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Determination of the disappearance rate of iodine-125 labelled oils from the injection site after intramuscular and subcutaneous administration to pigs

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Abstract

The rate of disappearance of clinically used vegetable oils, Viscoleo, sesame oil, castor oil and isopropyl myristate, from the injection site after intramuscular (i.m.) or subcutaneous (s.c.) administration to pigs were determined by using a non-invasive gamma-scintigraphy method. All the oil vehicles were spiked with 2.5% (v/v) ^{125}I-triolein and six injections of 1.9 ml were given to each of 12 pigs. No significant difference (ANOVA) in disappearance rate of each individual oil vehicle from the different injection sites was observed after administration of the oils: i.m. in the lower back, s.c. in the neck and s.c. in the mid-back. Likewise, no inter-individual difference between the pigs was observed. The half-life of 14 days for Viscoleo was significantly smaller than those of the other oil vehicles ($P < 0.0001$), i.e. 23, 20, 20 days for sesame oil, castor oil and isopropyl myristate, respectively. Due to the spreading effect of the oils and reflux of the oils through the injection canal, the half-lives were calculated omitting the data for the first sampling day.

Keywords: Parenteral depots; Disapperance rates; Pig; I.m administration; S.c. administration; Gamma-scintigraphy

1. Introduction

Many long-acting injectables are formulated in the form of lipophilic prodrugs dissolved in oil vehicles. The drug release rate has been suggested to be controlled by the partition coefficient of drug between the oil vehicle and the tissue fluid (Chien, 1981; Hirano et al., 1982).

However, recently we have suggested that for highly lipophilic compounds, the duration of action may not solely be explained by drug partitioning into the tissue fluid (Larsen et al., 2001a). To this end, several mechanisms may contribute to the absorption of a drug substance from an oil

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vehicle (Luo et al., 1997, 1998). Highly lipophilic drugs with strong affinity to the oil vehicle might be released concurrently with the disappearance of the oil vehicle from the injection site. Previously, the rate of disappearance of oil vehicles from the injection site has been investigated in different animal models by using various analytical techniques. The local clearance method, i.e. excision and analysis of muscle tissue at various time points after administration, has been used to estimate the disappearance of methyl oleate (Tanaka et al., 1974), castor and olive oil (Deanesly and Parkes, 1933), sesame oil and Viscoleo (Larsen et al., 1998; Svendsen and Aaes-Jørgensen, 1979). The non-invasive gamma-scintigraphy technique has been employed for determination of the clearance of ethyl oleate and arachis oil (Howard and Hadgraft, 1983), sesame oil and Viscoleo (Larsen et al., 1998; Schultz et al., 1998). The majority of these studies has used the rat or rabbit as the animal model. Since the pig is expected to be a representative model for man, the primary aim of the present study was to estimate the rate of disappearance of four commercially available oil vehicles, Viscoleo, sesame oil, castor oil and isopropyl myristate, from the injection site after intramuscular (i.m) and subcutaneous (s.c.) administration to pigs, using a simple non-invasive gamma-scintigraphy technique. A further aim was to set up a design allowing proper assessment of inter-individual variations in disappearance rates.

2. Materials and methods

2.1. Materials

Fractionated coconut oil (Viscoleo®) was obtained from P. Broeste A/S, Denmark. Sesame oil, triolein, castor oil, isopropyl myristate were purchased from Sigma Chemical Co. Sudan Black B was obtained from Merck.

2.2. Experimental animals

Twenty-seven female pigs (Danish land race from Danish bacon and meat council, Research station no. 3) weighing 26–38 kg were used in this study. Twelve of the pigs were used for the experiments with the radiolabelled oil vehicles and 15 pigs were used for the experiments with Sudan Black. They were housed in groups of three in solid concrete floor pens covered with straw bedding and kept at approximately 21 °C. The ventilation provided a total air exchange rate of 8–10 times/h and the relative humidity was 55–80%. There were no specific light cycles. The pigs were fed twice a day with a standard pelleted diet and provided with water ad libitum. The pigs were killed by stunning (bolt pistol) and subsequent bleeding.

2.3. Labelling procedure

Triolein was radioiodinated directly by a process involving the addition of ^{125}I to a double bond in the fatty acid chain of the triglyceride. The labelling procedure was performed according to the method of Lubran and Pearson (1958) without further purification. Viscoleo, sesame oil, castor oil and isopropyl myristate were spiked with radiolabelled triolein. In each case, triolein constituted 2.5% (v/v) of the total dose volume. The radioactivity of the doses for i.m. and s.c. administration were 10 and 5 μCi per dose, respectively.

2.4. Administration of oil vehicles

Prior to injection, the area around the injection site was shaved and the injection site and a circular area with af radius of 2 cm around the injections were marked. Thus, in this study, the injection site is defined as the marked area around the injection site. The injection volumes for both i.m. and s.c. administration were 1.9 ml. The i.m. injections were given with a $19G \times 1''$ (1.1×25 mm) cannula introduced perpendicular to the skin and inserted in its full length ensuring administration of the dose in the middle of the muscle. The s.c. injections were given with the cannula inserted in its full length parallel to the skin (s.c. tissue). Three identical injections were given at the right and left side of each pig: i.m. (m. longissimus dorsi) in the lower back, s.c. in the neck and s.c.

in the mid-back. Thus, three different injection sites were investigated and each oil vehicle was injected six times for each of the three injection sites in different pigs. The administration schedule was designed ensuring that each pig received at least one injection of each individual oil vehicle.

2.5. Data collection

Radioactivity at the injection sites was measured by using a model 44-17 low energy gamma scintillator connected via a 10 foot cable to a model 2200 scater ratemeter (Ludlum Measurements, Sweetwater, TX). The number of counts per 6 s was recorded. In most cases, the cpm values presented are the average of triplicate measurements. Radioactivity was monitored immediately after administration of the oil vehicles and at specified sampling times by placing the scintillator probe directly on the skin surface precisely on the pre-marked injection site. Data collection was continued for 35 days. The background radioactivity in the air of the animal room and at the thigh of the pig was measured immediately after each sampling sequence.

2.6. Determination of spreading of the oil vehicle at the injection site

The initial spreading of the oil vehicles at the injection site was investigated visually after s.c. (neck) and i.m. (lower back) administration of oil solutions containing 0.5% (w/v) Sudan Black. The experiments were carried out in duplicate. Pigs were sacrificed at time 2, 6 and 24 h after injections and the distribution area was estimated.

2.7. Statistics

The half-lives of disappearance from the injection site were tested for normality by means of the Anderson–Darling test and subsequently the half-lives were subjected to statistical analysis employing multifactor ANOVA. P-values less than 0.05 were considered statistically significant.

3. Results and discussion

During the 35 experiment days the pigs gained 46–85% in body weight. The relative standard deviation of radioactivity measurements carried out in triplicate was usually less than 10%. The injected volume of oil vehicles was 1.9 ml ($\pm$ 0.1 ml). Since each animal functions as its own control, it appears unlikely that this minor variation of the injection volume has significant influence on the disappearance rates. Previously, comparable disappearance rates were found after i.m. administration of different oil volumes to rabbits (Howard and Hadgraft, 1983; Schultz et al., 1998).

Labelling of oils for gamma-scintigraphy measurements involves addition of the gamma emitter (^{131}I or ^{125}I) to double bonds in the fatty acid chain of the triglyceride and consequently only oil vehicles containing unsaturated fatty acids can be iodinated directly. Labelling of oils consisting of saturated triglycerides have been done by spiking the oil vehicle with radioiodinated triglyceride. Various unsaturated triglycerides are available, but selection of a representative triglyceride might not be straightforward since clinically used vegetable oils consist of various triglycerides, the fatty acid content of which differs considerably. Also in the case of the local clearance method selection of a suitable, labelled triglyceride is necessary. The disappearance rate of Viscoleo after i.m. injection has been investigated after spiking with glycerol tri[^{14}C]-palmitate (Larsen et al., 1998) and glycerol tri[^{14}C]-octanoate (Svendsen and Aaes-Jørgensen, 1979), respectively. Similar clearance rates were found taking into consideration that different animal models were used. Moreover, comparable muscle disappearance rates were found for Viscoleo when spiked with either ^{14}C-palmitate or ^{131}I- triolein (Larsen et al., 1998). These observations indicate that the labelled triglyceride is leached from the muscle concomitantly with the oil vehicle. Since Viscoleo and isopropyl myristate cannot be radioiodinated directly it was decided to spike all the investigated oils with equal amounts (2.5% v/v) of radioiodinated triolein.

The background radioactivities (mean ± S.D.) in the air and at the thigh of the pig were 1280 ± 30 and 1640 ± 20 cpm, respectively. For far the majority (95%) of the cpm values reported, the radioactivity measured at the injection sites was at least five times that of the background. It was ensured that counting of radioactive oil vehicle remaining at the one side of the pig was not influenced by the radioactive dose injected on the other side. After injection of ^{125}I-labelled oils at the right side measurement of radioactivity was also done directly at the opposite left side. Counts in the range 1670 ± 48 cpm were observed, thus being comparable to that of background radioactivities found at the thigh of the pig (1640 ± 20 cpm).

Radioactivity measured in the defined area around the injection site was corrected for the decay of radioactivity for ^{125}I (half-life of 60 days) and the background radioactivity measured. The radioactivity at each sampling time was expressed as percentage of the radioactivity determined at time zero (% remaining). Disappearance–time profiles, in the form of plots of the natural logarithm of % remaining radioactivity at the site of administration against time (Fig. 1), were constructed for all the injection sites. The data for the individual profiles were treated according to first order kinetics excluding data for the initial part of the profiles (day 1) due to spreading effects as discussed later. This procedure appears reasonable since correlation coefficients of the obtained straight line portions in the range 0.87–1.00 were calculated. The disappearance rates of the oil vehicles, expressed as half-lives, were derived from following the remaining radioactivity at the injection site for 1.5–2.5 half-lives. In total 72 determinations of the disappearance rate of oil vehicle from the injection site were performed with 70 data set included in the statistical analysis. Two sets of experiments have been omitted due to experimental error. The Anderson–Darling normality test of the half-lives indicates that the half-lives were normal distributed and therefore ANOVA was performed.

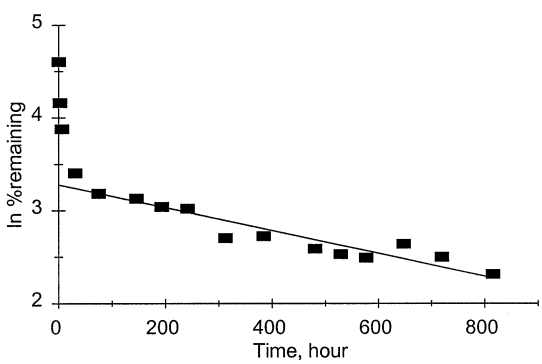

Fig. 1. Disappearance–time profile for sesame oil after subcutaneous administration at the mid-back of a pig. (% remaining: radioactivity measured at the injection site expressed as percentage of the radioactivity determined at time zero). The full line has been drawn from linear regression of the data collected in the time interval 30–820 h.

Table 1
Mean values ($t_{1/2}$) and standard deviations (S.D.) of half-lives

Oil vehicle	Site of injection	$t_{1/2}$ (days)	S.D. (days)
Sesame oil	i.m. back	21.4	5.5
Sesame oil	s.c. neck	24.1[a]	10.6[a]
Sesame oil	s.c. back	22.3	4.8
Viscoleo	i.m. back	11.6	1.8
Viscoleo	s.c. neck	13.1	2.5
Viscoleo	s.c. back	17.0	3.9
Castor oil	i.m. back	19.0	2.9
Castor oil	s.c. neck	18.6[a]	6.1[a]
Castor oil	s.c. back	23.3	6.4
Isopropyl myristate	i.m. back	18.3	3.5
Isopropyl myristate	s.c. neck	20.8	5.3
Isopropyl myristate	s.c. back	20.1	5.0

Obtained from the disappearance–time profiles for sesame oil, Viscoleo, castor oil and isopropyl myristate after intramuscular injections in the lower back (i.m. back), subcutaneous injections in the neck (s.c. neck) and in the mid-back (s.c. back), respectively. Mean values and standard deviations have been calculated from half-lives of six injections ($n = 6$).

[a] $n = 5$.

Investigation of the inter-individual differences between the pigs was performed. After subcutaneous administration of Viscoleo on the mid-back in six pigs the relative standard deviation of the half-lives was 23% (Table 1). As apparent from this table S.D.s of the same order of magnitude

were found for the other oil vehicles at the various injection sites. In addition, no significant difference (ANOVA) in individual half-lives determined for the 12 pigs was seen ($P = 0.456$). Thus, the inter-individual differences between the pigs can be excluded in the interpretation of the half-lives calculated.

The half-lives determined after administration of sesame oil s.c. at the mid-back, s.c. at the neck and i.m. in the lower back were 22, 24 and 21 days, respectively (Table 1). Thus, no significant difference between the half-lives calculated from the three injection sites was observed. Likewise, no systematic difference in half-lives was seen after injection of Viscoleo, castor oil and isopropyl myristate and no significant difference (ANOVA) in the half-lives obtained from the three different administration routes was observed ($P = 0.112$). This conclusion is in accordance with previous findings (Howard and Hadgraft, 1983) showing that the clearance rates of ethyl oleate and arachis oil in rabbits were independent of the injection site (i.m. and s.c). Consequently, the half-lives of each of the four oils presented in Table 2 are mean values calculated from half-lives obtained from different pigs and injection sites (17 or 18 injection sites in total per oil). The $t_{1/2}$-values determined for Viscoleo, sesame oil, castor oil and isopropyl myristate are 14, 23, 20 and 20 days, respectively, with the coefficient of variation in the range 23–30%. The results from the analysis of variance show that the disappearance rate of Viscoleo is significantly higher than those of the other oil vehicles ($P < 0.0001$) whereas no significant difference between the half-lives of sesame oil, castor oil and isopropyl myristate was observed.

In Table 2 previously reported disappearance rate of oil vehicles are presented. The data given have been obtained employing different experimental techniques. The local clearance method is based on measurement of the radioactivity (^{14}C-labelled oil vehicle) remaining in the muscle tissue excised from groups of animals sacrificed at different times after injection (Larsen et al., 1998; Svendsen and Aaes-Jørgensen, 1979; Tanaka et al., 1974). Deanesly and Parkes (1933) investigated the disappearance of coloured oil vehicle by visual estimation of the amount of oil remaining at the administration site at various time points following subcutaneous injection. In the non-invasive gamma-scintigraphy technique, the oil vehicle is labelled with a gamma emitter allowing each animal to function as its own control. The measurements of the radioactivity can be performed by using a gamma camera (Howard and Hadgraft, 1983; Larsen et al., 1998; Schultz et al., 1998) or by the use of a thallium activated NaI scintillation crystal (de Meijer et al., 1989).

Table 2
Half-lives (days) for clearance of various oil vehicles using different animals

Oil vehicle	Pig	Rabbit	Dog	Rat	Rat and mouse
Viscoleo	14[f,g]	8[a]	2[c]	7[c]	
Sesame oil	23[f]	27[a]	35[c]	63[c]	3–14[e]
Ethyl oleate		10[b]			
Arachis oil		25[b]			
Castor oil	20[f]				10–30[e]
Olive oil					2–7[e]
Methyl oleate				5–7[d]	
Isopropyl myristate	20[f]				

[a] Schultz et al., 1998 (average of i.m. administrations).
[b] Howard and Hadgraft, 1983 (average of i.m and s.c. administrations).
[c] Svendsen and Aaes-Jørgensen, 1979 (average of i.m. administrations).
[d] Tanaka et al., 1974 (average of i.m. administrations).
[e] Deanesly and Parkes, 1933 (average of s.c. administrations).
[f] Present study (average of i.m and s.c. administrations).
[g] $P < 0.0001$ compared to sesame oil, castor oil and isopropyl myristate. No differences between the latter three oils were observed.

The disappearance rate of the oil vehicles obtained in the present study has been compared with the previous data despite differences in analytical method, animal model and injection sites (Table 2). The half-life for clearance of Viscoleo from the injection site in pigs, rabbits, rats and dogs is 14, 8, 7 and 2 days, respectively. In spite of these apparent discrepancies, the disappearance rate of Viscoleo from the injection site is generally faster than those of the other oil vehicles investigated; independent of animal model used. Reasonably consistent disappearance rates of sesame oil from the injection site have been found in pigs, rabbits and dogs with a $t_{1/2}$ of 4–5 weeks. Although considerable variation in the estimation of the clearance rate of castor oil from the injection site was observed by Deanesly and Parkes (1933) the results appear to be in accordance with the half-life of castor oil of 20 days found in the present study. The muscle disappearance rates of the synthetic oils isopropyl myristate and ethyl oleate differ by a factor of two. Possibly, this observation reflects differences in hydrolysis rates of the two fatty acid esters. To this end, the half-life of a non-degradable mineral oil emulsion was estimated to 3 months in rats and monkeys using a ^{14}C-*n*-hexadecane tracer (Bollinger, 1970).

Previously, the relative spreading of oil vehicles after i.m. administration to rabbits has been shown to occur predominantly during the 1st day after administration (Schultz et al., 1998). This is in accordance with the results of the present study where the initial rapid decline of the disappearance–time profiles most likely can be ascribed to a spreading effect (Fig. 1). After subcutaneous administration of the oil vehicles containing Sudan Black, they were distributed over an area of approximately 4×6 cm and no difference in the distribution area between the oil vehicles were apparent. For subcutaneous injections, the radioactivity usually decreased very fast the 1st day accompanied by an increase in the radioactivity 4–8 cm around the injection site. However, after i.m. administration an initial decrease was not always seen. In some cases an initial increase in radioactivity was observed which might be explained by a reflux of the oil through the injection canal (Fig. 2). In addition, section of the muscle

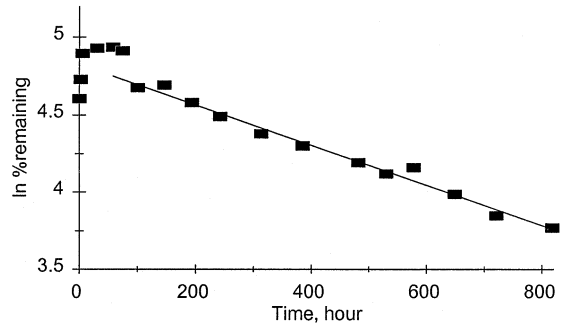

Fig. 2. Disappearance–time profile for isopropyl myristate after intramuscular administration at the lower back of a pig. (% remaining: radioactivity measured at the injection site expressed as percentage of the radioactivity determined at time zero). The full line has been drawn from linear regression of the data collected in the time interval 100–820 h.

tissue after i.m. injection of the four oil vehicles containing Sudan Black showed oil along the injection canal and a part of the oil was located subcutaneous.

Different mechanisms have been suggested to contribute to the disappearance of oil vehicles from various injection sites. Absorption of oil vehicles in the form of microdroplets through blood vessels into systemic circulation might occur, since muscle and subcutaneous tissue are well supplied with capillary vessels. Absorption by blood vessels was suggested to be the major factor in the disappearance of methyl oleate from the injection site (Tanaka et al., 1974). Lymphatic absorption of oil vehicles has been observed. A maximum of 5% of the injected dose of sesame oil and Viscoleo in rats and dogs was accounted for via lymphatic absorption (Svendsen and Aaes-Jørgensen, 1979). A small amount of methyl oleate was observed in the thoracic lymph after i.m. administration to rats (Tanaka et al., 1974). Also mineral oil administered as an oil-in-water emulsion was reported to be absorbed by the lymphatic system after i.m. or s.c. administration (Bollinger, 1970). Lymphatic absorption might be expected to take place more efficiently from the subcutaneous layers than from the intramuscular injection sites, since the lymphatic system is better developed in the former region (Ballard, 1968). Although some absorption into the lymphatic sys-

tem may occur it appears less likely that this route of absorption plays a dominant role in the clearance of oil vehicles. The surface area of the oil depot is likely to affect the clearance of the oil vehicle from the injection site. Thus, the distribution of the oil vehicle at the injection site can be an important variable. The spreading characteristics of the oil vehicle appears to be influenced by the viscosity of the oil (Howard and Hadgraft, 1983). Based on the viscosity and half-lives obtained for ethyl oleate and arachis oil Howard and Hadgraft (1983) suggested that the more viscous oil the more resistant to spreading at the injection site and consequently a slower clearance rate would be expected. In contrast, while the difference in the viscosity of isopropyl myristate and castor oil is substantial (Table 3) the disappearance rates from the injection site were similar (Table 2). Thus, including the data for all oil vehicles from the present study, no correlation between viscosity (Table 3) and half-lives (Table 2) was observed. Biological and physiological factors such as vascularisation (Zuidema et al., 1988) and body movement (Ballard, 1968) might also influence the absorption rate of the oil vehicles. Phagocytosis might constitute another possible absorption mechanism (Ballard, 1968). Phagocytosis is likely to be related to the tissue response to the injected oil material (Ballard, 1968). Metabolic degradation of oil vehicles has been suggested by Svendsen and Aaes-Jørgensen (1979) to play a role in the removal of oil vehicles from the site of injection. As a result of the inflammatory response, several enzymes might be present at the injection site. Degradation of oil vehicles mediated by lipases might therefore also contribute to the disappearance rate of oil vehicles where the rate of degradation might be influenced by the composition of the oil vehicles. Interestingly, Viscoleo is a vegetable oil consisting of triglycerides containing only medium chain saturated fatty acids (Table 3) whereas sesame oil and other vegetable oils previously used consist of triglycerides with long chain saturated and unsaturated fatty acids (Table 3). Except for olive oil, the magnitudes of the disappearance rate of the latter type of oils from the injection site were comparable. For the synthetic oil vehicles, the composition might also be related to the clearance rate. The half-lives of isopropyl myristate, ethyl and methyl oleate have been determined to 20 (the present study), 10 (Howard and Hadgraft, 1983) and 5–7 (Tanaka et al., 1974) days, respectively. The alcohol component of isopropyl myristate might give rise to steric hindrance, thereby accounting for the lower clearance rate. Investigations into the role

Table 3
The viscosity and the fatty acid content of the oil vehicles

Oil vehicle	Viscosity (cP)	Fatty acid content	
Viscoleo	12[a]	0.5%	C6
		58%	C8
		40%	C10
		1%	C12
Sesame oil	33[a]	13.4%	C16 and C18
		45.4%	C18:1
		40.4%	C18:2
		0.8%	C20
Ethyl oleate	3.9[b,c]	100%	C18:1
Arachis oil	38[a]/35.2[b,c]	11.4%	C16 and C18
		56%	C18:1
		26%	C18:2
		6.6%	C20, C22 and C24
Castor oil	283[a]	2.4%	C12–C18
		7.4%	C18:1
		3.1%	C18:2
		87%	C18:1, 1OH
Olive oil	36.3[e]	12.3%	C16, C18 and C20
		83.5%	C18:1
		4.0%	C18:2
Methyl oleate	4.1[d]	100%	C18:1
Isopropyl myristate	5–7[c]	100%	C14

C6, caproic acid; C8, caprylic acid; C10, capric acid; C12, lauric acid; C14, myristic acid; C16, palmitic acid; C18, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:1, 1OH, ricinoleic acid; C20, arahidic acid; C22, behenic acid; C24, lignoceric acid.

[a] Fredholt et al., 2000 (determined at 37 °C).

[b] Howard and Hadgraft, 1983 (determined at 25 °C).

[c] Handbook of Pharmaceutical Excipients, 1994 (determined at 25 °C).

[d] Tanaka et al., 1974 (determined at 37 °C).

[e] CRC Handbook of Chemistry and Physics 67th ed., 1986–1987 (determined at 40 °C).

of lipase mediated degradation of oil vehicles are presently ongoing in our laboratory.

The data of the present study can be used for the selection of the right oil vehicle for parenteral oil depot formulations. Viscoleo exhibiting a relatively fast disappereance rate from the injection site is not an adequate candidate if a duration of action of 2–3 months is required. However, the selection between castor oil, sesame oil and isopropyl myristate with approximately equal disappearance rate from the injection site has to be based on other criteria. The syringeability and injectability of the formulation are important parameters and has shown to be inversely related to the vehicle viscosity (Dexter and Shott, 1979). To this end, sesame oil and in particular isopropyl myristate exhibit a feasible syringeability. Castor oil constitutes a less convenient vehicle owing to a high viscosity (Larsen et al., 2001b). However, by addition of a less viscous vehicle to castor oil the viscosity can be decreased ensuring proper syringeability (Larsen et al., 2001b). Since the drug partitioning from the oil vehicle to the tissue fluid in many cases determines the absorption process, factors altering the partitioning coefficient must also be taken into consideration. Previously, a significant decrease in the in vitro rate of drug release from oil vehicles into an aqueous media was observed by adding hydrogen bond donating excipients including castor oil to an oil vehicle (Larsen et al., 2001b). Thus, for proper choice of oil vehicle for a parenteral oil depot formulation the chemical composition of the oil vehicle have to be considered.

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