.626	–3.12	15.5
	r^2	0.998	0.981	0.986	0.992
	Standart error	0.157	0.712	0.537	1.88
Released %/cm² at 6th hour		10.7±0.511	1.53+0.0193	13.2±0.0322	85.8±11.6

showed an initial moderate release phase followed after 2-6 h by a plateau. The partitioning of kinetin from the lipid bilayer into the phosphate buffer solution is not thermodynamically favorable. Being a lipophilic molecule, kinetin can be incorporated within the lipophilic phase. This can be considered as the principal factor controlling the kinetin release rate from the vesicles. Thus, the release rate of kinetin from liposome gel was dependent on its lipophilicity.

Release of kinetin from liposomes embedded into the Carbopol gel base was significantly slower (1.53% released within 6 h) than the release from Carbopol gel (13.2% within 6 h), which confirmed that the encapsulation of kinetin into liposomes resulted in a prolonged kinetin release rate. At 6^{th} hour, the cumulative release of kinetin from emulsion gel was 7 fold higher (Q_6= 10.7%) than from liposome gel (Q_6= 1.53%) (Figure 5). The significant difference in kinetin release between emulsion gel and liposome gel was probably due to the solubility of kinetin in the formulations. In the o/w-type emulsion gel, the dissolved kinetin molecules moved from oil droplets to the surrounding aqueous phase and were released through the hydrophilic membrane easily. On the other hand, kinetin molecules were entrapped between the bilamellar structures and could not move to the surrounding aqueous phase from liposome gel. The higher permeability rate of kinetin from the emulsion formulation may be observed because of the surfactants and the oil phase which act as penetration enhancers (39).

The plots of the cumulative amount of kinetin released from emulsion gel and liposome gel against the square root of time ($h^{1/2}$) was found to be linear (coefficients of determination (r^2) are 0.998 and 0.981, respectively) indicating a direct relationship between this factor. This implies that kinetin release is governed by a passive (Fickian) diffusion mechanism, which is described at the Higuchi diffusion model.

Although in vitro release profile of kinetin from liposome gel was lower than emulsion gel, liposome gel may enhance kinetin penetration into the skin through several mechanisms of vesicle-skin interactions described in the literature (40-42). In the case of drugs that should act topically, liposome-based formulations are known to provide a localized and controlled delivery, acting as a reservoir (43, 44). Therefore, it is possible to use the liposome formulation as local depot for the sustained release of incorporated kinetin over a prolonged period of time.

The release study was performed through artificial membrane in this study. However, it is known that the synthetic membranes can be used for assessing product performance in quality assurance but give little indication of its performance in vivo. Therefore, in vivo studies are needed to evaluate the actual skin accumulation and penetration properties of kinetin from the examined formulations.

CONCLUSION

In conclusion, topical kinetin formulations prepared as conventional pharmaceutical cream and lotions may not deliver kinetin through intact skin due to the barrier function of the stratum corneum effectively. This study suggests that the developed emulsion and liposome gels containing kinetin could perform therapeutically better effects than the conventional formulations. The prolonged and controlled release topical dosage forms may lead to improve efficiency, acting as a drug reservoir for continuous drug delivery. However, this emulsion system can be further homogenized with a high pressure homogenizer to achieve NEs of extremely low droplet size. The relevance of this study needs further in vivo investigation to extrapolate these results to human condition.

REFERENCES

1. Kaminek M. Progress in cytokinin research. *Trends Biotechnol* 10:159-164, 1992.
2. Binns AN. Cytokinin accumulation and action: biochemical, genetic, and molecular approaches. *Annu Rev Plant Physiol Plant Mol Biol* 45: 173-196, 1994.
3. Gan S, Amasino RM. Cytokinins in plant senescence: From spray and pray to clone and play. *BioEssays* 18: 557-565, 1996.
4. Kaminek M, Motyka V, Vankova R. Regulation of cytokinin content in plant cells. *Physiol Plant* 101:

689-700, 1997.
5. Barciszewski J, Massino F, Clark BFC. Kinetin-a multiactivate molecule. *Int J Biol Macromolecules* 40: 182-192, 2007.
6. Rattan SIS, Clark BFC. Kinetin delays the onset of ageing characteristics in humans fibroblasts. *Biochem Biophys Res Commun* 201: 665-672, 1994.
7. Barciszewski J, Rattan SIS, Siboska G, Clark BFC. Kinetin-45 years on. *Plant Science* 148: 37-45, 1999.
8. McCullough JL, Weinstein GD. Clinical study of safety and efficacy of using topical kinetin 0.1% (Kinerase) to treat photodamaged skin. *Cosmet Dermatol* 15: 29-32, 2002.
9. Chiu PC, Chan CC, Lin HM, Chiu HC. The clinical anti-aging effects of topical kinetin and niacinamide in Asians: a randomized, double-blind, placebo-controlled, split-face comparative trial. *J Cosmet Dermatol* 6:243-249, 2007.
10. Holck DE, Ng JD. Facial skin rejuvenation. *Curr Opin Ophthalmol* 14: 246-252, 2003.
11. Dragicevic-Curic N, Winter S, Stupar M, Milic J, Krajišnik D, Gitter B, Fahr A. Temoporfin-loaded liposomal gels: viscoelastic properties and in vitro skin penetration. *Int J Pharm* 373: 77-84, 2009.
12. Zhu W, Guo C, Yu A, Gao Y, Cao F, Zhai G. Microemulsion-based hydrogel formulation of penciclovir for topical delivery. *Int J Pharm* 378: 152-158, 2009.
13. Lee W-C, Tsai T-H. Preparation and characterization of liposomal coenzyme Q10 for in vivo topical application. *Int J Pharm* 395: 78-83, 2010.
14. Patel MR, Patel RB, Parikh JR, Bhatt KK, Solanki AB. Investigating the effect of vehicle on in vitro skin permeation of ketoconazole applied in O/W microemulsions. *Acta Pharmaceutica Sciencia* 52: 65-77, 2010.
15. Sznitowska M, Zurowska-Pryczkowska K, Dabrowska E, Janicki S. Increased partitioning of pilocarpine to the oily phase of submicron emulsion does not result in improved ocular bioavailability. *Int J Pharm* 202 (1-2): 161-164, 2000.
16. Fang J-Y, Leu YL, Chang CC, Lin CH, Tsai YH. Lipid nano/submicron emulsions as vehicles for topical flurbiprofen delivery. *Drug Deliv* 11: 97-105, 2004.
17. Subramanian B, Kuo F, Ada E, Kotyla T, Wilson T, Yoganathan S, Nicolosi R. Enhancement of anti-inflammatory property of aspirin in mice by a nano-emulsion preparation. *Int Immunopharmacol* 8 (11): 1533-1539, 2008.
18. Fernandez P, Andre V, Rieger J, Kühnle A. Nano-emulsion formation by emulsion phase inversion. *Colloid Surface A* 251: 53-58, 2004.
19. Calvo P, Vila-Jato JL, Alonso MJ. Comparative in vitro evaluation of several colloidal systems, nanoparticles, nanocapsules and nanoemulsions, as ocular drug carriers. *J Pharm Sci* 85: 530-536, 1996.
20. Bouchemal K, Briançon S, Perrier E, Fessi H. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization. *Int J Pharm* 280: 241-251, 2004.
21. Egbaria K, Weiner N. Liposomes as a topical drug delivery system. *Adv Drug Deliv Rev* 5: 287-300, 1990.
22. Lasic D. Liposomes. *American Sci* 80: 20-31, 1992.
23. El-Ridy MS, Khalil RM. Free versus liposome-encapsulated lignocaine hydrochloride topical application. *Pharmazie* 54: 682-684, 1999.
24. Kirjavainen M, Urtti A, Jääskeläinen I, Suhonen TM, Paronen P, Valjakka-Koskela R, Kiesvaara J, Mönkkönen J. Interaction of liposomes with human skin in vitro-the influence of lipid composition and structure. *Biochim Biophys Acta* 1304: 179-189, 1996.
25. Coderch L, Pera M de, Perez-Cullell N, Estelrich J, Maza A de la, Parra JL. The effect of liposomes on skin barrier structure. *Skin Pharmacol App Skin Physiol* 12: 235-246, 1999.
26. Muramatsu K, Maitani Y, Takayama K, Nagai T. The relationship between the rigidity of the liposomal membrane and the absorption of insulin after nasal administration of liposomes modified with an enhancer containing insulin in rabbits. *Drug Dev Ind Pharm* 25: 1099-1105, 1999.
27. Tırnaksız F, Akkuş Ş, Çelebi N. Nanoemulsions as drug delivery systems. In: Fanun M, editor. Colloids in drug delivery. FL-USA: CRC Press; 2010. p. 221-45.
28. Akkuş Ş. Development of nanoemulsion system containing kinetin for cosmetic usage. [Dissertation]. Ankara (Turkey): Gazi University Institude of ealth Sciences, 2007.
29. Kelmann RG, Kuminek G, Teixeira HF, Koester LS.

Carmazepine parenteral nanoemulsion prepared by spontaneous emulsification process. *Int J Pharm* 342: 231-239, 2007.

30. Akkuş Ş, Tırnaksız F, Değim T. Development of o/w emulsions gelled containing kinetin with spontaneous emulsification method. Proceed. 13th International Pharmaceutical Technology Symposium (IPTS), Antalya-Türkiye. 10-13 Eylül 2006. Poster No: PP-1-ET12SEP
31. Sintov AC, Shapiro L. New microemulsion vehicle facilitates percutaneous penetration in vitro and cutaneous drug bioavailability in vivo. *J Control Rel* 95: 173-183, 2004.
32. Kogan A, Garti N. Microemulsions as transdermal drug delivery vehicles. *Adv Colloid Interface Sci* 123-126: 369-385, 2006.
33. Xie Y, Ye R, Liu H. Microstructure studies on biosurfactant-rhamnolipid/n-butanol/water/n-heptane microemulsion system. *Colloid Surface A* 292: 189-195, 2007.
34. Mura P, Maestrelli F, González-Rodríguez ML, Michelacci I, Ghelardini C, Rabasco AM. Development, characterization and in vivo evaluation of benzocaine-loaded liposomes. *Eur J Pharm Biopharm* 67: 86-95, 2007.
35. Müller M, Mackeben S, Müller-Goymann CC. Physicochemical characterisation of liposomes with encapsulated local anaesthetics. *Int J Pharm* 274: 139-148, 2004.
36. Ramachandran S, Chen S, Etzler F. Rheological characterization of hydroxypropyl cellulose gels. *Drug Dev Ind Pharm* 25: 153-161, 1999.
37. Mortazavi SA, Tabandeh H. The influence of various silicones on the rheological parameters of AZG containing silicone-based gels. *Iran J Pharm Res* 4: 205-211, 2005.
38. Lee CH, Moturi V, Lee Y. Thixotropic property in pharmaceutical formulations. *J Control Release* 136: 88-98, 2009.
39. Lawrence MJ, Rees GD. Microemulsion-based media as noved drug delivery systems. *Adv Drug Deliv Rev* 45: 89-121, 2000.
40. Cevc G, Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. *Biochim Biophys Acta* 1104: 226-232, 1992.
41. Cevc G, Schätzlein A, Blume G. Transdermal drug carriers: basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. *J Control Release* 36: 3-16, 1995.
42. Vrhovnik K, Kristl J, Sentjurc M, Smid-Korbar J. Influence of liposome bilayer fluidity on the transport of encapsulated substance into the skin, studied by EPR. *Pharm Res* 15: 525-530, 1998.
43. Perugini P, Genta I, Pavanetto F, Conti B, Scalia S, Baruffini A. Study on glycolic acid delivery by liposomes and microspheres. *Int J Pharm* 196: 51-61, 2000.
44. Glavas-Dodov M, Goracinova K, Mladenovska K, Fredro-Kumbaradzi E. Release profile of lidocaine HCl from topical liposomal gel formulation. *Int J Pharm* 242: 381-384, 2002.

ACKNOWLEDGEMENT

This research is financially supported by a grant from research fund of Gazi University (code: 02/2006-07) and by a grant from Novartis/Turkey (2005).