The Effects of Internal and Receptor pH on the Rate of Drug Release from Water-in-Oil Emulsions

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We evaluated the effects of internal phase and receptor solution pH on the rate of drug release from water-in-oil emulsions using methylene blue as a model drug. The water-in-oil emulsions were prepared using an aqueous solution of methylene blue, squalene, and a non-ionic-lipophilic surfactant. The methylene blue release rate was strongly dependent on both internal phase and receptor solution pH. Methylene blue dissolved in squalene in the presence of a surfactant. The water–squalene distribution of methylene blue changed with pH, whereas its ionic state did not. The pH dependence of the methylene blue release rate may have been due to this distribution change. We also investigated the pH dependence in terms of the mobility of water molecules using time-domain NMR. The mobility of water in water-in-oil emulsions was also dependent on the internal phase pH. Water-in-oil emulsions that showed high water mobility also released drug more rapidly. These results suggest that methylene blue is released from the water-in-oil emulsion through a reverse micelle mechanism. Methylene blue moves from the internal phase to a soluble reverse micelle of the surfactant, diffuses through the oil phase within this reverse micelle, and is transferred to the receptor solution. It appeared that the reverse micelles could diffuse in oil more freely than water droplets of the water-in-oil emulsion because the micelles were much smaller than the droplets. We found that the drug release rate from a water-in-oil emulsion comprising squalene and a non-ionic surfactant could be controlled by pH optimization.

Key words: water-in-oil emulsion; drug release rate; non-ionic surfactant; pH optimization; squalene

Reducing the frequency of medication for parenteral drugs is highly desirable because it improves the patient’s quality of life. Sustained release technologies, which can substantially reduce medication frequency, are being studied for application to parenteral drugs. Emulsion technologies, which have multiple applications, are among those being studied. Typical emulsions contain an aqueous phase and an oil phase; they are frequently stabilized through the addition of a surfactant. Dispersion of an oil phase in an aqueous matrix produces an oil-in-water (o/w) emulsion, whereas dispersion of an aqueous phase in a matrix of oil results in a water-in-oil (w/o) emulsion. W/o emulsions have been studied as a sustained-release technology for hydrophilic drugs, which are commonly dissolved in the internal aqueous phase of the w/o emulsion.\cite{1,2} The objectives of these studies were protection of the drug against degradation caused by external factors and sustained release of the drug.\cite{3} Since the oil and surfactant phases constitute a barrier to the release of hydrophilic drugs dissolved in the internal aqueous phase, the drugs can be released sustainably over a prolonged period.\cite{4} Local retention is an important parameter for prolonged drug release from w/o emulsions when the emulsion is administered subcutaneously or intramuscularly.\cite{5} Rheological parameters such as viscosity are also factors affecting retention ability.

Many researchers have evaluated the stability and drug release characteristics of w/o emulsions and w/o/w emulsions by measuring the rate at which a drug is release from the internal phase.\cite{6} Additional studies have evaluated the effects of the physical properties of w/o emulsions on the drug release rate. Physical properties examined have included the types of surfactant and oil, the concentration of surfactant, the phase volume ratio, the size of the internal droplet, the osmotic pressure, and the molecular weight of the drug.\cite{6,7,8,9,10}

The pH of aqueous formulations must be controlled within a limited range near neutral pH because of bio-compatibility issues, such as local irritation. As a result, drugs with insufficient solubility at near-neutral pH have often been dropped as drug development targets. Although w/o emulsions may appear to be a way to circumvent this issue, pH is still an important physical property because the drug is initially dissolved in the internal aqueous solution. If the drug is dissolved at significantly lower or higher pH and then formulated in a w/o emulsion, the acidic and alkali components would also be sustainably released from the w/o emulsion. Because the w/o emulsion must also not lead to or reduce local irritation caused by a highly acidic or alkaline internal phase, it would be useful to have a more complete understanding of the effect of pH on the rate of drug release. However, there have been no previous reports about the relationship between pH and drug release rate (or stability of w/o emulsion) over a wide pH range. This study was designed to elucidate the effects of internal phase pH on the drug release rate to judge the possibility of using w/o emulsions as sustained-release carriers. We examined the effects of internal phase pH on the drug release rate using a simple w/o emulsion formulation, particularly focusing on the relationship between internal phase pH and drug release rate. We also discuss the effects of pH on the molecular mobility of water in w/o emulsions using findings from

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{chemical_structure.png}
\caption{Chemical Structure of Methylene Blue}
\end{figure}

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Experimental

Materials  Methylene blue (MB), methyl red (MR), bromophenol blue (BPB), and sorbitan monooleate (Span® 80) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acid red 52 (AR), acid blue 9 (AB), and sorbitan Sesquioleate (Span® 83) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Amaranth and sunset yellow (SY) were obtained from San-Ei Gen F.F.I., Inc. (Osaka, Japan). Squalene was purchased from Sigma-Aldrich (Missouri, U.S.A.). Other chemicals were of reagent grade.

Disposable syringes (product name: Injekt Luer Lock Solo 5mL) purchased from B·BRAUN (Melsungen, Germany) and disposable syringe connectors (product name: GP syringe connector) purchased from GreenPeptide Co., Ltd. (Fukuoka, Japan) were used for preparing the emulsions. Dialysis membrane tubing (product name: Spectra/Por 4) was obtained from Spectrum Laboratories Inc. (California, U.S.A.). The molecular weight cut-off (MWCO) of the membrane was 12–14 kDa; the membrane did not block the passage of drug molecules.

Preparation of W/O Emulsions  The aqueous and oil phases were prepared separately. The oil phase solution was prepared by adding the specified surfactant to squalene at a concentration of 10 wt%, and the aqueous phase solution was prepared by dissolving 0.5 mMB in an isotonic buffer.

The w/o emulsion was manually prepared using the following emulsification procedure: (1) a 5-mL syringe containing 1mL of the oil and a 5-mL syringe containing 1mL of the aqueous solution were connected through a GP syringe connector; (2) the syringe plungers were depressed alternately, 6 times, at a speed that resulted in complete emptying of the syringes over 5s each; (3) the syringe plungers were alternately depressed 60 times at a speed that resulted in complete emptying of the syringes over 0.5s each. The speed of the emulsion passing through the connector reached approximately 4.0cm/s. The internal droplet size of the w/o emulsion was dependent on this emulsifying shear stress (passing speed). The mixed solution was confirmed to be not dispersed in water.

Droplet Size Determination  The average mean droplet size (D₉₀) of the dispersed aqueous phase was determined using a laser diffractionmeter (Mastersizer2000; Malvern Instruments Ltd., U.K.).

Calculation for pKₐ and Log D  The acid dissociation constant (pKₐ) and the distribution coefficient (Log D) were calculated using Percepta software (Advanced Chemistry Development, Inc., Canada).

In Vitro Release Experiments  The w/o emulsion, prepared as described above, was encapsulated in dialysis membrane tubes, and the membrane tube and isotonic receptor solution were added into a conical tube. The receptor solution—emulsification interfacial areas were fixed at approximately 1600mm². A shaking bath (BW101; Yamato Scientific Co., Ltd., Japan) shook the conical tube at a constant speed of 75rpm and a constant temperature of 37°C, which was controlled by a constant temperature unit (BF200; Yamato Scientific Co., Ltd., Japan).

After a specified time period, 1mL of the receptor solution was sampled from the conical tube, and then 1mL of fresh receptor solution was added to the conical tube. This sampling method was repeated for up to 24h. The absorbance of the sample solution was measured with a UV–Visible spectroscope (U-3900H; Hitachi High-Technologies Corporation, Japan) and drug concentration was calculated. This release experiment was repeated thrice. The following equation can be used to calculate the amount of drug release Rₙ of the sample n in terms of percentage.

\[
Rₙ = \frac{Vₛ \sum_{k=1}^{n-1} Cₛ \times Vₛ Cₙ}{A_{Initial}} \times 100
\]

\(Vₛ\) and \(Vₙ\) are the volumes of sample and receptor solution, respectively. \(Cₛ\) is the drug concentration in sample n and \(A_{Initial}\) is the total amount of drug in the emulsion initially. Sample n is the solution withdrawn from the receptor solution, and n is the number of the withdrawal.

It has been shown that the amount of drug released from a w/o emulsion with a high volume fraction of the dispersant phase is linearly dependent on the square root of time. In our study, the volume fraction of the dispersant phase was also high, and the amount of drug released from the w/o emulsion was linearly dependent on the square root of time. We obtained drug release rates using the least-squares method.

T₁ and T₂ Relaxation Time Measurement  T₁ and T₂ relaxation times of H in w/o emulsions were determined using a 20MHz TD-NMR (the Minispec, Bruker Corporation, Germany) to evaluate the molecular mobility of water in w/o emulsions. The pulse sequences used to measure the T₁ and T₂ relaxation times were the inversion recovery sequence and the Carr–Purcell–Meiboom–Gill sequence, respectively. T₁ and T₂ relaxation times showed two phases, a shorter phase and a longer phase. Each value (shorter T₁, longer T₁, shorter T₂, and longer T₂) was obtained using the least-squares method.

Results and Discussion

Drug Selection  In order to evaluate the capability of the w/o emulsion as a sustained-release vehicle, we designed experiments to evaluate the relationship between pH and drug release rate. We started by selecting a series of model drugs that could be easily evaluated in a short period of time. We then selected a model drug from among the list: MR, MB, riboflavin, SY, amaranth, AR, BPB, and AB, all of which could be determined using visible light spectrophotometry. These drugs were individually dissolved in pH 7.4 Dulbecco’s phosphate buffer saline(-) [D-PBS(-)] at a concentration of 0.5mm. W/o emulsions were prepared with these drug solutions and squalene that contained 10wt% Span 83. Then the amount of drug released from these w/o emulsion to a pH 7.4 receptor solution, D-PBS(-), was evaluated. The rate of drug release from w/o emulsions is related to the molecular weight of the drug, with the release of a small-molecule drug being faster than the release of a large-molecule drug. Therefore, we selected an appropriate model drug after careful consideration of the ease of measurement and the effect of molecular weight.

Figure 1 shows the relationship between the amount of drug released, the molecular weight of the drug, and the calculated Log D of the drug at pH 7.4. The molecular weights of MR, MB, riboflavin, SY, amaranth, AR, BPB, and AB in D-PBS(-), calculated as ionic forms, are 269.3, 284.4, 376.4, 406.4, 535.5, 557.7, 668.0, and 746.9, respectively. The calculated Log D of these drugs at pH 7.4 are 1.45, 3.55, −1.14, −4.24, −5.96, −1.03, −0.29, and −3.35, respectively. Drugs
with molecular weights of \(<400\ \text{g/mol}\) were released within 48h, whereas drugs with molecular weights of \(\geq400\ \text{g/mol}\) were not released. As the molecular weight of the drug decreased, release rate increased. This result suggests that a drug of \(>400\ \text{g/mol}\) would be released extremely slowly or not at all. Since a drug of approximately 6500 g/mol molecular weight has been reported to be released from a w/o emulsion comprising saline, Span 80, triglycerol polyricinolate-6, and medium chain triglyceride-oil, \(^6\) we conclude that the boundary observed in our study (400 g/mol) was dependent on the components of the w/o emulsion. Log \(D\) was also an important drug release parameter because drugs with Log \(D\geq0\) were released, but those with Log \(D<0\) were not released. We selected MB as the typical model drug because it demonstrated a rapid release rate and good stability over a wide range of pH, and it has one \(pK_a\) value and did not change color as the pH shifted.

**Effects of Receptor Solution pH** Our main objective was to evaluate the effects of internal phase pH on drug release rate from a w/o emulsion. When dealing with controlled release using w/o emulsion, internal aqueous phase pH must be controlled using various pH buffers as the internal aqueous phase. However, if substantial changes are made to the internal phase, other properties of the w/o emulsion, such as the internal droplet size or the viscosity, may change, which may inhibit accurate evaluation of the pH effect. Therefore, we decided to start our evaluation of the MB release rate using essentially identical w/o emulsions, to receptor solutions at various pH levels, for understanding the effect of pH in the absence of differences between the w/o emulsions. The w/o emulsions were prepared using 0.5 mM MB in D-PBS(−) and squalene containing 10 wt% Span 83. The receptor solutions were 100 mM solutions of citrate buffer at pH 4 (CB4), citrate buffer at pH 6 (CB6), phosphate buffer at pH 6 (PB6), phosphate buffer at pH 8 (PB8), and glycine and sodium hydroxide buffer at pH 10 (GB10). Because a separate study reported that a gradient of osmotic tension between the internal phase and the receptor solution has an effect on drug release rate, \(^9\) all buffers were prepared as isotonic buffers (approximately 290 mOsm) by adding sodium chloride (NaCl). We determined the amounts of MB released from the emulsions described above to the isotonic receptor solutions at various pH values.

Figure 2 shows the amount of MB released to the various pH receptor solutions, plotted as a function of the square root of time. The internal buffer and the continuous phase solution were D-PBS(−) at pH 7.4 and squalene with 10 wt% Span 83. Drugs, their molecular weights, and Log \(D\) values at pH 7.4 were as follows: methyl red (molecular weight 269.3 g/mol, 1.45); methylene blue (284.4 g/mol, 3.55); riboflavin (376.4 g/mol, −1.14); sunset yellow (406.4 g/mol, −4.24); amaranth (535.5 g/mol, −5.96); acid red 52 (557.7 g/mol, −1.03); bromophenol blue (668.0 g/mol, −0.29); and acid blue 9 (746.9 g/mol, −3.35).
The components and the preparation procedure of all w/o emulsions were the same; therefore, the physical and chemical parameters of the emulsion had no influence on the MB release rate. The average internal droplet size ($D_{50}$) of the w/o emulsion was $0.465 \pm 0.026 \mu m$. The solubility of MB did not influence the MB release rate because the solubility of MB in buffers of pH 4–10 is much greater than $0.5 \text{mM}$, which was the concentration of the internal solution.

To confirm the relationship between receptor solution pH and drug release rate from w/o emulsions, we also evaluated the dependence of the MB release rate on receptor solution pH when other lipophilic surfactants (Table 1) were used as the only surfactant in the w/o emulsion. The relationship was much stronger than expected. Figure 3 shows the relationship between MB release rate and pH using w/o emulsions with various surfactants. Rates of MB release were affected by both the type of surfactant used to form the emulsion and receptor solution pH. The pH dependence with each of the various surfactants was essentially the same as that shown in Fig. 2. Thus, acceleration of the release rate by low pH receptor solutions occurred for all the non-ionic-lipophilic surfactants shown in Table 1. These results show that receptor solution pH influences the MB release rate from w/o emulsions.

**Effects of Internal Buffer pH**

We speculated that the internal buffer pH would also have an effect on the MB release rate and drug release rate from w/o emulsions. Table 2 shows the internal droplet size of the w/o emulsion with various pH internal buffers and rates of MB release from these internal buffers. The internal buffer was D-PBS(−) at pH 7.4. CB4, citrate buffer at pH 4; CB6, citrate buffer at pH 6; PB6, phosphate buffer at pH 6; PB8, phosphate buffer at pH 8; GB10, glycine and sodium hydroxide buffer at pH 10.

![Fig. 3. Relationship between the Rate of MB Release from W/O Emulsions (Mean±S.D., n=3) with Various Non-ionic-lipophilic Surfactants and the pH of the Receptor Solution](image)

The internal buffer was D-PBS(−) at pH 7.4. CB4, citrate buffer at pH 4; CB6, citrate buffer at pH 6; PB6, phosphate buffer at pH 6; PB8, phosphate buffer at pH 8; GB10, glycine and sodium hydroxide buffer at pH 10.

![Fig. 4. Relationship between MB Release Rate (Mean±S.D., n=3) and Internal Phase pH](image)

The internal buffers were CB4, CB6, PB6, PB8, and GB10. The receptor solution was D-PBS(−) at pH 7.4. CB4, citrate buffer at pH 4; CB6, citrate buffer at pH 6; PB6, phosphate buffer at pH 6; PB8, phosphate buffer at pH 8; GB10, glycine and sodium hydroxide buffer at pH 10.

<table>
<thead>
<tr>
<th>Internal phase buffer</th>
<th>Span 80</th>
<th>Span 83</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Internal droplet size: $D_{50}$ [µm]</td>
<td>Release rate [%/h$^{1/2}$]</td>
</tr>
<tr>
<td>CB4</td>
<td>0.556±0.027</td>
<td>0.8±0.0</td>
</tr>
<tr>
<td>CB6</td>
<td>0.517±0.017</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td>PB6</td>
<td>0.475±0.051</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>PB8</td>
<td>0.371±0.063</td>
<td>7.2±0.3</td>
</tr>
<tr>
<td>GB10</td>
<td>0.442±0.050</td>
<td>8.0±0.3</td>
</tr>
</tbody>
</table>

*a) CB4, citrate buffer at pH 4; CB6, citrate buffer at pH 6; PB6, phosphate buffer at pH 6; PB8, phosphate buffer at pH 8; GB10, glycine and sodium hydroxide buffer at pH 10.*
lease rate and evaluated the effect of internal buffer pH on MB release rate. The internal phase solutions were prepared by dissolving MB in the various pH buffers shown in Table 2 at a concentration of 0.5 mM. The oil phase solutions were prepared by dissolving Span 83 or Span 80 in squalene at a concentration of 10 wt%. The emulsions were then prepared using the appropriate internal phase solution and oil phase solution. Table 2 shows the internal droplet sizes of the w/o emulsions, and the rate of MB release from these emulsions to a D-PBS(−) receptor solution at pH 7.4. Drugs in the internal phase of emulsions have been shown to be more easily released if the emulsions have a small internal droplet size. However, as shown in Table 2, the difference in droplet size did not affect MB release rate in our study.

Figure 4 shows the relationship between the MB release rate and the internal phase pH. In this experiment, MB release rate was dependent on pH. Because the internal droplet size did not have a significant effect on the MB release rate and both surfactants showed the same effect, we considered that it was the change in pH that was responsible for the effect seen in Fig. 4. Variation of the internal aqueous phase from CB4 to GB10 increased the MB release rate approximately 10-fold. The fact that the rates of MB release from the emulsions containing CB6 and PB6 were essentially identical suggested that the type of buffer had no effect on the MB release rate.

Drugs are released from the internal aqueous phase of w/o emulsions using the two mechanisms outlined in Fig. 5. In the first mechanism, the drug, originally present in a water droplet, dissolves in the oil phase as a surfactant-coated reverse micelle, diffuses in the oil, and is transferred to the receptor solution. In the second mechanism, the entire droplet diffuses in the oil phase and the drug is transferred from the droplet directly to the receptor solution, without dissolving in the oil phase, when the droplet reaches the interface between the oil and the receptor solution. In mechanism 2, the oil phase may be regarded as a semipermeable membrane allowing transport of drug molecules and water molecules. We presumed that the pH influenced these release mechanisms.

We evaluated the water–squalene distribution of MB to identify the mechanism influenced by pH. MB was initially dissolved in buffers CB4, CB6, PB6, PB8, and GB10. MB transport from the buffer (bottom layer) to squalene (top layer) was observed when squalene was gently added to the MB solution. Figure 6 indicates the appearance of MB transfer from the aqueous phase to the oil phase. The transfer occurred in the presence of 10 wt% Span 83, but was not observed in the absence of surfactant. These results suggest that MB dissolves in squalene as a soluble reverse micelle of Span 83. Therefore, we concluded that mechanism 1 is a viable model for what was occurring in our w/o emulsions. MB transfer began as soon as oil was added to GB10. As the aqueous phase pH decreased, MB transfer rate also decreased. Almost no MB transfer was observed for CB4. Since the water–oil distribution of MB was affected by pH, we supposed that the change in MB release rate from the w/o emulsion was due to a pH-dependent change in mechanism 1.

Both the rate of MB release from the internal phase to the receptor solution (Fig. 4) and the rate at which MB diffuses from an aqueous buffer into an oil phase (Fig. 6) increase dramatically with increasing pH. These results suggest that the rate determining step of MB release is the diffusion of MB from the internal phase into the oil phase as a surfactant-coated reverse micelle. This suggestion is further supported by the slight dependence of the maximum MB release rate on the chemical structure of the surfactant (Fig. 3). The results also suggest that the driving force for MB release is the difference in drug concentration between the oil phase and the receptor solution. The MB release rate increase as the receptor solution pH decreased (Fig. 2), and then plateaued around pH 6, suggesting that the partition of MB between oil and water shows the same pH dependence.

**Effect of Internal Phase pH on the Molecular Mobility of W/O Emulsion**

We attempted to investigate the pH-dependent change in MB release rate from the w/o emulsion with respect to the molecular mobility of the w/o emulsion without MB. The measurement of T1 and T2 relaxation times were adopted as the evaluation technique. Figure 7 shows the T1 and T2 values for water molecules in buffers CB4, CB6, PB6, PB8, and GB10, which have essentially identical viscosities. The T1 and T2 values remained constant, regardless of the solution pH. Emulsions were prepared by dispersing these buffers into an oil phase consisting of squalene that included 10 wt% Span 80. The T1 and T2 values of these w/o emulsions were measured as a binary system because a w/o emulsion is a binary system consisting of an aqueous phase and an oil phase. A shorter relaxation time reflects the mobility of squalene in the continuous oil phase, and a longer relaxation time reflects the mobility of water in the internal phase.

Figure 8a shows T1 and T2 values for squalene, and Fig. 8b shows T1 and T2 values for water. T1 and T2 values of the oil were constant, suggesting that internal phase pH had no effect on the mobility of the oil phase of the w/o emulsion. The T1 of the water also did not change. However, a pH-dependent change in the T2 of the water was observed. The T2 of the water was the shortest at pH 6, and increased when the pH

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**Fig. 5. Mechanisms of MB Release from the Internal Aqueous Phase of W/O Emulsion**

(a) In mechanism 1, MB (1) dissolves in the oil phase as a reverse micelle of the surfactant, (2) diffuses in the oil, and (3) is transferred to the receptor solution. (b) In mechanism 2, (4) the internal droplet diffuses in the oil phase and (5) MB is transferred from the droplet to the receptor solution when the droplet reaches the interface between the oil and the receptor solution.
shifted to either higher or lower values. We suspect that the T1 of the water in w/o emulsions was also dependent on pH and that the change in T1 was not noticed because T1 was too long. The viscosities of these w/o emulsions were almost the same, and no pH-dependent change in viscosity was observed (data not shown). We supposed that pH did not cause a mobility change in the entire w/o emulsion, but only changed the mobility of the water molecules in it. Interestingly, the mobility of water in buffer alone was not changed by pH, but its mobility in a w/o emulsion was changed. This may be because the mobility of water molecules in w/o emulsions and the ionic state of the drug defines drug release rate. The details are presented below.

When the pH increases from pH 6 to pH 10, the water molecules in the w/o emulsion become highly mobile and drug release becomes faster. In w/o emulsions, the oil phase may be regarded as a semipermeable membrane allowing transport of drug molecules and water molecules. 10) MB and water can transfer from an internal droplet to another droplet and then transfer to the receptor solution without dissolving into the oil phase. Moreover, MB and water can transfer to oil and disperse in oil as a part of a reverse micelle. The observed mobility of the water molecules results from a combination of the following four processes outlined in Fig. 9: (a) the dispersion of the water droplets in the continuous oil phase; (b) the dispersion of water molecules in the water droplets; (c) the dispersion of water molecules in the continuous oil phase as a dissolving state or a part of a reverse micelle; and (d) the

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**Fig. 6.** Photographs of MB Transport from Water to Squalene

The initial top layer is squalene with 10 wt% Span 83. The initial bottom layer is 0.5 mM MB dissolved in the following buffers: (a) CB4, (b) CB6, (c) PB6, (d) PB8, and (e) GB10. All buffers were prepared at a concentration of 100 mM, and NaCl was included in the buffers as an isotonic agent. CB4, citrate buffer at pH 4; CB6, citrate buffer at pH 6; PB6, phosphate buffer at pH 6; PB8, phosphate buffer at pH 8; GB10, glycine and sodium hydoxide buffer at pH 10.

**Fig. 7.** T1 and T2 Relaxation Times of the Various pH Buffers (n=1)

CB4, citrate buffer at pH 4; CB6, citrate buffer at pH 6; PB6, phosphate buffer at pH 6; PB8, phosphate buffer at pH 8; GB10, glycine and sodium hydoxide buffer at pH 10.
transportation of water molecules between the aqueous phase and oil phase. The speed of (a) and (c) was constant, regardless of the pH change, because the dispersion in the oil phase was constrained by the mobility of the oil phase. Moreover, the speed of (a) may be approximately zero because the viscosity of or the emulsion is high and phase separation does not occur. The speed of (b) is also constant because the T1 and T2 values of water in the buffers were the same. From the above considerations, we supposed that the high mobility of the water molecules may indicate that many water molecules are diffusing in the oil and/or being transferred between water droplets and between the aqueous phase and the oil phase. Thus, MB moves along with the movement of water molecules. This agrees with the results presented in Fig. 6, which demonstrates that the amount of MB diffused in oil was dependent on the aqueous phase pH.

Between pH 4 and pH 6, as the internal phase pH increased, the mobility of water in the w/o emulsion became slower, but drug release rate becomes faster. We have to consider that the pK_a of MB is approximately 3.7. In pH 4 buffer, both the ionic form of MB and the molecular form of MB exist. At pH 6 or higher, almost all MB is in the molecular form. Thus, more MB tends to be distributed in water at pH 4 than at pH 6 or higher. The w/o emulsion with an internal phase pH of 4 showed slow MB release regardless of the high mobility of the water molecules.

**Conclusion**

We examined the relationships between the drug release rate from a w/o emulsion and both the internal phase pH and the receptor solution pH, using a pH range of 4–10, the drug mimic MB, and an oil phase consisting of squalene and a non-ionic-lipophilic surfactant. MB could dissolve in squalene in the presence of a non-ionic-lipophilic surfactant. We suppose that MB is released from the internal aqueous phase of a w/o emulsion can proceed through two distinct mechanisms. In the first, MB in the internal phase dissolves in the oil phase as a part of a reverse micelle, diffuses in the oil phase, and moves to the receptor solution. When the reverse micelle reaches the interface between the oil phase and the receptor solution, MB diffuses into the receptor solution. In the second mechanism, the water droplets diffuse in the oil phase and MB finally transferred to the receptor solution when the droplet reaches the interface between the oil and the receptor solution. These mechanisms appear to be promoted by the difference in MB concentration. MB dissolved in an aqueous solution can be distributed to the oil in the presence of surfactant, supporting the existence of mechanism 1. It was confirmed that MB release rate from the w/o emulsion could be controlled by optimizing internal phase pH.

We also investigated the relationship between internal phase pH and drug release rate with respect to molecular mobility. The mobility of water molecules in w/o emulsions was found to change depending on the pH, even in the absence of a change in the mobility of water molecules in the buffer. These results suggest that the mobility of water molecules is indicative of the amount of water dissolved in the oil such as through reverse micelles. However, the reason why the molecular mobility changes of water in w/o emulsions was dependent on pH is unclear. Further studies are required.

It is expected that the drug release rate from w/o emulsions can be well controlled by optimizing internal buffer pH, even
if the composition of the w/o emulsion, such as the type of oil and surfactant and/or the phase volume ratio, is left unchanged. This controlled release technique by pH optimization of the internal phase and/or receptor solution can be easily applied to w/o/w emulsions as a technique of long-term drug inclusion.

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