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Preparation and evaluation of a titrated extract of *Centella asiatica* injection in the form of an extemporaneous micellar solution

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Abstract

The micellar solution of titrated extract of *Centella asiatica* (TECA) was formulated by solubilizing TECA into a mixture of propylene glycol and ethoxylated hydrogenated castor oil. An extemporaneous TECA micelle was then successfully prepared by diluting this original micellar formulation with water, saline or isotonic glucose solution. A mixture of propylene glycol and ethoxylated hydrogenated castor oil achieved an acceptable solubilization of TECA up to 180 mg/ml via a formulation of micelle. The drug content of the original micellar formulation stored at 55°C over 60 days was almost constant. The extemporaneous TECA micelle prepared by diluting the original micellar formulation was physically stable for up to 23 h with shaking and 55 h without shaking depending on dilution ratio and medium. The estimated distribution of mean particle size was between 15.9 and 32.6 nm. The osmotic pressure and erythrocytic hemolysis were measured for the formulation with various media. The osmotic pressure of the formulation was found to be much lower than that in a commercially formulated propylene glycol-based injection. The erythrocytic hemolysis by the micellar solution was much more reduced compared with that by the propylene glycol-based preparation. Pain score after the peritoneal injection of the micellar solution was assessed by counting the number of writhe in ICR mice and compared with that in the conventional injection. The injection of extemporaneous TECA micellar solution did not show any single writhe in mice as like as saline. This indicates that pain is reduced dramatically.

These results indicated that a micellar solubilization, followed by an extemporaneous dilution, might be a choice of formulation for TECA injection with reducing the pain arising from the intramuscular injection.

Keywords: *Centella asiatica*; Solubilization; Micellar solution; Extemporaneous dilution; Pain reduction; Writhing test

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1. Introduction

Titrated extract of *Centella asiatica* (TECA) is a poorly water-soluble drug extracted from *Centella asiatica*. TECA is composed of asiaticoside, asiatic acid and madecassic acid (Nakajima and Akiyoshi, 1972; Boiteau et al., 1949), and these components are known to be clinically effective on systemic scleroderma, abnormal scar formation and keloids (Sasaki et al., 1972; Tallat and Abbas, 1971; Kiesswetter, 1964).

However, despite the excellent wound healing properties of TECA, the intramuscular injection of commercial preparation (Madecassol®) causes severe pain. This TECA solution for intramuscular injection is simply formulated by dissolving the TECA in propylene glycol because of poor solubility of TECA in aqueous media. The intramuscular injection of propylene glycol-based preparation produces swelling, pain and stiffness on the injection site due to hypertonicity and tissue damage. Therefore, the patient compliance of TECA injection is low, hindering the clinical use of this drug.

In this study, it is pursued to change the organic solvent-based preparation to the water-based preparation of TECA and optimize it to formulate the TECA injection and reduce the pain caused by the intramuscular injection. A mixture of propylene glycol and ethoxylated hydrogenated castor oil was employed to solubilize TECA via a formulation of micelle and from this original solution an extemporaneous TECA micelle was formulated using saline, isotonic glucose solution or distilled water.

For optimizing the extemporaneous TECA micelle system and determining the maximum solubilizing capacity of the system, the phase behaviour study and the solubility test were performed. And then the physicochemical characteristics such as osmotic pressure, precipitation time, physical and chemical stability were investigated to optimize the formulation. Finally, the erythrocytic hemolysis and writhing test were performed to evaluate the extent of pain arising from the intramuscular injection.

2. Materials and methods

2.1. Materials

Titrated extract of *Centella asiatica* (TECA, 40.4% (w/w) asiaticoside, 57.2% (w/w) asiatic acid and madecassic acid) was provided from Dong-Kook Pharmaceutical (Seoul, Korea) and used as received. Propylene glycol (Yakuri Pure Chemical, Osaka, Japan), glucose (Sigma, St. Louis, MO, USA) and sorbitol (Junsei Chemical, Tokyo, Japan) were of first grade and used without further purification. Ethoxylated hydrogenated castor oil (HCO-30, Nikkol, Tokyo, Japan) and propylene glycol-based TECA solution (Madecassol®, Laroche Navarron, Paris, France) were used. ICR mice were obtained from the Experimental Animal Breeding Center of Seoul National University (Seoul, Korea).

2.2. Solubilization of TECA

Propylene glycol was blended with ethoxylated hydrogenated castor oil (HCO-30) in various mass ratios and TECA was added into the propylene glycol/HCO-30 blends. The phase behaviour of these three component-system was observed, and the phase diagram was constructed. The solubility of TECA in the blend solution was measured by analysing the content of TECA.

2.3. Preparation of TECA micelle

Based on the phase diagram and the solubility data, the TECA micelle was prepared as follows. In brief, TECA was mixed with propylene glycol and HCO-30 and dispersed with a vortex mixing. The TECA mixture was then sonicated at 40°C for 2–3 h until the solution becomes transparent and forms micelles. The TECA micellar solution for physicochemical and biological characterization is composed of 100 mg of TECA, 300 mg of HCO-30 and 600 mg of propylene glycol. A TECA extemporaneous micelle was prepared by diluting this original solution with distilled water or one of 0.9% NaCl, 5.0% glucose and 5.5% sorbitol aqueous solutions.

2.4. Analysis of drug content

The amount of asiaticoside (as reference) in the original micelle solution consisting of TECA, propylene glycol and HCO-30 was measured. The samples kept at 4, 21 and 55°C were analyzed after 0, 15, 30 and 60 days by HPLC equipped with a UV detector at 214 nm. For the HPLC analysis, 1 g of TECA micelle solution was diluted with 1000 ml of methanol. The mixture of acetonitrile, methanol and distilled water (1:1:2) was prepared as a carrier phase. The injection volume was 20 μl and the flow rate was 1.0 ml/min. The μ-Bondarpak C_{18} column (3.9 × 300 mm, 10 μm particle size, Water Associates, Milford, MA, USA.) was used.

2.5. Droplet size measurement

The size distribution and the average droplet diameter of TECA micelle were measured at 26°C with varying dilution ratio by a dynamic light scattering method. The droplet size of extemporaneous TECA micelle was also observed with designated time interval using a laser particle analyzer (LPA-3000, Otsuka Electronics, Kyoto, Japan).

2.6. Precipitation test

The precipitation test of TECA extemporaneous micelle solution was performed by visual observation of samples with various aqueous media and with varying dilution ratio. The samples were kept either in the shaker of the water bath or in the air at room temperature and then the precipitation time was recorded.

2.7. Osmotic pressure and erythrocytic hemolysis measurement

The osmotic pressure of TECA micelle was measured by microosmometer (Precision Systems, Natick, MA, USA) for various aqueous media and compared with those of the propylene glycol-based preparations of TECA (Madecassol®) and propylene glycol by itself.

The erythrocytic hemolysis of TECA micellar solution was studied. The human erythrocytes (2.5%, v/v) were dispersed in an isotonic phosphate buffer. Two milliliters of this solution was added to 2 ml of TECA extemporaneous micellar solution which was diluted with saline and then incubated in a water bath at 37°C for 40 min. The incubated solution was then centrifuged at 250 × g for 10 min. The hemolyzed upper solution was separated, diluted with an isotonic phosphate buffer and then spectrophotometrically measured at 545 nm. The absorption of a distilled water, treated in the same way as TECA micellar solution, was also measured. The %hemolysis was calculated as follows (Ansel and Carbe, 1970):

$$\%\text{Hemolysis} = \frac{\text{The absorbance of sample}}{\text{The absorbance of distilled water}} \times 100$$

2.8. Writhing test

The writhing test (Parreca et al., 1987) in ICR mice was performed to obtain pain score for TECA extemporaneous micellar solution and propylene glycol-based TECA solution. Phenylquinone was known to cause pain when injected intraperitoneally and produce a constant number of writhes in mice depending on its injection amount. Thus, for control experiment, phenylquinone (4.5 mg/kg) was injected into the peritoneal cavity of a group of ICR mice and writhes/10 min were counted as an indication for pain. In another group of mice, morphine (5 mg/kg) was also injected subcutaneously 30 min prior to the administration of phenylquinone to make sure if the number of writhes by phenylquinone was reduced by morphine pretreatment. For test groups, TECA extemporaneous micellar solution diluted with saline (100 mg of TECA/ml) or propylene glycol-based TECA solution was injected to peritoneal cavity of ICR mice and writhes due to the injection per 10 min in each group was counted. The number of writhes due to TECA extemporaneous micellar solution (0.34 mg/kg) was compared with that obtained by propylene glycol-based TECA solution.

3. Results and discussion

3.1. Solubilization and formulation

In order to obtain the solubilized TECA system, the phase behaviour of TECA/propylene glycol/HCO-30 system was examined. The ternary phase diagram is shown in Fig. 1. The most of area in the phase diagram showed the suspension phase along with some drug-precipitation (marked P). The system with small amount of TECA produced a transparent and homogeneous solution (indicated as L). Nontransparent suspension area is also observed and marked S. The micellar solution phases (indicated as M) are found mostly in the region with 10–75% (w/w) of HCO-30 and low concentrations of TECA. The solutions represented as X, Y, and Z (Fig. 1) were selected and diluted with saline solution (micellar solution:saline solution = 9:1) in order to investigate the droplet size. The average diameters for diluted solutions of X, Y, and Z components were 15.9 ± 3.37, 16.7 ± 3.32 and 33.1 ± 6.97 nm, respectively.

The solubility of TECA in the propylene glycol/HCO-30 system was measured and plotted in Fig.

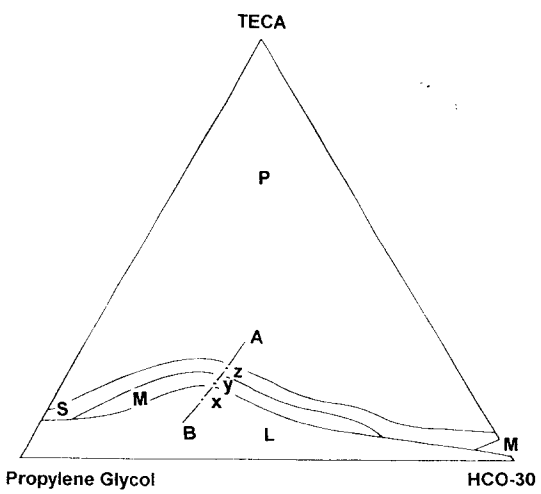

Fig. 1. Phase diagram of propylene glycol/HCO-30/TECA system showing transparent homogeneous solution (L), translucent homogeneous solution (M), nontransparent suspension (S) and suspension with drug precipitation (P) regions at 20°C.

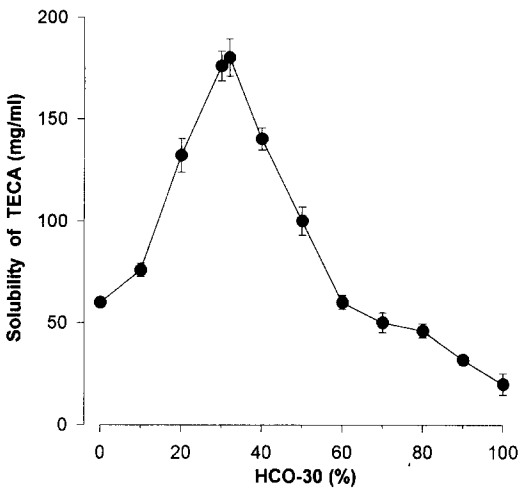

Fig. 2. Solubility of TECA in the mixture of propylene glycol and HCO-30.

2. The solubility of TECA in distilled water, propylene glycol and HCO-30 was 0.1, 60 and 22 mg/ml, respectively. As shown in Fig. 2, the solubility of TECA in the mixture of propylene glycol and HCO-30 increases as increasing the amount of HCO-30, reaches the maximum value when the ratio of propylene glycol/HCO-30 is about 7/3 and then decreases as increasing the amount of HCO-30 more than 30% (w/w) in the system. The maximum solubility of TECA attained was 180 mg/ml in the mixture containing 67% (w/w) propylene glycol and 33% (w/w) HCO-30. Thus, compared to the formulation using propylene glycol alone, this system could increase the solubility three fold higher. Based on the phase diagram and the solubility data, the TECA micellar solution was formulated with 100 mg of TECA, 300 mg of HCO-30 and 600 mg of propylene glycol for following examinations.

3.2. The drug content and the size distribution

There were no significant changes in the percentage drug content (asiaticoside) of TECA micellar formulation kept at 4, 21 and 55°C after 60 days. Even though the sample kept at 55°C, the drug content was almost constant after 60 days.

Generally, the dispersed system as a drug carrier induces the embolism and does not deliver the

Table 1
The mean diameter of TECA micelle with various dilution ratios ($n = 3$)

Dilution ratio[a]	Diameter (nm)
3	32.6 ± 7.37[b]
6	22.6 ± 5.52
12	21.6 ± 4.53
18	24.1 ± 4.38
21	20.6 ± 3.91

[a] Vol. of saline/1 g of TECA in ml.
[b] The values represent the mean ± S.D.

drug to the target area unless the size of the dispersed component is less than 2 μm, since the system is accumulated to the reticuloendothelial system (RES) by the phagocytosis (Tarr et al., 1987; Boyett and Davis, 1989). As shown in Table 1, the droplet size did not much change with increasing the volume of diluents except for the sample with the low dilution ratio. However, the mean diameter was slightly increased with respect to time as shown in Table 2. But the mean diameter increased after 4.5 h and the size distribution range was gradually broadened.

3.3. Measurement of precipitation time

The original micellar formulation was diluted with various dilution ratios and then the precipitation time was observed. The results are summarized in Table 3. Depending on the formulation, the system was stable against the precipitation for up to 55 h. As increasing the amount of aqueous phase, the precipitation occurs slowly since the molar volume of solute in the system decreases (Fahelelbome et al., 1993). When the micelles contact the aqueous phase, the activity of surfactant is affected by the distance of the drug molecule and reactivity (Florence, 1981).

Table 2
The change of mean diameter and particle size range of TECA micelle with respect to time ($n = 3$)

Time (h)	Mean diameter (nm)	Size range (nm)
0	15.9 ± 3.37[a]	13–22
1.5	17.8 ± 3.28	13–34
3.0	19.8 ± 5.58	14–48
4.5	32.5 ± 6.36	26–66

[a] The values represent the mean ± S.D.

The surfactant dispersed in propylene glycol solubilizes the drug in the hydrophobic part of micelle and the system of TECA micelle and external aqueous phase equilibrates. It is considered that the precipitation occurs as the surfactant moves from the solvent phase to the aqueous phase with some drug and the aqueous phase is saturated (Strickley and Anderson, 1993). Saline, sorbitol and glucose solution as aqueous phases show the longer precipitation time, compared with a distilled water, due to the decreased critical micelle concentration of surfactant in these solutions (Attwood and Florence, 1989). For the sample kept with shaking, the precipitation accelerated since the molecules move irregularly and collide more (Frieberg et al., 1989). Nevertheless, the extemporaneous TECA micelle prepared by diluting the original micellar formulation with shaking was physically stable for up to 23 h depending on diluting ratio and medium.

3.4. The osmotic pressure and the erythrocytic hemolysis of TECA micellar solution

The osmotic pressures of TECA micelle, propylene glycol-based TECA solution and propylene glycol alone diluted with various aqueous media are plotted as a function of dilution ratio in Fig. 3. As increasing the dilution ratio, the osmotic pressure decreases. The osmotic pressures of saline and red blood cell in serum are 308 and 306 mΠ/kg, respectively.

By the extrapolation in Fig. 3, the osmotic pressure of the propylene glycol-based preparation of TECA or propylene glycol is expected to be about 3000–4000 mΠ/kg. In order to prevent the pain occurring from the injection, the osmotic pressure of the formulation should be reduced. The ideal osmotic pressure of the injectable solution is 250–350 mΠ/kg, but it is hardly achieved in the formulation of insoluble drugs (Demorest, 1984). As shown in Fig. 3, the osmotic pressure of TECA micellar formulation is markedly lower than that of the diluted propylene glycol-based preparation of TECA or diluted propylene glycol.

Table 3
The precipitation time for various formulations of TECA extemporaneous micelle with and without shaking (h, $n = 3$)

Dilution ratio[a]	Aqueous phase							
	Distilled water		Saline		5.5% Sorbitol		5% Glucose	
	With	Without	With	Without	With	Without	With	Without
3	0.5 ± 0.15[b]	0.6 ± 0.14	0.5 ± 0.05	0.5 ± 0.16	0.8 ± 0.16	1.0 ± 0.33	0.8 ± 0.12	0.5 ± 0.12
6	0.8 ± 0.08	1.5 ± 0.38	1.0 ± 0.06	1.3 ± 0.36	1.5 ± 0.76	1.5 ± 0.21	1.3 ± 0.21	1.8 ± 0.13
9	1.3 ± 0.15	3.5 ± 0.25	2.1 ± 0.41	3.3 ± 0.84	1.8 ± 0.23	5.5 ± 1.25	2.5 ± 0.27	3.3 ± 0.31
12	1.0 ± 0.14	8.0 ± 2.94	3.0 ± 0.62	5.0 ± 0.26	2.3 ± 0.18	23.0 ± 7.05	2.8 ± 0.18	11.3 ± 3.51
15	2.5 ± 0.38	10.5 ± 3.44	3.5 ± 0.25	25.3 ± 5.92	3.0 ± 0.26	13.0 ± 2.97	3.8 ± 0.32	15.5 ± 4.42
18	3.0 ± 0.42	11.0 ± 4.38	22.0 ± 6.84	28.0 ± 8.17	18.3 ± 4.26	31.5 ± 5.90	13.3 ± 2.36	29.3 ± 10.93
21	3.5 ± 0.18	17.5 ± 5.71	22.3 ± 5.75	55.3 ± 17.52	17.8 ± 3.49	48.8 ± 12.74	23.3 ± 4.28	46.5 ± 11.63

[a] The amount of aqueous phase added to 1 g of TECA original micellar solution (ml).
[b] The values represent the mean ± S.D.

The osmotic pressure of 1 g of TECA micellar solutions diluted with 9 and 12 ml of distilled water are about 650 and 460 mΠ/kg, respectively. These results implicate that TECA extemporaneous micellar solutions make much less swelling, pain and stiffness on the injection site compared with propylene glycol-based preparation of TECA.

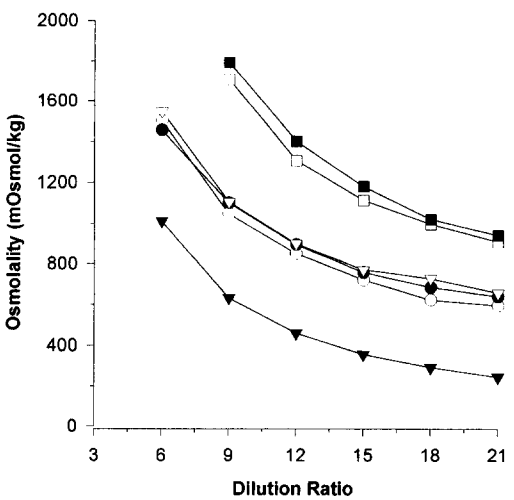

Fig. 3. Osmotic pressure of various formulations. Propylene glycol-based TECA solution diluted with distilled water (■), propylene glycol diluted with distilled water (□), TECA micelle diluted with 5.5% sorbitol solution (▽), 5% glucose solution (●), normal saline (○), and distilled water (▼).

The erythrocytic hemolysis of TECA micellar solution, propylene glycol-based preparation of TECA and saline is shown in Table 4. If the hemolysis occurs, the oxyhemoglobin drains out and the amount of oxyhemoglobin is linear to the number of blood cells hemolyzed (Cadwallader and Ansel, 1965). Standardizing the erythrocytic hemolysis of distilled water as 100%, propylene glycol-based preparation of TECA and propylene glycol alone accelerate the hemolysis much greater than distilled water under the current experimen-

Table 4
The erythrocytic hemolysis of TECA micellar solutions, propylene glycol-based TECA solution and saline

Solution	Dilution Ratio[a]	%Hemolysis
Distilled Water	—	100
Saline	—	3.2
Propylene glycol	—	>100
Propylene glycol-based TECA solution	—	>100
TECA micelle solution	3	10.8
TECA micelle solution	6	7.2
TECA micelle solution	9	6.3
TECA micelle solution	12	5.8
TECA micelle solution	15	4.8
TECA micelle solution	18	5.0
TECA micelle solution	21	4.5

[a] The amount of saline added to 1 g of TECA original micellar solution (ml).

tal condition. However, the hemolysis of TECA micellar solutions was dramatically reduced compared with those of propylene glycol-based TECA solution or distilled water. The hemolysis of TECA micellar solution was 4–11%, which was approached to the hemolysis of saline (3.2%). These results indicate that TECA extemporaneous micellar solution make much less damage of erythrocyte membrane compared with TECA solution using propylene glycol alone as a solvent.

3.5. Writhing test

The writhing test (Parreca et al., 1987) in mice has been developed and used to test analgesics by counting the number of writhes/unit time induced by a certain treatment. Many algogenic substances such as acetic acid or phenylquinone are used to evoke a peculiar pain reflex, writhe. This pain reflex is adopted not only for analgesic response but also hyperalgesic response like many other pain reflex tests such as tail-flick test or paw-withdrawal test. For example, hyperalgesia can be elicited in writhing test by the intraperitoneal injection of prostaglandins (Akarsu et al., 1989; Smith et al., 1985), bradykinin (Emele and Shanaman, 1963) or endothelin (Raffa and Jacoby, 1991). Similarly, in the present study we injected propylene glycol-based TECA solution intraperitoneally to determine if this form of the solution was painful.

The pain score after the injection of TECA micellar solution was assessed by a writhing test and compared with that of propylene glycol-based TECA solution. To address whether the writhing test is suitable for tests for hyperalgesic response, phenylquinone (4.5 mg/kg), a conventional pain causing substance, was injected intraperitoneally. As shown in Table 5, the intraperitoneal injection of phenylquinone produced a high incidence of writhe. In other group of mice, morphine (5 mg/kg) was injected subcutaneously 30 min prior to the phenylquinone injection. The pretreatment of morphine prevented the writhing response to phenylquinone in mice (Table 5). Similar protocol was used to test whether injection of propylene glycol-based TECA was hyperalgesic. As shown in Table 5, the intraperitoneal injection of propylene glycol-based TECA solution produced an appreciable number of writhes in mice, indicating that the solution was hyperalgesic. In contrast, injection of TECA extemporaneous micellar solution did not show any single writhe in mice. For a control test, saline in the same volume was injected intraperitoneally. The saline injection did not exhibit a single writhe in mice (Table 5). Thus, these results clearly indicate that extemporaneous micellar form of TECA solution is much less painful than propylene glycol-based TECA solution (Madecassol®).

Table 5
Writhing test ($n = 6$)

Drugs	Number of Writhes
Phenylquinone[a]	36.75 ± 8.19[e]
Phenylquinone + Morphine[b]	0
Saline	0
Propylene glycol-based TECA solution[c]	10.25 ± 2.64
Extemporaneous TECA micelle[d]	0

[a] Peritoneal injection of phenylquinone (4.5 mg/kg).
[b] Subcutaneous injection of morphine (5 mg/kg) 30 mm prior to phenylquinone injection.
[c] Peritoneal injection of TECA (0.34 mg/kg).
[d] Original TECA micellar solution was diluted with saline (100 mg of TECA/ml).
[e] The values represent the mean ± S.D.

4. Conclusion

In conclusion, the mixed system of propylene glycol and HCO-30 could improve the solubility of TECA (180 mg/ml). The TECA in micelle was chemically stable and also physically stable for up to 23 h. The average diameter of TECA extemporaneous micellar solution was less than 40 nm that was acceptable size for injection. The extemporaneous TECA micellar solutions take place much lower osmotic pressure and make much less erythrocyte hemolysis and pain compared with propylene glycol-based preparation of TECA. The present study suggests that the micellar solubilization, followed by an extemporaneous dilution, might be a choice of formulation for TECA injection with reducing the pain arising from the intramuscular injection.

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References

Akarsu, E.S., Ayhan, I.H. and Palaogu, O. Iloprost-induced writhing in mice and its suppression by morphine. *Methods Find. Exp. Clin. Pharmacol.*, 11 (1989) 273–275.

Ansel, H.C. and Carbe, G.E.J. Influence of antimicrobial preservatives I. *J. Pharm. Sci.*, 59 (1970) 478–481.

Attwood, D. and Florence, A.T., *Solibilization in Surfactant Systems*, Chapman and Hall, London, 1989, pp. 229–292.

Boiteau, P., Buzas, A., Lederer, E. and Polonsky, J. Chemical constitution of asiaticoside, a heteroside formerly used again leprosy. *Bull. Soc. Chim. Biol.*, 31 (1949) 46–51.

Boyett, J.B. and Davis, C.W. Injectable emulsions and suspensions. In Lieberman, H.A., Rieger, M.M. Banker and G.S. (Eds.), *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 2, Marcel Dekker, New York, 1989, pp. 379–397.

Cadwallader, D.E. and Ansel, H.C.J. Hemolysis of erythrocytes by antibacterial preservatives II. *J. Pharm. Sci.*, 54 (1965) 1010–1012.

Demorest, L.J. Formulation for large volume parenterals. In Avis, K.E., Lachman, L and Lieberman, H.A. (Eds.), *Pharmaceutical Dosage Forms: Parenteral Medications*, Vol. 2, Marcel Dekker, New York, 1984, pp. 55–84

Emele, J.F. and Shanaman, J. Bradykinin writhing: a method for measuring analgesia. *Proc. Soc. Exp. Biol. Med.*, 114 (1963) 680–682.

Fahelelbome, K.M.S., Timoney, R.F. and Corrigan, O.I. Micellar solubilization of clofazimine analogues in aqueous solutions of ionic and nonionic surfactants. *Pharm. Res.*, 10 (1993) 631–634.

Florence, A.T. Drug solubilization in surfactant system. In Yalkowsky, S.H. (Ed.), *Techniques of Solubilization of Drugs*, Marcel Dekker, New York, 1981, pp. 15–87.

Frieberg, S.E., Goldsmith, B. and Hilton, M.L. Theory of emulsions. In Lieberman, H.A., Rieger, M.M. and Banker, G.S. (Eds.), *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Marcel Dekker, New York, 1989, pp. 49–91.

Kiesswetter, H. Treatment of wounds with asaticoside (Madecassol). *Wien. Med. Wochenschr.*, 114 (1964) 124–126.

Nakajima, H. and Akiyoshi, K. Studies on the treatment of keloids and hypertrophic scars with madecassol. *Med. Consultat. New Remedy*, 9 (1972) 433–444.

Parreca, F., Mosberg, H.I., Omnaas, J.R., Burks, T.F. and Cowan, A. Suprapinal and spinal potency of selective opioid agonists in the mouse writhing test. *J. Pharmacol. Exp. Ther.*, 240 (1987) 890–894.

Raffa, R.B. and Jacoby, H.I. Endothelin-1, endothelin-2 and endothelin-3 directly and big-endothelin-1 indirectly elicit an abdominal constriction response in mice. *Lift Sci.*, 48 (1991) 85–90.

Sasaki, S., Shinkai, H., Akashi, Y. and Kishihara, Y. Studies on the mechanism of action of asiaticoside (Madecassol®) on experimental granulation tissue and cultured fibroblasts and its clinical application in systemic scleroderma. *Acta Dermatovenerolog. (Stockholm)*, 52 (1972) 141–150.

Smith, T.W., Follenfant, R.L. and Ferreira, S.H. Antinociceptive models displaying peripheril opioid activity. *Int. J. Tiss. React.*, 7 (1985) 61–67.

Strickley, R.G. and Anderson, B.D. Solubilization and stability of an anti-HIV thiocarbamate, NSC 629243, for parenteral delivery, using extemporaneous emulsions. *Pharm. Res.*, 10 (1993) 1076–1082.

Tallat, H.A. and Abbas, M.E. The effect of titrated extract of *Centella Asiatica* on abnormal scar formation. *J. Egypt. Surg. Soc.*, 6 (1971) 408–419.

Tarr, B.D., Sambandan, T.O. and Yalkowsky, S.H. A new parenteral emulsion for the administration of taxol. *Pharm. Res.*, 4 (1987) 162–165.