Hydrophobic drugs become more practical for treatments by being encapsulated in micelle compositions for increasing solubility. Micelle compositions may include an excipient tocopherol and/or prodrug formulations of the drug. Micelles extend the time period the drug remains in the micelles to improve drug circulation time and thereby drug delivery. Hydrophobic drugs for micelle encapsulation may include rapamycin, geldanamycin, and paclitaxel. Administration of these micelle compositions does not require Cremophor EL or Tween 80, avoiding serious side effects associated with these products which would previously accompany such drug administration.

17 Claims, 26 Drawing Sheets
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Fig. 1

Fig. 2

Fig. 3

Relative polarity

H₂O 1.9
Hexane 0.6

Pyrene

\[ \log P_{ow} = 5.16 \]
**FIG 12**

![Graph showing changes in λ339/λ334 with [PEG-DSPE], M. The graph includes different ratios of lipid to tocopherol: 1:0.1, 1:0.5, 1:2, and 1:1. Each ratio has a distinct line indicating its trend.]

**FIG 13**

![Bar chart showing relative viscosity, T_m/T_e, for different PEG-DSPE concentrations and ratios.](chart)

- **PEG-DSPE**
- 1:0.1 lipid:tocopherol
- 1:0.5 lipid:tocopherol
- 1:2 lipid:tocopherol
- 1:1 lipid:tocopherol

The chart indicates a decrease in relative viscosity as the ratio of lipid to tocopherol increases.
FIG. 17

FIG. 18
Fig. 19

Drug:Unimer (molar ratio)

PEG-phospholipid, additives, and drug in solvent (MeOH, chloroform mixture)

Fig. 20

Filtration (centrifugation and filtration)
Dissolve drug and polymer in water miscible solvent (e.g. acetone)

Add, drop-wise, to vigorously stirred aqueous solution

Evaporate solvent

Nano-filter / centrifuge to remove unincorporated drug

**FIG. 21**

**FIG. 22**

Loading efficiency

Weight % drug

Drug:Unimer (Molar Ratio)

PEG-DSPE micelle size: 14±2 nm (DLS)

rapamycin (2:1) 16±2 nm
Fig. 23

<table>
<thead>
<tr>
<th>Drug Formulation</th>
<th>D $\times 10^{20}$ (cm$^2$/s)</th>
<th>$t_{50%}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-DSPE micelle</td>
<td>$0.55 \pm 0.10$</td>
<td>$35 \pm 7$</td>
</tr>
<tr>
<td>+ 0.23 mg/ml BSA</td>
<td>$2.3 \pm 0.2$</td>
<td>$8.3 \pm 0.8$</td>
</tr>
<tr>
<td>+ 40 mg/ml BSA</td>
<td>$7.7 \pm 1.4$</td>
<td>$2.4 \pm 0.4$</td>
</tr>
</tbody>
</table>

Fig. 24
FIG. 25

Hydrodynamic diameter, nm

PEG-DSPE 1:0.1 lipid:choo 1:0.5 1:1 1:1.5 1:2

* p<0.05

FIG. 26

UV Absorbance

Refractive Index

Time, min

micelles
rapamycin
unimers
Crank\textsuperscript{1} solution for Fickian diffusion from sphere for short time periods

\[ \frac{M_t}{M_\infty} = 6 \left( \frac{D t}{r^2 \pi} \right)^{1/2} - \frac{3D t}{r^2} \quad M_t/M_\infty = 0.6 \\
C_{\text{bulk}} = 0 \\
(t=0) C_r = \text{constant} \]

Impenetrable sphere model of micelle \textsuperscript{2}

\[ r_{\text{core}} = \left[ \frac{3M_{\text{micelle}} W_{\text{core}} \gamma_{\text{core}}}{4\pi N_A \Phi_{\text{core}}} \right]^{1/3} \]


FIG. 27

<table>
<thead>
<tr>
<th>D x 10\textsuperscript{20} (cm\textsuperscript{2}/s)</th>
<th>(t_{50%}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-DSPE micelle</td>
<td>0.55 ± 0.10</td>
</tr>
<tr>
<td>+ 1:2 tocopherol</td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

\(p > 0.05\)
Drug loading of 20% wt/wt and > 5 mg/ml rapamycin
**FIG. 31**

<table>
<thead>
<tr>
<th>PEG-PCL 5:10 kDa</th>
<th>D x 10^{20} (cm^2/s)</th>
<th>t_{50%} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no tocopherol</td>
<td>7.4 ± 0.7</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>1:20 tocopherol</td>
<td>11 ± 0.0</td>
<td>33 ± 2</td>
</tr>
</tbody>
</table>

**FIG. 32**

<table>
<thead>
<tr>
<th>PEG-PCL 5:10 kDa</th>
<th>D x 10^{20} (cm^2/s)</th>
<th>t_{50%} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no toco</td>
<td>7.4 ± 0.7</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>no toco + 4% BSA</td>
<td>11 ± 0.0</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>1:20 toco</td>
<td>18 ± 4</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>1:20 toco + 4% BSA</td>
<td>9.0 ± 1.9</td>
<td>39 ± 6</td>
</tr>
</tbody>
</table>
FIG. 33

FIG. 34
FIG. 35

Rapamycin Concentrations at 1 minute

FIG. 36
FIG. 37

FIG. 38
Fig. 39

Fig. 40
- Very poor water solubility (< 10 mg/ml)
- Hepatotoxic (MTD <100 mg/m² in dog)
- C-17 derivatives are less hepatotoxic and retain high potency
- 17-AAG and 17-DMAG are in multiple human trials

MW 560
XLog P o/w 1.36

17-AAG

17-AAG

IC₅₀ 33 nM (SKBr3 cells)²
Solubility: 0.1 mg/ml
MTD: 500 mg/m² (dog)¹

IC₅₀ 24 nM²
Solubility: 1.4 mg/ml
MTD: 8 mg/m² (dog)³


**Fig. 41**

<table>
<thead>
<tr>
<th>Micelle</th>
<th>Loading (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-DSPE₂₀₀₀</td>
<td>1.7%</td>
</tr>
<tr>
<td>+ 1:2 tocopherol</td>
<td>1.9%</td>
</tr>
<tr>
<td>PEG-b-PCL 5:10 kDa</td>
<td>0.2%</td>
</tr>
<tr>
<td>+ 1:20 tocopherol</td>
<td>1.3%</td>
</tr>
</tbody>
</table>

- Geldanamycin poorly loaded in PEG-b-PCL and PEG-DSPE micelles
- Not lipophilic enough?

**Fig. 42**
Fig. 43

17-aminomethyl-ester acyl-17-GA

<table>
<thead>
<tr>
<th>n</th>
<th>Prodrug</th>
<th>Log P&lt;sub&gt;ow&lt;/sub&gt;</th>
<th>PEG-b-PCL loading (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Geldanamycin</td>
<td>2.77 ± 0.04</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>3</td>
<td>hydroxethylamino</td>
<td>2.75 ± 0.02</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>hexanoate</td>
<td>3.90 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>bromohexanoate</td>
<td>3.91 ± 0.04</td>
<td>2.8 ± 0.0%</td>
</tr>
<tr>
<td>9</td>
<td>dodecanoate</td>
<td>4.20 ± 0.05</td>
<td>21 ± 2%</td>
</tr>
<tr>
<td>9</td>
<td>bromododecanoate</td>
<td>4.20 ± 0.04</td>
<td>21 ± 2%</td>
</tr>
<tr>
<td>13</td>
<td>palmitate</td>
<td>4.35 ± 0.21</td>
<td>22 ± 5%</td>
</tr>
<tr>
<td>13</td>
<td>bromopalmitate</td>
<td>4.54 ± 0.06</td>
<td>25 ± 2%</td>
</tr>
<tr>
<td>13</td>
<td>aminohexidacyl</td>
<td>4.34 ± 0.02</td>
<td>20 ± 2%</td>
</tr>
</tbody>
</table>

Fig. 44
FIG. 45

C\textsubscript{17}

\begin{align*}
\text{1} & \rightarrow \text{2} \\
\text{10 eq } & \text{H}_2\text{N-CH}_2\text{OH} \\
\text{CHCl}_3, & 1-4 \text{ h, RT} \\
\text{workup:} & \text{hexane/acetone ppt}
\end{align*}

\begin{align*}
\text{2} + \text{3 eq DCC, 1 eq DMAP} \\
\text{CHCl}_3, & 2-6 \text{ h, RT} \\
\text{workup:} & \text{cold filter, MeOH:CHCl}_3 \text{ flash on silica}
\end{align*}

FIG. 46

\begin{align*}
\text{1} & \rightarrow \text{2} \\
\text{5 eq } & \text{H}_2\text{N-CH}_3 \text{X} \\
\text{CHCl}_3, & 1-4 \text{ h, RT} \\
\text{MeOH:CHCl}_3 \text{ flash on silica}
\end{align*}
1) 1 eq NaOCH₂CH₃ in EtOH, reflux 1 h
2) 0.95 eq 2-bromo-isopropane, reflux 4 h

Workup: add 2 vol cold H₂O,
extract 3x ether, vac. distill

1) 1:1 KOH : H₂O, reflux, 8 h
2) add water until solids gone
3) extract water from oil
4) add conc. HCl until no more solid
5) extract ether 3x, reduce in vacuo
6) heat 180 °C 3 h, vac distill

$\text{2 + 3} \rightarrow$
3 eq DCC, 1 eq DMAP
$\text{CH₂Cl₂, 2-6 h, RT}$
workup:
cold filter,
MeOH : CHCl₃ flash on silica

Fig. 47
Fig. 48

\[
\begin{align*}
\text{Fmoc-CH₂} & \text{-O-} \text{NH-CH₂-CO-} \text{N-CH₂-CO-OH} \\
& + 2 \\
\end{align*}
\]

3 eq DCC, 1 eq DMAP

\[
\begin{align*}
\text{CH₂Cl₂, 2-6 h, RT} \\
\text{workup:} \\
1) \text{cold filter,} \\
\text{MeOH:CHCl₃ flash on silica} \\
2) \text{2:8 piperidine : DMF} \\
1-2 \text{ h, RT} \\
3) \text{filter, MeOH:CHCl₃ flash}
\end{align*}
\]
Fmoc\text{OH} \rightarrow 1 \text{ eq Dess-Martin} \rightarrow \text{CH}_2\text{Cl}_2, 20 \text{ min} \rightarrow \text{workup:} 7 \text{ eq thiosulfate, NaHCO}_3 20 \text{ min} \rightarrow \text{extract ether, HCl, H}_2\text{O} \rightarrow \text{flash: 99:1 EtOAc : TEA} \rightarrow \text{overnight} \rightarrow \text{filter} \rightarrow \text{flash 89:10:1 CHCl}_3 : \text{MeOH} : \text{TEA}

1 + 5 \rightarrow \text{DMF, 2-6 H, RT} \rightarrow \text{workup:} \rightarrow \text{cold filter} \rightarrow \text{MeOH : CHCl}_3 \rightarrow \text{flash on silica}

1 \text{ eq palmitic acid hydrazide} \rightarrow \text{EtOH, reflux overnight} \rightarrow \text{workup:} 7 \text{ eq thiosulfate, NaHCO}_3 20 \text{ min} \rightarrow \text{extract ether, HCl, H}_2\text{O} \rightarrow \text{flash: 89:10:1 CHCl}_3 : \text{MeOH} : \text{TEA}

Fig. 49
Fig. 50
Fig. 51

<table>
<thead>
<tr>
<th>Compound</th>
<th>$t_{50%}$ (days)</th>
<th>Equation Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>dodeconate</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>bromododeconate</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>aminohexyldecyl</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>palmitate</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>bromopalmitate</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>aminohexyldecyl</td>
<td>6.8</td>
<td></td>
</tr>
</tbody>
</table>

1 Release at 37°C, pH 7.4
2 Calculated from Crank equation
<table>
<thead>
<tr>
<th>Compound</th>
<th>$\bar{D} \times 10^{23}$ (cm$^2$/s)</th>
<th>$t_{50%}$ (days)</th>
<th>Log $P_{o/w}$</th>
<th>Loading (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-aminoethyl-bromohexonate-17-GA</td>
<td>3.9</td>
<td>6.6</td>
<td>3.91</td>
<td>2.8</td>
</tr>
<tr>
<td>17-aminoethyl-dodeconate-17-GA</td>
<td>12</td>
<td>2.2</td>
<td>4.20</td>
<td>21</td>
</tr>
<tr>
<td>17-aminoethyl-bromododeconate-17-GA</td>
<td>4.8</td>
<td>5.3</td>
<td>4.20</td>
<td>21</td>
</tr>
<tr>
<td>17-aminoethyl-palmitate-17-GA</td>
<td>6.5</td>
<td>3.9</td>
<td>4.35</td>
<td>22</td>
</tr>
<tr>
<td>17-aminoethyl-bromopalmitate-17-GA</td>
<td>2.7</td>
<td>9.5</td>
<td>4.35</td>
<td>25</td>
</tr>
<tr>
<td>17-aminoethyl-decyl-17-GA</td>
<td>3.7</td>
<td>6.8</td>
<td>4.34</td>
<td>20</td>
</tr>
</tbody>
</table>

**Fig. 52**
1

MICELLE COMPOSITION OF POLYMER AND PASSENGER DRUG

RELATED APPLICATIONS

This application is a continuation application of U.S. patent application Ser. No. 11/402,639, filed Apr. 12, 2006, which claims the benefit of U.S. Provisional Patent Application No. 60/670,460, filed Apr. 12, 2005, and U.S. Provisional Patent Application No. 60/716,000, filed Sep. 9, 2005, which are incorporated herein by reference. This invention was made with United States government support awarded by the National Institutes of Health (NIH) under grant number AI043346. Accordingly, the United States has certain rights in this invention.

BACKGROUND

1. Field of the Invention

This invention is directed generally to micelle compositions, methods of making micelles, and the use of micelle compositions with drugs for treatment of disease.

2. Description of the Prior Art

Cancer is a very deadly disease. Various cytotoxic chemotherapy agents have been used to eradicate cancer and/or prevent the spread of the cancer. Alkylating agents, such as cisplatin and chlorambucil, crosslink DNA to prevent cell division. Antitumor antibiotics, such as dactinomycin and bleomycin, bind DNA and thus prevent DNA separation and mRNA synthesis. Antimetabolites, such as purine and pyrimidine analogs and 5-fluorouracil, may mimic cell nutrient and prevent normal DNA synthesis. Plant alkaloids, such as paclitaxel and vinblastine, block cell division by blocking microtubule formation. Topoisomerase inhibitors, such as camptothecin, topotecan, and irinotecan, inhibit DNA supercoiling and block transcription and replication. Many drugs that are potentially efficacious for treating diseases such as cancer have poor solubility that limits their usefulness.

Rapamycin is a large, highly hydrophobic compound with applications in chemotherapy, immunosuppression, anti-restenosis, fungal infections, and neurological disorders. Rapamycin as an anti-cancer agent is generally formed as ester analogs which are quickly hydrolyzed and sequestered into the red blood cells thereby reducing the effectiveness of rapamycin at tumor sites. Rapamycin is currently used as an immunosuppressant for kidney transplant patients, Rapamune (Wyeth-Ayerst), and has shown long term clinical safety. However, rapamycin is a poorly water soluble drug, creating difficulties in drug administration in patients.

Geldanamycin is also a hydrophobic compound with applications including the treatment of cancer. Geldanamycin is a member of the new class of compounds known as heat shock protein inhibitors, having both anti-tumor and neurological disease applications. The mode of action is by inhibiting heat shock protein 90 (Hsp90), strongly binding to Hsp90 (K_a=1.2 µM), and preventing interaction with downstream components. Hsp 90 is a molecular chaperon responsible for the folding, stability, and function of numerous client proteins. Inhibition of Hsp 90 leads to the destabilization and eventual ubiquitination of many oncogenic client proteins. By targeting multiple oncogenic proteins, geldanamycin may be efficacious against a broad range of tumors and may increase the chances of overcoming drug resistance. In addition, the inhibition of Hsp90 leads to an up-regulation of Hsp70, which reduces the formation of abnormal tau species, the primary component of plaque deposits in Alzheimer’s and Parkinson’s disease.

Paclitaxel is another hydrophobic compound with applications including the treatment of cancer. Paclitaxel belongs to a group of medicines called antineoplastics, which inhibit cellular growth. The inhibition is accomplished by disrupting microtubule function by binding to the beta subunit of tubulin. The disrupted microtubule looses the ability to disassemble, a necessary function, for example, in chromosomal migration during cell replication. Additionally, research has indicated that paclitaxel induces apoptosis, programmed cell death, by binding to an apoptosis stopping protein called Bcl-2 and stopping its function.

Various techniques for solubilizing poorly soluble compounds exist, such as the formation of emulsions, liposomes, or micelles, all of which may have multiple phases, some of which may be unstable and may tend to separate.

Micelle systems based on amphiphilic polymers using block copolymers (ABC’s) have been used to formulate such challenging drugs. ABC’s comprised of a hydrophobic, such as polypropylene glycol, and hydrophilic block, such as polyethylene glycol (PEG), can assemble into a microphase separated, core/shell architecture in a selective solvent. PEG-poly(e-caprolactone) (PEG-PCL) and PEG-poly(amine acids) can form these polymeric micelles. Alternatively, phospholipids can be used, such as, PEG-diesterarylphosphatidylethanolamine (PEG-DSPE) to form these polymeric micelles. In an aqueous environment, the hydrophobic drug can be encapsulated into the hydrophobic core of the micelle and have aqueous solubility provided by a poly(ethylene glycol) (PEG) and corona (shell). Due to their nanoscopic dimensions and stealth properties imparted by a PEG corona, micelles may have long-term circulation capabilities. During the circulation period, the micelle may gradually release drug and eventually dissociate and be eliminated from circulation.

Excipients and co-excipients have been used to solubilize poorly soluble compounds. Alpha-tocopherol, commonly known as Vitamin E or simply tocopherol, has been used as an excipient because of its ring and alkyl chain structures common to many poorly-soluble drugs. Vitamin E is not toxic to living organisms. Additionally, tocopherol stabilizes biological membranes. Tocopherol, however, is not soluble in water and therefore it has had limited usefulness in intravenous solutions.

SUMMARY OF THE INVENTION

A micelle composition may comprise an amphiphilic polymer, a hydrophobic excipient, and a hydrophobic passenger drug. In one aspect, the amphiphilic polymer is PEG-DSPE. In another aspect, the excipient is tocopherol. In yet another aspect, the ratio of tocopherol to PEG-DSPE is between about 0.1 and about 3.

In one aspect, a micelle composition comprises an amphiphilic polymer and paclitaxel. In another aspect, the micelle composition may have an amphiphilic polymer, rapamycin and tocopherol. In yet another aspect, the concentration of PEG-DSPE may be between about 1 and about 10 mM, the concentration of tocopherol may be between about 2 and about 20 mM, and the concentration of rapamycin may be between about 0.1 and 1.0 mg/ml.

A micelle composition may comprise an amphiphilic polymer and geldanamycin. The geldanamycin may be a geldanamycin prodrug with increased hydrophobic properties. A micelle composition may comprise an amphiphilic polymer and paclitaxel. The paclitaxel may be a paclitaxel prodrug with increased hydrophobic properties.

A process for forming micelle compositions may include mixing amphiphilic polymer, hydrophobic excipient, and
hydrophobic drug into an organic solvent to form a solution, removing substantially all of the organic solvent from the solution to leave a substantially solvent-free mixture, and resuspending the solvent-free mixture in water or buffer. A process may also include adding said solution to a substantially water solution before removing substantially all of said organic solvent from said solution to leave a substantially solvent-free mixture.

A process and resulting prodrug composition made for improving micelle encapsulation efficiency of hydrophobic drugs. In another aspect, a process for making geldanamycin prodrugs for encapsulation. In yet another aspect, a process for making paclitaxel prodrugs for encapsulation.

A method of treatment for a disease or condition in a human or an animal may comprise administering an effective amount of a micelle composition comprising an amphiphilic polymer, a hydrophobic excipient and a hydrophobic passenger drug.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 is a schematic showing a micelle structure for drug delivery, including a hydrophobic core and a hydrophilic corona.

FIG. 2 is a schematic showing a depiction of micelle formation by unimers above critical micelle concentration through hydrophobic interaction.

FIG. 3 is a graph showing polarity as a function of micelle concentration.

FIG. 4 is a schematic showing micelles being administered intravenously, and the uptake by tumors due to their leaky vasculature.

FIG. 5 depicts the structure of PEG-DSPE.

FIG. 6 depicts the structure of PEG-PCL.

FIG. 7 depicts the structure of tocopherol.

FIG. 8 is a schematic showing tocopherol incorporation into PEG-DSPE.

FIG. 9 depicts the structure of rapamycin.

FIG. 10 depicts the structure of geldanamycin.

FIG. 11 depicts the structure of paclitaxel.

FIG. 12 shows a graph of critical micelle concentration at different PEG-DSPE to tocopherol ratios as a function of the concentration of the PEG-DSPE micelles.

FIG. 13 is a bar graph of relative core viscosity as a function of the PEG-DSPE to tocopherol ratio.

FIG. 14 is a bar graph showing the increasing aggregate number within the core as a function of various PEG-DSPE to tocopherol ratios.

FIG. 15 is a graph showing the stability of PEG-DSPE micelles in phosphate buffered saline and in 4% bovine serum albumin as a function of time.

FIG. 16 is a graph showing the stability of PEG-PCL micelles in 4% bovine serum albumin as a function of time.

FIG. 17 is a graph showing the stability of PEG-DSPE micelles in 4% bovine serum albumin as a function of time.

FIG. 18 is a graph showing the core polarity of PEG-DSPE micelles for various PEG-DSPE to tocopherol ratios and PEG-DSPE concentrations.

FIG. 19 is a graph showing the rapamycin loading efficiency by diffusion-evaporation as a function of rapamycin to amphiphilic polymer ratio, for ratios of PEG-DSPE:tocopherol at 1:2, 1:1 and no tocopherol.

FIG. 20 is a schematic of a method of forming PEG-DSPE micelles.

FIG. 21 is a schematic of a drop wise method of forming polymer micelles.

**FIG. 22** is a graph showing rapamycin loading efficiency in micelles as a function of the ratio of rapamycin to amphiphilic polymer.

**FIG. 23** is a graph showing rapamycin release in the presence of albumin as a function of time in different bovine serum albumin concentrations.

**FIG. 24** is a bar graph showing the interaction of serum albumin, fibrinogen, and bovine pancreatic trypsin inhibitor with PEG-DSPE micelles.

**FIG. 25** is a graph showing how tocopherol incorporation affects the size of resulting micelles.

**FIG. 26** is a graph showing the incorporation of rapamycin in micelles through size exclusion chromatography.

**FIG. 27** is an analysis of release kinetics based on Fickian diffusion from sphere for short time periods.

**FIG. 28** is a graph showing the effect of tocopherol on rapamycin release from PEG-DSPE micelles in phosphate buffered saline solution.

**FIG. 29** is a graph showing the effect of tocopherol on rapamycin release from PEG-DSPE micelles in 4% bovine serum albumin.

**FIG. 30** shows the stability of PEG-PCL micelles in the presence of tocopherol.

**FIG. 31** is a graph showing the release of rapamycin from PEG-PCL micelles with incorporated tocopherol as a function of time in phosphate buffered saline.

**FIG. 32** is a graph showing the release of rapamycin from PEG-PCL micelles with incorporated tocopherol as a function of time in 4% bovine serum albumin.

**FIG. 33** is a graph showing rapamycin control formulation disposition in whole blood following intravenous administration.

**FIG. 34** is a graph showing rapamycin PEG-PCL formulation disposition in whole blood following intravenous administration.

**FIG. 35** is a graph showing rapamycin PEG-PCL+α-tocopherol formulation disposition in whole blood following intravenous administration.

**FIG. 36** is a bar graph showing rapamycin concentration in plasma or red blood cells for rapamycin control formulation, rapamycin PEG-PCL, and rapamycin PEG-PCL+α-tocopherol formulation at 1 min after intravenous administration.

**FIG. 37** is a bar graph showing plasma RBC ratios of rapamycin control formulation, rapamycin PEG-PCL, and rapamycin PEG-PCL+α-tocopherol formulation at 1 min after intravenous administration.

**FIG. 38** is a bar graph showing rapamycin concentration in plasma or red blood cells for rapamycin control formulation, rapamycin PEG-PCL, and rapamycin PEG-PCL+α-tocopherol formulation at 12 hours after intravenous administration.

**FIG. 39** is a bar graph showing plasma RBC ratios of rapamycin control formulation, rapamycin PEG-PCL, and rapamycin PEG-PCL+α-tocopherol formulation at 12 hours after intravenous administration.

**FIG. 40** is a schematic showing the targets of geldanamycin (in boxes) (Miyata Y. Curr. Pharm. Design. 11:1131-8 (2005)). The heat shock protein 90 inhibitor a) binds strongly (Kd 1.2 μM) to Hsp90; b) causes ubiquitination and down-regulation of a broad range of oncogenic client proteins and subsequent degradation; c) inhibits telomerase activity; d) induces apoptosis with antitumor activity (IC50=3 nM in MB-468); and e) leads to up-regulation of Hsp 70 — reducing abnormal tau species (the primary component of plaque deposits in Alzheimer’s and Huntington’s diseases (Petrucci et al. Hum. Mol. Genet. 13, 703-714 (2004))).
Polymeric micelles are spherical and may have nanoscopic structures having a core-shell form. Micelle formation is entropy driven. See FIG. 2. Water molecules are excluded into a bulk phase. \( \Delta G^{\text{mic}} = RT \ln(CMC) \) informs the formation of micelles. When above critical micelle concentration (CMC), amphiphilic unimers aggregate into structured micelles. Polymeric micelles are spherical and may have nanoscopic dimensions typically in the 20-100 nm range. This is advantageous as circulating particles should be less than about 200 nm to avoid filtering by the interendothelial cell slits at the spleen. Polymeric micelles have been shown to circulate in the blood for prolonged periods and capable of targeted delivery of poorly water-soluble compounds. Upon disassociation, micelle unimers are typically \( \leq 50,000 \) g/mol, permitting elimination by the kidneys. Ideally, this allows prolonged circulation with no buildup of micelle components in the liver that could lead to storage diseases.

The key benefits of micelle compositions include ease of storage and delivery; compositions may be lyophilized and reconstituted before intravenous administration. This lowers the risk of drugs precipitating and causing an embolism. Micelle compositions are capable of long blood circulation, low mononuclear phagocyte uptake, and low levels of renal excretion. Also, micelle compositions have enhanced permeability and retention (EPR) to increase the likelihood of the chemotherapeutics reaching tumors. As shown in FIG. 4, tumors have high vascular density as well as defective vasculature so high extravasation occurs. There may be impaired lymphatic clearance. The endocytosis and subsequent drug release increases the effect of the chemotherapeutics on the tumor.

Initial studies have focused on PEG-DSPE (FIG. 5) and the block co-polymers and PEG-PCL (FIG. 6) for drug solubilization. PEG-DSPE may be a safe and effective micelle carrier for both chemotherapeutic agents. PEG-PCL is biodegradable and may have biocompatibility.

The principal difference between neutral PEG-DSPE and negatively charged PEG-DSPE membranes is the electrostatic force between the two charged membranes. Membrane charges affect the adsorption of acidic and basic proteins on charged and neutral membranes. This may alter the interactions of various proteins with the bilayers. These differences may be responsible for the differences in opsonization and phagocytosis of neutral versus charged liposomes. The phosphate group at the hydrophobic head of PEG-DSPE may affect the tightness of the PEG-DSPE’s at the core-water interface due to electrostatic repulsion. Also, this charged nature may influence protein interaction with the hydrophobic core should the protein penetrate the PEG corona. Tocopherol (FIG. 7) has been shown to interpolate between the phospholipid head groups and the ring-structure at the head of the tocopherol may prevent further protein penetration and interaction. See FIG. 8. Also, the tocopherol head group and hydroxyl group have been shown to act as an antioxidant and may prevent protein disruption of the phospholipid layer. PEG-b-PCL may be biocompatible and biodegradable. PEG-b-PCL may be biocompatible and biodegradable.
Micelles have a loading capacity >20% (w/w). Therefore, tumors through the EPR effect, reducing the likelihood of immunosuppression. In addition, micelle delivery allows targeted treatment to therapeutic dosages of this drug without chemical modification. Mycophenolic acid, a 300-fold increase. In addition, using prodrugs of the micelles so as to induce higher loading of drugs which are otherwise poorly soluble in the micelle of study.

2.0 Passenger Compounds

In accordance with the invention, drugs can be passenger compounds in polymer carriers. Such drugs include: rapamycin (FIG. 9), geldanamycin (FIG. 10), and paclitaxel (FIG. 11). These drugs are potent small molecule chemotherapeutic agents with unique targets of action. Studies of these compounds and the development of clinical products have been hampered by their extremely low water solubilities, for example, rapamycin ~2.6 µg/ml and geldanamycin ~1.5 µg/ml. Using combinations of the above polymeric compounds and integrating tocopherol into the micelle structure, stable micelle solutions of these compounds were achieved incorporating up to about 5 mg/ml of rapamycin, a 1900-fold increase in solubility, and up to about 500 µg/ml of geldanamycin, a 300-fold increase. In addition, using prodrugs of geldanamycin or paclitaxel significantly increase solubilities.

The promise of these compounds as chemotherapeutics merits their further evaluation with in vitro and in vivo tumor models. The successful formulation of these compounds using the phospholipids and poly-caprolactone/tocopherol systems merits investigating their application to other hard-to-solubilize drug compounds.

The choice of polymeric micelle carrier can be highly dependent on the structural relationship between the target drug compound and the hydrophobic core of the carrier. Less than 3% (w/w) paclitaxel may be loaded into PEG-PCL micelles. However, PEG-poly(D,L-lactide) micelles have a loading capacity ~20% (w/w). Therefore, conditions of polymeric micelle carriers must be optimized for loading a desired passenger compound.

2.1 Rapamycin

The formulation of these compounds, especially rapamycin, for intravenous delivery without the use of co-solvents, e.g., ethanol or polyethylene glycol, permits them for therapeutic usage. The use of micelle carriers allows delivery of therapeutic dosages of this drug without chemical modification. In addition, micelle delivery allows targeted treatment to tumors through the EPR effect, reducing the likelihood of immunosuppression, a side-effect of free rapamycin and its water soluble derivatives.

Rapamycin (FIG. 9) is a large, highly hydrophobic compound with applications in chemotherapy, immunosuppression, anti-restenosis, fungal infections, and neurological disorders, e.g., Alzheimer’s and Huntington’s disease. Rapamycin has a unique target of action, binding the immunophilin FKBP12 and inhibiting the mammalian target of rapamycin (mTOR) pathway, which prevents cell cycle G1 to S phase transition. Rapamycin has demonstrated impressive activity against a broad range of human tumor xenograft models including lymphocytic leukemia, melanocarcinoma, ependymoblastoma, and various solid tumors with a typical IC50 of 10⁻⁸ M.

A novel mechanism may have rapamycin binding to FK506-12, in which rapamycin inhibits mTOR growth regulators, prevents G1 to S phase transition, and inhibits NF-kB and enhances apoptosis.

Unfortunately, rapamycin is practically insoluble in water (~2.6 µg/ml) and has no ionizable groups. The targeted delivery and retention of rapamycin to tumor sites, using the EPR effect, may substantially increase its potency. In addition, targeted delivery may attenuate the side effects of rapamycin treatment including immunosuppression. The retention of rapamycin’s native hydrophobic nature may be important in neurological applications where modification (to increase water solubility) may hinder crossing of the blood brain barrier.

Using polymeric micelles, rapamycin can be solubilized in large quantities—well within the range required for clinical feasibility. Rapamycin has been solubilized using PEG-PCL and PEG-DSPE micelles with the addition of tocopherol. Results are summarized in Example 2.

2.2 Geldanamycin

Geldanamycin (FIG. 10) is a member of the new class of compounds known as heat shock protein inhibitors, having both anti-tumor and neurological disease applications. The mode of action is inhibiting heat shock protein 90 (Hsp90), strongly binding to Hsp90 (Kₐ = 1.2 µM), and preventing interaction with downstream components. This in turn leads to ubiquitination of a broad range of oncoprotein clinical proteins and their subsequent degradation.

Hsp90 inhibitors may be useful in drug resistant cancers by inducing different pathways, such as in rapamycin resistant tumors. Despite the promise of Hsp90 inhibitors, such as geldanamycin, the clinical progression of these therapies has been slow due to the lack of a suitable formulation. Radicicol, an Hsp90 inhibitor, is also unstable in vivo. Geldanamycin has extremely poor water solubility, and is hepatotoxic in vivo (MTD dog<100 mg/m²). Geldanamycin prodrugs such as 17-AAG have slightly better solubility and lower hepatotoxicity (MTD dog 500 mg/m²), but are still difficult to formulate, requiring toxic excipients such as Cremophor, Tween 80, and DMSO. Water soluble prodrugs of geldanamycin, such as 17DMAG (MTD dog 8 mg/m²), may avoid these formulation problems, but the wide biodistribution and increased toxicity of these prodrugs may present additional difficulties.

For clinical formulations, a solubility of at least about 1 mg/ml is desirable. Phase I results found GI toxicity to be dose limiting for 17-AGG, with a suggested Phase II dosing of 40 mg/m². Preclinical trials found severe hepatotoxicity to be dose limiting for the parent compound, geldanamycin (4 mg/kg).

By targeting multiple oncogenic proteins, geldanamycin promises efficacy against a broad range of tumors and increases the chances of overcoming drug resistance. In addition, the inhibition of Hsp90 leads to an up-regulation of Hsp70, which reduces the formation of abnormal tau species, the primary component of plaque deposits in Alzheimer’s and Parkinson’s disease.

Because of the extremely low water solubility of geldanamycin, ~1.5 µg/ml, formulations have used various soluble analogs such as 17-AAG. As with rapamycin, the targeted delivery of geldanamycin to tumor sites and the EPR effect are expected to substantially increase its potency. In addition, prolonged circulation time and reduced liver retention should dramatically reduce hepatotoxicity. Finally, the possible advancement of geldanamycin as a treatment in neurological diseases will require the highly hydrophobic nature of the parent compound, which is attenuated in soluble analogues, in order to cross the blood-brain barrier.

2.3 Paclitaxel

Paclitaxel is another hydrophobic compound with applications including the treatment of cancer. Paclitaxel belongs to a group of medicines called antineoplastics, which inhibit...
cellular growth. The inhibition is accomplished by disrupting microtubule function by binding to the beta subunit of tubulin. The disrupted microtubule looses the ability to disassemble, a necessary function, for example, in chromosomal migration during cell replication. Additionally, research has indicated that paclitaxel induces apoptosis, programmed cell death, by binding to an apoptosis stopping protein called Bcl-2 and stopping its function.

3.0 Excipients

Multi-component excipients may be used in drug formulations, where a poorly water soluble component solubilizes the drug compound in addition with a second excipient or co-solvent. The solubilization capacity and stability of polymeric micelles may be enhanced by the inclusion of a co-excipient highly compatible with both the hydrophobic micelle core formed by the micelle unimers and the loaded drug.

Multi-component excipients may be used in drug formulations, where a poorly water soluble component solubilizes the drug compound in addition with a second excipient or co-solvent, e.g., risperidone oral formulation containing benzoic acid, tartaric acid, and sodium hydroxide. The solubilization capacity and stability of polymeric micelle compositions may be enhanced by the inclusion of a co-excipient highly compatible with both the hydrophobic micelle core formed by the micelle unimers and the loaded drug.

Excipients may have a high Po/w, preferably greater than about 3.5, and a low molecular weight, preferably less than 1000 Da. Excipients may improve biocompatibility and may improve drug-carrier compatibility or increase the drug loading and release time from the carrier.

3.1 Tocopherol

The ring and alkyl chain structure of α-tocopherol (FIG. 7), the most common isomer tocopherol, is a feature common to many poorly-soluble drugs, hence tocopherol’s long history as an excipient for many difficult to formulate drugs. Tocopherol may also be a modifying agent to micelle structures. Drug loading capacities of PEG-DSPE and PEG-PCL micelles are significantly enhanced by the addition of tocopherol. See Example 2.

The inclusion of tocopherol may also enhance the stability of micelles. For example, PEG-DSPE micelles can be formed with up to about 4 mg/ml of rapamycin, however, the micelles quickly “crush” causing the drug to come out of solution (typically <2 hours). The same micelles with the incorporation of tocopherol are stable for at least several days. See Example 3 and 6. The critical micelle concentration increases with the incorporation of tocopherol into the micelle compositions, thereby increasing the kinetic stability of the micelle composition. See FIG. 13.

The phytol chain of tocopherol interpolates between phospholipid acyl chains. When a phase has a tocopherol:phospholipid ratio greater than 0.2:1 then the phase is a tocopherol-rich phase. FIG. 8 shows the tocopherol incorporation between PEG-DSPE chains. Tocopherol incorporation results in the formation of separate tocopherol phase. The mobility of mixed acyl and phytol chains are decreased after tocopherol incorporation. There is a kinetic contribution of polymers to micelle composition stability. The micelle unimer exchange rate is slow with a highly viscous, or rigid, core. A reduced core viscosity, or rigidity may increase diffusion rate of the passenger drug. FIG. 13 shows the core rigidity data. As the tocopherol to PEG-DSPE ratio increases, the core rigidity generally decreases. An increase in the hydrophobic core size, influenced by the addition of tocopherol, may modulate the drug diffusion rate. The increased core size causes the drug to travel a further distance, but the less viscous core allows the drug to travel faster. If there is not optimized interaction between the tocopherol and the drug, then diffusion may be slowed. Tocopherol and drug incorporation into a micelle composition may affect the size of the micelle and thus affect extravasation at the tumor site. See Example 9 and FIG. 14. As shown in FIG. 15, PEG-DSPE micelles are stable in phosphate buffered saline solution, but are unstable in 4% bovine serum albumin which approximates in vivo conditions. FIG. 16 shows PEG-PCL is stable in a 4% albumin serum. As shown in FIG. 17, PEG-DSPE micelle compositions with incorporated tocopherol (at about 2:1 ratio of tocopherol:PEG-DSPE) stay about 60% solubilized in 4% bovine serum albumin for about 25 hours. See Example 6.

As seen in Example 3, the critical micelle concentration (CMC) increases with the incorporation of tocopherol into the micelle composition. Micelles composed of 10 mM PEG-DSPE and 1000 Da tocopherol ratio and the effect on the CMC are described in Example 3.

As shown in FIG. 18, the core polarity of a micelle composition with incorporated tocopherol also changes with the proportion of tocopherol. The core polarity decreases with the greater incorporation of tocopherol.

Rapamycin and tocopherol are both very hydrophobic and have similar structural components. Both have ring structures and long alkyl chains. Both may increase stability of drug incorporation within micelle compositions.

As shown in FIG. 19, rapamycin loading efficiency increases with the incorporation of tocopherol at all rapamycin to PEG-DSPE ratios. The most effective tocopherol to PEG-DSPE ratio is about 2 and about 4, both ratios leading to a loading efficiency around 25%.

4.0 Result of Micelle and Drug Incorporation

Tocopherol may have effects on the structure and properties of PEG-DSPE and PEG-PCL micelles. Briefly, PEG-DSPEmicelles were prepared according to the solvent film method of Lukyanov et al. (as summarized in FIG. 20), wherein, phospholipids, additives, and drug were dissolved in an organic solvent, evaporated to produce a dry film, and micelles were formed by the addition of water. Micelles were then filtered and/or centrifuged to remove unincorporated drug aggregates and drug incorporation verified by Size Exclusion Chromatography (SEC). PEG-DSPEmicelles used in this process may have a concentration between about 1 mM and about 20 mM, preferably between about 1.5 mM and about 10 mM, and most preferably about 5 mM. Tocopherol used in this process may have a concentration between about 1 mM and about 20 mM, preferably between about 2 mM and about 15 mM, and more preferably about 10 mM. The phospholipids, additives, and drug dissolved in an organic solvent may be spun at about 50 rpm and about 200 rpm, preferably about 50 rpm and about 150 rpm, and most preferably about 100 rpm. Solvent may be removed by vacuum at about 1 and about 500 µbar, preferably between about 5 and about 200 µbar, and most preferably between about 10 and about 100 µbar.

As described in FIG. 21, PEG-PCL micelles were also prepared by the drip-wise addition of drug and PEG-PCL dissolved in a miscible solvent, acetone, to vigorously stirred water, followed by removal of the solvent by N₂ purge, and 0.2-µm filtration and/or centrifugation. The final solvent to water ratio is between about 0.1 and about 5, preferably between about 0.5 and about 4, and more preferably about 2. The micelle solution should be delivered at a rate of between about 2 s/drop and about 60 s/drop, preferably between about
5 s/drop and about 30 s/drop, and more preferably between about 10 s/drop and about 20 s/drop.

As shown in FIG. 22, rapamycin loading by the solvent film method had a loading efficiency of between about 30% and about 50%, preferably between about 32% and about 47% and more preferably about 40% at a rapamycin to PEG-DSPE ratio of about 2:1. The weight % of rapamycin at the ratio of 2:1 is between about 10% and about 40%, preferably between about 15% and about 30%, and more preferably about 20%.

Rapamycin, as shown in FIG. 23, stays solubilized for a longer period of time when loaded into a micelle composition compared to a free drug under in vivo conditions. As shown in FIG. 24, PEG-DSPE is unstable in the presence of human serum albumin.

4.1 Micelle Composition Properties with the Incorporation of Tocopherol

Tocopherol alters the core structure of PEG-DSPE as expected based on studies with unpegylated DSPE micelles. As shown in Example 3, the addition of up to a 2:1 molar ratio of tocopherol to PEG-DSPE increases the critical micelle concentration (CMC) from 2.1 µM to 28 µM, but this CMC range is still indicative of a very stable micelle. Likewise, PEG-PCL micelles retained very low CMC’s at 10 and 20:1 ratios of tocopherols to PEG-PCL unimers. As shown in FIG. 18, tocopherol incorporation decreases core polarity and may increase the loading of lipophilic molecules.

The addition of tocopherol did not increase the size of micelles formed with PEG-DSPE. This may be due to the incorporation of tocopherol into the alkyl chains and minimal swelling of the hydrophobic core (Example 6). However, the PEG-PCL micelles increased in size with the addition of tocopherol. As shown in FIG. 25, tocopherol incorporation does not affect the size of the micelle composition significantly. As shown in FIG. 14, the increasing aggregate number of incorporation also reflects an increasing size of the core. At a tocopherol to lipid ratio of 0.5, the change in aggregate number became statistically significant. This may in part be due to the greater loading of tocopherol into the PEG-PCL micelles.

4.2 Micelle Properties with Incorporation of Tocopherol and Passenger Drugs

Rapamycin or geldanamycin may be loaded into PEG-DPSE and PEG-PCL micelles with varying amounts of tocopherol. See Example 1. As shown in FIG. 26, rapamycin may be loaded into PEG-DSPSE micelles. The loading of rapamycin may be increased by between about 2 and about 7 fold, preferably between about 4 and about 6 fold, and more preferably over 3-fold by the addition of tocopherol to PEG-DSPSE and PEG-PCL micelles. In addition, in the absence of tocopherol, precipitation may be observed after 1–4 hours; this indicated that tocopherol may increase the stability of drug loaded PEG-DSPSE micelles. See Example 10. Tocopherol increased the loading of geldanamycin into PEG-DSPSE micelles by between about 1 and about 4 fold, preferably between about 1 and about 3 fold, and more preferably about 2 fold and the loading into PEG-PCL micelles by between about 7 and about 15 fold, preferably between about 8 and about 12 fold, and more preferably about 10 fold.

The human body is like a perfect sink. As shown in FIG. 27, Crank’s solution for Fickian diffusion informs the diffusion of the drug from the micelle composition. The benefits of tocopherol were most dramatic in the case of geldanamycin and PEG-PCL. Without the addition of tocopherol, PEG-PCL may be ineffective as a solubilization agent. The maximal loading concentration of between about 0.2 and about 0.8 mg/ml, preferably between about 0.4 and about 0.6 mg/ml, and more preferably 0.5 mg/ml may be achieved with the 1:20 PEG-PCL:tocopherol. See Example 11 and 12. Further optimization of the carrier and additives may be required. Also, the EPR effect of micelle composition formulations may reduce the dosage requirements for chemotherapy.

As shown in FIG. 28, tocopherol increases the time over which rapamycin is released in a phosphate buffered solution, but not significantly so. In FIG. 29, tocopherol is shown as having a significant effect on the increased time over which rapamycin is released in a 4% bovine albumin solution.

PEG-PCL micelle compositions are capable of loading more rapamycin when incorporated with tocopherol. See FIG. 30. Furthermore, as shown in FIGS. 31 and 32, PEG-PCL keeps rapamycin solubilized longer in both phosphate buffered saline solution and 4% bovine serum solution.

Early results demonstrate the potential these polymers have as carriers for chemotherapeutic compounds. Results with tocopherol demonstrate that structurally similar additives can substantially increase drug loading capacity.

4.3 Dosage for Micelle Administration

The dose of rapamycin through micelle a micelle delivery system can be similar to doses used in clinical trials for rapamycin analogues: CCI-779, RAD-001, and AP-23573. The doses for CCI-779 is about 7.5 to 220 mg/m²/week i.v., about 0.75 to 20 mg/m²/day i.v. for about 5 days every 2 to 3 weeks, about 25 to 100 mg/day p.o. for about 5 days every 2 weeks. For RAD-001, about 5 to 60 mg/week p.o. For AP-23573, about 6.0 to 100 mg/week i.v., about 3 to 30 mg/day i.v. for about 5 days every 2 weeks. These doses should be easily attained by PEG-b-PCL micelles, given solubilization of rapamycin at about 1 to 4 mg/ml. The content of rapamycin in PEG-b-PCL micelles is about 10 to 20% wt drug/wt polymer. PEG-b-PCL micelles can reach at least about 40 mg/ml.

The dose of geldanamycin prodrugs can be about 100 to 1000 mg/m² at about 1 to 7 mg/ml, preferably about 200 to 700 at about 2 to 6 mg/ml, even more preferably at about 100 ml at about 4.0 mg/ml.

4.4 Geldanamycin Prodrugs Loading into Micelles

As shown in FIG. 42, geldanamycin loads poorly into PEG-b-PCL micelles and into PEG-DSPSE micelles due to not being lipophilic enough. As shown in FIGS. 43 and 44, fatty acid (ester) prodrugs of geldanamycin may increase lipophilicity. As shown in FIG. 14, increasing the log Po/w increases the loading percentage by weight of a geldanamycin prodrug. See Example 18.

In the design of a nanocarrier, a major concern must be drug-carrier interaction. Initial studies found that geldanamycin may not be sufficiently encapsulated by nanocarriers such as PEGylated phospholipids and PEG-b-polycaprolactone (PEG-PCL) micelles. Encapsulation of Hsp90 inhibitors may be dependent on hydrophobicity of the drug molecule. The octanol-water partition coefficient of geldanamycin was determined by microemulsion electrokinetic chromatography. As a comparison, rapamycin, which was loaded to high levels (>10% w/w) in PEG-PCL micelles, has a log Po/w of 3.77, as determined by MEEKC.

Several prodrugs were synthesized by DMAP/DCC chemistry, as shown in FIG. 44. As shown in FIGS. 45 and 46, extending the fatty acid chain length increases the hydrophobicity of the resulting molecule, resulting in a higher value log Po/w. The addition of a bromine adjacent to the carbonyl of the ester acts as an electron withdrawing group, destabilizing the ester bond. However, bromine (Br) is extremely hydrophobic and increases the molecule’s overall log Po/w coefficient. The addition of the Br may also increase loading into...
the nanocarrier, but may reduce the accessibility of hydro­
ion and hydroxide ions to the ester bond, decreasing the
hydrolysis rate of the encapsulated esters. In turn, slow
hydrolysis may prolong the drug release rate if the prodrug
partitions into the micelle core significantly better than the
parent drug. A highly partitioned drug, with a stable ester
bond, may be realized if the Br is replaced with a hydrophobic
group which is not electron withdrawing, such as an isopropyl
group, shown in FIG. 47.

As shown in Table 1, geldanamycin prodrugs are highly
hydrophobic, as evidenced by the high log P<sub>ow</sub> values.
Unmodified geldanamycin has a log P<sub>ow</sub> value of about
2.77, which is not hydrophobic enough to be encapsulated
by PEG-b-PCL. Effective encapsulation by PEG-b-PCL may
occur when the carrier has a hydrophobicity of about 3.5
or higher. The compound 17-aminoethyl-hexonate-17-
dermethylgeldanamycin has a log P<sub>ow</sub> of about 3.87,
which is enough to allow the molecule to be substantially encapsu-
lated into a micelle, such as PEG-b-PCL. The compound
17-aminoethyl-bromohexonate-17-demethoxygeldanamycin
is a very hydrophobic molecule with a log P<sub>ow</sub> at about
4.49 and should encapsulate into a micelle, such as PEG-b-
PCL.

FIG. 45 shows the process for formulating 17-aminoethyl-
hexonate-17-demethoxygeldanamycin, 17-aminooethyl-
dodeconate-17-demethoxygeldanamycin, 17-aminooethyl-
bromopalmitate-17-demethoxygeldanamycin, as shown in Table 1. In formulating 17-aminoethyl-hexonate-17-
dermethylgeldanamycin, n=9 and X=H. In formu-
lating 17-aminoethyl-bromohexonate-17-demethoxygeldana-
mycin, n=13 and X=H. In formulating 17-aminoethyl-bromo-
hexonate-17-demethoxygeldanamycin, n=13 and X=Br.

FIG. 45 shows an extension of a fatty acid chain. In the first
step, the addition of ethanol amine to geldanamycin (shown
as 1 in FIG. 45) may be accomplished by dissolving geldana-
mycin in chloroform with about 10 equivalents of ethanol
amine for between about 2 and about 6 hours until complete. The
reaction is monitored by thin layer chromatography (TLC) until
complete. The organic layer is washed with sodium bicarbonate
(NaHCO<sub>3</sub>) and then brine. The organic
layer is then dried over sodium sulfate (NaSO<sub>4</sub>) and
then the solvent is removed by rotary evaporation.

In the second step of FIG. 45, a fatty acid chain is added
to the geldanamycin prodrg structure shown as 2, by a DMAP/
DCC reaction. A fatty acid is added with a hydrophobic entity
(such as Br or H) adjacent to the carbonyl of the ester. In the
second step, the geldanamycin prodrg from 2 is suspended
in about 10 ml of dichloromethane having about 1.5 equiv­
ulents of the fatty acid, about 3 equivalents of DCC and about
1 equivalent of DMAP. The reaction is monitored by TLC for
between about 2 and about 6 hours until completion. The
solution is chilled and filtered. The solution is then purified
by flash chromatography on silica loaded with about 1:9 methan-
ol/chloroform. The solution is then rotovapped to obtain
the product.

FIG. 46 shows the process for formulating 17-amino-hexy-
deceyl-17-demethoxygeldanamycin. FIG. 46 shows a differ-
cent first step from FIG. 45, but the same second step. In the
first step, the addition of NH<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub> amine to geldana-
mycin (shown as 1 in FIG. 45) may be accomplished by
dissolving geldanamycin in chloroform with about 5 equiv­
lents of NH<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub> for between about 1 and about
4 hours. The reaction is monitored by thin layer chromatogra-
phy (TLC) until complete. The organic layer is washed with
sodium bicarbonate (NaHCO<sub>3</sub>) and then brine. The organic
layer is then dried over sodium sulfate (NaSO<sub>4</sub>) and
then the solvent is removed by rotary evaporation.

FIG. 47 shows the process for formulating 17-hydroxy-
ethylnitro- 1 (1-isopropyl-palmitate)-17-demethoxygeldana-
mycin. This is made by suspending diethyl malonate in about
1 equivalent of NaOCH<sub>2</sub>CH<sub>3</sub> in ethanol and refluxing
for about 1 hour. Then about 0.95 equivalents of 2-bromo-isop-
propane is added dropwise and refluxed for about 4 hours.
Twice the volume of cold water is added to the solution. The
product is extracted three times by ether and then vacuum
distilled. The isopropylmalonate diester is mixed with about
1 equivalent of NaOCH<sub>2</sub>CH<sub>3</sub> in ethanol and refluxed for
about 1 hour. Then about 0.95 equivalents of 1-bromotetrad-
cane is added and the solution is refluxed for about 4 hours
or until complete by TLC. Above twice the volume of cold
water may be added to the solution. The product may be
extracted three times by ether and then vacuum distilled.

Then 2-isopropyl-2-tetradecane-malonatediester may be
dissolved in about 1:1 KOH:water and refluxed for about
8 hours. Then water is added until the solids are gone. The
aqueous layer is extracted. Concentrated hydrochloric acid is
added until there are no more solids. The solution is extracted
with ether three times, and reduced in a vacuum. The product
is then heated to about 180 degrees C. for about 3 hours
and then vacuum distilled. This results in the fatty acid with
isopropyl shown as 3 in FIG. 2. Then the geldanamycin pro-
drug in 2 in FIG. 10 is mixed with 3 in FIG. 2. The geldana-
mycin prodrg is mixed with about 1.5 equivalents of the fatty
acid containing isopropyl with about 3 equivalents of DCC
and about 1 equivalent of DMAP in about 10 ml of dichlo-
romethane for about 2 and about 6 hours. The solu-
tion is chilled and filtered. The solution is then purified
by flash chromatography on silica loaded with about 1:9 methan-
ol/chloroform. The solution is then rotovapped to obtain
geldanamycin-C<sub>17</sub>-aminoethyl-2-isopropyldodecanate.

FIG. 48 shows the process for formulating geldanamycin-
C<sub>17</sub>-aminoethylmalonate-Phe-Leu-Phe-amine. The hydropho-
bic peptide is added to the geldanamycin prodrg shown as 2
in FIG. 45. Three equivalents of DCC and 1 equivalent of
DMAP are added along with about 10 ml of dichloromethane.
The reaction time may be between about 2 and about 6 hours.
The solution is chilled and filtered. The solution is then puri-
fied by flash chromatography on silica loaded with about 1:9 methan-
ol/chloroform and then rotovapped. The resulting
product is mixed with about 2:8 piperidine/DMF and then reacted
for about between about 1 and about 2 hours. The solution is then
purified by flash chromatography on silica loaded with about 1:9 methan-
ol/chloroform. The solution is then rotovapped to obtain
-geldanamycin-C<sub>17</sub>-aminoethylmalonate-Phe-Leu-Phe-
amine.
FIG. 49 shows the process for formulating geldanamycin-C17-aminooxyethylidene-palmitohydrazide. Fmoc-ethanolamine may be converted to the aldehyde using about 1 equivalent of Dess-Martin in DCM. After about 20 minutes, the reaction may be diluted with about 1 volume of saturated sodium bicarbonate and about 7 equivalents of saturated sodium thiosulfate. The reaction may be stirred for about 20 minutes and extracted about 3 times with substantially equal volumes of diethyl ether. The organic then may be washed with about 1M HCl and H2O, dried over sodium sulfate, and the solvent removed by rotary evaporation. The product was purified by flash chromatography on silica and eluted with about 99:1 EtOAc:TEA. The Fmoc-ethylaldehyde may be mixed with about 1 equivalent of palmitic acid hydrazide and refluxed overnight in EtOH.

The Fmoc-hydrazone product may be purified by flash chromatography on silica and eluted with about 89:10:1 chloroform:MeOH:TEA. The Fmoc-hydrazone may be deprotected in about 2.2:98 DBU:piperidine:DMF overnight at room temperature. The product ([E]-N(2-aminooxyethylidene) palmitohydrazide may be filtered and purified by flash chromatography with about 89:10:1 chloroform:MeOH:TEA. The hydrazide was then conjugated to geldanamycin in DMF by nucleophilic attack at the C17-methoxy. The product, 17-(2-aminooxyethylidene)palmitohydrazide-17-geldanamycin, was purified by flash chromatography on silica eluted with 1:9 MeOH:chloroform.

FIG. 50 shows the process for formulating PEO-b-PEGA. PEO-b-PBLA is aminolysed with HOOC(CH2)5NH2 in DMF and 2-hydroxypropyridines, thus incorporating a hydroxyl moiety. The product is then conjugated to 17-hydroxyethylamino-17-geldanamycin using DCC/DMAP chemistry in DCM. The product may be purified by cold filtering and ether precipitation.

Increasing the hydrophobicity of geldanamycin may increase the nanocapsulation of the compound. Prodrugs of geldanamycin at the 17 carbon have been shown to have less impact on bioactivity of geldanamycin than other positions; however, derivatization often leads to a decrease in activity, especially large groups (Sasaki et al, U.S. Pat. No. 4,261,989 (1981)).

Sasaki showed that the β-hydroxyethylamino-17-demethoxygeldanamycin prodrug had minimal impact on bioactivity in vitro. This prodrug provides a hydroxyl group allowing esterification. Ester prodrugs may hydrolyze into the active form of the parent compound.

Modifications to geldanamycin are not limited to those listed above. Instead of fatty acids, hydrophobic peptide sequences could be used, and, for example, attached via the terminal C-group using an ester bond. For example, a sequence of phenylalanines and leucines may be used. The sequence may alternate between amino acids to prevent the formation of extensive secondary structures. A representative prodrug, C17-aminooxyester-Phe-Leu-Phe is shown in FIG. 48. Amino acids may be assembled using standard solid phase peptide chemistry, e.g. Fmoc protected amino acids, with HATU/HOBt activated coupling. The resulting N-protected peptide may be conjugated using by DMAP/DCC chemistry as in FIG. 47. After conjugation, the terminal amino acid Fmoc protecting group may be removed.

Other groups besides esters may be used for attachment of hydrophobic groups, for example hydrazine linkers may be used that have the advantage of stability at neutral pH and enhanced hydrolysis at acidic conditions. Tumors may present an acidic environment that may enhance release of the drug, while the drug may be stable in the nanocarrier JM plasma, reducing non-specific release and resulting toxicity. An example of one linker is shown in FIG. 44.

The Hsp90 drug may also be linked using other bonds such as acetyl and disulfide bonds, elavable peptide bonds (e.g. Ala-Val), or a combination of these linkers. For example, a tumor selectively-cleaved linker (e.g. Ala-Val peptide) may be attached via the C-terminus to a fatty acid or hydrophobic peptide. The N-terminus may be linked directly to the Hsp90 inhibitor (e.g. via the C17 carbon of geldanamycin) or via a spacer linker such as an aminooxyethanol or aminohexanol. The N-terminus may also be linked via another elavable linker. The resulting compound may show reduced non-specific toxicity after nanocarrier release due to the bulky Ala-Val-(drug linker) groups reducing drug affinity to Hsp90. After tumor specific cleavage of the Ala-Val, the resulting compound may show sufficient Hsp90 binding for inhibition.

The Hsp90 inhibitor may also be linked to the nanocarrier. If linked reversibly, the drug may release from the nanocarrier and become bioactive. If linked irreversibly or reversibly, the presence of the bound drug may increase the partitioning of free drug into the micelle. An example is shown in FIG. 45 using PEO-b-PEGA as the carrier.

These modified Hsp90 inhibitors may show sustained release from the carrier. The release kinetics of several of these carriers are shown in Table 2. Drugs were loaded into 0.5 mM PEG-b-PCL (5000:10000 Da) micelles to achieve a 25% wt loading (or 1.9 mg/ml solution). These data were obtained by measuring release from 10000MWCO dialysis cassettes into pH 7.4 phosphate buffer under perfect sink conditions at 37° C. Drug diffusion was calculated as described in Forrest and Kwon, 2005 (Journal of Controlled Release).

PEG-PCL micelles are prepared by the drop-wise addition of geldanamycin produg and PEG-PCL dissolved in a miscible solvent, acetone, to vigorously stirred water, followed by removal of the solvent by N2 purge, and 0.2-μm filtration. Alternatively, the solution may be centrifuged to remove unincorporated and aggregated drug. The final solvent to water ratio is between about 0.1 and about 5, preferably between about 0.5 and about 4, and more preferably about 2. The micelle solution should be delivered at a rate of between about 2 s/drop and about 60 s/drop, preferably between about 5 s/drop and about 30 s/drop, and more preferably between about 10 s/drop and about 20 s/drop.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Difff Coef, cm²/s</th>
<th>Calc'd release (%)</th>
<th>w/w drug/carr.</th>
<th>Conc, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-aminooxyethyl-bromohexonate-17-demethoxygeldanamycin</td>
<td>2.14 × 10⁻²⁰</td>
<td>6.7 days</td>
<td>2.8 ± 0.0%</td>
<td>0.21</td>
</tr>
<tr>
<td>17-aminooxyethyl-dodeconate-17-demethoxygeldanamycin</td>
<td>2.55 × 10⁻²⁰</td>
<td>5.6</td>
<td>21 ± 2%</td>
<td>1.6</td>
</tr>
</tbody>
</table>

TABLE 2

Geldanamycin produg characteristics
**Geldanamycin prodrug characteristics**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Diff Coef, cm²/s</th>
<th>Calc’d release t½</th>
<th>w/w drug/carrier</th>
<th>Conc, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-aminoethyl-bromododecanoate-17-demethoxygeldanamycin</td>
<td>1.65 x 10⁻⁷⁰</td>
<td>8.7</td>
<td>21 ± 2%</td>
<td>1.6</td>
</tr>
<tr>
<td>17-aminoethyl-palmitate-17-demethoxygeldanamycin</td>
<td>3.61 x 10⁻⁷⁰</td>
<td>4.0</td>
<td>22 ± 5%</td>
<td>1.7</td>
</tr>
<tr>
<td>17-aminoethyl-bromopalmitate-17-demethoxygeldanamycin</td>
<td>1.51 x 10⁻⁷⁰</td>
<td>9.5</td>
<td>25 ± 2%</td>
<td>1.9</td>
</tr>
<tr>
<td>17-amino-hexyl-decyl-17-demethoxygeldanamycin</td>
<td>1.69 x 10⁻⁷⁰</td>
<td>8.5</td>
<td>20 ± 2%</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geldanamycin</td>
<td>5</td>
</tr>
<tr>
<td>17-hydroxyethylaminoo-17-demethoxygeldanamycin</td>
<td>73</td>
</tr>
<tr>
<td>17-aminoethyl-hexanate-17-demethoxygeldanamycin</td>
<td>240</td>
</tr>
<tr>
<td>17-aminoethyl-palmitate-17-demethoxygeldanamycin</td>
<td>350</td>
</tr>
<tr>
<td>17-aminoethyl-bromopalmitate-17-demethoxygeldanamycin</td>
<td>120</td>
</tr>
</tbody>
</table>

**4.5 Paclitaxel Prodrugs Loading into Micelles**

A Cremophor® and solvent free formulation of paclitaxel was prepared using amphiphilic block co-polymer micelles of poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-PCL). The poor loading of paclitaxel in micelles of PEG-PCL (<1% w/w) was overcome by forming hydrolysis-fatable fatty acid produgs of paclitaxel. Paclitaxel produgs had solubilities in excess of 5 mg/mL in PEG-PCL micelles. Drug loaded PEG-PCL micelles were prepared by a co-solvent extraction technique. Resulting PEG-PCL micelles contained 17-22% w/w produg and were less than 50 nm in diameter. PEG-PCL micelles released paclitaxel produgs over several days, t₁/₂ > 3 d.

**5.0 Different Aspects of the Invention**

In summary, a micelle composition may comprise an amphiphilic polymer, a hydrophobic excipient, and a hydrophobic passenger drug. The amphiphilic polymer may be a peglated phospholipid, such as PEG-DSPE, or a block copolymer, such as PEG-b-PCL and PEG-b-amino acids. The hydrophobic excipient may have a log P-o/w greater than about 3.5 and a molecular weight less than about 1000 Da. The hydrophobic excipient may be Vitamin E, which has many isomers, including: alpha-tocopherol, beta-tocopherol, gamma-tocopherol, delta-tocopherol, alpha-tocotrienol, beta-tocotrienol, gamma-tocotrienol, delta-tocotrienol. The hydrophobic passenger drug may be geldanamycin, geldanamycin prodrug, rapamycin, paclitaxel, or a paclitaxel prodrug.

A micelle composition may be an amphiphilic polymer and a hydrophobic passenger drug may be utilized for a micelle. The hydrophobic passenger drug may be geldanamycin, geldanamycin prodrug, rapamycin, paclitaxel, or a paclitaxel prodrug. The amphiphilic polymer may be PEG-DSPE, PEG-PCL, or PEG-polyamino acid. A hydrophobic excipient may be included, preferably, Vitamin E. A micelle composition may have a concentration of between about 1 and about 50 mM, Vitamin E may have a concentration of between about 2 and about 100 mM, and a rapamycin concentration of between about 0.1 and about 10.0 mg/mL. A micelle composition may also have the amphiphilic polymer concentration of between about 3 and about 7 mM, the Vitamin E concentration and removing substantially all of the solvent from the solution to leave a substantially solvent-free mixture. The process may further include resuspending the substantially solvent-free mixture in water or buffer. The process may also include adding the solution to a substantially water solution before removing substantially all of the solvent from the solution to leave a substantially solvent-free mixture. The process may further include removing the drug that has not incorporated into said micelle compositions. The process may be having the mixing step be spinning the solution at between about 50 and about 1000 rpm.

As characteristics of the final aqueous solution, the amphiphilic polymers may have a concentration of between about 0.1 mM and about 60 mM, and the hydrophobic excipients may have a concentration of between about 0.1 mM and about 600 mM, and the drugs may have a concentration of between about 0.1 mg/ml and about 100 mg/ml. Almost any
organic solvent may work in the process that all the components are soluble, for example, but not exclusively, MeOH, acetone, THF, ACN. The solvent may be about a 50:50 chloroform:methane solution. Additionally, the spinning step and the removing step of the process may occur simultaneously and the resuspending step may be combined with ultrasonification for between about 3 and about 20 minutes. The hydrophobic passenger drug may be rapamycin, paclitaxel, paclitaxel prodrugs, geldanamycin, and geldanamycin prodrugs.

A process for solubilizing rapamycin may comprise: dissolving amphiphilic polymer, a hydrophobic excipient, and rapamycin into an organic solvent to form a solution; mixing said solution; removing solvent from said solution to form a substantially solvent-free composition; and resuspending said substantially solvent-free mixture in water or buffer. The resuspending step may form micelle compositions. The polymers may be PEG-DSPE. A ratio of hydrophobic excipient to PEG-DSPE may be between about 0.1 and about 3. The hydrophobic excipient may be Vitamin E.

A micelle composition may comprise amphiphilic polymers and geldanamycin. The micelle composition may also include a hydrophobic excipient. The hydrophobic excipient may be Vitamin E. The geldanamycin may be between about 200 and about 800 µg/ml.

A prodrug composition may have a log P o/w of at least about 3.5. The prodrug may be of geldanamycin or paclitaxel. A geldanamycin prodrug may have an amino linker group and a hydrophobic passenger drug. The hydrophobic passenger drug may be geldanamycin, geldanamycin prodrugs, rapamycin, paclitaxel, or paclitaxel prodrugs. A process for forming micelle compositions may include a paclitaxel prodrug having a log P o/w of at least about 3.5.

A paclitaxel prodrug may have an amino linker group and an R group adjacent said linker group. The amino linker group may be at the C7 or C2 position. The paclitaxel prodrug may have a log P o/w of at least about 3.5. The R group may be a carbon chain between about 4 and about 24 carbons, more preferably between about 6 and about 16 carbons. The chain may be saturated or partially unsaturated. The R group may be an ester, bromoester, aminoethyl-hexonate, aminoethyl-dodeconate, aminoethyl-palmitate, aminoethyl-bromopalmitate, or amino-hexadecyl. A micelle composition may comprise an amphiphilic polymer and one of these geldanamycin prodrugs. The geldanamycin prodrug may have a log P o/w of at least about 3.5.

A paclitaxel prodrug may have an amino linker group and an R group adjacent said linker group. The amino linker group may be at the C7 or C2 position. The paclitaxel prodrug may have a log P o/w of at least about 3.5. The R group may be a carbon chain between about 4 and about 24 carbons, more preferably between about 6 and about 16 carbons. The chain may be saturated or partially unsaturated. The R group may be an ester, bromoester, aminoethyl-hexonate, aminoethyl-dodeconate, aminoethyl-palmitate, aminoethyl-bromopalmitate, or amino-hexadecyl. A micelle composition may comprise an amphiphilic polymer and one of these geldanamycin prodrugs. The geldanamycin prodrug may have a log P o/w of at least about 3.5.


A process for forming micelle compositions with a paclitaxel prodrug may comprise or produce: 7-palmitate-paclitaxel, 7-palmitate-paclitaxel, 2-TBS-paclitaxel, 2-palmitate-paclitaxel, 2-TBS-7-palmitate-paclitaxel. A process for forming micelle compositions may comprise: formulating a paclitaxel prodrug having a log P o/w of at least about 3.5; mixing amphiphilic polymer and said paclitaxel prodrug into an organic solvent to form a solution; removing solvent from said solution to leave a substantially solvent-free mixture; and resuspending said solvent-free mixture in water or buffer.

A method of treatment for a disease or a condition in a human or an animal comprising administering a micelle composition comprising an amphiphilic polymer, a hydrophobic excipient and a hydrophobic passenger drug. The hydrophobic passenger drug may be geldanamycin, geldanamycin prodrugs, rapamycin, paclitaxel, or paclitaxel prodrugs. The amphiphilic polymer may be PEG-DSPE, PEG-PCL, or PEG-polyamino. The hydrophobic excipient may be Vitamin E. Human or animal diseases or conditions may: cancer, neurological disorder, Alzheimer's disease, Huntington's disease, restenosis, fungal infection, immunosuppression. The fungal infection may be Candida albicans.

Although the invention has been described with reference to preferred embodiments and examples thereof, the scope of the present invention is not limited only to those described embodiments. As will be apparent to persons skilled in the art, modifications and adaptions to the above-described invention can be made without departing from the spirit and scope of the invention, which is defined and circumscribed by the appended claims. The following examples are provided for the intent of illustrating embodiments and advantages of the invention and are not intended to limit its scope.

EXAMPLE 1

Formation of Micelles and Passenger Drugs

Docorubicin and paclitaxel can be incorporated into micelle compositions to be delivered to targeted tumors. PEG-poly(aspartic acid), PEG-poly(aspartate), PEG-poly(lactide), PEG-DSPE are a few of the micelle carriers that can encapsulate passenger drug compounds. See Table 1.

<table>
<thead>
<tr>
<th>Passenger Drugs</th>
<th>Target</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-poly(aspartic acid) conjugated doxorubicin</td>
<td>Metastatic pancreatic</td>
<td>Phase II</td>
</tr>
<tr>
<td>PEG-poly(aspartate) entrapping paclitaxel</td>
<td>Various solid tumors</td>
<td>Phase I</td>
</tr>
<tr>
<td>PEG-poly(lactide) entrapping paclitaxel</td>
<td>Various solid tumors</td>
<td>Phase I</td>
</tr>
<tr>
<td>Pluronic entrapping doxorubicin</td>
<td>Various solid tumors</td>
<td>Phase I/II</td>
</tr>
</tbody>
</table>

EXAMPLE 2

Rapamycin Loading Efficiency

Loading of rapamycin into micelle compositions, which has a solubility of 2.6 µg/ml in water. The loading efficiency of rapamycin into PEG-DSPE increases proportionally with the increase of incorporated tocopherol. The loading efficiency of rapamycin into PEG-PCL also increases proportionally with the increase of incorporated tocopherol. See Table 2.
rapamycin in 0.5 ml of acetone and load into a syringe. Use a water should be used. 

Incorporation of tocopherol into the micelle compositions, thereby easily formed systems (e.g. PEG-DSPE) the rate may be increased to 10 s/drop.

Examples include: MeOH, acetone, EtOH, acetonitrile, THF, dioxane, and IPA.

According to FIG. 19, amphiphilic polymers and the desired passenger drug are dissolved in a highly water miscible solvent for which they have excellent solubility. Examples include: MeOH, acetone, EtOH, acetonitrile, THF, dioxane, and IPA.

For example to make a 0.5 ml solution of drug at 1 mg/ml and 2.5 mM PEG-DSPE and 1:2 tocopherol:

Dissolve stated quantities of tocopherol, PEG-DSPE, and rapamycin in 0.5 ml of acetone and load into a syringe. Use a syringe pump to deliver the solution to solution of water at 55°C. The water (or other aqueous buffer [e.g. PBS]) is placed in 60 ml of a 50:50 chloroform:MeOH solution. Place flask on a rotary evaporator, or rotovap, and spin at about 100 rpm and place under vacuum to remove the solvent. It is important to control the vacuum so that the solvent does not “bump” or violently evaporate/boil and backflow into the rotovap condenser.

After delivery is done, the vial is placed under a stream of nitrogen or other dry non-reactive gas (e.g. purified dry air, argon, helium) and the solvent is evaporated. If necessary the solution can be concentrated by the continuing the evaporation past the point that the water is all gone. A benefit of using acetone verses azetrope forming solvents (e.g. EtOH) is that all of the solvent can be removed under these conditions. Also a solvent such as DMSO or DMF would not evaporate before the water. In addition, the vial can be allowed to sit overnight or longer (maybe without a purge gas) to allow the solvent to slowly evaporate. This may be important for long hydrophobic chain polymers such as the PEG-PCL that may swell in the presence of the acetone and would require slow removal of the acetone to allow micelle stability.

After all of the organic is removed (and if the desired the solution is further concentrated) the solution can be sterile filtered (e.g. through a 0.2 µm or 0.45 µm syringe filter) to remove an aggregates of unincorporated drug or other non-micelle, >200 nm sized particles. Alternatively, the solution can be centrifuged to get rid of aggregates of drugs. (e.g. 16000xg for 5 minutes).

Thin Film Evaporation Method of Forming Micelle Compositions.

Thin film evaporation method for forming micelle compositions example is as follows:

1. Dissolve the desired passenger drug, tocopherol, and amphiphilic polymer in a highly volatile organic solution in which they are soluble. See FIG. 18.

2. To make 1 ml of a final 5 mM of PEG-DSPE, 10 mM of tocopherol, 0.5 mg/ml rapamycin solution, dissolve the components in a 10 ml 50:50 chloroform:MeOH solution. Place in a 50-100 ml round bottom vacuum flask. Place flask on a rotary evaporator, or rotovap, and spin at about 100 rpm and place under vacuum to remove the solvent. It is important to control the vacuum so that the solvent does not “bump” or violently evaporate/boil and backflow into the rotovap condenser.

3. After all of the solvent is evaporated, place under a very high vacuum (10-100 µbar) to remove all trace solvent. This is especially important in the case of high tocopherol loading because tocopherol is an oily viscous substance and the solvent may be slow to evaporate from the tocopherol containing film.

4. Add the appropriate volume of water or buffer. In this case 1 ml. Agitate vigorously and the micelles will form. This can be assisted by ultrasonification for 5-15 minutes.

According to FIG. 18, the loading efficiency of the drug increased until the drug to amphiphilic unimer ratio reached 2:1. The loading efficiency was about 40% of the desired

<table>
<thead>
<tr>
<th>Drug</th>
<th>Carrier</th>
<th>Drug load, mg/ml</th>
<th>Drug weight %</th>
<th>Loading efficiency</th>
<th>Loading Improvement %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapamycin</td>
<td>5 mM tocopherol</td>
<td>&lt;0.01</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5 mM PEG-DSPE&lt;sub&gt;5000&lt;/sub&gt;</td>
<td>+ tocopherol (1:1)</td>
<td>1.5</td>
<td>10%</td>
<td>75%</td>
<td>7%</td>
</tr>
<tr>
<td>2.5 mM PEG-DSPE</td>
<td>+ tocopherol (1:2)</td>
<td>1.7</td>
<td>12%</td>
<td>80%</td>
<td>53%</td>
</tr>
<tr>
<td>0.05 mM PEG-DSPE&lt;sub&gt;2000&lt;/sub&gt;</td>
<td>+ tocopherol (1:3)</td>
<td>3.9</td>
<td>21%</td>
<td>79%</td>
<td>160%</td>
</tr>
<tr>
<td>PC&lt;sub&gt;5000&lt;/sub&gt;</td>
<td>+ tocopherol (1:10)</td>
<td>0.34</td>
<td>44%</td>
<td>74%</td>
<td>70%</td>
</tr>
<tr>
<td>+ tocopherol (1:20)</td>
<td>0.41</td>
<td>34%</td>
<td>90%</td>
<td>105%</td>
<td></td>
</tr>
<tr>
<td>1.7 mM PEG&lt;sub&gt;3&lt;/sub&gt;PC&lt;sub&gt;10k&lt;/sub&gt;</td>
<td>+ tocopherol (1:15)</td>
<td>4.9</td>
<td>14%</td>
<td>59%</td>
<td>—</td>
</tr>
</tbody>
</table>

After delivery is done, the vial is placed under a stream of nitrogen or other dry non-reactive gas (e.g. purified dry air, argon, helium) and the solvent is evaporated. If necessary the solution can be concentrated by the continuing the evaporation past the point that the water is all gone. A benefit of using acetone verses azetrope forming solvents (e.g. EtOH) is that all of the solvent can be removed under these conditions. Also a solvent such as DMSO or DMF would not evaporate before the water. In addition, the vial can be allowed to sit overnight or longer (maybe without a purge gas) to allow the solvent to slowly evaporate. This may be important for long hydrophobic chain polymers such as the PEG-PCL that may swell in the presence of the acetone and would require slow removal of the acetone to allow micelle stability.

After all of the organic is removed (and if the desired the solution is further concentrated) the solution can be sterile filtered (e.g. through a 0.2 µm or 0.45 µm syringe filter) to remove an aggregates of unincorporated drug or other non-micelle, >200 nm sized particles. Alternatively, the solution can be centrifuged to get rid of aggregates of drugs. (e.g. 16000xg for 5 minutes).

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3. After all of the solvent is evaporated, place under a very high vacuum (10-100 µbar) to remove all trace solvent. This is especially important in the case of high tocopherol loading because tocopherol is an oily viscous substance and the solvent may be slow to evaporate from the tocopherol containing film.

4. Add the appropriate volume of water or buffer. In this case 1 ml. Agitate vigorously and the micelles will form. This can be assisted by ultrasonification for 5-15 minutes.

According to FIG. 18, the loading efficiency of the drug increased until the drug to amphiphilic unimer ratio reached 2:1. The loading efficiency was about 40% of the desired
rapamycin that was dissolved into the volatile solution. The loading efficiency of the desired rapamycin then decreased after the drug:unimer ratio increased beyond 2:1 to a drug loading efficiency of less than 20% at drug:unimer ratios of 3:1 and 4:1. The PEG-DSPE micelle-tocopherol-rapamycin composition may have a size of about 16±2 nm. Thus, the rapamycin does not increase the micelle composition to be beyond EPR standards.

EXAMPLE 5
Rapamycin Incorporation into Micelle Compositions

The incorporation of rapamycin into the micelle compositions can be detected by SEC. As shown in FIG. 24, the micelles and rapamycin both come off the column at the same time, thus showing that they are incorporated into one compound. Unincorporated amphiphilic unimers do not form micelle compounds and come off the column at a later time. This example was conducted in a Shodex 804 SEC column, at 0.75 ml/min, and 37 degrees C., and RI and 277 nm UV detection.

EXAMPLE 6
Instability of PEG-DSPE Micelles Alone

As shown in FIG. 14, within a phosphate buffered saline solution, PEG-DSPE micelles are very stable. When PEG-DSPE micelle compositions are mixed in a phosphate buffered solution with 4% bovine serum albumin (BSA), the micelle compositions are much less stable and the passenger compound crashes out of the drug within 1 hour. The micelle compositions were released into 37 degrees Celsius deionized water from a 7500 molecular weight cutoff dialysis.

TABLE 7

<table>
<thead>
<tr>
<th>No BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mM PEG-DSPE2000, 0.5 mg/ml loading with rapamycin</td>
</tr>
<tr>
<td>micelle size: 14.3 nm ± 1.9 nm</td>
</tr>
<tr>
<td>micelle core size: 1.5 nm</td>
</tr>
<tr>
<td>release into 37 C. dH2O from 7500MWCO dialysis cassette</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diff coef t50%</th>
<th>5.5±0.21 cm2/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>time, h</td>
<td>45</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.02939325</td>
</tr>
<tr>
<td>2</td>
<td>0.09035507</td>
</tr>
<tr>
<td>4</td>
<td>0.16267111</td>
</tr>
<tr>
<td>6</td>
<td>0.21671948</td>
</tr>
<tr>
<td>8</td>
<td>0.281604594</td>
</tr>
<tr>
<td>10</td>
<td>0.35689325</td>
</tr>
<tr>
<td>20</td>
<td>0.494470473</td>
</tr>
<tr>
<td>40</td>
<td>0.701218068</td>
</tr>
<tr>
<td>60</td>
<td>0.701218068</td>
</tr>
</tbody>
</table>

Stability of PEG-DSPE Micelles when Incorporated with Tocopherol

As shown in FIG. 28, when micelle compositions are incorporated with tocopherol, the compositions are more stable over time and the drugs do not crash out. In the presence of 4% BSA, the 5 mM PEG-DSPE without tocopherol crashed out within the first 20 hours, but the 5 mM PEG-DSPE micelle composition with 10 mM tocopherol composition held together in solution for almost 60 hours.

As shown in FIG. 16, about 60% of the micelle compositions stayed intact for at least 25 hours.

TABLE 8-continued

Rapamycin Release with 40 mg/ml BSA

2.5 mM PEG-DSPE2000, 0.5 mg/ml loading with rapamycin |
micelle size: 14.3 nm ± 1.9 nm |
micelle core size: 1.5 nm |
release into 37 C. dH2O from 7500MWCO dialysis cassette |

<table>
<thead>
<tr>
<th>Diff coef t50%</th>
<th>7.7±0.2 cm2/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>time, h</td>
<td>2.4</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.233381472</td>
</tr>
<tr>
<td>2</td>
<td>0.491290641</td>
</tr>
<tr>
<td>4</td>
<td>0.652144744</td>
</tr>
<tr>
<td>6</td>
<td>0.758615201</td>
</tr>
<tr>
<td>8</td>
<td>0.850808031</td>
</tr>
<tr>
<td>13.5</td>
<td>0.951785345</td>
</tr>
<tr>
<td>25</td>
<td>0.951785345</td>
</tr>
</tbody>
</table>

TABLE 9

Rapamycin Release with 40 mg/ml BSA

2.5 mM PEG-DSPE2000, 0.5 mg/ml loading with rapamycin |
micelle size: 14.3 nm ± 1.9 nm |
micelle core size: 1.5 nm |
release into 37 C. dH2O from 7500MWCO dialysis cassette |

<table>
<thead>
<tr>
<th>Diff coef t50%</th>
<th>7.7±0.2 cm2/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>time, h</td>
<td>2.4</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.233381472</td>
</tr>
<tr>
<td>2</td>
<td>0.491290641</td>
</tr>
<tr>
<td>4</td>
<td>0.652144744</td>
</tr>
<tr>
<td>6</td>
<td>0.758615201</td>
</tr>
<tr>
<td>8</td>
<td>0.850808031</td>
</tr>
<tr>
<td>13.5</td>
<td>0.951785345</td>
</tr>
<tr>
<td>25</td>
<td>0.951785345</td>
</tr>
</tbody>
</table>

TABLE 10

Free Drug Release

Release of 0.083 mg/ml from 7500MWCO at 37 C. |

<table>
<thead>
<tr>
<th>time, h</th>
<th>ave</th>
<th>stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.090232675</td>
<td>0.01274166</td>
</tr>
<tr>
<td>2</td>
<td>0.38168865</td>
<td>0.03853122</td>
</tr>
<tr>
<td>4</td>
<td>0.603494152</td>
<td>0.039794768</td>
</tr>
<tr>
<td>6</td>
<td>0.872656301</td>
<td>0.08263598</td>
</tr>
<tr>
<td>8</td>
<td>0.880369048</td>
<td>0.011406282</td>
</tr>
<tr>
<td>12</td>
<td>0.964583972</td>
<td>0.0766255</td>
</tr>
</tbody>
</table>

As shown in FIG. 28, when micelle compositions are incorporated with tocopherol, the compositions are more stable over time and the drugs do not crash out. In the presence of 4% BSA, the 5 mM PEG-DSPE without tocopherol crashed out within the first 20 hours, but the 5 mM PEG-DSPE micelle composition with 10 mM tocopherol composition held together in solution for almost 60 hours.

As shown in FIG. 16, about 60% of the micelle compositions stayed intact for at least 25 hours.
EXAMPLE 7
Core Rigidity of Micelle Compositions with Tocopherol

As shown to FIG. 13, the core viscosity, or rigidity, of a micelle composition decreases slightly when tocopherol is incorporated. PEG-DSPE without any tocopherol has a relative core viscosity of a little less than about 3 L/m. The core viscosity decreases when tocopherol is added to the micelle composition. The core viscosity does not decrease linearly, but holds steady at about 1 L/m when the PEG-DSPE:tocopherol ratio increases past 1:1. The decrease in micelle composition core rigidity may decrease micelle stability and increase drug diffusion.

EXAMPLE 9
Increasing Size of Micelle Compositions with Tocopherol

The size of the micelle compositions is important because of the extravasation into tumor site. The micelles should ideally be less than about 400 nm in diameter in order to reach tumor sites. As shown in FIG. 24, the incorporation of tocopherol into micelle compositions does not increase the size of the resulting micelle compositions beyond 400 nm in diameter.

EXAMPLE 10
Increasing Aggregate Number with Incorporation

As shown in FIG. 14, the aggregate number of polymers increases with the incorporation of tocopherol into micelle compositions. The increased aggregate number may indicate an enlarged core. The core increased in size from 5 to 6 nm radius for the PEG-PCL 1:0 tocopherol to the 1:20 tocopherol. The core increased from 1.5 nm to 3 nm radius for the PEG-DSPE 1:0 tocopherol to the 1:2 tocopherol. At a PEG-DSPE:tocopherol ratio of 1:0.5, then the difference in aggregate numbers within the micelle composition becomes statistically significant.

EXAMPLE 11
Rapamycin Loading by Diffusion-Evaporation

The weight percent of rapamycin in the micelle compositions when there is tocopherol incorporated, showing the benefit of tocopherol incorporation. As shown in FIG. 18, when there is no tocopherol incorporated, at a rapamycin:micelle unimer ratio of 2:1, there is about 20 weight% rapamycin in the micelle composition. When there is either 1:1 or 1:2 PEG-DSPE:tocopherol ratios, then the weight % of rapamycin increases past 25%.

EXAMPLE 12
Tocopherol Effect on Rapamycin Release

As shown in FIG. 27, tocopherol increases the time over which rapamycin is released in a polar buffer solution, but not significantly so. The difference in drug retention between PEG-DSPE micelle without tocopherol and PEG-DSPE with incorporated tocopherol is not statistically significant.

TABLE 11
Rapamycin Release in 0.23 mg/ml BSA

<table>
<thead>
<tr>
<th>Diff coef (50%)</th>
<th>2.78E-20</th>
<th>cm2/s h</th>
<th>time, h</th>
<th>fract. Total drug released</th>
<th>stddev</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.041998035</td>
<td>0.06873</td>
<td>0.0478</td>
<td>0</td>
<td>0.054491</td>
</tr>
<tr>
<td>4</td>
<td>0.18249538</td>
<td>0.054491</td>
<td>0.054491</td>
<td>0</td>
<td>0.054491</td>
</tr>
<tr>
<td>8</td>
<td>0.256874804</td>
<td>0.084345</td>
<td>0.052919</td>
<td>0</td>
<td>0.052919</td>
</tr>
<tr>
<td>11</td>
<td>0.391461017</td>
<td>0.09244</td>
<td>0.035194</td>
<td>0</td>
<td>0.035194</td>
</tr>
<tr>
<td>24</td>
<td>0.451203982</td>
<td>0.03157</td>
<td>0.03157</td>
<td>0</td>
<td>0.03157</td>
</tr>
<tr>
<td>48</td>
<td>0.61898303</td>
<td>0.030605</td>
<td>0.029581</td>
<td>0</td>
<td>0.029581</td>
</tr>
<tr>
<td>72</td>
<td>0.751619835</td>
<td>0.020531</td>
<td>0.020531</td>
<td>0</td>
<td>0.020531</td>
</tr>
<tr>
<td>96</td>
<td>0.806581887</td>
<td>0.02144</td>
<td>0.02144</td>
<td>0</td>
<td>0.02144</td>
</tr>
<tr>
<td>120</td>
<td>0.913998387</td>
<td>0.012044</td>
<td>0.012044</td>
<td>0</td>
<td>0.012044</td>
</tr>
</tbody>
</table>

TABLE 12
Critical micelle concentration of polymer systems

<table>
<thead>
<tr>
<th>Micelle components</th>
<th>CMC, µM (µg/ml)</th>
<th>Diameter, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-DSPE2000</td>
<td>2.1 (5.9)</td>
<td>14.3 ± 1.9</td>
</tr>
<tr>
<td>PEG-DSPE2000/tocopherol (1:0.1 molar)</td>
<td>3.0 (8.5)</td>
<td>16.0 ± 1.8</td>
</tr>
<tr>
<td>PEG-DSPE2000/tocopherol (1:0.5 molar)</td>
<td>8.2 (23)</td>
<td>18.9 ± 3.0</td>
</tr>
<tr>
<td>PEG-DSPE2000/tocopherol (1:1 molar)</td>
<td>17 (49)</td>
<td>16.4 ± 4.3</td>
</tr>
<tr>
<td>PEG-DSPE2000/tocopherol (1:2 molar)</td>
<td>21 (79)</td>
<td>19.3 ± 3.0</td>
</tr>
<tr>
<td>PEG5000-PCL4000</td>
<td>1.2 (13)</td>
<td>14.3 ± 2.5</td>
</tr>
<tr>
<td>PEG5000-PCL4000/tocopherol (1:10 molar)</td>
<td>2.0 (22)</td>
<td>20.4 ± 3.4</td>
</tr>
<tr>
<td>PEG5000-PCL4000/tocopherol (1:20 molar)</td>
<td>2.8 (31)</td>
<td>24.6 ± 5.5</td>
</tr>
</tbody>
</table>

TABLE 13
Rapamycin Loading by Diffusion-Evaporation

<table>
<thead>
<tr>
<th>Diff coef (50%)</th>
<th>2.30E-20</th>
<th>cm2/s h</th>
<th>time, h</th>
<th>fract. Total drug released</th>
<th>stddev</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.228699276</td>
<td>0.016892</td>
<td>0.016892</td>
<td>0</td>
<td>0.016892</td>
</tr>
</tbody>
</table>
As shown in FIG. 28, the effect of tocopherol on drug retention of PEG-DSPE micelle compositions when in solution with 4% BSA is statistically significant. 4% BSA is the concentration of albumin in the human spinal cord. Tocopherol helps keep PEG-DSPE micelle compositions stable in vivo conditions for improved drug delivery.
### TABLE 19

<table>
<thead>
<tr>
<th>Drug</th>
<th>Carrier</th>
<th>Drug load, mg/ml</th>
<th>Drug weight %</th>
<th>Loading efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapamycin</td>
<td>5 mM tocopherol</td>
<td>&lt;0.01</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.05 mM PEG5000</td>
<td>0.20</td>
<td>18%</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td>+ tocopherol (1:10)</td>
<td>0.34</td>
<td>44%</td>
<td>74%</td>
</tr>
<tr>
<td></td>
<td>1.7 mM PEG5-PCL10k</td>
<td>0.41</td>
<td>34%</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>+ tocopherol (1:15)</td>
<td>0.56</td>
<td>69%</td>
<td>95%</td>
</tr>
<tr>
<td>Geldan-700</td>
<td>5 mM tocopherol</td>
<td>&lt;0.01</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.5 mM PEG-PCL</td>
<td>0.018</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>+ tocopherol (1:20)</td>
<td>0.15</td>
<td>15%</td>
<td>54%</td>
</tr>
</tbody>
</table>

### EXAMPLE 15

**PEG-PCL Rapamycin Release in BSA Solution**

As shown in FIG. 30, tocopherol incorporation into PEG-PCL micelles also help the resulting micelle composition retain rapamycin in 4% BSA solution. This shows the stabilizing effect of tocopherol incorporation into PEG-PCL micelles in vivo conditions.

### TABLE 20

<table>
<thead>
<tr>
<th>Diff coef</th>
<th>7.40E-20 cm²/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>time, h</td>
<td>ave</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.11248528</td>
</tr>
<tr>
<td>0.75</td>
<td>0.203232673</td>
</tr>
<tr>
<td>1</td>
<td>0.21672982</td>
</tr>
<tr>
<td>2</td>
<td>0.22833588</td>
</tr>
<tr>
<td>4</td>
<td>0.239403519</td>
</tr>
<tr>
<td>8</td>
<td>0.297813974</td>
</tr>
<tr>
<td>11.5</td>
<td>0.323172267</td>
</tr>
<tr>
<td>24</td>
<td>0.48818767</td>
</tr>
<tr>
<td>48</td>
<td>0.509983519</td>
</tr>
<tr>
<td>72</td>
<td>0.64377228</td>
</tr>
<tr>
<td>96</td>
<td>0.641765258</td>
</tr>
<tr>
<td>120</td>
<td>0.725612619</td>
</tr>
<tr>
<td>144</td>
<td>0.781049617</td>
</tr>
<tr>
<td>168</td>
<td>0.8391109855</td>
</tr>
<tr>
<td>192</td>
<td>0.87063727</td>
</tr>
<tr>
<td>216</td>
<td>0.901749452</td>
</tr>
<tr>
<td>244</td>
<td>0.938011184</td>
</tr>
<tr>
<td>300</td>
<td>1.00000000</td>
</tr>
</tbody>
</table>

### TABLE 21

<table>
<thead>
<tr>
<th>Diff coef</th>
<th>1.80E-19 cm²/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>time, h</td>
<td>ave</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.120305267</td>
</tr>
<tr>
<td>2</td>
<td>0.186453694</td>
</tr>
<tr>
<td>4</td>
<td>0.249108455</td>
</tr>
<tr>
<td>6</td>
<td>0.345588083</td>
</tr>
<tr>
<td>8</td>
<td>0.59139283</td>
</tr>
<tr>
<td>12</td>
<td>0.566728869</td>
</tr>
<tr>
<td>24</td>
<td>0.657608843</td>
</tr>
<tr>
<td>48</td>
<td>0.78127577</td>
</tr>
<tr>
<td>72</td>
<td>0.872134244</td>
</tr>
<tr>
<td>96</td>
<td>0.936092462</td>
</tr>
</tbody>
</table>

### TABLE 22

<table>
<thead>
<tr>
<th>Free drug - Rapa release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Release of 0.083 mg/ml from 7500MWCO at 37 C.</td>
</tr>
<tr>
<td>time, h</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>12</td>
</tr>
</tbody>
</table>

### TABLE 23

<table>
<thead>
<tr>
<th>Diff coef</th>
<th>1.10E-19 cm²/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>time, h</td>
<td>ave</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.25</td>
<td>0.071170037</td>
</tr>
<tr>
<td>2.25</td>
<td>0.11057262</td>
</tr>
<tr>
<td>4</td>
<td>0.186902748</td>
</tr>
<tr>
<td>6</td>
<td>0.204138959</td>
</tr>
<tr>
<td>8</td>
<td>0.231392203</td>
</tr>
<tr>
<td>11.5</td>
<td>0.323172267</td>
</tr>
<tr>
<td>24</td>
<td>0.496117959</td>
</tr>
<tr>
<td>48</td>
<td>0.657608843</td>
</tr>
<tr>
<td>72</td>
<td>0.725849917</td>
</tr>
<tr>
<td>96</td>
<td>0.767562492</td>
</tr>
<tr>
<td>120</td>
<td>0.820292777</td>
</tr>
<tr>
<td>144</td>
<td>0.881377101</td>
</tr>
</tbody>
</table>
Male Sprague-Dawley rats (200-240 g) were obtained from Simonsen Labs (Gilroy, Calif., USA) and given food (Purina Rat Chow 5001) and water ad libitum in our animal facility for at least 3 days before use. Rats were housed in temperature-controlled rooms with a 12 h light/dark cycle. The day before the pharmacokinetic experiment the right jugular veins of the rats were catheterized with sterile silastic cannula (Dow Corning, Midland, Mich., USA) under halothane anesthesia. This involved exposure of the vessel prior to cannula insertion. After cannulation, the Intramedic PE-50 polyethylene tubing (Becton, Dickinson and Company, Franklin Lakes, N.J., USA) connected to the cannula was exteriorized through the dorsal skin. The cannula was flushed with 0.9% saline. The animals were transferred to metabolic cages and were fasted overnight. Animal ethics approval was obtained from The Institutional Animal Care and Use Committee at Washington State University.

12 male Sprague Dawley rats (average weight: 220 g) were cannulated as described in the previous section. Each of the animals were placed in separate metabolic cages, allowed to recover overnight, and fasted for 12 h before dosing. On the day of experiment, the animals were dosed intravenously with rapamycin (10 mg/kg) dissolved either in DMA, PEG, and Tween 80 (control formulation), poly(ethylene glycol)-β-poly(ε-caprolactone) (PEG-PCL formulation), or PEG-PCL co-incorporated with α-tocopherol (PEG-PCL + α-tocopherol formulation) (N=4 for each treatment group). Serial blood samples (0.25 ml) were collected at 0, 1 min, 0.5, 1, 2, 4, 6, 12, 24, and 48 h. Each blood sample was divided into two 0.1 ml fractions, the first one was collected into regular polypropylene microcentrifuge tube and labeled as whole blood sample and stored at -70°C until analyzed. The second fraction was collected in heparanized tubes (Monoject, Mansfield Mass.) and following centrifugation, the plasma and red blood cell (RBC) fractions were collected and stored at -70°C until analyzed.

The protocol previously described by Amnesle and Clayton, 2004 [1] was slightly modified. For our purpose, 10 ul of whole blood, plasma, calibrator or control was added in a regular polypropylene microcentrifuge tube. Then, 250 ul of deionized water, 250 ul of aqueous 0.1 mol/L zinc sulfate, and 500 ul methanoll containing the internal standard were added. The mixture was vortexed for 30 seconds, and the tubes were left at room temperature for 5-10 minutes. Then, the tubes were centrifuged for 4 minutes, and the colorless supernatant was analyzed. A 60 mg, 3 ml Oasis HLB column was utilized for the solid phase extraction (SPE) clean up of the samples. The column was conditioned with 1 ml methanol followed by 1 ml of water. The prepared supernatant was passed slowly through the column (1-2 ml/min), then the column was washed with 1 ml of water and air-dried for about 30 seconds. The LC/MS analyses were carried on a Agilent 1100 system. In the positive-ion mode the monitored multiple-reaction monitoring transition (m/z) was: rapamycin 931.6-864.5. Separation was performed with a Waters Xterra MS 18 2.1 x 100 mm maintained at 40°C. The injection volume was 25 ul with a flow rate of 0.4 ml/min. The mobile phases were (A) 10 mM ammonium acetate and 0.1% formic acid in water and (B) 10 mM ammonium acetate and 0.1% formic acid in methanol. The gradient program was 50% A and 50% B for the whole run (15 minutes).

Pharmacokinetic analysis was performed using WinNONLINE® software (Ver. 1). Summary data were expressed as mean±standard error of the mean (S.E.M.). The elimination rate constant (λz) was estimated by linear regression of the plasma concentrations in the log-linear terminal phase. The AUC(0-t) was calculated using the combined log-linear trapezoidal rule for data from time of dosing to the last measured concentration, plus the quotient of the last measured concentration divided by λz. Non-compartmental pharmacokinetic
methods were used to calculate clearance (CL) and volume of distribution (Vd) after iv dosing. The blood distribution of rapamycin was calculated by dividing the rapamycin concentration detected in plasma by the concentration detected in RBC at different time points after intravenous dosing with the different rapamycin formulations.

Following intravenous administration of the rapamycin control formulation, a small increase in rapamycin concentration was evident at 12 hours indicating the possibility of enterohepatic recycling (FIG. 1). The total clearance of rapamycin was determined to be 1.12±0.14 L/h/kg (Table 1). The volume of distribution of rapamycin is 20.94±3.65 L/kg, which is greater than total body water, suggesting rapamycin is highly distributed in tissue. The concentrations of rapamycin appeared to slowly decline rapidly with a mean elimination half-life of 11.52±0.57 h. The mean area under the curve (AUC), representing the total amount of drug exposure in the blood over time, was 8.34±0.91 µg/ml.

Following intravenous administration of the rapamycin PEG-PC formulation (FIG. 2), the total clearance of rapamycin was determined to be 1.11±0.07 L/h/kg (Table 1). The volume of distribution of rapamycin is 24.85±2.10 L/kg, which is greater than total body water, suggesting rapamycin is highly distributed in tissue. The concentrations of rapamycin appeared to decline slowly with a mean elimination half-life of 15.55±0.71 h. The mean area under the curve (AUC), representing the total amount of drug exposure in the plasma over time, was 9.23±0.71 µg/ml.

Following intravenous administration of the rapamycin PEG-PCI+a-tocopherol formulation (FIG. 3), the total clearance of rapamycin was determined to be 0.84±0.03 L/h/kg (Table 1). The volume of distribution of rapamycin is 17.74±1.27 L/kg, which is greater than total body water, suggesting rapamycin is highly distributed in tissue. The concentrations of rapamycin appeared to decline slowly with a mean elimination half-life of 14.63±0.81 h. The mean area under the curve (AUC), representing the total amount of drug exposure in the blood over time, was 11.93±0.41 µg/ml.

The plasma/RBC ratios were calculated at 1 min (FIG. 4) and 12 hours (FIG. 5) after intravenous dosing of the different rapamycin formulations. The plasma/RBC ratios after 1 min and 12 hr i.v. dosing of rapamycin control formulation are 2.21 and 0.41 respectively. The ratios after i.v. dosing of rapamycin PEG-PCI formulation are 3.44 and 0.48 respectively, and the ratios after i.v. dosing of rapamycin PEG-PCI+a-tocopherol are 4.80 and 0.76 respectively.

After i.v. dosing there was 40% mortality of the rats after the rapamycin control formulation which occurred 0-2 hours after drug administration. Control animals consistently appeared listless. There was no mortality with either of the rapamycin micellar formulations. The rats were held in metabolic cages and urine collected for 24 hour intervals and volume measured. There was no difference in renal output between groups.

Rapamycin pharmacokinetics has been studied extensively in different species including rat, monkey, rabbit, and human. These studies have characterized rapamycin to be a drug with a relatively long half-life of more than 5 hours, with volume of distribution values that indicates a substantial proportion of the drug residing extravascularly, and rapidly absorbed in the body [2-5]. Rapamycin is a lipophilic compound with a partition coefficient (XLogP) of 5.773 and is highly distributed into the tissue as evidenced by the high volume of distribution (Vd) of rapamycin from 20.94 L/kg in the control formulation to 17.75 L/kg in the tocopherol formulation respectively. Similarly the two formulations offer an increase in the half-life from 11.52 h (control) to 15.55 and 14.63 h for PEG-PCI and PEG-PCI+tocopherol respectively. There is also an increase in AUC values and a decrease in clearance values with the two formulations compared to the control. All these pharmacokinetic parameter changes show an eventual higher residence time of rapamycin in the body and an increase in plasma residence suggests less distribution into the RBC which may facilitate better distribution to possible target sites, which eventually will exert a higher pharmacological effect than the control formulation considering that all the formulations were applied at the same dose (10 mg/kg). Thus, the further study of the pharmacokinetic and pharmacodynamic effects of these formulations is warranted.

The blood distribution of rapamycin was also studied in vivo, and the plasma/RBC ratios were calculated at two time points (1 min and 12 h) after intravenous dosing of the different rapamycin formulations. These results show a higher distribution of rapamycin in plasma than red blood cells at 1 minute in all the formulations. However, after 12 hours rapamycin has a higher distribution in red blood cells than plasma. This change in blood distribution among time could be explained by the fact that rapamycin binds to FKBP [FK506 binding protein] in red blood cells [6]. This protein binding could make the clearance of rapamycin out of the red blood cells slower than the clearance out of the plasma giving this biodistribution change. The two formulations (PEG-PCI and PEG-PCI+tocopherol) at both time points (1 minute and 12 hours) show a higher plasma/RBC ratio than the control formulation. This would represent a higher concentration of rapamycin not bound to RBC proteins making it more available to exert its pharmacological effects.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters of Rapamycin Formulations in Rat Whole Blood.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>AUC (µg · h/ml)</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
</tr>
<tr>
<td>CL (L/h/kg)</td>
</tr>
<tr>
<td>KE (h⁻¹)</td>
</tr>
<tr>
<td>t½ (h)</td>
</tr>
</tbody>
</table>

EXAMPLE 18

Release Data of Geldanamycin Prodrugs in Micelles

As shown in Table 27, geldanamycin prodrugs loaded into micelles are pretty stable. Micelles loaded with 17-aminoethyl-palmitate-17-demethoxygeldanamycin or 17-aminoethyl-dodecanote-17-demethoxygeldanamycin release almost all the drug after about 8 days. Micelles loaded with 17-aminoethyl-bromododecanote-17-demethoxygeldanamycin or 17-amino-hexyldecyl-17-demethoxygeldanamycin release substantially all the drug after about 12 days. Micelles loaded with 17-aminoethyl-bronheoxencate-17-demethoxygeldanamycin or 17-aminoethyl-bronpalmitinate-17-demethoxygeldanamycin release substantially all the drug after about 14 days.
EXAMPLE 19
Paclitaxel Prodrug Formulations

<table>
<thead>
<tr>
<th>R₁ (C₂)</th>
<th>R₂ (C₇)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
</tr>
<tr>
<td>2</td>
<td>S((tert-buty)</td>
</tr>
<tr>
<td>3</td>
<td>COCH₂CH₃</td>
</tr>
<tr>
<td>4a</td>
<td>COCH₂COCH₃</td>
</tr>
<tr>
<td>4b</td>
<td>H</td>
</tr>
<tr>
<td>4c</td>
<td>H</td>
</tr>
<tr>
<td>5a</td>
<td>COCH₂</td>
</tr>
<tr>
<td>5b</td>
<td>H</td>
</tr>
<tr>
<td>5c</td>
<td>COCH₂</td>
</tr>
</tbody>
</table>

Synthesis of 7-palmitate-paclitaxel 4c. The method for synthesis of 2-palmitate-paclitaxel 4c is described infra. Substitution of 4a-b were according to the same procedure, with 1-M HCl (5 ml/mol) in 1 ml dry toluene was added palmitic anhydride (83.8 mg, 0.0774 mmol). The reaction mixture was stirred at room temperature for 1 h. The resulting solution was reduced to dryness in vacuo, redissolved in 2 ml CH₂Cl₂, washed with saturated NH₄Cl (5 ml/mol) and the organic layer dried over Na₂SO₄. Removal of the solvent followed by preparatory TLC on silica (1:1 EtOAc:hexane) provided 4c as a white solid (25 mg, 41% yield).

1H NMR (400 MHz, CDCl₃) δ 0.88 (t, 3H, tert-buty l), 1.22 (s, 3H, H₁), 1.76 (s, 3H, H₁), 1.93 (s, 3H, H₁), 1.92-2.14 (m, 2H, H₆), 2.3 and 2.56 (m, 2H, H₄), 2.58 (s, 3H, 4-Ac), 3.91 (d, J = 6.9 Hz, H₁H₃), 4.23 (d, J = 8.1 Hz, H₁H₂), 4.30 (d, J = 1.8 Hz, H₁H₂), 4.35 (d, J = 8.1 Hz, H₁H₂O), 4.42 (dd, J = 6.6 and 10.8 Hz, H₁H₇), 4.68 (dd, J = 2.1 Hz, H₁H₂), 4.98 (dd, J = 1.5 and 9.3 Hz, H₁H₅), 5.13 (d, J = 1.8 Hz, H₁H₁₀), 5.69 (d, J = 6.9 Hz, H₁H₁₂), 5.73 (dd, J = 1.8 and 9 Hz, H₁H₁₃), 6.34 (t, J = 8.7 Hz, H₁H₁₃), 7.11 (d, J = 9 Hz, H₁H₁₂), 7.33-8.16 (m, H₁H₁₃).

2-TBS-7-palmitate-paclitaxel 3. To a solution of 2 (50 mg, 0.053 mmol) in 1 ml dry toluene was added palmitic anhydride (38.3 mg, 0.0774 mmol). The reaction mixture was stirred at 90°C for 18 h. The resulting solution was washed with 1-M HCl (5 ml/mol) followed by water (5 ml/mol), and the organic layer was dried over Na₂SO₄. Removal of the solvent followed by preparatory TLC on silica (1:1 EtOAc:hexane) provided 3 as a white solid (25 mg, 41% yield).

1H NMR (400 MHz, CDCl₃) δ 0.5 (s, 9H, tert-buty l), 0.88 (t, 3H, CH₃), 1.0 (s, 3H, H₁H₁), 1.22 (s, 3H, H₁), 1.76 (s, 3H, H₁), 1.93 (s, 3H, H₁), 1.92-2.14 (m, 2H, H₆), 2.3 and 2.56 (m, 2H, H₄), 2.58 (s, 3H, 4-Ac), 3.91 (d, J = 6.9 Hz, H₁H₃), 4.23 (d, J = 8.1 Hz, H₁H₂), 4.30 (d, J = 1.8 Hz, H₁H₂O), 4.35 (d, J = 8.1 Hz, H₁H₂O), 4.42 (dd, J = 6.6 and 10.8 Hz, H₁H₇), 4.68 (dd, J = 2.1 Hz, H₁H₂), 4.98 (dd, J = 1.5 and 9.3 Hz, H₁H₅), 5.13 (d, J = 1.8 Hz, H₁H₁₀), 5.69 (d, J = 6.9 Hz, H₁H₁₂), 5.73 (dd, J = 1.8 and 9 Hz, H₁H₁₃), 6.34 (t, J = 8.7 Hz, H₁H₁₃), 7.11 (d, J = 9 Hz, H₁H₁₂), 7.33-8.16 (m, H₁H₁₃).

7-palmitate-paclitaxel 4c. To a solution of 3 (25 mg, 0.211 mmol) in 1 ml of THF was added 5 drops of 1-M TBAF (tetraethylammoniumfluoride) in THF. The reaction mixture was stirred at room temperature for 1 h. The resulting solution was reduced to dryness in vacuo, redissolved in 2 ml CH₂Cl₂, washed with water (5 ml/mol), and the organic layer was dried over Na₂SO₄. Removal of solvent followed by preparatory TLC on silica (1:1 EtOAc:hexane) provided 4c as a white solid (20 mg, 90% yield).

1H NMR (400 MHz, CDCl₃) δ 0.88 (t, 3H, CH₃), 1.10 (s, 3H, H₁H₁), 1.22 (s, 3H, H₁), 1.76 (s, 3H, H₁), 1.93 (s, 3H, H₁), 1.92-2.14 (m, 2H, H₆), 2.3 and 2.56 (m, 2H, H₄), 2.58 (s, 3H, 4-Ac), 3.91 (d, J = 6.9 Hz, H₁H₃), 4.23 (d, J = 8.1 Hz, H₁H₂), 4.30 (d, J = 1.8 Hz, H₁H₂O), 4.35 (d, J = 8.1 Hz, H₁H₂W), 4.42 (dd, J = 6.6 and 10.8 Hz, H₁H₇), 4.68 (dd, J = 2.1 Hz, H₁H₂), 4.98 (dd, J = 1.5 and 9.3 Hz, H₁H₅), 5.13 (d, J = 1.8 Hz, H₁H₁₀), 5.69 (d, J = 6.9 Hz, H₁H₁₂), 5.73 (dd, J = 1.8 and 9 Hz, H₁H₁₃), 6.34 (t, J = 8.7 Hz, H₁H₁₃), 7.11 (d, J = 9 Hz, H₁H₁₂), 7.33-8.16 (m, H₁H₁₃).

<table>
<thead>
<tr>
<th>TABLE 27</th>
<th>Geldanamycin produgs release data</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-aminoethylbromohexenoate-17-geldanamycin</td>
<td>17-aminoethylbromopelmatilacte-17-geldanamycin</td>
</tr>
<tr>
<td>17-aminoethylbromodecanoate-17-geldanamycin</td>
<td>17-aminoethyldecanoate-17-geldanamycin</td>
</tr>
<tr>
<td>time, fraction</td>
<td>released</td>
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<tr>
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</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>4</td>
<td>0.611639</td>
</tr>
<tr>
<td>6</td>
<td>0.781088</td>
</tr>
<tr>
<td>8</td>
<td>0.952778</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>TABLE 28</th>
<th>Paclitaxel Prodrugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁ (C₂)</td>
<td>R₂ (C₇)</td>
</tr>
<tr>
<td>1</td>
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<td>2</td>
<td>S((tert-buty)</td>
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<tr>
<td>3</td>
<td>COCH₂CH₃</td>
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<td>4a</td>
<td>COCH₂COCH₃</td>
</tr>
<tr>
<td>4b</td>
<td>H</td>
</tr>
<tr>
<td>4c</td>
<td>H</td>
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<tr>
<td>5b</td>
<td>H</td>
</tr>
<tr>
<td>5c</td>
<td>COCH₂</td>
</tr>
</tbody>
</table>

Synthesis of 7-palmitate-paclitaxel 4c. The method for synthesis of 2-palmitate-paclitaxel 4c is described infra. Substitution of 4a-b were according to the same procedure, with 1-M HCl (5 ml/mol) in 1 ml dry toluene was added palmitic anhydride (83.8 mg, 0.0774 mmol). The reaction mixture was stirred at room temperature for 1 h. The resulting solution was reduced to dryness in vacuo, redissolved in 2 ml CH₂Cl₂, washed with saturated NH₄Cl (5 ml/mol) followed by water (5 ml/mol), and the organic layer was dried over Na₂SO₄. Removal of the solvent followed by preparatory TLC on silica (1:1 EtOAc:hexane) provided 3 as a white solid (25 mg, 41% yield).
PEG-b-PCL micelle produg release studies. Release experiments were based on the methodology of Eisenberg and coworkers (Soo, P. L., et al., 2002) with modifications for temperature and pH control. Micelle produg solutions were prepared at 0.5 mM (PEG-b-PCL basis) with 20% w/w produg as above, and 0.5 ml of each solution was diluted to 2.5 ml with ddH₂O and injected into 10000 MWCO dialysis cassettes (Pierce, Rockford, Ill.) (n=4). Dialysis cassettes were placed in a well-mixed temperature controlled water bath at 37°C, overfilled with ddH₂O so that the bath volume was refreshed every 15 to 20 min. Peristaltic pumps under computer control separately injected 50-µL solutions of tribasic and monobasic phosphate to maintain pH at 7.4±0.05 (apparatus built in-house). At fixed time points, dialysis cassette volumes were made up to 2.5 ml with ddH₂O, 100-4 aliquots withdrawn, and produg concentrations determined by reverse-phase HPLC (see supra) [24].

Diffusion constants and release half-lives were determined as described previously by modeling release as Fickian diffusion from an impenetrable sphere using the Crank solution for short time periods [1]. Linear regression of release data was performed in Sigma Plot 9.0 (Systat Software, Inc.). Diffusion constants were determined for independent samples (n=3) and reported as the average±standard deviation. Release half-lives were determined using the calculated diffusion constant in the Crank solution for 50% drug release.

Octanol-water partition coefficients. Octanol-water partition coefficients (log P₀∞) of paclitaxel produgs were determined indirectly by microemulsion electrokinetic chromatography (MEEKC) based on the technique of Klotz et al. (22). Running buffer was prepared by titration of 25-mM sodium phosphate monobasic with 50-mM sodium tetraborate to pH 7.00, and 1.44 g of sodium dodecyl sulfate, 6.49 g of 1-butanol, and 0.82 g of heptane were made up to 100 ml with phosphate-borate buffer. The running buffer was ultrasonicated for 30 min in a closed 250-ml flask in ice water (G1128P1 Special Ultrasonic Cleaner, Laboratory Supplies Company Inc., Hicksville, N.Y.). Longer times may be required to obtain a stable emulsion with lower power ultrasonicators. Compounds and standards (n=3) were dissolved in the running buffer (0.05 mg/ml) with 0.5 µL of nitromethane, and 0.5 µL of 1-phenyldecane by ultrasonication (10 min) in a closed tube and centrifuged (16000 x g, 3 min) to degas. A BioFocus 3000 capillary electrophoresis system (Bio-Rad, Hercules, Calif.) equipped with a 50-µm ID×37-cm uncoated fused-silica column (Polymerion Technologies LLC, Phoenix, Ariz.) was used for MEEKC experiments. The column was prewashed with 1-M NaOH for 5 min and before runs with 0.1-M NaOH for 1 min, ddH₂O for 1 min, and running buffer for 1 min at 100 psi (690 kPa). Running conditions were 10 kV (ca. 30-35 µA, 30 min/run) at 20°C. With 1-psi-s injections (6.9 kPa·s) and detection at 210 and 232 nm. Log P₀∞ and retention factors, k', were calculated using the equations:

$$\log P_{0∞} = a \cdot \log k' + b$$

$$k' = \frac{t_r - t_o}{t_{ow} - t_o}$$

where tₙ, t₀, and tₚ are retention times of the produg, nitromethane, and 1-phenyldecane, respectively. Fitting parameters a and b were determined by linear regression of known standards: pyridine, phenol, benzoic acid, aniso.
benzene, toluene, dodecaneoic acid, benzopyrene, and pyrene (R²=0.996, Excel® 2003, Microsoft Corp.). Cytotoxicity
determination.
MCF-7 and MDA-MB-231 human breast cancer cells (American Tissue Type Collection) were plated at 96-well
plates at an initial density of 5000 cells per well in 50 µL of
RPMI 1640 (MCF-7) or DMEM (MDA-MB-231) supple-
mented with 10% fetal bovine serum, 100 IU penicillin, and
100 µg/ml streptomycin, 2 mM L-glutamine, and maintained
at 37°C in a 5% CO₂ atmosphere. After 24 h, the test
compounds in DMSO were diluted 10-fold with growth media
and added to wells (2 wells in triplicate, n=6) as 10-µL al-
iquots (1% v/v final DMSO concentration). Cells were incu-
bated with compounds for 96 h and the metabolic rate was
determined using an XTT assay. Briefly, 20 µL of freshly
prepared assay solution (1 mg/mL XTT and 0.1 mg/mL N-me-
thylphenazonium methyl sulfate in PBS) was added to each
well, cells were incubated for 4 h, and absorbances measured
at 550 nm with background subtraction at 630 nm. The con-
centrations inhibiting cell growth by 50% (IC50) were deter-
 missed by fixed Hill slope regression with Sigma Plot 2004

TABLE 29

<table>
<thead>
<tr>
<th>Prodrug</th>
<th>Diameter (intensity), nm *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>4b</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>4c</td>
<td>44 ± 2</td>
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<tr>
<td>5a</td>
<td>32 ± 0</td>
</tr>
<tr>
<td>5b</td>
<td>28 ± 0</td>
</tr>
<tr>
<td>5c</td>
<td>37 ± 6</td>
</tr>
</tbody>
</table>

*Hydrodynamic diameters from DLS with Gaussian intensity weighting of drug loaded micelles prepared at 20% w/w drug. Actually loadings are in Table 2 below. Table 2: Solubility parameters of paclitaxel produgs and PEG-6-PCL solubility.

TABLE 30

<table>
<thead>
<tr>
<th>Prodrug</th>
<th>Sдвиж</th>
<th>Vp/g</th>
<th>% drug-PCL</th>
<th>log P&lt;sub&gt;400&lt;/sub&gt;</th>
<th>Caprolactone mmol/mol</th>
<th>Prodrug w/w %</th>
<th>Solubilized mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.7</td>
<td>498</td>
<td>8.59</td>
<td>4.40 ± 0.06</td>
<td>&lt;1</td>
<td>—</td>
<td>&lt;0.2</td>
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<tr>
<td>4a</td>
<td>24.5</td>
<td>604</td>
<td>4.55</td>
<td>4.43 ± 0.06</td>
<td>36.5</td>
<td>17.1</td>
<td>1.55 ± 0.04 (5.1 ± 0.5)</td>
</tr>
<tr>
<td>4b</td>
<td>23.5</td>
<td>700</td>
<td>3.14</td>
<td>4.59 ± 0.18</td>
<td>31.8</td>
<td>16.4</td>
<td>1.47 ± 0.03 (2.2 ± 0.5)</td>
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<tr>
<td>4c</td>
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<td>4.48 ± 0.06</td>
<td>33.3</td>
<td>21.6</td>
<td>1.62 ± 0.03 (3.0 ± 0.9)</td>
</tr>
<tr>
<td>5a</td>
<td>24.5</td>
<td>604</td>
<td>4.45</td>
<td>4.45 ± 0.03</td>
<td>33.4</td>
<td>17.8</td>
<td>1.42 ± 0.11 (&gt;3)</td>
</tr>
<tr>
<td>5b</td>
<td>23.5</td>
<td>700</td>
<td>3.14</td>
<td>4.49 ± 0.03</td>
<td>34.0</td>
<td>17.3</td>
<td>1.57 ± 0.02 (&gt;3)</td>
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<tr>
<td>5c</td>
<td>23.0</td>
<td>765</td>
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<td>4.51 ± 0.04</td>
<td>40.0</td>
<td>19.8</td>
<td>1.85 ± 0.05 (&gt;3)</td>
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</table>

*Solubility and encapsulation based on 20% w/w prodrug loading in 0.5mM PEG-b-PCL micelles. Results are given ± standard
deviation (n = 3).

What is claimed is:

1. A micelle composition comprising a plurality of
micelles, wherein the micelles comprise a pegylated phos-
pholipid, Vitamin E, and a hydrophobic passenger drug;

2. The micelle composition of claim 1 wherein the hydro-
phobic passenger drug is selected from the group
consisting of rapamycin, paclitaxel, paclitaxel produgs
comprising a carbonyloxy-linked or silyloxy-linked
moiety at one or both of the paclitaxel positions C2 and
C7, geldanamycin, geldanamycin produgs comprising
a nitrogen-linked moiety at the geldanamycin C17 posi-
tion in place of the C17 methoxy group of geldanamyc-
in, and combinations thereof.

3. The micelle composition of claim 1 wherein the mol %
ratio of the Vitamin E to the pegylated phospholipid is about
1:1 to about 3:1.

4. The micelle composition of claim 1 wherein the pegy-
lated phospholipid is PEG-distearoylphosphatidyl ethanolol-
mine (PEG-DSPE).

5. The micelle composition of claim 1 wherein the Vitamin
E is selected from the group consisting of alpha-tocoipherol,
beta-tocopherol, gamma-tocopherol, delta-tocopherol,
alpha-tocotrienol, beta-tocotrienol, gamma-tocotrienol, and
delta-tocotrienol.

6. The micelle composition of claim 1 wherein the concentra-
tion of the Vitamin E is about 2 mM to about 20 mM.

7. The micelle composition of claim 1 wherein the hydro-
phobic passenger drug is one or more of rapamycin, pacli-
taxel, or geldanamycin.

8. The micelle composition of claim 1 wherein said pacli-
taxel produg comprises in increased log Po/w as compared to
paclitaxel and wherein the geldanamycin produg has an
increased log Po/w as compared to geldanamycin.

9. The micelle composition of claim 1 wherein the hydro-
phobic passenger drug is rapamycin and the rapamycin
comprises at least 11 wt. % of the micelles.

10. The micelle composition of claim 1 wherein the hydro-
phobic passenger drug is rapamycin and the concentration of
rapamycin is about 0.1 mg/mL to about 4 mg/mL.
the concentration of the Vitamin E is about 2 mM to about 100 mM; 41
the Vitamin E and the hydrophobic passenger drug are located within the micelles; and 42
the hydrophobic passenger drug is rapamycin.
14. The micelle composition of claim 13 wherein the rapamycin in the micelles is about 10% wt. drug/wt. micelles to about 20% wt. drug/wt. micelles.
15. A micelle composition comprising a plurality of micelles, wherein the micelles comprise a pegylated phospholipid, Vitamin E, and a hydrophobic passenger drug; wherein
the Vitamin E and the pegylated phospholipid are present at a mol % ratio of about 0.1:1 to about 3:1; the concentration of the Vitamin E is about 2 mM to about 100 mM; 5
the hydrophobic passenger drug is selected from the group consisting of rapamycin, paclitaxel, paclitaxel prodrugs comprising a carbonyloxy-linked or silyloxy-linked moiety at one or both of the paclitaxel positions C2 and C7, geldanamycin, geldanamycin prodrugs comprising a nitrogen-linked moiety at the geldanamycin C17 position in place of the C17 methoxy group of geldanamycin, and combinations thereof.
16. A process for forming a micelle composition of claim 1 comprising: combining a pegylated phospholipid, Vitamin E, and a hydrophobic passenger drug, in an organic solvent to form a solution, wherein the hydrophobic passenger drug is selected from the group consisting of rapamycin, paclitaxel, paclitaxel prodrugs comprising a carbonyloxy-linked or silyloxy-linked moiety at one or both of the paclitaxel positions C2 and C7, geldanamycin, geldanamycin prodrugs comprising a nitrogen-linked moiety at the geldanamycin C17 position in place of the C17 methoxy group of geldanamycin, and combinations thereof;
removing substantially all of the organic solvent from the solution to leave a substantially solvent-free mixture; and
resuspending the substantially solvent-free mixture in water or buffer, to provide the micelle composition wherein Vitamin E and the pegylated phospholipid are present at a mol % ratio of about 0.1:1 to about 3:1, the concentration of the Vitamin E is about 2 mM to about 100 mM, and the Vitamin E and the hydrophobic drug are located within the micelles.
17. The process of claim 16 wherein the hydrophobic drug is rapamycin, paclitaxel, or geldanamycin.