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Preparation and in-vitro release rate of fentanyl–cyclodextrin complexes for prolonged action in epidural analgesia

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Abstract

Fentanyl was complexed with cyclodextrin derivatives with the intention to obtain parenteral solutions able to provide prolonged analgesia following epidural administration. Three cylodextrins (CDs) suitable for parenteral use were used: hydroxypropyl-β-cyclodextrin (HP-β-CD), sulfobutylether-β-cyclodextrin (SBE-7-β-CD), and maltosyl-β-cyclodextrin (malt-β-CD). Analysis of fentanyl was done with HPLC-UV. The inclusion capacity of HP-β-CD was determined from phase-solubility diagrams at pH 6.5, 7.2 and 8.0, and those of SBE-7-β-CD and of malt-β-CD at pH 8.0. Solubility of fentanyl increased linearly (i) as a function of the CD concentration, and (ii) with decreasing pH. Complexation was highest with HP-β-CD and malt-β-CD, much higher than with SBE-7-β-CD, with stability constants at pH 8.0 of 801, 729 and 1309 M^{-1}, respectively. The CD concentration was calculated to obtain a fentanyl–CD formulation, with the desired amount free fentanyl as loading dose in solution and the rest complexed with CD, as reservoir for prolonged action. A suitable membrane and a release-rate apparatus were selected for in-vitro release-rate studies. Best results were obtained with Spectrapor membranes and a home-made release-rate apparatus. Release rate was evaluated in static and dynamic conditions. For both modes, the release rate of fentanyl decreased as a function of CD concentration, due to complex formation of fentanyl, which suggests the possibility to provide prolonged pharmacodynamic effects in vivo.

Keywords: Fentanyl; Cyclodextrins; Epidural use; Inclusion complexation; Prolonged-action formulation; In-vitro release studies in static and dynamic conditions

1. Introduction

Fentanyl, a synthetic opioid, about 100 times more potent than morphine, is used for its high analgetic effect following parenteral administration. The drug has a rapid onset of effect but with short duration. Oral administration of fentanyl is hampered by its extensive first-pass hepatic metabolism. Transdermal fentanyl delivery systems (Durogesic®) provide sustained analgesia in chronic pain syndromes, but are not appropriate for acute postoperative pain treatment because of the long delay needed to achieve therapeutic plasma concentrations (Gupta et al., 1998).

Spinal injections of narcotics are known to produce profound analgesia. To increase the availability of lipid soluble drugs, and to prolong the duration of analgesia, several approaches can be considered: e.g. reducing

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vascular uptake and physical or chemical sequestration (Meert et al., 1992). To reduce the rapid vascular redistribution large diluent volumes and the addition of adrenaline are used (Klepper et al., 1987; Vercauteren et al., 1987, 1992; Meert et al., 1989). In molecular sequestration liposomes, lipid solutions or cyclodextrins are used. Prolonged duration of analgesia was demonstrated by intrathecal administration of alfentanil encapsulated in liposomes (Bernards et al., 1992) and of meperidine in a lipid solution (Langerman et al., 1991).

Cyclodextrins form molecular complexes with, for instance, drug substances. Substituted cyclodextrins, such as hydroxypropyl-β-cyclodextrin (HP-β-CD), sulfobutylether-β-cyclodextrin (SBE-7-β-CD) and maltosyl-β-cyclodextrin (malt-β-CD) are very soluble in water. Due to their very hydrophilic character and large effective radius, only very small amounts of cyclodextrin molecules and drug–cyclodextrin complexes are able to pass through biological membranes (Szejtli, 1987; Gerloczy et al., 1990; Loftsson et al., 1994a,b). If the active compound is complexed, there will be less drug available, resulting in reduced diffusion into the lipid-rich systems such as epidural fat and vessels. The active compound might remain longer available in the cerebro-spinal fluid (CSF), resulting in a prolonged effect. At the same time, fewer systemic side-effects may occur. HP-β-CD–sufentanil complexes increased the effectiveness of sufentanil after epidural and intrathecal administration in rats (Meert et al., 1992). Maximal activity depended on the cyclodextrin concentration. A still longer analgesia was noted for the combination of bupivacaine with sufentanil–cyclodextrin complexes (Meert and Melis, 1992). Jang et al. (1992) demonstrated that the use of HP-β-CD as a delivery vehicle for intrathecal opioid administration in the rat resulted in prolongation of the opioid spinal analgesic effect and reduction in systemic side effects. However, the prolonged action of opioids was not confirmed in monkeys (Bernards, 1994).

The purpose of this work is choosing a cyclodextrin for a well-defined dose of fentanyl—in a limited infusion volume—for epidural administration. Therefore, we investigated the complex formation of fentanyl with the three above mentioned cyclodextrins, suitable for parenteral use (Irie and Uekama, 1997), in order to determine the free and complexed fractions as a function of the cyclodextrin concentration. We studied the in-vitro release as a function of cyclodextrin concentration, both in static and dynamic conditions.

2. Materials and methods

2.1. Materials

Fentanyl base (Mr: 336.5) was kindly supplied by Janssen Research Foundation, Beerse, Belgium. HP-β-CD (Mr: 1380; Roquette, Lestrem, France), SBE-7-β-CD (Captisol®, Mr: 2200, Cydex, Overland Park, KS, USA), malt-β-CD (Mr: 1796, Cyclodextrin Technology Development, High Springs, FL, USA) were used as cyclodextrins for parenteral use.

KH_2PO_4 crystals extra pure, NaOH and perchloric acid pro-analyse were purchased from Merck (Darmstadt, Germany), dodecanol from Merck-Schuchardt (Hohenbrunn, Germany), acetonitrile, Hiper Solv for HPLC from BHD Laboratory Supplies (Poole, England, UK).

The membranes used were polyethersulfone membranes (PES) (Merck), TE 35 (Schleicher and Schuell, Dassel, Germany), silastic membranes (Dow Corning, Seneffe, Belgium) and Spectrapor membrane tubing (standard cellulose dialyse tubing, cutoff: 12,000–14,000 Da, Spectrum Medical Industries, Los Angeles, CA, USA). The membranes were immersed in phosphate buffer pH 8.0 or in dodecanol during the experiments.

2.1.1. HPLC apparatus

The equipment used for the fentanyl HPLC assay included a L-6000 pump (Merck-Hitachi, Tokyo, Japan), equipped with a Rheodyne injector fitted with a 20 or 100 μl loop, a variable wavelength ultraviolet (UV) Perkin-Elmer LC 90 UV Spectrophotometric detector (Shelton, CT, USA) and a D-2500 Chromato Integrator (Merck-Hitachi). The stationary phase consisted of a C_8-column (LiChrospher 100, RP-8, 125 mm × 4 mm × 5 μm, Merck), while a mobile phase, composed of aqueous perchloric acid–acetonitrile (65:35 v/v) was used. Ultraviolet detection at 215 or at 206 nm was performed. The mobile phase was prepared in two steps: 2 ml perchloric acid was diluted to 1000 ml with water. From this solution 650 ml was

taken and 350 ml acetonitrile was added. The flow rate was 1.0 ml/min.

The buffers pH 6.5, 7.2 and 8.0 were prepared as described in the USP 24.

2.1.2. Other apparatus

For weighing low masses a microbalance (Mettler-Toledo, Zürich, Switzerland) was employed and otherwise an analytical balance (Sartorius, Göttingen, Germany). The water used in all experiments was mQ water, obtained from a milli-Q water purification system (Millipore, Molsheim, France). To shake samples, a water bath shaker (Branson, Danbury, CT, USA) was placed in a thermostatically controlled room at 25 °C. Centrifugation of the samples was performed with an IEC Centra centrifuge (International Equipment Company, Bedfordshire, UK). All pH measurements were performed using a Radiometer Copenhagen PHM 26 pH meter (Copenhagen, Denmark), calibrated using pH 4.0, 7.0 and 10.0 standard buffers (Merck). The pH of small sample amounts was measured by a WTW Multical pH meter (Metro Parkway, FL).

2.2. Methods

2.2.1. High-performance liquid-chromatography analysis

Different calibration lines were measured depending on the type of experiment considered. A calibration line for analysis of fentanyl in phase-solubility experiments was made from a stock solution containing 0.80 mg fentanyl/ml mobile phase. From the stock solution working solutions were prepared in a concentration range of 0.01–0.80 mg fentanyl/ml mobile phase and analysed at 215 nm with HPLC using a loop of 20 μl.

Calibration lines for analysis of fentanyl in release-rate experiments were also made. In static conditions, HPLC-analysis is executed at 215 nm with a loop of 20 μl for a concentration range of 0.5–2.5 μg/ml, obtained by diluting a stock solution with 0.50 mg/ml fentanyl base in mobile phase. In dynamic conditions, HPLC-analysis was performed at 206 nm with a loop of 100 μl. A stock solution of 40 mg fentanyl base in 1000 ml buffer, pH 8.0 and an intermediate stock solution consisting of a 20 times, with buffer pH 8.0 diluted stock solution, were prepared. From this latter solution five working solutions between 0.08 and 0.20 μg/ml were diluted.

2.2.2. Phase-solubility diagrams

A range of HP-β-CD concentrations (0, 1, 2, 5, 9, 12%, w/v) was prepared in the buffers pH 6.5, 7.2 and 8.0. For the two other CDs, the same CD concentration range was made, but only in buffer pH 8.0. An excess of solid fentanyl was added to 5 ml of each CD-solution in screw-capped test tubes. The preparations were shaken during one week in a thermostatically controlled water bath at 25 °C. When fentanyl dissolved the pH slightly changed, and was adjusted regularly by adding 0.1 N HCl. The corresponding dilution of CD was taken into account during later calculations. After equilibrium, the obtained suspensions were centrifuged at $4575 \times g$ for 20 min. The supernatant was removed carefully with a micropipet. Still a minimum of undissolved activum might be present in the supernatant. Therefore, a second centrifugation under the same conditions was performed. The final supernatant was diluted with its respective buffer to ensure that the fentanyl concentration is situated in the calibration line (0.01–0.80 mg/ml). The dilution factor used was taken into account when calculating the amount of complexed fentanyl. The solutions without CD were used to determine the solubility, S_0, of fentanyl in aqueous buffer.

2.3. Release-rate studies of fentanyl

2.3.1. Enhancer cell experiment

The conditions of the release-rate study in static conditions using the "Enhancer cell" (Pharma Test, Hainburg, Germany) were as follows. The donor compartment (Enhancer cell) was filled with 7 ml of a 0.16 mg/ml fentanyl solution (without CDs) in buffer pH 8.0 and was not rotated. The acceptor compartment contained 150 ml of phosphate buffer pH 8.0, equivalent to the volume of the CSF. Donor and acceptor compartments were separated by a semi-permeable membrane with a surface of 10.75 cm^2. The "Enhancer cell" was just immersed in the acceptor fluid, which was stirred. The whole was maintained at 25 °C. At specified time intervals, 1 ml sample was taken from the acceptor compartment and replaced by buffer. The sample was analysed with HPLC at 215 nm using a loop of 20 μl.

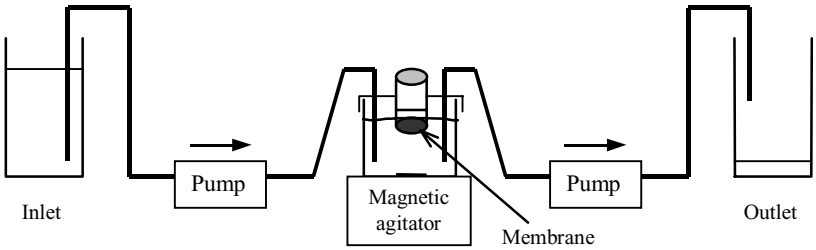

Fig. 1. Home-made set-up for static and dynamic release-rate experiments.

2.3.2. Home-made cell experiments

Adsorption of fentanyl on donor compartment (tube) and tubings of the release rate instrument was checked. To select the donor compartment two tubes, a plastic Falcon (polypropylene) (Becton Dickinson Labware Europe, Meylan, France) and a glass tube, were filled with 7 ml of a 0.16 mg/ml fentanyl solution. After 1 and 3 days, the concentration in the solution was determined and compared with the original solution. The inlet and outlet tubings (PVC, Beldico, Marche en Famenne, Belgium) were also filled with the fentanyl solution and analysed for fentanyl concentration after 3 days of storage.

For the release-rate study in static conditions, a home-made cell was used as donor compartment: an open glass tube. A screw cap with a hole of 8.04 cm^2 surface was put on one side of the tube, maintaining the semi-permeable membrane. The acceptor compartment was a beaker of 250 ml, the acceptor solution 150 ml of buffer which was stirred to avoid gradients. To avoid evaporation, the tube was closed with parafilm and the acceptor compartment was protected by a cover (Fig. 1).

For the dynamic release-rate study, the same glass tube was used, however, now in flow through conditions, as schematically drawn in Fig. 1. For that purpose two pumps (Argus, Lameris, Heimberg, Switzerland), an inlet and outlet pump, were used to ensure the continuous flow-through of the solution. The flow of the pumps was 23 ml/h. The donor compartment was filled with 7 ml fentanyl solution. The volume in the acceptor compartment was 150 ml.

At specified time intervals, a sample of 1 ml eluting from the acceptor compartment was collected and analysed for fentanyl.

2.3.3. Membranes

To select the membrane for release-rate studies, the highest possible fentanyl concentration, i.e. about 8 mg/50 ml was dissolved in buffer pH 8.0 and 7 ml of this solution was used to select the membrane using the home-made cell in static conditions. Membranes must fulfil some important requirements. Fentanyl must pass the membrane, but it must prevent the passage of CD and CD–fentanyl complex. Therefore, the permeation of CDs through the membrane also is investigated. The donor compartment contained a fentanyl–cyclodextrin solution, the acceptor compartment contained only buffer. The membrane used was a Spectrapor membrane. After ending the release-rate study, the acceptor compartment was analysed for cyclodextrin with the method of (Vikmon, 1981).

3. Results and discussion

3.1. High-performance liquid-chromatography analysis

The method was based on Lambropoulos et al. (1999, 2000). A chromatogram is presented in Fig. 2. Retention time of fentanyl was ca. 9 min. Linearity was obtained from 0.01 to 0.8 mg/ml for the phase-solubility studies calibration line. The calibration line for release-rate studies in static conditions (detection at 215 nm, loop 20 μl) and in dynamic conditions (detection at 206 nm, loop 100 μl) showed linearity from 0.5 to 2.5 μg/ml and from 0.08 to 0.20 μg/ml, respectively with corresponding respective equations: $y = 74883x + 5914.7$ and $y = 585868x + 3637$, with y is the peak area and x is the concentration in μg/ml. The calibration line at

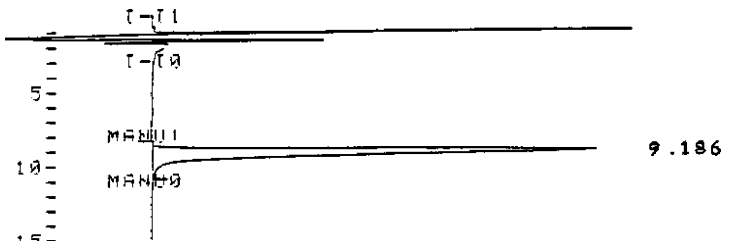

Fig. 2. Chromatogram of fentanyl at 206 nm using a mobile phase composed of aqueous perchloric acid–acetonitrile (65:35 v/v). Conditions: see text.

206 nm and a loop of 100 μl were necessary to allow analysis of the low concentrations of fentanyl in the dynamic-release-rate experiments. The quantification limits, measured at 215 and 206 nm, were 0.5 and 0.08 μg/ml with %RSD of 0.7 and 3.4% ($n = 6$), respectively.

The interferences of the different CDs in the fentanyl determination were examined. In the phase-solubility studies, the samples had to be diluted for analysis with HPLC and the highest concentration of CD present in the samples was 1.5%. For that reason, 1.5% solutions of the three CDs in buffer were injected. No interference was noted: only a small peak appeared around 4 min, well separated from the fentanyl peak (ca. 9 min).

The possible change in molar absorbance coefficients of fentanyl after complex formation with CD, was investigated. After diluting the fentanyl–CD samples, a proportional quantity of fentanyl was recovered, suggesting no change in the absorption coefficients of fentanyl due to inclusion (a few examples are given in Table 1).

3.2. Phase-solubility diagrams

To draw the phase-solubility diagrams at different pH values, it was necessary to control it. Indeed, as fentanyl dissolves, the pH slightly changes. A change in pH due to dissolution of the active compound as a function of time was also noted by McCandless and Yalkowsky (1998). Phase-solubility diagrams, i.e. the experimentally determined concentrations of fentanyl (mg/ml) as a function of the HP-β-CD concentration (g/100 ml), were determined at three different pH values (6.5, 7.2 and 8.0) (see Fig. 3a). The pH values of 6.5 and 8.0 were choosen, as they are the pH extremes for epidural use. The pH value of 7.2 is an intermediate pH. Each sample was injected three times and the peak areas were averaged. It can be noted that the solubility of fentanyl is increased with decreasing pH. The targeted concentration 0.429 mg/ml, which is derived from 3 mg fentanyl/7 ml solution for injection (see Section 3.3), can be obtained at the two low pH values without using CDs (Table 2). At pH 8.0 a 1% HP-β-CD is sufficient to solubilise the required

Table 1
Estimated and theoretical fentanyl concentrations in fentanyl–CD complexes after dilution

% CD	pH	Fentanyl (mg/ml)	Diluting factor	Estimated fentanyl concentration (E) (mg/ml)	Theoretical fentanyl concentration (T) (mg/ml)	E/T (%)
HP-β-CD						
9.02	7.2	7.185	1.77	4.109	4.059	101.2
12.45	8.0	8.931	6.23	1.427	1.433	99.6
SBE-7-β-CD						
12.6	8.0	5.303	2.13	2.539	2.490	102.0
12.6	8.0	5.303	3.98	1.324	1.332	99.4
Malt-β-CD						
8.95	8.0	6.801	2.07	3.229	3.286	98.3
8.95	8.0	6.801	4.49	1.507	1.515	99.5

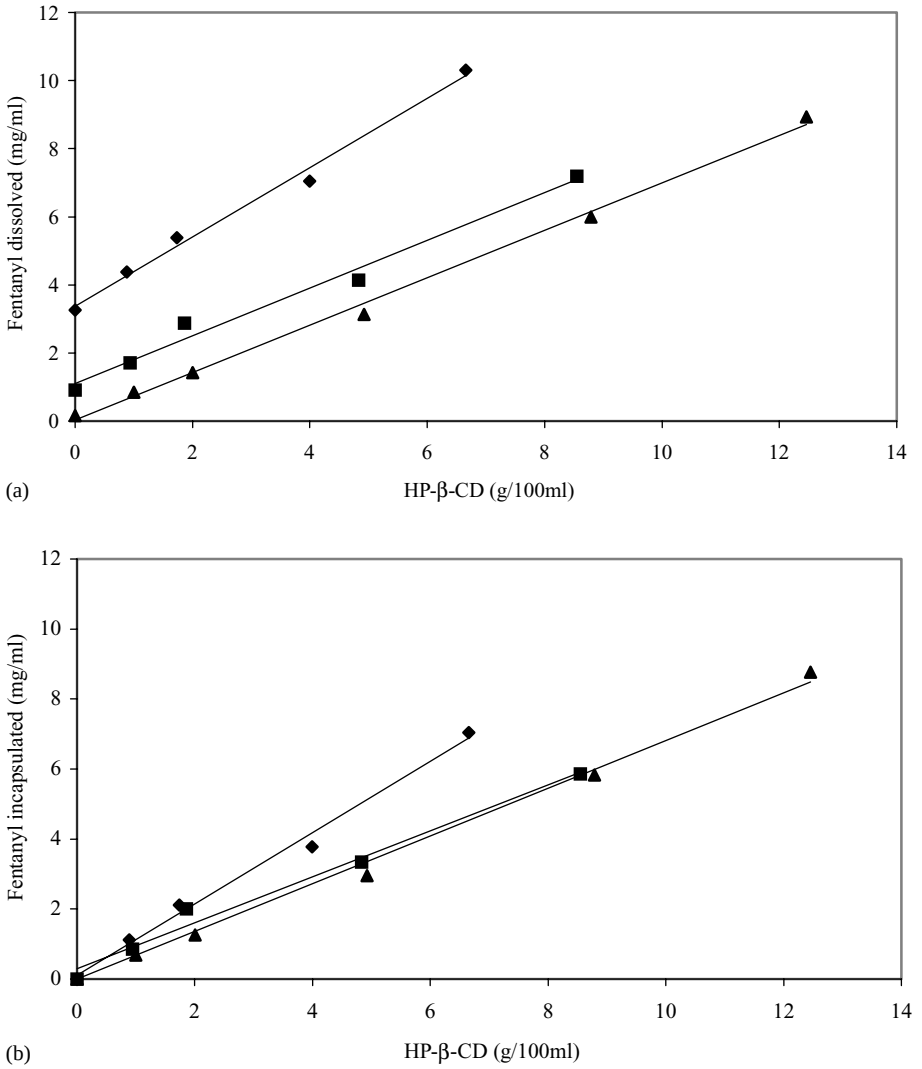

Fig. 3. (a) Phase-solubility diagram of fentanyl and (b) inclusion of fentanyl, both expressed as mg/ml, as a function of the HP-β-CD concentration (g/100 ml) at three different pH values at 25 °C (◆: pH 6.5; ■: pH 7.2; ▲: pH 8.0).

amount. When subtracting the solubility of fentanyl in the three buffers (Fig. 3b), the inclusion of fentanyl is found to be highest at pH 6.5, much higher than at pH 7.2 and 8.0, at which the complexation seems rather comparable. The complexation of fentanyl with SBE-7-β-CD and malt-β-CD was only tested at pH 8.0 (Fig. 4), as the K_c for HP-β-CD was higher at pH 8.0 than at pH 7.2 or 6.5 (Table 2). The higher the K_c, the more fentanyl is complexed, the less CDs are needed to obtain a given amount of complexed fentanyl. The aim of this study is to obtain a prolonged action. Therefore, preparations with a high K_c value are preferable (see Section 3.3).

For a given concentration of CD, the complexation capacity of malt-β-CD was comparable to HP-β-CD, which is much higher than for SBE-7-β-CD (Fig. 4). The phase-solubility diagrams were also drawn with the molar fentanyl concentrations and CDs (Fig. 5). The curves are linear, indicating 1:1 complexes (Higuchi and Connors, 1965). The stability constants

Table 2
Solubility (S_0) of fentanyl in aqueous buffer and stability constants (K_c) of fentanyl–cyclodextrin complexes at different pH and for different cyclodextrins

CD	pH	S_0[a] (mg/ml)	Slope[b]	S_0 ($\times 10^{-3}$ M)	K_c values (M^{-1})
HP-β-CD	6.5	3.2619	0.4171	9.694	72
HP-β-CD	7.2	0.8579	0.2691	2.549	108
HP-β-CD	8.0	0.1674	0.2848	0.497	801
SBE-7-β-CD	8.0	0.1674	0.2660	0.497	729
Malt-β-CD	8.0	0.1674	0.3944	0.497	1309

[a] Experimentally determined solubility value ($n = 3$).
[b] Slope of the linear curve of Fig. 5.

(K_c) for the 1:1 complexes were estimated from the equation:

$$K_{c_{1:1}} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

for the line, describing the linear increase in solubility of activum as a function of CD concentration (Higuchi and Connors, 1965). S_0 refers to the solubility of the drug in the absence of cyclodextrin, for which we used the experimentally determined solubility value ($n = 3$), while 'slope' is the slope of the line (Fig. 5). The results are presented in Table 2. The K_c values increase as a function of pH. By increasing the pH, fentanyl becomes more lipophilic and the S_0 values decrease. At pH 8.0, the stability constants are comparable for the fentanyl–SBE-7-β-CD (729 M^{-1}) and the fentanyl–HP-β-CD complexes (801 M^{-1}). The constant for the fentanyl–malt-β-CD complex (1309 M^{-1}) is higher.

3.3. Selection of the cyclodextrin–fentanyl formulation with prolonged action

Current epidural analgesia techniques with fentanyl in humans start with a loading dose of 1.5 μg/kg body weight, followed by a maintenance dose of 1 μg/kg/h (van Lersberghe et al., 1994). This means that for a person of 70 kg, a loading dose of 0.105 mg is necessary. Aiming at a prolonged analgesic action of 2 days, the CD–fentanyl complex should provide an additional dose of 3.36 mg fentanyl. Thus, the selected solution should contain about 3.5 mg fentanyl base in

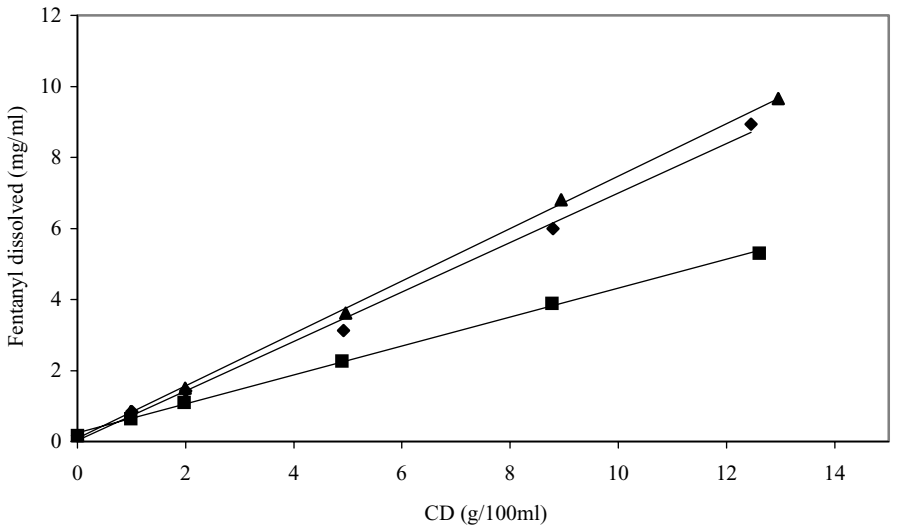

Fig. 4. Phase-solubility diagrams of fentanyl, expressed as mg/ml, as a function of the concentration of different cyclodextrins (g/100 ml) at pH 8.0 and at 25 °C (◆: HP-β-CD; ■: SBE-7-β-CD; ▲: malt-β-CD).

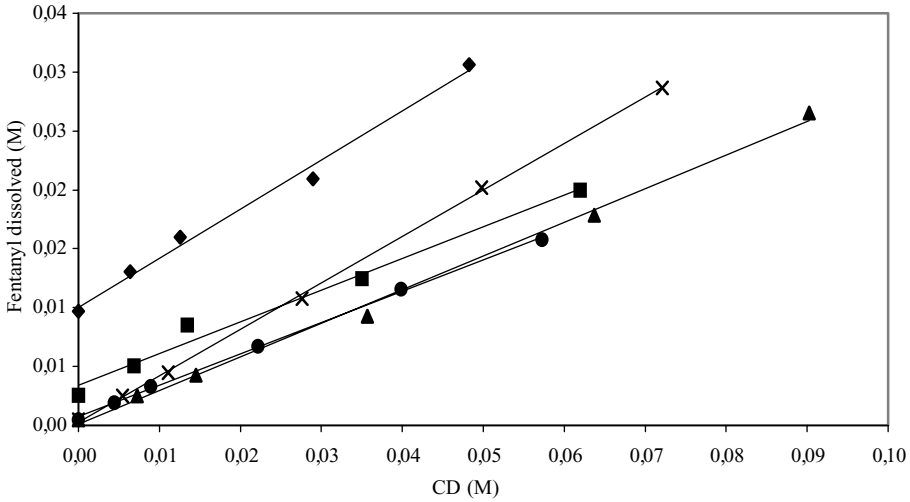

Fig. 5. Phase-solubility diagrams of fentanyl, expressed in M, as a function of the concentration of different cyclodextrins (M) at different pH for HP-β-CD and at pH 8.0 for SBE-7-β-CD and for malt-β-CD and at 25 °C (◆: HP-β-CD, pH 6.5; ■: HP-β-CD, pH 7.2; ▲: HP-β-CD, pH 8.0; ●: SBE-7-β-CD, pH 8.0; ×: malt-β-CD, pH 8.0).

7 ml buffer, the maximal volume for epidural injection. Therefore, we aimed to prepare a formulation containing 3 mg fentanyl base in 7 ml buffer. To select the CD concentration for a free fentanyl concentration of 0.105 mg/7 ml (loading dose), the solubility data are transformed according to following equations:

$$K_c = \frac{[L\text{–CD}]}{[L_f][\text{CD}_f]} \quad (2)$$

with [*L*–CD], the 1:1 complex fentanyl–CD concentration, which is our unknown concentration *x*; $[L_f]$, the free fentanyl concentration and $[\text{CD}_f]$, the free cyclodextrin concentration. This equation can be rewritten as

$$K_c = \frac{x}{([L_t] - x)([\text{CD}_t] - x)} \quad (3)$$

with $[L_t]$, the total fentanyl concentration and $[\text{CD}_t]$, the total CD concentration. All concentrations are in mol/l. With 3 mg/7 ml the total dose of fentanyl, $[L_t]$ is 1.27×10^{-3} M and when K_c is known, the unknown concentration [*L*–CD] can be calculated for every CD_t concentration, and consequently also the L_f and CD_f concentrations. In Figs. 6 and 7, the calculated free fentanyl concentrations are plotted as a function of the cyclodextrin concentration. From Fig. 6, it can be noted that at a given concentration of HP-β-CD, the fraction of complexed fentanyl is higher with increasing pH. The free fraction is also decreasing as a function of the CD concentration, which happens much faster at high pH values. At a given CD concentration at pH 8.0 (Fig. 7), higher fractions of fentanyl are complexed with HP-β-CD and malt-β-CD than with SBE-7-β-CD.

A given parenteral preparation of 7 ml would be diluted in the CSF. Therefore, the above calculations can also be done for a given dilution of the preparation. The fractions free fentanyl as a function of the dilution (up to dilution factor 20) are presented in Fig. 8 for different concentrations of HP-β-CD. The higher the initial concentration of CD, the higher the complex formation, the smaller is the influence of dilution on the percentage free fentanyl. This is also the case for the two other CDs (results not shown).

From the above data, a practical selection of the CD and its required concentration can be made. For a loading concentration of 0.105 mg free fentanyl/7 ml solution in a preparation with a total amount of 3 mg fentanyl, the required CD concentrations are summarised in Table 3. The required amount of HP-β-CD is too high to be feasible at pH 6.5 and 7.2. At pH 8.0, two solutions can be selected: 3 mg fentanyl in 7 ml of a 5% HP-β-CD or of a 4% malt-β-CD. Although malt-β-CD shows the highest stability con-

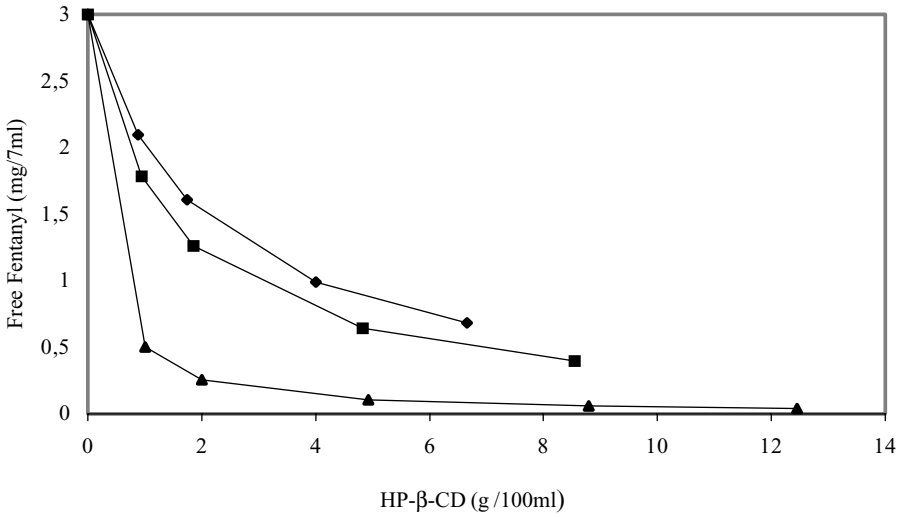

Fig. 6. Calculated free fentanyl concentrations (mg/7 ml), present in a preparation with 3 mg fentanyl/7 ml, expressed as a function of the HP-β-CD concentration (g/100 ml) at different pH values and at 25 °C (◆: pH 6.5; ■: pH 7.2; ▲: pH 8.0).

stant and therefore also the most complex formation at a given cyclodextrin concentration, the release-rate studies were only performed with HP-β-CD. For this derivative, namely safety profiles are best described. It shows very low toxicity when administered by the parenteral route (Pitha, 1985; Brewster, 1991), no adverse effects have been seen in humans (Carpenter et al., 1987; Vandewoude et al., 1997) and it is registered for parenteral use.

3.4. Release-rate studies

The membranes used in these studies must fulfil some important requirements. As mentioned before, fentanyl should pass the membrane, while the passage of CD and CD–fentanyl complex is prevented. The passage through the membrane may not be the rate limiting step. Sink conditions in the acceptor compartment must be maintained during the whole ex-

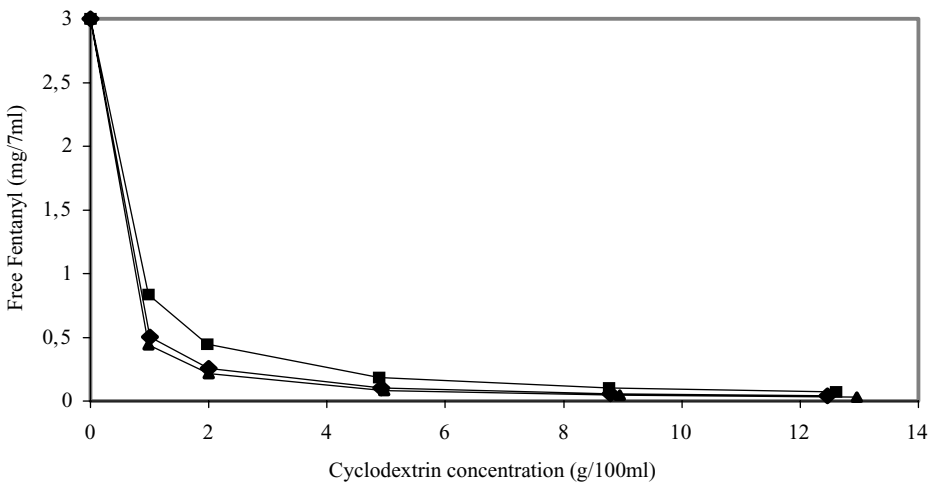

Fig. 7. Calculated free fentanyl concentrations (mg/7 ml), present in a preparation with 3 mg fentanyl/7 ml, expressed as a function of the concentration of different cyclodextrin concentrations (g/100 ml) at pH 8.0 and 25 °C (◆: HP-β-CD; ■: SBE-7-β-CD; ▲: malt-β-CD).

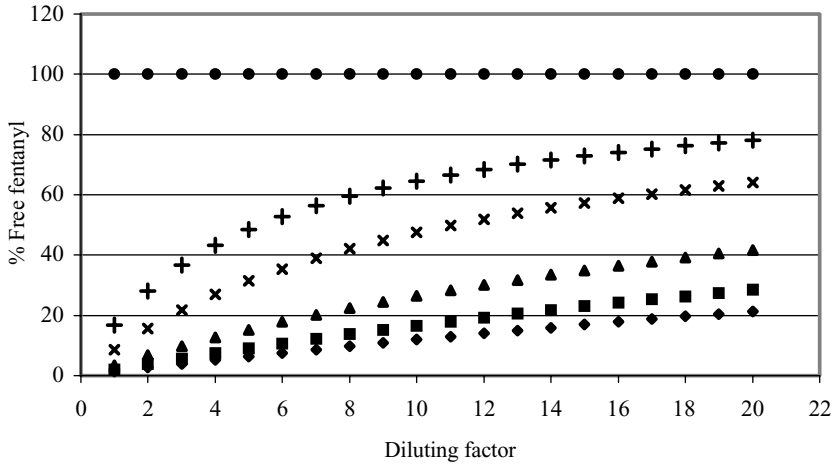

Fig. 8. Percent of free fentanyl as a function of the dilution factor for different initial HP-β-CD concentrations (●: 0%; +: 1%; ×: 2%; ▲: 5%; ■: 9%; ◆: 12.5%, m/v) at pH 8.0.

periment. To simulate the behaviour in spinal fluid the release-rate device also must fulfil specific criteria. The donor compartment must contain 7 ml of the fentanyl–CD solution, the acceptor compartment (simulating the volume of the human CSF) contains 150 ml of solvent, and should be renewed at the rate of 23 ml/h.

Only few experiments of release rates with CD were found in the literature. Some authors (Barthélémy et al., 1986; Bettini et al., 1992) have used polyethylene membranes impregnated with Mygliol and tributylphosphate. However, this artificial lipid membrane was found to be permeable for β-CD, while it was not known whether the complex could permeate the membrane (Bettini et al., 1992). Spectrapor membranes were used by Loftsson et al. (1994b) and Loftsson and Siguroardottir (1994), who noted that both products, free drug and inclusion complex, permeated the membrane, the free drug at a respective 24 and 130 times faster rate than the complex. Other authors used dimethyl polysiloxane membranes (Nakano et al., 1976).

Table 3
Required CD concentrations to obtain a loading dose of 0.105 mg free fentanyl/7 ml solution in a preparation with a total of 3 mg fentanyl

CD derivative	pH	Required CD concentration (%, m/v)
HP-β-CD	6.5	53.0
HP-β-CD	7.2	35.4
HP-β-CD	8.0	4.9
SBE-7-β-CD	8.0	8.6
Malt-β-CD	8.0	4.0

The dissolution apparatus of the *European Pharmacopeia* could not be used as release-rate apparatus, since neither the paddle nor the basket apparatus can serve for a liquid. Other apparatus for release-rate experiments, such as the Franz diffusion cell and a Sartorius apparatus also were not suitable, since they do not allow 150 ml solvent in the acceptor compartment. The "Enhancer cell", normally used for release-rate experiments of semi-solid preparations, potentially might be used here. The cell can contain 7 ml solution and can be immersed in a recipient with 150 ml solvent. Both static and dynamic release-rate experiments potentially are possible with this cell, which therefore was tested first.

Static release-rate experiments were first executed to select a suitable semi-permeable membrane. When putting the membrane onto the "Enhancer cell" or when closing the cell, often an air bubble was enclosed, which only extremely difficulty could be removed. Air bubbles diminish the diffusion surface and lead to erroneous results. Due to the large diameter of the "Enhancer cell", the choice of membranes was also limited. For these reasons, the home-made glass cell was used. The required 7 ml solution could eas-

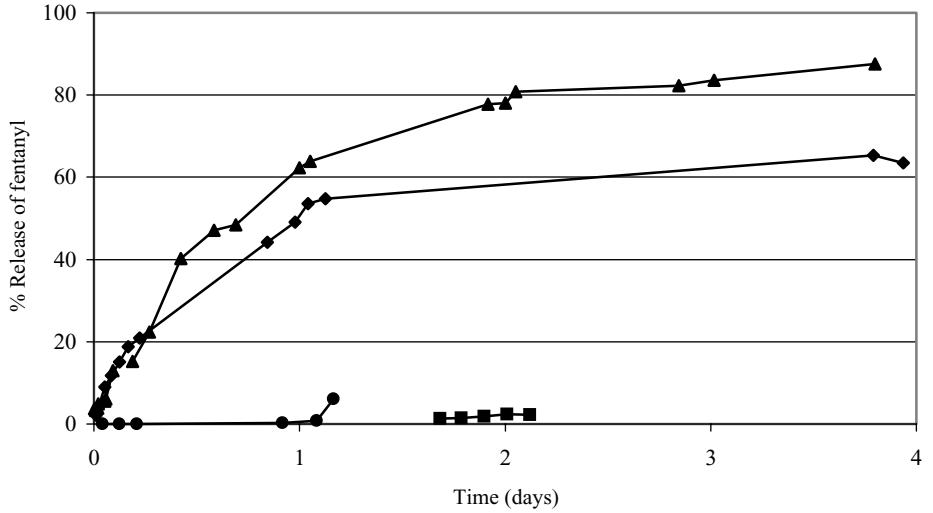

Fig. 9. Percent of a solution with 0.16 mg/ml free fentanyl diffused through different membranes (◆: silastic; ▲: Spectrapor; ■: PES; ●: TE 35).

ily be placed upon the membrane, without the risks of air bubbles. Four different membranes were tested during several days. A cumulative release-rate curve was drawn (Fig. 9). The percent release was calculated taking into account the initial fentanyl concentration, the concentration in the acceptor compartment and the withdrawn sample volumes. The experiments were reproducible (%RSD of the released percentages fentanyl was between 1.4 and 2.1% ($n = 3$)). From Fig. 9, it can be noted that the Spectrapor membranes ensured the fastest release rate. These membranes were used in the following release experiments. Impregnation of the membranes with dodecanol did not improve the release rate.

No adsorption was observed at the donor compartment when using a glass tube, while adsorption on a plastic tube occurred: 97.5 and 93.2% fentanyl were recovered after 1 and 3 days, respectively.

Before starting the release-rate studies with formulations containing different concentrations of CD, the permeation of cyclodextrins through the selected Spectrapor membrane was investigated. After 3 days, no cyclodextrin could be detected in the acceptor compartment, with the method of Vikmon (1981). The limit of detection for β-CD with this method is circa 1×10^{-4} M. When CD is present in the donor compartment the volume of this compartment increased with time due to Donnan equilibria (Martin, 1993). When using 12% HP-β-CD, for instance, the volume increased from 7 to 15.6 g at the end of the experiment. This introduces large errors due to a two-fold dilution of the CD and the fentanyl concentrations in the donor compartment and a change in the free fentanyl concentration. In an attempt to eliminate the Donnan equilibrium, 1 and 5% NaCl were added, both to donor and acceptor compartments. The problem was still not resolved: an increase with 6.15 and 6.11 g, respectively, was noted in the donor compartment. Therefore equal CD concentrations were added to the acceptor and donor compartment. With this set up, Donnan equilibria were finally eliminated.

3.4.1. Release-rate experiments in static conditions

Different concentrations of CD were tested and each experiment three times repeated. The results were very repeatable (%RSD of the released percentages fentanyl was between 1.5 and 2.4%). The averaged values ($n = 3$) of the release-rate experiments as a function of HP-β-CD are presented in Fig.10a. It can be noted that the release rate of fentanyl is negatively influenced by the cyclodextrin concentration and thus by complex formation. After 50 h only about 20, 40 and 60% fentanyl is released from the 12, 6 and 2% HP-β-CD containing solutions, respectively, meaning that fentanyl was released around 4, 2 and 1.5 times slower than for the solution without cyclodextrins.

3.4.2. Release-rate experiments in dynamic conditions

The same experiments were repeated but now in dynamic conditions. The acceptor compartment was now continuously renewed with buffer at a rate of 23 ml/h.

Due to the continuous renewel of solvent, the concentrations of fentanyl in the acceptor compartment were low. Therefore it was necessary to use a 100 μl loop and to analyse the samples at 206 nm. The release-rate cumulative curve was drawn using the trapezoidal rule (Ritschel, 1986). From the results, given in Fig. 10b, the same observations can be made as in the static release-rate experiment: a decreasing release rate with increasing CD concentration.

3.4.3. Comparison of static and dynamic release-rate results

When both experiments, static and dynamic, are compared, the absolute release rates were somewhat lower at dynamic conditions than at static ones. The reason for this remains unknown to us. At the end of each experiment, maximally 0.5% fentanyl was found to be adsorbed to the membrane. Both in static and dy-

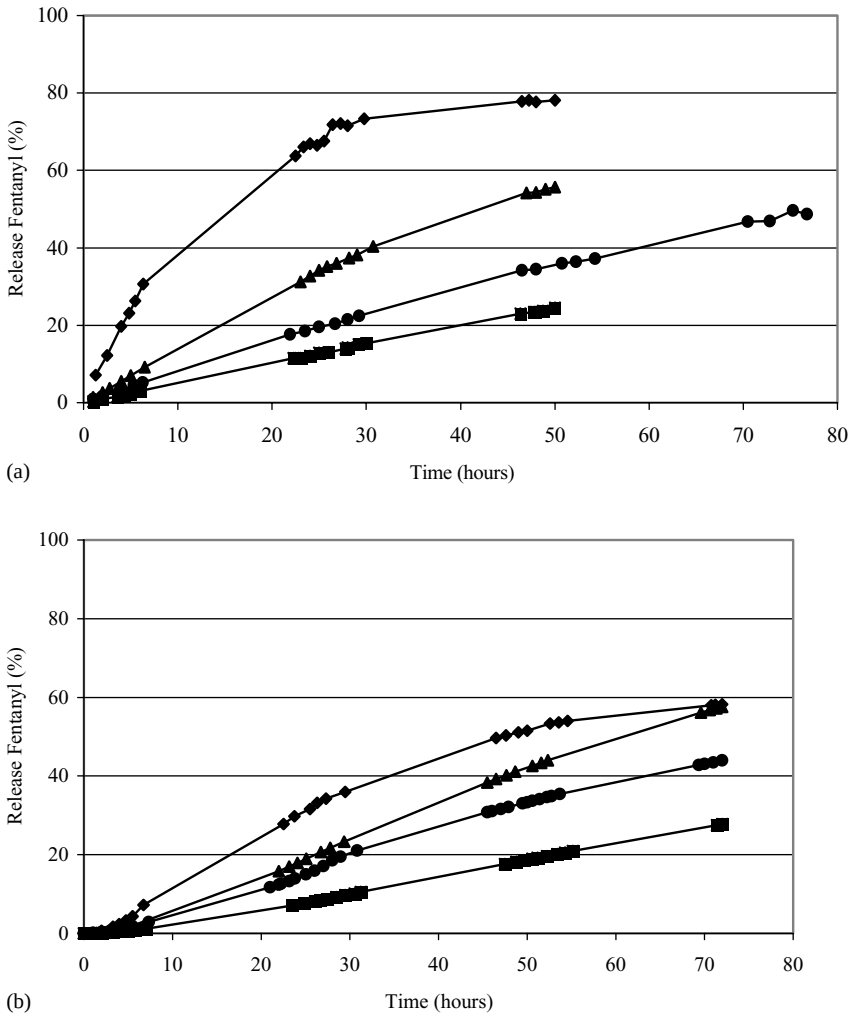

Fig. 10. Percent of released fentanyl as a function of time for different HP-β-CD concentrations in phosphate buffer, pH 8.0, using Spectrapor membranes: (a) static conditions; (b) dynamic conditions (◆: 0%; ▲: 2%; ●: 6%; ■: 12% of HP-β-CD).

namic conditions, the total fentanyl amount was recovered by detecting the remaining fentanyl in the donor compartment. However, what is even more important in the context of preparing and evaluating a formulation with a prolonged action is to compare the ratio of released amounts in situations with CD relative to those without. From such comparison (see Fig. 10) it can be observed that the ratio is higher in dynamic than in static conditions, i.e. 0.82, 0.65, 0.37 against 0.71, 0.46, 0.31 for 2, 6 and 12% HP-β-CD, respectively. This would indicate that evaluation of the prolonged release formulation from static conditions might overestimate the duration of action. However, the essential observation: a decreased release rate in the presence of given CD concentrations could be made from both types of release-rate experiments. This suggests the possibility to create a formulation with a prolonged action due to complex formation.

4. Conclusion

By drawing phase-solubility diagrams and calculating the complex formation stability constants, it is possible to select for each CD a solution where the required free fentanyl loading dose is present, while the rest of fentanyl is complexed with CD as a reservoir for prolonged action. For the in-vitro release experiments a suitable membrane and apparatus were selected. From the release-rate experiments, both in static and dynamic conditions, the release rate was negatively influenced by an increasing CD concentration, suggesting the possibility to create a formulation with a prolonged action due to complex formation.

Further investigation from in-vivo experiments will confirm whether the in-vitro in-vivo correlationship can be established.

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