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Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability

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Abstract

The poorly soluble drug buparvaquone is used in experimental clinics against the gastrointestinal persisting parasite *Cryptosporidium parvum*. It was produced as nanosuspension by high pressure homogenisation. Main advantages of nanosuspensions (amongst others) are their increase of saturation solubility and dissolution velocity, improving the bioavailability of drugs. The buparvaquone nanosuspension had a bulk population of about 600 nm (analysed by photon correlation spectroscopy (PCS)). The additional analysis performed with laser diffraction showed that only a very small content of microparticles occurred, which is, for the special features of nanosuspensions, negligible because they were still below 3 μm. Another feature of nanosuspensions is the adhesion properties to surfaces, e.g. mucosa. To further increase the adhesion time of the buparvaquone nanosuspension to *C. parvum*, the nanosuspension was formulated with hydrogels made from mucoadhesive polymers, e.g. different types of Carbopol® and chitosan. Only a small increase of the particle size of the bulk population occurred directly after the incorporation of buparvaquone nanosuspension into the hydrogels. The nanosuspension/hydrogel systems were physically long-term stable over a period of 6 months as indicated by the unchanged particle sizes.

Keywords: Buparvaquone; High pressure homogenisation; Nanosuspensions; Hydrogels; *Cryptosporidium parvum*; Mucoadhesion

1. Introduction

Buparvaquone is a naphtoquinone antibiotic structurally related to atovaquone (Wellvone®). It is used in experimental clinics for the treatment of *Cryptosporidium parvum*, a protozoan parasite that persists in the entire gastrointestinal tract (GIT). The infection with *C. parvum* causes watery diarrhoea, cramps and nausea, especially immunocompromised patients suffer from this parasite over months or even years. Until now there is no efficient therapy to treat cryptosporidiosis (Hoepelman, 1996; Laing, 1999).

The solubility properties of buparvaquone are very poor, i.e. solubility in water is < 1 mg/l. Therefore, it is consequently poorly soluble in biological media, like gastric fluids, too. Thus the bioavailability is very low when given orally,

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treatment efficiency in *C. parvum* infections is not sufficient. One way to improve solubility and dissolution behaviour of drugs is to reduce the particle size down to the nanometer range (Müller et al., 2000; Nyström, 1998; Liversidge and Cundy, 1995; Mosharraf and Nyström, 1995). Another feature of nanoparticulate systems is their general adhesivness to the intestinal wall (Tur and Chang, 1998; Henriksen et al., 1996; Ponchel et al. 1997). This leads to a prolonged residence and contact time in the GIT. For further increase of contact time of the nanosuspension and *C. parvum*, buparvaquone nanosuspension was combined with mucoadhesive polymers like different Carbopols®, Noveon® (Mortazavi and Smart, 1995; Lueßen et al. 1997) and chitosan (Henriksen et al., 1996).

A very easy and simple method to produce nanosuspensions is high pressure homogenisation (Müller and Böhm, 1998). It is a particle size reduction process well known from food, pharmaceutical industry (emulsions for parenteral nutrition) and cosmetic industry. With high pressure homogenisation particle size reduction from μm to nm can be achieved (Müller and Peters, 1998; Peters and Müller, 1996; Jahnke, 1998; Müller et al. 1999).

The aim of this study was to create a buparvaquone nanosuspension for oral application. This nanosuspension was incorporated in mucoadhesive hydrogels for further increase of the mucoadhesive properties. The combined systems were investigated on their physical properties and long term stability.

2. Materials and methods

The drug buparvaquone was provided by Dr S.L. Croft (London School of Hygiene and Tropical Medicine, London, UK). Soya lecithin (Lipoid S 75) was supplied by Lipoid GmbH (Ludwigshafen, Germany). Polyvinylalcohol (PVA) (MW 30 000–70 000) was purchased from Sigma (Germany), poloxamer 188 (Lutrol F68®) from BASF (Ludwigshafen, Germany). Carbopol® 934, Carbopol® 971, Carbopol® 974, Carbopol® 980 and Noveon® AA-1 were kindly provided by BF Goodrich Chemical Deutschland GmbH (Neuss, Germany), chitosan chloride was purchased from Pronova (Oslo, Norway) and chitosan from Primex Ingredients ASA (Bremen, Germany).

2.1. Preparation of nanosuspensions

The surfactants were dissolved or dispersed in warm (approx. 40 °C) bidistilled water by using an Ultra Turrax T 25 (Jahnke und Kunkel GmbH, Staufen, Germany). The drug powder was dispersed in the aqueous surfactant solution using again the Ultra Turrax (1 min at 9500 rpm). The obtained pre-mix was homogenised using an APV Gaulin Micron LAB 40 homogeniser (APV Deutschland GmbH, Lübeck, Germany). At first, 2 cycles at 150 bar and 2 cycles at 500 bar as a kind of premilling were applied, then 15 homogenisation cycles at 1500 bar were run to obtain the nanosuspension.

2.2. Preparation of hydrogels

Preparation of polymer gels (all 0.5% w/w), Carbopol® 934, 971, 974, 980 and Noveon® AA-1 was dispersed in water for injection with mortar and pestle and left to equilibrate for 24 h. The samples were neutralised by addition of triethanolamine (adjusted to pH 7.0–7.5). Chitosan chloride was dispersed in water for injection only and chitosan was dissolved in water for injection by addition of acetic acid (pH 4). The nanosuspension was incorporated into the hydrogels with mortar and pestle.

2.3. Rheological measurements of hydrogels

The rheological measurements were performed with a Rheo Stress RS 100 (Haake, Karlsruhe, Germany), cone and plate fixture (diameter 20 mm, angle 4°). The samples were given onto the plate and kept there for 10 min before performing the measurement. All measurements were carried out at a temperature of 25 °C; the viscosity was measured at 20/s.

2.4. Particle size analysis

The bulk particle populations of the nanosuspensions were analysed by photon correlation spectroscopy (PCS) with the Malvern Zetasizer 4 (Malvern Instruments, UK) yielding the mean particle diameter of the suspension and the polydispersity index. This index indicates the width of a particle distribution (e.g. 0.0 for a narrow, 0.5 for a very broad distribution). Additional particle size analysis was performed by laser diffraction using a LS 230 from Coulter Electronics (Krefeld, Germany). The diameters were calculated using the volume distribution. Diameters 50 and 99% mean that 50% (respectively, 99%) of the particles are below the given size. With this method particles up to 2000 μm can be detected. It should be noted, that the LD data are volume based, the PCS mean diameter is the light intensity weighted size. Therefore the PCS mean diameter and the diameter 50% from the LD are not identical, LD data are generally higher. For the performance of LD and PCS measurements the nanosuspension had to be diluted to a distinct concentration, also the nanosuspension/hydrogel system, because the original concentration of nanoparticles were too high. The nanosuspensions were simply diluted with deionised water. The nanosuspension/hydrogel systems were diluted with deionised water by mortar and pestle and ultra sonication to achieve a uniform dilution (without remaining viscous lumps of the gel). All measurements were performed in triplicate.

2.5. Zeta potential measurements

To determine the zeta potential a Malvern Zetasizer 4 (Malvern Instruments) was employed. The analyses were performed using the large bore capillary cell, a field strength of 20 V/cm was applied. The electrophoretic mobility was converted to the zeta potential via the Helmholtz–Smoluchowski equation. The zeta potential of the nanosuspensions was measured in three different media: (a) in distilled water with conductivity adjusted to 50 μS/cm by addition of sodium chloride, (b) in original dispersion medium (for measurement dilution of the nanosuspension with dispersion medium = surfactant solution) for the estimation of the long-term stability and (c) in original dispersion medium but the conductivity of the surfactant solution was adjusted to 50 μS/cm with sodium chloride for better comparison of the formulations in order to minimise ionic effects on the zeta potential. The measurements were performed in triplicate.

3. Results and discussion

3.1. Screening of different surfactants for the optimal stabilisation of buparvaquone nanosuspension

Three formulations of buparvaquone were examined on their capability in stabilising buparvaquone as nanosuspension. The composition of the screening formulation F1–F3 is shown in Table 1. Surfactants, regarded as suitable for oral administration were chosen (Marti-Mestres and Nielloud, 2000). The formulations F4 and F5, with a drug content of 10.0%, were produced after the screening. The higher concentrated nanosuspensions with a 10% drug content were required

Table 1
Composition of buparvaquone nanosuspensions (w/w%)

Formulation	Buparvaquone	Lecithin	Poloxamer 188	PVA
F1	1.0%	0.5%	1.0%	
F2	1.0%		2.0%	1.0%
F3	2.5%		0.5%	
F4	10.0%		2.0%	1.0%
F5	10.0%		0.5%	

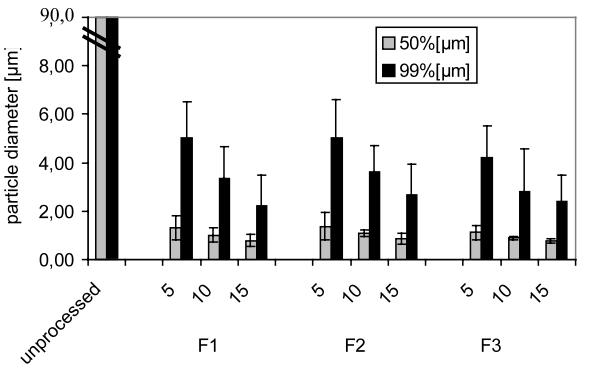

Fig. 1. LD diameters 50 and 99% of unprocessed buparvaquone and the three buparvaquone nanosuspensions F1–F3, as a function of cycle numbers (homogenisation pressure: 1500 bar).

for the in vitro and in vivo studies (Kayser, 2001) and were also used for the REM analysis. Fig. 1 shows the LD diameters 50 and 99% of formulation F1–F3 as a function of homogenisation cycles at the day of production in comparison to the unprocessed material.

All three formulations showed quite similar results. The data of the LD 50%, characteristic for the bulk population of the three formulations were close from each other, i.e. 0.78, 0.87, and 0.79 μm for F1, F2 and F3 formulations, respectively. The LD 99%, a sensitive parameter for large particles or aggregates, was below 3.0 μm for all three formulations after 15 homogenisation cycles. This was a satisfying result because it shows that the quality in regard to the homogeneity of the nanosuspension is very high. Formulation F1 had the lowest diameter LD 99% with 2.23 μm and F2 the highest with 2.69 μm; the LD 99% for F3 was 2.39 μm. As the LD diameter is very sensitive to large particles, and only a small amount of microparticles can shift this diameter very easily, one can say that the particle size distributions of the three formulations are practically identical. This is confirmed when plotting the LD size distribution curves (data not shown).

Additionally the PCS diameters were measured for further characterising the bulk population. The PCS results shown in Fig. 2 are well in agreement with the LD data. First of all formulation F2 showed smallest particle sizes at cycle 5 with 630 nm (PI of 0.384) versus F1 with 820 nm (PI of 0.458) and F3 811 nm (PI of 0.364). After cycle 15, the mean PCS of F2 diameter was about 589 nm (PI 0.280), the data of F3 were 663 nm (PI 0.240). Formulation F1 reached also a low mean PCS diameter with 558 nm but showed a PI 0.347, which indicates a broader particle size distribution. To sum up: it was possible with all three formulations to obtain a buparvaquone nanosuspension with very similar particle sizes, though the compositions of surfactants were different with regard to the kind of stabilisation mechanism.

Formulation F1 contains lecithin and poloxamer 188. Lecithin stabilises a suspension by electrostatic repulsion because it is an amphoteric surfactant. Poloxamer as a non-ionic surfactant stabilises suspensions sterically. Formulation F2 consists of two non-ionic surfactants (poloxamer 188 and PVA), this nanosuspension is stabilised sterically only. F3 containing only poloxamer 188 but at a low concentration, is stabilised sterically too.

The achievable particle size reduction depends on the hardness of the drug and the applied homogenisation parameters as pressure and number of applied cycles. They determine power den-

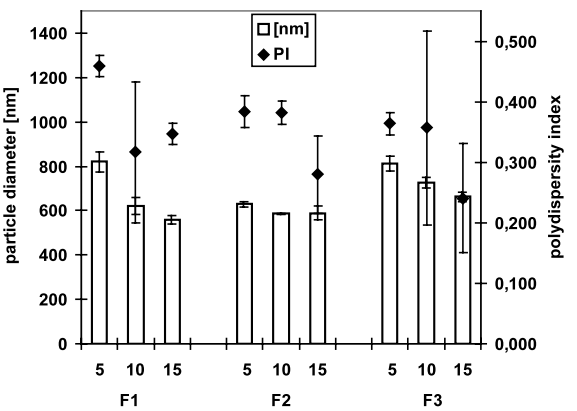

Fig. 2. Mean PCS diameter and polydispersity index (squares) of the three buparvaquone nanosuspensions F1–F3 (from Fig. 1), as a function of cycle numbers.

sity and total energy input for disintegration (Jahnke, 1998). The determining factor to get a finely dispersed, non-aggregated nanosuspension is the stabilising surfactant, respectively surfactant combination. The surfactants are used as wetting agents and can prevent the aggregation of the produced ultra fine nanoparticles. During the homogenisation process aggregates can form due to insufficient coverage of the newly generated particle surfaces by the stabilisers or—despite coverage—insufficient stabilising effect (e.g. too low zeta potential or too thin sterically stabilising layer, i.e. $\ll 10$ nm). The coverage of these newly generated surfaces is achieved through diffusion of the surfactants, which requires a distinct time and varies between surfactants (i.e. diffusion velocity is a function of molecular weight, hydration, viscosity of medium). Whether the production of a nanosuspension is successful, that means the bulk population is in the nanometer range and the amount of microparticles is very small, depends on the properties of the surfactants such as diffusion velocity and affinity to the particle surface (e.g. functional groups responsible for hydrophobic/hydrophilic interactions of surfactant and drug for firm anchoring on the particle surface). They are also important for the physical long-term stability. In this study, all three stabiliser combinations proved similar efficiency in stabilising nanosuspensions in the production process (= short term stability).

From regulatory aspects the number and concentration of ingredients accompanying the therapeutic compound should be kept to a minimum to minimise registration hurdles. These results show that it is possible to stabilise buparvaquone nanosuspension by different mechanisms of stabilisation, with different types and also at low concentrations of surfactants (e.g. 0.5%, F5). This knowledge can be used when buparvaquone should be formulated for other application ways, e.g. for pulmonary delivery against *Pneumocystis carinii* (activity of buparvaquone against this parasite was also shown). Surfactants of choice are stabilisers approved for pulmonary inhale products, e.g. lecithin and Span 85®.

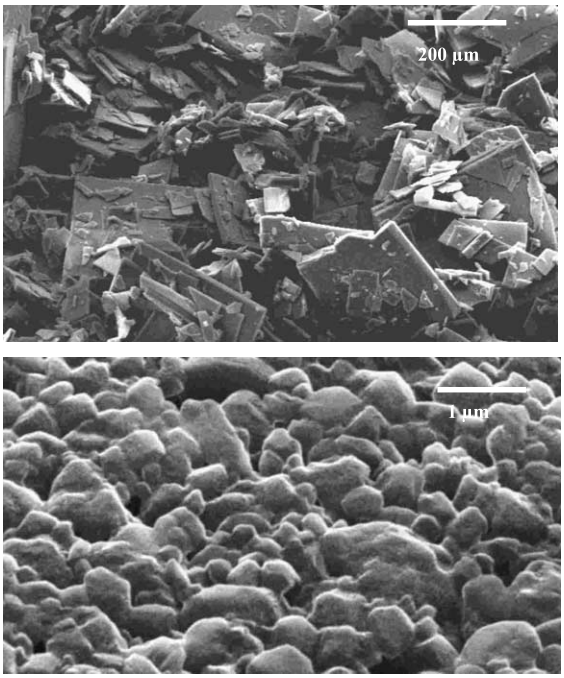

Fig. 3. Scanning electron micrograph of buparvaquone unprocessed (upper) and of buparvaquone formulation F4 (10% drug content) (lower).

3.2. Raster electron micrographs

Raster electron micrographs of buparvaquone unprocessed (Fig. 3, upper) and formulation F4 after 15 homogenisation cycles (Fig. 3, lower) are shown. Before homogenisation buparvaquone powder consisted of large crystals. After homogenisation, the large crystals were transformed into small round or cubic nanoparticles. This phenomenon was previously shown with other drugs (Müller et al. 1996; Böhm 1999). After homogenisation the nanoparticles have a more rounded surface appearance than the pure substance. This could be attributed to the particles being coated with a surfactant/stabiliser layer. In addition, creation of an amorphous surface layer by the high homogenisation pressure can also contribute to the change in appearance of the surface.

3.3. Zeta potential measurements

To investigate the surface properties of the nanosuspensions, the zeta potential of the three formulations were analysed. First the zeta potential was measured in bidistilled water (conductivity was adjusted to 50 μS/cm) to measure potential differences in surface charge (Helmhotz potential (Müller, 1996)). The nanoparticles of formulation F1 had a zeta potential of −34 mV, F2 of −15 mV and formulation F3 −32.7 mV (Fig. 4a).

For the estimation of the long-term stability of the nanosuspensions, they were measured in original dispersion medium because that is the medium, particles are surrounded during storage. Under this measurement condition, the zeta potential is an indirect measure of the thickness of the diffuse layer, i.e. can be used to predict long-term stability (Müller 1996).

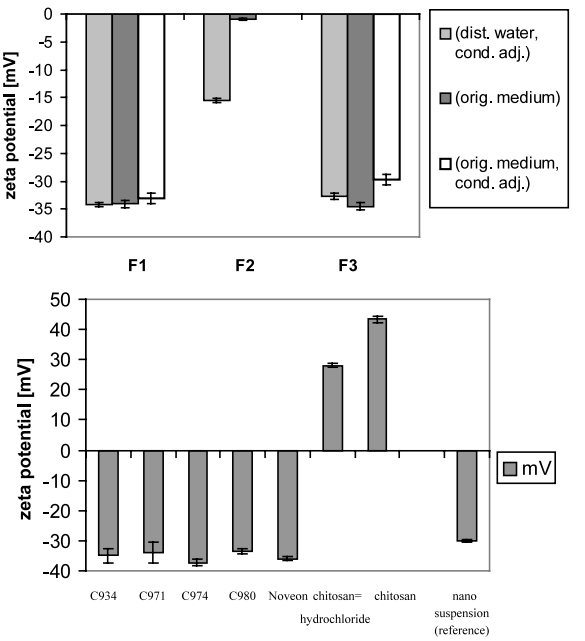

Fig. 4. (a) Zeta potential of the buparvaquone nanosuspension formulation F1–F3 measured in bidistilled water (conductivity adjusted to 50 μS/cm), in original dispersion medium, and original medium conductivity adjusted to 50 μS/cm (F1 and F3 only, cf. text) (upper) and (b) Buparvaquone nanoparticles after incorporation into different hydrogels (C for Carbopol), and pure buparvaquone nanosuspension as reference (measured in bidistilled water with adjusted conductivity to 50 μS/cm) (lower).

For a better comparison of the zeta potentials, a third measurement series was performed. For this measurement the conductivity of the surfactant solution (= original dispersion medium) was adjusted to 50 μS/cm with sodium chloride, too. This was done to minimise the ionic effects on the zeta potential when the conductivity of the media is different (the measuring media of F2 had already a conductivity of 118 μS/cm, therefore, no adjustment could be made). The data are shown in Fig. 4a. Formulation F1 and F3 were between −30 and −35 mV in all media, only the zeta potential of F2 was very low at −0.9 mV in original dispersion medium, i.e. particles are practically uncharged. This indicates that this nanosuspension will not be physically long-term stable, in case it has a too thin sterically stabilising poloxamer layer on the particle surface.

The zeta potential depends on the kind and concentration of employed stabiliser. The two main stabilising effects are electrostatic repulsion achieved with ionic surfactants/stabilisers and steric stabilisation by non-ionic surfactants/stabilisers (see Section 3.1). For example, a suspension stabilised with uncharged surfactants only, will have a lower zeta potential (e.g. $\ll -20$ mV), than a suspension which is stabilised with charged surfactants (e.g. of −35 to −50 mV in case of lecithin emulsions). However, despite a zeta potential below the critical value of −30 mV, both can have the same long-term stability, in case the sterically stabilising layer is sufficiently thick.

Suspensions can also be stabilised by combination of an ionic surfactant and a non-ionic surfactant, the zeta potential of these suspension will be between the ones of electrostatically and sterically stabilised suspensions. All three kinds of stabilisation can exhibit the same long-term stability though they have different zeta potentials.

A zeta potential of at least −30 mV for electrostatic and about −20 mV for sterically stabilised systems is desired to obtain a physically stable suspension according to the literature (Müller, 1996; Ney, 1973; Riddick, 1968). The zeta potential of formulation F1 and F3 are theo-

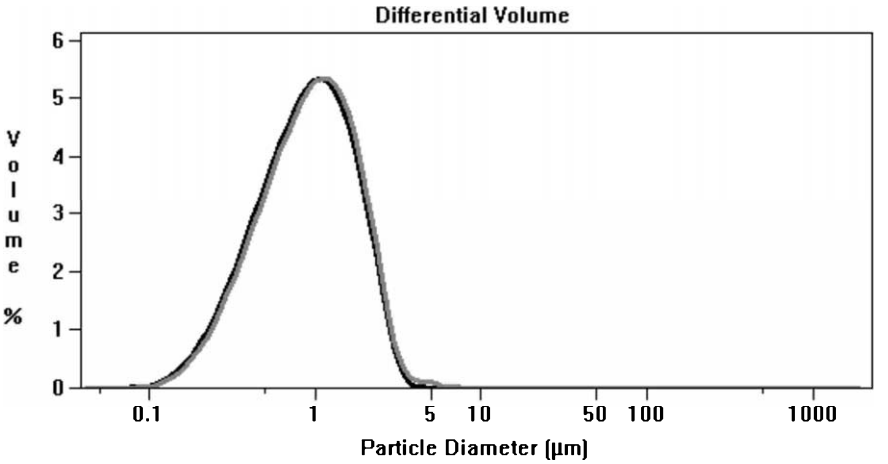

Fig. 5. LD size distribution of buparvaquone pure nanosuspension formulation F1. Analysed after 1 week and 3 months (redispersed after several min of intense hand shaking), stored at room temperature.

retically sufficiently high to obtain a long-term nanosuspension. As shown in Section 3.5 about long-term stability, all three formulations were stable over a period of 3 months. However, after this time caking of the nanosuspension occurred. The sediment was fully redispersable, but only after intense hand shaking for several minutes or by ultra sonic treatment (160 W, 3 min). In such cases, for commercial products lyophilisation of the nanosuspensions is sensible (Böhm, 1999).

3.4. Incorporation into mucoadhesive polymers

To raise the adhesivness to the GIT wall, the nanosuspensions were incorporated into hydrogels (Carbopol® 934, 971, 974, 980 and Noveon®AA-1, chitosan chloride and chitosan). One ml of the nanosuspension formulation F5 (drug content of 10%) was added to 13.93 g polymer gel. The dose was chosen because it was the desired dose for the in vivo testing (100 µl containing 0.67 mg buparvaquone drug).

3.5. Long-term stability

After production all three nanosuspension formulations showed a similar size distribution. In Fig. 5 exemplary for all three nanosuspension formulations, the LD distribution of F1 (pure nanosuspension) 1 week after production and 3 months later is shown. A narrow size distribution is essential to prevent particle growth due to Ostwald ripening being caused by different saturation solubilities in the viscinity of differently sized particles. In these three formulations only a small amount of microparticles occurred (see data in Fig. 1). The problem with Ostwald ripening can be pronounced when a particle fraction is above 10 µm, because above this size the intrinsic dissolution rate is much lower than for particles $\ll 10$ µm (Nyström, 1998; Grau, 2000).

The problem with all three formulations was the redispersivity after 3 months, especially when the concentration of drug was higher (F4 and F5 10%). After 6 months the suspension was hardly redispersable by hand shaking. The relatively solid sediment vanished only after several min of intense shaking, or by ultra sonic treatment (160 W, 3 min).

When the nanosuspensions were incorporated into hydrogels the caking problem was circumvented, the systems were physically stable, no caking occurred anymore. Fig. 6 shows the LD 50% and the LD 99%, which is an extremely sensitive parameter for microparticles, only a few aggregates will shift this diameter to larger values. Compared with the nanosuspension (= reference), the LD 99% increased in all gel formula-

tions to 4–13 μm. The 6 months values were generally below the day 0 values. The increase in LD 99% was attributed to aggregate formation during the dilution for analysis (bridging by gel polymer) and not due to crystal growth as proven by light microscopy. The lower values at 6 months might be explainable by reaching an equilibrium condition of mucoadhesive polymers adsorption onto the particle surface, i.e. the gel polymer having also simultaneously a sterically stabilising effect. This effect was obviously less pronounced when the samples were diluted for analysis directly after incorporation of the nanosuspension into the gels.

Fig. 7 shows, exemplary for all employed hydrogels, the micrograph of formulation F3 incorporated into Carbopol® 934 and into chitosan gels in comparison to the pure nanosuspension (light microscopy magnification 63 × 10). The micrographs show that aggregates occurred only after diluting the systems for measuring purposes (with deionised water) but when the suspension incorporated into the gel was analysed, no aggregates could be detected. That no caking occurred anymore can be explained by the Stokes equation. The sedimentation of particles is limited due to the higher viscosity of the dispersion medium. The viscosity of the different Carbopols® and Noveon® AA-1 0.5% are between 4000 and 60 000 mPa s (at 20 rpm Brookfield, pH 7.5), respectively, 2000 and 15 000 mPa s. The viscosity of chitosan is much lower. The viscosity data of the employed hydrogels are listed in Table 2. The excellent long-term stability of the chitosan/buparvaquone nanosuspension can be explained by the high zeta potential. The sample was diluted and measured in bidistilled water (conductivity 50 μS/cm), the result was a zeta potential of + 43 mV (conductivity 51 μS/cm, pH 5.3). Fig. 4 (lower) shows the zeta potentials of all hydrogel/nanosuspension formulations. The Carbopol®/nanosuspension system reached a maximum zeta potential of − 35 mV. These measurements were performed in bidistilled water only because it is not possible to measure in original medium due to the high viscosity of the hydrogels (conductivity 50 μS/cm, this value did not change when gel/nanosuspensions was added for the measurement, the pH was between 6.9 and 7.6).

Another way to circumvent the caking problem is to lyophilise the nanosuspension, which was successfully carried out with formulation F2 (unpublished data). F2 was taken for the lyophilisation, because this formulation contained PVA, which acts as a cryoprotector.

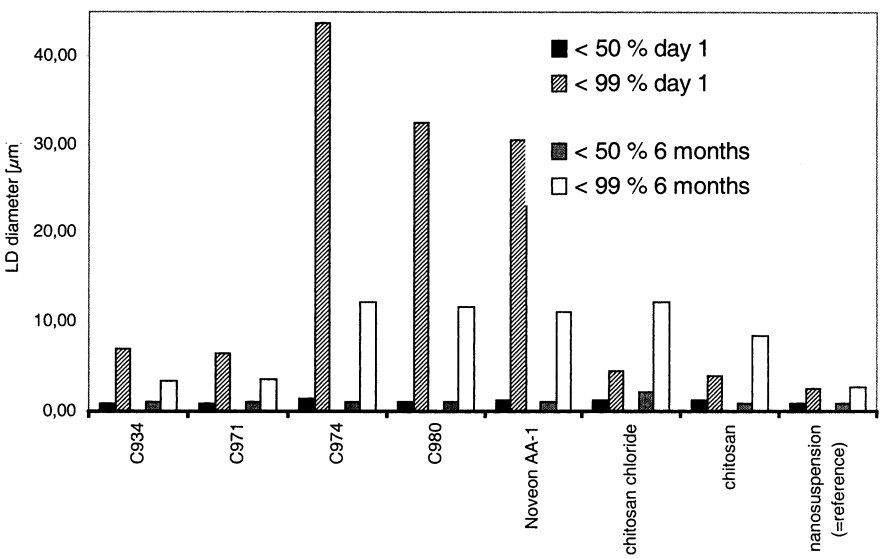

Fig. 6. LD diameter 50 and 99% of nanosuspension formulation F3 incorporated into hydrogels (C for Carbopol) 1 day after production and after 6 months stored at room temperature.

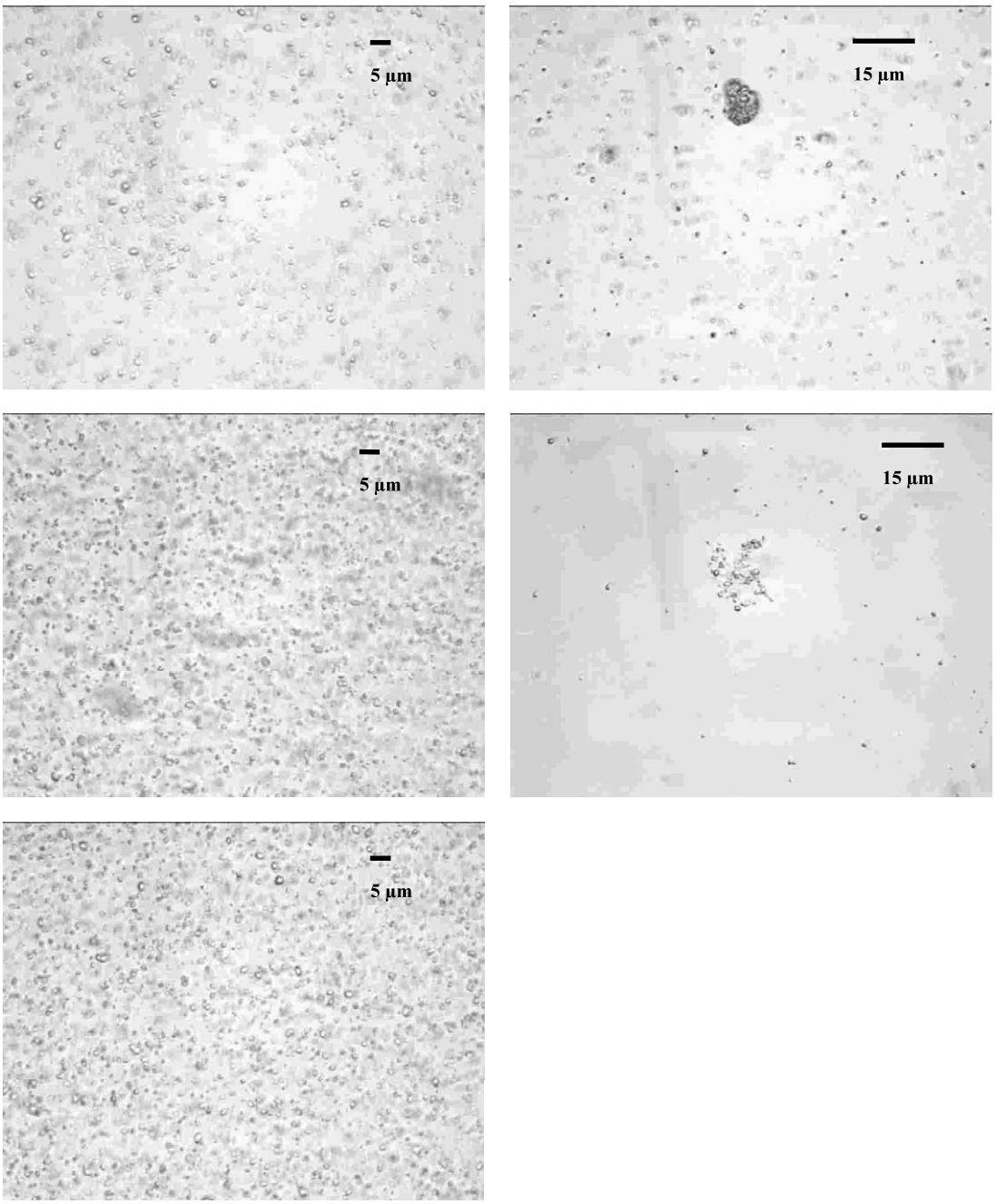

Fig. 7. Micrograph (light microscopy) of buparvaquone nanosuspension F3 incorporated into Carbopol® 934 (upper left) and diluted (upper right), chitosan (middle left) and diluted (middle right) and as comparison pure nanosuspension (lower) after 6 months.

Table 2
Viscosity of the employed mucoadhesive polymer gels

Polymer 0.5% solution (w/w)	Viscosity (mPa s) 20 °C	Viscosity (mPa s) 25 °C
Carbopol 934	30 500–39 400[a]	5160
Carbopol 971	4000–11 000[a]	1970
Carbopol 974	29 400–39 400[a]	5520
Carbopol 980	40 000–60 000[a]	15 200
Noveon AA-1	N.D.	7470
Chitosan chloride	12[b]	44
Chitosan	465[c]	53

[a] Product specification from BF Goodrich.
[b] Product specification from Pronova.
[c] Product specification from Primex.

4. Conclusion

It was shown that it is possible to obtain a buparvaquone nanosuspension stabilised with different components suitable for oral administration. By incorporation of the nanosuspension into mucoadhesive hydrogels, the physical stability of this system could be increased compared with the caking nanosuspensions, leading to long-term stable systems. Of special interest is that the combination of the nanosuspension and mucoadhesive polymers can increase the anticryptosporidial activity of buparvaquone allowing more efficient therapy compared with the present formulations (Kayser, 2001; Müller et al., 2001).

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