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Il Farmaco 56 (2001) 533–539

IL FARMACO

Changes in the flow properties of phospholipid dispersions induced by procaine hydrochloride. Effect of pH and temperature☆

Concepcion Arias *, Enrique López-Cabarcos, Pedro Galera, Carmen Rueda

Departamento de Fisicoquímica Farmacéutica, Facultad de Farmacia, Universidad Complutense, Ciudad Universitaria, E-28040 Madrid, Spain

Received 31 October 2000; accepted 10 January 2001

Abstract

Since local anaesthetics are known to interact with membrane lipids, we have examined the changes taken place by procaine hydrochloride in lipid matrices as a function of pH. Rheological methods might give useful information on the association of this anaesthetic with soybean lecithin. The procaine interacted with negatively charged phospholipid polar head groups at pH 4. This system exhibits a loosening in the tight arrangement of phospholipid molecules caused by the addition of procaine as a function of this anaesthetic's concentration.

The flow enthalpy values as a function of procaine–lipid ratio shows biphasic behaviour and suggests a phase transition when the anaesthetic concentration goes from 10 to 14 mM and temperatures dip below 10°C. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: Procaine hydrochloride; Soybean lecithin; Pseudoplastic behaviour; Flow enthalpy

1. Introduction

Studies on the interaction of local anaesthetics with phospholipid membranes are important, because they provide useful information for understanding the modes of action of these compounds in the phospholipid region of biomembranes. However, despite extensive studies [1–4] it is not yet fully understood which region of phospholipid bilayers interacts with local anaesthetics, or what type of modification of the membrane structure is important for induction of their effects on the membrane. Studies on these problems are important not only per se, but also for understanding the factors that govern the stable structure of biomembranes.

Tertiary amine anaesthetics are typical amphiphilic molecules which at physiological pH bind to phospholipid membranes through hydrophobic and electrostatic interactions, which are a function of pH, ionic strength, temperature and fatty acid composition [5]. The hydrophobic interactions involve the aliphatic fatty acyl moieties of the phospholipids and the aromatic ring systems of the anaesthetics [6]. The electrostatic interactions require the charged polar head groups of the phospholipids and the amine functions of the anaesthetics.

Current evidence supports a model of interaction between anaesthetic and membrane phospholipid molecules based upon the hydrophobic aromatic nucleus of the anaesthetic penetrating well into the internal fatty acyl chain region of the bilayer and the charged, alkyl-substituted amine moiety associating with the external head group region [7].

Fernandez [8] thinks that this type of local anaesthetic does not act unimolecularly, but rather as micellar aggregates, because of their amphipathic character, which allows it readily to form aggregates through hydrophobic interactions at relatively low concentrations. Verteuil et al. [9] found evidence for this in the fact that only at concentrations above the critical micelle concentration (CMC) the anaesthetic increased the fluidity of phospholipids chains.

☆ XXIV Int. Congress of the Latin-Mediterranean Pharmaceutical Society, Assisi (Italy), 20–23 Sept. 2000.

* Corresponding author.

E-mail address: carias@eucmax.sim.ucm.es (C. Arias).

0014-827X/01/$ - see front matter © 2001 Éditions scientifiques et médicales Elsevier SAS
PII: S0014-827X(01)01105-3

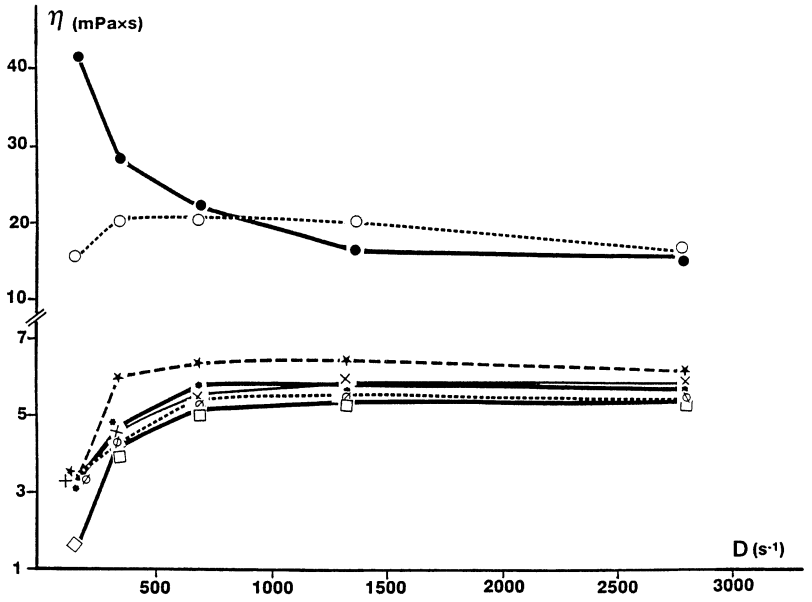

Fig. 1. Apparent viscosity versus shear rate at various procaine concentrations (mM): 1.8 (*); 5.49 (□); 9.16 (×); 14 (★); 27 (○); 42 (●) and without anaesthetic (∅). Temperature 37°C and pH 4.

The exact level to which the anaesthetic binds to the phospholipid is rather controversial [9–11]. The experimental data indicated that charged tetracaine molecules were attached to the bilayer at two different sites. At the first site, the tetracaine molecules are in the aqueous medium and seem to interact with the polar head groups of the lipids through their charged $-N^{+}H(CH_3)_2$ group and fast exchange with the aqueous medium takes place. At the second site, the tetracaine molecules are oriented parallel to the lipid hydrocarbon chains and hence their aromatic groups lie in the hydrophobic region of the bilayer. Only slow exchange with the aqueous medium may occur at this site [9].

Shimooka et al. [11] found that tetracaine cations interacted with negatively charged phospholipid polar head groups and this interaction loosened the tight arrangement of phospholipid molecules. The importance of the hydration layer at the membrane surface in stabilising the membrane structure was suggested. The membrane integrity is governed mainly by the electrical charge of phospholipid polar head groups when phospholipid bilayers are in the highly fluid state, and that the positively charged tetracaine molecule neutralise the negative surface charge, lowering the barrier for water permeation through phospholipid bilayers.

Boulanger et al. [12] believe that the changes observed at low pH values in the ^{2}H and ^{31}P NMR spectra of the phospholipid head group resemble those caused by ions and suggest that the head-group portion (trimethylamino) of the molecule undergoes a conformational change upon interaction with the anaesthetic. This induces the exchange of water molecules with the aqueous medium, with changes in the overall organisation of the bilayer.

Others think that the interaction takes place through van der Waals forces arising from the penetration of the anaesthetic molecules into the lipid bilayer, with minimal interaction with the aqueous medium [13].

The purpose of this paper is to examine the physical interaction between the local anaesthetic procaine and aqueous dispersions of soybean lecithin. The importance of the polar head group of phospholipids was suggested. This binding is involved in the modification in the adjacent aqueous phase and is dependent on temperature and concentration. At pH 7 (near physiological pH) and pH 4 (the conditions used in clinical applications range from 3 to 5), differences in rheological properties were observed.

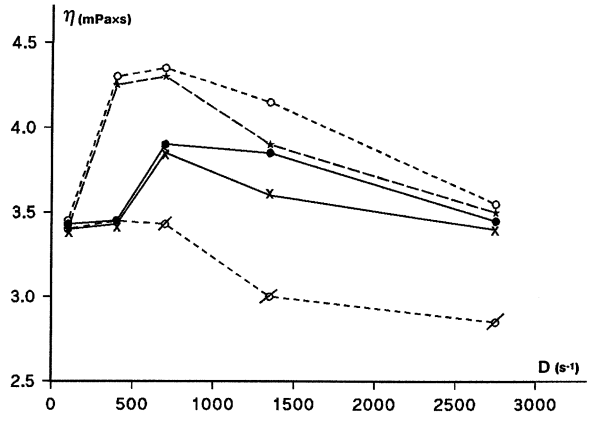

Fig. 2. Apparent viscosity versus shear rate at various procaine concentrations (mM): 9.16 (×); 14 (★); 27 (○); 42 (●) and without anaesthetic (∅). Temperature 37°C and pH 7.

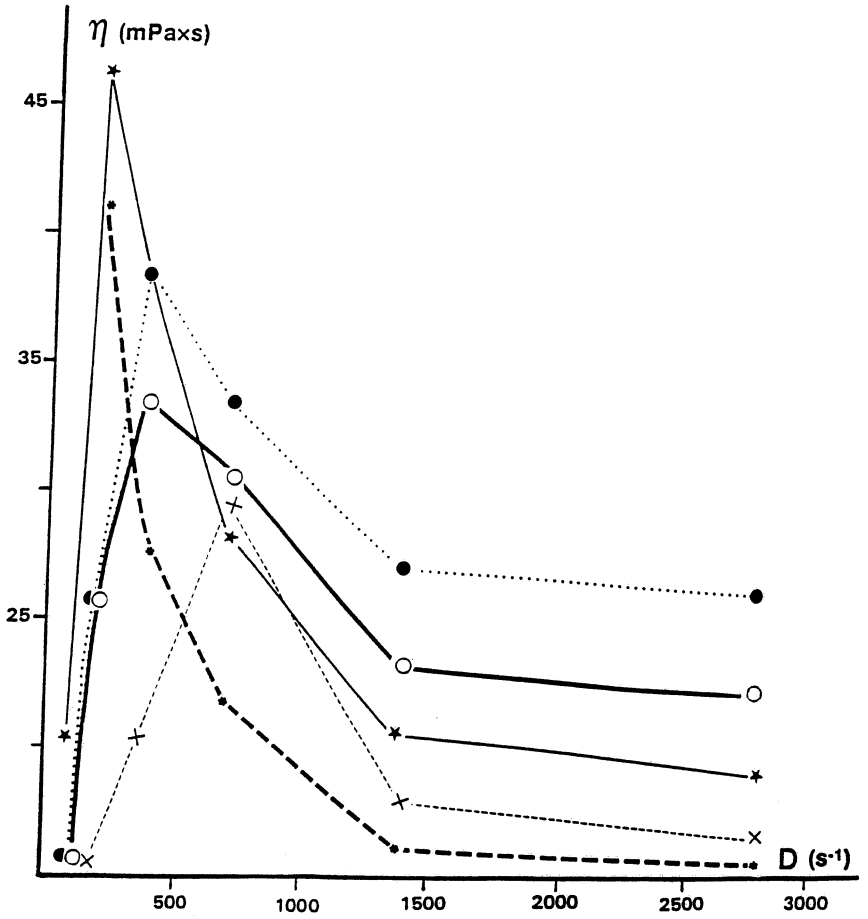

Fig. 3. Apparent viscosity versus shear rate for lecithin–procaine systems at various temperatures (°C): 20 (●); 25 (○); 30 (★); 35 (×) and 37 (*). Anaesthetic concentration 42 mM and pH 4.

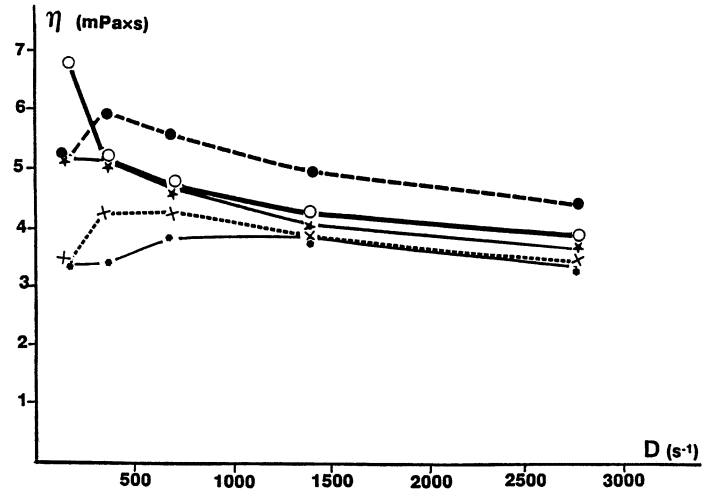

Fig. 4. Apparent viscosity versus shear rate for lecithin–procaine systems at various temperatures (°C): 20 (●); 25 (○); 30 (★); 35 (×) and 37 (*). Anaesthetic concentration 42 mM and pH 7.

2. Materials and methods

Soybean lecithin (L-α-phosphatidylcholine) was purchased from Sigma. It had the following composition certified by the manufacturer and checked by thin-layer chromatography at our laboratory: lysolecithin and phosphatidylethanolamine in small proportions and a higher percentage of phosphatidylcholine. Fatty acid

composition was $C_{16:0} = 17.9\%$, $C_{18:0} = 3.4\%$, $C_{18:1} = 9.5\%$, $C_{18:2} = 60.5\%$, $C_{18:3} = 7.6\%$.

Procaine hydrochloride ($M = 272.8$) ($pK_a = 8.9$) was purchased from Sigma.

Lipid solutions in chloroform were evaporated under nitrogen and placed under vacuum overnight to remove the residual solvent. The dry phospholipid was then dispersed in acetic–acetate and hydrogen disodium–dihydrogen potassium phosphate buffers of pH 4 and 7, respectively, that were used as purchased from Merck, after adjusting their ionic strength with NaCl.

The lecithin concentration was determined by phosphorus analysis according to Bartlett [14].

Prior to the rheological study of the soybean lecithin–procaine hydrochloride system we carried out the determination of the stability in an alkaline medium of the anaesthetic. It was determined by UV spectroscopy at 290 nm ($\varepsilon = 680$) on a Beckman DU-7 spectrophotometer using 0.001% (w/v) anaesthetic solutions of pH 6, 7, 8, 9, 10 and 11, which were adjusted from a starting solution in phosphate buffer of pH 7 by adding HCl and NaOH as required. The ionic strength was adjusted with NaCl.

The rheological measurements were obtained on a Couette-type viscometer which afforded shear rates between 5.41 and 2769.92 s^{-1} and temperatures from 20 to 37°C.

Table 1
The flow enthalpy (ΔH) and entropy factor (β) values for the soybean lecithin and the procaine–lecithin system at pH 4 and 7 [a]

Substrate concentration (mM)	pH 4		pH 7	
	ΔH (kJ mol^{-1})	$\beta \times 10^3$	ΔH (kJ mol^{-1})	$\beta \times 10^3$
Lecithin 90	12.9	35.2	12.7	20.9
Lecithin–Procaine 1.83	16.2	10.5		
Lecithin–Procaine 5.49	12.3	45.7		
Lecithin–Procaine 9.16	11.3	72.4	10.0	69.2
Lecithin–Procaine 14	11.6	66.1	8.3	141.3
Lecithin–Procaine 27	28.9	0.2	13.8	15.8
Lecithin–Procaine 42	23.2	2.0	11.7	35.5

[a] Shear rate $D = 2770$ s^{-1}; temperature between 20 and 37°C.

Table 2
The flow enthalpy (ΔH) and entropy factor (β) values for the soybean lecithin and the procaine–lecithin system at pH 4 and shear rate $D = 135.8$ s^{-1} [a]

Substrate concentration (mM)	ΔH (kJ mol^{-1})	$\beta \times 10^3$
Lecithin 90	20.4	11.0
Lecithin–Procaine 1.83	13.7	363.0
Lecithin–Procaine 5.49	8.8	4265.0
Lecithin–Procaine 9.16	−12.9	7.3×10^7
Lecithin–Procaine 14	−68.5	1.5×10^{18}
Lecithin–Procaine 27	18.9	5.4
Lecithin–Procaine 42	21.9	1.7

[a] Temperatures between 10 and −5°C.

3. Results and discussion

The stability of procaine hydrochloride in alkaline media as reflected in the absorbance did not vary significantly during the first 2.5 h, and only a small difference was observed in the solutions of pH 9, 10 and 11 after 24 h.

3.1. Rheological behaviour

Preliminary work was concerned with soybean lecithin at a concentration of 7% (w/v) at pH 4. At 37°C and low shear rate these suspensions show an increase in viscosity (3.5 at 5.5 mPa s). When the shear rate is higher than 800 s^{-1}, the suspensions of lecithin exhibit newtonian behaviour [15]. At pH 7 the viscosity curves are similar to those obtained at pH 4. No hysteresis loops were obtained on subjecting the phospholipid suspension to gradual increasing and decreasing shear rate at either pH.

3.2. Effect of procaine hydrochloride

We studied the influence of anaesthetic concentrations between 1.8 and 42 mM (i.e. including the range of concentrations used in clinical practice, 1–10 mM) at a constant temperature of 37°C and two pH values (4 and 7).

The viscosity curves obtained at pH 4 (Fig. 1) indicate that the viscosity only increases significantly with respect to that of the starting phospholipid suspension at a concentration above 14 mM. The highest anaesthetic concentration assayed, 42 mM, caused the medium to rearrange and develop a pseudoplastic behaviour. The molecular order was not modified at pH 7 (Fig. 2), so the presence of the anaesthetic only increased the system viscosity. At this pH, the suspension can be considered as mixed aggregates of the cationic form plus some proportion of the neutral form of anaesthetic. Probably this behaviour, which forwards the penetration into the acyl chain region of the lipid, is faster for the uncharged than for all the charged form, while at pH 4 only charged forms penetrate into the head group region of the bilayer [16–18].

The effect of temperature (20–37°C) at an anaesthetic concentration of 42 mM is reflected in Fig. 3. At pH 4 and with a shear rate greater than 800 s^{-1} there was no net influence from this variable. With a smaller

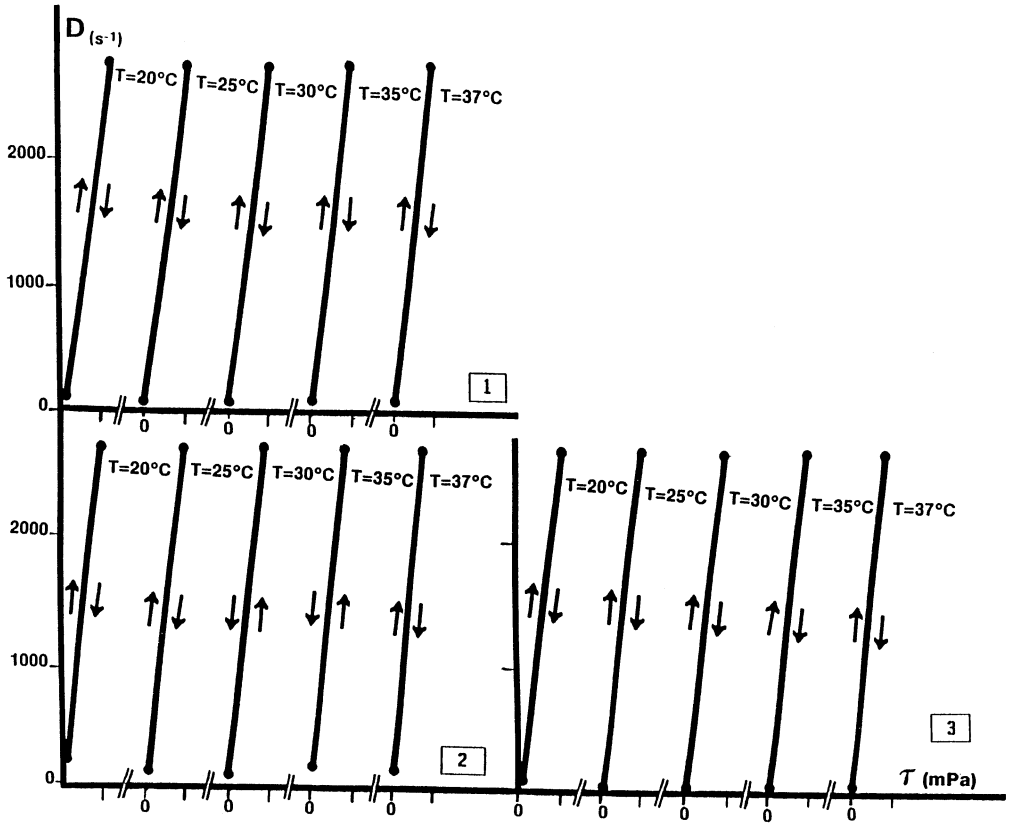

Fig. 5. The hysteresis loop for lecithin–procaine systems at various procaine concentrations (mM): (1) 14; (2) 27; (3) 42. Temperatures between 20 and 37°C. pH 7.

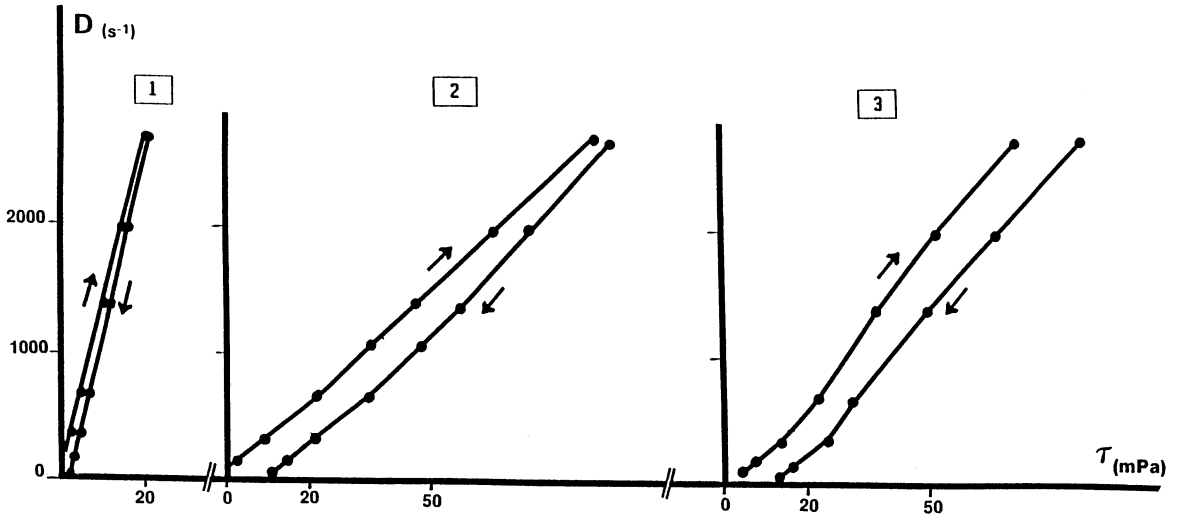

Fig. 6. The hysteresis loop for lecithin–procaine systems at various procaine concentrations (mM): (1) 14; (2) 27; (3) 42. Temperature 20°C and pH 4.

shear rate, the effect of the temperature becomes evident at 37°C, where the solutions behave pseudoplastically.

At pH 7 (Fig. 4), viscosity increased with the shear rate at all temperatures except 25°C below 800 s^{-1}, above which it increased with this variable. In no case did the variations exceed 2 mPa s.

The variation of viscosity with temperature is expressed by: $\eta = \beta\, e^{E/RT}$, where β and E are constants for the given system. As the temperature increases, the

number of molecules increases in proportion to the Boltzmann factor $e^{-E/RT}$ and hence the resistance to flow. The E values are indicative of flow enthalpy (ΔH) and the values were estimated from the slope of the curves, log η against the reciprocal of absolute temperature. The β values that correspond to the entropy factor were calculated from the y-intercept of the curves. This parameter may be interpreted to mean the degree of order of the system as described by equation: $\beta = (hN/V)\ e^{-\Delta S/R}$.

At pH 4 (Table 1), an increase of flow enthalpy is observed that indicates an increase of the interaction forces for the concentrations of 27 and 42 mM. Also high concentrations of this local anaesthetic lead to a decrease of the order of the membrane lipids as a consequence of the water molecules displaced from the internal medium. The anaesthetic's presence does not modify these parameters at pH 7 (Table 1).

The study of interactions at low temperatures (10 to -5°C) present negative value of flow enthalpy which suggests the phase transition for the anaesthetic concentrations of 9.16 and 14 mM (Table 2). Our results are in line with the report by Inoue et al. [19]. Local anaesthetics may affect the phase transition of the membrane lipids from the gel to the liquid-crystalline state that takes place at these temperatures. Table 2 shows an increase of the parameter β due to change of the packing density of the lipid molecules with a tendency to form ordered structures according to the hexagonal structures cited by De Paula et al. [20].

Of all the factors analysed, pH plays a major role in the mechanism of action of anaesthetics. The charged form may interact with the polar head groups by ionic interactions and cause marked changes in lipid matrices, while these are scarcely altered by the uncharged form.

The hysteresis loop can lead to a deeper understanding of this interaction. At pH 7 the effect of the local anaesthetic was not detected (Fig. 5). At pH 4, the addition of procaine caused an increase in the area of the hysteresis loop, showing a rheopexy phenomenon, which is the result of an increased interparticular bonding in time, as shown in Fig. 6. This area of hysteresis decreased with temperature (Fig. 7). This behaviour is possibly attributed to the new molecular arrangement that takes place with the exchange of water molecules with the aqueous medium which is only possible by interaction through the polar head groups of the phospholipid molecule. However, the perturbation takes place at a high concentration of procaine–HCl. Interactions through this group were not influenced by the heterogeneity of the lecithin sample used. These results

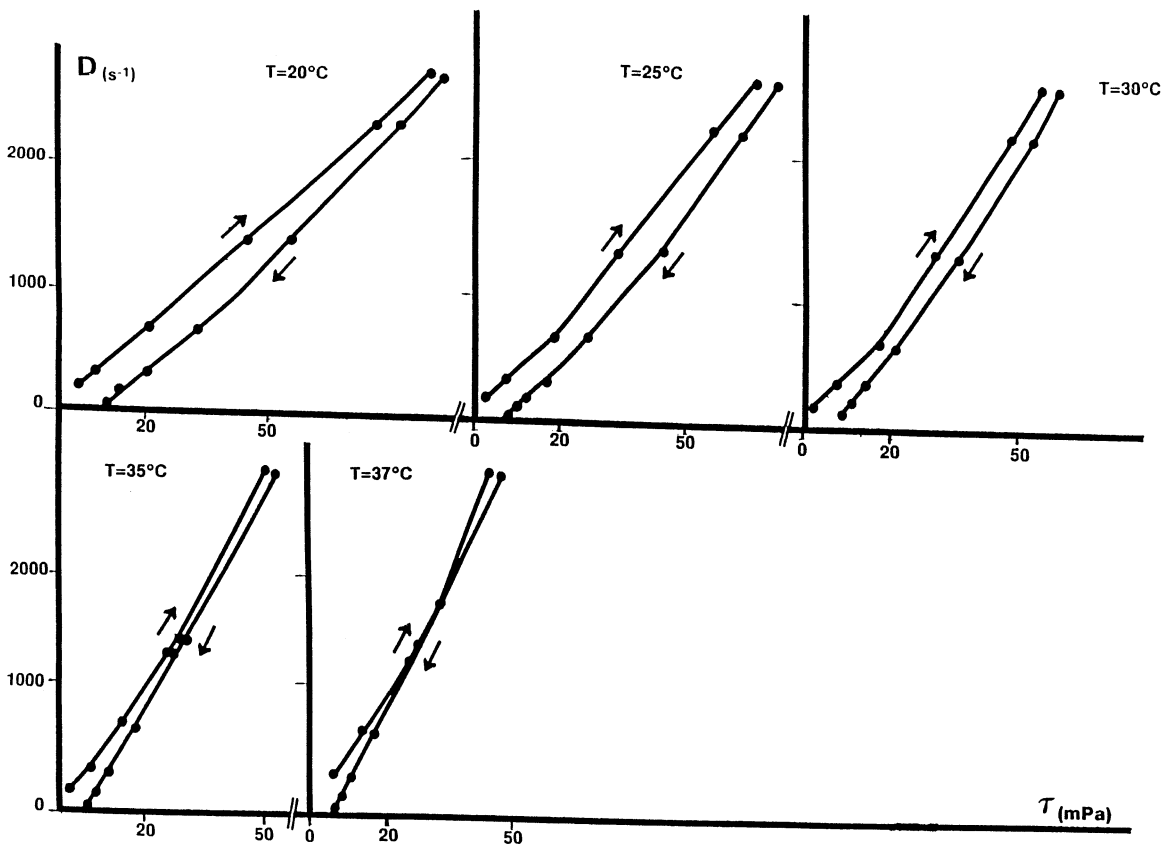

Fig. 7. The influence of temperature on the hysteresis loop. Anaesthetic concentration 27 mM and pH 4.

corroborate the conclusions obtained by Boulanger [12] using phospholipid mixture and De Paula and Schreier [20].

At pH 7 and concentrations above 9 mM, the suspensions only show increased viscosity revealing that the anaesthetic may have entered the lipid bilayer without altering its molecular arrangement.

The membrane perturbation induced by procaine hydrochloride takes place above pharmacological concentrations of the drug (doses up to 10 mM). However, the effect could be of interest in the encapsulation of therapeutic agents by lipid vesicles.

Acknowledgements

This study was financially supported by the Dirección General de Investigación (PGC2000-2246-E).

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