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(54) Title: FRAGRANCED CHITOSAN

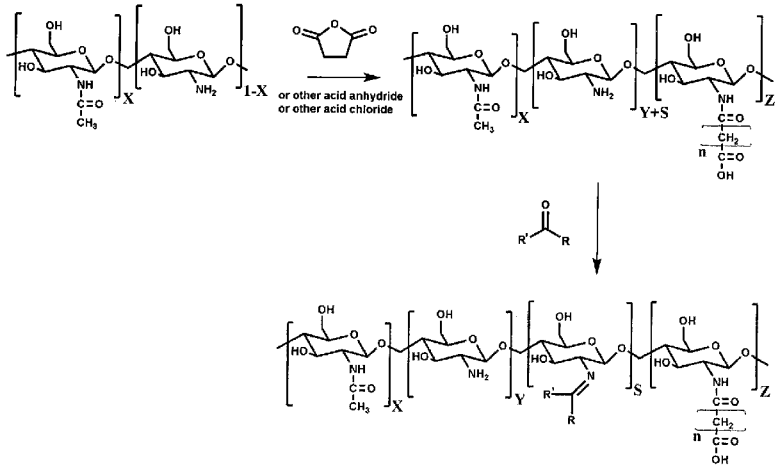

Figure 1

(57) Abstract: This invention involves the synthesis and fabrication of long lasting fragranced chitosan. The chemical synthesis and structures of chitosan derivatives in various forms, e.g. nano/microspheres, colloidal suspension of nano/microspheres, etc., are disclosed. Said chitosan derivatives are capable to slowly release aldehydes and/or ketones, thus suitable for fragrance controlled release materials.

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FRAGRANCED CHITOSAN

TECHNICAL FIELD

This invention is related to a fragranced chitosan and a process for making the fragranced chitosan.

This invention involves the synthesis and fabrication of long lasting fragranced chitosan. The chemical synthesis and structures of chitosan derivatives in various forms, e.g. nano/microspheres, colloidal suspension of nano/microspheres, etc., are disclosed. Fragrance molecules are chemically bonded to the chitosan backbone and at the same time embedded into the particles' core. Said chitosan derivatives are capable to slowly release aldehydes and/or ketones, thus suitable for fragrance controlled release materials.

BACKGROUND OF THE INVENTION

Various unique odorous molecules are being synthesized or isolated from natural sources and used as fragrance components in various industries. Fragranced chemicals are used worldwide not only in spa, cosmetics and toiletries, but also in many scented household and occupational products. However, many of these fragrance molecules are unstable due to their reactive functionalities, such as aldehyde, ketone and terpenes, resulting in degradation. The degradation not only causes changes in their sensory characteristics, but also, in many cases, creates allergenic products. It has been known that control of the volatilization rate and degradation is an essence of prolonging the sensory characteristics of fragrance materials.

One way of doing so is encapsulation, which provides both stabilization and a controlled release of the entrapped materials. Other benefits of encapsulation include ease of handling (e.g. a stable solid encapsulated product instead of an unstable volatile liquid), improved safety (e.g. reduced flammability) and an increased applicability to various products (e.g. water dispersible essential oil-encapsulated spheres can be easily applied in water based formulations). However, the fragrance release properties are the key issue in selecting a particular encapsulation technology.

The existing fragrance encapsulation technologies includes double emulsion preparation, molecular inclusion into a host, such as cyclodextrin, incorporation into solid lipid nanoparticles using appropriate lipids and surfactants, coacervation with various carbohydrates with and without the use of crosslinking agents, interfacial polymerization based on various polymers, such as polyurethane-urea (PUU) and phenol-formaldehydes and *in situ* polymerization, such as the synthesis of fragrance encapsulated

mesoporous silica spheres. Amongst theses, interfacial polymerization and complex coacervation are the two most popular choices.

Examples of inventions relating polymeric encapsulated fragrance materials are *US Patent No. 5,112,688* discloses microcapsules prepared using coacervation processes and containing perfume, especially desirable for inclusion in fabric softener compositions; *US Patent No. 5,145,842* discloses perfume particles comprising perfume dispersed within relatively low molecular weight nonpolymeric carrier materials, and encapsulated with a friable coating material, e.g. aminoplast shell, used in cleaning and fabric conditioning composition; *US Patent No. 7,125,835* discloses the fragrance encapsulated by a first polymer material to form a fragrance encapsulated polymer, then the polymer encapsulated shell being coated with a mixture of cationic polymers where the coating polymers are a reaction product of polyamides and (chloromethyl) oxirane or (bromomethyl) oxirane; and *US Patent No. 7, 294,612* discloses similar process to US Patent No. 7,125, 835 where the preferred cationic polymers are starch and guar

Partial solubility in water of many essential oils' components usually causes instability in the microencapsulation by interfacial reactions because of the change in the hydrolytic stability of the particle during polymerization reaction. Moreover, side reactions between the monomers with several reactive functionalities of the essential oil's components can lead to some alteration of the encapsulated products, especially in the PUU system. To avoid such drawbacks, the environmental unfriendly phenol-formaldehyde (melamine-formaldehyde) has been used. The system is neither biocompatible nor biodegradable which limits its applications.

Complex coacervation has been reported and patented by several authors as a method for fragrance prolongation. However, the coacervated products generally have a weak mechanical resistance, due to water solubility of the polymer, making them inappropriate for many applications where a long shelf-life and a good mechanical strength are required. The use of crosslinking agents to improve the stability, although it has been demonstrated, possesses the drawbacks of side reactions between the encapsulated material and the residues of the crosslinking agents.

The above controlled release systems are classified as physical barrier system in which the diffusion of active molecules is controlled by encapsulating them into polymeric matrix. However, there is another type of controlled release system, a chemical barrier system, in which active molecules are modulated

via chemical derivatization into more robust forms of which the original active molecules can be reversibly generated in a controllable manner.

Various labile chemical bonds have been employed for the controlled release of various functional molecules and their examples include the controlled release of alcohol using neighboring-group-assisted ester hydrolysis, the use of Schiff base to help controlling the release of aldehyde and ketone, the use of Norrish type-II photofragmentation to controllably deliver of alkene and acetophenone, carbonyl compounds, aldehyde and ketones, the use of aminal or acetal for the controlled release of aldehyde, the use of hydrazone for the delivery of aldehyde and ketone, the photo-assisted release of aldehyde from α- acetoxy ethers and the slow release of enones through Retro-Michael addition reaction. In addition to the labile covalent bonds, non-covalent complexation between active molecules and cyclodextrin has been used on the controlled release objective. Besides the use of labile chemical bonds directly linked to the active molecules, the cleavable bonds were also used in the construction of carriers in which the breaking of such bonds triggered the carrier degradation and thus released out the entrapped molecules.

This invention involves a fabrication of double barrier systems in which the active fragranced molecules are chemically linked to the polymeric matrix of the carriers and at the same time embedded at the particles' core.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a synthesis scheme of chitosan derivatives according to this invention.

Figure 2 represents SEM micrograph of the first step chitosan derivative (FSCD).

Figure 3 represents TEM micrograph of the first step chitosan derivative (FSCD).

Figure 4 is a graph of FSCD hydrodynamic size using light scattering technique.

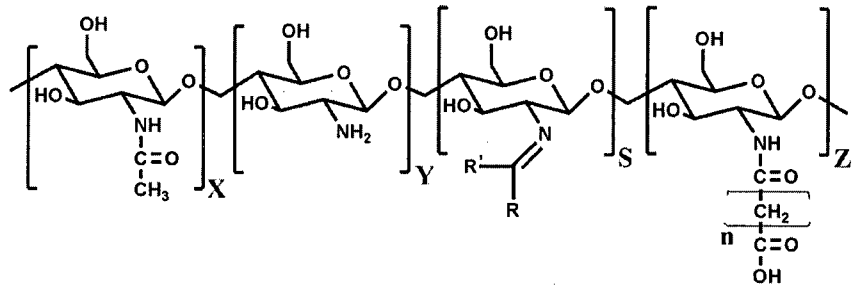
Figure 5 represents atomic force microscopic images of a) N,N'-vanillidene-succinylchitosan nanospheres, b) N,N'-cinnamylidene-succinylchitosan nanospheres, c) N,N'-citronellalidene-succinylchitosan nanospheres, and d) N,N'-citralidene-succinylchitosan nanospheres.

Figure 6 represents X-ray photoelectron spectroscopy of N,N'-vanillidene-succinylchitosan nanospheres.

Figure 7 represents graphs of release of fragranced aldehydes from the four obtained products (red squares) comparing to their corresponding free aldehydes (blue rectangles). The four products tested include a) N,N'-citronellalidene-succinylchitosan nanospheres, b) N,N'-cinnamylidene-succinylchitosan nanospheres, c) N,N'-citralidene-succinylchitosan nanospheres, and d) N,N'-vanillidene-succinylchitosan nanospheres.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The chemical structure of fragranced chitosan derivatives according to this invention is described as follows:



Wherein X = 0-0.6; Y = 0-0.8; S = 0.05-0.80; Z = 0.01-0.80; n = 1-8; R = any chemical moieties; and R' = Hydrogen (H) or any chemical moieties.

A synthesis of the fragranced chitosan derivative with simultaneous formation into nano/microspheres is revealed herein. The fragrance molecules are chemically bonded to the chitosan backbone and at the same time embedded into the particles' core. The fragranced chitosan according to this invention has a particle size in a range of 20 nm (nanometer) to 0.1 mm (millimeter) where the particle distributes remarkably in the water, i.e. an aqueous colloidal suspension form.

In this invention the fragranced molecules are chemically linked to the chitosan derivative backbone through imine linkages *via* the reaction between aldehydes or ketones and amine moieties on the chitosan chains.

The first step chitosan derivative (FSCD) is synthesized as followed: chitosan is dissolved in diluted aqueous acetic acid; succinic anhydride or other anhydride or acid chloride is dissolved in an appropriate amount of acetonitrile or acetone or ethanol or methanol; the anhydride (or acid chloride) solution is dropped into the chitosan solution and the mixture is stirred at appropriate

temperature (0-80^{O}C) for an appropriate time (1-48 hours). Then the mixture is precipitated with appropriated solvent, filtered, washed and dried to obtained white powder of FSCD.

Then the aqueous FSCD colloid is simply prepared by dispersing the material in water and then allowed the colloid to react with perfumery aldehyde or perfumery ketone, at the weight ratio of perfumery molecules: FSCD of 1:1-1:10, by adding dropwise alcoholic solution of the perfume (10-50% v/v) to the FSCD suspension under ultrasonic condition. The mixture should be further ultrasonicated (20-100 KHz at 4-60^{O}C) for another 1-12 h. The colloidal suspension of fragrance-grafted chitosan nanospheres is obtained afterwards. Any aldehydes or ketones can be used. Chitosan of various molecular weights and of various deacetylation degrees can be used as starting material for the synthesis of the chitosan derivatives according to this invention.

An example of the synthesis and fabrication process is presented in Figure 1. The invention will be further elucidated with reference to the following examples. These examples should not be construed as limiting the present invention.

Examples

The first step chitosan derivative (FSCD) was synthesized as followed: Chitosan (2.01 g) was dissolved in 70 mL of 2%v/v acetic acid. Succinic anhydride (0.25 g) in 10 mL of acetone was dropped into chitosan solution and the mixture was stirred at room temperature overnight. Then the mixture was precipitated, filtered and repeatedly washed with an excess amount of acetone to obtained white powder. Precipitation could also be carried out with sodium hydroxide and washed with water. The product was dry under vacuum. The product, FSCD, was then subjected to Infrared spectroscopic (IR), nuclear magnetic resonance (NMR) and UV-Vis spectroscopic analyses.

FSCD spectroscopic data (80% yield), ^{1}H NMR (D_2O, 400 MHz, δ, ppm): 2.01 (H of acetyl groups), 2.42-2.50 (methylene protons of the succinyl), 2.80 (H2 of glucosamine, GlcN), 3.50-3.92 (H2' of N-acetylglucosamine, GlcNAc, H3, H4, H5 and H6 of GlcNAc and GlcN), 4.54 (H1 of GlcNAc and GlcN). ATR-FTIR (cm^{-1}): 3282 (N-H stretching and O-H stretching vibration), 2864 (C-H stretching vibration), 1652 (amide I (C=O stretching)), 1555 (amide II), 1406 (symmetric stretching vibration of COO^{-} and amide III), 1319 (amide III (C-N stretching)), 1143 (C-O-C stretching vibration), and 1027 (C-O stretching vibration). UV-Vis (distilled water, 25°C) λ max: 252 nm, ε: 0.0278 $M^{-1}cm^{-1}$/monomeric unit.

Transparent colloidal suspension of FSCD could be easily obtained by simple dispersing the material in water. FSCD water suspension was left to dry at room temperature before being subjected to the morphology analyses: *scanning electron microscopic* (SEM), *transmission electron microscopic* (TEM) and *X-ray photoelectron spectroscopic* (XPS). The aqueous suspension of FSCD was subjected to the determination of hydrodynamic diameter and zeta potential values using dynamic scattering technique.

As presented in Figure 2, the hydrodynamic diameter and zeta potential of the FSCD nanoparticles are 46.32±0.24 nm (PDI of 0.185) and 22.3±2.6 mV, respectively. However, the size and zeta potential are dependent on various factors and this invention is not limited by such numbers. This is only an example, not the limit of the invention. Morphology of the FSCD is shown below in Figures 3 and 4. However, it should be noted here that the morphology also varies with various factors and the morphologies shown here (Figures 3 and 4) are for the purpose of helping the reader to understand this patent, thus should not be interpreted as the limit of the invention.

The above FSCD product is allowed to react with perfumery aldehyde or ketone. The example here shows the reaction with aldehyde at the weight ratio of aldehyde: N-SCS of 1:3. The procedure involved adding dropwise alcoholic solution of aldehyde (4 mL, 20% v/v) to the aqueous FSCD particle suspension (16 mL, 60 mg) under ultrasonic condition and the mixture was further ultrasonicated (40 KHz at 30^{O}C) for 4 h. Aromatic aldehydes used included vanillin and cinnamaldehyde, while aliphatic aldehydes used included citral and citronellal. Each product suspension was dropped onto glass slide and dry under nitrogen before subjected to ATR-FTIR, AFM, and XPS analysis. However, it should be stated here that this invention also includes other aldehydes and ketones.

N,N'-vanillidene-succinylchitosan nanospheres - Degree of vanillin substitution: 0.34. ATR-FTIR (cm^{-1}): 3282 (N-H stretching and O-H stretching vibration), 2867 (C-H stretching vibration), 1635 (C=N stretching vibration), 1592 and 1512 (C=C stretching vibration of aromatic), 1555 (amideII), 1143 (C-O-C stretching vibration), and 1027 (C-O stretching vibration).

N,N'-cinnamylidene-succinylchitosan nanospheres - Degree of cinnamaldehyde substitution: 0.29. ATR-FTIR (cm^{-1}): 3282 (N-H stretching and O-H stretching vibration), 2867 (C-H stretching vibration), 1632 (C=N stretching vibration), 1592 (C=C stretching vibration of

aromatic), 1552 (amideII), 1147 (C-O-C stretching vibration), and 1024 (C-O stretching vibration).

N,N'-citronellalidene-succinylchitosan nanospheres - Degree of citronellal substitution: 0.38. ATR-FTIR (cm^{-1}): 3282 (N-H stretching and O-H stretching vibration), 2870 (C-H stretching vibration), 1638 (C=N stretching vibration), 1612 (C=C stretching vibration), 1552 (amideII), 1147 (C-O-C stretching vibration), and 1024 (C-O stretching vibration).

N,N'-citralidene-succinylchitosan nanospheres - Degree of citral substitution: 0.38. ATR-FTIR (cm^{-1}): 3282 (N-H stretching and O-H stretching vibration), 2874 (C-H stretching vibration), 1638 (C=N stretching vibration), 1612 (C=C stretching vibration), 1555 (amideII), 1143 (C-O-C stretching vibration), and 1027 (C-O stretching vibration).

Materials with variations in substitution degree can also be applied in the scope of this invention. Morphology of all the above products is shown in Figure 5 and Table1.

Table 1: Size and zeta potential of the four products

Materials	Grafted aldehydes	Hydrodynamic diameter (nm)	PDI	Zeta potential (mV)
N,N'-vanillidene-succinylchitosan	Vanillin	163.42±2.05	0.1776	48.8±1.7
N,N'-cinnamylidene-succinylchitosa	Cinnamaldehyde	130.86±4.94	0.3334	57.3±4.6
N,N'-citronellalidene-succinylchitosan	Citronellal	109.56±1.59	0.1986	38.3±2.4
N,N'-citralidene-succinylchitosan	Citral	80.89±1.70	0.1668	42.4±0.6

The X-ray photoelectron spectroscopic (XPS) technique which usually gives the particular chemical composition data at the surface layer thickness of less than 8 nm from the surface is employed to analyze the location of the grafted fragrance moieties of the obtained spheres. Here only the analysis of N,N'-vanillidene-succinylchitosan nanospheres is shown. From XPS high resolution spectrum of N,N'-vanillidene-succinylchitosan nanospheres (Figure 6), the C 1s spectrum shows only three peaks assigned to C-H, C-OH and O-C-O/HN-C=O. Very importantly, its N 1s spectrum shows only three peaks for primary amine ($-NH_2$), amide (-HN-

C=O) and protonated amine ($-NH_3^+$), with no observable imine peak (while the imine functionality is confirmed in the FT-IR spectrum). This clearly indicates that all the imine functionality are covered with polymeric matrix. Since AFM and DLS indicate clearly the spherical morphology of the material, it can be concluded that the grafted imine groups are not at the spherical surfaces. Such groups must be embedded into the particle core, with the depth from the surface of more than 8 nm. This corresponds well to the scenario that the amine groups have been changed into the imine functionality through the reaction with vanillin. Therefore, the fragranced chitosan nanospheres in which the grafted perfumery aldehydes or ketones are embedded inside the spherical cores are successfully fabricated.

Since the materials according to this invention can be used as fragrance controlled release materials, here such release is demonstrated. The release of citronellal from N,N'-citronellalidene-succinylchitosan nanospheres, cinnamaldehyde from N,N'-cinnamylidene-succinylchitosan nanospheres, citral from N,N'-citralidene-succinylchitosan nanospheres and vanillin from N,N'-vanillidene-succinylchitosan nanospheres are demonstrated below in Figure 7. Each release profile is also shown with the release of free aldehyde mixed with the unmodified chitosan.

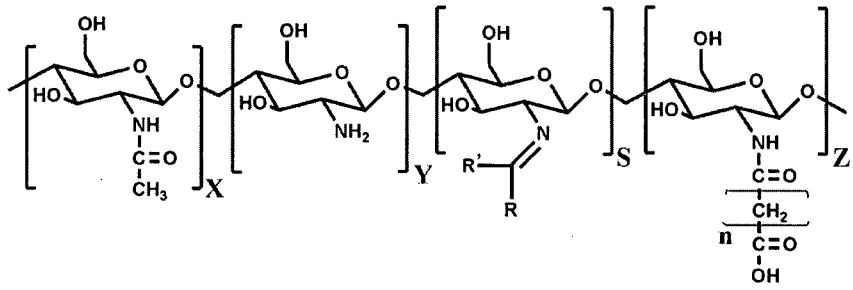
It can be seen clearly from the release profile that the materials according to this invention can slowly release out the grafted aldehydes or ketones. The release from the material is significantly slower than the mixture between ungrafted aldehydes and the unmodified chitosan.

Although the demonstration is carried out on only four fragranced aldehydes, it should be noted here that this invention covers all others aldehydes and ketones as well. The demonstration was carried out only for helping the readers to understand this invention, and thus, this the materials according to this invention does not limit to only the four examples shown above.

The materials according to this invention can be used with various products, for example, cream, lotion, fabric softener, shampoo, hair rinse, etc.

CLAIMS

1. A fragranced chitosan using chitosan derivative having the chemical structure:



Wherein X = 0-0.6; Y = 0-0.8; S = 0.05-0.80; Z = 0.01-0.80; n = 1-8; R = any chemical moieties; and R' = Hydrogen (H) or any chemical moieties.

2. The fragranced chitosan of claim 1, wherein said fragranced chitosan has a particle size from 20 nm (nanometer) to 0.1 mm (millimeter).

3. The fragranced chitosan of claim 1 or 2, wherein said fragranced chitosan is in an aqueous colloidal suspension form.

4. The fragranced chitosan of any one of the preceding claims, wherein said fragranced chitosan is prepared from a reaction between succinyl chitosan and aldehydes or ketones.

5. The fragranced chitosan of claim 4, wherein said aldehydes or ketones are chemically linked to said chitosan derivative backbone through imine linkages.

6. A process for making fragranced chitosan derivative comprising the steps of:
   (a) dissolving chitosan in acetic acid to form chitosan solution;
   (b) dissolving anhydride or acid chloride in any suitable solvents; then adding into the chitosan solution;
   (c) stirring a mixture at appropriate temperature for an appropriate time; and
   (d) precipitating, filtering, and washing the mixture with any suitable solvents to obtain first step chitosan derivative (FSCD) powder.

7. The process of claim 6, wherein said anhydride is succinic anhydride.

8. The process of claim 6, wherein said solvent is selected from the group consisting of acetonitrile, acetone, ethanol, and methanol.

9. The process of claim 6, wherein the appropriate temperature is from 0°C to 80°C.

10. The process of claim 6, wherein the appropriate stirring time is between 1-48 hours.

11. The process of claim 6, further comprising the steps of

(e) dispersing the FSCD powder in water forming aqueous FSCD colloidal;

(f) dissolving perfumery aldehyde or ketone in alcohol (10-50% v/v) to form fragranced solution; and

(g) adding the fragranced solution into the colloidal suspension under ultrasonic condition for 1-12 hours.

12. The process of claim 11, wherein a weight ratio of said perfumery aldehyde or ketone molecules to FSCD is from 1:1 to 1:10.

13. The process of claim 11, wherein a suitable ultrasonic condition is at temperature 4-60°C and 20-100 kHz.

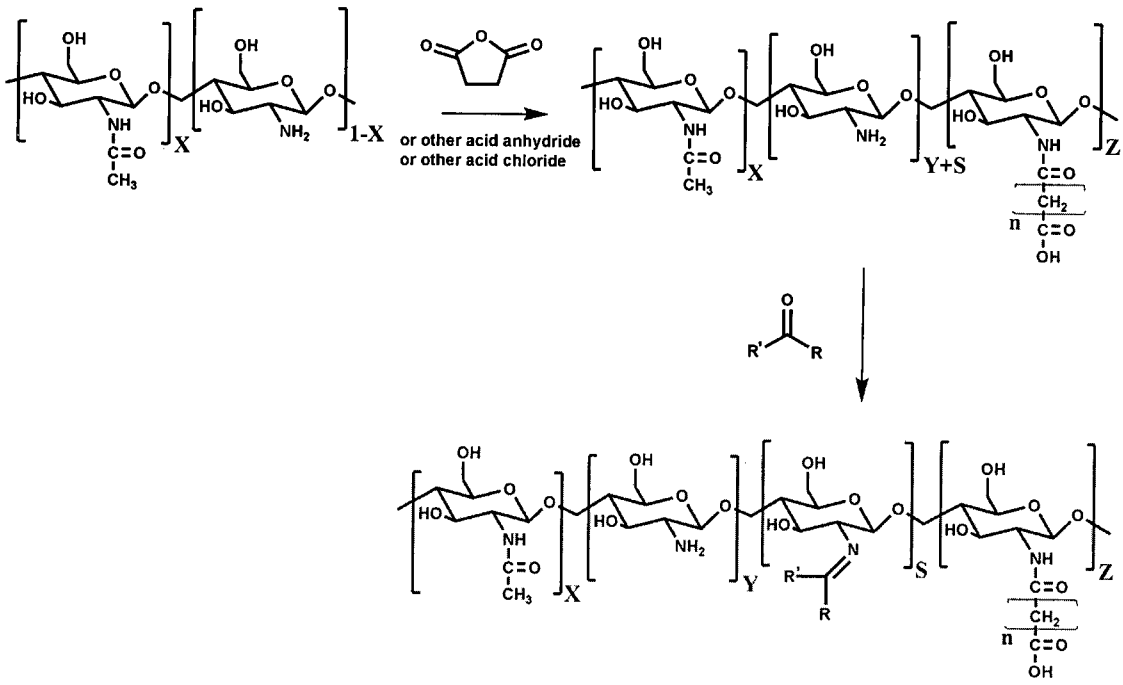


Figure 1

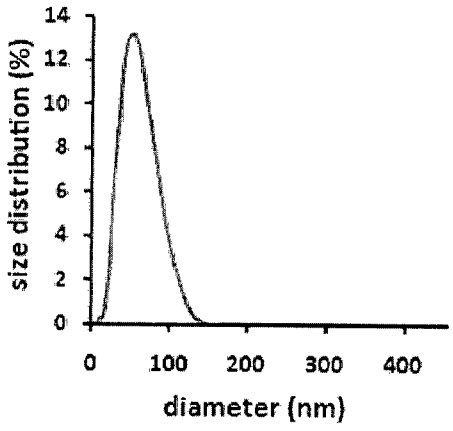


Figure 2

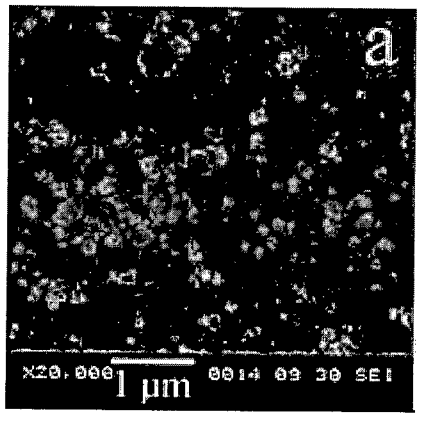


Figure 3

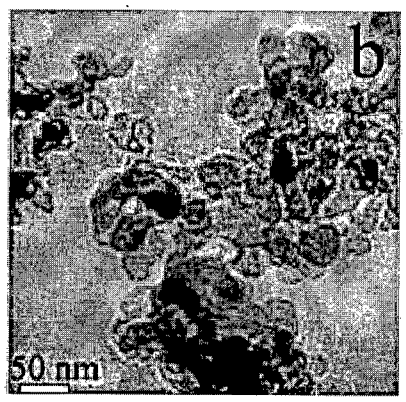


Figure 4

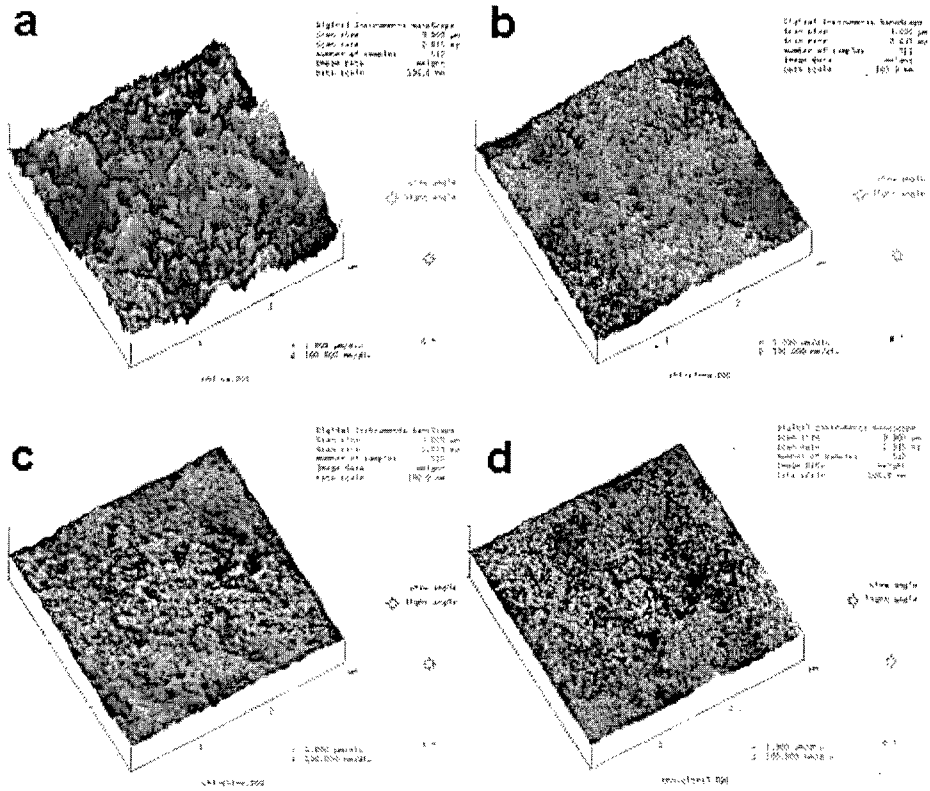


Figure 5

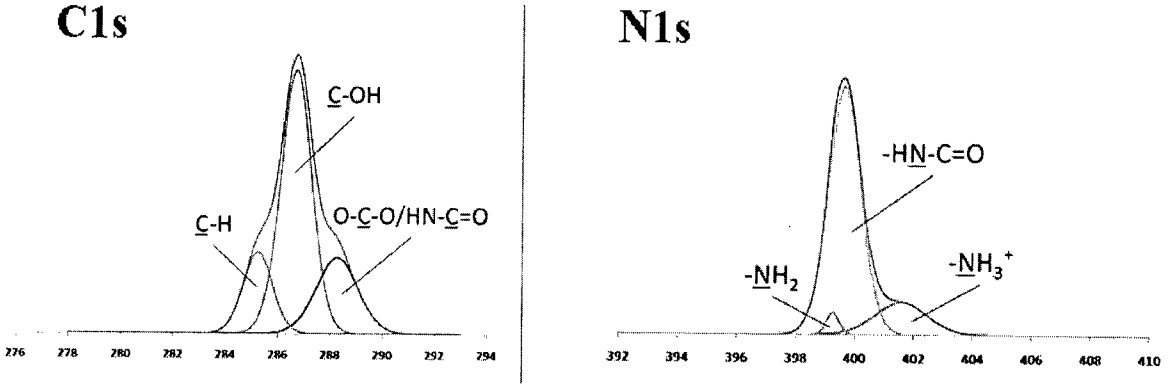


Figure 6

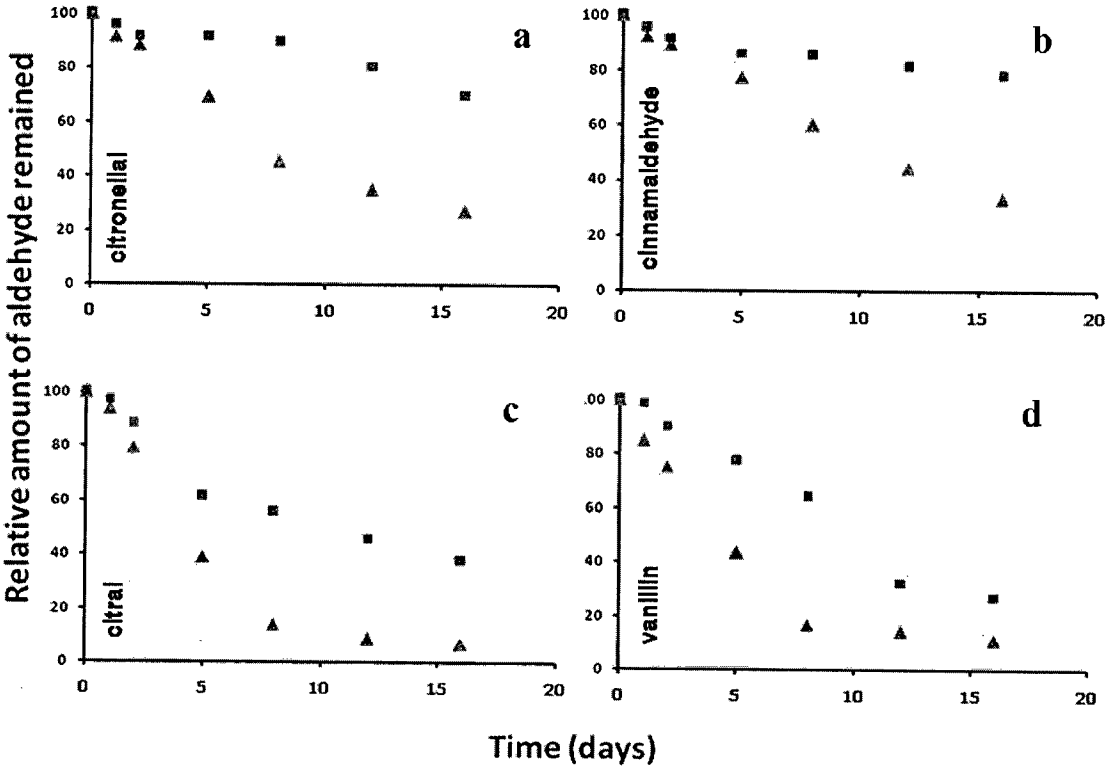


Figure 7