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Formulation of poorly water-soluble drugs via coacervation – a pilot study using febantel

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Abstract

In this study febantel was dissolved under increased temperature in a non-ionic surfactant Lutrol L44®, and subsequently mixed into an aqueous maltodextrin solution. After 8h under static conditions, coacervation or phase separation took place. $^1$H-NMR spectra and HPLC analysis showed that the upper phase contained mainly all febantel, while no febantel was detected in the lower phase. Fluorescent microscopy showed that maltodextrin is distributed in the lower phase. Coacervation proved to be a promising formulation technology for certain poorly water-soluble drugs, such as febantel. The coacervate phase showed an increase in in vitro dissolution kinetics, compared to Rintal® granules. These results were confirmed in an in vivo study performed on dogs. Febantel and fenbendazole showed a significant increase in plasma concentration compared to Rintal® granules. Further studies have to be performed to transform coacervates into a solid dosage form and to prove broad applicability to other poorly soluble drugs.

Keywords: Coacervation, phase separation, poorly water-soluble drugs, febantel, non-ionic tensioactive agent
1 INTRODUCTION

As approximately 40% of the novel drug candidates are classified as class II and class IV drugs in the biopharmaceutical classification system, their development is hindered by poor water-solubility, limiting their bioavailability. Therefore, technologies aimed at improving their rate and extent of dissolution of poorly water-soluble drugs are continuously being developed [1]. Different methods to improve the water-solubility have been described in literature, such as the inclusion of drugs in cyclodextrins [2-5], solid dispersions [6], self-emulsifying drug delivery systems [7], nanocrystals [8, 9] and coacervation. Coacervation is defined as the separation of colloidal systems into two liquid phases (IUPAC). It can be distinguished from precipitation, which is observed in the form of coagulum or flocs and occurs in colloidal unstable systems [10]. Bungenberg et al. [11] defined coacervation as a process in which aqueous colloidal solutions separate, upon alteration of the thermodynamic condition of state, into two liquid phases, one rich in colloid, i.e. the coacervate, while the other contains little colloid.

Drug formulation based on coacervate systems have already been reported, i.e a hydrogel-based or foam-based aqueous coacervate system including a water-insoluble drug, an amphoteric surfactant and an edible mono-or polysaccharide acting as rigidifying agent for the gel or foam material [12, 13]. Another, biological application of a coacervate system, is a gelatin-based synthetic whole blood useful as a replacement for whole mammalian blood [14]. The aqueous two-phase coacervate system evaluated in this study consisted of the combination of a poorly water-soluble drug with a non-ionic tensioactive agent and a water-soluble polysaccharide maltodextrin.

The first purpose of this study was to examine the distribution of the poorly water-soluble drug in the two-phase coacervate system. The second purpose was to examine the in-vivo and in-vitro performance of the coacervate system as a formulation strategy for poorly water-soluble drugs. For this purpose, febantel was used as an extremely poorly water-soluble (1-2 ppm in water pH 5-9) broad-spectrum probenzimidazole that is widely used against
gastrointestinal nematodes and lungworms in livestock. It is a prodrug that is metabolized in vivo to fenbendazole and further to oxfendazole, 4-hydroxyfenbendazole, 4-hydroxyfendazole, and an inactive metabolite fenbendazolesulfone [15-17].
2 MATERIALS AND METHODS

2.1 Materials
Febantel was purchased from UTAG (Amsterdam, The Netherlands). Maltodextrin, with a dextrose equivalent of 8 (Pharmadry C*01983) was obtained from Cargill (Mechelen, Belgium). Lutrol L44® was obtained from BASF (Ludwigshafen, Germany). Rhodamine B Octadecyl ester was obtained from Fluka (Buchs, Switzerland). The deuterated solvents D₂O and MeOH-d₄ were obtained from Acros Organics (Geel, Belgium).

2.2 Preparation of febantel coacervate
For the preparation of the coacervate, Lutrol L44® (a block copolymer of ethyleneoxide and propyleneoxide, used as a non-ionic surfactant) (BASF, Ludwigshafen, Germany) was heated to 130°C, and febantel (UTAG, Amsterdam, The Netherlands) was dissolved in the heated Lutrol L44® phase in a 1/7 (w/w) ratio. The febantel/Lutrol solution (about 80°C) was added to an aqueous maltodextrin solution (42 %, w/w) and homogenized during 5 min using a high shear mixer (Silverson L4R, Chesham, England) operating at 6000 rpm. Finally, the liquid was allowed to stand under static conditions at room temperature for 8h to allow coacervate formation and phase separation. The batch size produced in these experiments was 100 g. TGA measurements (Hi-res TGA 2950, TA Instruments, Leatherhead, UK) were employed to investigate the thermal stability of febantel and Lutrol L44®. Samples (± 15 mg) were equilibrated at 50°C and heated to 300°C (febantel) or 500°C (Lutrol L44®) at heating rate of 10°C/min while recording weight loss.
2.3 Characterization

2.3.1 Fluorescence microscopy

Fluorescence microscopy was used to determine the distribution of the respective components in the different phases of the coacervate system. For this purpose, the amount of material was downscaled with a factor 10. Fluorescent labelling of the febantel/Lutrol L44® phase was performed by addition of 1 μl of a 10 mg/ml rhodamine B octadecyl ester solution in ethanol, after complete dissolution of febantel in Lutrol L44®. The aqueous maltodextrin phase was fluorescently labelled by addition of 10 μl of a 1 mg/ml FITC-dextran solution in water. Using these phases a fluorescent coacervate system was formed as described in section 2.2. Fluorescence microscopy images were recorded on Leica DM2500P microscope equipped with 10 x and 63 x oil immersion objectives and a DFC360FX CCD camera. Hot stage microscopy was performed on the same microscope, equipped with a THMS600 heating stage.

2.3.2 $^1$H-NMR spectroscopy

$^1$H-NMR spectra were recorded separately in D$_2$O and/or MeOH-d$_4$, depending on the solubility of the components. The $^1$H-NMR spectra were recorded on a 300 MHz Varian Inova spectrometer. Spectra were acquired with a 45° pulse of 5 μs, a spectral width of 4800 Hz, an acquisition time of 3 s, a preparation delay of 2 s and 64 accumulations. The chemical shift axis was calibrated by means of the D$_2$O resonance at 4.64 ppm from tetramethylsilane.

2.4 Release of febantel

Hard-gelatin capsules (n°00) were loaded with 115 mg of febantel formulated as (a) physical mixture at room temperature of febantel and 1.39 g Lutrol L44®, (b) 1.5 g of coacervate and (c) 1.15 g of Rintal® granules(i.e. a commercially available granule formulation containing 10% febantel and sodium docusate). Febantel release from these formulations was subsequently determined using a USP Apparatus 1 (basket, 100 rpm) in 900 ml demineralized water (at
37.5 °C). Samples of 5 ml were taken at 5, 10, 15, 20, 30, 45, 60 and 120 min. Samples were analysed via high-pressure liquid chromatography (HPLC). The HPLC system (Merck-Hitachi D-7000, Tokyo, Japan) consisted of a pump (Merck-Hitachi L-7200), an autosampler (Merck-Hitachi L-7250), a Halo Phenyl-Hexyl column (4.6 x 50 mm, 2.7 µm) (Advanced Materials Technology, Wilmington, USA), and an UV-detector (Merck-Hitachi L-7400) set at 295 nm. For the preparation of the mobile phase 0.01 M ammonium dihydrogen phosphate (Merck, Darmstadt, Germany) was mixed with acetonitrile (Biosolve, Valkenswaard, the Netherlands) in a ratio 50/50 (vol/vol). The analyses were performed at ambient temperature and the flow rate was set at 1 ml/min. A volume of 2 µl was injected onto the HPLC system.

### 2.5 Bioavailability of febantel

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine (Ghent University, Belgium). A cross-over study in dogs (n=4, body weight varying between 20 and 40 kg) was performed to determine the bioavailability of febantel after oral administration of the febantel coacervate phase and Rintal® granules. The dogs received a hard-gelatin capsule containing 15 mg febantel per kg, formulated as the coacervate phase or Rintal® granules. The washout period between sessions was 2 weeks. Blood samples (2 ml) were taken prior to each treatment day (= blank sample) and 0.5, 1, 2, 4, 8, 16, 24, 48 and 72 h after administration by puncturing the vena jugularis. The blood samples were collected in dry heparinized tubes and within 1h after collection blood was centrifuged for 10 min at 1500 g and kept frozen at -20°C until analysed.

### 2.6 Determination of febantel plasma concentration

Febantel, fenbendazole and oxfendazole plasma concentrations were determined based on the HPLC-MS method developed by De Ruyck et al.[18] using mebendazole as internal standard. The chromatographic system consisted of a Kontron instrument (Zurich,
Switzerland). Separation was carried out using a Luna C18 Column (50 mm x 2.0 mm, particle size 3μm; Grace, Columbia, MD, USA). The injection volume was 10μl. Gradient elution was performed with a flow rate of 0.2 ml/min starting at 75% eluent A (water containing 0.1% formic acid). Eluent B (acetonitrile containing 0.1% formic acid) was linearly increased from 25% to 75% during 12 min. The initial conditions were regained over a 0.1 min time interval, followed by a 5 min equilibration time prior to the next injection. This resulted in an overall run time of 17 min. Detection was performed using a Quattro II triple quadrupole mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization source in the electrospray positive ion mode (ESI+). Nitrogen was used as both drying and nebulizing gas. Product ions were detected using the multiple reaction-monitoring (MRM) mode, using argon as collision gas. The capillary voltage and source temperature were optimized at 3.6 kV and 120°C, respectively. The collision energy and cone voltage were optimized for each compound individually. The collision energy varied from 17 eV to 30 eV and the cone voltage varied from 35 V to 50 V. Data were collected and processed using the MassLynx and QuanLynx software 4.0 (Waters, Manchester, UK). The peak plasma concentration (C max), the time to reach C max (T max) and the extent of absorption (AUC 0-12h) were determined.
3 RESULTS AND DISCUSSION

A first key step in our formulation strategy was the dissolution of the poorly water-soluble drug in a liquid surfactant phase. Febantel (Figure 1) was dissolved in Lutrol L44® by heating the liquid slightly above the melting point of febantel (i.e. 129 °C). TGA indicated an onset of thermal degradation of 160°C and 200°C for febantel and Lutrol L44®, respectively. These temperatures are well above the temperature of the coacervation process (data not shown). Figure 2A show photographs of test tubes filled with (A1) a Lutrol L44®/Febantel suspension prior to heating and (A2) a homogeneous liquid after heating. Further evidence that febantel effectively dissolved in a liquid Lutrol L44® phase was gained via hot stage microscopy under brightfield and polarized light. As shown in Figure 2B, the crystalline febantel gradually dissolves in the Lutrol L44® medium with no residual crystallinity remaining. The liquid febantel/Lutrol L44® formulation was stable over several weeks without drug precipitation to occur. However, when this liquid was poured in an aqueous medium a translucent gel was formed that readily became opaque (Figure 2A3). Figure 2C4 shows optical microscopy images taken at 1 min time intervals of this gelation process. Drug precipitates were formed that gradually grow over time. The optical microscopy image in Figure 2C4, recorded under polarized light, clearly demonstrated the crystalline nature of these precipitates. Most likely, Lutrol L44® micelles are unable to quickly solubilize the amount of febantel present.

A second step in the formulation strategy, aimed to resolve the issue of instantaneous drug precipitation upon contact of the febantel/Lutrol L44® solution with water, via coacervation. Therefore, the febantel/Lutrol L44® solution was poured into a concentrated (i.e. 42 % (w/w)) aqueous maltodextrin solution. It is known that at elevated concentration, aqueous solutions of macromolecular and/or amphiphillic species (as are Lutrol L44® and maltodextrin) phase separate, forming a so-called simple coacervate [19]. The febantel/Lutrol L44® solution was added to a 42 % (w/w) aqueous maltodextrin solution under vigorous stirring. Immediately an opaque liquid formed (Figure 3A1), although maltodextrin and Lutrol L44® are both readily
water-soluble. Optical microscopy revealed that the opacity of the liquid is due to the formation of two immiscible liquid phases and not due to precipitation of febantel. When left to stand under static conditions, both phases demixed (Figure 3A2-3) and a translucent lower phase (about 35 % of the total volume) and a slightly opaque upper phase (about 65 % of the total volume) were formed. This two-layer system was stable at room temperature (25 ± 2°C) over a period of several months without precipitation of febantel to occur. It is noteworthy that in absence of febantel similar phase behaviour was observed. Importantly, when the two-phase liquid is diluted in aqueous medium, a clear solution is readily obtained (Figure 3B) while no drug precipitation is observed. The formulation used in this study contained 5% febantel dissolved in a system with 35% Lutrol L44® and 60% aqueous phase (consisting of 58% water and 42% maltodextrin).

In order to get a better insight into the nature of the two phases, we spiked before mixing the febantel/Lutrol L44® and the aqueous maltodextrin phase, with two different fluorescent dyes. Rhodamine B octadecyl ester (red fluorophore) was used as hydrophobic dye and is expected to be distributed in the same way as febantel while FITC-dextran (green fluorophore) was used as hydrophilic dye that is expected to be distributed in the same way as maltodextrin. Upon mixing of both phases, an opaque liquid forms (Figure 4A) which slowly phase-separates over time, similarly as described above for the non-fluorescent labelled sample. After 8h, two distinct phases were formed with the lower phase stained yellow and the upper phase stained red. This gives a strong indication that maltodextrin will be preferentially distributed in the lower phase while febantel will be distributed in the upper phase. Further insight into the formation of both phases was gained via optical microscopy by recording images immediately upon mixing of the febantel/ Lutrol L44® and the maltodextrin phases. As shown in Figure 4B, a two-phase system is formed, composed of a discrete phase containing the hydrophobic (red) fluorescent marker and a continuous phase containing the hydrophilic (green) fluorescent marker.
These observations indicated indeed that upon contact with the aqueous maltodextrin phase, the febantel/Lutrol L44® phase withdraws water from the aqueous maltodextrin phase, forming a colloidal gel with febantel distributed within its hydrophobic domains. It is known that at elevated concentration macromolecular species tend to phase separate [20]. In this particular case, the maltodextrin aids the formation of the coacervate by reducing the solubility of the Lutrol L44®. To confirm this hypothesis we analysed the composition of the lower and upper phase by ¹H-NMR spectroscopy. Figure 5 shows the NMR spectra of the upper and lower phase as well as the spectra of the individual components. As febantel is poorly water-soluble, but soluble in methanol, and maltodextrin is soluble in water but not in methanol, we recorded the respective ¹H-NMR spectra either in deuterated water (D₂O) or deuterated methanol (d₄-MeOH), respectively. From the respective spectra in Figure 5, it is clear that febantel is exclusively distributed in the upper phase. Lutrol L44® is predominantly found in the upper phase, but also, to a lesser extent in the lower phase. Furthermore, maltodextrin was abundantly present in the lower phase but also to a limited extent in the upper phase. Also D₂O was observed in the spectrum of the upper phase that was recorded in d₄-MeOH, this attributed to the hypothesis that Lutrol L44® was present as a colloidal gel with febantel solubilized in the hydrophobic micellar domains. Quantification of the febantel concentration in the upper coacervate phase via HPLC indicated that the drug concentration was 7.5%.

For dissolution experiments, hard-gelatin capsules were filled with coacervate and added to dissolution vessels containing demineralized water thermostated at 37.5 °C. Worthwhile to note is that these capsules were stable at room temperature without changes over a period of several weeks, likely due to the small amount of water that is contained within the coacervate phase. The dissolution data (Figure 6) showed clear differences between the release rate of febantel from the coacervate system and from the Rintal® granules: after 20 min 83.4 ± 1.6 % of the febantel load was released from the capsules containing the coacervate formulation, whereas febantel release was limited to 19.9 ± 6.0 % from capsules
containing the granules (Figure 6). The poor performance of Rintal® in the experimental in vitro set-up is likely due to the crystalline nature of febantel within this formulation, whereas febantel is solubilised in the coacervate-based formulation. The reduction in cumulative drug release at 20 min is possibly due to supersaturation of the drug in the dissolution vessel.

Figure 7 shows the mean plasma concentration-time profiles of febantel and the active metabolites (fenbendazole and oxfendazole) after oral administration of both formulations to dogs, while the pharmacokinetic parameters are reported in Table 1. The coacervate system enhanced absorption and improved bioavailability of febantel in dogs as the AUC\(_{0-24h}\) values of febantel and fenbendazole after administration of the coacervate formulation were significantly different (P < 0.05) from the Rintal® granules. However, AUC\(_{0-24h}\) of oxfendazole was not significantly different (P > 0.05) between both formulations.
4 CONCLUSIONS

Coacervation proved to be promising as formulation strategy for poorly water-soluble drugs. The *in-vitro* results showed a significant increase in febantel release from the coacervate system compared to conventional granules. This was confirmed by the *in-vivo* study, as a significant increase in febantel concentration and fenbendazole was observed from the coacervate system compared to the granules. Further studies are required to transform the coacervate system into a solid dosage form and to assess the applicability of this concept for other poorly water-soluble drugs.
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5 LITERATURE