Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Application of Spin Labels for Research of Vanadyl Acetylacetonate Concentration in Model Bilayer Membranes by EPR Spectroscopy

W. W. Sułkowski [a], A. Hałas [a], A. Sułkowska [b], S. Maślanka [a] & R. Gryboś [c]

[a] Department of Environmental Chemistry and Technology, Institute of Chemistry, University of Silesia, Szkolna 9, 40-006, Katowice, Poland

[b] Department of Physical Pharmacy, Medical University of Silesia, Jagiellońska 4, 41-200, Sosnowiec, Poland

[c] Faculty of Chemistry, Jagiellonian University, R. Ingardena 3, 30-060, Kraków, Poland

Available online: 12 Jan 2012

To cite this article: W. W. Sułkowski, A. Hałas, A. Sułkowska, S. Maślanka & R. Gryboś (2012): Application of Spin Labels for Research of Vanadyl Acetylacetonate Concentration in Model Bilayer Membranes by EPR Spectroscopy, Molecular Crystals and Liquid Crystals, 554:1, 195-208

To link to this article: http://dx.doi.org/10.1080/15421406.2012.634321

Mol. Cryst. Liq. Cryst., Vol. 554: pp. 195–208, 2012

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421406.2012.634321

Taylor & Francis
Taylor & Francis Group

Application of Spin Labels for Research of Vanadyl Acetylacetonate Concentration in Model Bilayer Membranes by EPR Spectroscopy

W. W. SUŁKOWSKI,[1,*] A. HAŁAS,[1] A. SUŁKOWSKA,[2]
S. MAŚLANKA,[1] AND R. GRYBOŚ[3]

[1]Department of Environmental Chemistry and Technology, Institute of Chemistry, University of Silesia, Szkolna 9, 40-006 Katowice, Poland
[2]Department of Physical Pharmacy, Medical University of Silesia, Jagiellońska 4, 41-200 Sosnowiec, Poland
[3]Faculty of Chemistry, Jagiellonian University, R. Ingardena 3, 30-060 Kraków, Poland

The compounds and complexes of vanadium are used to treat diabetes and cancer. Research on the effectiveness and mechanism of action of new derivatives of vanadium, and their toxicity is currently very intense. The research shows that the vanadium(IV) acetylacetonate complex [$VO(acac)_2$] shows a synergism with insulin in treating diabetes, high pharmacological activity and low toxicity. In order to improve the effectiveness of drugs and minimize their toxicity, the active compounds are often closed in the liposome membranes. The objective of the work was preparation of bilayer liposomes from egg yolk phosphatidylcholine (EYPC), closing the complex $VO(acac)_2$ in these membranes and estimating the concentration of vanadium complex after incorporation into liposomes membranes. Due to the paramagnetic properties of vanadium(IV) the concentration of this metal complex can be determined directly by EPR. Entering the spin label CTPO in the water phase into the studied arrangement allows for the indirect measurement of the concentration of complex, on the basis of changes of the EPR spectrum of the spin label caused by the presence of the vanadium(IV) complex. In the work the dependence of the α parameter based on the analysis of CTPO EPR spectra on the concentration of $VO(acac)_2$ was determined. To demonstrate the presence of the complex in the membrane directly by measuring the EPR sulfate(IV) sodium was used in order to remove the EPR signal of vanadium(IV) from the water phase. The presence of vanadium(IV) in the membrane was also demonstrated indirectly using a spin label 12-SASL. Based on the results of EPR spectroscopy the concentration of the complex in the membrane was determined together with the partition coefficient of $VO(acac)_2$ between the membrane and outer water environment of the membrane.

Keywords Concentration; liposomes; spin labels; vanadium(IV) acetyloacetonate complex

*Address correspondence to W. W. Sułkowski, Department of Environmental Chemistry and Technology, Institute of Chemistry, University of Silesia, Szkolna 9, 40-006 Katowice, Poland. Tel./Fax: +48 32 359 1371; E-mail: wieslaw.sulkowski@us.edu.pl

1. Introduction

Vanadium compounds exhibit insulinomimetic properties in model studies conducted on cultured rat adipocytes, as well as in model studies of the application of vanadium salts as a drug for standardizing the clinical symptoms of diabetes induced in animals [1–4]. The first received organic compound containing vanadium was *naglivan* (compound of vanadyl with cysteine). Because the use of *naglivan* alone was not effective in reducing the level of glucose, it was administered in combination with insulin [5]. The next step towards understanding the activity of vanadium in treating diabetes with insulin and the ability to use its compounds was the synthesis of the compounds that would be water-soluble, electrically neutral and have low molecular weight. The vanadium(IV) acetyloacetonate complex [$VO(acac)_2$] was characterized by a high pharmacological activity, greater stability (less susceptibility to oxidation) and low toxicity [6–8]. With all the positive impact regulating the physiological and biochemical processes, vanadium compounds in the cation and anion form used in quantities above 0.05 mg/kg for compounds of vanadium(IV) and 0.1 mg/kg for compounds of vanadium(V) are toxic to living organisms. Tests done in the years 1993–2004 mainly on mice and rats have shown the emergence of various toxic effects on both the short (several days) and long (months) period of the administration of vanadium compounds [9]. There were hematologic and biochemical changes such as decrease in the number of erythrocytes, hemolysis, reduced hemoglobin and hematocrit levels, a decrease in enzyme activity (aminotransferase, lactate dehydrogenase, alkaline phosphatase), damage to the nervous system and damage to reproductive developmental abilities (embryotoxicity and teratogenicity) and morphological and functional damage to the liver, kidney, bone, spleen, and leukocytes [10,11]. The physiological symptoms of side effects of vanadium include: increased mortality, diarrhea, vomiting, weight loss, weight loss of organs in relation to body weight, bleeding from the nose, dehydration, changes in renal function [12]. Vanadium affects the proper functioning of the nervous system, respiratory, digestive and circulatory systems [13,14]. In animal model studies the regulation of diabetic symptoms was obtained using a dose of vanadium from 5 to 35 mg/(kg · day) [15]. In order to provide for a longer period therapeutic effect or partial normalization of clinical symptoms of diabetes vanadium ion was administered to animals at concentrations ranging from 0.25 to 1 mg/mL as liquid for drinking [16]. The first attempts to use vanadium as a drug in humans took place in 1899 [17]. As a result of administration of 4–5 mg of sodium metavanadate before a meal three times a week with a 24-hour breaks a reduction in blood sugar levels occurred in two of three patients [15]. Toxicity of vanadium compounds in diabetic patients is not widely known because of the short length of time of treatment, usually up to 4 weeks. The prolonged administration of vanadium compounds to 5 months in patients resulted in anorexia, weight loss and stomach pain [12].

In cases where excessive doses can cause toxic effects a solution is to close them in liposomes and use them for treatment in this form. The main advantages of liposomes are: a gradual release of active substances, protection of sensitive tissues and organs from high concentration of an active compound, reduction or elimination of the by - effects of drugs [18,19]. The concentration of complexes of vanadium(IV) in aqueous phase (buffer) is determined by EPR. The spectra of aqueous solutions of vanadium(IV) compounds are isotropic. Eight resonance signals in the EPR spectra are visible, which is associated with interaction of vanadium(IV) with nuclear spin $I = 7/2$ with an external magnetic field. The concentration of paramagnetic substances in the aqueous phase can be determined directly or indirectly by the introduction of 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxyl (CTPO) to the test system [20]. In the EPR spectrum of the spin label CTPO apart from the

hyperfine structure, arising from the interaction of free electrons with the protons of methyl groups a superhyperfine structure is also visible. This structure is related to the interaction of the unpaired electron with protons of the aromatic ring. If in an aqueous system of spin labels there are present paramagnetic substances, they affect the EPR spectrum of spin label, causing a decay of its superhyperfine structure. Quantitatively, the changes in the spectra are expressed by the parameter α and depend on the concentration of paramagnetic substance in the system. Spin label CTPO was previously used for measuring the concentration of oxygen [20] as well as to measure the concentration of copper(II) and nickel(II) in the complexes $Cu(H_2O)_6^{2+}$, Cu – EDTA, $Ni(H_2O)_6^{2+}$ Ni – EDTA in aqueous solutions [21]. There was also conducted research on oxygen, iron and nickel ions in hydrophobic models membranes [22]. For this type of research there were used stearic acid derivatives: 5-doxyl stearic acid spin labels (5 SASL), 16-doxyl stearic acid spin labels (16 SASL) and sterol derivatives: cholestane spin label (CSL), androsterone spin label (ASL), spin-labeled in different positions of the carbon chain [22,23]. The spin labels n SASL have the ability to incorporate into the membranes between the chains of phosphatidylcholine liposomes formed with EYPC lipids. The nitroxyl group of spin label with a single spin electron connected to the corresponding portion of hydrophobic lipid, allows for the study of the effect of the spin label with other paramagnetic substances in a specific area of the membrane by EPR spectroscopy.

The aim of our study was to demonstrate the use of spin labels to determine the concentration of vanadium complex in the aqueous phase and in the lipid membranes of liposomes. It was also necessary to check if the concentration of vanadium(IV) complex after incorporation into liposomes is sufficient to the therapeutic application.

2. Experimental

The research used: spin labels 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxyl (CTPO) (Sigma Aldrich), 12-doxyl stearic acid (12-SASL) (Sigma Aldrich), phosphatidylcholine from egg yolk (EYPC) (Sigma Aldrich), a complex of vanadium(IV) with acetylacetone [$VO(acac)_2$] (98%) (Jagiellonian University Department of Inorganic Chemistry), high-purity chloroform (POCH), borate buffer pH 7.4 (6.18 mg of boric acid was dissolved in 1 L double-distilled water, 4.76 mg of borax dissolved in 125 mL doubly distilled water, then slowly combined solutions and used immediately after preparation.) manganate(VII) potassium (POCH), sulfate(IV) sodium (POCH).

2.1. Preparation of Solution of the Complex $VO(acac)_2$ in Borate Buffer pH = 7.4

The solution of vanadium complex $VO(acac)_2$ at a concentration of 0.0055 M was prepared by dissolving 66.20 mg of solid complex in 50 ml of borate buffer at pH = 7.4. Then, using a quantitative dilution the complex samples for the EPR measurements were prepared at concentrations from 0.0006 M to 0.0055 M.

2.2. Preparation of Water Solutions of Mixture Spin Label CTPO with vanadium(IV) Acetyloacetonate Complex in Borate Buffer pH = 7,4

The stock $VO(acac)_2$ solution of spin label CTPO at the concentration of 0.0001 M was prepared by dissolving 3.78 mg of spin label probe in 10 mL borate buffer pH = 7.4. The mixtures of the spin label with a complex of vanadium were prepared by adding the

vanadium complex in concentration of 0.00008 M 0.00080 M to 1 mL of the spin label CTPO stock solution. Prepared samples deoxygenated with nitrogen for 5 minutes were collected into quartz capilares for EPR measurements.

2.3. Preparation of Liposomes of EYPC Bilayer Lipid Film Hydration Technique

140 μL of a solution of lipid phosphatidylcholine from egg yolk (EYPC) in chloroform at a concentration of 50 mg/mL were placed in quartz tubes [28]. Lipid solutions without or with spin label (5 μL chloroform solution of the spin label 12-SASL of a concentration of 0.001 M) were first dried with nitrogen and then in a exsiccator for 12 h. 250 μL of solution of vanadium complex $VO(acac)_2$ at a concentration of 0.005 M in borate buffer was added to the dried lipid. For the dried lipid with spin label 250 μL of solution of vanadium complex at a concentration of 0.001 M (mixture 1) and 0.0005 M (mixture 2) in borate buffer or 250 μL borate buffer (mixture 3) was added. The resulting mixtures were shaken at temperature above the lipid phase transition (T = 22°C). Then, in order to thicken the suspension it was centrifuged for 0.25 h (7000 rpm) at 4°C. The precipitate of liposomes for EPR measurements were collected.

2.4. Preparation of Bilayer Liposomes of EYPC for Determination of the Partition Coefficient of Vanadium Complex Between the Aqueous Phase and the Liposome Membrane

30 mL of chloroform solution of EYPC at a concentration of 50 mg/mL was placed in five quartz tubes of 5 mL each. Lipids solutions were dried in test tubes with nitrogen and then in a exsiccator for 12 h. For each tube of dried lipid residue 250 mL of solution of vanadium complex $VO(acac)_2$ at a concentration of 0.005 M in borate buffer was added. The mixture in each tube was shaken at 22°C to thicken the obtained bilayer liposomes it was centrifuged for 0.25 h (7000 rpm) at 4°C. To the liposome residue 250 mL of sodium sulphate(IV) in borate buffer at a concentration of 0.02 M was added.

EPR spectra were performed on a BRUKER EMX spectrometer at 293 K in quartz capillaries with a diameter of 1 mm.

Apparatus measuring parameters of EPR spectra of complexes of vanadium(IV) with acetylacetone: microwave power: 50 mW, modulation amplitude 5 Gs, time constant: 1 s, range of fields: 1000 Gs, center field: 3350 Gs, sweep time: 480 s.

Apparatus measuring parameters of EPR spectra of superhyperfine structure of the spin label CTPO: microwave power: 2 mW, modulation amplitude: 0.05 Gs, time constant: 0.1 s, the scope of the field: 10 Gs, center field: 3335 Gs, sweep time : 480 s.

Apparatus measuring parameters of signal saturation of EPR spin label 12-SASL: microwave power: from 0.26 mW to 200 mW, modulation amplitude: 1 Gs, the scope of the field: 100 Gs, sweep time: 480 s.

EPR spectra of solutions of the complex $VO(acac)_2$ in borate buffer at concentrations from 0.0055 M to 0.0002 M were recorded. The amplitude of the fourth spectral line was measured and on its basis the dependence of the amplitude [mm] on the concentration of the complex in aqueous solutions [M] was determined. We recorded the effect of the complex of vanadium(IV) with the concentration range from 0.00080 M to 0.00008 M on the EPR spectra of spin label CTPO at a concentration of 0.001 M. The analysis of obtained EPR spectra enabled to determined the value of the parameter α and to find the dependence of this parameter on the concentration of vanadium(IV) complex.

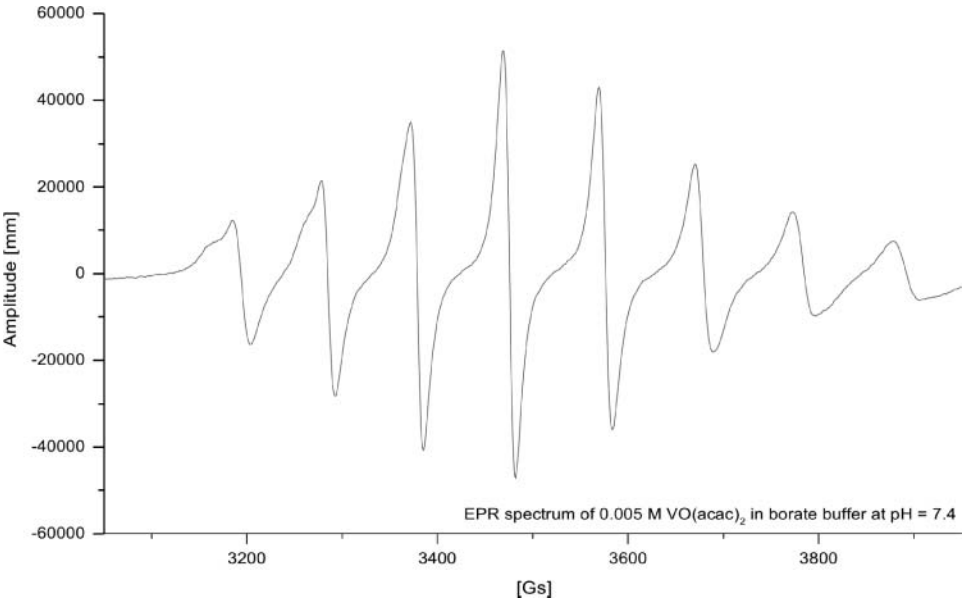


Figure 1. EPR spectrum of 0.005 M $VO(acac)_2$ in borate buffer at pH = 7.4.

The presence of the complex $VO(acac)_2$ in the membranes of liposomes can be shown directly by the recording EPR spectrum. The research relies in the quenching of the EPR signal of vanadium(IV) taken from the aqueous phase. The presence of the complex $VO(acac)_2$ in the membrane was also demonstrated indirectly using spin label 12 SASL. EPR spectrum of the spin label when incorporated into the phospholipid membrane is presented in (Fig. 1). In order to determine saturation curve of the spin label 12-SASL with microwave power the EPR spectrum of the center line spin label for increasing microwave power given to the sample was recorded. The measurements were performed for the samples of 12-SASL-liposomes in which vanadium complex of two different concentrations: 0.001 M $VO(acac)_2$ and 0.0005 M $VO(acac)_2$ was closed and for the samples of 12-SASL-liposomes without vanadium complex.

EPR spectra of the complex of vanadium(IV) taken from the supernatant collected after centrifuged liposomes vesicles for four independent preparations of liposomes were recorded. The amplitude of the fourth line of the spectrum was measured. Based on the dependence of amplitude of EPR spectrum [mm] on the concentration of the complex in aqueous solutions [M] the concentration of the complex in the supernatant was determined. Then, knowing the initial concentration of the complex and the concentration in the aqueous phase the complex concentration in the precipitate of the EYPC liposomes was calculated. EPR spectrum of the complex from the liposome residue after the addition of 0.02 M Na_2SO_3 at 22°C was recorded.

3. Results and Discussion

Hyperfine splitting constant (A_0) of vanadium(IV) compounds equals to 98.438. Spectral line width is constant for all concentrations of complex $VO(acac)_2$ in borate buffer and amounts $\Delta B = 10.71$ Gs (Fig. 1). The amplitude of the fourth line of the spectrum of the complex at concentrations from 0.0006 M to 0.0055 M was measured (Fig. 2).

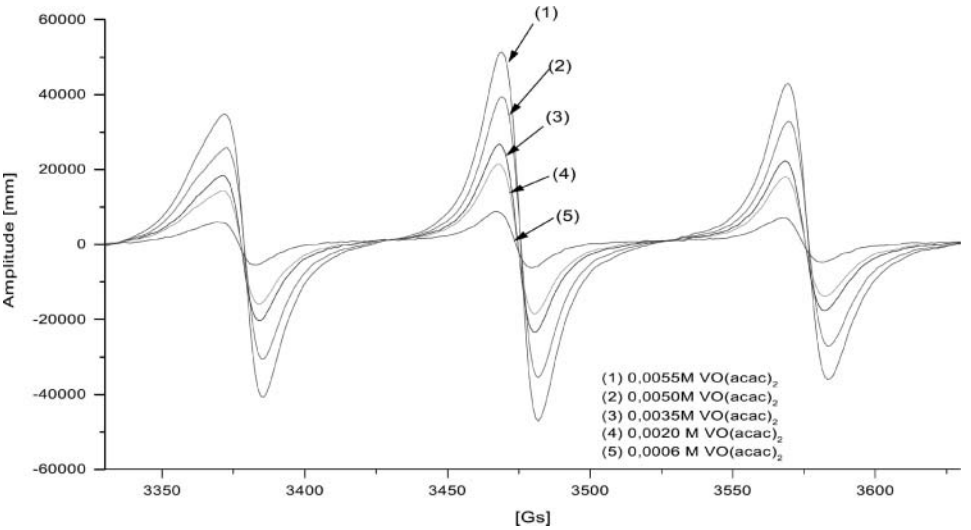


Figure 2. EPR spectrum of the fourth (central) line of the complex $VO(acac)_2$ in borate buffer at concentrations ranging from 0.0006 M to 0.0055 M.

It was found that this method allows for recording the EPR spectra of the complex at concentrations greater than 0.0006 M. The calibration curves equation: $y = 64914x + 0.2054$ was determined. The concentration of the complex of vanadium(IV) in the aqueous phase of liposomes (x) using calibration curve, i.e. the dependence of amplitude of EPR signal on the concentration of vanadium(IV) complex from aqueous phase of liposome was determined. To determine the concentration of the complex of vanadium(IV) $VO(acac)_2$ in the aqueous phase an indirect method was also used. This applies the changes of the spin label spectrum caused by the presence of the paramagnetic complex of vanadium(IV) in the solution of spin label CTPO. The central line of EPR spectra of spin label CTPO at a concentration of 0.0001 M in borate buffer was shown in (Fig. 3). The complex of vanadium(IV) present in the solution of the spin label CTPO causes the disappearance of superhyperfine structure of spin label EPR spectrum. EPR spectra of spin label CTPO at a concentration of 0.0001 M solution of the complex after the addition of vanadium(IV) at a concentration from 0.00008 M to 0.00080 M in borate buffer at pH = 7.4 (Fig. 4). As a result of particle collisions $VO(acac)_2$ with the spin label CTPO the superhyperfine structure of spin label CTPO EPR spectrum disappears. On the basis of changes in EPR spectra of spin label CTPO caused by varying amounts of the complex $VO(acac)_2$ of known concentration the concentration of $VO(acac)_2$ complex in the aqueous phase of liposomes can be determined.

The parameter α of the EPR spectra of spin label was determined from the formula:

$$\alpha = \frac{b + c}{2\alpha} \tag{1}$$

where: α - the parameter used in calibration charts, a - amplitude of the central line of the hyperfine structure of EPR spectra of the spin label, b, c - amplitude of the central line of the superhyperfine structure of EPR spectra of the spin label

The calibration curves equation: $y = -0{,}5266\ x + 401{,}32$ was determined. Application of spin label CTPO to the measurements of the concentration of $VO(acac)_2$ in

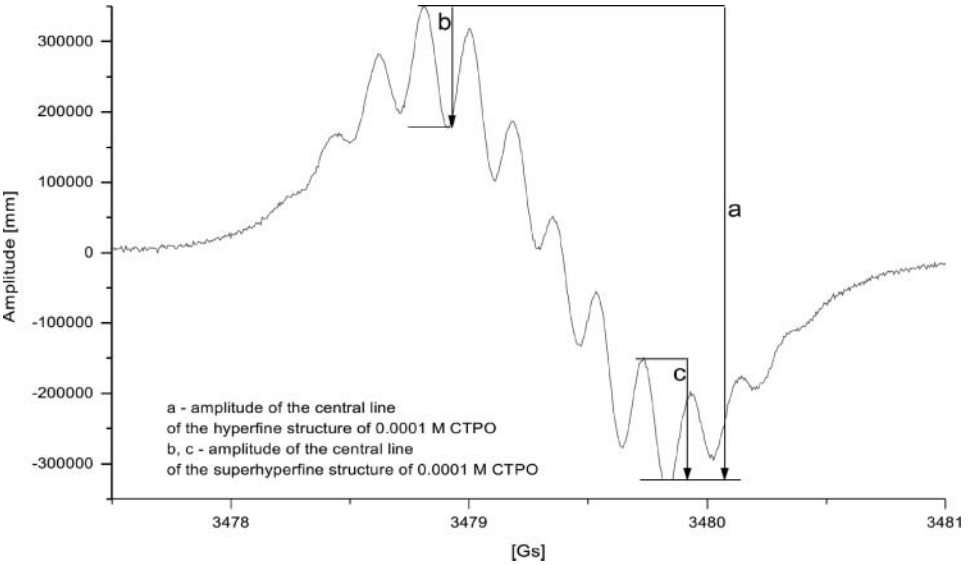


Figure 3. The central line on EPR spectrum of spin label CTPO of a concentration of 0.0001 M, where: a - amplitude of the central line of the hyperfine structure of EPR spectra of the spin label, b, c - amplitude of the central line of the superhyperfine structure of EPR spectra of spin label.

aqueous solutions allow for reduction of the volume of the sample of vanadium complex to 0,02 mL and increase of an accuracy of the measurement. This is important in the case of the vanadium complex encapsulated in liposomes.

In order to confirm the incorporation of vanadium complex $VO(acac)_2$ to the lipid layer of liposome EPR spectra of spin label 12 SASL embedded into bilayer liposomes prepared

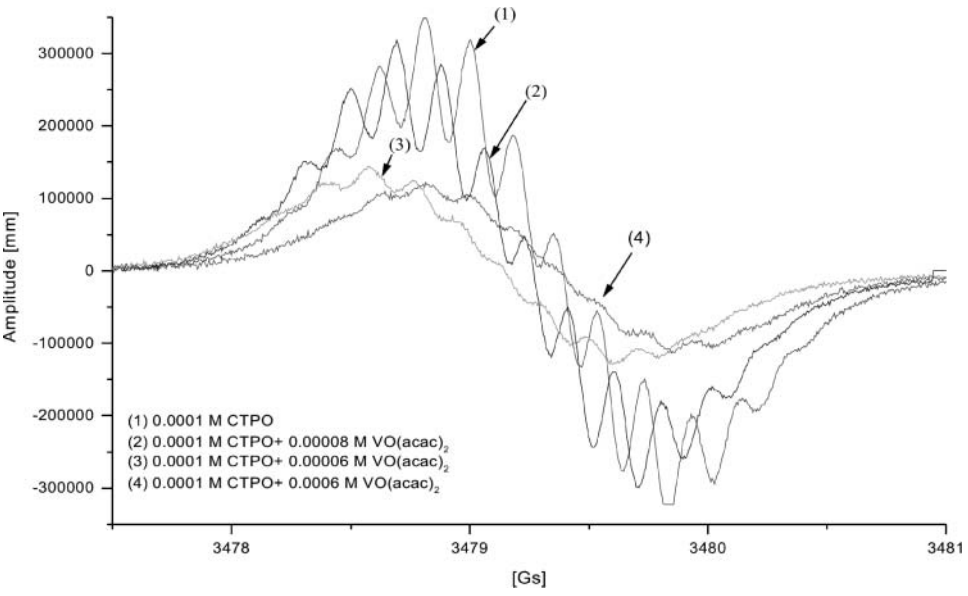


Figure 4. Effect of vanadium complex on the EPR spectra of spin label CTPO.

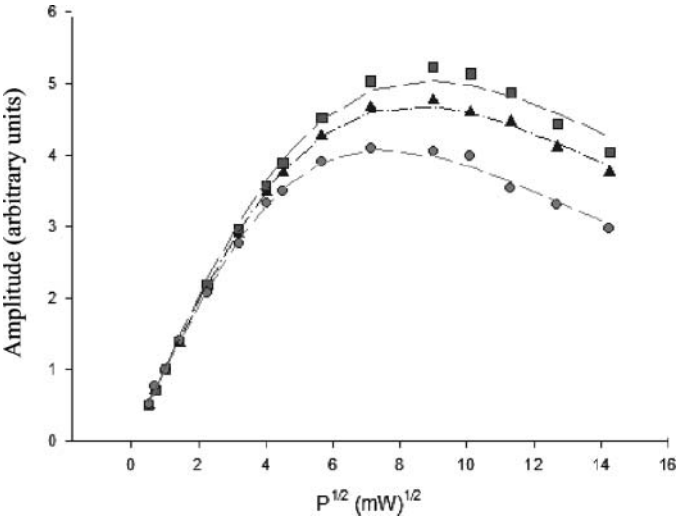


Figure 5. Influence of complex $VO(acac)_2$ on the power saturation curves of the microwave spectrum of the central EPR line spin label 12 SASL built into the liposome membrane.

from EYPC for increasing microwave power P given on the sample under anaerobic conditions. The change of the amplitude of the central line of the spin label EPR spectrum with the change of microwave power was measured. EPR spectra of spin label when incorporated it into the membrane and the EPR spectra of the spin label in the membrane in the presence of the complex $VO(acac)_2$ at a concentration of 0.0005 M and 0.001 M were recorded (Fig. 5, Table 1). Based on experimental data obtained for the spin label and the spin label in the presence of the complex $VO(acac)_2$ concentration of 0.001 M and 0.0005 M (Fig. 7) was calculated by minimizing the matching parameter c (Table 2) from the dependence:

$$h_0' = A\frac{\sqrt{P}}{(1 + cP)^{3/2}} \tag{2}$$

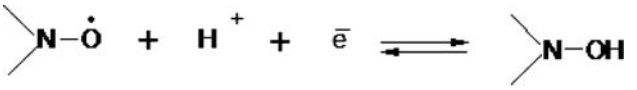
where: h_0 - amplitude central line of the EPR spectra of the spin label [*arbitrary units*], P - microwave power supplied on the sample [mW], A - coefficient of normalization, c – matching parameter.

The fit parameter c is related to the spin labels relaxation time T_1. When the parameter c has a lower value, it means that the relaxation time of spin label is shorter (the signal saturation of spin label occurs at higher microwave power). From the literature it is known that the presence of another paramagnetic substances in the system (oxygen, metals ions) causes the shortening of the spin-relaxation time T_1 of spin label and the decrease of the value of the matching parameter c [21,23]. The presence of vanadium ions VO^{2+} in the system at a concentration of 0.0005 M causes a drop in the value of the parameter c. In the presence of vanadium ions at a concentration of 0.0010 M an unexpected increase in the value of the parameter c can be observed (Table 2). The results allow for a suggestion that in the liposomal membrane (EYPC) vanadium ions can interact with spin label 12 SASL. The increase of the value of matching observed for the 0.0010 M of the complex $VO(acac)_2$ probably derives from an another type of interaction of vanadium(IV) with a spin label than

Table 1. The amplitude of the central line of the EPR spectra of spin label 12-SASL for microwave power supplied on the sample in the range from 0.26 mW to 203 mW, where: h_0 amplitude of the central line of the EPR spectra of spin label 12 SASL, P microwave power supplied on the spin label sample or the spin label sample with vanadium complex

	h_0 [arbitrary units]		
P [mW]	0,001 M 12-SASL + 0,0005 M $VO(acac)_2$	0,001 M 12-SASL + 0,001 M $VO(acac)_2$	0,001 M 12-SASL
0,26	0,7708	1,0478	0,9912
0,51	1,0691	1,4974	1,3793
1,02	1,506	2,1064	1,9108
2,03	2,0836	2,9364	2,7654
5,10	3,1855	3,2456	3,7650
10,2	4,1323	4,7685	4,9751
16,1	4,8808	5,5789	6,7313
20,3	5,1955	5,9791	7,3985
32,2	5,7801	6,6663	8,1089
51,0	6,1256	6,8975	8,2354
80,9	5,9827	7,1990	8,2908
102	5,8129	6,7208	7,8437
128	5,4305	6,1378	7,2489
161	4,7303	5,3218	6,6754
203	3,125	4,6915	6,1438

a physical mechanism of shortening the spin label relaxation time T_1. The spin label may be involved in the reaction of oxidation of vanadium(IV) to vanadium(V), resulting yielding in its reduction or modification leading to loss of its paramagnetic properties, manifested by a decrease in the amplitude of the spectrum or the lack of the EPR spectrum. The nitroxyl free radical group transformed into no paramagnetic hydroxylamine according to the reaction equation [24]:



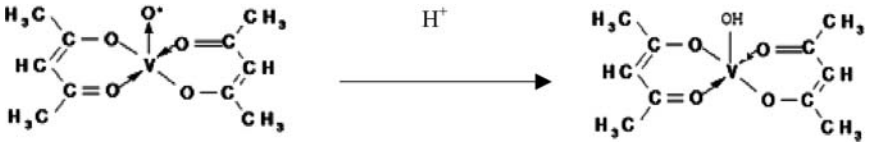
(3)

It can be assumed that the $VO(acac)_2$ reacts with the spin label participating in redox reactions. In the first stage of reaction an attachment of proton derived from the dissociation

Table 2. Values of the matching parameter c for samples $VO(acac)_2$ at the determined concentration

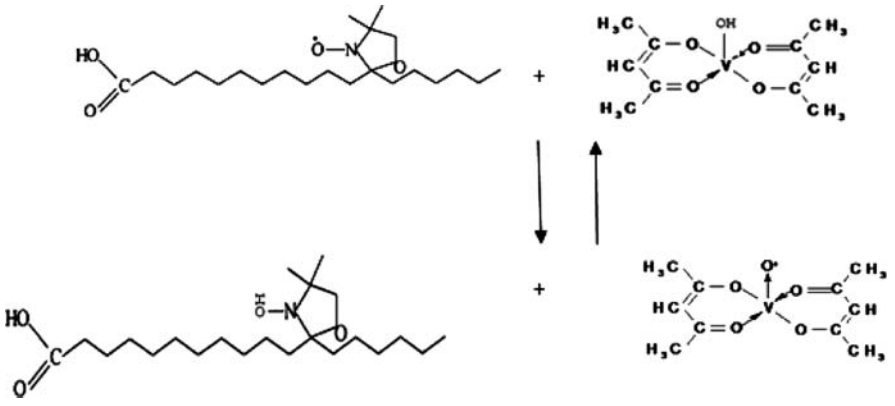
Concentration of $VO(acac)_2$ [M]	Matching parameter c
0	0,044
0,0005	0,040
0,001	0,056

of water molecules coordinatively bound to the vanadium atom of oxygen atom (4) may follows:



(4)

In the second stage the transfer of the hydroxyl group of proton to nitroxyl group of proton to pin label (5) occurs:



(5)

The analysis based on measurements of saturation of EPR signal of spin label 12-SASL demonstrates the impact of the vanadium complex on the EPR spectra of spin label. The observed changes in the EPR spectra of the spin label (a change in the amplitude of the signal for increasing microwave power supplied on the sample) are consistent with expectations for the concentration of $VO(acac)_2$ and 0.0005 M are associated with the shortening of relaxation time spin-spin or is likely to have a different character for the sample of spin label with complex of vanadium at a concentration of 0.0010 M. In both cases, however, they constitute the evidence of the incorporation of the complex to the liposome membrane.

To suppress the EPR signal of vanadium(IV) in aqueous solutions of $VO(acac)_2$ outside the liposomes sulfate(IV) sodium were used. This study was carried out in order to vanadium(IV) in to the no-paramagnetic form performed to axtinguish the signal of vanadium from the aqueous phase and to confirm an incorporation of the complex of vanadium(IV) to membranes. From the literature it is known that sodium sulfate(IV) reduces transition metals such as chromium(VI), iron(III) in complexes [25,26]. This compound can also reduce the vanadium(V) in VO_2^+ to vanadium(IV) ion VO^{2+}. It was stated that sodium sulfate(IV) of sodium added at a concentration of 0.02 M to the solution of $VO(acac)_2$ in borate buffer reduces vanadium(IV) to vanadium(III). This corresponds to the changes in the EPR spectrum of the complex after the addition of sodium sulfate(IV) (Fig. 6). EPR spectrum of the complex $VO(acac)_2$ obtained from residue of the EYPC liposomes after addition of Na_2SO_3 (Fig. 7) is highly anisotropic and difficult to analyze. Because the signal of vanadium complex $VO(acac)_2$ in the aqueous phase disappears anisotropic spectrum of the complex can be an evidence that for its encapsulation into the liposome lipid membrane.

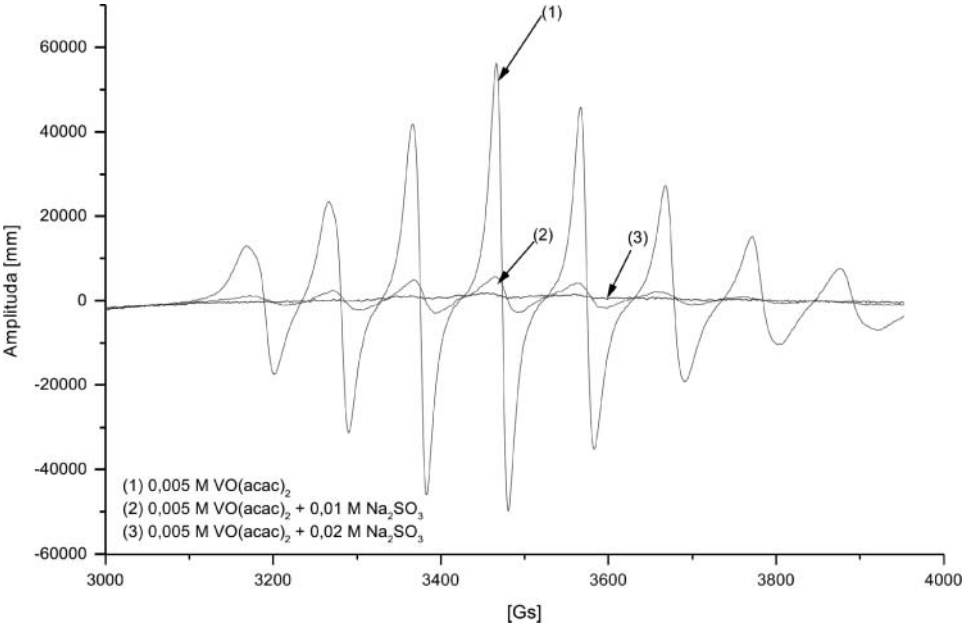


Figure 6. EPR spectra of 0.005 M $VO(acac)_2$ before and after the addition of sodium sulfate (IV) as a reductant.

In order to determine the partition coefficient of $VO(acac)_2$ between the aqueous phase and multi-layered liposome membrane EPR spectra of complex samples collected from the supernatant and residue of the liposomes after the addition of sodium sulfate(IV) were recorded and compared with EPR spectrum of the starting solution without sodium sulfate (Fig. 8). The concentration of $VO(acac)_2$ in the supernatant was determined directly from

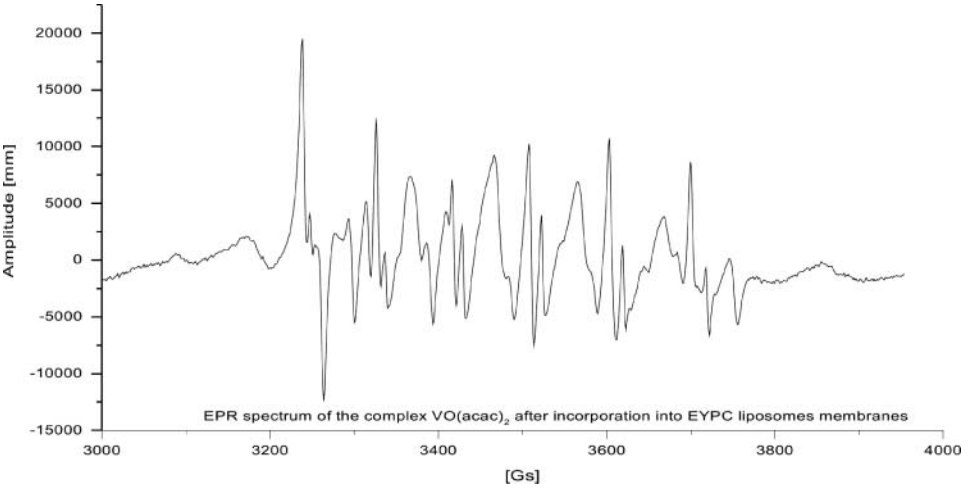


Figure 7. EPR spectrum of the complex $VO(acac)_2$ after incorporation into EYPC liposomes membranes.

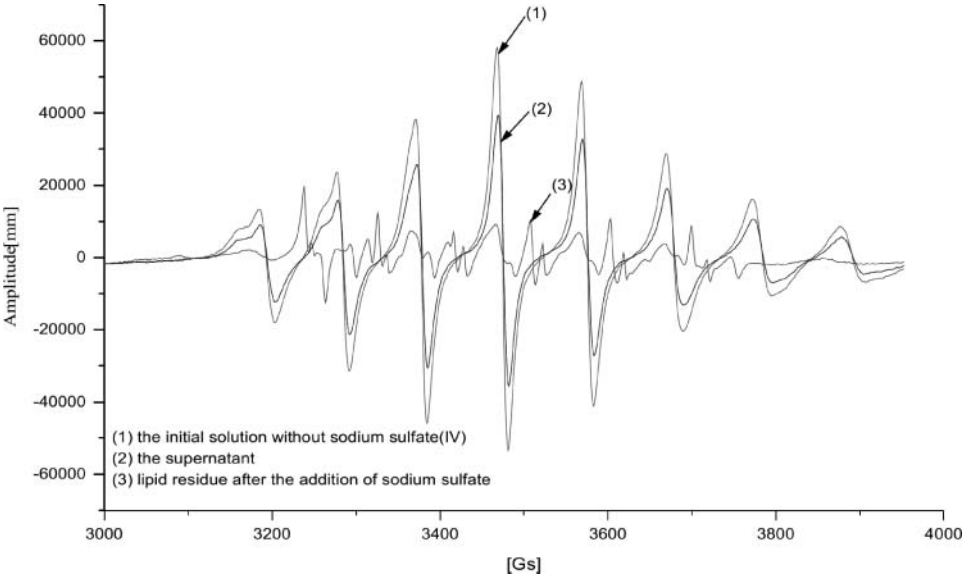


Figure 8. EPR spectra of samples of the complex $VO(acac)_2$ taken from: (1) the initial solution without sodium sulfate(IV), (2) the supernatant, (3) lipid residue after the addition of sodium sulfate.

the EPR spectra by measuring the amplitude of the central line of the complex. Because of the EPR spectrum of vanadium complex obtained from the liposomes residue is anisotropic, it is difficult to estimate on the basis the concentration of the complex in liposomes. Therefore, the concentration of the complex in multilayer liposome was determined from the difference between the initial concentration of the complex (0.005 M) used for preparation of liposomes and its concentration in the aqueous phase. Based on the values of the complex concentration in the supernatant and in the residue of liposomes partition coefficient K = 0,27 of the vanadium(IV) complex between the liposome membrane and the aqueous

Table 3. The concentrations of $VO(acac)_2$ in the supernatant determined from the amplitude A of fourth line of the EPR spectrum of vanadium(IV) complex and in membranes. and the values of partition coefficients K of the complex between the membrane and the aqueous phase of liposomes. Table presents the results of measurements for four independent samples

Supernatant		EYPC Membranes	Partition Coefficient
A	$[VO(acac)_2]_{buffer}$	$[VO(acac)_2]_{initial\ solution} - [VO(acac)_2]_{buffer}$	K
255 mm	0,0038 M	0,0012 M	0,31
260 mm	0,0040 M	0,0010 M	0,25
265 mm	0,0041 M	0,0009 M	0,21
255 mm	0,0038 M	0,0012 M	0,31
		Average Value	
259 mm	0,0039 M	0,0011 M	0,27

phase was determined (Table 3). It can be concluded that the complex $VO(acac)_2$ was incorporated into the membranes of liposomes. The average concentration of the vanadium(IV) incorporation into the membranes of liposomes (0.0011 M) is sufficient to use liposomes for the transport of vanadium compounds. From the literature it is known that in the treatment of diabetes in rats with type II diabetes-induced the effects of the action of vanadium(IV) complex with acetylacetonate were noticeable after its administration to animals to drink at a dose of 0.05 mmol/kg (13 mg/kg) [27]. The value of the partition coefficient is high enough to meet the demand of using liposomes as carriers of the vanadium(IV) compounds in the treatment of diabetes.

4. Conclusions

The efficacy of the application of spin labels to determine the spin concentration of vanadium(IV) complex $VO(acac)_2$ in the aqueous phase and to demonstrate its presence in the lipid membranes of liposomes was proved. It was found that the indirect method of the determination of the vanadium(IV) complex concentration in aqueous solutions is more sensitive than the direct method. Indirect method based on the analysis of the change of the parameters of the spin label EPR spectrum in the presence of vanadium(IV) in solution allows for the detection of low concentrations of the complex above 0,00008 M. The direct method based on signal amplitude of central line of EPR spectrum of the vanadium(IV) complex allows for the determination its concentration above 0,0006 M. Concentration of vanadium(IV) complex $VO(acac)_2$ in the supernatant of liposomes was determined by both direct and indirect methods. On the basis of changes in the nature of the saturation curves of EPR spectra of spin label 12-SASL cause by the presence of the paramagnetic complex of vanadium(IV) we proved the incorporation of complex in lipid membrane. The presence of the vanadium(IV) complex in the membrane was also demonstrated directly by EPR, by the use of sodium sulfate(IV) that suppresses EPR signal of the complex of vanadium(IV) in aqueous phase. It was also found that complex of vanadium(IV) occurs in the aqueous phase and in the lipid bilayer of liposomes. The studies show that 78% of the initial amount of the vanadium(IV) complex used for the preparation in the aqueous phase and 22% of the complex was incorporated in the lipid bilayer of liposome. Concentration of vanadium(IV) complex after incorporation into liposomes seems to be sufficient to trigger the desired therapeutic effect.

Acknowledgements

This work was supported by the Committee of Scientific Research, University of Silesia (BW/ICh/09/10) and Medical University of Silesia (KNW-1-050/P/1/0), Poland.

References

[1] Mehdi, M., Pandey, S., Theberge, J., & Srivastava, A. (2006). *Cell Biochem Biophys.*, *44*, 73.
[2] Shechter, Y. (1990). *Diabetes*, *39*, 3.
[3] Ramanadham, S., Brownsey, R., Cros, G., Mongold, J., & Mcneill, J. (1989). *Metabolism.*, *38*, 1022.
[4] Crans, D., Smee, J., & Gaidamauskase, L. (2004). *Pol. J. Pathol.*, *55*, 25.
[5] Cam, M., Cros, G., Serrano, J., Lazaro, R., & Mcneill, J. (1993). *Diabetes Resarch And Clinical Practice*, *20*, 111.
[6] Yang, X. g., Yang, X. d., Yuan, L., Wang, K., & Crans, D. (2004). *Pharmacol Res.*, *21*, 1026.
[7] Xiaogai, Y., Kui, W., Jingfen, L., & Crans, D. (2003). *Coord. Chem. Rev.*, *237*, 103.

[8] Hesheng, O., Limei, Y., Devkumar, M., Marvin, W., Makinen, M., & Brady, J. (2005). *J. Biol. Inorg. Chem.*, *10*, 874.
[9] Domingo, J. (2000). *Mol. Cell Biochem.*, *203*, 185.
[10] Dąbroś, W., Goc, A., Turyna, B., & Kordowiak, A. (2006). *Pol. J. Pethol.*, *57*, 91.
[11] Dąbroś, W., Goc, A., Turyna, B., & Kordowiak, A. (2004). *Pol. J. Pathol.*, *55*, 25.
[12] Badmaev, V., Prakash, S., & Majeed, M. (1999). *J. Altern. Complement Med.*, *5*, 273.
[13] Li, Z., Carter, J., Dailey, L., & Huang, Y-Ct. (2004). *Environ Health Perspect.*, *112*, 201.
[14] Mukherjee, B., Patra, B., Mahapatra, S., Banerjee, P., Tiwari, A., & Chatterjee, M. (2004). *Toxicol Letters*, *150*, 135.
[15] Thompson, K., & Orvig, C. (2006). *J. Inorg. Biochem.*, *100*, 1925.
[16] Mcneill, J., Yuen, V., Dari, S., & Orvig, Ch. (1995). *Molecular And Cellular Biochemistry*, *153*, 175.
[17] Lyonnet, B., & Martin, E. (1899). *La Presse Med.*, *7*, 190.
[18] Mufamadi, M. *et al.* (2010). *J. of Drug Delivery*, *2011*, 939851.
[19] Pezeshk, A., Pezeshk, V., Firlej, A., Wojas, J., & Subczyński, W. (1993). *Life Sciences*, *52*, 1071.
[20] Subczyński, W. K., & Hyde, J. S.(1983). *Biophys.*, *41*, 283.
[21] Froncisz, W., Ching-San, L., & Hyde, J. (1987). *Journal Of Magnetic Resonance*, *71*, 313.
[22] Wiśniewska, A., & Subczyński, W. (1999). *Current Topics In Biophysics.*, *23*, 79.
[23] Wiśniewska, A., & Subczyński, W. (1998). *Biochim. Biophys. Acta*, *1368*, 235.
[24] Raguz, M., Widomska, J., Dillon, J., Gaillard, E., & Subczyński, W. (2008). *Biochim. Biophys. Acta*, *1778*, 1079.
[25] Pettine, M., Tonnina, D., & Millero, F. (2006). *Marine Chemistry*, *99*, 31.
[26] Conkllnt, M., & Hoffmann, R. (1988). *Environ. Sci. Technol.*, *22*, 899.
[27] Crans, D. (2000). *J. Inorg. Biochem.*, *80*, 123.
[28] Kusumi, A., Subczynski, W., Pasenkiewicz-Gierula, M., Hyde, J., & Merkle, H. (1986). *Biochim. Biophys. Acta*, *854*, 307.