CLAIMS

What is claimed is:

1. A method of making lyophilized Annamycin comprising the steps of:

making a solution comprising one or more lipids, one or more non-ionic surfactants, and one or more solvents and having a pH of 4.8 – 5.9;

adding to the lipid solution an Annamycin solution comprising about 8 - 12 wt. % Annamycin in dimethyl sulfoxide (DMSO) to provide a lipid containing solution of Annamycin;

sterile filtering the lipid containing solution of Annamycin; and

lyophilizing the lipid containing solution of Annamycin to provide a preliposomal Annamycin lyophilizate.

2. A method of making lyophilized Annamycin comprising the steps of:

making a first solution comprising water and *t*-butanol wherein the ratio of water to *t*-butanol is from 8:2 to 9:1;

adding one or more lipids and one or more non-ionic surfactants to the first solution to form a second solution;

adjusting the pH of the second solution such that the pH is 4.8 – 5.9;

adding a third solution comprising 8 - 12 wt. % Annamycin in DMSO to the second solution to provide a lipid containing solution of Annamycin;

sterile filtering the lipid containing solution of Annamycin; and

lyophilizing the lipid containing solution of Annamycin to provide a preliposomal Annamycin lyophilizate.

3. The method of claim 2 wherein the second solution comprises dimyristoyl phosphatidylcholine (DMPC), 1,2-dimyristoyl-sn-glycero-3-[phosphor-rac-(1 glycerol)] sodium salt (DMPG), and a polyoxyethylene sorbitan surfactant.

4. The method of claim 2 or 3 wherein the second solution comprises DMPC, DMPG, and polyoxyethylene sorbitan monolaurate.

5. The method of any one of claims 2-4 wherein the pH of the second solution is adjusted to 5.3± 0.2 with a pharmaceutically acceptable acid selected from the group consisting of HCl and H_2SO_4.

6. The method of any one of claims 2-5 wherein the third solution comprises about 10 wt. % Annamycin in DMSO.

7. The method of any one of claims 2-6 wherein each of the lipids and each of the surfactants are added individually.

8. The method of any one of claims 2-7 wherein DMPC, DMPG, and polyoxythylene sorbitan monolaurate are added sequentially to the first solution.

9. The method of any one of claims 2-8 wherein the preliposomal Annamycin lyophilizate comprises: 1.8 – 2.2 wt. % Annamycin; 3.0 – 3.4 wt. % Polysorbate 20; and 94.4 - 95.2 wt. % of lipids selected from DMPC and DMPG.

10. The method of claim 9 wherein the Annamycin in the lyophilizate produced is at least 98% pure.

11. The method of claim 10 wherein the Annamycin in the lyophilizate produced is at least 99% pure.

12. The method of any one of claims 4-11 further comprising:

after initially adjusting the pH of the second solution to pH 5.3± 0.2, then allowing the solution to equilibrate for at least 15 minutes;

measuring the pH and adjusting the pH with acid if the pH is above 5.3

allowing the solution to equilibrate for at least 15 minutes; and

repeating the steps of measuring the pH, adjusting the pH, and allowing the solution to equilibrate until the pH of the solution remains at pH 5.3± 0.2.

13. The method of claim 12 wherein the acid used to adjust the pH is selected from the group consisting of HCl, and H_2SO_4.

14. The method of claim 12 or 13 wherein said second solution is allowed to equilibrate for at least 30 minutes after each addition of acid.

15. The method of any one of claims 2-14 wherein each solution is maintained at a temperature of from 38 °C to 42 °C until lyophilization is started.

16. The method of claim 1 or 9 wherein said preliposomal Annamycin lyophilizate comprises 1.8 – 2.2 wt % Annamycin; 3.0 – 3.4 wt. % Polysorbate 20; and 94.4 - 95.2 wt. % of lipids selected from DMPC and DMPG.

17. The method of claim 16 wherein the DMPC is 65.3 -67.3 wt. % and the DMPG is 27.1 – 29.9 wt. %.

18. A method of producing preliposomal Annamycin lyophilizate comprising the steps of:

adding DMPC, DMPG and polysorbate 20 to a mixture of pre-warmed water and *t*-butanol to prepare a first solution, wherein the prewarmed temperature is from 35 °C to 42 °C, and wherein the ratio of water to *t*-butanol is from 8:2 to 9:1;

adjusting the pH of the first solution with one or more pharmaceutically acceptable acids, such that the pH of the solution remains constant for 15 – 90 minutes;

adding a second solution comprising approximately 8- 12 wt. % of Annamycin in DMSO to the first solution, to provide a lipid containing solution of Annamycin;

sterile filtering the lipid containing solution of Annamycin; and

lyophilizing the lipid containing solution in individual aliquots to provide a preliposomal Annamycin lyophilizate.

19. The method of claim 18, wherein the second solution comprises approximately 10 wt. % of Annamycin in DMSO, and the pH is 5.3 ± 0.2.

20. A composition of preliposomal Annamycin lyophilizate prepared by the method of claim 1, comprising: Annamycin, one or more lipids, and one or more non-ionic surfactants.

21. The composition of claim 20 comprising:
1.8 – 2.2 wt % Annamycin; 3.0 – 3.4 wt. % Polysorbate 20; and 94.4 - 95.2 wt. % of lipids selected from DMPC and DMPG.

22. The composition of claim 20 wherein the DMPC is 65.3 -67.3 wt. % and the DMPG is 27.1 – 29.9 wt. %.

23. The composition of any one of claims 20-22 wherein the Annamycin in the lyophilizate produced is at least 98 % pure.

24. Use of an effective amount of liposomal Annamycin prepared by reconstituting the preliposomal Annamycin lyophilizate made according to any one of claims 1-19, in the manufacture of a medicament for treating cancer in a patient in need thereof.

25. The use of claim 24 wherein said patient is human and the cancer is selected from the group consisting of leukemias and lymphomas.