# MEAN VISCOSITIES IN MICROSCOPIC SYSTEMS AND MEMBRANE BILAYERS

## A semi-empirical general basis applicable to different kinds of extrinsic probes

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## 1. Introduction

Severe limitations to semi-empirical uses of Stokes-Einstein relation for estimations of microviscosities in membranes have recently arisen. Studies on the motions of a rod-like fluorescent molecule by the time-resolved components of its polarized light emission [1–6] cast some doubt on the previous estimations. When a probe of this shape is inserted in a bilayer and submitted to a pulsed excitation, a stationary light anisotropy remains at long times; this effect lacks in homogeneous viscous liquids which have been studied until now and probably reflects a hindrance of rotation. This restriction must be looked for in the peculiar space order of the lipidic chains.

We have shown [7] that when studying a wide range of viscous liquids by combining steady state polarization and life time measurements of the precedent probe, the method [8–10] was unable to predict even the viscosity of an oil from those of the others: with still more reason, a mean viscosity in membranes seemed difficult to be reached by this way.

However, the different behavior of the rod-like probe in homogeneous liquids is also set up on the various local orders and this situation is not actually different from the comparison between one of the liquids and a bilayer.

In fact the problem is to determine if an average viscosity may still have any meaning inside the bilayer, despite the transversal order gradient which has been long known [11,12].

We further experimented with such systems of various viscosities and local order: we can now propose a classification which allows us to solve the difficulty semi-empirically.

Moreover the proposed method seems applicable to many kind of extrinsic probe molecules; polarized in fluorescent emission, paramagnetic nitroxides and excimer-forming.

## 2. Materials and methods

### 2.1. *Chemicals*

As in many studies [2–10] the chosen rod-like probe is the 1,6-diphenyl hexatriene (DPH); it was supplied by Aldrich Chem. Co. (Milwaukee, WI).

Glycerol esters were the following: tributyrin (from Eastman Org. Chem.), trilaurin and triolein from Fluka (puriss. grade). The melting point of trilaurin was checked before use. Triolein was supplied and always handled under nitrogen flow.

Bis-(2-ethyl)-hexyl Sebacate (BEHS) was a product from Merck (chemicals for gas chromatography).

Penta- and hexadecane were supplied by Fluka (purum grade), while 4-methyl pentadecane and 2,2,4,4,6,8,8-heptamethyl nonane were from KEK (ICN Pharm.); 6-pentyl undecane was a gift of Dr P. Tancrede to our Research Center.

Other products are as in [7], except the phospholipidic system; in this report, it was constituted by multibilayers (obtained by dispersion in water) and not by calibrated vesicles.

### 2.2. *Physical measurements*

Similarly the details of measurements can be found in [7]: here again the viscosities were obtained by

two different methods, when they were consistent with the time scale of experiments.

Two optical parameters were determined:

(i) $\tau$ : fluorescence lifetime of the probe in each medium (for at least three temperatures in each case).

(ii) $r$ : anisotropy of fluorescence polarization by the mean of the parallel and perpendicular light components.

With these data an experimental rotational diffusion coefficient can be evaluated:

$$\bar{D}_r = \left(\frac{r_o}{r} - 1\right) \times \frac{1}{6\tau}$$

($r_o$ : fundamental anisotropy, obtained by extrapolation to infinite viscosity).

## 3. Results

### 3.1. *Glycerol esters, BEHS and C16 isomers*

Figure 1 shows the thermal variations of $(\bar{D}_r/T)$ for C16 isomers and the esters. It also contains the results precedently obtained with viscous hydrocarbons. The corresponding variations of the macroscopic viscosities $\eta$ are plotted in fig.2.

The values for the esters and C16 isomers lie in a range which seems a good continuation of the precedent experiments. A noticeable fact must be considered: the same sequence is found in the oils series, as well for the viscosity as for $(\bar{D}_r/T)$, except when comparing the C16 isomers with each other.

Figure 3 is a log/log plot of the viscosities versus the corresponding values of $(\bar{D}_r/T)$ obtained in the same solvent. Since there is no inversion in the classification of liquids of fig.1,2, no curve (dashed lines) intersects another one in fig.3.

We precedently suggested that the place of a given liquid in this series could reflect its local order [7]. Except when comparing a C16 isomer with the others, the above sequence is confirmed; but the figures give evidence that at low viscosity (as it is found in C16 isomers group) this order is no more respected. The conclusions below will be unapplicable inside the hexadecane group.

### 3.2. *Use of precedent plots: reference lattice of isotherms*

We note that the only way to deduce the macroscopic viscosity of a liquid from those of the other ones by the mean of microscopic data is to draw the isotherms (full lines).

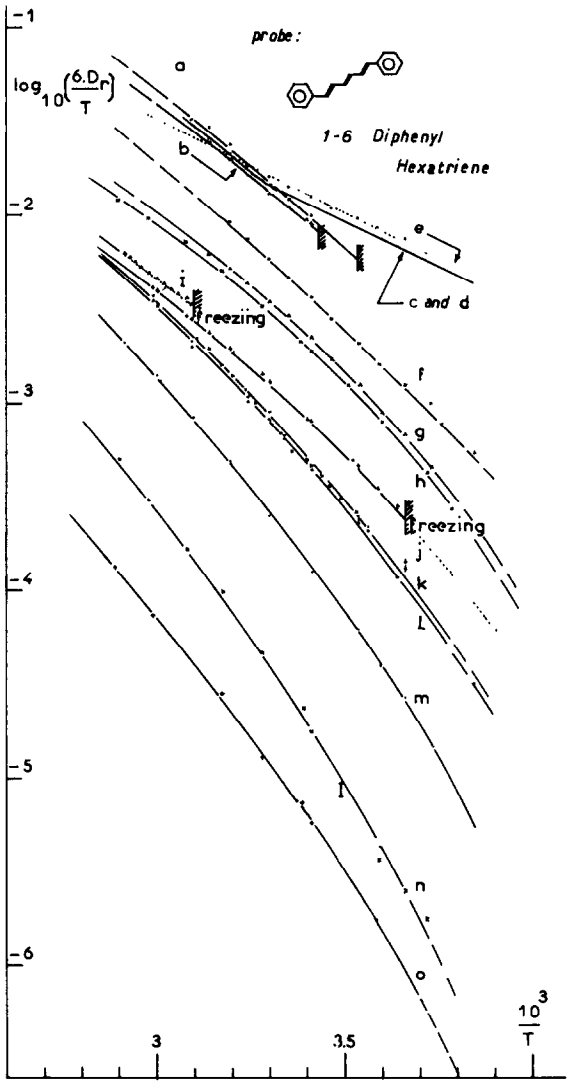


Fig.1. Arrhenius plot of the ratios: rotational diffusion constant $\bar{D}_r$ to absolute temperature for the whole range of the studied liquids. (For mineral oils, cf. [7]). Dividing by $T$ is a correction of the $(kT)$ effect. (a) *n*-Pentadecane; (b) *n*-hexadecane; (c) 4-methyl-pentadecane; (d) 6-phenyl-undecane; (e) 2,2,4,4,6,8,8-heptamethylnonane; (f) glycerol tributyrin; (g) bis(2-ethyl)hexyl sebacate; (h) squalane; (i) glycerol trilaurine; (j) glycerol trioleine; (k) mineral oil Primol 342; (l) mineral oil USP 35; (m) mineral oil Cargille B (purified); (n) polyisobutene, type II; (o) polyisobutene, type IV.

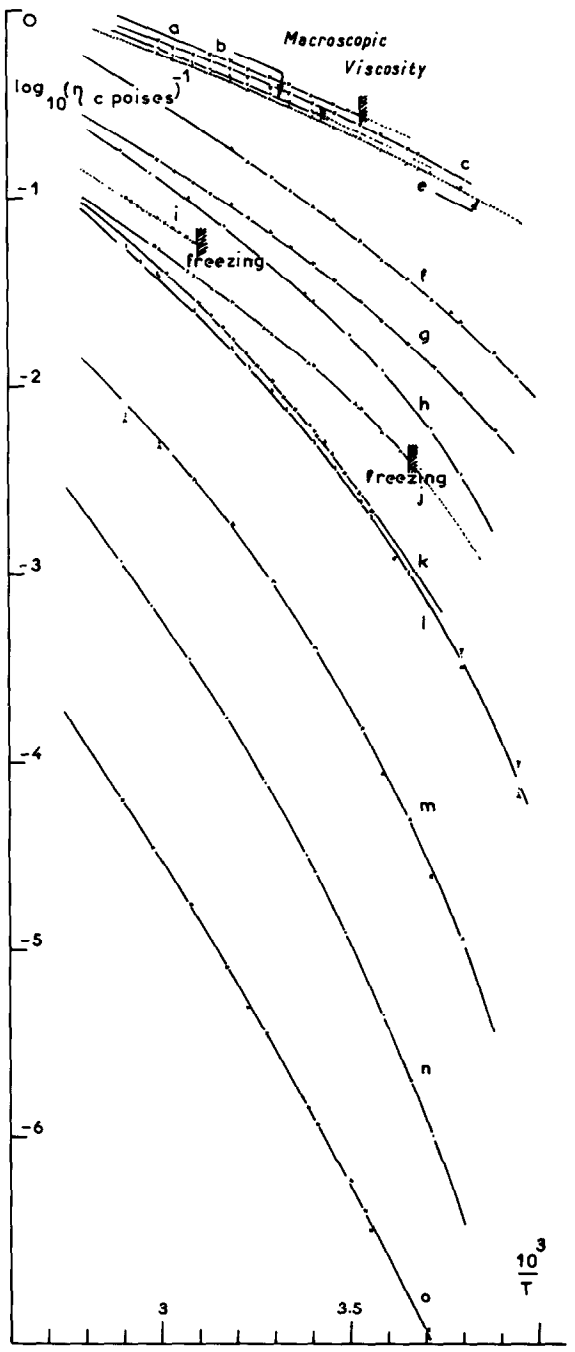


Fig.2. Arrhenius plot of the macroscopic viscosities for the same oils as in fig.1.

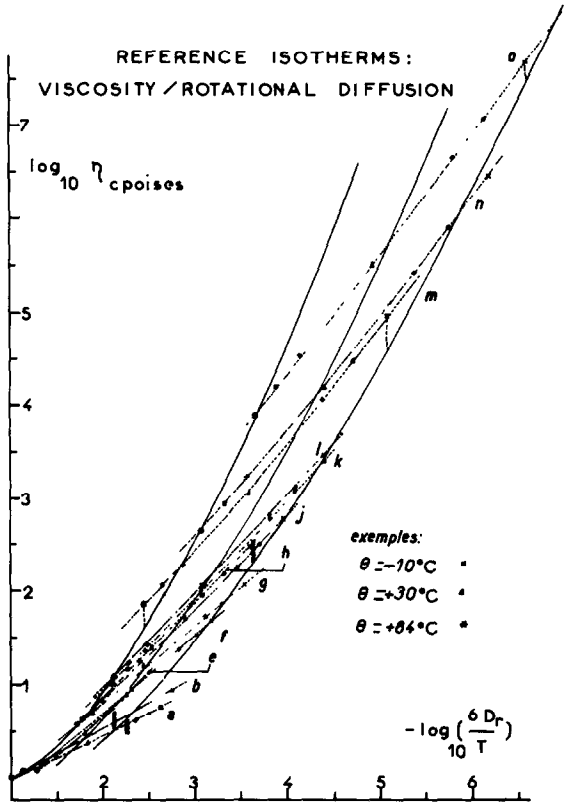


Fig.3. Log/log variations of macroscopic viscosity $\eta$ versus the diffusion coefficient after correcting the $(kT)$ effect $(\bar{D}_r/T)$. The dashed curves are directly deduced from fig.1,2 by eliminating the foregoing abscissa. The full lines are isotherms: we propose to use them instead of a single reference curve [8–10].

On this condition, the method becomes valuable again: every time the fluorescence parameters are measured for the probe in an unknown medium, every time the temperature of the measure settles the corresponding isotherm: the value of $(\bar{D}_{rot}/T)$ determines the abscissa and the 'equivalent' macroscopic viscosity can now be deduced.

For the first time, the results on different liquids are self-consistent.

We propose:

1. Firstly, to apply this consistency of behaviours to some media whose degree of order is particularly high, i.e., to estimate the 'equivalent' viscosities of membrane bilayers either above or below the gel-

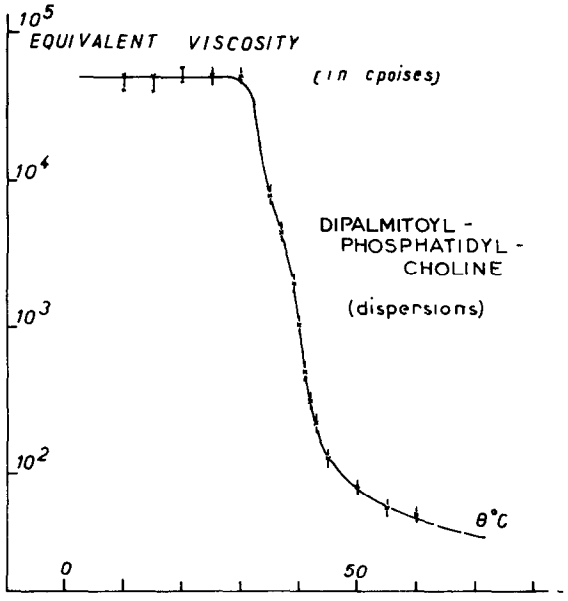


Fig.4. The values of 'equivalent' macroscopic viscosities obtained for a DPPC dispersion from the measure of $(\bar{D}_r/T)$ through the gel–liquid crystal transition when using the isotherms of fig.3 as references. The gel state gives values consistent with a solid state.

phase transition temperature by this way.

Figure 4 shows the result in the case of a DPPC dispersion:

The viscosities found for the gel state are much more consistent with a 'solid' state of the aliphatic chains than those estimated in [8–10]. Reciprocally, the estimated values above the transition are, at least at high temperatures, lower than those obtained with a single reference oil such as USP 35 or glycerol trioleine.

Such a method is applicable to other macroscopic or microscopic systems in which the macroscopic viscosity is not directly measurable. Obviously, the average viscosity only, and not the degree of order, must be wanted to describe the local system.

2. For each {temperature, $(D_r/T)$} pair the method uses a settled reference liquid which is assumed to have the 'good' degree of order. It will be strongly supported if very different kinds of molecular probes lead to the same values of the 'equivalent' viscosity.

An ESR study has been undertaken to check this point: the measurements give very similar results until now, for instance a largely distributed lattice of isotherms.

## 4. Discussion

When the objection is raised that a bilayer and a homogeneous liquid must not be likened, one assumes by implication that the liquid is a continuum.

In fact, a membrane (or micellar) system exhibits a long distance order which lacks in a liquid. But a short distance order is present in both the systems; the probe motion, particularly its rotation, can be principally governed by this order.

Although the probe motion is probably restricted inside a cone in the bilayer [1–6] the relative methods as the Shinitzky's one (modified as here) could give indirect but more sure results than the direct evaluations.

Three points are to be considered before to throw back any theoritical ground of our semi-empirical approach:

(i) As long as we know, no measure of DPH polarized decays has been done with liquids as much viscous as polyisobutenes: until these measures will be done and the corresponding 'long time' anisotropies will be evaluated, one cannot conclude whether they are correct references for the gel state of membranes or not.

(ii) The short distance order in liquids can be estimated by other techniques: for instance, the Rayleigh depolarized scattering [13] supplies a molecular parameter, the optical anisotropy whose relative variations between the pure liquid and infinitely diluted solutions measure the degree of orientational correlations in liquids. NMR relaxation times would yet be a better method and if it would be applied to the extrinsic probe nuclei, it would probably lead to results comparable with the foregoing ones.

(iii) If the transversal order gradient of membranes is thought to be the major restriction for using the method, further results in EPR work would bring forth a partial answer: there are few reasons for getting the same values of viscosity with two very different probes if they do not stay on the average in the same region of the bilayer.

Then, we will try to extend the method to some extrinsic probes sensitive to motions of an other nature: translational diffusion in fluorescence quenching and excimer formation.

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