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Solubilization of fluocinolone acetonide by cosolvents and surfactants for buccal solution preparation

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ABSTRACT

Objective: Fluocinolone acetonide (FA) buccal solution was prepared for treatment of oral lichen planus using cosolvents and surfactants to enhance FA solubility. **Results:** Based on solubility power (σ) in the concentration range of 0–40% w/w, cosolvents enhanced FA solubility in the order; polyethylene glycol 400 ($\sigma = 3.06 \times 10^{-2}$)> propylene glycol ($\sigma = 2.92 \times 10^{-2}$)> glycerin ($\sigma = 0.95 \times 10^{-2}$). The order of FA solubility in surfactant solutions was 1% polysorbate 80 (1.04 × 10−2 %w/v)>0.05% cetylpyridinium chloride (0.69×10^{-2} %w/v)>0.1% sodium lauryl sulfate (SLS) (0.27×10^{-2} %w/v). Cosolvents increased the critical micelle concentration, especially for ionizable surfactants (SLS and cetylpyridinium chloride [CPC]). These effects of cosolvents on micellization impeded the ability of the surfactants to increase FA solubility. Both polysorbate 80 and CPC induced degradation of FA. **Conclusion:** We conclude that a 0.01% FA buccal solution should be prepared in a cosolvent system without use of surfactants.

INTRODUCTION

Fluocinolone acetonide (FA) is a potent corticosteroid that produces disease remission in patients with oral lichen planus (OLP).[1,2] OLP is a chronic inflammatory mucocutaneous disorder that causes soreness and a burning sensation in the atrophic and erosive forms.[3,4] A suggested concentration range of FA is 0.0025–0.025% w/w in external preparations while FA is commercially available as a gel, cream, lotion, ointment, and scalp application at higher strength than the suggested concentration.[5,6] These preparations are intended for external administration and not for oral mucosa due to chemical effects on the mucosal membrane. However, *Candida* and fungal infections are a drawback after long-term use of corticosteroid drugs.[7,8] FA is practically insoluble in water (0.005% w/v at 25°C) and undergoes degradation by hydrolysis,[9-11] with the lowest degradation at pH 4.[10]

Micellar solubilization, cosolvency and complexation are commonly used to improve the solubility of poorly water-soluble compounds.[12,13] While the use of cosolvents and surfactants has shown to improve aqueous solubility of nonpolar compounds, the type and amount of these solubilizers are a major concern for buccal use. In the pharmaceutical preparations, glycerin (Gly), propylene glycol (PG), and polyethylene glycol 400 (PEG) are widely used as cosolvents. Polysorbate 80 (P80), a nonionic surfactant, is used as a solubilizing agent in oral, parenteral and topical preparations. These compounds are relatively non-toxic and nonirritant materials.[14] Cetylpyridinium chloride (CPC), a quaternary ammonium cationic surfactant, has been used as an antiseptic agent for oral and throat care. This effect of CPC may overcome the drawback of long-term use of corticosteroids. Sodium lauryl sulfate (SLS), an anionic surfactant, is used in oral care and topical preparations. However, CPC and SLS at high concentration are moderately toxic, including irritation to skin and mucous membrane.[14]

The aim of this study was to develop a 0.01% w/v FA buccal solution using a cosolvent or micellar solubilization to enhance the solubility of FA. The effects of the cosolvents on micellization behavior were examined and the chemical and physical stability of FA in the solutions was determined.

MATERIALS AND METHODS

Materials

FA of USP grade was a gift from Siam Pharmaceutical Co. Ltd., Thailand, and was used as received. FA (98.7%, secondary standard), CPC, PG, and PEG were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA. Gly was purchased from Ajax Aldrich, Finechem Pty Ltd, Seven Hills, NSW, Australia. Acetic acid, sodium acetate and P80 were purchased from Merck Schuchardt OHG, Hohenbrunn, Germany. SLS was purchased from Carlo Erba Reagent, Rodano, Italy. Acetonitrile was purchased from RCI Labscan Limited, Bangkok, Thailand. All experiments used ultrapure water passed through a Millipore water purification system (>18 MΩ resistance, ELGA 15).

High-performance Liquid Chromatography (HPLC) Analysis of FA

FA was analyzed using HPLC, as described in the British Pharmacopeia.[9] The HPLC system (Shimadzu LC 20 AD/ SPD 20 A/SIL 20 AHT) was equipped with an Apollo® C18 column (5 μ 250 × 4.6 mm, Alltech, Los Altos, CA, USA) and a spectrophotometric detector working at 238 nm. The mobile phase was 45% v/v acetonitrile in water, and the flow rate was set at 1 ml/min with an injection volume of 20 μL. FA standard was dissolved in the mobile phase to prepare FA solutions in the concentration range of 0.01×10^{-2}–1.00×10^{-2}% w/v. The solutions were injected into the HPLC system to construct a standard curve.

Critical Micelle Concentration (CMC) Determination of Surfactants in Cosolvents

CMCs of CPC, SLS, and P80 (cationic, anionic, and nonionic surfactants, respectively) were determined. A series of solutions of each surfactant was prepared in cosolvent systems (PG, PEG, and Gly in water) with concentrations of cosolvents from 0% to 40% w/w. The concentration ranges of CPC, SLS, and P80 were 0–2.50, 0–1.44, and 0–1.05% w/v, respectively. Surface tension of the solutions was measured using a tensiometer (dataphysics DCAT11, Germany) at 25 ± 1°C. A plot of surface tension versus log surfactant concentrations was constructed for each surfactant and the CMC was determined from the breakpoint of the plot. Each experiment was run in triplicate.

Solubility Determination of FA

Solubility determination in cosolvent systems

FA solubility was determined in single and combined cosolvent systems. The single cosolvent systems were composed of Gly, PG or PEG in the range 0–40% w/w and buffered aqueous solution at pH 4 (0.1 M acetate buffer). Combinations of cosolvents containing 1:1 of Gly: PG or Gly: PEG were prepared in the same concentration range in buffered aqueous solution at pH 4. An excess amount of FA (approximately 0.1 g) was added to each cosolvent system, and the sample was sealed in a 10-ml amber glass vial. All vials were rotated at 20 rpm using an end-over-end tube rotator (Glas-Col Laboratory Rotator, Terre Haute, IN, USA) at 25°C for 24 h. The samples were then filtered through disposable membrane filters (0.45 μm 13 mm Nylon, Agela Technologies, Wilmington, DE, USA) and the filtrates were diluted using mobile phase before HPLC analysis.

In Yalkowsky's model, a log-linear relationship between the solubilities and the concentrations of a cosolvent (C) is described by Equation 1:

$$\log S_{mix} = \log S_0 + \sigma C \quad (1)$$

Where S_{mix} and S_0 are the FA solubilities in cosolvent-buffer mixture and buffer solution, respectively. The slope of the plot is the solubilization power (σ) of the cosolvent. Solubility curves of FA in aqueous media containing Gly, PG, or PEG were constructed, and the relationship was determined using Equation 1. The Yalkowsky's model can be extended to multiple water-miscible cosolvents. The log-linear relationship between solubilities and concentrations of combined cosolvents is shown in Equation 2:

$$\log S_{mix} = \log S_0 + \sigma_1 C_1 + \sigma_2 C_2 \quad (2)$$

Where C_1 and C_2 are concentrations of cosolvents in the solvent system.[12] The relationship of FA solubilities and concentrations of Gly: PG or Gly: PEG was determined using Equation 2.

Solubility determination in combined cosolvents containing surfactants

Solvent systems contained 0.05% w/v CPC, 0.1% w/v SLS, or 1% w/v P80 and combined cosolvents in buffered aqueous solution. The combined cosolvents were 1:1 Gly: PG or Gly: PEG and the concentrations were in the range of 0–40% w/w. An excess amount of FA (approximately 0.1 g) was added to each solvent system. The samples were prepared and analyzed as described above and the log-linear relationship of FA solubilities and cosolvent concentrations was determined.

Stability of 0.01% w/v FA Solution Containing Cosolvents and Surfactants

FA stability was determined in combined cosolvent (Gly: PEG, 1:1) with and without surfactants. CPC or P80 was added into 40% w/w combined cosolvents in buffered aqueous solution (pH 4, acetate buffer) to give final concentrations of CPC and P80 of 0.05% and 1.0% w/v, respectively. FA was dissolved in the solvents until a concentration of 0.01% w/v was obtained. The solutions were then kept in aluminum-sealed amber vials in 30°C and 40°C ovens. At multiple time points during storage for 2 months, FA remaining in the samples was analyzed by HPLC and calculated using Equation 3.

$$\%\,\text{Remaining} = \frac{\text{Concentration of FA at sampling time}}{\text{Concentration of FA at initial time}} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

CMCs of Surfactants in Cosolvents

A typical plot of SLS concentration versus surface tension is shown in Figure 1. The breakpoint in the plot represents the CMC of the surfactant. CMCs of CPC, SLS, and P80 with and without cosolvents are shown in Figure 2. The CMCs of CPC, SLS, and P80 in pure water at 25°C were 3.13×10^{-2}, 8.02×10^{-2}, and 0.54×10^{-2}% w/v, respectively.

Surfactant molecules have polar head groups and hydrophobic groups. Aggregation of these molecules into clusters to form so-called micelles occurs when the surfactant concentration reaches CMC. The CMCs of CPC, SLS, and P80 found in the current study are similar to reported values of 3.58×10^{-2} (25°C), 8.36×10^{-2}(20°C), and 0.16×10^{-2}(25°C)% w/v, respectively.[12,14] The small differences between these CMCs values and our results could be due to differences in the determination method and temperature.

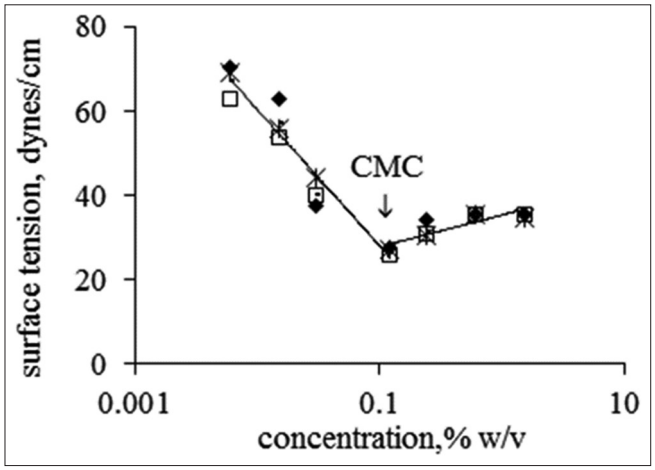

Figure 1: Typical plot for determination of critical micelle concentration of sodium lauryl sulfate in water using the surface tension method. Different symbols indicate separate experiments

The CMCs of all surfactants tended to increase with an increase in the cosolvent concentration. This effect was most clearly observed with charged surfactants (CPC and SLS) in solvents containing PEG and PG. Gly had the least effect on micellization. Higher CMCs of the surfactants were obtained when more cosolvent was added in the aqueous solvents, as also found previously.[15] This may be because the cosolvents decreased water self-association or disrupted water structure and lowered the polarity of the solvent system. Consequently, squeezing out of the surfactant molecules by water molecules would be reduced. In other words, the cosolvents modified solvent-surfactant interactions. Moreover, since the entropy increase on micellization is decreased when the water structure is altered, the driving force to orient hydrophobic groups of the surfactants away from the solvent is reduced, and a higher bulk surfactant concentration is required for micelle formation (i.e., CMC is increased).[16] Based on the dielectric constant (ε) and logarithm of partition coefficient (log K_{ow}), the solvent polarities have the following order: Water (ε = 78.5, log K_{ow} = −4.00)> Gly (ε = 42.5, log K_{ow} = −2.60)> PG (ε = 37.7, log K_{ow} = −1.40)> PEG (ε = 13.6, log K_{ow} = −1.30).[12] Thus, PEG and PG decrease the polarity of the solvent system to a greater extent than Gly and, consequently, greater CMCs were obtained in a solvent containing PEG or PG. The greater effects on ionic surfactants are likely to be due to the cosolvents reducing the dielectric constant of the

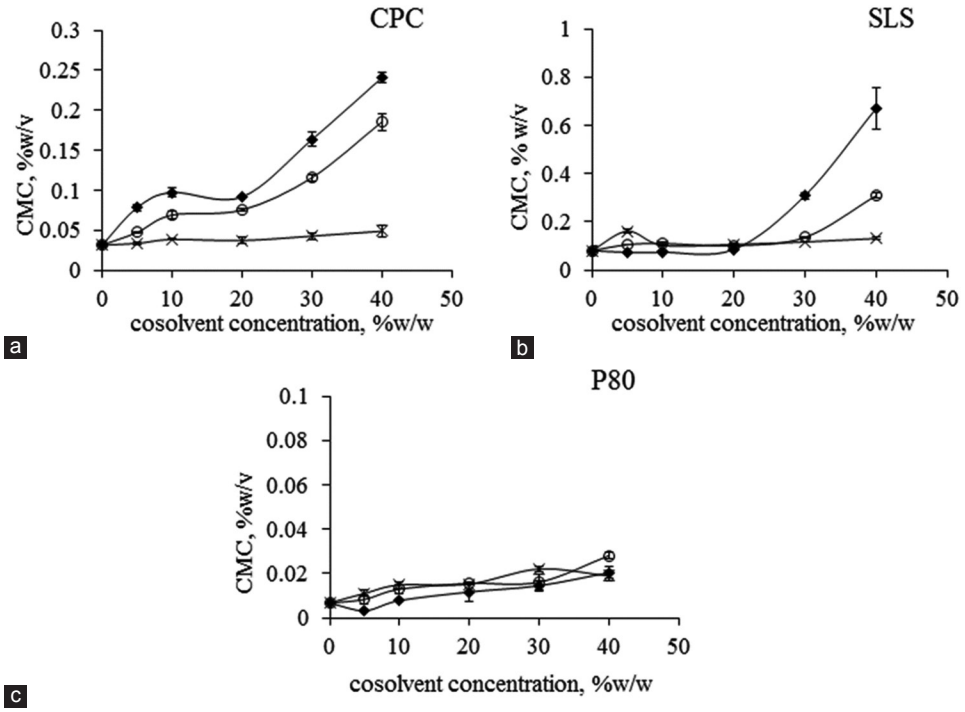

Figure 2: Critical micelle concentrations of (a) cetylpyridinium chloride, (b) sodium lauryl sulfate and (c) P80 in cosolvent systems. ♦PEG, ○PG, ×Gly

aqueous bulk phase, and thus increasing repulsion among the ionic head groups in the micelle, which would lead to reduced micellization and an increase in CMC.[16]

Solubility Determination of FA

Solubility determination in cosolvent systems

The log-linear relationship of FA solubility and cosolvent concentration (Equation 1) is shown in Figure 3, and related parameters (σ and correlation coefficient) are presented in Table 1. The solubility of FA in the buffer was 0.002% w/v, and clear solution of 0.01% w/v FA could be prepared in 22% w/w PEG or 23% w/w PG, as estimated from the log-linear relationship.

The solubility of FA was determined in multiple water-miscible cosolvents. The log-linear relationship between FA solubilities and combined cosolvent concentrations is shown in Figure 4 and Table 1. From this relationship, the solubility of FA at 0.01% w/v was obtained at 32% w/w PG: Gly (1:1) and 30% w/w PEG: Gly (1:1) at 25°C.

FA solubility was enhanced with higher concentrations of cosolvents. The exponential increase in the solubility of FA with a linear increase in cosolvent concentration reflects the non-polar properties of the compound. FA has log K_{ow} of 2.48 and is classified as a nonpolar solute that is insoluble in water.[17] The ability of a cosolvent to dissolve a solute is defined by the solubilization power (σ), which depends on the polarity of the solute and cosolvent. The σ values for PEG and PG were approximately 3 times greater than Gly, indicating that PEG and PG increased the solubility of FA to a greater extent than Gly. The log K_{ow} values of PEG, PG, Gly, and water are −1.30, −1.40, −2.60, and −4.00, respectively. The addition of PEG decreases the polarity of an aqueous system to a greater extent than the addition of PG and Gly. Thus, PEG produced the greatest increase in the solubility of FA. However, the log K_{ow} for PG is close to that of PEG and the σ value for PG was similar to that for PEG.

Since the objective of this study was to prepare a buccal solution of FA, taste of the solution was considered. Among the cosolvents, Gly is sweet, and it is reported to be about 0.6 times as sweet as sucrose.[14] While PEG has a slight but characteristic odor with a bitter, slightly burning taste, PG has a slightly acrid and faintly sweet taste.[14] To decrease the adverse effects and unacceptable taste with a large amount of a cosolvent, a combination of cosolvents was considered for buccal solution preparation. Combination of PEG and PG was dropped off due to its unacceptable bitter and acrid taste.

In the extended Yalkowsky model, the log-linear relationship of combined cosolvents can be calculated using σ_{PEG}, σ_{PG}, and σ_{Gly} obtained from single cosolvent systems. The

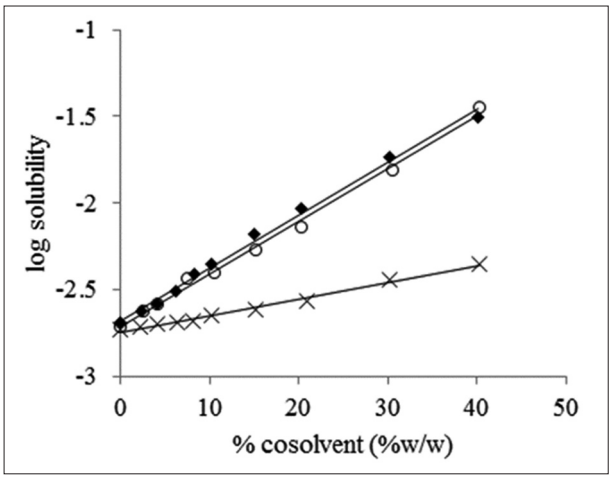

Figure 3: Solubility of fluocinolone acetonide in 0–40% cosolvents in buffered solution. ♦PEG, ○PG, ×Gly

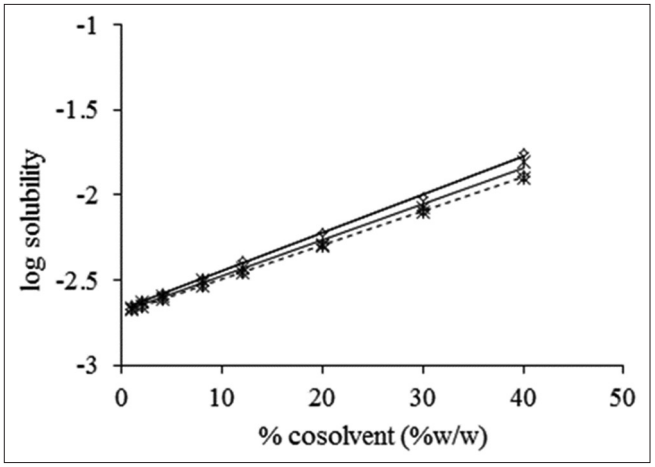

Figure 4: Solubility of fluocinolone acetonide in 0–40% combined cosolvents in buffer solution. ◊Gly:PEG, *Gly:PG (observed data shown as solid lines and calculated values as dashed lines)

Table 1: Log-linear relationship parameters for fluocinolone acetonide solubility and cosolvent concentration

Cosolvent	Concentration range (%w/w)	σ_{exp}	Correlation coefficient (r^2)
PEG	1–40	3.06×10^{-2}	0.995
PG	1–40	3.04×10^{-2}	0.963
Gly	1–40	0.95×10^{-2}	0.991
Gly:PEG (1:1)	1–40	2.24×10^{-2}	0.999
Gly:PG (1:1)	1–40	2.11×10^{-2}	0.995
Gly:PEG (1:1) with 0.05% CPC	1–40	0.76×10^{-2}	0.752
Gly:PEG (1:1) with 0.1% SLS	1–40	1.94×10^{-2}	0.452
Gly:PEG (1:1) with 1% P80	1–40	1.04×10^{-2}	0.988

CPC: Cetylpyridinium chloride, SLS: Sodium lauryl sulfate

calculated solubilities were lower than the experimental values [Figure 4]. This could be due to the total hydrogen bonding in the mixture being less than the predicted level; that is, PEG-Gly-water and PG-Gly-water interactions were less than the average of PEG-PEG, PG-PG, Gly-Gly, and water-water interactions.[12] Therefore, the ability of the solvent systems to dissolve FA was better than expected. PEG-Gly (1:1) was chosen for subsequent work because this mixture enhanced FA solubility more effectively than PG-Gly (1:1).

Solubility determination in cosolvent systems containing surfactants

FA solubilities determined in systems containing cosolvents and surfactants are shown in Figure 5 and Table 1. Without cosolvent, the FA solubilities at 25°C in surfactant solutions of 0.05% CPC, 0.1% SLS, and 1% P80 were 0.69×10^{-2}, 0.27×10^{-2}, and 1.04×10^{-2}% w/v, respectively.

P80 has been used as solubilizing agent at a concentration of 1% w/v.[14] CPC is commonly added in mouthwashes as a bactericidal antiseptic agent and is considered to be a non-toxic compound at a concentration of 0.05% w/v.[14] SLS has also been widely used in mouthwashes and toothpastes, but it has been suggested that SLS should be eliminated from these products due to irritation of skin and mucous membrane to an extent that correlates with the concentration of SLS.[18-21] Products without SLS also prevent or decrease recurrent aphthous stomatitis (RAS) and reduce the pain in RAS.[22-24] Therefore, a low concentration of SLS (0.1% w/v) was added in the cosolvent mixtures.

Surfactants increased FA solubility at concentrations higher than their CMC. The suitable concentration of P80 to enhance the solubility of FA was suggested at 1% w/v which was higher than the CMC of P80 (0.54×10^{-2}% w/v at 25°C).[14] Among these surfactants, since the interaction between the cosolvent and P80 was the least, thus, good linearity of the log-linear relationship between the solubility and the cosolvent concentration in the presence of P80 was retained. As the cosolvent concentration increased, the abilities of the surfactants to enhance solubility were reduced because micellization was less favorable, as shown by the increase of CMCs at high concentrations of cosolvents. When the cosolvent concentration reached the point at which the surfactant concentrations were less than their CMCs, the FA solubilities with and without surfactants were not different, as shown in solvent systems of CPC and SLS at 30% w/w cosolvent at 25°C. The correlation coefficient of the log-linear relationship also decreased in these systems, which may be due to surfactants disrupting the cosolvent structure and the cosolvent disturbing micellization. To ensure that FA did not precipitate during storage, a stability test of 0.01% FA was conducted in a 40% w/w PEG: Gly (1:1) solvent system. The use of SLS was subsequently discontinued because 0.1% w/v SLS provided neither FA solubility enhancement nor antiseptic activity in this cosolvent system, but could induce irritation.

Stability of 0.01%w/v FA Solution Containing Cosolvents and Surfactants

To study the effect of the solvent systems on the FA stability, solutions of 0.01% w/v FA were prepared in combined cosolvents with a surfactant (1% w/v P80 or 0.05% w/v CPC) and without surfactant. There was no precipitation in the solutions throughout the experiment and amount of FA in the solutions was monitored using HPLC. Within 30 days at 30°C, 10% w/v FA degradation was observed in all solvent systems [Figure 6]. FA was clearly more stable in the solvent system without surfactant at 40°C.

Transition metals and hydroxide and hydrogen ions catalyze degradation of corticosteroids through oxidative and hydrolytic reactions.[10] In this study, surfactants accelerated FA degradation, which may be explained by the positive charge on CPC activating degradation of FA, and the P80 microenvironment in the micellar structure facilitating the degradation. Therefore, P80 at 1% w/v increases FA solubility in the presence of cosolvents, but the instability of FA with this surfactant is a drawback. CPC also induces FA degradation and did not provide a solubilization advantage at high concentrations of cosolvents.

CONCLUSION

A solution of 0.01% w/v FA was successfully prepared in 40% w/w Gly: PEG (1:1) in the absence and presence of surfactants. Modification of water structure by cosolvents increased CMCs of surfactants, especially ionic surfactants, and diminished the ability of the surfactants to increase FA

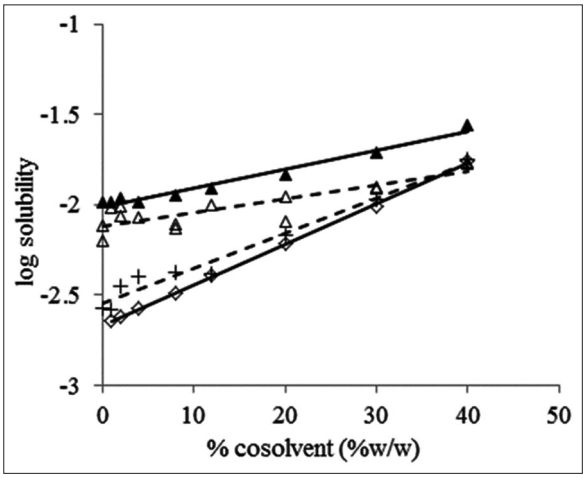

Figure 5: Solubility of fluocinolone acetonide in various concentrations of combined cosolvents (Gly: PEG) with and without surfactant. —▲— 1% P80, - - - Δ - - - 0.05% CPC, - - - + - - - 0.1% sodium lauryl sulfate, —◊— without surfactant

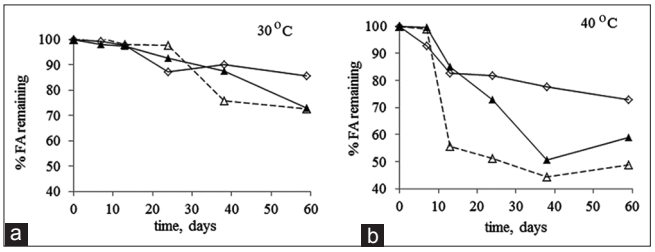

Figure 6: Percent remaining of fluocinolone acetonide in combined cosolvent (Gly: PEG) with and without surfactant. —▲— 1% P80, - - - Δ - - - 0.05% CPC, —◊— without surfactant; (a)30°C, and (b)40°C

solubility. Since both CPC and P80 have shown to induce the degradation of FA as evidenced by <60% remaining of FA after 2-month storage time at 40°C, a 0.01% FA buccal solution should be prepared in a cosolvent system without surfactants.

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