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Production and characterisation of mucoadhesive nanosuspensions for the formulation of bupravaquone

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Abstract

Bupravaquone is a new naphthoquinone antibiotic against *Cryptosporidium parvum* and other parasites. It has attracted interest for the treatment of *C. parvum* infections, because of the lack of a drug in the treatment of mostly AIDS patients. The bioavailability of bupravaquone is limited when given orally. To overcome the problem of the high elimination rate caused by diarrhoea, typical for *C. parvum* infections, bupravaquone was formulated as a mucoadhesive nanosuspension, i.e. combining the properties of mucoadhesive drug delivery systems, in this case hydro gels, with nanosuspensions. In this study different polymers/hydro gels were employed to create a prolonged retention time for the drug in the infected gastrointestinal tract (GIT). The second step to improve the bioavailability of bupravaquone was the formulation as nanosuspension. Therefore various concentrations of bupravaquone with different surfactants were tested. The production of these nanosuspensions was carried out by high pressure homogenisation. In addition to the classical stepwise production, about a new one step production method is described. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bupravaquone; Carbopol; Chitosan; Cryptosporidium; Nanosuspension; Mucoadhesion

Bupravaquone is a naphthoquinone antibiotic, structurally related to atovaquone (Wellvone®), and it is used in experimental clinics against *Cryptosporidium parvum* and other parasitic infections. *C. parvum* has recently been recognised as a serious health problem in immunocompromised people, like AIDS patients. In healthy people *C. parvum* infections also occur, but the pathogen is cleared in the gastrointestinal tract (GIT) rapidly, and infected patients show only mild diarrhoeic symptoms. *C. parvum* acts in HIV patients as an opportunistic parasite. Here diarrhoea as the main symptom persists over months up to years, sometimes also leading to death. There is an urgent need for new drugs and drug delivery systems as no rational therapy is known. As many newly developed drugs (up to 40%), bupravaquone is poorly soluble in biological media, therefore its bioavailability is limited when given orally.

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One approach to overcome bioavailability problems of poorly soluble drugs is their formulation as nanosuspension. Nanosuspensions are drug nanoparticles dispersed in a liquid phase, e.g. water. The drug nanoparticles can also be transferred to a dry product, e.g. by spray-drying, lyophilisation or using nanosuspensions as wetting agents in a granulation process to formulate tablets or pellets. The increase in bioavailability is due to the special feature of nanosuspensions. Similar to other nanoparticles they show an increased adhesiveness to the wall of the gut (Ponchel et al., 1997). Additional features are an increased saturation solubility and an increased dissolution velocity. In general, saturation solubility is described as a compound-specific constant which depends on the temperature. However, due to an increased dissolution pressure (Kelvin equation) the saturation solubility increases below a particle size of approx. 1 μm (Müller and Böhm, 1998; Nyström, 1998). In addition, the increase in surface area also contributes to an increased dissolution rate. Increases observed in bioavailability in vivo are quite remarkable. For example, the bioavailability of danazole formulated as drug nanoparticles increased from 5 to 82% compared to the danazole suspension (Müller, 1998). Considering these general advantages, the poorly soluble drug bupravaquone was formulated as a nanosuspension.

However, the poor solubility is not the only problem associated with bupravaquone for the treatment of *C. parvum* infections. The infection leads to severe diarrhoea increasing the passage velocity of the administered drug in the GIT. To increase the retention time mucoadhesive microspheres are described in the literature. Therefore the approach was chosen to combine the nanosuspension technology with the mucoadhesive principle to achieve a sufficiently high bioavailability for bupravaquone.

To achieve mucoadhesiveness, the particles can be dispersed in a solution of a mucoadhesive polymer which adsorbs onto the particle surface. Alternatively, microparticles can be produced directly in the polymer solution e.g. by solvent evaporation. However, a polymer with good mucoadhesive properties is not necessarily an optimum stabiliser to obtain a physically stable nanosuspension. Especially in the milling process by high pressure homogenisation a fast coverage of the newly created surfaces by fast diffusing molecules is required. Therefore in this study an alternative system was developed.

A bupravaquone nanosuspension was produced using an optimal stabiliser combination and incorporating the nanosuspension in a mucoadhesive gel. In addition, a one step production process for this mucoadhesive formulation was developed. Drug nanoparticles can be produced by a milling process. The product Nanocrystals® by the company Nanosystems is produced using a pearl mill. The general problem associated with such types of mills is the erosion from the pearls being a possible contamination of the product. In contrast nanosuspensions are produced by high pressure homogenisation of a drug suspension. This avoids contamination of the product by the equipment, metal contamination was found to be distinctly below 10 ppm (Krause et al., 2000). The production process is described in detail elsewhere (Böhm et al., 1998, Müller et al., 2000). Briefly, the crude drug powder was wetted by the surfactant solution and dispersed in the water phase by using an ultra turrax (Jahnke und Kunkel GmbH, Staufen, Germany) for 1 min at 9500 rpm. The obtained premix was homogenised using an APV Gaulin Micron LAB 40 homogeniser (APV Deutschland GmbH, Germany). First two cycles at 150 bar and two cycles at 500 bar as a kind of premilling were applied, followed by 15 homogenisation cycles at 1500 bar to obtain the final product. For the screening for an optimised stabilisation, different surfactants in different concentrations were tested to obtain a long-term stable nanosuspension. One of the most suitable surfactant combinations for bupravaquone was 1.0% poloxamer 188 (Lutrol F68, purchased by BASF, Ludwigshafen, Germany) and 0.5% lecithin (Lipoid E 80, obtained by Lipoid KG, Ludwigshafen, Germany). This formulation was able to stabilise a drug content up to 10.0% (Jacobs and Kayser, 2000a).

The nanosuspensions were characterised in terms of particle size and size distribution and measurement of the zeta potential. Particle size

analysis was performed by laser diffraction using a Coulter LS 230 (Coulter Electronics, Germany). In addition photon correlation spectroscopy (PCS) and zeta potential measurements were performed using a Zetasizer 4 (Malvern Instruments, UK). Storage stability was assessed by monitoring the size as a function of time (up to 3 months).

In general, four parameters regarding high pressure homogenisation influence the product quality:

1. power density (homogenisation pressure);
2. number of homogenisation cycles;
3. percentage of solid content;
4. surfactant combination and concentration.

The power density is defined as applied energy per volume unit and time unit in the homogenisation gap. In general, for piston gap homogenisers the hydraulic pumping power (Watt) can be used to calculate the power density, that means it increases with the pressure. To obtain particles as small as possible maximum power density was applied for the production of the nanosuspension, that means 1500 bar.

To reach maximum dispersitivity multiple homogenisation cycles need to be applied. As shown in Fig. 1, if more cycles are applied one will reach smaller particles up to a certain degree, dependent on the nature respectively of the hardness of the drug. For some drugs 4 cycles of 1500 bar were sufficient enough to obtain a suspension where the particle size of the bulk population is in the range

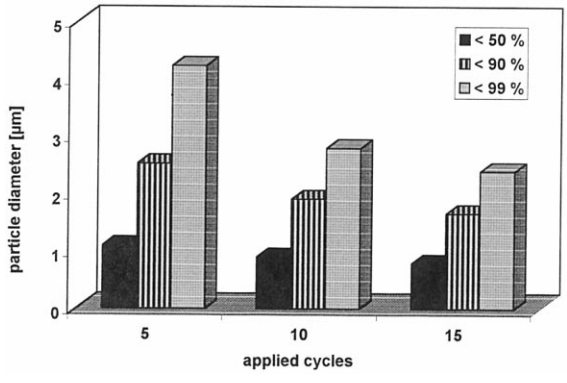

Fig. 1. Influence of the cycle numbers on particle size reduction for bupravaquone 1.0% suspension diameters 50 to 99%, determined by laser diffraction.

of ± 400 nm (Jacobs et al., 2000). For other drugs 20 cycles had to be applied to obtain a nanosuspension. After having reached the maximum dispersitivity, additional cycles will not lead anymore to size reduction, the remaining drug crystals possess such a hardness that they cannot be disintegrated further at the given power density.

Concentrations ranging from 1% solid to 10% were investigated. An increase in the solid concentration leads to an increased probability of particle collision leading to an additional milling effect. Therefore, increasing the solid concentration is beneficial for the disintegration process. In most cases, similarly small particles are achieved at identical cycle number, when moving from 1% to 10% solid — despite the fact that the input disintegration energy remains unchanged but the amount of drug powder to be disintegrated is 10-fold. For bupravaquone nanosuspensions, even a decrease in the PCS diameter from approximately 800 nm to about 600 nm was observed. This demonstrates nicely the contribution of an increased solid content to the disintegration result.

The particle size obtained in the production process is mainly a function of the power density and cycle number. The role of the surfactants is to physically stabilise the ultrafine nanosuspension. That means surfactants, surfactant concentrations and surfactant mixture differ in their ability to avoid the formation of particle aggregates. This was confirmed in previous studies showing that particle size and shape is a function of drug and power density, not a function of the surfactants used (Müller et al., 1996). In addition, the stabilisers need to stabilise the nanosuspension not only during formation in the production process but also long-term. Adhesive polymers are not necessarily the optimal stabilisers for the nanosuspensions leading to our concept of incorporating an optimal stabilised nanosuspension into a mucoadhesive gel. As mentioned above the mixture of 1.0% poloxamer 188 and 0.5% lecithin was able to stabilise the nanosuspension up to 10%.

To investigate the physical stability during storage, particle size was monitored over a period of 3 months (data by now). The diameter 50%, char-

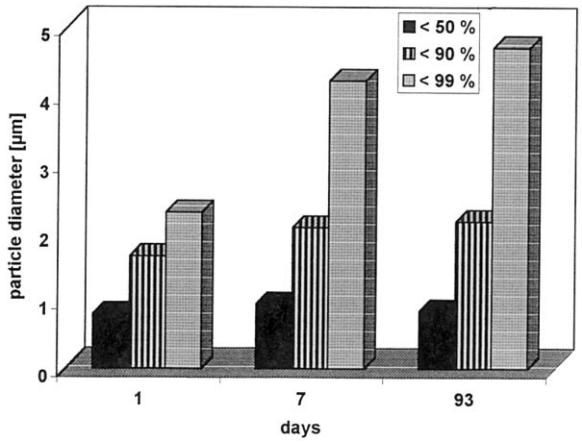

Fig. 2. Storage stability of bupravaquone nanosuspension containing bupravaquone 1.0%, poloxamer 1.0%, and lecithin 0.5% (modified after Jacobs and Kayser, 2000b).

acterising the bulk population of particles showed no change during storage, the diameters 90 and 99% being very sensitive to particle aggregation showed only negligible increase (Fig. 2). An unchanged dispersitivity is the prerequisite for an unchanged bioavailability because the particle size affects the adhesion to the gut wall — a bioavailability determining factor.

Mucoadhesive gels were prepared by hydrating the polymers in water for 24 h. The polyacrylate gels were then neutralised by addition of triethanolamine (Carbopol 934, 971, 974, 980, and Noveon AA-1). Gels based on chitosan were produced by dissolving chitosan chloride in water or by dissolving chitosan by addition of acetic acid. The polymer content was 0.5% for all gels. The concentrated nanosuspensions were incorporated into the gels using mortar and pestle and subsequent treatment with an ultra turrax. Based on the required single dose and administration volume for the envisaged in vivo study (Kayser, 2000), 1 ml of 10% nanosuspension was added to 13.93 g polymer gel. The administered dose in vivo was 100 µl containing 0.67 mg bupravaquone in form of a nanosuspension.

As mentioned above, an unchanged dispersitivity is the prerequisite for a constant bioavailability during the storage time of the product. Therefore it was necessary to prove that incorporation of the nanosuspension into the mucoadhesive gels did not lead to particle aggregation and that also aggregation was absent during storage. Aggregation of drug nanoparticles can occur by e.g. polymer bridging or anchoring phenomena. In addition, displacement effects cannot be excluded which might — in the case of an unfavourable coverage — also lead to particle aggregation. Particle size analysis was performed after diluting the gel with water and subsequent ultrasonic treatment for perfect dissolution of the polymer molecules in the water (formation of a sol). Analysis was performed by laser diffraction. Fig. 3 shows the LD diameter (volume based) of the drug particles in the different mucoadhesive gels after 3 months. The diameter 50% characterising the bulk population remained unchanged compared to the nanosuspension itself (columns at the right). In some gels the diameter 99% showed no or little change proving the absence of particle aggregation (e.g. gel with Carbopol 971 and 980). Only in two gels distinct aggregation was observed, that means Carbopol 934 and 974. The two Carbopols are chemically identical and only of a different quality (way of production, regulatory status for administration). Obtaining the same results with the two chemically identical polymers shows the reproducibility of this aggre-

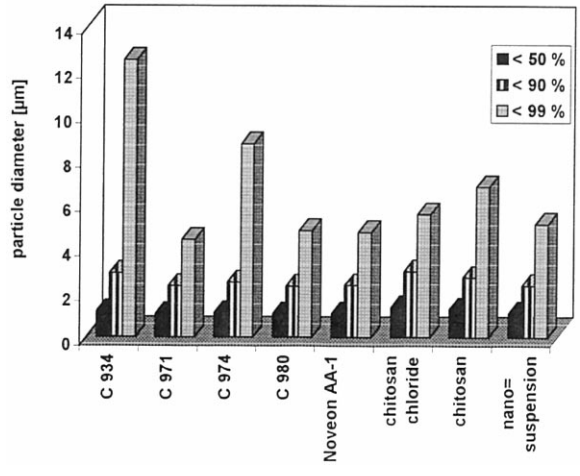

Fig. 3. Particle diameters of bupravaquone nanosuspension incorporated into different polymer gels (C for Carbopol), determined by laser diffraction (modified after Jacobs and Kayser, 2000b).

gation phenomenon. The extent is slightly different (different values for diameter 99%) because aggregation is a random event which can vary in its extent.

Preparation of this mucoadhesive nanosuspension gel in the 'classical way' requires a three step production process, that means producing the nanosuspensions, producing the gel and finally mixing the two components. Alternatively, a more straightforward process for the general production of mucoadhesive nanosuspension gels was developed. The drug powder was dispersed in the surfactant solution as described above, then the polymer was added and partially hydrated by addition of triethanolamine (adjustment of pH to approximately 5). This leads to a slightly viscous system which could still be passed through the homogeniser without any problem. The complete system was then homogenised at two cycles 150 bar, two cycles 500 bar (premilling process) and then for 15 cycles applying 1500 bar. Then the remaining triethanolamine was added to adjust to a pH of 7 to form the highly viscous gel (addition of triethanolamine under stirring using an ultra turrax).

The mucoadhesive delivery systems prepared by the three step and by the one step method were compared regarding the size of the drug nanoparticles. The sizes were comparable showing that the added polymer did not interfere with the production process. For both systems storage stability was documented so far for 3 months. Similar stability was found as determined by laser diffraction and PCS. These results prove, that the one step production method can be used as a simple and fast alternative to the more complicated previously applied production process.

To summarise: Nanosuspension technology and the mucoadhesive principle were combined to develop a formulation for the poorly soluble drug bupravaquone, especially for the treatment of *Cryptosporidium parvum* infections. The production method was optimised by developing a one step production process. The delivery system proved to be physically stable for 3 months and was successfully investigated in vivo (Kayser, 2000).

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