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Tremblay et al.

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[54] PROCESS FOR PURIFICATION OF PHOSPHOLIPIDS

[75] Inventors: Paul A. Tremblay, Mercerville; John J. Kearns, Princeton, both of N.J.

[73] Assignee: The Liposome Company, Inc., Princeton, N.J.

[21] Appl. No.: 698,668

[22] Filed: Feb. 6, 1985

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 579,535, Feb. 13, 1984, abandoned.

[51] Int. Cl.[4] C11C 1/00
[52] U.S. Cl. 260/403; 260/412.4
[58] Field of Search 260/403, 412.4

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Primary Examiner—J. E. Evans
Attorney, Agent, or Firm—Pennie & Edmonds

[57] ABSTRACT

A process for the separation and purification of individual phospholipids, especially phosphatidylcholine or lecithin and phosphatidylethanolamine, from mixtures containing members of the sub-class of phosphatides, incorporating methods of solvent extraction appropriate to the scale of the sample and utilizing an acetonitrile, acetonitrile-hydrocarbon, or acetonitrile-fluorocarbon solvent, which exhibit differential solubility properties towards the individual phospholipids.

44 Claims, 3 Drawing Figures

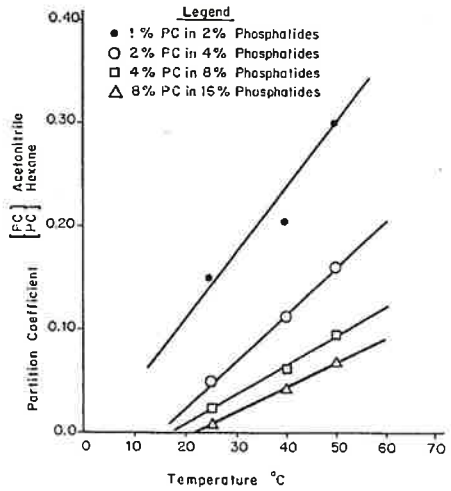

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FIG. 1

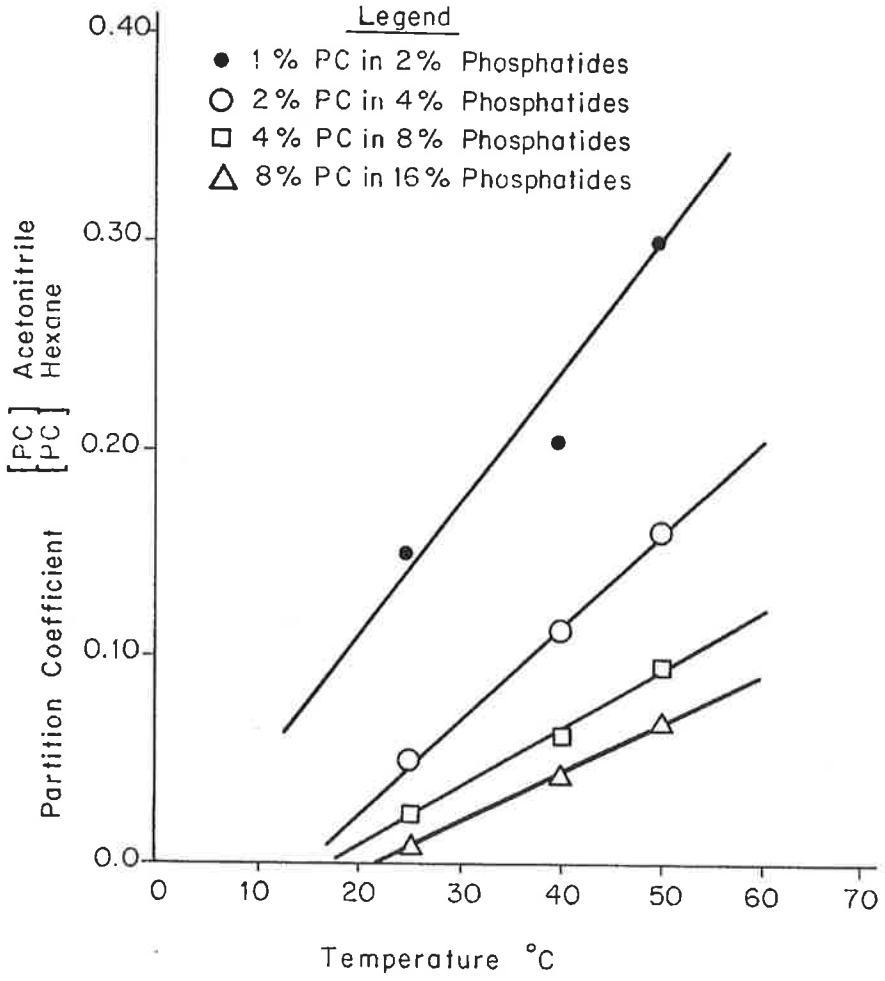


FIG. 2

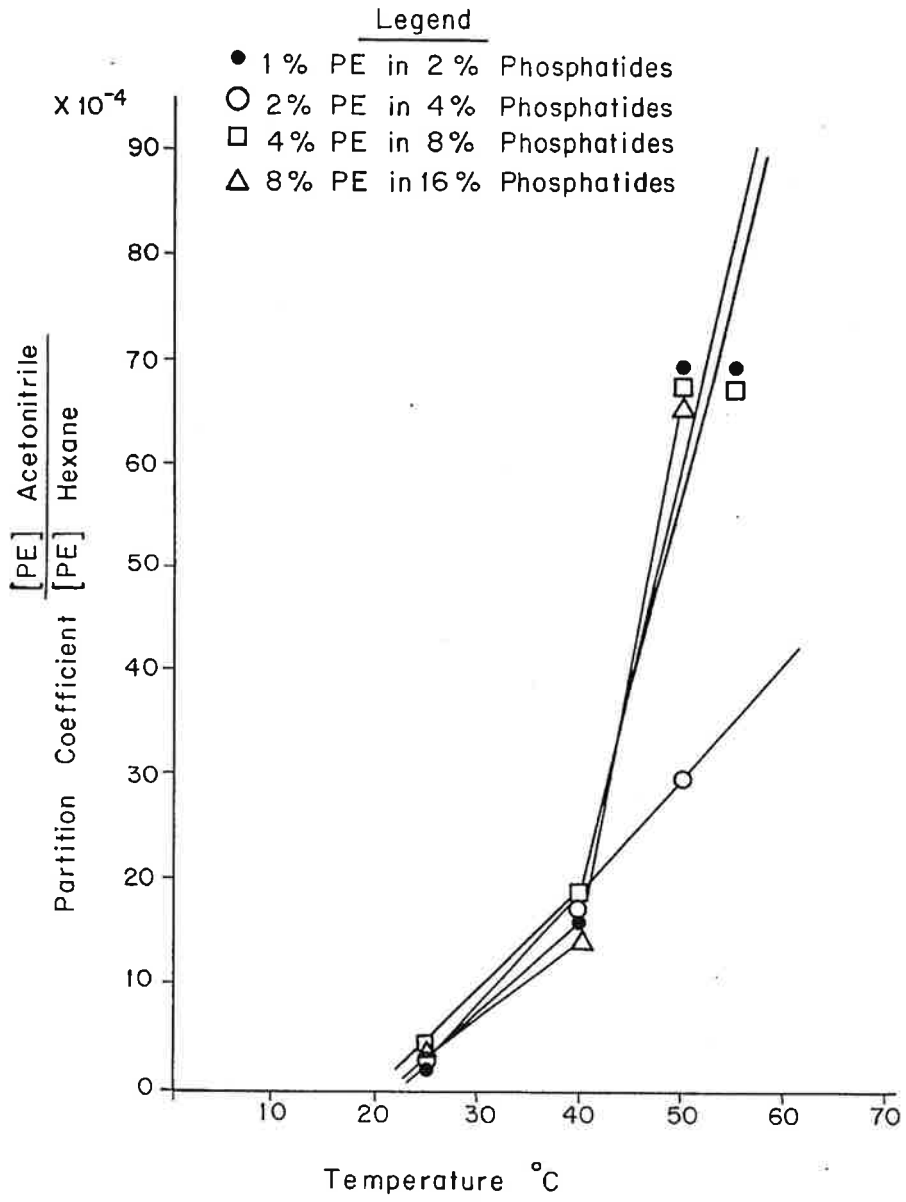
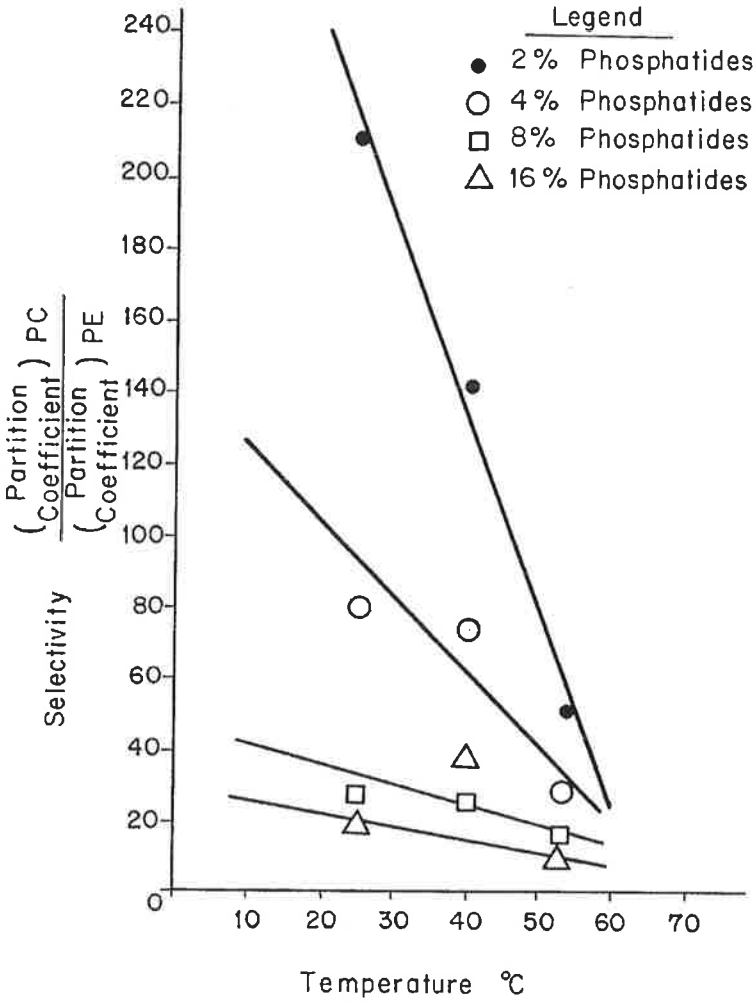


FIG. 3



PROCESS FOR PURIFICATION OF PHOSPHOLIPIDS

The present application is a continuation-in-part of prior copending application Ser. No. 579,535, filed Feb. 13, 1984, now abandoned.

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1. FIELD OF THE INVENTION

The present invention relates to a process for the production of high-purity, individual phospholipids from mixtures thereof, by means of separation techniques utilizing solvents novel for this purpose. More specifically, this invention concerns a process for separating and purifying phospholipids, especially those of the sub-class of phosphatides, including, but not limited to the variant fatty acid chain members of the phosphatidylcholine ("PC") or lecithin, phosphatidylethanolamine ("PE"), phosphatidylserine ("PS") and phosphatidylglycerol ("PG") groups.

Particular embodiments of this invention incorporate various known solvent-based separation methods using the solvent systems here disclosed to be most effective in this novel application. Specific phospholipids can be extracted in high purity from mixtures of phospholipids derived from egg yolks, soya beans or other sources because of the different degrees of solubility of the phospholipids in the solvent used. This invention teaches the novel use of a solvent selected from the group consisting of acetonitrile, and mixtures of acetonitrile and one or more hydrocarbons of the group consisting of pentane, hexane, isohexane, heptane and octane, and mixtures of hydrocarbons such as petroleum ether or mixtures of acetonitrile and fluorocarbons.

The present invention is advantageous in that it is both less time consuming and less costly than other known methods.

2. BACKGROUND OF THE INVENTION

2.1 Phospholipids

Phospholipids, including PC, which is commonly known as lecithin, are members of the class of phosphatides. They are of significant commercial importance because of their wetting and emulsifying properties. They are widely used as ingredients in food products, cosmetics, pharmaceuticals, insecticides, paints, plastics and textiles, and have also found numerous applications in the petroleum industry. Because of its widespread occurrence in nature, PC is known colloquially as "nature's emulsifier." The occurrence of PC as a component of cell membranes has been the subject of much recent scientific research. Emphasis in this research has been on the determination of the physical properties and functional characteristics of PC.

Purified egg phospholipids are currently used as a starting material to synthesize other compounds such as glycerophosphocholine; saturated, unsaturated, single and mixed fatty acids, phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylserines, phosphatidic acids, and diether lipids, etc.

2.2 Phospholipid Purification

At present, high purity PC is typically obtained by time consuming, expensive methods such as high pressure liquid chromatography (HPLC), solid-liquid column chromatography (SLCC), flash chromatography, and thin layer chromatography (TLC).

These methods involve the separation of the lipids, typically by solvent extraction or by other solvent-based techniques. Neutral lipids can be separated from the phospholipid class by precipitation with cold acetone. A form of chromatography is then used to separate the individual lipid components. HPLC and flash chromatography on silica gel or alumina represent the state of the art in chromatography. For example, Jungalwala et al. [Biochem. J. 155:55 (1976)] have described HPLC in silica-gel, using a mixture of acetonitrile, methanol and water as eluant, to separate phosphatidylcholine from sphingomyelin. These methods, because they are relatively faster than conventional column chromatography, permit higher solvent flow-rates through the column (throughput) than are attainable with slow conventional column chromatography. Chromatographic means are, however, generally slow and costly. On a large scale, especially, the large quantity of column packing required and the high associated instrumentation costs limit the use of column chromatography to the separation and purification of only the most valuable and expensive compounds.

U.S. Pat. No. 2,651,646, issued to Goldsmith, discloses a method of purifying monoglycerides from diglycerides, using multiple solvent systems including methanol-hydrocarbon, methanol-water-hydrocarbon, and ethanol-water-hydrocarbon. These systems, how-

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