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TUNNELING WITHIN LOCALIZED STATES
IN NITROSYL MYOGLOBIN

by

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Abstract:The temperature (T) dependence of the Spin Lattice Relaxation time of nitrosyl myoglobin (MbNO) was measured in the range from 9K to 310K. Both powdered and solution samples exhibit an unexpected dependence. Experimental data is well fitted by a T^n function with $n=2.2\pm 0.3$ in this large temperature range. This result is interpreted within the tunneling localized states model associated with the existence of conformational equilibrium in MbNO.

Key-words: Nitrosyl myoglobin; Spin lattice relaxation; E.P.R.

I. INTRODUCTION

Spin Lattice Relaxation (SLR) from metalloproteins has been widely studied¹⁻⁸. The relaxation rate was characterized by a Raman mechanism with an anomalous T^n temperature(T) dependence in copper and pure low spin state iron containing proteins. Stapleton et al³ have first proposed a correlation between the n exponent and the protein conformation through the fractal dimension. The results have led to different theoretical models for the SLR properties of proteins that yield relationships between the n exponent and the fractal structure^{3,9-12}.

Nevertheless some deviations from a power law temperature based on the fractal dimension have been observed for the SLR time (T_1) of proteins. At low temperatures where a linear temperature dependence is expected for a direct process, some hemoproteins present a T^2 dependence^{1,5}. Type V cytochrome C show a T^2 dependence up to a temperature of 22.6K³ with no evidence of a Raman mechanism. In the presence of a high spin state population metmyoglobin (metMb) and methemoglobin (metHb) present an Orbach like mechanism for the iron low spin state⁸. Recently small deviations from a simple T^n behavior were observed for the blue-copper protein plastocyanin⁷. Despite the large amount of data on this subject it is not yet sufficient to give a direction to the theoretical discussion, and there are still several points to be explained about the subject concerning the anomalous behavior of the temperature dependence of SLR rates (T_1^{-1}) in

proteins.

More recently investigations on the role of water content in Hb and Mb have shown dependence of conformation, spin and valence states on the degree of hydration^{13,14}. Very few studies have been done on the relaxation mechanism using powdered Hb and Mb⁸.

Measurements over a wide temperature range help to understand complex processes occurring in proteins¹⁵ and can provide useful information on SLR rates in iron and copper containing proteins⁷. This study aims to clarify the SLR mechanism of nitrosyl Mb (MbNO) in a large temperature range from (9K to 310K) and to verify the effect of protein hydration by comparing solution and powder with a small hydration level (10% in weight) samples of MbNO. It reveals one more deviation of the T_1^{-1} temperature dependence from the fractal power law.

II. EXPERIMENTAL

Sperm whale (Mb) from Sigma Chemical Co. was used for sample preparations. Protein solutions were completely oxidized with a 20-fold excess of potassium ferricyanide followed by equilibration with phosphate buffer 0.1M pH 7.0 in a Sephadex G-25 (Sigma Chemical Co) column. Powdered samples were dried from the commercial form without further oxidation under vacuum in the presence of P_2O_5 . Nitrosylation was obtained by introducing nitric oxide (NO) gas directly into the previously deaerated EPR (Electron

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Paramagnetic Resonance) sample tube.

EPR measurements were performed in a E-9 Varian X-band spectrometer between 9K and 310K using a helium flux cryostat (Helitran LTD-3-110). Temperatures were measured with a Au-Fe versus chromel thermocouple placed just above the sample.

The half-saturation powers $P_{1/2}$ and the SLR rate T_1^{-1} were estimated as previously described, using the continuous saturation method⁸. The intensities of the $g=2.064$ and $g=2.003$ EPR lines in powdered samples and $g=2.007$ in solution were monitored as shown in the spectra of Fig.1a and 1b respectively.

The experimental curves of T_1^{-1} versus T (Fig.2-4) are fitted with the simple expression

$$T_1^{-1} = AT^n \quad (1)$$

The exponent n was determined by a Monte Carlo like method minimizing the mean square root error.

III. RESULTS AND DISCUSSION

In Fig.1 the EPR spectra obtained for MbNO in solution (Fig.1a) and powder (Fig.1b) are presented. In the powdered form the nitrogen hyperfine interaction characteristic of the pentacoordinated iron¹⁶ is clearly observed, while for the solution sample a broad unresolved line is obtained.

The temperature dependence of the SLR rates estimated from the EPR lines indicated by arrows in Fig.1 are shown in Fig2-4.

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The log-log plot evidences a T^n power law with $n=2.2\pm 0.3$. The values of n for each magnetic field position are shown in the figures. This is an unusual T_1^{-1} behavior. One should expect $T_1^{-1}\propto T$ for the low temperature range with the Direct process dominant and $T_1^{-1}\propto T^n$ with $7\geq n\geq 5$ related to the fractal dimension for temperatures where a Raman mechanism is the relevant one, that changes to T^2 at high temperatures. Muench and Stapleton⁴ have described a similar result for nitrosyl ferrous Mb solution in the restricted temperature range from 4.2K to 20K. They attributed a linear ($n=1$) temperature dependence to their SLR data but they made no attempt to identify the relaxation mechanism. We observed an unusual behavior with a different exponent value that persists for temperature as high as 310K for powdered MbNO and 200K in MbNO solution. In this temperature range we can not attribute this temperature dependence to a strong bottleneck effect. Nevertheless we can look for the interpretation in non biological systems with similar results.

Amorphous materials possess physical proprieties strikingly different from those of crystalline solids^{17,18}. Several papers¹⁹⁻²¹ have established that those materials exhibit anomalously large SLR rates and unusual temperature dependence given by T^n with $n=1-3$. This result was also described for molecular radicals embedded in matrices²². The usual Raman process is not observed. In glassy systems it appears that modulation by tunneling modes involving one phonon is much more effective than

modulation by lattice phonon in SLR mechanism for temperatures lower than 100K. The tunneling localized state (TLS) model requires the identification of the tunneling unit and the assumption concerning the interaction that couples the spin to the TLS. The TLS is a two level system with splitting energy $E \gg \hbar\omega$ the Zeeman energy. It is strongly coupled to the phonons. The spin system relaxes due to mutual TLS-spin transitions induced by a weak coupling spin-TLS.

This model was first suggested by Lyo and Orbach²⁴ and quantitative studies of SLR via TLS-phonon mechanism were presented by Kurtz and Stapleton¹⁹ and Deville et al.²⁰ The two calculations differ mainly by the spin-TLS interaction description. For irradiated β -alumina the interaction assumed is a superhyperfine one¹⁹ and a phenomenological operator describes the crystal field modulation for the vanadium system²⁰.

The model predicts a temperature dependence of the form

$$T_1^{-1} \propto T^{2+\lambda} \quad T \ll E_{\max}/k \quad (2a)$$

$$T_1^{-1} \propto T \quad T > E_{\max}/k \quad (2b)$$

where E_{\max} is the maximum energy of the TLS^{5,19,20} or the cut off in the distribution of the asymmetry parameter of the double-well potential in eq. 2b if a more accurate calculation is done²⁰.

The experimental results presented here for MbNO could be

explained by the mechanism described above, with a λ value of 0.2. An interesting task is to identify the tunneling system and its interaction with the paramagnetic center. EPR temperature dependence studies^{25,26} in frozen solutions and crystal of MbNO evidence the existence of at least two different binding conformations of the NO relative to the heme plane. We suggest that these two NO orientations could be described by a two level system as shown for other heme proteins²⁷. In this case the relaxation mechanism could be induced by the TLS modulation of the crystal field since the paramagnetic center belongs to the tunneling system²⁰. Our T_1^{-1} result does not show the linear dependence in the high temperature limit. The low value of λ (0.2) may reflect a lower characteristic energy E_{\max}^{19} , when compared to inorganic amorphous materials.

Previous papers^{22,23} have analysed the T_1^{-1} temperature dependence of atomic radicals in glass matrices using the expression $T_1^{-1} = AT + B \exp(-\Delta/kT)$. The first term was interpreted as due to the relaxation by tunneling motions characteristic of the disordered state. The second term is a convenient functional form to describe various relaxation mechanisms at higher temperatures. However we used the simpler expression given by Eq.1 which gives a good fit to the experimental data for MbNO. Fig.2 shows that the fittings with both expression can not be distinguished significantly one from each other. The experimental errors do not justify the use of a more complicated function than that with the

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exponential contribution.

Although the values of T_1^{-1} are estimated from the continuous wave saturation technique used, this is adequate to determining the temperature dependence and relative values. We estimate that at 20K, $T_1^{-1}=1.1 \times 10^5 \text{ s}^{-1}$, and $1.8 \times 10^4 \text{ s}^{-1}$ for $g=2.003$ and $g=2.064$ of the powdered sample respectively and $5.6 \times 10^3 \text{ s}^{-1}$ for frozen solution. For the MbNO the order of magnitude of T_1^{-1} presented is in good agreement with those published⁴. SLR occurs due to a time dependent perturbation of electron spin by its environment. Anything that affects the disordered structure and the distribution of tunneling modes should have a large effect on the magnitude of T_1 . The effect of dehydration of MbNO is to increase the SLR rate relative to the solution sample by a factor of at least 3. On the other hand the difference in the T_1 values of the two lines in the powdered sample could be explained by an anisotropic behavior of T_1 .

We propose that in MbNO there is a coupling of the spin system to a localized tunneling system strong enough to be more efficient than the usual spin-phonon interaction which makes the Raman mechanism dominant in comparison with other Mb derivatives. This observation strengthens the similarity between proteins and glasses. Other fundamental properties were shown to be shared by both systems^{28, 29}. We believe that these results will stimulate more experimental work and more detailed calculations on SLR of proteins. In these systems it is possible to get information on

excitations and dynamics of the structure in large temperature range.

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Figure Captions.

Fig 1. MbNO EPR spectra. (a) Powdered sample, (b) solution. Vertical lines indicate the line intensities used for determining T_1 .

Fig 2. Temperature dependence of the spin lattice relaxation of powdered MbNO at $g=2.003$. Solid line is the fit with $T_1^{-1} \propto T^n$, with $n=2.1 \pm 0.2$.

Fig 3. Temperature dependence of the spin lattice relaxation of powdered MbNO at $g=2.064$. Solid line is the fit with $T_1^{-1} \propto T^n$, with $n=2.4 \pm 0.1$.

Fig 4. Temperature dependence of the spin lattice relaxation of MbNO solution at $g=2.007$. Solid line is the fit with $T_1^{-1} \propto T^n$, with $n=2.4 \pm 0.1$.

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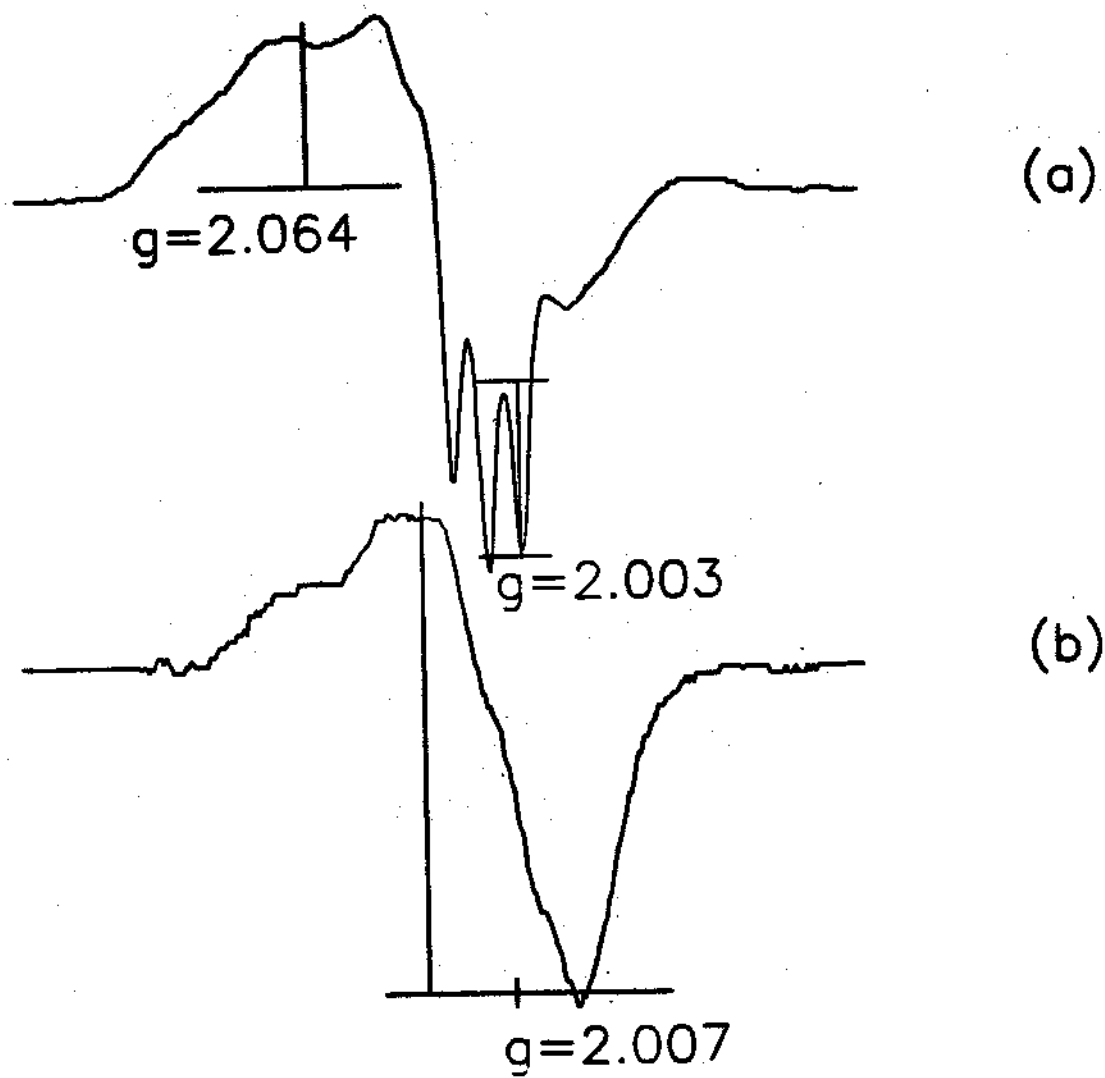


Figure 1

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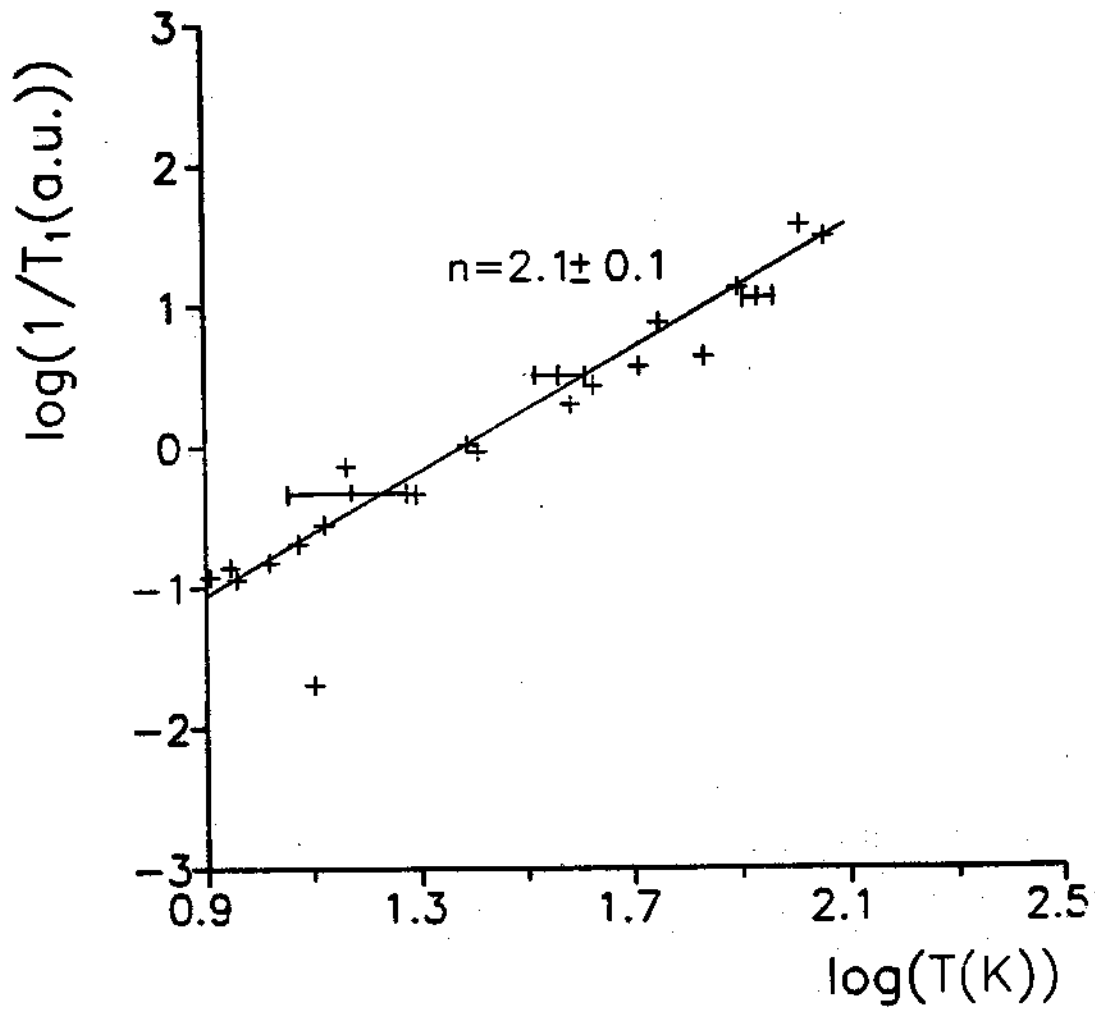


Figure 2

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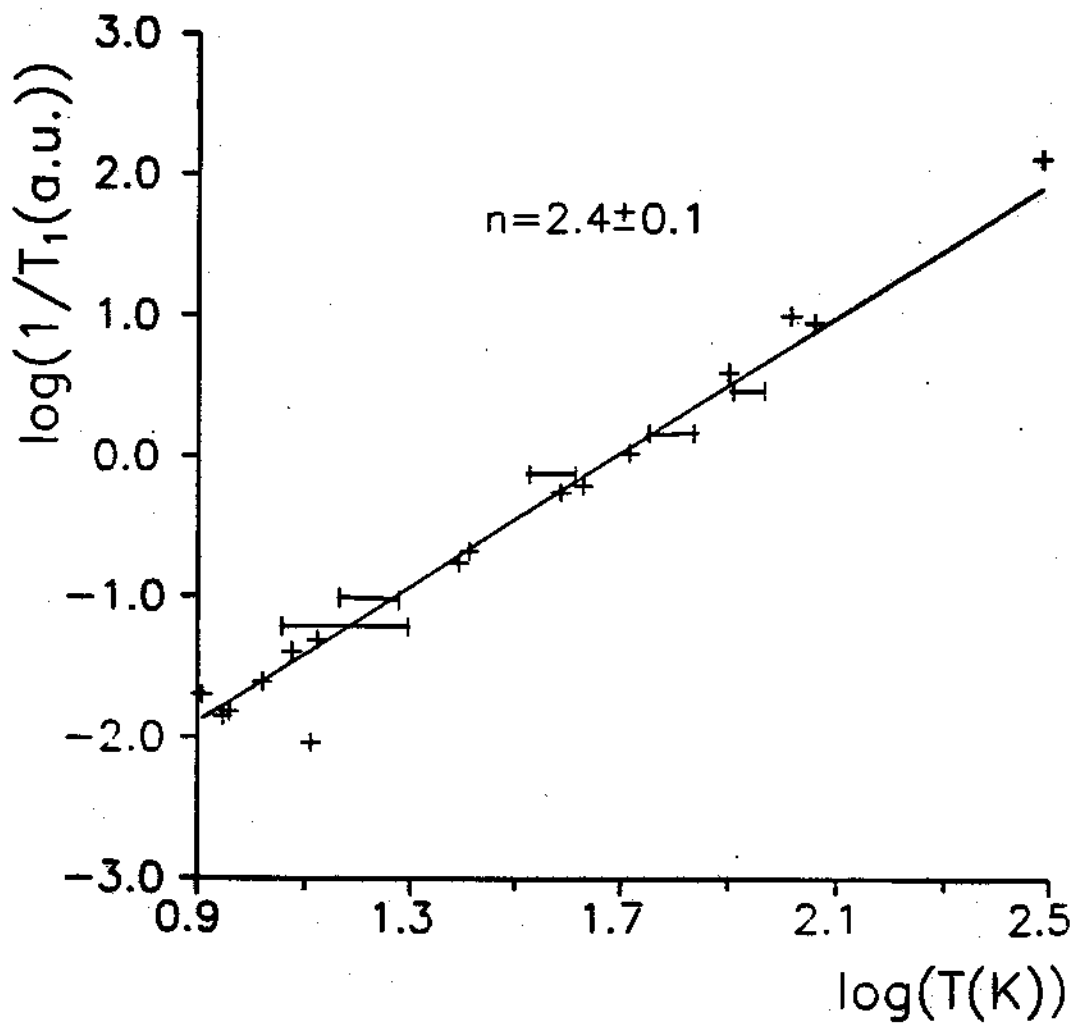


Figure 3

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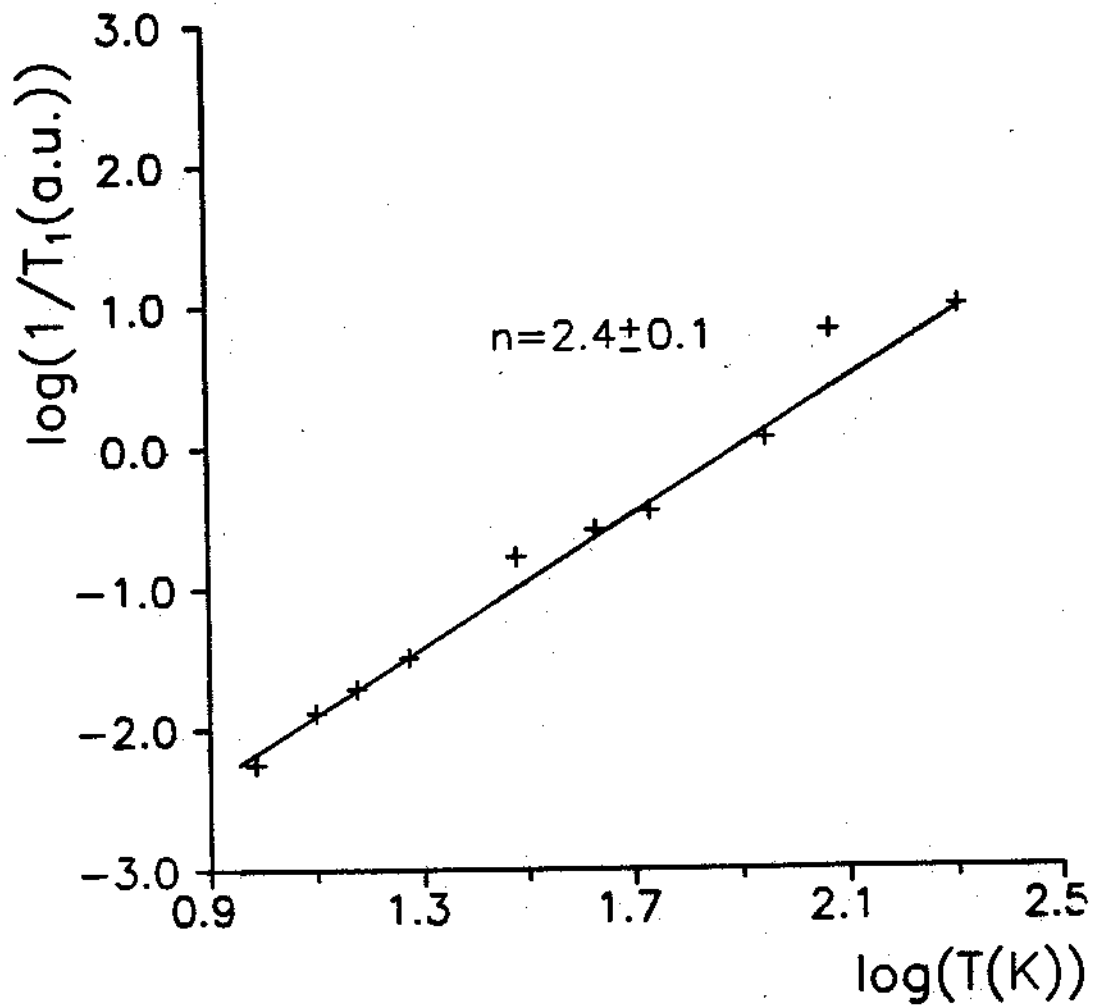


Figure 4

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