

- [54] PROCESS FOR PURIFICATION OF PHOSPHOLIPIDS
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- [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.<sup>4</sup> ..... C11C 1/00
- [52] U.S. Cl. .... 260/403; 260/412.4
- [58] Field of Search ..... 260/403, 412.4

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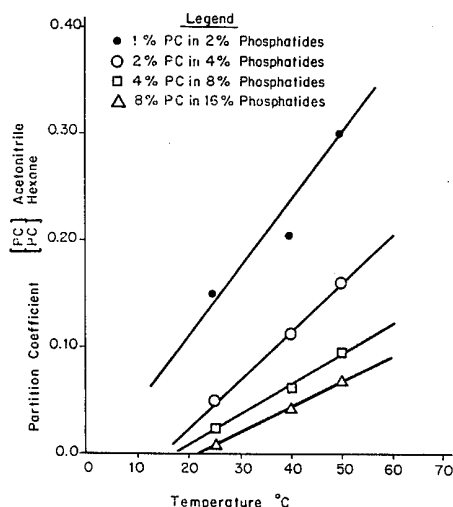
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[57] **ABSTRACT**

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**



Petition for Inter Partes Review  
 Of U.S. Patent 8,278,351

Exhibit

**ENZYMOTEC - 1036**

FIG. 1

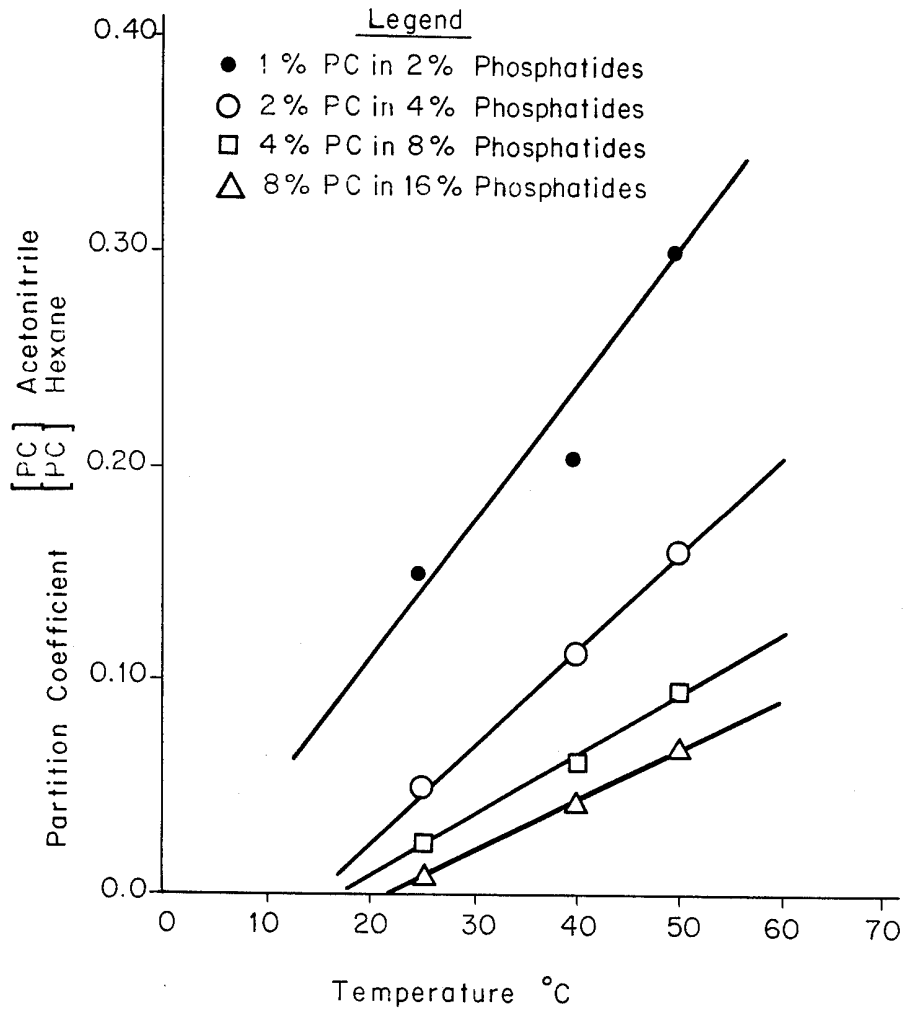


FIG. 2

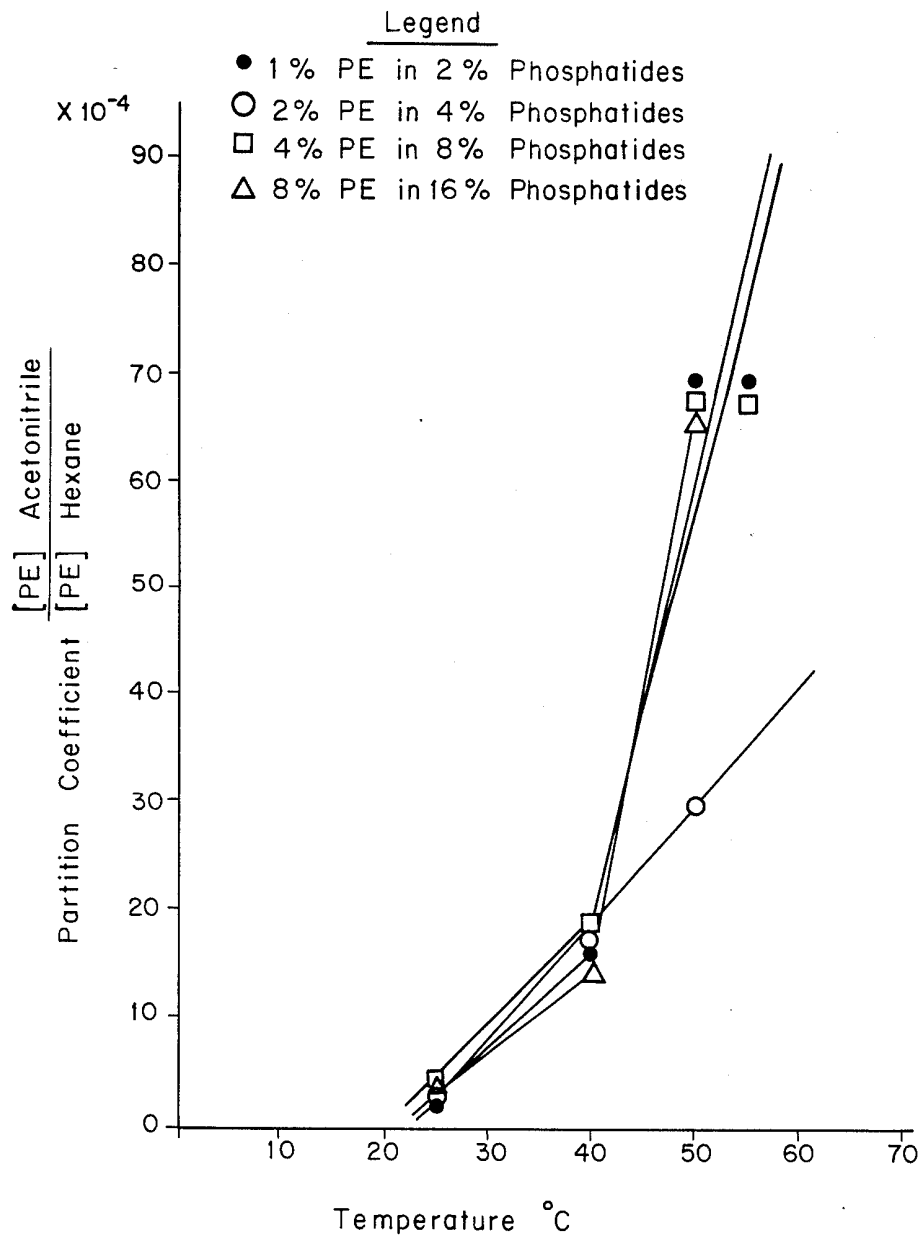
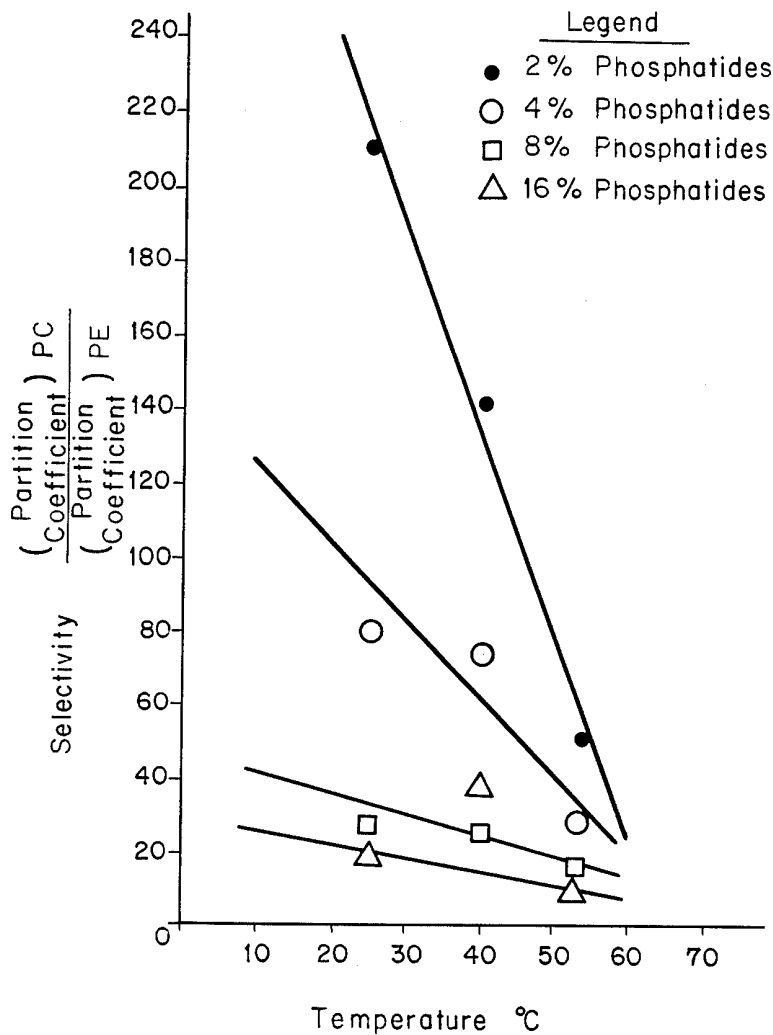


FIG. 3



## PROCESS FOR PURIFICATION OF PHOSPHOLIPIDS

The present application is a continuation-in-part of 5  
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 50  
production of high-purity, individual phospholipids  
from mixtures thereof, by means of separation tech-  
niques utilizing solvents novel for this purpose. More  
specifically, this invention concerns a process for sepa-  
rating and purifying phospholipids, especially those of  
the sub-class of phosphatides, including, but not limited 55  
to the variant fatty acid chain members of the phos-  
phatidylcholine ("PC") or lecithin, phosphatidyletha-  
nolamine ("PE"), phosphatidylserine ("PS") and phos-  
phatidylglycerol ("PG") groups.

Particular embodiments of this invention incorporate 60  
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 65  
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 aceto-  
nitrile and one or more hydrocarbons of the group  
consisting of pentane, hexane, isohexane, heptane and  
octane, and mixtures of hydrocarbons such as petro-  
leum ether or mixtures of acetonitrile and fluorocar-  
bons.

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 phospha-  
tides. 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 "na-  
ture's emulsifier." The occurrence of PC as a compo-  
nent 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, phos-  
phatidylethanolamines, phosphatidylglycerols, phos-  
phatidylserines, 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 pres-  
sure liquid chromatography (HPLC), solid-liquid col-  
umn 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 ace-  
tone. A form of chromatography is then used to sepa-  
rate the individual lipid components. HPLC and flash  
chromatography on silica gel or alumina represent the  
state of the art in chromatography. For example, Jun-  
galwala et al. [Biochem. J. 155:55 (1976)] have de-  
scribed HPLC in silica-gel, using a mixture of aceto-  
nitrile, methanol and water as eluant, to separate phos-  
phatidylcholine from sphingomyelin. These methods,  
because they are relatively faster than conventional  
column chromatography, permit higher solvent flow-  
rates through the column (throughput) than are attain-  
able with slow conventional column chromatography.  
Chromatographic means are, however, generally slow  
and costly. On a large scale, especially, the large quan-  
tity of column packing required and the high associated  
instrumentation costs limit the use of column chroma-  
tography to the separation and purification of only the  
most valuable and expensive compounds.

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

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