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EMULSIONS OF OIL FROM *Adenanthera pavonina* L. SEEDS AND THEIR PROTECTIVE EFFECT

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Abstract: In our previous study, we developed very stable formulations of submicron oil-in-water emulsions from *Adenanthera pavonina* L. (family Leguminosae, subfamily Mimosoideae) seed oil, stabilised with soybean lecithin (SPC). Continuing our research, we introduced an additional co-emulsifier, Tween 80, to those formulations in order to decrease the size of the emulsion particles and improve their stability. Formulations with a mean particle size ranging from 43.6 to 306.5 nm and a negative surface charge from -45.3 to -28.5 mV were obtained. Our stability experiments also revealed that most of the tested formulations had a very good degree of stability over a 3-month storage period, both at 4ºC and at room temperature.
Since many intravenous injectable drugs exhibit lytic activity against erythrocytes, we examined this activity for the emulsion form of cardol, a natural compound with already proven hemolytic properties. The incorporation of this agent into the emulsion caused an evident decrease in hemolytic activity (97-99%). This highly protective effect, observed against sheep erythrocytes, was independent of both the composition and the particle size of the emulsions used. Our studies suggest that nonionic surfactant/phospholipid-based emulsions containing this edible oil of *A. pavonina* L. may be useful as an alternative formulation matrix for pharmaceutical, nutritional or cosmetic applications of otherwise membrane-acting components.

Key words: *Adenanthera pavonina* L., Submicron oil-in-water emulsion, Hemolysis, Cardol

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Abbreviations used: H – hemolysis; PI – polydispersity index; SPC – soybean lecithin

INTRODUCTION

At present, there is a huge renewed interest in the lipid emulsions of vegetable oils for the delivery of drugs. Emulsions are defined as dispersions of one liquid inside another, where those liquids are immiscible. Submicron lipid emulsions are biocompatible, biodegradable and physically stable. They minimize the side effects of the incorporated drugs and can easily be produced on a large scale. The drug-induced hemolysis of erythrocytes can also be avoided via incorporation into a lipid emulsion [1]. In our previous study, we demonstrated that the oil from the seeds of the tropical deciduous tree *Adenanthera pavonina* L. (Leguminosae) could be used as a valuable matrix for forming stable lipid emulsions [2]. *A. pavonina* L. is endemic to southern China and India. It has been widely naturalized in Malaysia, western and eastern Africa, and on most islands of both the Pacific and Caribbean regions. Extracts from the seeds of this extensively cultivated plant are under investigation in numerous laboratories [3-5], and the anti-inflammatory and analgesic effects of the *A. pavonina* extract have been demonstrated [6]. *A. pavonina* is also known as a food tree, because its seeds are often eaten by people. Nutritional studies showed that one quarter of the seed is oil with a high percentage of proteins [7]. Instrumental analyses of this oil revealed a lack of hemolytic compounds like polyamines or resorcinolic lipids. A further detailed examination of the properties (particle size, Zeta potential values, stability over time) of emulsions from oil from *A. pavonina* indicated that the tested oil could be used as a carrier of active ingredients [2].

Submicron oil-in-water (o/w) emulsions, stabilised mainly by phospholipids, have been in use for many years. They have important potential as carriers of drugs that are insoluble in the aqueous phase. Several drugs have already been successfully introduced into emulsions and are commercially available, including diazepam (Diazemuls®), propofol (Diprivan®) and cyclosporin A (CIPOL Inj.®, Sandimmun Neoral®). Other poorly water-soluble drugs have been formulated in o/w emulsions and are currently being extensively studied, including such drugs as paclitaxel [8-10] and cyclosporin A [11]. Emulsions with phosphatidylcholine as the major emulsifier have also been widely used as a source of energy in parenteral nutrition (fat emulsions). Phospholipids obtained from soybeans are generally used in the formulation of submicron emulsions, liposomes and mixed micelles for intravenous drug administration. The non-ionic surfactant Tween 80 is another potent emulsifier exploited in many pharmaceutical preparations.

Lipid emulsions are well accepted as intravenous delivery systems due to their ability to incorporate hydrophobic drugs or compounds of poor water solubility. This is very important: many newly developed drugs are poorly water-soluble as this reduces side effects. Submicron emulsions are also used in oral drugs to increase bioavailabity and to prolong the pharmacological effects of drugs with poor oral absorption or a short biological half-life, or in ocular delivery systems

[12, 13]. Direct contact of the drug with body fluids and tissues can be avoided, which means a minimization or significant decrease in side effects.
The erythrocyte toxicity assay is especially important in the evaluation of the safety of a given emulsion. Inducing erythrocyte hemolysis *in vitro* is a simple and reliable measure for estimating the membrane damage that may occur *in vivo*. The behaviour of emulsions *in vivo* can be predicted by investigating the degree of hemolysis in suspensions of isolated erythrocytes [14].
The aim of this study was to investigate the correlation between various ratios of the non-ionic emulsifier Tween 80, soybean lecithin and *A. pavonina* seed oil, and the physicochemical properties of those emulsions. These experiments allowed us to optimise the appropriate concentrations of the emulsifiers in order to obtain as low as possible size ranges of particles (around 50-300 nm), to guarantee an ameliorated tissue tolerance, uptake and transfer, and no foreign body reactions. As larger particles may cause embolism, the particle size distribution of parenteral fat emulsions is a critical factor for patient safety. We also report on the effects of emulsification upon the hemolytic behaviour of the model compound. The hemolytic activity of cardol, a highly lytic resorcinolic lipid [15], was compared in free and emulsion formulations. The extent of hemolysis induced by various emulsion formulations with or without cardol, prepared with various amounts of emulsifying agents, was assessed. The minimization of possible toxic side effects is of clinical relevance.

MATERIALS AND METHODS

Materials

Polyoxyethylene-(20)-sorbitan monooleate (Tween 80), glycerol and sorbitol were obtained from Sigma-Aldrich, Poznan, Poland. Soybean lecithin (Lipoid S75) was supplied by Lipoid, Ludwigshafen, Germany. Cardol (1,3-dihydroxy-5-*n*-pentadecenylbenzene) was isolated from technical cashew nut shell liquid (CNSL) via normal-phase chromatography on a silica gel column equilibrated with chloroform/ethyl acetate (90:10, v/v) [16]. Acrodisc® filters were provided by Gelman Sciences, Ann Arbor, MI. All the other reagents used were of the highest commercially available quality (POCh, Gliwice, Poland). Deionized double distilled water was used for all the experiments.

Plant material and extraction

Mature seeds of *Adenanthera pavonina* were collected in the Kandy district of Sri Lanka. The extraction was performed as described elsewhere [17]. A volume of acetone sufficient to soak a 750-g sample of seeds completely was used. The extraction was carried out three times for 24 h at room temperature each time using a fresh solvent. The obtained extracts were combined, filtered through filter paper to remove any solid contaminating particles, and concentrated under reduced pressure. This procedure yielded about 30 ml of the oil, which was stored at -20ºC for further laboratory analyses.

Preparation of emulsion
Emulsions were prepared as described previously [2]. Appropriate amounts of soybean lecithin and Tween 80 were dissolved in 50 mg of oil extracted from *A. pavonina* seeds. For the hemolytic experiments, the oily phase contained dissolved resorcinolic lipid (cardol). 2 ml of an aqueous phase consisting of 2.25% (w/v) glycerol or 4.5% (w/v) sorbitol (for adjustment to isotonicity) were added and vigorously mixed with the oil phase, then gently heated and vortexed for 5 min. The sample was further sonicated with an ultrasonic cell disruptor (Microson™) for 10 min at 4 W. The sonicated preparations were filtered through sterile Acrodisc® 0.45 μm filters. All the emulsion preparations were prepared at room temperature.

Evaluation of the stability of the formulations
The formulations were protected from light, and submitted to a 3-month long stability test at 4ºC and at room temperature. Determinations of the size of the emulsions were made at 14-day intervals.

Measurements of particle size and Zeta potential
The mean particle size, polydispersity index (PI) and surface charge (Zeta potential) of the emulsion dispersions were determined using a ZetaSizer 5000 (Malvern, Warsaw, Poland) and PCS software. The analysis of particle size and PI, determined by photon correlation spectroscopy, was done using the volume distribution algorithm. The polydispersity index qualifies the particle size distribution, which here ranged from 0 for monodispersed to 1.0 for entirely heterodispersed emulsions. In order to obtain the optimum light scattering intensity and for the size analysis, approximately 15 μl of the emulsion was added to 2 ml of 2.25% aqueous glycerol solution. For the surface charge (Zeta potential) determination, 3 μl of the emulsion was diluted with 2 ml of 2.25% aqueous glycerol solution. All the measurements were carried out at 25ºC.

Preparation of erythrocyte suspensions
Freshly collected blood from a healthy sheep was mixed with anticoagulant solution (74.8 mM sodium citrate/38.07 mM citric acid/124.3 mM glucose 1:1.19 v/v) and centrifuged at 650 × *g* for 10 min. The supernatant was discarded and the erythrocytes were resuspended in phosphate buffered saline (PBS; 5 mM phosphate, 150 mM NaCl, pH = 7.4). Next, the erythrocytes were washed three times with an isotonic buffer (PBS), and the upper phase with a buffy coat containing precipitated debris and serum proteins was carefully removed at each wash step. After the last washing, the packed cells were suspended in a buffer to a hematocrit of 50%. All the erythrocyte suspensions used in the experiments were prepared daily.

Determination of hemolytic activity
To determine the hemolytic effect, 700 μl of each emulsion containing cardol in 4.5% sorbitol was added to 20 μl of erythrocyte suspension (50% hematocrit)

and adjusted to a 4-ml volume with PBS. The final concentration of resorcinolic lipid was 100 μM in all the samples. The samples were stirred and incubated for 30 min at 37ºC. Debris and intact erythrocytes were removed by centrifugation at 650 × *g* for 10 min. The hemoglobin released into the supernatant was detected spectrophotometrically at 540 nm against a corresponding blank sample.

The hemolytic effect, measured as the percentage of hemolysis (H), was determined on the basis of released hemoglobin, according to the following formula:

$$H\,[\%] = \frac{A_s - A_{c1}}{A_{c2} - A_{c1}} \times 100$$

where *A* is the absorbance, *s* is for the sample, *c1* is for the mechanical hemolysis (erythrocytes in PBS), and *c2* is for 100% hemolysis (erythrocytes in double distilled water). Control emulsions were prepared under identical experimental conditions, but without cardol.

RESULTS AND DISCUSSION

In this study, we examined the correlation between various ratios of non-ionic emulsifier (Tween 80), soybean lecithin and A. *pavonina* seed oil and the physicochemical properties of the emulsions obtained. Fig. 1 demonstrates how the different compositions of those emulsions affected the mean particle size and polydispersity index (PI) values (pH in the range 5.6-6.0). According to our previous report [2], the particle size in emulsions containing 2.4% (w/w) oil from A. *pavonina* seeds and 0.7% (w/w) soybean lecithin was 209.8 nm. In this study, we used a formulation containing the same amount of oil, but stabilised with the same dose of the non-ionic detergent Tween 80, which had a substantially larger size of about 306.5 nm (Fig. 1). This may indicate that soybean lecithin has better stabilizing properties for this type of formulation.

The presence of both emulsifiers resulted in an evident decrease in the emulsion size and PI. This was explained as the result of the formation of a closely packed mixed film by intercalation of the non-ionic surfactant molecules between phospholipid monolayers [18, 19]. For emulsions stabilised with a fixed amount of Tween 80 (0.7% w/w), the introduction of larger doses of SPC (0.9 or 1.9% w/w) led to a decrease in the particle diameter (52.1 and 44.6 nm, respectively). When both emulsifiers were present at equal concentrations (0.9/0.9% w/w), the resulting colloidal carrier possessed the smallest of the sizes observed – only 43.6 nm. For all the described formulations containing 0.7-0.9% w/w Tween 80 and 0.9-1.9% w/w SPC, the polydispersity indexes varied in a range from 0.235 to 0.258. When the amount of non-ionic surfactant was reduced to 0.35% w/w, the emulsion containing 0.9% w/w SPC exhibited a particle size about 55% higher than that for emulsions stabilised with 0.7% w/w Tween 80 and the same amount of SPC. Surprisingly, this formulation exhibited the lowest PI value (0.208).

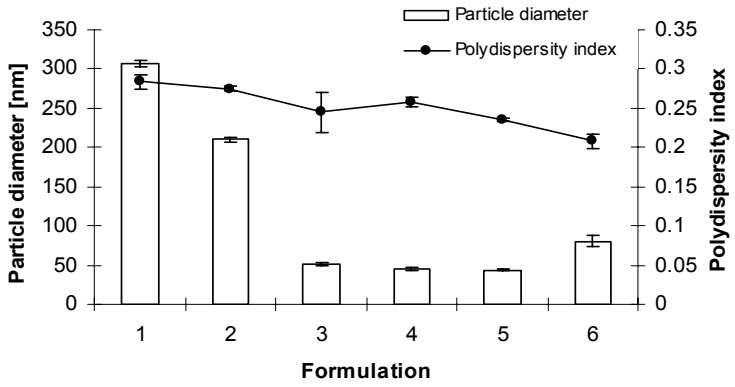

Fig. 1. The effect of emulsion composition on the particle size and polydispersity index. The amounts of Tween 80 and soybean lecithin used were (% w/w): 0.7/0 (1), 0/0.7* (2), 0.7/0.9 (3), 0.7/1.9 (4), 0.9/0.9 (5) and 0.35/0.9 (6). Each formulation contains 2.4% w/w oil from *A. pavonina* seeds. Each point represents the mean ± S.D. (n = 3).*Data taken from [2].

All the formulations had negative, relatively large Zeta potential values (Tab. 1). It could be clearly seen that an increased SPC content consequently resulted in higher negative values of the surface charge. The highest charge (-45.3 mV) was determined for the emulsion with the highest soybean lecithin content (1.9% w/w). Formulations containing fixed 0.9% w/w doses of SPC and diverse amounts of Tween 80 were characterised by very similar Zeta potentials, between -35.6 and -39.7 mV. The lowest value (-28.5 mV) was observed in an emulsion devoid of lecithin. It could be concluded that a decrease in the size of the particles in the emulsion tested was associated with increasing values of the negative Zeta potential. This observation concurs well with results previously reported for chitosan-lipid emulsions (a decrease in particle size followed by an increase in the positive Zeta potential) [20]. However, the obtained Zeta potential values might be changed during sterilization by autoclaving.

Tab. 1. Comparison of Zeta potential for various emulsion formulations.

Tween 80 [% w/w]	SPC [% w/w]	Zeta Potential [mV]
0.7	-	-28.5 ± 1.9
0.7	0.9	-35.6 ± 1.3
0.7	1.9	-45.3 ± 2.9
0.9	0.9	-39.7 ± 2.3
0.35	0.9	-38.8 ± 2.1

Each emulsion contained 2.4% w/w of oil from *A. pavonina* seeds. Each value represents the mean ± S.D. (n = 3).

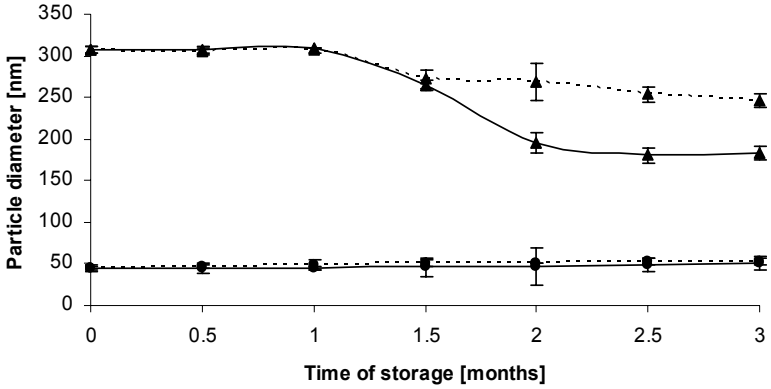

Fig. 2. A comparison of particle size changes in emulsions at 4ºC (—) and at room temperature (---). The emulsions were prepared with Tween 80 and SPC (% w/w): 0.7/1.9 (●) and 0.7/0 (▲), together with 2.4% w/w oil from *A. pavonina* seeds. Each point represents the mean ± S.D. (n = 3).

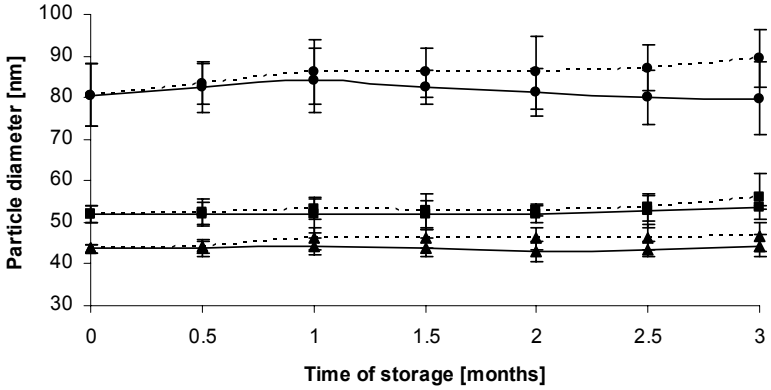

Fig. 3. A comparison of particle size changes in emulsions at 4ºC (—) and at room temperature (---). The emulsions were prepared with Tween 80 and SPC (% w/w): 0.35/0.9 (●), 0.9/0.9 (▲) and 0.7/0.9 (■) together with 2.4% w/w oil from *A. pavonina* seeds. Each point represents the mean ± S.D. (n = 3).

As the physical stability of colloidal carriers is a prerequisite for the development of stable and effective formulations, the stability of preparations was tested at two different thermal conditions: at 4ºC and at room temperature. It is shown in Fig. 2 that the concentrations of the emulsifiers played a crucial role in stabilizing the emulsions during the 3-month long stability experiments. Emulsions containing only 0.7% w/w Tween 80 exhibited rather unsatisfactory behaviour at both temperatures, as the particle size decreased after one month. In this case, the possibility that larger particles underwent coalescence and their

size was beyond the measuring scope of the instrument should be taken into account. Emulsions with the same amount of the studied detergent and with 1.9% w/w SPC showed an excellent stability and no considerable changes in their size were recorded. Since the first emulsion exhibited a Zeta potential value of -28.5 mV (306.5 nm) and the latter -45.3 mV (44.6 nm), the variability observed might be explained as a consequence of the existence of varying surface charge potentials in those formulations. The results presented in Fig. 3 also indicated a clear correlation between size and stability. In emulsions containing 0.35/0.9% w/w Tween 80 and 0.9% w/w SPC, particle size measurements performed just after emulsion preparation and 3 months later did not show any significant changes, with an average particle size of 80.6 nm. Emulsions with a smaller diameter (52.1 and 43.6 nm) were very stable, with only a slight increase in size observed during storage at room temperature. These results concur well with other studies on the stability of emulsions performed on eighteen different natural oils, which also demonstrated the connection between the initial size of the particles and their stability [21]. The stabilizing properties of free fatty acids present in the oil from *A. pavonina* seeds (9.7% of the total lipids) [2] should not be ignored, as they might act as co-surfactants. Such a phenomenon has been previously reported on for castor oil [22].

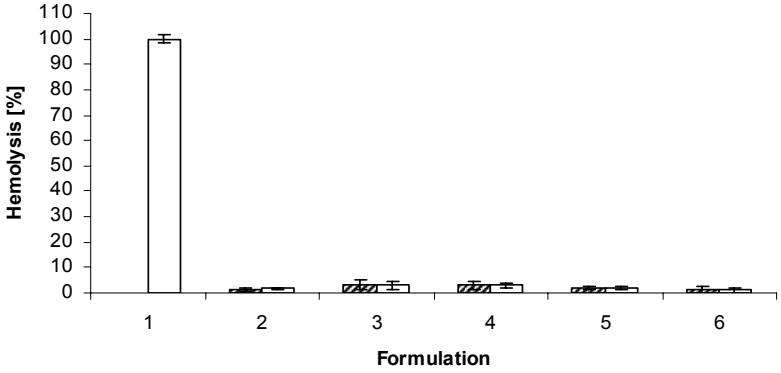

Fig. 4. Hemolysis of sheep erythrocytes induced by free cardol (1) and by various emulsion formulations with (2-6) (open bars) or without cardol (2-6) (dashed bars). The amounts of Tween 80 and soybean lecithin used were (% w/w): 0.7/0 (2), 0.7/0.9 (3), 0.7/1.9 (4), 0.9/0.9 (5), and 0.35/0.9 (6). Each formulation contained 2.4% w/w oil from *A. pavonina* seeds. Cardol was present at a concentration of 100 μM. Each point represents the mean ± S.D. (n = 3).

The determination of *in vitro* erythrocyte hemolysis is a test frequently used to establish the potential *in vivo* side effects of formulations. In this study, we used 4.5% sorbitol as an isotonizing emulsion agent instead of 2.25% glycerol, which may exhibit highly hemolytic behaviour [23]; sorbitol did not influence the size of the tested emulsions. We compared the extent of membrane damage induced by emulsions containing cardol; the presence of cardol caused an increase in the

Zeta potential, data not shown. At 100 μM, this compound, applied in its free form, showed a very high hemolytic activity and caused lysis of all the tested red blood cells. As shown in Fig. 4, the encapsulation of cardol in the formulations studied resulted in an almost complete loss of its hemolytic action, and only a very low-level lytic activity (~1-3%) against sheep red blood cells was observed. This indicates that the encapsulation of cardol neutralized its lytic activity and resulted in the protection of cells against its deleterious effect. However, there was no direct correlation between the size and the extent of this protection. Due to the small sizes of the particles in the formulations tested, they might be recommended for parenteral application. As demonstrated by Nagasaka [24], the percentage of hemolysis in this type of experiment is related to the PC content in the lecithins used as emulsifying agents. Therefore, this assay was also carried out with the same emulsions, but prepared without cardol. The values obtained were almost identical, indicating a negligible level (lower than 3%) of hemolysis after a 30-min incubation at 37ºC. Cardol, like other hemolytic amphiphilic compounds, is probably incorporated into the lipophilic core of oil droplets or into the interface. It is also possible that solubilization occurs in the phospholipid structures like the micelles or liposomes present in the aqueous phase [25]. Consequently, cardol has limited direct contact with the tested blood cells, and is not easily partitioned into them, resulting in the loss of its hemolytic activity. Such a mechanism has been suggested for other lytic agents [1].

The studied formulations, which contained only a 2.4% w/w oil phase, are sufficient cardol carriers. We are aware that such a low oil content might be not suitable for other lipophilic drugs. There are some publications where very similar compositions (o/w emulsions) containing non-ionic surfactants and phospholipid (egg PC) were proposed as carriers for lipophilic drugs [26]. Kan *et al.* also reported that increasing the oil/water ratio in such emulsion compositions beyond 10% (v/v) led to an enlargement in particle size. These compositions were also not stable in stability experiments. The acceptable oil/water ratio should be equal to or below 10% (v/v).

Since cardol was not observed to have toxic effects or irritation potential, the results reported here suggest that the presented emulsion systems are safe, and that those formulations containing the *A. pavonina* oil should be further investigated as potential carriers of other bioactive compounds. Further studies into whether the incorporation of polyethylene glycol derivatives (e.g. PEG-PE) into those emulsions would increase their blood circulation time *in vivo,* as reported earlier [27, 28] are the next logical step.

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REFERENCES

1. Jumaa, M. and Müller, B.W. Lipid emulsions as a novel system to reduce the hemolytic activity of lytic agents: mechanism of the protective effect. **Eur. J. Pharm. Sci.** 9 (2000) 285-290.
2. Zarnowski, R., Jaromin, A., Certik, M., Czabany, T., Fontaine, J., Jakubik, T., Iqbal, M.C.M., Grandmougin-Ferjani, A., Kozubek, A. and Pietr, S.J. The oil of *Adenanthera pavonina* L. seeds and its emulsions. **Z. Naturforsch.** 59c (2004) 321-326.
3. Lam, J.M., Pwee, K.H., Sun, W.Q., Chua, Y.L. and Wang, X.J. Enzyme-stabilizing activity of seed trypsin inhibitors during desiccation. **Plant Sci.** 142 (1999) 209-218.
4. Santos, I.S., Da Cunha, M., Machado, O.L.T. and Gomes, V.M. A chitinase from *Adenanthera pavonina* L. seeds: purification, characterisation and immunolocalisation. **Plant Sci.** 167 (2004) 1203-1210.
5. Ali, M.S., Ahmed, F., Azhar, I. and Pervez, M.K. Pavonin: a new five-membered lactone from *Adenanthera pavonina* Linn. (Mimoaceae). **Nat. Prod. Res.** 19 (2005) 37-40.
6. Olajide, O.A., Echianu, C.A., Adedapo, A.D. and Makinde, J.M. Anti-inflammatory studies on *Adenanthera pavonina* seed extract. **Inflammopharmacology** 12 (2004) 196-202.
7. Burkill, I.H. A Dictionary of the Economic Products of the Malay Peninsula. 2nd edition., vol. 1, A-H. Government of Malaysia and Singapore, Kuala Lumpur, 1966.
8. Kan, P., Chen, Z.B., Lee, C.J. and Chu, I.M. Development of nonionic surfactant/phospholipid o/w emulsion as a paclitaxel delivery system. **J. Control. Release** 58 (1999) 271-278.
9. Lundberg, B.B., Risovic, V., Ramaswamy, M. and Wasan, K.M. A lipophilic paclitaxel derivative incorporated in a lipid emulsion for parenteral administration. **J. Control. Release** 86 (2003) 93-100.
10. Kang, B.K., Chon, S.K., Kim, S.H., Jeong, S.Y., Kim, M.S., Cho, S.H., Lee, H.B. and Khang, G. Controlled release of paclitaxel from microemulsion containing PLGA and evaluation of anti-tumor activity in vitro and in vivo. **Int. J. Pharm.** 286 (2004) 147-156.
11. Kim, S.J., Choi, H.K. and Lee, Y.B. Pharmacokinetic and pharmacodynamic evaluation of cyclosporin A O/W-emulsion in rats. **Int. J. Pharm.** 249 (2002) 149-156.
12. Klang, S.H., Baszkin, A. and Benita, S. The stability of piroxicam incorporated in a positively-charged submicron emulsion for ocular administration. **Int. J. Pharm.** 132 (1996) 33-44.
13. Tamilvanan, S. and Benita, S. The potential of lipid emulsion for ocular delivery of lipophilic drugs. **Eur. J. Pharm. Biopharm.** 58 (2004) 357-368.
14. Pape, W.J.W., Pfannenbecker, U. and Hoppe, U. Validation of the red blood cell test system as *in vitro* assay for the rapid screening of irritation potential of surfactants. **Mol. Toxicol.** 1 (1987) 525-536.

15. Kozubek, A. and Tyman J.H. Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. **Chem. Rev.** 99 (1999) 1-26.
16. Kozubek, A. Isolation of 5-n-alkyl-, 5-n-alkenyl- and 5-n-alkdienyl-resorcinol homologs from rye grains. **Acta Aliment. Polon.** 9 (1985) 185-198.
17. Zarnowski, R. and Kozubek, A. Alkylresorcinol homologs in *Pisum sativum* L. varieties. **Z. Naturforsch.** 54c (1999) 44-48.
18. Weingarten, C., Magahaes, N.S.S., Baszkin, A., Benita, S. and Seiller, M. Interaction of non-ionic ABA copolymer surfactant with phospholipid monolayers: possible relevance to emulsion stabilization. **Int. J. Pharm.** 75 (1991) 171-179.
19. Jumaa, M. and Müller B.W. Influence of non-ionic surfactant PEG-660-12-hydroxy stearate on the surface properties of phospholipid monolayers and their effect on lipid emulsion stability. **Col. Polym. Sci.** 277 (1999) 347-353.
20. Jumaa, M. and Müller, B.W. Physicochemical properties of chitosan-lipid emulsions and their stability during the autoclaving process. **Int. J. Pharm.** 183 (1999) 175-184.
21. Chung, H., Kim, T.W., Kwon, M., Kwon, I.C. and Jeong, S.Y. Oil components modulate physical characteristics and function of the natural oil emulsions as drug or gene delivery system. **J. Control. Release** 71 (2001) 339-350.
22. Yamaguchi, T., Nishizaki, K., Itai, S., Hayashi, H. and Ohshima, H. Physicochemical characterization of parenteral lipid emulsion: influence of cosurfactants on flocculation and coalescence. **Pharm. Res.** 12 (1995) 1273-1278.
23. Jumaa, M. and Müller, B.W. *In vitro* investigation of the effect of various isotonic substances in parenteral emulsions on human erythrocytes. **Eur. J. Pharm. Sci.** 9 (1999) 207-212.
24. Nagasaka, Y. and Ishii, F. Interaction between erythrocytes from three different animals and emulsions prepared with various lecithins and oils. **Col. Surf. B: Biointerfaces** 22 (2001) 141-147.
25. Sznitowska, M., Dąbrowska E.A. and Janicki, S. Solubilizing potential of submicron emulsions and aqueous dispersions of lecithin. **Int. J. Pharm.** 246 (2002) 203-206.
26. Kan, P., Chen, Z.B., Kung, R.Y., Lee, C.J. and Chu, I.M. Study on the formulation of o/w emulsion as carriers for lipophilic drugs, **Col. Surf. B: Biointerfaces** 15 (1999) 117-125.
27. Liu, F. and Liu, D. Amphipathic polyethylene glycol stabilized emulsions (o/w): physical characterization and *in vivo* distribution. **Int. J. Pharm.** 125 (1995) 73-80.
28. Lundberg, B.B., Mortimer, B.C. and Redgrave, T.G. Submicron lipid emulsions containing amphipathic polyethylene glycol for use as drug-carriers with prolonged circulation time. **Int. J. Pharm.** 134 (1996) 119-127.