Albendazole solid dispersions against alveolar echinococcosis: a pharmacotechnical strategy to improve the efficacy of the drug

Julia Fabbri1,2, Patricia Eugenia Pensel1,2, Clara María Albani1,2, Lurdes Milagros Lopez1, Analia Simonazzi3,2, José María Bermudez3,2, Santiago Daniel Palma4,2 and María Celina Elissondo1,2

1Laboratorio de Zoonosis Parasitarias, Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), Facultad de Ciencias Exactas y Naturales (FCEyN), Universidad Nacional de Mar del Plata (UNMdP), Mar del Plata, Buenos Aires, Argentina; 2Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; 3Instituto de Investigaciones para la Industria Química, Universidad Nacional de Salta (UNSa), Salta, Argentina and 4Laboratorio de Farmacotecnia, Facultad de Ciencias Químicas (FCdQ), Universidad Nacional de Córdoba (UNC), Córdoba, Argentina

Abstract
Alveolar echinococcosis is a neglected parasitic zoonosis caused by Echinococcus multilocularis. The pharmacological treatment is based on albendazole (ABZ). However, the low water solubility of the drug produces a limited dissolution rate, with the consequent failure in the treatment of the disease. Solid dispersions are a successful pharmacotechnical strategy to improve the dissolution profile of poorly water-soluble drugs. The aim of this work was to determine the in vivo efficacy in ABZ solid dispersions using poloxamer 407 as a carrier (ABZ:P407 solid dispersions (SDs)) in the murine intraperitoneal infection model for secondary alveolar echinococcosis. In the chemoprophylactic efficacy study, the ABZ suspension, the ABZ:P407 SDs and the physical mixture of ABZ and poloxamer 407 showed a tendency to decrease the development of murine cysts, causing damage to the germinal layer. In the clinical efficacy study, the ABZ:P407 SDs produced a significant decrease in the weight of murine cysts. In addition, the SDs produced extensive damage to the germinal layer. The increase in the efficacy of ABZ could be due to the improvement of water solubility and wettability of the drug due to the surfactant nature of poloxamer 407. In conclusion, this study is the basis for further research. This pharmacotechnical strategy might in the future offer novel treatment alternatives for human alveolar echinococcosis.

Introduction
Alveolar echinococcosis (AE) is a severe neglected parasitic zoonosis caused by Echinococcus multilocularis, which represents an important public health threat. This parasite is predominantly maintained in a wildlife cycle, with carnivores as definitive hosts and small mammals as intermediate hosts. Humans acquire the infection by ingesting eggs shed in the feces of a definitive host and develop the metacestode stage, which is characterized by a tumour-like and infiltrative growth. If not appropriately treated, parasite expansion will eventually lead to organ failure and death of the patient (Kern et al., 2017).

The metacestode stage is composed of numerous small vesicles with a wall structure formed by an outer acellular laminated layer and an internal cellular layer called germinal layer (Eckert and Deplazes, 2004). A special cell type in the germinal layer, the germinative cells, are responsible for the high regenerative potential of the parasite (Kern et al., 2017).

There are several approaches to the management of AE. In patients with viable cysts, the treatment of choice is the total removal of the cystic lesion combined with oral treatment with 15 mg kg−1 day−1 of albendazole (ABZ) for 2 years. In inoperable patients, prolonged treatment with ABZ should be carried out to decrease the proliferation of E. multilocularis. In cases of calcified or negative lesions by fluorodeoxyglucose (FDG) positron emission tomography (PET), the patient should be periodically monitored (watch and wait) (Wen et al., 2019).

For an effective treatment in systemic infections, the drug must be sufficiently soluble in water to easily reach the cell membrane, but also hydrophobic enough to cross it (Thompson, 1997). The biopharmaceutical classification system categorizes ABZ as a class 2 drug due to its low aqueous solubility and high permeability (Kasim et al., 2004). These characteristics produce a limited dissolution rate resulting in poor and erratic bioavailability of ABZ (Marriner et al., 1986; Edwards and Breckenridge, 1988; Castro et al., 2009). Due to the low concentration of drug reaching the parasite, ABZ acts as a parasitostatic rather than as a parasitocidal agent for many cases, and the recurrence rates after interruption of therapy are high (Reuter et al., 2004). Consequently, the treatment must be carried out with high daily doses of ABZ for prolonged periods, with the risk of low adherence to the treatment and the possibility of adverse effects (Bardonnet et al., 2013; Kern et al., 2017). Moreover, another explanation for the parasitostatic effect of ABZ on germinative cells is that they may
specifically express a β-tubulin isoform with limited affinity to benzimidazoles (Brehm and Koziol, 2014).

The development of new ABZ formulations that improve its solubility is essential to increase the effectiveness of pharmacological treatment. Until now, several pharmacotechnical strategies to increase bioavailability and, consequently, the effectiveness of ABZ have been evaluated in murine models of cystic and alveolar echinococcosis: incorporation of ABZ into liposomes (Dvorožňáková et al., 2004; Lv et al., 2013), ABZ loaded in lipid nanocapsules (Pensel et al., 2015; Ullio Gamboa et al., 2019), nanocrystal and nanocrystalline formulations of ABZ (Pensel et al., 2018; Hu et al., 2020), solid dispersions of ABZ with poloxamer 188 (Pensel et al., 2014) and ABZ-chitosan microspheres (Abulaihaiti et al., 2015).

The solid dispersions (SDs) are a successful strategy to improve the dissolution profile of poorly water-soluble drugs. This strategy is currently widely used in therapeutics, which is reflected in numerous commercialized products. For example, Sporanox®, Onmel® and Gris-PEG® are used as antifungals, whereas Kaletra®, Intuclese® and Norvir® are indicated in combination with other antiretroviral agents for the treatment of HIV. On the other hand, Isoptin SR®, Nivadil®, Adefibat CR® and Adalat-XL® are indicated for the treatment of heart conditions, Cesaemé® is used as antiemetic and Kalydeco® is indicated for cystic fibrosis (Cid et al., 2019).

The SDs are molecular mixtures of drugs and inert carriers, prepared by the fusion method and/or solvent method (Chiou and Riegelman, 1971). According to the physical state of the carrier, SDs are classified into four generations (Vasconcelos et al., 2007). In the third generation of SDs, surfactants or emulsifiers are used as carriers, which improve the dissolution profile and the physical and chemical stability of the drug (Desai et al., 2006). These SDs were more stable mainly due to a reduction of drug recrystallization (Vasconcelos et al., 2007).

Poloxamers, nonionic surfactants with solubilizing properties, are suitable for most of the standard procedures used to prepare SDs because of their polymeric nature. In addition, they are not metabolized in the body (Collett and Popli, 2000). Poloxamer 407 (P407) is accepted by the FDA as an inactive ingredient for different types of preparations (e.g., intravenous, inhalation, oral solution, suspension, ophthalmic or topical formulations) (Rowe et al., 2005). Simonazzi et al. (2018) designed ABZ SDs using P407 as carrier (ABZ:P407 SDs). These SDs markedly improved ABZ solubility and dissolution rate compared with pure ABZ and a commercial formulation. These drug-related factors affect the gastrointestinal absorption thus improving the bioavailability. In this context, the aim of the current work was to determine the in vivo efficacy of ABZ:P407 SDs in the murine model of AE.

Materials and methods
Preparation of solid dispersions and physical mixtures
The ABZ:P407 SDs were prepared by the fusion method as reported by Simonazzi et al. (2018) ensuring quality in terms of physicochemical properties and dose adjustment. Briefly, ABZ (Pharmaceutical grade, Parafarm, Argentina) was homogeneously dispersed in the molten P407 (BASF®, Germany) at 63°C (1:1), by stirring. The preparation was rapidly cooled in liquid nitrogen, pulverized and sieved. The 210μm particle size fraction was kept in a glass vial at room temperature until use.

Physical mixtures were prepared from ABZ and P407 previously sieved (210μm particle size fraction). The components were mixed in equal proportions in a laboratory-scale V-blender for 5 min. The powders were stored in a glass vial at room temperature until use.

Preparation of ABZ formulations
The suspension of ABZ (3.08 mg mL⁻¹) was prepared by dispersion of pure ABZ in distilled and deionized water (pH = 7.0) with carboxymethylcellulose (CMC, Todo Drogas, Córdoba, Argentina) (0.5% w/v, pH = 6.0). The suspension was shaken for 5 h and sonicated for 1 h. The ABZ:P407 SDs (6.16 mg mL⁻¹), physical mixture (6.16 mg mL⁻¹) and P407 (3.08 mg mL⁻¹) suspensions were prepared by dissolution in distilled and deionized water (pH = 7.0) under shaking (5 h). All formulations were stored at 4°C and were vigorously shaken before administration to mice.

Parasite material
The studies were carried out using E. multilocularis isolate J2012 (kindly provided by Klaus Brehm, Institute for Hygiene and Microbiology, University of Würzburg, Germany). To establish the murine intraperitoneal infection model for secondary AE, the parasite was propagated in the peritoneum of CF-1 mice and was processed as described by Albani et al. (2015), with some modifications. Briefly, the metacestodes obtained from the peritoneal cavity of the animals were cut to obtain a parasitic suspension. The suspension was passed through a metallic strainer and washed several times with phosphate-buffered saline (PBS). Finally, 0.5 vol of PBS and 12μg mL⁻¹ of ciprofloxacin (Roemmers, Argentina) were added to parasite tissue and incubated overnight at 4°C (Spiliotis and Brehm, 2009).

Experimental design and evaluation of in vivo efficacy of ABZ: P407 SDs against the murine model of AE
For chemoprophylactic and clinical efficacy studies, 100 female CF-1 mice were intraperitoneally infected with 0.3 mL of homogenized parasitic material of E. multilocularis in PBS (n = 50 for each study). In the chemoprophylactic efficacy study, the dosage of the animals began 1-day post-infection, while in the clinical efficacy study the treatment began 6 weeks post-infection. In each study, the experimental groups were: (1) water control group, mice received distilled and deionized water as a placebo; (2) P407 control group, mice received P407 suspended in distilled and deionized water; (3) ABZ-CMC group, mice were treated with a suspension of ABZ in distilled and deionized water with CMC; (4) physical mixture group, the animals received a suspension of physical mixture (ABZ and P407, 1:1); (5) ABZ:P407 SDs group, animals were treated with a suspension of ABZ:P407 SDs. The animals were randomly distributed into the treatment groups (10 animals/group) with 5 mice per cage.

In both studies, treatments were performed daily for 30 days by intragastric administration in a volume of 0.3 mL. For groups 3, 4 and 5 the dose of ABZ was 25 mg kg⁻¹ day⁻¹.

Approximately 10 weeks post-infection, the mice were anesthetized with 100 mg kg⁻¹ of ketamine and 10 mg kg⁻¹ of xylazine and subsequently euthanized by cervical dislocation and necropsy. The cystic masses were obtained from the peritoneal cavity of each mouse and weighed. The median cysts weight from each group and ultrastructural study of the germinal layer of cysts by scanning electron microscopy were used to determine the efficacy of each treatment (Albani et al., 2015).

Scanning electron microscopy
Samples of cysts obtained from animals involved in both in vivo efficacy studies were processed for scanning electron microscopy as described by Elissondo et al. (2007). Briefly, samples were fixed in 3% glutaraldehyde (Sigma-Aldrich, St. Louis, USA) in 0.1 M sodium cacodylate buffer pH 7.4 (Sigma-Aldrich, St. Louis,
USA) for 72 h at 4°C. Then, several washes in 0.1 M sodium cacodylate buffer were made. After that, the specimens were dehydrated by sequential incubations of 10 min in increasing concentrations of ethanol (Cicarelli, Argentina): 50, 70, 80, 90, 95% and twice in 100%. Finally, samples were immersed in hexamethyldisilazane (Sigma-Aldrich, St. Louis, USA) for 5 min, 1 h, and overnight. They were then sputter-coated with gold (100-Å thickness) and inspected on a JEOL JSM-6460 LV scanning electron microscope operating at 15 kV.

**Statistical analysis**

Cysts weights of the different groups, reported as median and interquartile range (IQR), were compared by Kruskal–Wallis Test (nonparametric method) followed by Dunn’s Multiple Comparisons Test. The analysis was carried out using Instat 3.0 software program (GraphPad Software, San Diego, CA, USA).

In all cases, P values less than 0.05 (P < 0.05) were considered statistically significant.

**Results**

**Chemoprophylactic efficacy study of ABZ:P407 SDs against the murine model of AE**

All the infected mice belonging to the chemoprophylactic efficacy study developed cystic masses in the abdominal cavity. No significant differences were found (P > 0.05) between the median weight of the cysts of the water and P407 control groups. Although the median weight of cysts recovered from mice treated with all formulations of ABZ was lower in relation to the control groups, no significant differences were detected (P > 0.05, Table 1).

The ultrastructural study of the germinal layer of metacestodes recovered from control and treated groups is shown in Fig. 1. The germinal layer of cysts obtained from control mice showed the characteristic multicellular structure (Fig. 1A). The decrease in the weight of the cysts belonging to treated groups was correlated with ultrastructural alterations observed by scanning electron microscopy. Areas without cells in the germinal layer were observed in treated cysts (Figs 1B–D).

**Clinical efficacy study of ABZ:P407 SDs against the murine model of AE**

Table 2 summarizes the cyst weights (median and IQR) recorded after treatments of the different experimental groups involved in the therapeutic efficacy study. There were no statistically significant differences (P > 0.05) between the median cyst weights of control groups (i.e. water and P407 control groups). Although the median weight of cysts recovered from ABZ-CMC and physical mixture groups were lower than those observed in the control groups, no differences were found between treated groups and unmedicated controls.

| Table 1. Chemoprophylactic efficacy study. Median weight (g) and interquartile range (IQR) of the E. multilocularis cysts recovered from artificially infected mice from the unmedicated control and treated groups |
|----------------|----------------|
| **Median weight of cysts (g)** | **Interquartile range (IQR)** |
| Water control | 3.62 | 2.53 |
| P407 control | 2.91 | 4.81 |
| ABZ-CMC | 1.72 | 0.91 |
| Physical mixture | 1.05 | 1.53 |
| ABZ:P407 SDs | 0.95 | 1.78 |

Twenty-four hours post-infection, daily treatments were performed by intragastric administration of different formulations of ABZ at the dose of 25 mg kg⁻¹ of ABZ over a period of 30 days.

---

![Fig. 1](image_url). Scanning electron microscopy of E. multilocularis cysts recovered from infected mice belonging to the chemoprophylactic efficacy study. (A) Control cyst with an intact germinal layer (gl). (B) Cyst recovered from mice treated with ABZ-CMC. Note the loss of cells in the germinal layer. (C) Cyst obtained from treatment with the physical mixture. Observe the areas without cells. (D) Germinal layer of metacestode recovered from the ABZ:P407 SDs treated group. Areas with extensive loss of cells can be observed. Scale bar = 50 μm.
In contrast, ABZ:P407 SDs treatment caused a significant decrease in the weight of the cysts compared with control groups ($P < 0.05$). Metacestodes recovered from treated mice showed damage in the germinal layer, in relation to the control groups. However, the damage extension appears to be greater after ABZ:P407 SDs compared to the ABZ-CMC treatment (Fig. 2).

**Discussion**

The drug of choice for the pharmacological treatment of human echinococcosis is ABZ. As this drug was developed primarily to target parasites in the gastrointestinal tract, a low bioavailability outside the intestine was considered important for its optimal performance. However, this feature is considered undesirable for a systemic parasitic disease as echinococcosis (Shuhua et al., 2002). The expression of a $\beta$-tubulin isoform with limited affinity to benzimidazoles by germinative cells and the low concentrations of ABZ reaching the parasite produces a parasitostatic effect and relapses after chemotherapy have been reported (Reuter et al., 2004; Brehm and Koziol, 2014).

The gastrointestinal permeability and solubility of some drugs are limiting conditions for oral absorption, directly affecting their bioavailability. Although permeability is an intrinsic property of a drug, different strategies have been developed for improving the dissolution rate to design suitable formulations for oral administration (Vo et al., 2013). Scientific evidence indicates that a higher drug bioavailability correlates with improved efficacy of benzimidazoles against murine echinococcosis (Mingjie et al., 2002; Shuhua et al., 2002; Dvorozňáková et al., 2004; Ceballos et al., 2006, 2008, 2009; Liu et al., 2012; Abulaihaiti et al., 2015; Hu et al., 2020).

The in vitro dissolution of a drug can be correlated with its bioavailability in vivo (Amidon et al., 1995). Simonazzi et al. (2018) demonstrated that the use of P407 as the carrier in ABZ SDs markedly improved its solubility and dissolution rate compared with pharmaceutical-grade ABZ and a commercial formulation. In addition, it was observed that the polymer maintained a desirable level of a supersaturation state in the dissolution medium. This was reached by preventing solvent-mediated crystallization over the time period necessary for the absorption process. The results observed in vitro with the ABZ:P407 SDs could be correlated with the efficacy obtained in the present study in the murine model of AE.

During the chemoprophylactic efficacy study, all formulations of ABZ showed a tendency to decrease the development of *E. multilocularis* cysts. The ultrastructural study of metacestodes supports these results, showing the loss of cells of the germinal layer. However, no significant differences were detected between the median weight of cysts recovered from the treated mice. In contrast, Morris and Taylor (1988) reported that a significant protection against protoscoleces of *E. granulosus* was achieved in gerbils by 1-month treatment of ABZ (10 mg kg$^{-1}$ day$^{-1}$).

**Table 2.** Clinical efficacy study. Median weight (g) and interquartile range (IQR) of the *E. multilocularis* cysts recovered from artificially infected mice from the unmedicated control and treated groups.

|                      | Median weight of cysts (g) | Interquartile range (IQR) |
|----------------------|----------------------------|--------------------------|
| Water control        | 4.27                       | 2.41                     |
| P407 control         | 3.44                       | 2.25                     |
| ABZ-CMC              | 0.69                       | 0.72                     |
| Physical mixture     | 0.57                       | 0.27                     |
| ABZ:P407 SDs         | 0.28*                      | 0.66                     |

Six weeks post-infection, daily treatments were performed by intragastric administration of different formulations of ABZ at the dose of 25 mg kg$^{-1}$ of ABZ over a period of 30 days.

*Statistically significant differences with the control groups ($P < 0.05$).
In the clinical efficacy study, the ABZ·P407 SDs achieved a statistically significant decrease in the weight of cysts, with an efficacy of 86%. In addition, the extent of damage caused by ABZ·P407 SDs was greater compared to the other treated groups. The ultrastructural alterations in the germinal layer were similar to those observed in mice infected with *E. granulosus* treated with other benzimidazoles (Ceballos et al., 2009, 2010). Our results are consistent with those reported by Pensel et al. (2014), who demonstrated a greater *in vivo* efficacy of ABZ formulated as SDs using P188 in the murine model of cystic echinococcosis.

The SDs increase the dissolution rate of low water-soluble drugs (Vo et al., 2013). The enhanced efficacy obtained after oral administration of ABZ·P407 SDs could be explained by an increase in ABZ dissolution rate caused by the surfactant nature of the drugs (Vo et al., 2013). The oral administration of ABZ·P407 SDs could be explained by an increase in the dissolution rate caused by the surfactant nature of the drugs (Vo et al., 2013). The oral administration of ABZ·P407 SDs could be explained by an increase in the dissolution rate caused by the surfactant nature of the drugs (Vo et al., 2013).

**References**

Abulaihaiti M, Wu XW, Qiao L, Lv HL, Zhang HW, Aduwaiy N, Wang YJ, Wang XC and Peng XY (2015) Efficacy of albendazole-chitosan microsphere-based treatment for alveolar echinococcosis in mice. *PLoS Neglected Tropical Disease* 9, e003950.

Albani CM, Pensel PE, Elissondo N, Gambino G and Elissondo MC (2015) In vivo activity of albendazole in combination with thymol against *Echinococcus multilocularis*. *Veterinary Parasitol* 212, 193–199.

Amidon GL, Lennernäs H, Shah VP and Crison JR (1995) A theoretical basis for a biopharmaceutical drug classification: the correlation of *in vitro* drug product dissolution and in vivo bioavailability. *Pharmaceutical Research* 12, 413–420.

Bardonnent K, Vuitton DA, Grenouillet F, Mantion GA, Delabrousse E, Blagosklonov O, Miguet JP and Bresson-Hadni S (2013) 30-yr Course and favorable outcome of alveolar echinococcosis despite multiple metastatic organ involvement in a non-immune suppressed patient. *Annals of Clinical Microbiology and Antimicrobials* 12, 1.

Brehm W and Koziol U (2014) On the importance of targeting parasite stem cells in anti-echinococcosis drug development. *Parasite* 21, 72.

Brunetti P, Kern P and Vuitton DA (2010) Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Tropic* 114, 1–16.

Castro N, Márquez-Caraveo C, Brundage RC, González-Esquível D, Suárez AM, Góngora F, Jara A, Urizar J, Lanao JM and Jung H (2009) Population pharmacokinetics of albendazole in patients with neurocysticercosis. *International Journal of Clinical Pharmacology and Therapeutics* 47, 679–685.

Ceballos L, Alvarez L, Sánchez Bruni S, Elissondo MC, Dopchiz M, Denegri G, Torrado J and Lanusse CE (2006) Development of a cyclodextrin-based flubendazole formulation to control secondary echinococcosis: pharmaco-kinetics, hydatid cyst morphology and efficacy in mice. *Journal of Veterinary Pharmacology and Therapeutics* 29, 85–86.

Ceballos L, Elissondo MC, Moreno L, Dopchiz M, Sánchez Bruni S, Denegri G, Alvarez L and Lanusse CE (2008) Albendazole treatment in cyst echinococcosis: pharmacokinetics and clinical efficacy of two different aqueous formulations. *Parasitology Research* 103, 355–362.

Ceballos L, Elissondo MC, Sánchez Bruni S, Denegri G, Alvarez L and Lanusse CE (2009) Flubendazole in cyst echinococcosis therapy: pharmaco-parasitological evaluation in mice. *Parasitology International* 58, 354–358.

Ceballos L, Elissondo MC, Sánchez Bruni S, Confalonieri A, Denegri G, Alvarez L and Lanusse CE (2010) Chemoprophylactic activity of flubendazole in *E. multilocularis*. *Veterinary Parasitology* 163, 363–382.

Chen Y, Zhang GGZ, Neilly J, Marsh K, Mawhinney D and Sangirzi YD (2004) Enhancing the bioavailability of ABT-963 using solid dispersion containing fluronic F-68. *International Journal of Pharmaceutics* 286, 69–80.

Chioi WL and Riegelman S (1971) *Clinical Microbiology Reviews*. 73, 1003–1010.

Cid AG, Simonazzi A, Palma SD and Bermúdez JM (2019) Solid dispersion technology as a strategy to improve the bioavailability of poorly soluble drugs. *Therapeutic Delivery* 10, 363–382.

Collett JH and Popli H (2000) Poloxamer. In Kibbe AH (ed.), *Handbook of Pharmaceutical Excipients*. London: Pharmaceutical Press, pp. 385–388.

Desai J, Alexander K and Riga A (2006) Characterization of polymeric dispersions of dimethyldihydrazine in ethyl cellulose for controlled release. *International Journal of Pharmaceutics* 308, 115–123.

Dvoržáčková E, Hříčková G, Borosková Z, Velebný S and Dubínský P (2004) Effect of treatment with free and liposomized albendazole on selected immunological parameters and cyst growth in mice infected with *Echinococcus multilocularis*. *Parasitology International* 53, 315–325.

Eckert J and Deplazes P (2000) Biological, epidemiological, and clinical aspects of *Echinococcus multilocularis*. *Clinical Microbiology Reviews* 13, 107–135.

Edwards G and Breckenridge A (1988) Clinical pharmacokinetics of anthelmintic drugs. *Clinical Pharmacokinetics* 15, 67–93.

Elissondo MC, Ceballos L, Dopchiz M, Andresiu V, Alvarez L, Sánchez Bruni S, Lanusse C and Denegri G (2007) *In vitro* and *in vivo* effects of flubendazole on *Echinococcus granulosus* metacestodes. *Parasitology Research* 100, 1003–1009.

Horton RJ (1997) Albendazole in treatment of human cystic echinococcosis: 12 years of experience. *Acta Tropic* 64, 79–93.

Hu C, Liu Z, Liu C, Zhang Y, Fan H and Qian F (2020) Improvement of antialveolar echinococcosis efficacy of albendazole by a novel nanocrystalline formulation with enhanced oral bioavailability. *ACS Infectious Diseases* 6(5), 802–810. doi: https://doi.org/10.1021/acsinfecdis.9b00231.
Kabanov AV, Battrakova EV and Alakhom VO (2002) Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery. *Journal of Controlled Release* 82, 189–212.

Kasim NA, Whitehouse M, Ramachandran C, Bermejo M, Lennermäki H, Hussain AS, Junginger HE, Stavchansky SA, Midha KK, Shah VP and Amidon GL (2004) Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Molecular Pharmaceutics* 1, 85–96.

Kern P, Menezes da Silva A, Akhan O, Müllhaupt B, Vizcaícho CA, Budke C and Vuitton DA (2017) The echinococcoses: diagnosis, clinical management and burden of disease. In Thompson RCA, Deplazes P and Marriner E, 8th Edn. Washington, DC, USA: National Academies Press.

Pensel PE, Pensel PE, Paredes A, Albani CM, Allemandi D, Sanchez Bruni S, Palma SD and Elissondo MC (2018) Albenzadole nanocrystals in experimental alveolar echinococcosis: enhanced chemoprophylactic and clinical efficacy in infected mice. *Veterinary Parasitology* 251, 78–84.

Reuter S, Buck A, Manfras B, Kratzer W, Seitz HM, Darge K, Reske SN and Kern P (2004) Structured treatment interruption in patients with alveolar echinococcosis. *Hepatology* 39, 509–517.

Rowe R, Sheskey P and Owen S (2005) *Handbook of Pharmaceutical Excipients*, 5th Edn. Washington, USA: Pharmaceutical, London UK and American Pharmaceutical Association.

Shuhua X, Jiqing Y, Mingjie W, Pieling J, Fanghua G, Junjie C, Wei J and Hotze P (2002) Augmented bioavailability and cysticidal activity of albendazole reformulated in soybean emulsion in mice infected with *Echinococcus granulosus* Or *Echinococcus multilocularis*. *Acta Tropica* 82, 77–84.

Simonazzi A, Cid AG, Paredes AJ, Schofs L, Gonzo EE, Palma SD and Bermúdez JM (2018) Development and *in vitro* evaluation of solid dispersions as strategy to improve albendazole biopharmaceutical behavior. *Therapeutic Delivery* 9, 623–638.

Spiliotis M and Brehm K (2009) Axenic *in vitro* cultivation of *Echinococcus multilocularis* metacestode vesicles and the generation of primary cell cultures. In Rupp S and Sohn K (eds), *Host-Pathogen Interactions*. *Critical Reviews in Therapeutic Drug Carrier Systems* 14, 104.

Ullio Gamboa G, Pensel PE, Elissondo MC, Sanchez Bruni S, Benoit JP, Palma SD and Allemandi DA (2019) Albenzadole-lipid nanocapsules: optimization, characterization and chemoprophylactic efficacy in mice infected with *Echinococcus granulosus*. *Experimental Parasitology* 198, 79–86.

Vasconcelos T, Sarmiento B and Costa P (2007) Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. *Drug Discovery Today* 12, 1068–1075.

Vo CLN, Park C and Lee BJ (2013) Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. *European Journal of Pharmaceutics and Biopharmaceutics* 85, 799–813.

Wen H, Vuitton L, Tuxun T, Li J, Vuitton DA, Zhang W and McManus DP (2019) Echinococcosis: advances in the 21st century. *Clinical Microbiology Reviews* 32, e00075–18.