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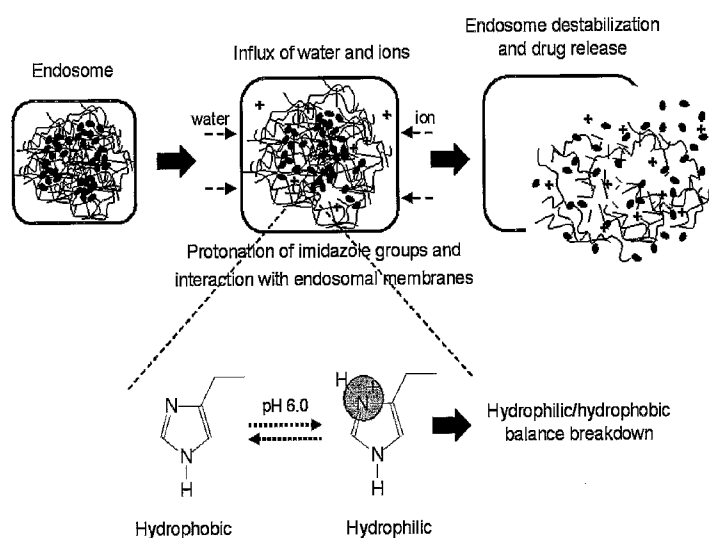
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(54) Title: CHITOSAN COMPLEX CONTAINING PH SENSITIVE IMIDAZOLE GROUP AND PREPARATION METHOD THEREOF



(57) Abstract: Disclosed herein a chitosan complex containing a pH-sensitive imidazole group and a method for the preparation thereof. The amino group of glycol chitosan is linked via amide bond to the histidine of the imidazole, which is hydrophobic in a neutral range, but hydrophilic in a weak acid region an imidazole group via an amide bond between the amino group of glycol chitosan. The chitosan complex can be loaded with various hydrophobic drugs, such as anticancer agents and gene, and labeled with traceable radioisotopes, so that it is useful as a drug delivery system, a scintigraphical tracer and a gene carrier.

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Description

CHITOSAN COMPLEX CONTAINING PH SENSITIVE IMIDAZOLE GROUP AND PREPARATION METHOD THEREOF

Technical Field

- [1] The present invention relates to a chitosan complex containing pH-sensitive imidazole group and a preparation method thereof.

[2]

Background Art

- [3] In most cases, medicines are administered at larger doses than are necessary for suitable efficacy on diseases or disorders. Effective as this is in treating diseases or disorders, overdoses are likely to produce side effects. For this reason, most pharmaceutical companies have great interest in dosage forms that can guarantee high medicinal effects with little side effects.
- [4] In this regard, great attention has been paid to drug delivery systems (hereinafter referred to as "DDS"). Developed to maximize pharmaceutical efficacy and effects as well as minimizing side effects, DDS are useful in increasing the selectivity of drugs which must be administered at low doses or are difficult to administer. Physico-chemically or pharmacokinetically unusual properties of drugs to be administered, such as high water solubility, high oil solubility or insolubility, require the use of special DDS. In addition, drugs having special requirements, for example, highly toxic drugs for single injection, unstable cytotoxic drugs, high clearance drugs, drugs apt to be inactivated in vivo, and drugs for topical application must be accompanied by suitable DDS.
- [5] Since 1970's, DDS techniques have been under active study in advanced countries. It is known that the time period and cost necessary for the development of new drugs amounts to 15 years and two hundred million for each drug on average, respectively, which is three times as much as those for the amelioration of conventional drugs with new DDS. This is also found to have a high success rate compared to the DDS. With an increasing sense of impending crisis according to the introduction of the product patent system in Korea in 1987, many domestic pharmaceutical companies focused their resources on the development of new DDS. In Korea, active research has been conducted on DDS since 1990, with a gradual increase in the number of patent applications from 1992.
- [6] Chitosan can be produced through the deacetylation of chitin, the second most abundant polysaccharide in nature after cellulose, and is a linear polysaccharide

composed of 2-amino-2-deoxy- β -D-glucopyranose. In contrast to many naturally occurring polysaccharides, chitosan has primary amines in its backbone chain and thus finds useful applications in a variety of fields including environmental, agricultural, medicinal fields, etc. thanks to its unique characteristics. Superior in biocompatibility and biodegradability, chitosan is an intensive research target of great interest for use in gene and drug carriers, scaffolds for tissue engineering, and injectable hydrogel (Polym. Int. 1999, 48, 732~734).

[7]

[8] Having the main theme of controlling matter on a scale smaller than one micrometer, normally between 1 and hundreds of nanometers, nanotechnology is a field of applied science and technology covering a broad range of topics. Particularly, nanotechnology is intensively applied to nanoscale carriers for transferring therapeutically useful ingredients, such as chemical components, oligonucleic acid, siRNA, DNA, proteins, etc., into cells. In order to afford the obvious effects thereof, the nanoscale carriers release drugs within cells after passing through lipid bilayers of cell membranes via specific or general passages.

[9]

[10] In an aqueous phase, amphophilic macromolecules, both hydrophilic and hydrophobic, form into micelles or self-aggregates to obtain stable interfacial energy as a result of interaction between hydrophobic blocks. Depending on the hydrophilicity and hydrophobicity thereof, micelles of amphophilic macromolecules change in size, size distribution, rheology, and thermodynamic stability. Recently, micelles having hydrophobic drugs entrapped therein have been used as carrier vesicles for the selective and effective transfer of drugs (Adv. Drug. Deliv. Rev. 2001, 47, 113-131).

[11]

[12] As such, a block copolymer consisting of polyethyleneoxide and polyaspartic acid was developed into micelles with the anticancer agent adriamycin combined chemically therewith, by Prof. Kataoka, Tokyo University (J. Control. Release. 2001, 74, 295-302).

[13]

Okano, et al., developed an amphiphilic block copolymer consisting of N-isopropylacrylamide and styrene, which was reported as a targeting drug carrier vesicle using the thermo-responsive behavior by which it forms into 20 nm-size micelles at room temperature in an aqueous phase and a large aggregate at 32°C or higher by interaction between micelles (J. Control. Release. 1997, 48, 157-164).

[14]

Sunamoto et al. developed cholesterol-bearing polysaccharides which are reportedly used as protein drug carrier vesicles (J. Am. Chem. Soc. 1996, 118, 6110-6115).

[15]

[16] However, no conventional techniques describe drug delivery systems which are

selective for differences in pH from other loci within the same organ, for example, tumors.

[17]

Disclosure of Invention

Technical Problem

[18]

It is therefore an object of the present invention to provide a chitosan complex containing a pH-sensitive imidazole group, which is not only superior in biocompatibility and biodegradability, but also shows pH-dependent micelle formation wherein nano-self-aggregates form in neutral or basic conditions and disintegrate in acidic conditions in order to release drugs entrapped therein into cells, and a method of preparing the same.

[19]

Technical Solution

[20]

In accordance with an aspect of the present invention, there is provided a chitosan complex, capable of forming nano self-aggregates in aqueous phases, comprising a water-soluble chitosan chain grafted with a pH-sensitive imidazole group.

[21]

In accordance with another aspect of the present invention, there is provided a method for preparing a chitosan complex, comprising reacting a pH-sensitive imidazole group with water-soluble chitosan to form an amide group therebetween.

[22]

Advantageous Effects

[23]

The present invention provides a chitosan complex pH-sensitive containing imidazole group and a method for preparing the same. The chitosan complex is superior in biocompatibility and biodegradability, and forms globular aggregates ranging in size from tens to hundreds of nanometers, which consist of hydrophilic glycol chitosan on the surface and hydrophobic histidine inside. The chitosan complex can be loaded with various hydrophobic drugs, such as anticancer agents and genes, and can be labeled with traceable radioisotopes, so that it is useful as a drug delivery system, a scintigraphical tracer and a gene carrier.

[24]

Brief Description of the Drawings

[25]

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

[26]

FIG. 1 is a diagram illustrating the intracellular behavior of the chitosan complex according to the present invention;

[27]

FIG. 2 is a graph showing the particle size distribution of nano self-aggregates of the

chitosan complex according to an embodiment of the present invention, measured using dynamic light scattering (DLS);

[28] FIG. 3 shows cell cycles of cancer cells, obtained by FACS (fluorescence activated cell sorting) when cancer cells are treated with a chitosan complex loaded with paclitaxel in accordance with an embodiment of the present invention; and

[29] FIG. 4 provides photographs showing the selectivity of the chitosan complex of the present invention for tumors, obtained through nuclear scintigraphy with mice injected with a radiolabeled (^{131}I) chitosan complex through the tail vein.

[30]

Best Mode for Carrying Out the Invention

[31] Below, a detailed description is given of the present invention.

[32] In accordance with an aspect thereof, the present invention pertains to a chitosan complex containing a pH-dependent imidazole group, which can form into self-aggregates.

[33] The formation of the chitosan complex of the present invention into self-aggregates is based on the presence of both a hydrophobic moiety and a hydrophilic moiety which are linked to each other via the linkage of the amino group of water-soluble chitosan to the carboxylic moiety of the pH-sensitive imidazole group.

[34] Water-soluble glycol chitosan, chitosan oligomer, or acetylated chitosan may be used as the water-soluble chitosan.

[35] Examples of the pH-sensitive imidazole group useful in the present invention include, but are not limited to, histidine, N-acetyl histidine, and polyhistidine.

[36] Preferably, the pH-sensitive imidazole group is substituted in an amount of 1 ~ 30% based on the number of moles of chitosan monomer in order to form self-aggregates optimal for entrapping drugs.

[37] Having amphiphilicity resulting from the hydrophobicity of the pH-sensitive imidazole group and the hydrophilicity of chitosan, the chitosan complex containing a pH-sensitive imidazole group can form into globular self-aggregates in an aqueous phase.

[38] Preferably, the chitosan complex ranges in size from 50 nm to 500 nm in order to migrate to target cells.

[39]

[40] In accordance with another aspect thereof, the present invention pertains to a chitosan-nano-paclitaxel comprising a chitosan complex with paclitaxel entrapped therein, the chitosan complex, capable of forming into self-aggregates, consisting of a water-soluble chitosan chain grafted with a pH-sensitive imidazole.

[41] While the chitosan nano-paclitaxel undergoes endocytosis, the histidine group in the

self-aggregate changes from hydrophobicity to hydrophilicity because the pH condition of the endosome is of acidity. As the equilibrium between hydrophobicity and hydrophilicity in the self-aggregate collapses, the paclitaxel entrapped therein is released. Therefore, the aggregate of the present invention with pH-sensitive imidazole groups introduced therein can solve the problem in which conventional aggregates, although able to enter cells, release drugs too slowly or with great difficulty.

[42] Paclitaxel is entrapped at an efficiency of 30 ~ 95% in the chitosan complex. This increased entrapment efficiency leads to an increased anticancer effect of the chitosan nanoparticle.

[43] As long as it can be physically entrapped in the chitosan complex containing a pH-sensitive imidazole group, any hydrophobic anticancer agent can be employed. Examples of hydrophobic anticancer agents useful in the present invention include adriamycin, cis-platin, mitomycin-C, daunomycin, and 5-fluorouracil.

[44]

[45] A typical micelle in aqueous solution forms an aggregate of amphiphilic molecules with the hydrophilic "head" regions in contact with the surrounding solvent, sequestering the hydrophobic tail regions in the micelle center [Adv. Drug Deliv. Rev., 1996, 21, 107]. Hence, micelles are widely used as drug delivery systems for effectively carrying hydrophobic anticancer agents. The DDS based on self-aggregates of amphiphilic molecules exhibits highly sufficient selectivity for target cells with greatly reduced toxicity on normal cells and the sustained release of drugs, and thus can be applied to new paradigm therapy for serious diseases, such as cancer.

[46] Thanks to the enhanced permeability and retention (hereinafter referred to as "EPR") thereof, the chitosan complex containing a pH-sensitive imidazole in accordance with the present invention, when entrapping an anticancer agent, shows higher selectivity for cancer tissues than do low molecular weight anticancer agents alone. Thus, when delivered by the chitosan complex of the present invention, anticancer agents can accumulate in larger amounts in cancer tissues, resulting in more effective anticancer activity. In an aqueous phase, the chitosan complex with a hydrophilic chitosan backbone linked to pH-sensitive histidine spontaneously forms globular self-aggregates, ranging in size from tens to hundreds nanometers, which can be used as target-directed carriers of anticancer agents and release the entrapped anticancer agents over a long period of time.

[47] In accordance with a further aspect thereof, the present invention pertains to a method for preparing a chitosan complex, comprising linking a pH-sensitive imidazole group to water-soluble chitosan via an amide bond.

[48] The amide bond between a water-soluble chitosan backbone and a pH-sensitive imidazole group may be further induced in the presence of

1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (hereinafter referred to as "EDC") and N-hydrosuccinimide (hereinafter referred to as "NHS").

[49] The examples of the pH-sensitive imidazole group useful in the present invention include histidine, N-acetyl histidine, and polyhistidine, but are not limited thereto.

[50] For the preparation of the chitosan complex according to the present invention, water-soluble chitosan is dissolved in phosphate buffered saline.

[51] Also, the pH-sensitive imidazole group, which is a histidine based compound, is dissolved in phosphate buffered saline.

[52] The pH-sensitive imidazole group may be grafted to chitosan in an amount of 1~30 mol% based on the total number of moles of chitosan monomers in order for the chitosan complex to readily form self-aggregates.

[53] The method may further comprise entrapping nano-paclitaxel within the core formed by the hydrophobic pH-sensitive imidazole group of the chitosan complex.

[54]

[55] Capable of effectively entrapping various hydrophobic drugs, such as anticancer agents and genes, therein, as described above, the chitosan complex of the present invention is useful as DDS.

[56]

[57] A better understanding of the present invention may be obtained through the following examples which are set forth to illustrate, but are not to be construed as the limit of the present invention.

[58]

Mode for the Invention

[59] <EXAMPLE 1> Preparation of Chitosan Complex containing histidine

[60] To a suspension of 0.5 g of glycol chitosan (Mw: 250 kDa) in 100 ml of phosphate buffered saline, histidine was added dropwise in an amount of 5~30 moles per 100 moles of the glycol chitosan. EDC and NHS were also added dropwise in an amount 3 times as great as the number of moles of the histidine added, followed by stirring at room temperature for 24 hours. After completion of the reaction, the reaction mixture was dialyzed against de-ionized water for 4 days using a semi-permeable membrane. The dialysate was freeze-dried to yield chitosan nano self-aggregates containing histidine.

[61]

[62] <EXAMPLE 2> Preparation of Chitosan Complex containing N-Acetyl Histidine

[63] Nano self-aggregates of chitosan complex containing an N-acetyl histidine were prepared in a manner similar to that of Example 1, with the exception that N-acetyl histidine in an amount of 5~30 moles per 100 moles of the glycol chitosan monomers

was added to the same suspension as in Example 1, followed by the addition of EDC and NHS in an amount three times as large as that of N-acetyl histidine.

[64]

[65] <EXAMPLE 3> Chitosan Complex Containing Histidine Labeled with *In-Vitro* Traceable Radioisotope

[66]

[67] To a suspension of 0.5 g of the chitosan complex of Example 1 in 100 ml of de-ionized water, N-acetyl tyrosine was added dropwise in an amount of 1 ~ 20 moles per 100 moles of the glycol chitosan monomers, followed by the introduction of the radioisotope ¹³¹I. After the completion of the reaction, the reaction mixture was dialyzed against de-ionized water for 4 days using a semi-permeable membrane. The dialysate was freeze-dried to yield chitosan nano self-aggregates containing histidine labeled with a radioisotope.

[68]

[69] <EXAMPLE 4> Chitosan Complex Containing N-Acetyl Histidine Labeled with *In-Vivo* Traceable Radioisotope

[70]

[71] Nano self-aggregates of chitosan complex containing an N-acetyl histidine labeled with radioactive ¹³¹I were prepared in a manner similar to that of Example 3, with the exception that the chitosan complex of Example 2 was used.

[72]

[73] <EXPERIMENTAL EXAMPLE 1> Measurement of Degree of Substitution of N-Acetyl Histidine in Chitosan Complex

[74]

[75] The content of N-acetyl histidine in the chitosan complex of the present invention was measured as follows.

[76]

The chitosan complex prepared in Example 2 was dissolved at a concentration of 1 mg/ml in a mixture solvent of heavy water (D₂O)/dimethylsulfoxide (d-6) (1/4, v/v) and analyzed with ¹H-NMR, followed by measuring the degree of substitution of N-acetyl histidine from the histidine characteristic peaks around 7 ~ 8 ppm and the C-2 hydrogen characteristic peaks of chitosan around 3.0 ~ 3.3 ppm.

[77]

The results are summarized in Table 1 below.

[78]

Table 1

[Table 1]

[Table]

	Molar RatioN-Acetyl Histidine/ 100 moles of Chitosan Monomer	Degree of Substitution of N-Acetyl Histidine (%)
Example 2	5	3.5
	10	6.7
	20	12.3
	30	17.8

[79] As is apparent from the data of FIG. 1, when higher molar ratios of N-acetyl histidine to chitosan monomer are used, higher degrees of substitution thereof in chitosan complex result.

[80]

[81] <EXPERIMENTAL EXAMPLE 2> Conformational Change of Chitosan Complex According to pH

[82]

[83] The sizes of nano self-aggregates of the chitosan complex in aqueous phases were measured in the following experiments.

[84] The chitosan complex containing N-acetyl histidine of Example 2 was dissolved at a concentration of 1 mg/ml in phosphate buffered saline at a pH of 7.4 to form self-aggregates which were then analyzed for size using dynamic light scattering (hereinafter referred to as "DLS"). The results are shown in FIG. 2.

[85] As seen in FIG. 2, the self-aggregates formed in aqueous phase at pH 7.4 were nano-sized. That is, the chitosan complex of the present invention can form nano-size self-aggregates in neutral aqueous phases.

[86]

[87] <EXPERIMENTAL EXAMPLE 3> Cytotoxicity of AntiCancer Agent-Trapped Nano-sized Self-Aggregate Against Cancer Cell (In Vitro)

[88]

[89] The nano self-aggregates with an anticancer agent entrapped therein were assayed for cytotoxicity against cancer cells as follows.

[90] After the anticancer agent paclitaxel was loaded into the inside thereof, the nano self-aggregates of Example 2 were applied to a human lung cancer cell line (A549) and a human breast cancer cell line (MDA-MB231). In order to determine the anticancer effect of the nano self-aggregates, these cells were observed for change in cell cycle using FACS (fluorescence activated cell sorter). The results are shown in FIG. 3.

[91] As shown in FIG. 3, neither the human lung cancer cell line nor the human breast

cancer cell line showed any significant difference in G_0/G_1 phase from a control within 1 hour after treatment with the anticancer agent-loaded nano self-aggregates. However, both the cell lines were observed to start to increase in G_2/M phase 6 hours after the treatment and to greatly increase in G_2/M phase 24 after the treatment compared to the control. This is believed to be attributed to the fact that, when transferred inside cancer cells, the nano self-aggregates loaded with the anticancer agent were disintegrated due to the low pH of cancer cells to thus release the anticancer agent within plasma, which caused cell cycle arrest leading to the necrosis of cancer cells.

[92] Consequently, the nano self-aggregates were proven to be an excellent drug delivery system.

[93]

[94] <EXPERIMENTAL EXAMPLE 4> Selectivity of Nano Self-Aggregate for Tumor (*In vivo*)

[95]

[96] The nano self-aggregates were assayed for selectivity for tumors as follows.

[97] The chitosan complex of Example 2 was labeled with an *in-vivo* traceable radioisotope as in Example 4 and injected into cancer cell-implanted mice via the tail vein. The nano self-aggregates were observed for tumor selectivity through images acquired using a gamma camera. The resulting nuclear scintigraphs are shown in FIG. 4.

[98] As seen in FIG. 4, the images of the radioisotope (^{131}I)-labeled nano self-aggregates, taken with a gamma camera, show the focal location of the radioisotope in cancer regions, demonstrating that the nano self-aggregates are highly selective for tumors and traceable *in vivo*.

[99]

Industrial Applicability

[100] As described hitherto, the present invention provides a chitosan complex pH-sensitive containing imidazole group and a method for preparing the same. The chitosan complex is superior in biocompatibility and biodegradability, and forms globular aggregates ranging in size from tens to hundreds of nanometers, which consist of hydrophilic glycol chitosan on the surface and hydrophobic histidine inside. The chitosan complex can be loaded with various hydrophobic drugs, such as anticancer agents and genes, and can be labeled with traceable radioisotopes, so that it is useful as a drug delivery system, a scintigraphical tracer and a gene carrier.

[101] Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of

the invention as disclosed in the accompanying claims.

[102]

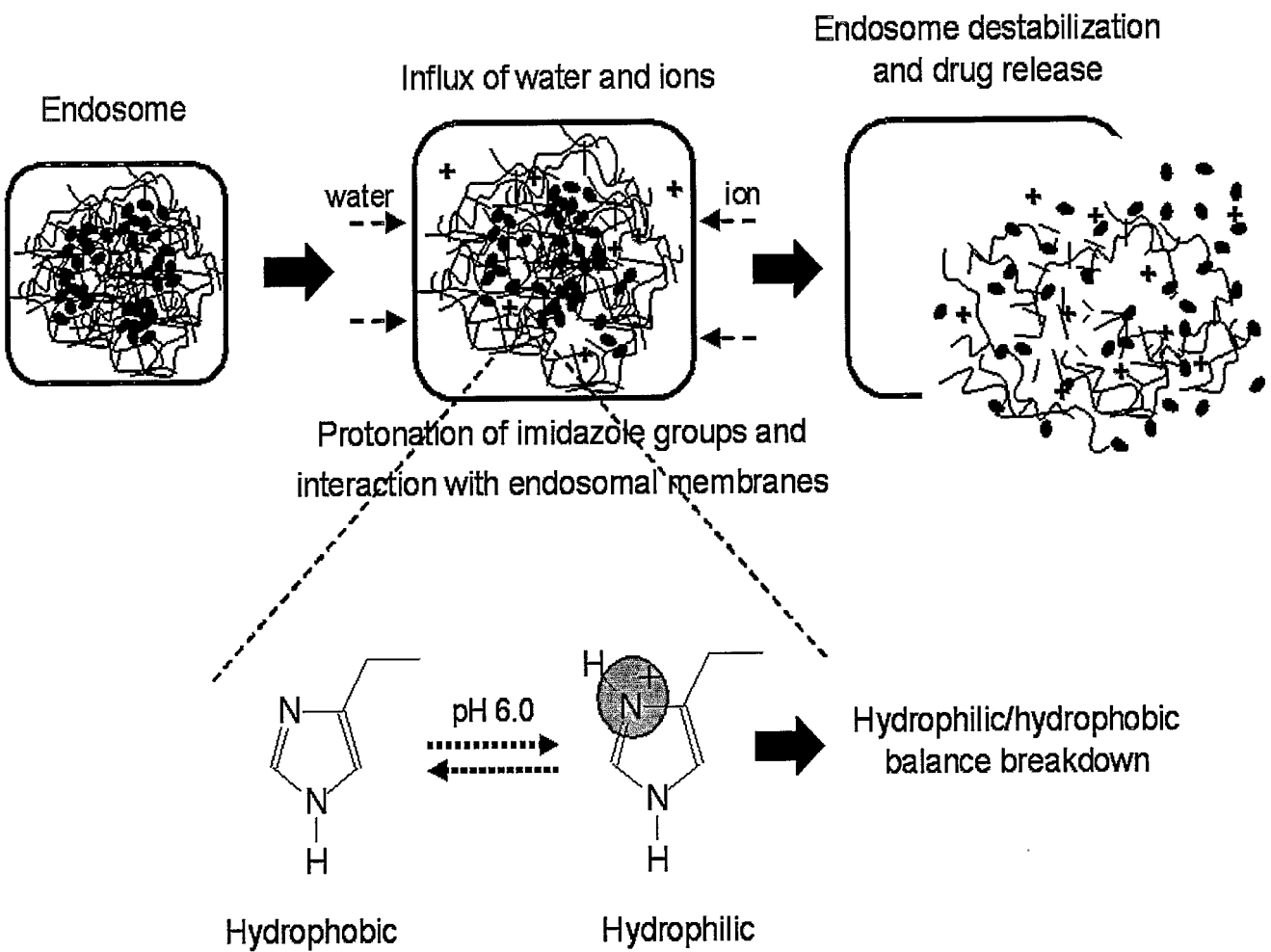
Claims

- [1] A chitosan complex, capable of forming self-aggregates in aqueous phases, comprising a water-soluble chitosan chain grafted with a pH-sensitive imidazole group.
- [2] The chitosan complex according to claim 2, wherein the water soluble chitosan chain is linked to the pH-sensitive imidazole group via a linkage between the amino group of chitosan and the carboxylic group of the imidazole group.
- [3] The chitosan complex according to claim 1 or 2, wherein the water-soluble chitosan is selected from a group consisting of water-soluble glycol chitosan, a chitosan oligomer, acetylated chitosan and a combination thereof.
- [4] The chitosan complex according to claim 1 or 2, wherein the pH-sensitive imidazole group is selected from a group consisting of histidine, N-acetyl histidine, polyhistidine and a combination thereof.
- [5] The chitosan complex according to claim 1 or 2, wherein the pH-sensitive imidazole group is grafted in an amount of 1 ~ 30 % based on a number of moles of chitosan monomer.
- [6] The chitosan complex according to claim 1 or 2, wherein the chitosan complex ranges in size from 50 to 500 nm.
- [7] Chitosan nano-paclitaxel in the form of self-aggregates in aqueous phases, said self-aggregates comprising a water-soluble chitosan chain grafted with a hydrophobic, pH-sensitive imidazole group with paclitaxel entrapped therein.
- [8] A method for preparing a chitosan complex, comprising reacting a pH-sensitive imidazole group with water-soluble chitosan to form an amide group therebetween.
- [9] The method according to claim 8, wherein the reacting is conducted in presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and N-hydroxysuccinimide.
- [10] The method according to claim 8, wherein the pH-sensitive imidazole group is selected from a group consisting of histidine, N-acetyl histidine, polyhistidine and a combination thereof.
- [11] A method for preparing chitosan nano-paclitaxel, comprising allowing the chitosan complex of claim 8 or 9 to aggregate in the presence of paclitaxel in an aqueous phase to entrap the paclitaxel in the aggregates.
- [12] Use of the chitosan complex of one of claims 1 to 7 in anticancer therapy (delivery of an anticancer agent).
- [13] The chitosan complex for use in anticancer therapy, prepared by the method of one of claims 8 to 11.

DRAWINGS

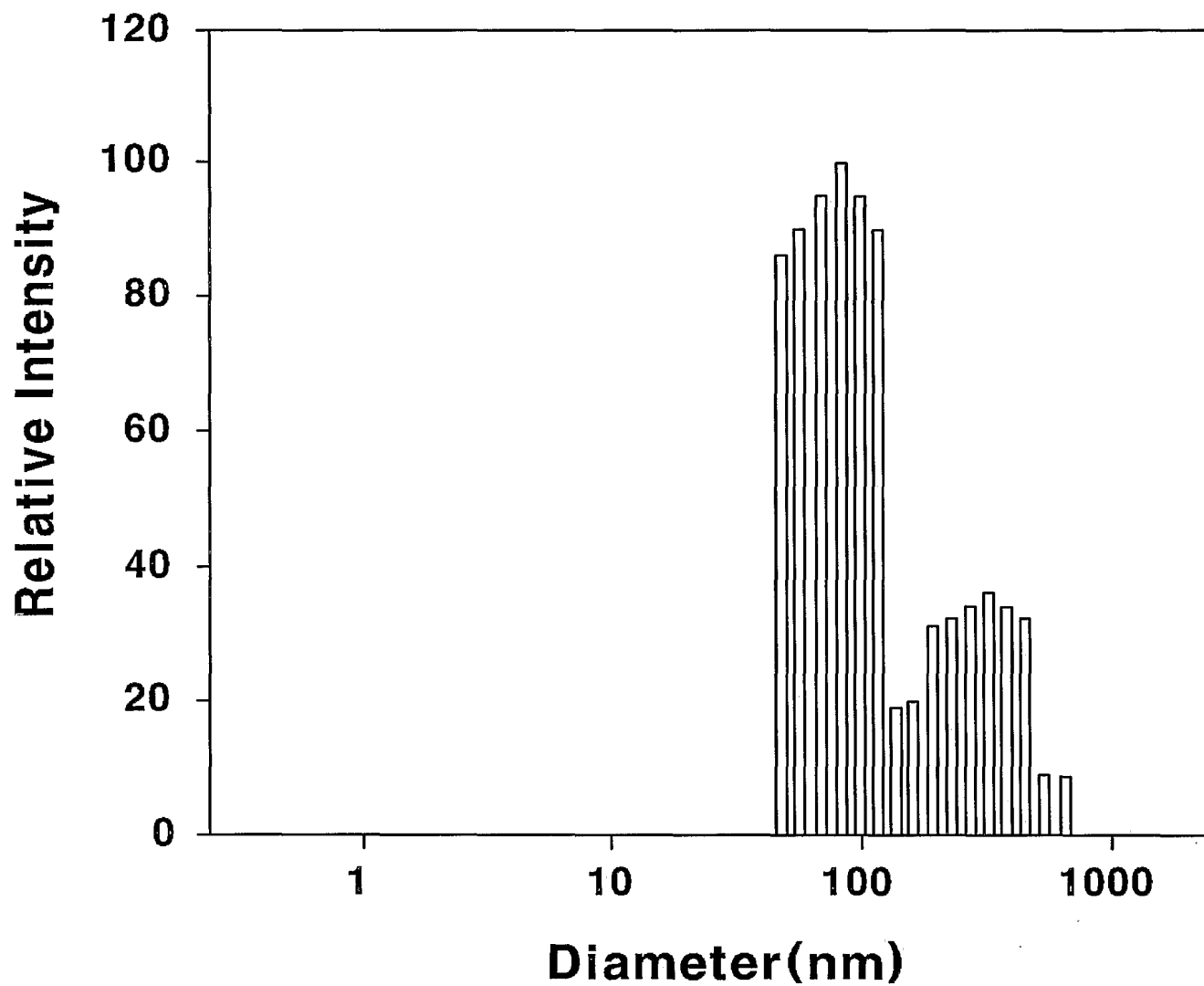
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FIGURE 1



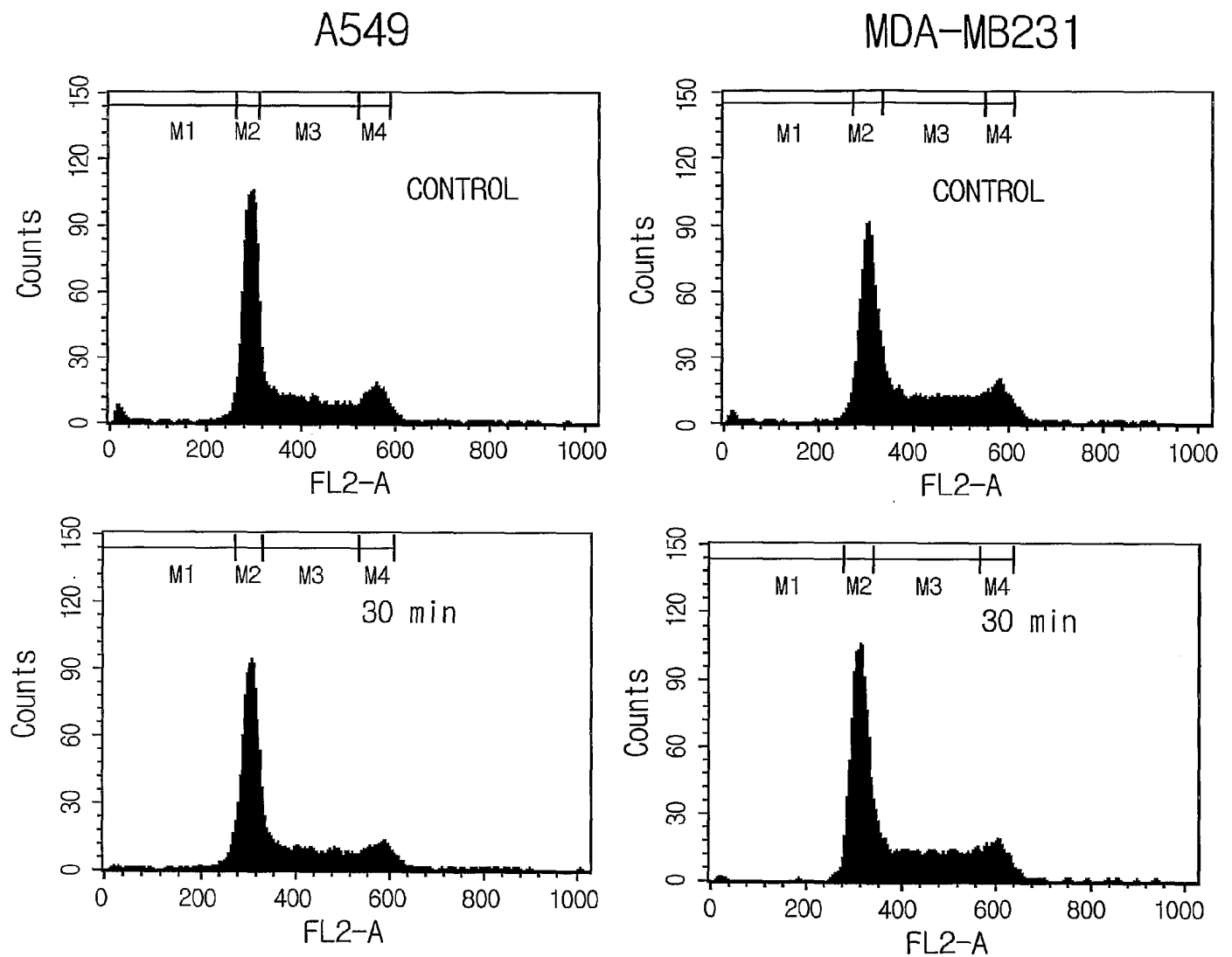
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FIGURE 2



3/4

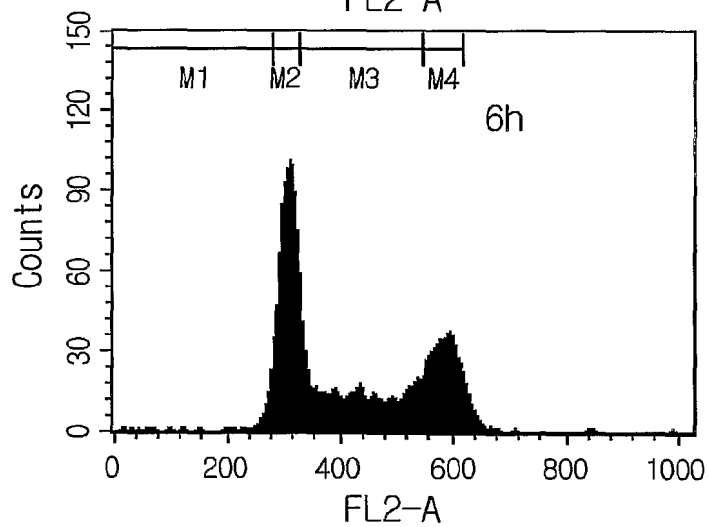
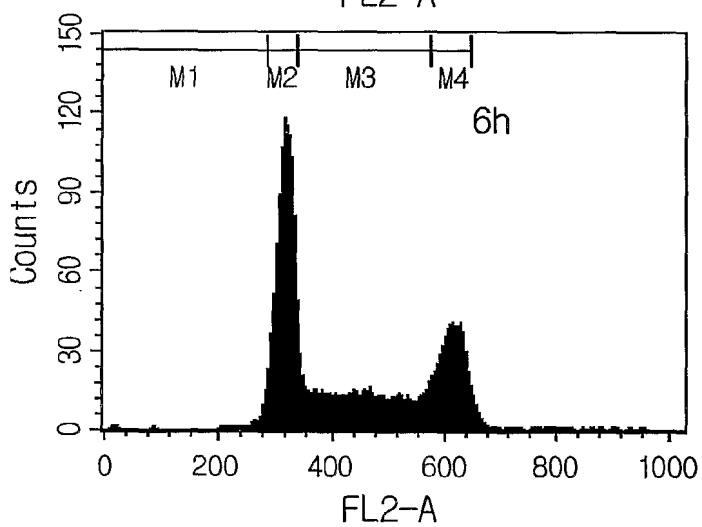
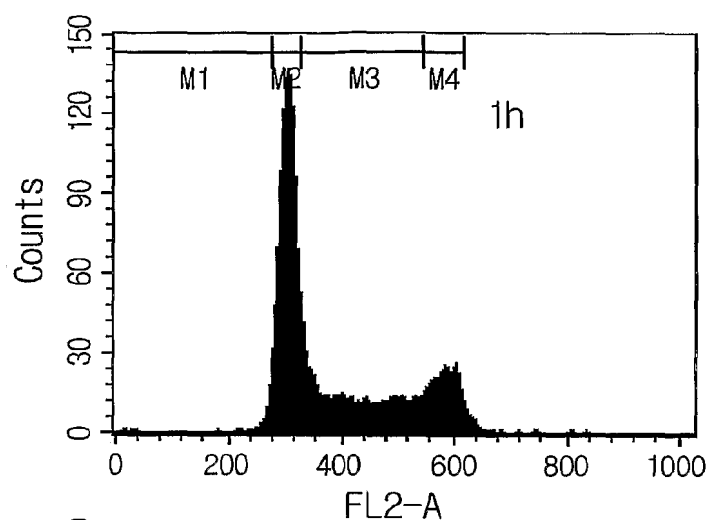
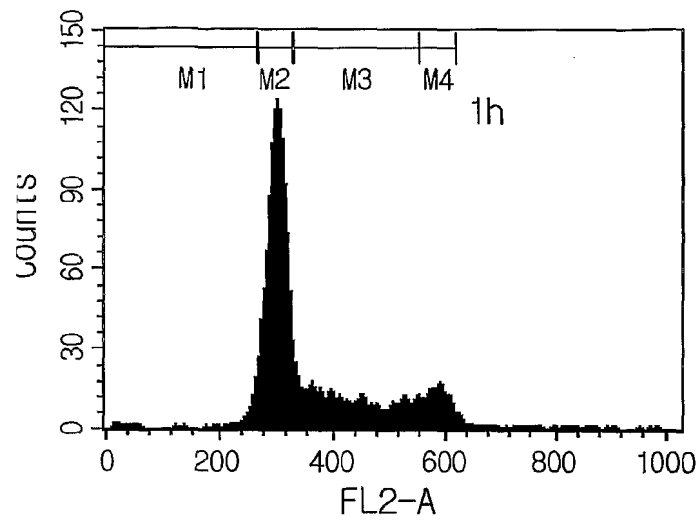
FIGURE 3



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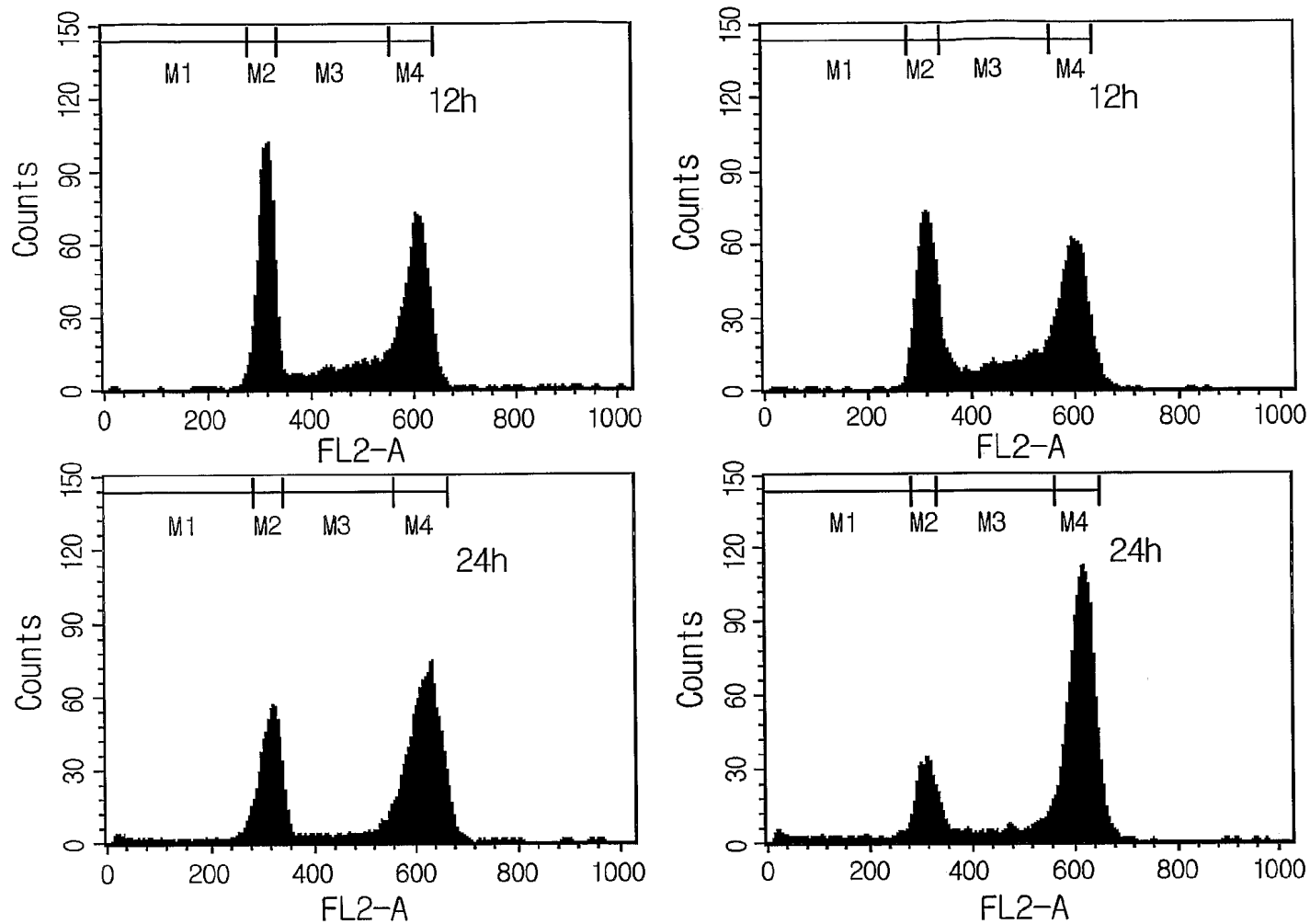
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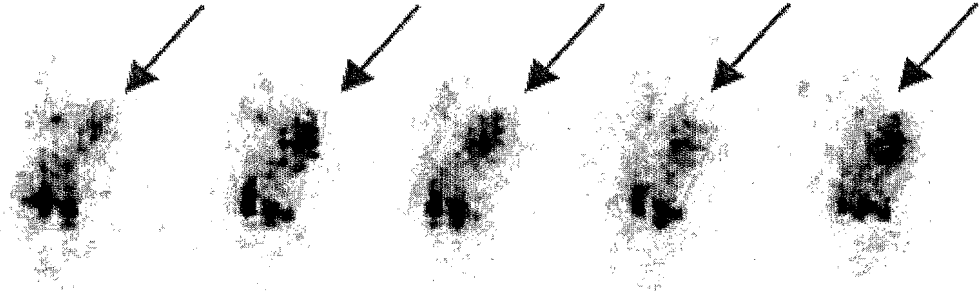
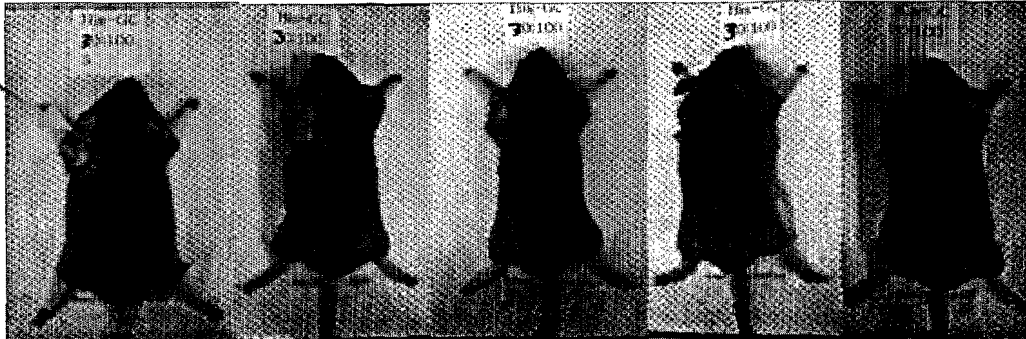
Continuous page of FIGURE 3



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FIGURE 4

tumor



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2007/003447

A. CLASSIFICATION OF SUBJECT MATTER <i>A61K 47/36(2006.01)i, A61K 45/00(2006.01)i, C08B 37/08(2006.01)i</i> According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 8 : A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) eKIPASS(KIPO Internal), CAS(ON LINE), DELPHION		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KR1020050097189 A (SEOUL NATIONAL UNIVERSITY INDUSTRY FOUNDATION) 07 October 2005 See the abstract and claims 1-4.	1-13
A	LOMADZE, N. and SCHNEIDER, H-J., 'A chitosan-based chemomechanical polymer triggered by stacking effects with aromatic effectors including aminoacid derivatives.' Tetrahedron, July 2005, Vol.61, No.36, pp8694-8698. See the scheme 2.	1-13
A	WO 2005/021514 A1 (THE UNIVERSITY OF TOKYO) 10 March 2005 See the whole document.	1-13
A	US 5912000 A (ZONAGEN, INC.) 15 June 1999 See the whole document.	1-13
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2007/003447

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 2005097189 A	07. 10. 2005	None	
WO 2005021514 A1	10. 03. 2005	JP 17075765 A2	24. 03. 2005
US 5912000 A	15. 06. 1999	None	