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Rotating dialysis cell as in vitro release method for oily parenteral depot solutions

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Abstract

The purpose of the study was to investigate an in vitro release method based on a rotating dialysis cell for parenteral oil depot formulations using different model conditions and test formulations. The total amount of drug released from the rotating dialysis cell was in accordance with the theoretical values calculated from the partition coefficients. The release rates were shown to depend on the total amount of drug available for the release process and to follow first order kinetics. The rotating dialysis cell has a potential as in vitro release method for characterization of oily depot formulations for parenteral administration. © 1997 Elsevier Science B.V.

Keywords: In vitro release; Oily solutions; Parenteral depots; Dialysis cell

1. Introduction

Many parenteral depot formulations are formulated as lipophilic prodrugs dissolved in oil vehicles with the release rate suggested to be controlled by the chain length of the lipophilic promoiety. Dose intervals ranging from 2–3 days up to 3 weeks are reported (Armstrong and James, 1980).

Official in vitro release methods are not available for parenteral depot formulations. Apparatus developed for other types of formulations, for instance suppositories and topical preparations, might under certain circumstances be applied as in vitro release methods for oily parenteral depots. The latter methods can be divided into two groups depending on whether the formulation is in direct contact with the release medium or separation of the two phases is afforded by a membrane. Non membrane systems (Crommelin and de Blaey, 1980; Radd et al.,

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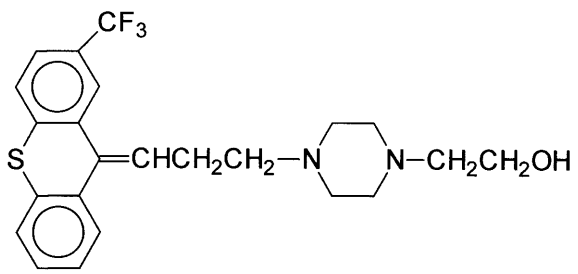
1985; Gido et al., 1993) are only applicable to formulations with densities lower than that of the release medium. In most membrane systems (Benita et al., 1986; Realdon et al., 1996), the lack of stirring of the lipophilic and often viscous vehicle makes the release process too slow for practical use. However, the dialysis method earlier described for determination of in vitro release from suppositories (Dibbern and Wirbitzki, 1983; Lootvoet et al., 1992) and from transdermal systems (Yamaguchi et al., 1996) might be advantageous, because the dialysis membrane provides a well defined surface area and mixing is accomplished by rotating the dialysis cell.

As regards formulation development and quality control of parenteral oily solutions, a need exists for a technique enabling characterisation of the in vitro drug release from such formulations. The aim of this study was to evaluate the feasibility of the rotating dialysis cell as an in vitro relase method for oily depot formulations using a weak base (flupentixol) and a weak acid (naproxen) as model drug substances (Fig. 1).

Flupentixol, pKa 4.7 and 7.4



Naproxen, pKa 4.2

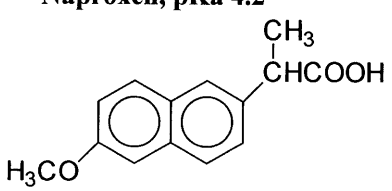


Fig. 1. Molecular structures of model drugs.

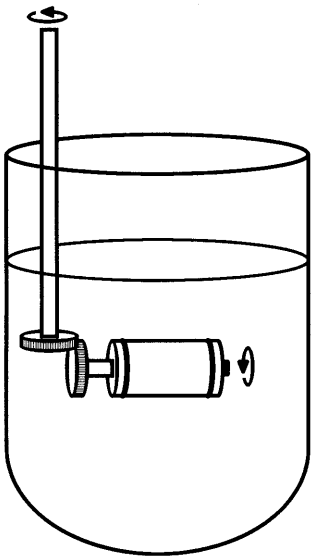


Fig. 2. Schematic illustration of rotating dialysis cell.

2. Materials and methods

2.1. Materials

Flupentixol and fractionated coconut oil (Viscoleo) were supplied by H. Lundbeck A/S. Naproxen was purchased from Sigma Chemical Co. Chemicals for preparation of buffers and HPLC mobile phases were of analytical grade. Demineralized and ultra pure water was used throughout. Visking Dialysis Tubing size 27/32, 30 mm with a cut off at 12 000–14 000 Da was employed for the dialysis cell.

2.2. Release experiments

The dialysis apparatus was build as a slightly modified version of an equipment previously described (Dibbern and Wirbitzki, 1983). Minor changes in the design of the stirring wings were implemented. The apparatus (Fig. 2) consists of a rotating dialysis cell, mounted on a standard tablet dissolution apparatus (VK 7000, VanKel

Industries Inc.). The distance from the bottom of the vessel to the rotating cell is 5.6 cm. Samples were taken by using a fraction collector (VK 8000, VanKel Industries Inc.), the sampling port being placed immediately below the surface of the media. Drug content was measured by HPLC or UV spectrophotometry.

Throughout the study, 1000 ml release media controlled at 37 ± 0.5°C was used. The revolution speed of the cell was 50 rpm. The dialysis membranes were rinsed several times with boiling water in order to remove UV absorbing plasticizer from the material. The formulation was placed inside the cell at time zero. The vessels were covered with a lid and tightened with laboratory film in order to avoid evaporation of the release medium. Ten samples of 2.0 ml (for HPLC detection) or 5.0 ml (for UV detection) were withdrawn during 96 h. For solutions of flupentixol and naproxen in fractionated coconut oil release media of pH 3.00 ± 0.01 (0.05 M phosphate buffer), pH 5.00 ± 0.01 (0.05 M acetate buffer) and pH 7.00 ± 0.01 (0.05 M phosphate buffer), were used respectively.

The amount of drug released from the solutions was calculated (corrected for sampling) according to Eq. (1):

$$\% \text{ released} = \frac{(V_s \cdot \sum_{n=1}^{n} C_{n-1} + V_m \cdot C_n)}{M} \cdot 100 \quad (1)$$

where V_s and V_m are the volumes of sample and release medium respectively, C_n is the drug concentration in sample n and M represents the total amount of drug initially applied to the dialysis cell.

2.3. Determination of partition coefficients

Solutions of drug in oil vehicles were allowed to equilibrate at 37 ± 0.5°C with the aqueous buffers for at least 24 h. Drug concentrations in the aqueous phase were measured by HPLC. The partition coefficients were calculated according to Eq. (2):

$$P = \frac{M/V_{aq} - C_{aq}}{C_{aq}} \cdot \frac{V_{aq}}{V_o} \quad (2)$$

where M is the total amount of drug, V_{aq} and V_o represent the volume of the aqueous and the oil phase, respectively. C_{aq} is the drug concentration found in the aqueous buffer at equilibrium.

2.4. HPLC and UV analyses

Flupentixol was analysed by an HPLC method. The column was a YMC-Pack C-4, 5 μm, 100×4.6 mm. The mobile phase consisted of 0.01 M phosphate buffer pH 7.0, acetonitrile and tetrahydrofurane (30:65:5). The flow rate was maintained at 1 ml/min and the oven temperature was set at 30°C. The column effluent was monitored at 254 nm.

Naproxen was analysed by HPLC or UV spectrophotometry. The HPLC system was based on a MOS HYPERSIL column, 5 μm, 100×4.6 mm (Hewlet Packard) using a mobile phase consisting of 0.05 M phosphoric acid and acetonitrile (55:45). The flow rate was 1 ml/min and the oven temperature was set at 30°C. The effluent was monitored at 235 nm. UV measurements were done at 230 nm using a Shimadzu UV-1601PC spectrophotometer.

3. Results and discussion

Initial experiments revealed that identical release data were obtained by using membranes with cut off values of 8000 and 12 000–14 000 Da, respectively. In addition, variation of the revolution speed of the dialysis cell (25, 50 and 100 rpm) was found to be without influence on the release process, indicating that sufficient stirring was provided by the rotating cell. Based on these observations further experimental runs were performed by using the 12 000–14 000 Da membrane type and a revolution speed of 50 rpm. All release experiments were done in triplicate. In most cases standard deviations below 4% were observed.

Release profiles were determined from variation of the following experimental conditions: (1) drug concentration in fractionated coconut oily solutions, (2) volume of oily solution applied to the dialysis cell and (3) pH of the aqueous release medium. Typical plots of amount of drug released (calculated as a percentage of the total amount initially applied to the dialysis cell) versus time are

shown in Figs. 3 and 4. The release profiles are characterised by the total amount of drug delivered to the release medium at equilibrium and the rate of release of drug from the vehicle. The former parameter is controlled by the partition coefficient between oil vehicle and release medium (Table 1). The drug concentration in the aqueous phase at equilibrium can be calculated from the partition coefficient and the mass balance equation. Values of the calculated (M_{cal}) and experimentally determined (M_{obs}) total amount released of flupentixol and naproxen, respectively, at different test conditions appear to be comparable (Tables 2 and 3).

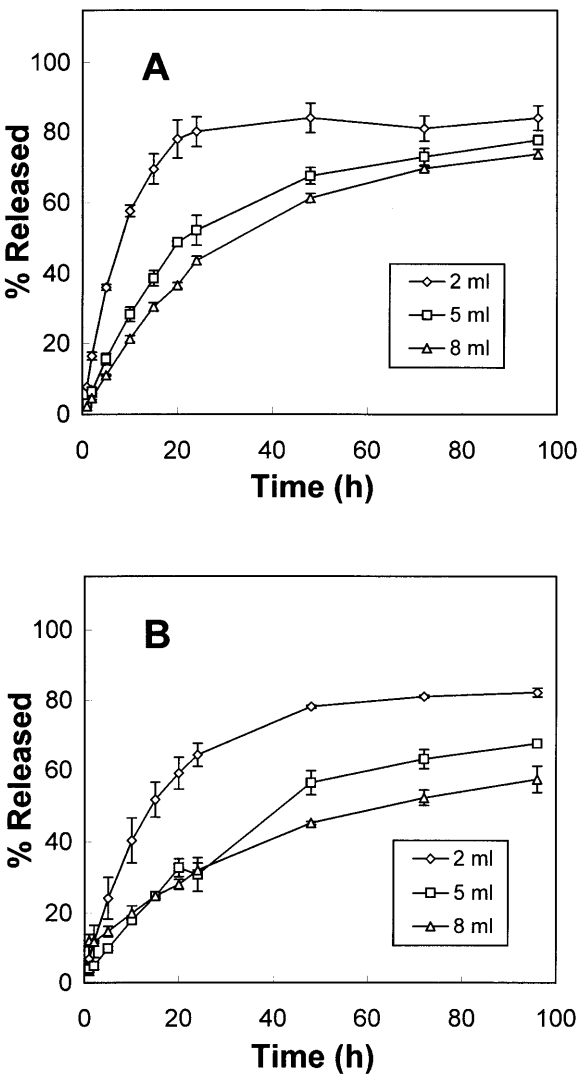


Fig. 3. Release profiles at pH 5.00 for three different volumes of formulation. A: flupentixol, 5 mg/ml; B: naproxen, 2 mg/ml. Mean values of three determinations and S.D. are shown.

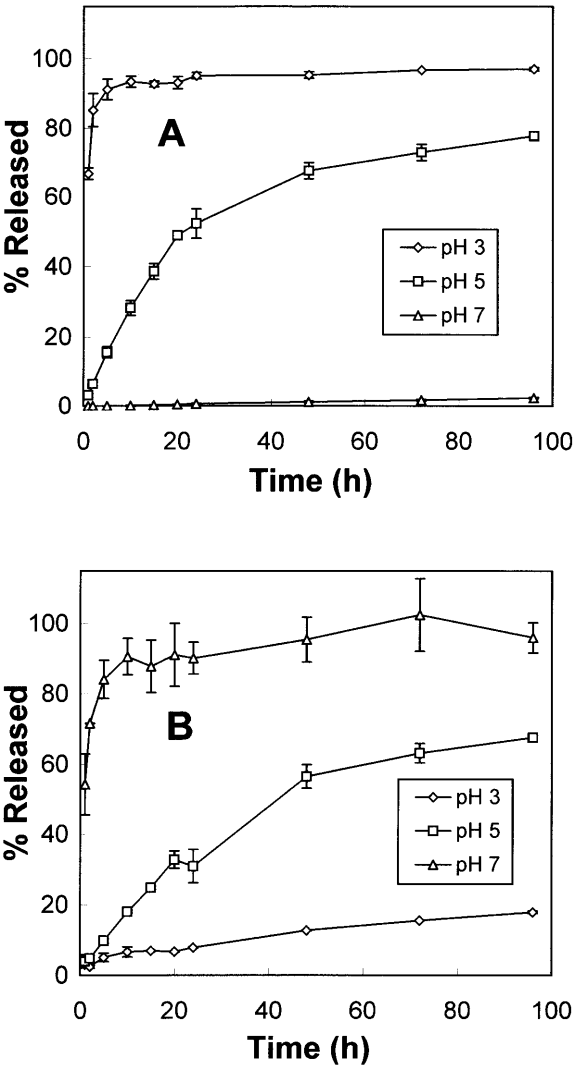


Fig. 4. Release profiles for 5 ml of formulation at three different pH values. A: flupentixol, 5 mg/ml; B: naproxen, 2 mg/ml. Mean values of three determinations and S.D. are shown.

Ratios of M_{obs}/M_{cal} slightly below unity might reflect drug adsorption to the dissolution assembly or that equilibrium has not been totally established. Since no further release of drug occurs,

Table 1
Partition coefficients determined at 37°C for flupentixol and naproxen between oil vehicle and aqueous buffer

Model drug	pH 3.00	pH 5.00	pH 7.00
Flupentixol	0.4	29.9	1440.5
Naproxen	404.1	74.9	1.0

Mean values of two determinations are shown.

when the experimental period is extended to 168 h, the low ratio is suggested to be due to adsorption. This is in agreement with ratios close to unity at pH 7.00 for naproxen and pH 3.00 for flupentixol assuming that adsorption predominantly takes place for the unionized form of the drug compounds. As previously reported by Petty and Cunningham (1974), we observed that addition of small amounts of serum albumin to the release medium prevents adsorption of drugs. Presumably albumin adsorbs to the surface itself, thus displacing the drug from the adsorption sites. The rate of drug release from the oil vehicle has been expressed by the mean release time (Brockmeier, 1986). In order to enable comparison of the individual profiles MRT values have been derived by plotting the amount of drug released relative to the total amount at 96 h as a function of time (Tables 2 and 3).

Three concentrations of flupentixol in fractionated coconut oil, (2, 5 and 10 mg/ml) were tested applying identical volumes to the dialysis cell. Within the drug concentration range investigated no statistical significant difference (analysis of variance) in the release characteristics was observed (Tables 2 and 3). Likewise, almost identical relative release rates resulted from experiments with oily solutions of naproxen (1, 2 and 3 mg/ml). However, enhanced absolute release rates (mg drug released per time unit) resulted from increasing the concentration of the drug in the oil vehicle, indicating that transport through the dialysis membrane is not rate limiting for the phase transfer process.

The shape of the release profiles was influenced by variation of the test volumes (2, 5 and 8 ml) of oily solutions of flupentixol or naproxen (Fig. 3). With the volume of the aqueous phase kept constant the total percentage of drug released (M_{obs}) increased with decreasing volume of oily solution in the dialysis cell (Tables 2 and 3). Thus, M_{obs} was enhanced 14% (flupentixol) and 44% (naproxen) by a 4-fold reduction of the test oil volume. This observation is consistent with the fact that naproxen under the experimental conditions used, exhibits a larger partition coefficient than flupentixol. Also, drug release as expressed by mean release times (Tables 2 and 3) was faster from small oil volumes compared to those from larger volumes. The observed difference in MRT indicates that the influence of the relative amount of formulation in direcd contact with the dialysis membrane decreases with increasing test volumes in the dialysis cell.

Table 2
Total amounts of drug released, release rates and curve parameters for experiments with flupentixol

Drug conc. (mg/ml)	Volume of formulation (ml)	pH of medium	M_{obs} (%)	M_{obs}/M_{cal}	MRT (h)	$k' \cdot 10^2$ (h^{-1})	r^2
2	5	5.00	79	0.91	20.9	4.7	−0.99
5	5	5.00	78	0.90	23.1	3.9	−1.00
10	5	5.00	80	0.92	19.0	5.2	−1.00
5	2	5.00	84	0.89	9.6	14.8	−1.00
5	8	5.00	74	0.92	26.7	4.0	−1.00
5	5	3.00	97	0.97	2.7	4.8	−0.90
5	5	7.00	2	0.16	45.2	1.8	−0.99

M_{obs} is the observed percentage of drug released after 96 h. M_{obs}/M_{cal} is the ratio between the observed and calculated total percentages of drug released, respectively. Rate constants (k') and correlation coefficients (r^2) are derived from plotting data according to Eq. (5). Three levels of each variable parameter, drug concentration, volume of formulation and pH of the medium, respectively, were tested.

Table 3
Total amounts of drug released, release rates and curve parameters for experiments with naproxen

Drug conc. (mg/ml)	Volume of formulation (ml)	pH of medium	M_{obs} (%)	M_{obs}/M_{cal}	MRT (h)	$k' \cdot 10^2$ (h^{-1})	r^2
1	5	5.00	63	0.87	27.6	3.9	−1.00
2	5	5.00	68	0.93	29.0	3.8	−0.99
3	5	5.00	64	0.88	30.3	3.7	−0.99
2	2	5.00	82	0.94	16.2	5.9	−1.00
2	8	5.00	57	0.91	27.7	3.1	−1.00
2	5	3.00	18	0.54	32.7	2.5	−0.99
2	5	7.00	96	0.96	4.0	7.7	−0.94

M_{obs} is the observed percentage of drug released after 96 h. M_{obs}/M_{cal} is the ratio between the observed and calculated total percentages of drug released, respectively. Rate constants (k') and correlation coefficients (r^2) are derived from plotting data according to Eq. (5). Three levels of each variable parameter, drug concentration, volume of formulation and pH of the medium, respectively, were tested.

Aqueous buffers of pH 3.00, 5.00 and 7.00 were tested as release media for oily solutions of the model drug compounds. Both the release rate and the total amount of drug released after 96 h were highly affected by the pH value (Fig. 4). As expected, the release process became very slow when employing a buffer system favouring the unionized form of the drug. Significant differences between M_{cal} and M_{obs} values at pH 3.00 for naproxen and pH 7.00 for flupentixol were observed (Tables 2 and 3) suggesting that the release experiments had not reached equilibrium within 96 h.

As apparent from the release profiles the rate of release of drug substance is diminishing as the aqueous concentration approaches the equilibrium concentration, which is in accordance with the fact that the release process is governed by a concentration gradient. Consequently, MRT values should be inversely proportional to the corresponding M_{obs} values. In Fig. 5, MRT is plotted as a function of M_{obs} clearly showing the correlation, although only a correlation coefficient of -0.87 was calculated. Therefore, the rate with which the drug leaves the oil vehicle might tentatively be described by Eq. (3):

$$\frac{dw}{dt} = k \cdot (C_{equilibrium} - C_t) \quad (3)$$

where dw/dt is the amount of drug released per time unit, $C_{equlibrium}$ and C_t are the drug concentrations in the release medium at equlibrium and at time (t), respectively, and k is a constant including the interfacial area, the diffusion coefficient and the thickness of the diffusion layer. The equation bears resemblence to the Noyes-Whitney equation for dissolution of solids:

$$\frac{dw}{dt} = \frac{D \cdot A}{h} \cdot (C_s - C_t) \quad (4)$$

where D is the diffusion coefficient, A is the surface area exposed to the solvent, h is the thickness of the diffusion layer and C_s is the solubility of drug substance in the dissolution

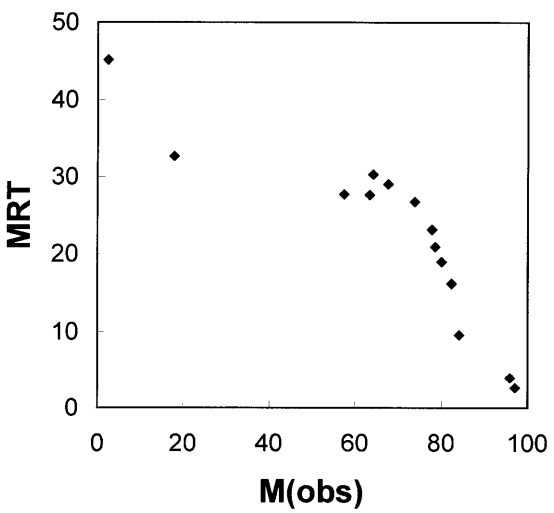


Fig. 5. Correlation between the release rate (MRT) and the maximum percentage of drug released (M_{obs}).

medium. In the dialysis experiment the interfacial area between the two phases is constant, while in dissolution of drug substances A varies with time. The main parameters controlling the processes are $C_{\text{equilibrium}}$ in the dialysis cell system and the solubility in the dissolution process, respectively.

Integration of Eq. (3) gives:

$$\ln(1 - \frac{\%\ \text{released}_{t=t}}{M_{\text{obs}}}) = -k' \cdot t \quad (5)$$

The release data were treated according to Eq. (5) and the calculated apparent first order rate constants are presented in Tables 2 and 3. Correlation coefficients close to unity imply that Eq. (3) adequately describes the release profiles. As seen from Tables 2 and 3 the rate constants are inversely proportional to the MRT values. Changes in the geometrical design of the dialysis cell in order to vary the interfacial area between oil phase and aqueous phase will influence the rate constants directly because of incorporation of interfacial area in the constant.

The rotating dialysis cell was assessed as an in vitro release method to be used in formulation development work and quality control of parenteral oily depot solutions. Thus, no specific efforts were made to develop systems potentially feasible for establishment of in vitro-in vivo correlations. The release characteristics can be optimised particularly by selection of a suitable pH of the release medium. The release data obtained suggest that it is possible to design sufficiently discriminative systems that at the same time allow the release process to finalise within a convenient period of time. The applicability of the present method is, however, confined to drug compounds possessing reasonable aqueous solubilities. Evaluation of various solubility enhancing excipients added to the release medium is ongoing in this laboratory.

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