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Research Article

Yiwen Rao, Lele Zhou, Zejing Fan, Hongya Li*, Biao Yan, and Xiaoli Zhang

Study on the preparation of glycerylphosphorylcholine by transesterification under supported sodium methoxide

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Abstract: Glycerylphosphorylcholine (GPC) was prepared by transesterification using supported sodium methoxide as catalyst and natural lecithin as raw material. Sodium methoxide has been proved to be an effective catalyst for the preparation of GPC, which is easy to recover and reuse. After six repeated uses, its stability is satisfactory. The effects of agitation speed, catalyst dosage, and reaction temperature on the reaction were investigated, respectively, and the optimum conditions for preparing GPC catalyzed by supported sodium methoxide were found: the concentration of phosphorylcholine was $0.1\,\text{mol}\cdot\text{L}^{-1}$, the stirring speed was 600 rpm, the amount of catalyst was $7.5\,\text{g}\cdot\text{L}^{-1}$, the reaction temperature was 45°C, and the reaction time was 4 h; then, the conversion rate of phosphatidylcholine could reach 99%. At the same time, the reaction kinetic model was established based on the mechanism of the transesterification, and the experimental data were compared with the calculated values; it was found that the experimental data fitted the model well. Finally, the reaction activation energy obtained by the Arrhenius equation is $41.6\,\text{kJ}\cdot\text{mol}^{-1}$, which indicates that the supported sodium methoxide has good catalytic performance in this reaction system.

Keywords: supported catalyst, sodium methoxide, glycerylphosphorylcholine, kinetics

1 Introduction

Glycerylphosphorylcholine (GPC) is a water-soluble phospholipid precursor that is found in the human body, and it is very important for treating brain diseases such as acute cerebrovascular accident, Alzheimer's disease, multi-infarct dementia, and cerebral circulation decline, and it can also improve the attention, cognition, and memory of all ages [1–3]. In addition, GPC has the largest effects on protecting the liver and kidney, maintaining the normal secretion of reproductive hormones, and reducing high blood lipid. So, it is widely used in food, health products, medicine, and other industries, which has greatly improved people's quality of life [4–7]. However, the content of GPC in nature is relatively small, and the domestic research on GPC started late and developed slowly; there is a certain difference in quality scale with foreign products. Therefore, many scholars and companies have invested in the research of preparing and producing high-purity GPC, with the hope that it will bring great medicinal value and economic value [8,9].

There are several methods for preparing GPC, the biological extraction method [10,11], in which the raw material is limited, the process is complicated, and the cost is high; in the chemical synthesis method [12,13], which has many side reactions, the intermediate products are unstable and the product yield is lower, while the transesterification method has attracted widespread attention of scholars at home and abroad due to its natural and reliable raw materials in recent years [14–20]. Chadha [21] used egg yolk lecithin as raw material, added cadmium chloride in the alcoholysis process, and finally obtained a complex of GPC and cadmium chloride through recrystallization, in order to obtain pure product; it still needed further treatment. Kangyi et al. [22] hydrolyzed soybean lecithin powder using phospholipase as a catalyst to obtain GPC, and the product was separated using a chromatography column. The purity and recovery of the final GPC were 99.8% and 69.8%, respectively. Our research group has carried out a series of studies on GPC preparation under different

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*** Corresponding author: Hongya Li,** School of Chemistry and Chemical Engineering, Yulin University, Yulin, Shaanxi 719000, China, e-mail: lihongya0521@163.com
Yiwen Rao, Lele Zhou, Zejing Fan, Biao Yan: School of Chemistry and Chemical Engineering, Yulin University, Yulin, Shaanxi 719000, China
Xiaoli Zhang: School of Chemical Engineering, Northwest University, Xi'an, Shaanxi 710069, China

Table 1: Comparison of the performance of several catalysts

Literature number	Catalyst used	Reaction conditions	Conversion of PC	Advantage and disadvantage
[23]	Low-boiling point organic amines	Catalyst content: 2.4% of the total reaction volume Lecithin concentration: 0.10 mol·L^{-1} Reaction temperature: 60°C Reaction time: 4 h	90%	Advantage: (1) Good activity (2) Easy to separate (3) Little deactivation Disadvantage: Low conversion rate
[24]	Propylamine	Catalyst content: 2.4% of the total reaction volume Lecithin concentration: 0.05–0.10 mol·L^{-1} Reaction temperature: 60°C Reaction time: 4 h	98%	Advantage: (1) Easy to separate (2) Easy to recycle Disadvantage: Short lifespan
[25]	*Tert*-butylamine	Catalyst content: 2.4% of the total reaction volume Lecithin concentration: 0.10 mol·L^{-1} Reaction temperature: 60°C Reaction time: 4 h	98%	Advantage: (1) High purity (2) Little deactivation (3) Long service life Disadvantage: Not easy to recycle
[15]	Calcined sodium silicate	Catalyst content: 6 wt% Lecithin concentration: 10.5 mmol·L^{-1} Reaction temperature: 65°C Reaction time: 2 h	99.50%	Advantage: (1) Easy to separate (2) Repeated use Still maintain high activity (3) High conversion rate

catalysts [23–25], and the properties of several catalysts are shown in Table 1. In the preparation of GPC, the catalyst used affects the difficulty of separation and purity of product directly, and the solid catalyst can be recycled many times and has long service life [26–28], so the choice of catalyst and its preparation process are very important for the preparation of GPC by transesterification.

In this work, GPC is prepared by transesterification using lecithin as raw material, catalyzed by the supported sodium methoxide. The optimal conditions for the reaction were obtained, and the reaction kinetics was discussed in order to provide theoretical basis for its practical application.

2 Experimental sections

2.1 Materials and chemicals

Lecithin was purchased from Tianjin Heowns Biochemical Technology Co., Ltd. All the chemicals such as sodium methoxide, methanol, and chloroform are of analytical grade, and water used is deionized water.

2.2 Preparation of catalyst

A certain amount of activated silica was added to a round-bottomed flask, and the sodium methoxide solution (2.2 mol·L^{-1}) in methanol was poured into it at the ratio of 0.25 g·mL^{-1}, then stirred, and refluxed at 60°C for 4 h, and filtered after cooling. Finally, the supported sodium methoxide catalyst was obtained after dried.

2.3 Transesterification reaction

The transesterification was performed in a 100 mL three-necked flask equipped with a reflux condenser and magnetic stirrer. When the methanol solution of lecithin was heated to the setting temperature, the supported sodium methoxide catalyst was added and the stirrer was started, and liquid samples about 0.5 mL were withdrawn by a syringe in predefined time intervals; the sample solutions were collected in small vials for further analysis.

2.4 Analysis method

Thin-layer chromatography (TLC) was used to analyze the sampling and calculate the conversion of phosphatidylcholine

(PC). Each sample with equal volume was spotted on the TLC plate, which was prepared from our laboratory by silica gel GF254. After drying for several minutes, the plate was developed in the solvent of chloroform:methanol:water = 13:5:0.8 (volume ratio), the developed plate was dried in an oven for about 10 min and detected in the iodine chamber, and then yellow spots with white background appeared after several minutes. The spots were quantified by densitometric scanning at 450 nm using a Shimadzu dual-wavelength chromato scanner CS-930.

3 Results and discussion

3.1 Effect of stirring speed

For heterogeneous reaction processes, the stirring speed affects the mixing, diffusing, and contacting with catalyst of the reactants. Increasing the speed of the stirrer can reduce the effect of external diffusion on the reaction. The concentration of PC is 0.1 mol·L^{-1}, the catalyst dosage is 10 g·L^{-1}, the reaction temperature is 40°C, and the reaction time is 4 h; the effect of stirring speed on the transesterification was examined at 200, 400, 600, and 800 rpm, respectively, and the results are shown in Figure 1.

It was found that with increasing stirring speed, the conversion of PC increased, but when the stirring speed was greater than 600 rpm, the stirring speed had little effect on the conversion of PC, indicating that the catalyst was distributed satisfactory in the reactor at the high stirring speed, and this was considered to be the minimum stirring speed required to eliminate the effect of external diffusion, so the 600 rpm was selected at the subsequent experiments.

3.2 Effect of catalyst amount

As an important factor affecting the chemical reaction, the catalyst not only affects the speed of the reaction and the yield of the product, but also affects the difficulty of purification and separation of subsequent products seriously. Under the conditions of PC concentration of 0.1 mol·L^{-1}, stirring speed of 600 rpm, reaction temperature of 40°C, and reaction time of 4 h, the catalyst amount was investigated at 5, 7.5, 10, and 12.5 g·L^{-1}, and the results are shown in Figure 2.

It can be seen that the conversion of PC increased with the increase in catalyst amount. When the amount of catalyst exceeded 7.5 g·L^{-1}, the amount of catalyst had no significant effect on the conversion of PC. At the end of the reaction, the conversion of PC under catalyst amount of 12.5 g·L^{-1} was only 4% higher than that at 7.5 g·L^{-1}. Therefore, the appropriate amount of catalyst used in the subsequent experiments was 7.5 g·L^{-1}.

3.3 Effect of reaction temperature

The reaction temperature was examined at 30°C, 35°C, 40°C, 45°C, and 50°C, respectively, to discuss its effect on the transesterification process, under the conditions of a

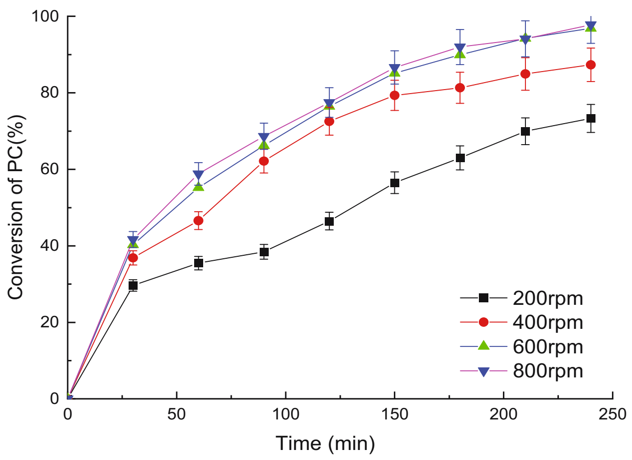


Figure 1: Effect of stirring speed on the conversion of PC. Reaction conditions: the concentration of PC was 0.1 mol·L^{-1}, the catalyst dosage was 10 g·L^{-1}, the reaction temperature was 40°C, and the reaction time was 4 h.

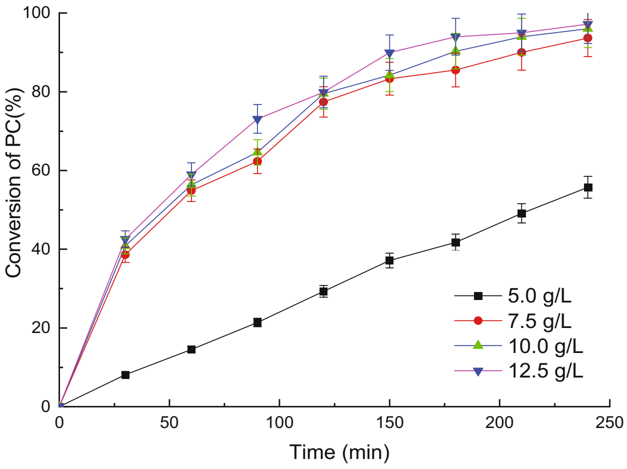


Figure 2: Effect of catalyst amount on the conversion of PC. Reaction conditions: concentration of PC was 0.1 mol·L^{-1}, stirring speed was 600 rpm, reaction temperature was 40°C, and reaction time was 4 h.

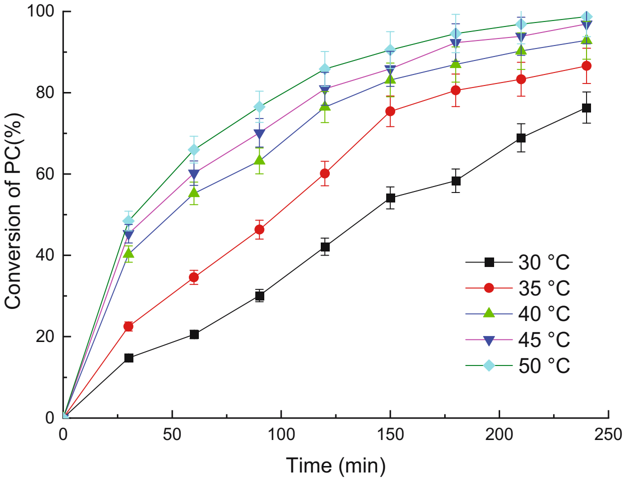


Figure 3: Effect of temperature on the conversion of PC. Reaction conditions: concentration of PC was 0.1 mol·L^{-1}, stirring speed was 600 rpm, catalyst amount was 7.5 g·L^{-1}, and reaction time was 4 h.

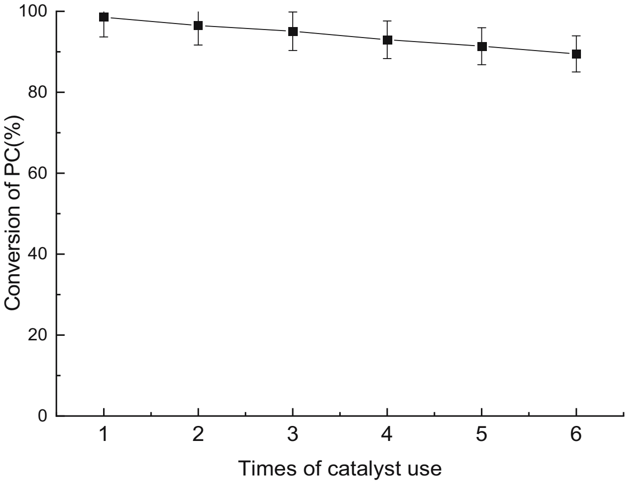


Figure 4: Stability of the catalyst.

concentration of PC of 0.1 mol·L^{-1}, a stirring speed of 600 rpm, a catalyst amount of 7.5 g·L^{-1}, and a reaction time of 4 h. The results are shown in Figure 3.

As can be seen, the conversion of PC increased with increasing temperature. When the temperature rose above 40°C, the increase in the conversion of PC gradually decreased. When the reaction temperatures were 45°C and 50°C, the conversion of PC was basically the same at the end of the reaction. Therefore, 45°C was selected as the most suitable experimental temperature.

3.4 Stability of catalyst

When the transesterification completed under the suitable conditions, the catalyst was filtered off and reused after washing with anhydrous methanol (Figure 4). It was found that only 9% decrease in PC conversion was observed after recycling six times under the same conditions, and the catalyst stability was satisfactory during the experiment.

3.5 Reaction kinetic analysis

The mechanism of the transesterification can be interpreted as shown in Scheme 1. First, sodium methylate dissociates the methoxy ion. In the second step, the nucleophilic methoxy group attacks the carbonyl carbon atom on the PC molecule and forms an intermediate with a tetrahedral structure. In the third step, this intermediate undergoes electron transfer and rearranges to produce lysophospholipid (LPC) negative ions, which combine with protons provided by methanol to form the intermediate LPC. Finally, the methoxy group continues to attack the acyl carbon atoms on the lecithin molecule, and the process is repeated to produce GPC.

The entire transesterification can be expressed as follows:

$$\mathrm{PC} + \mathrm{MeOH} \underset{k_1'}{\overset{k_1}{\rightleftarrows}} \mathrm{LPC} + \mathrm{Ester} \tag{1}$$

$$\mathrm{LPC} + \mathrm{MeOH} \underset{k_2'}{\overset{k_2}{\rightleftarrows}} \mathrm{GPC} + \mathrm{Ester} \tag{2}$$

The first step is assumed to be much faster than the second step, so the intermediate LPC is in equilibrium with the reactants PC and MeOH at any time. Using the steady-state approximation, the concentration of LPC can be expressed as:

$$\frac{\mathrm{d}C_\mathrm{L}}{\mathrm{d}t} = k_1 C_\mathrm{P} C_\mathrm{M} - k_1' C_\mathrm{L} C_\mathrm{E} = 0 \tag{3}$$

so,

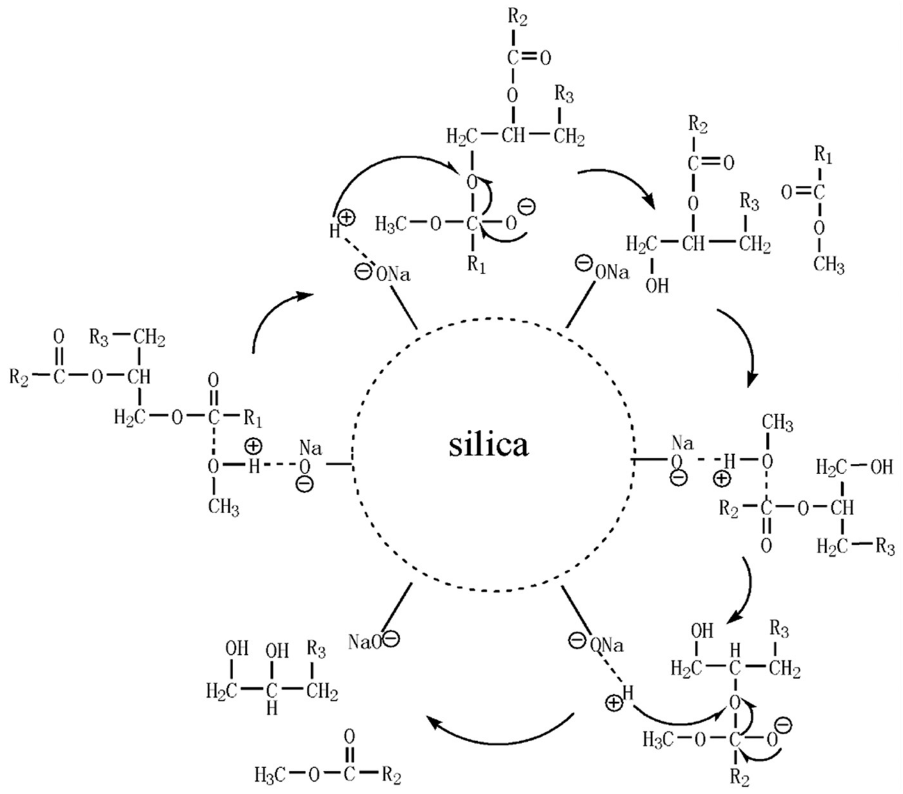
$$C_\mathrm{L} = \frac{k_1 C_\mathrm{P} C_\mathrm{M}}{k_1' C_\mathrm{E}} \tag{4}$$

During the entire reaction process, methanol is greatly excessive, so the reverse reaction can be ignored. The formation rate of GPC can be expressed as:

$$\frac{\mathrm{d}C_\mathrm{G}}{\mathrm{d}t} = k_2 C_\mathrm{L} C_\mathrm{M} \tag{5}$$

Expressing the concentration of each substance by the conversion rate of PC, x_p, so the formula 5 can be changed to:

$$\frac{\mathrm{d}x_\mathrm{P}}{\mathrm{d}t} = \frac{k_1 k_2 (1 - x_\mathrm{P})(C_\mathrm{M0} - 2C_\mathrm{P0} x_\mathrm{P})^2}{k_1' C_\mathrm{P0} 2 x_\mathrm{P}} \tag{6}$$



Scheme 1: Mechanism of transesterification catalyzed by the supported sodium methoxide.

Defined C_{M0}/C_{P0} = M, $K' = k_1 k_2 / k_1'$, then

$$\frac{dx_P}{dt} = \frac{K'C_{P0}(1 - x_P)(M - 2x_P)^2}{2x_P} \tag{7}$$

By integrating the aforementioned formula, we get

$$K'C_{P0}t = Kt = \frac{2}{M-2} \cdot \left[\frac{1}{M-2}\ln\left(\frac{M-2x_P}{1-x_P}\right) - \frac{M}{2(M-2x_P)}\right] \tag{8}$$

The experimental data under different catalyst amounts were substituted into the right part of formula 8 and plotted against the reaction time. It was found that the data fit the equation model well (Figure 5).

Similarly, the experimental data at different reaction temperatures were substituted into the right part of formula 8 and plotted against the reaction time (Figure 6). The linear correlation coefficient R^2 of each fitted line is above 0.97, which indicates that the kinetic equation model is

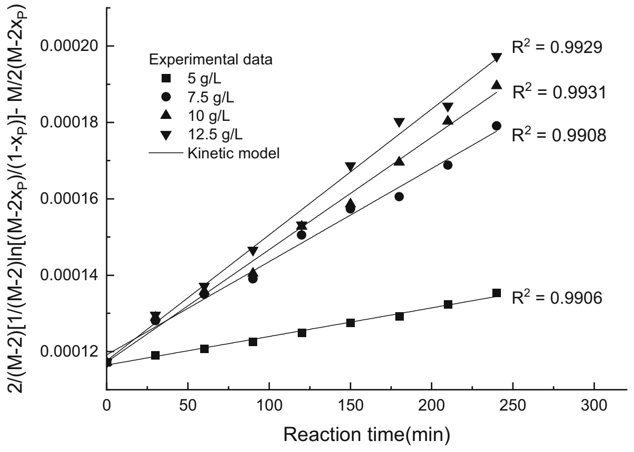


Figure 5: Fitting of reaction kinetics at different catalyst amounts.

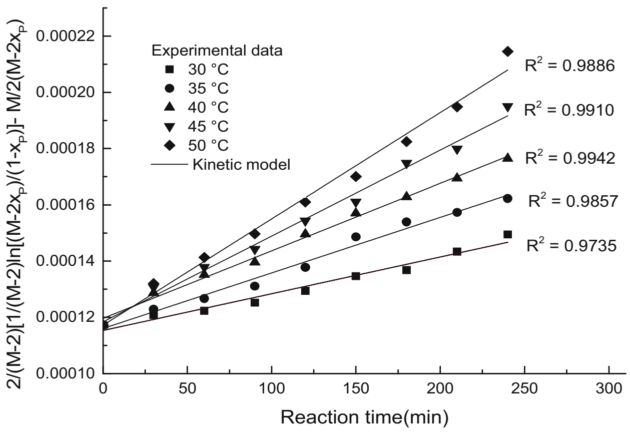


Figure 6: Fitting of reaction kinetics at different reaction temperatures.

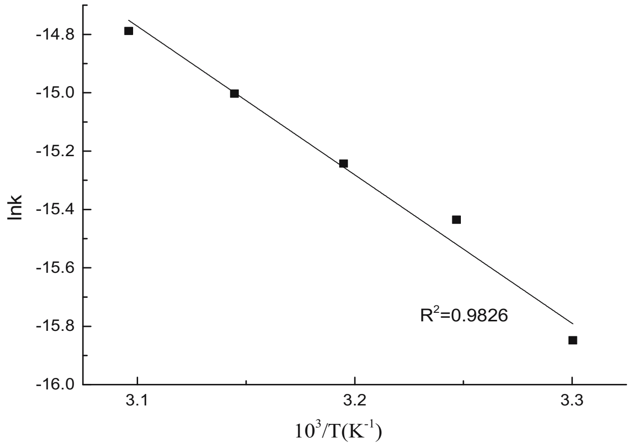


Figure 7: Arrhenius plot of ln *K* vs $10^3/T$.

equally applicable for the reaction systems at different temperatures.

According to the Arrhenius equation, the logarithms of the slope *k* for the dynamic fit curves at different temperatures were plotted against $1/T$ (Figure 7). From the slope of the straight line, the activation energy E_a is 41.6 kJ·mol^{-1}, indicating that the catalyst has good catalytic performance in their action system.

4 Conclusions

The supported sodium methoxide catalyst has achieved significant results in the preparation of GPC by transesterification. Under the optimum reaction conditions, the amount of catalyst is 7.5 g·L^{-1}, the reaction temperature is 45°C, stirring rate is 600 rpm, and reaction time is 240 min, and the conversion rate of PC is 98.9%. The reaction kinetic model was established, and it was found that the experimental data fitted the model well. The activation energy of the reaction was 41.6 kJ·mol^{-1}, and the catalytic performance of the supported sodium methoxide in this reaction system is satisfactory.

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Conflict of interest: The authors state no conflict of interest.

Data availability statement: All data generated or analysed during this study are included in this published article.

References

[1] Wang Z, Hazen J, Jia X, Org E, Zhao Y, Osborn LJ, et al. The nutritional supplement L-alpha glyceryl phosphorylcholine promotes atherosclerosis. Int J Mol Sci. 2021;22(24):13477. doi: 10.3390/IJMS222413477.

[2] Catriona S, Stephanie P, Jean S, Michal A, Gabriel L, Michel P, et al. Visceral fat-related systemic inflammation and the adolescent brain: a mediating role of circulating glycerophosphocholines. Int J Obes. 2019;43(6):1223–30. doi: 10.1038/s41366-018-0202-2.

[3] Ferry L, Massa A, Mola AD, Diehl B. An effective method for the determination of the enantio-purity of l-α-glycerylphosphorylcholine (l-α-GPC). J Pharm Biomed. 2020;183:113152. doi: 10.1016/j.jpba.2020.113152.

[4] Feng J, Yang W, Lu Y, Li H, Xu S, Wang X, et al. Enhancing soluble expression of phospholipase B for efficient catalytic synthesis of L-alpha-glycerylphosphorylcholine. Catalyst. 2022;12(6):650. doi: 10.3390/CATAL12060650.

[5] Lee G, Choi S, Chang J, Choi D, Son J, Kim K, et al. Association of L-α Glycerylphosphorylcholine with Subsequent Stroke Risk After 10 Years. JAMA Netw Open. 2021;4(11):e2136008. doi: 10.1001/jamanetworkopen.2021.36008.

[6] Che H, Zhou M, Zhang T, Zhang L, Ding L, Yanagita T, et al. Comparative study of the effects of phosphatidylcholine rich in DHA and EPA on Alzheimer's disease and the possible mechanisms in CHO-APP/PS1 cells and SAMP8 mice. Food Funct. 2018;9(1):643–54. doi: 10.1039/C7FO01342F.

[7] Reynolds S, Calvert SJ, Paley MN, Pacey AA. 1H magnetic Resonance Spectroscopy of live human sperm. Mol Hum Rep. 2017;23(7):441–51. doi: 10.1093/molehr/gax025.

[8] Shi T, Wang Z, Liu Y, Jia S, Changming D. Removal of hexavalent chromium from aqueous solutions by D301, D314 and D354 anion-exchange resins. J Hazard Mater. 2008;161(2–3):900–6. doi: 10.1016/j. jhazma.2008.04.041.

[9] Ichihara K, Iwasaki H, Ueda K, Takizawa R, Naito H, Tomosugi M, et al. Synthesis of phosphatidylcholine: An improved method without using the cdmium chloride complex of Sn-glycero-3-phosphocholine. ChemPhys Lipids. 2005;137(1–2):94–9. doi: 10.1016/j.chemphyslip.2005.06.001.

[10] Schmidt G, Hershman B, Thannhauser SJ. The isolation of glycerylphosphorylcholine from Incubated Beef. Pancreas: its significance for the intermediary metabolism of Lecithin. J Biol Chem. 1945;161(2):523–36. doi: 10.1016/S0021-9258(17)41488-8.

[11] Wallace JC, Wales RG, White IG. The respiration of the rabbit epididymis and Its synthesis of glyceryl phosphorylcholine. Aust J Biol Sci. 1966;19(5):849–56. doi: 10.1071/BI9660849.

[12] Longhui W, Binglin L, Xiaoli Z, Kai T, Wenwen Z, Dan C, et al. Choline derivatives immobilized on silica to catalyze transesterification reaction for production of glycerophosphocholine. Turkish J Chemisrty. 2020;44(1):249–60. doi: 10.3906/kim-1907-43.

[13] Song YS, Song ES, Kang DS, Song IW. P. G. A process for preparation of L-alpha-glycerylphosphorylcholine. World Intellect Property Organ. 2007;WO145476(A1).

[14] Zhang T, Zhang X, Li H, Bai W, Zhao B. Kinetic evaluation of the ethanolysis of phosphatidylcholine catalyzed by choline hydroxide. React Kinet Mech Cat. 2013;110(1):31–9. doi: 10.1007/s11144-013-0579-9.

[15] Li B, Li H, Zhang X, Fan P, Liu L, Li B, et al. Calcined sodium silicate as an efficient and benign heterogeneous catalyst for the transesterification of natural lecithin to L-α-glycerylphosphorylcholine. Green Process Synth. 2019;8(1):78–84. doi: 10.1515/gps-2017-0190.

[16] Li H, Zhang X, Yan B, Zhao B, Gao J. Preparation and catalytic performance of quaternary ammonium base resin for methanolysis of natural phosphatidylcholine. Chem Pap. 2016;70(6):706–12. doi: 10.1515/chempap-2016-0023.

[17] Eszter T, Renáta G, Olias MI, Kamilla G, Bernadett K, Gerda S, et al. L-Alpha-glycerylphosphorylcholine can be cytoprotective or cytotoxic in neonatal rat cardiac myocytes: a double-edged sword phenomenon. Mol Cell Biochem. 2019;460(1):195–203. doi: 10.1007/s11010-019-03580-1.

[18] Bang HJ, Kim IH, Kim BH. Phospholipase A1-catalyzed hydrolysis of soy phosphatidylcholine to prepare l-α-glycerylphosphorylcholine in organic-aqueous media. FoodChem. 2016;190:201–6. doi: 10.1016/j.foodchem.2015.05.093.

[19] Li H, Zhang X, Zhang J, Zhang T, Zhao B. Preparing l-α-glycerylphosphorylcholine in a phase-transfer catalytic reaction: kinetic study. Int J Chem Kinetic. 2014;46(3):169–75. doi: 10.1002/kin.20840.

[20] Li H, Yan B, Ma Y, Ma X, Zhang X, Zhao B. Adsorption of L-α-glycerophosphocholine on ion-exchange resin: Equilibrium, kinetic, and thermodynamic studies. Green Process Synth. 2020;9(1):275–82. doi: 10.1515/gps-2020-0032.

[21] Chadha JS. Preparation of crystalline L-α-glycerophosphoryl-choline-cadmium chloride adduct from commercial egg lecithin. Chem Phys Lipids. 1970;4(1):104–8. doi: 10.1016/0009-3084(70)90067-8.

[22] Kangyi Z WangX, Huang J LiuY. Purification of l-alpha glycerylphosphorylcholine by column chromatography. J Chromatogr A. 2012;1220:108–14. doi: 10.1016/j.chroma.2011.12.003.

[23] Li HY, Zhang XL, Bai WL, Zhang JB, Zhang TT, Zhao BX. Study on the preparation of L-α-glycerophosphocholine catalyzed by low boiling point organic amines. Reac Kinet Mech Cat. 2013;108(2):305–16. doi: 10.1007/s11144-012-0509-2.

[24] Li HY, Zhang TT. L-α-glycerophosphocholine from natural lecithin via transesterification catalyzed by propylamine. Adv Mater Res. 2014;997:73–6. doi: 10.4028/www.scientific.net/AMR.997.73.

[25] Li HY, Zhang XL, Bai WL, Zhao BX. Preparation of L-α-glycerophosphocholine from natural lecithin catalyzed by tert-butylamine. AMR. 2013;2216(641–642):148–51. doi: 10.4028/www.scientific.net/AMR.641-642.148.

[26] Hirokazu K, Atsushi F. Development of solid catalyst–solid substrate reactions for efficient utilization of biomass. Bull Chem Soc Jpn. 2018;91(1):29–43. doi: 10.1246/bcsj.20170263.

[27] Changmai B, Sudarsanam P, Rokhum SL. Biodiesel production using a renewable mesoporous solid catalyst. Ind Crop Products. 2020;145:111911. doi: 10.1016/j.indcrop.2019.111911.

[28] Krishnan SG, Pua FL, Zhang F. A review of magnetic solid catalyst development for sustainable biodiesel production. Biomass Bioenergy. 2021;149:106099. doi: 10.1016/j.biombioe.2021.106099.