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PHARMACEUTICAL CHARACTERIZATION AND PHARMACOKINETICS OF FLORFENICOL-LOADED ALGINATE DRIED BEADS IN RABBITS

Lilia Gutierrez*, Xelhua Marcos*, Perla García-Guzmán*, Minerva Monroy-Barreto†, Héctor Sumano*

*Departamento de Fisiología y Farmacología. Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autonoma de Mexico. Ciudad de México 04510, México.
†Departamento de Química Analítica. Facultad de Química. Universidad Nacional Autonoma de México. Ciudad de México 04510, México.

Abstract: The pharmacokinetic variables of a new formulation of florfenicol included in dried bean of alginate (FADBs), its acceptance as in food medication, and its relationship with theoretical minimum inhibitory concentration (MIC) values of the main pathogens in rabbits, are presented. FADBs sought to mask the unpleasant taste of florfenicol while enhancing sustained absorption in a day to facilitate and optimise its dosage in this species. The entrapment efficiency was determined to be 94-98% and 73.56±3.26% of drug loading. No reduction in food consumption was detected, nor selectivity when choosing from their usual food. The elimination half-life was 1.23 to 2.4 h slower than the one previously reported in the literature. Possible flip-flop pharmacokinetics is proposed for FADBs in rabbits, thus complying better with the key pharmacokinetics/pharmacodynamics (PK/PD) ratio of t≥MIC. Also, if a $MIC_{2.0\ \mu g/mL}$ is taken as the cut-off point for florfenicol in rabbits, then *ad libitum* intake of FADBs in their standard diet is sufficient to maintain plasma concentrations of florfenicol above this level during the whole dosing interval of 24 h. Additionally, FADBs are a low-cost and attractive drug delivery system for the oral controlled release of florfenicol in rabbits.

Key Words: florfenicol, alginate, dried beads, rabbits, pharmacokinetics.

INTRODUCTION

Florfenicol is a potent antibacterial drug derivative of chloramphenicol and is considered a time-dependent (t-d) antibiotic from the pharmacokinetics/pharmacodynamics perspective (Martínez *et al*., 2013; Toutain *et al*., 2019). Hence, optimal antibacterial efficacy *in vivo* occurs when the drug is administered in a pharmaceutical presentation capable of achieving initial serum concentrations of 2 to 4 times the minimum inhibitory concentration (MIC) of the pathogen to be treated and remain at or above the MIC level for at least 50-90% of the dosing interval. This active principle is found undissociated in a pH range of 3 to 9. It is almost insoluble in acidic or alkaline aqueous media and is soluble in polar organic solvents such as polyethylene glycols and N-methyl pyrrolidone, as it has a high solubility in lipid matrices (Wang *et al.,* 2011). It acts against Gram+ and Gram– microorganisms and exhibits superior antibacterial activity as compared to thiamphenicol and chloramphenicol. It is active *vs*. the main bacterial pathogens that affect rabbits such as *Pasteurella multocida, Staphylococcus aureus, Bordetella bronchiseptica, Moraxella catarrhalis, Salmonella* sp., *Yersinia enterocolitica* and *Streptococcus* spp. (Espinoza *et al*., 2020). In most species, florfenicol is efficiently absorbed from the GI tube and is widely distributed in tissues and organs such as the lung, heart, pancreas, skeletal muscle, spleen and synovial fluid. Concentrations are relatively high in bile, kidney, small intestine and urine. It can be considered a drug with zero-order kinetics, with a very moderate tissue accumulation

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rate (Adams et al., 1987). The pharmacokinetic studies of florfenicol that have been carried out in rabbits were done by administration of the drug by intravenous and intramuscular routes (Koc *et al.,* 2009), and after a single forced oral bolus dose (Park *et al.*, 2007). These studies were aimed at defining the basic pharmacokinetics (PK) of the drug, but their pragmatic value was not tested, as only on rare occasions individual oral dosing is chosen in rabbits, even in those considered pets. Administration of florfenicol *ad libitum* through drinking water is difficult given the unpleasant taste of florfenicol, and rabbits tend to diminish water intake or even reject it completely. Likewise, a certain reduction in food intake is observed if florfenicol is administered as in-feed medication. Rabbits are known to have extra taste buds compared to humans and it has been proposed that rabbits have well-developed taste buds to detect bad-tasting, potentially toxic food (McBridge *et al*., 2004; Brewer, 2006). These predicaments diminish the usefulness of florfenicol in rabbit medicine and were the impetus to develop a pharmaceutical preparation of florfenicol as dried beads of alginate (FADB) and to define its oral pharmacokinetics when administered as a bolus dose or *ad libitum* within their ration.

MATERIAL AND METHODS

Materials

Alginate sodium salt ($C_6H_9NaO_7$) and calcium chloride ($CaCl_2$) were obtained from Sigma-Aldrich and used as received. Florfenicol was obtained from Hangzhou Think Chemical Co., Ltd. (Hangzhou, China). The *in vitro* release study was performed with regenerate cellulose dialysis bags (MWCS 12-14 kDa) obtained from Spectra/Por, USA. All solutions were prepared with ultrapure water of quality 18.2 MΩ cm at 25.0±1°C, obtained from distilled water with a Barnstead Nanopure diamond system. All reagents and solvents were analytical grade and all solutions were prepared according to the USP30-NF25.

Preparation of alginate beads

Alginate sodium salt and calcium chloride were purchased from Sigma-Aldrich and used as received. Enrofloxacin was obtained from HiMedia Laboratories Pvt. Ltd., India. Ultrapure water of quality 18.2 MΩ cm at 25.0±1°C was obtained from distilled water with a Barnstead Nanopure diamond system. All solutions were prepared according to the USP30-NF25 and all other reagents and solvents were analytical grade. A sodium alginate solution (2.5% w/v) was prepared containing enrofloxacin (5 and 7% w/v). The solution was dispersed by constant stirring and dropped through a syringe needle into a 2.5% (w/v) $CaCl_2$ solution with constant magnetic stirring to produce alginate beads. The fully formed beads were collected by filtration and air-dried at room temperature for 48 h.

Characterization of Alginate beads

Entrapment efficiency (EE %) and drug loading (%DL)

Entrapment efficiency (%) of florfenicol was determined by direct and indirect methods. For the indirect method, freshly prepared alginate beads were separated from the aqueous solution by filtration through a hydrophilic cellulose esters 0.45 µm filter, and the supernatant was analysed by UV-VIS spectrophotometric method. The direct method was performed analysing the drug content in the dried alginate beads. The florfenicol beads were dispersed in a monobasic potassium phosphate buffer solution pH 7.8±0.1 with gentle magnetic stirring for 24 h to release the entire entrapped drug. The solution was filtered through a 0.22 µm filter and the florfenicol concentration was determined in the supernatant by UV-VIS spectrophotometric method. This experimental method was also used for the drug loading (%DL) determination. All the UV-VIS spectrophotometric determinations were made with an S2000 spectrometer using a DT1000 deuterium light source, a SAD500 serial port interface (Ocean Optics, Inc.), with 10 mm path length quartz cuvette (Prolab, Mexico City, MX). All measurements were performed in triplicate at room temperature and the data were presented in mean±standard deviation (SD). The entrapment efficiency (%) was calculated as follows:

Entrapment Efficiency (%)=(Amount Florfenicol)/(Total amount of Florfenicol)×100 (1)

The drug loading (%) (Equation 2) was expressed as the mass fraction of the entrapped drug relative to the mass of the dried alginate beads, and was calculated as follows:

Drug Loading (%)=(Mass of loaded florfenicol)/(Mass of alginate beads)×100 (2)

Fourier-transform infrared (ATR-FTIR) spectroscopy

Florfenicol-alginate beads and their compounds (florfenicol powder and alginate sodium salt) were characterized by ATR-FTIR spectroscopy. The infrared spectra were measured over a wavelength range of 400-4000 cm^{-1} with an FTIR-FIR Spectrum 400 spectrophotometer (Perkin Elmer de México S.A. de C.V., Mexico City MX).

Differential scanning calorimeter (DSC)

DSC scans were performed using a DSC1 Mettler Toledo system from 25 to 400°C with a scan rate of 10°C/min. The samples were crimped in a standard aluminium pan under a nitrogen atmosphere.

In vitro drug release studies

The *in vitro* release studies were conducted by the dialysis bag method. An amount of florfenicol-loaded alginate beads was loaded into a pre-swelled dialysis bag. The dialysis assembly was suspended in a hydrochloric acid buffer solution pH 3.0±0.1 and kept for 1.5 h to simulate gastric conditions. Then, it was transferred into a phosphate buffer solution pH 7.8±0.1 and kept for 7.5 h to simulate intestinal conditions. During all the studies, the release medium was maintained with constant magnetic stirring (500 rpm) at 37.0±1°C, and at determined time intervals (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 3.0, 3.5, 4.0, 4.5, 5.5, 6.5, 7.5, 9.0 h), a volume of the release medium (10.0 mL) was withdrawn and an equal amount of fresh medium was added after each sample drawing. All the experiments were performed in triplicate and the data were presented in mean±1 SD. A spectrophotometric UV-VIS analysis of florfenicol was carried out at a wavelength of maximum absorbance (λ_{max}=267.67 nm) (Elimam *et al.*, 2016). The analysis was performed in an S2000 spectrometer using a DT1000 deuterium light source, SAD500 serial port interface (Ocean Optics, Inc. FL, USA), and 10 mm path length quartz cuvette (Prolab, Mexico City, MX). The profile release from alginate dried beads was analysed to evaluate the release kinetics and mechanism, through fitting the experimental data to zero-order, first order, Higuchi and Korsmeyer-Peppas model according to the following equations:

Zero-order	$M_t=M_0+K_0t$	(3)
First-order	$\ln M_t=\ln M_0-Kt/2.303$	(4)
Higuchi	$M_t=K_Ht^{½}$	(5)
Korsmeyer-Peppas	$M_t/M_\infty=K_{KP}t^n$	(6)

Where M_t and M_t/M_∞ is the drug fraction released at time t, M_0 is the initial amount of the drug, K is the release rate constant according to each model and in the Korsmeyer-Peppas model n is the release exponent, which indicates information on whether the release mechanism is Fickian (n=0.5), non-Fickian (0.5>n<1.0), or case-II transport mechanism (n≥1.0) (Jitendra and Ashwini, 2014). The drug content to determine the entrapment efficiency (EE%), drug loading (%DL) and profile release was quantified by referring to a florfenicol calibration curve. A florfenicol calibration curve was prepared in triplicate, and it was linear over the concentration range of 0.03-0.3 mg/mL in water (R^2=0.9999) (n=3). Another calibration curve was prepared in hydrochloric acid buffer solution pH 3.0±0.1 (R^2=0.9998) (n=3), and phosphate buffer solution pH 7.8±0.1 (R^2=1) (n=3) at room conditions.

Pharmacokinetics (PK) set-up

All study procedures and animal care activities were carried out following the Institutional Committee for Research, Care and Use of Experimental Animals of the National Autonomous University of Mexico (UNAM), under Official Mexican Regulation NOM-062-ZOO-1999 with a project approval number of MMVZ-2018/2-1. A group of 24 healthy New Zealand rabbits (12 males and 12 females) was included in this trial. All rabbits were from 15 to 17 wk old,

weighed a mean of 3.2±0.4 kg, and were clinically healthy based on physical examination, complete blood count and serum chemistry panel results. Animals were then randomly divided into two groups, blocking their sex to end up with the same number of males and females. Groups were: bolus dose ($FADB_{bd}$) and ad libitum ($FADB_{al}$). Considering that the FADBs have an inclusion rate of 0.8 mg of florfenicol/dried bead, an approximate dose of 20 mg/kg of florfenicol was administered as follows: for $FADB_{bd}$ the dose was forced administered by two persons, one of them restraining the rabbit tightly wrapped with a towel, while the other delivered the dose of FADBs in shredded alfalfa as with a morsel, added by a plastic dozer. The aspect of this paste-like medicated morsel is shown in Figure 1. After dosing, food was allowed immediately after treatment and water was freely available at all times. When rabbits were medicated *ad libitum,* FADBs were also included in shredded alfalfa and then mixed with half their standard commercial pelleted food. When food was completely consumed, they were given the rest of their daily food assignment. This was repeated for three days. Rabbits destined to be part of the single forced-dose were caged individually and their floor was slotted to avoid re-ingestion of their caecotrophs and rabbits that were part of the *ad libitum* group were housed in groups of 5 and their droppings were removed only after 24 h (McBridge *et al.*, 2004; Brewer, 2006).

Blood samples were taken from the marginal ear vein, sampling each rabbit no more than 3 to 4 times using a paediatric needle-wing Gauge 23 (Becton Dickinson, Mexico City) attached to a 3 mL syringe containing 10 IU of heparin as an anticoagulant (Inhepar®, Pisa Pharmaceutics, Guadalajara, Mexico). For the bolus dose, samplings were performed before florfenicol administration and at 1, 2, 4, 5, 8, 12, and 24 h after FADBs administration, and for the group receiving florfenicol *ad libitum,* blood samplings were carried out at 4 h intervals up to 12 h and at 24 h for 3 consecutive days.

Non-compartmental PK analysis was performed with Phoenix WinNonlin (Certara, Ma, USA) software. Results presented include C_{max}, maximum plasma concentration; T_{max}, time to reach C_{max}; T½λ, elimination half-life; AUC_{0-24}, area under the plasma concentrations versus time curve in 24 hr; $AUC_{0-\infty}$, area under the plasma concentrations versus time curve from 0 to ∞; $AUMC_{0-\infty}$, area under the moment curve from 0 to ∞; MRT, mean residence time. Also, the most relevant pharmacokinetics/pharmacodynamics (PK/PD) ratio for florfenicol was established i.e., T≥MIC. This is the time at which plasma concentrations are at or above the MIC considered as a breakpoint for florfenicol and florfenicol-amine, which in this case was 2.0 µg/mL (Bretzlaff *et al.*, 1987; Ueda and Suenaga, 1995; Park *et al.*, 2007).

Analytical procedure

Concentrations of florfenicol and its active metabolite florfenicol-amine were determined in plasma samples by HPLC, using the method described by Kowalski et al. (2005), with thiamphenicol as an internal standard. Briefly, the extraction procedure was initiated by thawing the plasma samples at 20-25°C laboratory temperature. Then, to 0.5 mL plasma aliquots thiamphenicol was added as internal standard (0.5 µg in 0.2 mL), as well as 0.2 mL of 1.0 M

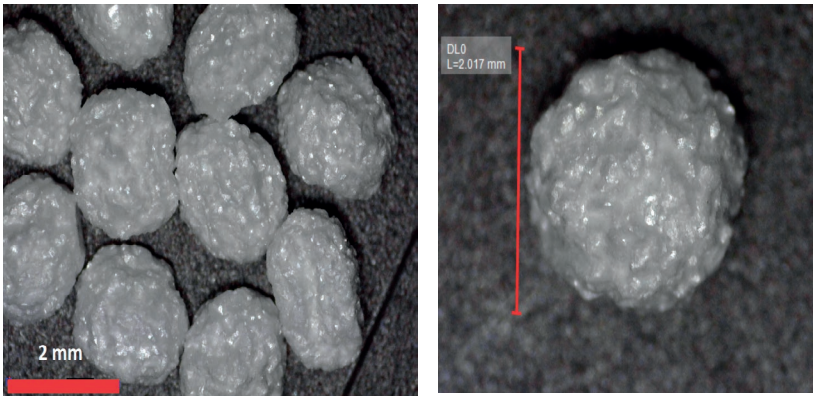

Figure 1: Optical microscope aspect of florfenicol-loaded alginate dried beads.

sodium hydroxide, and 3 mL of ethyl acetate. Each sample was vortex mixed and centrifuged at 5000 *g* for 15 min, and the organic layer was carefully transferred to another tube. The supernatant was dissolved in 0.5 mL of the mobile phase. Then, the samples were filtered through a membrane (nylon 0.45 µm) and injected into the high performance liquid chromatography (HPLC), with a 0.6 mL/min flow. Acetonitrile–water (25:75, v/v) adjusted to a pH of 2.7 with 85% orthophosphoric acid, was utilised as mobile phase. Detection and quantitation were performed at 224 nm for excitation wavelength and 290 nm for emission wavelength. Calibration curves for florfenicol and florfenicol-amine were prepared from 0.05 to 20.48 µg/mL (n=5).

The apparatus used was a Jasco XLC HPLC system (LC-2000Plus; Jasco Benelux, the Netherlands) with a Symmetry-C18 column (4.6 mm×100 mm, 3.5 µm; Waters, USA) and equipped with a fluorescence detector. Data were analysed using Empower-3 software from Waters (Mexico). The chromatographic method was validated and the analytical procedure was demonstrated as specific. The method produced a linear result from 0.05 to 20.48 µg/mL (r^2=0.984; y=500030x–107 046). Recovery of florfenicol and florfenicol-amine was calculated by applying linear regression analysis. Precision was demonstrated by the inter-day coefficient of variance (3.0) and inter-assay error value (<3.8). The lower quantification limit for florfenicol and florfenicol-amine in plasma was 0.05 µg/mL with a detection limit of 0.008 µg/mL, and linearity was established from 0.05 to 20.48 µg/mL for both drug analytes.

RESULTS

Entrapment efficiency (EE %) and drug loading (%DL)

The EE% determined by the direct method was 94.56±6.276% (n=3) and 98.32±0.21% (n=3) by indirect method. The %DL was 73.56±3.26% (n=3), indicating the % mass of the bead that is due to florfenicol.

Fourier-transform infrared (ATR-FTIR) spectroscopy

The ATR-FTIR spectra of different samples are displayed in Figure 2. The alginate spectrum, black line, presents broadband at 3278 cm^{-1} for OH^-, and two bands at 1596 cm^{-1} and 1397 cm^{-1} for COO^-, symmetric and asymmetric stretching vibration (Sarmento, 2006; Karp, 2019). For florfenicol, red line in Figure 2b, we can see the typical signal: 3447 cm^{-1} for OH^-, stretching vibration; 3312 cm^{-1} for –NH, stretching vibration; 2900 cm^{-1} for $–CH_2$ and $–CH_3$ vibration; 1676 cm^{-1} for –C=O, stretching vibration; 1531 cm^{-1} for –NH, bending vibration and –CN, stretching vibration; 1268 cm^{-1} -CN, bending vibration and –NH stretching vibration (Zhang, 2020; Arriagada, 2019; Marciniec, 2008; Sun, 2014). The blue line in Figure 2c corresponds to florfenicol-loaded alginate dried beads spectra. It presents signals that correspond to both components: florfenicol (red arrows) and alginate (black arrows).

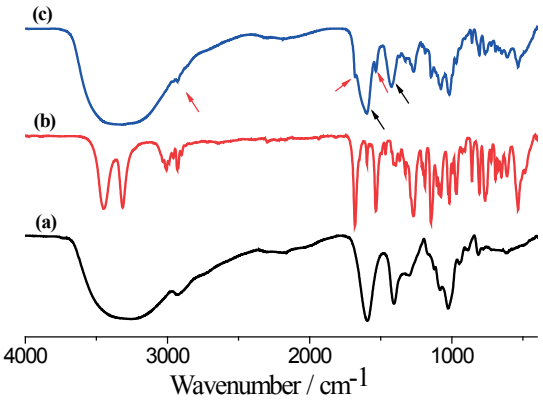

Figure 2: Fourier-transform infrared spectra of (a) alginate, (b) florfenicol, and (d) florfenicol-loaded alginate dried beads.

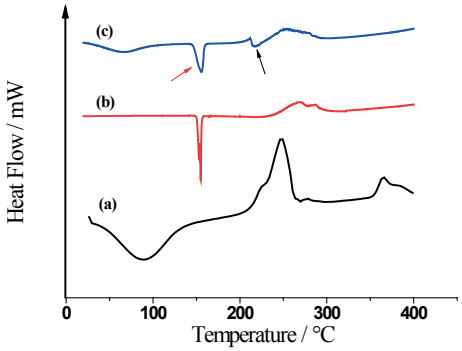

Figure 3: Differential scanning calorimeter thermograms of (a) alginate, (b) florfenicol, and (c) florfenicol-loaded alginate dried beads.

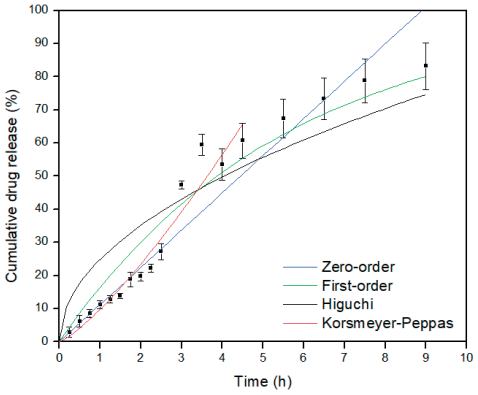

Figure 4: *In vitro* release profile of florfenicol-alginate dried beads (FADBs) ($\bar{x}\pm1SD$; $\eta = 3$). Symbols correspond to experimental data; blue line corresponds to *Zero-order*, green line corresponds to *First-order*, black line corresponds to *Higuchi* model, and red line corresponds to the 60% drug release fitted to *Korsmeyer-Peppas* model.

Differential scanning calorimeter (DSC)

Figure 3, presents the thermograms of different samples. The typical thermogram for pure alginate is present in the black line; the endothermic peak at 88.86°C has been correlated with dehydration of hydrophobic groups of the polymer; the second and third exothermic peaks, 247°C and 365°C, were of degradation of biopolymer due to polymerization reactions. The florfenicol thermogram (red line) presents an endothermic peak near 150°C, corresponding to the melting point, and two broad exothermic peaks at 250°C, corresponding to thermal decomposition (Karp *et al.*, 2019). Finally, the thermogram corresponding to florfenicol-loaded alginate dried beads is shown with a blue line.

In vitro drug release studies

The drug release behaviour of alginate dried beads was studied to simulate the orally administered conditions including pH, temperature, and transit time during 9 h. *In vitro* release profile is shown in Figure 4. The dried beads displayed a biphasic pattern with a slow-release in gastric conditions, releasing only 13.83±0.86% within 1.5 h. At intestinal condition, it showed a controlled release of 83.16±6.99% for 5% w/w within 7.5 h.

No rejection of the FADBs concealed in alfalfa morsels was detected when the mixture was force-fed to the rabbits. Also, no rejection of the FADBs-medicated feed was detected and the animals consumed their ration without differentiating the FADBs from the rest of the food.

Pharmacokinetics

Figures 5 and 6 present the plasma concentrations of florfenicol and florfenicol-amine after the bolus dose of FADBs concealed in alfalfa shredding and after the *ad libitum* consumption of FADBs-medicated food, respectively. Table 1 shows the pharmacokinetic parameters obtained, including the PK/PD ratio of $T\geq MIC_{0.2\mu g/mL}$, considering a dosing interval (DI) of 24 h.

DISCUSSION

Alginate is a polysaccharide extracted from brown seaweed, with several advantages such as ease of preparation, biocompatibility, biodegradability and non-toxicity. Alginate is one of the most popular polymers for hydrogel drug delivery systems, typically processing through crosslinking by ionotropic gelation with divalent cations like Ca^{2+}, making the typical "egg-box" model (Hariyadi and Islam, 2020). Alginate hydrogels for drug delivery systems can be formulated in several physical forms such as microparticles, nanoparticles, films and beads (Lupo *et al.*, 2015; Arriagada *et al.*, 2019; Zhang *et al.*, 2020). The air-dried alginate beads were prepared using the method according to Gutierrez *et al.* (2020) based on a combination of extrusion and ionic gel formation, where a florfenicol/alginate

solution is dropped using a syringe into a $CaCl_2$ solution under gentle stirring. The beads are formed immediately as ionic induced gelation occurs. Alginate thus forms the polymeric matrix, trapping the florfenicol inside (Lupo *et al*., 2015; Gutierrez *et al*., 2020). Florfenicol has been formulated in several drug delivery systems for veterinary medicine such as chitosan nanoparticles, solid lipid nanoparticles and silica nanoparticles (Li *et al*., 2016; Karp *et al*., 2019; Youssef *et al*., 2019). However, alginate beads have been demonstrated to be an easy and low-cost method to prepare a controlled release delivery system (Tønnesen and Karlsen, 2002; Gutierrez *et al*., 2020).

Entrapment efficiency (EE%) and drug loading (%DL) play an important role in the preparation of drug delivery systems and affect their therapeutic effect, since a high EE% and %DL are desirable to minimise the number of dried beads required to deliver the antibacterial drug. In this study, high efficacy to load florfenicol (EE%=94-98% and %DL=73.56±3.26%), was obtained. The high drug loading and entrapment efficiency may be due to the preferential localisation of the drug inside the polymer matrix which is less hydrophilic than the external aqueous environment since florfenicol has been reported as a broad-spectrum antibiotic with the drawback of poor aqueous solubility (Wang *et al.,* 2011). Other authors suggested that the poor solubility allowed drug precipitation inside the beads leading to an increased %EE (Tønnesen and Karlsen, 2002; Karp *et al*., 2019). In this study, the EE% obtained was higher as compared to the florfenicol-loaded alginate-Eudragit®RS blended matrix with an EE%=60-80% (Karp *et al*., 2019) and doxycycline/florfenicol PVP microparticles with an EE% ~89% (Li *et al*., 2016).

To study the drug release mechanism, the release profile was fitted to *zero-order* (R^2 = 0.9074), *first-order* (R^2=0.9312), *Higuchi* (R^2=0.8099), and *Korsmeyer-Peppas* (R^2=0.9428) equation. The highest R^2 value was obtained for the *Korsmeyer-Peppas* model (0.9428), and an *n* value of 1.26 was attributed to the case-II transport mechanism (n≥1.0). The case-II anomalous transport considers relaxational mechanism, which may refer to a combination of mechanisms, as the alginate beads are exposed to the dissolution medium and the drug release system undergoes a swelling-dissolution-erosion process, where the osmotic pressure gradient and the pH of the release medium comprise important factors in the swelling process (Sarmento *et al*., 2006). In this study, gastric conditions were mimicked (pH=3.0±0.1), and swelling is limited due to the protonated carboxyl groups, and this causes a releasing of only 13.83±0.86% under this condition. At this point, the florfenicol release will be mainly related to its diffusion through the insoluble matrix. However, in intestinal conditions (pH=7.8±0.1) the matrix is structurally more relaxed because of carboxylate

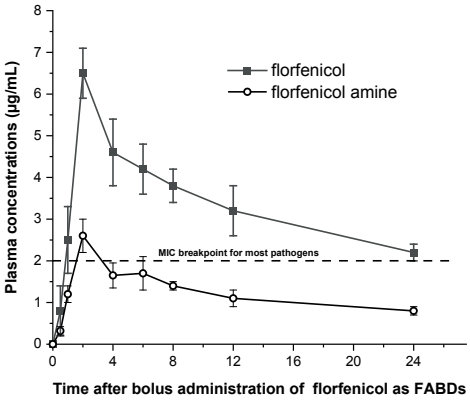

Figure 5: Plasma profiles of florfenicol and florfenicol-amine in New Zealand rabbits after administration of the antibiotic orally as a forced bolus and at a dose of 20 mg/kg and as florfenicol-alginate dried beads (FADBs) and concealed in fresh alfalfa shredding (bars: standard deviation).

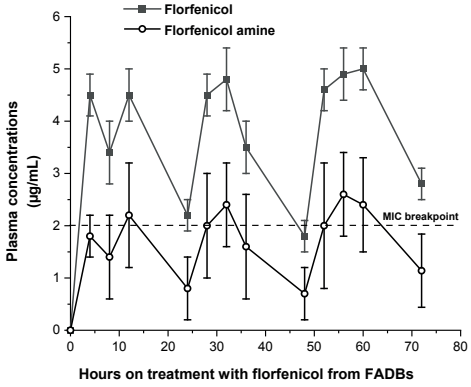

Figure 6: Plasma profiles of florfenicol and florfenicol-amine in New Zealand rabbits receiving an approximate dose of 20 mg/kg of florfenicol administered as in-feed medication employing florfenicol-alginate dried beads (FADBs), concealed in fresh alfalfa shredding and their standard food assignment in pellets (bars: standard deviation).

Table 1: Pharmacokinetic variables of florfenicol in New Zealand rabbits after its administration in food (calculated dose of 20 mg/kg) using florfenicol-loaded alginate in dried beads (FADBs).

Parameter	Florfenicol	Florfenicol-amine
$AUC_{0-\infty}$ (µg·h)/mL)	76.3±5.6	32.08±3.1
AUC_{0-24} (µg·h)/mL)	48.4±1.8	26.44±0.4
λ (1/h)	2.2±0.1	2.4±0.6
T½λ (h)	3.8±0.6	4.1±0.08
C_{max} (µg/mL)	6.08±0.3	2.4±0.6
T_{max} (h)	2.06±0.4	2.45±0.04
MRT (h)	6.90±1.4	7.2±1.4
PK/PD ratio		
$\%T>MIC_{2.0\ \mu g/mL}$ (ID_{24h})[1]	100%	almost zero

η=14 New Zealand rabbits weighing approximately 2 kg; C_{max}=maximum plasma concentration; T_{max}=time to reach C_{max}; λ=elimination rate constant; T½λ=elimination half-life; AUC_{0-24}=area under the curve of plasma concentrations *vs.* time in 24 h; $AUC_{0-\infty}$=area under plasma concentrations *vs.* time from 0 to ∞; MRT=mean residence time; MIC=minimum inhibitory concentration.
[1]$MIC_{2.0\ \mu g/mL}$=cut-off point for most pathogens of importance in veterinary medicine (Bretzlaff *et al.*, 1987; Ueda and Suenaga, 1995, Park *et al.*, 2007).

ionisation and high chain repulsion. In consequence, polymer chains gain flexibility, and mean porous size increases, releasing ~70% of the drug. Due to the high lipid solubility of florfenicol, absorption occurs easily in the intestinal region (USP, 2007). It is then here proposed that the dried beads allow a controlled release of florfenicol lasting 9 h, with a pH-dependent pattern. Consequently, possible flip-flop pharmacokinetics is occurring in the gastrointestinal tract of the rabbit. In turn, this may comply better with the key PK/PD ratio of t≥MIC as pharmacokinetic analysis revealed as T½λ of 3.8±0.6 for florfenicol and 4.1±0.08 for florfenicol-amine. In contrast, a parenteral dose of florfenicol resulted in a T½β of 1.49±0.23 h (Koc *et al.,* 2009), and T½λ after a forced oral bolus dose of florfenicol was reported to be 1.42±0.56 h (Park *et al.*, 2007) or 2.57 h (Abd EL-Aty *et al.*, 2004).

Administering an antibacterial drug with such an unpleasant taste as florfenicol to rabbits can be frustrating, because food consumption is reduced and with it, the calculated dose, hampering the clinical outcome (McBridge *et al.*, 2004; Brewer, 2006). With FADBs, no reduction in food consumption was detected, nor was any selectivity detected concerning their usual food. This aspect is of great importance to achieve therapeutic or metaphylactic efficacy in rabbits, particularly at the commercial level where large populations require proper antibacterial medication. When a respiratory disease outbreak is diagnosed in a rabbit farm, rapid metaphylactic medication through water or food can solve the problem, as rabbits' health rapidly deteriorates (Lennox, 2010). Injected antibacterial drugs can cause considerable stress and rabbits often suffer muscular lesions (Lennox, 2010; Sailer-Fleeger, 2021). Hence, oral dosing of antibacterial drugs is preferred and appropriate dosing is essential to succeed. The FADBs described here may be adequate, considering that affected animals are likely to reduce food consumption and water intake. So, the tasteless or amiable nature of FADBs for rabbits may become important. Favourable unpublished results in clinical outbreaks have already been obtained, but formal-controlled trials are now needed.

CONCLUSIONS

The florfenicol-loaded alginate beads (FADBs) were prepared with high drug entrapment efficiency (>94%) and high drug loading (>70%). Composition, interactions and stability of the components were defined, and FADBs showed an *in vitro* controlled drug release of florfenicol during 9 h pH-dependant following swelling–dissolution-erosion sequence of alginate. FADBs are a low-cost and attractive drug delivery system for oral controlled release and pharmacokinetic parameters confirm good bioavailability and a longer half-life as compared to previous studies in which conventional florfenicol as a solution was force-administered. Considering a $MIC_{2.0\ \mu g/mL}$ as the cut-off point for florfenicol in rabbits, *ad libitum* intake of FADBs in their standard diet maintained plasma concentrations of florfenicol above this level during the whole dosing interval of 24 h.

Conflict of interest: The authors declare that they have no conflict of interest.

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