ABSTRACT: Capillary flow techniques have been used to determine the translational diffusion constant, $D$, of squalene in seven alkanes and five cyclohexanes. The alkanes are $n$-hexane, $n$-octane, $n$-decane, $n$-dodecane, $n$-tetradecane, 2,2,4,4,6,8,8-heptamethylnonane (isocetane), and 2,6,10,14-tetramethylpentadecane (pristane). The cyclohexanes are cyclohexane, $n$-butylcyclohexane, $n$-hexylcyclohexane, $n$-octylcyclohexane, and $n$-dodecylcyclohexane. When combined with published data in CD$_2$Cl$_2$, ethyl acetate, $n$-hexadecane, squalane, $n$-octane−squalane mixtures, and supercritical CO$_2$, the 35 diffusion constants and viscosities, $\eta$, vary by factors of $\sim 230$ and $\sim 500$, respectively. A fit to the modified Stokes−Einstein equation (MSE, $D/T = A_{SE}/\eta p$) gives an average absolute percentage difference (AAPD) of 7.72% between the experimental and calculated $D$ values where $p$ and $A_{SE}$ are constants, $T$ is the absolute temperature, and the AAPD is the average value of $(10^2 |D_{calc} - D_{exp}| / D_{exp})$. Two other MSE fits using subsets of the 35 diffusion constants may be useful for (a) estimating the viscosity of the hydrophobic core of lipid droplets, where squalene is a naturally occurring component, and (b) providing estimates of the $D$ values needed to design extraction processes by which squalene is obtained from plant oils. The Wilke−Chang equation also was considered and found to give larger AAPDs than the corresponding MSE fits.

■ INTRODUCTION

Squalene is a triterpene with a 24-carbon backbone, six methyl groups, and six isolated double bonds (Figure 1). In addition to its antioxidant and anti-inflammatory properties, it is one of the major lipids on the surface of human skin and its reaction with ozone has been studied because of the role it plays in indoor air quality. An intermediate in the biosynthesis of cholesterol, squalene is found in the hydrophobic core of lipid droplets. In ref 5, we suggested that if its translational diffusion constant, $D$, was measured in a lipid droplet, our $D$ values in five alkanes could be used to make a label-free estimate of the droplet’s viscosity, $\eta$. This was worth pursuing because lipid droplet viscosities depend on the cell type; the $D$ values of the probe coumarin 153 (C153) indicated that the droplets of a human lung cancer cell were 66% more viscous than those in a non-cancer lung fibroblast cell.6

Lipid droplet viscosities also can differ from those of other cell components. C153 showed that the droplets of a Chinese hamster ovary cell were $\sim 2.4$ times more viscous than its nucleus and cytoplasm. Additionally, viscosity would show how readily triacylglycerides and sterol esters move through a droplet to its surface, where they participate in the reactions that play a primary role in intercellular defense. Measuring the viscosity as a function of temperature could give information about phase transitions such as the liquid−liquid crystal transition that has

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The transitions have been studied in low-density lipoproteins and are reviewed in ref 12. These separations have become more important as a function of composition and pressure and are reviewed in ref 12. A fit to the modified Stokes–Einstein equation (MSE)5 gave an average absolute percentage difference (AAPD) of 7.72% between the experimental and calculated D values, less than that of the Wilke–Chang equation (11.2%), an oft-employed correlation in analytical chemistry. The AAPD is the average value of $\langle10^{D_{\text{calculated}} - D_{\text{experimental}}}/D_{\text{calculated}}\rangle$.

Two other MSE fits have been made that may be useful for estimating the viscosities of lipid droplets and the diffusion constants needed to design the supercritical and pressurized extractions by which squalene can be obtained from plant sources. These separations have become more important because international regulations reduced the supply of squalene’s traditional source, shark liver oil.1,13 Our diffusion constants for squalene also may be useful for checking molecular dynamics (MD) computer codes16,17 and machine learning (ML) diffusion constant predictions.18,19

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### Table 1. Squalene Diffusion Constants

| solvent          | $T$, °C | $10^5D$, cm$^2$/s | $10^5D_T$, cm$^2$/s | $r$, Å | % difference |
|------------------|---------|-------------------|---------------------|-------|--------------|
|                  |         |                   |                     |       | no CO$_2$    | no HPSM $^d$ | all 35 $^e$ |
| n-C$_6$          | 23.0    | 0.1650            | 0.306               | 4.30  | −11.2        | −4.51       | −4.51       |
| n-C$_8$          | 24.0    | 0.107            | 0.514               | 4.20  | −4.99        | −1.88       | 0.17        |
| n-C$_{10}$       | 24.0    | 0.627            | 0.850               | 4.08  | 0.70         | −0.006      | 4.15        |
| n-C$_{12}$       | 24.0    | 0.418            | 1.41                | 3.70  | −0.52        | −5.01       | 0.95        |
| n-C$_{14}$       | 24.0    | 0.291            | 2.14                | 3.50  | 1.28         | −6.38       | 1.15        |
| n-C$_{16}$       | 22.75   | 0.210            | 3.18                | 3.24  | 0.38         | −10.0       | −1.26       |
|                  |         |                   |                     |       |              | 0.90        | 0.96        |
|                  |         |                   |                     |       |              | 1.50        | 1.50        |
|                  |         |                   |                     |       |              | 2.50        | 2.50        |
|                  |         |                   |                     |       |              | 5.00        | 5.00        |
|                  |         |                   |                     |       |              | 10.0        | 10.0        |
|                  |         |                   |                     |       |              | 20.0        | 20.0        |
|                  |         |                   |                     |       |              | 50.0        | 50.0        |
|                  |         |                   |                     |       |              | 100.0       | 100.0       |
|                  |         |                   |                     |       |              | 500.0       | 500.0       |
|                  |         |                   |                     |       |              | 1000.0      | 1000.0      |
|                  |         |                   |                     |       |              | 5000.0      | 5000.0      |
|                  |         |                   |                     |       |              | 10000.0     | 10000.0     |
|                  |         |                   |                     |       |              | 50000.0     | 50000.0     |
|                  |         |                   |                     |       |              | 100000.0    | 100000.0    |
|                  |         |                   |                     |       |              | 500000.0    | 500000.0    |
|                  |         |                   |                     |       |              | 1000000.0   | 1000000.0   |
|                  |         |                   |                     |       |              | 5000000.0   | 5000000.0   |
|                  |         |                   |                     |       |              | 10000000.0  | 10000000.0  |
|                  |         |                   |                     |       |              | 50000000.0  | 50000000.0  |
|                  |         |                   |                     |       |              | 100000000.0 | 100000000.0 |
|                  |         |                   |                     |       |              | 500000000.0 | 500000000.0 |
|                  |         |                   |                     |       |              | 1000000000.0| 1000000000.0|
|                  |         |                   |                     |       |              | 5000000000.0| 5000000000.0|
|                  |         |                   |                     |       |              | 10000000000.0| 10000000000.0|
|                  |         |                   |                     |       |              | 50000000000.0| 50000000000.0|
|                  |         |                   |                     |       |              | 100000000000.0| 100000000000.0|
|                  |         |                   |                     |       |              | 500000000000.0| 500000000000.0|
|                  |         |                   |                     |       |              | 1000000000000.0| 1000000000000.0|
|                  |         |                   |                     |       |              | 5000000000000.0| 5000000000000.0|
|                  |         |                   |                     |       |              | 10000000000000.0| 10000000000000.0|
|                  |         |                   |                     |       |              | 50000000000000.0| 50000000000000.0|
|                  |         |                   |                     |       |              | 100000000000000.0| 100000000000000.0|

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*From the refs in the Experimental Methods section. *Calculated from the D values using eq 1. *Percentage differences between 30 calculated and experimental D values using fit to eq 2 for 29 D values excluding HPSM data. *Same as footnote c using fit to eq 2 for 35 D values excluding CO$_2$ data. *From ref 5 ($x_i$ = mole fraction squalane in n-C$_6$–squalane mixed solvents). *From ref 12. *All CO$_2$ D values are from ref 14. *All EtOAc D values are from ref 13.
EXPERIMENTAL METHODS

Chemicals and Sample Preparation. In this and the following sections, n-Ci is used for the n-alkanes and n-CiC6H11 is used for the cyclohexanes. Chemicals were obtained and used as received from (a) Sigma-Aldrich: pristane (98%), n-C6 (≥99%), and n-C10 (99+%); (b) Aldrich: HPMN (98%), n-C8 (99+%), n-C16 (99+%), and n-C6C8H11 (99+%); (c) Sigma: squalene (≥98%) and n-C12 (99%); (d) Fisher: cyclohexane (99.9%); and (e) TCI: n-C6C8H11 (>98.0%), n-C6C8C18H35 (>98.0%), and n-C12C12H14 (>98.0%). Squalene was stored in a cooler at 4 °C. Samples were prepared and profiles were taken with the laboratory lights off to minimize the possibility of photo-oxidation.21

Profile Acquisition and Analysis. The sigmoidal elution profiles used to determine squalene’s D values were obtained21 using a Thermo Separation Products SC100 variable wavelength detector, Chrom Perfect software (Justice Innovations), and a fused silica microcapillary (Polymicro Technology, 76.5 μm i.d.). The detector wavelength was 198 nm. Profiles were taken at room temperature (Table 1), which varied by no more than ±0.25 °C during a given acquisition. The experimental profiles were compared with those calculated using Taylor’s equations.5,22–25 The D values, with uncertainties of ±5%, are given in Table 1, along with the average values in squalane,5 n-C12,6 the n-C6C8=squalane mixtures,5 CD3Cl2,12 EtOAc13, and supercritical CO2.14

Solvent Viscosities. The viscosities for squalene’s solvents are given in Table 1. Those for n-Ci (i = 6, 8, 10, 12, 14, and 16) are from ref 26. Those for HPMN, pristane, and squalane are from refs 27, 28, and 29, respectively. The viscosities for the other solvents were determined by interpolation from the following sources: cyclohexane, ref 30; n-C6C8H13, n-C6C8H15, n-C6C8C18H35, and n-C12C12H14, ref 31; supercritical CO2, ref 32; and CD3Cl2, ref 33. The viscosities for the n-C6C8=squalane mixed solvents with squalane mole fractions $\chi_i$ = 0.291, 0.508, and 0.708 are from ref 5. Those for EtOAc are from ref 13.

RESULTS

Viscosity and Temperature Dependence of D Values. The analysis of squalene’s diffusion constants starts with the Stokes–Einstein relation34,35

$$D = k_B T / (6\pi\eta r)$$

(1)

where $k_B$ is Boltzmann’s constant, $T$ is the absolute temperature, and $r$ is the solute’s hydrodynamic radius. The Stokes–Einstein limit, which would give a common $r$ value for a given solute in a series of solvents, requires the solute to be much larger than the solvent.35 Squalene is not in this limit; its $r$ values in the n-alkanes, cyclohexanes, n-C8=squalane mixed solvents, and methyl-substituted alkanes (HPMN, pristane, and squalane) decrease as the viscosity increases (Table 1). This dependence of a solute’s size parameter on viscosity31,36 has been attributed to solute–solvent interactions.37 A larger value indicates stronger coupling of the solute’s motion to the solvent’s flow.37

When $r$ decreases as $\eta$ increases, following the early work of Chen, Davis, and Evans,38 we,5,21,36 and others39,40 have fitted the $D$ values for a given solute in a series of solvents to the MSE5,38

$$D / T = A_{SE} / \eta^p$$

(2)

where $p$ and $A_{SE}$ are constants and $p = 1$ for the Stokes–Einstein limit. The $p$ values for 26 hydrocarbons in n-alkanes and squalane21 showed the expected increase in $p$ as the solute size increased. Representative values21 ranged from 0.656 ± 0.017 for 1-hexene to 0.953 ± 0.020 for rubber.41

Squalene’s $D$ values were used for three fits to eq 2. All had ranges of viscosity and diffusion constants larger than those in ref 5. The values of $p$, $A_{SE}$, and $R^2$ for the fits are given in Table 2.

Table 2. Values of $p$, $-\log A_{SE}$, and $R^2$ for the Fits of Squalene’s $D$ Values to eq 2

| solvents      | $p$      | $-\log A_{SE}$ | $R^2$ |
|---------------|----------|----------------|-------|
| all $^a$      | 0.865 ± 0.007 | 9.449 ± 0.017 | 0.995 |
| no CO$_2$ $^b$ | 0.827 ± 0.009 | 9.385 ± 0.021 | 0.997 |
| no HPSMs $^c$ | 0.905 ± 0.008 | 9.549 ± 0.018 | 0.997 |

$^a$All 35 of the D values in Table 1 are included. $^b$The five D values in CO$_2$ are excluded. $^c$The six D values in the HPSMs are excluded.

The differences between the experimental $D$ values and the values calculated for each solvent for each fit are given in Table 1. The AAPD by the solvent group and the total AAPD for each fit are given in Table 3.

Table 3. AAPD between Experimental and Calculated Diffusion Constants for the Three Fits to eq 2

| solvent type | no. of solvents | no CO$_2$ $^a$ | no HPSMs $^a$ | all $^a$ |
|--------------|-----------------|----------------|---------------|----------|
| HPSM         | 6               | 8.33           | 19.7          | 12.2     |
| CO$_2$       | 5               | 3.38           | 7.28          | 12.7     |
| n-C$_6$      | 6               | 3.18           | 4.63          | 2.03     |
| cyclohexanes | 5               | 6.80           | 3.91          | 6.59     |
| CD$_3$Cl$_2$ | 1               | 2.10           | 1.62          | 3.48     |
| EtOAc        | 12              | 1.13           | 6.25          | 7.07     |
| AAPD for included | 3.96$^b$ | 5.53$^c$ | 7.72$^d$ |

$^a$AAPD between experimental and calculated $D$ values for each solvent group. $^b$AAPD for 30 solvents excluding CO$_2$ data. $^c$AAPD for 29 solvents excluding HPSM data. $^d$AAPD for all 35 solvents.

The first fit, shown in Figure 2, used all 35 of the diffusion constants and gave an AAPD of 7.72%. This is reasonably good agreement for $D$ values and viscosities that vary by factors of ~230 and ~500, respectively, in solvents with different shapes, sizes, and structures. The fit (Table 3) gave an AAPD of 12.7% for the least viscous CO$_2$ solutions and 12.2% for the most viscous group, the HPSMs (HPMN, pristane, squalane, and the n-C$_8$=squalane mixtures). The largest AAPD of the other groups was 7.07% for the EtOAc solutions. Nine of the individual
differences (Table 1) had absolute differences greater than 10%, the largest was −18.8% in pristane. The values of D calculated from this fit are plotted against the experimental values as shown in Figure 3.

![Figure 3. Plot of calculated vs. experimental diffusion constants for squalene. The calculated values were obtained using the MSE fit to eq 2 for all 35 of squalene’s D values.](Image)

The other two fits focused on the high and low ends of the viscosity range. The CO₂ viscosities are clearly the lowest of those given in Table 1. The other 30 solutions have values of D and η that vary by factors of ∼50 and ∼100, respectively, and their fit to eq 2 gave an AAPD of only 3.96% (Table 3). The AAPD for the HPSMs, 8.33%, was smaller than that for the all-inclusive fit and that for the excluded CO₂ diffusion constants was predictably higher, 23.3%. The absolute differences for only four of the 30 D values used in the fit were >10% (Table 1). The largest was +17.0% for HPMN.

The third fit omitted the D values for the six HPSMs and improved the agreement for the low viscosity CO₂ solutions. Their AAPD, 7.28%, was smaller than that for the 35-solution fit, 12.7% (Table 3). The AAPD for the 29 solutions used in the fit (5.53%) also was relatively small. Their diffusion constants and viscosities varied by factors of ∼70 and ∼110, respectively (Table 1). The largest AAPD of the other groups was 6.25% for EtOAc. Except for the excluded HPSMs (AAPD of 19.7%, Table 3), only CO₂ at 17 MPa (−11.5%) and n-C₆ (−10.0%) had absolute differences ≥10% (Table 1). The agreement for the D values in CO₂ is probably the best that can be expected, given that they were taken at the same temperature with small differences in the supercritical pressure and do not follow the viscosities (Table 1).

## DISCUSSION

### Lipid Droplets

In ref 5, we noted that a viscosity close to that of squalane had been reported in a lipid droplet. Bhattacharyya and co-workers used fluorescence correlation spectroscopy and C153 to determine η = 34 cp at 25 °C in the droplet of a live Chinese hamster ovary cell, a value 12% higher than that of squalane (Table 1). Bhattacharyya’s group then used fluorescence correlation spectroscopy at 20 °C to determine diffusion constants for C153 in the lipid droplets of a non-cancer lung fibroblast cell (W138) and a human lung cancer cell (A549). When used with eq 1, the D values give viscosities 1.2, and 2.1 times that of squalane, respectively.

The previous section’s MSE fits and squalene’s D values also were used to calculate the difference between the solvents’ experimental and calculated viscosities. The AAPDs are given by solvent in Table 4 and solvent group in Table 5. For the viscosities, the AAPD is the average value of (10²) (η_{calcd} − η_{expd})/(η_{expd}).

| solvent | T, °C | 10ⁿ | D² | no CO₂ | no HPSM | all 35 ² |
|---------|-------|-----|-----|--------|---------|---------|
| n-C₆  | 23.0  | 0.306 | −13.4  | −4.97  | −5.19  |
| n-C₆  | 24.0  | 0.514 | −6.00  | −2.08  | 0.195  |
| n-C₁₀ | 24.0  | 0.850 | 0.841 | −0.007 | 4.81   |
| n-C₁₂ | 24.0  | 1.41  | −0.611 | −5.51  | 1.11   |
| n-C₁₄ | 24.0  | 2.14  | 1.55  | −7.02  | 1.34   |
| n-C₁₆ | 22.75 | 3.18  | 0.472 | −11.0  | −1.43  |
| x₁ = 0.291e | 22.5 | 2.97 | −6.75 | −16.4 | −7.95 |
| x₁ = 0.508e | 22.75 | 7.24 | −4.54 | −20.9 | −9.47 |
| x₁ = 0.708e | 22.5 | 13.8 | −7.55 | −27.3 | −14.6 |
| squalane ² | 23.0 | 30.3 | −2.87 | −28.9 | −13.6 |
| pristane | 22.25 | 7.28 | −17.7 | −30.9 | −21.4 |
| HPMN ² | 24.0 | 3.52 | 21.0 | 4.51 | 17.2 |
| cyclohexane | 24.0 | 0.910 | 1.67 | 0.154 | 5.32 |
| n-C₆C₆H₁₁ | 24.0 | 1.23 | 4.97 | 0.514 | 7.18 |
| n-C₆C₆H₁₁ | 24.5 | 2.02 | 10.3 | 0.753 | 9.94 |
| n-C₆C₆H₁₁ | 23.25 | 3.24 | 16.3 | 1.50 | 13.2 |
| n-C₁₂C₁₂H₁₄ | 24.0 | 6.53 | 8.25 | −10.5 | 2.57 |
| CDCl₃ ² | 10.0 | 0.482 | −2.61 | 1.71 | 3.94 |
| CO₂ 18 MPa ² | 41.35 | 0.0728 | −29.4 | −10.8 | −17.0 |
| CO₂ 17 MPa | 41.35 | 0.0709 | −31.1 | −12.6 | −18.4 |
| CO₂ 16 MPa ² | 41.35 | 0.0688 | −22.5 | 2.47 | 9.04 |
| CO₂ 15 MPa ³ | 41.35 | 0.0665 | −26.6 | −6.85 | −13.5 |
| CO₂ 13 MPa | 41.35 | 0.0602 | −27.6 | −7.27 | −14.3 |
| EtOAc, 1 bar ⁴ | 30.0 | 0.399 | 3.57 | 9.17 | 11.0 |
| EtOAc, 75 bar | 30.0 | 0.433 | 3.17 | 8.03 | 10.2 |
| EtOAc, 150 bar | 30.0 | 0.464 | 1.37 | 5.67 | 7.99 |
| EtOAc, 1 bar | 40.0 | 0.359 | 1.90 | 8.54 | 9.75 |
| EtOAc, 75 bar | 40.0 | 0.390 | 0.390 | 6.31 | 7.81 |
| EtOAc, 150 bar | 40.0 | 0.419 | 0.790 | 6.04 | 7.88 |
| EtOAc, 1 bar | 50.0 | 0.325 | 0.702 | 8.29 | 8.99 |
| EtOAc, 75 bar | 50.0 | 0.354 | −0.028 | 6.79 | 7.84 |
| EtOAc, 150 bar | 50.0 | 0.381 | −0.280 | 5.87 | 7.23 |
| EtOAc, 1 bar | 60.0 | 0.295 | −1.49 | 7.02 | 7.17 |
| EtOAc, 75 bar | 60.0 | 0.323 | −1.89 | 5.79 | 6.33 |
| EtOAc, 150 bar | 60.0 | 0.348 | −1.34 | 5.66 | 6.56 |

²From the refs in the Experimental Methods section. ³Percentage differences between calculated and experimental viscosities using fit to eq 2 excluding CO₂ data. Same as footnote c using fit to eq 2 excluding HPSM data. ⁴Same as footnote c using fit to eq 2 for all 35 experimental D values. ⁵From ref 5 (x₁ = mole fraction squalane in n-C₆−squalane mixed solvents). ⁶From ref 12. ⁷All CO₂ D values are from ref 14. ⁸All EtOAc D values are from ref 13.

The fit that excluded the CO₂ data appears to be preferable for the lipid droplet viscosities. It gave the smallest AAPD between the experimental and calculated values for the HPSMs (10.1%, Table 5) and gave a small AAPD of 4.82% for the 30 non-CO₂ solutions. The AAPD for the five CO₂ solutions, the most viscous of which is a factor of ∼4.1 outside the range of the viscosities used in the fit, was 27.4%. The three largest non-CO₂ viscosity differences were for HPMN, +21.0%; pristane, −17.7%; and n-C₆C₆H₁₁, +16.3%; squalane’s was −2.87%. Figure 4 shows a comparison of the fit’s experimental and calculated viscosities. The fit that used all 35 solutions gave a larger AAPD of 14.0% for the HPSM viscosities (Table 5).
values is the uncertainty in viscosities a factor of sitosterol, campesterol, and cycloartenol. The calculated values were obtained using the fit that omitted the HPSMs (7.28%), a value higher than the corresponding values for the fit that omitted the HPSM diffusion constants (7.28%).

Wilke–Chang Correlation. The Wilke–Chang equation (WCE), widely used for estimating D values in liquid chromatography, is given by

\[
D_{AB} = (7.4 \times 10^{-8})[T(\phi M_B)^{1/2}/(\eta_B V_A^{0.6})]
\]

where \(D_{AB}\) (cm² s⁻¹) is the diffusion constant of solute A in solvent B, \(\phi\) is the solvent’s association factor, \(\eta_B\) (cP) is the solvent’s viscosity, \(M_B\) (g mol⁻¹) is the solvent’s molar mass, and \(V_A\) (cm³ mol⁻¹) is the solute’s molar volume at its normal boiling point, determined using the Le Bas group contribution method.\(^{47}\)

Calculations were carried out using \(V_A = 629\) cm³ mol⁻¹.\(^{47}\) \(\phi = 1\) for our non-associated solvents,\(^{15,46}\) and \((M_B)_{mixed} = \sum x_i M_{B,i}\) for the molar masses of the three \(n\)-C₆–squalane mixtures. \(x_i\) and \(M_{B,i}\) are the mole fraction and molar mass of solvent \(i\), respectively. As seen in Table 6, the WCE gave an AAPD of 11.2% for the “without \(CO_2\)” entry which is the “AAPD for included” entry in the “no \(CO_2\)” column in Table 3.

| solvent type | no. of solvents | WCE | MSE |
|--------------|-----------------|-----|-----|
| HPSM         | 6               | 10.9| 12.2|
| CO₂₂         | 5               | 32.4| 12.7|
| \(n\)-C₆      | 6               | 5.53| 2.03|
| cyclohexanes  | 5               | 7.24| 6.59|
| CD₂Cl₂       | 1               | 13.6| 3.46|
| EtOAc        | 12              | 6.77| 7.07|
| all solvents | 35              | 11.2| 7.72|
| without CO₂   | 30              | 7.65| 3.96|

“AAPDs for this column are from the “all” column” in Table 3 except for the “without CO₂” entry which is the “AAPD for included” entry in the “no CO₂” column in Table 3.

11.2% between the experimental and calculated diffusion constants, a value larger than 7.72% for the corresponding MSE fit. As also seen in Tables 6 and 3, the WCE fit gave an AAPD of 10.9% for the HPSMs, slightly better than the 12.2% from the 35-solution MSE fit but worse than the 8.33% for the fit that excluded the CO₂ solutions. When the CO₂ data (AAPD = 32.4%) are excluded, the AAPD for the WCE fit decreases from 11.2 to 7.65% whereas the AAPD for the MSE fit without the CO₂ data was 3.96% (Table 6). The 35 diffusion constants calculated using eq 3 are compared with the experimental values as shown in Figure 5. The WCE cannot be used for lipid droplets’ viscosities because the solvent’s molar mass is required and their cores have variable compositions. It could be used for estimating squalene’s D values for solvents involved in extraction processes but the MSE fit gives better overall agreement with experiment.

Other Possible Uses for Squalene’s Diffusion Constants. There have been MD studies of squalene’s (a) dynamics in a monolayer on graphite,\(^{16}\) (b) conformational dynamics in solution,\(^{17}\) (c) properties at the air/squalene interface, and (d) orientation and phase preference in an H₂O/CCl₄ system.\(^{49}\) No diffusion constants have been calculated, however, and the

Table 5. AAPD between Experimental and Calculated Viscosities for the Three Fits to eq 2

| solvent type | no. of solvents | no CO₂    | no HPSMs  | all  |
|--------------|-----------------|-----------|-----------|------|
| HPSM         | 6               | 10.1      | 21.5      | 14.0 |
| CO₂₂         | 5               | 27.4      | 8.00      | 14.5 |
| \(n\)-C₆      | 6               | 3.81      | 5.10      | 2.35 |
| cyclohexanes  | 5               | 8.30      | 2.68      | 7.64 |
| CD₂Cl₂       | 1               | 2.61      | 1.71      | 3.94 |
| EtOAc        | 12              | 1.41      | 6.93      | 8.23 |
| AAPD for included |           | 4.82     | 5.82      | 8.90  |

“AAPD between experimental and calculated viscosities for each solvent group. AAPD for 30 solvents excluding CO₂ data. AAPD for 29 solvents excluding HPSM data. AAPD for all 35 solvents.

Figure 4. Plot of calculated vs. experimental viscosities for squalene’s solvents. The calculated values were obtained using the fit that omitted squalene’s D values in supercritical CO₂.

Table 6. AAPD between Experimental and Calculated Diffusion Constants for the WCE and MSE Correlations by the Solvent Group

| solvent type | no. of solvents | WCE | MSE |
|--------------|-----------------|-----|-----|
| HPSM         | 6               | 10.9| 12.2|
| CO₂₂         | 5               | 32.4| 12.7|
| \(n\)-C₆      | 6               | 5.53| 2.03|
| cyclohexanes  | 5               | 7.24| 6.59|
| CD₂Cl₂       | 1               | 13.6| 3.46|
| EtOAc        | 12              | 6.77| 7.07|
| all solvents | 35              | 11.2| 7.72|
| without CO₂  | 30              | 7.65| 3.96|

“AAPDs for this column are from the “all” column” in Table 3 except for the “without CO₂” entry which is the “AAPD for included” entry in the “no CO₂” column in Table 3.
values given here should provide an adequate test of MD force fields. The MD simulations in refs 48 and 49 are in agreement with our earlier study5 that indicated squalene has a relatively extended conformation in nonpolar solutions. The calculations and Figure 4 of ref 48 showed that approximately 94% of 343 molecules were relatively extended; ~45% had all-anti methylene bridges, ~37% had one gauche defect, and ~12% had two. The percentages in bulk squalene and the interface were essentially the same. The calculations in ref 49 gave an elongated conformation in CCl4. Squalene’s conformation and molecular motion in solution are discussed in more detail in ref 5.

Machine learning studies of diffusion have focused on solutes in polar,16 nonpolar,19 and supercritical CO2 solvents.18 The gradient boosted ML algorithm gave the best agreement of 2.58% for 1476 D values in CO2, 5.07% for 430 D values in polar solvents,19 and 5.86% for 342 D values in nonpolar solvents.19 Our D values in the alkanes and cyclohexanes are candidates for the solute-nonpolar solvent data set.

A machine learning analysis that determines viscosities from D values using single solvent properties has not been carried out but it would present problems for a lipid droplet because its interior is not a single solvent. The MSE fit requires only squalene’s diffusion constant and no other MD force fields.

**SUMMARY AND CONCLUSIONS**

Capillary flow techniques have been used to determine the translational diffusion constant, $D$, of squalene in five $n$-alkanes, two methyl-substituted alkanes, and five cyclohexanes. The $D$ values show deviations from the Stokes–Einstein relation, as do published data for squalene in CDCl3,12 n-C10,5 squalane,3 n-C8–squalane mixtures,4 supercritical CO2,14 and ethyl acetate.13 Three fits of squalene’s $D$ values to the MSE, $D/T = A_0/\eta_p$, were made. One was motivated by the possibility of using it to make a label-free estimate of the viscosity in the core of lipid droplets. It included the data for the least viscous CO2 solutions because the viscosities in lipid droplets are likely to be near or beyond that of our most viscous solvent, squalane. The fit gave an AAPD of 3.96% between the 30 experimental and calculated diffusion constants in the non-CO2 solvents.

Another fit included the five $D$ values in CO2 but excluded the diffusion constants in the more viscous HPMN, pristane, squalane, and $n$-C8–squalane mixtures. It could be useful for estimating squalene’s $D$ values in the low-viscosity solutions used in extractions from plant sources. The AAPD between the 29 experimental and calculated $D$ values was 5.53%. The third fit included all 35 $D$ values and gave an AAPD of 7.72%. Fits using the Wilke–Chang correlation with and without the CO2 diffusion constants were less successful than those using the MSE although the agreement improved when the CO2 data were not included.

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**Notes**

The author declares no competing financial interest.

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**Figure 5.** Plot of calculated vs. experimental diffusion constants (in cm2 s−1) for squalene. The calculated values were obtained using the Wilke–Chang correlation, eq 3, for all 35 of squalene’s D values.
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