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Green biocatalytic synthesis of panthenyl monoacyl esters in Ionic Liquids and Deep Eutectic Solvents

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The enzymatic synthesis of six panthenyl monoacyl esters (PMEs) was carried out by the direct esterification of fatty acids (*i.e.* capric, lauric, myristic, palmitic, oleic and linoleic acids, respectively) with panthenol in different ionic liquids (ILs) based on cations with a long alkyl side-chain (*e.g.* 1-dodecyl-3-methylimidazolium tetrafluoroborate [C_{12}mim][BF_4], etc.). All the assayed ILs were seen to be suitable reaction media for Novozym 435-catalyzed synthesis of PMEs (*i.e.* up to 90% conversion and 100% selectivity), enabling easy recovery and the reuse of both biocatalyst and IL. Alternatively, mixtures of panthenol with free fatty acids were seen to act as deep eutectic solvents (DES), that were excellent reaction media for the biocatalytic synthesis of PMEs (*i.e.* up to 83% conversion and 98% selectivity in the case of the panthenyl monolaurate), the enzymatic activity remaining unchanged for seven consecutive cycles of reuse. The enzymatic synthesis of PMEs by direct esterification using the DES approach can be considered as a clean and useful process for the sustainable industrial scaling up of panthenyl acyl ester production.

1. Introduction

Panthenol (2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethyl-butanamide, see Fig. 1), also called pro-vitamin of B_5, is a bioactive molecule of great relevance for the pharmaceutical and cosmetic industries. It is a common component in creams, shampoos, hair conditioners and skin treatment formulations, and is considered as a cosmeceutical product.[1,2] However, as it is highly soluble in water, panthenol has a relatively short time of interaction when used for hair conditioners, as shampoo or lotions. In this context, panthenol esters are of high interest for pharmacological and cosmetic preparations, because of their modified physical properties (being for example hydrophobicity, amphiphilicity among others character, etc.) which improve their interactions with skin and/or hair. Panthenol esters remain after washing and drying hair, considerably increasing their regenerative effects on the hair.[3]

In spite of the industrial interest of these products, very few synthetic approaches to obtain panthenyl esters have been reported.[4-8] In this respect, panthenyl triacetate is a molecule usually contained in cosmetic formulations. It is obtained by an exothermic reaction between panthenol with acetic anhydride in the presence of a heterogeneous catalyst, based on dimethylaminopyridineanhydride groups, at 110ºC, providing up to 80% product yield.[4] However, the selective esterification of the hydroxyl groups of panthenol to synthesize mono- or di- panthenyl esters by this approach has not been described. Alternatively, the synthesis of panthenyl docosahexaenoate has been reported following a classical protocol of organic synthesis based on three steps, such as, *i*) the selective chemical protection of two hydroxyl groups of panthenol by trimethylchlorosilane, *ii*) followed by the esterification of the remaining free hydroxyl group

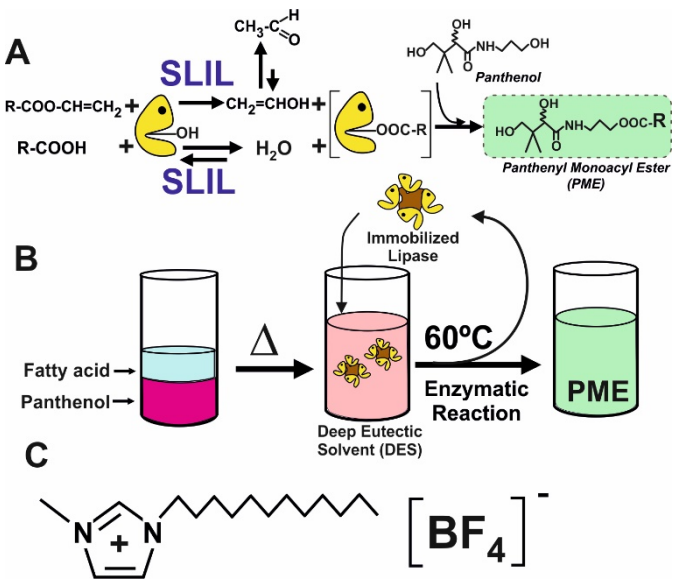

Fig. 1. A. Scheme of the immobilized lipase-catalysed synthesis of panthenyl monoacyl esters (PMEs) by direct esterification of fatty acids with panthenol in SLIL. **B.** Scheme of the enzymatic synthesis of PMEs in deep eutectic solvents (DESs). **C**. Structure of the [C_{12}mim][BF_4], as an example of sponge-like ionic liquids used as reaction media for the enzymatic synthesis of PMEs.

with docosahexaenoic acid in the presence of an amine as catalyst in acetone, and *iii*) the final deprotection of the two protected OH functional groups, leading to a 54% final product yield.[5] A further step for tailoring the synthesis of panthenol esters was carried out using lipases that permits the controlled esterification of their two primary hydroxyl groups, giving rise to the PMEs as amphipathic panthenol derivatives that are highly appreciated for use in cosmetic formulations. The biocatalytic synthesis of both the panthenyl monoacyl esters and the panthenyl diacyl esters was carried out by transesterification reactions using short aliphatic esters (*e.g.* isopropyl acetate,[6] ethyl acrylate,[7] etc.) as acyl donors, and volatile organic solvents (VOSs, *e.g.* acetonitrile, acetone, *tert*-butyl methyl ether, etc.) as reaction medium. As a function of the reaction parameters (*e.g.* nature and concentration of the acyl donor, reaction time, etc.), a final mixture with variable yield of both the PME and PDE products was obtained (60 - 96% PME and 4 - 40% DME) and dissolved in the corresponding organic solvent. However, this approach is outside the Green Chemistry principles,[8] because of the use of substrate derivatives (*i.e.* aliphatic esters) and VOSs.

Sustainability plays a fundamental role in the cosmetic and personal care industry of today, leading to the search for new production methods based on clean approaches at industrial scale (*e.g.* catalysis, solvent-free approaches, recovery and reuse of solvents, pure products, etc).[9] The excellent suitability of enzymes for developing selective chemical transformations, combined with clean and straightforward separation technical approaches able to provide pure products, is key axis to build sustainable cosmetic industries.[10] Furthermore, the combination of biocatalysts with ionic liquids (ILs) resulted in synergic platforms for the synthetic processes, enabling the development of easy, cheap and/or sustainable approaches for pure product extraction.[11]

In this context, hydrophobic ILs based on cations with long alkyl side-chains are temperature switchable ionic liquid/solid phases that behave as sponge-like systems (Sponge-Like Ionic Liquids, SLILs).[12] These ILs are able to dissolve the substrates, and forming monophasic liquid systems upon heating above their respective melting points (at moderate temperatures). After the reaction, it was observed how these perfectly clear solutions became solid upon cooling to room temperature. At this point, the solid mixture can be separated into two phases by simple centrifugation at a temperature below room temperature. This resulted in an upper liquid phase of nearly pure product, while the bottom phase was the recovered solid IL, which is ready for reuse.[13] Although ILs have been described as being not fully green solvents because of their low biodegradability and high (eco)toxicological properties,[14] the insolubility in water of the SLILs, together with their displayed melting points higher than room temperature, allows their immediate precipitation as solids, as well as their easy full recovery after a possible accident during handling. The reasonable criticism regarding the non-green character of ILs against an environmental contamination produced by an accident are clearly mitigated. Additionally, the non-volatile character of these ILs can be regarded as an important advantage for a final step of products refining, *e.g.* classical distillation process. These SLILs permit the separation of nearly pure products by using centrifugation/filtration approaches on the solid system obtained after cooling. The SLIL reaction/separation systems represent as a new sustainable platform for the preparation of pure chemicals. [12]

Another neoteric solvent which have gained a lot of interest recently are deep eutectic solvents (DESs) which are novel reaction media with properties similar to those of ionic liquids (ILs).[15] According to the theory, a DES is created by an adequate mixture of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) which can self-associate through hydrogen-bonding interactions, resulting in a strong depression of the freezing-melting point of the mixture.[16]

Among the different types of DES, the Hydrogen Bond Donor (HBD)-organic salt based-DES are recently gaining interest as reaction media for biocatalysis, because of the use of renewable resources (*e.g.* carboxylic acids, carbohydrates, amines or polyols), which provide suitable environments for enzymatic transformations.[17] Therefore, in comparison to traditional ILs, these kind of DESs have a number of important advantages including low toxicity, low precursor cost, simple synthesis, negligible volatility, and high biodegradability.[18]

This paper shows for first time the comparative enzymatic synthesis of six different fatty acid panthenyl monoacyl esters by using two different sustainable reaction media, such are ILs and DESs. The PME products were firstly obtained by direct esterification of free fatty acids (*i.e.* capric, lauric, myristic, palmitic, oleic acid and linoleic, respectively) with panthenol, as well as by transesterification using different esters as acyl donors (*e.g.* vinyl laurate, methyl laurate, etc.) by using different SLILs (1-dodecyl-3-methylimidazolium tetrafluoroborate, [C_{12}mim][BF_4]; 1-decyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide [C_{10}mim][NTf_2]; 1-dodecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, [C_{12}mim][NTf_2]); 1-tetradecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_{14}mim][NTf_2]; 1-hexadecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, [C_{16}mim][NTf_2] or 1-octadecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide [C_{18}mim][NTf_2], respectively), as reaction media. In an attempt to find alternative green reaction media, this work demonstrates by first time the ability of panthenol and free fatty acid mixtures to form deep eutectic solvents (DESs) after melting. The suitability of these substrates/DESs mixtures for the enzymatic synthesis of PMEs, as solvent-free media, has successfully been demonstrated. The influence of several reaction parameters (*e.g.* nature of acyl donor, acid/panthenol ratio, etc.), as well as the recovery and reuse of the biocatalyst, are studied for all the described conditions. The better suitability of the substrate/DESs-based reaction media with respect the ILs is demonstrated, as a straightforward and sustainable procedure for the large scale production of panthenyl monoacyl ester in fully solvent-free systems.

2. Experimental

2.1 Chemicals

Immobilized *Candida antarctica* lipase B (Novozym® 435, EC 3.1.1.3) was a gift from Novozymes S.A. (Spain). D,L-Panthenol (99% purity, m.p. 69ºC), capric acid (98% purity, m.p. 31.6ºC), lauric acid (98% purity, m.p. 43.2ºC), myristic acid (99% purity, m.p. 54.4ºC), palmitic acid (99% purity, melting point m.p. 62.9ºC), oleic acid (99% purity, m.p.13.4ºC), linoleic acid (99%, m.p. -5ºC), vinyl caprate (99% purity), vinyl laurate (99% purity), ethyl caprate (99% purity), molecular sieves 13x (MS13x; 270 mg H_2O/g adsorption capacity), BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) and TMCS (trimethylchlorosilane) reagent, solvents and other chemicals were acquired from Sigma-Aldrich-Fluka (Spain). The ILs 1-dodecyl-3-methylimidazolium tetrafluoroborate ([C_{12}mim][BF_4], 99% purity), 1-decyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_{10}mim][NTf_2], 99% purity), 1-dodecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_{12}mim][NTf_2], 99% purity), 1-tetradecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, ([C_{14}mim][NTf_2] 99% purity, 1-hexadecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, ([C_{16}mim][NTf_2], 99% purity), 1-octadecyl-3-methylimidazolium

bis(trifluoromethylsulfonyl)imide ([C_{18}mim][NTf_2], 99% purity) were obtained from IoLiTec GmbH (Germany).

2.2. Enzymatic synthesis of panthenyl monoacyl esters in ionic liquids

Into 3-mL screw-capped vials with teflon-lined septa, 0.5, or 1 mmol of acyl donor (capric acid, lauric acid, myristic acid, palmitic acid, oleic acid, linoleic acid, vinyl caprate, vinyl laurate, ethyl caprate or methyl laurate) were mixed with the corresponding amount of D,L-panthenol to finally achieve 1:2 or 1:3 acyl donor:panthenol molar ratio. Then, the corresponding amount of IL ([C_{10}mim][NTf_2], [C_{12}mim][NTf_2], [C_{14}mim][NTf_2], [C_{16}mim][NTf_2], [C_{18}mim][NTf_2] or [C_{12}mim][BF_4], respectively) was added to reach a final concentration of 50% (w/w) IL with respect to the overall mass. The resulting reaction mixtures were pre-incubated at 60ºC for 60 min, leading to monophasic systems, and then 240 mg MS13x were also added. The reaction was started by adding Novozym 435 (60 mg per mmol of acyl donor) and the reaction mixture was shaken (250 rpm) at 60ºC for 4 h under vacuum conditions. To obtain time-course profiles, 15 μL aliquots were taken at regular intervals and suspended in 485 μL of a dodecane/isopropanol (95:5 v/v) solution, and the resulting biphasic mixtures were strongly shaken for 3 min, and then centrifuged at 15,000 rpm for 15 min at 6ºC to precipitate the IL. Finally, 300 μL of the dodecane/THF liquid phase (upper phase containing panthenyl monoacyl ester product) were collected for further GC analysis.

2.3. Enzymatic synthesis of panthenyl monoacyl esters in DES

Into 10-mL screw-capped tubes with teflon-lined septa, 2, or 4 mmol of free fatty acid (capric acid, lauric acid, myristic acid, palmitic acid, oleic acid or linoleic acid, respectively) were mixed with the corresponding amount of solid panthenol to finally achieve 1:1, 1:2 or 1:3 free fatty acid:panthenol molar ratio. The resulting mixtures were sealed under vacuum, and then shake (600 rpm) for 60 min at 80ºC, leading to viscous and fully clear monophasic liquid systems. The resulting DES was cooled to 60ºC, and then MS13x molecular sieves (5% w/w) were also added. The reaction was started by adding Novozym 435 (60 mg per mmol of free fatty acid) and the reaction mixture was shaken (250 rpm) at 60ºC for 4 h under vacuum conditions. To obtain time-course profiles, 15 μL aliquots were taken at regular intervals and suspended in 985 μL of a dodecane/THF (1:1, v/v) solution, then analysed by GC.

2.4. Separation of unreacted panthenol from DESs

Panthenyl monolaurate was chosen as representative example for separation of unreacted panthenol from DES-based reaction media. Into a 2-mL vial, a sample (100 μL) of the DES-based medium after enzymatic reaction (see Table 2, entry 3) was dissolved in limonene (1 mL). The resulting mixture was strongly shaken for 60 min at 60ºC, cooled into an ice bath for 30 min, and then centrifuged (15000 rpm) for 10 min at 0ºC, resulting in two phases: a gelled bottom-phase containing unreacted panthenol, and a limonene liquid phase containing the panthenyl monolaurate product. Samples of this limonene phase were collected for GC analysis (see ESI). Finally, the panthenyl monolaurate was recovered from the limonene phase by precipitation after cooling for 6 hours at -20ºC, followed by centrifugation (15,000 rpm, 5 min) at 0ºC. Samples (20 mg) of this precipitated fraction were dissolved in acetone-δ_6 (1 mL), and used for ^{1}H NMR and ^{13}C NMR analyses.

2.5. Extraction of PMEs from [C_{12}mim][BF_4] IL

The panthenyl monoacyl ester/[C_{12}mim][BF_4] reaction mixture (0.3 g) was placed into a 2-mL vial, and then incubated at 60ºC until a fully clear and homogeneous phase was observed. Then, water (0.5 mL) and dodecane (0.5 mL) were added, and the resulting mixture was strongly shaken for 30 min at 60ºC, then cooled to 0ºC into an ice bath, and followed to centrifugation at 15,000 rpm (15 min) at 0ºC, resulting in three phases: a solid bottom-phase containing the IL, a middle aqueous phase containing the unreacted panthenol (hydrophilic compound insoluble in non-polar solvents), and a dodecane phase containing PME product (top-phase). The top-phase was collected, and the residual IL content was analysed by ^{19}F NMR, by using a [C_{12}mim][BF_4] solution in acetone-δ_6 containing TFA, as standard (see ESI).

2.6 GC analysis

Before GC analysis, samples (25 μL) were added into 1.5-mL screw-capped vial with teflon-lined septa containing dodecane/THF (1:1 v/v) solution (275 μL) and BSTFA+TMCS (99:1, v/v) reagent (200 μL). The resulting mixture was incubated for 1 h at 70ºC to carry out the trimethylsilylation of alcohols groups of panthenol and PMEs.[19] Derivatized samples (1 μL injection volume) were analysed by gas chromatography (GC) with a Shimadzu GC-2010 (Shimadzu Europe, Germany) equipped with an FID detector and automatic injector, by using a TRB-biodiesel capillary column (10 m x 0.28 mm x 0.1 μm, Teknokroma, Spain), in the following conditions: carrier gas (He) at 28.6 kPa (40 mL/min total flow), temperature injector, 290ºC; temperature programme: 100ºC, 10ºC min^{-1}, 200ºC, 15ºC min^{-1}, 370ºC, variable split ratio (80:1 to 10:1); detector temperature, 370ºC. Peak retention times (min) were as follows: ethyl decanoate (internal standard), 1.1; panthenol, 5.0 and 5.3; capric acid, 1.9; lauric acid, 2.7; myristic acid, 4.3; palmitic acid, 6.2; oleic acid, 7.5; linoleic acid, 7,0 ;vinyl caprate, 2.4; vinyl laurate, 3.3; methyl laurate, 1.9, panthenyl monocaprate, 11.6; panthenyl dicaprate, 16.5; panthenyl monolaurate, 12.6; panthenyl dilaurate, 18.2; panthenyl trilaurate, 22.3; panthenyl monomyristate, 14.0; panthenyl dimyristate, 20.4, panthenyl monopalmitate, 15.4; panthenyl dipalmitate, 22.1; panthenyl monooleate, 16.2; panthenyl dioleate, 23.5; panthenyl trioleate, 28.1; panthenyl monolinoleate, 16.7; panthenyl dilinoleate, 20.9.

2.7 ^{1}H NMR and ^{13}C NMR analysis

Panthenyl monolaurate was identified from ^{1}H NMR and ^{13}C NMR spectra. ^{1}H homonuclear correlation experiments as well as ^{1}H,^{13}C heteronuclear experiments were performed on a Bruker Avance 600

MHz. Panthenyl monolaurate samples, collected from the limonene phase (see section 2.4), were dissolved in acetone-δ_6 (1 mL). The assignment of almost the totality of protons and carbons was achieved using TOCSY and NOESY homonuclear experiments, as well as ^{1}H,^{13}C HSQC spectra. ^{1}H NMR δ(ppm): 0.88 (s, 3H, H_4 or H_5); 0.89 (t, 3H, H_{24}); 0.96 (s, 3H, H_4 or H_5); 1.27-1.33 (from H_{17} to H_{23}, indistinguishable); 1.59 (m, 2H, H_{16}); 1.69 (m, 2H, H_{11}); 2.05 (m, 2H, H_{15}); 2.31 (t, 2H, H_{14}); 3.38 (m, 2H, H_{10}); 3.43 (d, 2H, H_2); 3.96 (d, 1H, H_6); 4.11 (t, 2H, H_{12}); 4.18 (t, 1H, H_1); 4.83 (d, H1, H_7); 7.64 (d, H1, H_9). ^{13}C NMR δ(ppm): 13.7 (C_{24}); 20.1 (C_5); 20.8 (C_4); 25.5 (C_{16}); 29.6-32.3 (from C_{17} to C_{23}, indistinguishable); 30.0 (C_{15}); 33.1 (C_{11}); 34.1 (C_{14}); 36.0 (C_{10}); 61.9 (C_{12}); 70.0 (C_2); 75.4 (C_6). See ESI.

2.8. Identification of panthenyl esters by HPLC-MS

An Agilent 1200 HPLC instrument coupled to an Agilent 6220 ESI-TOF detector (Agilent, USA) were used to perform HPLC-MS analyses of the reaction media. Separation was carried out in a reversed phase C18 column (250 mm x 5 μm). The mobile phase was comprised by: A, 20 mM ammonium acetate in water; and B, 20 mM ammonium acetate in acetonitrile-. These phases were used in gradient mode: 0-5 min 40% A, 60% B; 5-8 min: 11% B min^{-1}; 8-30 min 10% A, 90% B. UV detection 205 nm. TOF-MS (gas temperature, 350ºC; gas flow, 12 L/min). Each sample (20 μL) of FFA, panthenol or the reaction mixture containing PME and PDE, was diluted in 980 μL acetonitrile (10 μL injection volume). Total ion signal from the sample was obtained by scanning the range of mass corresponding to m/z values ranging from 100 to 1000 in negative ion mode. Each peak of panthenyl ester was identified by comparison of their mass spectra with those in a computer library (NIST Library). Panthenol, retention time (Rt, min): 4.72; positive ion (m/z): 204.12; capric acid, retention time, Rt: 8.6; positive ion (m/z): 171.14; lauric acid, Rt: 11.7; positive ion (m/z): 199.1; myristic acid, Rt: 14.22; positive ion (m/z): 227.2; palmitic acid RT: 17.82; positive ion (m/z): 256.2; oleic acid, RT: 18.7; positive ion (m/z): 281.2; linoleic acid, RT: 15.4; positive ion (m/z) 279.3, panthenyl monocaprate, Rt: 10.6; positive ion (m/z): 358.26; panthenyl dicaparate, Rt: 18.9; (m/z): 512.40; panthenyl monolaurate, Rt, 14.02; (m/z): 386.29; panthenyl dilaurate, Rt: 36.6; (m/z): 568.46; panthenyl monomyristate, Rt: 15.0; (m/z): 414.32; panthenyl monopalmitate, Rt: 16.8; (m/z): 442.36; panthenyl monooleate, Rt: 18.6; (m/z): 468.37; panthenyl monolinoleate, Rt: 16.28; (m/z): 466,3.

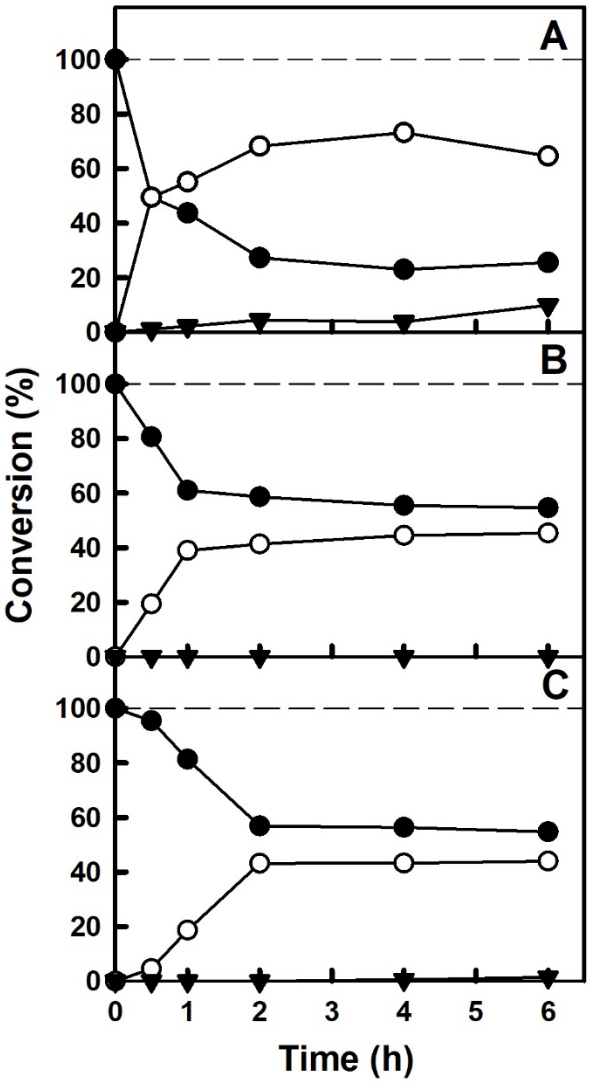

Fig. 2. Time-course profiles for the Novozym 435-catalysed synthesis of panthenyl monolaurate and (○), panthenyl dilaurate (▼) using vinyl laurate (A), methyl laurate (B) or lauric acid (C) (●), respectively, as acyl donor for the esterification of panthenol in [C_{12}mim][BF_4] at 60ºC. Acyl donor : panthenol mole ratio, 1:2 (mol/mol)

3. Results and discussion

3.1. Enzymatic synthesis of panthenyl monoacyl esters in ionic liquids.

The synthesis of different PMEs catalysed by immobilized *Candida antarctica* lipase B was studied in seven different SLILs (*i.e.* [C_{12}mim][BF_4], [C_{10}mim][NTf_2], [C_{12}mim][NTf_2], [C_{14}mim][NTf_2], [C_{16}mim][NTf_2] and [C_{18}mim][NTf_2]), as reaction media at 60ºC. Both the direct esterification of the FFAs (*i.e.* capric acid, lauric acid, myristic acid and palmitic acid), as well as the transesterification of fatty acid esters (*i.e.* ethyl caprate, vinyl caprate, methyl laurate and vinyl laurate), with panthenol were tested as usual synthetic approaches, leading to the synthesis of PMEs (see Table 1). As a representative example, Fig. 2 depicts the time-course profiles for the enzymatic synthesis of panthenyl monolaurate by using vinyl laurate (Fig. 2A), methyl laurate (Fig. 2B) and lauric acid (Fig. 2C) as acyl donors for the esterification of panthenol in [C_{12}mim][BF_4], at a 1:2 (mol/mol) acyl donor:panthenol ratio. As can be seen in Fig 1A, the reaction mechanism of lipases occurs via a covalently linked acyl-enzyme intermediate, which deacylates through the nucleophilic attack of either water (reversible hydrolytic pathway), or another nucleophile, such as panthenol (synthetic pathway). When using activated acids as acyl-donors, such as vinyl esters, the vinyl alcohol released in the enzyme acylation step tautomerizes to acetaldehyde, which

Table 1. Biocatalytic synthesis of panthenyl monoacyl esters (PME) and panthenyl diacyl esters (PDE) by using different acyl donors after 6 h of reaction in ionic liquids at 60ºC. See experimental section for further details).

Entry	IL	Acyl donor[a]	Conv.[b] (%)	PME (%)	PDE (%)	PME[c] (% w/w)
1	$[C_{12}mim][BF_4]$[d]	VL	74.5	86.6	13.4	21.1
2	$[C_{12}mim][BF_4]$[d]	ML	45.5	100	0	15.9
3	$[C_{12}mim][BF_4]$[d]	LA	45.4	96.9	3.1	15.5
4	$[C_{12}mim][BF_4]$[d]	VC	83.1	91.5	8.5	24.4
5	$[C_{12}mim][BF_4]$[d]	EC	45.8	100	0	14.7
6	$[C_{12}mim][BF_4]$[d]	CA	45.4	97.3	2.7	16.5
7	$[C_{12}mim][BF_4]$[d]	MA	54.3	100	0	19.8
8	$[C_{12}mim][BF_4]$[d]	PA	54.1	100	0	20.6
9	$[C_{10}mim][NTf_2]$[e]	LA	90.0	82.8	17.2	16.0
10	$[C_{12}mim][NTf_2]$[e]	LA	92.9	88.3	11.7	17.6
11	$[C_{14}mim][NTf_2]$[e]	LA	91.1	87.2	12.8	16.9
12	$[C_{16}mim][NTf_2]$[e]	LA	90.1	89.5	10.5	17.2
13	$[C_{18}mim][NTf_2]$[e]	LA	87.0	87.3	12.7	16.3

[a]Acyl donor: lauric acid (LA); capric acid (CA); myristic acid (MA); palmitic acid (PA); ethyl caproate (EC); vinyl caprate (VC); methyl laurate (ML); vinyl laurate (VL); [b]Conversion (yield) with respect the offered acyl donor. [c]PME content in the reaction medium; [d]Acyl donor:panthenol ratio, 1:2 (mol/mol); [e]Acyl donor:panthenol ratio, 1:3 (mol/mol).

cannot act as a substrate for the enzyme. This synthetic pathway can be regarded as a kinetically controlled process, where the rapid accumulation of the acyl-enzyme intermediate, the absence of water, and the high alcohol concentration are essential.[20] When using free carboxylic acids as acyl donors, the enzyme acylation reaction is in equilibrium (thermodynamics control), and it is necessary to eliminate the water by-product molecules released from the enzyme acylation step (*e.g.* by using dehydrating agents, etc.) to shift the reaction equilibrium towards the synthetic pathway.[13c] As a function of these reaction mechanisms (see Fig. 1A), the use of an acyl-donor concentration higher than that of panthenol leads to the di- and tri-esterification of the same panthenol molecule. Thus, a 1:2 (mol/mol) acyl donor:panthenol ratio was selected to favour the selective synthesis of PMEs against the PDEs undesired target.

As can be seen in Fig 2 A, the use of laurate vinyl ester as acyl donor led to the highest PME reaction rate during the first reaction hour (18.4 μmol/min), reaching a maximum conversion level (70%) at 4 hours, before decreasing with the concomitant increase in the PDE product up to 13.4%. When the methyl laurate was used as acyl donor, the reaction rate was lower (6.7 μmol/min), giving 45.5% panthenyl monolaurin conversion at 6 h, without the synthesis of the PDE product. In the same way, the direct esterification of panthenol with lauric acid resulted in the slowest reaction rate (3.7 μmol/min), leading to a 45.5% PME conversion after 6 h, although the PDE content was increased up to 3.1% (see Table 1, entries 1-3). Similar results were obtained for the synthesis of panthenyl monocaprate when using vinyl caprate (entry 4), methyl caprate (entry 5) and capric acid (entry 6) in the same conditions ($[C_{12}mim][BF_4]$ at 60ºC). By increasing the alkyl chain length of the acyl donor (*i.e.* myristic and palmitic acids), both the conversion (up to 53.4%) and the PME content (up to 20.6% w/w) were slightly improved with respect lauric and capric acids (see entries 7-8).

These results should also be related with both the diffusional mass-transfer phenomena around the enzyme active-site, as well as the reaction rate displayed by the enzyme for each case. When a fast biocatalytic reaction rate is combined with a low diffusion rate of the PME product from the enzyme microenvironment to the bulk reaction medium, PDE synthesis will be enhanced. In this context, the PDE content obtained (Fig 2A) could be related to the fast reaction rate that the use of vinyl esters as acyl donors usually induces. The hydrophobic character of the IL based on a C_{12}-alkyl chain in the cation could be involved in a possible low mass-transfer rate of the hydrophilic panthenol, leading the enzyme to a second acylation of the PME product molecule. These possibilities were also corroborated when ILs, based on the hydrophobic anion $[NTf_2]$, were assayed as reaction media for the enzymatic synthesis of panthenyl monolaurate by the direct esterification of lauric acid with panthenol at a 1:3 (mol/mol) acyl donor:panthenol ratio (see Table 1 entries 9 to 13). For these cases, the presence of molecular sieves, as dehydrating agent, as well as the use of vacuum conditions, in this esterification approach are involved in the shift of the reaction equilibrium towards the esterification products, because of the elimination of water by-product.[13c] As can be seen, the increase in the acyl donor:panthenol molar ratio leads to the increase in the conversion parameter was observed for all $[NTf_2]$ ILs (*i.e.* up to 92.9% for the $[C_{12}mim][NTf_2]$ case) with respect the obtained for the $[BF_4]$ ILs, although the PDE (panthenyl dilaurate) content was also enhanced. Both the enzymatic reaction rate and the hydrophobicity of the IL are key parameter to control the selectivity of the enzymatic synthesis of PMEs.

On the other hand, it should be noted the all these experimental approaches for the biocatalytic synthesis of PMEs were carried out in 50% (w/w) SLILs, leading to 14-24% (w/w) PME content in the full reaction medium. Besides the efficiency and selectivity of any chemical transformation accomplished, it is necessary to demonstrate that all the elements involved in the process can be recovered for reuse when developing sustainable processes. Thus, the excellent suitability of ILs with a long alkyl-chain in cation, and based on $[NTf_2]$ or $[BF_4]$ anions, providing an excellent operational stability to Novozym 435, has been widely demonstrated for the synthesis of terpene esters,[13a] biodiesel (*i.e.* half-lives of up to 1370 d under operational conditions during biodiesel synthesis),[13b] oxygenated biofuels,[21] and even monoglycerides.[13c] Furthermore, it has also been reported how this extremely ordered supramolecular structure of ILs in liquid phase might also be able to act as a ''mould'', stabilizing the active 3-D structure of the enzyme in these non-aqueous nano-environments.[11,22]

The development of clean experimental approaches for the straightforward separation of products, including the recovery of the IL for reuse, is an essential milestone in this field. For example, the separation of panthenyl monolaurate from the $[C_{12}mim][BF_4]$ reaction media (see Table 1, entry 3) was carried out by a common cooling/centrifugation protocol based on the

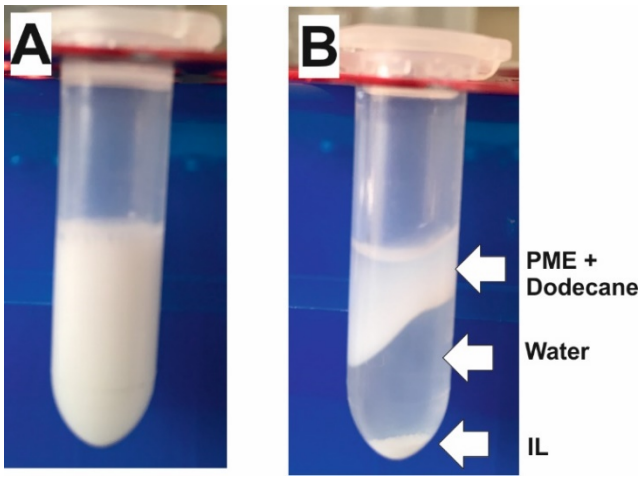

Fig. 3. Phase behaviour of panthenyl monolaurate/[C_{12}mim][BF_4] reaction mixture (Table 1, entry 3) after addition of 0.5 mL water and 0.5 mL dodecane (**A**), and then centrifugation (15,000 rpm, 15 min) at 15ºC (**B**) . See experimental section for further details.

characteristic of SLILs.[11,12] The addition of dodecane and water into the reaction medium at 60ºC, followed by cooling to 0ºC and centrifugation (15,000 rpm for 15 min at 0ºC) leaded to the precipitation of a solid phase (mainly containing the IL), a middle aqueous phase containing the unreacted panthenol (as hydrophilic compound insoluble in non-polar solvents), and a dodecane top-phase containing PME product (see Fig. 3B). By ^{19}F NMR, it was detected a 3.5% (w/w) residual IL content in this top-phase. By this approach, the IL is easily recovered for further reuse, while the residual IL content in PME-dodecane phase could be reduced by increasing the number of washing-by-water steps. This result was similar to those obtained for the separation of monolaurin from the same IL,[13c] allowing the recovery IL for further reuse.

3.2. Enzymatic synthesis of panthenyl monoacyl esters in deep eutectic solvents (DES).

Six different panthenol-based DES were prepared with capric acid (CA), lauric acid (LA), myristic acid (MA), palmitic acid (PA), oleic acid (OA) and linoleic acid (LA), as reported in the experimental section. As can be seen in Fig 4, the initial mixture of solids becomes a biphasic liquid system after heating at 80ºC, which can be transformed into a monophasic liquid system by strong shaking for 1 h at the same temperature, remaining unchanged with time after cooling to room temperature.[23] The suitability of these viscous and fully transparent panthenol-based DES as reaction media for the Novozym 435-catalyzed synthesis of PMEs was studied (see Table 1).

As a representative example, Fig. 5 depicts the time-course profiles for the enzymatic synthesis of panthenyl monolaurate in the panthenol-based DES with lauric acid (2:1 mol/mol) at 60ºC. As can be seen, this panthenol-based DES was shown as an excellent reaction medium for the enzymatic synthesis of panthenyl monolaurate, leading to a reaction rate of 18.4 μmol/min during the first reaction hour, reaching a maximum

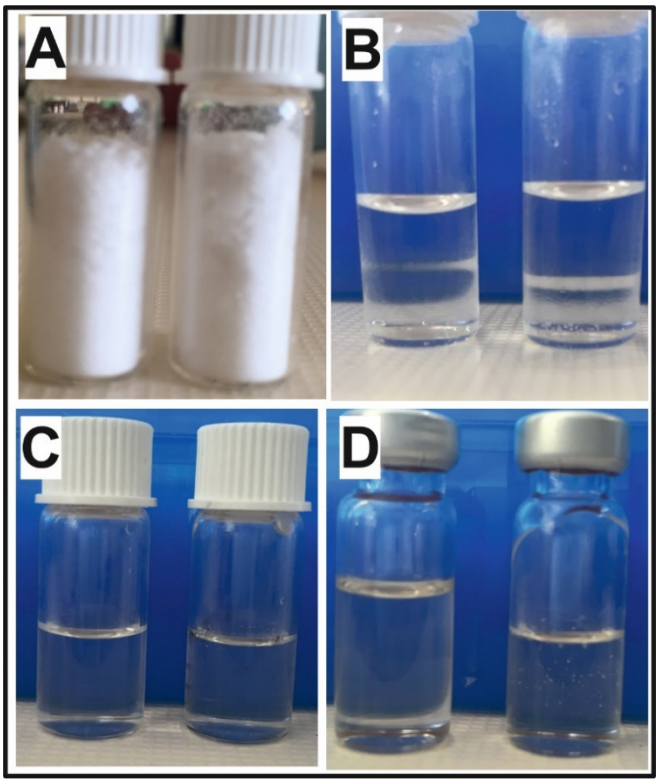

Fig. 4. Phase behaviour of a 1:2 (mol/mol) lauric acid:panthenol (left) and palmitic acid:panthenol (right) mixtures at room temperature (**A**), after heating at 80ºC (**B**), after shaking for 1h at 80ºC (**C**), after enzymatic reaction at 60ºC (**D**). See experimental section for further details.

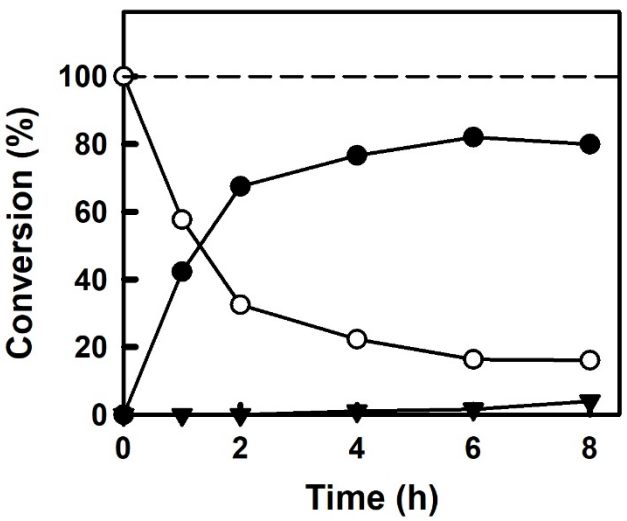

Fig. 5. Time-course profiles for the Novozym 435-catalysed synthesis of panthenyl monolaurate and (●), panthenyl dilaurate (▼) by direct esterification of lauric acid (○) with panthenol in a panthenol-based DES at 60ºC. Lauric acid: panthenol ratio, 1:2 (mol/mol).

Table 2. Biocatalytic synthesis of panthenyl monoacyl esters (PME) and panthenyl diacyl esters (PDE) by using different acyl donors after 6 h of reaction in panthenol-based DES at 60ºC. See experimental section for further details).

Entry	Acyl donor[a]	Panth./AD (mol/mol)	Conv.[b] (%)	PME (%)	PDE (%)	PME[c] (% w/w)
1	LA	1:1	59.1	96.5	3.5	54.4
2	LA	3:2	68.9	98.1	1.9	51.1
3	LA	2:1	80.3	95.0	5.0	48.4
4	LA	3:1	83.6	98.1	1.9	38.5
5	CA	2:1	83.0	93.0	7.0	47.1
6	MA	2:1	79.9	95.1	4.9	49.4
7	PA	2:1	73.0	94.2	5.8	45.7
8	OA	2:1	80.9	97.7	1.7	53.6
9	LnA	3:1	77,3	99,5	0,4	40.3

[a]Acyl donor (AD): lauric acid (LA); capric acid (CA); myristic acid (MA); palmitic acid (PA); oleic acid (OA); linoleic acid (LnA); [b]Conversion (yield) with respect the offered acyl donor. [c]PME content in the reaction medium.

conversion level of 83% at 6 hour, before slightly decreasing with the concomitant increase in panthenyl dilaurate production (up to 3.9%) at 8 h reaction.

As can be seen in Table 2, all the proposed panthenol-based DES with fatty acids were suitable reaction media for Novozym-435 catalysed PME synthesis at the different mole ratio assayed. Taking the use of lauric acid as example (entries 1-4), it should be noted how the increase in panthenol concentration with respect to this acyl donor (*i.e.* from 1:1 to 3:1) resulted in an increase in the conversion of lauric acid to panthenyl monolaurate (*i.e.* from 59.1 to 83.6%), although the final content of this target product inside reaction mixture was reduced (*i.e.* from 54.4 to 38.5%). Alternatively, the increase in the alkyl chain length in the acyl donor (*i.e.* capric, lauric, myristic, palmitic and oleic acids, see entries 5 to 8) at the same acyl donor:panthenol molar ratio leaded to an improvement in the selective synthesis of PMEs, although the enzymatic conversion of the acyl donor in PME was practically the same. Thus, the PME yield was increased from 93% (for the panthenyl monocaprate) to 97.7% in the case of panthenyl monooleate. The highest selectivity (99.7%) was obtained for panthenyl monolinolenate. For all the assayed panthenol-based DES at 2:1 molar ratio, it should also be emphasised that the enzymatic syntheses led to around a 50% (w/w) PME content with respect to the overall weight of the reaction medium, a value practically three-times higher than that obtained for reaction media based on ILs.

Furthermore, even though both panthenol and panthenyl monoacyl esters are active biomolecules used in the preparation of cosmetic products, an increase in the PME content, as a result of enzyme catalysis in these DES-based reaction media, could be obtained by selective precipitation of the unreacted panthenol (e.g. by adding a nonpolar solvent). Thus, it was observed how the addition of limonene to the reaction mixture at 60ºC,

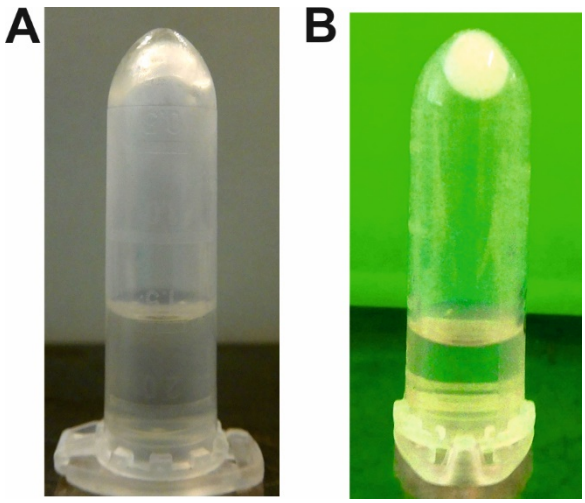

Fig. 6. **A** Separation of unreacted panthenol (see top picture) from the panthenyl monolaurate reaction mixture (see Table 2, entry 3) dissolved in limonene, after incubation at 60ºC for 1 h, then cooled in ice bath, and finally centrifuged (15,000 rpm, 10 min) at 0ºC. **B**. Separation of panthenyl monolaurate (see top picture) from the resulting liquid limonene phase of previous picture, after cooling for 6 h at -20ºC, limonene, followed by centrifugation (15,000 rpm, 5 min) at 0ºC

followed by a cooling/centrifugation protocol at 0ºC (see Experimental section), lead to the precipitation of the unreacted panthenol as a gel, while the PME product remained as dissolved in the limonene phase. As example, for the panthenyl monolaurate case (see Table 2, entry 3), a 89.2% of the unreacted panthenol was precipitated after the cooling/centrifugation protocol. Then, the panthenyl monolaurate can be separated from limonene by precipitation after a second cooling step at

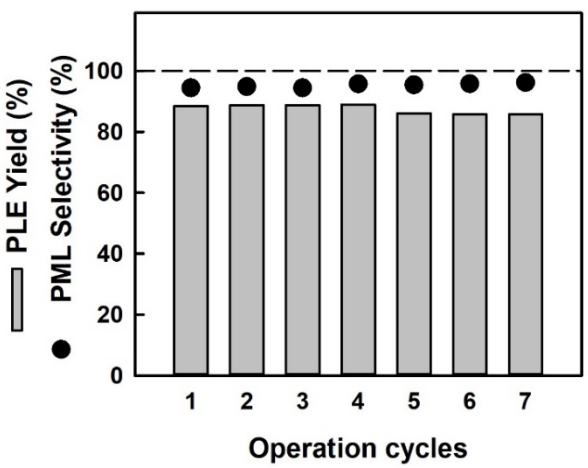

Fig. 7. Panthenyl laurate esters (PLE) yield (grey) and panthenyl monolaurate (PML) selectivity (●) during continuous operation cycles for the Novozym 435-catalyzed esterification of lauric acid with panthenol in DES-based reaction medium at 60ºC. Lauric acid:panthenol ratio, 1:2 (mol/mol).

-20ºC, followed to centrifugation (see Fig. 6). The NMR analysis of this precipitated fraction confirmed panthenyl monolaurate as the synthetic product (see ESI).

Hummer *et al* reported how menthol-based DES with fatty acids were suitable reaction media for the lipase-catalyzed esterification of the DES compounds itself to synthesize menthol fatty acid esters, obtaining conversions of up to 83% after 7 d reaction time.[24] The menthol:fatty acid DES acted as solvent and substrate at the same time, as in the case of choline chloride:sugar based DES, which were used for the enzymatic synthesis of glycolipids.[25] The advantage of using substrate-based DES as reaction media is that solid substrates are liquefied and made available for the enzymatic reaction. However, in contrast to choline chloride-based DESs, the developed panthenol:fatty acid DES reaction systems solely consists of the neat reactants and a substrate-liquefying additive is not required.[24]

The excellent suitability of these panthenol-based DES with fatty acids as reaction media for the enzymatic synthesis of panthenyl monoacyl esters was also observed when the immobilized enzyme was reused (Fig. 7). As can be seen, the operational stability profile of the Novozym 435 biocatalyst in panthenol-based DES with lauric acid showed an unchanged yield and selectivity for the synthesis of panthenyl monolaurate during seven consecutive operation cycles of reuse. Polyols (*e.g.* xylitol, sorbitol, etc.)[26] and DES (*e.g.* urea: choline chloride mixture)[27] have been reported to be excellent media for protecting biocatalysts against deactivation as a result of the hydrogen binding net formed around the enzymes, maintaining the solvophobicity that plays a key role in supporting the active conformation of the enzyme molecule. The excellent operational stability displayed by the enzyme at 60ºC in this panthenol-based DES could be attributed to the same phenomena.

Conclusions

The development of easy and green chemical processes for the synthesis of bioactive molecules is a key target for both cosmetic and pharmaceutical industries, where the implementation of new integrated reaction/separation systems must be simple, effective and sustainable at the same time. This paper comparatively describes for the first time two straightforward and green approaches for the selective synthesis of panthenyl monoacyl esters, as target molecules for the cosmeceutical industries by combining the efficiency of enzyme catalysis with the excellences of reaction media based on ILs and DES technologies.

Ionic liquids with a long alkyl side chain in the cation (SLILs) used as non-aqueous reaction media are widely recognised to preserve catalytic properties of enzymes,[11-13] and make excellent reaction media for dissolving FFA and panthenol substrates. A proper selection of the cation and anion structures in these SLILs also helps to control mass transfer processes into and from the active site of the enzyme, favouring the achievement of selective synthetic processes for the preparation of PMEs (*e.g.* aprox. 100% selective synthesis of panthenyl monolaureate in $[C_{12}mim][BF_4]$. Furthermore, the characteristics of SLILs facilitate the development of a straightforward protocol, based on cooling and centrifugation steps, which allows the separation of PMEs products, and the recovery of the IL for further reuse.

Furthermore, the ability of panthenol:fatty acid mixtures to form DES, shown for the first time in this work, was successfully applied for the lipase-catalysed selective synthesis of panthenyl monoacyl esters by direct esterification in a solvent-free approach. The reaction yields (*i.e.* up to 83.9%) and PME selectivity (*i.e.* 93-99%), together with the fact that no solvents are involved, make this approach as a useful way to prepare panthenyl monoacyl esters.

Regarding the greenness of both processes, the DESs-based approach seems to be more sustainable than that based on SLILs, because only the mixture of both pure substrates and the immobilized biocatalyst are involved, without using any kind of solvent. Furthermore, as both the panthenol and the panthenol monoacyl esters are ingredients for commercial formulations, the resulting raw mixture after the enzymatic reaction (*e.g.* 51.1% w/w panthenyl monolaurate mixed with unreacted panthenol, see Table 2 entry 3) could be directly used for the preparation cosmetic products.

Once again, it was demonstrated how the combination of enzymes with sustainable non-aqueous reaction media, such are SLILs or DESs, provides synergic opportunities and opens up the way to new green platforms for developing green chemical processes of industrial interest.

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