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# The Effect of Contrast Medium SonoVue® on the Electric Charge Density of Blood Cells

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**Abstract** The effect of contrast medium SonoVue® on the electric charge density of blood cells (erythrocytes and thrombocytes) was measured using a microelectrophoretic method. We examined the effect of adsorbed $H^+$ and $OH^-$ ions on the surface charge of erythrocytes or thrombocytes. Surface charge density values were determined from electrophoretic mobility measurements of blood cells performed at various pH levels. The interaction between solution ions and the erythrocyte's or thrombocyte's surface was described by a four-component equilibrium model. The agreement between the experimental and theoretical charge variation curves of the erythrocytes and thrombocytes was good at pH 2–9. The deviation observed at a higher pH may be caused by disregarding interactions between the functional groups of blood cells.

**Keywords** SonoVue · Erythrocyte · Thrombocyte · pH measurement · Surface charge density · Association constant

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## Introduction

Hepatic hemangiomas (congenital vascular malformations) are the most common benign tumors, accounting for 9.4–22.7% of all diagnosed hepatic tumors (Numata et al. 2006; Tranquart et al. 2009; Tani et al. 2010; von Herbay et al. 2010). Most of them are asymptomatic and require no treatment; therefore, differentiation between hepatic hemangiomas and hepatic malignant lesions is a frequent challenge. Radiologists can use numerous methods to diagnose hepatic tumors. Ultrasound is considered to be inferior in comparison to computed tomography (CT), and magnetic resonance imaging (MRI) using contrast agents allows for an evaluation of its nature (Soyer et al. 1998; Herman et al. 2005; Dietrich et al. 2007; Zviniene et al. 2010). The introduction of contrast medium for diagnosis of various medical conditions has been associated with a number of adverse effects such as nephropathy, thrombosis and nephrogenic systemic fibrosis (Carr et al. 1995; Broome 2008; Feldkamp and Kribben 2008; Van Linden et al. 2011). Currently, a growing number of centers perform contrast-enhanced ultrasonography (CEUS) with the contrast agent SonoVue®. Although the use of SonoVue is characterized by a small amount of contraindications, compared to other contrast media it has a good safety profile in abdominal applications, myocardial perfusion assessment and transcranial ultrasound diagnostic (Kaps et al. 1999; Bokor et al. 2001; Piscaglia and Bolondi 2006; Dijkmans et al. 2009; Gaibazzi et al. 2009; Beaton et al. 2010). CEUS achieves comparable sensitivity and specificity to CT and MRI (Bartolotta et al. 2007; Dietrich et al. 2007; Li et al. 2007; Tranquart et al. 2009; Beaton et al. 2010; von Herbay et al. 2010). We have used this method since 2006, especially in people in whom CT or MRI is contraindicated. No complications have been observed.

This study was designed to assess the effects of SonoVue on erythrocytes and thrombocytes.

SonoVue is for diagnostic use only. It is a contrast agent (it helps make internal body structures visible during imaging tests). SonoVue is used in tests that measure how ultrasound travels within the body because it improves the ability of the blood to create an echo. It is only used when the results of the test without a contrast agent are inconclusive.

SonoVue is used in the following:

- Echocardiography (a diagnostic test where an image of the heart is obtained), to obtain a clearer scan of the chambers of the heart, especially of the left ventricle, in patients with suspected or confirmed coronary artery disease.
- Doppler (a diagnostic test that measures the speed of blood flow), in tests for large blood vessels, such as those in the head and those leading to the head or the main vein to the liver, or for smaller blood vessels, such as those in lesions (areas of disease) in the breast or liver.

A microscopic picture SonoVue microbubbles and a schematic representation of the SonoVue microbubble structure are presented in Fig. 1.

The total amount of sulfur hexafluoride ($SF_6$) administered in a clinical dose is extremely small (in a 2-ml dose the microbubbles contain 16 µl of gas). $SF_6$ is an inert, innocuous gas, poorly soluble in aqueous solutions. It dissolves in the blood and is subsequently exhaled. More than 80% of the administered $SF_6$ was recovered in exhaled air within 2 min after injection and almost 100% after 15 min (Schneider et al. 1995; Schneider 1999a, 1999b; Greis 2004). SonoVue is a contrast agent of the second generation that contains phospholipid-stabilized microbubbles filled with $SF_6$ with a diameter <8 µm (mean 2.5 µm). The physicochemical parameters of SonoVue are presented in Table 1.

Biological membranes are characterized by a markedly ordered structure and can be considered to be a distinct phase from the physical point of view, separated from surrounding cytoplasm or intermolecular biological fluid. Thus, the membrane surface can be approximated to an interface. The membrane–medium interface is the site where some physicochemical processes occur which are characteristic of a typical interface, e.g., asymmetric distribution of electric charge (Nalecz and Wojtczak 1982).

Examining the electrical charge could reveal substantial information about the balance between membrane components but also between membrane components and the surrounding solution. The electrical charge is determined by structural positive charge carriers (free amino groups of proteins and aminophospholipids) and by negative charge carriers (some phospholipids, especially phosphatidylserine, sialic acid, glycophorins and free carboxy groups of polypeptide chains) (Szachowicz-Petelska et al. 2010).

The effect of contrast medium SonoVue on the electric charge density of blood cells (erythrocytes and thrombocytes) was measured using a microelectrophoretic method. We describe the changes of electric charge density of the

**Table 1** SonoVue physicochemical parameters

| | |
|---|---|
| Concentration | 1–5 × $10^8$ $ml^{-1}$ |
| Mean microbubble diameter | 2.5 (µm) |
| $SF_6$ encapsulated volume | 8 (µl/ml) |
| $SF_6$ volume in a 2.4-ml dose | 0.02 (ml) |
| Osmolarity | 290 (mOs/kg) |
| Viscosity | 2 (mPa s) |
| pH | 6.0–6.5 |
| Stability after reconstitution | 6 (h) |

Data from Schneider et al. 1995; Schneider 1999a, 1999b

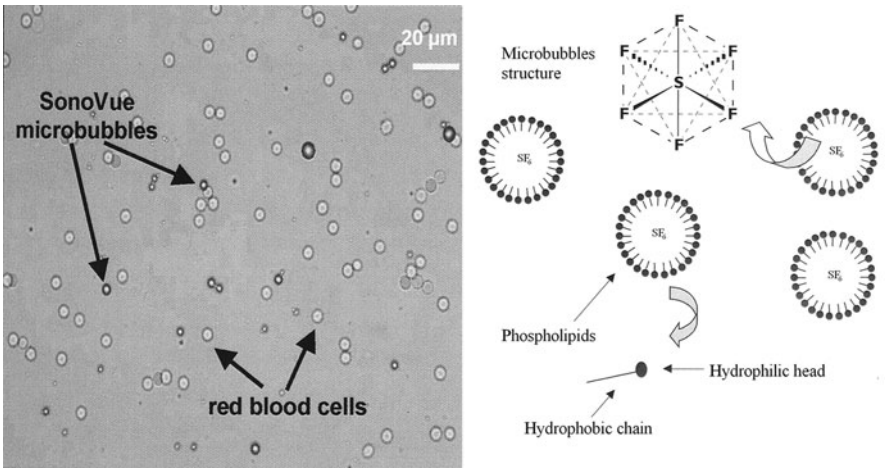


**Fig. 1** Microscopic picture and schematic representation of SonoVue microbubble structure (Schneider et al. 1995; Schneider 1999a, 1999b; Greis 2004)

erythrocytes and thrombocytes after the SonoVue effect over the pH range 2–11.

## Theory

The dependence of the surface charge density of erythrocytes or thrombocytes as a result of SonoVue on the pH of the electrolyte solution can be described using four equilibrium equations. Two are connected with positive groups (e.g., phospholipids or proteins and sodium and hydrogen ions), and two concern the negative species of phospholipids or proteins and hydroxide and chloride ions. The $H^+$, $OH^-$, $Na^+$ and $Cl^-$ ions are adsorbed at the cell membranes (erythrocytes or thrombocytes), and the adsorption equilibria can be presented in the following form (Dobrzynska et al. 2006, 2007):

$$A^- + H^+ \Leftrightarrow AH \quad (1)$$

$$A^- + Na^+ \Leftrightarrow ANa \quad (2)$$

$$B^+ + OH^- \Leftrightarrow BOH \quad (3)$$

$$B^+ + Cl^- \Leftrightarrow BCl \quad (4)$$

Therefore, the association constants of the $H^+$, $Na^+$, $OH^-$ and $Cl^-$ ions with functional groups are expressed in the following manner (Dobrzynska et al. 2006, 2007):

$$K_{AH} = \frac{a_{AH}}{a_{A^-} \cdot a_{H^+}} \quad (5)$$

$$K_{ANa} = \frac{a_{ANa}}{a_{A^-} \cdot a_{Na^+}} \quad (6)$$

$$K_{BOH} = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}} \quad (7)$$

$$K_{BCl} = \frac{a_{BCl}}{a_{B^+} \cdot a_{Cl^-}} \quad (8)$$

where $K_{AH}$, $K_{ANa}$, $K_{BOH}$ and $K_{BCl}$ are association constants; $a_{AH}$, $a_{ANa}$, $a_{A^-}$, $a_{BOH}$, $a_{BCl}$ and $a_{B^+}$ are surface concentrations of corresponding groups on the membrane surface; and $a_{H^+}$, $a_{Na^+}$, $a_{OH^-}$ and $a_{Cl^-}$ are volume concentrations of solution ions. The concentration balances are expressed as follows (Dobrzynska et al. 2006):

$$C_A = a_{A^-} + a_{AH} + a_{ANa} \quad (9)$$

$$C_B = a_{B^+} + a_{BOH} + a_{BCl} \quad (10)$$

where $C_A$ is the total surface concentration of the membrane acidic groups and $C_B$ is the total surface concentration of the membrane basic groups.

Surface charge density of the membrane is given by the equation presented by Dobrzynska et al. (2006):

$$\delta = (a_{B^+} - a_{A^-}) \cdot F \quad (11)$$

where $F = 96,487\,\mathrm{C/mol}$ is the Faraday constant.

Elimination of $a_{AH}$, $a_{ANa}$, $a_{A^-}$, $a_{BOH}$, $a_{BCl}$ and $a_{B^+}$ from the above equations yields the following formula (Dobrzynska et al. 2006):

$$\frac{\delta}{F} = \frac{C_B}{1 + K_{BOH}a_{OH^-} + K_{BCl}a_{Cl^-}} - \frac{C_A}{1 + K_{AH}a_{H^+} + K_{ANa}a_{Na^+}} \quad (12)$$

Determination of the searching parameters requires a simplification of the above equation to a linear form at high $H^+$ ($a_{H+} \to \infty$) and low $H^+$ ($a_{H+} \to 0$) concentrations, which were presented by Dobrzynska et al. (2006). In the former case Eq. 12 was rewritten as a decreasing exponential function of $H^+$ concentration (Eq. 13) and in the latter case, as an increasing exponential function of $H^+$ concentration (Eq. 14) (Dobrzynska et al. 2006).

$$\frac{\delta}{F} = \frac{C_B a_{H^+}}{a_{H^+}(1 + K_{BCl}a_{Cl^-}) + K_{BOH}K_W} - \frac{C_A}{K_{AH}a_{H^+} + K_{ANa}a_{Na^+} + 1} \quad (13)$$

$$\frac{\delta}{F} = \frac{C_B a_{H^+}}{K_{BOH}K_W + a_{H^+}(1 + K_{BCl}a_{Cl^-})} - \frac{C_A}{K_{ANa}a_{Na^+} + 1 + K_{AH}a_{H^+}} \quad (14)$$

The numerator of each term in Eq. 13 was divided by the denominator to yield two terms. These operations resulted in a linear equation in the $a_{H+}$ and $(\delta a_{H^+})/F$ coordinate system, which was correct for high hydrogen ion concentrations ($a_{H+} \to \infty$) (Dobrzynska et al. 2006).

$$\frac{\delta a_{H^+}}{F} = \frac{C_B}{1 + K_{BCl}a_{Cl^-}} a_{H^+} - \left( \frac{C_B K_{BOH} K_W}{(1 + K_{BCl}a_{Cl^-})^2} + \frac{C_A}{K_{AH}} \right) \quad (15)$$

Applying the same procedure to Eq. 14 resulted in a linear equation in the $1/(a_{H^+})$ and $\delta/(Fa_{H^+})$ coordinate system, which was correct for low hydrogen ion concentrations ($a_{H+} \to 0$) (Dobrzynska et al. 2006):

$$\frac{\delta}{Fa_{H^+}} = -\left( \frac{C_A}{1 + K_{ANa}a_{Na^+}} \right) \frac{1}{a_{H^+}} + \left( \frac{C_B}{K_{BOH}K_W} + \frac{C_A K_{AH}}{(1 + K_{ANa}a_{Na^+})^2} \right) \quad (16)$$

The coefficients describing these linear functions may be easily obtained using linear regression and subsequently applied to calculate the parameters. Calculation of $C_A$, $C_B$, $K_{AH}$ and $K_{BOH}$ is possible owing to knowledge of the association constants $K_{ANa}$ and $K_{BCl}$ obtained for phosphatidylcholine liposome membrane (Dobrzynska et al. 2007). Defining the value of these parameters permits calculation of the theoretical cell membrane surface charge from Eq. 12 for comparison to experimental data.

## Materials and Methods

### Contrast Medium SonoVue

SonoVue (Bracco, Milan, Italy) contains the active substance $SF_6$ gas. It is available as a kit containing one vial of gas and powder and one prefilled syringe containing the solvent. SonoVue solution was prepared according to the manufacturer's instructions by mixing the solvent with the powder and gas. The solution contains $SF_6$ gas as microbubbles.

### Blood Cell Preparation

Approval for this study was granted by the Ethics Review Board of Medical University of Bialystok, and informed consent was obtained from all patients at the time of scanning after the nature of the procedure had been fully explained.

The examination was based on 20 selected individuals (12 women and 8 men; mean age 33.2 years, range 18–49) with hepatic hemangioma size up to 30 mm (19 ± 7.5 mm), without any other diseases. The size was limited to 30 mm because hemangiomas of at least 4 cm in diameter are defined as giant hemangiomas and in some cases give rise to symptoms because of Kasabach–Merritt syndrome (tumor leads to decreased platelet counts) (Tani et al. 2010).

There were two control groups. Both consisted of 20 healthy volunteers. The first (14 women and 6 men; mean age 33.3 years, range 18–50) was used to assess the impact of only ultrasound on the red blood cells and platelets, and the second (12 women and 8 men; mean age 33.2 years, range 18–49) was used to compare blood cells.

Blood in the amount of 2 ml was taken immediately before and 10 min after administration of SonoVue into biologically clean glass containers containing anticoagulant (sodium citrate) from a separate puncture antecubital vein without stasis.

All sonographic examinations were performed by a senior radiologist using Toshiba Aplio (Toshiba Medical Systems, Otawara, Japan) equipped with pulse subtraction harmonic imaging software, with the use of a wide-band, multifrequency convex array abdominal transducer (3.5 MHz PVT 375BT). A bolus of 2.4 ml of SonoVue was administered with a 21-gauge peripheral intravenous cannula, followed by a 10-ml saline flush. Dynamic real-time CEUS was then performed using a low mechanical index (MI 0.08) to avoid microbubble disruption. After SonoVue injection, the lesion was scanned continuously for up to 8 min until the enhancement effect began to subside. CEUS recognition of hemangiomas was based on enhancement and washing and washout patterns of the lesion relative to normal hepatic parenchyma during the three vascular contrast phases: arterial, portal-venous and late (Lencioni 2006; Correas et al. 2009). Static image captures as well as dynamic video clips were stored on digital video disc.

#### *Preparation of Erythrocytes from Blood*

Erythrocytes were isolated from 2 ml of anticoagulated whole blood by centrifugation at $900\times g$ for 8 min at room temperature. The supernatant, thrombocyte-rich plasma was removed and saved for subsequent processing, while the erythrocytes were washed three times with isotonic saline (0.9% NaCl) at $3{,}000\times g$ for 15 min. After the final wash, the erythrocyte pellet was resuspended in isotonic saline for electrophoretic measurement.

#### *Preparation of Thrombocytes from Plasma*

The thrombocyte-rich plasma was centrifuged at $4{,}000\times g$ for 8 min. The supernatant plasma was removed and discarded. The thrombocyte pellet was washed three times with isotonic saline by centrifugation at $3{,}000\times g$ for 15 min. After the final wash, thrombocytes were resuspended in isotonic saline for electrophoretic measurement.

All solutions and cleaning procedures were performed with water purified using a Milli-Qll system (18.2; Millipore, Billerica, MA).

### Microelectrophoretic Mobility Measurements

The electrophoretic mobility of erythrocyte or thrombocyte cells in suspension was measured using laser Doppler velocimetry and a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) apparatus. Measurements were carried out as a function of pH. Cell membranes were suspended in NaCl solution and titrated to the desired pH using (HCl + NaCl or NaOH + NaCl). The reported values represent the average of at least six measurements performed at a given pH.

From electrophoretic mobility measurements the surface charge density was determined using the following equation (Alexander and Johnson 1949):

$$\delta = \frac{\eta \cdot u}{d} \tag{17}$$

where $\eta$ is the viscosity of solution, $u$ is electrophoretic mobility and $d$ is the diffuse layer thickness. The diffuse layer thickness was determined from the following formula (Barrow 1996):

$$d = \sqrt{\frac{\varepsilon \cdot \varepsilon_0 \cdot R \cdot T}{2 \cdot F^2 \cdot I}} \tag{18}$$

where $R$ is the gas constant, $T$ is the temperature, $F$ is the Faraday number, $I$ is the ionic strength of 0.9% NaCl and $\varepsilon_o$ is the permeability of the electric medium.

## Results and Discussion

The electrophoretic mobility measurements of the blood cells containing SonoVue formed the basis for investigations of ion/membrane association phenomena. Experiments were performed at several pH values using 0.155 M NaCl as a supporting electrolyte. The electrophoretic mobility values were converted to surface charge density using Eq. 17. The theoretical values of the surface charge density were determined by applying Eq. 12 to the experimental data. The association constants of blood cell surface groups with $Na^+$ and $Cl^-$ [$K_{A_1Na} = 0.230$ ($m^3$/mol), $K_{B_1Cl} = 0.076$ ($m^3$/mol)] have been previously reported (Dobrzynska et al. 2007). The calculated association constants were substituted into Eq. 12 to produce a theoretical curve. The surface charge densities of the control and SonoVue erythrocytes are plotted as a function of pH in Fig. 2. The points denote the experimental values, and the continuous lines represent the theoretical values obtained from Eq. 12. The theoretical and experimental surface charge density values agree between pH 2 and 9 but diverge slightly in the high pH range. Deviations from the theoretical curve may be caused by interactions between the functional groups of the blood cell components.

If we considered an acid solution, a small decrease in positive charge was observed in erythrocytes after SonoVue use in comparison to control erythrocytes. In basic solutions we also observed a small change in the negative charge in erythrocytes after SonoVue in comparison to control erythrocytes and a small shift of the isoelectric point of the membrane to high pH values.

The surface charge densities of the control and SonoVue thrombocytes are plotted as a function of pH in Fig. 3. The points denote experimental values, and the continuous lines represent theoretical values obtained from Eq. 12. The theoretical and experimental surface charge density values agree between pH 2 and 9 but diverge slightly in the high pH range.

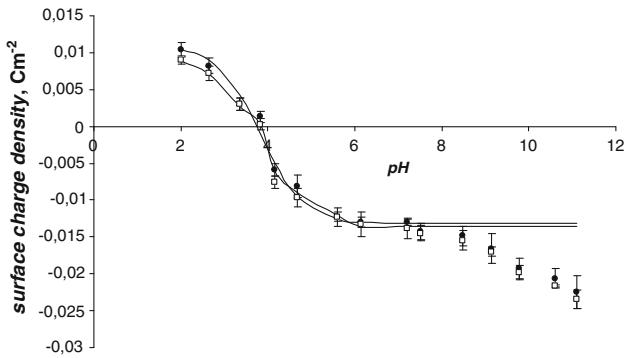


**Fig. 2** pH dependence of surface charge density of erythrocytes: *filled circle* control; *open square* SonoVue effect (experimental values are indicated by *points* and theoretical values, by the *curve*)

If we consider an acid solution, any change in positive charge is observed in thrombocytes with SonoVue in comparison to control thrombocytes. In basic solutions we observed an increase of the negative charge in thrombocytes with SonoVue in comparison to control thrombocytes and a small shift of the isoelectric point of the membrane to low pH values.

The isoelectric point and surface charge density values for human erythrocytes and thrombocytes determined using electrophoresis are presented in Tables 2 and 3, respectively. Data are expressed as mean ± standard deviation. These data were analyzed using standard statistical analysis.

The surface charge density values at low pH values in erythrocytes with SonoVue decreased compared with control groups (14%); however, at high pH values they increased compared with the control group (3%, Table 2). The isoelectric point of SonoVue erythrocyte membranes (3.60) compared to controls (3.80) slightly shifted to low pH values.

The surface charge density values at low pH values as well as at high pH values in thrombocytes with SonoVue decreased compared with controls (15%); however, at high pH values they increased compared to controls (33%), which is presented in Table 3. The isoelectric point of SonoVue thrombocyte membranes (4.40) compared with the control group (4.65) showed a considerable shift to low pH values.

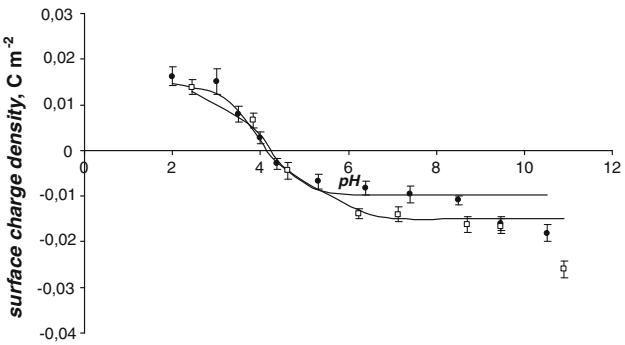


**Fig. 3** pH dependence of surface charge density of thrombocytes: *filled circle* control; *open square* SonoVue effect (experimental values are indicated by *points* and theoretical values, by the *curve*)

**Table 2** Surface charge density and isoelectric point values for human erythrocytes (control and SonoVue effect)

| Groups | Isoelectric point | Surface charge density ($10^{-2}$ C/$m^2$) | |
|---|---|---|---|
| | | Low pH values | High pH values |
| Control | 3.80 | 1.04 ± 0.11 | –1.31 ± 0.08 |
| SonoVue effect | 3.60 | 0.89 ± 0.09 | –1.35 ± 0.09 |

**Table 3** Surface charge density and isoelectric point values for human thrombocytes (control and SonoVue effect)

| Groups | Isoelectric point | Surface charge density ($10^{-2}$ C/m$^2$) | |
|---|---|---|---|
| | | Low pH values | High pH values |
| Control | 4.65 | 1.63 ± 0.10 | –1.09 ± 0.19 |
| SonoVue effect | 4.40 | 1.39 ± 0.13 | –1.62 ± 0.32 |

**Table 4** Acidic and basic functional group concentrations and association constants for erythrocyte functional groups and $H^+$ and $OH^-$ ions

| Groups | Parameters | | | |
|---|---|---|---|---|
| | $C_A$ ($10^{-6}$ mol/m$^2$) | $C_B$ ($10^{-6}$ mol/m$^2$) | $K_{AH}$ ($10^2$ m$^3$/mol) | $K_{BOH}$ ($10^7$ m$^3$/mol) |
| Control | 7.06 ± 0.42 | 1.54 ± 0.47 | 3.39 ± 1.12 | 3.65 ± 0.84 |
| SonoVue effect | 6.82 ± 0.10 | 1.46 ± 0.08 | 3.95 ± 0.73 | 4.07 ± 0.60 |

Acidic ($C_A$) and basic ($C_B$) functional group concentrations and their average association constants with hydrogen ($K_{AH}$) and hydroxyl ($K_{BOH}$) ions of erythrocyte and thrombocyte surface groups were determined as described previously by Dobrzynska et al. (2006). These values are presented in Tables 4 and 5, respectively. Data are expressed as mean ± standard deviation. These data were analyzed using standard statistical analysis.

The concentration of acidic (4%) functional groups in erythrocytes with SonoVue decreased compared to controls, and the basic (5%) groups in erythrocytes also decreased compared to controls (Table 4). The $K_{AH}$, the association constant of the acidic groups of the erythrocyte membrane, increased (15%) with SonoVue compared to controls; and the $K_{BOH}$ value was only 10% higher compared with the control group.

The experimental results indicate that SonoVue caused a small decrease in negative and positive charge numbers at the erythrocyte surface. This corresponded to a slightly decreased surface concentration of acidic groups ($C_A$) and basic groups ($C_B$). Changes in functional group composition on the membrane surface are due to the appearance of new functional groups and/or to the disappearance of existing ones as the result of a reaction of SonoVue with erythrocyte components. Variations in the number and kind of functional groups bring about variations in $C_A$ and $C_B$ and, in turn, in their association constant values. SonoVue increases the association constants of negatively charged ($K_{AH}$) and positively charged ($K_{BOH}$) groups.

In thrombocytes with SonoVue we observed an increase of the acidic group's concentration, $C_A$ (3%), and an increase of the basic functional concentration, $C_B$ (11%), compared with the control group (Table 5). SonoVue induces an increase in $K_{AH}$ (6%) and $K_{BOH}$ values (11%) in the thrombocytes compared with the control groups.

**Table 5** Acidic and basic functional group concentrations and association constants for thrombocyte functional groups and $H^+$ and $OH^-$ ions

| Groups | Parameters | | | |
|---|---|---|---|---|
| | $C_A$ ($10^{-6}$ mol/m$^2$) | $C_B$ ($10^{-6}$ mol/m$^2$) | $K_{AH}$ ($10^2$ m$^3$/mol) | $K_{BOH}$ ($10^7$ m$^3$/mol) |
| Control | 3.67 ± 0.79 | 1.17 ± 0.21 | 2.81 ± 1.70 | 2.04 ± 0.59 |
| SonoVue effect | 3.98 ± 0.08 | 1.31 ± 0.07 | 2.98 ± 0.43 | 2.29 ± 0.56 |

The experimental results indicate that SonoVue causes a small increase in negative and positive charge numbers at the thrombocyte surface. This corresponded to a slightly increased surface concentration of acidic groups ($C_A$) and basic groups ($C_B$). Changes in functional group composition on the membrane surface are due to the appearance of new functional groups and/or to the disappearance of existing ones as the result of a reaction of SonoVue with thrombocyte components. Variations in the number and kind of functional groups bring about variations in $C_A$ and $C_B$ and, in turn, in their association constant values. SonoVue increases the association constants of negatively charged ($K_{AH}$) and positively charged ($K_{BOH}$) groups.

The current results demonstrate that SonoVue causes a small change in the levels of all membrane components (phospholipids and integral membrane proteins) in erythrocytes and thrombocytes (Tables 4, 5). In addition, our results have shown that the electrical properties of erythrocyte and thrombocyte membranes are not affected by SonoVue. An essential property of the electric double layer is its electrokinetic potential, the potential difference between the membrane and its environment and the surface charge. Changes in cell membrane charge are connected with changes in membrane composition (Szachowicz-Petelska et al. 2008; Dobrzynska et al. 2008). An increase in the amount of specific phospholipids results in the appearance of additional functional groups, both positively and negatively charged, at the membrane surface (Dobrzynska et al. 2010).

In our results we did not observe any changes in surface charge density of erythrocytes after addition of SonoVue, but in thrombocytes we observed changes of surface charge density at high pH values. The changes of negative surface charge density of thrombocytes probably are connected with adsorption of the remaining phospholipids from the SonoVue contrast agent. In our opinion the problem could be studied by means of model experiments (e.g., bilayer lipid membranes) as well as in vitro examination of the SonoVue effect in blood cells.

Our CEUS results are in agreement with the literature that typical small hemangiomas show peripheral nodular enhancement in the arterial phase and from portal to late phase nodules have progressive centripetal enhancement (Dietrich et al. 2007; von Herbay et al. 2010; Zviniene et al. 2010). There have been no adverse events or technical difficulties encountered in this patient population, and many authors have also reported no adverse effects of SonoVue (Bokor et al. 2001; Piscaglia and Bolondi 2006; Gaibazzi et al. 2009; Tranquart et al. 2009; Beaton et al. 2010).

The normal blood pH is tightly regulated between 7.35 and 7.45. Virtually all degenerative diseases, including cancer, heart disease, osteoporosis, arthritis, kidney and gall stones and tooth decay, are associated with excess acidity in the body. We observed no large changes of the surface charge density of blood cells in a wide pH range. We see no reason for limiting the use of SonoVue contrast medium in clinical diagnostics.

## Conclusions

The interaction between blood cell membranes and solution ions has been well characterized. The dependence of the surface charge density of the erythrocytes and thrombocytes as a SonoVue effect on pH was described using a mathematical model derived from experimental electrophoretic data. The theoretical estimates of electric charge enabled the determination of association constants for the functional groups of erythrocytes or thrombocytes and electrolyte ions. The agreement of the experimental and theoretical charge variation curves of the erythrocytes as well as thrombocytes is good between pH 2 and 9. The deviation observed at a higher pH may be caused by disregarding interactions between the functional groups of blood cells.

No adverse events or technical difficulties were encountered in the patient population presented in this study. In our opinion, SonoVue is a safe contrast medium.



## References

Alexander AE, Johnson P (1949) Colloid science. Clarendon Press, Oxford

Barrow GM (1996) Physical chemistry. McGraw-Hill, New York

Bartolotta TV, Midiri M, Galia M, Rollandi GA, Cademartiri F, Lagalla R, Cardinale AE (2007) Characterization of benign hepatic tumors arising in fatty liver with SonoVue and pulse inversion US. Abdom Imaging 32:84–91

Beaton C, Cochlin D, Kumar N (2010) Contrast enhanced ultrasound should be the initial radiological investigation to characterise focal liver lesions. Eur J Surg Oncol 36:43–46

Bokor D, Chambers JB, Rees PJ, Rees PJ, Mant TGK, Luzzani F, Spinazzi A (2001) Clinical safety of SonoVue, a new contrast agent for ultrasound imaging, in healthy volunteers and in patients with chronic obstructive pulmonary disease. Invest Radiol 36:104–109

Broome DR (2008) Nephrogenic systemic fibrosis associated with gadolinium based contrast agents: a summary of the medical literature reporting. Eur J Radiol 66:230–234

Carr ME Jr, Carr SL, Merten SR (1995) Effects of ionic and nonionic contrast media on clot structure, platelet function and thrombolysis mediated by tissue plasminogen activator in plasma clots. Haemostasis 25:172–181

Correas JM, Tranquart F, Claudon M (2009) Guidelines for contrast enhanced ultrasound (CEUS)—update 2008. J Radiol 90:123–138

Dietrich CF, Mertens JC, Braden B, Schuessler G, Ott M, Ignee A (2007) Contrast-enhanced ultrasound of histologically proven liver hemangiomas. Hepatology 45:1139–1145

Dijkmans PA, Juffermans LJ, van Dijk J, Musters RJP, Spreeuwenberg MD, Kamp O (2009) Safety and feasibility of real time adenosine myocardial contrast echocardiography with emphasis on induction of arrhythmias: a study in healthy volunteers and patients with stable coronary artery disease. Echocardiography 26:807–814

Dobrzynska I, Skrzydlewska E, Figaszewski Z (2006) Parameters characterizing acid–base equilibria between cell membrane and solution and their application to monitoring the effect of various factors on the membrane. Bioelectrochemistry 69:142–147

Dobrzynska I, Kotyńska J, Figaszewski Z (2007) Changes in electrical charge of phosphatidylcholine and phosphatidylserine liposomal membranes caused by adsorption of monovalent ions. Chem Anal 52:931–944

Dobrzynska I, Szachowicz-Petelska B, Skrzydlewska E, Figaszewski ZA (2008) Effects of green tea on physico-chemical properties of liver cell membrane of different age rats intoxicated with ethanol. Pol J Environ Stud 17:327–333

Dobrzynska I, Szachowicz-Petelska B, Skrzydlewska E, Figaszewski ZA (2010) Effect of L-carnitine on liver cell membranes in ethanol-intoxicated rats. Chem Biol Interact 188:44–51

Feldkamp T, Kribben A (2008) Contrast media induced nephropathy: definition, incidence, outcome, pathophysiology, risk factors and prevention. Minerva Med 99:177–196

Gaibazzi N, Squeri A, Ardissino D, Reverberi C (2009) Safety of contrast flash-replenishment stress echocardiography in 500 patients with a chest pain episode of undetermined origin within the last 5 days. Eur J Echocardiogr 10:726–732

Greis C (2004) Technology overview: SonoVue (Bracco, Milan). Eur Radiol Suppl 14:P11–P15

Herman P, Costa ML, Machado MA et al (2005) Management of hepatic hemangiomas: a 14-year experience. J Gastrointest Surg 9:853–859

Kaps M, Seidel G, Bokor D, Modrau B, Algermissern C (1999) Safety and ultrasound-enhancing potentials of a new sulfur hexafluoride-containing agent in the cerebral circulation. J Neuroimaging 9:150–154

Lencioni R (2006) European federation of societies for ultrasound in medicine and biology (EFSUMB). Impact of European federation of societies for ultrasound in medicine and biology (EFSUMB) guidelines on the use of contrast agents in liver ultrasound. Eur Radiol 16:1610–1613

Li R, Guo Y, Hua X, He Y, Ding J, Guo A, Luo M (2007) Characterization of focal liver lesions: comparison of pulse-inversion harmonic contrast-enhanced sonography with contrast-enhanced CT. J Clin Ultrasound 35:109–117

Nalecz MJ, Wojtczak L (1982) Surface charge of biological membranes and its regulatory functions. Post Biochem 28:191–225

Numata K, Isozaki T, Morimoto M, Sugimori K, Kunisaki R, Morizane T, Tanaka K (2006) Prospective study of differential diagnosis of hepatic tumors by pattern-based classification of contrast-enhanced sonography. World J Gastroenterol 12:6290–6298

Piscaglia F, Bolondi L (2006) Italian society for ultrasound in medicine and biology (SIUMB) study group on ultrasound contrast agents. The safety of Sonovue in abdominal applications: retrospective analysis of 23188 investigations. Ultrasound Med Biol 32:1369–1375

Schneider M (1999a) Characteristics of SonoVue™. Echocardiography 16:743–746

Schneider M (1999b) SonoVue, a new ultrasound contrast agent. Eur Radiol 9:S347–S348

Schneider M, Arditi M, Barrau MB, Brochot J, Broillet A, Ventrone R, Feng Y (1995) BR1: a new ultrasonographic contrast agent based on sulfur hexafluoride-filled microbubbles. Invest Radiol 30:451–457

Soyer P, Dufresne AC, Somveille E, Lenormand S, Scherrer A, Rymer R (1998) Differentiation between hepatic cavernous hemangioma and malignant tumor with T2-weighted MRI: comparison of fast spin-echo and breathhold fast spin-echo pulse sequences. Clin Imaging 22:200–210

Szachowicz-Petelska B, Dobrzynska I, Skrzydlewska E, Figaszewski ZA (2008) Changes in phospholipids composition studied by HPLC and electric properties of liver cell membrane of ethanol-poisoned rats. Toxicol Mech Methods 18:525–530

Szachowicz-Petelska B, Dobrzynska I, Sulkowski S, Figaszewski ZA (2010) Characterization of the cell membrane during cancer transformation. J Environ Biol 31:845–850

Tani A, Yoshida H, Mamada Y, Taniai N, Kawano Y, Uchida E (2010) Extrahepatic portal venous obstruction due to a giant hepatic hemangioma associated with Kasabach–Merritt syndrome. J Nippon Med Sch 77:269–272

Tranquart F, Correas JM, Ladam Marcus V, Manzoni P, Vilgrain V, Aube C, Elmaleh A, Chami L, Claudon M, Cuilleron M, Diris B, Garibaldi F, Lucidarme O, Marion D, Beziat C, Rode A, Tasu JP, Trillaud H, Bleuzen A, Le Gouge A, Giraudeau B, Rusch E (2009) Real-time contrast-enhanced ultrasound in the evaluation of focal liver lesions: diagnostic efficacy and economical issues from a French multicentric study. J Radiol 90:109–122

Van Linden A, Kempfert J, Rastan AJ, Holzhey D, Blumenstein J, Schuler G, Mohr FW, Walther T (2011) Risk of acute kidney injury after minimally invasive transapical aortic valve implantation in 270 patients. Eur J Cardiothorac Surg 39:835–843

von Herbay A, Westendorff J, Gregor M (2010) Contrast-enhanced ultrasound with SonoVue: differentiation between benign and malignant focal liver lesions in 317 patients. J Clin Ultrasound 38:1–9

Zviniene K, Zaboriene I, Basevicius A, Jurkiene N, Barauskas G, Pundzius J (2010) Comparative diagnostic value of contrast-enhanced ultrasonography, computed tomography, and magnetic resonance imaging in diagnosis of hepatic hemangiomas. Medicina (Kaunas) 46:329–335