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# Basic principles for evaluation of less deformable erythrocyte subpopulations with the Microfiltrometer

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The Microfiltrometer consists of a filtration system for diluted erythrocyte suspensions, through a filter containing 30 cylindrical micropores, 5 μm in diameter, under the influence of a driving pressure. A feeding sinusoidal alternating current of 40 kHz, 300 μA is delivered to the filter. The change in impedance is collected for each temporary flow of erythrocytes through a given micropore. Two main parameters are measured for individually explored erythrocytes: the entry time $\tau$ in the micropore and the maximal variation of impedance $\Delta Z$ occurring for the transitory flow. The slope $\Delta Z/\tau$ defines the velocity of pore blockage. A "Microfiltrometer Deformability Index" (MDI) is established by using this slope. When MDI $\geq 1$, the erythrocyte is considered to be deformable and, conversely, when MDI $< 1$, the erythrocyte is considered to be undeformable. Using this procedure, less than 2% undeformable erythrocytes in healthy blood samples are identified, with a specificity of 99% and a sensitivity of 97.5%.

*Key words:* Bioimpedance; erythrocyte deformability; index; microcirculation; MicroFM; RBC; red cell; thresholding

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## 1. INTRODUCTION

The Microfiltrometer (MicroFm) was designed in France in 1997 and the hardware has been described elsewhere [1]. The MicroFm is a micro-filtration device which uses an Oligopore filter for the purpose of assessing erythrocyte deformability on a cellular level. The device exploits the insulating properties of the erythrocytes. In order to improve the efficiency of the MicroFm, another study of the Oligopore filters was carried out earlier [2]. In the present paper, we present the procedure of erythrocyte deformability qualification by measuring the

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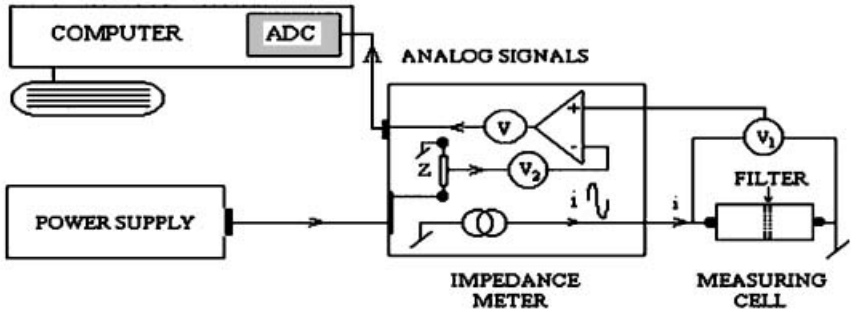


FIG. 1. The Microfiltrometer block diagram.

velocity of Oligopore filter impedance change during individual erythrocyte flow.

## 2. MATERIALS AND METHODS

### 2.1. Functioning principle of the Microfiltrometer

The functioning principle of the MicroFm, summarized in Figure 1, comprises a measuring cell divided into two compartments, A and B, by an 11-micron-thick Oligopore filter that contains 30 identical cylindrical micropores, 5 μm in diameter. Compartments A and B are designed to hold a diluted suspension of erythrocytes and the physiological serum, respectively. Under the influence of a driving pressure of ΔP of a few centimetres of water, each erythrocyte is forcibly passed through a micropore.

The measuring cell is fed by an alternating current of frequency 40 kHz and amplitude 300 μA. Each passage of an erythrocyte induces a voltage drop across the filter and the corresponding change of filter impedance occurring from the temporary passage of this erythrocyte is recorded. The dynamic change in the impedance of the filter is called an elementary signal and two examples of such elementary signals are shown in Figure 2.

### 2.2. Description of the elementary signal

Each elementary signal shows the dynamic change in the electrical impedance of the filter due to individually explored erythrocytes. The signal can be characterized by five marks: Mark *A* indicates the beginning of the signal, mark *C* the maximum of the signal, mark *E* the end of the signal and marks *B* and *D*, the maximum and the minimum of the first derivative of the elementary signal, respectively.

Each elementary signal consists of two distinct phases: a rising phase bordered by marks *A* and *C*, corresponding to the entering phase of the erythrocyte into the micropore and a decreasing phase between marks *C* and *E* which corresponds to the exit phase of the erythrocyte from the micropore.

At this point in the description, it is useful to define two further parameters, $\tau_e$ and ΔZ, where $\tau_e$ is the duration of the entering phase between marks *A* and *C* and ΔZ is the height of the elementary signal, namely the magnitude between marks *A* and *C* (or the magnitude between *C* and *E*) representing the impedance change caused by the temporary passage of the considered erythrocyte through one micropore.

### 2.3. The parameter of interest

We assume that the slope $Z' = \frac{dZ}{d\tau}$ of the entering phase may be a parameter that is sensitive enough to discriminate erythrocytes according to their state of deformability. In fact, on the one hand, the change *dZ* of impedance owing to an undeformable erythrocyte has to be greater than that occurring for a deformable erythrocyte; on the other hand, the time duration of micropore blockage by an undeformable erythrocyte has to be shorter than that of a deformable one. Based on the above assumption, the parameter of interest is identified to be the slope *Z'* between marks preceding and succeeding *B*.

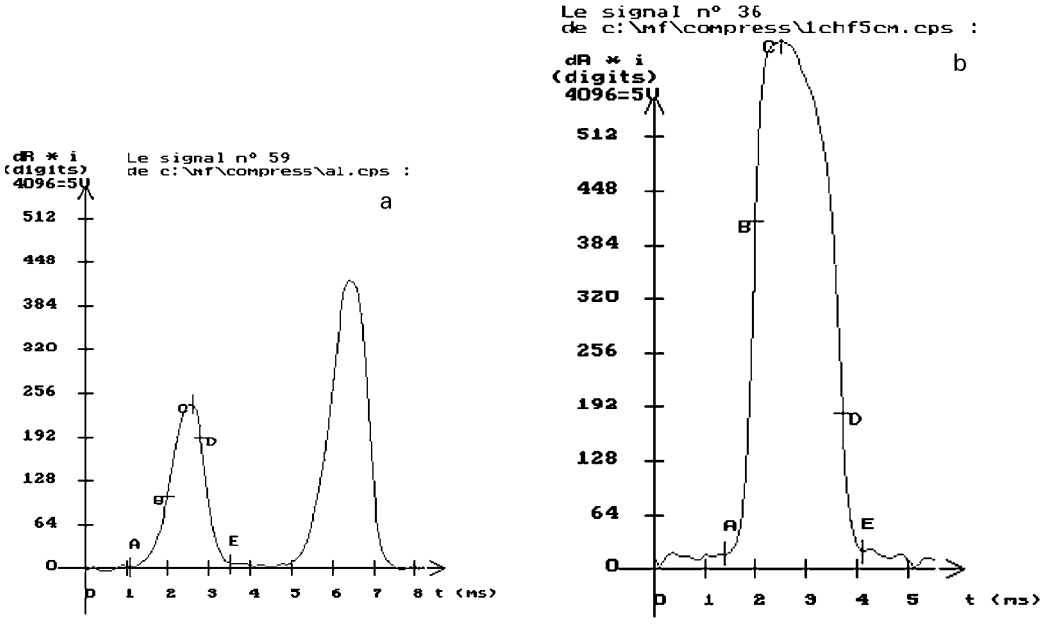


FIG. 2. a: Example of two elementary signals for two successively explored fresh erythrocytes. b: Example of one elementary signal for a heat-treated erythrocyte.

### *2.4. Limiting value of Z'*

We pre-calculate a limiting value $Z'_L = \frac{\Delta Z_L}{\tau}$ of the slope when one micropore is blocked by an erythrocyte entering in the latter during time $\tau$. When a micropore is blocked by an erythrocyte of which the impedance is $Z_C$, a simple calculation of the maximum increase in impedance $\Delta Z_L$ of an Oligopore filter containing 30 identical cylindrical micropores in parallel is given by the equation

$$\Delta Z_L = \frac{Z_E * Z_C - 30 * Z_E^2}{30 * Z_E + 29 * Z_C}$$

where $Z_E$ is the impedance of the 5-micron Oligopore filter filled with saline.

### *2.5. Thresholding*

According to our considerations in section 2.3., the slope Z' of an undeformable erythrocyte signal has to be more abrupt than that of a deformable one, and by extension, the slope of an undeformable erythrocyte signal may be greater than the limiting value $Z'_L$. Subsequently, we define a Microfiltrometer Deformability Index (MDI) equal to $\frac{Z'_L}{Z'}$ and we define that an erythrocyte for which the MDI $<1$ is considered to be undeformable and that for which the MDI $\geq 1$ is deformable.

### *2.6. Samples*

To evaluate the sensitivity and the specificity of the procedure of erythrocyte deformability measurement with the MicroFm, we used one Oligopore filter and explored two suspensions of erythrocytes of one healthy subject. The first suspension is a fresh sample of erythrocytes and the second one is the same sample of erythrocytes rigidified by heat treatment up to 49°C for one minute [3]. The erythrocyte deformability of intermediary samples between a fresh sample and a sample heat treated up to 49°C would have to be evaluated in further studies. For each case, 2 μL whole blood obtained by finger capillary puncture was immediately suspended in 5 mL isotonic saline solution (NaCl, 300 mOsmol/L, 25°C, conductivity $\sigma = 1.98$ S/m, viscosity $\acute{\eta} \approx 1$ mPoiseuille) to obtain a suspension with a hematocrit of around 0.02%. Before each run, the filter is cleaned by ultrasound for 30 s. The measurements were performed under a driving pressure of $\Delta P = 5$ cm $H_2O$. Each exploration entails

acquiring 500 individual elementary signals, 10 min after the capillary puncture.

## 3. RESULTS

### *3.1. Examples of elementary signals*

In Figures 2a and 2b, we present an example of two elementary signals for two successively explored fresh erythrocytes and an example of an elementary signal for one heat-treated erythrocyte.

### *3.2. Entering slope values Z' according to the erythrocyte suspension sample*

Distributions of the entering slope for each of the 500 fresh erythrocytes and for the 500 heat-treated erythrocytes are presented in Figure 3. Notice that the distribution is in accordance with our assumption in section 2.3.

### *3.3. Limiting value $\Delta Z_L$ of the considered filter*

One 5-micron Oligopore filter has been tested in the present study. For this filter, two different values of impedance may be considered: the impedance $Z_i$ of one individual micropore filled with saline and the impedance $Z_E$ of the whole filter containing 30 identical micropores in parallel. The impedance $Z_E$ of the considered Oligopore filter is given by the equation $Z_E = \frac{V}{i_R} = \frac{V}{i^* \cos\theta}$. The value of $\theta$ has been studied in a preceding paper [2] by measurements performed with a Solartron 1260; for a feeding current of 40 kHz, the value of $\theta$ is about 12°. So, with the value of voltage $V = 3.4$ Volts measured for $i = 0.3$ mA (peak to peak), $Z_E \approx 11\,600\ \Omega$ and $Z_i \approx 348$ kΩ. The theoretical distribution of $\Delta Z$ according to the electrical impedance $Z_C$ of one erythrocyte is presented in Figure 4.

Note that the limiting value of $\Delta Z_L$ for this filter is approximately 400 Ω.

Another important value is the mean value duration of the entering phase. When putting together entering times of all signals of the two samples, we calculate the mean value $\tau = 1.3$ ms. From then on, the limiting value of $Z'_L = 307.7$ Ω/ms.

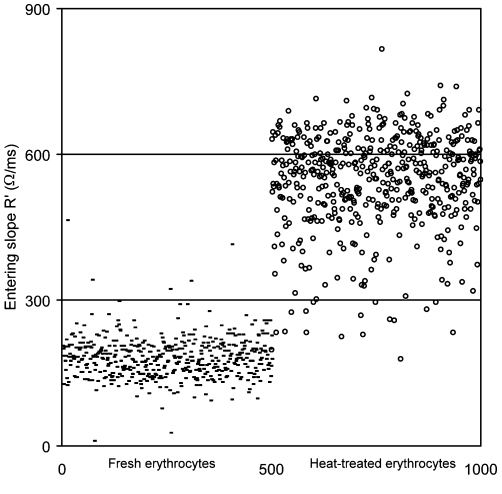


FIG. 3. Distribution of entering slopes R' for the explored erythrocytes.

### *3.4. Thresholding and result of exploration*

We counted the rate of undeformable erythrocytes in each explored sample using the thresholding as described in section 2.5. The MDI data are plotted in Figure 5.

Based on this threshold, we counted 1% undeformable erythrocytes in the normal blood sample explored, and 97.5% undeformable erythrocyte in the heat-treated blood sample explored, which leads to a sensitivity and specificity of the qualification procedure of 97.5% and 99%, respectively.

## 4. CONCLUSION

The development of a new device for acquisition of elementary signals induced by the temporary passage of erythrocytes through micropores measuring 5 μm in diameter has provided relevant and reliable information on the change in electrical impedance of the filter. In addition, this new filtration device calculated the slope of electrical change during the entering phase, on a cellular level. The definition of the MDI and the procedure of classification are simple and straightforward.

By comparing the MDI of each explored erythrocyte with 1, we counted 1% undeformable erythrocytes in a fresh, healthy, blood sample. The same threshold shows 97.5% undeformable erythrocytes in a blood sample heat treated up to 49°C during one minute. The ease with which the MicroFm assesses the rate

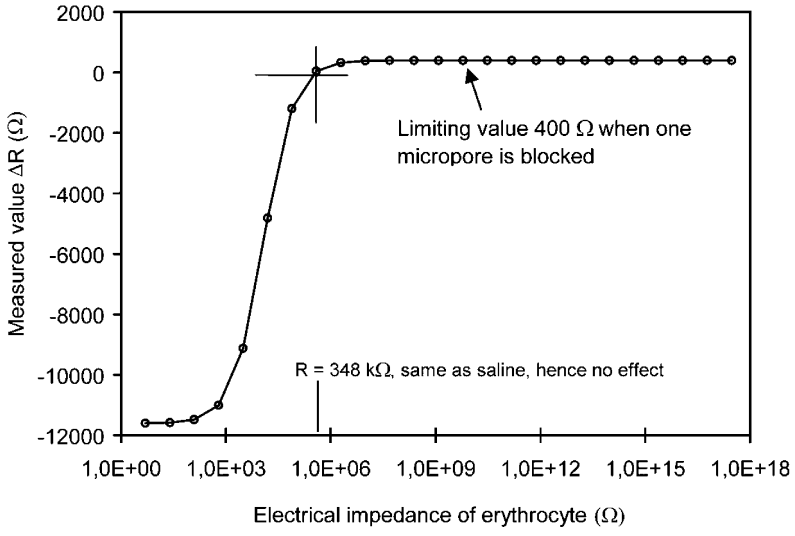


FIG. 4. Theoretical distribution of ΔR as a function of erythrocyte impedance.

of undeformable erythrocytes in normal or abnormal samples and the high specificity and sensitivity of the evaluation procedure constitute a significant improvement compared with the performance of the existing devices. Hence, the MicroFm is recommended for use in clinical routine with the intention of detecting impaired erythrocyte deformability in some diseases such as human haemolytic anaemias [4], Raynaud's phenomenon [5], cerebrovascular stroke [6], malaria [7, 8], sickle cell anaemia [9], diabetes mellitus [10] and primary hypertension.

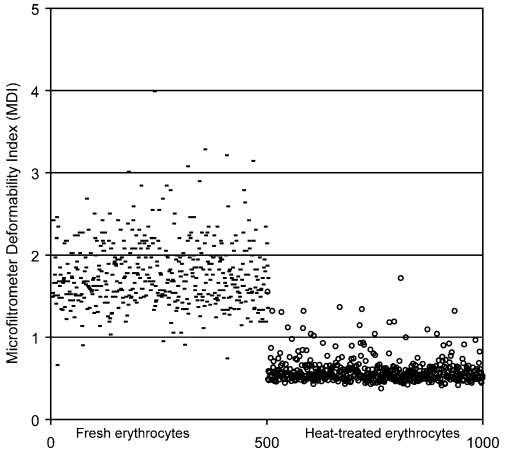


FIG. 5. Distribution of the Microfiltrometer Deformability Index (MDI) for fresh and heat-treated erythrocytes.

Furthermore, this device may be useful in clinical research, for instance in microrheological tests in pharmacology [11, 12] and in cell physiology, for example by treating erythrocytes with ionophore 23187 under various conditions before measurements.

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