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Lipase catalysed transesterification of 2-*o*-benzylglycerol with vinyl acetate: solvent effect

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Abstract

The *Pseudomonas cepacia* lipase catalysed synthesis of (S)-2-*o*-benzylglycerol-1-acetate by transesterification of 2-*o*-benzylglycerol with vinyl acetate was studied in 10 different solvents in order to deduce the solvent effect through an attempt to correlate the initial reaction rate, selectivity for monoester formation and enantiomeric excess (ee) with such solvent properties as hydrophobicity ($\log P$), water solubility ($\log S_w$), electron pair acceptance (E_T^N) and donation abilities (D_N^N), polarisability and dielectric constant. The initial rate was found to exhibit reasonable correlation with $\log P$, $\log S_w$, $E_T^N + D_N^N$, $\log P$/polarisability and dielectric constant. The correlations for selectivity and ee were also found to be reasonable excepting deviation of data points of certain solvents. Probable explanation for the deviation has been put forwarded based on established hypothesis. The study revealed, in general that polar solvents favour the initial reaction rate, selectivity and enantiomeric excess for transesterification of prochiral diol to chiral monoester.

Keywords: Lipase; 2-*o*-Benzylglycerol; Vinyl acetate; *Pseudomonas cepacia*

1. Introduction

One of the most important characteristic features of enzymes is the ability to discriminate enantiomers. Accordingly, the high enantioselectivity of enzymes makes their applications to optical resolutions of recemates an increasingly growing field in both preparative organic synthesis and biotechnology [1–3]. There are many potential advantages for chemical biosynthesis with enzymes in organic solvents as the nature of the solvent can have a profound effect on substrate specificity, regioselectivity, and enantioselectivity of enzymes [4–6]. Asymmetric esterification or transesterifications catalysed by hydrolytic enzymes in nonaqueous media has emerged as a method of choice for facile kinetic resolution of racemic alcohols, acids and their derivatives [7].

Chiral-acetyl-2-*o*-benzylglycerol, (*R*) or (*S*) is a very useful building block for the preparation of enantiomerically pure, biologically active molecules such as phospholipids, PAF (platelet aggregation factor), phospholipase A2 inhibitors, sphinoglycolipids and many others [8]. The compound can be advantageously synthesised by transesterification of prochiral 2-*o*-benzylglycerol with vinyl acetate (Scheme 1) catalysed by lipase from *Pseudomonas cepacia*. This lipase is reported to give good yield and stereoselectivity for the above and analogous transesterification reactions in organic solvent [8–10]. However, a systematic investigation on the effect of solvent and a comprehensive kinetic analysis of the reaction have not been reported in the literature. In view of this, we present here a study of solvent effect on the initial reaction rate and selectivity of monoester formation and enantiomeric excess which we feel, is important from process point of view.

2. Experimental

2.1. Materials and methods

Lipase from *Pseudomonas* sp. (Amano "PS", specific activity 34 IU/mg solid) was obtained from Amano Pharmaceutical Co. (Nogoya, Japan), 2-*o*-benzylglycerol and vinyl acetate were procured from FLUKA. Silica gel 60, the solvents and 3 Å molecular sieves were obtained from CDH Pvt. Ltd., New Delhi, India. The solvents were purified by distillation and dried over 3 Å molecular sieves prior to use.

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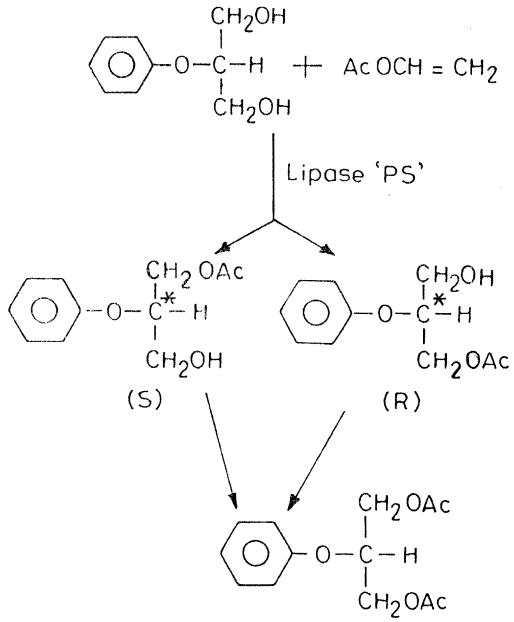

Scheme 1.

2.2. Transesterification reaction, analysis of product and preparation of standard

Standard 2-*o*-benzylglycerol-1-acetate was prepared by treating 50 mmol of 2-*o*-benzylglycerol with 100 mmol vinyl acetate in presence of 10 mg of lipase 'PS' and in absence of any extraneous solvent. The reaction mixture was taken in an iodine flask of 500 ml capacity and shaking the mixture at 120 rpm and 10 °C for 4 h in a water bath shaker (MURHOPYE Scientific Company, India). After the reaction the enzyme was separated from the reaction mixture by filtration and the enzyme free reaction mixture was concentrated by using vacuum rotatory evaporator (EYELA, Japan). The monoacetate formed in the reaction mixture was separated by column chromatography using silica gel (60–120 mesh) column (1 m × 2 mm). The solvent system used was dichloromethane, ethyl acetate and *n*-hexane at the ratio of 42.5:15.0:42.5 (v/v). The purified 2-*o*-benzylglycerol-1-acetate was concentrated by VRE and the purity and quantity was determined by GLC. The operating condition of the GLC (Varian 3700) was as follows: oven temperature 150 °C, injector temperature 220 °C, column temperature (programmed) 150–220 °C at the rate of 2 °C/min, FID temperature 230 °C. The chart speed of the recorder was 0.5 cm/min. The flow rate of the carrier gas N_2, 30 ml/min, H_2, 30 ml/min and O_2, 60 ml/min. Injector volume 1 μl. The structure of the molecule was verified by GC–MS (Finnigun, UK) and 300 MHz NMR spectroscopy (Brucker, Germany). Mass spectra of the compounds were taken using SLD PROBE (50–280 °C).

The optical purity of the purified monoacetate was determined by HPLC (Waters 510, 486 Tunable absorbance UV detector, recorded on 746 data module) using chiral column (0.46 cm × 25 cm, chiralcel o.d.) with 2-propanol in *n*-hexane (1:8) as the solvent at a flow rate of 1 ml/min and a wave length of 254 nm. The pressure was maintained at 158 psi at ambient temperature (25 °C). The retention times for (*S*)-monoacetate and (*R*)-monoacetate were 17.20 and 15.80, respectively. The enantiomeric configuration and optical purity of the monoacetate was determined by H^1 NMR spectroscopy in presence of tris[3-[(heptafluoropropyl)-hydroxy-methylene]-(+)-camphorato]curopium(III) derivative ($Eu(hfc)_3$).

2.3. Studies on solvent effect

Anhydrous solvents for the reaction were prepared by using 3 Å molecular sieve. In the reaction mixture (50 mmol 2-*o*-benzylglycerol and 100 mmol vinyl acetate) a 20 ml volume of the anhydrous solvent was added and the reaction was carried out following the procedure stated above. Aliquots of the samples were withdrawn from the reaction mixture at 30 min intervals and the samples were analysed by GC and HPLC according to the procedure described above. The organic solvents used in this study include aliphatic and aromatic hydrocarbons, esters, alcohols and ether and their pertinent properties are given in Table 1. The effect of solvents

Table 1
Characteristics of the solvents used for this study

S.N.	Solvent	log *P*	log S_w	E_T^N	D_N^N	Dielectric constant	Polarisability ($\times 10^2$)
1	Dichloromethane	0.60	−0.84	0.321	0.03	8.93	8.49
2	Ethyl ether	0.85	−0.24	0.120	0.49	4.34	6.14
3	2-Methyl-2-pentanol	1.80	−0.44	0.100	0.40	2.5	5.4
4	Isopropyl ether	1.90	−0.64	0.114	0.49	3.88	6.02
5	Chloroform	2.00	−1.12	0.260	0.10	4.81	7.55
6	Benzene	2.00	−1.51	0.127	0.00	2.27	5.21
7	Toluene	2.50	−1.80	0.096	0.00	2.27	5.21
8	Carbon tetrachloride	3.00	−1.93	0.090	0.00	2.24	4.87
9	Cyclohexane	3.20	−2.25	0.075	0.00	2.0	4.5
10	Hexane	3.50	−2.39	0.070	0.00	1.88	3.44

Note: log *P* is the logarithm of the partition coefficient in *n*-octanol–water system. log S_w is the saturated solubility of water in the solvent on the molar basis. E_T^N is the normalised electron pair acceptance index, D_N^N is the normalised Gutmann donor number. Source of data: references [20,21,32,33].

will be assessed from the probable relationship of their properties with experimentally determined initial reaction rate, selectivity of monoester formation and enantiomeric excess (ee). The initial reaction rate (r_i) was calculated from the substrate concentration versus time profile taken for the first 10% conversion below which the profiles in all cases were found to be linear and expressed as mM per minute per gram of lipase (mM/(min g)). The analysis was done in triplicate and the reproducibility was found to be ±10%.

3. Results and discussions

3.1. Initial reaction rate

3.1.1. Effect of solvent hydrophobicity

The solvents have been selected on the basis of their hydrophobicity (log *P*) values which range from 0.6 to 3.5 because the activity and stability of enzyme can be maximum in this range [11]. Since the enzyme activity is strongly dependent on the amount of water present in the reaction mixture and the maximum degree of transesterification can be achieved at an optimum water activity of 0.25 [12], thus the experimental data generated under the conditions of such water activity control were considered so as to ensure meaningful interpretation of our results.

The relationship between r_i and hydrophobicity (log *P*) is shown in Fig. 1 which indicates that initial rate decreases almost linearly with increasing log *P* values and the following empirical relation could be deduced:

$$r_i = -0.198 \log P + 1.038 \quad (1)$$

with a standard deviation of ±5%. Our finding is identical to that obtained for *Subtilisin curlsberg*, *Aspergillus oryzae* and *Subtilisin BPN′* catalysed transesterification reactions [13] in the range of log *P* studied. However, the rate was found to increase with increase of log *P* with a plateau in a s-shaped curve for several other lipase catalysed esterification [11,14] and transesterification [15–17] reactions.

In the present transesterification reaction, less hydrophobic dichloromethane exhibits the highest initial rate probably due to preferential partitioning behaviour of the substrate between the reaction medium and the active sites of the lipase [3]. This partitioning is likely to diminish as the substrate and solvent hydrophobicities increase [5]. In general, catalytic efficiency of lipase decreases as the substrate hydrophobicity is increased [18] and a linear free energy relationships exist between the catalytic efficiency and both substrate and solvent hydrophobicities. Parida and Dordick [19] indicated for enantioselective esterification of 2-hydroxy acids with primary alcohol using lipase from *Candida cylindracea*, a branched substrate in some hydrophobic solvents such as toluene cannot fit properly into enzyme active site as a result of which catalytic efficiency may be lost. Furthermore hydrophobic solvents may not be easily accessible to the relatively polar phase around the hydrolytic enzyme for contact with the catalytic surface. There may also be an effect of product solvation: the polar monoester formed in polar

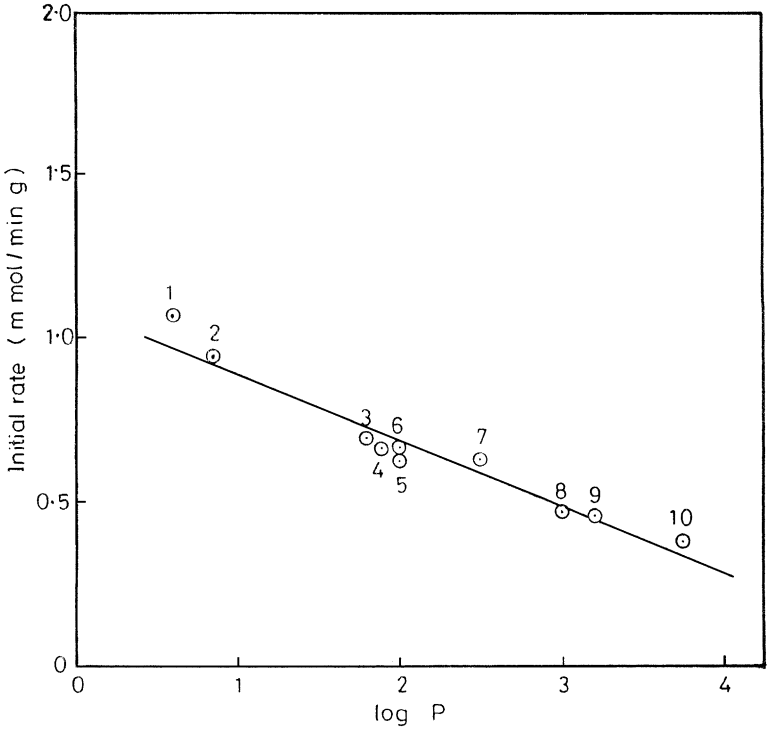

Fig. 1. Initial rate as a function of solvent hydrophobicity.

solvent is expected to be highly solvated. This can result in a high yield of monoester at a faster rate in agreement with the result reported for acylglycerol synthesis by *Chromobacterium viscosum* lipase [20]. Thus in the present work, the observed variation of rate with solvent hydrophobicity may be considered reasonable.

3.1.2. Effect of water solubility

The water solubility of the solvent has also been recognised as an important polarity index for correlating lipase activity in ester synthesis [14,20,21] and racemic resolution of ibuprofen [22] and glycerol [23]. Valivety et al. [21] reported a reasonable correlation between $\log P$ and $\log S_w$ for many solvents except a few like dichloromethane, 1,2-dichloroethane and the group of tertiary alcohol. To our knowledge, there is no published literature on an attempt to correlate initial rate with $\log S_w$ for transesterification reactions as such.

In order to substantiate our findings on the effect of solvent hydrophobicity ($\log P$), the relationship of initial rate with $\log S_w$ has also been considered and is shown in Fig. 2. The figure shows that the rate tends to increase with $\log S_w$ inferring favourable effect of polar solvent for the transesterification reaction commensurate with the effect deduced from Fig. 1. However, the rate versus $\log S_w$ relationship is not linear like that obtained with $\log P$. A rather sigmoidal nature of the relationship with $\log S_w$ can be visualised from the data points if the marginal deviation shown by benzene and carbon tetrachloride and significant deviation by dichloromethane can be justifiably ignored. While the marginal deviation small (∼15%) is attributed to analytical error for the sake of brevity, the significant deviation (≈50%) of the data point for dichloromethane may be attributed to its exceptional property signified by the high value of dielectric constant. Reexamining the data presented in Figs. 1 and 2, it may be reasonable to infer that hydrophobicity rather than the water solubility is a better choice of solvent property to predict the initial rate of the transesterification reaction under study.

3.1.3. Effect of electron pair donor–acceptor interaction of solvent

It is believed that the more fundamental basis for interpreting solvent polarity affect is the donor–acceptor interaction of the solvent including hydrogen bonding capability. Solvation of water requires both donation and acceptance of hydrogen bonds (or electron pairs) or other dipole–dipole interaction. An accepted measure of such an interaction is the sum of electron pair acceptance index (E_T^N) and Guttmann's donor number (D_N^N). Based on an established relationship between $\log S_w$ and $E_T^N + D_N^N$, Valivety et al. [21] could correlate well the later parameter with equilibrium position of esterification reactions. However, such a correlating approach has not been reported for transesterification reactions.

In view of the above, we attempted to correlate the initial rate with $E_T^N + D_N^N$ and the data plotted are shown in Fig. 3. Excepting a little more deviation of the some data points, the sigmoidal nature of the curve of Fig. 2 is retained also in Fig. 3 which, however indicates a conservative correlation. The deviation of these data points is attributable to uncertainties in the values of $E_T^N + D_N^N$. However, the values of $E_T^N + D_N^N$ for many solvents for Fig. 3 were obtained by extrapolating the line correlating $\log S_w$ and $E_T^N + D_N^N$ following the approach also adopted by Valivety et al. [21].

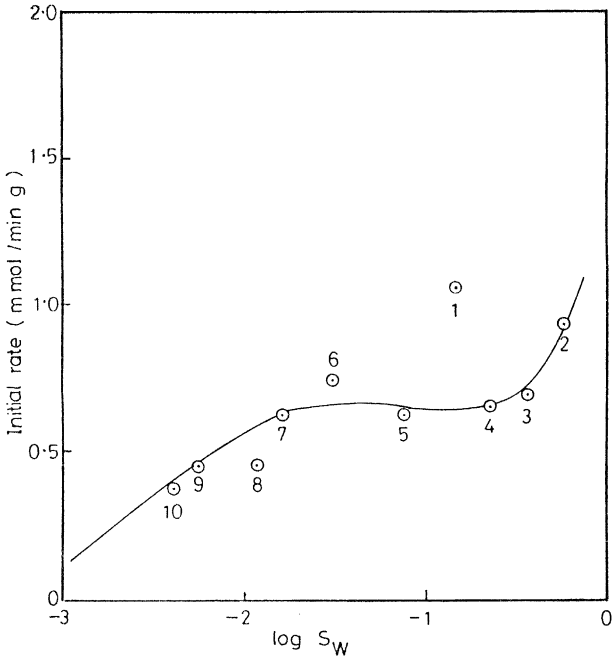

Fig. 2. Initial rate as a function of water solubility of the solvent.

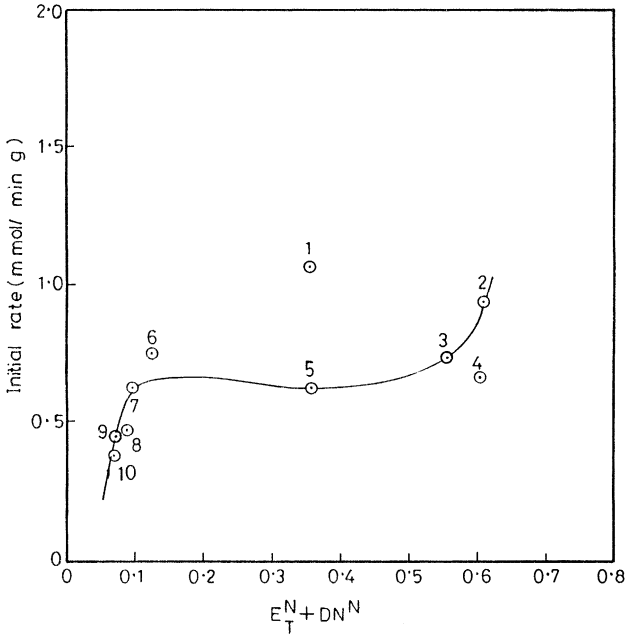

Fig. 3. Initial rate as a function of $(E_T^N + D_N^N)$.

However, the observed increase of initial rate with $E_T^N + D_N^N$ seems to be reasonable and may be explained from the solvation effect of the ester. The differential solvation which would affect the equilibrium position involves additional acceptor and donor interactions. Solvent capable of either or both of these interactions would favour hydrolysis and transesterification products. The correlation of transesterification rate with $\log S_w$ and $E_T^N + D_N^N$ perhaps indicates the role of the bulk behaviour of the solvent and functional group with specific interaction often referred to as "chemical" effects by the liquid state theories as suggested also for some other esterification reactions [21].

3.1.4. Effect of solvent polarisability

Polarisability represents the ability of a solvent to stabilise the charge of a dipole in solution by virtue of its dielectric constant. Since it is a function of dielectric constant and refractive index which can be easily measured, their values are known for almost all the solvents. In order to understand the effect of all the important solvent properties on reaction rates, we have attempted a correlation with polarisability also. As such no good correlation between initial rate and polarisability could be obtained (correlation not shown). When combined with $\log P$ and expressed in terms of $\log P$ divided by polarisability, a reasonably good correlation is obtained as shown in Fig. 4, indicated the important role of solvent hydrophobicity on initial rate. Similar correlation was observed for other reactions also [14,21]. However, since the correlating parameter covered in this study is of limited range, more data on various other solvents will be required to precisely establish the correlation. Yet, we feel that better fit of data on rate versus $\log P$ combined with a polarisability parameter provides further evidence that solvent hydrophobicity is the most important solvent property for prediction of transesterification rate.

3.1.5. Effect of dielectric constant

Since dielectric constant is a function of polarisability, an attempt has also been made to deduce a correlation of initial rate with dielectric constant as an independent parameter and the correlation is shown in Fig. 5. It is apparent that the initial rate increases with increase of dielectric constant values of the solvent. The probable explanation for such on observation in enzyme activity may be forwarded based on high conformational rigidity of enzyme in anhydrous media. This rigidity is the result of non covalent interactions which are essentially of the electrostatic origin [3]. According to Coulomb's law, the strength of the interactions is inversely proportional to the dielectric constant which is generally higher for water than organic solvents [24]. It may also be suggested that enzymes are much more rigid in anhydrous solvents of low dielectric constant than in those of high dielectric constant. This conformational rigidity leads to higher lipase activity and the observed correlation of Fig. 5 seems to be reasonable. However, the deviation of the data point of isopropyl ether from the correlating line may be attributed to branched nature of the solvent. Considering also the nearly equal initial rates of benzene and toluene whose dielectric constant values are equal, the correlation obtained seems to be reasonable.

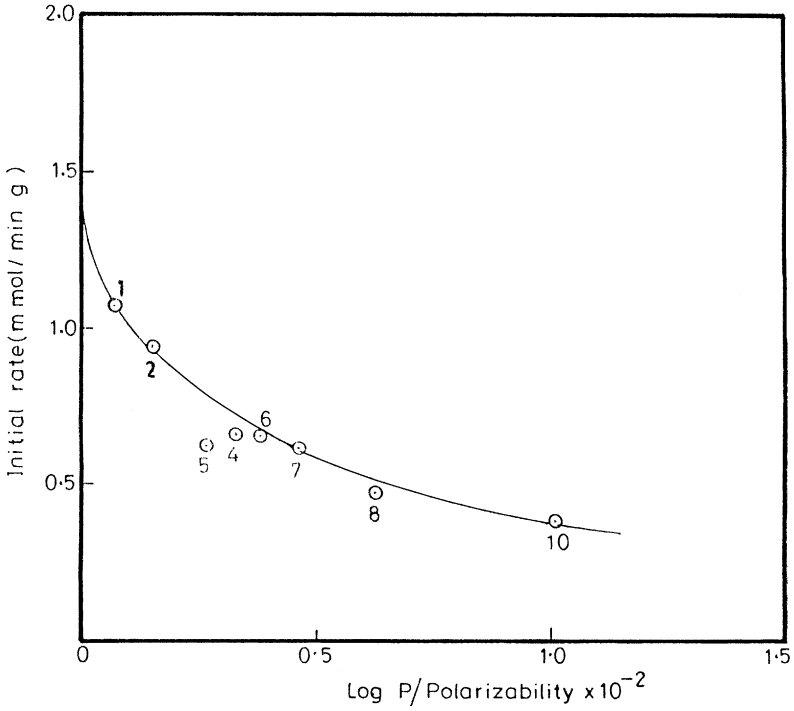

Fig. 4. Initial rate as a function of $\log P$/polarisability.

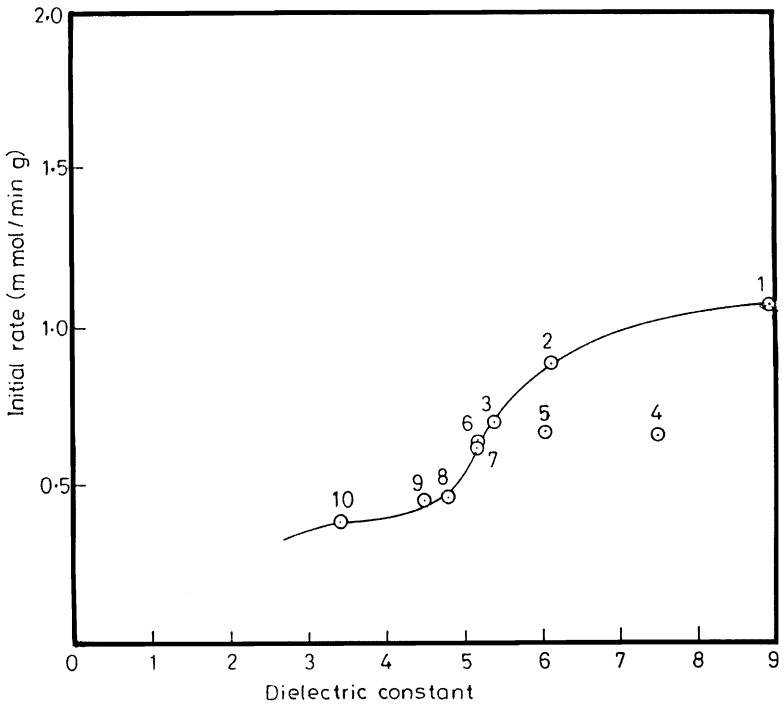

Fig. 5. Initial rate as a function of dielectric constant.

3.2. Selectivity and enantiomeric excess (ee)

Since the substrate specificity as well as enantioselectivity is sometimes affected by organic solvents [25], we made an attempt to correlate the selectivity and ee with the pertinent solvent properties in the reaction medium. Selectivity versus solvent is the consequence of thermodynamic effect and the solvent changes the thermodynamic activities of the reactant. Different solvents would exhibit different abilities to solvate the substrate and thus may influence the thermodynamic

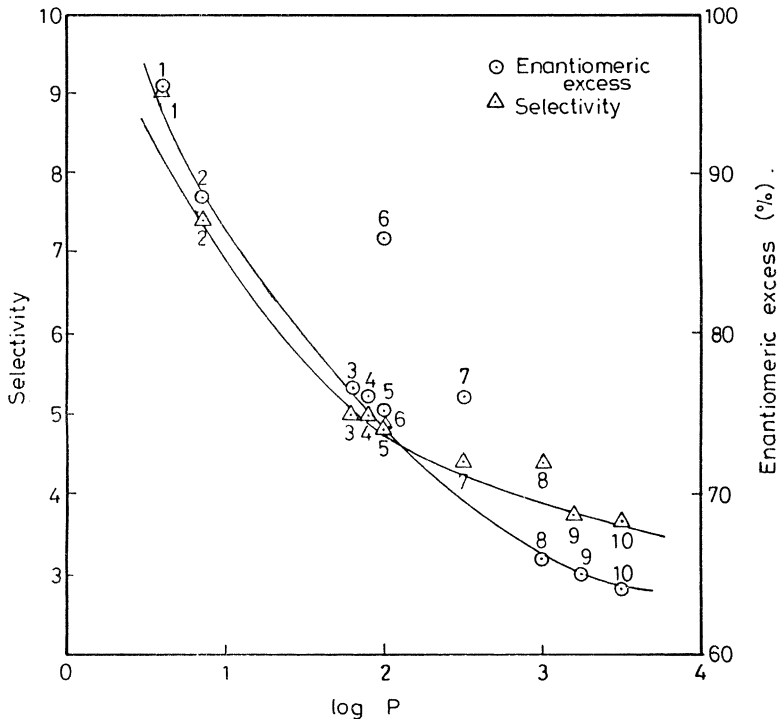

Fig. 6. Enantiomeric excess and selectivity as a function of solvent hydrophobicity.

activity of the substrate, the measured enzyme activity and the partition coefficients of the substrate as well as the products [14]. Predictable and rational control of enantioselectivity of enzymes by changing the reaction media is recognised as an alternative strategy for designing enzyme catalysed stereoselective synthesis. For instance, Watanabe et al. [26] recently established that Subtilisin catalysed transesterification of (*RS*)-2-(4-ethylphenoxy)-propionate and methyl mendate with *n*-butylalcohol in organic solvents and in presence of polar additive, i.e. water and dimethyl sulfoxide exhibit *S*-enantioselectivity ascribable to the enzymes conformational flexibility which was confirmed by ESR measurement.

Selectivity here is defined as the ratio of the percentage of monoester to that of diester formed in the reaction for a constant reaction time. Thus, an attempt has made to analyse the probable effects of the pertinent solvent properties on selectivity and enantiomeric excess as discussed below.

3.2.1. *Effect of solvent hydrophobicity*

Typical relationships of selectivity and ee with log *P* are shown in Fig. 6 which indicates decreasing trend of both the reaction parameters with increase of log *P*. However, the data points for benzene and toluene exhibit marked deviation from the correlating line of ee whereas there is a marginal deviation of the carbon tetrachloride data point from the line of selectivity. The general inference is that polar solvents favour both selectivity and ee, an effect analogous to that observed for initial rate. Our observation of lower enantioselectivity in hydrophobic solvent is consistent with that obtained for *Subtilisin carlsberg* [25,27], *A-oryzae* [17] and *P. cepacia* [15] catalysed transesterification reactions. But in case of α-*chymotrypsin* catalysed transesterification reaction, the organic solvents affected both rates and enantioselectivity differently [13,28].

For the transesterification reaction under study, the rate of formation of diacetate from *R*-isomer is greater than that from the *S*-isomer [8] and in highly apolar solvents such as cyclohexane, the reactivity of *R*-isomer increases relative to that of *S*-isomer and hence greater amount of diacetate was obtained [19]. For branched substrates in apolar solvents, the *S*-isomer cannot fit properly into the active site of the enzyme and hence both selectivity and ee drop. For the *R*-isomer to be reactive, it must bind to the enzyme incorrectly. This incorrect binding is much less affected by the branched nature of the substrate, as the branched group of *R*-isomers would not occupy the same spatial position in the active site of the lipase as the branched groups of the *S*-isomers. Similarly, hydrophilic solvents must impart the ability of the *S*-isomer to bind well to the active site of the lipase. The *R*-isomers do not bind well to the active site and hence is less effective. Consequently, small amount of diacetate and greater amount of monoacetate was obtained in hydrophilic solvents reflecting in high selectivity and ee in such solvents. It is evident from Fig. 6 that the selectivity and ee values are higher in benzene and toluene than in chloroform, although log *P* values for both the solvents are the same. This implies different features of selectivity and ee in aromatic and nonaromatic solvents like that of cyclic and acyclic solvents as reported by Nakamura et al [29] for other transesterification reactions. In fact, the effect of organic solvent on lipase activity and selectivity is highly dependent on the nature of the enzyme itself and the solvent used for the reaction. For instance, α-*chymotrypsin* catalysed transesterification of 1-propanol with racemic-*N*-tryfluoroacetylphenylalanine

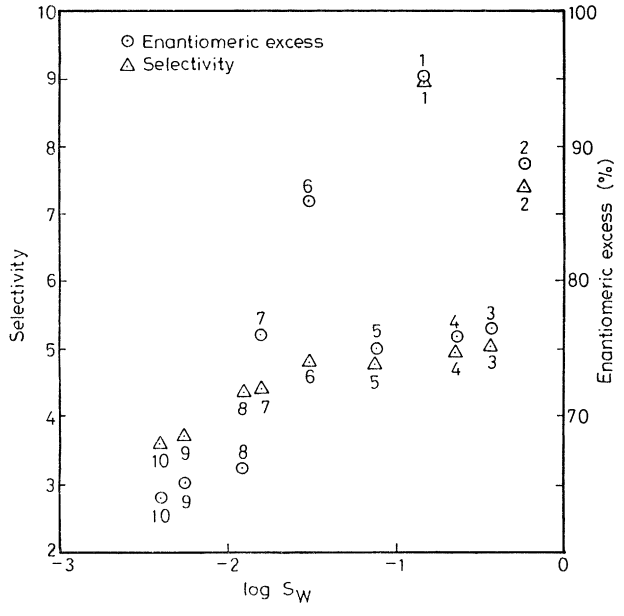

Fig. 7. Enantiomeric excess and selectivity as a function of water solubility of the solvent.

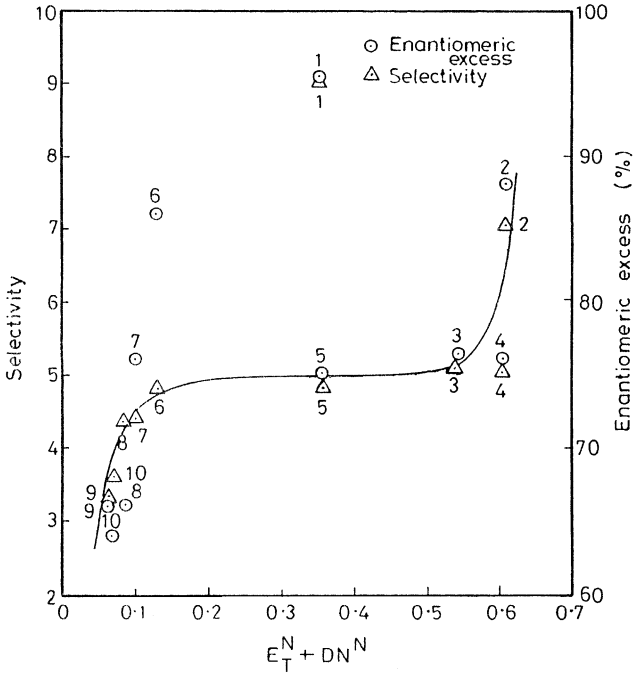

Fig. 8. Enantiomeric excess and selectivity as a function of $(E_T^N + D_N^N)$.

ester exhibited high reaction rate and low enantioselectivity in hydrophobic solvent, but L-selectivity was reversed with *Subtilisin carlsberg* lipase [13].

3.2.2. Effect of water solubility

As shown in Fig. 7, which is a plot of selectivity and ee versus $\log S_w$, following a s-shaped appears to increase selectivity with $\log S_w$ and implying favorable effect of polar solvent. However, the majority of the data points falls in the Plateau region of the curve whose selectivity values are almost the same order of magnitudes and differs only marginally from the maximum and minimum selectivities corresponding to the most polar and apolar solvents, respectively. Dichloromethane exhibit significantly high values of selectivity probably due to its relatively high dielectric property. Apparently the correlation of selectivity with $\log S_w$ may be considered rather conservative and as shown in Fig. 7, the correlation for ee seems to be even less significant with substantial deviation of data points for dichloromethane, ethyl ether and benzene. Yet the finding in general, of low selectivity and ee for apolar solvent may be considered reasonable in view of the established relationship of $\log P$ and $\log S_w$ [21] and the observed relationship with $\log P$ as presented in Fig. 5.

3.2.3. Effect of electron pair donor–acceptor interaction of solvent

As shown in Fig. 8, selectivity and ee tend to increase with increase in the value of $E_T^N + D_N^N$. This result seems to be almost consistent with the effect of $E_T^N + D_N^N$ on initial rate (Fig. 3), however with significantly high values of selectivity and ee for dichloromethane, ethyl ether and benzene indicating substantial deviation of the data points from the visualised correlating line. While the observed high selectivity of the monoester formation in more polar solvent may be interpreted from solvation effect involving additional electrons acceptor and donor interactions. The increased ee may perhaps be explained from the conformational flexibility of the enzyme. It is known that *P. cepacia* and *PS lipoprotein* lipases behave differently from other lipases in transesterification reactions [5]. Furthermore, *P. cepacia* lipase has a hydrophobic cleft or binding pocket in the vicinity of the catalytic sites [30] in which, group with specific configuration only can fit easily. The high ee for *S*-isomer in more polar solvent may thus be attributed to poor fit of the *R*-isomer into the small binding pocket of the enzyme. This result is consistent with that reported by Wang et al. [8] for the reaction using chloroform as the solvent in which *S*-monoacetate was predominant and the optical purity of the *S*-monoacetate was shown to increase with an increase of the substrate conversion.

3.2.4. Effect of polarisability and dielectric constant

As shown in Fig. 9, both selectivity and ee increase with polarisability following approximately a s-shaped curve. The relationship with dielectric constant (Fig. 10) also seems to be identical in nature, however with substantial deviation of data points for isopropyl ether and chloroform in both the figures which cannot be explained at ease from the currently available hypothesis. It is apparent that the order

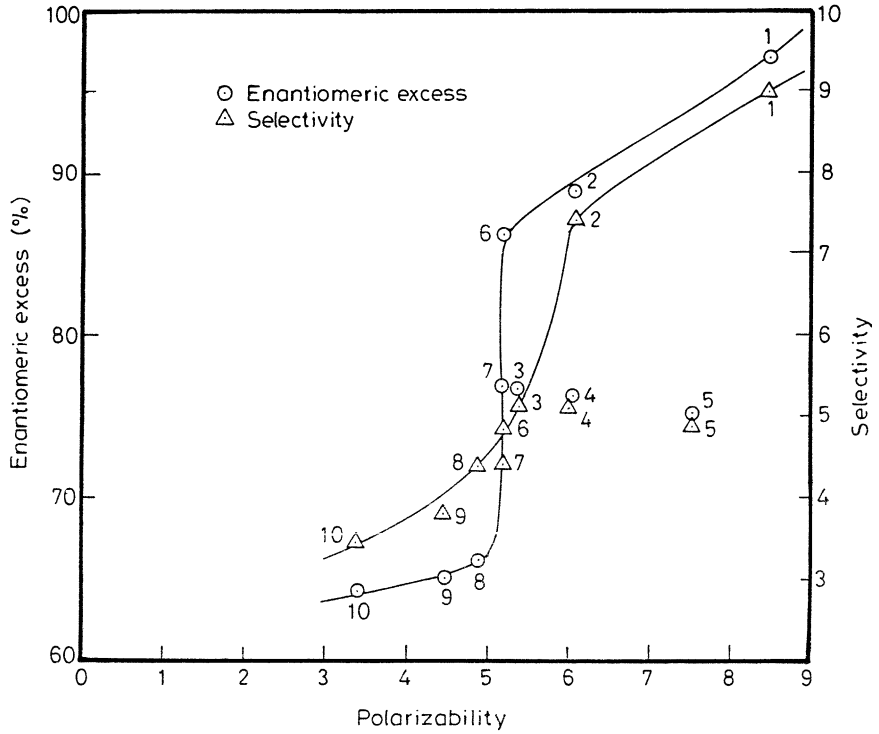

Fig. 9. Enantiomeric excess and selectivity as a function of polarisability.

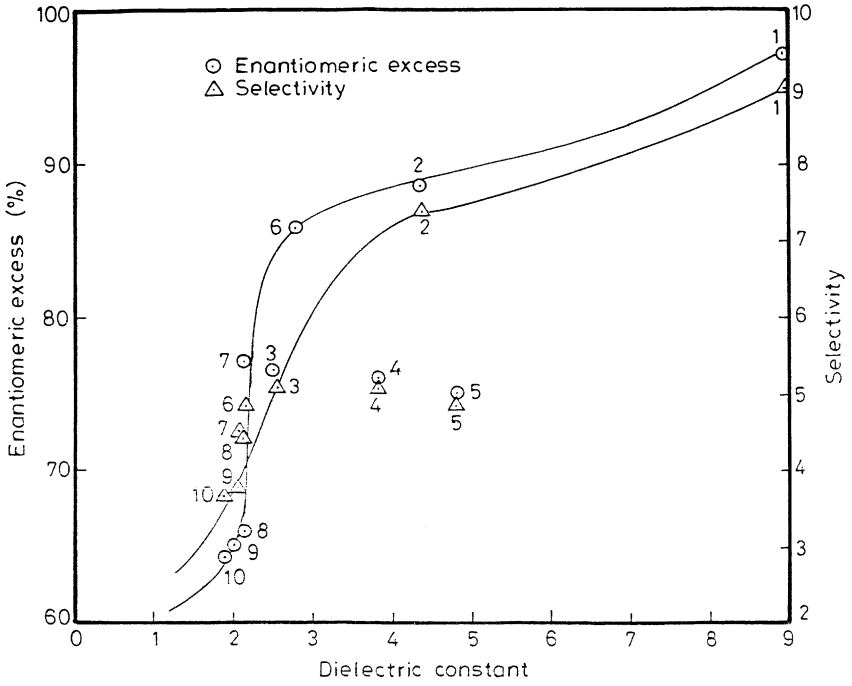

Fig. 10. Enantiomeric excess and selectivity as a function of dielectric constant.

of magnitude increase of both selectivity and ee is quite significant for the solvents with higher values of polarisability and dielectric constant in the range studied in this work. This finding contradicts that obtained for the resolution of mucolytic drug, (+)-*trans* sobrerol using the same lipase [31]. For *subtilisin carlsberg* catalysed transesterification of chiral sec-phenethyl alcohol with vinyl butyrate [27], an unequivocal relation of enantioselectivity with dielectric constant and dipole moment was obtained. However, for the aforesaid reaction system the enantioselectivity (*S*-isomer) was found to decrease with the solvent parameters. Conceptually, in our reaction the ee refers to product specificity in contrast to most of the reported transesterification reactions where enantioselectivity refers to substrate specificity of the enzyme. Thus, our observation of increase of ee with the above solvent parameters may be considered reasonable and the same reasoning given based on the conformational rigidity of the enzyme to explain the effect of dielectric constant on initial rate (Section 3.1.4) may be applied for ee too.

4. Conclusion

The catalytic activity and product enantioselectivity of lipase from *P. cepacia* for transesterification of 2-*o*-benzylglycerol with vinyl acetate are realised differently in different solvents. An attempt has been made to correlate the initial reaction rate, selectivity for monoester formation and enantiomeric excess (*S*-isomer) with the pertinent solvent properties such as hydrophobicity, water solubility, electron pair acceptance and donation ability, polarisability and dielectric constant. While the initial reaction rate, selectivity and ee exhibited reasonable correlations with almost all the solvent properties excepting deviation of only a few data points, the most important property that could reliably predict the reaction parameters is the hydrophobicity. Polar solvent is most favoured for the transesterification reaction. The high catalytic activity of *P. cepacia* lipase in polar solvent is due to the small hydrophobic cleft of the enzyme in which the group with specific configuration can fit easily. Thus, the stereochemical behaviour of the lipase catalysed transesterification can be fundamentally altered by properly selecting the solvent. The stereochemistry of the reaction seems to be influenced by a local solvent–enzyme interaction at the close vicinity of the active site instead of the change in the bulk conformation of the enzyme. Therefore, the enzyme activity and enantioselectivity in such system can perhaps be predicted solely on the basis of the physicochemical properties and structure of the solvents as well as the structure and properties of the products.

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