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Emulsifiers from solid and liquid polyols: different strategies for obtaining optimum conversions and selectivities

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Abstract

The present paper provides a general overview of the factors involved in both the kinetics and the selectivity of partial acylation reactions of polyols (sugars). Different kinetic strategies for maximum production of intermediate esters of various polyols and monosaccharides are reported and discussed. Physicochemical requirements for obtaining maximum selectivities and complementary strategies for reducing reaction times are discussed. The reactions studied include glycerol, glucose, fructose, mannose, sorbitol and an alkyl glucoside as precursors. The high selectivity towards the monoglyceride in the presence and absence of a solvent has been attributed to a combination of the precipitation of the desired ester, use of glycerol in excess and the relatively low solubility of the fatty acid in the system. Unlike the reaction in the presence of a solvent, the reaction in a solvent-free medium produces the diester first. The monoester only accumulates in the medium as a consequence of disproportionation and glycerolysis reactions of the diester formed earlier. Selective esterification of solid sugars (polyols) which have an intermediate solubility in acetone is favored at low temperatures at which a sufficient amount of polyol dissolves and concurrent precipitation of the desired product can be achieved. By contrast, use of elevated temperatures is more appropriate for selective partial esterification of polyols, which are the most soluble in the solvent employed. Polyols (sugars) which are the less soluble into the liquid reaction phase cannot be easily esterified. Diffusional limitations on the rate of dissolution of the solid precursor can be minimized by increasing the surface area of the solid polyol.

Keywords: Lipase; Emulsifier; Sugar; Polyol; Sorbitol

1. Introduction

Non-ionic surfactants obtained by partial esterification of solid and liquid polyols, sugars, and sugar alcohols with fatty acids have very good emulsifying, stabilizing or conditioning effects. They find wide application as emulsifiers in the food, cosmetic, pharmaceutical and detergent industries. Both technical and regulatory (FDA, European Union, etc.) factors provide incentives for developing improved processes for the synthesis of emulsifiers, especially those used in food-related and pharmaceutical applications.

Selective manufacture of non-ionic emulsifiers via consecutive esterification reactions frequently requires optimization of the production of a desired

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reaction intermediate. Although a selective biocatalyst is not necessarily essential, reaction conditions that maximize the production of the desired intermediate are required. The mild conditions under which enzymes function should permit one to obtain strict kinetic control of reactions occurring in series, e.g., sequential acylation of both solid and liquid polyols and sugars.

Enzymatic synthesis of emulsifiers via direct esterification of solid polyols and sugars can be problematic because of differences in the polarities of the precursor reagents (fatty acid and polyol). Complete incorporation of the polyol in the liquid reaction medium (from the beginning of the reaction [1,2] or during the process [3,4]) is required to obtain maximum conversion of this material.

In this paper, different kinetic strategies for the maximum production of the desired species are reported and discussed. Relevant differences in the requirements for reaction of various solid and liquid polyols (sugars) are addressed. Physicochemical requirements for obtaining maximum selectivities and complementary strategies for reducing reaction times and requirements for product purification/extraction are discussed. The reactions studied include glycerol, glucose, fructose, mannose, sorbitol and an alkyl glucoside as precursors. Determinations of their corresponding requirements have been carried out to find relationships between the characteristics of precursors/products and the corresponding requirements for selective partial esterification.

2. Experimental

2.1. Materials

Lipase from *Candida antarctica* (lipase B, a non-specific lipase immobilized on a macroporous acrylic resin, designated «Novozym 435») was kindly provided by Novo Nordisk (Bagsvaerd, Denmark). Lipase PS was supplied from Amano Pharmaceuticals (Nagoya, Japan). Celite for gas–liquid chromatography [30–80 mesh (0.18–0.59)] from BDH (Poledorset, England). Anhydrous glucose, fructose, *n*-hexane and 2-propanol of high-performance liquid chromatography (HPLC) quality were purchased from Merck (Darmstadt, Germany). Acetonitrile, acetone, methanol and dioxane were of HPLC quality and were supplied by Scharlau (Barcelona, Spain). Prior to use, all solvents were dried with molecular sieves. Glycerol, sorbitol, L-lactic acid ethyl ester, *n*-octyl β-D-glucopyranoside, molecular sieves (effective pore diameter = 4 Å), and caprylic, lauric, palmitic and oleic acids (more than 99% pure) were from Sigma (St. Louis, MO). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Lipase-catalyzed esterification reactions

Reactions were carried out by mixing the indicated amounts of the corresponding reagents (fatty acid and glycerol or monosaccharide or sorbitol or *n*-octyl β-D-glucopyranoside) in stoppered glass bottles. After addition of 1 ml of an appropriate solvent and enzyme, the bottles were shaken at 200 rpm on a thermoconstanter orbital shaker (Stuart Scientific, Surrey, England) for the desired time at a specified temperature. When indicated, molecular sieves were added to the reaction mixture to increase the yields of the desired product. The molar yields indicated in all figures in this paper were calculated with respect to the less concentrated reactant.

2.2.2. Purification of the esters

Monoesters of glycerol, glucose, and *n*-octyl β-D-glucopyranoside and diesters of sorbitol and fructose were prepared in large amounts, purified, and used as standards for the HPLC analyses. Mono- and diesters of glycerol, monosaccharides and sorbitol were purified as previously described [5–8].

Purification of *n*-octyl β-D-glucopyranoside mono- and dilactate. The enzyme and the molecular sieves were removed from the reaction mixture via filtration through a 0.1-mm sieve, and the solvent was then evaporated. The resulting solid was dissolved by addition of sufficient dimethyl formamide (DMF) to produce a final concentration of 30 mg/ml. Further purification was accomplished in a semi-preparative HPLC fitted with a Lichrocart RP-18 (250 × 10 mm) column and a fraction collector L-7650.

Other components of the HPLC system were the same as those indicated below. The injection volume was 1 ml and the column temperature was 25°C. The eluant consisted of acetonitrile/methanol/water [25/25/50 (v/v)]. The retention time of the product at a flow rate of 4.72 ml/min was 17.8 min.

2.2.3. Analysis of the reaction mixtures

The progress of the enzymatic reactions was monitored using HPLC at 22°C, using an apparatus with an L-7100 isocratic pump connected to a Kromasil RP-18 (250 × 4.6 mm) column, a RI-71 refractive index detector and a D-7500 integrator. All components were from Merck-Hitachi (Germany and Japan).

2.2.3.1. Glycerides. At the desired time, 2 ml of DMF was added to the reaction mixtures. The solution was then heated 50°C for 2 min to obtain complete dissolution of both substrates and products in DMF. The solid enzyme and molecular sieves were then separated by centrifugation and filtration. The volume of the resulting transparent solution was increased to 5 ml by addition of DMF. HPLC analysis of the products used the mobile phases indicated in Table 1, all of which contained acetic acid (0.1% v/v). The retention times of the different mono- and diglycerides are also indicated in Table 1. Calibration analyses were performed with standards purchased from Sigma (monoesters of oleic and caprylic acids and diesters of oleic, lauric, palmitic, and caprylic acids) and standards prepared and purified in our laboratory (monoesters of lauric and palmitic acids, > 99% pure).

2.2.3.2. n-Octyl β-D-glucopyranoside mono- and dilactate. At the indicated reaction time, 30 μl of the reaction mixture was extracted and diluted with 0.5 ml of DMF. The enzyme and molecular sieves were removed by filtration and centrifugation. A 20 μl of the resulting transparent solution was injected in the HPLC apparatus. The eluant consisted of acetonitrile/methanol/water 35/25/40 (v/v). At a flow rate of 1 ml/min, the retention times for *n*-octyl β-D-glucopyranoside, *n*-octyl β-D-glucopyranoside lactate and *n*-octyl β-D-glucopyranoside dilactate were 7.2, 9.1, and 11.48 min, respectively.

2.2.3.3. Esters of monosaccharides and sorbitol. The corresponding mobile phases and retention times have previously been reported [6–8].

3. Results and discussion

3.1. Glycerides

3.1.1. Effect of the solvent

The reaction courses of the direct esterification of glycerol with lauric acid were compared in acetone, acetonitrile, and dioxane (Fig. 1A) at 5°C. Glycerol (75 mg) and lauric acid (33 mg) were added to 1 ml of each of these three solvents. Under these condi-

Table 1
Retention times and mobile phases for the HPLC analyses of fatty acids and their corresponding mono- and diglycerides. ACN: acetonitrile

Fatty acid	Developing system (v/v)	Retention time monoester/acid (min)
Caprylic	METOH/H_2O (70:30)	6.32/8.78
Lauric	ACN/acetone/H_2O (40:40:20)	2.99/4.14
Palmitic	ACN/acetone/H_2O (46:47:7)	3.25/4.34
Oleic	ACN/acetone (95:5)	3.78/4.83

Fatty acid	Developing system (v/v)	Retention time diester (min)
Caprylic	Acetone/ACN (5:95)	2.65
Lauric	Acetone/ACN (60:40)	2.82
Palmitic	Acetone/ACN (90:10)	2.73
Oleic	Acetone/ACN (90:10)	2.85

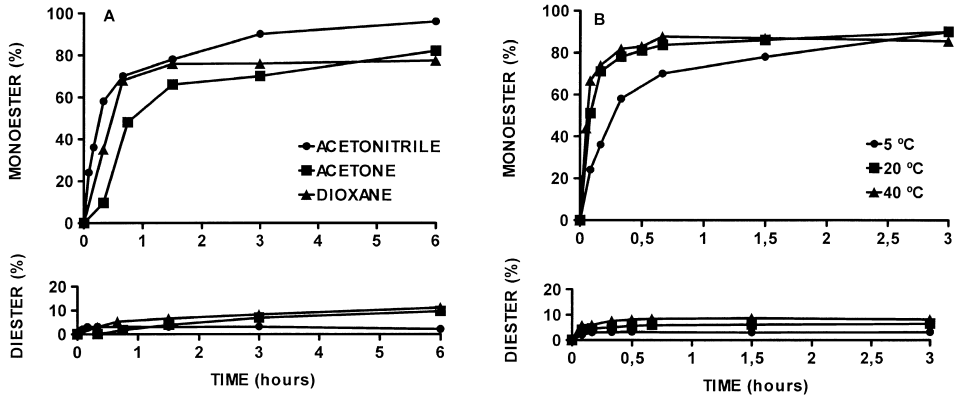

Fig. 1. (A) Effect of the solvent on the synthesis of monolauroyl glycerol. Conditions: lauric acid (33 mg, 0.165 mmol), [acid]/[glycerol] = 1/5, Novozym 435 (50 mg), solvent (1 ml), 5°C, molecular sieves (100 mg), 200 rpm. (B) Influence of temperature on the synthesis of monolauroyl glycerol. Conditions: same as for (A) except acetonitrile (1 ml).

tions, this quantity of lauric acid was completely dissolved in both dioxane and acetone. The corresponding solubilities at 5°C are 175 ± 0.7 and 84 ± 0.35 mg/ml, respectively. However, only a fraction of the lauric acid dissolved initially in the acetonitrile (the solubility of lauric acid at 5°C is 12 ± 0.28 mg/ml). In spite of this, the lauric acid was converted to an ester derivative to a similar extent (see Fig. 1A). The reaction rate in acetonitrile was similar to that in dioxane and faster than in acetone. The conversions of lauric acid achieved in 1.5 h were 82%, 81%, and 70% in dioxane, acetonitrile, and acetone, respectively. These results indicate that in addition to the solubility of the precursor reagents, the effect of the solvent on the activity of the enzyme affects the rate at which the limiting precursor reagent is converted. Evidence for variations in enzyme activities as the solvent is varied have been previously reported [9].

Values of solubility of monolauroyl glycerol in acetonitrile, acetone, and dioxane at 5°C were also determined (6 ± 0.32, 32 ± 0.58, and 77 ± 1.04 mg/ml, respectively), since the solubility seems to be directly related to the selectivity of the reaction towards the monoester. Precipitation of the desired monoester was observed only in acetonitrile. Nevertheless, the selectivity of the reaction towards this intermediate product was high for these three solvents. This fact must be associated with the excess of glycerol employed. Use of a five-fold excess of glycerol favors conversion of the acid to the monoester via a direct esterification reaction or glycerolysis of any diesters that might form in the course of reaction (no triglyceride was formed). Nevertheless, the selectivity of the reaction in acetonitrile was higher than in the other two solvents. The higher selectivity towards the monoacyl derivative in acetonitrile can be attributed to a combination of the precipitation of the monoester (which impedes further esterification) and the relatively low solubility of lauric acid in this solvent (which decreases the amount of fatty acid available to participate in further acylation reactions in the liquid phase).

3.1.2. Effect of temperature

The reaction temperature affects the rate constants and has a major influence on the solubilities of both the reactants and products (i.e., the fatty acid and monoglyceride).

The extent of conversion of lauric acid to the monoglyceride in acetonitrile at different temperatures (5–40°C) is shown in Fig. 1B. Continuous precipitation of the monoester permits one to obtain nearly complete conversion of lauric acid in 6 h at 5°C. Higher temperatures increase the rate of the process (95%, 92%, and 81% conversion of the acid in 1.5 h at 40°C, 20°C, and 5°C, respectively.) However, higher temperatures decrease the selectivity towards the desired intermediate ester. The higher selectivity observed at 5°C is a consequence of the low solubility of the monoester at this temperature

(6 ± 0.32 mg/ml). Under the reaction conditions reported here, 86% (w/w) of the total amount of monoester produced precipitates in the reaction medium. At temperatures above 5°C, the solubilities of both the fatty acid and the corresponding monoester increase. As has been demonstrated in the previous section, both increases in solubility favor further acylation of the desired monoester in the liquid phase loading to a concomitant decrease in the reaction selectivity.

3.1.3. Effect of the fatty acid

The nature and structure of the fatty acid lead to different interactions with the solvent and the enzyme. Hence, variations in the values of solubilities of both the precursor fatty acids and the product glycerides are observed as the structure of the precursor acid is changed. The discussion above indicates that two factors of particular importance are the length and degree of unsaturation of the alkyl group in the fatty acid.

In order to extend the procedure described above to different fatty acids, direct esterification reactions of glycerol with several fatty acids (C8, C12, C16 and C18:1) were compared (see Fig. 2). All the reactions were carried out at 5°C in acetonitrile (1 ml) at a molar ratio of glycerol to acid of 5/1.

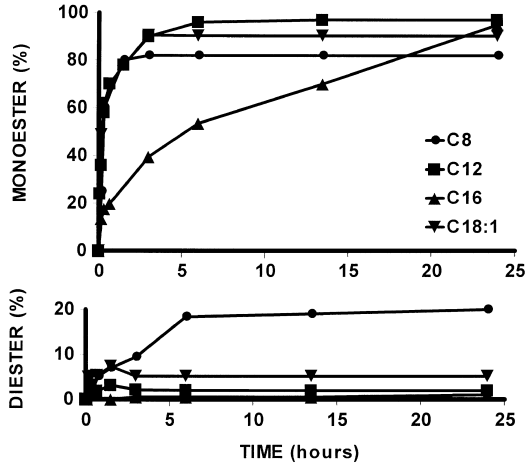

Fig. 2. Esterification of glycerol with different fatty acids. Conditions: acid (0.165 mmol), [acid/glycerol] = 1/5, Novozym 435 (50 mg), acetonitrile (1 ml), 5°C, molecular sieves (100 mg), 200 rpm.

Different results were obtained depending on the acid studied (Fig. 2). The rate at which the fatty acid was converted decreased in the order oleic (C18:1) > lauric (C12) > caprylic (C8) > palmitic (C16) acid (conversions of 53%, 36%, 25% and 13% of the corresponding acids were observed in 10 min). This rank order can be attributed in part to differences in the solubilities of these fatty acids at 5°C (> 200, 22 ± 1.5, 12 and 2 ± 0.3 mg/ml for C8, C18:1, C12 and C16, respectively). However, the rank order is also a consequence of the selectivity of this enzyme for long chain fatty acids rather than for short chain fatty acids. In particular, this enzyme prefers C18:1 and C12 rather than C8. Hence, fatty acids which are less soluble than C8 (i.e., C18:1 and C12) react more rapidly.

Nearly quantitative conversions to the corresponding monoesters were found for long chain saturated acids (lauric and palmitic acids). However, unsaturated (C18:1) and short chain (C8) fatty acids produce larger amounts of the corresponding diesters. At 5°C, the solubilities of monocaprylin and monoolein in acetonitrile (> 200 and 19.5 ± 0.9 mg/ml, respectively) are much higher than those of monolaurin and monopalmitin (6 ± 1 and 1.3 ± 0.3 mg/ml, respectively). Again, continuous precipitation of the desired intermediate (the monoester) minimizes further acylation of this product in the liquid phase. The higher solubilities of monoesters of short and/or unsaturated fatty acids at 5°C provide evidence for the requirement that the monoester has a lower solubility than a critical value (i.e., ≤ 7 mg/ml for the conditions herein employed) in order to avoid formation of significant amounts of diesters in the liquid phase.

In order to reduce the long reaction time (25 h) required for the selective synthesis of monopalmitoyl glycerol (C16), the reaction temperature was increased to 20°C. At this temperature, precipitation of most of the monopalmitin formed (90% (w/w), considering the solubility of this product at 20°C, namely 3.9 mg/ml) still occurs, and a greater amount of this fatty acid is dissolved in the liquid phase. Consequently, complete conversion of the acid to the monoester takes place in only 6 h. Similarly, temperatures higher than 5°C can be also employed for selective esterification of glycerol with saturated fatty acids which are longer than C14. Above 20°C, sig-

nificant yields of the diacylderivative were observed because of the increased solubility of the monopalmitoyl glycerol [only 26% (w/w) of the monoester precipitates at 40°C; the solubility of this product at 40°C is 32 ± 0.6 mg/ml]. Reaction temperatures higher than 20°C are especially recommended for esterifications involving saturated fatty acids longer than C16.

3.1.4. Glycerides in solvent-free systems

Liquid glycerol can be also esterified with liquid free fatty acids in the absence of solvents. Solvent-free systems eliminate the need for solvent removal from the reaction mixture.

Esterification of glycerol with oleic acid (140 mg) at a molar ratio ([glycerol]/[acid]) of 5 in the presence of a lipase from *Pseudomonas* sp. adsorbed on Celite (75 mg of biocatalyst) and 2.5 wt.% of 0.1 M sodium phosphate buffer (pH 7.0) was carried out at 3°C. Under these conditions, complete conversion of fatty acid is achieved. However, unlike the reaction in the presence of a solvent, the reaction in a solvent-free medium produces first the diester (67% of the maximum molar percentage with 6% monoester after 1 day). The monoester only accumulates in the medium as consequence of disproportionation and glycerolysis reactions of the diester formed earlier (93% by weight of the monoester after 4 days with no formation of by-products). In solvent-free systems, higher temperatures diminished the selectivity of the process towards the monoester (not shown), because of the relatively high melting point of the monoglyceride [5,10].

In the absence of a solvent, the direct esterification of glycerol with fatty acids which are solids at temperatures below 5°C does not occur. In these media, the reaction temperature must necessarily be higher than the melting point of the fatty acid. Saturated fatty acids higher than C12 are solids at 20°C.

3.2. Solid polyols

3.2.1. Effect of the solvent

Direct esterification of polyols (sugars) such as glucose, fructose, mannose, sorbitol, glucosides, etc.; which have high melting points requires the addition of a solvent to the reaction mixture. Only after their dissolution in the liquid phase can the required interactions of these precursors with both the enzyme and the fatty acid take place at the active site.

Direct esterification of polyols and sugars with fatty acids are frequently carried out in different polar solvents because of their ability to dissolve both reagents. However, variations in both conversion and selectivity from one solvent to another have not been sufficiently clarified in the literature.

The reaction of glucose (60 mg) with lauric acid (three-fold excess, 200 mg) as catalyzed by Novozym 435 (100 mg) was studied at 40°C in acetonitrile, acetone, and dioxane (2 ml). The reaction mixtures contained 200 mg of molecular sieves as a desiccant. Under these conditions, only a fraction of the sugar was dissolved in the liquid phase at the beginning of the process. Nevertheless, complete conversion of the sugar was obtained after an appropriate time of reaction, a result indicating that the remainder of the sugar dissolves in the solution as the reaction progresses. Examination of Table 2 shows that glucose reacts faster in dioxane than in acetonitrile and acetone. At 20°C, glucose is three and eight times more

Table 2
Partial acylation of glucose in different solvents. Conditions: glucose (60 mg), lauric acid three times in excess (200 mg), Novozym 435 (100 mg), solvent (2 ml), molecular sieves (200 mg) and 40°C

Time (h)	Conversion					
	Dioxane		Acetonitrile		Acetone	
	Monoester (%)	Diester (%)	Monoester (%)	Diester (%)	Monoester (%)	Diester (%)
1	55	1	43	0	40	1
2	81	2	84	0	78	1
3	86	4	97	1	96	2
6	90	7	98	2	97	3

soluble in dioxane than in acetone and acetonitrile, respectively (Table 3). Hence, the higher solubility of the solid precursor in the liquid reaction phase accelerates the esterification reaction. The selectivity of the process towards the glucose monoester decreases slightly as the solubility of this product increases. The order of the selectivities in various solvents is acetonitrile > acetone and dioxane. (The corresponding solubilities at 20°C are 0.6, 3.8, and 30 mM, respectively.)

3.2.2. Effects of the nature of the acidic residue and temperature

As is the case for glycerol, selective synthesis of an intermediate ester of glucose is favored by continuous extraction of the desired product from the liquid reaction phase. In polar media, the solubilities of glucose monoesters of different saturated fatty acids increase as the chain length of the acid residue decreases. Values of the optimum temperatures for complete conversion of glucose (60 mg, in the presence of a three-fold molar excess of the acid) in acetone are shown in Fig. 3. Analysis of these data indicates that the selective preparation of the more soluble monoesters (those prepared with C8, C10, and C18:1) requires use of a low temperature in order to precipitate the desired product, thereby minimizing its potential to undergo further reaction to form the diester.

Reactions at low temperature are more selective but require longer times than reactions conducted at higher temperatures [5,6,8]. In order to achieve an appropriate balance between selectivity and time requirements, the temperature can be varied during the course of the reaction decreasing the temperature as the reaction proceeds. This strategy permits one to obtain a significant reduction in the time required for selective production of intermediate esters (e.g., acylation of two primary hydroxyl groups of sorbitol and fructose), while maintaining the selectivity of the process. Basically, the first stage of the reaction is carried out for a short time at elevated temperature (e.g., 40°C for 6 h) while the second stage is accomplished at a low temperature (e.g., below 10°C). Before significant amounts of higher esters are formed, precipitation of the monoester is not required. As an example, this strategy reduces the reaction time in acetone for complete biotransformation of sorbitol (60 mg, in the presence of a five-fold excess of oleic acid) to the corresponding dioleoyl esters to 6 days (10 days shorter than the isothermal process at 10°C).

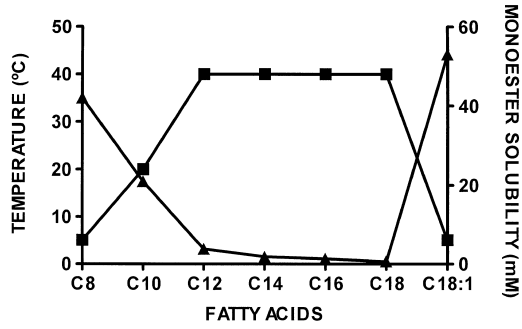

Fig. 3. Optimum temperatures for selective monoacylation of glucose and solubilities of the corresponding monoesters at 20°C, as a function of the type of fatty acid involved.

Table 3
Solubilities of polyols (sugars)

Species	Solvent	Temperature (°C)	Solubility (mg/ml)
Glucose	Acetonitrile	20	0.03
Glucose	Acetone	20	0.08
Glucose	Dioxane	20	0.24
Galactose	Acetone	20	0.01
Galactose	Acetone	40	0.03
Sorbitol	Acetone	20	0.29
Mannose	Acetone	20	0.35
Fructose	Acetone	20	0.39

3.2.3. Influence of the polyol (sugar)

Monosaccharides such as glucose, fructose, mannose and galactose have similar structures and molecular weights. However, they can exhibit very different reactivities in different media. Unlike other sugars, galactose could not be esterified in acetone. Solubility values of different polyols and sugars are shown in Table 3. The tabular entries indicate that only a negligible amount of this precursor dissolves in acetone where the reaction must take place.

The opposite situation prevails for mannose, which is much more soluble in acetone than glucose (4.3 and 3.8 times more soluble at 20°C and 40°C, respectively). Because of the difference in solubilities, mannose reacts faster than glucose. However, man-

nose cannot be selectively converted to the corresponding monoester (not shown) via a strategy based on the continuous precipitation of this product because of its high solubility. The solubilities of monolauroyl mannose and monolauroyl glucose at 20°C are 180 and 3.8 mM, respectively. Nevertheless, the relatively high solubility of mannose permits one to exploit another strategy for increasing the selectivity of the process toward the desired ester. Much more selective production of monlauroyl mannose (90%with 10% diester) can be obtained at 55°C as the presence of a three-fold excess of the precursor acid (60 mg polyol, 200 mg molecular sieves and 100 mg of Novozym 435). In this case, the increased temperature favors two aspects of the first esterification: the rate constant and the solubility of the precursor sugar. However, the elevated temperature only increases the rate constant of the second acylation. Consequently, the process is more selective with respect to the formation of the monoester at a temperature of 55°C.

An alternative strategy to that described for mannose involves increasing the solubility of the precursor polyol by using alkyl glucosides instead of monosaccharides. For example, selective esterification of the primary hydroxyl residue of *n*-octyl β-D-glucopyranoside (30 mg; 0.1 mmol) with L-lactic acid ethyl ester (100 mg; 0.8 mmol) was obtained in the presence of Novozym 435 (50 mg), acetone (1 ml) and molecular sieves (800 mg). A molar yield of 85% in *n*-octyl β-D-glucopyranoside lactate (90% conversion) was obtained after 16 h at 60°C. This precursor (*n*-octyl β-D-glucopyranoside) dissolves completely in the reaction medium, the desired ester also remains soluble, and the lactate reagent was present in molar excess with respect to the precursor sugar. Moreover, in both cases (mannose and the alkyl glucoside), differences in the activation energies for acylation of primary and secondary alcohols in the alkyl glucopyranoside are sufficiently high to permit selective monoesterification of this material under these conditions.

4. Conclusions

Non-ionic emulsifiers formed from liquid and solid polyols and sugars are partial esters of these materials whose selective synthesis requires careful control over process conditions. Strict kinetic control of sequential esterification reactions can be achieved at atmospheric pressure and mild temperatures (i.e., below 60°C). Under these conditions, one can employ solvents, which can be easily eliminated after completion of the reaction (those with low boiling points). Acetone is a particularly attractive solvent because it is accepted by EEC directives (88-344-CEE) as an extraction solvent in the manufacture of food products and additives [6–8].

Unlike inorganic catalysts, enzymes function under mild conditions. However, optimization of processes, which employ the various solid and liquid polyols discussed in this paper will require different strategies if nearly complete conversion of the precursor sugar (polyol) is to be achieved with high selectivity. Temperatures as low as 3–5°C or as high as 55°C may be required for the complete conversion of different polyols to their intermediate esters [11]. Reactions at low temperatures are relatively slow, but faster processes in which a programmed temperature variation with time is employed are indicated in some cases. Strategies based on precipitation of the product facilitates recovery and purification of the desired product by simple filtration of the reaction mixture [5–8].

The present work analyzes the requirements for selective esterification of one or two of the primary hydroxyl groups of various solid and liquid polyols. Selective synthesis of monoglycerides requires the use of excess glycerol and are favored by precipitation of the monoester products. Activation energies for first and second esterification steps do not differ sufficiently to permit one to employ an equimolar ratio of reagents. The process is more selective at temperatures at which more than 85% of the desired product precipitates.

Fatty acids employed for esterification of glycerol in the absence of solvents must be liquid at the reaction temperature. As is the case for reactions in the presence of a solvent, the selective synthesis of an intermediate ester requires the use of excess glycerol and is favored at a temperature at which the desired product precipitates and/or which minimizes esterification of the second primary hydroxyl group.

Reaction times are longer in the absence than in the presence of a solvent and are similar to those

required for esterification of solid polyols in solvents. These reaction times can be substantially reduced by using an appropriate reactor design, which promotes contact between the two precursors.

Selective esterification of solid sugars (polyols) which have intermediate solubility in acetone is favored at low temperatures at which a sufficient amount of polyol dissolves and concurrent precipitation of the desired product can be achieved [6,7]. By contrast, use of elevated temperatures is more appropriate for selective partial esterification of polyols, which are most soluble in the solvent employed. The corresponding partial esters are difficult to precipitate [11]. In that case, it is essential that the increased temperature sufficiently favor dissolution of the precursor polyol in the liquid reaction phase. In this manner, the first esterification is favored over subsequent acylations of the polyol, so that precipitation of the desired product is not required. Examples of the first and second cases are glucose and mannose, respectively (see Table 3). Moreover, polyols (sugars) which are the less soluble into the liquid reaction phase cannot be easily esterified. Monosaccharides such as galactose and disaccharides are not soluble in such polar solvents as acetonitrile, acetone, and dioxane. 2-Methyl-2-butanol is a better solvent for sugars (polyols) [12]. Pyridine and DMF have been used extensively as good solvents for disaccharides [1,13]. However, the toxicity of these materials provides a major incentive to seek new synthetic methods. The present paper provides a general overview of the factors involved in both the kinetics and the selectivity of acylation reactions of polyols (sugars). It describes a general basis for further development of selective synthetic methods.

Unlike acylation reactions involving glycerol, acylations of solid polyols (sugars) are carried out in the presence of excess acid. The fact that the sugar gradually dissolves in the solution while the acid is completely soluble in this medium indicates that the acid is always present in excess in the liquid phase, regardless of the initial molar ratio of reactants employed.

Selective diacylation of sorbitol requires continuous precipitation of the diester prepared via consecutive esterification of its two primary hydroxyl groups. In this case, differences in reactivities of the primary and secondary hydroxyl groups are not sufficient to impede accumulation of triesters in the reaction mixture containing the acid in excess [7]. This situation applies when the precursor sugar (polyol) can be dissolved in sufficient quantity at the reduced temperature at which the ester of interest precipitates (see Table 3).

Selective partial esterification of the primary OH groups of a solid polyol does not always require in situ precipitation of the desired product. This case occurs when the solubility of solid precursor is sufficiently great to provide access of all of the unreacted material to the enzyme before esterification of the secondary hydroxyl groups takes place (e.g., for fructose, see Table 3 [7]). Alternatively, complete solubilization of alkyl glucosides can be exploited for selective esterification of the single hydroxyl group present.

The unreacted polyol(sugar) which originally did not dissolve must subsequently dissolve rapidly as soon as the dissolved fraction of the original polyol has been transformed by reaction. Diffusional limitations on the rate of dissolution of the solid precursor can be minimized by increasing the surface area of the solid polyol. Use of sufficiently small size particles become more important as the temperature is decreased (i.e., at 5–20°C).

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References

[1] S. Riva, J. Chopineau, A.P.G. Kiebom, A.M. Klibanov, J. Am. Chem. Soc. 110 (1988) 584.
[2] A. Ducret, A. Giroux, M. Trani, R. Lortie, Biotechnol. Bioeng. 48 (1995) 214.
[3] G. Ljunger, P. Adlercreutz, B. Mattiasson, Biotechnol. Lett. 16 (1994) 1167.
[4] L.Q. Cao, A. Fischer, U.T. Bornscheuer, R.D. Schmid, Biocatal. Biotransform. 14 (1997) 269.
[5] J.A. Arcos, C. Otero, J. Am. Oil Chem. Soc. 73 (1996) 673.

[6] J.A. Arcos, M. Bernabé, C. Otero, Biotechnol. Bioeng. 57 (1998) 505.
[7] J.A. Arcos, M. Bernabé, C. Otero, Enzyme Microb. Technol. 22 (1998) 27.
[8] J.A. Arcos, M. Bernabé, C. Otero, Biotechnol. Bioeng. 60 (1998) 53.
[9] C.R. Wescott, A.M. Klibanov, Biochim. Biophys. Acta 1206 (1994) 1.
[10] G.P. McNeill, S. Shimizu, T. Yamane, J. Am. Oil Chem. Soc. 67 (1990) 779.
[11] J.A. Arcos, M. Bernabé, C. Otero, J. Surfactants Deterg. 1 (1998) 345.
[12] N. Khaled, D. Montet, M. Farines, M. Pina, J. Graille, Oleagineux 47 (1992) 181.
[13] T. Polat, H.G. Bazin, R.J. Linhardt, J. Carbohydr. Chem. 16 (1997) 1319.