Supplementary Material (ESI) for Analyst

A versatile approach for assaying in vitro metallodrug metabolism using CE hyphenated with ICP-MS

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Experimental

Apparatus

The instrumental setup consisted of an Agilent HP^{3D} CE system (Waldbronn, Germany) and an Agilent 7500 mass spectrometer (Tokyo, Japan) interfaced via a microconcentric nebulizer CEI-100 (CETAC, Omaha, USA) and a custom-machined low-dead-volume conical spray chamber. A PC equipped with ICP-MS Chromatographic Software was used for data acquisition. The ICP-MS and interface instrumental settings and operating conditions (subjected to the optimisation with regard to signal intensity) are presented in Table 1. All electropherograms were recorded using the total ion counts of ^{69}Ga. The stability of ICP-MS performance was controlled by measuring the signal of ^{72}Ge, incorporated into the make-up solution as continuous internal standard, during the analysis and the capillary post-run washing.

A 60-cm fused-silica capillary with an inner diameter of 75 μm purchased from Composite Metal Service (The Chase Hallow, UK) was utilised. The samples were introduced into the capillary by applying a pressure of 25 mbar for 5 s (injection volume 10.6 nL). The separations were generated at 17 kV with a negative polarity at the inlet end of the capillary (typical current value was 22.0±1.5 μA) and at 37^{o}C using a thermostated capillary cassette. To attain repeatable migration times over the measurement series taking several days, the capillary post-run conditioning was carefully optimised and the following washing protocol adopted: water (1 min), 1 M NaOH (3 min), water (1 min) and the running electrolyte (5 min).

Reagents and solutions

All chemicals were of reagent grade and Millipore water (Molsheim, France) was used throughout. Human serum albumin (min. 96%; lot No. 035K7560), apotransferrin (ca. 98%, lot No. 062K1644) and transferrin (min. 98%) were obtained from Sigma (St. Louis, USA) and used as 0.1 mM standard solutions. Arsenobetaine (Fluka, Buchs, Switzerland) was used as the marker of electroosmotic flow. tris(8-Quinolinolato)gallium(III) was donated by the Institute of Inorganic Chemistry, University of Vienna. Gallium nitrate (hydrate) was received from Across (Geel, Belgium). Human serum (from male AB plasma, sterile-filtered) was purchased from Sigma. A 10 mM phosphate buffer (pH 7.4), containing 100 mM sodium chloride, served for making all standards and samples. The electrolyte solutions were prepared daily from stock solutions of 1 M HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) or 50 mM phosphate buffer and 1 M sodium chloride (all chemicals were the products of Sigma, Steinheim, Germany). After optimization trials, 10 mM phosphate buffer, containing 10 mM NaCl, and 40 mM HEPES have been chosen for intestine juice and protein-binding studies, respectively. The final (physiological) pH-value of the electrolyte solutions (7.4) adjusted with 1 M NaOH (purum p.a., Fluka) was checked using a pH-meter model CP-401 Elemetron (Zabrze, Poland) and compared with a certified reference material of pH 7.41 (Labstand, Poland). All electrolyte solutions were filtered through 0.45-mm membrane filters (Q3, Brzeziny, Poland) and degassed in an ultrasonic bath for 5 min prior to use.

Sample preparation

Simulated intestine juice was prepared by dissolving 3% (w/v) pancreatin enzyme (from porcine pancreas, Sigma) in 20 mM phosphate buffer (pH 6.8) containing 140 mM sodium chloride and mixed (1:1) with the saturated solution of the drug. All protein-drug binding experiments were carried out in incubation solution, comprising 10 mM phosphate buffer (pH 7.4) and 100 mM NaCl, which was also used for serum dilution (4–20-fold). The initial protein concentration in the reaction mixture was 5×10^{-5} M unless stated otherwise. The gallium drug was treated as a saturated solution in physiological buffer and after careful filtration added to the protein(s) solution or serum so as to provide its initial concentration of 1×10^{-5} M. All reaction mixtures were thermostated at 37^{o}C for the designated time and aliquots were continuously taken for CE-ICP-MS analysis.

Table 1 Optimum ICP-MS and interface operating parameters used in this work

ICP-MS settings	
Plasma gas flow rate, L/min	14.9
Auxiliary gas flow rate, L/min	0.9
Sampling cone	Pt (18 mm insert)
Skimmer cone	Pt
Plasma Rf power, W	1200
Isotopes monitored (abundance %)	^{69}Ga (60%)
	^{57}Fe (2%)
	^{72}Ge (27%)
Integration time, s:	0.6 for ^{69}Ga
	0.3 for ^{57}Fe
	0.01 for ^{72}Ge
Interface conditions	
Spray chamber volume, mL	5
Nebulizer gas flow rate, L/min	1.08
Make-up solution	2 mM phosphate buffer (pH 7.4), 20 μg/L Ge

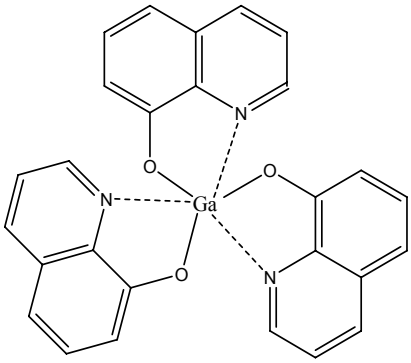

Fig. S1 Structure of the gallium drug.

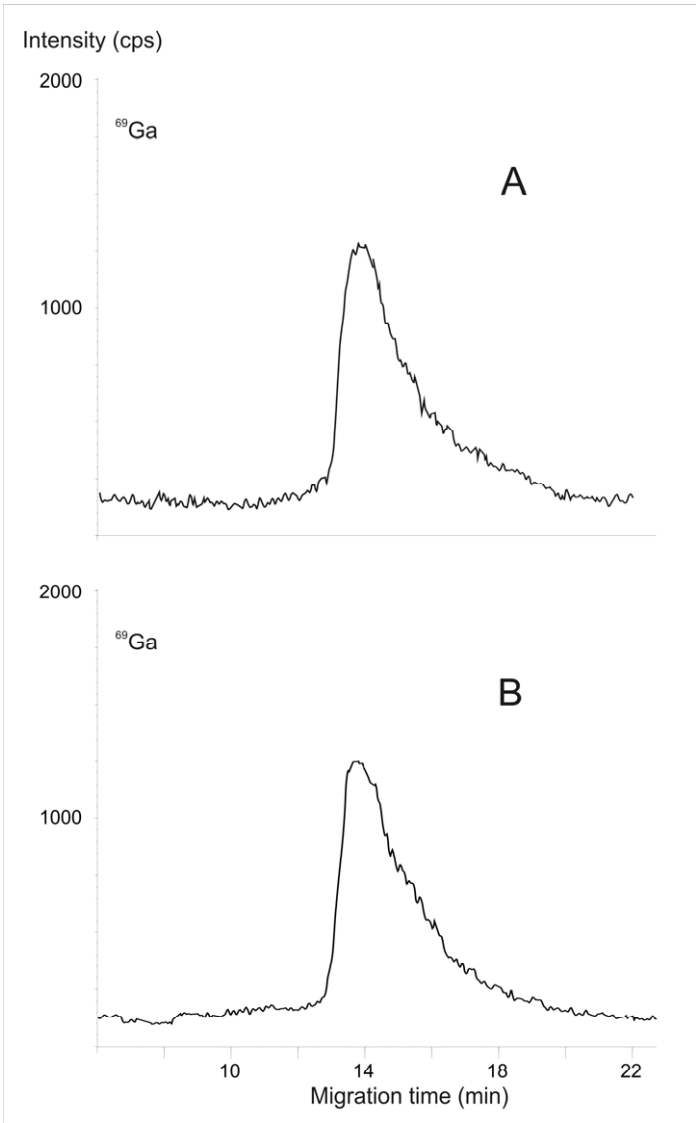

Fig. S2 Electropherograms of the parent drug (A) before and (B) after incubation in simulated intestine juice.

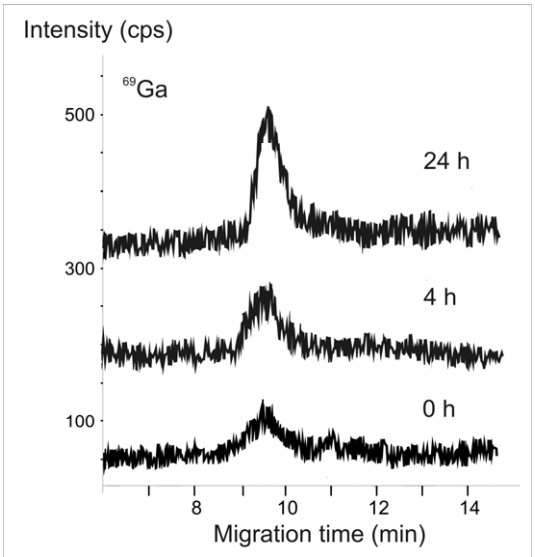

Fig. S3 ICP-MS electropherograms illustrating the evolution of drug-albumin adduct at a 1:10 ratio.