

International Atomic Energy Agency
and
United Nations Educational Scientific and Cultural Organization
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS

**POSITRON AND POSITRONIUM REACTIVITIES
WITH HEMIN COMPOUNDS
IN ALKALINE WATER/ETHYLENEGLYCOL MIXTURE (50%)**

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ABSTRACT

Positron lifetime experiments have been performed in 50% water/ethyleneglycol with various hemin concentrations at pH 9. Due to the time resolution of the spectrometer, the short lifetimes were not significant separately. We only discussed the long lived component ascribed to the orthopositronium annihilation. A critical hemin concentration was observed. We described the annihilation characteristics in relation with the formation of hemin aggregates. An analytical model for positron and positronium chemistry is proposed in terms of interaction with various compounds in the solution, with the reaction rates forming a continuous set within boundaries.

MIRAMARE - TRIESTE

September 1993

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1 Introduction

Hemin or ferriprotoporphyrin IX is a porphyrin with an iron(III) atom in its center. Hemin results from the degradation of hemoglobin as derivative of heme or ferroprotoporphyrin IX which is the natural prosthetic group of the protein. Heme serves as the prosthetic group of a variety of important hemoproteins that are essential for hemopoietic processes [1].

Proteins generally lose their biological properties when heated above 323 K or irradiated with UV rays, or when dissolved in organic solvents or in aqueous solutions of very high pH. In extreme conditions the compact globular structure of the protein is spread out and heme is expelled from its hydrophobic unit, leading to hemin production [2,3,4].

In humans hemin can result from degradation of hemoglobin in erythrocytes infected by the malarial parasite plasmodium. Both heme and hemin play an important physiological role. Heme constitutes the binding site of oxygen for hemoglobin which is known as the oxygen carrier in blood. Free hemin has been suggested recently to play a role in malarial chemotherapy. Some authors proposed hemin to be the principal drug receptor in infected erythrocytes [5,6]. The formation of complexes with hemin in vitro has been demonstrated with various techniques as equilibrium dialysis, spectrophotometric titration and NMR [5,7,8,9], but quantitative information is rather scarce.

In aqueous solutions hemin has the tendency to form dimeric species or species of high size depending on the concentration of hemin, the ionic strength or pH of the solution [10]. Both dimerization and formation of aggregates of hemin can be effectively modified by the addition of alcohols [11]. In alkaline water ethyleneglycol mixture (50 % v/v) at low ionic strength the dimerization or the formation of hemin aggregates occurs at relatively high concentration [10,12].

We think that understanding chemical dynamics of positrons and positronium atoms is a prerequisite to quantitative studies of heme-drug interactions by positron annihilation spectroscopy. Positron annihilation spectroscopy was shown to be a powerful technique to probe a wide variety of materials. In solids positron techniques are used to study defects, surfaces, electronic structures and phase transitions. In fluids, it is experimentally possible to study positron interactions with gas molecules. Positron methods serve to better understand the behaviour of positronium atoms in liquids, and then allow the investigation of various kinds of systems: micelles, microemulsions, mixtures, etc...[13].

Hemin in water/ethyleneglycol (water/EGOH) mixture is certainly a complex system for positron studies. Without hemin the mixture already presents some questions which remain unsolved. For example, still now we don't know the nature of positron sites in this system. The influence of each kind of compounds present in mixtures (water or EGOH molecules) on the annihilation characteristics had not been well analyzed. In despite of the complexity of the subject, we hope in this study to provide further information to those available by others methods like UV/Vis spectrophotometry or Nuclear Magnetic Resonance. Furthermore, the water/EGOH milieu has been found to be favorable for the study of reactivities of hemin with some antimalarial drugs [14].

In a previous paper [15], we reported a positron annihilation study in solutions with relatively low concentration of hemin. It has been shown that hemin weakly affects positronium annihilation characteristics. This was probably due to the low values of the concentration. In the present paper, we are interested in more concentrated solutions (greater than 10^{-3} M) in order to obtain observable interactions of positronium with hemin. Free

positrons were seen to interact with hemin molecules, however due to the time resolution of the spectrometer the behaviour of the short lived components were not discussed. After the description of the experimental results, the study of chemical dynamics of positronium atoms is introduced through a model with competing reactions for both positron and positronium. We deduce the annihilation characteristics using the concept of continuum in the hemin solution.

2 Experimental

We have prepared hemin from sheep's blood adjusting the well known method [16]. Some of the studied hemin had been purchased from Aldrich-Chemical. The characterization of the prepared hemin was made using an UV/Vis spectrophotometer Perkin Elmer Lambda 2.

In positron annihilation experiments the sample holder consisted of two pyrex compartments separated by thin foils of Kapton (7.5 micrometers) used to protect the positron emitter (6 μCi Na-22). The lifetime experiments were performed using a conventional fast-slow time spectrometer equipped with BaF_2 scintillators mounted on XP2020Q phototubes.

Alkaline hemin stock solutions (pH = 9) have been prepared in 50 % water/ethyleneglycol mixture, containing NaOH. Concentrations of hemin solutions were measured by spectrophotometric method. Lifetime measurements were performed in 50 % water/ethyleneglycol with various concentrations of hemin obtained by dilution. Each measured spectra contained about 10^6 counts. The lifetime spectra were analyzed into three lived components using the PATFIT-88 programs [17]. Analyses were performed without constraint subtracting the annihilation contribution of 382 ps in the Kapton foils. The time resolution (FWHM) as determined from the fitting of the resolution function in the measured spectra varied between 305 ps and 325 ps. Measurements were performed at room temperature (293 K).

3 Results and Discussion

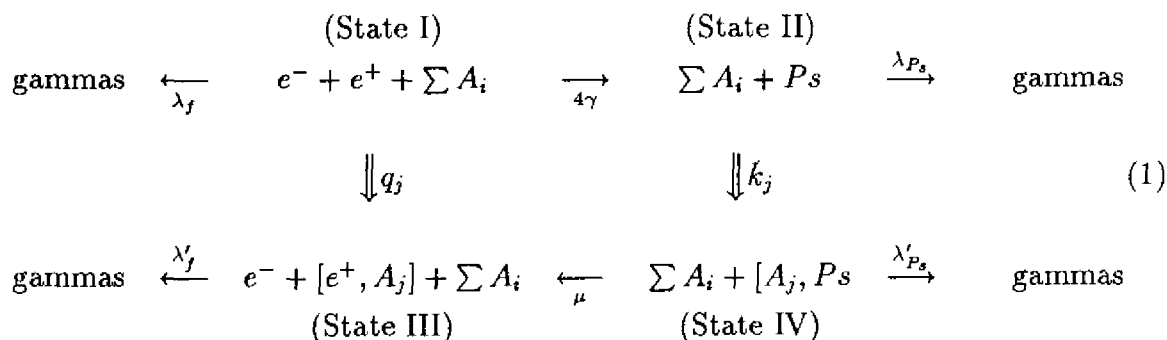
The measured long lifetime component τ_3 varied between 1870 ps and 2200 ps with the hemin concentration. Such values are in good agreement with those generally observed in aqueous solutions [18,19]. This component is ascribed to the pick-off annihilation of ortho-positronium atoms (o-Ps).

The shortest measured lifetime τ_1 varied between 32 ps and 87 ps, while the intermediate one (τ_2) lied between 371 ps and 393 ps. As the τ_1 values could hardly be explained, we were more interested to the behaviour of the mean short lifetime (τ_m) computed from the τ_1 and τ_2 values. As one can see in figure 1, τ_m remains constant in the full range of the studied hemin concentration. This observation is in good agreement with the previous observation [15]. τ_m contains contributions of both the annihilation of free and bounded positrons and the annihilation of para-positronium atoms (p-Ps). As τ_m does not vary with the hemin concentration, we only discuss the o-Ps annihilation characteristics.

Chemical experiments [10,12] showed that at concentration below 10^{-3} M only monomeric and dimeric hemin molecules predominate in alkaline solution. In this range of concentration no observable effect could be measured (figure 2 and figure 3). τ_3 remains constant at

concentrations below $c_0 = 4. \times 10^{-3}$ M, then it decreases with the increasing of the hemin concentration. The same behaviour is observed in the corresponding intensity I_3 . This behaviour of both I_3 and τ_3 had been discussed through various annihilation models. We concluded that positronium is not only quenched, but also inhibited by hemin compounds in the water/ethyleneglycol mixture.

To explain the behaviour of annihilation characteristics, we propose that both positrons and positronium atoms interact with various hemin compounds in the water/EGOH mixture according to the following scheme :



The indexes i and j always take different values and are referred to every hemin compound in the solution. k_j and q_j would vary with the hemin concentration. The A_j stands for an hemin compound of the j th compound. The above reactions would compete with the quasi-free electron scavenging in the positron spur.

The first and the second states (state I and state II) are respectively positron and positronium freelike states in the "milieu". The annihilation rates λ_f and λ_{Ps} are those measured in the water/EGOH mixture without hemin. In the third state (state III) positron has formed a complex with an A_j in the solution, while in the fourth state (state IV) positronium has reacted with a compound of hemin. The reaction from state II to state IV is positronium complexation (or oxydation), while that from the state I to state III is positron scavenging.

Since there are various species of hemin compounds, several possibilities of reaction may occur with positron (or positronium). Therefore, we can suppose that the different values q_j or k_j are limited and form a continuum within boundaries:

$$q_{min} \leq q_j \leq q_{max} \quad \text{and} \quad k_{min} \leq k_j \leq k_{max} \quad (2)$$

The q_j and k_j are then considered as well defined values of the variables q and k in the continuum.

Let $g(q)dq$ be the number of states III obtained from positron interactions with a reaction rate q_j lying between q and $q + dq$. Let us define $f(k)dk$ in the same way, i.e. the number of states IV obtained from positronium reaction. The quenching rate of the freelike states I and II can be expressed by:

$$\Gamma_f = \lambda_f + 4\gamma + \int_{q_{min}}^{q_{max}} qg(q)dq \quad (3)$$

$$\Gamma_{Ps} = \lambda_{Ps} + \int_{k_{min}}^{k_{max}} kf(k)dk \quad (4)$$

Experimentally we have found for the o-Ps lifetime $\tau_3 = \frac{1}{\Gamma_{o-Ps}}$ that:

$$\Gamma_{o-Ps} = \lambda_{o-Ps} \quad \text{for} \quad c < c_0 \quad (5)$$

$$\Gamma_{o-Ps} = \lambda_{o-Ps} + \beta[\log(\frac{c}{c_0})] \quad \text{for} \quad c \geq c_0$$

(the o-Ps lifetime in the mixture without hemin is $1/\lambda_{o-Ps} = (2155 \pm 10)ps$, $\beta = 2.0 \times 10^{-5}/ps$, and $c_0 = (0.004 \pm 0.0006) M$). The reaction rate of positronium atoms with hemin compounds is proposed to be proportional to the hemin concentration, $k(c) = \sigma c$. Then the density of the states IV can be expressed as:

$$f(k) = \frac{\beta}{k^2} \quad (6)$$

The total amount $N(c)$ of states IV in the solution can be deduced:

$$N(c) = \int_{k_{min}}^{k_{max}} f(k)dk = \frac{\beta}{\sigma} \left(\frac{1}{c_0} - \frac{1}{c} \right) \quad \text{for} \quad c \geq c_0 \quad (7)$$

$N(c) = 0$ for $c < c_0$. Because positron and positronium interact with the same compounds in the solution, the functions $g(q)$ and $f(k)$ which describe these interactions can be considered as identical. This means that:

$$\Gamma_f = \lambda_f + 4\gamma \quad \text{for} \quad c < c_0 \quad (8)$$

$$\Gamma_f = \lambda_f + 4\gamma + \beta[\log(\frac{c}{c_0})] \quad \text{for} \quad c \geq c_0$$

As it is proposed $q(c) = \sigma_f c$, and $\beta/\sigma = \beta_f/\sigma_f$. With these statements I_3 can be expressed as:

$$I_3 = \frac{I_{30}}{1 + a[\log(\frac{c}{c_0})] + b[\log(\frac{c}{c_0})]^2} \quad (9)$$

Successful data reduction made using a simplex method have given $a = 0.074$, and $b = 0.029$. I_{30} is the I_3 value in the mixture without hemin. The constants a and b depend on the reaction rates stated in the equation (1).

The invariance of the τ_3 up to c_0 can be interpreted as resulting from the solvation of hemin by surrounding water and EGOH molecules in the solution. As long as hemin compounds were fully screened by water and alcohol dipoles, they remained inactive towards positrons and positronium atoms. Due to the hemin solvation positrons and positronium atoms can also be considered as localized out of the sites of hemin. This is in good agreement with the statement reported by J.Ch.Abbé et al.[20] which proposed two pathways for positronium formation in aqueous solutions: either by reaction of quasi-free positrons and electrons, or through localized particles. The later process seems to better explain the results of our experiments. The presence of hemin molecules in relatively low concentration could not significantly modify the abundance of reactive species in the terminal spur, so that positrons and positronium atoms remain inefficient to probe hemin compounds in their sites.

When the hemin concentration is increased above c_0 , hemin aggregates of higher size are formed, producing the modification of solvation structure. In the same way positrons and positronium atoms became roughly delocalized and can probe hemin compounds in their sites.

The modification of solvation structure of hemin compounds must be understood in terms of decreasing of the ratio of surrounding water/EGOH molecules. This can be explained by the occurrence of more unpolar hemin compounds of greater size, i.e. hemin aggregates in the solution. The molar fraction of water around hemin compounds decreases because of the polarity difference between water and EGOH and the size increasing of hemin compounds due to aggregation. It is well known that water is more polar than EGOH. In diluted solutions small size compounds (monomeric and dimeric hemin) are fully surrounded by alcohol and water molecules. The reaction of these small compounds with particles in the positron spur is hindered. While with the appearance of greater size compounds above c_0 , there also occurs an alteration of their hydrophilic properties. This results in the change of water fraction around these aggregates. This facilitates reactions with particles in the positron spur. In fact there are competitions between reactions with hemin compounds and those with solvent entities. This agrees some suggestions made conformly to the spur model[21,22]. According to the proposed reactions the characteristic parameter β contains the rate of aggregate formation in the solution.

4 Conclusion

The present study has shown that the positron lifetime measurements can give twofold of informations, concerning the solvation of hemin compounds and the chemical behaviour of both positrons and positronium atoms in solutions with various compounds of hemin. Regarding the solvation of hemin, we have demonstrated that there exists a critical concentration above which aggregates of hemin are formed. With the appearance of great size hemin compounds the water fraction is modified around them. This results in the change of solvation properties in the water/EGOH mixture. For the chemical behaviour the study provides a model which successfully describes various competing reactions of both positrons and positronium atoms using the concept of continuum. Results of this study constitute an important prerequisite for further investigations in the field of reactivities of hemin with drugs by positron spectroscopy.

5 Acknowledgments

The authors are grateful to Professor Dr. Maurice Dorikens and his staff (State University of Ghent) for their continuous assistance. They are also indebted to DAAD (Deutscher Akademischer Austauschdienst) for the support. They would like to thank the Risø National Laboratory (Dr. Morten Eldrup and his group) for the PATFIT-88 programs. (M.T.) would like to thank Professor Abdus Salam, the International Atomic Energy Agency and UNESCO for hospitality at the International Centre for Theoretical Physics, Trieste, and the Swedish Agency for Research Cooperation with Developing Countries, SAREC, for financial support during his visit at the ICTP under the Associateship scheme. One of the authors (L.O.R.) is indebted to BEPUZA for a grant.

References

- [1] J.D. Lutton, R.D. Levere and N.G. Abraham, in: *Heme-Cytochrome P-450*, Society for Experimental Biology and Medicine, New York 1991, p.260
- [2] T.T. Herskovits, B. Gadegbeku and H. Jaillet, *The Journal of Biological Chemistry* 245 (1970) 2588.
- [3] B. Mavakala-Kiazolua and Z.Y. Gushimana, Master Thesis, Faculty of Sciences, University of Kinshasa (1991).
- [4] H. Neu, Doctoral thesis, University of Koln (1987).
- [5] A.C. Chou, R. Chevli and C.D. Fitch, *Biochemistry* 19 (1980) 1543.
- [6] J.D. Sattertlee and J.A. Shelnutt, *J. Phys. chem.* 88 (1984) 5487.
- [7] D. Warhurst, *Biochemical Pharmacology* 30 (1981) 3323.
- [8] D. Behere and H. Goff, *J. Am. Chem. Soc.* 106 (1984) 4945.
- [9] I. Constantinides, J. Satterlee, *J. Am. Chem. Soc.* 110 (1988) 927.
- [10] K.M. Smith, *Porphyrin and Metalloporphyrins*, (Elsevier, Amsterdam, 1985).
- [11] Y. Gushimana, B. Doepner, E. Martinez-Hackert and G. Ilgenfritz, to be published.
- [12] Reinhard Prinzen, Diplomarbeit, Universtät zur Köln (1988).
- [13] L. Dorikens-Vanpraet, M. Dorikens and D. Segers, eds., in: *Positron Annihilation, Proc. of the 8th Int. Conf. on Positron Annihilation, Gent, Belgium, 29 Aug. - 3 Sept. 1988* (World Scientific, Singapore, 1989)
- [14] K. Tshimanga, Master Thesis, University of Kinshasa (1991).
- [15] Mbungu-Tsumbu, Gushimana Yav, Kanyinda Malu, Lohalo O. Rostha and C. Dauwe, in: *Positron Annihilation, Proc. of the 9th Int. Conf. on Positron Annihilation, August 26 - 31, 1991, Szombathely, Hungary*, eds. Zs. Kajcsos and Cs. Szeles, *Materials Science Forum Vols. 105-110* (1992) p.1657 (Trans Tech Publications, Switzerland, 1992)
- [16] E. Antonini and M. Brunoni, *Hemoglobin and myoglobin in their reactions with ligands* (North-Holland Publishing Co., 1971).
- [17] P. Kirkegaard, N.J. Pedersen and M. Eldrup, Risø National Laboratory, DK-4000 Roskilde, Denmark (1989).
- [18] R. Zana, S. Millan, J.Ch. Abbé, and G. Duplâtre, *J. Phys. Chem.* 86 (1982) 1457.
- [19] G. Duplâtre, A. Haessler and A. Marques Netto, *Journal of Radioanalytical and Nuclear Chemistry* 82/2 (1984) 219.
- [20] J.Ch. Abbé, G. Duplâtre, A.G. Maddock and A. Haessler, *Chemical Physics* 49 (1980) 165.

[21] O.E. Mogensén, J. Chem. Phys. 60 (1974) 998.

[22] Y. Ito, in: Positron and Positronium Chemistry, eds. D.M. Schrader and Y.C. Jean (Elsevier, Amsterdam, 1988)

FIGURE CAPTIONS

- Fig.1 The variation of the mean short lifetime, τ_m , with the hemin concentration in the solution.
- Fig.2 The o-Ps lifetime, τ_3 , as a function of the hemin concentration in the 50% water/ethyleneglycol micture.
- Fig.3 Variation of the o-Ps intensity, I_3 , as a function of the hemin concentration in the 50%water ethyleneglycol mixture.

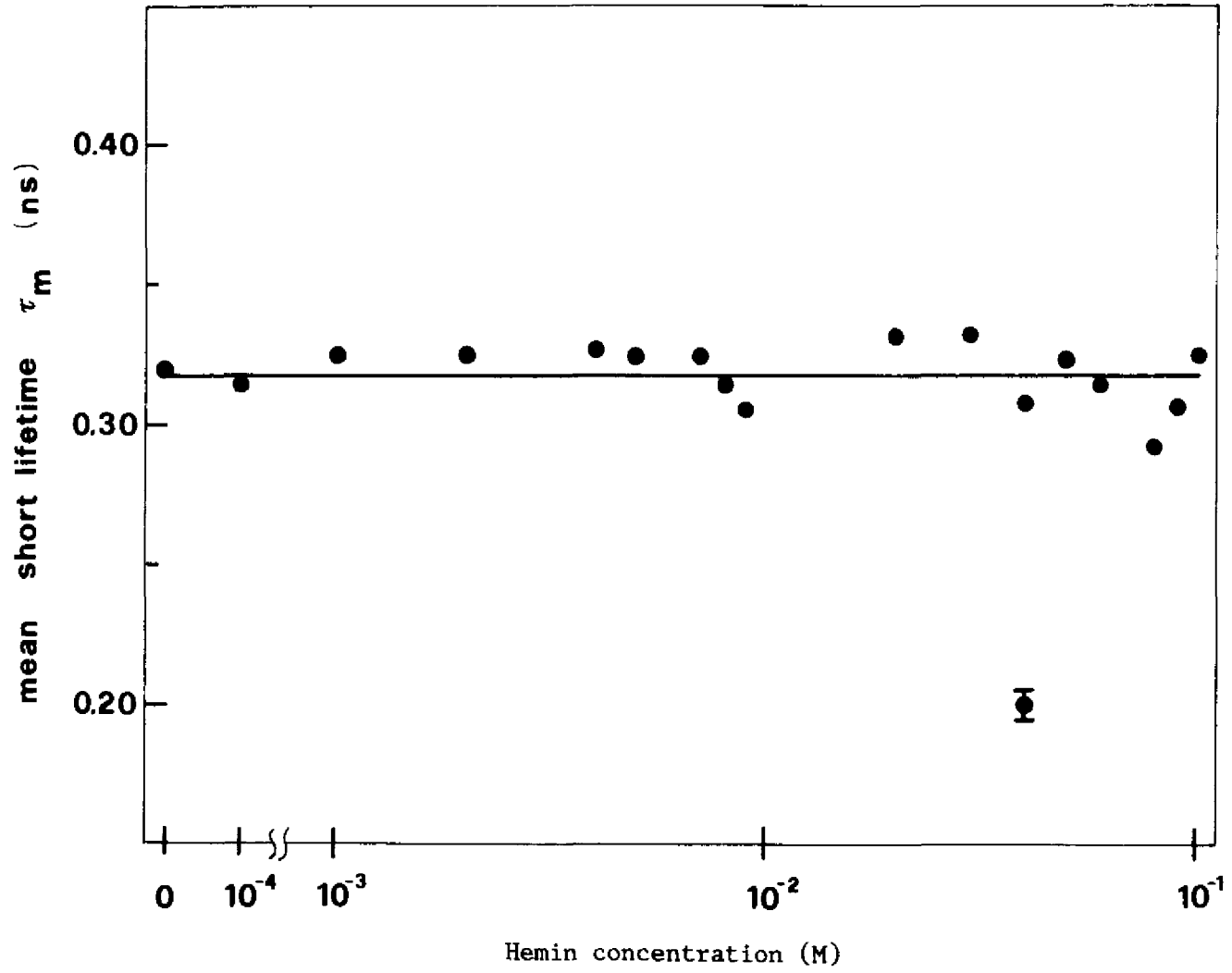


Fig.1

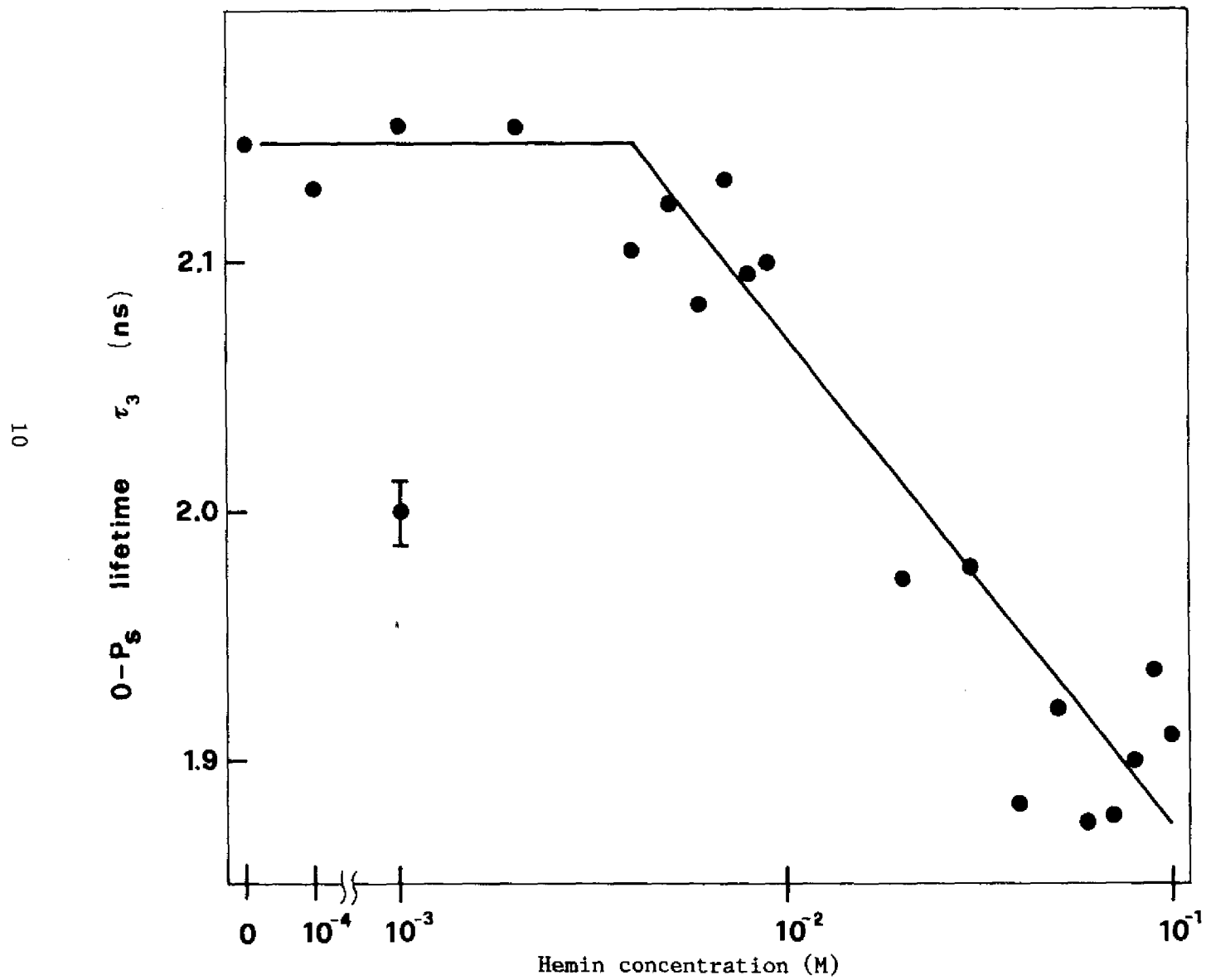
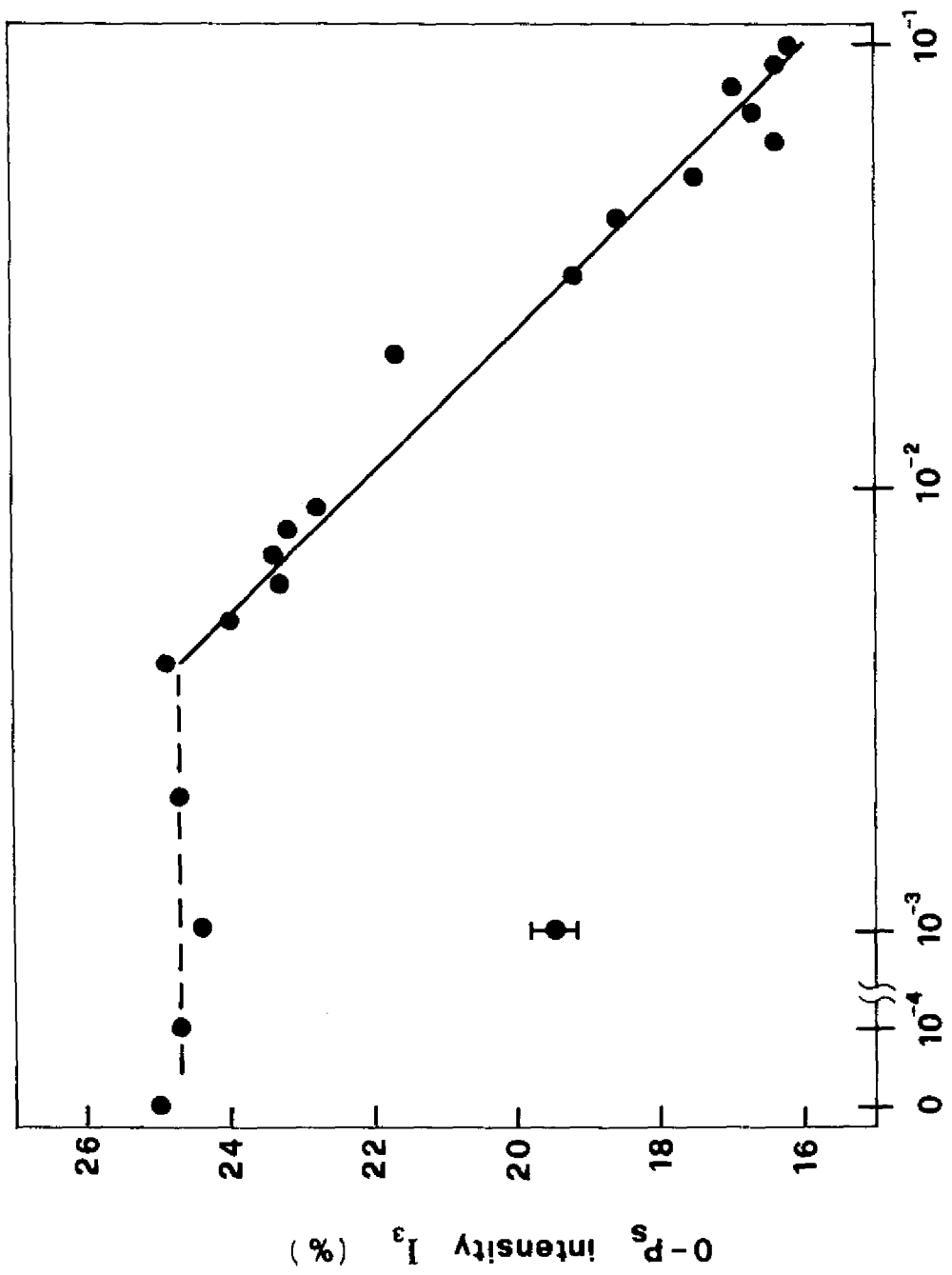


Fig.2



Hemin concentration (M)

Fig. 3