Biochimica et Biophysica Acta, 1153 (1993) 243–248

BBAMEM 76153

Electrical impedance and erythrocyte sedimentation rate (ESR) of blood

Tian-Xian Zhao [a,*] and Dieter Lockner [b]

[a] *Department of Medical Engineering, Karolinska Institute, F60 Novum, S-141 86 Huddinge (Sweden) and* [b] *Department of Medicine, Karolinska Institute, Huddinge Hospital, S-141 86 Huddinge, Sweden*

(Received 10 June 1993)

Key words: Blood; Membrane; Electric conductivity; Erythrocyte sedimentation rate; Sedimentation

The electrical impedance of blood is primarily determined by plasma resistance R_p, cell interior resistance R_i, and cell membrane capacitance C_m. These impedance parameters were measured for 62 samples with various erythrocyte sedimentation rates (ESR = 1–150 mm/h). A formula for estimating ESR by R_p and C_m was obtained by linear regression as: $\ln(ESR) = 10.16 - 0.016 \cdot f_0$, ($r = 0.974$, $P < 0.001$), where $f_0 = 10^9/(2\pi \cdot R_{p100} \cdot C_{m100})$, defined as the effective characteristic frequency in kHz, $R_{p100} = R_p/h$ in Ωcm, $C_{m100} = C_m/h$ in pF/cm, h is the haematocrit in decimal. The 95% confidence intervals for the coefficients in the above equation were 9.73 to 10.59 and −0.017 to −0.015. The origin of the association was found reasonable since factors increasing the ESR, i.e., the concentration of some plasma proteins and the size of the blood cells, also elevate the capacitance. The results imply that the impedance measurement might be an alternative method for fast determination of the ESR.

Introduction

The erythrocyte sedimentation rate (ESR), mainly reflecting the acute phase inflammatory response, is still a useful method in the clinic although it lacks specifity [1–3]. It is useful for determining prognosis of patients with Hodgkin's disease [4] or prostatic cancer [5] and to monitor disease activity, as in rheumatoid arthritis [6], temporal arteritis [7], congestive heart failure [8], etc. It is also useful for the diagnosis and monitoring of some infection and cancers [3].

The clinically commonly used method for measuring ESR is based on that of Westergren, recommended by The International Committee for Standardisation in Haematology [9]. Acute phase protein (APP) methods, such as determination of C-reactive protein (CRP), are increasingly being recommended [10]. The Westergren method is simple and relatively cheap, but takes some time and requires a relatively large volume of blood. APP methods are quick, specific and sensitive but somewhat expensive and need sophisticated equipment [10].

The electrical impedance of blood is determined primarily by the plasma resistance (R_p), cell interior fluid resistance (R_i) and cell membrane capacitance (C_m). The membrane capacitance (C_m) from patients with high ESR has been found significantly higher than that of blood with low ESR [11,12]. It seemed that C_m and ESR were related to each other. Hence the aim of this study is to explore the possible relationships between ESR and the electrical impedance parameters, which may be an alternative method for determining ESR indirectly.

Materials and Methods

Theory and measuring system

The theory and the measuring system have been described in detail in a previous paper [12]. In brief, the electrical impedance of blood can be approximately simulated by a three-element circuit in which R_p is in parallel with R_i and C_m which are connected in series. For such a circuit, if three impedance amplitudes $Z(\omega_0)$, $Z(\omega_1)$, $Z(\omega_2)$ at different frequencies ω_0, ω_1, ω_2 are measured, the values of the three elements can be obtained:

$$R_p = Z(\omega_0)\sqrt{K_0}$$

$$R_i = \frac{R_p}{\sqrt{K_0 \dfrac{K_1 - K_0 - F_1(1-K_0)}{K_1 - K_0 - F_1 K_1 (1-K_0)}} - 1}$$

$$C_m = \frac{1}{\omega_0}\sqrt{\frac{1-K_0}{K_0 R_i^2 - (R_p + R_i)^2}}$$

* Corresponding author. Fax: +46 8 7795550.

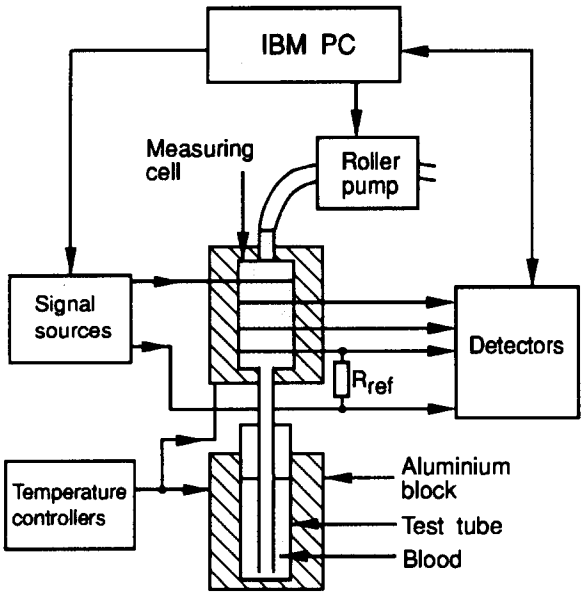

Fig. 1. Block diagram of the measuring system.

where

$$F_1 = \left(\frac{\omega_1}{\omega_0}\right)^2, \quad F_2 = \left(\frac{\omega_2}{\omega_0}\right)^2,$$

$$K_1 = \left(\frac{Z(\omega_1)}{Z(\omega_0)}\right)^2, \quad K_2 = \left(\frac{Z(\omega_2)}{Z(\omega_0)}\right)^2,$$

$$K_0 = \frac{F_1K_2(1-K_1)(1-F_2) - F_2K_1(1-K_2)(1-F_1)}{F_1(1-K_1)(1-K_2F_2) - F_2(1-K_2)(1-K_1F_1)}.$$

The diagram of the measuring system is shown in Fig. 1. Blood is sucked by the roller pump from the test tube into the specially designed flow-through measuring cell which utilises tetrapolar technique to reduce the errors caused by polarisation and was carefully calibrated using saline solutions. Three constant currents with frequencies 100 kHz, 800 kHz and 1.2 MHz are consecutively exerted to the blood sample and the reference resistor, R_{ref}. The ratio of the voltages detected from blood and R_{ref} is then a measure of blood impedance. The temperatures of the test tube and the measuring cell are adjusted to 37 ± 0.1°C by two temperature controllers. The maximum measuring errors are estimated to 0.5% for R_p, 5% for C_m, and 10% for R_i in the haematocrit range from 20% to 60%.

Samples

To explore the possible relations between the *ESR* and the electrical impedance parameters, measurements were made on 62 samples from patients with various *ESR*s (1–150 mm/h) and haematocrits (*Hct* = 22–43%). Each sample was drawn into a 6.25-ml vacuum plastic tube with 1.25 ml buffered sodium citrate as anticoagulant. First the *ESR* of each sample was determined by the Westergren method following the standard procedures used in the clinic. Then the impedance measurements were made according to the procedures described later within 6 h after the blood had been drawn from the subjects.

For comparing high and normal *ESR* samples, the impedances of 20 normal samples from blood donors were measured. The haematocrits of the samples were adjusted to the same range (22–43%) of the patient samples by reconstituting the proportions of plasma and blood cells after centrifugation at about $1000 \times g$ for 10 min. The results were compared with those of 34 high *ESR* samples (> 30 mm/h) extracted from the patient group.

In order to study the effects of plasma on the capacitance and *ESR*, measurements were also made on washed samples from normal subjects and high *ESR* patients. Washing was made by centrifuging and then mixing the packed blood cells with about twice as much phosphate-buffered saline (PBS; pH 7.4) and centrifuging again. The washed cells were finally mixed with a certain volume of PBS to obtain a sample with a desired haematocrit value of 45–57%. Twenty-two subjects were used for the normal group, and 18 for the high *ESR* group (*ESR* = 44–108 mm/h).

Ten normal samples were measured to study the influence of fibrinogen on the electrical impedance. From each subject, 20 ml of blood was drawn into two tubes (Becton Dickinson Vacutainer, 10 ml each), one plain (with no anticoagulant) and the other with dried sodium heparin as anticoagulant. The serum, obtained from the plain tube after centrifugation, was put into an unused tube with sodium heparin to eliminate the possible influence of the anticoagulant. The cells and the original plasma were separated by centrifuging the other tube of anticoagulated blood. After being washed twice with PBS, the packed cells were divided into two parts, which were then mixed with plasma and serum, respectively. The haematocrits of the samples were controlled to within 46–52%.

Procedures of measurement

Before measurement, the samples, contained in 5-ml plastic tubes, were placed inside an incubator with a temperature controlled within ±1.5°C, and agitated for at least 20 min by a roller mixer. The haematocrit of each sample was determined by centrifuging two capillary tubes with the blood at 12000 rpm for 5 min in a micro-centrifuge. Four measurements were then made on each 2-ml blood sample. For each measurement, 0.5 ml blood was sucked into the measuring cell by a roller pump and the measurement started after a delay of 45 s to allow the blood to reach thermal equilibrium and the blood cells to settle. The sample tube was removed and agitated between each of the four measurements. The first two measurements were

ignored, however, since they were merely used for checking that the sample had reached a constant temperature and that the rinsing water had been sufficiently removed from the cell. The means of the last two measurements were used as the results for the sample.

To study the possible influence of washing on the surfaces of erythrocytes, six samples were examined by a scanning electron microscope (SEM). Each sample was divided into two portions, one was washed twice with PBS and the other was not washed. The cells were fixed with 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), washed with 0.15 M cacodylate buffer and dehydrated in a series of ethanol solutions of increasing concentrations. Finally the cells were dehydrated in acetone. An SEM micrograph was then taken for each sample with a voltage of 10 kV and a magnification of 10000.

Normalisation for haematocrit

For comparison between different samples, it is necessary to eliminate the influence of *Hct* with which R_p, C_m and R_i are almost linearly related. When h was introduced to represent haematocrit in decimal, i.e., $h = Hct/100$, R_p, C_m and R_i could be normalised to 100% haematocrit by calculating the ratios $R_{p100} = R_p/h$, $C_{m100} = C_m/h$ and the product $R_{i100} = R_i \cdot h$, respectively.

Results

The natural logarithm of *ESR* was found to be correlated with R_{p100}, R_{i100} and C_{m100}, as shown in Figs. 2, 3 and 4. R_{p100} and C_{m100} were directly and R_{i100} inversely proportional to $\ln(ESR)$. By performing least-squares regression, linear equations were obtained as:

$R_{p100} = 8.7 \cdot \ln(ESR) + 328$ ($r = 0.783$, $P < 0.001$)

$R_{i100} = -6.6 \cdot \ln(ESR) + 176$ ($r = 0.725$, $P < 0.001$)

$C_{m100} = 102.0 \cdot \ln(ESR) + 728$ ($r = 0.935$, $P < 0.001$)

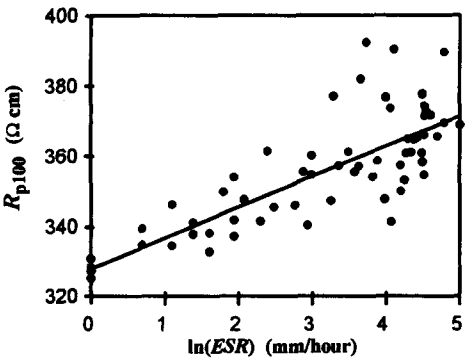

Fig. 2. Scatter plot of plasma resistance versus the natural logarithm of sedimentation rate. The regression line is: $R_{p100} = 8.7 \cdot \ln(ESR) + 328$, $r = 0.78$, $P < 0.001$.

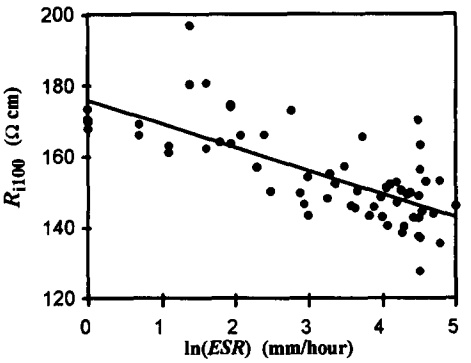

Fig. 3. Scatter plot of interior resistance versus the natural logarithm of sedimentation rate. The regression line is: $R_{i100} = -6.6 \cdot \ln(ESR) + 176$, $r = 0.73$, $P < 0.001$.

Using multiple non-linear regression, the following relationship was obtained:

$$\ln(ESR) = 0.021 R_{p100} - 9 \cdot 10^{-6} \cdot C_{m100}^2 + 0.026 C_{m100} - 21.44$$

($r = 0.974$, and $P < 0.001$ for all four coefficients)

The contribution of R_{i100} was not significant. The 95% confidence intervals for the coefficients, from left to right in the above equation, were 0.0134 to 0.0287, 0.0023 to 0.0037, 0.0176 to 0.0350 and −25.433 to −17.437, respectively. The mean relative error of the predicted *ESR* according to the above formula was 9% with a standard deviation of 35%.

A simpler equation was obtained by performing linear regression on the *ESR* against the reciprocal of the product of R_{p100} and C_{m100}:

$$\ln(ESR) = 10.16 - 0.016 \cdot f_0$$

($r = 0.974$, and $P < 0.001$ for both coefficients)

where $f_0 = 10^9/(2\pi \cdot R_{p100} \cdot C_{m100})$, defined as the effective characteristic frequency in kHz. The 95% confidence intervals for the coefficients in the above equation were 9.73 to 10.59 and −0.017 to −0.015, respec-

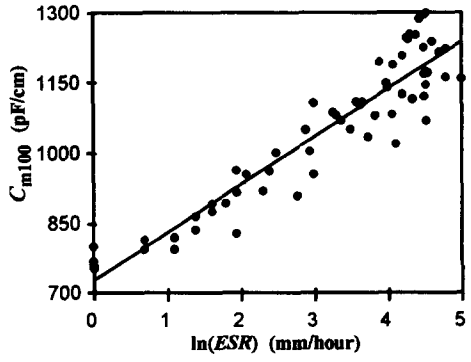

Fig. 4. Scatter plot of membrane capacitance versus the natural logarithm of sedimentation rate. The regression line is: $C_{m100} = 102 \cdot \ln(ESR) + 728$, $r = 0.94$, $P < 0.001$.

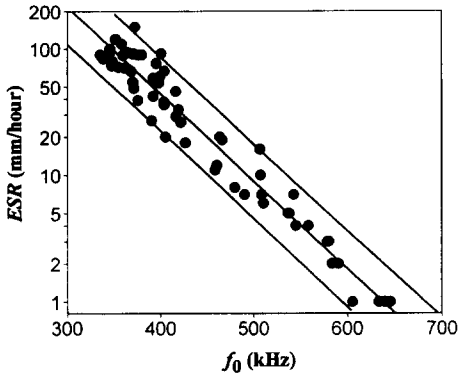

Fig. 5. Scatter plot of *ESR* versus the effective characteristic frequency, $f_0 = 10^9/(2\pi \cdot R_{p100} \cdot C_{m100})$, in kHz. The middle line is the fitted: $\ln(ESR) = 10.16 - 0.016 \cdot f_0$, $R = 0.97$, $P < 0.001$; the outer two are the 95% confidence limits for predicting individual observations.

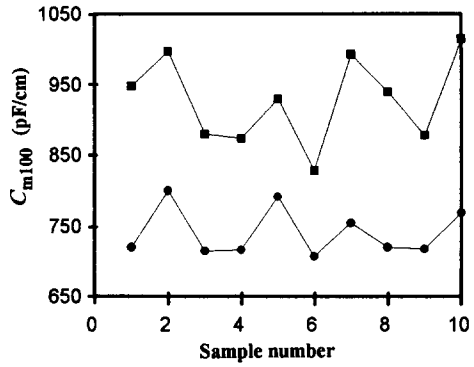

Fig. 6. Comparison of capacitance between samples with plasma (■) and serum (●) as suspending solutions.

tively. A scatter plot with the fitted curve and the 95% confidence intervals for predicting individual observations is shown in Fig. 5. The mean relative error and standard deviation of the predicted *ESR* were 2.6% and 33%, respectively.

The mean C_{m100} of the high *ESR* group, 1167 pF/cm (S.D. = 76 pF/cm, $n = 34$) was significantly higher ($P < 0.001$) than that for the normal samples, 834 pF/cm (S.D. = 53 pF/cm, $n = 20$), Table I. After washing, however, no significant difference was found between the two groups, $P > 0.3$. Between original and washed groups, the capacitance was significantly decreased after washing for both normal and high *ESR* groups ($P < 0.001$).

By performing a paired *t*-test, the capacitance of the washed blood cells resuspended with serum was significantly lower (20%) than that of the plasma samples ($P < 0.001$) (Fig. 6).

Two SEM micrographs for the original and washed specimens of the same sample are shown in Fig. 7. It is obvious that some particles were adsorbed on the surfaces of the original cells, while those of the washed cells were clean.

Discussion

The *ESR* was significantly correlated with both R_p and C_m, but the association of *ESR* was much closer with C_m than with R_p. Hence the following discussion is concentrated on understanding the inherent relation between *ESR* and C_m by taking into account the factors influencing both parameters.

Factors influencing ESR

The procedures of the erythrocyte sedimentation is divided into three phases: aggregation, precipitation and packing. The forming speed and the final size of aggregates in the aggregation phase are critical for the outcome of the *ESR*. There are two main factors which

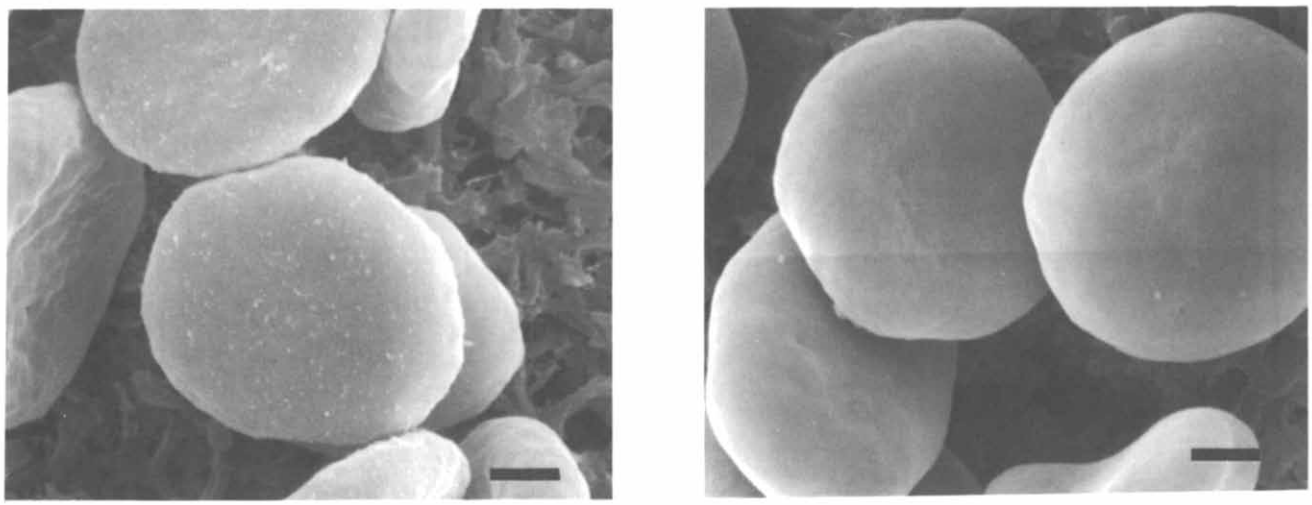

Fig. 7. SEM micrographs for erythrocytes before (left) and after (right) washing with PBS. Bar = 1 μm.

TABLE I

Comparison of C_{m100} (pF / cm) between normal and high ESR samples before and after washing

	Normal (mean ± S.D.)	High *ESR* (mean ± S.D.)	*P* (normal vs. high *ESR*)
Original	834 ± 53 ($n = 20$)	1 167 ± 76 ($n = 34$)	< 0.001
Washed	582 ± 17 ($n = 22$)	588 ± 22 ($n = 18$)	> 0.3
P (original vs. washed)	< 0.001	< 0.001	

may influence the aggregation process, i.e., certain high molecular weight components of the plasma and the structure of the blood cells [3,13–15]. Normally, the cells have negative charges and repel each other, while many plasma proteins are positively charged and neutralise the surface charges of erythrocytes, reducing the repulsive forces and promoting aggregation. Hence a high concentration of certain plasma proteins causes a high *ESR*. The relative contribution of the plasma proteins to aggregation, on a scale of 10, is fibrinogen, 10; β-globulin, 5; α-globulins, 2; γ-globulins, 2; and albumin, 1 [3].

On the other hand, *ESR* is directly proportional to the mass of the erythrocyte but inversely proportional to the area of its surface. Large cells have a smaller surface-to-volume ratio and therefore less charge in relation to their mass than microcytes. Thus macrocytes sediment more rapidly than normal cells, and microcytes do so more slowly [3].

Factors influencing the capacitance

Blood cells

The electrical capacitance of blood is determined mainly by the properties of the cell membranes. A single cell may be regarded as a parallel plate capacitor since the thickness of the membrane is small compared to the diameter of a cell [16]. The capacitance of such a plate capacitor is expressed as: $C = \epsilon s / d$, where ϵ is the dielectric constant of the membrane; s, the area of the plate; and d, the thickness of the membrane. The formula shows that the capacitance of an individual cell is directly proportional to the surface area of the cell membrane. Big cells have a larger area and hence a larger capacitance than small ones.

For a suspension of such cells, the overall capacitance normalised to 100% haematocrit can be expressed as $C_{100} = C_0 \alpha q$, where C_0 is the capacitance per cm^2 of a cell surface and depends on the chemical structure and the thickness of the membrane; α is the form factor of a cell; and $2q$ is the major axis of a cell [17]. For cells with the same shape (equal α) and the same structure of the membrane (equal C_0), a suspension with big cells has a larger q value and hence a larger overall capacitance than that with small cells.

Plasma

Apart from the static capacity of the thin poorly conducting membranes, the blood capacitance can be influenced by the polarisation at the interface of the cell and the suspending medium [17]. As shown in Table I, the capacitance decreased after washing for both normal and high *ESR* groups, indicating that the components of plasma elevate the capacitance. The difference of the capacitance between the high *ESR* and normal groups disappeared after washing, implying that the difference in both *ESR* and capacitance between the two groups were caused by plasma components, instead of changes in the structure of the blood cells. The influence of plasma proteins is further confirmed by the results in Fig. 6. The measurement on the pure plasma showed no contribution to C_m at the frequencies used in this study. When mixed with erythrocytes, however, fibrinogen could elevate the capacitance by 20%.

The interfacial polarisation, caused when two different phases of matters contact to each other, can largely influence the dielectric characteristics of the system [18,19]. The electric properties of the blood cells and the suspending medium like plasma are dramatically different. Around an erythrocyte, there exists an ionic atmosphere: a bound layer and a diffuse layer. The bound layer consists of the inner Helmholtz plane (IHP) and the outer Helmholtz plane (OHP). The IHP is the locus of partially desolvated (negative) ions, adsorbed from the solution by covalent binding, dispersion forces, etc. The OHP is the locus of solvated and positively charged counter-ions, attracted to the negatively charged surface by Coulombic forces. Beyond this bound layer is the diffuse region of the double layer, containing loosely bound and highly solvated counter-ions.

Such an ion atmosphere will influence the dielectric property mainly in two ways. First the accumulated surface charges give rise to the effective dipole, and hence to the observed dielectric constant and capacitance. Secondly, the bound and the diffuse counter-ions can move tangentially along the surfaces of the cells when an external electric field is exerted. Such a counter-ion polarisation or surface conductance will also increase the effective dielectric constant and capacitance [18,19].

The plasma components could be adsorbed on the surface of the erythrocyte membrane. This was confirmed by the electron micrographs which showed some particles adsorbed on the surfaces of the original cells, but not on the washed ones. As a needle-shaped and positively charged macromolecule, fibrinogen would be attracted to the counter-ion region by the negative surface charges, enhancing the counter-ion polarisation and increasing the effective dielectric constant and capacitance of the suspension.

In conclusion, the factors increasing *ESR* can also elevate capacitance, i.e., *ESR* and C_m are mutually related. Hence, C_m might be used to measure *ESR* indirectly. The accuracy for estimating *ESR* was somewhat better by including R_p into the regression. The origin of the association between R_p and *ESR* might be that the conductivity of fibrinogen is lower than that of serum. Hence, the resistivity of the plasma will be elevated slightly with an increased concentration of fibrinogen.

The accuracy of the method is at present not satisfactory in the high *ESR* region. For example, if the estimated value is 50 mm/h, the 95% confidence limits are about 25 to 100 mm/h. For low *ESR*, however, it is reasonably accurate. If the estimated value is 5 mm/h, the 95% confidence limits are 2.5 to 10 mm/h. Hence the method might be used as means for screening purposes.

Schwan [20] and Nelson and Wilkinson [21] reported an electric method for measuring *ESR*. The resistivities of plasma and blood cells are dramatically different at low frequency. While the blood cells are sedimenting along a vertically placed measuring cell, the resistance is changing between the electrodes on two opposite sides of the tube. It can be used to monitor and record the whole process of sedimentation. But similar to the Westergren method, it is an 'effect measuring' method, and has similar disadvantages. For instance, the results are influenced by the haematocrit to some extent, and they can only be obtained after a delay needed to allow the blood cells to sediment.

The potential advantages of the electrical impedance method presented in this study for estimating the *ESR* are that it is very quick, possible for automation, requires only 1 ml of blood, and can be easily combined with other haematological instruments.

Acknowledgements

We appreciate the support of professor Håkan Elmqvist, head of the Department of Medical Engineering. We also like to thank Kjell Hultenby, in The Clinical Research Centre in Huddinge Hospital, for making the SEM micrographs. The project was supported by a grant from the Swedish National Board for Industrial and Technical Development (No. 623-90-1169).

References

1 Dinant, G.J., Knottnerus, J.A., Van Aubel, P.G.J. and Van Wersch, J.W.J. (1989) Scand. J. Prim. Health Care 7, 231–235.
2 Grønlie, M. and Hjortdahl, P. (1991) Scand. J. Prim. Health Care 9, 97–102.
3 Sox, H.C. and Liang, M.H. (1986) Ann. Intern. Med. 104, 515–523.
4 Haybittle, J.L., Hayhoe, F.G.J., Easterling, M.J., Jelliffe, A.M., Bennett, M.H., Vaughan Hudson, G., Vaughan Hudson, B. and MacLennan, K.A. (1985) Lancet i, 967–972.
5 Imai, K., Suzuki, T., Kobayashi, M., Yamanaka, H., Tomaru, Y. and Sato, J. (1990) Jpn. J. Cancer Res. 81, 971–974.
6 Bull, B.S., Westengard, J.C., Farr, M., Bacon, P.A., Myer, P.J. and Stuart, J. (1989) Lancet ii, 965–967.
7 Wise, C.M., Agudelo, C.A., Chmelewski, W.L. and McKnight, K.M. (1991) Arthritis Rheumatism 34, 1571–1574.
8 Haber, H.L., Leavy, J.A., Kessler, P.D., Kukin, M.L., Gottlieb, S.S. and Packer, M. (1991) New Engl. J. Med. 324, 353–358.
9 International Committee for Standardization in Haematology (1977) Am. J. Clin. Pathol. 68, 505–507.
10 International Committee for Standardization in Haematology (1988) J. Clin. Pathol. 41, 1203–1212.
11 Zhao, T.X. (1992) Proc. 8th Int. Conf. Bio-imped., July 28–31, Kuopio, Finland, pp. 185–187.
12 Zhao, T.X., Jacobson, B. and Ribbe, T. (1993) Physiol. Meas. 14, 145–156.
13 Märki, H.H. (1984) in Geigy Scientific Tables, 8th Edn. (Lentner, C., ed.), pp. 162–164, CIBA-GEIGY, Basle, Switzerland.
14 Phear, D. (1957) J. Clin. Pathol. 10, 357–359.
15 Reinhart, W., Singh, A. and Straub, W. (1989) Br. J. Haematol. 73, 551–556.
16 Ruch, T.C. and Fulton, J.F. (1960) in Medical Physiology and Biophysics, W.B. Saunders, Philadelphia and London.
17 Fricke, H. (1925) Phys. Rev. 26, 678–681.
18 Pohl, H.A. (1978) in Dielectrophoresis: The Behavior of Neutral Matter in Nonuniform Electric Fields, Cambridge University Press, Cambridge, London, New York, Melbourne.
19 Takashima, S. (1989) in Electrical Properties of Biopolymers and Membranes, Adam Hilger, Bristol and Philadelphia.
20 Schwan, H. (1948) Kolloid Ztschr. 3, 53–60.
21 Nelson, C.V. and Wilkinson, A.F. (1972) J. Maine Med. Assoc. 63, 160–163.