

## РЕЗЮМЕТА НА ПУБЛИКУВАНИТЕ ТРУДОВЕ, СЛЕД ЗАЩИТА НА ДОКТОРСКА ДИСЕРТАЦИЯ

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### **Role of Plasma Membrane at Dielectric Relaxations and Intermembrane Interaction in Human Erythrocytes**

Ivan T. Ivanov and Boyana K. Paarvanova  
Membranes 2023, 13, 658

**Abstract:** Dielectric relaxations at 1.4 MHz ( $\beta_{sp}$ ) and 9 MHz ( $\gamma_{1sp}$ ) on the erythrocyte spectrin network were studied by dielectric spectroscopy using dense suspensions of erythrocytes and erythrocyte ghost membranes, subjected to extraction with up to 0.2% volume Triton-X-100. The step-wise extraction of up to 60% of membrane lipids preserved  $\gamma_{1sp}$  and gradually removed  $\beta_{sp}$ -relaxation. On increasing the concentration up to 100 mM of NaCl at either side of erythrocyte plasma membranes, the  $\beta_{sp}$ -relaxation was linearly enhanced, while the strength of  $\gamma_{1sp}$ -relaxation remained unchanged. In media with NaCl between 100 and 150 mM  $\beta_{sp}$ -relaxation became slightly inhibited, while  $\gamma_{1sp}$ -relaxation almost disappeared, possibly due to the decreased electrostatic repulsion allowing erythrocytes to come into closer contact. When these media contained, at concentrations 10–30 mg/mL dextran (MW 7 kDa), polyethylene glycol or polyvinylpyrrolidone (40 kDa), or albumin or homologous plasma with equivalent concentration of albumin, the  $\gamma_{1sp}$ -relaxation was about tenfold enhanced, while  $\beta_{sp}$ -relaxation was strengthened or preserved. The results suggest the Maxwell–Vagner accumulation of ions on the lipid bilayer as an energy source for  $\beta_{sp}$ -relaxation. While  $\beta_{sp}$ -relaxation appears sensitive to erythrocyte membrane deformability,  $\gamma_{1sp}$ -relaxation could be a sensitive marker for the inter-membrane interactions between erythrocytes.

### **Thermal dielectroscopy study of human erythrocytes with hereditary spherocytosis**

B. K. Paarvanova, B. B. Tacheva, M. I. Karabaliiev, I. Chakaarov, P. Chakaarova and I. T. Ivanov  
Current Topics in Electrochemistry, 24, 69-75.

**Abstract:** Using the threshold changes in the electric admittance of human red blood cells (RBCs) at the temperature of spectrin dissociation and denaturation, two single-time dielectric relaxations,  $\beta_{sp}$  (1.4 MHz) and  $\gamma_{1sp}$  (9 MHz), were detected on the spectrin network (sub-membrane skeleton). For the first time, these relaxations were here studied in RBCs with hereditary anemia. The strengths of  $\beta_{sp}$  and  $\gamma_{1sp}$  relaxations and the characteristic frequency,  $f\gamma_{1sp}$ , of  $\gamma_{1sp}$  relaxation

had the same values in RBCs of healthy patients (n=9) and of patients with hemoglobinopathy (n=5). Compared to the RBCs of healthy patients, the strengths of  $\beta_{sp}$  and  $\gamma_{1sp}$  relaxations in RBCs of children with severe signs of hereditary spherocytosis, due to spectrin deficiency (n=2), were reduced by about 70%, while  $f\gamma_{1sp}$  was reduced by 33%. In RBCs of children with milder signs of hereditary spherocytosis, due to spectrin deficiency (n=6), the diminutions of the strengths of  $\beta_{sp}$  and  $\gamma_{1sp}$  relaxations and of  $f\gamma_{1sp}$  were weaker and occupied intermediate values between those of control RBCs and RBCs with severe signs of hereditary spherocytosis. Compared to the RBCs of healthy patients, the strengths of  $\beta_{sp}$  and  $\gamma_{1sp}$  relaxations in RBCs of patients with anemia due to band 3 deficiency (n=2) were reduced by about 50%, while  $f\gamma_{1sp}$  was not changed. In conclusion, the  $\beta_{sp}$  and  $\gamma_{1sp}$  dielectric relaxations on RBC spectrin network represent a useful approach in studying the RBC membrane of patients with different membranopathy anemia.

### **Dielectric relaxations on erythrocyte membrane as revealed by spectrin denaturation**

I.T. Ivanov, B. Paarvanova  
Bioelectrochemistry 110 (2016) 59–68

**Abstract:** We studied the effect of spectrin denaturation at 49.5 °C ( $T_A$ ) on the dielectric relaxations and related changes in the complex impedance,  $Z^*$ , complex capacitance,  $C^*$ , and dielectric loss curve of suspensions containing human erythrocytes, erythrocyte ghost membranes (EMs) and Triton-X-100 residues of EMs. The loss curve prior to, minus the loss curve after  $T_A$ , resulted in a bell-shaped peak at 1.5 MHz. The changes in the real and imaginary components of  $Z^*$  and  $C^*$  at  $T_A$ , i.e.,  $\Delta Z_{re}$ ,  $\Delta Z_{im}$ ,  $\Delta C_{re}$  and  $\Delta C_{im}$ , calculated in the same way, strongly varied with frequency. Between 1.0 and 12 MHz the  $-\Delta Z_{im}$  vs  $\Delta Z_{re}$ , and  $\Delta C_{im}$  vs  $\Delta C_{re}$  plots depicted semicircles with critical frequencies,  $f_{cr}$ , at 2.5 MHz expressing recently reported relaxation of spectrin dipoles. Between 0.02 and 1.0 MHz the  $-\Delta Z_{im}$  vs  $\Delta Z_{re}$  plot exhibited another relaxation whose  $f_{cr}$  mirrored that of beta relaxation. This relaxation was absent on Triton-X-shells, while on erythrocytes and EMs it was inhibited by selective dissociation of either attachment sites between spectrin and bilayer. Considering above findings and inaccessibility of cytosole to outside field at such frequencies, the latter relaxation was assumed originating from a piezoelectric effect on the highly deformable spectrin filaments.

### **Differential dielectroscopic data on the relation of erythrocyte membrane skeleton to erythrocyte deformability and flicker**

Ivan T. Ivanov, Boyana K. Paarvanova  
European Biophysics Journal (2021) 50:69–86

**Abstract:** Two dielectric relaxations,  $\beta_{sp}$  (1.5 MHz) and  $\gamma_{1sp}$  (7 MHz), have been detected on spectrin-based membrane skeleton (MS) of red blood cells (RBCs) using the plot of admittance changes at the spectrin denaturation temperature (Ivanov and Paarvanova in Bioelectrochemistry 110: 59–68, 2016, Electrochim Acta 317: 289–300, 2019a). In this study, we treated RBCs and RBC ghost membranes with agents that make membranes rigid and suppress membrane flicker, and studied the effect on  $\beta_{sp}$  and  $\gamma_{1sp}$  relaxations. Diamide (diazene dicarboxylic acid bis-(N,N-dimethylamide)) (up to 0.85 mM), taurine mustard (tris(2-chloroethyl)amine) (up to 2 mM), known to specifically cross-link and stiffen spectrin, and glutaraldehyde (up to 0.044%) all inhibited the relaxations in RBC ghost membranes. Similar inhibition was obtained resealing RBC

ghost membranes with 2,3-diphosphoglycerate (up to 15 mM), binding WGA (wheat germ agglutinin) (up to 0.025 mg/ml) to exofacial aspect of RBCs, incubating RBCs in hypotonic (200 mOsm) and hypertonic (600–900 mOsm) media and depleting RBCs of ATP. By contrast, concanavalin A (1 mg/ml) and DIDS (4,4'-diiso-thiocyanato stilbene-2,2'-disulfonic acid) (75  $\mu$ M, pH 8.2), both known to bind specifically band 3 integral protein of RBCs without effect on RBC membrane rigidity, did not affect the relaxations. We conclude there might be a relation between the strength of dielectric relaxations on MS spectrin and the deformability and flicker of RBC membrane.

### **Mild laboratory-induced metabolic disorder in rats. Effect on erythrocyte membrane according to a dielectroscopic study**

B. K. Paarvanova, B. Tacheva, A. Tolekova, P. Hadzhibozheva, T. K. Georgiev, M. Karabaliev, and I. T. Ivanov

AIP Conference Proceedings **2186**, 110006 (2019)

**Abstract:** This study concentrates on the effect of mild metabolic disorder (MMD) in fructose-fed rats on the submembrane skeleton MS of their erythrocytes relying on the relationship between membrane structure and its dielectric properties. The segmental flexibility of MS and its attachment to the lipid membrane of erythrocytes is expressed by the MS intrinsic dielectric polarization. The latter was assessed by the difference in dielectric properties of erythrocytes (complex impedance,  $Z^*$ , and capacitance,  $C^*$ ) prior to and after the thermal denaturation of spectrin assuming the dielectric activity of denatured spectrin nil. The erythrocytes of control (n=4) and fructose-fed (n=5) rats were isolated, washed, suspended in isotonic 10 mM NaCl/mannit solution (hematocrit 0.45) and heated (1.5 °C/min). At the denaturation temperature of spectrin (49°C) the dielectric properties of erythrocyte suspension sustained threshold changes  $\Delta Z^* = \Delta Z_{re} + j\Delta Z_{im}$  and  $\Delta C^* = \Delta C_{re} - j\Delta C_{im}$ . The frequency analysis of  $\Delta Z_{re}$ ,  $\Delta Z_{im}$ ,  $\Delta C_{re}$  and  $\Delta C_{im}$  indicated strongly increased contribution of spectrin to the dielectric properties of erythrocyte membrane. Thus, spectrin contribution to the static capacitance of erythrocyte membrane was 83 % greater and that to the dipole loss of erythrocyte membrane was 38 % greater in erythrocytes from MMD rats, compared to control RBCs. This finding is suggested to be due to the moderate increase in glucose level and related increase in phosphorylation of membrane proteins in MMD rats.

### **Thermal Dielectroscopy - a New Method for Studying the Membrane Skeleton of Human Erythrocytes**

Boyana Paarvanova, Bilyana Tacheva, Miroslav Karabaliev and Ivan T. Ivanov

AIP Conference Proceedings **1906**, 150004 (2017)

**Abstract:** The structure and mechanical properties of erythrocyte plasma membrane are strongly affected by both the dephosphorylation and thermal denaturation (49.5°C) of erythrocyte under-membrane spectrin skeleton. Here, the dielectric loss (DL) of suspensions, containing native erythrocytes or erythrocyte ghost membranes (EGMs), was determined applying a mathematical method to remove the conductive loss from the imaginary capacitance,  $C_{im}$ , of the suspensions. The DL frequency profile of spectrin skeleton was obtained subtracting the DL data collected prior to, and after the denaturation of spectrin at 49.5°C. Spectrin skeleton exhibited narrow bell-shaped DL frequency curve, centered at 1.5 MHz, presumably reflecting the segmental mobility of

spectrin. The area of this curve was reduced by 30 % after mild dephosphorylation (starvation of erythrocytes at 37°C for 5 h) and reduced to zero at EGMs resealed with alkaline phosphatase (full dephosphorylation). These results, combined with others, indicate the relevance of dielectric analysis for the study of dynamics and separation of membrane skeleton from the lipid membrane of erythrocytes.

### Effect of Permeant Cryoprotectants on Membrane Skeleton of Erythrocytes

I.T. Ivanov, B.K. Paarvanova

Probl Cryobiol Cryomed 2019; 29(3):237–245

**Abstract:** The impact of formamide (FA), N-methylformamide (MFA), N,N-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) on the spectrin-based membrane skeleton of human erythrocytes was studied by thermal dielectroscopy. Upon heating the erythrocyte suspension, spectrin denatures at 49.5°C causing suspension impedance to change by  $\Delta Z^* = \Delta Z' + j\Delta Z''$ . The  $-\Delta Z''$  vs.  $\Delta Z'$  plot reveals two dielectric relaxations on spectrin, called beta (0.05–1.0 MHz) and gamma (1.0–10 MHz) relaxations. At concentrations up to 5 M (relevant to cryoprotection) DMF and DMSO specifically subdued gamma-relaxation, while FA and MFA specifically inhibited beta-relaxation. Each effect was eliminated by preliminary washing of cryoprotectant and presumably did not depend on the osmotic pressure produced. Applied in combination, DMSO and FA neutralized their effects. Based on previous study, the results indicate that tested cryoprotectants detached the membrane skeleton from the lipid membrane, thereby compromising cell integrity.

### Drug Exchange between Albumin Nanoparticles and Erythrocyte Membranes

Bilyana Tacheva, Boyana Paarvanova, Ivan T. Ivanov, Boris Tenchov, Radostina Georgieva and Miroslav Karabaliev

Nanomaterials 2019, 9, 47; doi:10.3390/nano9010047

**Abstract:** The effects of thioridazine (TDZ) and chlorpromazine (CPZ) and bovine serum albumin nanoparticles (BSA-NPs) on erythrocyte membranes have been investigated. Two kinds of hemolytic assays were used; hemolysis under hypotonic conditions and hemolysis in physiological conditions. Under hypotonic conditions for 50% hemolysis, both TDZ and CPZ have a biphasic effect on membranes; namely, stabilization at low concentrations and destabilization after reaching a critical concentration. In physiological conditions, there are other critical concentrations above which both drugs hemolyse the erythrocytes. In each case, the critical concentrations of TDZ are lower than those of CPZ, which is consistent with the ratio of their partition coefficients. When BSA-NPs are added to the erythrocyte suspension simultaneously with the drugs, the critical concentrations increase for both drugs. The effect is due to the incorporation of a portion of drug substances into the BSA-nanoparticles, which consequently leads to the decrease of the active drug concentrations in the erythrocyte suspension medium. Similar values of the critical concentrations are found when the BSA-NPs are loaded with the drugs before their addition to the erythrocyte suspension in which case the events of the partition are: desorption of the drug from BSA-NPs, diffusion through the medium, and adsorption on erythrocyte membranes. This result suggests that the drugs are not influenced by the processes of adsorption and desorption onto and out of the BSA-NPs, and that the use of BSA-NPs as drug

transporters would allow intravenous administration of higher doses of the drug without the risk of erythrocyte hemolysis.

### Thermal dielectroscopy study on the vertical and horizontal interactions in erythrocyte sub-membrane skeleton

I.T. Ivanov, B. Paarvanova  
Electrochimica Acta 317 (2019) 289e300

**Abstract:** Thermal dielectroscopy was used to study the spectrin-based sub-membrane skeleton (MS) and its attachment to the lipid bilayer of human erythrocyte membrane. At the spectrin denaturation temperature,  $T_A$ , the complex impedance,  $Z^*$ , and capacitance,  $C^*$ , sustain sigmoid, frequency-dependent changes,  $\Delta Z^* = \Delta Z' + j\Delta Z''$  and  $\Delta C^* = \Delta C' - j\Delta C''$ , respectively. The impedance change plot,  $-\Delta Z''$  vs.  $\Delta Z'$ , depicts two semicircles revealing two dielectric relaxations on MS, here designated as beta and gamma relaxations on MS [Ivanov and Paarvanova, 2016]. We report here that ortho-vanadate (5 mM), alkaline cytosole (pH 9.2), N-ethylmaleimide (5 mM), diamide (1 mM) and cell shrinkage, all known to disrupt the band 3 tetramer-spectrin attachment site, inhibited gamma relaxation, which represents the direct interaction of high frequency field with spectrin. By contrast DNAase I, diphosphoglycerate (10 mM) and urea (1-2M), all known to disrupt the actin-spectrin junction, inhibited beta relaxation, which represents the piezo effect on spectrin, powered by the low frequency electrostriction of lipid bilayer. The frequency curves of  $\Delta C_d''$  (dielectric component of  $\Delta C''$ ) and of  $\Delta C'$  both determined the critical frequencies of beta and gamma relaxations as 1.4 and 6 MHz at 46 °C (1.1 and 4.5MHz at 37 °C), respectively. Based on the presented results, the effect of MS attachment on the dynamics and elasticity of MS was discussed.

### Effects of heat and freeze on isolated erythrocyte submembrane skeletons

Ivan T. Ivanov, Boyana K. Paarvanova, Veselin Ivanov, Kathrin Smuda, Hans Bäumler and Radostina Georgieva  
Gen. Physiol. Biophys. (2017), 36, 155–165

**Abstract:** In this study we heated insoluble residues, obtained after Triton-X-100 (0.1 v/v%) extraction of erythrocyte ghost membranes (EGMs). Specific heat capacity, electric capacitance and resistance, and optical transmittance (280 nm) sustained sharp changes at 49°C ( $T_A$ ) and 66°C ( $T_C$ ), the known denaturation temperatures of spectrin and band 3, respectively. The change at  $T_A$  was selectively inhibited by diamide (1 mM) and taurine mustard (1 mM) while its inducing temperature was selectively decreased by formamide in full concert with the assumed involvement of spectrin denaturation. In the residues of EGMs, pretreated with 4,4'-diisothiocyanato stilbene-2,2'-disulfonic acid (DIDS), the change at  $T_C$  was shifted from 66 to 78°C which indicated the involvement of band 3 denaturation. The freeze and rapid thaw of EGM residues resulted in a strong reduction of cooperativity of band 3 denaturation while the slow thaw completely eliminated the peak of this denaturation. These effects of freeze-thaw were prevented in residues obtained from DIDS-treated EGMs. The freeze-thaw of residues slightly affected spectrin denaturation at 49°C although an additional denaturation appeared at 55°C. The results indicate preserved molecular structure and dynamics of the membrane skeleton in Triton-X-100

extracts of EGMs. The freeze-thaw inflicted strong damage on band 3 and spectrin-actin skeleton of EGM extracts which is relevant to cryobiology, cryosurgery and cryopreservation of cells.

### **Impact of Permeant Cryoprotectors on Under-Membrane Skeleton of Human Erythrocytes**

B. Paarvanova, M. Karabaliev, B. Tacheva and I. Ivanov  
AIP Conference Proceedings **2075**, 170012 (2019)

**Abstract:** Formamide (FA), N-methylformamide (MFA), N,N-dimethylformamide (DMFA) and dimethyl sulfoxide (DMSO) are frequently used as cryoprotectors of cells. We applied thermal dielectroscopy to study the impact of these membrane-permeable cryoprotectors on the segmental mobility and attachment of spectrin-based skeleton to the lipid membrane of human erythrocytes. Upon heating a suspension of erythrocytes, spectrin denatures at 49.5 °C causing sigmoid frequency-dependent changes in the complex impedance,  $\Delta Z^* = \Delta Z_{re} + j.\Delta Z_{im}$ , of suspension. The  $-\Delta Z_{im}$  vs  $\Delta Z_{re}$  plot depicts two semicircles which correspond to the beta (0.05-1.0 MHz) and gamma (1.0-10 MHz) dielectric relaxations on spectrin skeleton. At concentrations (1 to 4 M), relevant to cryoprotection, DMFA and DMSO subdued gamma relaxation, while FA and MFA strongly inhibited the beta relaxation. These effects were eliminated on washing and did not depend on osmotic pressure produced. Applied in combination, DMSO and FA neutralized their opposite effects on the dielectric relaxations. These results are discussed in light of possible impact of tested cryoprotectors on the attachment sites of spectrin skeleton to the lipid membrane.

### **Spectrofluorimetric Study of the Membrane Permeability Disturbance in *Listeria Monocytogenes* at Hyperthermic Temperatures**

B. Paarvanova, T. Stoyanchev, I. Zlatanov, V. Georgieva, I. Ivanov  
*Bulg. J. Vet. Med.*, 17, No 4, 285-292

**Abstract:** Thermal resistance of pathogenic bacteria, determined by the colony forming ability after thermal stress, is important for the microbial inactivation by heat. It is related to the ion permeability barrier disturbance at a high temperature ( $T_g$ ). The mechanism of this membrane disturbance is not clear. Our goal was to study this disturbance in the plasma membrane of *L. monocytogenes*. After cultivation the bacteria were washed in a low-salt medium and heated to  $T_g$ . Hyperthermia induced out leakage of cytosolic electrolytes with sigmoid temperature profile, centered at  $T_g$ , as detected by conductometry (10 kHz). The  $T_g$  temperature correlated the thermal resistance of bacteria, acclimated at various temperatures of growth. To this end, we used fluorescent probes Pyrene and N-(3-Pyrenyl) maleimide (PyM). While Pyrene intercalates in the lipid region of plasma membranes PyM binds covalently the SH-groups of membrane proteins. The excimerisation of Pyrene reporting on the microfluidity of lipid bilayer did not change at  $T_g$ , while excimerisation of PyM raised indicating immobilisation and association of integral proteins. Overall, the ion permeability rise at  $T_g$  could be ascribed to changes in the tertiary and quaternary structure of integral proteins.

## Species-dependent variations in the dielectric activity of membrane skeleton of erythrocytes

Ivan T. Ivanov, Boyana K. Paarvanova, Bilyana B. Tacheva and Todor Slavov  
Gen. Physiol. Biophys. (2020), 39

**Abstract:** Previously detected  $\beta_{sp}$  and  $\gamma_{1sp}$  dielectric relaxations on the spectrin-based membrane skeleton (MS) of human red blood cells (RBCs) have been shown sensitive to the attachment of MS to the lipid-protein membrane. Such relaxations were now detected on the MS of mammal (rat, horse, bovine, sheep and goat) and “unstrained” chicken RBCs. To become “unstrained” chicken RBCs were subjected consecutively to cold (4°C, >20 h) and either colchicine (15 mM) or vinblastine (30  $\mu$ M) (4°C, 1 h) that led to irreversible disassembly of their marginal band and an additional portion of their cytoskeleton. With the exception of bovine RBCs, the critical frequency ( $f_c$ ) of either relaxation increased, although at different rates, with the decrease in the volume of RBC species. The strong increase in  $f_c$  of  $\gamma_{1sp}$  relaxation from 2.5 MHz (“unstrained” chicken RBCs) to 13 MHz (goat RBCs) could indicate denser state of MS in smaller RBC species. The low values of  $f_c$  of  $\gamma_{1sp}$  relaxation in “unstrained” chicken RBCs (2.5 MHz) and bovine RBCs (4.5 instead of 9 MHz) could be related to their extraordinary thermal stability at the temperature of spectrin denaturation.

## Kinetics of hemolysis induced by thioridazine

Bilyana Tacheva, Boyana Paarvanova, Stanislav Bozhikov, Ivan T. Ivanov, and Miroslav Karabaliev  
AIP Conference Proceedings 2186, 110007 (2019)

**Abstract:** The process of hemolysis of erythrocyte suspension induced by thioridazine (TDZ) was investigated by measuring the UV-Vis absorbance spectrum in the range 200 nm – 700 nm. The time needed to obtain the spectrum in this range was 12.5 s, so the spectrum was measured each 15 s. This permitted to follow the changes in absorbance at different wavelengths of the spectra during hemolysis. It was found that at most of the wavelengths measured the absorbance decreased which was related to the decrease of the light-scattering by the intact erythrocytes. In contrast, the absorbance peak of the hemoglobin (Hb) at 416 nm increased significantly during hemolysis. This was attributed to smaller light absorption by Hb when the erythrocytes were still intact and the Hb is still inside the erythrocytes. This phenomenon was due to the forward-scattered light that did not penetrate the erythrocytes and did not interact with Hb. When Hb was released from erythrocytes it could already interact with the light, resulting in increase of the absorption peaks of Hb and especially of the peak at 416 nm. The kinetics of the decrease of light-scattering at 700 nm and 500 nm and the increase of Hb absorption at 416 nm were compared and it was found that they are directly related. The kinetics of hemolysis induced by different concentrations of TDZ were measured. It was found that the time for hemolysis depended on the TDZ concentration as the inverse power of 5.

### **Multiplicative scatter correction and principal component analysis of UV-Vis absorption spectra during acid hemolysis of erythrocyte suspension**

Miroslav Karabaliev, Boyana Paarvanova, Bilyana Tacheva, Mitko Mitev, Radoslav Ginin, and Stefka Atanassova

AIP Conference Proceedings **2343**, 070008 (2021)

**Abstract:** The process of hemolysis of erythrocyte suspension induced by hydrochloric acid (HCl) is investigated by measuring the UV-Vis absorption spectrum in the range 250 nm – 750 nm at interval 1 nm. The spectrum is measured each 15 s to the end of hemolysis. The methods of Multiplicative Scatter Correction (MSC) and Principal Component Analysis (PCA) were applied for analysis of spectra. Multiplicative Scatter Correction of spectra allowed removing effects of scattering and observing the changes related to the hemoglobin absorption in the course of hemolysis – shift of Soret band from 420 to 412 nm, decreasing of two peaks of oxyhemoglobin at 543 and 577 nm and disappearing of globin-hem interaction band at 344 nm. The Principal components of transformed spectra were more informative compared to Principal components of raw spectra and contained features related to absorption of hemoglobin.

### **Numerical analysis of absorption spectrum peak wavelength**

Miroslav Karabaliev, Boyana Paarvanova, Stanislav Bozhikov, Radoslav Ginin, Stefka Atanassova, and Bilyana Tacheva

AIP Conference Proceedings 2343, 070007 (2021)

**Abstract:** The process of hemolysis of erythrocyte suspension induced by hydrochloric acid (HCl) is investigated by measuring the UV-Vis absorption spectrum in the range 250 nm – 750 nm at interval 1 nm. The spectrum is measured each 15 s which permitted to follow the changes in absorbance at different wavelengths during hemolysis. The kinetics of the hemolysis is monitored by the changes in the absorbance at 700 nm, and by the changes in the wavelength of the main absorbance peak in the spectrum due to the hemoglobin. Because of the finite number and discrete values of the wavelengths used in the measurement the real value of the peak wavelengths in the spectra must be found by additional processing of the experimental data. The procedure uses the first derivative of the spectrum ( $dA/dt$ ) and a geometrical approach to calculate the value of the wavelength at which the first derivative  $dA/dt$  is equal to 0. The procedure smoothed the kinetic curve of the peak wavelength to a great extent. This permit to compare it to the curve of the absorbance at 700 nm and to make assumptions of the process that occurs during the acid hemolysis. The procedure could be applied to any process where there is a shift in the peaks' wavelength.

### **Electrostriction techniques for preparation of thin lipid films on different solid supports**

M. Karabaliev, B. Paarvanova, I. Ivanov, and B. Tacheva

AIP Conference Proceedings **2075**, 170010 (2019)

**Abstract:** Two types of electrostriction techniques that apply constant (DC) or alternate (AC) voltage are used in the work to prepare thin lipid films on three different types of electrodes – glassy carbon (GC), gold and SiO/SiO<sub>2</sub>. The films are obtained by a self-thinning of lipid organic solution placed between the electrode and an electrolyte solution. This process leads to relatively thick films with thicknesses in the range 100-1000 Å depending on the solid support. Further



thinning is achieved by electrostriction that generates additional external pressure  $P \sim U_{LF}^2/h^2$ , where  $U_{LF}$  is the voltage drop across the lipid film and  $h$  is the film thickness.  $U_{LF}$  depends on the applied external voltage  $U_{EXT}$  and the voltage drops across the other elements in the overall circuit that represents the electrochemical cell consisting of the film-supporting electrode, the lipid film, the electrolyte solution and the reference electrode. On the other hand, the voltage drop across each element depends on the type of the applied voltage - constant (DC) or alternate (AC). In the case of glassy carbon and gold electrodes, the most insulating element is the lipid film and the voltage drop  $U_{LF}$  is close to the applied  $U_{EXT}$ , which permits to obtain easily thin films by DC-electrostriction. In the case of Si/SiO<sub>2</sub> wafers, the insulating SiO<sub>2</sub> layer represents an almost ideal capacitor in series with the other elements in the circuit. Applying DC-electrostriction leads to a predominant voltage drop across the SiO<sub>2</sub> layer and small voltage drop  $U_{LF}$ . To obtain sufficiently high  $U_{LF}$  that would lead to the thinning of SiO/SiO<sub>2</sub>-supported lipid layer, an AC-electrostriction is used. At higher frequencies of AC-voltage, the impedance of the SiO<sub>2</sub> layer becomes small and the voltage drops across the SiO<sub>2</sub> layer and across the lipid film become comparable. Thus, according to the type of the support either DC-electrostriction or AC-electrostriction could be used to obtain very thin lipid films.

### **Segmental flexibility of spectrin reflects erythrocyte membrane deformability**

Ivan T. Ivanov and Boyana K. Paarvanova  
Gen. Physiol. Biophys. (2022), **41**, 87–100

**Abstract:** The frequency-dependent contribution of spectrin, the main cytoskeletal protein of red blood cell (RBC) membrane, to the complex admittance and capacitance of suspended RBCs have revealed two dielectric relaxations,  $\beta_{sp}$  (1.4 MHz) and  $\gamma_{1sp}$  (7 MHz). The strength of these relaxations was related to the ability of RBC membrane to deform. In this study the two relaxations were inhibited by N-ethylmaleimide (up to 5 mM), known to impair the RBC deformability, and the degree of inhibition, i.e., the number of accessible SH-groups on spectrin, depended on the deformation of RBC membrane. Dithiothreitol (up to 5 mM), which does not affect RBC deformability, did not affect the above dielectric relaxations in line with the absence of S-S groups on spectrin. Phenylhydrazine (up to 3 mM) and hydrogen hydroperoxide (up to 450  $\mu$ M) are known to denature the haemoglobin of RBCs producing nanoparticles (globins) that bind to spectrin turning the RBC membrane rigid. At the same concentrations they were shown to inhibit progressively the two relaxations on spectrin. The results are in line with the involvement of some globin-sized segments of spectrin in the dielectric activity of spectrin and in the ability of RBC plasma membrane to deform.

### **Impedance dispersion analysis of drug-membrane interactions**

Bilyana Tacheva, Boyana Paarvanova, Ivan T. Ivanov, and Miroslav Karabaliev  
Citation: AIP Conference Proceedings **1906**, 150005 (2017)

**Abstract:** Thin lipid films modified glassy carbon electrodes (GCE) were used in this work as model system for studying the interactions between two antipsychotic phenothiazine drugs, chlorpromazine and thioridazine, and the lipid fraction of the biomembranes. The lipid films on the electrode surface were obtained through the thinning of film-forming lipid solution deposited between an electrolyte phase and the working GC electrode. The effects of the drugs on the lipid

film structure were investigated by electrochemical impedance spectroscopy (EIS). To characterize the electric properties of the lipid film the impedance of the working GCE is modeled with an equivalent circuit consisting of parallel capacitance  $C_p$  and resistance  $R_p$ . These capacitance and resistance are not frequency independent but could be calculated as equivalent  $C_p$  and  $R_p$  for each measured frequency of the impedance spectrum and presented as functions of the frequency  $f$ ,  $C_p = C_p(f)$  and  $R_p = R_p(f)$ . For the lipid films used in this work, it is demonstrated that both  $C_p(f)$  and  $R_p(f)$  are well approximated with power-law functions. This behavior implies that the impedance  $Z$  of the films could be analysed in terms of the well-known constant-phase angle element (CPE), which is often used to describe the interfacial impedance of solid working electrodes.

### Using Trajectory Log Files as Additional Tool for Dosimetry Verification Plan. A Case in Practice

Stanislav Bozhikov, Filippa Vassileva, Karina Mitarova, Boyana Paarvanova, Bilyana Tacheva and Miroslav Karabaliev  
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**Abstract:** In this survey, we investigate the usage of Trajectory log data in a single case of a patient undergoing Stereotactic Radiosurgery (SRS) treatment. A dedicated linear accelerator machine Varian TrueBeam STX was used. The software was developed for analyzing the log files which were created by the machine when a certain plan was performed in treatment mode or QA mode. Four plans were created with 7 no-coplanar fields. Additional structures must be added by the physicist in the contouring section in order to optimize the plans. Dosimetry equipment ArcCHECK was used to verify the dose distribution for the created plan. The evaluation of  $\gamma$  passing rate (%GP) from the Trajectory log was done for each field separately using Pylinac v.2.2.6 API (application programming interface) for Machine quality control. It was shown that for one of the plans the values of %GP in some of the fields were very low with about less than 15% passing and at the same time the ArcCHECK shows a good %GP of about 90%. When verifying the plans using the analyzed Trajectory log files it can be detected if there are problems with the rotation of the collimator, the positioning of the collimator jaws or with over-modulating of the plan.

### Hemolysis by Saponin is Accelerated at Hypertonic Conditions

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**Abstract:** Saponins are large group of organic amphiphilic substances (surfactants) mainly extracted from herbs with biological activity, considered as one of the main ingredients in numerous remedies used in traditional medicine since ancient times. Anti-inflammatory, antifungal, antibacterial, antiviral, anti-parasitic, antitumor, antioxidant and many other properties have been confirmed for some. There is increasing interest in elucidation of the mechanisms behind effects of saponins on different cell types at the molecular level gain increasing interest. In this regard, erythrocytes are very welcome model having very simple structure with no organelles. They react to changing external conditions and substances by changing shape or volume, with

damage to their membrane ultimately leading to hemolysis. Hemolysis can be followed spectrophotometrically and provides valuable information about the type and extent of membrane damage. We investigated hemolysis of erythrocytes induced by various saponin concentrations in hypotonic, isotonic and hypertonic media using measurements of real time and end-point hemolysis. The osmotic pressure was adjusted by different concentrations of NaCl, manitol or a NaCl/manitol mixture. Unexpectedly, at fixed saponin concentration, hemolysis was accelerated at hypertonic conditions but much faster in NaCl compared to mannitol solutions at the same osmotic pressure. These findings confirm the colloid-osmotic mechanism behind saponin hemolysis with pore formation with increasing size in the membrane.

Изготвил:



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