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Research Article

Transdermal Delivery of an Anti-Cancer Drug via W/O Emulsions Based on Alkyl Polyglycosides and Lecithin: Design, Characterization, and *In Vivo* Evaluation of the Possible Irritation Potential in Rats

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Abstract. The purpose of this work was to develop w/o emulsions that could be safely used to promote transdermal delivery of 5-fluorouracil (5-FU). Two pseudo-ternary phase diagrams comprising oleoyl-macrogol glycerides, water, and a surfactant/co-surfactant (S/CoS) mixture of lecithin, ethanol, and either coco glucoside or decyl glucoside were investigated for their potential to develop promising 5-FU emulsions. Six systems were selected and subjected to thermodynamic stability tests; heat–cool cycles, centrifugation, and finally freeze–thaw cycles. All systems passed the challenges and were characterized for transmission electron microscopy, droplet size, rheological behavior, pH, and transdermal permeation through newly born mice skin in Franz diffusion cells. The systems had spherical droplets ranging in diameter from 1.81 to 2.97 μm, pH values ranging from 7.50 to 8.49 and possessed Newtonian flow. A significant ($P<0.05$) increase in 5-FU permeability parameters as steady-state flux, permeability coefficient was achieved with formula B5 comprising water (5% *w*/*w*), S/CoS mixture of lecithin/ethanol/decyl glucoside (14.67:12.15:18.18% *w*/*w*, respectively) and oleoyl-macrogol glycerides (50% *w*/*w*). When applied to shaved rat skin, this system was well tolerated with only moderate skin irritation that was recovered within 12 h. Indeed, minor histopathologic changes were observed after 5-day treatment. Further studies should be carried out, in the future, to investigate the potentiality of this promising system to promote transdermal delivery of 5-FU through human skin.

KEY WORDS: 5-fluorouracil w/o emulsions; alkyl polyglycosides; irritation potential; lecithin; transdermal delivery.

INTRODUCTION

5-Fluorouracil (5-FU) is an antineoplastic drug that is approved for palliative treatment of cancer of the colon, rectum, stomach, breast, and pancreas. Per-oral 5-FU administration results in poor drug absorption, variable first pass elimination by the gut and liver, and consequently erratic bioavailability (1). It was reported that only 20–30% of 5-FU-treated patients have drug levels that are in the appropriate therapeutic range. On the other hand, 40–60% of patients are under-dosed and 10–20% of patients are over-dosed (2). Following intravenous administration, the drug undergoes rapid clearance from plasma with a mean half-life of about 16 min. Consequently, doses up to 1 g should be administered daily (3). These high doses are usually accompanied by severe systemic toxic effects of gastrointestinal, hematological, neural, cardiac, and dermatological origin (4).

To find an alternative pathway, many attempts have been made to overcome the hydrophilic nature of 5-FU so that it could be possibly delivered via the transdermal route. Some of these investigations include (1) incorporation of 5-FU into w/o microemulsion (ME) systems (5,6), (2) design of prodrugs (7,8), and (3) the use of penetration enhancers (9,10).

Topical application of once daily 5-FU cream (0.5%, Carac®, Sanofi-aventis, USA) may be associated with skin reactions including redness, dryness, burning, pain, erosion of the upper layer of skin, and swelling. The irritation may continue two or more weeks after treatment is over. Therefore, an attempt has been made in the present work to develop safer 5-FU w/o emulsions depending on a Surfactant/Co-Surfactant (S/CoS) mixture of alkyl polyglycosides, lecithin, and ethanol (11).

Alkyl polyglycosides (sugar-based surfactants) emerged as an important class of natural surfactants that are made from renewable raw materials such as glucose and fatty alcohols (12). These nonionic surfactants have outstanding biodegradability (13), excellent dermatological properties (14,15). They were used in the design of topical vehicles for hydrocortisone (16) and other ME systems loaded with ascorbic acid (17) and insulin (18). As suggested by Rybinski *et al.* (19), alkyl polyglycoside-based microemulsions are potential systems that have very low interfacial tensions and are very largely electrolyte and temperature independent.

Lecithin is an integral part of cell membranes and thus is considered highly biocompatible. It is widely used as an emulsifier for food products, cosmetics, and in many transdermal drug delivery systems like organo-gels (20),

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ME systems (21), liposomes, niosomes (22), and ethosomes (23).

The current work aims to (1) design 5-FU-loaded w/o emulsions based on lecithin, ethanol, and a sugar-based surfactant like coco glucoside (Plantacare® 818 UP; P 818) or decyl glucoside (Plantacare® 2000 UP; P 2000) (2) promote an adequate penetration of 5-FU through a model membrane (newly born mice skin), and (3) explore the possible irritation potential of the best achieved system in rats.

MATERIALS AND METHODS

Materials

5-Fluorouracil was purchased from Fluka BioChemika (Buchs, Switzerland). Plantacare® 818 UP (Coco glucoside) and Plantacare® 2000 UP (decyl glucoside) were kindly provided by Henkel (Düsseldorf, Germany). Labrafil® M1944CS (oleoyl macrogol glycerides) was donated by Gattefossé (St-Priest, France). Soybean lecithin was obtained from Sigma Chemicals (St Louis, MO). Disodium hydrogen phosphate and potassium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany). Methyl alcohol, ethyl alcohol, absolute ethyl alcohol, sodium chloride, formaldehyde, and bromthymol blue were derived from El-Nasr Pharmaceutical Chemicals Co. (Abu Zaabal, Egypt).

Construction of Pseudo-Ternary Phase Diagrams

Pseudoternary phase diagrams of oil (labrafil® M1944CS), S/CoS mixtures and water were developed at room temperature using the water titration method. The S/CoS mixtures were prepared from lecithin, ethanol, and either P 818 (series A) or P 2000 (series B) at a ratio of 32.6%:27%:40.4% (*w*/*w*), respectively (11,18). Briefly, transparent and homogenous blends with varying ratios of labrafil and S/CoS mixtures ranging from 9:1 to 1:9 (*w*/*w*) were formed by vortexing (VSM-3 Variable speed vortex mixer, PRO Scientific Inc., Oxford, England) for 3 min. Each blend was then titrated with water. Samples were left for equilibration between each addition of water for 10 min and were visually observed for phase transparency and flowability. The concentration of water at which phase transitions occurred (w/o clear emulsion → one-phase clear gel → two-phase turbid gel → milky emulsion) was derived from weight measurements (18). Pseudoternary phase diagrams were constructed with Tri-plot software (David Graham and Nicholas Midgley, Loughborough University, Leicestershire, UK) Ver. 4.1.2.

Preparation of W/O Clear Emulsions Containing 5-FU

Two series of drug-free w/o clear emulsions were prepared using labrafil® M1944CS, water and S/CoS mixture 1 (series A) or S/CoS mixture 2 (series B) (Table I). Drug-loaded formulae (1.25 mg/g) were prepared by dissolving an accurate amount of 5-FU in the developed w/o clear emulsions with the aid of vortexing. The formulae were stored at room temperature until further use.

Thermodynamic Stability Studies

The thermodynamic stability of the developed emulsions was evaluated, on three phases, according to the protocol designed by Shafiq *et al*. (24). Initially, the formulae were subjected to six heat (45°C)–cool (4°C) cycles with storage at each temperature for 48 h. Then, they were centrifuged at 3,500 rpm for 30 min. Finally, they were allowed to three freeze (−21°C)–thaw (25°C) cycles with storage at each temperature for 48 h.

Characterization of the Developed Emulsions

Transmission Electron Microscopy

A drop of each drug-loaded sample was placed on a copper grid, and the excess was removed with a filter paper. One drop of 0.1% bromthymol blue solution was added onto the grid, and the excess was similarly removed. Finally, the grid was examined under a transmission electron microscope (Jeol JEM 1230, Tokyo, Japan) at 80 kV.

Droplet Size Determination

The droplet size of each drug-loaded sample was determined, in triplicate, using Mastersizer S laser diffractometer (Malvern Instruments, Malvern, Worcestershire, UK) at 25±0.5°C. The samples were examined using a 300-mm lens that can measure particles ranging in size from 0.5 to 900 μm. Three replicates were taken for each sample,

Table I. The Composition and Physicochemical Properties of the Investigated W/O Emulsions (Mean ± S.D., $n=3$)

	Composition of w/o emulsions (% *w*/*w*)							
Formulae[a]	Labrafil® M1944CS	S/CoS mixture (lecithin: ethanol: P 818 or P 2000)	Water	VMD (μm)	Span	Viscosity (cP)	Farrow's constant (*N* value)	pH
A3	30	63 (20.54:17.01:25.45)	7	2.97±0.11	2.59±0.09	26.16±1.66	0.94±0.04	8.49±0.05
A4	40	54 (17.60:14.58:21.82)	6	2.78±0.07	2.39±0.06	36.04±2.23	1.05±0.07	8.23±0.08
A5	50	45 (14.67:12.15:18.18)	5	2.50±0.12	2.25±0.03	42.03±4.25	1.16±0.09	7.95±0.04
B3	30	63 (20.54:17.01:25.45)	7	2.43±0.06	1.33±0.07	7.21±1.13	1.15±0.02	7.91±0.07
B4	40	54 (17.60:14.58:21.82)	6	2.17±0.11	1.21±0.07	18.26±2.04	1.11±0.06	7.78±0.08
B5	50	45 (14.67:12.15:18.18)	5	1.81±0.13	1.13±0.04	26.82±2.22	1.10±0.08	7.50±0.03

VMD volume mean diameter
[a] All formulae contain 5-FU (1.25 mg/g)

and polystyrene beads were used as a standard to check instrument performance. The droplet size of each formula was described by the volume mean diameter. The polydispersity, a measure of homogeneity, was expressed by the span (1)

$$\text{Span} = (D(v90) - D(v10)/D(v50) \quad (1)$$

where, D (v90), D (v50), and D (v10) are the equivalent volume diameters at 90%, 50%, and 10% cumulative volumes, respectively (25).

Rheological Measurements

Drug-loaded samples (1 ml) were tested for their rheological characteristics at 25±0.5°C using Brookfield viscometer (DV-III Programmable Rheometer, Brookfield Engineering LABS, Stoughton, MA) fitted with a cone spindle #52. The measurements were conducted, in triplicate, at a shear rate ranging from 10 to 300 rpm with 1 min between each two successive points. Sample flow behavior was studied according to Eq. (2)

$$\text{Log}S = N\text{Log}D - \text{Log}\eta \quad (2)$$

where D is the shear rate (per second), S is the shear stress (dyne per square centimeter), η is the viscosity (cP), and N (Farrow's constant) is the slope of log S against log D plot. It denotes deviation from Newtonian flow. When $N<1$, dilatant flow is indicated. If $N>1$, pseudoplastic flow is assured (26).

pH Determinations

The pH of each drug-loaded sample was determined, in triplicate, at 25±0.5°C using a bench-top pH meter (Jenway model 3510, Barloworld Scientific Ltd., Dunmow, UK).

In Vitro 5-FU Skin Permeation Studies

Experiments were run, in triplicate, at 37±0.5°C, using vertical Franz diffusion cells having an effective permeation area of 3.14 cm^2. The receptor medium (25 mL) was phosphate-buffered saline (pH 7.4) containing 0.11% (*w*/*v*) formaldehyde as a preservative and was stirred constantly by a magnetic stirrer (50 rpm).

The drug permeation studies were performed using the skin of newly born mice (age 6 days or younger) derived from the Cairo University Labs, Cairo, Egypt. All animals were treated in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care international's expectations for animal care and use/ethics committees. Mice skins were obtained after killing the animals by peeling the skin from the underlying cartilage (27). A preliminary wash of the skin was done with normal saline, followed by drying between two filter papers. The skin was used directly in the study w/o storage. Prior to sample application, the skin was checked for any damage then carefully mounted onto the diffusion cell, fastened with a rigid clamp with the stratum corneum side up and the donor compartment dry and open to the air, and floated on receiver solution for 24 h for equilibration and pre-hydration. As described by El Maghraby *et al.* (28), this approach was suggested to maintain a transepidermal hydration gradient. The receptor content was then replaced by fresh medium. One gram of each drug-loaded formula (1.25 mg/g) was applied to the skin surface (6). One milliliter of an aqueous 5-FU solution (1.25 mg/ml) was used as a control. One-milliliter samples of receptor medium were removed at appropriate intervals and immediately replaced with fresh medium. Drug concentration was determined spectrophotometrically (6) (Shimadzu, model-UV-1601 PC, Japan) at 266 nm, after precipitation of proteins using acetonitrile. Parallel blank experiments, using drug-free formulae, were conducted. At the same time intervals, samples were withdrawn from receptor medium and were treated as previously described.

Permeation Data Analysis

The cumulative amount of 5-FU permeated through the skin (micrograms per square centimeter) was plotted as a function of time (hours) for each formula. Drug flux (permeation rate) at steady-state (J_{ss}) was calculated from the slope of the linear portion of the graph (29). The permeability coefficient (K_p) was calculated from Eq. (3)

$$K_P = J_{SS}/C_0 \quad (3)$$

where, J_{ss} is the drug flux at steady state and C_0 is the initial drug concentration in the donor cell. Finally, the enhancement ratio (E_r) was calculated by dividing the J_{ss} of the respective formulation by the J_{ss} of the control solution (30)

The significance of results was checked statistically (SPSS statistics program, Release 14.0 for Windows, Chicago, IL) at $P<0.05$ applying a one-way ANOVA test. Post hoc multiple comparisons were carried out using the least square difference test.

Skin Irritation Studies

The skin irritation studies were conducted on rats to evaluate the possible irritation potential of the best achieved o/w emulsion (B5) on the skin. The protocol of the study was approved by the Research Ethics Committee in the Faculty of Pharmacy, Cairo University, Egypt. The rats (200–250 g) were housed in polypropylene cages of suitable size that allow freedom of movement, six per cage. The cages were kept in a room under standardized environmental conditions (20±2°C, 35–45% RH) and a constant day / night cycle. These conditions were checked daily to ensure their safety and well-being. They received standard laboratory diets and water *ad libitum* throughout the study.

Application of Treatments to Rats. The experiments were conducted according to the scheme designed by Jibry and Murdan (31). Six male Wistar rats were assigned to each treatment group as follows: group A received normal saline solution (negative control), group B received sodium lauryl sulfate (SLS) solution (5%, *w*/*v*; positive control) and group C received drug-loaded o/w emulsion.

Twenty-four hours prior to the first application, the animals' backs were shaved with clippers, their skin was checked for cuts, and they were allowed to rest overnight. On the first day of the study, a patch (2 cm^2) on their lower backs

was marked out. Each treatment (10 μL) (6) was carefully rubbed once (to test single insult challenge) onto the marked patch on each rat. For the following days of the study (2nd–5th), the application of each treatment was done twice daily at 3-h interval (to test repeated insult challenge).

As suggested by Jibry and Murdan (31), all treatment sites were covered with sterile gauze and secured with surgical tape to prevent grooming and removal of the formulation from the skin. At the end of the application time (1 h), the gauze was taken off and the treated area was gently wiped with water-soaked gauze to remove any residual vehicle.

Visual Assessment of Skin Irritation. The application sites were evaluated for their irritation degree on the first day of the study directly after the removal of the gauze, 3, 6, 9, and 12 h later by visual scoring using a modified method of Draize *et al.* (32). Erythema scores ranging from 0 to 4 were given depending on the degree of erythema as follows: no erythema 0, slight erythema (barely perceptible light pink) 1, moderate erythema (dark pink) 2, moderate to severe erythema (light red) 3, severe erythema (extreme redness) 4.

Histopathological Assessment of Skin Patches. At the end of the fifth day, the animals were sacrificed. Skin biopsies (1 cm^2) were taken from all treated patches and were preserved in 10% formalin solution for 48 h before processing for histopathological studies. The bodies and the remains of rats were frozen and transferred to be incinerated at the Faculty of Veterinary Medicine, Cairo University, Egypt.

Histopathological specimens were prepared according to the protocol designed by Banchroft *et al.* (33). Briefly, serial dilutions of alcohols (methyl alcohol, ethyl alcohol, and absolute ethyl alcohol) were used for dehydration. Specimens were cleared in xylene embedded in paraffin in a hot air oven (Heraeus, Hanau, Germany) adjusted at 56°C for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at 3–4-μm thickness by a sledge microtome (Leica Microsystems SM2400, Cambridge, England). The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin, and finally examined under a light microscope (Leica Microsystems DM3000, Cambridge, England), and the microphotographs were compared.

RESULTS AND DISCUSSION

Pseudoternary Phase Diagrams

The relationship between the phase behavior of a mixture and its composition can be investigated with the aid of a phase diagram (34). In the current study, two pseudoternary phase diagrams of labrafil® M1944CS, water, and S/CoS mixtures (mix 1; Fig. 1a or mix 2; Fig. 1b) were constructed. Four distinct zones (A, B, C, and D) could be identified within each diagram. Fluid and clear emulsions (Zone A) were developed spontaneously at ambient temperature when their components were brought into contact. This might be related to the ability of the investigated S/CoS mixtures to cause (1) a large reduction in the surface tension of the oil-water interface and (2) favorable entropic changes. As suggested by Lawrence and Rees (34), these actions would provide the negative free energy required for spontaneous development of emulsions. On the other hand, the development of clear transparent systems could be related to the presence of a cosurfactant (ethanol) which is suggested to penetrate the surfactant film, lower the fluidity and surface viscosity of the interfacial film, decrease the radius of curvature of the droplets, and form transparent systems (35).

A gradual increase in the water content of these systems leads to the formation of clear one-phase gels (Zone B), turbid two-phase gels (Zone C), and milky emulsions (Zone D), respectively. In a parallel line, the conversion of ME systems to gels was previously reported. It was confirmed, by polarizing light microscopy, that a lamellar liquid-crystalline gel region was adjacent to the ME region (18,36).

When 5-FU was incorporated into the developed systems (1.25 mg/g), no change in the phase behavior of these systems was observed. This could be related to the high stability of alkylpolyglucosides towards electrolyte addition (19). The

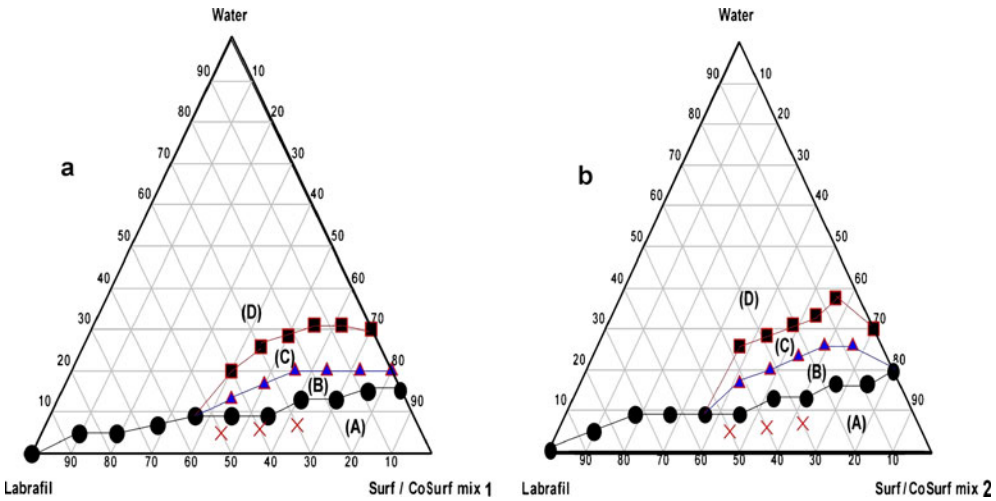

Fig. 1. Pseudo-ternary phase diagrams of labrafil, Surfactant/Co-Surfactant mixture 1 **a** or 2 **b** and water. Areas (*A*), (*B*), (*C*), and (*D*) represent clear w/o emulsion, clear one-phase gel, turbid two-phase gel and milky emulsion regions, respectively. Samples (*x*) were chosen for further investigations

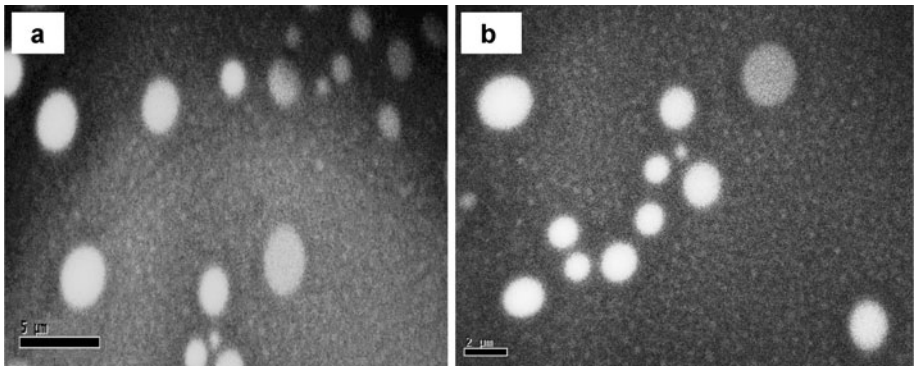

Fig. 2. Transmission electron micrographs, ×3,000, of formula A5 **a** and, ×6000, formula B5 **b**

phase behavior of both series is very similar despite the use of two different alkylpolyglucosides; P 818 (series A) or P 2000 (series B). However, the developed w/o clear emulsion zone in series B is slightly larger than that developed with series A. This could be an advantage for loading higher amounts of water soluble drugs, like 5-FU. This might be related to the ability of S/CoS mix 2 (series B) to cause more increment in the dispersion entropy, more reduction in the interfacial tension of oil–water interface, more increment in the interfacial area. Consequently, the system free energy would be decreased to a lower value than that achieved with S/CoS mix 1 (series A) (30,34).

Thermodynamic Stability Studies

The thermodynamic stability of investigated w/o emulsions (A3, A4, A5 and B3, B4, B5) was evaluated using heat–cool cycles, centrifugation, and freeze–thaw cycle stress tests. All emulsions passed the tests with no signs of phase separation, creaming, or cracking. This could indicate that the developed systems have good physical and thermodynamic stability.

Rybinski *et al.* (19) reported that the ability of alkyl polyglycosides to lower the interfacial tension showed a negligible temperature dependence. As an explanation to this behavior, Stubenrauch *et al.* (37) suggested that the interaction of the sugar unit of alkyl polyglycosides with water is only slightly influenced by temperature.

Characterization of the Developed Emulsions

Transmission Electron Microscopy

The shape and surface morphology of two representative samples (formula A5 and formula B5) is illustrated in Fig. 2. It was observed, in all samples, that the dispersed water droplets (the internal phase) have almost spherical shape. The droplet size of the former formula appears to be larger than that of the latter.

Droplet Size Distribution

The developed systems have a droplet size range of 1.81–2.97 μm (Table I). Consequently, these systems could be described as w/o emulsions rather than w/o microemulsions which usually have a droplet size range of 20–200 nm (38).

The mean droplet size of the investigated o/w emulsions is directly proportional to their S/CoS mixture content (%, *w*/*w*) and is inversely proportional to their oil content (%, *w*/*w*; Table I). The mean droplet size of formula B5, containing 50% oil, is the lowest (1.81 μm) while the mean droplet size of formula A3, containing 30% oil, is the highest (2.97 μm). These findings are in accordance with those reported by Graf *et al.* (18) who found that the droplet size of insulin-loaded (w/o) ME systems decreased with increasing their oil (isopropyl myristate) content.

The span value is a measure of polydispersity. It is used to indicate the uniformity of droplet size within the formula. The higher the span value, the lower the uniformity of the droplet size distribution. The mean span values of most formulae are low; ranging from 1.13 (formula B5) to 2.59

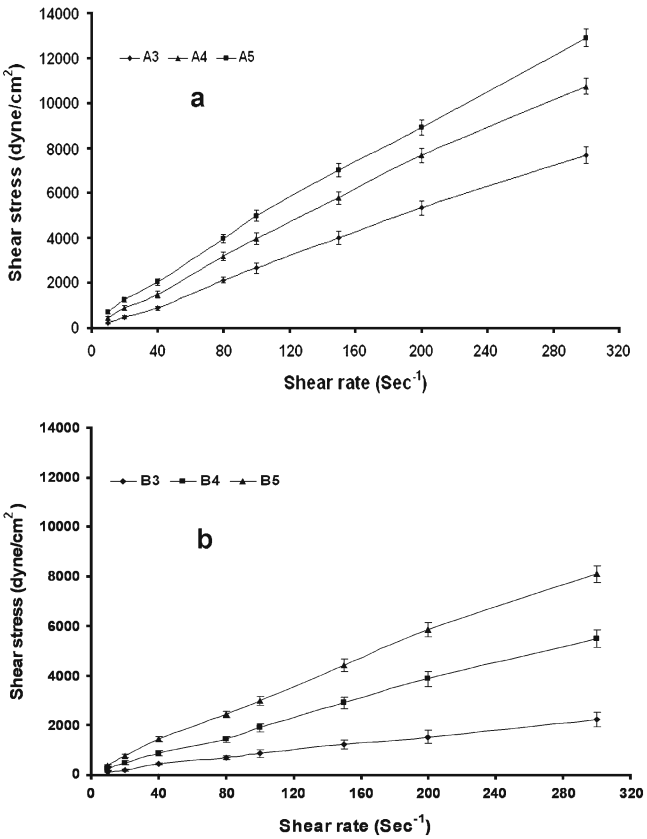

Fig. 3. Rheograms of the investigated w/o emulsions of series A **a** and series B **b** at 25±0.5°C, mean ± S.D., $n=3$

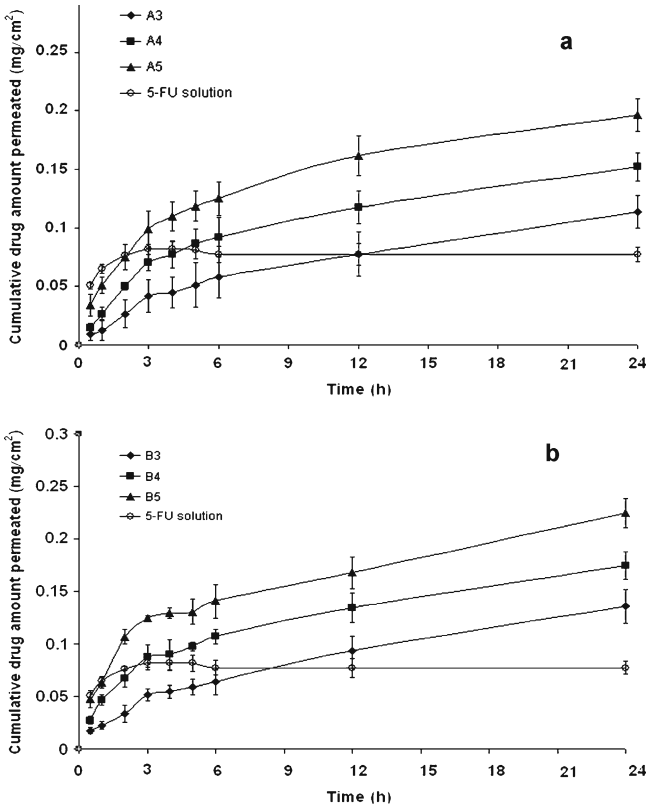

Fig. 4. *In-vitro* permeation of 5-FU from an aqueous solution and the investigated w/o emulsions of series A **a** and series B **b** through newly born mice skin in a Franz diffusion cell at 37±0.5°C, mean ± S.D., $n=3$

(formula A3). The higher span value of the latter formula could indicate the presence of a greater proportion of larger droplets.

Rheological Measurements

Viscosity measurements were examined as a function of shear rate. The viscosity values (cP) of different formulae is shown in Table I. It is clear that the selected emulsions of Series B have lower viscosity values than their corresponding formulae of series A. This might indicate that the former systems have weaker structures that would enable easier application to the skin. Within either series, the increase in viscosity could be directly correlated to the external phase (oil) content (%, *w*/*w*) rather than the S/CoS mixture content (%, *w*/*w*).

The flow behavior of the developed systems is illustrated in Fig. 3 where the shear rate was plotted against the shear stress. Systems that show proportionality between shear stress and shear rate are considered to be Newtonian fluids. Their viscosities can be obtained from the slopes of the linear plots of shear stress *vs.* shear rate (39). Newtonian behavior is suggested for all the investigated w/o emulsions due to the linearity of the rheograms (r^2 values$\geq$0.99).

To provide a measure of the degree of pseudoplasticity (deviation from Newtonian flow), Farrow's constant was determined for each system. The mean calculated "N" values are very close to 1; ranging from 0.94 (formula A3) to 1.16 (formula A5), indicating Newtonian flow.

pH Determinations

The mean pH values of the developed w/o emulsions varied from 7.50 (formula B5) to 8.49 (formula A3; Table I). It is clear that the selected emulsions of Series B have lower pH values than their corresponding formulae of Series A. Within either series, it is obvious that higher pH values are directly correlated to the S/CoS mixture content (%, *w*/*w*). As reported by their manufacturer, alkyl polyglycosides have pH values ranging from 11.5 to 12. Accordingly, increasing the content of S/CoS mixture would be expected to cause marked increases in pH values.

Drug Permeation Studies

The permeation profiles of 5-FU from the investigated formulae are shown in Fig. 4. For the developed formulae, the cumulative amount of drug permeated through newly born mice skin (micrograms per square centimeter) was plotted as a function of time (hours). The steady-state flux (J_{ss}) as well as the permeability coefficient (K_p) were calculated from the linear portion of graph (Table II).

The permeated percentages of 5-FU from the developed systems through the skin in 24 h were variable; ranging from 28.57% (formula A3) to 56.39% (formula B5). On the other hand, the permeated percentage of 5-FU from the aqueous solution was 20.53% only. The difference in the drug permeation patterns could be related to lower ability of the latter to penetrate skin layers. In fact, the J_{ss} and K_p of the aqueous drug solution (8.31 μg/cm^2/h and 6.65 cm/h, respectively) were the lowest among the investigated formulae.

Table II. *In Vitro* Permeation Data of 5-FU from the Investigated Formulae Through Newly Born Mice Skin (Mean ± S.D., $n=3$)

Formulae	Drug permeated in 24 h (%)	Cumulative amounts of drug permeated in 24 h (μg/cm^2)	Steady-state flux (μg/cm^2/h)	Enhancement ratio	Permeability Coefficient (cm/h)
5-FU solution	20.53±1.11	81.72±10.21	8.31±0.13	–	6.65±0.11
A3	28.57±1.27	113.73±16.24	13.38±0.22	1.60	10.70±0.18
A4	38.22±1.78	152.15±12.18	22.24±0.31	2.67	17.79±0.22
A5	49.28±2.77	196.17±14.66	25.41±0.45	3.05	20.32±0.31
B3	34.14±1.23	135.93±12.19	13.49±0.33	1.62	10.79±0.28
B4	43.89±2.56	174.72±15.87	23.14±0.51	2.78	18.51±0.37
B5	56.39±2.18	224.51±14.33	32.10±0.83	3.86	25.68±0.73

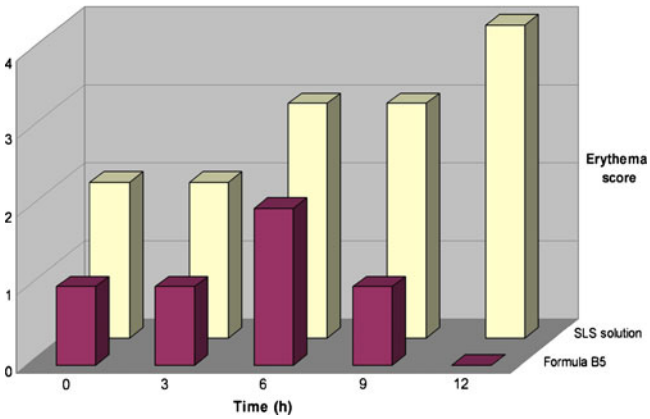

Fig. 5. The mean erythema scores developed in rat skin patches treated with formula B5 and SLS solution (5%, *w/v*)

After 24 h, it was clear that the formulae derived from series (B) have higher cumulative amounts of drug permeated, higher steady-state flux values, higher enhancement ratios, and higher permeability coefficients than their corresponding formulae derived from series (A). In either series, the formulae containing higher percentages of labrafil® (formulae B5 and A5) shows significantly ($P<0.05$) higher transdermal efficiency parameters than other formulae. These results are in accordance with those reported by Ammar *et al.* (40) who developed novel nanoemulsions of dorzolamide hydrochloride and found that those systems containing higher contents of triacetin (oil phase) showed significantly ($P<0.01$) higher release efficiency percentages. It was suggested that the increase in oil content, combined with a decrease in surfactant concentration, might cause an increase in the thermodynamic activity of the drug which acts as a driving force for its release (41,42).

Conclusively, formula B5 showed significantly ($P<0.05$) higher mean cumulative drug amounts permeated in 24 h (224.51 μg/cm^2), higher mean steady-state flux (32.10 μg/cm^2/h), and higher permeability coefficient (25.68 cm/h) than other emulsion-based formulae. When compared to the aqueous drug solution, the enhancement ratio of formula B5 (3.86-folds) was the highest. Consequently, formula B5 was suggested to be the most promising system in improving the transdermal efficiency parameters of 5-FU.

Evaluation of Skin Irritation by the Scoring System

Erythema is caused by increased blood flow in the dermis. It could be considered as a tool to monitor the response of that layer to topical preparations. The erythema scores upon exposure to formula (B5) as well as SLS solution (5%, *w/v*) are presented in Fig. 5.

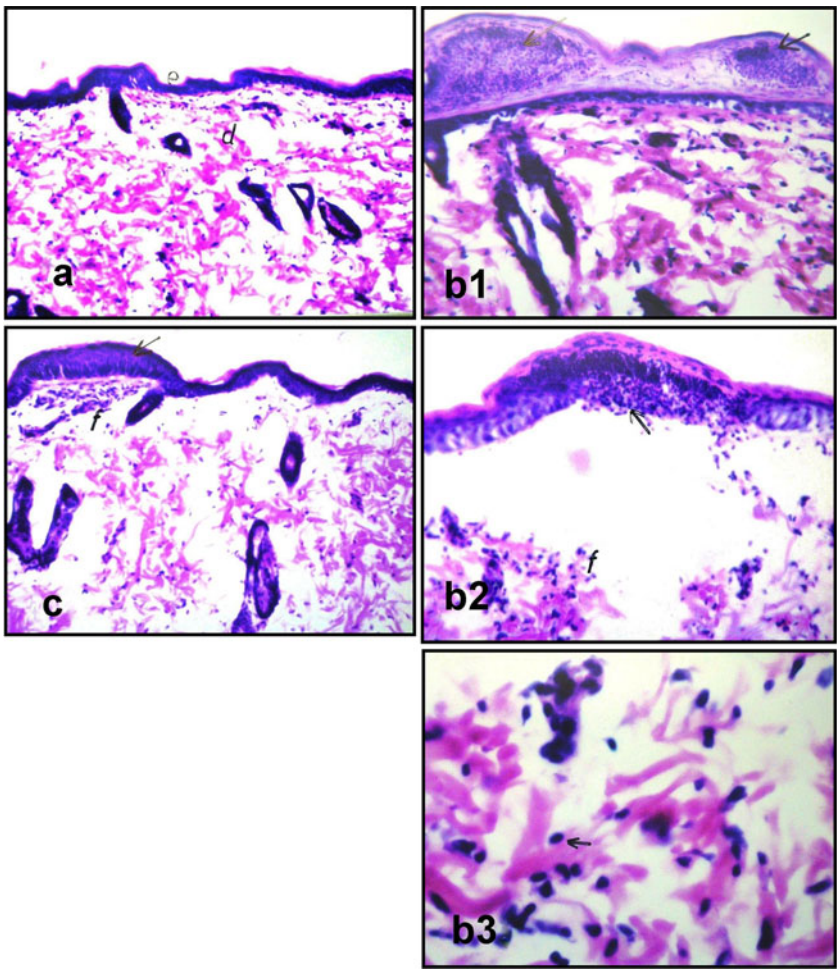

Fig. 6. Micrographs of rat skin patches treated with normal saline solution **a**, sodium lauryl sulfate solution (5%, *w/v*) **b1**, **b2**, and **b3** and formula B5 **c**

The rat skin patches treated with SLS solution suffered from much higher erythema levels all over the study period (12 h). Strong, infiltrated erythema with superficial erosions involving at least 50% of the test area was observed after 6 h and was scored as (3). At the end of 12 h, extensive erosions involving at least 50% of the test area were revealed and were scored as (4). In a previous study (31), the application of SLS solution (5%, *w*/*v*) for several days, as a positive control, caused extensive irritation in the form of erythema, wrinkling, and fissures on the skin surface of some mice. Under occlusion conditions, it caused inflammation, erythema, and significant changes in skin morphology (43).

On the other hand, formula (B5) was well tolerated by all rats. As the time progressed (3 h), the erythema level increased. Moderately intense erythema involving around 30% of the test area was evidenced after 6 h and was scored as (2). Within 12 h, the erythema diminished and skin recovery took place. The developed erythema scores, although minor, could be related to the drug itself rather than the components of formula (B5). In fact, 5-FU-associated dermatitis, erythema, pain, and desquamation of the skin of palms and soles were previously reported (44).

Histopathological Findings of Rat Skin Biopsies

No histopathological findings were observed in both the epidermal (basal cell layer, prickle cell layer, spinosum, lucidium, and cornium) and the dermal layers (connective tissue containing sebaceous gland and hair follicles) of the skin biopsies derived from all rats treated with normal saline solution; group A (Fig.6: a).

On the other hand, histopathological examination of the skin biopsies derived from two rats belonging to group B, treated with SLS solution, revealed the occurrence of necrosis in a focal manner all over the epidermis with raised areas. However, the necrosis did not involve the basal cell layer (Fig.6: b1). The skin biopsies derived from the remaining members of group B revealed that necrosis has extended to the basal cell layer and was covered externally by thick keratin layers (Fig.6: b2). In either case, marked focal inflammatory cells infiltration was observed in the underlying dermal tissues (Fig.6: b3).

Rats treated with formula B5 showed mild acanthosis in a focal manner in the prickle cell layer. This was associated with few inflammatory cells infiltration in the underlying dermal tissue (Fig.6: c). These findings suggest the safety of the developed system (formula B5) to rat skin.

CONCLUSIONS

W/o emulsions based on lecithin and alkyl polyglycosides (Coco glucosides or decyl glucosides) were successfully developed. Decyl glucoside-based systems (Series B) were more promising and showed higher cumulative amounts permeated of 5-FU in 24 h through newly born mice skin, higher steady state flux values and higher permeability coefficients than coco glucoside-based systems (series A). The best achieved formula (B5) was well tolerated by rats with only moderate skin irritation that was recovered in 12 h and showed only minor histopathologic changes in shaved rat skin after treatment for 5 days. Further studies should be carried out, in the future, to incorporate this promising emulsion in a suitable lipogel base and evaluate the potentiality of this patient-friendly dosage form in promoting the transdermal delivery of 5-FU through human skin.

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