Articles

Iron Determination in Rat Plasma Samples by Inductively Coupled Plasma Emission Spectrometry and Application to Pharmacokinetic Studies

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Received March 5, 2003

This paper describes an inductively coupled plasma emission spectrometric method for the analysis of Fe in rat plasma. Calibration curves were obtained in the range of 0.125-1.50 $\mu g \cdot mL^{-1}$. The relative standard deviation ranges from 5.93% to 6.80%, and accuracy was between 87.6 and 102.0%. Dilution with water had no influence on the performance of the method, which could then be used to quantify Fe concentration in plasma up to 0.50 $\mu g \cdot mL^{-1}$. The limit of quantification was 0.10 $\mu g \cdot mL^{-1}$. At this level, the average relative standard deviation was 6.8%. The results indicate that the method meets the accuracy and precision requirements for the pharmacokinetic studies. The Fe concentration in rat plasma was measured and the main pharmacokinetic parameters were calculated by Topfit 2.0 (GmbH, Shering AG, Godecke AG, Germany).

Key Words : Inductively coupled plasma emission spectrometry, Biological fluids, Pharmacokinetic

Introduction

Magnetic resonance imaging (MRI) has been used for the detection of liver tumors.[1,2] However, the MRI method has its own drawback, because of wide biological variations and considerable overlap of T_1 and T_2 values of tumors and normal livers. Also, the similarity between the signals of hepatic tumors and adjacent normal liver tissue made them hard to discern.[3] Use of iron oxide in the form of superparamagnetic ferrite crystals has recently been reported to improve the detection of hepatic metastasis with MRI.[4-6] Contrast agents consist of iron oxide nanoparticles magnetite (Fe_3O_4) or other ferrite, which are insoluble in water. Superparamagnetic agents are highly effective in MRI. Their relaxivities, R_1 and R_2, are higher than those of gadolinium chelates currently available.[7]

Few methods are available to quantify iron oxide nanoparticles in biological samples, using inductively coupled plasma (ICP) atomic emission spectrometry. So the objective of this work is to develop a method to validate an ICP method to quantify Fe concentration in rat plasma for the calculation of pharmacokinetic parameters in rats.

Experimental Section

Materials and Reagents. Dextran kD40 were supplied by Sigma Chemicals Ltd. and used as received. Water was doubly distilled and pure nitrogen gas was bubbled through it for more than 2 h just before used to remove dissolved oxygen. Nitrogen was purified of traces of carbon dioxide and oxygen by passing it through a basic pyrogallol solution. All other chemicals used were of analytical grade and were used as received.

For the validation of the method, rat plasma samples were obtained from Wistar rats from pooled heparinized blood samples and stored at -20 °C before use. The plasma was used for preparation of calibration and for quality control.

ICP operating conditions and wavelengths of emission lines examined.

Parameters
Incident power (KW) 1.3
Carrier gas (Ar) flow rate (l min^{-1}) 0.8
Auxiliary gas (Ar) flow rate (l min^{-1}) 0.2
Coolant gas (Ar) flow rate (l min^{-1}) 15
Observation height (mm) 10
Integration time (s) 40
Wavelength (nm) Fe: 238.204 nm

Preparation of Magnetite Nanoparticles Coated with Dextran (MD). A mixed solution of ferrous and ferric ions in the molar ratio of 1 : 2 was prepared by adding both 50 mL 0.10 mol $FeCl_2 \cdot 6H_2O$ and 50 mL 0.20 mol $FeCl_3 \cdot 4H_2O$ to a 100 mL aqueous solution of dextran at 60°C. Dextran, with an average molecular weight of 40,000, was used to obtain aqueous solution at concentrations up to 10% (w/w). Magnetite was precipitated by adding 50 mL of the above-mentioned mixed solution to 200 mL aqueous solution of 0.2 $mol \cdot L^{-1}$ NaOH at 80 °C while stirring at 1000 $rpm \cdot min^{-1}$ with an impeller. The mixture was subsequently aged at 80 °C for 50 min and cooled to room temperature. After the end of magnetite precipitation, pH was 5.0. Precipitates were

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separated by centrifugation at 10000 rpm·min^{-1} for 60 min, and then the precipitated magnetite was immobilized with a permanent magnet and the aqueous supernatant was discarded. The precipitated magnetite was dialyzed against running water for 15 h to yield an aqueous sol of magnetic iron oxide and then dried under vacuum at 80 °C for 3 h.

Characterization. More than 100 particles were observed under a transmission electron microscope (TEM) (JEG-2000EXII) to study the morphology and to determine the particle size. The average particle size in a supernatant fluid was determined by photon correlation spectroscopy (PCS) (Zetasizer 3000, Malvern Instruments, GB).

X-ray diffraction (XRD) was conducted on a Rigaku D/max-2500 PC X-ray diffractometer, using CuKα radiation, to determine the crystal structure.

The magnetic properties were determined by a vibration sample magnetometer (US PAR155) at room temperature with an external magnetic field of $8\,k \times 10^3/4\pi$ (A/m).

Sample Processing. Samples were directly nebulized; each determination was performed in replicate (n = 5).

The matrix effect was investigated using water, 25% water diluted rat plasma samples and pure rat plasma.

Instrument Calibration. Two calibration curves were performed in the matrices: the first one at the concentrations of 0, 0.125, 0.250, 0.375, 0.500, 0.625 and 0.750 μg·mL^{-1} of Fe; and the second one at concentrations of 0, 0.250, 0.500, 0.750, 1.000 and 1.250, 1.500 μg·mL^{-1} of Fe.

Data Analysis. Analyses were performed on an ICP atomic emission spectrometer 4300DV (ICP Optima Emission Spectrometer Per Kin-Elmer USA). The concentration of iron in the blank blood samples (C_b) was determined and then the total concentration of iron after i.v. administration (C_t) was determined. The real concentration (C_r) was computed as follows: $C_r = (C_b - C_t) \times 25$; where 25 is the dilution ratio.

Finally, pharmacokinetic analysis was performed using the Topfit 2.0 (GmbH, Shering AG, Gŏ decke AG, Germany).

Precision and Accuracy. Inter-day and intra-day reproducibility of the assay were assessed by performing replicate analyses of quality control samples in studied matrices [water and 25% (w/w) diluted plasma], containing iron at the concentrations of 0.05, 0.50, 5.00 μg·mL^{-1}. The procedure was repeated on different days of the same spiked standards to determine inter-day repeatability. Intra-day repeatability was determined by treating spiked samples in replicate the same day. The recovery test was also performed. The results are reported in Table 1.

Effect of Dilution. To study the application of the described method to samples with concentrations higher than the last calibration point, spiked samples were prepared in blank plasma (0.05-5.00 μg·mL^{-1}) and diluted with water to bring the concentration within the range of standard curve. Each analysis was performed in replicate and the concentration was determined using a calibration curve in 25% (w/w) diluted plasma. The concentration was compared with the nominal ones.

The direct injection of pure rat plasma was not plausible due to the risk of obstructing the nebulizer. Several unknown samples were diluted [25% (w/w)] in water before assay, and this dilution was validated using QC samples at 0.05, 0.50, 5.00 μg·mL^{-1} in pure plasma. The results are shown in Table 2.

Table 1. Intra-day and inter-day precision and accuracy for the analysis of MD in rat plasma (n = 3)

	Conc. (Added) μg·mL^{-1}	Conc. (Measured) μg·mL^{-1}	Recovery %	Mean ± SD %	RSD %
Intra-day	0.050	0.0505	101.0	92.67 ± 5.936	5.93
	0.500	0.438	87.6		
	5.00	4.471	89.4		
inter-day	0.050	0.0510	102.0	92.78 ± 9.62	6.80
	0.500	0.442	88.4		
	5.00	4.397	87.9		

Table 2. Effect of dilution

Theoretical concentration (μg·mL^{-1})	rat plasma		RSD (%)	Accuracy (%)
	n[a]	Experimental Concentration (μg·mL^{-1})		
0.05	6	0.049	2.93	98.6
0.60	6	0.592	2.94	98.7
1.00	6	1.031	6.32	96.1
5.0	6	5.066	6.56	101.1

[a] : number of replicate.

Determination of the Limits of Quantification and Detection. The limits of quantification were defined as the lowest drug concentrations that can be determined with an accuracy between 100 ± 20% and a relative standard deviation below or equal to 20% on a day-to-day basis. To determine the analytical error in the determination of the limits of quantification, spiked samples were used.

The limits of detection were defined as the sample concentration resulting in a mean corrected intensity of three times the noise level.

Pharmacokinetic Study. The inductively coupled plasma emission spectrometric method was used to quantify Fe concentration in rat plasma for pharmacokinetic study after i.v. administration of 0.5 mg·kg^{-1} body weight of MD.

Wistar rats weighing 180-200 g (7 weeks old) were housed in an environmentally controlled room with a 12 h light/dark cycle. Diet and tap water were provided ad libitum. MD dissolved in H_2O (11.2 mg·mL^{-1}) was i.v. administered, using a heparinized syringe at a dose of 3.54 mg·kg^{-1} body weight (n = 3). Blood was withdrawn from the jugular vein with a heparinized syringe at 0.167, 0.5, 1, 2, 4, 6, 8, 12h after administration of MD. The blood samples were immediately centrifuged at 4000 rpm·min^{-1} for 10 min (4 °C), and the plasma samples obtained were stored at -20 °C until analysis.

Results and Discussion

Characterization of MD. Superparamagnetic magnetite

nanoparticles were prepared by co-precipitation in a dextran aqueous solution. The formation of pure magnetite nanoparticles was confirmed by analyses of XRD and electron diffraction patterns. The TEM measurements indicated that the average diameter of the magnetite nanoparticles was about 7.2 ± 1.1 nm. (The average size of particles by PCS was estimated to be 56.7 nm, analyses by intensity, 36.5 nm by volume and 29.2 nm by number, respectively. Check this sentence. Is a value missing?) The magnetic properties of MD were determined by a vibration sample magnetometer. The saturation magnetization of the particles was about 36.4 emu·g^{-1}, which is lower than that of bulk magnetite particle size (σ_s = 92 emu·g^{-1}).[8,9] The reduction in σ_s might be due to the decrease in particle size and the accompanying increase in surface area. Another possible reason for the diminution in σ_s might be the incomplete crystallization of the Fe_3O_4 nanoparticles coated with dextran. The σ_s decreased when the relative crystallinity became smaller, and this decrease in σ_s was reported to occur when the particle size of magnetite decreased below 10 nm due to superparamagnetism.[10,11] The coercive force dropped sharply and became almost zero when the particle size decreased. Since the particle size of the magnetite prepared in the present study was much less than 10 nm, these samples were expected to exert superparamagnetism, in part, to the hindrance of thermal agitation at the surface due to the dextran adsorption.

Linearity. Intensity of Fe varied linearly with concentration over the range used. Peak intensity at analysis 238.204 nm for the MD was plotted against the concentration of the MD added. The determination coefficients (R^2) for calibration curves were equal to or better than 0.999 for water and 0.9978 for 25% diluted plasma. Line range was 0.05-5.00 μg·mL^{-1}.

Precision and Accuracy. Recovery was 101.0, 88.0, and 89.4%, respectively, and RSD was 5.6. Therefore, the accuracy was within an acceptable range. Results of the intra-day and inter-day precisions are presented in Table 1.

Effect of Dilution. The dilution had no influence on the performance of the method, which could then be used for concentrations up to 5.00 μg·mL^{-1} (Table 2).

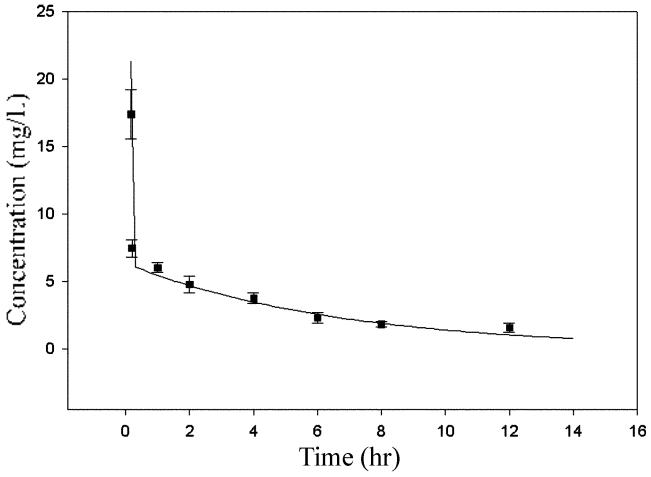

Figure 1. Time course for the concentration of MD in rats plasma after *i.v* administration of DM (3.53 mg·kg^{-1}, n = 4, mean ± SD).

Table 3. Pharmacokinetic Parameters

Parameters	unit	Value			mean	SD
		1	2	3		
MRT-disp	h	7.550	7.420	7.220	7.397	0.166
MRT-tot	h	7.690	7.550	7.360	7.533	0.166
MRT-abs	h	0.134	0.131	0.134	0.133	0.002
V_c	L	0.018	0.023	0.020	0.021	0.003
V_3	L	0.531	0.488	0.500	0.506	0.022
V_{ss}	L	0.549	0.511	0.520	0.527	0.020
Clearance	mL·min^{-1}	1.210	1.150	1.200	1.187	0.032
C(0)	mg·L^{-1}	194.000	152.000	176.000	174.000	21.071
AUC	mg·L^{-1}·h^{-1}	48.600	51.200	49.100	49.633	1.380
T_{max}	h	0.134	0.131	0.134	0.133	0.002
C_{max}	mg·L^{-1}	193.000	152.000	176.000	173.667	20.599
dosage	mg·kg^{-1}	3.530	3.530	3.530	3.530	0.000
$t_{1/2(\alpha)}$	h	0.008	0.011	0.009	0.010	0.002
$t_{1/2(\beta)}$	h	5.500	5.390	5.250	5.380	0.125

The limits of Quantification and Detection. The limit of quantification and detection under the described conditions was 0.1 μg·mL^{-1} and 0.05 μg·mL^{-1}, respectively, for water and for 25% in diluted plasma. At these concentrations, the relative standard deviation around the mean value did not exceed 15.0%.

In general, the detection limit of Fe in ICP-MS (inductively coupled plasma mass spectrometer) was much lower than that of ICP-OES (inductively coupled plasma optical emission spectroscopy).

Pharmacokinetic Study. The ICP method was used to analyze plasma samples containing MD obtained after *i.v.* administration to rats (3.53 mg·kg^{-1}). Concentration *vs.* time profile (Fig. 1) was constructed for up to 12 h for the analyses (n = 3). We conducted the pharmacokinetic analyses *via* Topfit 2.0 (GmbH. Shering AG, Gdecke AG, Germany). The statistical results based on SD, AIC and R^2 show that a two-compartment model is the best choice to fit the data. The pharmacokinetic parameters of the MD two-compartment model are given in Table 3. The figure of V_c (volume of drug in the central compartment) (0.021 ± 0.003) and V_3 (volume of drug in the peripheral compartment) (0.506 ± 0.022) show that MD in vivo aggregated in the peripheral compartment (tissue) and not in the central compartment (blood, extra cellular fluid and highly perfuse tissue). The data of $t_{1/2(\alpha)}$ (central compartment; 0.01 ± 0.002) and $t_{1/2(\beta)}$ (peripheral compartment; 5.38 ± 0.125 h) imply that MD eliminated faster from the central compartment than from the peripheral compartment. C_{max} was 166.67 ± 20.599 (mean ± SD) mg·L^{-1} in rats at 0.133 h. The total plasma clearance was 1.187 ± 0.032 (mean ± SD) mL·min^{-1}. And the plasma AUC of MD was 49.633 ± 1.380 (mean ± SD) mg·L^{-1}·h^{-1}. The advanced pharmacokinetic analysis will be performed in the future.

In this study, a method to quantity Fe in plasma, using plasma emission spectrometry, is described. Assay performance was assessed both on the basis of the statistical

characteristics of individual calibration lines and from the results of quality control samples. The matrix effect was studied using water, diluted plasma and plasma itself.

Inductively coupled plasma emission spectrometry was used to detect concentration of magnetite nanoparticles in rats for the first time. The quantitative analysis ability of the inductively coupled plasma emission spectrometry shows its high performance in determining the concentration of biological samples.

Acknowledgments. This work was partly supported by the National Nature Sciences Foundation of China (Grant Number: 39570843).

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