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TOPICAL EMULSIONS STABILIZED BY SILICA NANOPARTICLES: *IN VITRO* RELEASE AND ANTI-INFLAMMATORY STUDIES OF FLURBIPROFEN AND DICLOFENAC SODIUM

Hatem Sarhan[1], Mohamed A. Ibrahim[2*], Mohamed A. Amin[2] and Amro K. F. Dyab[3]

[1]*Department of Pharmaceutics, Faculty of Pharmacy, Minia University, Minia 61519, Egypt*
[2]*Department of Pharmaceutics, Faculty of Pharmacy, Al-Azhar University, Assuit, Egypt*
[3]*Department of Chemistry, Faculty of Science, Minia University, Minia 61519, Egypt*

تناولت هذه الدراسة تحضير المستحلب البسيط (زيت/ ماء) و المستحلب العديد (ماء/ زيت/ ماء) باستخدام نوعين من الزيت هما زيتي الخروع أو البارافين. ولضمان ثبات هذه المستحلبات، تم اضافة جزيئات السيليكا الغروية ذات الخصائص السطحية المحكمة.كما تم دراسة ثبات تلك المستحلبات المحتوية على عقاري فلوربيبروفين في الاملجل المحضر من المستحلب البسيط (زيت/ ماء) و عقار ديكلوفيناك الصوديوم في المستحلب العديد (ماء/ زيت/ ماء) كأحد مضادات الالتهاب غير الاستيرويدية. كما تم ايضا دراسة الانطلاق المعملى للعقارين من تلك المستحلبات. وقد تناولت الدراسة كذلك التقييم الحيوى للعقارين كمضادات الالتهاب من المستحلبات المحضرة باستخدام اختبار تورم القدم فى الفئران الناتج عن حقن الكاراجينين. وقد أظهرت النتائج أن المستحلبات تم تحسين ثباتها باضافة جزيئات السيليكا الغروية. كما كانت معدلات الانطلاق المعملى لعقار فلوربيبروفين من الاملجل وديكلوفيناك الصوديوم في المستحلب العديد المحضر باستخدام زيت البارافين قيما اعلى من تلك التي تم تحضيرها باستخدام زيت الخروع. كما أظهرت الدراسة تأثير تركيز الزيت على الانطلاق المعملى للعقار من تلك المستحلبات. بالاضافة الى أن المستحلبات المحضرة والمحتوية على عقاري فلوربيبروفين في الاملجل وديكلوفيناك الصوديوم في المستحلب العديد قد اعطت أفضل النتائج كمضادات للالتهاب باستخدام اختبار تورم القدم فى الفئران الناتج عن حقن الكاراجينين، وأن هذه الصبغ الصيدلية تميزت بأن لها تأثير طويل المفعول إذا ما قورنت بالدواء الذي تم تعاطيه عن طريق الفم.

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*Correspondance author e-mail address: abbma71@yahoo.com

Simple and multiple emulsions have a wide range of pharmaceutical applications. Therefore, the stabilization of such emulsions is a challenge to ensure a stable formulation along the period of storage, usage and at the same time to conserve the efficacy of the incorporated medicament. Simple o/w and multiple w/o/w emulsions were prepared using castor and paraffin oils as oil phases and stabilized solely by silica nanoparticles of well-controlled surface properties. Two non-steroidal ant-inflammatory drugs, namely flurbiprofen and diclofenac sodium were incorporated in the stabilized simple and multiple emulsions, respectively. The stability of emulsions and the in vitro release of the drugs from the prepared emulsions were studied. In addition, the anti-inflammatory activity of the drugs from these liquid formulations was assessed using carageenan-induced hindpaw edema in rats. The results indicated that the prepared liquid emulsions, which stabilized with silica nanoparticles, were highly stable. The in vitro release of flurbiprofen and diclofenac sodium from these simple and multiple emulsions showed higher rates compared with those preapred from paraffin oil due to their lower viscosities. The results revealed also that the percentage of oil has a pronounced effect on the in vitro release rates of the drugs from the emulsions. Furthermore, topical flurbiprofen and diclofenac sodium emulsions exhibited a potent local anti-inflammatory activity compared with the orally adminstered drugs in the suspension form and this activity reached its peak (57-84%) 3 hrs after carrageenan injection and persisted for 5 hrs, the period of study.

INTRODUCTION

Simple and multiple emulsions are vesicular and complex systems[1]. Multiple emulsions (w/o/w) consist of smaller aqueous droplets separated from the outer aqueous phase by an oily phase layer. The presence of at least two surfactants is required. One of them is predominantly lipophilic for stabilizing the primary w/o emulsion and the other is hydrophilic for the secondary o/w emulsion. However, the application of multiple emulsions has been limited because of thermodynamic instability and their complex structure.

The stability and release characteristics of drugs from these emulsions are influenced by different factors such as surfactant type, surfactant ratio and certain physical properties of the system (globule size, viscosity, conductivity, phase volume ratio, etc)[1-3]. The role of finely divided insoluble solid particles in stabilizing emulsions is now well-established[4]. Particles of colloidal

dimensions that are wetted more by water than by oil can act as an emulsifying agent for o/w emulsions by residing at the oil/ water interface. However, several years earlier, it was concluded that the stability of many emulsions may be attributed, in part, to the presence of solids or highly viscous matters at the interface of the two liquids[5]. Since then, several investigators have studied the stability of solid-stabilized emulsions. These selective particulate emulsifiers offer a number of potential advantages over conventional surfactants such as imparting improved stability against coalescence and a reduced rate and extent of creaming/sedimentation owing to the enhanced viscosity of the continuous phase[5]. Inorganic particles such as silica, carbon black, barium sulfate, and calcium carbonate have been widely used as particulate emulsifiers. Solid particles have been formulated in emulsions for many years, including those of the food, paint, agrochemical, and pharmaceutical and oil industries. Their presence is thought to contribute to enhanced stability by certain mechanisms.

Certain emulsions have been formulated as cosmetics such as skin moisturizer[6-8]. Also, they have potential pharmaceutical applications including taste masking, adjuvant vaccines, an immobilization of enzymes, sorbent reservoir of over dose treatments, and for enhancement of interal or dermal absorption[9-12]. Furthermore, a prolonged release from diffrerent drug delivery systems of drugs can also be obtained by means of formulating multiple structure emulsions[13-16]. These systems, in addition to their applications in controlling the drug release rates, have certain advantages, such as the protection of entrapped substance from hydrolysis or oxidation in the continuous phase[17&18].

The purpose of this study was: i) to investigate the usage of silica for stabilizing simple o/w and w/o/w multiple emulsion formulations, ii) to study the influence of the type and the percentage of oil phase on the release properties of flurbiprofen and diclofenac sodium from such emulsion formulations and iii) to evlauate the anti-inflammatory activity of the drugs from these emulsion systems.

EXPERIMENTAL

Materials

- Standard cellophane membrane, molecular weight cut off ≈ 12,000 (Sigma chemical Co., USA).
- Diclofenac sodium (Dic) was kindly supplied by El-Nasr Co., Abu-Zabal, Egypt.
- Flurbiprofen (Flu) was kindly supplied by Kahira Pharmaceutical Co., Cairo, Egypt.
- Castor oil and paraffin oil (El-Nasr Co., Abu-Zabal, Egypt).
- Carrageenin (Sigma chemical Co., USA).
- Urethane (Sigma chemical Co., USA).

- Carbopol 941, (Goodrich chemical Co., England).
- Albino rats of both sexes, weighing 90-120 gm were obtained from the animal house, Assiut University.

- **Silica particles**

The amorphous fumed silica powders were a gift from Wacker-Chemie (Munich, Germany) with primary particle diameters quoted of between 5 and 35 nm, which can aggregate into larger units of 100 nm in diameter due to the formation of hydrogen bonds between the silanol groups on adjacent particles. The hydrophilic silica (100% SiOH) is prepared by flame hydrolysis of silicon tetrachloride in a hydrogen/oxygen flame at about 1200 ºC. The silica is hydrophilic due to the silanol groups (SiOH) present on its surface. The surface also contains siloxane bridges (Si-O-Si). The hydrophobic silica powders were prepared by Wacker-Chemie using the hydrophilic silica (100% SiOH) with surface area of 200 m^2 g-1 and reacting with dichlorodimethylsilane (DCDMS) reagent to varying extents in the presence of water followed by drying at 300°C for 2 hours according to the reaction described by the authers[1&2].

This silanisation process gives a desired density of silanol groups (SiOH) on the silica surface. For example, hydrophobic silica (50% SiOH) has a surface density of silanol groups of around 1/nm^2, i.e., half of the surface contains silanol (SiOH) and half contains Si-O-Si$(CH_3)_2$ groups. The hydrophilic particles SLM contain 83% SiOH.

Equipment

- Shaking water bath thermostatically controlled (Kötterman Labortechnik, Germany).
- Centrifuge (Merlin-502, England).
- Thermostatically controlled water bath (Julabo TWB1411, Julabo Labortechnik GMBH, Germany).
- Janke and Kunkel Ultra Turrax T25 homogenizer (rotor-stator), IKA-Labortechnik, Staufen, Germany)
- Vernier caliper (Shanghai, China).
- Nikon Labophot microscope fitted with a DIC-U camera (Walt Whitman Road Melville, NY, U.S.A.).
- UV Spectrophotometer (UV. 1601, Shimadzu Co., Japan).

Methods

Preparation of colloidal dispersions

The various types of silica particles were prepared by dispersing a known weight of powder (expressed in wt.% of the dispersion medium) into a known volume of the desired liquid using a high intensity ultrasonic vibracell processor (Sonics & Materials) with tip diameter 3 mm operating at 20 KHz and up to 10 W for 2 mins. The resulting dispersions were colourless or bluish in appearance.

Preparation of Flu emulgel

The composition of the tested simple o/w emulgel formulations containing Flu is listed in Table 1. A typical simple o/w emulsion in which

the volume fraction of oil (ϕo) equals 0.2 and 0.5 was prepared by adding 2.0 or 0.5 gm of castor or paraffin oil into 8 or 5 gm of water, respectively containing 1% hydrophilic silica particles and 1% carbopol 941. Flu (1% weight per total emulsion weight) was incorporated into the oil phase. The mixture was then homogenized with an 18 mm head operating at 6000 rpm for 2 mins. The head was immersed in the liquid mixture so that when the rotor blades turn, the liquids were forced between the slits in the stator. Uniform homogenization was achieved by moving the container vertically. The emulsion type was inferred by observing what happened when a drop of each emulsion was added to a volume of either oil or water alone. Water continuous (oil continuous) emulsions dispersed in water (oil) and remained as drops in oil (water). Carbopol 941 was then neutralized by sodium hydroxide (0.4 gm sodium hydroxide/ 1 gm carbopol) to get the emulgel.

Preparation of multiple w/o/w emulsion

A typical primary w/o emulsion in which the volume fraction of water (ϕw) equals 0.2 (as described in Table 2) was prepared by adding 2.0 gm of water containing Dic (1% weight per total emulsion weight) and also contained 0.01 M sodium chloride into 8 gm of castor or paraffin oil containing 1% of hydrophobic silica particles. The mixture was then homogenized as mentioned in the previous section.

In the second stage, the w/o/w multiple emulsions were prepared by re-homogenizing the primary w/o emulsion (ϕw/o = 0.2) into the outer aqueous phase containing 2% hydrophilic silica particles and the 0.0183 M concentration of D-glucose to maintain the osmotic pressure caused by the salt in the inner water drops of the multiple emulsions to load the inner water phase with 0.01 M sodium chloride the outer aqueous phase should contain 0.0183 M D-glucose to give an osmolality of 0.0185 Os kg^{-1}.

Table 1: Composition of the tested simple o/w emulgel formulations containing Flurbiprofen.

Ingredients	Weight % of ingredients used in preparing the following formulae			
	F1	*F2*	*F3*	*F4*
Flurbiprofen (Flu)	1	1	1	1
Paraffin oil	20 (ϕ o = 0.2)	50 (ϕ o = 0.5)	-	-
Castor oil	-	-	20 (ϕ o = 0.2)	50 (ϕ o = 0.5)
Carbopol 941	1	1	1	1
Hydrophilic silica (SiOH 85%)	1	1	1	1
Water to	100	100	100	100

Table 2: Composition of the tested Simple w/o/w multiple emulsion formulations containing Diclofenac sodium.

Ingredients	Weight % of ingredients used in preparing the following formulae	
	F5	*F6*
1- Primary w/o Emulsion		
Diclofenac sodium (Dic)	1	1
Water	20*	20 *
Hydrophobic silica (SiOH 50%)	1	1
Paraffin oil	80	-
Castor oil	-	80
2- Secondary w/o/w Emulsion		
Primary w/o Emulsion	20(ϕ w/o = 0.2)	20(ϕ w/o = 0.2)
Hydrophilic silica (SiOH 85%)	2	2
Water to	100	100

Water phase volume (ϕ w) = 0.2.

Microscopic examination of the prepared emulsion systems

Optical micrographs were taken to characterize the prepared emulsions and to compare the droplet size distribution obtained by light diffraction method. The method involved adding a small sample of the emulsion, diluted with the continuous phase in the ratio 2:1, to a glass slide with a dimple in the middle and covering with a cover slip. The samples were viewed at 4X, 10X, 40X and 100X magnification with a Nikon Labophot microscope fitted with a DIC-U camera (World Precision Instruments).

***In vitro* release studie**

The release of Dic and Flu from the tested emulsuions was followed through a semi-permeable cellophane membrane in saline buffer at 37± 0.5°C. The membrane was cut into 4 x 4 cm pieces and soaked in distellid water for 24 hr just before its use in the release study. The water was removed from the membrane by pressing it between two filter papers. One gram of the emulsion, in question, was weighed over the center of the membrane. The membrane was placed over the lower end of the dialysis tube by means of a cotton theard. The upper part of the tube was coverd with a thin perforated nylon membrane, so as to minimize the evaporation of the liquids, which was incarporated in the base.

The assembly was placed in a glass vessel containing 20 ml of phosphate buffer saline (pH 7.4) and then submerged in a water-bath shaker previously adjusted to 37°C and 50 strokes/min. At predetermined time intervals, one ml sample was withdrawn and the amount of the drug release into the buffer was determend spectrophotometerically at 277 nm and 247 for Dic and Flu,

respectively after apprioparte dilution with the buffer. It should be noted that, at each sampling time, one ml of saline buffer, at the same temprature, was added to the medium to keep the volume constant.

The anti-inflammatory activity of Flurbiprofen and Diclofenac formulated in emulsions

The anti-inflammatory activity of diclfenac sodium and flurbiprofen multiple emulsions was evaluated using carrageenin-induced paw edema model[19-25]. The experiment was conducted on 50 albino rats of both sexes weighing 90-120 gm fasted for 18 hr with free access to water. They were equally and randomly allocated in 10 groups. The rats were anesthetized with urethane (0.5 ml intraperitonial) and the topical formulations; 100 mg of the tested formulation were applied to the surface of the right hind paw. 0.1 ml of 1% w/v carrageenin physiologic solution was injected subcutaneously into the treated area one hour after the treatment. The treated area was immediately covered by thin vinyl sheet and gauze. One hour latter, the covers were removed and the edema thickness was measured by the micrometer at suitable time intervals (1, 2, 3, 4 and 5 hrs). The percentage of edema inhibition was calculated as follow[26]:

$$\% \ Inhibition = \frac{T_o - T_t}{T_o} \times 100$$

where T_0 and T_t are the average edema thickness (mm) of the control and the tested groups, respectively. The time course of the anti-edemal effect either oral or topical of the following treatments was determined: control group or no medication (group 1), oral Dic suspension (group 2), oral Flu suspension [5 mg/Kg in 1% carboxy methylcellulose for Dic and Flu given by an oesophageal tube] (group 3), topical non-mediacted emulgel prepared using (group 4, 100 mg), 1% topical Flu emulgel prepared using paraffin oil castor oil, *F1* (Φo= 0.2) (group 5, 100 mg), 1% topical Flu emulgel prepared using paraffin oil, *F2* (Φo= 0.5) (group 6, 100 mg), 1% topical Flu emulgel prepared using castor oil, *F3* (Φo= 0.2) (group 7 100 mg), 1% topical Flu emulgel prepared using castor oil, *F4* (Φo= 0.5) (group 8 100 mg), topical non-mediacted w/o/w emulsion (group 9, 100 mg) 1% topical Dic w/o/w emulsion prepared using paraffin oil (Φo= 0.2), *F5* (group 10 100 mg) and 1% topical Dic w/o/w emulsion prepared using castor oil (Φo= 0.2), *F6* (group 11 100 mg).

Statistical Analysis

Results were expressed as mean ± SEM. The results were analyzed for statistically significant differences using one-way ANOVA, followed by the Dunnett's post- test. *P values < 0.01 were considered significant. Groups (1-2) were compared to oral free suspension (a), groups (3-6) were compared to free emulgel base (b),

Groups (7-8) were compared to free w/o/w base (c). GraphPad Prism was used for statistical calculations (Version 4 for Windows, GraphPad Software, San Diego, Calif., USA).

RESULTS

Miocroscopical examinations

Microscopical examinations of the prepared simple w/o and multiple w/o/w emulsions using paraffin and castor oils taken after 10 days after manufacture are shown in Figure 1 (A&B). The images indicate that 1% of SiOH (50%) and 2% of SiOH (85%) are optimum for the stabilization of the w/o and w/o/w emulsions respectively. In addition, the figure reveals that after 10 days of the preparation, the emulsions were stabilized by silica particles as indicated from the homogeneous dispersion of the internal phases.

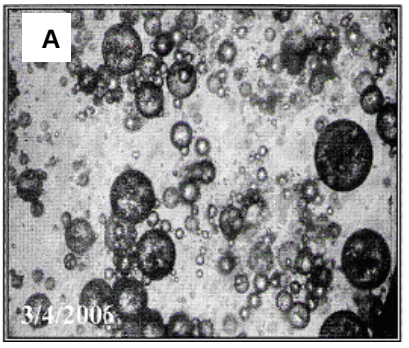

Fig. 1A: Optical micrograph of water-in castor oil-in-water multiple emulsion (w/o/w) stabilized by 1% of 50% SiOH silica particles in castor oil $\phi_{w/o} = 0.2$ and 2% of 83% silica particles in the water, $\phi_{w/o} = 0.2$.

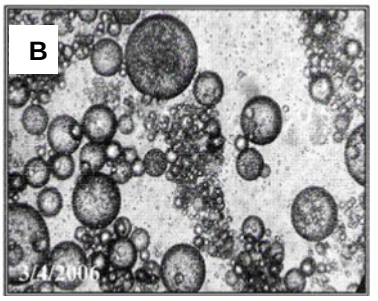

Fig. 1B: Optical micrograph of water-in paraffin oil-in-water multiple emulsion (w/o/w) stabilized by 1% of 50% SiOH silica paticles in paraffin oil $\phi_{w/o} = 0.2$ and 2% of 83% silica particles in the water, $\phi_{w/o} = 0.2$.

Release profiles from emulsion bases

The *in vitro* release rate of Flu from single emulsion formulations prepared by castor or paraffin oils and stabilized by silica nanoparticles is shown in Figures 2 & 3. Changing the volume of the oily phase controls the release rate of Flu from such emulsion formulations, i.e., increasing the percentage of the oily phase is accompanied by slowing the release rate of the incorporated Flu from such vehicles. For example, after 45 min., 40% of Flu was released from the emulsion prepared using 50% castor oil, while about 51% of the incorporated drug was released using 20% after the same period, Figure 2. The oil type has a significant role in controlling the release rate of Flu from the tested emulsion in which the emulsions prepared based on paraffin oil showed higher release rates than those manu-factured using castor oil, Figure 3.

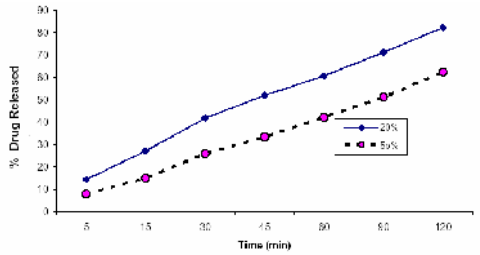

Fig. 2: Release profile of Flu from emulgel containing 20% and 50% paraffin oil stabilized by 1 wt% of 50 % SiOH silica particles in the oil phase.

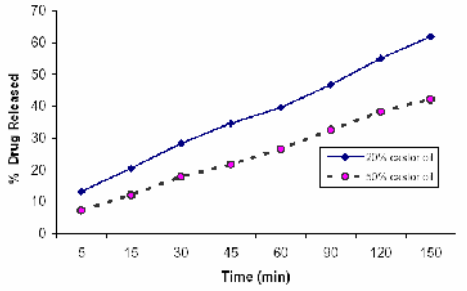

Fig. 3: Release profile of Flu from emulgel containing 20% and 50% castor oil stabilized by 1 wt% of 50 % SiOH silica particles in the oil phase.

Similarly, the *in vitro* release of Dic supports this finding, Figure 4, in which the oil type affect the release from the prepared multiple emulsion. This could be explained on the basis that the emulsions formulated from castor oil exhibit lower viscosities than those prepared using paraffin oil.

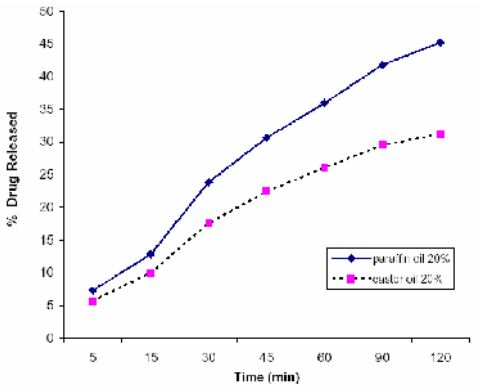

Fig. 4: Release profile of Dic from w/o/w multiple emulsions (prepared using castor and paraffin oils and stabilized by 1 wt% of 50% SiOH silica particles in the oil phase for the primary, ϕ_w = 0.2 and 2 wt% of 83% silica particles in the water, $\phi_{w/o}$ = 0.2).

The anti-inflammatory activity of Flurbiprofen and Diclofenac sodium from emulsion formulations

Emulgel and w/o/w emulsion formulations containing 1% w/w Flu or Dic, respectively, stabilized silica nanoparticles were studied for their anti-inflammatory activity via the carrageenin-induced edema method and the results were surveyed in Figures 5& 6 and listed in Table 3.

The edema swelling was significantly inhibited in all groups of rats either treated with the tested emulsion formulations or the group received the drug oral suspension. For example, at 4 hrs post carrageenin injection, the rats treated with the Dic multiple emulsion stabilized by

colloidal silica exhibited percentage of edema inhibition of 53.7% and 47.5% for the emulsions prepared from castor and paraffin oils, respectively. This is significantly different from the control group (group 1) and numerically close to that recorded in the group received Dic oral suspension (group 2) in which the percentage of edema inhibition was 64.7%. Furthermore, the rats treated with the the Flu emulgel stabilized by colloidal silica and prepared by castor and paraffin oils showed percentage of edema inhibition of 52.9 and 58.1%, respectively which is also significantly different from the control group. Moreover, the anti-inflammatory activity of all these emulsion formulations containing either Flu or Dic persisted for 5 hr, the end of the testing period, Table 3.

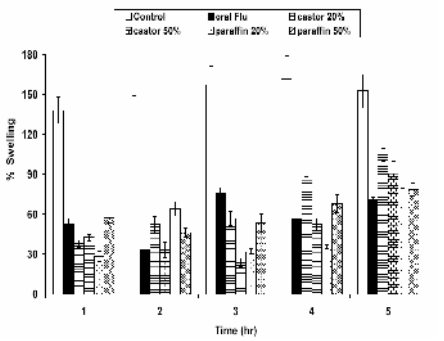

Fig. 5: Effect of Flu formulations on reducing the swelling of rat hind paw induced by carrageenin.

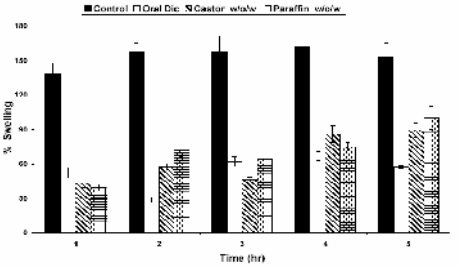

Fig. 6: Effect of Dic formulations on reducing the swelling of rat hind paw induced by carrageenin

Table 3: Anti-inflammatory activity of diclofenac sodium and flurbiprofen formulations using the carrageenan-induced rat paw oedema test.

Formulations	Anti-inflammatory effect (%) after				
	1 hr	2 hr	3 hr	4 hr	5 hr
Oral free suspension	7.03±1.8	8.8± 1.01	6.6±1.5	5.1±1.3	5.9±2.2
Oral Dic suspension, gp 2	62.03**±11.8	81.8**± 9.01	60.6**±10.5	55.1**±17.3	69.1**±20.2
Oral Flu suspension, gp 3	62.03**±11.8	78.8**±5.3	57.4**±10.4	64.7**±8.8	53.01**±1
Non-medicated emulgel base, gp 4	5.03±1.8	4.8± 1.01	3.6±1.5	2.1±1.3	1.1±2.2
F 1, gp 5	74.8**±6.8	59.1**±11.7	77.6**±18.4	78**±18.4	53.2**±25.4
F2, gp 6	58.6**±11.9	70.4**±4.56	65.9**±8.7	58.1**±11.1	48.5**±12.1
F3, gp 7	72.4**±6	66.2**±10.2	63.6**±9.1	47.0**±15.3	31.3**±5.5
F4, gp 8	69**±20.7	78.8**±18.9	84.8**±5.3	52.9**±13.5	44.1**±10.2
Non-medicated w/o/w base, gp 9	5.9±1.8	4.8± 1.01	5.6±1.5	5.1±17.3	4.1±20.2
F5, gp 10	71.5**±5.2	54.9**±0.75	59.1**±9.1	53.7**±11.1	34.8**±0.8
F6, gp 11	69.0**±16.9	63.6**±12.9	70.4**±8.7	47.2**±17.5	41.4**±8.9

DISCUSSION

Finely divided insoluble solid particles constitute an important class of emulsifying agents. Colloidal particles that are partially wetted by both the aqueous and the oleic phases are capable of effectively stabilizing emulsions. Finkle et al.[27] have studied the stability of oil-water emulsions by linking the wettability of the particles to the type of emulsion formed. They have observed that the more poorly wetting liquid becomes the dispersed phase of the emulsion. Particles which were completely wetted by water or oil became dispersed in either phases and were incapable of stabilizing emulsions. Tambe and Sharma[28] have shown that oil-water interfaces containing adsorbed surfactants and/or colloidal particles exhibit viscoelastic behavior. Such viscoelastic interfaces enhance emulsion stability by increasing the magnitude of steric hindrance (i.e., the energy required to displace particles away from the drop-drop contact region) and by decreasing the rate of film thinning between coalescing emulsion droplets. In addition, they have revealed that the effectiveness of these particle in stabilizing emulsions depends in part on the formation of sufficiently dense and stable monolayer of particles at the oil-water interface that can inhibit the coalescence of the emulsion droplets[29]. Moreover, several workers have studied the stability of solids-stabilized emulsions[29&30]. It has been found that the fine particles adsorbed at the droplet surface act as a barrier preventing droplets form coalescing[31]. Whitby et al.[32] have investigated the effect of adding weakly or strongly flocculated nanoparticles on the emulsion stability to coalescence and the emulsion drop size.they have found that adsorption of particles at the oil–water interface is thermodynamically favoured since the oil–water interfacial energy is larger than the difference between the particle–oil and particle–water interfacial energies. Thus a particle will adsorb onto a drop if they collide with sufficient energy to cause drainage of the liquid film between them. Particles that are preferentially wetted by one liquid phase tend to be adsorbed weakly than those wetted to an intermediate extent by both liquids[33]. In addition, Welin-Berger et al.[34] have shown that the rheological properties of the o/w emulsion are an important factor controlling the permeation of local anesthetic. Moreover,

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