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INFLUENCE OF HUMAN PROTEINS ON THE RELAXIVITY OF Gd(III) COMPLEXES

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Introduction

We proceeded to a short term stability evaluation of MAGNEVIST, OMNISCAN and GADODIAMIDE in protein containing aqueous solutions by measuring their proton longitudinal relaxivity over a period of 27 hours at variable magnetic fields (NMRD).

To unambiguously distinguish the effect of Gd(III) complex dissociation from its simple non-covalent association with the biological proteins, we also measured the rotationnal correlation time of the organic ligand throught the ^2H relaxation rate of the deuterated molecules (labelled on the α position of carboxylate group). These last experiments were limited to the MAGNEVIST and the GADODIAMIDE. The calculations were carried out on T_1 and T_2 data.

Preparation

Gd(DTPA) dimeglumine salt

MAGNEVIST- SCHERING 82004 300690 - 0.469mg/ml
Stock solution (468.9 ± 11.5)mM (checked by relaxometric method)

Gd(DTPA-BMA)

OMNISCAN - SQ-14-042288 S041 - 500mM - containing 25mM of Gd(DTPA-BMA)CaNa
Stock solution (447.9 ± 7.0)mM (checked by relaxometric method)

GADODIAMIDE - SQ-14-042288 S041 - 500mM
Stock solution (424.3 ± 8.0)mM (checked by relaxometric method)

^2H Labelled DTPA and (DTPA-BMA)

The synthesis and characterization of $DTPAd_{10}$ and $(DTPA\text{-}BMA)d_8$ will be described in a further report.

Human Serum Albumin (HSA)

Fraction V Sigma Chemical Co. A-1653 Batch 126F-9357
The solution 4% by weight was prepared in ditilled water

Globulins

Cohn Fraction IV-4 Sigma Chemical Co. G-3637 Batch 115F-9358

Mixed Biological Proteins

We mixed up HSA and globulin in physiological amounts (36g/l HSA, 24g/l G) in distilled water.

Lyophilized Serum

KONTROLLOGEN L from Behring Diagnostics

Freshly Uptaken Serum

The serum has been obtained after centrifugation of human blood freshly collected in an heparin tube.

Paramagnetic Samples

All the solutions were prepared by adding 5 µl of the paramagnetic complex into 2.5 ml of biological fluids (HSA 4%(wt), mixed biological proteins, lyophilized serum, fresh serum). The final gadolinium concentration of the samples has been checked by ICP (See Annex).

Measurements

The proton NMR experiments were performed at 39°C and 20MHz on a spin analyser BRUKER PC-20 and at 37°C on the IBM relaxometer working over a large range of proton Lamor frequencies (0.01 MHz up to 30 MHz). Between the experiments, the samples were kept at 37°C in a dry block thermostat. The following table summarises the follow up of the measurements.

	MAGNEVIST			OMNISCAN			GADODIAMIDE		
	HSA 4%	M.B.Prot	Kontrol.	HSA 4%	M.B.Prot	Kontrol.	HSA 4%	M.B.Prot	Kontrol
20MHz	00:05	00:15	NA	00:20	00:10	2:05	00:40	00:10	NA
	19:15	22:15	NA	21:25	21:50	23:35	22:00	18:00	NA
NMRD	00:45	00:15	NA	00:55	00:20	00:10	00:50	NA	00:35
	NA	NA	NA	NA	NA	23:35	NA	NA	25:50

	MAGNEVIST Fresh serum	OMNISCAN Fresh serum	GADODIAMIDE Fresh serum
20MHz	NA	NA	NA
	NA	NA	NA
NMRD	00:35	00:05	NA
	26:45	24:35	NA

NA : not available

The deuterium NMR experiments were achieved on a BRUKER MSL200 (4.7T). The longitudinal relaxation rates were determined by using the Inversion-Recovery sequence while the spin-spin relaxation rates were obtained from the line widths. The measurements were realised at 37°C by a thermostated air flow.

Results

Proton experiments

From the table 1, it can be seen that in aqueous mixture of albumin and globulins, the 20MHz relaxivities of Gd(III) complexes is slightly larger than those measured in distilled water but are not time dependent.
This increase is likely due to a microviscosity change and a water content reduction (4% -6%).

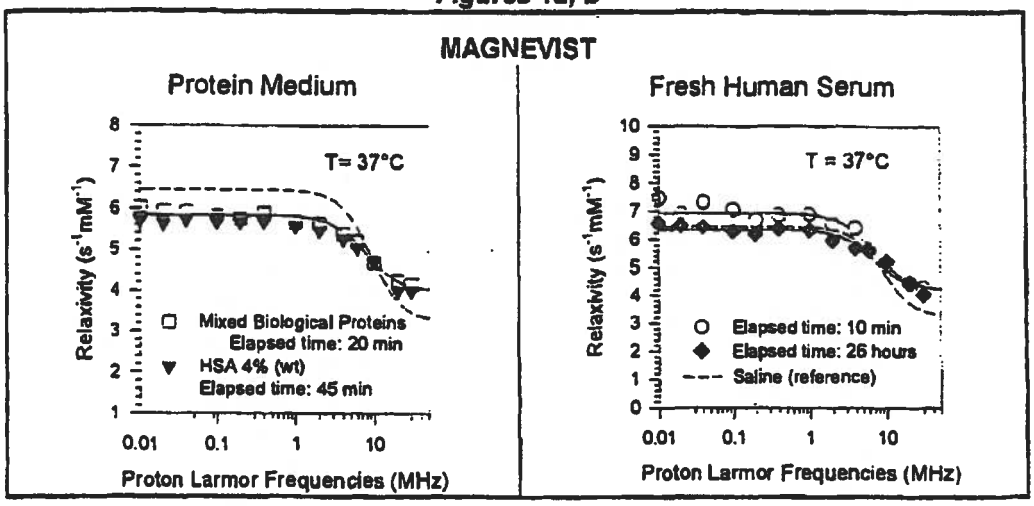
Table 1 - Relaxivities ($s^{-1}mM^{-1}$)

20MHz	MAGNEVIST			OMNISCAN			GADODIAMIDE		
39°C	HSA 4%	Mixed Prot	Kontrol.	HSA 4%	Mixed Prot	Kontrol.	HSA 4%	Mixed Prot	Kontrol.
± 00:30	4.20±0.14	4.40±0.19	NA	3.20±0.05	3.54±0.10	NA	3.53±0.09	3.79±0.27	NA
± 24:00	4.13±0.16	4.12±0.16	NA	3.18±0.07	3.36±0.07	NA	3.40±0.05	3.67±0.13	NA
WATER	3.84 ± 0.16			3.85 ± 0.19			3.85 ± 0.19		

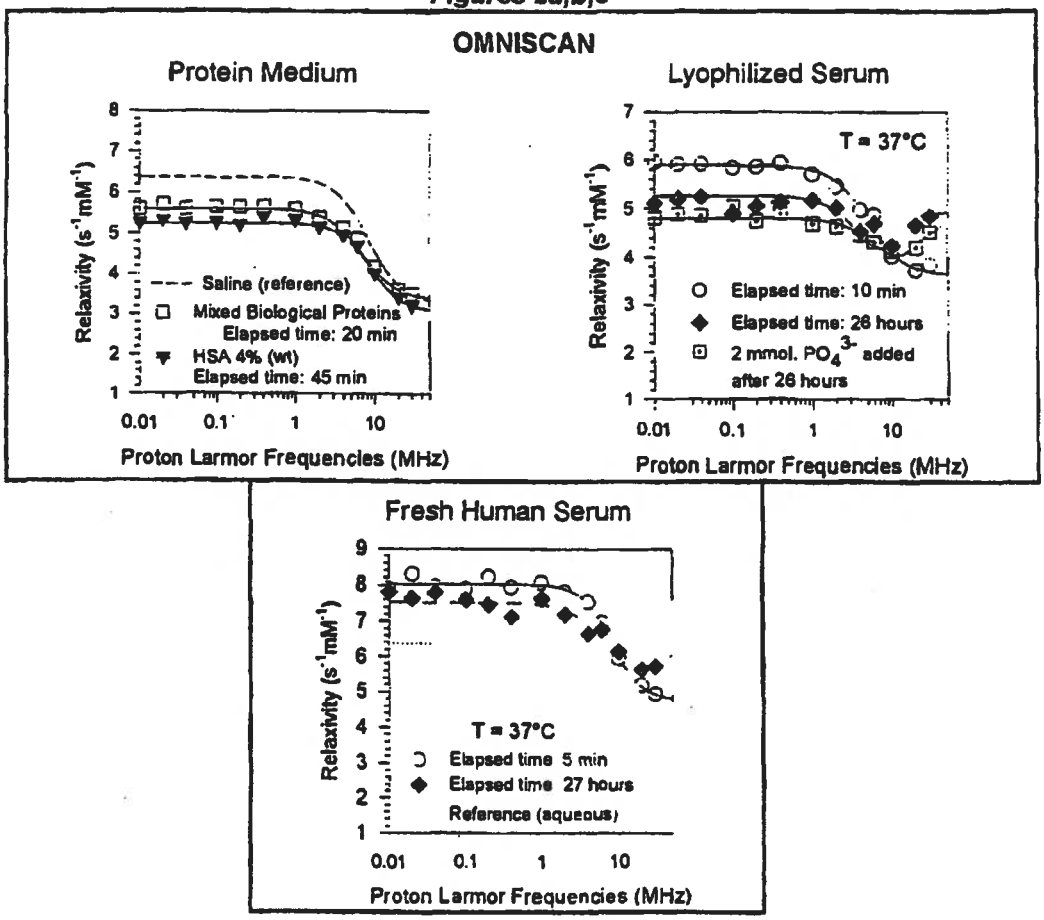
T_1 by IR pulse sequence -Mean values calculated on 8 measurements.

While the NMRD data (figures 1a,2a,3a) confirm the behaviour of the compounds in this protein containing medium, the relaxation profiles of OMNISCAN and GADODIAMIDE recorded in fresh or in lyophilized serum indicate a time dependence over ca.24 hours.
The figures 2b,c and 3b show a net increase of the high field relaxivities which is a consequence of a τ_R lengthening.
These results confirm previous studies which already suggested that a dissociation of the complex followed by the complexation of the released Gd(III) by the proteins was taking place. This is demonstrated by deuterium experiments developped in the next section.
We unsuccessfully tried to remove the Gd(III) from the proteins by adding inorganic phosphate in the 26 hours old seric samples. On formation of insoluble $GdPO_4$, the relaxivities shoud have dropped. Since no such evolution was observed within 2 hours, we may conclude that either the protein complex is very stable or the degradation reaction is kinetically slow (figures 2b,3b).

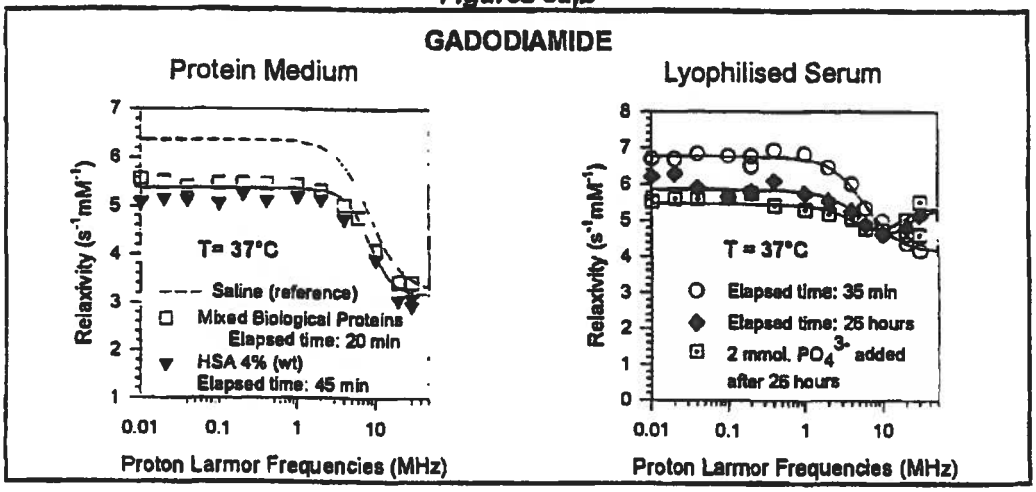
Figures 1a, b



Figures 2a,b,c



Figures 3a,b



Deuterium experiments

Knowing that Gd(DTPA) does not to interact with serum, the effect observed on R_1 when the complex is dissolved in serum must be due to microviscosity effects. Since $R_1 \equiv cst * \tau_R$, where τ_R is temperature dependent (eq.1), the ratio of R_1 observed in water and in serum over the explored temperature rangewill be proportional to the change of viscosity between water and serum.

Figure 4

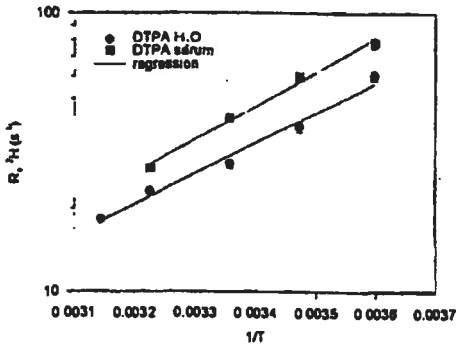


Table 2

Ratio of R_1 in water and serum calculated from the fitted curves (figure 4)

	5°C	15°C	25°C	37°C
$R_{1(serum)}/R_{1(water)}$	1.437	1.387	1.343	1.294

$$\tau_R = \frac{4\pi a^3 \eta}{3kT} = \tau_R^0 \exp\left[\frac{E_R}{RT}\right] \qquad \textit{Eq.1}$$

From these results and knowing the R_1 of the (DTPA-BMA) in water (fig.5), one can estimate its relaxation rate in serum at each temperature when only viscosity and microviscosity effects are considered and are assumed to be identical to those observed for DTPA solutions (fig.6)

Table 3

Estimated and observed values of R_1 of (DTPA-BMA) in serum (Fig 5, 6) - 50mM

	5°C	15°C	25°C	37°C
$R_{1\ est^*}$	93.06	66.53	48.27	33.7
$R_{1\ measured}$	82.6±4.1	67.1±3.2	47.95±1.4	36.2 ±1.2

$^*R_{1\ estimated} = R_{1(water)\ fitted} * R_{1(water)DTPA}/R_{1\ (SERUM)\ DTPA}$

Figure 5

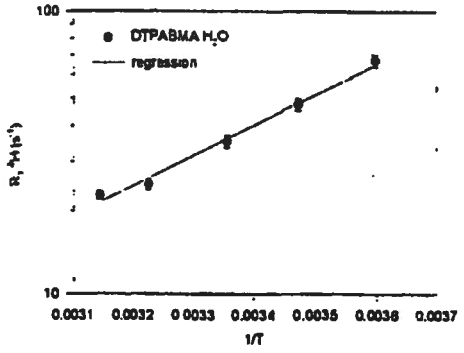
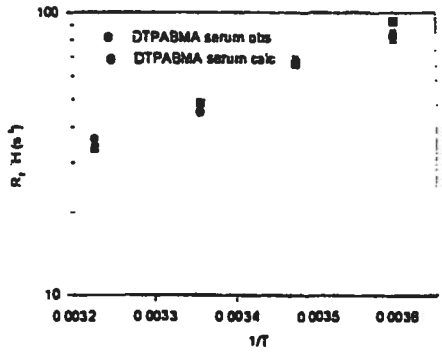


Figure 6



From the similitude of observed and calculated R_1 , one could indeed conclude that the effect seen on R_1 is due only to viscosity and preclude additionnal reduction of the mobility due to interactions with serum proteins.

The NMR line at half height is related to T_2 and thus also to τ_R. Consequently, its measurement allows a direct evaluation of the change of small ligands mobility induced by their interaction with the macromolecules.

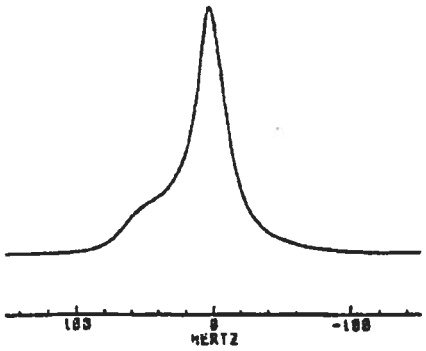
Deuterium linewidths of DTPA-d_{10} and (DTPA-BMA)-d_8 50 mM have been measured in saline solutions and serum (Kontrollogen L). The broadening of DTPA resonances in serum solution as compared to saline solution is ≈ 6.2 Hz (figure 7). Since DTPA is known not to bind to proteins, this increase is attributed to viscosity or microviscosity effect on τ_R. After two hours, (DTPA-BMA) linebroadening is of the same order of magnitude (≈ 7.5 Hz) (figure 8). After 24 hours in serum (6 hours at 310°K), the line broadening is ≈ 5 Hz. This confirms that (DTPA-BMA) does not bind to serum proteins.

It has to be noticed that a significant broadening has been observed for DTPA derivatives carrying lipophilic groups

Figure 7

DTPAd$_{10}$

Water



Serum

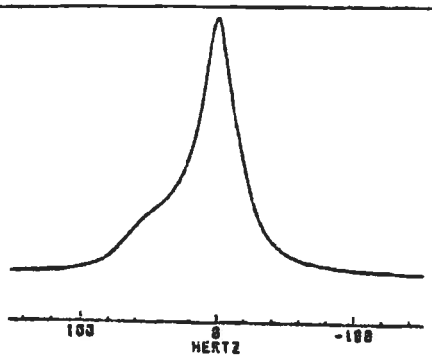
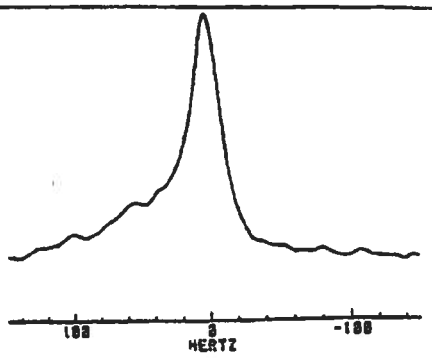


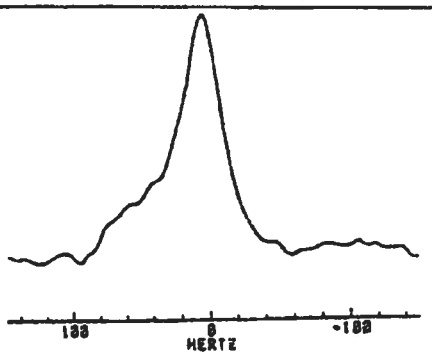
Figure 8

(DTPA-BMA)$_{d8}$

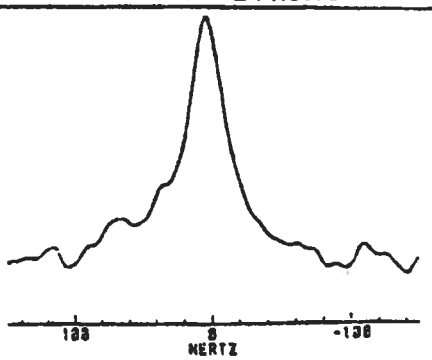
Water



Serum after 2 hours



Serum after 24 hours



Conclusion

None of the compounds evolves in aqueous mixture of albumin and globulins over a period 24H00. The higher high field relaxivities observed in those media are attributed to both the microviscosity change and the water content reduction.
In serum (both fresh and lyophilized material), the high field relaxivities of OMNISCAN and GADODIAMIDE significantly increase during period. The formulation of samples does not influence the stability of the gadolinium complex in those media.
Previous investigations concerning the interactions between Gd(III) ion and human proteins indicate that this behaviour is a consequence of a partial dissociation of complexes. This conclusion is confirmed by ^{2}H measurements.
It has to be mentionned that the time course of the dissociation process is long in comparison with the excretion kinetics of OMNISCAN.
The interesting question of the reason why the dissociation takes place in serum and not in the simple mixture of proteins remains unsolved but would deserve more research work.

Annex

ICP Measurements

The Gd(III) content was directly measured on samples involved in NMR experiments. The following table includes the actual Gd(III) concentration of samples and the correction factor which has to be only applied on NMRD relaxivities stored on the floppy disk and in the next tables[1].

Gd(III) Concentration (mM)						Correction factor		
MAGNEVIST			OMNISCAN			GADODIAMIDE		
HSA 4%	M.B.Prot	Kontrol.	HSA 4%	M.B.Prot	Kontrol.	HSA 4%	M.B.Prot	Kontrol.
1.077±0.027	1.107±0.039	NA	1.062±0.009	1.070±0.017	0.948±0.011	1.110±0.011	1.061±0.029	0.990±0.010
0.8690	0.8454	NA	0.8263	0.8355	0.9428	0.7631	0.7983	0.8559
MAGNEVIST Fresh serum			OMNISCAN Fresh serum			GADODIAMIDE Fresh serum		
1.138 ± 0.009			0.826 ± 0.011			NA		
0.8225			1.0823			NA		

NA : not available.

Additionnal NMRD Relaxivities concerning the lipphilized serum

"NYCOMED - KAREN - 0.847MM GADODIAMIDE IN KONTROLLOGEN L TEMP = 37.00"

0.010	7.832
0.020	7.815
0.040	8.000
0.100	7.934
0.200	7.920
0.200	7.802
0.400	8.116
1.000	7.987
2.000	7.571
2.000	7.564
4.000	7.048
6.000	6.268
10.000	5.850
10.000	5.432
20.000	5.436
20.000	5.137
30.000	4.902

"NYCOMED - KAREN - 0.847 MM GADODIAMIDE IN KONTROLLOGEN L - 24H00 TEMP = 37.00"

0.010	7.261
0.020	7.358
0.040	6.900
0.100	6.620
0.200	6.769
0.400	7.123
1.000	6.734
2.000	6.481
4.000	6.185
6.000	5.751
10.000	5.450
20.000	5.662
30.000	6.044

[1] By this way you will get the same NMRD profiles that we present in this report.

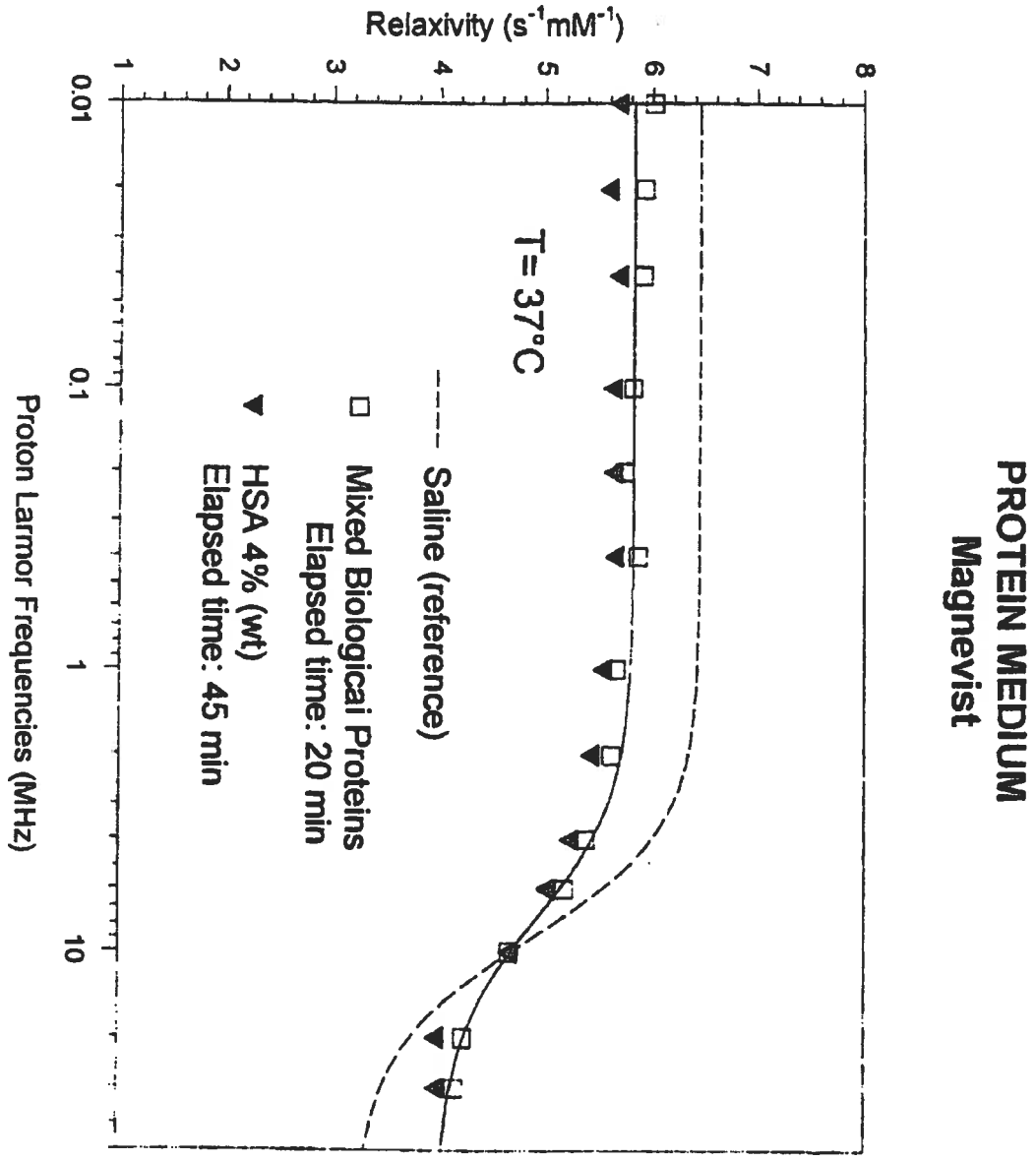
"NYCOMED - KAREN - 0.840 MM GADODIAMIDE IN KONTROLLOGEN L - 24H00 - 2.65 MM EXOGENEOUS (PO4)3- TEMP = 37.00"
0.010 6.454
0.020 6.544
0.040 6.550
0.100 6.824
0.200 6.732
0.400 6.344
1.000 6.210
2.000 6.085
4.000 5.938
6.000 5.614
10.000 5.557
20.000 5.900
30.000 6.467

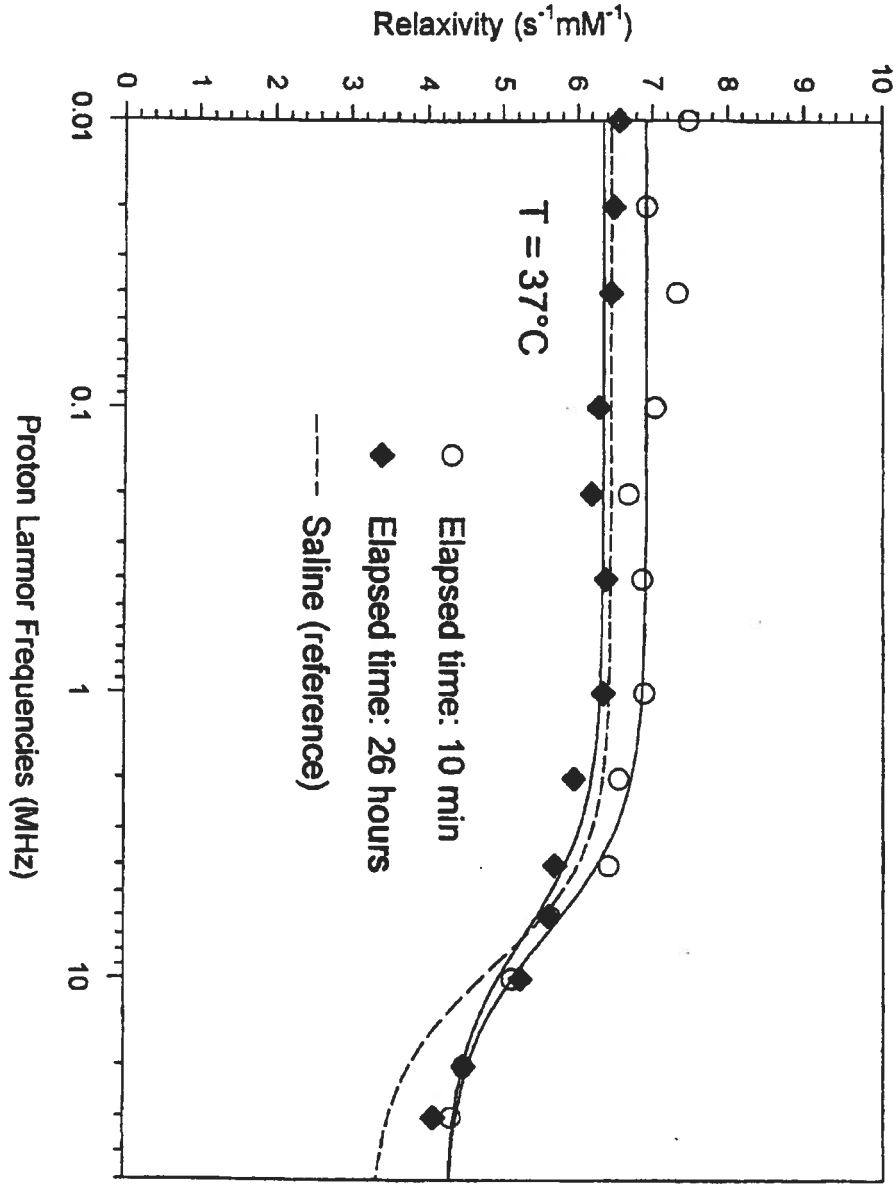
"NYCOMED - KAREN OMNISCAN 0.894MM IN KONTROLLOGEN L TEMP = 37.00"
0.010 7.125
0.020 7.060
0.040 7.078
0.100 6.978
0.200 7.008
0.400 7.102
1.000 6.830
2.000 6.521
4.000 5.954
6.000 5.827
10.000 4.801
20.000 4.456
30.000 4.597

"NYCOMED - KAREN - 0.894MM OMNISCAN IN KONTROLLOGEN L - 24H TEMP = 37.00
0.010 6.117
0.020 6.221
0.040 6.270
0.100 5.859
0.200 6.048
0.400 6.142
1.000 6.192
2.000 6.009
4.000 5.432
6.000 5.615
10.000 5.072
20.000 5.584
30.000 5.806
"NYCOMED - KAREN - 0.887MM OMNISCAN IN KONTROLLOGEN L - 24H00 - 2.65 MM EXOGENEOUS (PO4)3- TEMP = 37 00
"
0.010 5.752
0.020 5.843
0.040 5.814
0.100 6.038
0.200 5.679
0.400 5.870
1.000 5.611
2.000 5.546
4.000 5.335
6.000 5.156
10.000 4.950
10.000 4.956
20.000 5.032
30.000 5.404

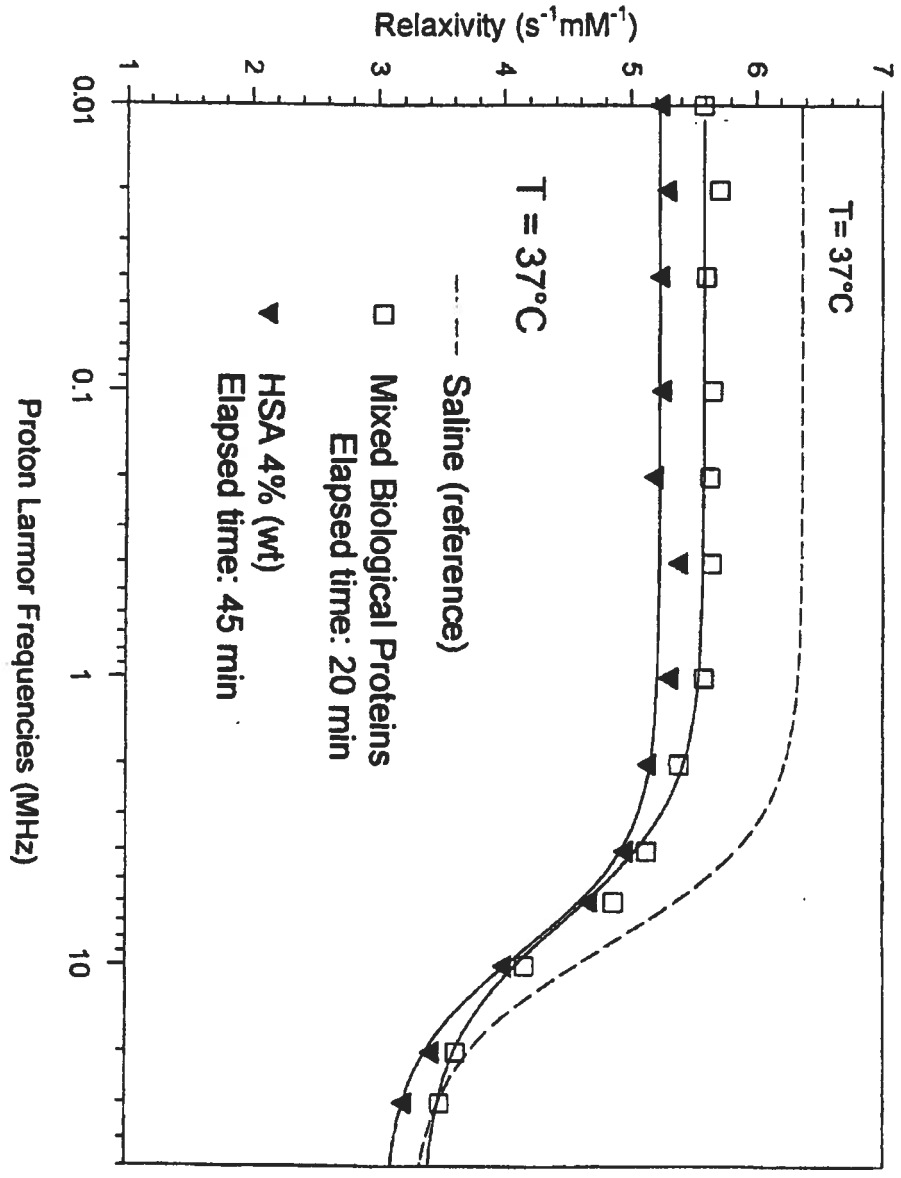
"NYCOMED - KAREN - 0.894 MM OMNISCAN IN PLASMA OF K. TEMP = 37.00"
0.010 7.293
0.020 7.666
0.040 7.342
0.100 7.287
0.200 7.594
0.400 7.330
1.000 7.457
2.000 7.215
4.000 6.968
6.000 6.438
10.000 5.494
20.000 4.801
30.000 4.565

"NYCOMED - KAREN - 0.894 MM OMNISCAN IN PLASMA OF K. - 24H00 TEMP = 37.00"
0.010 7.210
0.020 7.058
0.040 7.199
0.100 7.022
0.200 6.898
0.400 6.595
1.000 7.051
2.000 6.655
4.000 6.155
6.000 6.261
10.000 5.673
20.000 5.216
30.000 5.303

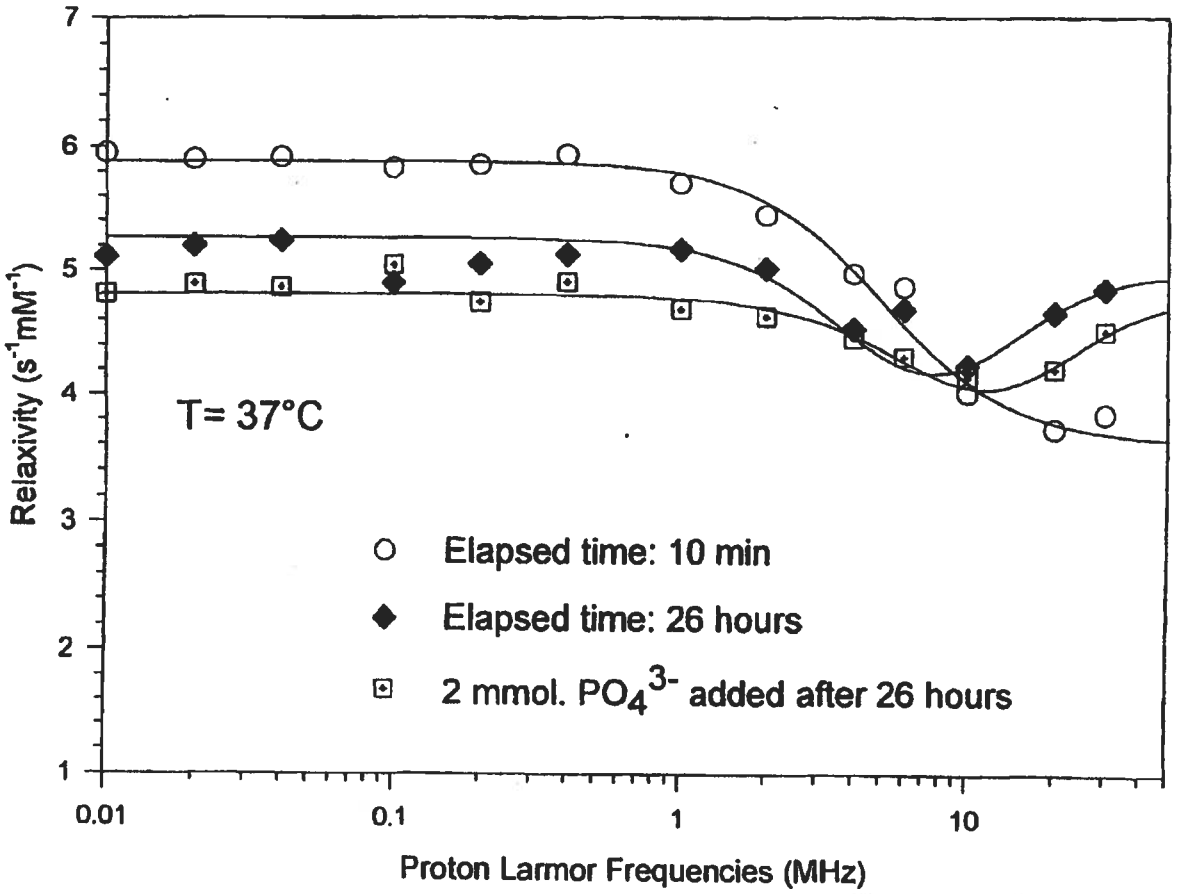




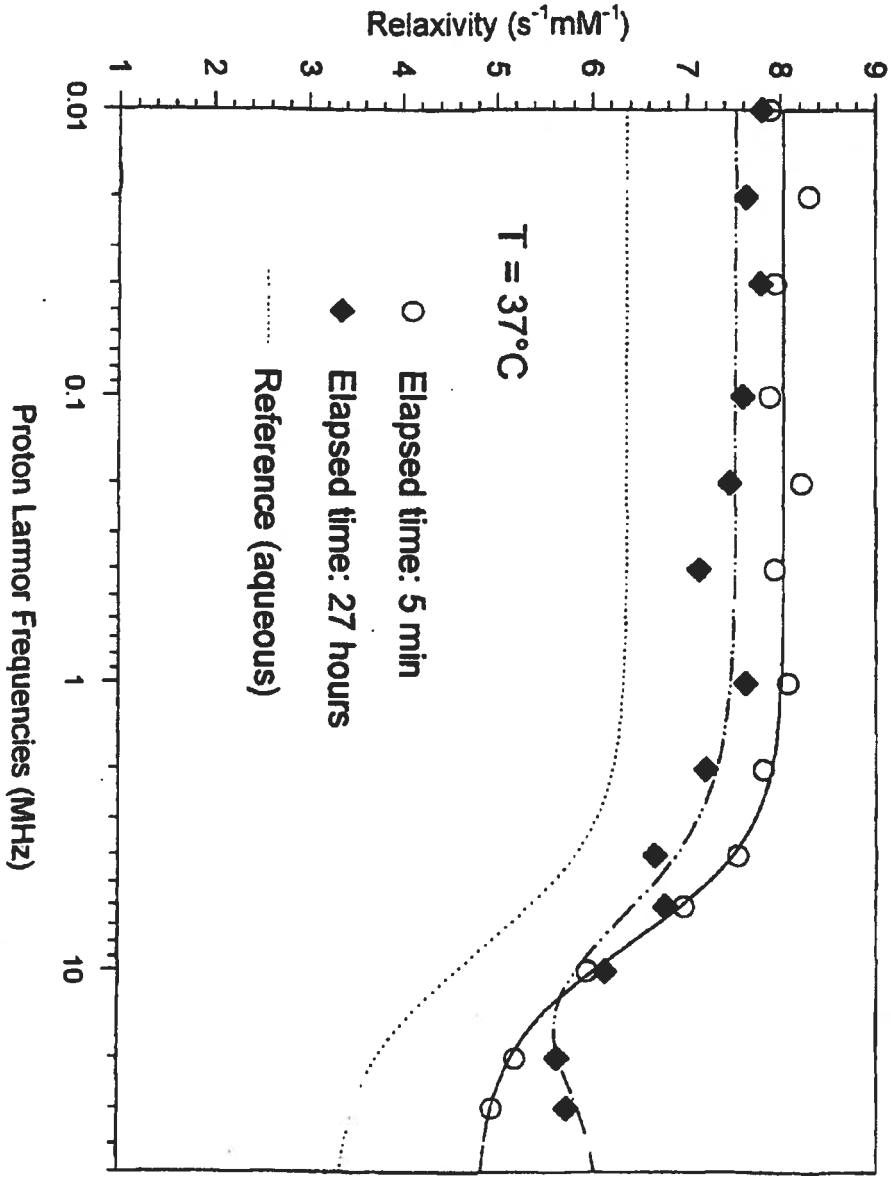
TIME DEPENDENCE OF THE RELAXIVITY
Magnevist
Fresh Human Serum

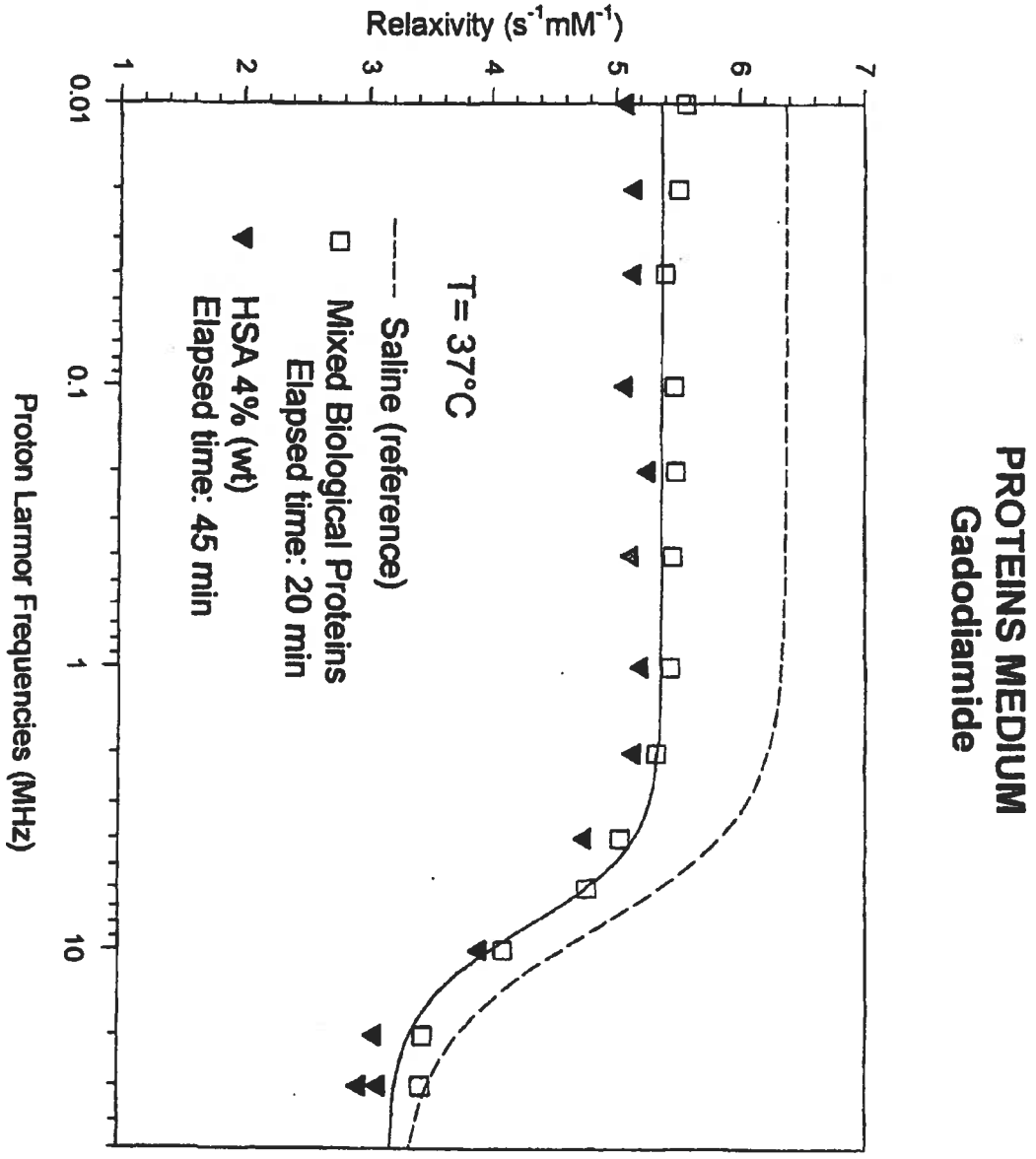


TIME DEPENDENCE OF THE RELAXIVITY
OMNISCAN
Serum Kontrollogen L

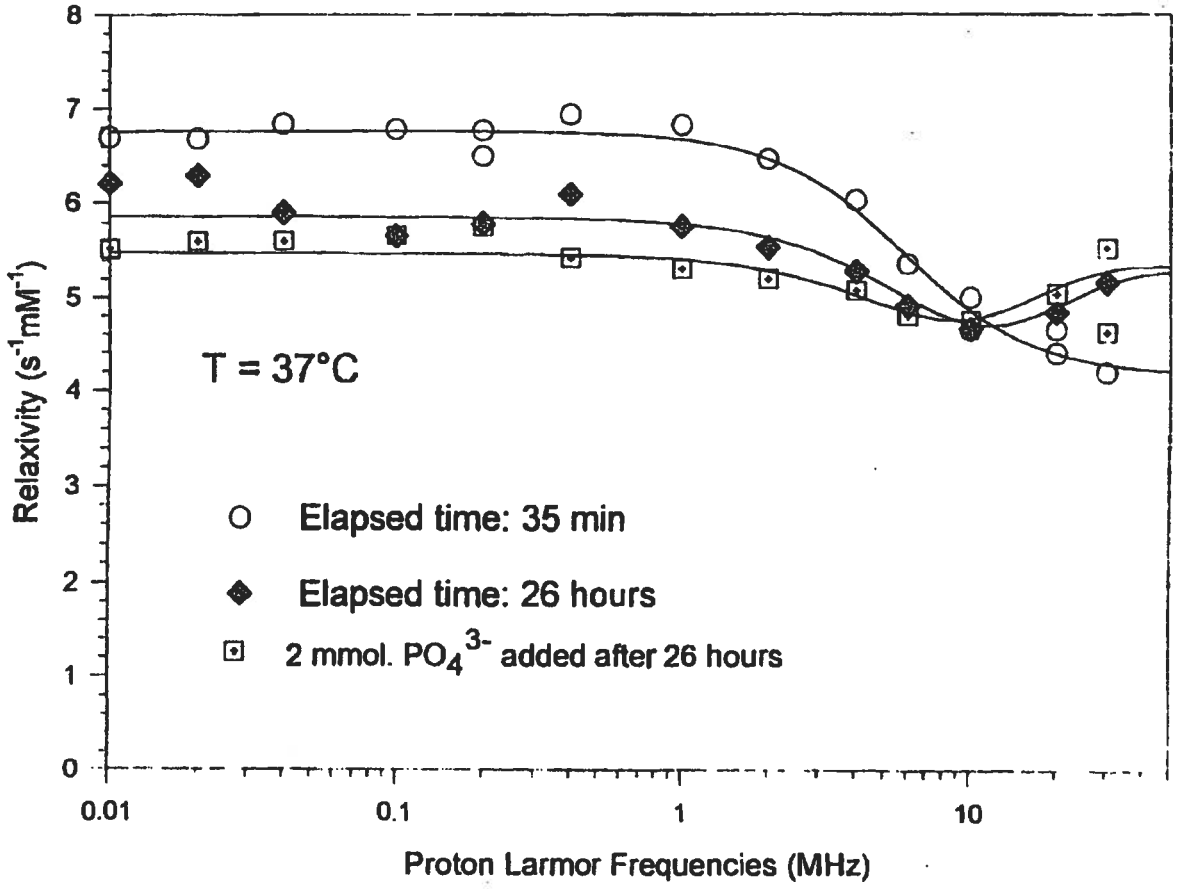


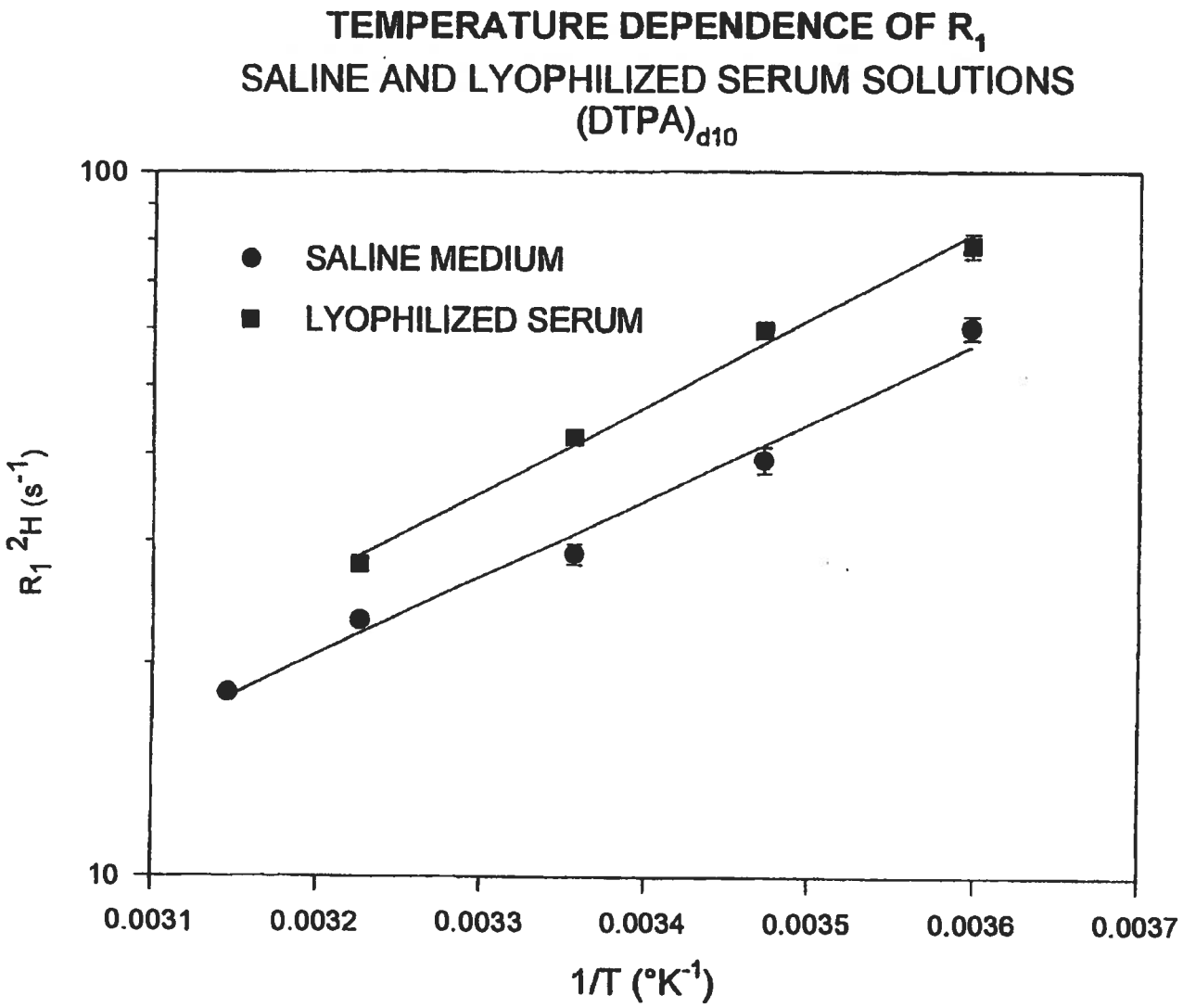
TIME DEPENDENCE OF THE RELAXIVITY
OMNISCAN
Human Fresh Plasma

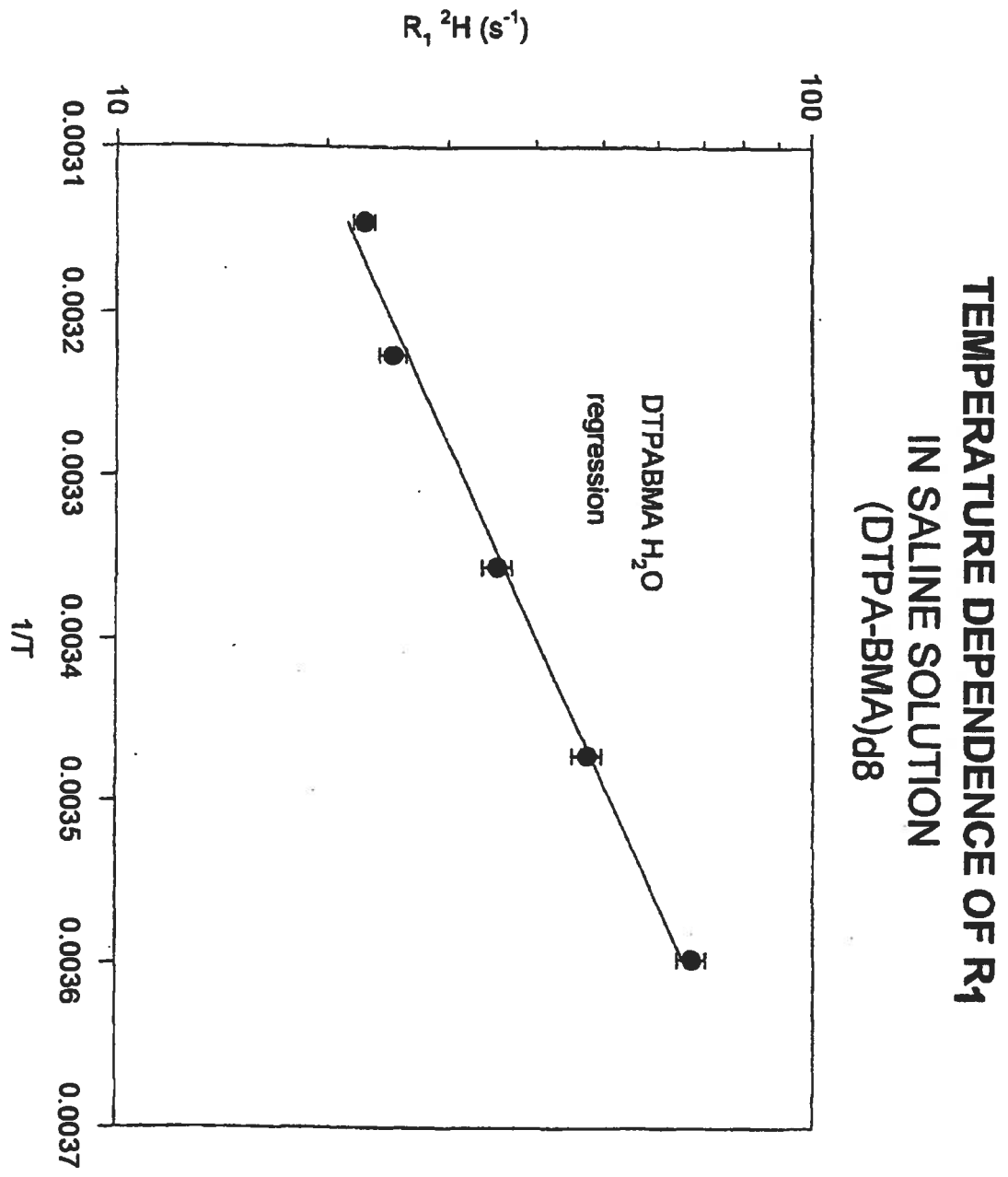




TIME DEPENDENCE OF THE RELAXIVITY
Gadodiamide
Serum Kontrollogen L







TEMPERATURE DEPENDENCE OF R_1
IN LYOPHILISED SERUM
Microviscosity Effects
$(DTPA\text{-}BMA)_{d8}$