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Stability of fenbendazole suspensions for veterinary use Correlation between zeta potential and sedimentation

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ABSTRACT

In this paper we have carried out a detailed investigation of the stability and redispersibility characteristics of fenbendazole aqueous suspensions, through a thermodynamic and electrokinetic characterization, considering the effect of both pH and ionic strength. The hydrophobic character of the drug, and the surface charge and electrical double-layer thickness play an essential role in the stability of the system, hence the need for a full characterization of fenbendazole. It was found that the drug suspensions displays "delayed" or "hindered" sedimentation, determined by their hydrophobic character and their low zeta potential (indicating a small electrokinetic charge on the particles). The electrostatic repulsion between the particles is responsible for the low sedimentation volume and poor redispersibility of the drug. However, only low concentrations of $AlCl_3$ induced a significant effect on both the zeta potential and stability of the drug, leading to a "free-layered" sedimentation and a very easy redispersion which could be of great interest in the design of an oral pharmaceutical dosage form for veterinary.

1. Introduction

The treatment of helmintic diseases consists in a public health subject of great importance. Fenbendazole (FBZ) [5-(phenylthio)-1H-benzimidazol-2-yl]carbamic acid methyl ester; methyl-5-(Phenylthio) benzimidazole-2-carbamate; see Fig. 1] is a broad spectrum benzimidazole antihelmintic indicated worldwide in the treatment and prevention of several endoparasitic diseases in veterinary (Iosifidou et al., 1997; Praslička et al., 1997; Garossino et al., 2005; Ghazaei, 2007; Willesen et al., 2007). This drug is thought to bind to tubulin and thereby preventing its polymerization to form microtubules, but it also inhibits fumarat reductase and glucose transport. As a result, parasites may die of starvation. FBZ also has an antifungal effect (McKellar, 1997).

Despite it is a widely used compound, little is known about its surface properties, and nothing has been published concerning its colloidal behaviour, which may be important to understand its adhesion and aggregation characteristics. Moreover, it must be considered that the thermodynamic properties and, the electrokinetic potential and the electrical double-layer thickness will play an essential role in the stability of the system. Hence, the need for the investigation of the surface thermodynamics and the electrical state of the particles if a full characterization of the system is sought. In this paper, our aim is to perform a simultaneous analysis of the hydrophobicity/hydrophilicity and the electrokinetic properties, and the stability of FBZ. The effects of both pH (an essential variable, given the extreme pH values of the gastrointestinal tract) and ionic content of the suspension will be

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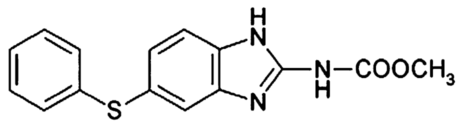

Fig. 1 – Chemical structure of fenbendazole.

investigated, in order to clarify the best formulation aspects of an oral dosage form that will facilitate the administration of exact dosages to animals.

2. Materials and methods

2.1. *Materials*

The drug powder was obtained from Roig Farma-Fagron (Spain) and used as received. All other chemicals were of analytical quality from Panreac (Spain) except for KOH and diiodomethane (Merck, Alemania) and formamide (Carlo Erba, Italy). Water used in the experiments was deionized and filtered (Milli-Q Academic, Millipore, France).

2.2. *Methods*

2.2.1. *Characterization methods*

Size and shape of FBZ particles were deduced from SEM pictures using a Zeiss DSM 950 (Germany) scanning electron microscope set at 80 kV accelerating voltage. Prior to observation, a dilute (≈0.1% (w/v)) drug suspension was sonicated for 5 min, and drops of the suspension were placed on copper grids with formvar film. The grids were then dried at 40.0 ± 0.5 °C in a convection oven. In order to confirm these results, the mean particle diameters were determined at 25.0 ± 0.5 °C by quasi-electric light scattering (QELS) using a Nanosizer (Coulter® N4MD, Coulter Electronics, Inc., Hialeah, FL, USA). The selected angle was 90° and the measurement was made after dilution of the aqueous suspensions.

A surface thermodynamic analysis of this antihelmintic drug was done by using the model developed by van Oss (2006) (see also Arias et al., 2007), according to which the total surface free energy of any material *i* is the sum of two contributions:

$$\gamma_i^{TOT} = \gamma_i^{LW} + \gamma_i^{AB} = \gamma_i^{LW} + 2\sqrt{\gamma_i^+\gamma_i^-} \quad (1)$$

one of which, γ_i^{LW}, is the non-polar Lifshitz–van der Waals component, and the second one, γ_i^{AB} or acid–base component, is related to the electron-donor (γ_i^-) and electron-acceptor (γ_i^+) characteristics of the material. Similarly, the interfacial solid/liquid free energy, γ_{SL}^{TOT}, and its LW and AB components (γ_{SL}^{LW} and γ_{SL}^{AB}, respectively) are related to the surface free energies of both the solid (subscripts S) and the liquid (subscripts L):

$$\gamma_{SL}^{TOT} = \gamma_{SL}^{LW} + \gamma_{SL}^{AB} = \gamma_{SL}^{LW} + 2\sqrt{\gamma_S^+\gamma_S^-} + 2\sqrt{\gamma_S^-\gamma_S^+} - 2\sqrt{\gamma_S^+\gamma_L^-} - 2\sqrt{\gamma_S^-\gamma_L^+} \quad (2)$$

These quantities can be related to the contact angle θ between the liquid and the solid, using the Young's equation (Adamson and Gast, 1997):

$$(1+\cos\theta)\gamma_L^{TOT} = 2\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + 2\sqrt{\gamma_S^+\gamma_L^-} + 2\sqrt{\gamma_S^-\gamma_L^+} \quad (3)$$

The three unknowns (γ_S^{LW}, γ_S^+ and γ_S^-) can be obtained by solving the resulting system of three equations if the contact angles of three liquids of known γ_L^{LW}, γ_L^+ and γ_L^-, are measured. In our case, we used water ($\gamma_L^{LW} = 21.8$, $\gamma_L^+ = \gamma_L^- = 25.5$ mJ/m^2), formamide ($\gamma_L^{LW} = 3.90$, $\gamma_L^+ = 2.28$, $\gamma_L^- = 39.6$ mJ/m^2) and diiodomethane ($\gamma_L^{LW} = 5.08$, $\gamma_L^+ = \gamma_L^+ = 0$ mJ/m^2), all data taken from van Oss (2006). The contact angles of the three liquids were determined at 25.0 ± 0.5 °C, using a Ramé-Hart 100–00 goniometer (USA), on pellets (radius: 1.3 cm) obtained by compressing the dry powders in a Spepac hydraulic press (UK) set to 8 ton during 5 min.

Finally, the surface electrical properties of FBZ suspensions (≈0.1% (w/v)), were analyzed by electrophoresis measurements as a function of both pH (adjusted with either HCl or NaOH) and electrolyte concentration (NaCl, $CaCl_2 \cdot 4H_2O$ or $AlCl_3 \cdot 6H_2O$), using a Zetasizer 2000 (Malvern Instruments, UK) electrophoresis device. Measurements were performed at 25.0 ± 0.5 °C, after 24 h of contact at this temperature under mechanical stirring (50 rpm). The experimental uncertainty of the measurements was below 5%. The theory of O'Brien and White (1978) was used to convert the electrophoretic mobility (u_e) into zeta potential (ζ) values.

2.2.2. *Stability of febendazole aqueous suspensions*

Apart from the electrokinetic evaluation, another method was used to determine the stability of FBZ suspensions, consisting in the measurement of the sediment volume, V_s, after keeping the suspensions in 100 mL cylinders (inner diameter: 2.4 cm) placed in a thermostatted bath at 25.0 ± 0.5 °C. The concentration of solids in the cylinders was 5% (w/v) in all cases. The flocculation ratio, *F*, was the quantity chosen for the characterization of the stability. This is defined as 100 (V_s/V_0), where V_0 is the initial volume of the suspension (Matthews and Rhodes, 1970). The redispersibility was ascertained by visual inspection of the suspensions after placing them for 2 min in an Branson 5200E4 ultrasonic bath (USA) operating at 40 kHz and with a sonic power of 100 W (Gallardo et al., 2005).

3. Results and discussion

3.1. *Particle size and morphology*

FBZ particles were of irregular shape, in the colloidal size range and moderately polydisperse. The average diameter (±S.D.) and polydispersity index were 235 ± 60 nm and 0.277, respectively. Fig. 2 includes an example of scanning electron microscope (SEM) pictures of the samples prepared and the size histogram (based on ≈200 particles counting).

3.2. *Surface thermodynamics*

The contact angles of water, diiodomethane and formamide measured on the FBZ pellets were 66 ± 1°, 29 ± 1° and