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COMPARATIVE STUDY OF LIPID SYSTEMS FROM VARIOUS SOURCES BY ROTATIONAL VISCOMETRY AND POTENTIOMETRY

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ABSTRACT

We carried out the analysis of lecithins with a common polar group and paraffin chains of different lengths and degree of saturation at two levels, namely: (a) that of the paraffin chain proper, by studying whether structural defects arising from the different mobility of the lipid chains affected their rheological properties; and (b) that of the polar group, which is known to be affected by perturbations to the paraffin chain, and so should its ionization.

INTRODUCTION

The use of lecithins from different sources is limited by the heterogeneous nature of the non-polar region of the molecule. The paraffin chains of different lengths and degree of saturation bear a

common polar group, which is normally accommodated at the interface between the hydrophobic region and the aqueous phase, so it plays a crucial role in interactions with other molecules that may induce changes in the physico-chemical properties of the lipids in the bilayer and hence alter biological functions.

Changes in the structure of the polar group of phosphatidylcholine on addition of metal ions or anaesthetics to an aqueous phase containing this substance were found by Hauser *et al.*[1] and Akutsu *et al.*[2] by NMR and Raman spectroscopy, respectively. Saris[3] and Reiss-Husson[4] later confirmed the occurrence of such an interaction, which was found not to be influenced by the heterogeneity of the lipids in the samples.

The variety of sizes and degree of saturation of lipid chains must affect their mobility; in fact, by using spectroscopic techniques for the estimation of segment mobility, Bourgoin[5] found these aliphatic chains to occur in a liquid state at room temperature, so variations in the mobility of the fatty segments should result in structural defects that in turn would give rise to the development of a higher-rank molecular level without affecting the structure of the lecithin-water system.

In this work we studied three types of soybean lecithin of different composition and phospholipid contents but the same polar group (trimethylamino) by analysing texture and ionization differences.

MATERIALS

The three soybean lecithins used were Sigma, Dianorm and Sigma purified.

Sigma soy lecithin (M=774.1). Thin-layer chromatography revealed the presence of lysolecithin and phosphatidylethanolamine in small proportions, and a higher content of phosphatidylcholine. The fatty acid contents, as certified by the manufacturer, were as follows: $C_{16:0}$ (palmitic) = 17.9%; $C_{18:0}$ (staric) = 3.4%; $C_{18:1}$ (oleic) = 9.5%; $C_{18:2}$ (linoleic) = 60.5%; $C_{18:3}$ (linolenic) = 7.6%.

Dianorm soy lecithin (M=768). The composition, as specified by the supplier was 90-96% phosphatidylcholine, no phosphatidylethanolamine or phosphatidylinositol, 6% max. lysolecithin and the following fatty acid contents: $C_{18:2}$ (linoleic) = 61-71%; $C_{18:1}$ (oleic) = 6-13%; $C_{18:3}$ (linolenic) = 4-7%; $C_{16:0}$ (palmitic) = 10-15%; $C_{18:0}$ (stearic) = 1.5-3.5%. This compositional data were checked by thin-layer chromatography.

Sigma purified lecithin was obtained by the authors from Sigma soybean lecithin (L-α-phosphatidylcholine) using the method of Singleton *et al.*[6] as modified by Hart[7] and Dervichian and Small[8].

Phosphatidylcholine was isolated from all other components by using a neutral alumina column activated at 110°C for 1 h and then allowed to cool *in vacuo*. *A 1:1 (v/v) chloroform/methanol mixture containing an anti-oxidant (BHT: 2,6-di-t*-butyl-p-cresol).

A second thin-layer chromatographic run revealed the persistence of some lysolecithin, which was found to be absent after new passage through the aluminum column and using the same eluent, but in a 9:1 (v/v) ratio.

All aqueous solutions were prepared in HAcO/NaAcO buffer of pH 4 (Merck) and their ionic strength was adjusted with NaCl, also from Merck.

METHODS

The lecithin concentration, expressed as a percent (p/v) of that of phosphatidylcholine, was determined by analysis for phosphorus according to Bartlett[9].

Texture analyses were carried out by rotational viscometry. Measurements were made with a Couette Rheomat 15 rotational viscometer by using the MSCd measuring system at shear rate between 9.742 and 612.9 s_{-1} and temperatures from 10 to -1°C. Measurements made over the temperature range 20-37°C were made with a Haake RV-12 instrument at shear rate between 5.41 and 2769.92 s_{-1}. The lecithin concentration used was 7 g%. The flow and viscosity curves yielded by the lecithins used were duly analysed.

Potentiometric measurements were made with a Standard pHM 82 pH-meter furnished with a TTT80 titration unit and an autoburette. Aqueous solutions of soybean lecithin of concentration 0.003g% were adjusted to pH 2 with 0.01 N HCl in order to be able to analyse the entire neutralization curves. Under these conditions, 0.01 N NaOH was used as titrant and the ionic strength was kept constant by addition of NaCl. The ionization of the three lecithin was studied comparatively from their neutralization curves and the Henderson-Hasselbach equation.

RESULTS AND DISCUSSION

Rotational Viscosimetry

At a concentration of 7% and over the temperature range 37 to -1°C, the soybean lecithins used (Sigma and Dianorm) showed an identical rheological behaviour. Figures 1 and 2 show the flow

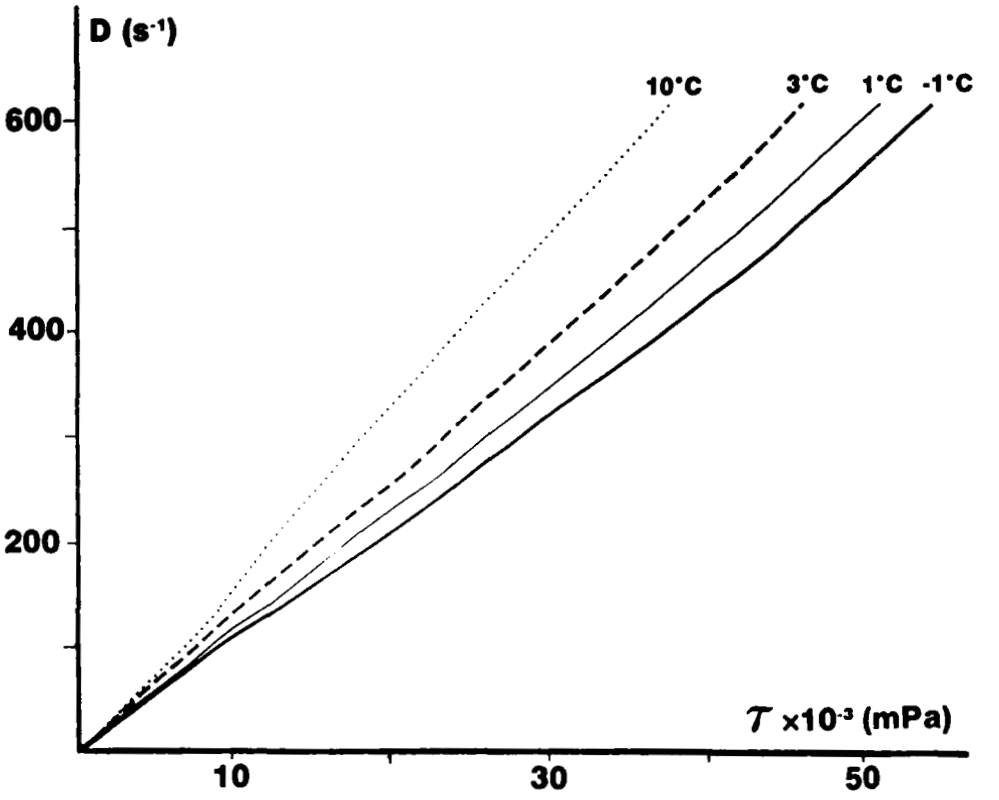

FIGURE 1

The flow curves of a Sigma soybean lecithin.

curves for temperature range 10 to -1°C [D=f(τ), where D is the shear rate and τ shear stress] yielded by the Sigma and Dianorm lecithin, respectively. Both conform to the Ostwald equation:

$$\tau^N = \eta' \times D \qquad (1)$$

where N, the structural number, is very close to unity (Table 1), consistent with a Newtonian fluid --this indicates that the phospholipids form a suspension of spherical micelles.

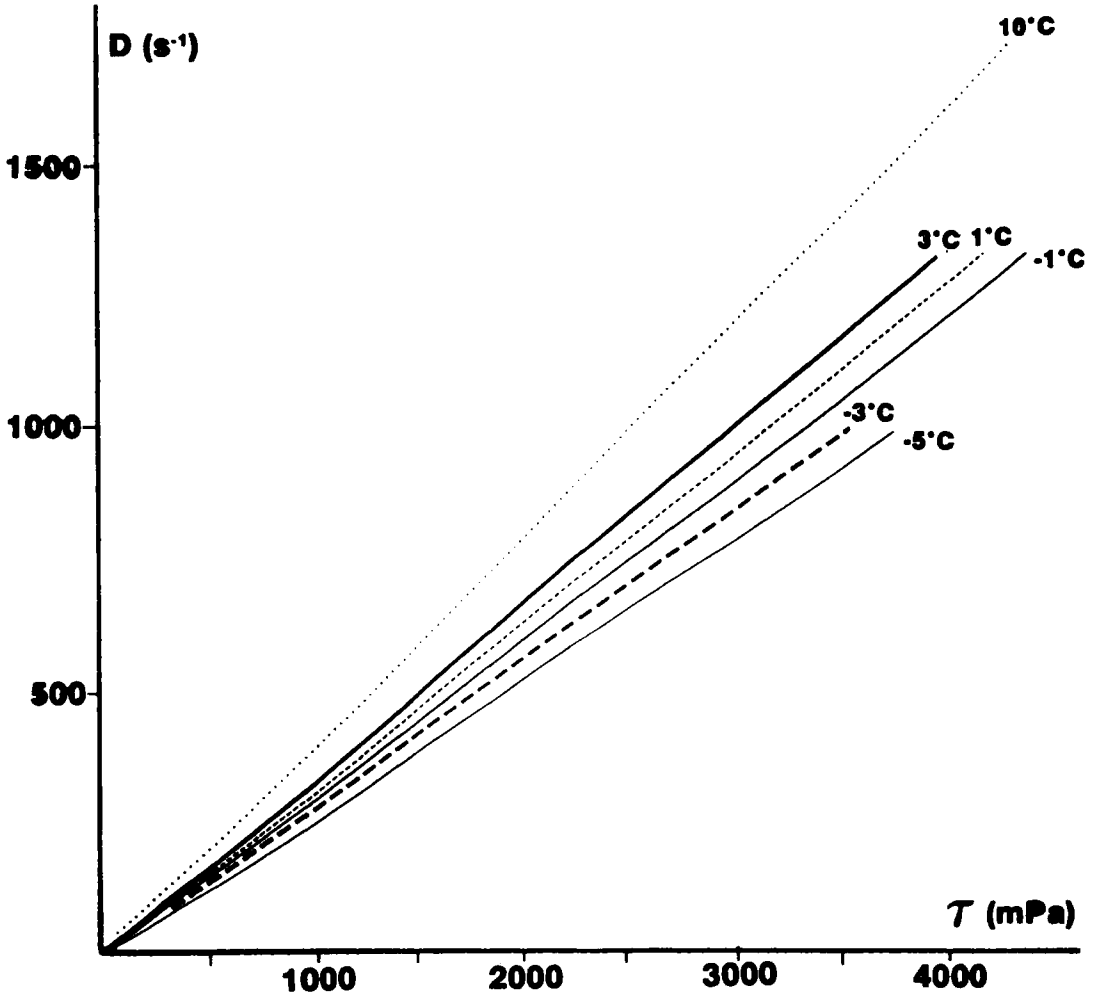

FIGURE 2

The flow curves of a Dianorm soybean lecithin.

The viscosity curves yielded by the two lecithins used reveal two types of behaviour depending on the temperature. Below 10°C and at low shear rate (Fig. 3), the phospholipid suspensions show decreased viscosity consistent with a pseudo-plastic fluid, which may be a result of the original laminar structure of lecithin. As the shear rate increases,

TABLE 1

The structural number the soybean lecithins at a concentration of 7% (p/v).

	Sigma		Dianorm	
Temperature	N	r	N	r
10°C	1.020	0.999	0.986	0.999
3°C	1.022	0.999	1.013	0.999
1°C	1.037	0.999	1.036	0.999
-1°C	1.052	0.999	1.038	0.999
-3°C	-----	-----	1.045	0.999
-5°C	-----	-----	1.061	0.999

the structure tends to break down and form a suspension of spherical aggregates. Above 20°C and at low shear rate (Fig. 4) these suspensions shown increased viscosity (i.e. a dilatance phenomenon). This behaviour can be ascribed to an increase in the mobility of the acyclic chains on the phospholipid resulting from the increasing temperatures, which induce a transition to a "fused-chain state" arising from the increased water adsorption; this in turn increases the lamellar spacing and hence results in an dilatant behaviour. As the shear rate increases, the laminar structure is broken and gives rise to a suspension of aggregates exhibiting the above-mentioned newtonian behaviour.

The viscosity values provided by the Sigma lecithin were somewhat higher than those of the Dianorm lecithin (Table 2). The lecithin composition

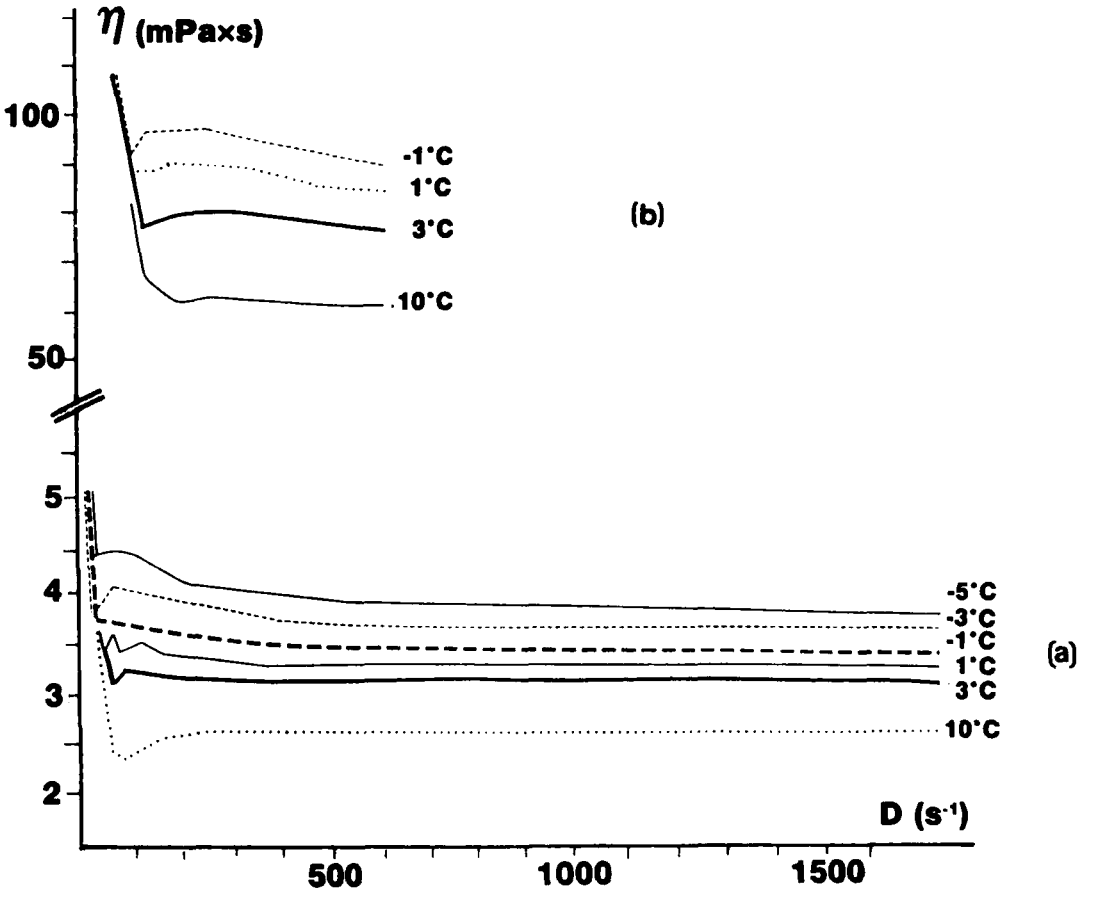

FIGURE 3

The viscosity versus shear rate for a low temperatures. a. Dianorm soybean lecithin. b. Sigma soybean lecithin.

influences the system viscosity, so the difference can be attributed to the presence of phosphatidylethanolamine in the Sigma lecithin.

Potentiometry

The potentiometric analysis of the Sigma, Sigma purified and Dianorm soybean lecithins (Fig. 5)

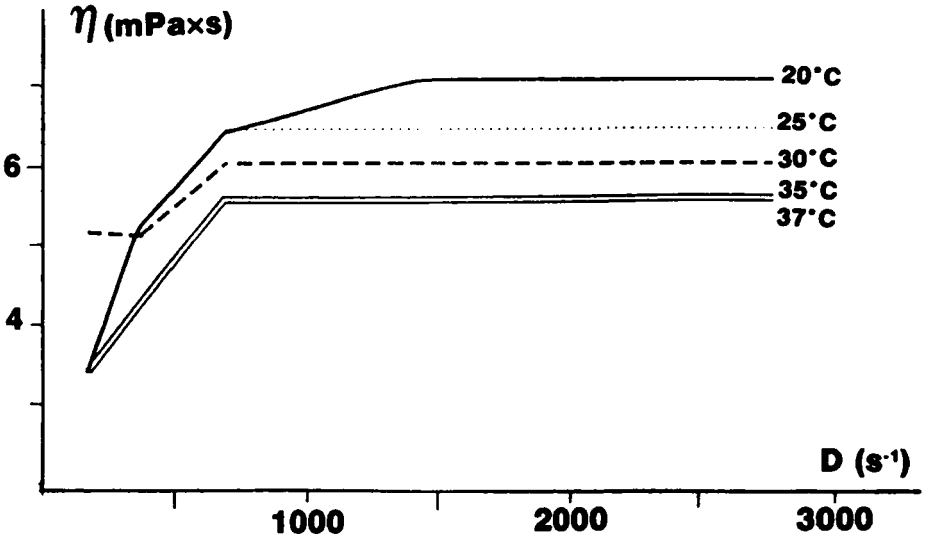

FIGURE 4

The viscosity versus shear rate over the temperature range 37 to 20°C. Sigma soybean lecithin.

TABLE 2

The viscosity value for Sigma and Dianorm lecithin.

	Sigma		Dianorm	
T(°C)	D<100s^{-1}	D=500s^{-1}	D<100s^{-1}	D=500s^{-1}
10°C	80 mPas	60 mPas	3.5 mPas	2.5 mPas
-1°C	110 mPas	90 mPas	5.0 mPas	3.5 mPas

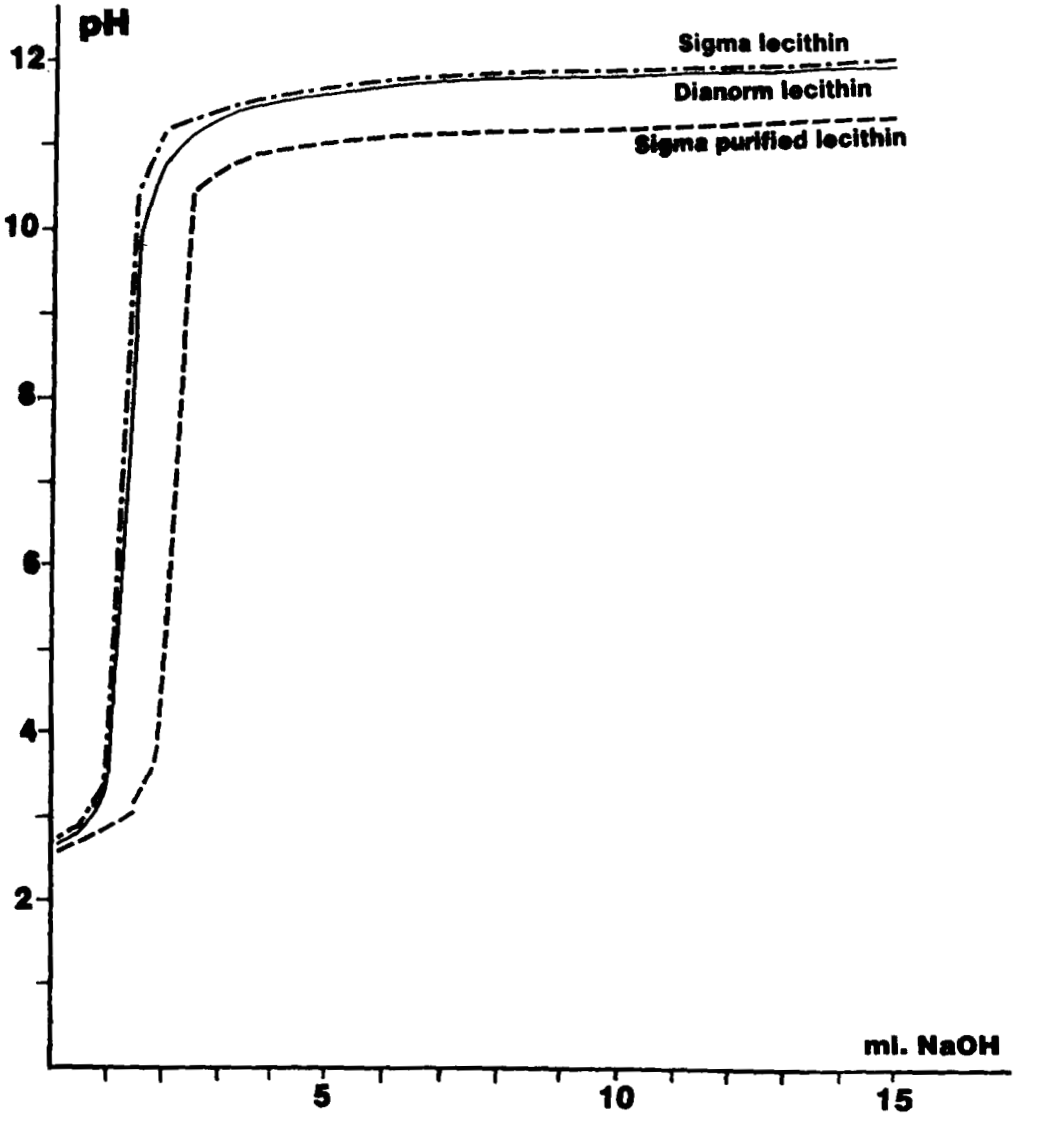

FIGURE 5

The neutralization curves of the Sigma, Sigma purified and Dianorm soybean lecithins.

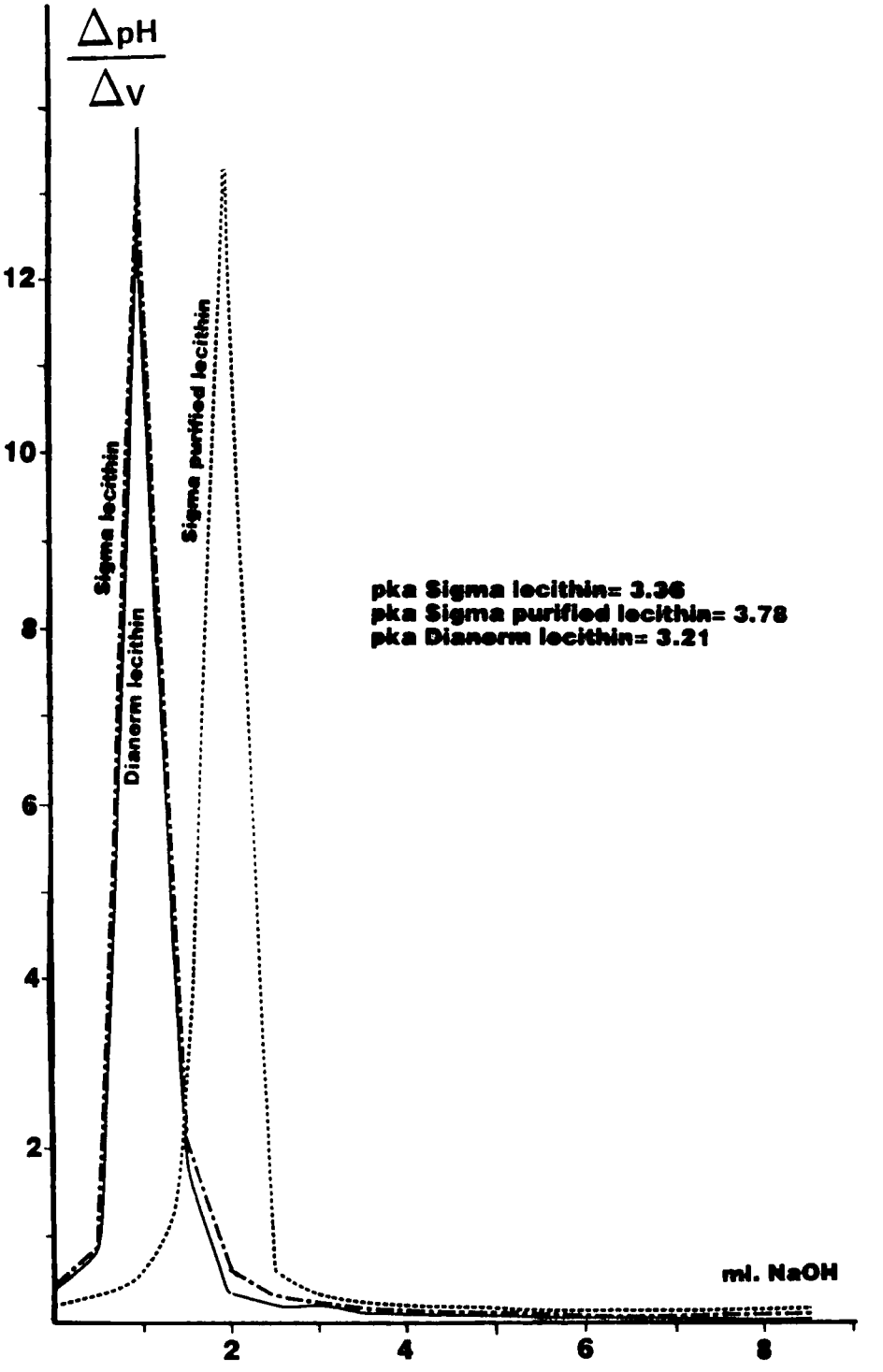

FIGURE 6

The derivative curves for the Sigma, Sigma purified and Dianorm soybean lecithins.

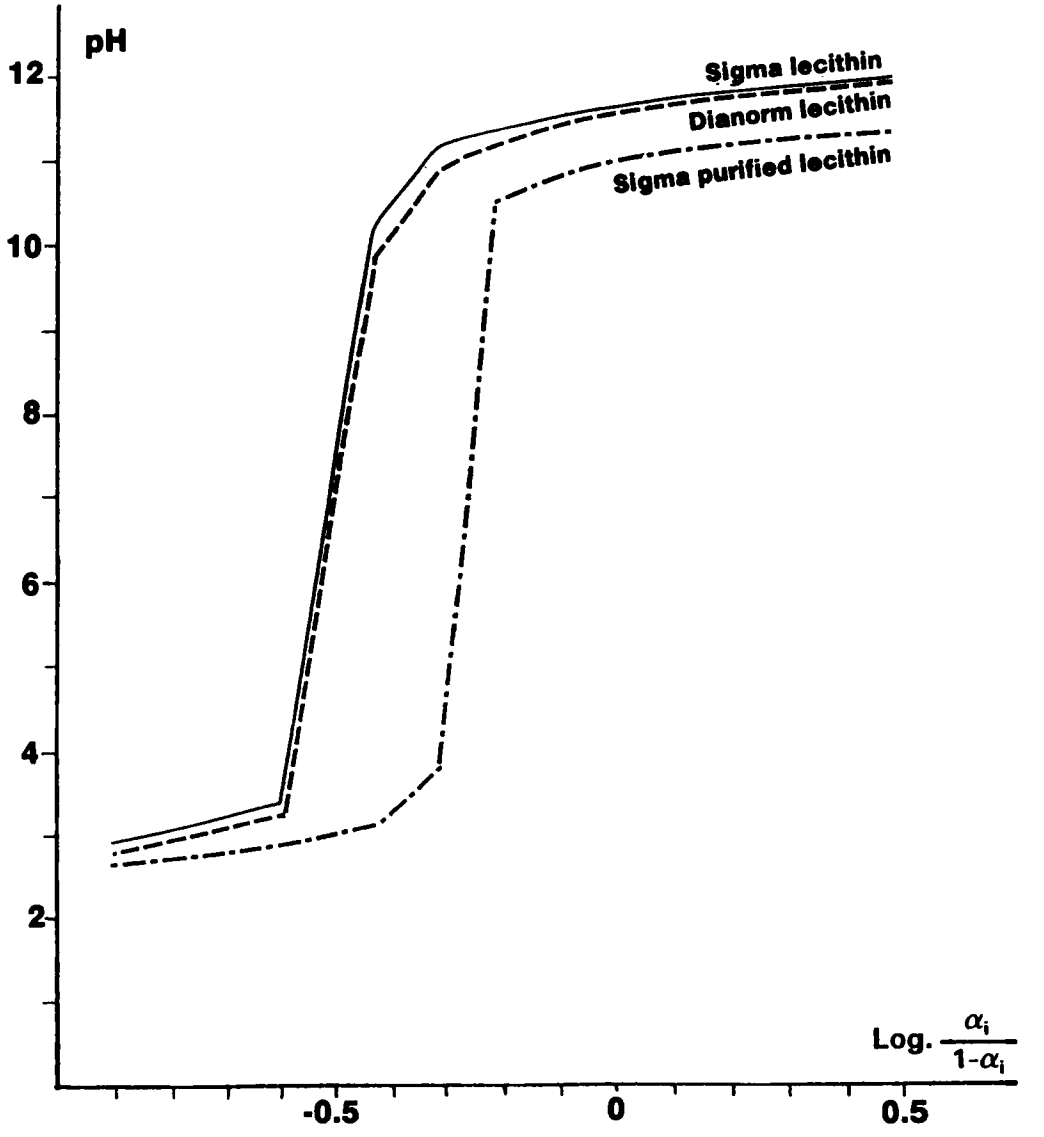

FIGURE 7

The curves Henderson-Hasselbach of Sigma, Sigma purified and Dianorm soybean lecithins.

reveals perfect correlation between unpurified Sigma and Dianorm lecithins, with roughly 96% of phosphatidylcholine, and that the phosphatidylcholine, present in the former does not modify its pka, as can be inferred from the derivative curve (Fig. 6). The lecithin in the purified Sigma sample differs from the other two in that its pKa undergoes a slight decrease (less than one unit) as a result of the absence of lysolecithin. The shape of the neutralization curve is identical in the three cases, as one would expect since, despite the heterogeneity in the phospholipid composition of the first sample, the polar group has common features in all three.

The curves in Fig. 7, plots of the Henderson-Hasselbach equation,

$$pH = pKa + n \log [\alpha_i/(1-\alpha_i)] \qquad (2)$$

are consistent with the behaviour of systems containing a single ionizable group; the slopes (n) are 1.52, 1.42 and 0.82 for the unpurified Sigma, Dianorm and purified Sigma lecithins, respectively. These values reveal the occurrence of only slight interactions with the ionizable group that increase with increasing lipid heterogeneity.

Consequently, the heterogeneity of lipids in the lecithin samples influences neither their rheological nor their potenciometric behaviour. However, for homologation the quantitative results, we believe lecithin should be purified by removal of phosphatidylethanolamine and lysolecithin in order to go deeper into the behaviour of these systems in aqueous solutions.

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