Supplementary Information

for

Interaction of macrocyclic gadolinium-based MR contrast agents with Type I collagen. Equilibrium and kinetic studies.

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Content:

I. Acid-base properties of Gd(BT-DO3A), Gd(HP-DO3A) and Gd(HB-DO3A) complexes at 37°C in 0.15 M NaCl solution.

The distributions of GdL and $GdLH_{-1}$ species formed by Gd(BT-DO3A), Gd(HP-DO3A) and Gd(BT-DO3A) as a function of pH are shown Figures S1 – S3.

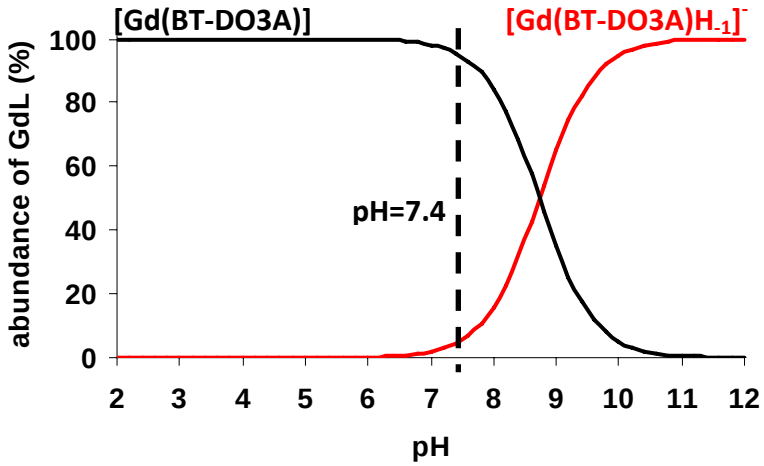

Figure S1. Distribution of [Gd(BT-DO3A] – [Gd(BT-DO3A)H_{-1}] species as a function of pH ([Gd(BT-DO3A)]=0.001 M, 37°C, 0.15 M NaCl)

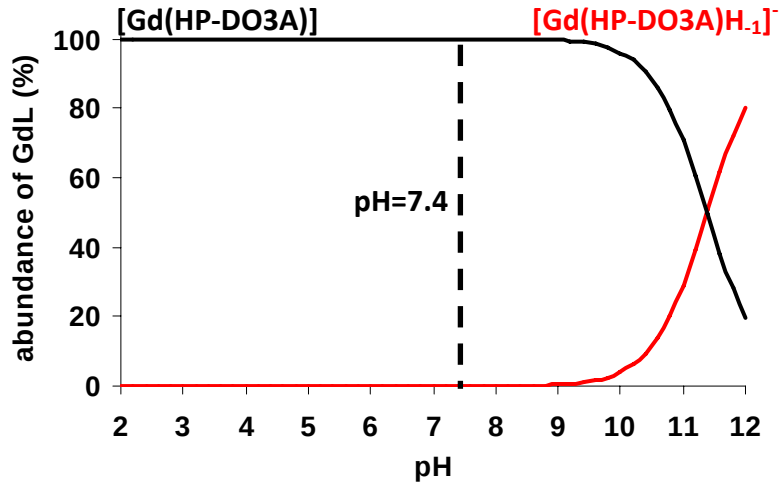

Figure S2. Distribution of [Gd(HP-DO3A] – [Gd(HP-DO3A)H_{-1}] species as a function of pH ([Gd(HP-DO3A)]=0.001 M, 37°C, 0.15 M NaCl)

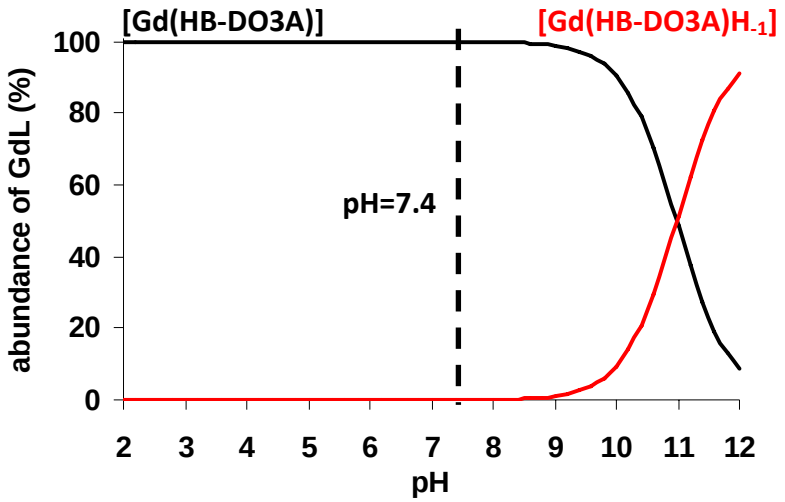

Figure S3. Distribution of [Gd(HB-DO3A] – [Gd(HB-DO3A)H_{-1}] species as a function of pH ([Gd(HB-DO3A)]=0.001 M, 37°C, 0.15 M NaCl)

The data presented in Table S1 demonstrate that the deprotonation of the alcoholic -OH group proceeds at much lower pH values for Gd(BT-DO3A) than for Gd(HP-DO3A) or Gd(HB-DO3A). Based on the species distribution, the deprotonation of the alcoholic –OH group of Gd(BT-DO3A) complex takes place via the formation of Gd(BT-DO3A)H_{-1} species (5.7 %) at physiological conditions (pH=7.4, 37°C, 0.15 M NaCl). Conversely, the acidity of the –OH proton of the Gd(HP-DO3A) and Gd(HB-DO3A) complexes is significantly lower and results in the formation of Gd(HP-DO3A)H_{-1} and Gd(HB-DO3A)H_{-1} species only at pH>9 in both cases, therefore outside the physiological range conditions.

II. Capillary electrophoresis

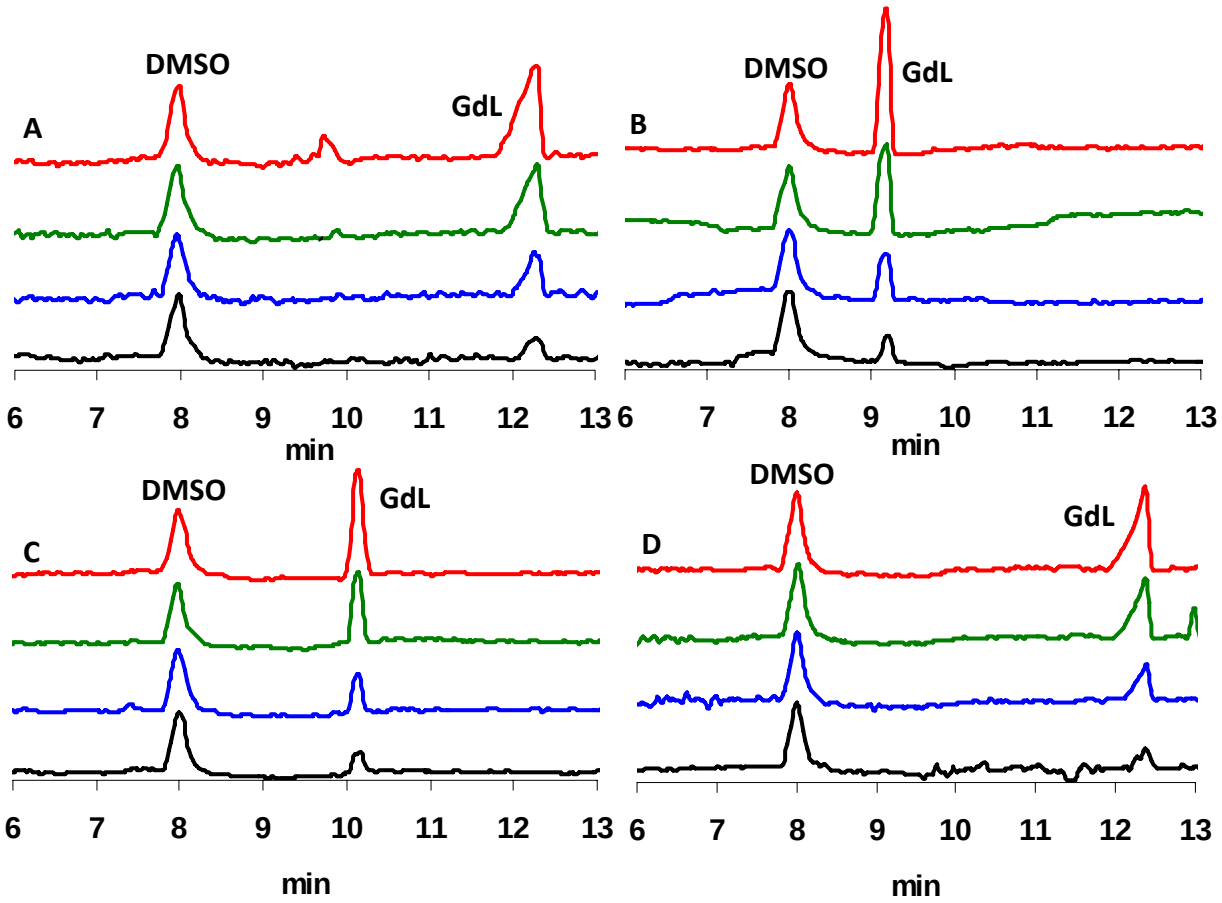

Figure S4. MEKC electropherograms of gadoterate meglumine (**A**), gadobutrol (**B**), gadoteridol (**C**) and Gd(HB-DO3A) (**D**) (Samples: [GdL]=**1.0**, **2.0**, **3.0** and **4.0** mM, pH=7.4, 1.0 mM Na_2HPO_4 0.15 M NaCl, 5.0 mM DMSO, Conditions: 20 kV, 50 mbar for 20 s, λ=200 nm; 25 Na_2HPO_4, 70 mM sodium dodecyl sulphate-SDS, pH=9.1, 12°C)

Table S1. Analytical performance data of MEKC determination of gadoterate meglumine, gadobutrol, gadoteridol and Gd(HB-DO3A)

	molar integral (mAu/M)	LOD[a] (μM)	Linear range (M)
gadoterate meglumine	$(9.9\pm0.3)\times10^4$	6.7	5.0×10^{-4} – 7.0×10^{-3}
gadobutrol	$(7.5\pm0.1)\times10^4$	5.8	
gadoteridol	$(6.2\pm0.1)\times10^4$	7.0	
Gd(HB-DO3A)	$(8.3\pm0.1)\times10^4$	6.2	

[a] LOD=3σ/μmolar integral

III. Diffusion of the macrocyclic Gd^{III}-complexes in the absence of collagen

In order to determine the effect of the membrane on the adsorption and desorption of macrocyclic Gd^{III} complexes on/from collagen, dialysis of 5 membranes with 1, 2 and 3 mL solutions (wash in: 1.0 mM Na_2HPO_4, 0.15 M NaCl, pH=7.4; wash out: 1.0 mM Gd^{III}-complex, 1.0 mM Na_2HPO_4, 0.15 M NaCl, pH=7.4) was performed in 500 mL buffer (pH=7.4 and 37°C in 1.0 mM Na_2HPO_4 and 0.15 M NaCl) in the presence (wash in) and absence (wash out) of 1.0 mM gadoterate meglumine, gadobutrol, gadoteridol or Gd(HB-DO3A). Rates of the wash-in and wash-out processes (Eq. (S1)) were obtained by the ICP-OES determination of the macrocyclic Gd^{III} complexes in the solutions within the membranes:

$$GdL_{buffer} \rightleftharpoons GdL_{membrane} \qquad (S1)$$

where GdL_{buffer} and $GdL_{membrane}$ are the macrocyclic Gd^{III} complexes in the buffer and in the membrane, respectively. The amounts of the Gd^{III} complexes in the membranes are shown in Figures S5 (wash in) and S6 (wash out).

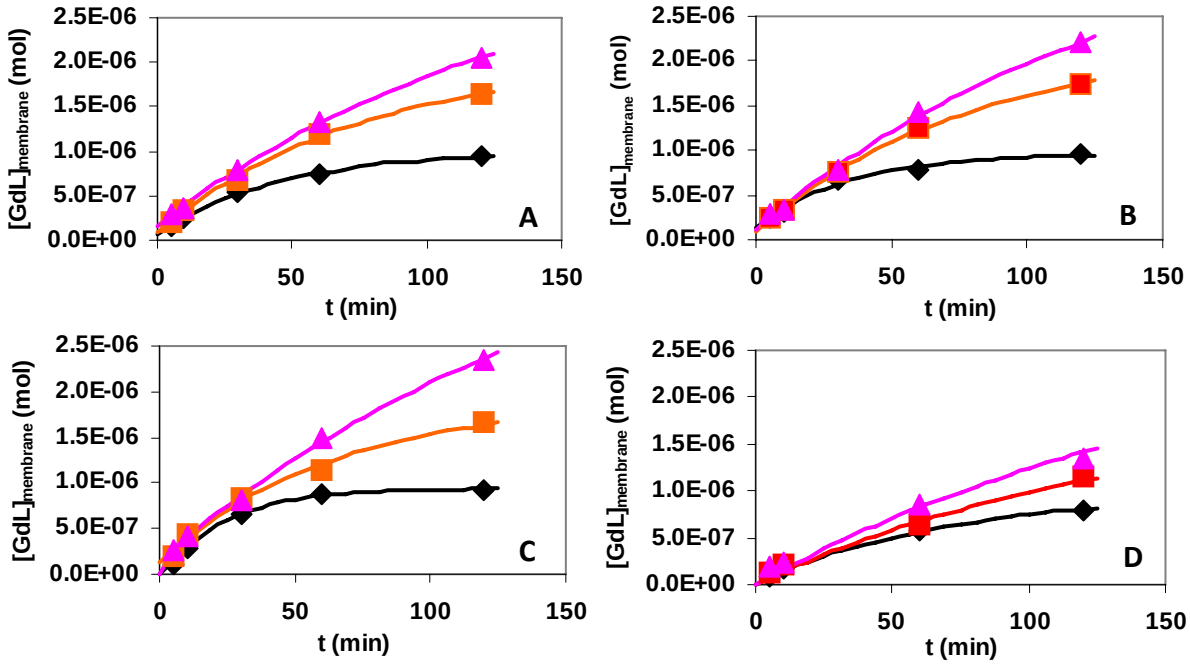

Figure S5. Amounts of Gd^{III} complexes in the membrane as a function of time (wash in). (gadoterate meglumine (**A**), gadobutrol (**B**), gadoteridol (**C**) and Gd(HB-DO3A) (**D**), 1 mL (◆), 2 mL (■) and 3 mL (▲), buffer: [GdL]=1.0 mM, pH=7.4, 37°C, 1.0 mM Na_2HPO_4, 0.15 M NaCl) Symbols and solid lines represent the experimental and the calculated [GdL] values, respectively.

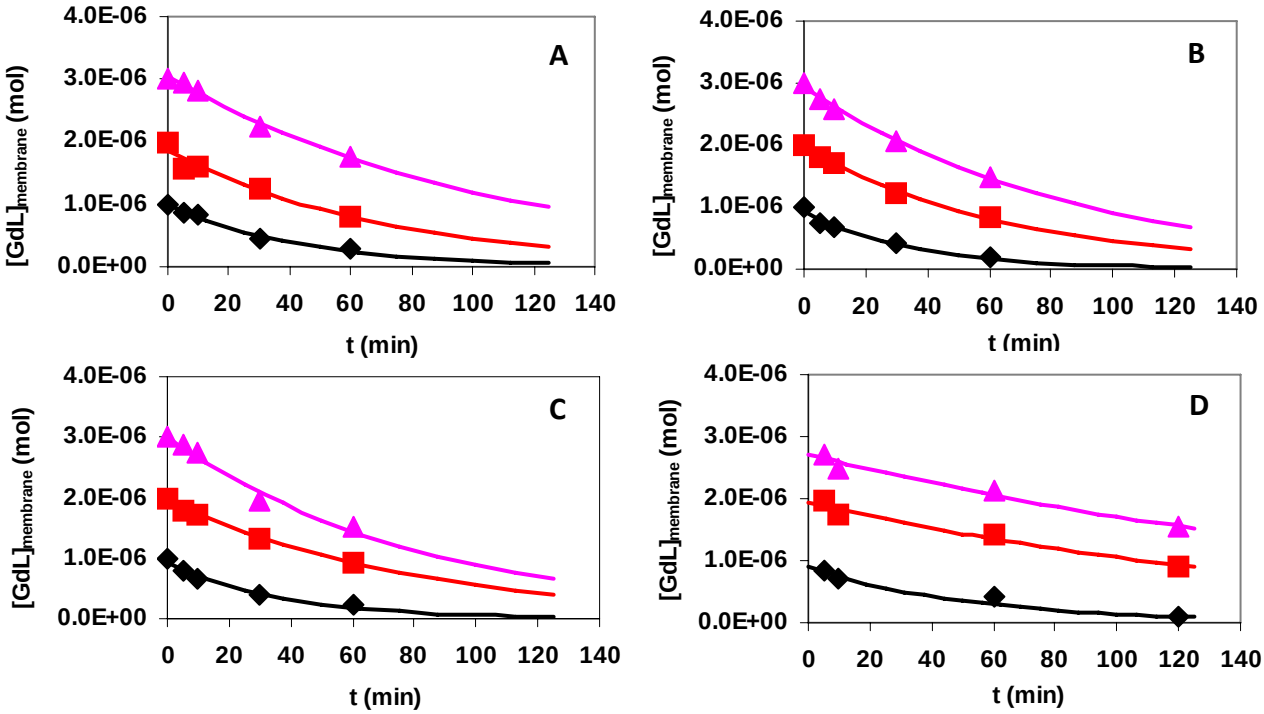

Figure S6. Amounts of Gd^{III} complexes in the membrane as a function of time (wash out). (gadoterate meglumine (**A**), gadobutrol (**B**), gadoteridol (**C**) and Gd(HB-DO3A) (**D**), 1 mL (◆), 2 mL (■) and 3 mL (▲), buffer: pH=7.4, 37°C, 1.0 mM Na_2HPO_4, 0.15 M NaCl). Symbols and solid lines represent the experimental and the calculated [GdL] values, respectively.

In the presence (wash-in) and absence (wash-out) of GdL excess in the buffer solution (500 mL), the wash-in and the wash-out can be treated as a pseudo-first-order process and the rate can be expressed with the Eqs. (S2) and (S2), where k_{obs} is a pseudo-first-order rate constant and $[GdL]_t$ is the total concentration of the macrocyclic Gd^{III} complex in the buffer (wash-in) and in the membrane (wash-out).

$$\frac{d[GdL]_t}{dt} = k_{obs}[GdL]_t \qquad (S2)$$

$$-\frac{d[GdL]_t}{dt} = k_{obs}[GdL]_t \qquad (S3)$$

The rate constants (k_{obs}) characterizing the wash-in and wash-out of Gd^{III} complexes were calculated by fitting the kinetic data in Figures S5 and S6 to Eq. (S4).

$$[GdL] = ([GdL]_0 - [GdL]_\infty)e^{(-k_{obs}t)} + [GdL]_\infty \qquad (S4)$$

where $[GdL]_0$, $[GdL]_\infty$ and [GdL] are the concentrations of GdL complex at the start, the end and at the time *t* of the reaction. The k_{obs} rate constants are shown in Table S2, with standard deviations.

Table S2. Rate constant characterizing the wash-in and wash-out of macrocyclic Gd^{III}-complexes (buffer: $[GdL]_t$=1.0 mM (wash in), 0.0 mM (wash out), pH=7.4, 37°C, 1.0 mM Na_2HPO_4, 0.15 M NaCl)

Wash-IN	**Dotarem**	**Gadovist**	**ProHance**	**Gd(HB-DO3A)**
k_{obs}/min^{-1} **(1 mL)**	0.022 ± 0.001	0.026 ± 0.003	0.030 ± 0.004	0.016 ± 0.001
k_{obs}/min^{-1} **(2 mL)**	0.014 ± 0.001	0.016 ± 0.001	0.018 ± 0.002	0.009 ± 0.0003
k_{obs}/min^{-1} **(3 mL)**	0.009 ± 0.002	0.010 ± 0.001	0.010 ± 0.001	0.007 ± 0.0003
k^{in} **($min^{-1}cm$)**	$(8.6 \pm 0.8)\times10^{-4}$	$(1.0 \pm 0.1)\times10^{-3}$	$(1.1 \pm 0.1)\times10^{-3}$	$(6.4 \pm 0.4)\times10^{-4}$
Wash-OUT	**Dotarem**	**Gadovist**	**ProHance**	**Gd(HB-DO3A)**
k_{obs}/min^{-1} **(1 mL)**	0.024 ± 0.002	0.027 ± 0.004	0.026 ± 0.004	0.018 ± 0.001
k_{obs}/min^{-1} **(2 mL)**	0.014 ± 0.001	0.014 ± 0.001	0.014 ± 0.003	0.010 ± 0.001
k_{obs}/min^{-1} **(3 mL)**	0.009 ± 0.001	0.011 ± 0.001	0.011 ± 0.001	0.006 ± 0.0004
k^{out} **($min^{-1}cm$)**	$(9.0 \pm 0.5)\times10^{-4}$	$(1.0 \pm 0.1)\times10^{-3}$	$(1.0 \pm 0.1)\times10^{-3}$	$(6.0 \pm 0.3)\times10^{-4}$

The k_{obs} rate constants characterizing the wash-in and wash-out process of gadoterate meglumine, gadobutrol and gadoteridol in the presence of 1.0 mM Gd^{III} complexes in the buffer (wash-in) and in the membrane (wash-out) are essentially identical and decrease with the increase of the volume in the membrane. However, the k_{obs} values of Gd(HB-DO3A) are somewhat lower than those of other complexes, which might be explained by the more hydrophobic character of the 2-hydroxy-butyl sidechain that results in the slower wash-in and wash-out processes of Gd(HB-DO3A) through the membrane. The rate of dialysis is directly proportional to the concentration of molecules and to the ratio of membrane surface area to membrane volume (specific surface of the membrane).[1] Since the wash-in and wash-out experiments were performed in the presence of 1.0 mM Gd^{III} complex in the buffer (wash-in) and in the membrane (wash-out), the k_{obs} can be expressed by the following equation:

$$k_{obs} = k^{in/out} \times \frac{area}{volume} \qquad (S5)$$

where $k^{in/out}$ is the rate constant characterizing the diffusion of the GdIII complexes in the presence of 1.0 mM Gd^{III} complex in the buffer (wash-in) and in the membrane (wash-out). Considering the surface area of the membrane ($2\times6.5\times2.3=29.9$ cm^2) and the volume of the buffer (1, 2 and 3 mL) in the membrane, k^{in} and k^{out} rate constants characterizing the

permeability of the membrane for the Gd^{III} complexes were calculated in the presence of 1.0 mM Gd^{III} complex in the buffer (wash-in) or in the membrane (wash-out) (Table S4). k^{in} and k^{out} rate constants characterizing the diffusion of the gadoterate meglumine, gadobutrol and gadoteridol in the presence of 1.0 mM Gd^{III} complex in the buffer (wash-in) and in the membrane (wash-out) are essentially identical, which reveals that the cellulose membrane (12 kD, *Sigma Aldrich*) has practically no influence on the rate of the adsorption and desorption of gadoterate meglumine, gadobutrol and gadoteridol on collagen.

IV. The distribution, adsorption/desorption and elimination of the GBCAs in an open three-compartment model.

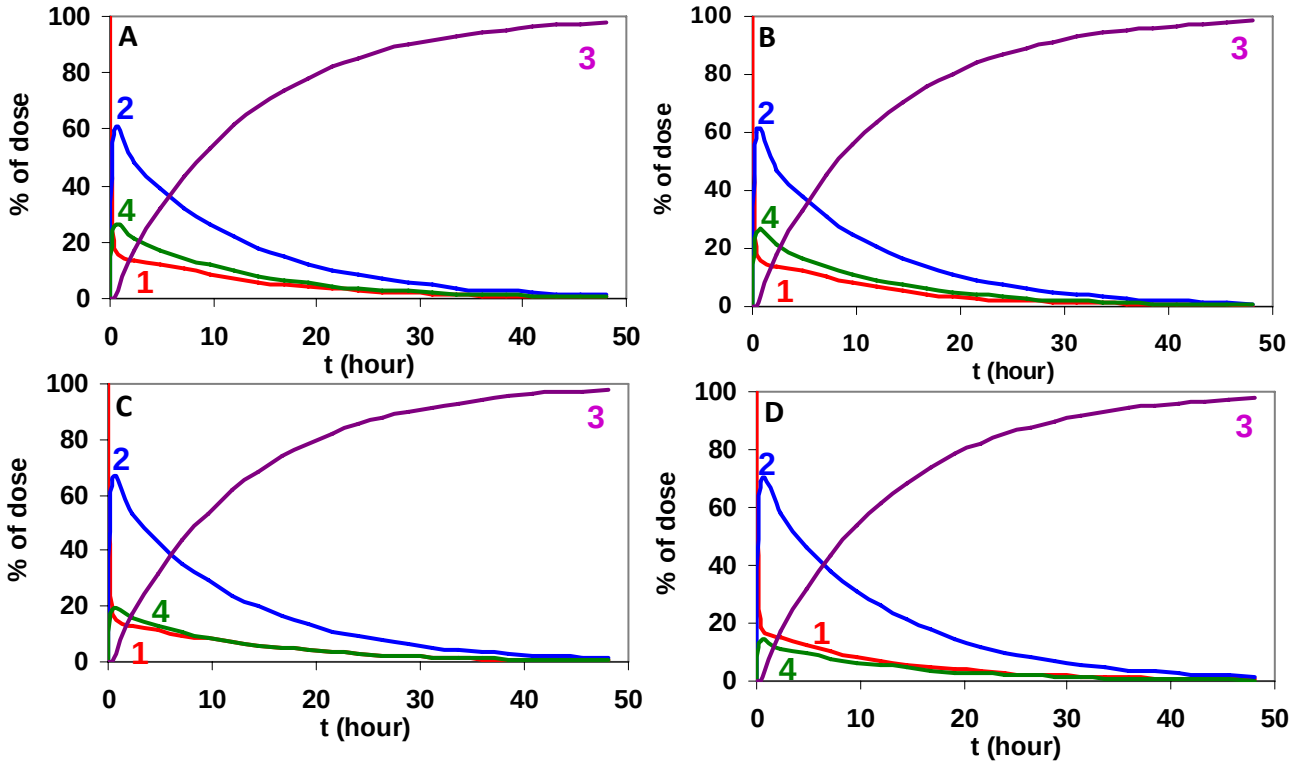

Figure S7. The distribution, adsorption and elimination of gadoterate meglumine (**A**), gadovist (**B**), gadoteridol (**C**) and Gd(HB-DO3A) (**D**) Amounts of GdL in the plasma (**1**), in the interstitium (**2**), the eliminated GdL (**3**) and the adsorbed GdL by collagen (**4**). The distribution volume was 0.25 L/kg body weight, the weight of total collagen was 2.65 kg, the volume of the plasma was taken 3.5 L and the dose of CA was 0.1 mmol/kg body weight for a 70 kg patient.

Table S3. The amount of retained Gd(III)-complexes (% of dose) for the whole body.

T (hours)	gadoterate meglumine	gadobutrol	gadoteridol	Gd(HB-DO3A)
24	85.251	82.898	85.266	85.283
48	97.832	97.084	97.834	97.837
168	100.000	99.999	100.000	100.000
720	100.000	100.000	100.000	100.000

V. References

1. P. W. Atkins, *Physical Chemistry*, fourth edition edn., Oxford University Press, Walton Street, Oxford OX2 6DP, Oxford, 1990.