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BIOCATALYSIS

Enzymatic Synthesis of Polyethylene Glycol-400 Esters

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Abstract—Esters of fatty acids (FAs) and polyethylene glycol-400 (PEG) are valuable chemically derived surfactants. In this work, it is shown that the chemical synthesis of PEG esters can be replaced by enzymatic synthesis under milder conditions and using simpler and more environmentally friendly technology. The main obstacles to enzymatic catalysis in a system of PEG and higher FAs are the high viscosity of the former and the low water solubility of the latter. These problems are solved by selecting organic media and other conditions of the process mediated by pancreatic lipase. The optimum conditions for the synthesis of PEG esters of FAs are determined: reaction medium, benzene/hexane in a ratio of 2 : 3; optimum temperature, 25°C; water content in the system, no more than 0.2%; FA : PEG molar ratio, 1 : 1.8; reaction time, 48 h. Under these conditions, the yields in synthesizing PEG esters of capric, lauric, and palmitic acids are 80, 78, and 44%, respectively.

Keywords: polyethylene glycol-400, fatty acids, biocatalyst in nonaqueous solvents, pancreatic lipase
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INTRODUCTION

Surfactants based on polyethylene glycol (PEG) are used on a large scale in the pulp-and-paper, textile, leather, fur, and chemical industries, in the production of pharmaceutical and cosmetic preparations, emulsion concentrates, and pesticide powders, in the manufacture of varnishes, dyes, and plastics, and for the purification and processing of metals. The strong cleansing and washing abilities of these surfactants are advantageously combined with moderate foamability; their wetting, emulsifying, dispersing, and stabilizing properties allow us to use these materials for a variety of purposes [1]. In addition, esters of fatty acids (FAs) and PEG form the basis for the synthesis of various chemical products.

In industry, FA esters are prepared via chemical synthesis at temperatures above 100°C and pressures of 1.3–2.0 kPa. Shifting to biotechnological methods allows us to implement these processes under milder conditions using simpler and more environmentally friendly technology [2–5]. However, the use of enzymatic synthesis in the esterification of PEG is hindered by the high viscosity of PEG and the low water solubility of FAs.

In this work, we present the results from investigating the synthesis of PEG esters in nonaqueous organic media, benzene and hexane. These solvents were used earlier to study the esterification and hydrolysis of vegetable oils using an enzyme preparation of pancreatic lipase. The choice of this enzyme was based on it being one of the most extensively studied lipolytic enzymes. It was shown that pancreatic lipase in organic media maintains its enzymatic activity and ensures the high-efficiency synthesis of esters and hydrolysis of vegetable oils [6–8].

EXPERIMENTAL

The following materials were used in this work: a commercial preparation of B pancreatic lipase (activity of 180 units/mg; manufactured at the Tukums Enzyme Plant (Tukums, Latvia)); PEG-400; palmitic, lauric, and capric acids (reagent grade); and hexane and benzene (reagent grade).

The lipolytic activity of pancreatic lipase was tested using the standard method proposed by Ota and Yamada in [9]. This method is based on using alkaline titration to determine the amount of FAs formed under the action of lipase, employing olive oil as a substrate.

One unit of lipase enzyme activity is defined as the amount of enzyme that releases 1 μmol of oleic acid from a 40% emulsion of olive oil at a pH of 7.0 and a rise in temperature of 37°C per hour.

The reaction system was a slurry of the enzyme preparation—pancreatic lipase—in an organic medium with a low water content. For repeated use, the enzyme can be separated from the reaction mixture by filtering it through Whatman filter paper (grade 1), or by centrifuging it at a speed of 3000 rpm for 10 min. However, these studies did not involve repeated use of the enzyme preparation.

In a typical experiment, the acid concentration was 0.05 M, while the PEG concentration was 0.05–5 M.

Table 1. Effect of the reaction medium on the yield of PEG ester of lauric acid in the presence of pancreatic lipase

Reaction medium	Ester yield, %
Benzene	33.0 ± 0.5
Benzene/hexane (1 : 4, vol/vol)	**55.0 ± 0.3**
Hexane	34.0 ± 0.5
Benzene/PEG (1 : 1, vol/vol)	14.0 ± 0.1
PEG	1.0

Reaction conditions: temperature, 25°C; synthesis time, 48 h.

The enzyme was introduced in doses of 5 mg per milliliter of reaction medium. The dry or dampened enzyme preparation (the amount of added water varied from 0.2 to 1%) was admixed with a solution of the reactants in an organic solvent: benzene, hexane, or a benzene–hexane mixture. The resulting slurry was stirred thoroughly and held without stirring for 24–100 h.

The amount of the resulting ester was determined via titration, according to the change in the amount of acid in the system. Titration was conducted with a 0.1 N alcoholic solution of NaOH (in 80% alcohol) until we obtained a constant pink color with a 1% alcohol solution of phenolphthalein as an indicator at a temperature of 20°C. The reference sample did not contain the enzyme preparation; the enzyme was added to it immediately before titration.

The ester yield (B, %) was calculated using the formula

$$B = \frac{(K - O)}{K} \times 100\%,$$

where O is the amount of the 0.1 N alcoholic solution of NaOH consumed for titration of the sample, mL, and K is the amount of the 0.1 N alcoholic solution of NaOH consumed for titration of the reference sample, mL. To isolate the product after the reaction, the suspended enzyme was filtered through Whatman filter paper (grade 1) and washed with double or triple the amount of solvent. The solution containing the product was boiled down, dried in a desiccator over calcined $CaCl_2$, and then used for analysis.

The formation of ester was confirmed via thin-layer chromatography (TLC), IR spectroscopy, and a qualitative test with bromthymol blue [10, 11]. Silufol plates were used for TLC. Chromatography was conducted using the ascending method in a hexane : diethyl ether : glacial acetic acid system of solvents in the ratio of 60 : 60 : 1.

All experiments were repeated 3–12 times; most of them, at least 6 times. The experiment was repeated 3 times only if further studies were considered unnecessary. The results were processed using the STATISTICA software package. The confidence level for differences between the reference and experimental data was estimated using Student's t-test. The measurement error did not exceed 7%.

RESULTS AND DISCUSSION

Selecting the Medium for Enzymatic Synthesis

At the first stage of this work, we explored the possible enzymatic synthesis of esters in a solvent-free PEG medium. However, our studies showed that the reaction in a PEG medium occurs at an extremely slow rate, due apparently to the high viscosity of the solution. Using the example of the synthesis of PEG ester of lauric acid, we therefore considered several solvents as possible reaction media: benzene, hexane, a benzene–hexane mixture (1 : 4), and a benzene–PEG mixture (1 : 1). The data are shown in Table 1.

Benzene is the best of the studied solvents for the precursors; however, the reaction occurs slowly in this medium, and the ester yield in a 48-h reaction is as low as 33%; this can be attributed to the strong inhibitory effect of pure benzene on pancreatic lipase. The inhibitory effect exerted on the enzyme by hexane is not as strong as that of benzene; however, the yield from the reaction in hexane is also low owing to the poor solubility of PEG in hexane. A mixed medium (benzene/hexane) was found to be best for the synthesis of PEG esters: the product yield was 55%. The use of a mixture of organic solvents, one of which thoroughly dissolves the precursors and the other helps maintain the enzymatic activity, thus allowed us to conduct the enzymatic processes under study.

The effect of the benzene/hexane solvent ratio on the yield of target product was then studied (Table 2).

Table 2. Effect of a reaction medium on the yield of PEG ester of lauric acid in the presence of pancreatic lipase

Reaction medium: benzene/hexane (vol/vol)	System	Ester yield, %
1 : 4	Emulsion	55.0 ± 2.0
2 : 3	Stable emulsion (does not separate within 60 min)	**77.0 ± 2.0**
1 : 1	Emulsion (does not separate for more than 24 h)	70.0 ± 2.0
3 : 2	Colloidal solution (opalescent)	53.0 ± 2.0
4 : 1	True solution (nonopalescent)	54.0 ± 2.0

Reaction conditions: temperature, 25°C; synthesis time, 48 h.

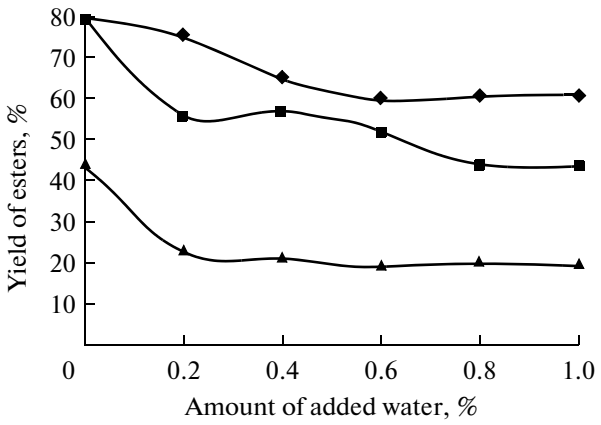

Fig. 1. Effect of the content of water in the system on the yield of PEG esters of (◆) capric, (■) lauric, and (▲) palmitic FAs. Reaction conditions: 25°C; FA : PEG ratio, 1 : 1.8; medium, benzene/hexane in a ratio of 2 : 3; process time, 48 h.

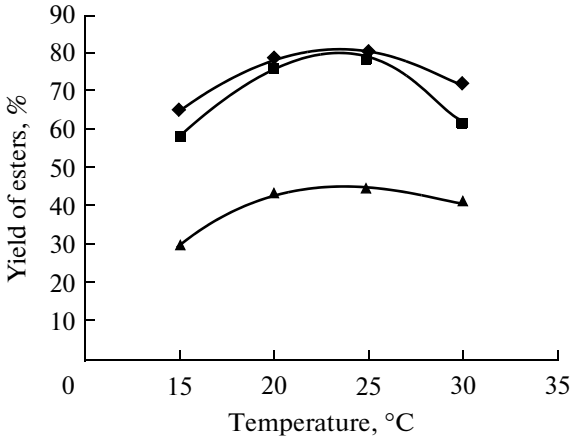

Fig. 2. Effect of the temperature on the yield of PEG esters of (◆) capric, (■) lauric, and (▲) palmitic FAs. Reaction conditions: FA : PEG ratio, 1 : 1.8; medium, benzene/hexane in a ratio of 2 : 3; process time, 48 h.

Our results showed that the best medium for the esterification of PEG with lauric acid was a benzene–hexane mixture with a ratio of 2 : 3. Even though use of this medium results in the formation of a stable emulsion (rather than the complete dissolution of the reactants), the ester yield in this medium is the highest: 77%. Based on these data, enzymatic synthesis in subsequent experiments was conducted using a benzene–hexane mixture with a ratio of 2 : 3.

The data of Table 2 clearly show that despite the improvement in the solubility of the substrates, increasing the benzene content in the medium lowers the yield of esters, due to the inhibitory effect of benzene on the enzyme.

Optimizing the Ratio of Reactants

Analysis of the effect of the reactant molar ratio (PEG : FA) on the efficiency of the esterification reaction showed that a ratio of 1 : (1.5–2.0) is optimal for synthesizing PEG esters of capric, lauric, and palmitic acids. The ester yield falls considerably as the molecular weight of the acid rises; this can be attributed to the lower solubility of our solvent system.

Esterification was conducted using excess PEG with respect to FA in order to ensure a higher yield of the target product. According to our studies, the synthesis of most of the esters catalyzed by lipases of different origin in organic media requires the excess carrier of the –OH group. This excess apparently protects the enzyme from the adverse effect of organic solvents [6–8].

To exhibit maximum activity, pancreatic lipase must have a certain hydration sheath. However, introducing water into the system lowers the yield of PEG esters (Fig. 1). The water contained in the enzyme preparation (humidity of the enzyme preparation, 10%) is evidently sufficient to maintain its catalytically active conformation, while increasing the water content in the system (0.2–0.4% added water) enhances the hydration of the PEG chains that wrap around the enzyme and thus hinders the progress of the enzymatic process. Introducing additional water into the system also shifts the reaction equilibrium toward the formation of the precursors.

Effect of the Temperature and Reaction Time on the Ester Yield

The temperature dependence of the yield of products from the reaction between FA and PEG catalyzed by pancreatic lipase is shown in Fig. 2. It is evident that the optimum temperature for the synthesis of lauric acid ester lies in the range of 20–25°C. In the synthesis

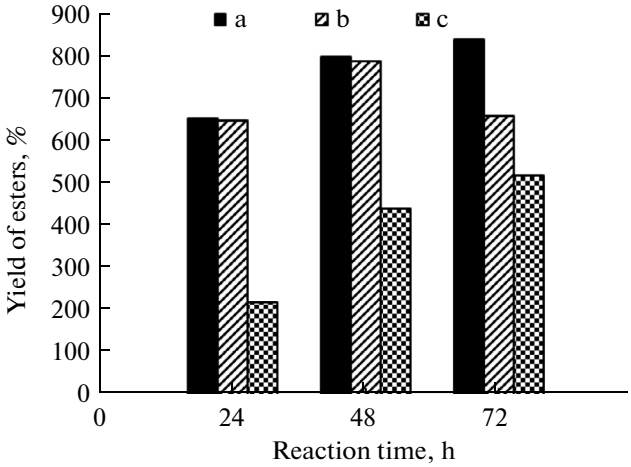

Fig. 3. Effect of the reaction time on the yield of PEG esters of (a) capric, (b) lauric, and (c) palmitic FAs. Reaction conditions: 25°C; FA : PEG ratio, 1 : 1.8; medium, benzene/hexane in a ratio of 2 : 3.

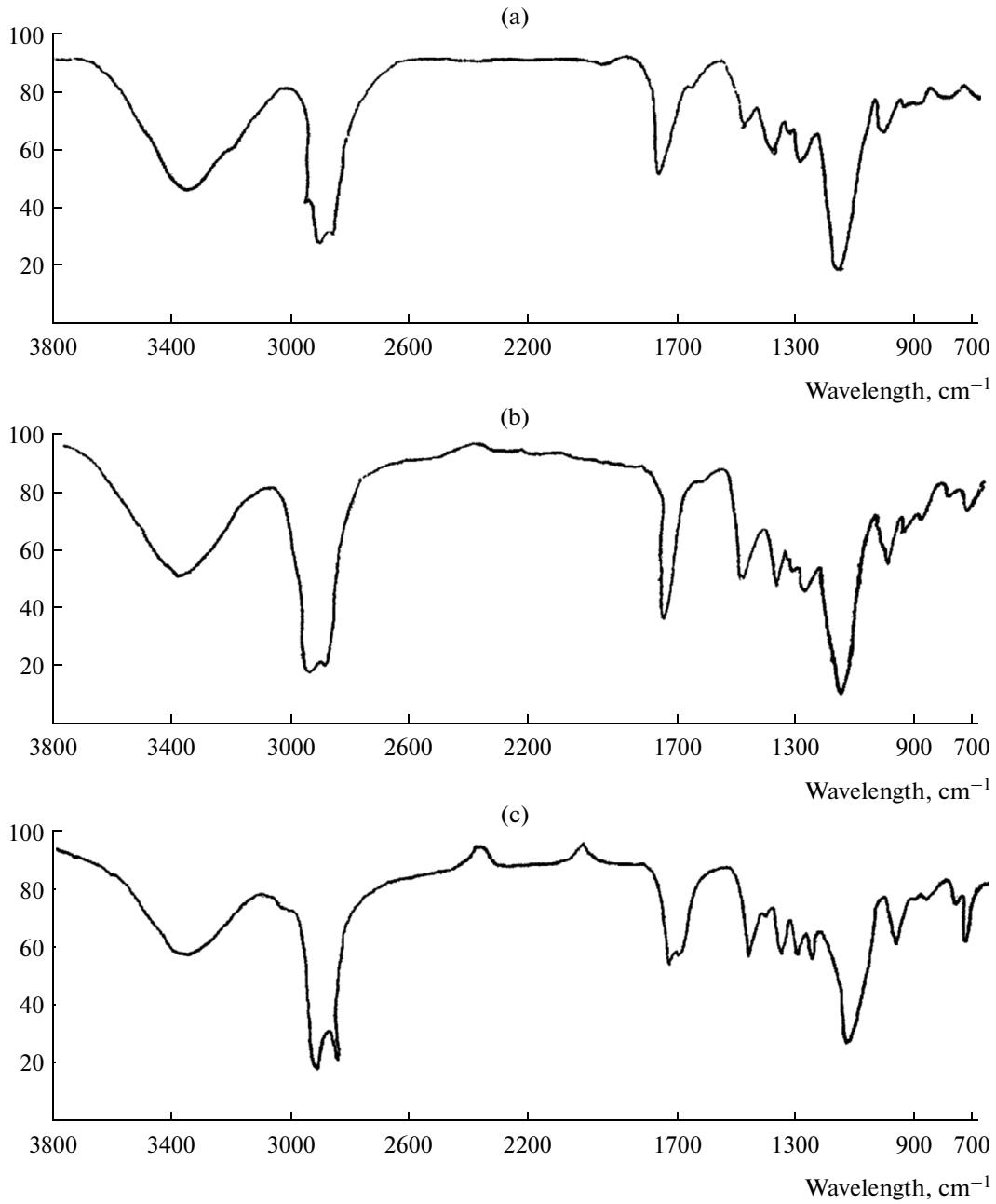

Fig. 4. IR spectra of PEG-400 esters of (a) capric, (b) lauric, and (c) palmitic FAs.

of other esters, the reaction rate in the temperature range of 20–30°C varies only slightly. This dependence can apparently be attributed to the complicated effect of the temperature and the organic solvent on the reactants.

Figure 3 shows the dependence of the yield of esters of capric, lauric, and palmitic acids on the time of the enzymatic reaction. Within 48 h, the yield of PEG esters of capric and lauric acids reaches high values of 80 and 78%, respectively. The synthesis of PEG ester of palmitic acid involving pancreatic lipase occurs much more slowly: in a 72-h reaction, the yield can be as low as 52%. In all cases, prolonging the reaction time from 48 to 72 h did not greatly increase the yield of esters; the recommended process time is therefore 48 h.

It was found that stirring has no considerable effect on the synthesis of PEG esters, so stirring was not used further; it was obvious that diffusion does not limit the synthesis of esters.

The formation of PEG esters of FAs was confirmed analytically, using a qualitative test with bromthymol

blue, TLC, and IR spectroscopy. The IR spectroscopy data are shown in Fig. 4.

Our qualitative test with bromthymol blue was based on the color of bromothymol blue changing from blue to yellow after adding a nonionic surfactant (an ethylene oxide derivative) to a bromothymol blue solution in the presence of a buffer solution (phosphate buffer; pH 8.4). This reaction is not typical of pure PEGs [10]. A qualitative test was conducted after isolating PEG esters of FAs. Adding the resulting esters to the bromothymol blue solution changed its color from blue to yellow. A reference sample containing pure PEG and the respective acid did not change color.

Analysis of the resulting samples by TLC showed that the test samples contained three new compounds not found in the reference samples. The presence of the three compounds in the test samples can be attributed to the PEG being characterized by chemical heterogeneity and containing molecules with different molecular weights. This accordingly led to the synthesis of esters with different molecular weights.

The IR spectra of the synthesized compounds exhibited a high-intensity band in the region of 1750–1730 cm^{-1} (peaks at 1740–1745 cm^{-1}) corresponding to ν(C=O) stretching vibrations. The high-intensity band characteristic of ν(C–O) stretching vibrations in esters is found in the region of 1200–1150 cm^{-1}. The intense band characteristic of ν(C–O–C) stretching vibrations is observed in the region of 1150–1060 cm^{-1} (peaks at 1130 cm^{-1}). A broad, complex medium-intensity band characterizing the vibrations of the associated OH groups is seen in a region of 3600–3400 cm^{-1} (peaks at 3370 cm^{-1}) [11]. The IR spectra thus indicate ester bonds were present in the resulting compounds, confirming the formation of PEG esters of FAs.

CONCLUSIONS

It was shown that PEG esters of FAs can be synthesized using pancreatic lipase in organic media. Optimum conditions for the synthesis of PEG esters of FAs were determined: reaction medium, benzene/hexane in the ratio of 2 : 3; optimum temperature, 25°C; water content in the system, no more than 0.2%; reaction time, 48 h; no stirring. Under these conditions, the yield in the enzymatic synthesis of PEG esters of capric, lauric, and palmitic acids was 80, 78, and 44%, respectively.

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