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A new formulation concept for drugs with poor water solubility for parenteral application

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The parenteral application of active substances with poor solubility in water is often bound to the use of stabilizing excipients or surfactants with serious undesired side effects. A new concept is introduced based on a drug concentrate, comprising the active substance dissolved in parenterally acceptable organic solvents, and an aqueous dilution medium, which are mixed in a special mixing device immediately prior to application and thus generating the applicable formulation directly prior to administration. Due to the requirement of formulation stability for only a few minutes, the amount of stabilizing agents can be reduced significantly. It can be shown that model drugs dissolved in a mixture of polyoxyethylen glycol, ethanol and soya lecithin as stabilizer may be mixed to an aqueous glucose solution resulting in a parenterally acceptable and administerable dispersion which is physically stable for several minutes. First *in vivo* data show good tolerability and blood plasma levels which are comparable to conventional solutions.

1. Introduction

Intravasal administration of drugs is becoming more and more important in pharmaceutical development. One reason is the poor enteral bioavailability of many modern drugs, which means that parenteral administration is often the only way to provide sufficiently high blood levels. Furthermore, a small therapeutic window and patient individual dosing, accompanied with high dose regimes favors the parenteral route of application.

However, the development of parenteral formulations is often hindered by the poor solubility of the active substances in water. This has led to the development of innovative formulation concepts such as the use of special emulgator systems (Sweetna, 1996; Buszello 2000), water soluble inclusion complexes (Brauns 1985), mixed micelles (Hammad 1998), nanoparticular suspensions (Borner, 1999), vesicular systems as e.g. liposomes and spherosomes (Bangham, 1993), and other systems (Klang, 1998). These are mostly based on the use of additional excipients that may reduce the risk of toxicological intolerability. Often these formulations are highly specialized and focus on the administration of only one drug substance or substance class. So the use of an emulsion system is limited by the saturation solubility of the drug in the 20% oil phase while the mixed micelle principle only works with diazepam and not with tetrazepam due to the strong interaction between drug molecule volume and micelle structure. At present only hydroxypropyl-β-cyclodextrin and macrogol-15-hydroxystearate as new excipients have become constituents of marketed products.

Especially in preclinical and early clinical studies a more universal system usable for a variety of drug substances and concentrations with an easy-to-use setup is highly appreciated. A crucial problem is the need for sufficient long term stability of the administered formulation. Whereas a short term stability of up to some hours can be reached by most of the current concepts of parenteral application of poorly soluble drugs, stability of more than 6 months seems unobtainable or can only be achieved with inconvenient storage conditions such as cooling chain.

A solution to this problem is offered by two-bottle systems. Here the drug is diluted from a concentrate to the application form with a physiological diluent prior to medication. Nevertheless, the time frame of administration is rather small (e.g. up to 12 h for some paclitaxel formulations) and the formulations contain stabilizing agents such as Cremophor® EL, that may lead to massive undesired side effects (Lang, 1990).

As toxicologically more tolerable two-bottle formulations may show a reduced short term stability due to the excipients selected, a new administration regime is required where the drug concentrate is diluted and a administered instantly. First attempts in this direction have been made by Sucker, Gaßmann, and List, using a static mixer and introducing the fluids by pumps (Gaßmann et al. 1994). As the flow rates needed for sufficient mixing exceed the limits for a direct injection of the mixture, the volume for one administration is produced, collected and subsequently administered. However, as the storage time for collection may exceed the period of formulation stability, this concept has not come to market.

To overcome this disadvantage, a two-bottle concept has been developed, using a mixing device integrated directly into the application system. As illustrated in the schematic

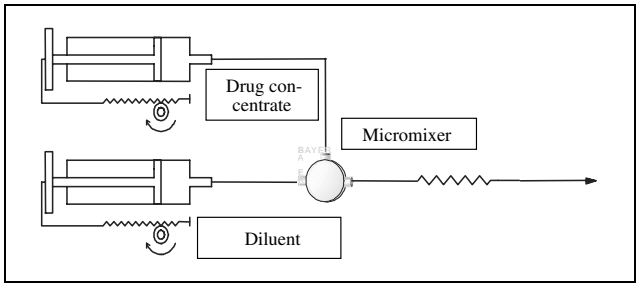

Fig. 1: Experimental setting up

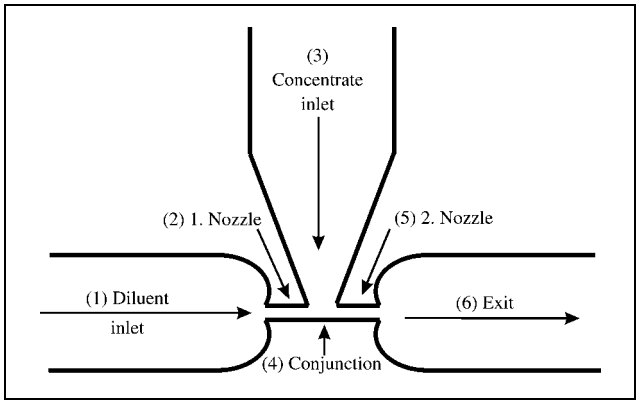

Fig. 2: Micromixer device

drawing in Fig. 1, two fluids, the drug concentrate and the physiological diluent, are pumped by two separate pumps into the mixing device, where they are intensively mixed and form the applicable formulation. This is led from the mixer to the patient's vein via conventional tubing and is directly administered.

The mixing device used is a microstructural mixer ("micromixer") (Kühn 1999), a prototype mixer designed as seen in Fig. 2. The two fluids are pumped into the mixer through the two inlets ((1) for the diluent and (3) for the drug concentrate), are unified in at the conjunction (4) and exit via the outlet (6). The mixer uses turbulent mixing conditions achieved by a nozzle system (nozzles (2) and (5)) for intensive, constant and reproducible mixing of the fluids. The turbulent mixing conditions depend on the flow rate and the properties of the fluids and are prerequisite for a successful mixing operation. (Jürgens 2002).

The drug concentrate consists of a mixture of ethanol and macrogol 400 (PEG 400) in which lecithin is dissolved.

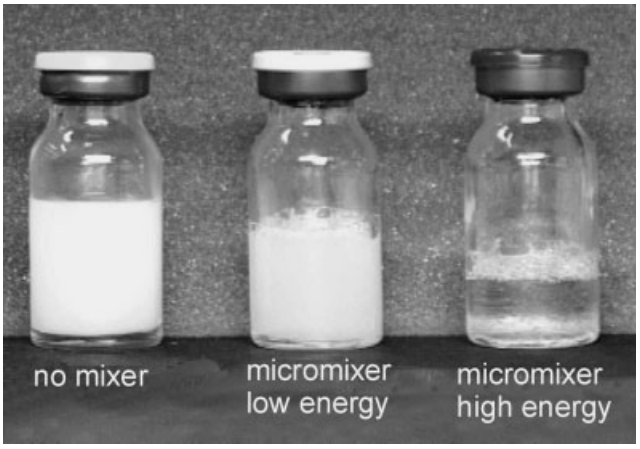

Fig. 3: Influence of mixing energy on the appearance of a test dispersion

When mixing this concentrate to the diluent (preferably water) at low mixing energy, a turbid, coarse dispersion of precipitated lecithin is obtained (cf. Fig. 3, left and middle). By increasing the mixing energy, the mixture becomes clear and transparent (Fig. 3, right), indicating the formation of a very fine dispersion.

2. Investigations, results and discussion

2.1. Development of the composition of drug concentrate

Different placebo drug concentrate formulations were assessed with regard to the formation of transparent, clear dispersions when mixed to water using the micromixer at high mixing energy. The concentrates consisted of the three components PEG 400, ethanol and the lecithin type Lipoid S75 in concentrations between 0 and 100% in 5% steps. The individual concentrates were prepared as described above and assessed regarding the visual appearance and viscosity. Subsequently each placebo drug concentrate was mixed with water ratio of 1 : 10 using the micromixer device and applying high mixing energy by choosing a total fluid flow rate, where turbulent mixing conditions are present. The appearance of the mixtures were assessed by turbidimetric measurement as described above.

2.2. Influence of different lecithin types

Using an optimized drug concentrate composition of 10% lecithin, 27% of ethanol and 63% of PEG 400, seven concentrates comprising different types of lecithin were prepared. The lecithins used were Phospholipon 90 (egg yolk lecithin with approx. 90% of phosphatidylcholine), Lipoid S 100 (soy lecithin with more than 94% of phosphatidylcholine), Lipoid EPC (egg yolk lecithin more than 98% of phosphatidylcholine), Lipoid E100 (egg yolk lecithin with more than 94% of phosphatidylcholine), Lipoid E 80 (egg yolk lecithin with approx. 80% of phosphatidylcholine), Lipoid S75 (soy lecithin with approx. 70% of phosphatidylcholine), and Epikuron 170 (egg yolk lecithin with approx. 70% of phosphatidylcholine). Subsequently each concentrate was mixed with water in a ratio of 1 : 10 using the micromixer device and applying high mixing energy by choosing a total fluid flow rate, where turbulent mixing conditions are present. The appearance of the mixtures was assessed by turbidimetric measurement as described above.

2.3. Influence of mixing energy and mixing ratio

An optimized placebo drug concentrate comprising 10% of Lipoid S75, 27% of ethanol and 63% of PEG 400 was used. It was mixed with water using the micromixer in ratios between 1 : 20 and 1 : 1 for the placebo drug concentrate. The mixing energy, defined as the total fluid flow rate of diluent and concentrate was varied between 40 and 140 ml/h for each mixing ratio. The appearance of the mixtures was assessed by turbidimetric measurement as described above.

2.4. Influence of different diluents

An optimized placebo drug concentrate comprising 10% of Lipoid S75, 27% of ethanol and 63% of PEG 400 was used. It was mixed to water, glucose solution 5%, and sodium chloride 0.9% with the micromixer in a ratio of

1 : 10. The mixing energy, defined as the total flow rate of diluent and concentrate was varied between 10 and 110 ml/h for each diluent. The appearance of the mixtures is assessed by turbidimetric measurement as described above.

2.5. Stability of formulations containing different drugs

Drug concentrate solutions of nimodipine (10 mg/ml), nifedipine (10 mg/ml), clotrimazol (5 mg/ml), paclitaxel (20 mg/ml), and the development substance WSC (5 mg/ml) were prepared as described above using a drug concentrate comprising 10% of Lipoid S75, 27% of ethanol p.A. and 63% of PEG 400. The solutions were mixed with water in a ratio of 1 : 10 with a high mixing energy providing turbulent mixing conditions. The appearance of the mixtures was assessed by turbidimetric measurement as described above. During the phase of stability the dispersion remained clear and transparent with a transmission value higher than 90%. With precipitation of the drug substance the dispersion became turbid and the transmission value decreased. The endpoint of stability was defined at 5% loss of transmission. In addition, the size of the dispersion was measured by photon correlation spectroscopy.

2.6. In vivo tolerability test on placebo on drug formulations

The tolerability of a placebo formulation was assessed by an *in vivo* test. The formulation consisted of the optimized placebo drug concentrate, comprising 10% Lipoid S75, 27% ethanol and 63% of PEG 400, prepared as described above. The concentrate was diluted 1 : 10 with dextrose solution 5% in the micromixer at high mixing energy, providing turbulent mixing conditions. The mixture was taken into a 1 ml syringe and applied without further treatment to the tail vein of 4 female CFW1-mice. The mice were treated with 10 ml of formulation per 1 kg of body weight, resulting with a medium weight of 22 g, in an applied dose of 0.22 ml per mouse.

WSC was chosen for the examination of formulations containing drugs. WSC is an anti-infective drug with poor solubility currently under development at BAYER AG, Leverkusen. The micromixer formulation consisted of a drug concentrate comprising the drug substance dissolved at a concentration of 5 mg/ml in the optimized drug concentrate of 10% Lipoid S75, 27% of ethanol and 63% PEG 400. Directly prior to administration the drug concentrate was diluted with water for injection in a ratio of 1 : 10. As the desired volume cannot be given directly, the mixture was produced in surplus in bulk and placed in a syringe for administration. The time from production to administration did not exceed the time of stability as this had previously been determined before.

In the *in vivo* study the micromixer formulation was compared to a conventional formulation consisting of 50% (m/m) PEG 400, 10% (m/m) ethanol and 40% (m/m) water. The drug concentrate is 5 mg/ml as well. For the preparation of the conventional formulation the amount of drug substance was dissolved in PEG 400 and ethanol. Subsequently, the water was added slowly, resulting in a clear solution, stable for several hours.

Each formulation was given to 3 Wistar-rats in a concentration of 1 mg/kg. With a body weight of approximately 200 g, the application volume was 0.4 ml. After the administration blood was drawn from each animal at 11 time points over 24 h to examine the drug level in the blood. After the experiment the animals were killed, and spleen, liver, lung, kidney, and heart were prepared for histological inspection.

2.7. Development of the drug concentrate

As shown in the phase diagram Fig. 4, mixing PEG 400, ethanol, and lecithin led to three different types of dispersions. At low concentrations of ethanol, the resulting mixture was a suspension of lecithin in PEG 400 and ethanol (I). At higher concentrations of ethanol, swelling of lecithin occurred due to the formation of a phase of high viscosity with an oily appearance (III). With increasing amounts of ethanol, the lecithin was dissolved completely, leading to clear solutions of low viscosity (II).

Figure 5 shows the phase diagram of the different compositions when mixed to water in a ratio of 1 : 10 at high mixing energy. The resulting mixtures can be qualified roughly into four classes: At high lecithin concentrations, neither clear concentrates nor clear mixtures with water can be reached (I). Swollen lecithin phases form turbid mixtures as well, which show thixotropic behavior (III). Lower lecithin concentrations may be dissolved clearly in ethanol and PEG 400, but do not lead to transparent mix-

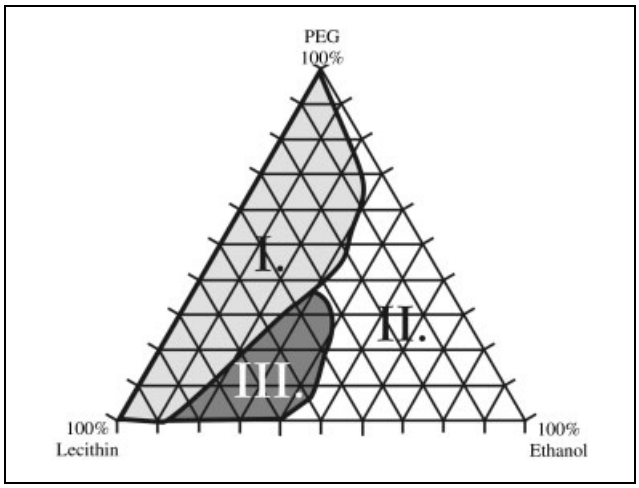

Fig. 4: Phase diagram with three different types of dispersions

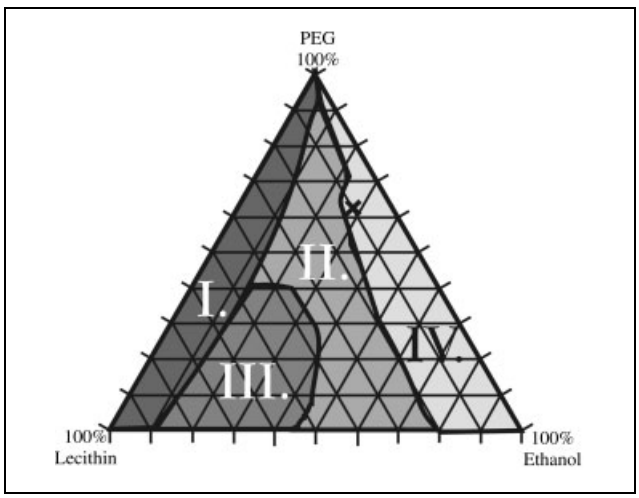

Fig. 5: Phase diagram of compositions shown in Fig. 4 after mixing with water (1 : 10)

tures with water (II). Only clear mixtures at rather small lecithin concentrations (IV) result in a clear solution after diluting with water.

As a higher amount of lecithin is regarded to have a better stabilizing effect on a drug solution and a lower concentration of ethanol is to be preferred for good local tolerability, the optimized concentrate contains 10% (w/w) of lecithin dissolved in 27% (w/w) of ethanol and 63% (w/w) of PEG 400. The composition of this placebo concentrate is indicated in Fig. 5 by a cross. When mixed to an aqueous medium in a ratio of 1 : 10 in the micromixer at sufficient mixing energy, a transparent dispersion resulted. The examination of this dispersion by photon correlation spectroscopy showed a mean particle size of less than 100 nm. The placebo mixture was stable for several days, characterized by the mean hydrodynamic diameter of the dispersed phase, which did not change significantly. The transparent dispersion was subjected to freeze-fraction TEM examinations, with an experimental preparation described elsewhere (Egelhaaf et al. 1996). The mixtures showed a non-characteristic fraction pattern, assuming the formation of small, low organized structures. Although the typical layer structures of vesicles were not found, a formation of vesicles cannot be excluded.

2.8. Influence of different lecithin types

The influence of the type of lecithin on the mixing result was evaluated by mixing concentrates with various lecithins with water. The types of lecithin differed in origin (i.e. egg yolk or soya bean) and degree of purification (i.e. concentration of phosphatidylcholine). Figure 6 shows the resulting transmission values with respect to the different types of lecithin. Best performances, resulting in the highest transmission values and hence clear dispersions, were obtained with blends of heterogeneous phospholipids (this means phospholipids with lower purification degree). This is explained by the spatial orientation of the different phospholipids, where a significant amount of hydrated phospholipids were found especially suitable for the formation of transparent dispersions. Concerning the choice of phospholipid blends of this experiment, Lipoid S75 and Epikuron 170 showed the best performance in forming transparent dispersions. These types of lecithin originated from soya beans contained about 70–75% of phosphatidylcholine. They are currently approved for parenteral nutrition.

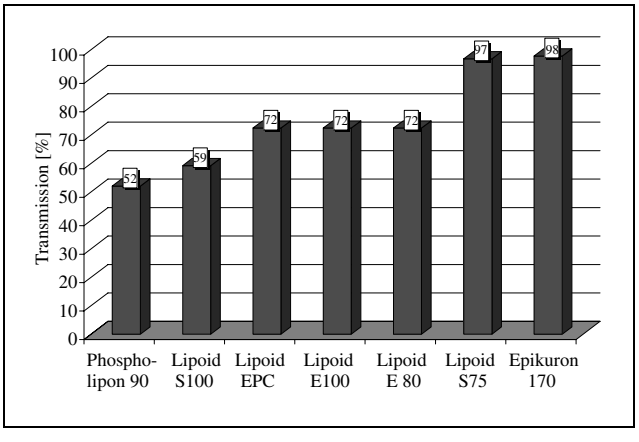

Fig. 6: Influence of phospholipid type on turbidity

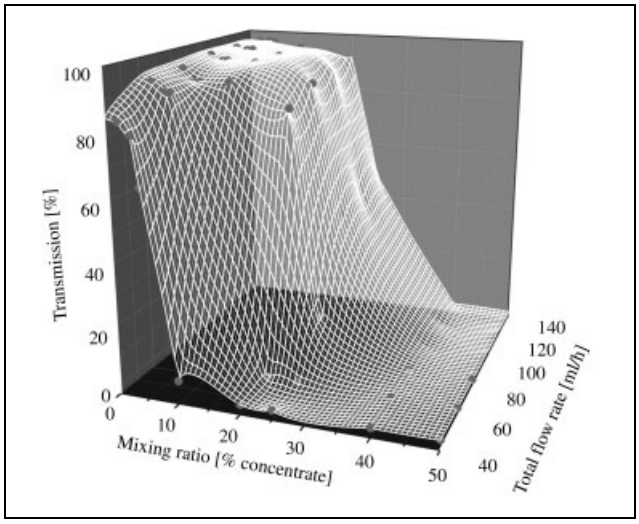

Fig. 7: Relationship between mixing ratio, total flow and turbidity

2.9. Influence of mixing energy and mixing ratio

Figure 7 shows the relation of mixing ratio, total flow rate and turbidity of the mixture. The mixing result depends on the flow rate, as it represents the mixing energy, and the mixing ratio. Mixing results that appear as clear and transparent dispersion have a transmission value of more then 90%. This was reached with a mixing ratio of less than 15% of concentrate and a minimum flow rate of 60 to 80 ml/h. The parameters for the use of the micromixer concept can be derived. These values depend strongly on the geometric layout of the mixer. The mixing process can be described by the following observation: When mixing the concentrate to water at turbulent mixing conditions, the resulting mixture contained the concentrate disrupted into small dispersed units. Following the gradient of concentration, ethanol and PEG 400 diffused into the surrounding water from the large surface of the small units. The remaining lecithin almost instantly precipitated into the non-solvent environment as small aggregates. The stability of these aggregates increased with the amount of hydrated phospholipids. The resulting mixtures showed a high degree of dispersion, and were transparent and stable over a comparatively long time. A reduction of the mixing energy below turbulent mixing conditions, led to a decrease in the degree of dispersion of the initial mixture. The concentrate was not disrupted into small units, but remained partly coherent. The diffusion of ethanol and

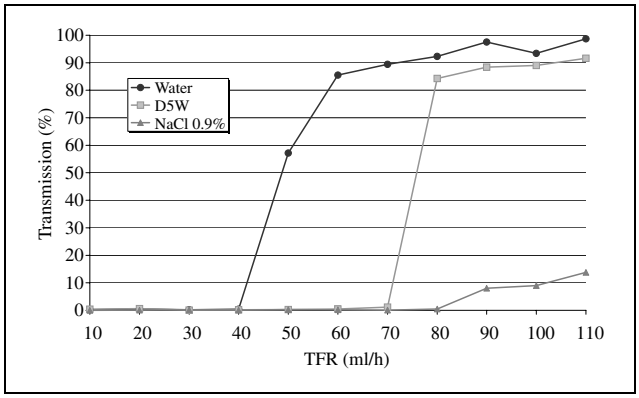

Fig. 8: Influence of the mixing energy as total flow rate (TFR) on turbidity using water, 5% glucose solution and 0.9% sodium chloride solution

Table: Test drugs used in the micromixer formulation

Substance	Solubility in concentrate (mg/ml)	Concentration examined (mg/ml)	Duration of stability (min)	Size of dispersion (PCS) (nm)
Nimodipine	100–120	10	ca. 16	80
Nifedipine	90–100	10	ca. 19	80
Clotrimazol	50–60	5	>15	110
Paclitaxel	>250	20	>5	100
WSC	7	5	ca. 17	100

PEG was slower due to the smaller surface of the units. So the phospholipids were able to form larger aggregates. This process was supported by lecithin types with a high amount of phosphatidylcholine. These phospholipids formed planar structures with a high radius of curvature. The resulting dispersions were turbid and less stable.

2.10. Influence of diluents

Figure 8 shows the turbidity of a mixture at different total flow rates (TFR), which represent different mixing energies. The best performance is reached with water for injection. To achieve a sufficiently clear dispersion with glucose solution 5% a slightly higher TFR is needed. Sodium chloride 0.9% was not suitable to be used with the micromixer device. The resulting mixture was turbid and coarse without any mixing energy being applied.

2.11. Stability of formulations containing different drugs

Experiments with formulations containing drugs were conducted on several model drug substances. The Table gives an overview of the substances examined, the concentration used and the duration of stability. All substances had poor solubility in water. However, they showed sufficient solubility in the placebo drug concentrate. The period of physical stability reached up to approx. 20 minutes depending on the substance and the drug concentration. With an increase in drug concentration the duration of stability decreased. The drug concentration of the formulation shown here was optimized for a stability of minimum 5 min.
Extending the perception of the structure of the placebo formulation, it is assumed that during the formation of the lecithin aggregates the drug substance remains in the vicinity of the phospholipids, is integrated into the aggregates and thus is protected from precipitation. This theory is based on the observation that the size of the dispersion does not change from placebo to verum and that electron microscopic examinations did not show any differences, but revealed comparably low organized lecithin structures as seen on placebo.

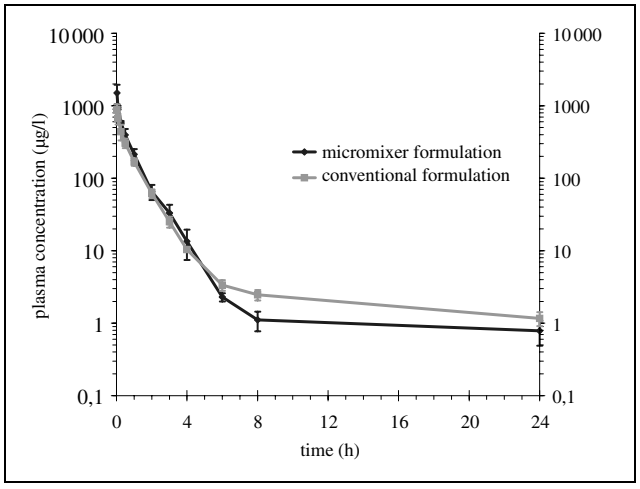

Fig. 9: Plasma concentrations of a test drug applied by the micromixer formulation versus a conventional formulation with PEG/ethanol/water

2.12. In vivo tolerability test on placebo and drug formulations

The placebo formulation applied to four mice was transparent and no particles could be seen. After application, all animals showed normal behavior over an observation time of 48 hours.
The two formulations containing drugs which were applied were transparent and no particles could be seen. During the time of inspection of 24 h all 6 rats showed normal behavior. Both formulations were well tolerated.
Figure 9 shows the kinetic profile of the micromixer formulation versus a conventional solution. The AUC of both formulations of WSC was basically comparable with 738 µg · h/L for the micromixer formulation and 598 µg · h/L for the conventional formulation. The plasma clearance was similar as well with 1.35 L/(h · kg) and 1.67 L/(h · kg), respectively. The significant body distribution of the drug substance with V_{ss} beyond 1.5 L/kg led to extended terminal half-life of 9 to 16 h. Comparing the blood plasma levels they did not reveal a statistically significant difference of the formulations, though the initial distribution volume of the micromixer formulation was slightly decreased compared to the conventional formulation (V_c: 0.603 vs. 0.960 L/kg). The kinetic data showed that the micromixer formulation was not being opsonized. Due to the blood levels an uptake into the reticulo-endothelial system would have been appeared as a fast drop in the blood level, compared to the conventional formulation with the drug substance in molecular distribution.
The histological examination showed a slight perivascular and granulocytotic infiltration of the surrounding tissue at the application site for all animals of both groups. In the lungs of one animal of the micromixer group a minimal focal activation of the endothelium of a blood vessel at the lobus was detected. The surrounding tissue showed slight granulocytotic infiltration. In the upper part of the right ventricle of another animal of the micromixer group another focal activation was found. However, no particles were found in both modified tissues. Though the abnormalities cannot be directly assigned to the use of the new formulation concept, the possibility of an influence of the micromixer formulation cannot be excluded.

3. Experimental

3.1. Materials

Glucose solution 5% and sodium chloride solution 0.9% were purchased from B. Braun Melsungen, Germany. Ethanol of analytical grade was obtained from Merck KG, Darmstadt; Germany, PEG 400 from Hoechst Frankfurt, Germany. The lecithins Lipoid S75, E80, E100, EPC, and E100 were acquired from Lipoid GmbH, Ludwigshafen, Germany. Epikuron 170 was provided by Lucas Meyer, Hamburg, Germany, and Phospholipon 90 by Nattermann Phospholipid GmbH, Cologne, Germany. Paclitaxel was purchased at Indena, Italy. Nimodipine, nifedipine, clotrimazol, and the development substance WSC were donated by Bayer AG, Leverkusen, Germany.

3.2. Methods

3.2.1. Preparation of the drug concentrate

The lecithin was dissolved in ethanol p.A. Then PEG 400 was added and stirred until a homogeneous mixture was obtained. Subsequently the drug substance was dissolved.

3.2.2. Preparation of the applicable formulation

The drug concentrate and the diluent were loaded into one syringe each and placed into syringe pumps. The application system including the micromixer was mounted to syringes and the flow rates were set. After the mixer had been flooded with diluent, the drug concentrate was started and the system was given approx. 5 min to stabilize the flow rates. Formulation samples were taken directly at the mixer outlet.

3.2.3. Assessment of the formulation by turbidimetric measurement

The formulation was put into a 10×10 mm cuvette, placed into a transmission spectrometer and the transmission was measured at 620 nm. The measurement was conducted time dependently by recording the transmission values via a XY-recorder over a time of minimum 5 min.

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