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Drug Targeting to Lungs by Way of Microspheres

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In many conventional drug delivery systems in vogue, failure to deliver efficient drug delivery at the target site/organs; is evident as a result, less efficacious pharmacological response is elicited. Microspheres can be derived a remedial measure which can improve site-specific drug delivery to a considerable extent. As an application, Lung-targeting Ofloxacin-loaded gelatin microspheres (GLOME) were prepared by water in oil emulsion method. The Central Composite Design (CCD) was used to optimize the process of preparation, the appearance and size distribution were examined by scanning electron microscopy, the aspects such as *in vitro* release characteristics, stability, drug loading, loading efficiency, pharmacokinetics and tissue distribution in albino mice were studied. The experimental results showed that the microspheres in the range of 0.32-22 μm. The drug loading and loading efficiency were 61.05 and 91.55% respectively. The *in vitro* release profile of the microspheres matched the korsmeyer's peppas release pattern, and release at 1h was 42%, while for the original drug, ofloxacin under the same conditions 90.02% released in the first half an hour. After i.v. administration (15 min), the drug concentration of microspheres group in lung in albino mice was 1048 μg/g, while that of controlled group was 6.77 μg/g. GLOME found to release the drug to a maximum extent in the target tissue, lungs.

Key words: Ofloxacin, Gelatin, Microspheres, *In vitro*, *In vivo*, Compartment model, Lung-targeting

INTRODUCTION

Pneumonia is one of the widely prevalent diseases of both developed and developing world. The incidence of pneumonia in most cases are found in endemic and epidemic proportions, currently making this one of the difficult disease to manage and is rated as the sixth highest disease in terms of contraction and fatality (Peng *et al.*, 2001). The current therapeutic practices to contain and cure pneumonia centers around anti-microbial therapy which in most cases is of empirical nature, since pneumonia is caused by a variety of bacterial flora and hence a low success rate is achieved in spite of advances in newer molecules available for treatment.

Ofloxacin of late has emerged as the gold standard in the treatment of pneumonia, nevertheless the conventional approaches adopted to administer ofloxacin by oral or parenteral routes does not provide the most efficacious mode of treatment (Yuk *et al.*, 1991).

Microspheres are solid colloidal particles ranging in size from 1 to 1000 μm, particles with diameter 7-15 μm when given through i.v. route are trapped by the first capillary system encountered. Exploring alternative routes of administration like microspheres to develop a targeted drug delivery system to act locally on the organ of infection will improve the therapeutic efficacy, reduce side effects and thereby provide patient compliance (Illum *et al.*, 1982; Delgado *et al.*, 2000; Dhanaraj *et al.*, 2001).

In the present work we report the microspheres of ofloxacin using biodegradable polymer, gelatin as carrier and injected into the vein of albino mice. The results showed that the microspheres were accumulated almost entirely in the lung after i.v. injection and have a good sustained release efficacy.

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MATERIALS AND METHODS

Materials

Gelatin (prepared by alkaline method-type-B bloom strength 250, Shree Ram Industry, Punjab) was used as carrier. Ofloxacin (content 99.85%, provided by Micro Labs, Hosur, India). The other reagents used were of analytical grade.

Preparation of ofloxacin gelatin microspheres

Ofloxacin gelatin microspheres, designated GLOME, were prepared by emulsion method (Brime *et al.*, 2000). Microspheres were prepared by varying parameter such as stirring rate (1000-5000 rpm), concentration of gelatin (0.5-2 g) and amount of emulsifying agent (0.5-2 mL). A solution of gelatin (gelatin dissolved in 10 mL of water) was prepared by warming and the drug was added to the gelatin solution. The concentration of drug was varied according to the varying concentration of gelatin (1:1 Drug: Gelatin). The solution was stirred for 20 min at 25°C and the solution was pre heated to 80°C and added to 30 mL of liquid paraffin previously warmed to the same temperature. This two-system, plus Tween 85 was added to obtain a w/o emulsion. The resulting microspheres were stabilized using 0.1 mL formaldehyde solution (25%w/v) for a period of 15 min. After 5 min of continuous stirring, the emulsion was rapidly cooled to 5°C and 100 mL of acetone added in order to dehydrate and flocculate the coacervated particles. Gelatin microspheres were then isolated by filtering the suspension through a sintered glass filter. Washing is necessary for washing off the liquid paraffin and excessive formaldehyde, though at the same time the drug on the surface and the drug loading rate will be also reduced. The preparation was carried out according to optimization procedure Table I.

Appearance and size distribution measurement

The surface morphology of the microspheres was observed by scanning electron microscope. Particle size distribution and measurement were carried out using optical microscopy (Lu and Wu., 1999).

Determination of ofloxacin drug loading and loading efficiency

A weighed quantity of microspheres was dissolved in 0.1N HCl. The ofloxacin content was assayed by spectrometry at 297nm using a calibration curve. The calibration curve: A = 0.314x + 0.0063. The method recovery was 91.53%. The experiments were conducted in triplicate. The average value of ofloxacin content is the drug loading. The loading efficiency was calculated using the following formula (Jia *et al.*, 1997).

$$\text{Loading efficiency } (\%) = (Q_m/W_m) \times 100$$

where Q_m actual is the actual amount of ofloxacin in each composite and W_m theoretical amount of ofloxacin in each composition calculated from the quantity added in the fabrication process (Lu *et al.*, 2003).

In vitro dissolution study of microspheres

In vitro release patterns were studied using conventional dialysis technique. Ofloxacin gelatin microspheres were placed in a dialysis bag and dialyzed against 200 mL of phosphate saline buffer (PBS), pH 7.4 at 37±1°C. Sink conditions were maintained and throughout the course of study and were stirred continuously using a magnetic stirrer. Aliquots were withdrawn at specific time intervals and the same volume of0 dissolution medium was added to the flask to maintain a constant volume. (Zhang *et al.*, 1994) The withdrawn aliquots were estimated through UV spectrometry method at 297 nm, and the data were used to calculate a cumulative drug release profile from the microspheres. The accumulated amount of drug released was calculated using a calibration curve.

The stability of GLOME

The microspheres powders were put into a bottle and stored for 12 months at 3-5°C, 15-25°C, and 37°C, respectively. The surface morphology and ofloxacin content were examined periodically.

In vivo pharmacokinetics of microspheres

Thirty six adult albino mice weighing between 20±2 g were selected at random and divided into 6 groups to test the drug release at the following time periods -10 min, 30 min, 1 h, 3 h, 6 h, and 12 h. Prior to the administration, the study groups were randomly divided into 6 groups, with 6 animals in each group. One group was administered 0.52 mg/kg ofloxacin injection *via* the tail route vein, while the other group received equivalent amount of drug in a GLOME suspension form. All the animals were kept on starvation 12 h before injection, with free access to water. At predetermined time intervals as stated earlier the animals were injected with the microspheres *via* the tail route vein; and were sacrificed by cervical dislocation. The organs, which were studied, for target action-namely lungs, liver and spleen were extracted. The tissue samples were stored for 24 h at -20°C. Then the concentrations of drug localized in each organ as the result of targeted release were determined through extraction method. (Zhang *et al.*, 1992)

The extracted organs were homogenized by adding saline at the ratio of 0.1 mg/mL. The protein in the mixture was precipitated (to prevent interference during the quantification process) by adding 2M perchloric acid (100 μL).

The mixture was then vortexed for 5 seconds to aid the mixing process and was then centrifuged at 5000 rpm for 15 min (Tadashi *et al.*, 1992; Masakazu *et al.*, 1994; Himangshu *et al.*, 2004; Gupta *et al.*, 1989). The prepared samples were used to determine the amount of ofloxacin present in the tissue samples quantitatively using the above HPLC method.

Tissue distribution of microspheres

Thirty six adult albino mice weighing between 20±2 g were selected at random and divided into 6 groups to test the drug release at the following time periods -10 min, 30 min, 1 h, 3 h, 6 h, and 12 h. Prior to the administration, the study groups were randomly divided into 6 groups, with 6 animals in each group. All the animals were kept on starvation 12 h before injection, with free access to water. A dose of 0.52 g/kg was administered to each of the animal as dispersion in saline with 1% of tween 80. The animals were injected with the gelatin microspheres *via* tail route vein; and were sacrificed by cervical dislocation. The organs, which were studied, for target action-namely lungs, liver and spleen were extracted. The tissue samples were stored for 24 h at -20°C, and then 1 g of tissue sample was collected, respectively, after surface water was dried. The drug concentration in tissues was determined by HPLC quantification method (Huo *et al.*, 2005).

RESULTS AND DISCUSSION

Optimization of microspheres preparation was performed by Response Surface Methodology (RSM) (specifically; randomized rotatable central composite designs-CCD) as it is the most suitable technique for the modeling and analysis of problems in which the response of interest is non-linear and influenced by several variables. The mathematical modeling was carried out by employing the software - *Design Expert* Ver. 6.0.5 © Statease Inc., U.S.A.

A central composite design was created to study the main effects and interactions of these three factors on the particle size. Fifteen microspheres formulations were prepared as per Table I to elucidate the effect of the three independent variables; stirring rate (rpm) concentration of polymer (Gelatin - g) and amount of emulsifying agent (Tween 85 - mL) and the dependent response variable measured was the particle size distribution. Experiments were conducted in random sequence in a face-centered manner in order to evaluate the interaction. The particle size optimization data is shown in the Table I.

The experimental output showed the particle size of microspheres to lie in a range of 0.32-22 μm, and is not affected by transformations and this followed a linear setup which indicated that the response measured holds good over a range of other factors namely stirring, polymer concentration and the amount of emulsifier used. This would in turn help in fixing the optimal particle size range, which has the maximum efficacy and the effect of other factors in arriving at this range.

Table I. Results for DOE for ofloxacin gelatin microspheres

		Factor 1	Factor 2	Factor 3	Response
Sl #	Run	A: Stirring Rate	B: Gelatin Conc.	C: Amt. of EA	Particle Size
		rpm	g	mL	μm
1	1	3000	1.25	1.25	2.1
2	5	3000	1.25	1.25	2.3
3	6	3000	1.25	1.25	2.3
4	13	3000	1.25	1.25	2.2
5	11	3000	1.25	1.25	2.3
6	3	5000	2	0.5	16.2
7	15	5000	0.5	2	0.32
8	10	1000	2	2	21.2
9	14	1000	0.5	0.5	5.1
10	7	1000	1.25	1.25	15
11	9	5000	1.25	1.25	1.3
12	8	3000	0.5	1.25	3.1
13	12	3000	2	1.25	22
14	2	3000	1.25	0.5	14
15	4	3000	1.25	2	1.2

rpm = rotation per minute, EA = Emulsifying Agent, g = grams, mL = milliliter, μm=micrometer

The polynomial equation giving the mathematical relationship between each factor was found to be:

Particle Size = (RPM) 5.44755 – 1.18010E-003
– (Gelatin Conentration) – 4.90412 × 10.0492
– (Amount of Emulsifying Agent) + 3.20294E-007
– $(RPM)^2$ + 10.09987
– $(Gelatin\ Concentration)^2$ + 1.29987
– $(Amount\ of\ Emulsifying\ Agent)^2$ – 4.30333E-003
– (RPM) – (Gelatin Concentration) + 9.70000E-004
– (RPM) – (Amount of Emulsifying Agent) – 7.83111
– (Gelatin Concentration) × (Amount of Emulsifying Agent).

The numerical optimization study was conducted to study the constraints on the design space and the vulnerability of the experimental model, this is important since it suggest factors, responses and the goal for each variable with respect to the measured response. By this study, a list of optimum solutions can be arrived at in quantitative terms, i.e. the likely behavior of the measured response in terms of analyzed factors in a random manner within the design space. The influences of stirring rate, polymer concentration and the amount of emulsifier on the particle size of gelatin microspheres in such a

Table II. Numerical optimization solutions of ofloxacin gelatin microspheres

Solutions Number	Stirring rpm	Gelatin Conc. g	Amt. of EA mL	Particle Size μm
1	1042.78	1.09	1.00	11.42
2	4242.69	1.82	1.00	11.06
3	2899.73	1.34	1.00	8.38
4	4896.62	1.94	1.00	9.85
5	2238.31	1.29	1.00	10.24
6	4640.52	1.83	1.00	9.13
7	4093.13	1.72	1.00	9.76
8	3551.97	1.58	1.00	9.66
9	3098.70	1.57	1.00	11.61
10	3879.52	1.66	1.00	9.60

EA = Emulsifying Agent

manner are shown in the Table II.

The optimized formulae were selected based on the numerical optimization data Table III suggested the closeness of the predicted results with that of the actual results.

From the numerical optimization solutions 8, 1 & 6 (See Table II) were randomly selected and further evaluated for the response *i.e.*, particle size. It was seen that the response was almost similar to the response predicted by the design expert software (See Table III).

Ofloxacin gelatin microspheres had different surface and drug loading according to different experimental conditions. In an attempt to identify the optimal conditions for the preparation of microspheres, the influence of polymer ratio, rate of stirring and the amount of emulsifier were examined.

Microspheres prepared by optimal experimental conditions were smooth with a regular spherical to near spherical shape and were free flowing. Examination using scanning electron photomicrographs showed spherical particles with smooth surfaces, which may be due to cross-linking of coacervate to stabilize emulsion droplets and hence form free flowing microspheres. (see Fig. 1). The average drug loading and the average percentage yield were (61.05 ± 0.514) % and (91.55 ± 0.312) %, respectively.

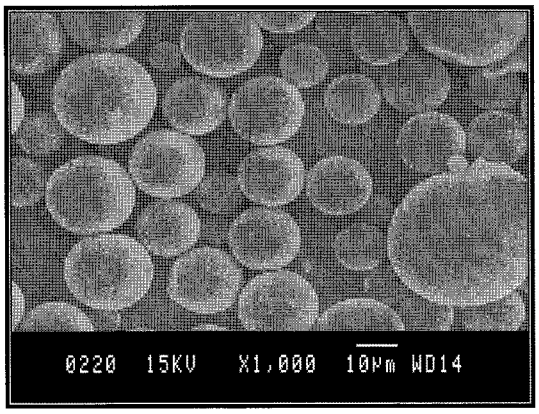

Fig. 1. Scanning electron microscopy of ofloxacin gelatin microspheres prepared by water in oil emulsion method. They had smooth surface.

The particle size distribution is important factor since it controls the tissue location of the microspheres after their intra-artery infusion. Previous reports pointed out that the microspheres with the size range of 5-25 μm have a notable lung-targeting (Kanke *et al.*, 1980). The size range of 0.32-22 μm were prepared and the average particle size was 9.6 μm. The results showed that the microspheres were mainly accumulated in the lung after i.v. injection to the albino mice Figs. 2(a) and 2(b).

The *in vitro* release study helps us to understand the behavior of these systems in terms of drug release. The pattern of GLOME was observed to be in a bi-phasic manner characterized by a burst effect followed by a slow release. The drug release profile of GLOME was 91.62% at 12 h.

Fig. 3 shows *in vitro* release for GLOME and ofloxacin respectively. About 42% of the total ofloxacin in GLOME released in the first one hour, which reflected the significant amount of ofloxacin adsorbed on or incorporated near the surface of the microspheres. In clinical practice this would lead to 'burst effect', which enables the preparation to show fast effect to the patients. However ofloxacin release from gelatin microspheres was completed 12 h. During the same period, the released amount was 91.62% respectively. In comparison with GLOME composite, the ofloxacin injection releases the ofloxacin

Table III. Optimized formulae of ofloxacin gelatin microspheres

Drug (g) Ofloxacin	Gelatin Concentration (g)	Emulsifying agent concentration (mL)	Rate of stirring (rpm)	Rounded-up rpm (to nearest -500s)	Particle size μm predicted	Particle size μm actual
1.58	1.58	1	3551.97	3500	9.66	9.6
1.09	1.09	1	1042.78	1000	11.4	11.35
1.83	1.83	1	4640.52	4500	9.13	9.1

rpm = rotation per minute, EA = Emulsifying Agent, g = grams,
ml = milliliter, ìm=micrometer

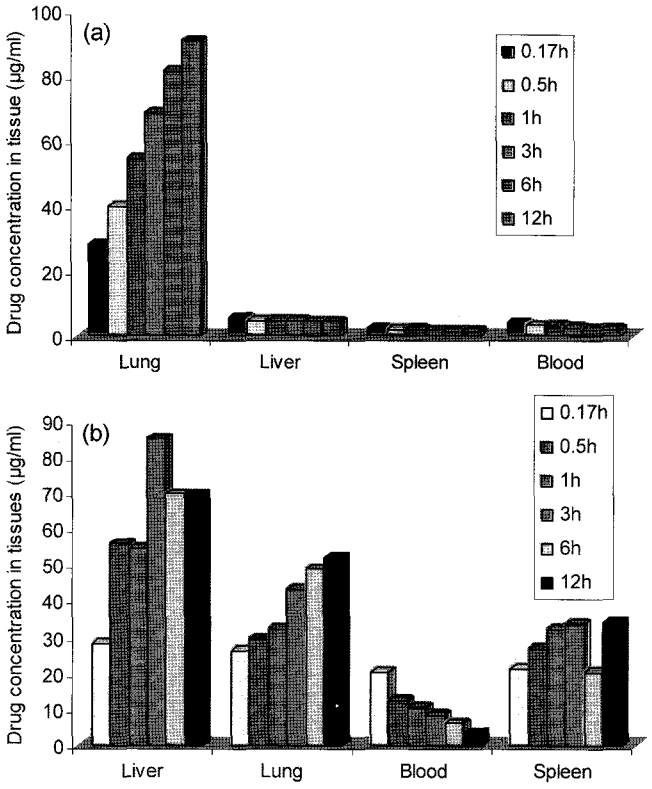

Fig. 2. (a) Distribution in tissue in albumin after injection of GLOME tissue (µg/g) and blood (µg/mL). (b) Distribution in tissue in albumin after injection of Ofloxacin tissue (µg/g) and blood (µg/mL).

very fast. In approximately 0.5 h, 90% of ofloxacin has been released. The results indicated that the GLOME had a well-controlled release efficacy.

The data obtained from *in vitro* release studies were fitted to various kinetic equations (*i.e.*, First order, Baker and Lonsdale, Hixon and Crowell, Korsmeyer's Peppas and Higuchi) to determine the mechanism of drug release and release rate using a macro written for graphing tool *sigma plot version 9.01* (see Fig 4). The correlation coefficient value R^2 is taken into account to decide upon the relevance of the model/curve fit which will best

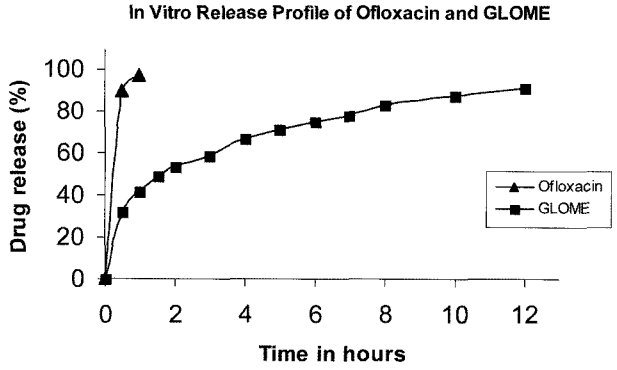

Fig. 3. Cumulative amount of drug release (▲) Ofloxacin (■) microspheres

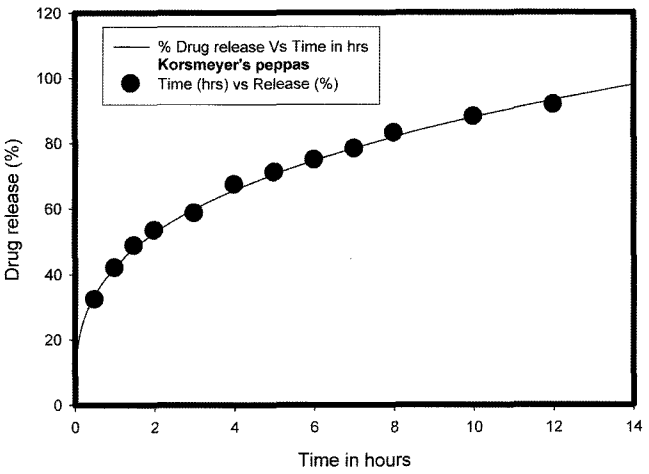

Fig. 4. *In vitro* release profile of GLOME - Curve fit for Korsmeyer's peppas model

describe the extent of fit. According to the R^2 values given by different data fits for GLOME, the Korsmeyer's Peppas model was to be an ideal fit having R^2= 0.9970. According to Korsmeyer's Peppas fit, the release of the drug is decided upon by the diffusion of the polymeric matrix and the drug release is governed by a variation of Fick's law of diffusion.

The release pattern showed classical Fickian diffusion the release was influenced by the initial swelling of the microspheres and releasing drug particles adsorbed in the surface aiding in a 'burst' release (Lu *et al.*, 2003). Later on the release pattern is more controlled and sustained for longer period of time due to diffusion.

During storage at 3-5°C or room temperature (15-25°C) for 12 months (Huo *et al.*, 2005), surface morphology and content of ofloxacin had no notable changes (see Fig. 5). However, at 37°C and RH 75% the agglutinative phenom-

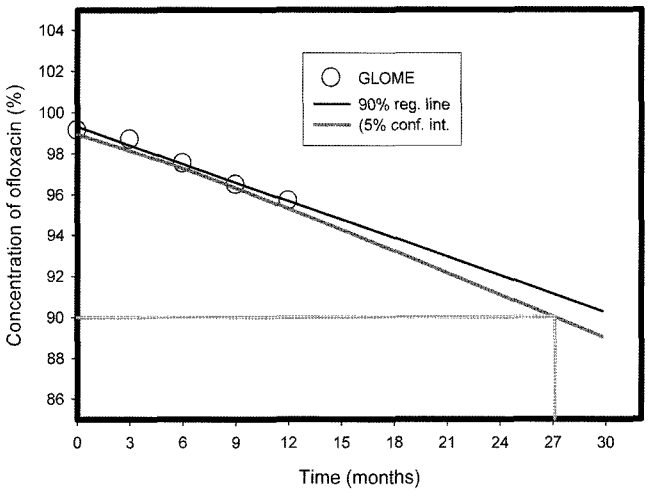

Fig. 5. The stability studies of GLOME

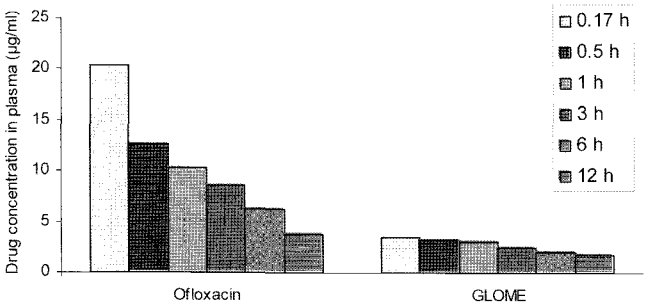

Fig. 6. Plasma drug concentration of Ofloxacin and GLOME at different time intervals

enon was observed.

The *in vivo* pharmacokinetics of microspheres was studied with using "WINNONLIN Version" and was fitted by one-compartment model, two-compartment model and three-compartment model, respectively. Based on the analysis of the model and parameters, it was concluded that the *in vivo* pharmacokinetics of microspheres in blood could be described by the model of two-compartment model with i.v. injection. The plasma concentration and pharmacokinetic parameters are reported in Fig. 6 and Table IV, respectively. From Table IV, one can see that in comparison with ofloxacin injection, GLOME altered the distribution of ofloxacin *in vivo* and the half-life after i.v. injection of GLOME ($t_{1/2(\alpha)}$ = 0.5784 h, $t_{1/2(\beta)}$ = 10.92 h) were prolonged remarkably than those ($t_{1/2(\alpha)}$ =0.3678 h, $t_{1/2(\beta)}$ = 10.68 h) after i.v. injection of ofloxacin injection. The result indicated that the GLOME had sustained release efficacy.

The drug concentration of tissues was determined by HPLC method. The results indicated that the microspheres could deliver ofloxacin mainly to lung after i.v. injection to albino mice and the concentration of ofloxacin in lung (1048 μg/g, 15min) was significantly than those in other tissue and blood. Compared with ofloxacin injection, the drug concentration of ofloxacin in lung after i.v. injection of GLOME enhanced from 6.77 to 1048 μg/g (15 min).

Table IV. Pharmacokinetics parameters of GLOME group and ofloxacin group in albino mice

Parameters	GLOME group	Ofloxacin group
AUC (μg·h/mL)	51.378	156.73
$t_{1/2}(\alpha)$ (h)	0.5784	0.3678
$t_{1/2}(\beta)$ (h)	10.92	10.68
K_{21} (L/h)	1.19	2.16
K_{10} (L/h)	7.5717	8.167
K_{12} (L/h)	0.063	0.532
CL (L/h)	0.101	0.083
Vss (L)	0.1558	0.103
Cmax (μg/mL)	4.703	2.306

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