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# Novel poloxamer-based nanoemulsions to enhance the intestinal absorption of active compounds

Carsten Brüsewitz [a,b,*], Andreas Schendler [a], Adrian Funke [a], Torsten Wagner [a], Ralph Lipp [b,c]

[a] *Schering AG, Pharmaceutical Development, Berlin 13342, Germany*
[b] *Freie Universität Berlin, Institute of Pharmacy, Kelchstr. 31, Berlin 12169, Germany*
[c] *Eli Lilly, Lilly Research Laboratories, Indianapolis, IN 46285, USA*

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**Abstract**

On the basis of Pluronic® P104 as primary emulsifier and Lauroglycol® 90 as amphiphilic oil phase, two nanoemulsion systems were developed with Pluronic® L62 or L81 as secondary emulsifiers. The possible nanoemulsion region of combinations of these excipients was described in ternary phase diagrams. Three formulations were selected from the nanoemulsion region and their potential impact on oral absorption was examined in the Caco-2 monolayer model of the small intestine. The apparent permeability of the BCS class III compound Atenolol was enhanced 2.5-fold, of BCS class II compound Danazol 3.2-fold and of BCS class I compound Metoprolol 1.4-fold. The three formulations were very well tolerated by the Caco-2 cells, which was confirmed by TEER measurements, a MTT test and a LDH release test.


*Keywords:* Absorption enhancer; Nanoemulsion; Poloxamer

## 1. Introduction

Nano- and microemulsions can be excellent vehicles for the oral delivery of poorly permeable and/or highly lipophilic drugs since they can be manufactured from excipients that have solubilizing or even permeation enhancing properties. Oral nanoemulsions, which are understood in this article as emulsion droplets of a size below 150 nm, are almost exclusively from the o/w type. Similar to ordinary emulsions, they promote enhanced gastrointestinal absorption and reduced inter- and intraindividual variability for a variety of drugs. Additionally, due to their very large interfacial area, they exhibit excellent drug release properties. Furthermore, nanoemulsions may offer a certain degree of protection against degradation or may improve difficult organoleptic properties of the active (Eccleston, 2002). Some nanoemulsions tend to self-emulsify in aqueous media, which makes them interesting for oral formulations. The self-emulsifying formulations can be administered as water-free preconcentrates that in situ form nanoemulsions in the fluids of the gastrointestinal tract. The preconcentrate can be easily manufactured at any scale and filled into softgels or sealable hard gelatine capsules. Another use of nanoemulsion preconcentrates can be found in early drug development. Nowadays, many new drug candidates show poor aqueous solubility (Strickley, 2004). They need to be solubilized in order to characterize their intestinal permeability. This task can be accomplished very well by nanoemulsion preconcentrates, if a good tolerability of the excipients in in vitro cell cultures like Caco-2, test animals and finally humans is assured.

Pluronics® are class of non-ionic surfactants that are well known for their very low toxicity, as shown on the example of Poloxamer 188 for injectables (e.g. Orringer et al., 2001) and ophthalmic solutions (e.g. Neosporin®, MedlinePlus website). The Pluronics®, also known as poloxamers, are triblock copolymers of poly(oxyethylene)–poly(oxypropylene)–poly(oxyethylene) [$(EO)_x(PO)_y(EO)_x$]. They are used as solubilizers, wetting agents, emulsifiers for microemulsions (Varshney et al., 2004; Siebenbrodt and Keipert, 1993) and as microcontainer for drugs after micellization (Kataoka et al., 1993). It has been demonstrated that Pluronics® may influence the carrier-mediated transport of drugs, depending on their structural composition (Batrakova et al., 2003). This effect might be advantageous for the treatment of drug-resistant tumors as well as to enhance the oral bioavailability of actives (Kabanov et al., 2002).

* Corresponding author. Tel.: +49 30 468 15120; fax: +49 30 468 95120.
*E-mail address:* carsten.bruesewitz@schering.de (C. Brüsewitz).

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It was the aim of this study to:

1. Manufacture nanoemulsion formulations based on Pluronic® P104, L62 and L81.
2. Investigate their effects on the passive transport of a BCS class I, II and III substance each.

Atenolol was selected as model compound for paracellulary transported drugs. Atenolol is a highly water soluble drug with a $c\log P$ of −0.1, a molecular weight ($M_{rel}$) of 266 Da, presenting in its molecular structure five H-bond acceptors and three H-bond donors and a polar surface area (PSA) of 95 (Vieth et al., 2004). Due to its high water solubility, the interaction with cellular membranes is limited, making it a class III drug (Vogelpoel et al., 2003) according to the Biopharmaceutics Classification System (BCS).

Metoprolol was chosen as model drug for transcellular diffusion, being the most common route of intestinal absorption. It has a $c\log P$ of 1.4, a $M_{rel}$ of 287 Da, four H-bond acceptors and two H-bond donors in its structure and a PSA of 60 (Vieth et al., 2004). Its higher lipophilicity, compared with Atenolol, facilitates membrane interactions. Metoprolol is classified as a BCS class I drug (Kasim et al., 2004).

Finally, Danazol was selected as model for BCS class II compounds (Dressman and Reppas, 2000), which have poor aqueous solubility but good membrane permeability. It has a $c\log P$ of 3.9, a $M_{rel}$ of 338 Da, three H-bond acceptors and one H-bond donor and a PSA of 49 (Vieth et al., 2004). Applying Lipinski's Rule of Five (Lipinski et al., 1997), this drug has no membrane permeation problem, its fraction absorbed will be mainly limited by its poor solubility. In contrast to Atenolol and Metoprolol, Danazol should therefore benefit the most from solubilizing formulations.

## 2. Materials and methods

### 2.1. Nanoemulsion excipients

Pluronic® P104 (HLB 13, pasty at 20 °C, av. $M_{rel}$: 5900 Da), Pluronic® L62 (HLB 7, liquid at 20 °C, av. $M_{rel}$: 2500 Da) and Pluronic® L81 (HLB 2, liquid at 20 °C, av. $M_{rel}$: 2750 Da) (BASF website, 2006) were a gift of BASF AG (Ludwigshafen, Germany). Transcutol® HP (liquid at 20 °C, $M_{rel}$: 134 Da) and Lauroglykol® 90 (liquid at 20 °C, $M_{rel}$: 261 Da) were obtained as samples from Gattefossé (Weil-am-Rhein, Germany).

### 2.2. Drugs

Atenolol, Metoprolol tartrate and Danazol were obtained from Sigma Aldrich GmbH (Schnelldorf, Germany).

### 2.3. Caco-2 experiments

Caco-2 is a human adenocarcinoma cell line derived from colon. The experiments were carried out with Caco-2 tissue culture ACC 169, obtained from the DSMZ being the German Collection of Human and Animal Cell Cultures (Braunschweig, Germany). Passage numbers 42–57 were used. Cells were seeded at a starting concentration of 100,000 cells/cm$^2$ and cultured for 21–28 days on Transwells No. 3460 from Corning Costar Co. (Cambridge, USA). The culture medium consisted of Dulbecco's Modified Eagle's Medium, containing 3.7 g/L sodium hydrogen carbonate and 4.5 g/L D-glucose and was supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 1% NEAA (non-essential amino acids), 2 mM glutamine and 10% fetal calf serum. All media components were purchased from Biochrom KG (Berlin, Germany). The medium was exchanged every second day. The cells were kept in an incubator at 37 °C and 90% relative humidity, supplied with 5% $CO_2$. During the experiment a HEPES–carbonate buffer at pH 7.2 was used as transport medium. Samples were drawn from the apical compartment (0.1 mL) at start and end of the experiment and from the basolateral compartment (1.0 mL) at 15, 30, 60, 90 and 120 min.

The samples were analyzed using HPLC, consisting of a Waters W717 autosampler together with a W474 fluorescence detector at excitation/emission wavelengths of 230/300 nm for Atenolol and Metoprolol and a W2996 PDA detector at 287 nm for Danazol. The data examination was performed using Millennium™ software. A Phenomenex Sphereclone® C6 column, 5 μm, 150 mm × 4.6 mm was used to analyze Atenolol and Metoprolol. The eluent consisted of 0.5 M $KH_2PO_4$ buffer/acetonitrile 87/13 (v/v) with 0.1% acetic acid at a flow rate of 1.5 mL/min.

For Danazol a Waters Symmetry® C18 column (3.5 μm, 150 mm × 4.6 mm) was applied with an eluent consisting of acetonitrile/purified water 60/40 (v/v). The flow was set to 1.5 mL/min.

Apparent permeabilities ($P_{app}$) were calculated according to the following equation (Chen et al., 2002):

$$P_{app} = \frac{\Delta Q/\Delta t}{60 \times A c_0} \quad (1)$$

where $\Delta Q$ is the cumulative drug amount (g); $\Delta t$ the sampling time (min), $A$ the transwell area (cm$^2$); $c_0$ is the drug starting concentration (g/cm$^3$).

### 2.4. Cell viability and monolayer integrity

Monolayer integrity was controlled by TEER measurements using an Ohmmeter and cell viability by a MTT test and a Cytotox96® assay from Promega (Mannheim, Germany). While the MTT test checks for mitochondrial dehydrogenase activity in living cells, the Cytotox96® assay reacts with lactate dehydrogenase (LDH) released from dead cells.

The Cytotox96® assay had to be adapted from 96- to 12-well inserts in the following way: 50 μL of each testing solution were transferred to a 96-well plate to calculate the background at the beginning of the experiment. Fifty microliters were removed apically from each test- and control-well after the end of the experiment. The control was used to calculate the spontaneous LDH release during the experiment. The control wells were emptied and 100 μL of lysis-solution (supplied in kit) were added, followed by 400 μL transport buffer to

adjust the volume. The control wells were incubated for 45 min, afterwards 50 μL samples were drawn to calculate the maximum LDH release. All 50 μL samples were spiked with 50 μL substrate-mix (supplied in kit) and incubated for 30 min. The conversion of a tetrazolium salt into a red formazan dye was stopped by addition of 50 μL stop-solution (supplied in kit). The plate was measured with a standard VIS-plate reader at 490 nm.

Results were calculated using the following equation (adapted from Promega, 2006):

$$\text{cytotoxicity (\%)} = \frac{(\text{testwell} - \text{background} - \text{spontaneous}) \times 100}{\text{maximum release} + \text{spontaneous}} \quad (2)$$

Monolayer integrity was controlled by TEER measurements using an Millicell-ERS® ohmmeter from Millipore (Billerica, USA) equipped with a chopstick electrode.

Cell viability was monitored by a MTT test utilizing the Biochrom-Alphakit from Biochrom (Berlin, Germany), which was adapted to match the well size as follows.

After end of the experiment, all wells were washed twice with phosphate buffered solution (PBS) and culture medium was added, then the cells were incubated for 12 h. The medium was washed off twice and 250 μL of MTT-solution (5 mg/mL) were added followed by an incubation of 2 h. The wells were washed twice and were allowed to dry for 2 h. The blue formazane crystals were dissolved in 500 μL ethanol/hydrochloric acid (99/1, v/v), transferred to a 96-well plate and measured with a VIS-plate reader at 570 nm.

### 2.5. Evaluation of self-emulsifying properties

The influence of separate components on self-emulsification was judged according to the following method. Four mixtures, containing Lauroglycol®:Pluronic® L62 or L81 in ratios 3:1, 2:1, 1:2 and 1:3 were prepared. An amount of 200 mg was dripped cautiously in a vessel containing 20 mL of purified water each. The vessels were left for 5 min without stirring. Afterwards, the turbidity resulting from emulsification was judged visually.

### 2.6. Ternary phase diagrams

Mixtures of the pasty primary emulsifier (P104) and ethanol/Transcutol® were prepared at ratios of 4.6:1:1.5 (L81 diagram) and 4.6:0.8:1 (L62 diagram) to gain pipettable surfactant solutions. Varying amounts of these solutions, a secondary emulsifier (Pluronic® L62 or L81) and Lauroglycol® 90 were mixed until a homogeneous phase was obtained. The final formulations (100 mg) were diluted with purified water to 25 mL in volumetric flasks, gently shaken by hand and allowed to equilibrate at room temperature for 45 min. The resulting emulsions were evaluated by visual observation and PCS measurement. The nanoemulsion region, including micelles, mixed micelles and swollen micelles, was defined as a system, which appeared transparent or translucent and showed particle sizes below 150 nm. Emulsions were cloudy white and displayed a particle size above 150 nm. The set-up of the ternary diagrams was changed to suite the requirements of oral formulation development. Usually, the phases depicted in each corner are primary emulsifier/oil/water. This set-up is practical for technical applications, e.g. lubricant development or for topical application development, to take an example from the pharmaceutical field. In peroral drug delivery, the relation of summed up primary emulsifier/oil to water is outbalanced to the waterside. On the one hand, the amount of preconcentrate deliverable in a capsule is roughly 1.0 g only. On the other hand, even in the fasted stomach, there are about 50–100 mL of gastric fluid (Abrahamsson and Lennernäs, 2003), the intake of water to aid swallowing of the capsule adds another 150–200 mL. So the area of interest (200–250 mL) is in the far corner of the water phase, close to 100% water. Furthermore, oral micro- and nanoemulsions usually comprise one or even more secondary emulsifiers, cosurfactants or solubilizers. The formulator is interested in the possible design space of these components, because they contribute to the overall loading capacity for drugs, have an influence on self-emulsifying properties of the preconcentrate and on surface rheological properties of nanoemulsion droplets that control drug release. Since there is no necessity to discuss water/formulation ratios for oral preconcentrate delivery in a ternary phase diagram, the volume of water was set constant to 250 mL. This corresponds with the volume proposed in the Guidance for Industry (FDA/CDER, 2000), in which a drug should completely

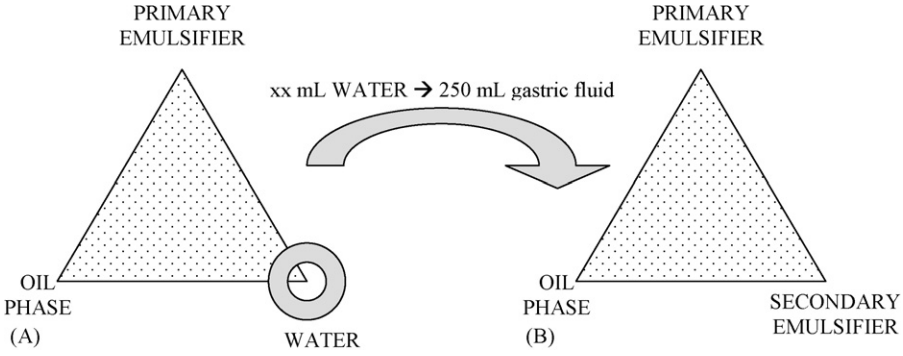


Fig. 1. Conversion of a standard ternary phase diagram (A) to a diagram suitable for oral formulation development of preconcentrates (B). The ring marks the area of interest due to dilution in a constant amount of e.g. gastric fluid. Influence of a secondary emulsifier becomes viewable in (B) by setting the water phase constant.

dissolve to be regarded as highly soluble. Instead of the water phase, the third phase now depicts the amount of a secondary emulsifier. The changes made to the diagram are also illustrated in Fig. 1.

### 2.7. Particle size measurement

The mean volume diameter of the nanoemulsion was determined by photon correlation spectroscopy (PCS), also known as dynamic light scattering (DLS), using Malvern Zetasizer Nano ZS (Malvern, UK). This instrument is able to measure sizes between 0.6 nm and 6 μm. The Zetasizer utilizes a 633 nm laser detecting scattered light at an angle of 173°. All measurements were performed at 25 °C in triplicate, each consisting of 14–20 runs.

## 3. Results

### 3.1. Influence of formulation components on mean volume particle diameter

To fill the grid of the ternary diagram, several formulations were prepared according to the method described under Section 2.6. Their compositions and resulting particle sizes can be seen in Table 1; working with Pluronic® L62 as secondary emulsifier, and Table 2 with Pluronic® L81. The ternary phase diagrams (Figs. 2 and 3) for the investigated formulations show that an increase in the primary emulsifier Pluronic® P104 leads to a decrease in particle size and to nanosized emulsions whereas raising the content of Lauroglycol® 90 results in an increasing

Table 1
Effect of formulation main components with Pluronic® L62 as a secondary emulsifier on the nanoemulsion mean droplet diameter in water

| Formulations | Components (%, w/w) | | | Particle size (nm) | |
|---|---|---|---|---|---|
| | Pluronic® P104 | Pluronic® L62 | Lauro-glycol® | Mean | S.D. |
| 1 | 100.0 | – | – | 4.4 | 0.6 |
| 2 | 86.5 | 13.5 | – | 4.3 | 0.6 |
| 3 | 74.1 | 25.9 | – | 3.8 | 0.6 |
| 4 | 62.5 | 37.5 | – | 3.6 | 0.4 |
| 5 | 51.9 | 48.1 | – | 4.0 | 0.6 |
| 6 | 41.8 | 58.2 | – | 3.8 | 0.5 |
| 7 | 32.4 | 67.6 | – | 3.1 | 0.4 |
| 8 | 23.5 | 76.5 | – | 3.0 | 0.5 |
| 9 | 15.2 | 84.8 | – | 3.0 | 0.5 |
| 10 | 7.4 | 92.6 | – | 2.8 | 0.3 |
| 11 | – | 100.0 | – | 2.8 | 0.2 |
| **12 (formulation A)** | **85.1** | – | **14.9** | **17.6** | **3.2** |
| 13 | 74.2 | – | 25.8 | 107.0 | 43.9 |
| 14 | 74.1 | 11.5 | 14.4 | 17.1 | 7.7 |
| 15 | 64.8 | 10.1 | 25.1 | 66.9 | 21.5 |
| 16 | 63.8 | 22.2 | 14.0 | 17.9 | 6.6 |
| **17 (formulation C)** | **56.0** | **19.5** | **24.4** | **48.5** | **20.5** |
| 18 | 53.9 | 32.3 | 13.8 | 18.0 | 3.6 |
| 19 | 45.1 | 41.9 | 13.0 | 18.8 | 3.7 |
| 20 | 36.5 | 50.8 | 12.7 | 19.8 | 4.9 |
| 21 | 28.4 | 59.3 | 12.3 | 22.1 | 6.1 |
| 22 | 20.7 | 67.4 | 11.9 | 30.1 | 9.96 |
| 23 | 13.4 | 74.8 | 11.7 | 40.7 | 21.1 |

S.D., standard deviation of three measurements (assumed Gaussian distribution).

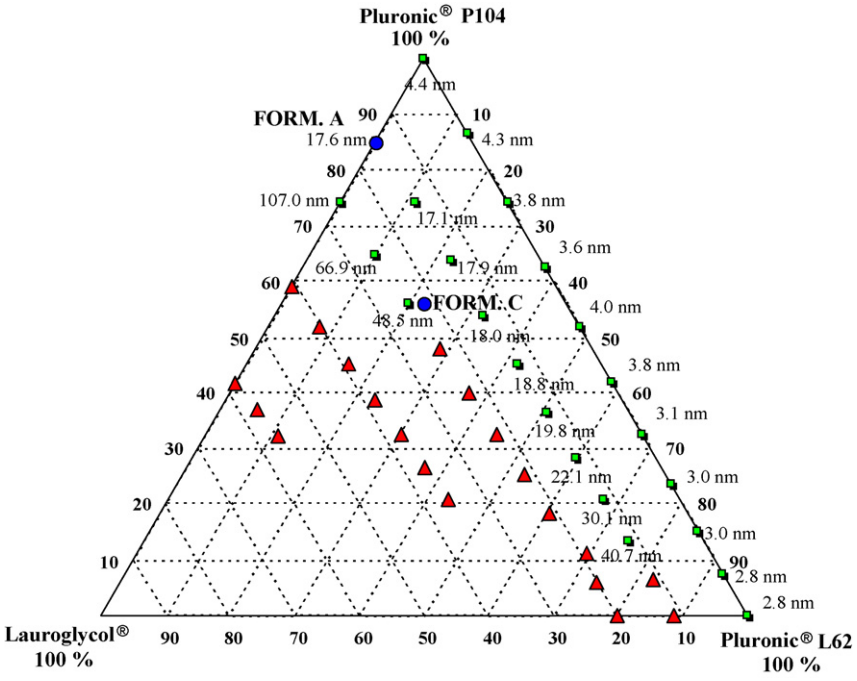


Fig. 2. Ternary phase diagram (wt.%) of compositions consisting of Pluronic® P104 as primary emulsifier, Lauroglycol® 90 as amphiphilic oil and Pluronic® L62 as secondary emulsifier in water. (□) Nanoemulsion; (Δ) macroemulsion; (◯) compositions tested in Caco-2. Numbers are mean volume diameters from PCS measurements.

Table 2
Effect of formulation main components with Pluronic® L81 as a secondary emulsifier on the nanoemulsion mean droplet diameter in water

| Formulations | Components (%, w/w) | | | Particle size (nm) | |
|---|---|---|---|---|---|
| | Pluronic® P104 | Pluronic® L81 | Lauroglycol® | Mean | S.D. |
| 1 | 100.0 | – | – | 4.2 | 0.6 |
| 2 | 85.4 | 14.6 | – | 3.2 | 0.4 |
| 3 | 72.1 | 27.9 | – | 3.62 | 0.7 |
| **4 (formulation A)** | **83.9** | – | **16.1** | **17.8** | **3.49** |
| 5 | 72.4 | – | 27.6 | 135.0 | 56.0 |
| 6 | 72.2 | 12.4 | 15.4 | 20.1 | 4.75 |
| **7 (formulation B)** | **61.5** | **23.7** | **14.8** | **23.2** | **5.73** |
| 8 | 71.0 | 11.0 | 18.0 | 35.7/107.0 | 6.36/33.1 |

S.D., standard deviation of three measurements (assumed Gaussian distribution).

size (Tables 1 and 2) and in a broader size distribution. The choice of the secondary emulsifier has an influence on the extension of the nanoemulsion region, which is broader using the more hydrophilic Pluronic® L62 (HLB 7) instead of the lipophilic L81 (HLB 2). Both Pluronic® L104 and L62 are hydrophilic enough to form very small swollen micelles (4 nm) without the aid of each other (formulations 1 and 11 in Table 1). Pluronic® L81 and Lauroglycol® can be applied to a maximum of 30%, with L81 still producing very small mixed micelles (formulation 3, Table 2) with a narrow distribution while Lauroglycol® micelles become rapidly larger (formulation 5, Table 2).

### 3.2. Formulation selection and preparation for in vitro experiments

From the nanoemulsion region lined out in the ternary phase diagrams, three nanoemulsion systems, one for each secondary emulsifier (formulations B and C) and one without them (formulation A), were selected for evaluation in Caco-2 experiments. The selection aimed at choosing the nanoemulsion formulations with the best self-emulsifying property since this would be a prerequisite for peroral application of preconcentrates in later use.

For formulation A, there was a choice between formulation 12 and 13 (Table 1) corresponding to formulations 4 and 5 (Table 2), respectively. Two hundred milligrams of each formulation were dripped cautiously in a vessel containing 20 mL of purified water each. The vessels were left for 5 min without stirring. Afterwards, the turbidity resulting from emulsification was judged visually. Formulation 12 (Table 1) corresponding to formulation 4 (Table 2) were found to disperse more readily and were, therefore, selected as formulation A.

Self-emulsification of formulations containing Pluronic® L62 and L81 was jugded according to the method described

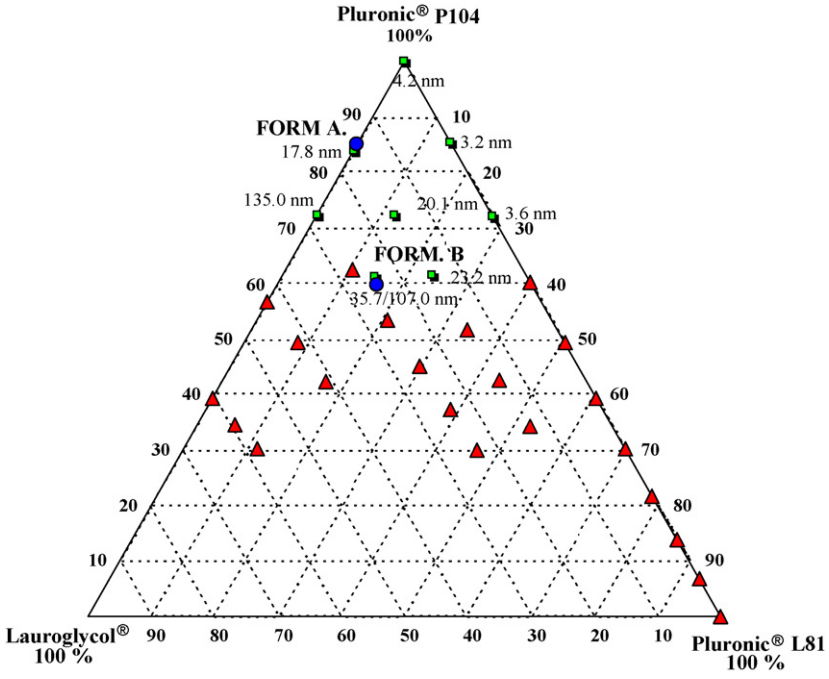


Fig. 3. Ternary phase diagram (wt.%) of compositions consisting of Pluronic® P104 as primary emulsifier, Lauroglycol® 90 as amphiphilic oil and Pluronic® L81 as secondary emulsifier in water. (□) Nanoemulsion; (Δ) macroemulsion; (◯) compositions tested in Caco-2. Numbers are mean volume diameters from PCS measurements.

Table 3
Composition of preconcentrates examined in the Caco-2 experiment and their ratios according to the ternary phase diagrams

| Formulation | Components (%, w/w) | | | | | |
|---|---|---|---|---|---|---|
| | Ethanol | Lauroglycol® 90 | Transcutol® HP | Pluronic® P104 | Pluronic® L81 | Pluronic® L62 |
| Formulation A | 16.4 | 8.0 | 30.2 | 45.4 | – | – |
| Ratio | – | 15 | – | 85 | – | – |
| Formulation B | 10.4 | 12.2 | 14.9 | 45.6 | 16.9 | – |
| Ratio | – | 16 | – | 61 | 22 | – |
| Formulation C | 9.3 | 17.8 | 9.9 | 44.8 | – | 18.2 |
| Ratio | – | 22 | – | 55.5 | – | 22.5 |

under Section 2.5. The two vessels containing a higher amount of Lauroglycol® showed self-emulsification by turning cloudy white, while the other two, containing a higher amount of Pluronic® L62 or L81, remained clear with a large, separate phase of surfactants at the bottom. Formulations 7 and 8 (Table 2, L81) and 15 and 17 (Table 1, L62) contained the highest possible fractions of Lauroglycol®. Formulation 8 was discarded since it already started to show polydispersity. Therefore, formulation 7 was chosen as formulation B. Formulation 17 produced a smaller droplet size than 15 and was selected as formulation C.

To decide on the influence of L81 and L62 in the Caco-2 experiment, the concentration of Pluronic® P104 was kept constant at approximately 46%. The resulting three compositions are listed in Table 3. The droplet size distribution of formulations A–C as obtained from PCS measurements can be seen in Fig. 4, showing that all are within the nanoemulsion range.

At first, a premix of the pastous P104 with ethanol and Transcutol® was prepared to gain a pipettable solution. Then, all components were simply mixed with a lab shaker. Amounts of 0.4 mg of Atenolol, Metoprolol tartrate and Danazol were dissolved in 400 mg of formulations A–C, then 100 mL of transport medium were added and put on a magnetic stirrer until a homogenous nanoemulsion had formed. About 0.6 mL were applied apically, leaving 0.5 mL during the permeation experiment after the initial starting concentration sample was taken.

### 3.3. In vitro permeation experiments

Atenolol absorptive transport was significantly enhanced by compositions formulations A and B, $P_{app}$ was increased 2.1- and 2.5-fold relative to control. Formulation C also improved permeation but the effect was too low to be regarded as significant ($p < 0.05$). Metoprolol is per se transported well via the transcellular route. All three formulations slightly, however statistically significantly, increased the already high permeation rate by factors 1.4, 1.2 and 1.1 (formulations A–C) relative to control. The lipophilic drug Danazol benefited the most from formulation A (factor 3.2), followed by formulation B (factor 2.0) and formulation C (factor 1.7). The actual apparent permeabilities and their standard deviations are given in Table 4.

### 3.4. Cell viability and monolayer integrity

On the one hand, TEER measurements are an indicator of physical damage to the monolayer during the experiment, which can be caused for example through contact of pipette tips with the membrane. On the other hand, components of the test solution that are cytotoxic or interfere with cellular adhesion, e.g. by forming complexes with $Ca^{2+}$ or $Mg^{2+}$, also cause a reduction of TEER. Therefore, a TEER reduction

Table 4
Influence of selected formulations on apparent permeabilities ($P_{app}$) of three different drugs

| Drug | Formulation | $P_{app} \pm$ S.D. (nm/s) | Total drug recovery[a] (%) | Enhancement factor[b] |
|---|---|---|---|---|
| Atenolol | Formulation A | 26.3* ± 3.1 | 102.4 | 2.1 |
| | Formulation B | 31.6* ± 5.9 | 107.6 | 2.5 |
| | Formulation C | 15.9 ± 1.9 | 105.8 | – |
| | Control | 12.8 ± 3.6 | 102.3 | – |
| Metoprolol | Formulation A | 335.3* ± 19.7 | 106.8 | 1.4 |
| | Formulation B | 289.3* ± 3.7 | 108.7 | 1.2 |
| | Formulation C | 256.6* ± 11.0 | 100.8 | 1.1 |
| | Control | 232.5 ± 19.5 | 101.9 | – |
| Danazol | Formulation A | 22.5* ± 1.3 | 103.6 | 3.2 |
| | Formulation B | 14.0* ± 1.6 | 102.6 | 2.0 |
| | Formulation C | 11.8* ± 0.9 | 104.0 | 1.7 |
| | Control | 7.1 ± 2.3 | 120.1 | – |

Each value represents the mean ± S.D. of three experiments (formulations) or nine experiments (control). Asterisks (*) indicate a significant difference from control at $p < 0.05$ (Welch's $t$-test, assumed Gaussian distribution).

[a] Sum of acceptor compartments and donor compartment after 120 min relative to starting concentration.

[b] Enhancement factor = $P_{app}$ (formulation)/$P_{app}$ (control).

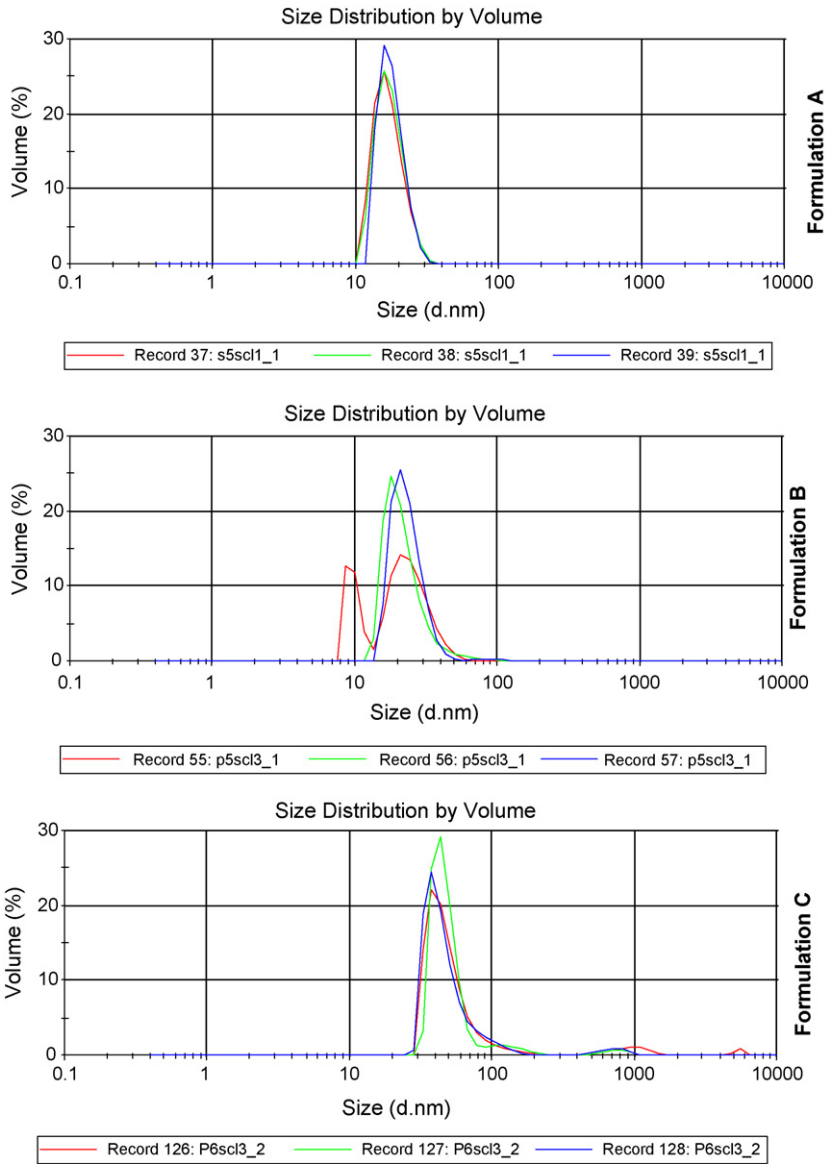


Fig. 4. Droplet size distribution by volume obtained from photon correlation spectroscopy at 25 °C of three formulations A–C applied in the in vitro test (depicted are three measurements per formulation, each consisted of 14–20 runs).

throughout all samples would denote a severe interaction of the test solution with the cells. All three formulations did not provoke a significant ($p < 0.05$) reduction of TEER, relative to control. See Table 5 for results.

TEER measurements can only be a rough indicator of cytotoxic effects, since they would not include damage caused to the membranes of individual cells. Measuring the LDH release from cytosol into the transport medium is an appropriate test method to detect the leaking of cells. The amount of LDH determined in all three test solutions at the end of the experiment was similar to the amount measured in the control solution, being within the standard deviation of control. The results are also given in detail in Table 5.

Finally, cytotoxic effects of the formulation can also be monitored by measuring mitochondrial activity after a 24 h reincubation of the wells. Damaged cells would show no or only reduced recovery during this time. All three formulations showed com-

Table 5
Cell viability and monolayer integrity after 2 h application of test compositions, results of three different test methods

| Formulation | Method | | |
|---|---|---|---|
| | TEER reduction (%) | MTT assay (%) | LDH release assay (%) |
| Formulation A | 6.7 | 3.5 | 1.4 |
| Formulation B | −1.2 | 0.1 | −0.5 |
| Formulation C | 9.6 | −1.3 | −0.4 |
| Control | 0.0 | 0.0 | 0.0 |

All values are % relative to control and were calculated on the basis of at least $n = 3$. Asterisks (*) indicate a significant difference from control at $p < 0.05$ (Welch's t-test, assumed Gaussian distribution).

parable mitochondrial activity to that of control, indicating a complete recovery. Also see Table 5 for results.

## 4. Discussion

In all three formulations tested, the amount of the primary emulsifier P104 is high, namely approximately 45% (w/w). This amount is needed to form stable droplets in the high aqueous dilutions that would arise in the proposed oral use. The resulting droplet size is small, being always below 150 nm. Droplets at this size are regarded as swollen micelles (Eccleston, 2002). The small micellar size is supposed to be advantageous since partitioning of drugs into the aqueous phase is more likely due to a maximized interfacial area and a high droplet curvature. But besides micellar size there are also other factors that control drug release. The surface rheological properties are of specific importance, since they influence the diffusion of the active through direct interactions. This is because drugs, especially in their ionized forms, are surface-active as well and can also become part of the interface. On the one hand, a high fluidity of the surfactant layer facilitates diffusion of actives out of the micelle. On the other hand, it can increase the risk of Ostwald ripening (Sarker, 2005). The fluidity can be raised by the addition of short chain alcohols like ethanol or Transcutol®. These components are also known to reduce o/w micellar size due to swelling of head regions and allow for spontaneous micellar formation (Fletcher and Parrott, 1988).

Looking at the composition of the three nanoemulsions tested in Caco-2, one could derive a trend towards formulation A being the strongest enhancing formulation and also containing the highest amounts of ethanol and Transcutol®. Regarding ethanol, the effect on Caco-2 permeability is discussed controversially. One group (Rao, 1998) found no effect on paracellular permeability of [2-(3)H]mannitol for concentrations up to 900 mM, being approximately 4% (w/v). Another group (Ma et al., 1999) reported a linearly dose-dependent increase of paracellular permeability at non-cytotoxic concentrations between 0 and 10% (w/v). Looking at Transcutol®, a doubling of $P_{app}$ was measured for transcellulary transported Propanolol and paracellulary transported Nadolol at a concentration of 10% (Takahashi et al., 2004). Formulation A, containing the highest concentrations of ethanol (0.07%) and Transcutol® (0.12%) of all three formulations, has a stronger effect on permeability than reported in literature although the concentrations of both solubilizers are considerably lower. It can be concluded that the solubilizers are not primarily responsible for the permeability enhancement, although they might contribute to the effect. The permeation differences between the formulations are more likely based on different partition coefficients, surface rheology and fluidity of the interface and cannot be attributed to the presence of single formulation components. Especially, the determination of the partition coefficient of drugs in nanoemulsions (Sarker et al., 1996) is difficult to accomplish and outside the scope of this work.

In summary, three nanoemulsion systems based on different Pluronics® have been found that can be used to solubilize lipophilic drugs. It has been discovered that the formulations have an active influence on intestinal permeation of both transcellulary and paracellulary transported drugs. They could, therefore, find their use for actives with either low solubility or low permeability or a combination of both problems (BCS class II, III and IV substances). Furthermore, since the Pluronics® are known to inhibit P-gp-mediated drug efflux (Batrakova et al., 1998), they might also be used in formulations of actively effluxed drugs like many cytostatics. Since the three formulations also showed no cytotoxicity, they could find their use in early formulation, e.g. dose escalation studies, especially for substances that could otherwise not be tested with in vitro/in vivo methods due to their low solubility.

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## References

Abrahamsson, B., Lennernäs, H., 2003. Application of the Biopharmaceutics Classification System now and in the future. In: Van de Waterbeemd, H., et al. (Eds.), Drug Bioavailability. Wiley–VCH, Weinheim.

Batrakova, E.V., Han, H.-Y., Alakhov, V.Y., Miller, D.W., Kabanov, A.V., 1998. Effects of Pluronic® block copolymers on drug absorption in Caco-2 cell monolayers. Pharm. Res. 15, 850–855.

Batrakova, E.V., Li, S., Alakhov, V.Y., Miller, D.W., Kabanov, A.V., 2003. Optimal structure requirements for Pluronic® block copolymers in modifying P-glycoprotein drug efflux transporter activity in bovine brain microvessel endothelial cells. J. Pharmacol. Exp. Ther. 304, 845–854.

BASF website, url: http://www.basf.com/performancechemical/bcperfpluronic_grid.html, information taken on 2006-03-22.

Chen, W., Tang, F., Horie, K., Borchardt, R.T., 2002. Caco-2 monolayers as model for studies of drug transport across human intestinal epithelium. In: Lehr, C.M. (Ed.), Cell Culture Models of Biological Barriers. Taylor & Francis, London, p. 153.

Dressman, J.B., Reppas, C., 2000. In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs. Eur. J. Pharm. Sci. 11, 73–80.

Eccleston, G.M., 2002. Emulsions and microemulsions. In: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology, vol. 2, 2nd ed. Marcel Dekker, New York, pp. 1066–1083.

Fletcher, D.I., Parrott, D., 1988. Protein partitioning between microemulsion phases and conjugate aqueous phases. In: Pileni, M.P. (Ed.), Structure and Reactivity in Reversed Micelles. Elsevier, New York, pp. 303–322.

Kabanov, A.V., Batrakova, E.V., Alakhov, V.Y., 2002. Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery. J. Control. Rel. 82, 189–212.

Kasim, N.A., Whitehouse, M., Ramachandran, C., Bermejo, M., Lennernas, H., Hussain, A.S., Junginger, H.E., Stavchansky, S.A., Midha, K.K., Shah, V.P., Amidon, G.L., 2004. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. Mol. Pharm. 1, 85–96.

Kataoka, K., Kwon, G.S., Yokohama, M., Okano, T., Sakurai, Y., 1993. Block copolymer micelles as vehicle for drug delivery. J. Control. Rel. 24, 119–132.

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Dev. Res. 23, 3–25.

Ma, T.Y., Nguyen, D., Bui, V., Nguyen, H., Hoa, N., 1999. Ethanol modulation of intestinal epithelial tight junction barrier. Am. J. Physiol. 276, G965–G974.

Neosporin® MedlinePlus website, url: http://www.nlm.nih.gov/medlineplus/druginfo/uspdi/202399.html, information taken on 2006-04-11.

Orringer, E.P., Casella, J.F., Ataga, K.I., Koshy, M., Adam-Graves, P., Luchtman-Jones, L., Wun, T., Watanabe, M., Shafer, F., Kutlar, A., Abboud, M., Steinberg, M., Adler, B., Swerdlow, P., Terregino, C., Saccente, S., Files, B., Ballas, S., Brown, R., Wojtowicz-Praga, S., Grindel, J.M., 2001. Purified Poloxamer 188 for treatment of acute vaso-occlusive crisis of sickle cell disease. JAMA 286, 2099–2106.

Promega Corporation, 2006. Technical Bulletin. Cytotox 96® Non-Radioactive Cytotoxicity Assay. Instructions for use of product G1780. Madison, March, p. 10.

Rao, R.K., 1998. Acetaldehyde-induced increase in paracellular permeability in Caco-2 cell monolayer. Alcohol Clin. Exp. Res. 22, 1724–1730.

Sarker, D.K., 2005. Engineering of nanoemulsions for drug delivery. Curr. Drug Deliv. 2, 297–310.

Sarker, D.K., Wilde, P.J., Clark, D.C., 1996. Enhancement of the stability of protein-based food foams using trivalent cations. Colloids Surf. A: Physicochem. Eng. Aspects 114, 227–236.

Siebenbrodt, I., Keipert, S., 1993. Poloxamer-systems as potential opthalmics II. Microemulsions. Eur. J. Pharm. Biopharm. 39, 25–30.

Strickley, R.G., 2004. Solubilizing excipients in oral and injectable formulations. Pharm. Res. 21, 201–230.

Takahashi, Y., Kondo, H., Yasuda, T., Watanabe, T., Kobayashi, S., Yokohama, S., 2004. Common solubilizers to estimate the Caco-2 transport of poorly water-soluble drugs. Int. J. Pharm. 246, 85–94.

U.S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), BP, 2000. Guidance for industry, Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. Guidance documents, p. 2.

Varshney, M., Morey, T.E., Shah, D.O., Flint, J.A., Moudgil, B.M., Seubert, C.N., Dennis, D.M., 2004. Pluronic® microemulsions as nanoreservoirs for extraction of bupivacaine from normal saline. J. Am. Chem. Soc. 126, 5108–5112.

Vieth, M., Siegel, M.G., Higgs, R.E., Watson, I.A., Robertson, D.H., Savin, K.A., Durst, G.L., Hipskind, P.A., 2004. Characteristic physical properties and structural fragments of marketed oral drugs. J. Med. Chem. 47, 224–232.

Vogelpoel, H., Welink, J., Amidon, G.L., Junginger, H.E., Midha, K.K., Moller, H., Olling, M., Shah, V.P., Barends, D.M., 2003. Biowaiver monographs for immediate release solid oral dosage forms based on biopharmaceutics classification system (BCS) literature data: verapamil hydrochloride, propranolol hydrochloride, and atenolol. J. Pharm. Sci. 93, 1945–1956.