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The enantioselectivity of *Candida rugosa* lipase is influenced by the particle size of the immobilising support material Accurel

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Abstract

When *Candida rugosa* lipase (CRL) was immobilised on the porous polypropylene carrier Accurel it was found that differences in the samples of the carrier influenced the enantiomeric ratio (*E*) when racemic 2-methylhexanoic acid was esterified with 1-decanol in organic solvent at a constant water activity (a_w) of 0.76. CRL immobilised on Accurel with smaller particles and smaller pore diameters gave higher enantiomeric ratio in the esterification. The reaction rate was influenced by the different Accurel materials and also by the amount of CRL exposed to the carrier. Characterisation of the different Accurel grades, with and without adsorbed CRL, was performed by light scattering analysis, SEM and FT-IR.

Keywords: *Candida rugosa* lipase; Immobilisation; Polypropylene carrier Accurel; Enantiomeric ratio; Enantioselective esterification; Morphology; Particle and pore size

1. Introduction

Considerable attention has been devoted to explore the use of enzymes in organic media and especially to use lipases as catalysts in acyl transfer reactions [1,2]. Enzyme immobilisation is an important technique which ensures recycling of the biocatalyst, permits easy product separation and improves the performance of the enzyme [3,4]. Immobilisation can also be used to purify crude commercial preparations of lipase as only a small amount of the crude enzyme preparation is the lipase of interest. Kinetic behaviour of immobilised enzyme is different from the free enzyme due to conformational changes, steric effects, partition effects, micro environmental effects and diffusion effects [3]. Various immobilisation techniques have been developed and the most commonly used techniques are entrapment, encapsulation, cross-linking, carrier bonding, flocculation and adsorption [3]. The adsorption of a protein onto a solid surface is a relatively simple and widely used method and porous polypropylene (Accurel) has been used by many research groups to immobilise different hydrolases [5–13]. It has previously been reported that immobilisation of lipases on Accurel influences the catalytic activity [9,12] and the enantioselectivity [8,14]. Published results show that esterification of oleic acid catalysed by lipase immobilised on smaller Accurel carrier particles results in higher reaction rates [15].

In this paper, we report our results when different granular grades of Accurel are used as support materials for adsorption of CRL. The immobilised lipase was then used in enantioselective esterification of racemic 2-methylhexanoic acid in organic solvent at a fixed a_w of 0.76 (see Scheme 1). To our knowledge it has never been reported that differences in particle size and pore size in different grades of the immobilising support Accurel influences the enantioselectivity of *Candida rugosa* lipase.

2. Experimentals

2.1. Chemicals

Different lots of *Candida rugosa* lipase-type VII was purchased from Sigma–Aldrich. Lot: 074K0685, activity: 1410 units/mg solid used in the immobilisation A and as crude lipase. Lot: 123K1334, activity: 706 units/mg solid used in the immobilisation B. Lot: 85H0629, activity: 835 units/mg solid used in the immobilisation C. Racemic 2-methylhexanoic acid

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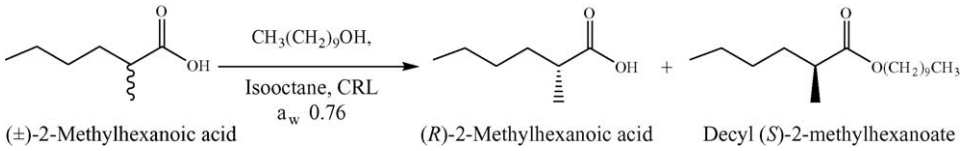

Scheme 1. CRL catalysed enantioselective esterification of (±)-2-methylhexanoic acid.

and 1-decanol were purchased from Sigma–Aldrich and isooctane was purchased from Fluka and used without further purification. Accurel EP 100 (<200 μm) and EP 100 (200–350 μm) were gifts by Accurel systems, AKZO Faser AG, Obernburg, Germany. Accurel MP 1001 (400–1000 μm) and MP 1000 (<1500 μm) were purchased from Accurel systems, Membrana GmbH, Obernburg, Germany.

2.2. Gas chromatographic (GC) methods

A Varian 3400 C_X gas chromatograph, equipped with a EC-1 column (Alltech, 30 m × 0.32 mm ID × 0.25 μm, N_2 13 psi) was used to monitor the progress of enzymatic reactions. Enantiomeric excesses were analysed by GC using a Varian 3300 equipped with a β-dex 225 column (Supelco, 30 m × 0.25 mm × 0.25 μm; He 18 Psi).

2.3. Fourier transform infrared (FT-IR) spectroscopy of Accurel samples

Immobilised CRL (2 mg) or pure Accurel (2 mg) was mixed and ground with 200 mg of potassium bromide and then compressed into a pellet under a pressure of 22 kp/cm^2. FT-IR spectra of the pellets were obtained with a Perkin Elmer 16 PC FT-IR instrument.

2.4. Light scattering analysis of Accurel samples

Particle size and size distribution were determined on a Malvern Mastersizer Hydro 2000 SM light scattering apparatus on dry samples using a Scirocco 2000 accessory. Calculations were made with the Mastersizer 2000 software using a general purpose model and a refractive index of polypropylene of 1.49.

2.5. Scanning electron microscopy (SEM) of Accurel samples

Particle morphologies were studied using a Philips SEM XL 20 scanning electron microscope. All samples were coated with a thin layer of gold prior to analysis using a BAL-TEC SCD 005 sputter-coater (0.1–0.01 mbar, 230 s at ∼35 mA).

2.6. Adsorption of CRL on different Accurel particle fractions and with three different amounts of lipase in the buffer (immobilisations A, B and C)

CRL was immobilised on Accurel <200, 200–350, 400–1000 and <1500 μm by adsorption. The following amounts of CRL powder per gram Accurel were used in the phosphate buffer: Immobilisation A: 20 g CRL powder. Immobilisation B: 8 g CRL powder. Immobilisation C: 2 g CRL powder. In all the cases the lipase was dissolved in 200 ml phosphate buffer (20 mM, pH 7.0) and centrifuged at 2700 rpm for 2 min to remove the insoluble impurities. The resulting supernatant was separated and used in the immobilisation procedure. Accurel was wetted with ethanol (3 ml per 100 mg of Accurel) and then most of the ethanol was removed under vacuum. The supernatant was added to the wetted Accurel and stirred at room temperature for 15 h and then filtered. The resulting residue was dried for 3 days over silica gel in an air tight desiccator.

2.7. General procedure for CRL catalysed enantioselective esterification

To 2-methylhexanoic acid (97.6 mg, 0.75 mmol) and 1-decanol (119 mg, 0.75 mmol) in isooctane (5 ml) was added the salt pair Na_2SO_4 (142 mg, 1 mmol) and $Na_2SO_4{\cdot}10H_2O$ (161 mg, 0.5 mmol) to maintain a water activity (a_w) of 0.76. The enantioselective esterification was started by addition of 86.2 mg of immobilised CRL or 500 mg crude CRL. After stirring for an appropriate time at 20 °C the reaction was stopped below 40% conversion by filtering off and washing the enzyme with several portions of Et_2O. The filtering off and the washing of the enzyme together with conversions calculated from ee_p and ee_s (using the formula by Chen et al. [16]) should avoid the problem, observed by others [17], that sorption of the substrate acid into the polymer matrix of the catalyst influences the determination of the *E*-value. The remaining substrate acid was separated from the product ester via liquid chromatography using an increasing gradient of distilled diethyl ether (0–100%) in distilled pentane as eluent. After reduction with $LiAlH_4$ in Et_2O the enantiomeric excesses (ee_s and ee_p) of obtained enantiomerically enriched *R*- and *S*-alcohols were determined using a β-dex 225 GC-column. The retention time for (*R*)-2-methylhexanol was 16.5 min and for (*S*)-2-methylhexanol it was 17.0 min. The *S*-(+)-2-methylhexanoic acid is the faster reacting enantiomer in the resolution and this was confirmed by the sign of optical rotation of the remaining substrate (*R*)-2-methylhexanoic acid obtained from the reaction [18].

3. Results and discussion

We have previously used immobilised (Accurel 350–1000 μm, EP 100) CRL in enantioselective esterifications of racemic 2-methylalkanoic acids and obtained excellent results [14,19]. When Accurel EP 100 (350–1000 μm) was no longer commercial available we started to use Accurel MP 1000 (<1500 μm) for the immobilisation of CRL by adsorption. When

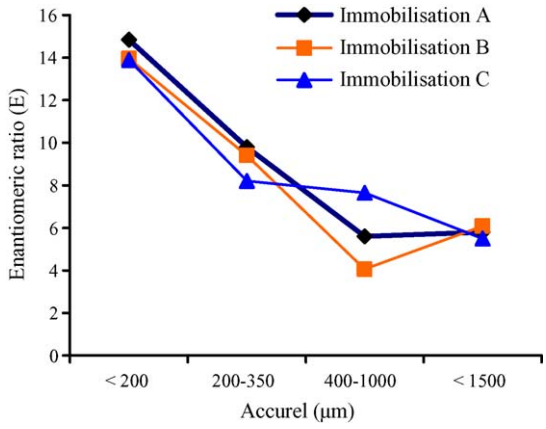

Fig. 1. Influence of different grades of Accurel with different particle size on the enantiomeric ratio of CRL catalysed enantioselective esterification of racemic 2-methylhexanoic acid. *E*-values are presented as an average of all trials corresponding to immobilisations A, B and C, respectively (see Table 1).

we used the new immobilised CRL in our esterification reactions we observed a small change in the enantiomeric ratio for 2-methylalkanoic acids as substrates [20]. It has been reported that the type of support chosen in the immobilisation procedure of CRL influences the enantioselectivity in the hydrolysis of (*R*,*S*)-methyl mandelate [21]. Thus, our hypothesis was that the change in the *E*-value might be due to some aspects of differences in the morphology of the Accurel samples. To examine this further we chose to study 2-methylhexanoic acid as a model substrate in esterification with *n*-decanol in *iso*-octane at $a_w = 0.76$ with CRL immobilised on different Accurel grades. Recently published results, in resolutions of secondary alcohols with high *E*-values, show that with Novozyme 525 F the enantioselectivity decreased by increasing conversion [22]. But, for resolutions with low *E*-values (~25) the enantiomeric ratio did not change significantly at different conversions [22]. Thus, we used a substrate that both crude and immobilised CRL resolves with low enantioselectivity in an attempt to confirm the influence of the morphology of the immobilising support material on the enantiomeric ratio and reaction rate. CRL was immobilised on four Accurel grades, <200, 200–350, 400–1000 and <1500 μm by exposing different amounts of CRL in phosphate buffer as described above (Immobilisations A, B and C).

Our results are presented in Table 1 and summarised in Figs. 1 and 9. The highest *E*-values were obtained when Accurel <200 μm was used in the three immobilisations with different amounts of CRL (*E* = 12.8–16.7, entries 1–5 in Table 1). Accurel grades with 200–350 μm gave *E*-values from 8.2 to 11.0 (entries 6–11 in Table 1). When Accurel material with 400–1000 and <1500 μm particles were used, the enantioselectivity in the reactions varied from 3.5 to 8.0 (entries 12–20).

We repeated the immobilisation method A on all Accurel grades (entries 2, 7, 13 and 18) using CRL from the same lot. Small changes (0.2–1.6) in *E*-values were registered compared to what was obtained with the first immobilisations (entries 1, 6, 12 and 17). Thus, the immobilisation A was repeated with two different Accurel grades (<200 and 200–350 μm) using a mixture of two different lots of CRL from the same manufacturer (Sigma). Each entry (entries 3 and 8) was repeated three times and interrupted at almost the same conversion and also now small differences in the *E*-values were registered between the three trials within entry 3 (*E* = 12.9–16.7) and entry 8 (*E* = 9.2–9.8), respectively. When repeating other entries we also obtained small differences between the trials even when using immobilised CRL from the same immobilisation fraction (see entries 4, 9, 14, 16 and 19).

Jacobsen et al. observed that the enantioselectivity decreased by increasing conversion for substrates resolved with high *E*-values which was not the case for substrates resolved with low *E*-values [22]. Our observations when interrupting reactions at different conversions (see entries 1–3, 6–8, 9–10, 12–13, 14–15 and 17–18 in Table 1) shows that the *E*-value does not differ more than between the trials within an entry (see for example entries 3–4).

The observed differences in *E*-values between and within entries (see Table 1) seem to be more pronounced when using Accurel EP 100 (<200 and 200–350 μm) grades. These two Accurel grades also contain a fraction of very small polypropylene particles together with the main fraction of particles (see Fig. 2). We concluded that the small *E*-value differences observed (see Table 1) are due to the wide range of particle sizes and pore sizes in the Accurel fractions. Thus, it is difficult to know if the particle distribution and the morphology of the used immobilised CRL is representative for each trial. It is therefore important to use supporting materials with a narrow distribution of particle sizes and pore sizes

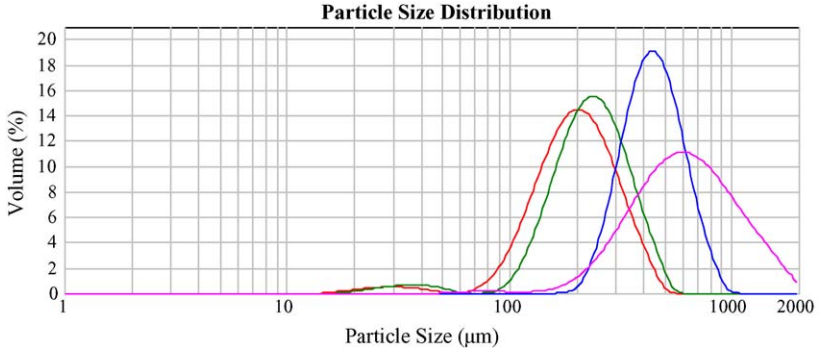

Fig. 2. Light scattering analysis of Accurel samples.

Table 1
Influence on the enantiomeric ratio (E) and the reaction rate when different Accurel grades were used as support material for immobilising CRL

Entry	Accurel grade[a]	Immobilisation	Conversion (%)	Reaction rate (%/h)	E[b]
1	Accurel EP 100, <200 μm	A	26.2	5.2	15.7
2		A[c]	28.2	4.7	14.1
3		A[d]	21.0, 20.7, 20.0	3.8, 3.8, 3.1	14.9, 16.7,12.9
4		B	31.6, 29.0, 30.0	6.3, 5.8, 6.0	12.8, 13.4, 15.7
5		C	21.0	0.40	13.9
6	Accurel EP 100, 200–350 μm	A	31.1	6.2	11.0
7		A[c]	31.8	5.3	9.8
8		A[d]	19.8, 20.0, 21.6	3.6, 3.6, 3.3	9.2, 9.8, 9.2
9		B	34.2, 34.2, 35.0	6.8, 6.8, 7.0	9.2, 9.3, 9.3
10		B[c]	24.5	4.1	9.8
11		C	24.8	0.48	8.2
12	Accurel MP 1001, 400–1000 μm	A	12.4	1.1	5.0
13		A[c]	21.0	0.90	6.2
14		B	23.4, 23.6	1.1, 1.1	3.7, 5.0
15		B	2.8	0.55	3.5
16		C	20.2, 16.6	0.39, 0.31	8.0, 7.3
17	Accurel MP 1000, <1500 μm	A	21.7	1.2	5.9
18		A[c]	16.3	0.90	5.7
19		B	15.9, 16, 12.8	0.74, 0.74, 0.60	6.2, 6.2, 5.9
20		C	16.6	0.32	5.5
21[e]	–	–	13.0	3.5	16.4

[a] It was confirmed by the supplier of Accurel that the polypropylene material is the same for EP and MP and that they differ only in the particle size distribution.
[b] Calculated according to the equation $E = \ln [1 - c\,(1 + ee_p)]/\ln [1 - c\,(1 - ee_p)]$ where $c = ee_s/(ee_s + ee_p)$ [16].
[c] The immobilisation procedure was repeated using the same lot of CRL and in some cases the reaction was consciously interrupted at the same or at different conversions.
[d] The immobilisation procedure was repeated using a mix of two different lots of CRL (with different activities) from the same manufacturer (Sigma).
[e] Crude CRL was used in the reaction without immobilisation.

or sieve the material to a very narrow particle size range before use.

The amount of lipase immobilised on Accurel has been found to influence the enantioselectivity [23] and the reaction rate [23–25] in other lipase catalysed reactions. Thus, we used different amounts of CRL in the buffer (immobilisation methods A, B and C) and we also found an influence on the E-value. Higher amount of CRL in the buffer most frequently resulted in somewhat higher enantiomeric ratios compared to when using lower amounts (see Fig. 1).

The reaction was also performed with crude CRL (entry 21) and this gave the next highest E-value ($E = 16.4$) when compared to what we obtained with immobilised CRL. Engel et al. also used crude CRL in the same reaction, but under slightly different reaction conditions, and they obtained an even higher E-value ($E = 25$) with crude CRL [26]. The commercially available CRL powder also consists of other proteins, polypeptides and polysaccarides which can function as carrier of the lipase and this might influence the selectivity.

Conformational changes of the immobilised enzyme can influence the catalytic properties of the enzyme to a large extent [27]. In our case, small conformational changes of CRL (that influences the enantioselectivity) might depend on the nature of the surface or the pores of the polypropylene material. Accurel is a hydrophobic polypropylene support material that adsorbs lipases with strong multipoint interactions [6] and immobilisation of CRL on different Accurel particle sizes and pore diameters can influence the preferred conformation of the lipase. It has also been reported that the pore sizes play an important role in the diffusion properties of the substrate [5,25,28,29]. Some years ago a Dutch research group reported that diffusion limitations causes decreased enantioselectivity in the esterification of 2-butanol catalysed by a commercial preparation of immobilised *Candida antarctica* lipase B [30]. They used sieved fractions (between 400 and 700 μm) of the immobilised lipase and experimentally obtained the same E-value ($E = 2.5$) for all tested particle sizes. Only when the carrier, with the adsorbed enzyme, was ground into very small (~1 μm) and undefined particles they obtained a slightly higher E-value ($E = 4.4$) [30]. When we adsorb CRL onto the polypropylene surface the carrier becomes a chiral environment for the substrate and thus, the diffusion of *S*-(+)-2-methylhexanoic acid might be more favoured with the Accurel grades with smaller particles and pores. Nevertheless, when all results in Table 1 are taken together and presented in Fig. 1 it gives a clear indication that the E-value is influenced by differences between the Accurel samples.

In an attempt to explain the obtained differences in the enantioselectivity of CRL when adsorbed onto different Accurel grades, our results prompted us to try to characterise the Accurel samples thoroughly. Others have characterised Accurel MP 1004 and reported that the Accurel consists of a wide range of particle sizes and pore sizes [28]. Thus, we measured the particle size distribution for the samples (see Table 2 and Fig. 2). In Fig. 2 the particle size distribution can be seen and the Accurel material with particles <200 and 200–350 μm consists of a large number of very small particles besides the main particle sizes. These very

Table 2
Granulometric analysis of different Accurel materials

Accurel sample	Particle size[a] (μm)	Span[b]	Average pore diameter (μm)	Range pore diameter (μm)
Accurel EP 100, <200 μm	200	1.1	9[c]	4–17[c]
Accurel EP 100, 200–350 μm	230	1.0	11[c]	8–15[c]
Accurel MP 1001, 400–1000 μm	440	0.81	23[d]	12–35[d]
Accurel MP 1000, <1500 μm	610	1.5	25[d]	12–35[d]

[a] Determined by laser light scattering, presented as the volume median diameter $d(0.5)$.
[b] Size distribution is expressed as span = $d(0.5)/[d(0.9) - d(0.1)]$.
[c] Measured manually on SEM picture with magnification 500×.
[d] Measured on SEM picture with magnification 100×.

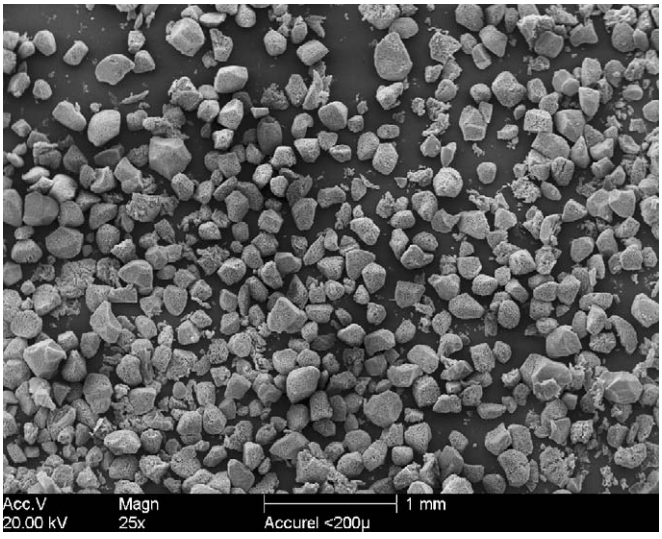

Fig. 3. SEM of Accurel <200 μm at a magnification of 25×.

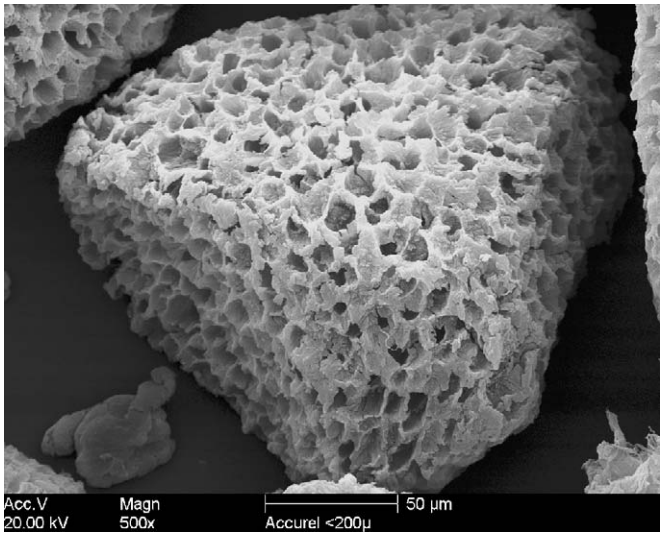

Fig. 5. SEM Accurel <200 μm at a magnification of 500×.

small particles are not found in the Accurel grades that consists of bigger particles (400–1000 and <1500 μm).

All grades of Accurel show different particle sizes and pore sizes within a sample and this was also confirmed by the SEM pictures (see Figs. 3–5, 7 and 8). Figs. 3 and 4 show SEM pictures of Accurel <200 and 400–1000 μm as an example. From our detailed SEM analysis we conclude that the Accurel particles, even from the same grade of Accurel, have very inhomogeneous morphologies (see Figs. 5, 7 and 8).

The diameters of 20 pores on a representative particle from the four Accurel samples were manually measured in some of the

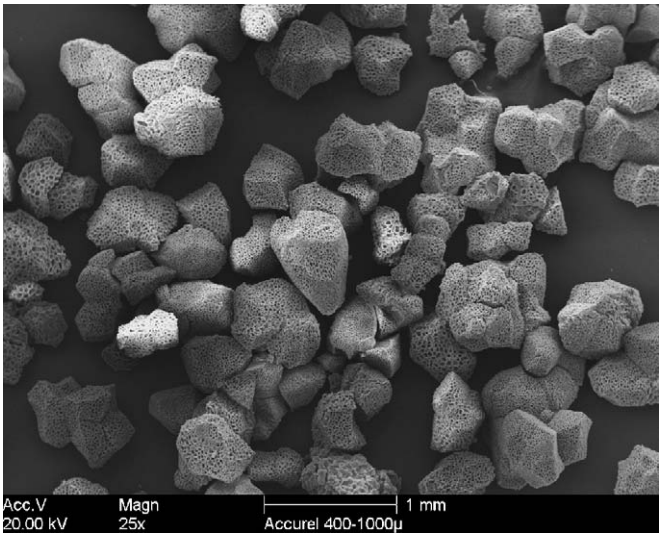

Fig. 4. SEM of Accurel 400–1000 μm at a magnification of 25×.

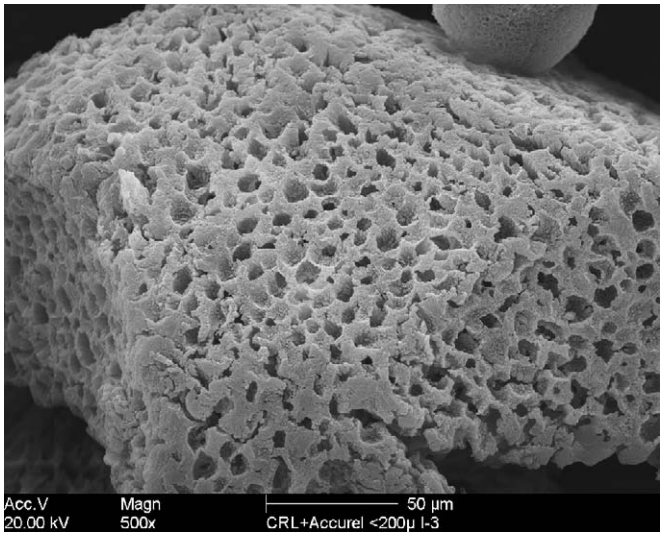

Fig. 6. SEM of CRL immobilised on Accurel <200 μm at a magnification of 500×.

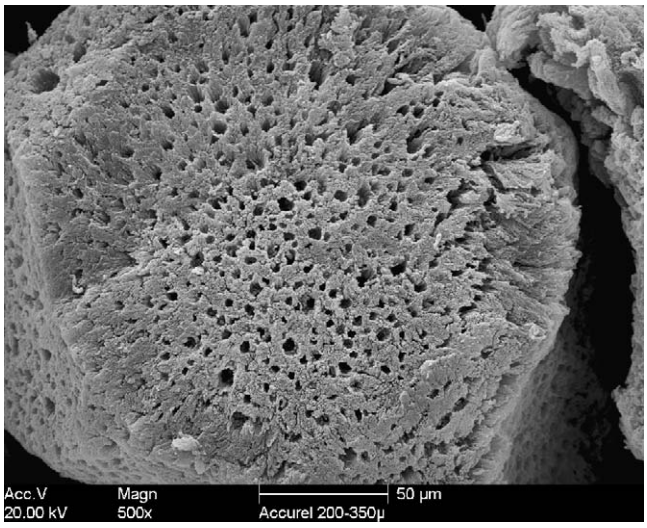

Fig. 7. SEM Accurel 200–350 μm at a magnification of 500×.

SEM pictures with an appropriate magnification and the conclusion is that the smaller the particle is the smaller is the diameter of the pores (see Table 2).

We performed a thorough investigation of the Accurel samples with the CRL adsorbed on the carrier using SEM. Fig. 5 shows the SEM picture of Accurel EP 100 (<200 μm) and Fig. 6 with CRL adsorbed on the same Accurel grade. The example shown in Fig. 6 is from the immobilisation A when the highest amount of CRL was used for adsorption onto the carrier. Foresti and Ferreira have reported to have visualised CRL immobilised on polypropylene by SEM analysis on a 20 μm scale [31]. They have used 600 mg CRL per 1 g polypropylene powder (210–1180 μm) which is less than the amounts we used in our immobilisation procedures (see immobilisations A, B and C). Typical diameter of a lipase has been reported to be approximately 50 Å (5×10^{-3} μm) [28] and therefore it should only be possible to visualise big aggregates of the lipase on the support. In our attempt to visualise CRL adsorbed on the support it

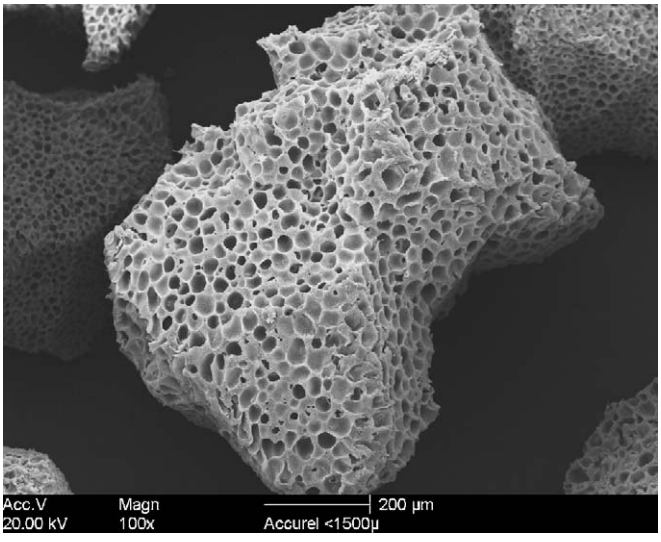

Fig. 8. SEM Accurel <1500 μm at a magnification of 100×.

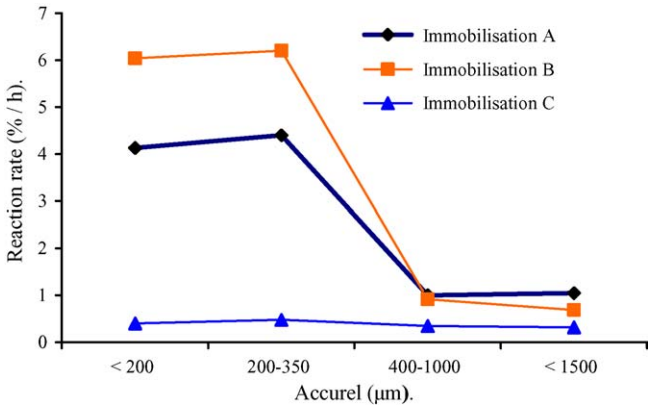

Fig. 9. Influence on reaction rate in the enantioselective esterification of 2-methylhexanoic acid when different Accurel fractions are used to adsorb CRL. The reaction rates are presented as an average of all trials corresponding to immobilisations A, B and C, respectively (see Table 1).

shows that SEM images of Accurel without CRL (Fig. 5) and with CRL (Fig. 6) look nearly the same. We also analysed immobilised CRL samples at high magnifications (5000×, results not shown) but even from these pictures we were not able to claim that the lipase was visualised.

When the lowest amount (immobilisation C) of CRL was used to immobilise the lipase on the different Accurel grades, essentially the same reaction rate was obtained independent of particle size used (see entries 5, 11, 16 and 20 Table 1 and Fig. 9). When using immobilisation B, more CRL should be adsorbed onto the carrier and the reaction rate increased as expected for the Accurel samples <200 μm and 200–350 μm (see Fig. 9). But, when Accurel 400–1000 and <1500 μm were used the reaction rate was ~6 times lower than the rates with the smaller particles in immobilisation B (see Fig. 9).

When increasing the lipase amount in the buffer solution even more (immobilisation A), the same pattern was revealed when it comes to the reaction rate. Surprisingly, the rate did not increase when compared to immobilisation B despite of the possibility for more lipase to be adsorbed in immobilisation A. When a porous material, as Accurel, is used as support for a lipase the diffusion of the substrate through the pores may be the rate-limiting step in a catalysed reaction [25,28]. Salis et al. observed a saturation behaviour for *Candida antarctica* lipase B and *Thermomyces lanuginosa* lipase at enzyme loadings above 10 mg/g Accurel [25]. They suggested that the reduced catalytic activity at high enzyme loading on the support might be due to mass transfer (diffusion) limitations. They also observed that internal diffusion becomes more rate-limiting as the diameter of the support increases [28]. We observed the same effect when using high amounts of CRL on the smaller particles of Accurel (see Table 1 and Fig. 9). In our case immobilisations A and B can be considered as high enzyme loading while immobilisation C can be compared with low enzyme loading. Our result might be explained by the fact that the specific surface area is larger on the smaller particles than the surface area of the bigger particles [6,7,28] and probably more surface area on the polypropylene is free for lipase adsorption. This together with mass transfer limitations [25] probably results

in the slower reaction rate that we observed for the bigger polypropylene particles (see Table 1 and Fig. 9). According to FT-IR analyses (see below) the amount of CRL immobilised on Accurel in immobilisation A was not more than that of immobilisation B though larger amount of CRL was exposed to the carrier in immobilisation A. Probably the polypropylene surface is already covered with lipase and the tendency of CRL to form multi layers is not so high. But, it has been reported that CRL forms multi-layers on Accurel material [8]. It has also been suggested in several papers that a reduced catalytic activity for some lipases at low enzyme loading on the support might be due to enzyme distortions arising from the strong interactions between the enzyme and the immobilising support [25,32,33]. We observed that the rate of enantioselective esterification decreased as the enzyme loading decreased, i.e. lower reaction rates with immobilisation C compared to immobilisations A and B. This might be a result of inactivation of the lipase as observed with the *Thermomyces lanuginosa* lipase [25]. Lopez et al. observed that CRL from different sources showed different adsorption patterns and concluded that the polysaccharide content in crude CRL play an important role in the adsorption of CRL onto Accurel and results in an influence on the reaction rate [10]. Thus, not surprisingly we also noticed that the reaction rate differed (see above and

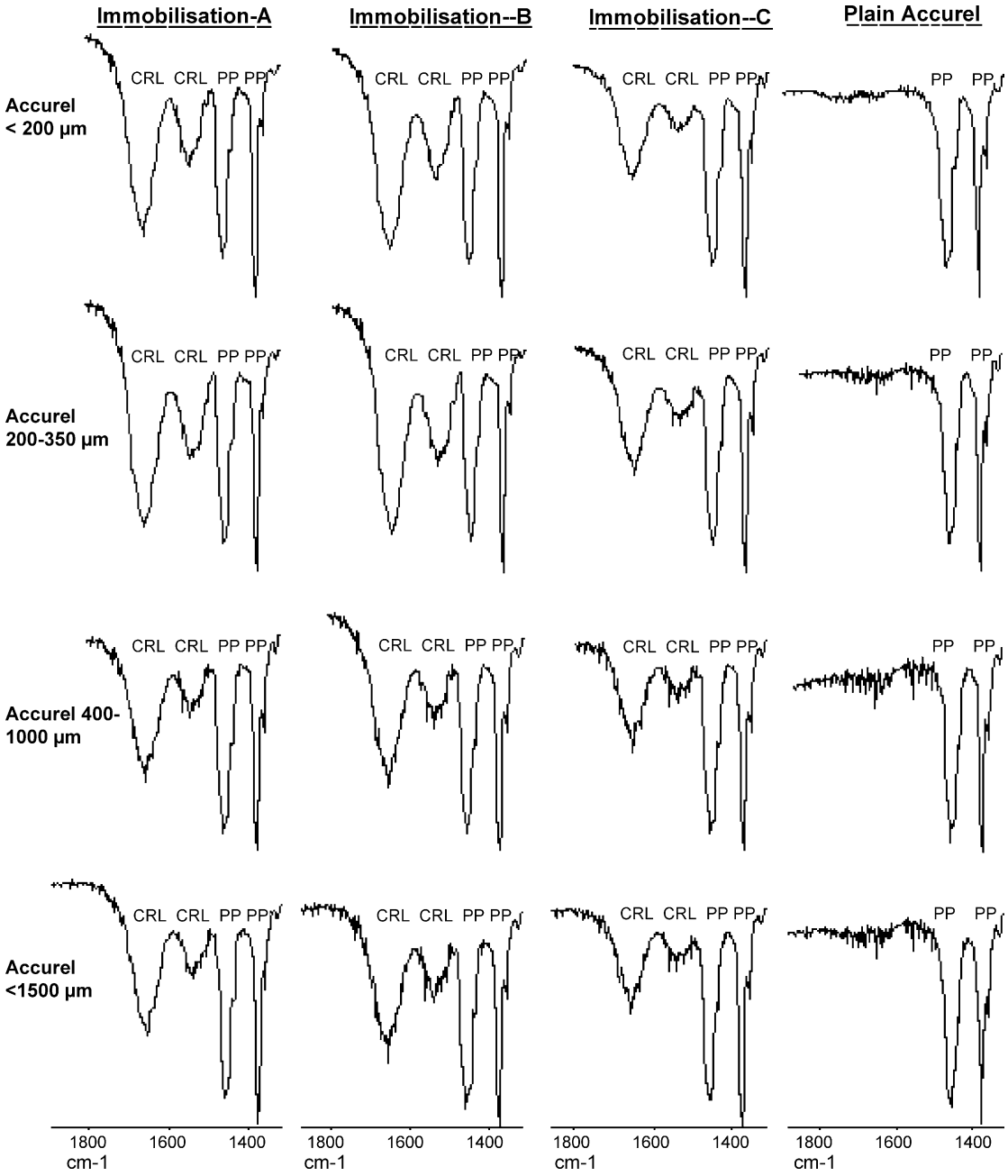

Fig. 10. Part of FT-IR spectra of CRL immobilised (immobilisations A, B and C with different amount of lipase added into the buffer) on different Accurel grades. CRL represents the peaks from carbonyl groups of *Candida rugosa* lipase and PP represents the normalised peaks from polypropylene (Accurel).

Table 1) when different lots of CRL was used in the immobilisation.

We analysed the immobilised CRL samples using FT-IR to study the amount of CRL adsorbed on to the Accurel particles. The ratio of peaks registered from the CRL compared to the ones that originate from the polypropylene support (see Fig. 10) was investigated. Fig. 10 shows a part of the normalised IR-spectra obtained from immobilised CRL. The immobilisations A and B (higher CRL loading) look very similar and nearly equal amounts of CRL seem to have been adsorbed on to the carrier. This might explain the similar reaction rate obtained as discussed above (see Fig. 9). Immobilisation C (low amount of CRL) shows a much lower ratio between the peaks but the ratio is the same for all grades of Accurel. This is also consistent with the obtained nearly uniform reaction rate independent of particle size. Different amounts of CRL have been adsorbed onto the different Accurel grades in immobilisations A and B. On bigger particle sizes (Accurel 400–1000 and <1500 μm) less adsorbed CRL was found compared with smaller particle sizes (Accurel <200 and 200–350 μm) (see Fig. 10). This also explains the differences in reaction rates discussed above (see Fig. 9).

We also determined the amount of CRL adsorbed on different particle sizes in immobilisation A. Thus, an exact amount of the carrier was weighed before immobilisation (see Section 2 for experimental details). After the immobilisation procedure the carrier with the adsorbed lipase was weighed. We found that the following amounts of CRL had been adsorbed per gram of Accurel; <200 μm; 150 mg, 200–350 μm; 200 mg, 400–1000 μm; 90 mg and <1500 μm; 40 mg. The result was in good agreement with the FT-IR analysis of the CRL **:** Accurel ratio and also the observed reaction rate is correlated to the amount of adsorbed CRL. Foresti and Ferreira [31] claim, by FT-IR and the reaction rate in ethyl oleate synthesis, that CRL is found to be present in larger amounts on bigger polypropylene particles. The polypropylene powder was in their case obtained by metallocene mediated polymerisation. Our results contradict this as we found that adsorption on smaller particles at high concentration of CRL results in more immobilised lipase and at low CRL concentration (see Fig. 10, immobilisation A) the same amount of enzyme is adsorbed on all the particle sizes.

4. Conclusions

Our results show that when CRL is immobilised on Accurel with different particle size distributions an influence in both the enantiomeric ratio and the reaction rate is noted. It was found that when higher amount of CRL was immobilised on Accurel with smaller particle sizes the highest reaction rate and the highest enantiomeric ratio was obtained in the enantioselective esterification. It is therefore important to characterise the immobilising support material obtained from the manufacturer and only use material with a narrow range of particle size and pore size in order to obtain desirable enantioselectivities, reaction rates and a high degree of reproducibility.

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