BIORHEOLOGY

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Contents

Summary

Biorheology is a science treating the characteristics of biological materials such as fluids circulating inside the human body or the artificial biocompatible materials. However the latter are mainly studied by other areas of rheology and, as a consequence, the main focus of biorheology is on human body fluids.

The measurement techniques for biofluids are critically discussed starting from the old capillary, and a rheometric approach based on the use of rotational rheometers is introduced. The importance of understanding how to treat the obtained data is recognized, and relative viscosity is introduced with reference to suspension systems, thereby proposing a distinction between the liquid and solid approach. Apparent shear viscosity measurement performed with rotational apparatus is presented for blood as a shear rate function, and the application of Casson's equation which provides the two material parameters of viscosity and yield stress is discussed in depth. Relative viscosity vs hematocrit plots are discussed in terms of some concentration dependent equations, showing the possibility of having an indirect way to quantify cell membrane flexibility, which is well known to be very important as an index of several illnesses. Following a

solid approach the oscillatory technique is presented as capable of measuring viscoelastic properties and in several cases it is possible to make a clear distinction between normal individuals and patients with different pathologies. The promising new technique of creep test is shown with its characteristic of giving a measure of solid-like behavior of blood in terms of specific elasticity. The most used non-rheometrical techniques capable of giving information about cell membrane flexibility are critically presented with specific reference to blood. Filtration, flow in microchannels and diffraction techniques are discussed and the main aspects are shown. The clinical use of rheology is discussed with reference to some typical disease or pathologies, showing the help that this science may give from the knowledge of the alteration of the flow properties of blood. Finally, a very brief comment is made about the extension of the presented techniques to other biofluids.

1. Introduction

The term "Biorheology", was first proposed by A.L. Copley at the first International Congress on Rheology (1972) as the name of “rheology of living systems or materials directly derived from living systems”, to describe the studies at the interface between biology and rheology. Since then, biorheology had a large development becoming an independent area of rheology (as confirmed by the International Society and the numerous scientific journals dedicated to this theme) and enlarging the treated topics involving also other materials related to biological applications. Nowadays biorheology is a very multidisciplinary matter, involving among other things, medical, biological and fluid-dynamic expertise. In the last few decades the fluid-dynamic and rheological study of biological fluids has received more and more attention for its influence on several phenomena linked to human health. It is well known that several diseases imply changes both in the properties of human biofluids and their components, and in the flow regimes occurring in the vascular ducts. Owing to interest, the research has been focused on two different fields: rheological characterization and flow modeling. The former has the aim to produce physical measurable quantities that may be linked to specific diseases, implying some alteration of the bio fluids and their components, whilst the latter deals with the flow pattern that are realized inside the vascular arteries, capillaries, etc. Thus, roughly speaking, one should divide this rather complex matter into two main arguments: finding rheological properties and constitutive equations characterizing the bio fluids, or using these equations to describe flow conditions and find, as a consequence, flow pattern inside the bio ducts. In principle Biorheology intends to treat the flow properties of all fluids present in our body, but obviously owing to its importance, many studies and researches deal with blood.

Another crucial point is the general use of rheology in the medical field, which should be seen as an interesting support, for instance, in diagnosis but not as a substitute. In the past the enthusiasm raised around the possibility of applying a very promising new technique, has led to some misunderstandings and even to some failure when trying to substitute rather solid-based techniques with rheological properties. This has depressed the initial expectations of a direct application, but nowadays, also, owing to the availability of better and more suitable measurement instruments, rheological properties are seen as a very important support to other techniques.

Whole blood is a circulating tissue composed of fluid plasma (55% vol) and cells often called formed elements (Red Blood Cells, White Blood Cells and Platelets, about 45% vol), but from a rheological point of view it may be modeled as a suspension, containing interacting deformable particles, having different shape and dimensions, dispersed in a complex solution called plasma. The precise composition and the dimensions of cells vary somewhat among individuals and according to their health conditions. Plasma is a solution containing a series of solutes (9.77 g/100ml) such as proteins, carbohydrates, electrolytes, organic acids, lipids, etc., very similar to other biofluids. The formed elements are essentially erythrocytes (about 95%), leukocytes and platelets, with diameters ranging up to 20 μm.

Flowing properties of materials are classically quantified by means of viscosity, a physical entity measuring cohesive energy during a shear flow. The latter is realized when material layers do not mix with each the other on more than the molecular scale, with the consequence that there is no deformation along flow trajectories, but only when passing from one trajectory to another. As a consequence, dependency on the rate of deformation is generally expected. According to a classical definition of viscosity, when dealing with suspension systems, the study of the effect of the suspended matter is made by the relative viscosity η_r defined as the ratio between the viscosity of the suspension η divided by the viscosity of the suspending solution η_S:

$$\eta_r = \frac{\eta(\dot{\gamma},\phi)}{\eta_S(\dot{\gamma})} = \frac{\eta(\phi)}{\eta_S} \qquad (1)$$

If the rate of deformation dependency of both suspension and suspending medium is absent (i.e. Newtonian behavior), or equally is the same, relative viscosity depends only on the particle volume fraction ϕ Obviously the use of the volume fraction is needed, because flow properties are affected by the real volume occupied by particles.

The dependency on the shape of the suspended particles has also some relevance: RBCs appear as biconcave discs which can deform into bullet-shaped particles, allowing passage through the small capillaries, WBC are generally round, and finally Platelets may assume different shapes but approximately they look about flat. Since viscosity is a measure of the resistance of any material to flow, it is obvious that it must change according to the actual shape of the particles that determine the friction. However, because particles are not rigid, also their deformability under the effect of the flow should be considered, as well as their tendency to aggregate or disaggregate. The erythrocytes may form a single aggregate called rouleaux or even networks of several rouleaux that may strongly influence the flow behavior of blood. Since these phenomena may drastically change as a consequence of some illness, rheological properties and viscosity will change too, thus their values and variation can be helpful in studying the health pattern and eventually suggesting proper actions in the case of disease.

It should be noted that at free surfaces clotting phenomena may occur, with the

consequence of a thin film formation making it impossible to perform correct measurement with any instruments where free surfaces are present. To prevent this phenomenon often some specific anticoagulants are added to the whole blood. However, free surfaces are always confined in a sort of small cover cup where saturated conditions, which greatly limit surface effects, are realized. The anticoagulant addition, even if limited to few quantities, sometime makes the rheological results questionable and a test on the anticoagulant concentration dependence must be always detected. This also applies to any addition aimed at stabilizing biofluids during rheological tests.

When dealing with such complex systems, rheological properties are often a mixture of solid and liquid behavior, therefore there are different methods to determine those properties. As a general rule it is possible to say that small time or high velocity give solid properties, large time or slow motion give fluid properties. This implies that for solids it may be assumed that stress depends on the deformation, while for liquids the stress depends on the rate of deformation, whatever the function is: linear or nonlinear. When dealing with materials showing both liquid and solid behavior, to evidence this complex behavior in liquid-like systems, it is necessary to make transient measurement by varying the shear rate during the experiment. In fact, when a constant shear rate is applied, no elastic effects may be directly detected, but only viscosity may be measured. This in turn implies that a constitutive equation for a rheologically complex system is very often a differential or integral equation because of the material time dependency.

2. Blood as a Liquid-Like Material

Previous studies on the rheology of biofluids as well as blood, were essentially based on the use of the Ostwald viscometer or similar capillary tube devices. This method may be seen as the first attempt to determine rheological properties, but it is limited to measuring only the liquid contribution to the flow behavior. In fact, these techniques consist in pushing the fluid contained in a reservoir through a glass capillary, thus the material flows under a driving force that often is just the weight of the fluid itself. The flow pattern in this case consists in a shear flow with radius variable shear rate, which as a consequence requires an averaging method. Thus knowing the pressure drop ΔP, the capillary radius R_c and its length L, by measuring the flow rate Q, it is possible from the well-known Hagen–Poiseuille law to determine the so-called viscosity η:

$$\eta = \frac{\pi R_c^{\,4}\left(\Delta P / L\right)}{8Q} \qquad (2)$$

It is difficult to apply this experimental technique to biofluids for several reasons. Generally speaking the amount of available samples is rather low, limited to some milliliters of volume, whilst to have a good reproducibility the apparatus needs larger quantities. In addition, the capillary radius is a very severe condition, because owing to the flow suspended particles it may deform and migrate from the solid walls toward the tube axis, thus eq.1 referring to a sort of overall behavior is no longer valid. To partially avoid that, a large radius should be used, but as a consequence the previous flow analysis fails, apart from the already mentioned need for some material quantities. Finally, capillary measurement may be classified as a one point measurement technique,

and therefore it cannot be adopted when materials show a no-constant viscosity, as it occurs with the majority of fluids.

A very important experimental development was the introduction of rotational rheometers, where the flow is ensured by the drag force of a rotating solid surface; two geometries are generally used: cone-and–plate or coaxial cylinders. The available small gaps allow the use of rather small quantities of materials to be tested; the viscosity is defined by the rheometer equation based on a proper analysis of the shear flow realized in the gap giving the deformation rate, and the measurement of the torque owing to the fluid friction. From this point of view, rotational rheometers are strain rate controlled while capillary rheometers are stress controlled. The flow pattern realized shows a constant shear rate in the case of the cone and plate geometry, while in the case of Couette geometry, the dependency on the gap requires some averaging methods, but less severe than those used in the capillary measurement.

Materials are called Newtonian or non-Newtonian when viscosity is shear rate constant or not respectively, thus from a constitutive point of view it could be better referring to apparent viscosity in the second case; however the commonly adopted term, also for non-Newtonian fluids is shear viscosity or simply viscosity. In the case of blood and other biofluids a shear rate dependency is found, depending on composition, therefore an apparent viscosity should be used. By changing the rotational speed it is possible to investigate shear rate dependent fluids and find data of the apparent viscosity, which in turn may be fitted or correlated by suitable material constitutive equations.

In the case of a cone-and plate geometry (radius R, cone angle θ), if N is the number of revolutions per minute, the rate of deformation $\dot{\gamma}$ is :

$$\dot{\gamma} = \frac{2\pi\left(N/60\right)}{\sin\theta} \qquad (3)$$

The advantage that $\dot{\gamma}$ is constant throughout the gap is evident. Then the apparent viscosity is obtained in a straightforward way from the measured torque M :

$$\eta = \frac{M}{\left(2/3\right)\pi R^2 \dot{\gamma}} = \frac{\sigma}{\dot{\gamma}} \qquad (4)$$

Where σ is called shear stress. In the case of coaxial cylinders some additional assumptions and mathematical derivations should be introduced, because flow analysis is more complex, owing to a no-constant rate of deformation inside the gap, leading at the end to a similar definition of the apparent viscosity.

Apparent viscosity results are reported in terms of shear stress versus shear rate (Figure 1), in the linear case a Newtonian behavior is found, a less than linear trend is associated with a pseudoplastic or shear thinning behavior, whilst sometimes a yield stress and a Bingham plastic behavior is evident.

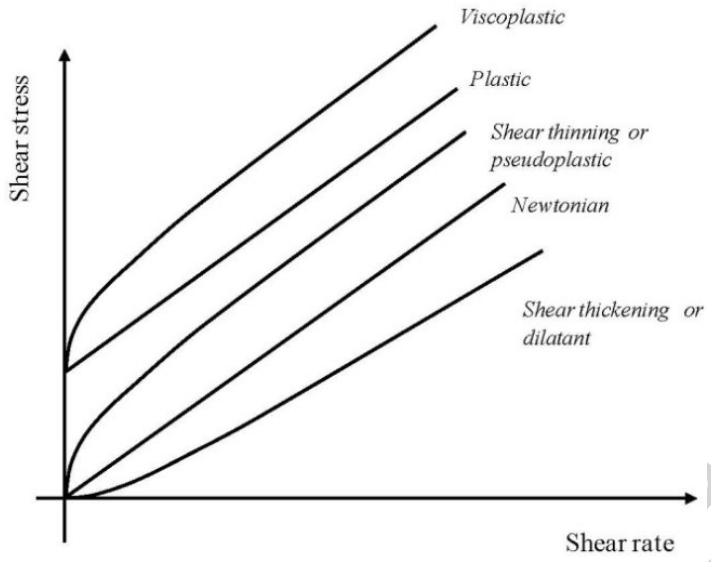

Figure 1. Nonlinear viscous behavior of rheologically complex systems

Several fitting equations are proposed to relate this data as for instance the power law

$$\sigma = k \cdot \dot{\gamma}^n \qquad (5)$$

where k is the consistency parameter and n the flow index. Another fitting equation very often used for blood is the Casson equation that reads:

$$\sigma^{1/2} = \sigma_y^{1/2} + S\dot{\gamma}^{1/2} \qquad (6)$$

where σ_y is the yield stress and S is a material parameter. Practically speaking both Eqs. (5) and (6) are two-parameter models, compared to Newtonian fluid that is a single parameter model, therefore they take into account the nonlinear behavior of the considered system, arising from either the no-constancy of the viscosity and from the existence of a finite value of the stress needed for flowing.

Biofluids often show viscous behavior that may be related to the two above-described viscous models. When considering plasma a Newtonian behavior is often assumed and the value of the shear viscosity is obtained by any of the experimental techniques cited above. Very often a relationship with the concentration of some relevant biochemical data is reported, such as cholesterol (*CHO*), fibrinogen (*FIB*), triglycerides (*TRI*), high density cholesterol (*HDL*)

$$\eta_P = a + b \cdot (CHO) + c \cdot (FIB) + d \cdot (TRI) + e \cdot (HDL) \qquad (7)$$

however the value is about 1.5 mPa·s.

Whole blood, as already said, is assumed to be a suspension of particulates in plasma, thus it is very dependent on both shear rate and the amount of cells. When factorizing

this dependency, two separate functions of shear rate and cell concentration are obtained:

$$\eta_B = f(\dot{\gamma}, \phi) = h(\dot{\gamma}) \cdot g(\phi) \qquad (8)$$

In such a way it is possible to study the two functions separately by assuming either constant concentration or shear rate.

Concerning shear rate dependency, the shear viscosity of whole blood is actually measured by means of either cone and plate or coaxial cylinders, showing a strong shear rate dependency. Capillary measurements are avoided because the measurements are affected by the tube diameter as a consequence of the deformation of the cells when pushed through small diameter tubes. This phenomenon is often referred to as the Fahraeus-Lindqvist effect. When using rotational rheometers a rim exposed to gas is present where stiff mono layers of different materials may form, for instance, denatured proteins and their relevant contribution to the torque causes errors. To prevent this problem a confined geometry is used, and in addition an anticoagulant must be used to prevent clotting, usually heparin, citrates or EDTA. Even though the addition is limited to few quantities, there is a sensible effect on the measured values of blood viscosity that has to be taken into account.

The shear rate dependency appears to be essentially shear-thinning with a yield stress, therefore it is well fitted by a Casson equation. This rheological behavior is due to the existence of flow dependent aggregates of different sizes. Since shear rate is linked to the shear stress, the less the shear rate the less the shear stress, consequently rouleaux and networks of RBCs are predominant at low shear rate, but are gradually broken by increasing the shear rate, because the corresponding shear stress is large enough to break the interaction aggregating individual cells. Nevertheless, a minimum value of the stress seems to be needed to flow. It is generally found that above a shear rate of about 50 s^{-1}, blood closely approaches a constant viscosity and aggregates disappear.

Concerning with viscosity dependency on cell concentration, several data are reported by varying the RBCs concentration, i.e. hematocrit (see for example Zydney et al., 1991). The latter is a very important biological parameter, often it is used as a first global index of a healthy state. When increasing the hematocrit, viscosity rises, but in a different way depending on the shear rate. This is essentially due to the strong influence of aggregate formation on the amount of RBC, but at high shear rates a constant value of the viscosity is approached.

To better understand the role of hematocrit, it is better to use relative viscosity (see Eq.(1)) referred to the plasma viscosity. It should be noticed that plasma is assumed shear rate constant, while blood depends on both the shear rate and RBCs concentration, but if the limit viscosity of blood is reported, a relative viscosity depending only on the volume fraction of RBC is obtained:

$$\eta_r = \frac{\eta_B(\dot{\gamma}, \phi)}{\eta_P} \qquad (9a)$$

$$\lim_{\dot{\gamma}\to\infty}\left(\frac{\eta_{\mathrm{B}}(\dot{\gamma},\phi)}{\eta_{\mathrm{P}}}\right)=\frac{\eta_{\mathrm{B},\infty}(\phi)}{\eta_{\mathrm{P}}} \qquad (9b)$$

A semilogarithmic plot reporting the relative viscosity data determined according to Eq.(9) versus the RBCs concentration shows that by increasing hematocrit, data tend to a linear behavior (Figure 2).

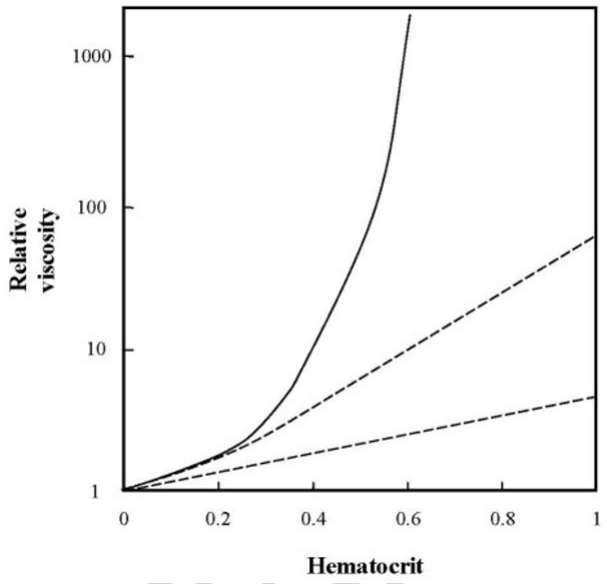

Figure 2. Typical trends for relative blood viscosity as hematocrit function. Area between dotted curves encloses values commonly observed for blood. The solid line represents the typical viscosity of a suspension of rigid particles.

It is well known that dilute suspensions of rigid particles follow the linear relationship

$$\eta_{\mathrm{r}}=1+\alpha\cdot\phi \qquad (10)$$

When dealing with rigid spheres, the Einstein rule predicts α=2.5. When increasing the concentration, packing effect leads to a diverging function approaching a vertical asymptote at the maximum value of the volume concentration $\phi_{\max}$, ranging between about 0.5-0.75 for a unimodal distribution of different shaped particles. One of the most used empirical equations is known as the Krieger-Dougherty equation:

$$\eta_{\mathrm{r}}=\left(1-\frac{\phi}{\phi_{\max}}\right)^{-n} \qquad (11)$$

RBCs are deformable cells, at a low value of the hematocrit less than 0.01, they show a behavior similar to Einstein's equation, but with a factor different from 2.5:

$$\eta_r = 1 + TKC \qquad (12)$$

where KC is the effective RBC volume fraction, C being the RBCs concentration, and T is known as the Taylor factor, taking into account cell deformability. The latter is a function of the internal viscosity of the RBCs η_I, therefore, if parameter p is defined as the ratio $p = \eta_I / \eta_P$, then the following equation is often used

$$T = \frac{p + 0.4}{p + 1} \qquad (13)$$

When $\eta_I >> \eta_P$, then $p \to \infty$ and T becomes 1 (RBCs membrane very hard), on the contrary when $\eta_I << \eta_P$ T tends to the empirical value 0.4 (RBCs membrane very flexible). Increasing erythrocytes concentration, the phenomenon of particle deformability avoids the existence of a maximum packing volume, and it should be noted that it is just this behavior that allows blood to flow inside the capillaries. Usually, the following equation is used:

$$\eta_r = (1 - TKC)^{-2.5} \qquad (14)$$

It should be noted that if for any reason RBCs become harder than normal values, p increases and relative viscosity tends to approach the hard sphere curve, becoming in such a way a sort of quantitative index of this phenomenon. This is shown in Figure 3 where relative viscosity is plotted for increasing values of the hardness of a sphere.

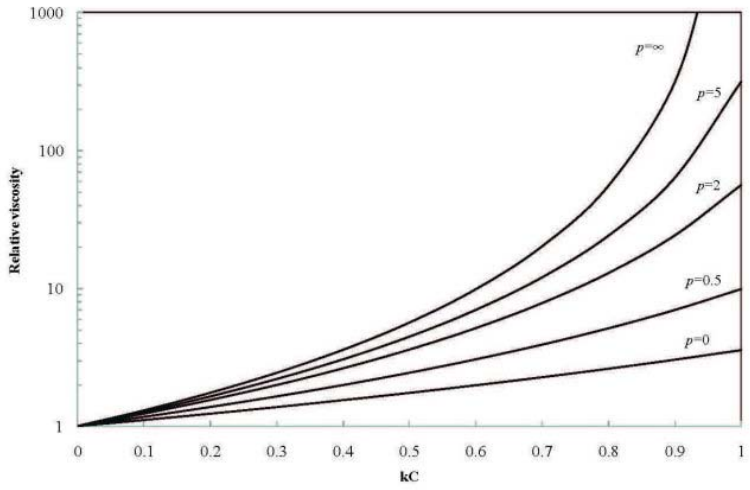

Figure 3. Changes of relative viscosity of human blood as a function of the volume fraction of red cells (kC) according to eq. 14. Parameter p is the ratio of the apparent internal viscosity of red cells and of viscosity of plasma. Rigid cells are described by $p = \infty$, the contribution of red cells to blood viscosity is negligible when $p = 0$

The obtained result looks very interesting because it may be used as a technique to

measure indirectly the RBCs deformability, and several works have been carried out using this approach since the early 1960's. Some improvements were made both from an experimental point of view and with fitting equations. However, the main frame remained the same, being based on the idea that the shear rate, applied to the continuous phase, is transported into it as a shear stress, inducing an internal flow in the RBC drops through the deformation of the interface: the amount of which depends on the deformability of the cell membrane. In this respect, changing the concentration of some components, well known for their strong influence on membrane mechanical properties (such as proteins), an evident effect on the relative viscosity function is found as evidenced since the early meetings in biorheology.

It is also very interesting to look at the yield stress behavior when Casson's law is used to fit the viscosity measurement. Usually the value of the square root of the shear stress is reported versus the square root of the shear rate, then by extrapolating the resulting curve to zero shear rate, a yield stress is obtained, albeit often a fairly low value. In principle, it should be noted that the Casson equation allows the insertion of a sort of solid like behavior through the yield stress, as already stated above. It is then expected that any concentration change of components, which are usually assumed to affect the solid contribution to the rheological behavior, should also affect the value. Yield stress is actually related to the formation of a weak physical gel of aggregates, which implies not only a high viscosity, but also just a solid behavior at rest. Some researchers do not agree with this statement, because it is not possible to verify experimentally, making a clear distinction between a very high viscosity and yield stress. However, if a pragmatic approach is used, it can be said that by Casson model extrapolation, a value of a material parameter can be determined and interpreted as an overall parameter, linked to the need for a finite stress to break network and rouleaux before the suspension starts flowing. By increasing the hematocrit the Casson yield stress also increases, and a cubic law seems to give a good fitting:

$$\sigma_y = \sigma_{y0}\phi^3 \qquad (15)$$

where σ_{y0} is a constant, equal to 26.87 mPa·s according to some works in the literature, and ϕ is the cellular volume fraction.

Finally, the dependency of the results on the individuals should be noticed, particularly due to the great variability of composition, so requiring in principle several measurement to fix, for instance, the health behavior. This obviously limits a deterministic use of the results and a statistical approach is necessary, making more difficult blood rheology, but this argument should always be applied. It should also be noted that despite the extensive previous work on the viscosity of red cell suspensions, none of the available constitutive equations is able to describe the complex dependence of the viscosity on red cell concentration, shear rate, and suspending phase viscosity over the full range of conditions of interest. In addition, it should be admitted that those equation have no strong physical meaning and therefore they must be used as a fitting equation.

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Biographical Sketches

Bruno de Cindio was born on June 30, 1946, in Napoli, Italy. He has a MSc in Chemical Engineering obtained from the University of Napoli "Federico II", Italy (1972).

He was Assistant Professor and Associate Professor at the Department of Chemical Engineering of the University of Napoli "Federico II", Italy, since 1977 to 1993; he was Associate Professor at University of Calabria since 1993 to 2001, and he is currently Full Professor at the Department of Engineering Modeling of the University of Calabria, where he has been a Faculty member since January 1993. He published more than 100 papers on national and international journals, more than 200 contributions in proceedings of International and National refereed conferences and he is co-author of 1 patent concerning a novel apparatus for rheological properties determination. Main areas of his scientific activity are: Rheology of complex systems (foods, emulsion, suspensions, biological fluids), setup and optimization of novel rheological techniques, Thermodynamics of complex systems, Food process engineering.

Prof. de Cindio is currently a member of the Italian Society of Rheology, SIR (of which he is the President), the Italian Group of University Chemical Engineers, Gr.I.C.U., the European Society of Rheology, ESR, the Society of Rheology, SoR.

Domenico Gabriele was born on May 30, 1971, in Cosenza, Italy. He has a MSc in Chemical Engineering obtained from the University of Calabria, Italy (1997), a PhD in Chemical Engineering, concerning Food Rheology, from the University of Napoli "Federico II", Italy (2000).

He is currently an Assistant Professor at the Department of Engineering Modeling of the University of Calabria, Italy, where he has been a Faculty member since January 2005. He is author or co-author of more than 15 papers on international journals, more than 80 contributions in proceedings of International and National refereed conferences, and 1 patent concerning a novel apparatus for rheological properties determination. Main areas of his scientific activity are: Rheology of complex systems (foods, emulsion, suspensions, biological fluids), setup and optimization of novel rheological techniques, Thermodynamics of complex systems, Food process engineering.

Dr. Gabriele is currently a member of the Italian Society of Rheology, SIR (of which he is the Secretary), the Italian Group of University Chemical Engineers, Gr.I.C.U., the European Society of Rheology, ESR, the Society of Rheology, SoR.