Appendix

This appendix describes the calculations for estimating the binding parameters of drug molecules with microemulsion particles. The number of nanoparticles, the partition coefficient of drug in microemulsion and bulk media, and the volume occupied by drug molecules into microemulsion nanoparticles were calculated.

The following three assumptions were made for the calculations:

1. The density of the oil-in-water microemulsion is approximately equal to water.
2. The typical diameter of the nanoparticles is equal to that of the average sized nanoparticle as determined by laser light scattering measurements.
3. The drug molecules occupy a maximally packed structure on the surface of the nanoparticles.

The following definitions were used.

1. Let the diameter of the nanoparticle equal N_p = X nm.
2. Let the concentration of ME equal Y mg/ml where Y is total mass of surfactant, co-surfactant and oil per ml of ME solution.
3. Let the diameter of drug equal Z_d nm.
4. Let the density of the ME equal $\rho \bullet 10^3$ mg/ml

Surface Area

The surface area of an individual nanoparticles was equal to:

$$S_{NP} = \pi X^2 \qquad (1)$$

Particle Density

The following intermediate steps were used to derive the formula for particle density (eq. 5). The volume of the ME (V_{ME}) was defined as:

$$V_{ME} = \frac{Y}{\rho} \bullet 10^{-3} cm^3 \qquad (2)$$

Then, the volume of a single nanoparticle (V_{Np}) was defined as:

$$V_{Np} = \frac{1}{6}\pi X^3 \bullet 10^{-21} cm^3 \qquad (3)$$

Therefore, the theoretical maximum number of nanoparticles per ml (NP_{max}) or particle density (particles/ml) was defined as follows:

$$NP\max = \frac{V_{ME}}{V_{Np}} \qquad (4)$$

Substituting equations 2 and 3 into equation 4:

$$NP\max = \frac{6Y}{\pi X^3} \bullet 10^{18} \qquad (5)$$

Total Interfacial Area

The aggregate, total interfacial area of the nanoparticles ($TotalSA_{NP}$) was calculated by multiplying the surface area of a single nanoparticle (eq.1) by the total number of nanoparticles (eq. 5). Thus:

$$TotalSA_{NP}(m^2/ml) = \frac{6Y}{X} \qquad (6)$$

Partition Coefficient of Drug

The partition coefficient (P_c) was defined as:

$$P_c = \frac{C_{ME}}{C_{Free}} \qquad (7)$$

Where C_{Free} is the concentration of drug as measured by HPLC following ultrafiltration from the microemulsions (i.e., not bound on or in microemulsions). C_{ME} is defined as follows:

$$C_{ME} = \frac{drugmoles_{ME}}{V_{ME}} \qquad (8)$$

Where $drugmoles_{ME}$ is the measured moles of drug in or on the ME and V_{ME} is defined in eq. 2. Combining eqs. 2 and 8 into eq. 7:

$$Pc = \frac{Drugmole_{ME}}{Drugmoles_{Free}} \cdot \frac{\rho}{Y} \cdot 10^3 \qquad (9)$$

Percentage Volume of Particle Occupied by Drug

The fractional occupancy of the nanoparticle volume by drug was calculated as:

$$Volume(\%) = 100 \bullet molecDrugperNP \bullet \frac{V_{Drug}}{V_{Np}} \qquad (10)$$

Where molecDrugperNP is the number of molecules of bupivacaine bound per nanoparticle as calculated by the measured mass of drug bound to nanoparticles:

$$molcDrugperNP = \frac{Drugmole_{ME} \bullet Avog.number}{NP_{\max}} \qquad (11)$$

Where Avog. Number is equal to $6.023 \cdot 10^{23}$, and NP_{max} is defined in eq. 4.

The volume of the drug (V_{Drug}) was calculated as using spherical geometry and a previously published diameter of 1.43 nm for bupivacaine. Thus:

$$V_{Drug} = \frac{\pi Z_d^{\ 3}}{6} \cdot 10^{-21} cm^3 \qquad (12)$$

Substituting eqs. 5, 11 and 12 into eq. 10, the following formula is derived:

$$Volume(\%) = 3.15 \cdot 10^{4} \frac{Drugmoles_{ME}}{Y} \cdot Z_{d}^{\ 3} \qquad (13)$$