For reprint orders, please contact: reprints@futuremedicine.com

Rifampicin-loaded 'flower-like' polymeric micelles for enhanced oral bioavailability in an extemporaneous liquid fixed-dose combination with isoniazid

Background: Coadministration of rifampicin (RIF)/isoniazid (INH) is clinically recommended to improve the treatment of tuberculosis. Under gastric conditions, RIF undergoes fast hydrolysis (a pathway hastened by INH) and oral bioavailability loss. **Aim:** We aimed to assess the chemical stabilization and the oral pharmacokinetics of RIF nanoencapsulated within poly(ε-caprolactone)-*b*-PEG-*b*-poly(ε-caprolactone) 'flower-like' polymeric micelles. **Materials & methods:** The chemical stability of RIF was evaluated *in vitro* under acid conditions with and without INH, and the oral pharmacokinetics of RIF-loaded micelles in rats was compared with those of a suspension coded by the US Pharmacopeia. **Results:** Nanoencapsulation decreased the degradation rate of RIF with respect to the free drug. Moreover, *in vivo* data showed a statistically significant increase of RIF oral bioavailability (up to 3.3-times) with respect to the free drug in the presence of INH. **Conclusion:** Overall results highlight the potential of this nanotechnology platform to develop an extemporaneous liquid RIF/INH fixed-dose combination suitable for pediatric administration.

Original submitted 6 April 2013; Revised submitted 7 August 2013

KEYWORDS: extemporaneous liquid rifampicin/isoniazid fixed-dose combination ■ improved oral pharmacokinetics ■ poly(ε-caprolactone)-*b*-PEG-*b*-poly(ε-caprolactone) 'flower-like' polymeric micelle ■ pediatric tuberculosis ■ rifampicin chemical stabilization

Marcela A Moretton[1,2], Christian Hocht[3], Carlos Taira[2,3] & Alejandro Sosnik*[1,2,4]

[1]The Group of Biomaterials & Nanotechnology for Improved Medicines, Department of Pharmaceutical Technology, Faculty of Pharmacy & Biochemistry, University of Buenos Aires, Buenos Aires, Argentina
[2]National Science Research Council, Buenos Aires, Argentina
[3]Department of Pharmacology, Faculty of Pharmacy & Biochemistry, University of Buenos Aires, Argentina
[4]Department of Materials Science & Engineering, Technion-Israel Institute of Technology, Haifa, Israel
**Author for correspondence: alesosnik@gmail.com*

Tuberculosis (TB) is the most deadly infection after HIV [1–3,101]. TB is regarded as a poverty-related disease because it mainly affects poor countries [4]. In 1993 WHO declared a global sanitary emergency [5].

The first-line pharmacotherapy of nonresistant TB is comprised of two phases that last 6 months. Both phases demand the coadministration of rifampicin (RIF) and isoniazid (INH; isonicotinylhydrazine) by the oral route [6,7,102]. Nonresistant TB is curable but it accounts for more than 25% of preventable and 24% of all deaths worldwide [8,103]. Under gastric conditions, RIF undergoes hydrolysis to 3-formyl RIF SV (3-FRSV), a derivative without activity *in vivo* due to negligible gastrointestinal absorption [9]. This degradation pathway is hastened by soluble INH [10–12]. The coadministration of RIF/INH fixed-dose combinations (FDCs) is clinically recommended to prevent the development of resistance. The significant decrease of the oral bioavailability of RIF in most RIF/INH FDCs is usually disregarded [13]. WHO has raised awareness of this therapeutic drawback and advised the use of quality-assured FDCs of proven RIF bioavailability [14,104].

TB is among the ten main causes of childhood mortality, with an estimated annual toll of 130,000 deaths [15]. The lack of commercially available pediatric medicines that enable fine-tuning of dosage and swallowing in poverty-related diseases is a remarkable hurdle for convenient therapy [16,17]. Single RIF and INH liquid formulations are commercially available in several countries. Conversely, liquid RIF/INH FDCs have not been developed. Macleods Pharmaceuticals (Mumbai, India) developed a series of double (RIF/INH) and triple (RIF/INH/pyrazinamide) FDC dispersible tablets that could be used to obtain extemporaneous suspensions for pediatric use [17,105]. These products are listed in the WHO List of Prequalified Medicinal Products; although oral bioavailability data are not currently available.

Novel drugs are expected to shorten the course of the treatment and to be effective against resistant strains. At the same time, the development of innovative formulations of approved drugs that could also lead to breakthroughs in pharmacotherapy is in constant progress and it is becoming complementary to drug discovery [18]. For example, Choonara *et al.* have recently reported on the production of super-stable nanoparticles for the sustained release of anti-TB drugs [19].

Polymeric micelles are one of the most versatile nanocarriers to enhance the water solubility, the physicochemical stability and the bioavailability of poorly water soluble and instable drugs [20,21] and diverse administration routes, such as oral, parenteral, ocular and intranasal, have been explored [22–26].

Future Medicine part of fsg

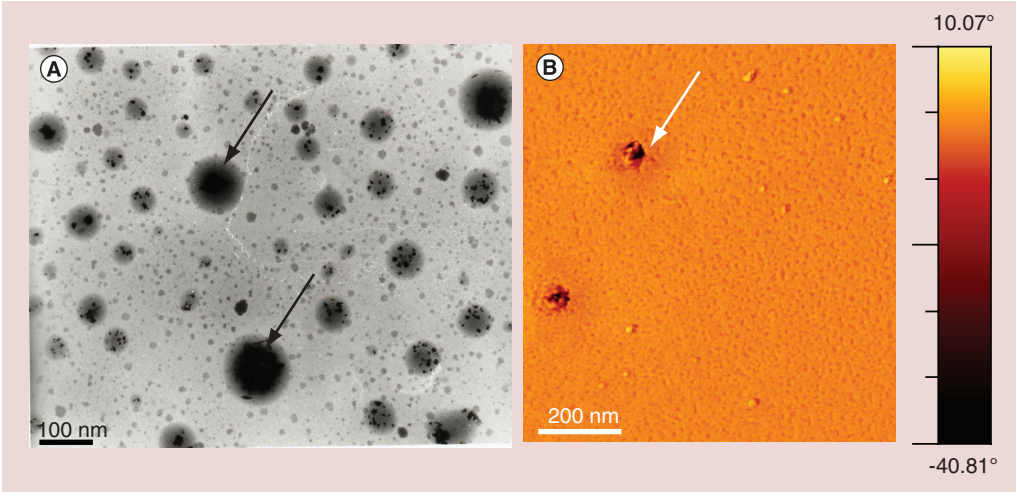

Figure 1. Morphology of rifampicin-loaded poly(ε-caprolactone)(4500) polymeric micelles. **(A)** Transmission electron microscopy micrograph of fresh 4% micelles and **(B)** tapping mode atomic force microscopy 2D micrograph of freeze-dried 1% micelles lyoprotected with hydroxylpropyl β-cyclodextrin 15%. The sample was diluted 1:20 before the analysis. Arrows indicate micelles.

The development of liquid pediatric FDCs is urgently needed to ease dose adjustment and swallowing and improve patient compliance [17]. At the same time, due to the detrimental RIF/INH interaction, liquid FDCs would need to be conceived as extemporaneous powders for redispersion where RIF is chemically stabilized. We previously described the encapsulation of RIF within 'flower-like' polymeric micelles of poly(ε-caprolactone)-*b*-PEG-*b*-poly(ε-caprolactone) (PCL-*b*-PEG-*b*-PCL) block copolymers and their physical stabilization by freeze drying [27,28].

The present work investigated the capacity of these nanocarriers to protect RIF from degradation under extreme acid conditions in the absence and presence of soluble INH and the pharmacokinetics of the drug in rats after oral administration. Overall results highlight the potential of this nanotechnology platform to develop an extemporaneous liquid RIF/INH FDC to treat childhood TB.

Materials & methods

Materials

PEG (molecular weight: 10 kg/mol, PEG10000), ε-caprolactone (CL; monomer), tin (II) 2-ethylhexanoate (catalyst), RIF, INH, and solvents of analytical or HPLC grade were used as received. Hydroxypropyl-β-cyclodextrin (HPβ-CD) was a gift of ISP Technologies Inc. (NJ, USA).

Methods

Copolymer synthesis

PCL-*b*-PEG-*b*-PCL copolymers were synthesized as described previously [27,29,30]. Two derivatives bearing terminal PCL blocks of average molecular weight of 3.7 (32 CL units/arm) and 4.5 kg/mol (40 CL units/arm) and theoretical molecular weights of 17.4 and 19.0 kg/mol, respectively, were used [27]. PCL3700-*b*-PEG10000-*b*-PEG3700 and PCL4500-*b*-PEG10000-*b*-PCL4500 copolymers are named PCL(3700) and PCL(4500), respectively. The hydrophilic–lipophilic balance of the copolymers was estimated by the ratio between the number of CL and ethylene oxide (EO) repeating units in the copolymer, designated the CL:EO ratio.

Preparation of RIF-loaded PCL-*b*-PEG-*b*-PCL polymeric micelles

RIF-loaded micelles of different copolymer concentrations (1, 4 and 6%) were prepared by means of the cosolvent/evaporation method [27]. RIF concentrations were determined by UV–visible spectrophotometry (Supplementary Information; see online at www.futuremedicine.com/doi/suppl/10.2217/nnm.13.154).

Freeze drying of RIF-loaded micelles

RIF-loaded micelles were freeze-dried employing HPβ-CD (10, 13, 15 and 20% w/v) as cryo-/lyo-protectant [28]. Samples were reconstituted in distilled water (1 ml) before use (Supplementary Information).

Morphology of RIF-loaded micelles

The morphology of RIF-loaded PCL(4500) micelles was studied by transmission electron microscopy and atomic force microscopy (Supplementary Information).

In vitro release of RIF

The release of RIF was assessed employing phosphate-buffered saline (pH 7.4) containing ascorbic acid (200 µg/ml) [31] as an external release medium (600 ml) under mechanical stirring (70 rpm) over 6.5 h, at 37°C (Supplementary Information). The goal of this assay was to study the role played by the release of RIF in the degradation process. These pH conditions were selected to prevent RIF degradation during the assay and to simplify the analysis. The addition of HPβ-CD as cryo-/lyo-protectant could alter the release of RIF. To study this effect, the release from RIF-loaded micelles (4%) containing 20% HPβ-CD was also assessed.

Assays were carried out in triplicate and the results are expressed as the mean ± standard deviation of the mean. Release data were fitted to the Korsmeyer–Peppas model [32,33], considering micelles as spheres [27,34] (Supplementary Information).

The analysis and fitting were conducted with SigmaPlot® software and Microsoft Excel® 2003 (both Microsoft, WA, USA).

Measurement of the micellar size & size distribution

The effect of RIF release on the size and size distribution (expressed by the polydispersion) of RIF-loaded micelles was studied by dynamic light scattering over 2 h, at 37°C (Supplementary Information).

Chemical stability of RIF

Fresh and freeze-dried RIF-loaded micelles (4%) were incubated in acid medium in the absence and presence of soluble INH. For the latter assays, INH was solubilized in hydrochloric acid (HCl) 0.1 N and the RIF:INH weight ratio was maintained at 3:2, as used in previous studies [9] and recommended in clinics [10,11,14].

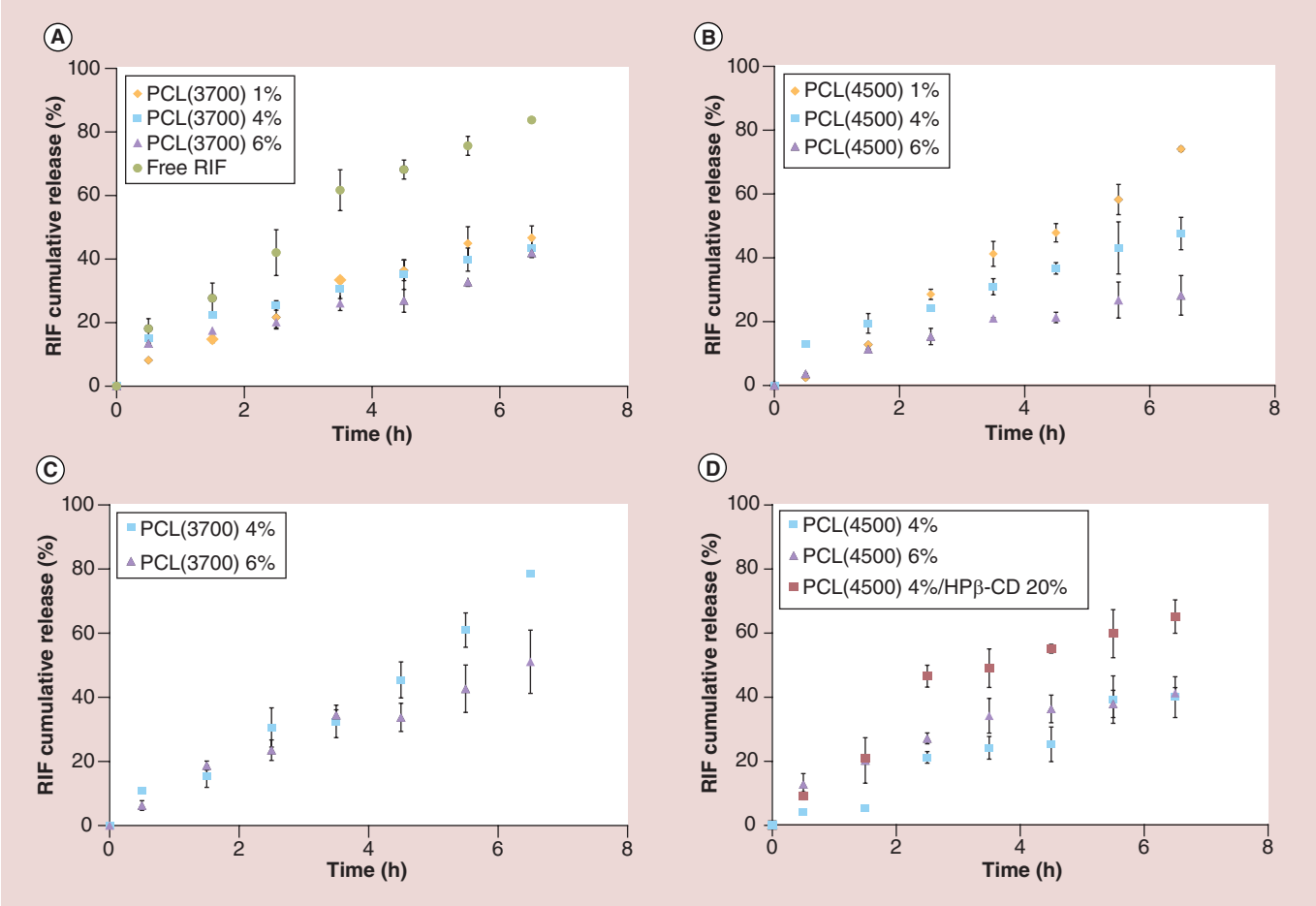

Figure 2. *In vitro* rifampicin release from poly(ε-caprolactone)-*b*-PEG-*b*-poly(ε-caprolactone) polymeric micelles containing different copolymer concentrations. (A) Fresh 1–6% PCL(3700) micelles containing 4.9 mg/ml of RIF, **(B)** fresh 1–6% PCL(4500) micelles containing 4.9 mg/ml of RIF, **(C)** fresh 4 and 6% PCL(3700) micelles containing 10.4 and 12.2 mg/ml of RIF, respectively, and **(D)** fresh and lyophilized 4% PCL(4500) micelles containing 10.4 and fresh 6% micelles containing 12.2 mg/ml of RIF. Each point represents the mean ± standard deviation of the mean of three independent experiments.
HPβ-CD: Hydroxylpropyl β-cyclodextrin; PCL: Poly(ε-caprolactone); RIF: Rifampicin.

doi:10.2217/NNM.13.154

Table 1. Curve-fitting analysis of rifampicin release data from poly(ε-caprolactone)-*b*-PEG-*b*-poly(ε-caprolactone) polymeric micelles over 3.5 and 6.5 h.

Copolymer	Copolymer concentration (%)	Rifampicin concentration (mg/ml)	Zero order$_{0–3.5\ h}$		Zero order$_{0–6.5\ h}$		First order$_{0–3.5\ h}$		First order$_{0–6.5\ h}$		Korsmeyer–Peppas model[§]		
			k_o (% *h*)	$R^2_{adjusted}$	k_o (% *h*)	$R^2_{adjusted}$	k_1 (h^{-1})	$R^2_{adjusted}$	k_1 (h^{-1})	$R^2_{adjusted}$	*k*	*n*	$R^2_{adjusted}$
PCL(3700)[†]	1	4.9	8.27	0.9674	6.81	0.9710	0.10	0.9436	0.10	0.9788	0.12	0.75	0.9787
	4		5.00	0.9599	4.63	0.9905	0.07	0.9682	0.07	0.9951	0.18	0.45	0.9772
	6		4.07	0.9655	4.39	0.9480	0.05	0.9562	0.06	0.9180	0.14	0.53	0.9405
	4	10.4	8.03	0.8688	11.04	0.9532	0.10	0.8666	0.22	0.8372	0.14	0.77	0.9274
	6	12.2	8.94	0.9629	6.88	0.9588	0.11	0.9642	0.10	0.9609	0.12	0.73	0.9690
PCL(4500)[†]	1	4.9	13.22	0.9915	11.61	0.9896	0.17	0.9785	0.21	0.9417	0.10	1.00	0.9803
	4		5.86	0.9953	5.83	0.9982	0.08	0.9924	0.09	0.9943	0.15	0.60	0.9789
	6		5.62	0.9768	3.95	0.9423	0.06	0.9833	0.05	0.9577	0.08	0.67	0.9854
	4	10.4	7.59	0.8282	6.40	0.9235	0.09	0.8315	0.08	0.9253	0.07	0.96	0.9241
	6	12.2	7.11	0.9996	4.64	0.9187	0.09	0.9984	0.06	0.9428	0.18	0.46	0.9806
Lyophilized PCL(4500)[‡]	4	10.4	14.51	0.8825	8.79	0.8451	0.21	0.8791	0.15	0.9132	0.20	0.67	0.9115

[†]*Fresh micelles.*
[‡]*Micelles lyophilized with 20% hydroxylpropyl* β*-cyclodextrin.*
[§]*Analysis by the Korsmeyer–Peppas model was conducted for M_t/M_∞ (fraction of drug released at a given time) ≤0.6.*
k: Kinetic constant; k_o: Zero-order release constant; k_1: First-order release constant; n: Release exponent; PCL: Poly(ε-caprolactone).

Saturated free RIF solutions (2.6 mg/ml) were used as control. Samples were diluted (1:10 and 1:40) in HCl 0.1 N and incubated at 37°C under continuous magnetic stirring (100 rpm) for 3 h [22,35]. These pH conditions and sampling intervals were selected to mimic the gastric transit time and acid environment where RIF degradation is maximal.

The addition of HPβ-CD could modify the chemical stability of RIF with respect to fresh samples. Thus, freeze-dried samples were resuspended in the original volume of distilled water and diluted with HCl 0.1 N. At different time points, samples (500 µl) were diluted with phosphate buffer saline of pH 7.0 to quench the acid degradation process and analyzed by reverse-phase HPLC (see below). RIF follows first-order degradation kinetics [10]. Thus, the degradation constant under the different conditions was determined by the following equation:

$$In(C_f) = In(C_0) - K_d t$$

where C_o is the initial concentration of RIF and C_f the concentration at a certain time, K_d the apparent degradation constant (min^{-1}) and t the time expressed in minutes. The percentage of RIF degraded at 3 h, D_{3h} (%), was also calculated. This time is clinically relevant because it is the maximum gastric transit of RIF. Assays were carried out in triplicate and the results are expressed as the mean ± standard deviation of the mean.

HPLC method for chemical stability assays

Analyses were carried out using an adapted reverse-phase HPLC validated method with a UV detector (λ = 254 nm) (Supplementary Information) [11].

Oral pharmacokinetics of RIF

The goal of this study was to evaluate the effect of RIF solubilization and chemical stabilization within the micelles on its bioavailability. The oral pharmacokinetics of RIF was assessed in male Wistar rats (weight of 300–350 g) in the absence and presence of INH (RIF:INH weight ratio 3:2) (Supplementary Information).

HPLC method for oral pharmacokinetics assays

RIF concentrations in plasma were determined by reverse-phase HPLC with UV (λ = 330 nm) (Supplementary Information).

Table 2. Micellar size and size distribution of rifampicin-loaded poly(ε-caprolactone)-*b*-PEG-*b*-poly(ε-caprolactone) micelles over time, at 37°C (n = 3).

Copolymer concentration (%w/v)	RIF payload (mg/ml)	Time (h)	PCL(3700)							PCL(4500)				
			Peak 1		*Peak 2*		*Peak 3*		*PDI (SD)*	*Peak 1*		*Peak 2*		*PDI (SD)*
			D_h; nm (SD)	%	D_h; nm (SD)	%	D_h; nm (SD)	%		D_h; nm (SD)	%	D_h; nm (SD)	%	
1	4.9	0	227.0 (8.2)	100.0	–	–	–	–	0.150 (0.017)	169.9 (7.1)	100.0	–	–	0.158 (0.016)
		1	84.1 (1.2)	100.0	–	–	–	–	0.172 (0.002)	66.6 (5.4)	100.0	–	–	0.193 (0.111)
		2	75.9 (0.8)	100.0	–	–	–	–	0.174 (0.010)	72.7 (1.0)	100.0	–	–	0.137 (0.009)
4	4.9	0	27.2 (5.6)	8.9	170.9 (3.1)	91.1	–	–	0.424 (0.061)	25.4 (4.8)	7.7	176.0 (11.2)	92.3	0.337 (0.061)
		1	32.9 (3.8)	9.7	171.9 (7.4)	90.3	–	–	0.360 (0.046)	27.6 (3.0)	7.1	173.3 (8.4)	92.9	0.284 (0.010)
		2	28.4 (4.6)	4.8	170.3 (3.7)	95.2	–	–	0.361 (0.047)	35.2 (4.7)	10.4	187.2 (14.8)	89.6	0.321 (0.040)
6	4.9	0	39.9 (4.8)	11.0	427.3 (18.2)	89.0	–	–	0.557 (0.009)	38.9 (7.5)	9.1	361.3 (27.9)	90.9	0.473 (0.014)
		1	39.2 (1.4)	19.3	506.6 (68.2)	80.7	–	–	0.616 (0.054)	47.1 (3.6)	11.5	481.0 (14.8)	88.5	0.585 (0.038)
		2	58.0 (7.3)	14.9	457.3 (19.8)	85.1	–	–	0.610 (0.040)	47.6 (5.9)	12.4	450.6 (26.5)	87.6	0.562 (0.041)
4	10.4	0	32.5 (4.0)	13.1	234.6 (5.3)	86.9	–	–	0.492 (0.031)	113.8 (13.0)	5.1	858.9 (36.1)	94.9	0.338 (0.037)
		1	39.6 (2.1)	14.3	293.4 (5.1)	85.7	–	–	0.540 (0.021)	50.5 (8.2)	7.9	365.0 (29.8)	92.1	0.472 (0.008)
		2	49.2 (3.3)	18.4	316.4 (3.7)	81.6	–	–	0.541 (0.005)	35.6 (1.2)	6.5	327.9 (9.0)	93.5	0.314 (0.007)
6	12.2	0	35.4 (7.9)	10.0	684.9 (57.4)	90.0	–	–	0.691 (0.010)	1289.3 (124.6)	100.0	–	–	0.154 (0.024)
		1	25.4 (2.8)	4.0	88.8 (9.5)	13.8	801.5 (46.0)	82.2	0.712 (0.017)	1264.3 (84.3)	100.0	–	–	0.209 (0.017)
		2	37.6 (2.0)	8.9	142.4 (5.4)	14.1	860.3 (61.3)	77.0	0.686 (0.021)	213.8 (39.7)	14.4	1464.3 (98.8)	85.6	0.627 (0.054)

D_h: Hydrodynamic diameter; PCL: Poly(ε-caprolactone); PDI: Polydispersion; RIF: Rifampicin; SD: Standard deviation of the mean.

doi:10.2217/NNM.13.154

Table 3. Rifampicin degradation parameters under gastric-like conditions for fresh and cryoprotected/lyophilized rifampicin-loaded 4% poly(ε-caprolactone)-*b*-PEG-*b*-poly(ε-caprolactone) polymeric micelles upon 1:10 and 1:40 dilution in the presence and absence of isoniazid.

Sample	HPβ-CD content (%)	RIF final concentration upon dilution; mg/ml (SD)	Copolymer final concentration upon dilution		INH content	K_d; min^{-1} (SD)	R^2 (SD)	D_{3h}; % (SD)
			%	*mM*				
Control	–	0.25 (0.03)	–	–	-	0.0031 (0.0001)	0.9769 (0.0023)	42.8 (1.5)
		0.24 (0.04)			+[†]	0.0041 (0.0001)	0.9933 (0.0018)	52.2 (0.1)
PCL(3700)	–	1.08[‡] (0.04)	0.4	0.23	-	0.0008[§] (0.0001)	0.9312 (0.0062)	14.2 (1.1)
		1.11[‡] (0.06)			+[†]	0.0018[§] (0.0001)	0.9799 (0.0175)	27.7 (1.8)
		0.26[¶] (0.01)	0.1	0.058	-	0.0015[§] (0.0002)	0.9549 (0.0271)	22.2 (4.0)
		0.28[¶] (0.01)			+[†]	0.0023[§] (0.0002)	0.9685 (0.0152)	33.9 (2.1)
PCL(4500)	–	1.15[‡] (0.02)	0.4	0.21	-	0.0007[§] (0.0001)	0.9800 (0.0285)	12.4 (1.8)
		1.12[‡] (0.04)			+[†]	0.0018[§] (0.0001)	0.9502 (0.0057)	27.7 (1.3)
		0.27[¶] (0.01)	0.1	0.053	-	0.0016[§] (0.0001)	0.9452 (0.0484)	24.1 (0.8)
		0.28[¶] (0.01)			+[†]	0.0024[§] (0.0003)	0.9531 (0.0646)	35.1 (2.3)
Lyophilized PCL(4500)[#]	15	0.98[‡] (0.05)	0.4	0.21	-	0.0008[§,††] (0.0001)	0.9776 (0.0274)	13.4 (0.1)
		0.95[‡] (0.05)			+[†]	0.0021[§,††] (0.0001)	0.9668 (0.0220)	31.5 (1.7)
		0.27[¶] (0.01)	0.1	0.053	-	0.0021[§] (0.0001)	0.9807 (0.0175)	31.5 (0.1)
		0.27[¶] (0.00)			+[†]	0.0028[§,††] (0.0001)	0.9954 (0.0059)	39.6 (0.1)
Lyophilized PCL(4500)[‡‡]	20	0.98[‡] (0.04)	0.4	0.21	-	0.0011[§,††] (0.0001)	0.9868 (0.0078)	17.2 (1.1)
		1.07[‡] (0.08)			+[†]	0.0021[§,††] (0.0003)	0.9560 (0.0411)	32.0 (4.3)
		0.24[¶] (0.01)	0.1	0.053	-	0.0022[§] (0.0001)	0.9736 (0.0094)	32.1 (0.9)
		0.25[¶] (0.01)			+[†]	0.0042 (0.0001)	0.9507 (0.0373)	52.6 (0.6)

Results are expressed as mean (SD of the mean); n = 3.
[†]Sample containing INH in a RIF:INH weight ratio of 3:2.
[‡]Final RIF concentration after the corresponding 1:10 dilution.
[§]K_d parameter of RIF-loaded micellar dispersions is significantly lower than that of free RIF controls.
[¶]Final RIF concentration after the corresponding 1:40 dilution.
[#]HBβ-CD was incorporated as cryo-/lyo-protectant before the lyophilization at a concentration of 15%.
[††]No statistically significant difference was observed for the K_d of lyophilized and fresh RIF-loaded micellar dispersions.
[‡‡]HBβ-CD was incorporated as cryo-/lyo-protectant before the lyophilization at a concentration of 20%.
D_{3h}: Percentage of RIF degradation at time point 3 h; HPβ-CD: Hydroxylpropyl β-cyclodextrin; INH: Isoniazid; K_d: Degradation constant; RIF: Rifampicin; SD: Standard deviation of the mean.

Evaluation on *in vivo* data

Noncompartmental analysis of RIF plasma concentrations was performed using the PKSolver program (China Pharmeutical University, Jiangsu, China). Pharmacokinetic parameters were log transformed for statistical analysis to reduce heterogeneity of the variance. The relative oral bioavailability (F_r) between the micellar formulations and the suspension (control) was determined by calculating the ratio between the area under the curve $(AUC)_{0-24h}$ of the RIF-loaded micelles and the RIF suspension after the administration of an identical dose, according to:

$$F_r(\%) = AUC_{mic}/AUC_{susp} \times 100$$

where AUC_{mic} and AUC_{susp} are the AUC_{0-24h} of micellar dispersion and suspension, respectively.

Statistics

Statistical analysis was performed by one-way analysis of variance using Microsoft Excel 2003 software. The results were considered statistically significant if $p < 0.05$.

Results

We have previously investigated the nanoencapsulation of RIF within different types of polymeric micelles [26,36]. However, due to its bulky structure, micelles need to display relatively larger cores to host it. In this framework, we synthesized three series of PCL-*b*-PEG-*b*-PCL block copolymers with PEG precursors of growing molecular weight and different CL:EO ratios and produced flower-like polymeric micelles [27]. Two derivatives with central PEG10000 block, PCL(3700) and PCL(4500), showed the greatest solubilization capacity in water and physical stability of all the copolymers. RIF-loaded flower-like polymeric micelles displayed a spherical morphology (Figure 1). The present work initially investigated how encapsulation affected the release and chemical stability of the drug *in vitro*. Finally, the oral pharmacokinetics was evaluated toward the development of an extemporaneous liquid RIF/INH FDC for pediatric use.

In vitro RIF release from polymeric micelles

Free RIF

RIF displays a relatively high molecular weight of 822.95 g/mol. To rule out the retention of the drug by the dialysis membrane, the release of free RIF in solution (2.6 mg/ml) was assayed.

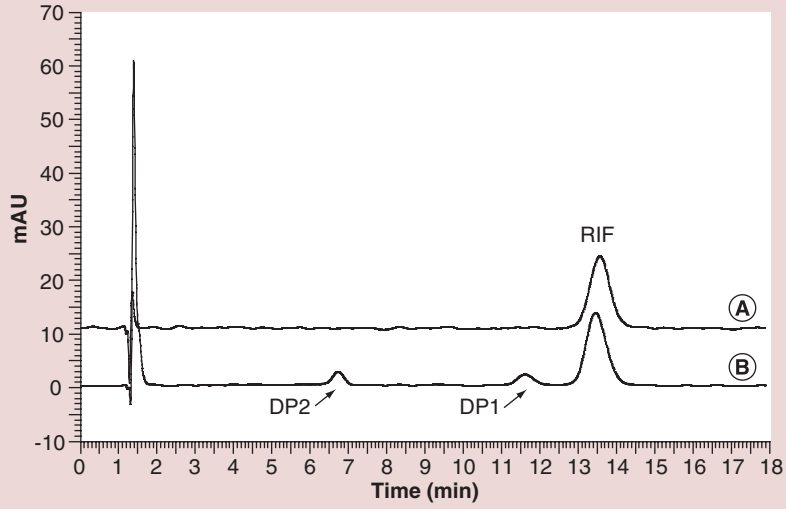

Figure 3. Chromatogram showing the degradation of free rifampicin in hydrochloric acid 0.1 N at 37°C. (A) At 0 h and **(B)** after 2 h, in the presence of isoniazid (RIF: isoniazid weight ratio of 3:2). The appearance of two additional peaks (DP1 and DP2) corresponded to RIF degradation products in acid medium. RIF: Rifampicin.

The release was almost completed within 6.5 h (released amount: 84%) (Figure 2A).

Effect of copolymer concentration

In the next stage, we investigated the effect of growing copolymer concentrations (1, 4 and 6% w/v), while maintaining a constant RIF payload (4.9 mg/ml). It is worth stressing that 1% micelles were saturated with RIF, while 4 and 6% micelles were not. All the PCL(3700) polymeric micelles released 43–47% of the drug cargo after 6.5 h (Figure 2A). For PCL(4500) micelles, the greater the copolymer concentration, the slower the release rate. Thus, 1, 4 and 6% micelles released 74, 48 and 28%, respectively, at 6.5 h (Figure 2B). Another parameter of consideration was the hydrophilic–lipophilic balance estimated by the CL:EO ratio. PCL(3700) and PCL(4500) present values of 0.29 and 0.35, respectively [27]. The 1% micelles of the more hydrophobic PCL(4500) showed a faster release rate than their PCL(3700) counterparts, while the opposite was true for unsaturated 6% systems (Figure 2A & B). Both 4% unsaturated micelles released the same amount of drug (~47–48%) after 6.5 h.

Effect of drug payload

To understand the effect of the drug payload on the release process, 4 and 6% micelles containing the maximum possible RIF payload (10.4 and 12.2 mg/ml, respectively) were also assayed. RIF-saturated PCL(3700) micelles released faster than their unsaturated counterparts

doi:10.2217/NNM.13.154

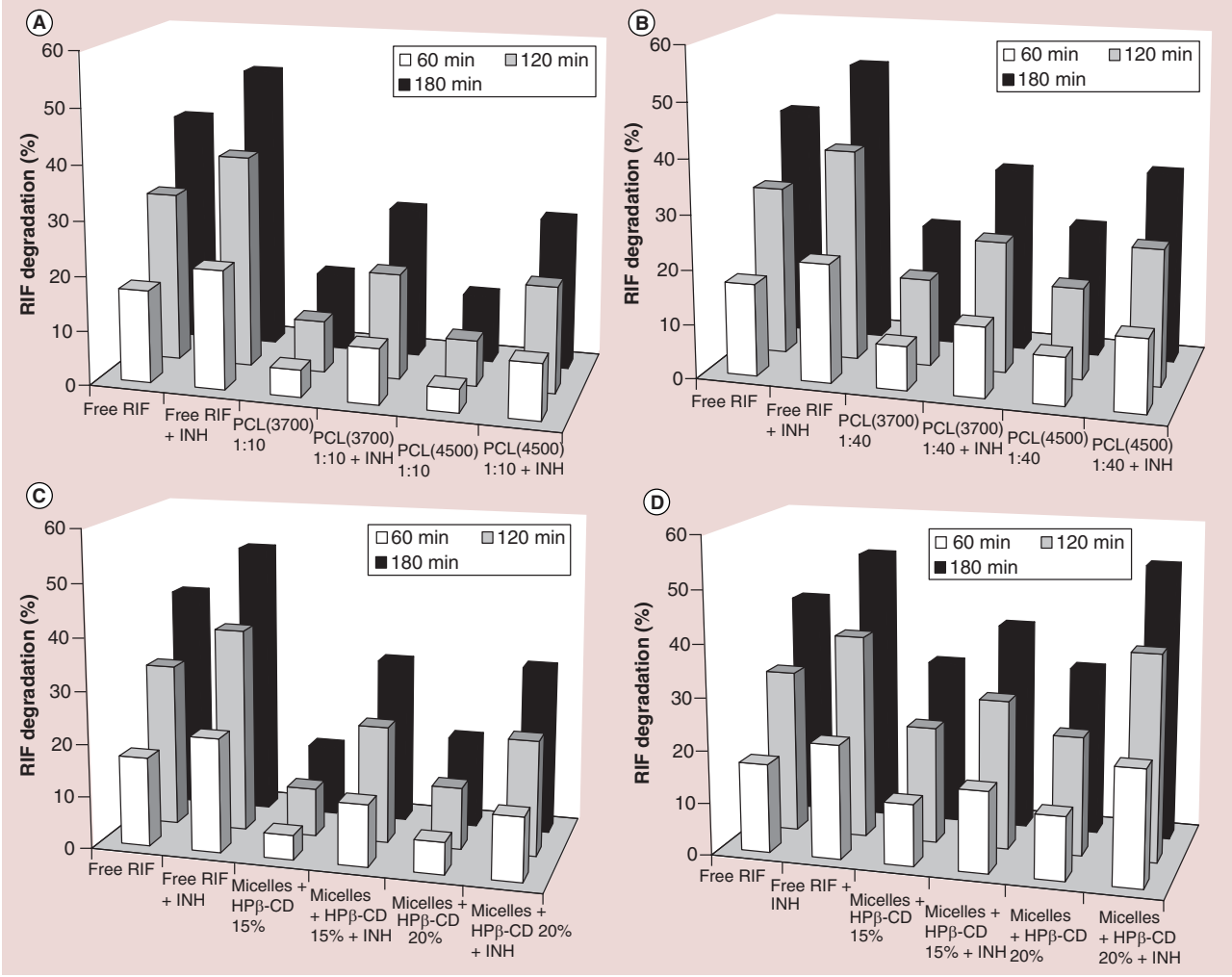

Figure 4. Percentage of rifampicin degradation in acid medium over 3 h. A free RIF solution was used as control. Fresh RIF-loaded micelles were diluted in hydrochloric acid 0.1 N **(A)** 1:10 and **(B)** 1:40 in the absence and presence of INH. PCL(4500) freeze-dried samples were diluted **(C)** 1:10 and **(D)** 1:40 in the presence and absence of INH. Different cryo-/lyo-protectant concentrations were used (20 and 15% w/v). Each bar represents the mean of three assays.
HPβ-CD: Hydroxylpropyl β-cyclodextrin; INH: Isoniazid; PCL: Poly(ε-caprolactone); RIF: Rifampicin.

(Figure 2C); for example, 79 and 51% for 4 and 6% systems, respectively. Conversely, the release from RIF-saturated PCL(4500) micelles was slower than that of the unsaturated micelles (Figure 2D).

Effect of cryo-/lyo-protection

To enhance their mid-to-long-term physical stability, RIF-loaded micelles need to undergo lyophilization with HPβ-CD [28]. However, this hydrophilic additive might increase the release rate. As expected, 4% PCL(4500) micelles containing 20% HPβ-CD released much faster (twofold) than the fresh ones (without cryo-/lyo-protectant) (Figure 2D).

Release data fitting

Drug release data were analyzed by different models (Table 1). The analysis was initially conducted between 0 and 6.5 h. For example, 1% micelles containing 4.9 mg/ml RIF payload fitted first-order kinetics, with k_1 values of 0.10 h^{-1} for PCL(3700) and 0.21 h^{-1} for PCL(4500). A similar trend was observed for unsaturated 4% micelles, although k_1 values were slightly smaller; 0.07 and 0.09 h^{-1} for PCL(3700) and PCL(4500), respectively. A further increase of the copolymer concentration to 6% led to a decrease in the release rate and a slight deviation from linearity. RIF-saturated 4 and 6% micelles showed faster release rates than their corresponding unsaturated counterparts. For example, k_1 values for 4 and 6% PCL(3700) were 0.22 and 0.10 h^{-1}, respectively. Release data were also analyzed between 0 and 3.5 h. All the systems fitted zero-order kinetics (Table 1).

When release data were analyzed by the Korsmeyer–Peppas model, 1, 4 and 6% saturated

PCL(3700) micelles showed n values of 0.75, 0.77 and 0.73, respectively (Table 1). A similar trend was followed by 4 and 6% RIF-unsaturated PCL(3700) micelles with n values of 0.45 and 0.53, respectively. PCL(4500) micelles showed a behavior that depended on both the drug payload and the copolymer concentration. Micelles containing a constant drug payload of 4.9 mg/ml and a growing copolymer concentration resulted in n values of 1.00 (1%), 0.60 (4%) and 0.67 (6%). Fresh RIF-saturated 4% PCL(4500) micelles showed an n value of 0.96. This value decreased after freeze drying to 0.67. Conversely, 6% PCL(4500) micelles showed a behavior similar to that of saturated 6% PCL(3700) micelles with an n value of 0.46.

Effect of RIF release on micellar size & size distribution

The release of RIF from drug-saturated 1% micelles was accompanied by a fast decrease in the micellar size (Table 2); for example, the size of 1% PCL(3700) and PCL(4500) micelles decreased from 227.0 and 169.9 nm to 84.1 and 66.6 nm, respectively, after 1 h. RIF-unsaturated 4 and 6% PCL(3700) and PCL(4500) micelles were smaller and larger, respectively, than 1% saturated ones. In addition, they showed a bimodal aggregation pattern. In both cases, sizes did not change much over time (Table 2). RIF-saturated 4 and 6% micelles were larger than their unsaturated counterparts and, at the beginning of the assay, they displayed a slight size growth (Table 2). Before, their size decreased (data not shown).

In vitro RIF chemical stability

The residence time of a formulation in the stomach depends on gastric emptying and is between 15 min and 3 h. Thus, we assessed the chemical stability of the encapsulated RIF *in vitro* over 3 h. In this time interval, all the systems followed zero-order release kinetics (Table 1 & Figure 2). Since 4% micelles showed a good balance between RIF encapsulation capacity, physical stability upon lyophilization and a relatively low release rate (<33%) over the first 3.5 h, stability studies were exclusively conducted on them. Free RIF (2.6 mg/ml) showed an apparent degradation rate constant (K_d) without and with INH of 3.1×10^{-3} and 4.1×10^{-3} min^{-1}, respectively (Table 3). INH increased the degradation rate by approximately 30%. The chromatogram of RIF in acid medium with INH presented a major peak of RIF and two secondary ones of equivalent area that probably corresponded to the main degradation products of RIF, 3-FRSV and isonicotinyl hydrazone (Figure 3). In the absence of INH, only one degradation product (probably 3-FRSV) was observed (data not shown).

In PCL(3700) micelles diluted 1:10 without INH, RIF degradation fitted first-order kinetics (Table 3) and K_d significantly decreased 3.9-fold

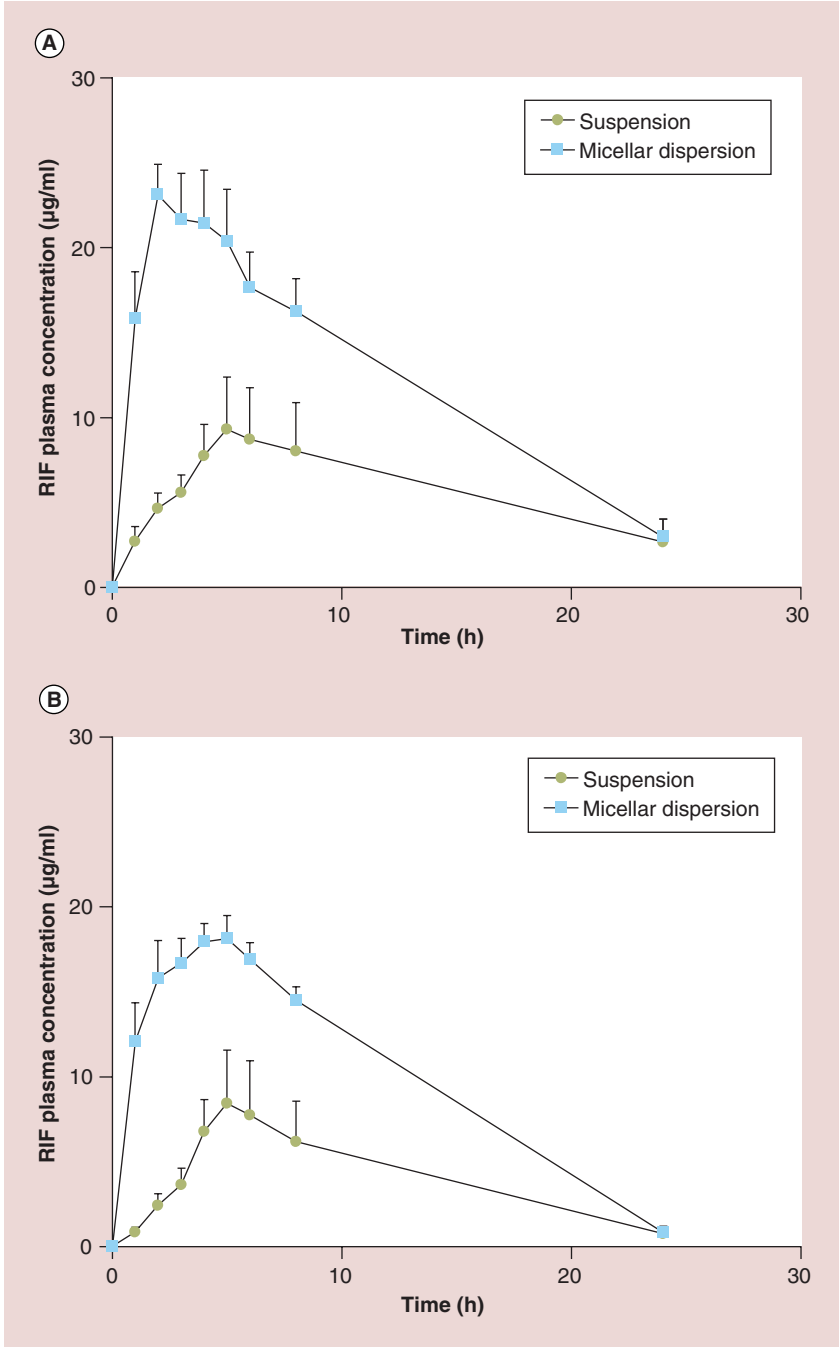

Figure 5. Rifampicin plasma concentrations upon oral administration of rifampicin suspension and fresh rifampicin-loaded micellar dispersion. RIF concentrations in the **(A)** absence and **(B)** presence of isoniazid. The RIF:isoniazid weight ratio was 3:2. Results are expressed as mean ± standard error of the mean (n = 6).
RIF: Rifampicin.

doi:10.2217/NNM.13.154

Table 4. Rifampicin pharmacokinetic parameters after the oral administration of the different formulations (n = 6).

Formulation composition	PK parameter	Formulation type					
		Suspension		*Fresh micellar dispersion†*		*Lyophilized micellar dispersion†‡*	
		Media	*SE*	*Media*	*SE*	*Media*	*SE*
RIF	C_{max} (μg/ml)	10.18	2.77	24.50*,**	2.24	25.50*,***	2.69
	t_{max} (h)	3.83	0.48	2.67	0.49	3.00	0.32
	AUC_{0-24} (μg/ml/h)	136.52	42.71	298.95*,**	31.06	243.14*,***	27.49
	k_e (h^{-1})	0.08	0.02	0.12	0.03	0.16	0.01
	F_r (%)	100.00	–	218.97	–	178.10	–
RIF/INH§	C_{max} (μg/ml)	9.58	2.85	19.93*	1.46	14.46	0.92
	t_{max} (h)	4.00	0.68	3.83	0.48	3.40	0.40
	AUC_{0-24} (μg/ml/h)	66.37	19.75	220.98*	25.32	146.29*,***	20.71
	k_e (h^{-1})	0.12	0.02	0.17	0.04	0.17	0.01
	F_r (%)	100.00	–	332.94	–	220.41	–

The rifampicin concentration was 10 mg/ml and the dose was 10 mg/kg.
†Copolymer concentration was 4% w/v.
‡Cryo-/lyo-protectant concentration in the micellar dispersion was hydroxylpropyl β-cyclodextrin 15% w/v.
§RIF:INH weight ratio was 3:2.
**The PK parameter was significantly higher ($p < 0.05$) for the micellar dispersion than for the suspension; **There was no significant difference ($p > 0.05$) between micellar dispersions before and after INH incorporation; ***There was no significant difference ($p > 0.05$) between the micellar dispersion before and after lyophilization.*
AUC_{0-24}: Area under the curve between 0 and 24 h; F_r: Relative oral bioavailability; INH: Isoniazid; K_e: Elimination rate constant; PK: Pharmacokinetic; RIF: Rifampicin; SE: Standard error of the mean; t_{max}: Time to the maximum concentration.

from 3.1×10^{-3} to 0.8×10^{-3} min^{-1}. A concomitant decrease of D_{3h} from 42.8 to 14.2% was observed (Figure 4A). PCL(4500) micelles diluted 1:10 showed a 4.4-fold decrease in their degradation rate ($K_d = 0.7 \times 10^{-3}$ min^{-1}) and a D_{3h} of 12.4%. In the presence of INH, degradation was reduced to a lesser extent; K_d and D_{3h} being 1.8×10^{-3} min^{-1} and 27.7%, respectively, for both copolymers (Table 3).

To study the stability under more extreme conditions, a 1:40 dilution was also assessed. RIF degradation also followed first-order kinetics and the degradation rates were significantly smaller than those of the control ($p < 0.05$), regardless of whether INH was present or absent (Table 3). However, the stability conferred by the micelle was partially curtailed (Figure 4B). For example, K_d values for PCL(4500) were 1.6×10^{-3} and 2.4×10^{-3} min^{-1} without and with INH, respectively, representing D_{3h} values of 24.1 and 35.1%.

Addition of HPβ-CD increased the release rate. Thus, we also expected a lower chemical stability. Lyophilized 4% PCL(4500) micelles containing 20% HPβ-CD and diluted 1:10 displayed a K_d of 1.1×10^{-3} and 2.1×10^{-3} min^{-1} without and with INH, respectively, which corresponded to D_{3h} of 17.2 and 32.0% (Figure 4C & Table 3). After 1:40 dilution, values without INH were still significantly better than the control with a K_d of 2.2×10^{-3} min^{-1} and D_{3h} of 32.1%. However, when the medium contained soluble INH, results were similar to the control (Figure 4D & Table 3).

To overcome this detrimental effect, we adjusted the concentration of the additive to attain a f_c value of ≤2, as previously established [28]. Micelles containing 10 and 13% cyclodextrin showed a f_c >2.0. Conversely, micelles with 15% HPβ-CD showed a f_c of 1.9 and redispersion was feasible, resulting in a completely translucent dispersion. Degradation rates upon 1:10 dilution without and with INH were significantly smaller than the control even without INH; K_d and D_{3h} being 0.8×10^{-3} and 2.1×10^{-3} min^{-1} and 13.4 and 31.5%, respectively (Figure 4C & D & Table 3). A similar trend was observed after 1:40 dilution, although degradation was faster than the fresh system, differences being nonstatistically significant (Figure 4C & D & Table 3). Furthermore, in freeze-dried micelles, RIF was more stable in the presence of INH than the free drug without INH.

Oral pharmacokinetics of RIF

Free RIF

Since both copolymers showed similar *in vitro* degradation kinetics, the pharmacokinetics were only evaluated for 4% PCL(4500) micelles. An INH-free RIF suspension resulted in a C_{max} of 10.18 μg/ml at 3.83 h. Thereafter, the RIF plasma concentration gradually decreased, with the drug being detectable for up to 24 h (Figure 5). In addition, the AUC_{0-24} was 136.5 μg/ml/h (Table 4). The coadministration of RIF and INH led to a slight decrease in the C_{max} to 9.58 μg/ml and a pronounced drop

in the AUC_{0-24} to 66.37 µg/ml/h (Table 4). Other parameters (e.g., k_e) remained almost unchanged (Table 4).

Fresh RIF-loaded polymeric micelles

Fresh RIF-loaded micelles without INH showed a significant ($p < 0.05$) increase in the C_{max} from 10.18 µg/ml (control) to 24.50 µg/ml (Table 4). t_{max} values showed a slight decrease from 3.83 to 2.67 h (Figure 5). The AUC_{0-24} for the RIF-loaded micelles also showed a significant increase ($p < 0.05$) of 2.2-times with respect to the suspension (Table 4). The coadministration of free INH and RIF-loaded micelles showed a significant 2.1-fold increase ($p < 0.05$) in the C_{max} (Table 4) from 9.58 to 19.93 µg/ml. The increase in the AUC_{0-24} was even more remarkable, from 136.52 to 220.98 µg/ml/h (Table 4). Furthermore, k_e and t_{max} values did not change substantially. A decrease in the C_{max} and AUC_{0-24} was observed in the presence of INH, with the differences not being significant ($p > 0.05$) (Table 4).

Freeze-dried RIF-loaded polymeric micelles

Freeze-dried micelles in the absence of INH showed a significant ($p < 0.05$) increase in C_{max} and AUC_{0-24} with respect to the suspension (control) (Table 4 & Figure 6). For example, RIF bioavailability increased almost 1.8 times (F_r [%] of 178.10). However, AUC_{0-24} values were lower than those observed for the fresh counterparts, with the difference not being significant (Table 4). Once more, differences in k_e and t_{max} were negligible. The coadministration of this formulation with INH also led to a decrease in both C_{max} and AUC_{0-24} with respect to the fresh system (Figure 6). At the same time, AUC_{0-24} values for the lyophilized micelles were significantly greater ($p < 0.05$) than the RIF suspension, with the F_r (%) being 220.41.

Discussion

The present work evaluated a platform of polymeric micelles to develop an extemporaneous liquid RIF/INH FDC for the therapy of non-resistant TB in children. Two amphiphiles that showed the best RIF encapsulation capacity were used [27]. The goal of the nanoencapsulation was to increase the aqueous solubility of the drug and to stabilize it in acid medium. Our hypothesis was that only the free drug would undergo hydrolysis. Thus, the release study was initially aimed to determine the amount of drug that was released in a time interval that fits the gastric (~3 h) and gastrointestinal (~6 h) transits. Release data indicated that the process was mainly controlled by the micelle and not by the dialysis membrane.

A parameter that governs the encapsulation process is the CL:EO ratio. An increase in the relative length of the PCL blocks may result

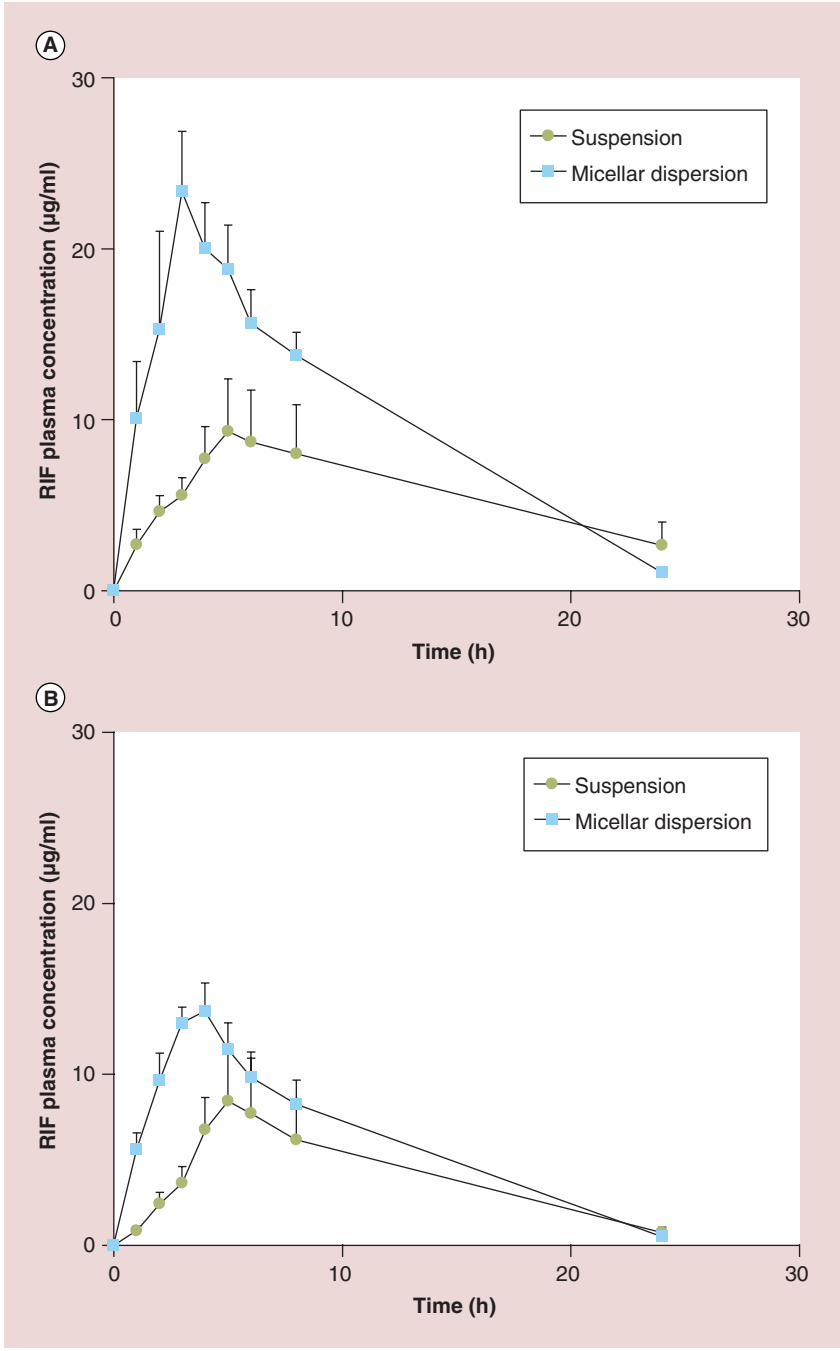

Figure 6. Rifampicin plasma concentrations upon oral administration of rifampicin suspension and lyophilized rifampicin-loaded micellar dispersion. RIF concentration in the **(A)** absence and **(B)** presence of isoniazid. The RIF:isoniazid weight ratio was 3:2 and the cryo-/lyo-protectant concentration was 15% w/v. Results are expressed as mean ± standard error of the mean (n = 6). RIF: Rifampicin.

doi:10.2217/NNM.13.154

in two competing effects: stronger copolymer–hydrophobic drug interactions and tighter packing of the core during self-aggregation. The former hinders the release, while the latter facilitates it [37]. In systems with constant RIF payloads (4.9 mg/ml) and 1% copolymer concentration, the second effect was more relevant and PCL(3700) released at a slower rate than PCL(4500). A total of 6% of unsaturated micelles followed the opposite trend and a slower release was observed for PCL(4500). The 4% systems showed very similar release profiles, suggesting that both effects would be counterbalanced. RIF-saturated micelles (4 and 6%) showed a faster release rate (**Figure 2C & D**), probably owing to the presence of a greater number of RIF molecules hosted in outer layers of the micelle that went through a shorter path to be released [38]. In addition, slightly longer PCL blocks led to stronger drug–core interactions and slower release rates [39].

The release kinetics from polymeric micelles are complex and involve various mechanisms [40]. In the Korsmeyer–Peppas model, n values of 0.43 indicate Fickian diffusion release [32,33]. Conversely, n values between 0.43 and 0.85 reveal mass transfer according to a non-Fickian release (anomalous transport) [40,41]; the release relies on a combination of drug diffusion and relaxation of the polymeric chains. Finally, $n \geq 0.85$ indicates supercase II transport where the drug release is purely controlled by polymer relaxation [32,33]. The release from 1% RIF-saturated systems was mainly controlled by copolymer chain relaxation ($n \geq 0.85$). In 4% micelles, the release was the result of both diffusion and copolymer chain relaxation mechanisms (anomalous transport). A further increase to 6% resulted in a release mainly controlled by diffusion. In addition, higher copolymer concentrations favored the generation of larger aggregates (**Table 2**), where the diffusion of RIF molecules would demand longer times compared with smaller micelles [42].

RIF disturbs the self-aggregation of PCL-*b*-PEG-*b*-PCL amphiphiles [27]. Depending on the CL:EO ratio, the behavior of 1% micelles was different or not to that of samples that contained the same drug payload, but a greater copolymer concentration (**Figure 1A & B**). Samples were also monitored by dynamic light scattering to assess the effect of drug release on the micellar size and size distribution. The release from RIF-saturated 1% systems led to a sharp micelle shrinkage even when the accumulated release was less than 20% (**Table 2**). These data confirmed that RIF enlarges the micelle upon encapsulation [27]. Saturated 4 and 6% micelles displayed a slight size growth and the occasional appearance of additional size populations probably due to some swelling during the initial stages of release. In a longer-term range (24 h), sizes always decreased (data not shown). The size of unsaturated PCL(3700) and PCL(4500) micelles did not change substantially. Irrespective of the CL:EO ratio, micelles of the same copolymer (and drug concentration) showed similar size and size distribution, indicating that the size of the aggregate is mainly governed by the copolymer concentration and not by the drug cargo.

Overall findings strongly suggested the ability of the micelles to sustain the release of RIF, a phenomenon that was expected to isolate and protect it from the acid medium and soluble INH.

The main goal of this work was to stabilize RIF in contact with an acid medium in the presence of INH and, by doing so, improve its oral bioavailability. Samples were diluted to mimic the dilution extent that liquid formulations usually undergo in gastric fluids (total volume of ~500 ml) [22,35]. Nanoencapsulation resulted in a significant decrease in the degradation rate with respect to the free drug. In addition, degradation always fitted a first-order model. Regardless of the fact that the final copolymer concentration after dilution was always above the corresponding critical micellar concentration [27], the generation of micelles that are more permeable to the external medium owing to dilution could be not be ruled out. Findings of release and chemical degradation *in vitro* were in good agreement and strongly suggested that only free RIF molecules underwent degradation.

CDs are cyclic oligosaccharides used to enhance the aqueous solubility of hydrophobic drugs, including RIF [43,44]. This interaction would explain the faster RIF release and higher degradation extent in lyophilized samples. Thus, the formation of a RIF/CD inclusion complex increased the release rate and jeopardized the stabilization capacity of the micelles. The reduction of the lyoprotectant concentration to 15% had a beneficial effect and the degradation parameters in the presence of INH remained significantly smaller than those measured for free RIF without INH (**Table 3 & Figure 4**). Differences for K_d and D_{3h} values between fresh and lyophilized samples were not significant. These findings support the hypothesis that the protective effect stems from the ability of micelles to isolate RIF from the surrounding medium and to prevent the diffusion of INH molecules into the RIF reservoir. It is

remarkable that the degradation rate of encapsulated RIF in the presence of INH was always slower than that of free RIF even without INH.

Since the oral bioavailability of RIF is a key parameter to achieve an effective TB treatment, the effect of RIF encapsulation was evaluated *in vivo*. The gastric and duodenal pH values in rats are approximately 3.9 and 5.9, respectively [45]. For a RIF suspension, the decrease in C_{max} and AUC_{0-24} with INH (Table 4) was in full agreement with the *in vitro* stability studies (Table 3). Extravascular drug administration might lead to incomplete drug absorption due to limited dissolution in the gastrointestinal fluids. Thus, the solubilization within the micelles increased the oral bioavailability of the drug. Moreover, drug presystemic elimination, particularly after oral administration, could be related to acid or enzymatic degradation, quelation and intestinal bacteria metabolization [46]. The capacity of the micelles to encapsulate RIF, increase its aqueous solubility and sustain its release (Figure 2) not only prevented degradation in acid medium (Figure 4), but also contributed to optimize the intestinal absorption of the drug (Figures 5 & 6). The slight decrease in t_{max} for the micelles could be explained by the greater solubility of the drug in the gut, while similar k_e values suggested that the elimination mechanisms were not altered by the micelles, which are mostly eliminated in the feces. Although the coadministration of drug-loaded micelles and INH led to a decrease in the C_{max} and AUC_{0-24} compared with formulations without INH, the relative oral bioavailability was significantly greater than that of the suspension. The highest oral bioavailability improvement (3.3-fold) was attained with fresh micelles coadministered with INH at a clinically relevant drug weight ratio. HPβ-CD showed some detrimental effects on the pharmacokinetics of RIF, probably owing to the formation of complexes [47–49]. For instance, this study also suggested that HPβ-CD alters the permeability of RIF *in vivo* and reduces oral bioavailability compared with the fresh dispersion (Table 4).

Conventional *in vitro* release studies from nanocarriers have been developed based on dissolution tests used for quality control of solid formulations, and they are of limited value to predict the performance of the nanodrug delivery system *in vivo* without the development and validation of an *in vitro–in vivo* correlation, a goal that was beyond the scope of this work. This phenomenon is particularly valid for the oral route, owing to the complexity of the GI tract. Conversely, in this study, *in vitro* release assays mainly aimed to shed light on the parameters that affect the chemical stabilization of RIF in acid medium. Findings *in vitro* confirmed the intimate association between release and degradation. At the same time, it should be stressed that the ability of the micelles to partially delay the release of RIF for 2–3 h and to minimize the direct contact of the drug with the gastric medium explains the improved bioavailability of RIF in the micelles compared with the suspension even under the most disadvantageous administration conditions, namely in the presence of soluble INH.

The increase in AUC_{0-24} could be related to the oral effectiveness of the drug. The antimicrobial activity of RIF is time and concentration dependent and the ratio between AUC and the minimum inhibitory concentration is a parameter that correlates well with the bactericidal efficacy [50]. Thus, the enhanced preclinical oral bioavailability attained with this innovative nanopharmaceutical product could enable a reduction in the administration frequency and increased patient compliance, and supports the potential of this strategy for the first-line therapy of pediatric TB.

Conclusion

This study demonstrated the feasibility of polymeric micelles to protect RIF from degradation in acid medium in the presence of INH. This process optimized the pharmacokinetics under a recommended clinical coadministration regimen. The addition of a cryo-/lyo-protectant was appropriately adjusted to minimize the release from the micelles, a phenomenon that, if not controlled well, could increase the degradation rate and reduce the permeability *in vivo*. Even under these less favorable conditions, polymeric micelles were shown to be a valuable platform to improve the performance of the FDC. In this context, our development would enable a fast redispersion of the RIF/INH powder in water and its administration as a limpid liquid formulation. Independent of the preliminary *in vivo* results, more advanced studies in an aerosol-infected TB animal model should be performed to correlate the improved pharmacokinetics with greater anti-TB efficacy.

Future perspective

The development of child-friendly formulations, including FDCs, is being addressed by few pharmaceutical companies in developing nations [105]. Although these products would make patients more compliant, the chemical stability of RIF in the stomach remains doubtful. In other words, considering that in these products RIF is not

doi:10.2217/NNM.13.154

isolated from INH, once the tablet disintegrates *in vivo* and drugs come into contact with the acid medium, the degradation of RIF will occur. In this regard, research of innovative pharmaceutical products that overcome this crucial biopharmaceutic disadvantage remains an urgent matter. At the same time, we must realize that the progresses made at the interface of poverty-related diseases and pharmaceutical development have been very limited. In this context, the foundation and consolidation of multidisciplinary and multisectoral initiatives that capitalize on the available infrastructures, economic and human resources, and technology platforms is crucial to reduce the development time and costs and, by doing so, to improve the access of specific patient subpopulations (e.g., children) to improved medicines.

Acknowledgements

The authors thank D Chiappetta for technical assistance.

Financial & competing interests disclosure

MA Moretton thanks PhD scholarships from Consejo Nacional de Investigaciones Científicas y Técnicas. The work was supported by grants from University of Buenos Aires (UBACyT 20020090200189) and Consejo Nacional de Investigaciones Científicas y Técnicas (PIP0220). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

In vitro *rifampicin release from polymeric micelles*

- Overall, research strongly suggests the ability of the micelles to sustain the release of rifampicin (RIF). The release from 1% RIF-saturated micelles is mainly controlled by copolymer chain relaxation ($n \geq 0.85$). In 4% micelles, the release is the result of both diffusion and copolymer chain relaxation mechanisms (anomalous transport). A further increase to 6% results in a release mainly controlled by diffusion.

Effect of RIF release on the micellar size & size distribution

- The release of RIF from drug-saturated 1% micelles is accompanied by a fast decrease in the micellar size, while the size of RIF-unsaturated 4 and 6% micelles does not change much over time. Conversely, RIF-saturated 4 and 6% micelles combined an initial slight size growth (2 h) with a later decrease (24 h).

In vitro *RIF chemical stability*

- The encapsulation of RIF significantly decreases the degradation rate compared with the free drug, and this protection is associated with the drug release kinetics. This protective effect is also observed in micelles that are freeze-dried with a cryo-/lyo-protectant.

Oral pharmacokinetics of RIF

- *In vivo* data indicate a statistically significant increase in the RIF oral bioavailability (up to 3.3-times) compared with a suspension containing free RIF and soluble isoniazid.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Kaufmann SH, McMichael AJ. Annulling a dangerous liaison: vaccination strategies against AIDS and tuberculosis. *Nat. Med.* 11, S33–S44 (2005).

2 Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. *Lancet* 362, 887–899 (2003).

3 Sharma S, Sharma PK, Kumar N, Dudhe R. A review on various heterocyclic moieties and their antitubercular activity. *Biomed. Pharmacother.* 65, 244–251 (2011).

4 Bertel Squire S, Thomson S. Tuberculosis and poverty. In: *Tuberculosis – A Comprehensive Clinical Reference.* Schaaf SH, Zumla AL, Grange JM *et al.* (Eds). Saunders Elsevier, London, UK (2009).

5 WHO declares tuberculosis a global emergency. *Soc. Prevent. Med.* 38, 251–252 (1993).

6 Rivers EC, Mancera RL. New antituberculosis drugs in clinical trials with novel mechanism of action. *Drug Discov. Today* 13, 1090–1098 (2008).

7 Matteelli A, Carvalho A, Dooley KE, Kritski A. TMC207: the first compound of a new class of potent anti-tuberculosis drugs. *Future Microbiol.* 5, 849–858 (2010).

8 Cole ST, Brosch R, Parkhill J *et al.* Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393, 537–544 (1998).

9 Singh S, Mariappan TT, Sharda N, Kumar S, Chakrabarti AK. The reason for an increase in decomposition of rifampicin in the presence of isoniazid under acid conditions. *Pharm. Pharmacol. Commun.* 6, 405–410 (2000).

10 Shishoo CJ, Shah SA, Rathod IS, Savale SS, Kotecha JS, Shah PB. Stability of rifampicin in dissolution medium in presence of isoniazid. *Int. J. Pharm.* 190, 109–123 (1999).

▪ **Good study of the degradation of rifampicin in the absence and presence of isoniazid.**

11 Sankar R, Sharda N, Singh S. Behavior of decomposition of rifampicin in the presence of isoniazid in the pH range 1–3. *Drug Dev. Ind. Pharm.* 29, 733–738 (2003).

12 Shishoo CJ, Shah SA, Rathod IS, Savale SS. Impaired bioavailability of rifampicin from fixed dose combination (FDC) formulations with isoniazid. *Indian J. Pharm. Sci.* 63, 443–449 (2001).

13 Singh S, Mariappan TT, Sankar R, Sarda N, Singh B. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combinations (FDC) products, and the likely solutions to the problem. *Int. J. Pharm.* 228, 5–17 (2001).

14 Blomberg B, Spinaci S, Fourie B, Laing R. The rationale for recommending fixed-dose combination tablets for treatment of tuberculosis. *Bull. World Health Organ.* 79, 61–68 (2001).

15 Swaminathan S, Rekha B. Pediatric tuberculosis: global overview and challenges. *Clin. Infect. Dis.* 50, S184–S194 (2010).

16 Trouiller P, Olliaro P, Torreele E, Orbinski J, Laing R, Ford N. Drug development for neglected diseases: a deficient market and a public-health policy failure. *Lancet* 359, 2188–2194 (2002).

17 Sosnik A, Seremeta KP, Imperiale JC, Chiappetta DA. Novel formulation and drug delivery strategies for the treatment of pediatric poverty-related diseases (PRDs). *Expert Opin. Drug Deliv.* 9, 303–323 (2012).

▪▪ **Comprehensive review on the state-of-the-art in innovative pharmaceutical product development in the context of poverty-related diseases.**

18 Sosnik A, Carcaboso AM, Glisoni RJ, Moretton MA, Chiappetta DA. New old challenges in tuberculosis: potentially effective nanotechnologies in drug delivery. *Adv. Drug Del. Rev.* 62, 547–559 (2010).

▪▪ **Thorough overview of the main nanotechnology approaches to improve the pharmacotherapy of tuberculosis.**

19 Choonara YE, Pillay V, Ndesendo VM *et al.* Polymeric emulsion and crosslink-mediated synthesis of super-stable nanoparticles as sustained-release anti-tuberculosis drug carriers. *Colloids Surf. B Biointerfaces* 87, 243–254 (2011).

20 Sosnik A, Carcaboso A, Chiappetta DA. Polymeric nanocarriers: new endeavors for the optimization of the technological aspects of drugs. *Recent Pat. Biomed. Eng.* 1, 43–59 (2008).

21 Chiappetta DA, Sosnik A. Poly(ethylene oxide)-poly(propylene oxide) block copolymer micelles as drug delivery agents: improved hydrosolubility, stability and bioavailability of drugs. *Eur. J. Pharm. Biopharm.* 66, 303–317 (2007).

22 Chiappetta DA, Hocht C, Taira C, Sosnik A. Efavirenz-loaded polymeric micelles for pediatric anti-HIV pharmacotherapy with significantly higher oral bioavailaibility. *Nanomedicine (Lond.)* 5, 11–23 (2010).

23 Chiappetta DA, Hocht C, Taira C, Sosnik A. Oral pharmacokinetics of efavirenz-loaded polymeric micelles. *Biomaterials* 32, 2379–2387 (2011).

▪ **Comprehensive study on the improvement of the oral bioavailability of a model drug, the antiretroviral efavirenz, by the oral route.**

24 Peroni RN, Di Gennaro SS, Hocht C *et al.* Efavirenz is a substrate and in turn modulates the expression of the efflux transporter ABCG2/BCRP in the gastrointestinal tract of the rat. *Biochem. Pharmacol.* 82, 1227–1233 (2011).

25 Chiappetta DA, Hocht C, Opezzo JA, Sosnik A. Intranasal administration of antiretroviral-loaded micelles for anatomical targeting to the brain in HIV. *Nanomedicine (Lond.)* 8, 223–237 (2013).

26 Ribeiro A, Sosnik A, Chiappetta DA, Veiga F, Concheiro A, Alvarez-Lorenzo C. Single and mixed poloxamine micelles as suitable nanocarriers for solubilization and sustained release of ethoxzolamide for topical glaucoma therapy. *J. R. Soc. Interface* 9, 2059–2069 (2012).

27 Moretton MA, Glisoni RJ, Chiappetta DA, Sosnik A. Molecular implications in the nanoencapsulation of the antituberculosis drug rifampicin within flower-like polymeric micelles. *Colloids Surf. B Biointerfaces* 79, 467–479 (2010).

▪ **First study of the nanoencapsulation of rifampicin within polymeric micelles.**

28 Moretton MA, Chiappetta DA, Sosnik A. Cryoprotection–lyophilization and physical stabilization of rifampicin loaded flower-like polymeric micelles. *J. R. Soc. Interface* 9, 487–502 (2012).

29 Gotelli G, Bonelli P, Abraham G, Sosnik A. Fast and efficient synthesis of high molecular weight poly(ε-caprolactone) diols by microwave-assisted polymer synthesis (MAPS). *J. Appl. Polym. Sci.* 121, 1321–1329 (2011).

30 Sosnik A, Gotelli G, Abraham GA. Microwave-assisted polymer synthesis (MAPS) as a tool in biomaterials science: how new and how powerful. *Prog. Polym. Sci.* 36, 1050–1078 (2011).

31 Sreenivasa Rao B, Ramana Murthy KV. Studies on rifampicin release from ethylcellulose coated nonpareil beads. *Int. J. Pharm.* 231, 97–106 (2002).

32 Ritger PL, Peppas NA. A simple equation for description of solute release I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *J. Control. Release* 5, 23–36 (1987).

33 Ritger PL, Peppas NA. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *J. Control. Release* 5, 37–42 (1987).

34 Hu Y, Ding Y, Li Y, Jiang X, Yang C, Yang Y. Physical stability and lyophilization of poly(caprolactone)-*b*-poly(ethyleneglycol)-*b*-poly(caprolactone) micelles. *J. Nanosci. Nanotech.* 6, 1–8 (2006).

35 Chiappetta DA, Hocht C, Sosnik A. Highly concentrated and taste-improved aqueous formulation of efavirenz for a more appropriate pediatric management of the anti-HIV therapy. *Curr. HIV Res.* 8, 223–231 (2010).

36 Moretton MA, Glisoni RJ, Chiappetta DA, Sosnik A. Synthesis and characterization of amphiphilic poly(ε-caprolactone)-poly(ethylene glycol) block copolymers. Optimization of the solubility and stability of rifampicin by means of encapsulation into polymeric micelles. Presented at: *BIOOMAT 2009, I Workshop on Artificial Organs, Biomaterials and Tissue Engineering.* Rosario, Argentina, 25–27 August 2009.

37 Agrawal SK, Sanabria-DeLong N, Coburn JM, Tew GN, Bhatia SR. Novel drug release profiles from micellar solutions of PLA–PEO–PLA triblock copolymers. *J. Control. Release* 112, 64–71 (2006).

38 Bastakoti BP, Guragain S, Yokoyama Y, Yusa S-I, Nakashima K. Incorporation and release behavior of amitriptylene in core–shell–corona type triblock copolymer micelles. *Colloids Surf. B Biointerfaces* 88, 734–740 (2011).

39 Lin WJ, Juang LW, Lin CC. Stability and release performance of a series of pegylated copolymeric micelles. *Pharm. Res.* 20, 668–673 (2003).

40 Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* 13, 123–133 (2001).

▪▪ **Wide review on the different mathematical models used to fit drug release data.**

41 Wang Q, Zhang J, Wang A. Preparation and characterization of a novel pH-sensitive chitosan-*g*-poly (acrylic acid)/attapulgite/sodium alginate composite hydrogel bead for controlled release of diclofenac sodium. *Carbohyd. Polym.* 78, 731–737 (2009).

42 Forrest ML, Won C-Y, Malick WA, Kwon GS. *In vitro* release of the mTOR inhibitor rapamycin from poly(ethylene glycol)-*b*-poly(-caprolactone) micelles. *J. Control. Release* 110, 370–377 (2006).

43 Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. *Adv. Drug Deliv. Rev.* 59, 645–666 (2007).

doi:10.2217/NNM.13.154

44 Tewes F, Brillault J, Couet W, Olivier J-C. Formulation of rifampicin–cyclodextrin complexes for lung nebulisation. *J. Control. Release* 129, 93–99 (2008).

45 McConnell EL, Basit AW, Murdan S. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for *in vivo* experiments. *J. Pharm. Pharmacol.* 60, 63–70 (2004).

46 Armijo JA. Absorción, distribución y eliminación de fármacos. In: *Farmacología Humana (3rd Edition)*. Florez J, Armijo JA, Mediavilla A (Eds). Masson, Barcelona, Spain (1997).

47 Loftsson T, Brewster ME. Cyclodextrins as functional excipients: methods to enhance complexation efficiency. *J. Pharm. Sci.* 101, 3019–3032 (2012).

▪ **Updated review on the most relevant pharmaceutical applications of cyclodextrins.**

48 Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: effects on drug permeation through biological membranes. *J. Pharm. Pharmacol.* 63, 1119–1135 (2011).

49 Jansook P, Loftsson T. CDs as solubilizers: effects of excipients and competing drugs. *Int. J. Pharm.* 379, 32–40 (2009).

50 Jayaram R, Gaonkar S, Kaur P *et al.* Pharmacokinetics–pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. *Antimicrob. Agents Chemother.* 47, 2118–2124 (2003).

Websites

101 WHO. Global Tuberculosis Report. www.who.int/tb/publications/global_report/2011/gtbr11_full.pdf (Accessed January 2013)

102 WHO. Treatment of tuberculosis: guidelines. http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf (Accessed January 2013)

103 WHO. The top ten causes of death. www.who.int/mediacentre/factsheets/fs310/en/index.html (Accessed August 2012)

104 WHO. Dosing instructions for the use of currently available fixed-dose combination TB medicines for children. www.who.int/tb/challenges/interim_paediatric_fdc_dosing_instructions_sept09.pdf (Accessed July 2013)

105 WHO. WHO list of prequalified medicinal products. http://apps.who.int/prequal/query/ProductRegistry.aspx?list=tb (Accessed March 2013)