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Effect of Solvent on Lipase-Catalyzed Transesterification in Organic Media

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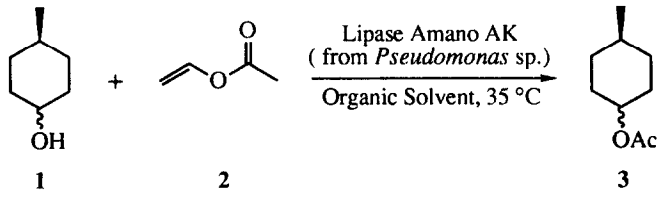
Abstract: Lipase-catalyzed transesterifications of *cis*- and *trans*-4-methylcyclohexanols with vinyl acetate in various organic solvents have been studied, and the effect of solvent on activity and stereoselectivity of lipase has been investigated. Initial rate of the reaction increases with the increase in hydrophobicity of the solvent exerting a good correlation with log*P*′, a corrected index of log*P*. However, since the stereoselectivity is also affected by molecularity of the solvent, the correlation between stereoselectivity factor, γ, and log*P*′ is poor. The solvent effect on the stereoselectivity has been correlated with excellent linearity by the aid of two-parameter relationship; $\gamma = a(\varepsilon_r-1)/(2\varepsilon_r+1) + bV_m$, where ε_r and V_m denote dielectric constant and molar volume of the solvent, respectively.

INTRODUCTION

Recently, biocatalysts have widely been applied to the syntheses of optically active compounds.[1,2] Especially, lipases have attracted much attention of organic chemists because of their high stability in organic solvents, and now they are usually treated in organic media as catalysts for asymmetric transesterifications.[2,3] Choice of reaction medium for a lipase-catalyzed reaction is known to be important, because stereoselectivity of an enzyme is affected largely by the reaction medium.[4-14] However, there has been predicted no reasonable explanation for the solvent-dependent reactivity and stereoselectivity of a lipase systematically. Before an enzyme is employed by organic chemists as an organic reagent, it is necessary to establish optimum reaction conditions for obtaining the best and reproducible result. We studied on this topic systematically, and the results will be reported in this paper.

RESULTS

Competitive acylation of *cis*- and *trans*-4-methylcyclohexanols (**1**) with vinyl acetate (**2**) catalyzed by a lipase (Amano AK from *Pseudomonas* sp.) in various organic solvents was investigated as a model reaction.



Scheme 1

In a typical experiment, 10 mg of powdered lipase and 50 mg of molecular sieves 4A (MS) were suspended in 1 ml of an organic solvent and stirred for 1 h at 35 °C. Then, 0.44 mmol of **1** (cis/trans ratio = 1/1) and 5.8 mmol of **2** in 1 ml of the same organic solvent were added to the suspension and the whole mixture was stirred at 35 °C. Periodically, an aliquot was taken off and analyzed by gas chromatography quantitatively. Initial rates of reaction in various organic solvents are summarized in Table 1. Usually, log*P* is employed as an index for solvent hydrophobicity in biocatalytic reactions.[3-9,13,15] In the present research, however, a modified log*P*, log*P*′, was employed initially in order to correlate the reactivity with a parameter to characterize the solvent. Log*P*′ denotes corrected solvent hydrophobicity in which a perturbation by the presence of vinyl acetate is taken into account and expressed by Eq. 1.

$$\log P' = A \log P + B \log P_{\text{vinyl acetate}} \quad (1)$$

, where A and B are mole fractions of the solvent and vinyl acetate in the medium, respectively. This modification of solvent hydrophobicity is necessary because relatively large amount of vinyl acetate exists in the reaction mixture. Fig. 1 shows correlations of the reaction rates with log*P*′.

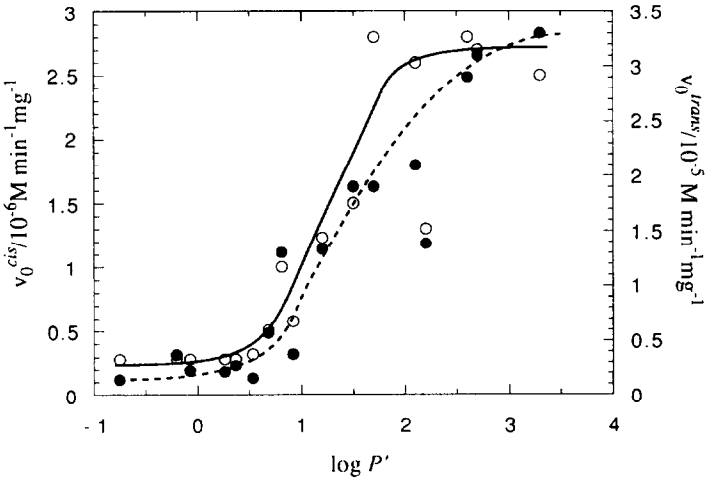


Figure 1. Initial rates of lipase-catalyzed transesterification between *cis*/*trans*-4-methylcyclohexanol and vinyl acetate in various organic solvents: v_0^{cis} and v_0^{trans} are the initial rates of the *cis*- and *trans*-esters, respectively. v_0^{cis} : —○— v_0^{trans} : - -●- -

Table 1. Initial Rate and Stereoselectivity of Lipase-Catalyzed Transesterification between 4-Methylcyclohexanol and Vinyl Acetate in Various Organic Solvents

No.	Solvent	log*P*	log*P*′	$(\varepsilon_r-1)/(2\varepsilon_r+1)$[a]	V_m[b]/l•mol^{-1}	v_0[c]/M•min^{-1}mg^{-1} cis	trans	γ
1	Dioxane	-1.1	-0.75	0.22	0.09	2.8×10^{-7}	1.4×10^{-6}	5
2	Acetonitrile	-0.33	-0.2	0.48	0.05	2.9×10^{-7}	3.7×10^{-6}	13
3	Acetone	-0.23	-0.07	0.46	0.07	2.8×10^{-7}	2.2×10^{-6}	8
4	Propionitrile	0.17	0.26	0.47	0.07	2.8×10^{-7}	2.1×10^{-6}	8
5	2-Butanone	0.29	0.37	0.46	0.09	2.8×10^{-7}	2.7×10^{-6}	10
6	THF	0.49	0.53	0.41	0.08	3.2×10^{-7}	1.6×10^{-6}	5
7	Ethyl acetate	0.68	0.68	0.38	0.10	5.1×10^{-7}	5.7×10^{-6}	11
8	Diethyl ether	0.85	0.81	0.34	0.10	1.0×10^{-6}	1.3×10^{-5}	13
9	2-MTHF[d]	0.99	0.92	—	0.10	5.8×10^{-7}	3.7×10^{-6}	7
10	*t*-BME[e]	1.35	1.2	0.35	0.12	1.2×10^{-6}	1.3×10^{-5}	11
11	Diisopropyl ether	1.9	1.5	—	0.14	1.5×10^{-6}	1.9×10^{-5}	13
12	Benzene	2.0	1.7	0.23	0.09	2.8×10^{-6}	1.9×10^{-5}	7
13	Toluene	2.5	2.1	0.24	0.11	2.6×10^{-6}	2.1×10^{-5}	8
14	Dibutyl ether	2.9	2.2	0.29	0.17	1.3×10^{-6}	1.4×10^{-5}	12
15	Cyclohexane	3.2	2.6	0.20	0.11	2.8×10^{-6}	2.9×10^{-5}	10
16	Hexane	3.5	2.7	0.18	0.13	2.7×10^{-6}	3.1×10^{-5}	12
17	Heptane	4.0	3.0	0.19	0.15	2.7×10^{-6}	3.1×10^{-5}	13
18	Octane	4.5	3.3	—	0.16	2.5×10^{-6}	3.3×10^{-5}	13

a) Polarity parameter of the solvent used in Kirkwood theory.[16] b) Molar volume of the solvent. c) Initial rate for the formation of the product ester. d) 2-MTHF: 2-methyltetrahydrofuran. e) *t*-BME: *tert*-butyl methyl ether.

Catalytic activity of the lipase for transesterification increases with the increase in log*P*′ of solvent. Similar phenomenon has already been observed in a lipase-catalyzed transesterification.[15] The reactivity of a lipase increases with the increase in hydrophobicity of the solvent, because the lipase in an organic solvent requires considerable amount of water to maintain its activity, probably its conformation, and a hydrophilic solvent deactivates the lipase by diffusing essential water on it into bulk solvent.[17]

To evaluate the effect of a solvent on the stereoselectivity of the lipase, a value of stereoselectivity, γ,[18] has been introduced as defined in Eq. 2:

$$\gamma = \ln([T]/[T]_0)/\ln([C]/[C]_0) \quad (2)$$

, where $[C]_0$ and $[T]_0$ are initial concentrations of *cis*- and *trans*-**1**, respectively, and [C] and [T] are the concentrations of *cis*- and *trans*-**1**, respectively, at an appropriate time interval. Fig. 2 shows a typical result

from the competitive reaction in hexane, which clearly demonstrates that the *trans*-isomer is approximately 12 times as reactive as the *cis*-isomer. Stereoselectivity, γ, obtained from the reaction in various organic solvents is plotted in Fig. 3 against logP'.

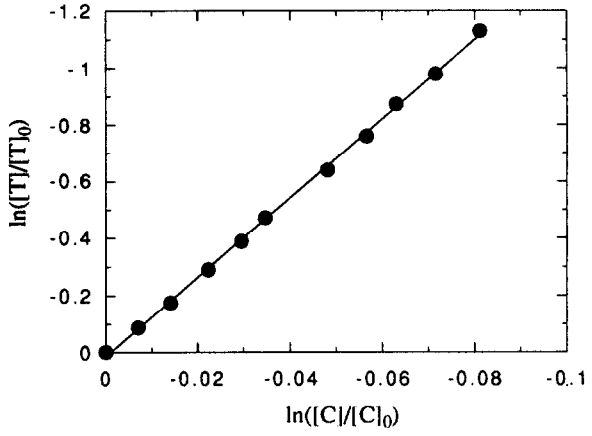


Figure 2. Linear correlation between v_0^{cis} and v_0^{trans}. The stereoselectivity factor, γ, is evaluated as the slope of this straight line.

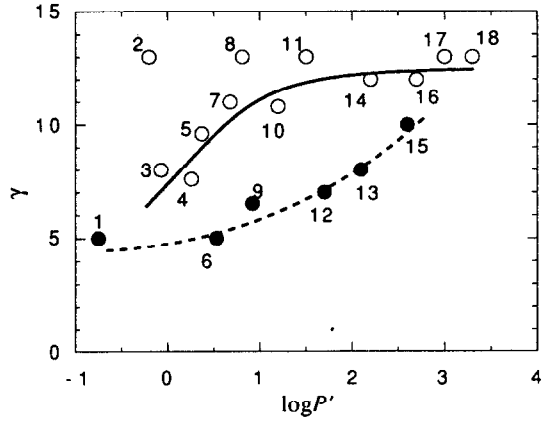


Figure 3. Correlation between γ and logP':
---●--- : Cyclic solvent —○— : Acyclic solvent.

DISCUSSION

Stereoselectivity of the lipase increases with the increase in hydrophobicity of the solvent. Interestingly, solvents plotted in Fig. 3 are classified into two groups; cyclic and acyclic solvents. These two groups show different effects on the stereoselectivity. Similar result was obtained recently by us for a lipase-catalyzed transesterification of a nitro alcohol,[8] while the mechanism of the solvent effect has been remained ambiguous. Organic solvents have many intrinsic properties such as dielectric constant, dipole moment, electron-pair donor

character and so on in addition to hydrophobicity.[19] Among them, hydrophobicity (or hydrophilicity) of a solvent is known to affect the stereoselectivity of enzyme-catalyzed reactions largely,[4-9,13,14] although this parameter alone cannot explain all of the classified stereoselectivity in the present reaction satisfactorily. That is, the stereoselectivity observed in the present reaction appears differently in cyclic and acyclic solvents of the same logP'. There must be an additional factor (or factors) to represent cyclic and acyclic properties of the solvent.

Another classification is possible for the solvents plotted in Fig. 3: those numbered 12, 13, 15, 16, 17 and 18 are hydrocarbon solvents and constitute a straight line: some ethers numbered 6, 9, and 14 locate on another straight line: there exists the third straight line for the remaining solvents except for No. 2. Interestingly, these three straight lines hold almost identical slopes. In other words, the plots shown in Fig. 3 suggest that the second parameter is required in order to elucidate a linear free-energy relationship for γ, a stereoselectivity index, and solvent property.

It is necessary to consider a site-specific interaction when an enzyme-solvent interaction is discussed, which is different from the discussion on a small molecule-solvent interaction. In this sense, we predict that molar volume of solvent, V_m,[20] plays an important role for the enzyme-solvent interaction. When V_m becomes larger, the number of solvent in effective contact with an enzyme will decrease and the enzyme will be released from unfavorable influence from an organic solvent. For reactions of small molecules, it has been reported that the bulkiness of solvent plays an important role for controlling the reactivity of the solute.[21-23] Although we are now dealing with the number of solvent molecules and no attention is paid for its bulkiness, these two physical properties may coincide each other: the bulkier the solvent molecule, the larger the molar volume and the smaller the number of solvent molecules that exist in an unit volume.

For representing solvation effect, the Kirkwood parameter, $(\varepsilon_r - 1)/(2\,\varepsilon_r + 1)$[16] may be physically better than the hydrophobicity parameter, log$P$, because the former is a direct measure of electrostatic interactions. The linearity in $(\varepsilon_r - 1)/(2\,\varepsilon_r + 1)$ - log$P$ relationship is satisfactory as shown in Fig. 4.

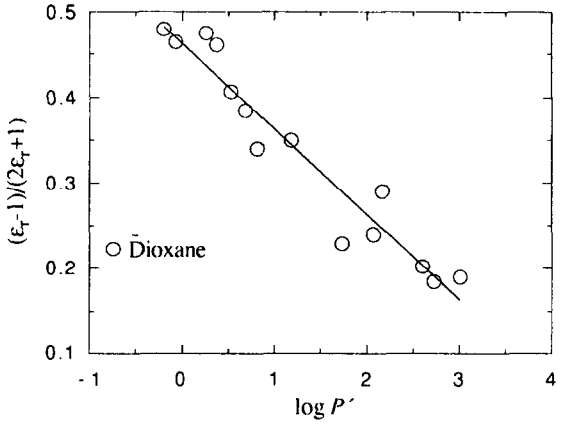


Figure 4. Correlation between logP' and $(\varepsilon_r-1)/(2\varepsilon_r+1)$: r = 0.965.

Thus, we plotted γ against two-parameter coordinate, $f(\varepsilon_r, V_m)$, and the result is demonstrated in Fig. 5.

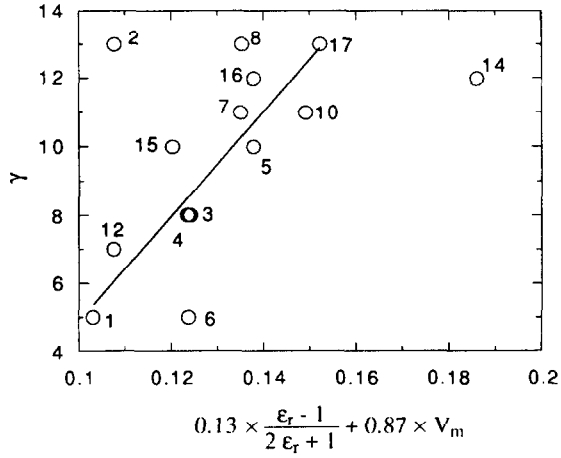


Figure 5. Linear relationship between $f(\varepsilon_r, V_m)$ and γ.

The linear relationship

$$\gamma = 153.27 \times (0.13 \times \frac{\varepsilon_r - 1}{2\varepsilon_r + 1} + 0.87 \times V_m) - 10.425 \qquad (3)$$

with correlation coefficient r = 0.811 is reasonably good except for dioxane and dibutyl ether. Although the coefficients 0.13 and 0.87 in the linear relationship seems to emphasize the importance of V_m, it is recognizable from the values of these parameters listed in Table 1 that these two terms operate almost equally.

When a two-parameter correlation is discussed, it is required to employ independent parameters. When two parameters are dependent on each other, there is no physical meaning of discussion based on these parameters. Independency of two parameters, $(\varepsilon_r - 1)/(2\varepsilon_r + 1)$ and V_m, has been tested as shown in Fig. 6.

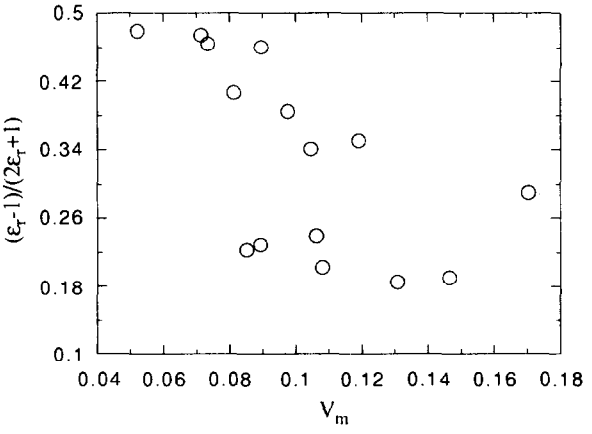


Figure 6. Independency between V_m and $(\varepsilon_r-1)/(2\varepsilon_r+1)$.

Although detailed mechanism for the enzyme-solvent interaction still remains ambiguous, the linear free-energy relationship in stereoselectivity, demonstrated in Fig. 5, predicts that site-specific interaction is important in solvent effect for enzyme reactions rather than a bulk medium effect. In other words, physical meaning of using log*P* (or log*P*') is not restricted for measuring the amount of water stripped off the enzyme as predicted previously[4] and mentioned above, but, rather positively, may evaluate the enzyme-solvent interaction.

When initial rates of the reaction are plotted against the two-parameter ordinate, we obtain Figs.7 and 8.

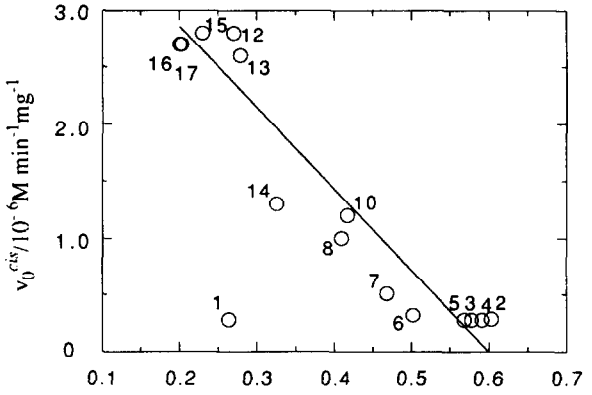


Figure 7. Linear relationship between $f(\varepsilon_r, V_m)$ and v_0^{cis}.

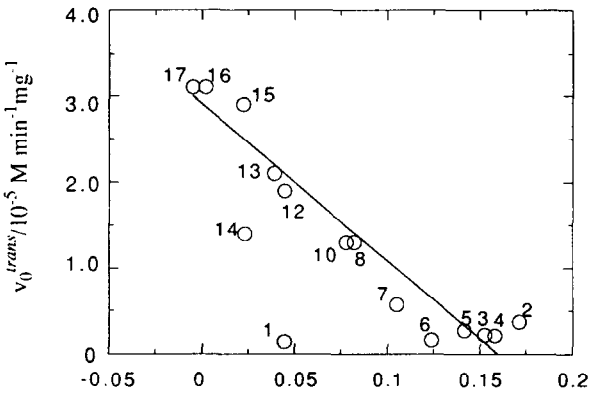


Figure 8. Linear relationship between $f(\varepsilon_r, V_m)$ and v_0^{trans}.

The relationship:

$$v_0^{cis} = -4.29 \times 10^{-6} \times (0.82 \times \frac{\varepsilon_r - 1}{2\varepsilon_r + 1} - 0.18 \times V_m) + 7.15 \times 10^{-6} \quad (4)$$

and

$$v_0^{trans} = -1.82 \times 10^{-4} \times (0.42 \times \frac{\varepsilon_r - 1}{2\varepsilon_r + 1} - 0.58 \times V_m) + 2.91 \times 10^{-5} \quad (5)$$

with r = 0.968 and 0.965, respectively, demonstrate reasonably good linearity between these parameters. The coefficients in Eqs. 4 and 5, however, emphasize that the reactivity is governed by a polar effect almost entirely in contrast with the stereoselectivity, as already has been observed for reactivity-hydrophobicity relationship illustrated in Fig.1. In other words, it has been elucidated that, although the polarity of solvent as a bulk reaction medium affects the reactivity of lipase primarily, detailed site-specific interactions between the enzyme and solvent molecules influence small perturbations on substrates of different shape: reaction with the *trans*-isomer is more affected by the site-specific interaction than the *cis*-isomer. Such a detailed information was unavailable from a one-parameter analysis.

Figs. 7 and 8 are equivalent to the plot shown in Fig. 1 in the sense that the reactivity is correlated with a polarity parameter. Nevertheless, we believe that the Kirkwood parameter is superior to the hydrophobicity parameter to test solvent effect on enzymatic reactions, because the former covers wider range of polarity than the latter. In Fig. 1, only a half of solvents employed for the present reaction exert linear relationships with the initial rates remaining the other half uncorrelated. In addition, stereoselectivity affords scattered plot with the hydrophobicity parameter.

Dioxane is an exceptional solvent in reactivity relationship. This solvent has smaller Kirkwood parameter than that expected from logP' (Fig. 4), which may be responsible to figure this solvent to be exceptional. The discrepancy seems worthy to be studied seriously in order to elucidate detailed mechanism of interaction between an enzyme and solvent. Dibutyl ether is another exceptional solvent in stereoselectivity relationship (Fig. 5). Detailed inspection reveals that the discrepancy stems from abnormally low reactivity for the *trans*-isomer (Fig. 8). Since both the Kirkwood and hydrophobicity parameters for this particular solvent are not abnormal, steric effect which characterizes this solvent seems to play an important role: two bulky butyl groups block the polar center, the oxygen atom, of this molecule and the site-specific interaction must be much smaller than that expected from a bulk electronic property, dielectric constant. Further inspection is required to obtain a conclusion for these exceptionalities.

In conclusion, when an enzymatic reaction is conducted in an organic solvent, chemists should pay attention to polarity and molar volume of the solvent. We are now investigating these effects of solvent on the kinetic resolution of chiral compounds.

EXPERIMENTAL

Instruments. Gas chromatographic (GC) analysis of reaction mixture was performed on a Shimadzu GC-14A using a Gasukuro Kogyo Inc. 25 m capillary column, Type PEG-20M. Data process of chromatograms was performed on a Hitachi D-2500 Chromato-Integrator.

Materials. cis- and trans-4-Methylcyclohexanols (> 98 % purities), vinyl acetate and dodecane were purchased from Tokyo Kasei Ltd. Methylcyclohexanols were used without further purification. Vinyl acetate was purified by distillation before the use and the purity was confirmed to be 99 %. Organic solvents and molecular sieves 4A were purchased from Nacalai Tesque, Inc. Before the use, molecular sieves were milled with a

mortar and a pestle, heated for 1 min in a microwave oven and cooled to room temperature in a desiccator spread with silica gel. All organic solvents were dried with sodium benzophenone ketyl, CaH_2, $MgSO_4$, or molecular sieves 4A. Lipase from *Pseudomonas* sp. (Amano AK) was provided from Amano Pharmaceutical Co., Ltd as a powdery form. The powder was dried in a desiccator over P_2O_5 under reduced pressure at room temperature for more than 1 day.

Standard Procedure for Kinetics of Lipase-catalyzed Transesterification in Organic Solvent. Into a test tube (1 5×125 m/m), 10 mg of a lipase, 50 mg of molecular sieves 4A, 1 ml of an organic solvent and a stirrer bar were placed and the test tube was covered with a rubber stopper. In another test tube of the same size, 0.22 mmol of *cis*-4-methylcyclohexanol, 0.22 mmol of *trans*-4-methylcyclohexanol, 5.8 mmol of vinyl acetate and 1 ml of an organic solvent were placed and the tube was covered with a rubber stopper. The tubes were immersed in a thermostat at 35 °C for 1 h with stirring in one tube. The content in the latter test tube was poured into the former, and the whole mixture was kept in a thermostat at 35 °C with stirring. Aliquots were taken out at regular time intervals (usually 30 min), diluted with ether, filtered and analyzed on GC. The reaction rates were determined as pseudo-first-order reactions. Up to 30 % conversion of the starting material was followed for obtaining an initial rate.

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20. Molar volume of a solvent, V_m ($l \cdot mol^{-1}$), is calculated according to the following equation:

$$V_m = \frac{MW}{1000 \times d}$$

, where d and MW are density and molecular weight of the solvent, respectively.

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