EVALUATION OF BLOOD VISCOSITY AT HIGH SHEAR RATE WITH A FALLING BALL VISCOMETER

C. FONS 1, 2, J.F. BRUN 2, L. SUPPARO 2, C. MALLARD 2, L. BARDET 1 and A. ORSETTI 2

1 Laboratoire de Physique Industrielle, Faculté de Pharmacie,
2 Département de Physiologie, Institut de Biologie, Faculté de Médecine,
34060 Montpellier, France

(Received 20.12.1992; accepted 16.4.1993)

ABSTRACT

We tested the accuracy and the usefulness of the MT90 (falling ball) viscometer as an alternative to more expensive and sophisticated devices for physiological and clinical investigations. This instrument measures viscosity from the velocity of a ball falling through a syringe filled with less than 1 ml of blood or plasma at a shear rate of 1000 s⁻¹, i.e. the range of the so-called 'newtonian' behavior of blood. We postulated that this shear rate allows us to measure RBC rigidity in a completely disaggregated structure. Four indices of RBC rigidity derived from high shear rate viscometry were used: μ45r (i.e. blood viscosity at corrected hematocrit 45% divided by plasma viscosity); 'T'k' (Dintenfass); 'k' (Quemada); 'μ'(RBC)' (Breugel and coll.). All were strongly correlated to each other (r=0.98 p<0.01) and easily detected in vitro rigidification of RBCs with (a) ionophore A23187 (10⁻⁵M); (b) heating at 56 °C during 10 min; (c) 30 min incubation in hypercalcemic-hyperosmolar buffer, when used together with Hanss' hemorheometer. At this shear rate, modifications of blood viscosity induced by changes (within a physiological range) of hematocrit and RBC rigidity were correctly described by both Quemada's and Dintenfass's equations for blood viscosity. 'k' values given by the equation of Quemada at this shear rate correlated with RBC rigidity indices calculated with the Carri-Med rheometer using both Quemada's and Wang's equations. Correlations between these indices of rigidity and those given by the hemorheometer were found only for in vitro experiments of RBC stiffening, while in clinical situations viscometric and hemorheometric indices gave rather different results. The coefficient of variation for viscometric measurements was 2% (plasma viscosity) and 3% (blood viscosity). We conclude that this viscometer gives results consistent with other methods. We suggest that it is useful and accurate for physiological and clinical studies.

Key words: Blood viscosity, hemorheology, erythrocyte deformability
INTRODUCTION

Viscometry at high shear rate allows the study of blood viscosity (μb) in a completely disaggregated structure. If the rate of shear is high enough, μb is believed to depend only on three parameters (1):
- plasma viscosity (μp);
- RBC rigidity
- hematocrit (h).

This so-called 'newtonian' behavior of blood has been accurately described by several mathematical models which allow a precise analysis of the data (1, 2, 3, 4).

Most studies of blood viscosity at high shear rate were performed at 100 or 200 s⁻¹ (1). However, current experience with devices measuring RBC aggregation have given evidence that many samples of blood are not completely disaggregated at such rates of shear (5). In addition, rates of shear tenfold higher than the above have been supposed to exist at the arteriolar level (6).

For these reasons, we tested the MT 90 viscometer (Medicatést, Poitiers, France) which works at 1000 or 2000 s⁻¹ as an alternative to more expensive and sophisticated devices for physiological and clinical investigations.

SUBJECTS AND METHODS

Subjects

Thirty-one control subjects (healthy volunteers for a physiological study on the glycemic index of three varieties of rice) underwent a blood sampling for the comparison between MT90 and Carri-Med rheometer. Blood was drawn on EDTA after a catheter was set in the cubital fossa. Blood samples for hemorheological measurements (7 ml) were obtained with a large bore needle (Luer adaptor Venoject, set into the catheter) to avoid shear damage to erythrocytes. A vacuum tube was used for blood withdrawal, with potassium EDTA as the anticoagulant. No tourniquet was used for sample drawing in order to minimize venous stasis (7).

Viscometric measurements

The falling ball viscometer MT 90 was used for measuring blood and plasma viscosities. It was purchased from Medicatést, 37 rue de l'Ermitage F-86280 Saint Benoit. (8, 9). This device has been specially designed for routine measurements of blood viscosity. A reusable glass syringe containing a stainless steel ball is filled with the blood sample and introduced in the viscometer. The viscosity is computed by a microprocessor from the fall time of the ball. The ball is automatically raised by a magnetic apparatus and the timing is detected by induction coils. A digital display indicates viscosity. A built-in thermostat maintains sample temperature at 37°C. The Carri-Med Rheometer 'CS 100' (Rhêo, 19 rue Ambroise Croizat, 91120 Palaiseau, France) was used for comparison of the results (10).
Mathematic treatment of viscometric data

We used in this study the following mathematical models for blood viscosity.

(a) QUEMADA (2): \( \mu b = \mu p \cdot (1 - 1/2 k h)^{-2} \)

Where \( k \) is a shear dependent structural parameter of blood which measures RBC rigidity at infinite shear rate \( (k_{\infty}) \) and RBC aggregation at an extrapolated value of "zero" s\(^{-1} \) \( (k_0) \). This relation between \( k \) and the shear rate \( (\dot{\gamma}) \) is given by the equation:

(b) \( k(\dot{\gamma}) = \frac{(k0 + k_{\infty}\sqrt{\dot{\gamma}/\dot{\gamma}_c})/(1+\sqrt{\dot{\gamma}/\dot{\gamma}_c})}{1} \)

which includes a term \( \dot{\gamma}_c \) which is a disaggregation parameter.

(c) WANG (4): \( \mu b = \mu p(\mu_{\infty} + \alpha \dot{\gamma}^{-0.9}) \)

where \( \mu_{\infty} \) is blood viscosity at infinite shear rate (an index of RBC rigidity) and \( \alpha \) the "curvature" of the rheogram (an index of RBC aggregation).

Accuracy of the method

The coefficient of variation of this method was determined with two procedures: (a) ten repetitive measurements of the same sample and (b) with ten aliquots of one sample measured in ten different syringes.

Comparison of viscosity measurements between MT90 and Carri-Med rheometer

The values of blood viscosity given by the MT90 were compared with those given at 1000 s\(^{-1} \) by the Carri-Med Rheometer. However the latter values were obtained by extrapolation with a software using the equations of Wang and Quemada from viscometric measurement made between 0.01 and 2000 s\(^{-1} \).

\[ r = 0.719 \ p < 0.001 \]

![Graph](image1)

**FIG.1**

Correlation of the values of blood viscosity measured at 1000 s\(^{-1} \) with the falling ball viscometer MT90 and the values interpolated with the equation of Quemada from measurements performed with the Carri-Med rheometer. \( r = 0.719, p < 0.01 \).
Measurement of erythrocyte rigidity

Four indices of RBC rigidity derived from high shear rate viscometry were used:

\( \mu_{45r} \) (i.e. blood viscosity at corrected hematocrit 45% divided by plasma viscosity).

'(d) \( Tk = (\mu r^{0.4} - 1).(\mu r^{0.4}h)^{-1} \)

'(e) \( k = 2.(1 - \mu r^{-0.5}).h^{-1} \)

'm(RBC)' according to Breugel and coworkers (3)

'(f) \( \mu = \mu p(1 + \mu(RBC).h) \)

'viscosity at infinite shear rate' according to Wang (4), equation (c) given above.

In all these equations, \( h \) is hematocrit, \( \mu p \) plasma viscosity, and \( \mu r \) is relative blood viscosity \( \mu b / \mu p l \).

They were used in the 31 samples. The Carri-med rheometer gave RBC rigidity indices using the equations (c) and (e). Equation of Quemada (a) gave after smoothing (personal software based on multiple regression analysis) a parameter \( k_{\infty} \) or \( Dv \) which is the limit of \( k \) at infinite shear rate. Equation of Wang (c) gave a parameter \( \mu_{\infty} \). For the MT 90 the values directly measured were used.

![Correlation graph](image)

**FIG. 2**

Correlations of two RBC rigidity indices: \( \mu_{\infty} \) (Wang's equation) measured with the Carri-Med rheometer and Quemada's 'k' measured at 1000 s\(^{-1} \) with the MT90 viscometer \( (r=0.0657; p=0.00226) \).
Detection of RBC rigidification in vitro

We studied also whether these indices detected in vitro rigidification of RBCs with classical procedures. The hemorheomètre (11) was used for comparison, with the results expressed as a 'viscosity of filtration' ($\mu_f$) with $\mu_f = (ts/tb)/h$ in which $ts$ is the time of passage of suspension, $tb$ the time of passage of buffer and $h$ is hematocrit. Firstly suspended RBCs ($n=11$ samples) were incubated during 30 min at 37°C in ionophore A23187 (10^{-4}M). Heating at 56 °C during 10 min was also used ($n=41$ samples). We also tested a 30 min incubation at 37°C in hypercalcemic-hyperosmolar buffer ($n=11$) according to Lowe (12).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{In vitro rigidification of RBCs measured with the MT90 ('Tk' coefficient) and the hemorheomètre. Suspended RBCs ($n=11$ samples) were incubated during 30 min at 37°C in ionophore A23187 (10^{-4}M).}
\end{figure}

RESULTS

The coefficient of variation for viscometric measurements was 2% (plasma viscosity) and 3% (blood viscosity) when ten aliquots of one sample were measured in ten different syringes. They fell to respectively 0.6 and 0.8 when ten repetitive measurements of the same sample were made on the same syringe.

Fig. 1 shows correlation between the values of blood viscosity measured with the MT90 and values at 1000 s^{-1} obtained by interpolation with Quemada’s equation with the Carri-Med rheometer in the 31 subjects tested. This curve shows higher values (1.3 fold) with the Carri-Med, and discrepancies (less than 10%) between the individual values. The 'r' coefficient indicates a strong correlation ($r=0.719$; $p<0.001$).

The four indices of RBC rigidity derived from high shear rate viscometry were strongly correlated to each other ($r=0.98$; $p<0.01$). Fig. 2 gives an example of the correlations between RBC rigidity measurements with the MT90 and the Carri-Med rheometer. The 'k' value calculated with equation (e) on the MT90 correlated with the indices of RBC rigidity obtained by
extrapolation to an 'infinite' shear rate with equation (c) ($r=0.657; \ p=0.00226$) and equations (a) and (b) of the model of Quemada used together ($r=0.489; \ p=0.011$).

![Graph showing rigidity index before and after filtration.]

**FIG.4**

*In vitro rigidification of RBCs measured with the MT90 ('Tk' coefficient) and the hemorheometre. Suspended RBCs (n=41 samples) were submitted to heating at 56 °C during 10 min.*

Fig. 3, 4 and 5 show that the MT 90 easily detected in vitro rigidification of RBCs with (a) ionophore A23187 (10^{-8}M); (b) heating at 56 °C during 10 min; (c) 30 min incubation in hypercalcemic-hyperosmolar buffer, when used together with Hanss' hemorheometre. In this case a correlation between hemorheometre and viscometric measurements of RBC rigidity could be detected ($r=0.623; \ p<0.01$) on all the samples included in these three experiments.

![Graph showing rigidity index before and after filtration.]

**FIG.5**

*In vitro rigidification of RBCs measured with the MT90 ('Tk' coefficient) and the hemorheometre. Suspended RBCs (n=11 samples) were incubated during 30 min at 37°C in hypercalcemic-hyperosmolar buffer.*
DISCUSSION

Our aim in this study was to define the reliability of this method, to compare it with the more conventional Couette viscometry, and to evaluate the usefulness of the RBC rigidity indices which can be derived from these measurements with theoretical equations.

The coefficient of variation calculated above (2-3% in the most unfavorable conditions of measurements) is satisfactory for a biological method.

This viscometer measures blood viscosity at a shear rate which has been evaluated in previous papers (8) to range (according to ball's diameter) between 1000 and 2000 s\(^{-1}\). Assuming that the ball does not roll and that the flow is laminar, the shear rate (\(\dot{\gamma}\)) is:

\[
\dot{\gamma} = 0.5 \frac{(V.(3R^2+r^2+2Rr))/(R+r)e^2)}
\]

where \(V\) is the velocity of the ball, \(e\) the difference between internal diameter of the syringe and the diameter of the ball, \(R\) the internal radius of the syringe, and \(r\) the radius of the ball. Thus the shear rate is influenced by the velocity of the ball which depends on the viscosity of the medium. However, the measurement of blood viscosity with the Carri-Med rheometer between 800 and 2000 s\(^{-1}\) shows that in this range of shear rates viscosity does not differ and remains quite constant. Thus, the imprecision in the exact value of shear rate is probably of minor importance.

Measurement of blood viscosity at 1000 or 2000 s\(^{-1}\) is not usually done. However, it is clear that these ranges of shear rate are physiologically relevant (6). At this shear rate, modifications of blood viscosity induced by changes (within a physiological range) of hematocrit and RBC rigidity were correctly described by Quemada's equation for blood viscosity, as previously reported by Garnaud (13). Since we indicate here that the results obtained with Dintenfass and Breugel equations are strongly correlated with those results, it can be hypothesized that these three equations allow a good description of the interrelationships between \(\mu_b\), \(\mu_p\), \(h\), and RBC rigidity when blood is completely disaggregated by shear. However, the limits of validity of these equations within this range of shear remain to be further determined by a specific study.

The correlation showed in fig.1 requires some comments. First, the values of \(\mu_b\) measured by the two devices are not the same: MT90 values are lower. Discrepancies (which do not exceed 10%) are observed. We think that rotational and falling ball viscometers do not provide to the blood the same shear conditions, although the theoretical value of shear rate calculated is the same. Nonetheless, a difference in magnitude of 30% is not acceptable, and we think that the quality control of the MT90 should include the use of such comparisons with Couette viscometry and the calculation of a correction coefficient allowing the comparison between viscosity data measured at the same shear rate with different devices. In our laboratory we find a correspondence factor of 1.3 for giving similar values of blood viscosity with both viscometers. Should this correction be used also for plasma viscosity? It has been shown that rotational viscometry is not the 'golden standard' for measuring plasma viscosity, since flow instability phenomena may occur (1). Thus, a capillary viscometer is generally used as reference method (7). However, the MT90 is not likely to induce the same phenomenon and may be a good measurement of plasma viscosity (14).

Viscometric measurements of RBC rigidity are attractive if one keeps in mind the complexity and the artifacts of filtration methods, which are currently used only for research studies. The data presented in this paper support the concept that the measurements of RBC rigidity derived from high shear rate viscometry on the MT90 are able to detect in vitro rigidification of RBCs.
with three usual procedures (ionophore A23187 (10⁻⁴M); heating at 56 °C during 10 min; 30 min incubation in hypercalcemic-hyperosmolar buffer). Correlations between these indices of rigidity and those given by the hemorheometre were found only for in vitro experiments of RBC stiffening. In other studies comparing these two methods of measurements (see for instance ref 15), there were strong discrepancies between them. This point has been already discussed by the team of JC Lelièvre (16). However, Reinhart and Straub (17) have demonstrated in a recent paper that high shear rate viscometry allowed the detection of rigidified subpopulations of RBCs, while low shear rate viscometry did not. These experiments, made with suspensions containing artificially hardened RBCs (with 0 to 0.03 % glutaraldehyde) used rates of shear ranging from 0.1 to 875 s⁻¹. In our opinion, they further confirm the interest of measuring RBC rigidity indices by viscometry at high shear rate.

The correlations between viscometric indices of RBC rigidity given by the MT90 from direct measurement at one shear rate and those given by the Carri-Med (by extrapolation to an 'infinite value' of shear rate as done by the promotors of these measurements) show some discrepancies. They are not surprising since the physical stress given to blood is different. In addition the indices obtained from theoretical models by extrapolation to an 'infinite' shear rate are a more indirect evaluation than a direct measurement at very high shear rate. They may be influenced by the shape of the curve at low shear rates, especially in the case of equation (c) which includes a coefficient of curvature. It is therefore not surprising to observe some differences between these two approaches which are quite different.

We conclude that, notwithstanding some minor discrepancies which result from differences in the procedure of measurement itself, the falling ball viscometer MT90 gives results consistent with other methods: Carri-Med rheometer, hémorhéomètre. This device is very simple to use in hospital or laboratory for multiple measurements with little volumes of blood (0.8 to 1 ml). Measurements are reproducible and the data can be treated by appropriate equations for a more precise analysis of blood rheology at very high shear rate.

ACKNOWLEDGEMENT

Our thanks are extended to Aguettant pharmaceuticals for their kind financial and technical support.

REFERENCES


11. HANSS, M. Erythrocyte filterability measurement by the initial flow rate method. Biohoreology 20,199-211, 1983.


