anionic functional group; and b) one or more cationic ions, wherein the anionic ion or the anionic functional group is ionically paired with the cationic ions.

[0044] The anionic ion or the anionic functional group can be selected from one or more of the following: citrate, sulfate, sulfonate, phosphate, pyrophosphate, tartrate, succinate, maleate, borate, carboxylate, glucoronate, chloride, hydroxide, nitrate, cyanate or bromide. In one embodiment, the anionic ion and the anionic functional group is selected from one or more of the following: citrate, sulfate, sulfonate, phosphate, pyrophosphate, or carboxylate.

[0045] In yet another embodiment, the entity linked to the anionic functional group can be a natural or synthetic, organic or inorganic compound. Examples of the entity include, but are not limited to, non-polymer such as benzene, oligonucleotide and monosaccharide, or polymer such as polyvinyl, polyol such as glycerol, sorbitol and mannitol, polysaccharide, polypeptides, glycoproteins and polynucleotide.

[0046] The cationic ion of the pharmaceutically acceptable salt can be selected from one or more of the following: calcium ion, magnesium ion, sodium ion, potassium ion, manganese ion, or NR_4^+, wherein R is H or an organic residue such as alkyl or aryl, or a mixture thereof. In one embodiment, the cationic ion is ammonium.

[0047] A second embodiment of the present invention provides for an amphipathic acidic therapeutic agent, and a monovalent counterion donor within the liposome may be selected from or includes a cationic ion or an entity which is covalently linked to a cationic functional group. The cationic ion or the cationic functional group has a valency of +1, +2, or +3.

[0048] The pharmaceutically acceptable salt of the monovalent counterion donor comprises a) a cationic ion or an entity which is covalently linked to a cationic functional group; and b) one or more anionic ion, wherein the cationic ion or the cationic functional group is ionically paired with one or more anionic ions.

[0049] In one embodiment, the monovalent counterion donor is ammonium sulfate. In another embodiment, the concentration of the monovalent counterion donor is about 100 to about 500 mM, or any value or ranges therebetween in 10 mM increments (*e.g.* 80mM, 320 mM). In yet another embodiment, the concentration of the monovalent counterion donor is about 150 to about 450 mM. In yet another embodiment, the concentration of the monovalent counterion donor is about 200 mM to about 400 mM. In yet another embodiment, the concentration of the monovalent counterion donor is about 300 mM.

forming component further comprises a hydrophilic polymer.

[0061] Advantageously, by combining a polyvalent counterion donor or a pharmaceutically acceptable salt thereof, and a monovalent counterion donor or a pharmaceutically acceptable salt thereof, the encapsulation efficiency and/or retention profile of the therapeutic agent can be adjusted to maintain therapeutic efficiency, yet minimize toxicity.

[0062] In one group of embodiments, the anionic functional groups of the polyvalent counterion donor or its pharmaceutically acceptable salt have a total equivalent of valency about 1 to about 160 milliequivalent (mEq), about 3 to 160 mEq, about 1 to about 320 mEq, about 1 to about 250 mEq, about 3 to about 250 mEq, about 160 to about 250 mEq, about 160 to about 320 mEq or any value or ranges between 1 to 320 mEq in 1 mEq increments (*e.g.* 23 mEq, 233 mEq). In another embodiment, the anionic functional group of the polyvalent counterion donor or its pharmaceutically acceptable salt is sulfate.

[0063] In another group of embodiments, the concentration of the polyvalent counterion donor or its pharmaceutically acceptable salt is about 2mM to less than 8mM, about 0.1mM to less than 8mM, about 0.1 mM to less than about 10mM, about 2mM to less than 10 mM, or any value or ranges between 0.1mM to 10mM in 0.1 mM increments (*e.g.* 1.5 mM, 8.3 mM).

[0064] The pharmaceutical composition is formulated for any suitable administering routes including intracranial, intracerebral, intraventricular, intrathecal, intraspinal, oral, topical, rectal, transdermal, subcutaneous, intravenous, intramuscular intranasal, intraperitoneum, intratumor and the like.

[0065] The dosage of the pharmaceutical composition of the present invention can be determined by the skilled person in the art according to the embodiments. Unit doses or multiple dose forms are contemplated, each offering advantages in certain clinical settings. According to the present invention, the actual amount of the pharmaceutical composition to be administered can vary in accordance with the age, weight, general condition of the subject to be treated, the type of cancer, toxicity, and depends on the discretion of medical professionals.

[0066] In some embodiments, at least a portion of the therapeutic agent (such as vinorelbine) forms a salt with the pharmaceutically acceptable salt of polyvalent counterion donor and precipitates in the intraliposomal aqueous core, as evident in FIGURE 1.

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depends on the concentration of the polyvalent counterion donor. 8mM of sodium dextran sulfate is associated with a higher percentage of vinorelbine retention at 24 hours (78.9%) than that of 2mM of sodium dextran sulfate (51.8%).

[0092] Table 3. Characteristics of liposomal vinorelbine with mono- and polyvalent counterion donor combination

Pharmaceutical Composition	Types of counterion donor and concentration (mM)		Particle size	Encapsulation Efficiency	% of encapsulated vinorelbine remaining after 24h plasma incubation
	Ammonium Sulfate	Sodium Dextran Sulfate	nm	%	%
LV301	300	2	106.2	101.6	51.8
LV302	300	4	104.5	106.3	67.3
LV303	300	8	101.5	104.6	78.9

[0093] **Example 5: Effect of Various Polyvalent Counterion Donor Salts**

[0094] An *in vitro* study was conducted to assess the effect of different polyvalent counterion donor salts on the retention profile of liposome vinorelbine.

[0095] Liposomes prepared according to Example 1 were mixed with 300mM of ammonium sulfate (AS) and two different polyvalent counterion donor salts: dextran sulfate (DS) sodium salt and DS ammonium salt.

[0096] The encapsulating efficiency and the retention profile of liposomal vinorelbine was assessed using 24-hour *in vitro* plasma release method and the results are summarized in Table 4.

[0097] Results: The data shows that sodium salt and ammonium salt of dextran sulfate were equally effective in retaining vinorelbine in the liposome after 24 hours of plasma incubation. In addition, when the concentration of polyvalent counterion donor or its salt is at 10mM, the retention profile of the mono- and polyvalent counterion donor combination (100% and 94.2% of vinorelbine remaining in the liposome at 24 hours) was similar to that of polyvalent counterion donor composition (98.7% of vinorelbine remaining in the liposome after 24 hours). This is in contrast with the data in Table 3, wherein when the concentration of the polyvalent counterion donor was less than 10mM, the retention profile of liposomal vinorelbine depends on the concentration of the polyvalent counterion donor.

(day x - day 0)

- day x was the time taken for the tumor volume to double compared with the staging size.
- Skin Toxicity Score, assessed and graded based on the parameters listed in Table 9.

[00120] Table 9. Skin Toxicity Score

Grade	Signs & Severity	
	Hair loss & edema around the eyelid	Hair loss around the groin
0	None	None
1	Slight	Slight
2	Moderate	Moderate
3	Severe	Severe
4	Very severe	Very severe

[00121] Result:

[00122] Table 10 shows the percentage of tumor growth change (% T/C) on day 8 was similar between NanoVNB and LV304 groups (-41.0% for NanoVNB and -42.4% LV304). The mean tumor doubling time (Mean TDT) was > 78 days in the NanoVNB group, 67.1 days in LV304 group and 7.6 days in the control group. In addition, mice received LV304 displayed less side effects (less weight loss and lower skin toxicity score) relative to mice received NanoVNB.

[00123] FIGURE 2 shows the mean tumor volume in the NanoVNB group, the LV304 group and the saline (control) group. The results indicate that the mean tumor volume in the NanoVNB and LV 304 groups were lower than 200 mm^3 throughout the study period, whereas the mean tumor volume in the control group exceeded 3000 mm^3 at day 40.

[00124] These results indicate that LV304 is an effective anti-cancer therapeutic agent relative to NanoVNB, but displayed less side effects.

[00125] Table 10. Anti-cancer evaluation of NanoVNB, LV304 and saline in HT-29 human colon cancer model

Treatment Group	% T/C (day)*	Max. % BW change (day)*	Skin Toxicity Score (day)*	Mean TDT
Saline	-	-10.3 (34)	-	7.0 ± 2.4
NanoVNB	-41.0 ± 15.4 (8)	-20.8 (13)	37 (17)	> 78
LV304	-42.4 ± 13.1 (8)	-4.7 (13)	20 (15)	67.1 ± 5.4

* no. of days after Day 0

Example 9: *In Vivo* Anti-Cancer Evaluation Using PC14PE6/AS2 Human Lung Adenocarcinoma Orthotopic Model

[00126] An *in vivo* anti-cancer evaluation of the LV304 pharmaceutical composition was performed using an orthotopic PC14PE6/AS2 lung tumor model in mice.

[00127] The study design involved 3 study groups as follows:

[00128] NanoVNB Group: 6 mice were given 50% of the maximum tolerated dose (MTD) of NanoVNB (1/2 MTD=7.5mg/kg of vinorelbine) as a single intravenous injection on day 0.

[00129] LV304 Group: 6 mice were given 50% of the MTD of LV304 pharmaceutical composition (1/2 MTD= 10mg/kg of vinorelbine) as a single intravenous injection on day 0.

[00130] Control Group: 6 mice were given a single saline intravenous injection on day 0.

[00131] During the study period, the following outcomes were measured:

- Maximum body weight change, compare to the body weight on Day 0.
- Mean survival time.

[00132] Results: Referring to Table 11, the mean survival time for mice was 33.8 days after a single NanoVNB injection, 34.2 days after a single LV304 injection and 21.4 days after a single saline injection. FIGURE 3 shows the survival time in NanoVNB group and LV304 group were significant longer from the saline (control) group ($p < 0.01$).

[00133] Table 11. Anti-cancer evaluation of NanoVNB, LV304 and saline groups in PC14PE6/AS2 human lung adenocarcinoma orthotopic model.

Treatment Group	Max. % BW change (day)*	Mean survival time±SD (days)
Saline	-3.6 (18)	21.4 ± 2.0
NanoVNB	-12.6 (9)	33.8 ± 6.6
LV304	-17.0 (9)	34.2 ± 4.8

* days after drug administration on day 0

[00134] Example 10: *In Vivo* Skin Toxicity Evaluation Using SCID Mouse Model

[00135] An *in vivo* skin toxicity evaluation of the LV304 pharmaceutical composition was performed using BALB/c mice. Mice had free access to drinking water and food at all time during this trial and were randomized into 3 study groups as follows:

[00136] NanoVNB Group: 6 mice received 7.5 mg/kg of vinorelbine as NanoVNB, through a daily IV injection on Day 0 and 9, 5 mg/kg on Day 3 and 6.

[00137] LV304 Group: 6 mice received 7.5mg/kg of vinorelbine as LV304, through a daily IV injection on Day 0 and 9, 5mg/kg on Day 3 and 6.

[00138] Control Group: 6 mice received once daily IV saline injection on Day 0, 3, 6 and 9.

[00139] During the study period, the skin toxicity was assessed and scored based on the grading system in Table 9.

[00140] Results: FIGURE 4 shows the skin toxicity scores in the NanoVNB group and the LV304 group. The skin toxicity in the LV304 group was significantly less compare to the NanoVNB group during the 60 day trial period.

[00141] When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations, and subcombinations of ranges specific embodiments therein are intended to be included.

[00142] The disclosures of each patent, patent application, and publication cited or described in this document are hereby incorporated herein by reference, in their entirety.

[00143] Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.