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THE PATENT OFFICE

जिस किसी से संबन्धित हो

TO WHOMSOEVER IT MAY CONCERN

मैं, अधोहस्ताक्षरी जो पेटेंट अधिनियम, 1970 की धारा 73(3) के तहत महानियंत्रक एकम्ब, अभिकल्प एवं व्यापार चिह्न की ओर से प्रमाणपत्र हस्ताक्षर व जारी करने के लिए प्राधिकृत अधिकारी हूँ एतद्वारा यह प्रमाणित करता/ती हूँ कि निम्नलिखित पेटेंट आवेदन के संबंध में फाइल दस्तावेज(जों) की सही प्रतिलिपि इसके साथ संलग्न है।

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क) आवेदन संख्या/ a) Application Number: 202311032522

ख) फाइल करने की तारीख/ b) Date of Filing: 08th May, 2023

ग) अनुरोधित दस्तावेज(जों) का नाम/ c) Name of the document(s) requested:

1) Application Form-1

2) Provisional Specification

3) Drawings

filed on 08th May, 2023 (As per e-filing record)

यह प्रमाणपत्र पेटेंट अधिनियम, 1970 की धारा 147(1) के अधीन मुझमें निहित शक्तियों के तहत जारी किया गया है। This certificate is issued under the powers vested in me U/S 147(1) of The Patents Act, 1970.

दिनांक/ Dated this 29th day of September 2025.

MSingh

नियंत्रक पेटेंट व डिजाइन/ Controller of Patents and Designs

(प्राधिकृत हस्ताक्षरी/ Authorised Signatory)

FORM 1 THE PATENTS ACT, 1970 (39 of 1970) & The Patents Rules, 2003 APPLICATION FOR GRANT OF PATENT <i>[See section 7,54 & 135 and rule 20(1)]</i>				(FOR OFFICE USE ONLY) Application No.: Filing Date: Amount of Fees Paid: CBR No: Signature:			
1. APPLICANT'S REFERENCE / IDENTIFICATION NO. (AS ALLOTTED BY OFFICE)							
2. TYPE OF APPLICATION							
Ordinary (✓)		Convention ()		PCT-NP ()			
Divisional ()	Patent of Addition ()	Divisional ()	Patent of Addition ()	Divisional ()	Patent of Addition ()		
3A. APPLICANT(S)							
NAME		NATIONALITY	COUNTRY OF RESIDENCE	ADDRESS			
Tidal Vision Products		US	US	3710 Iron Gate Rd, Bellingham, WA 98226, United States of America.			
3B. CATEGORY OF APPLICANT							
Natural Person ()			Other than Natural Person				
			Small Entity ()	Startup ()	Educational Institution(s) ()	Others (✓)	
4. INVENTOR(S)							
Are all the inventor(s) same as the applicant(s) named above?		Yes ()		No (✓)			
NAME		NATIONALITY	COUNTRY OF RESIDENCE	ADDRESS			
RALIYA, Ramesh		IN	IN	Apt. 1003 - B6 Waterlilly, Ahmedabad - 382421, Gujarat, India.			
5. TITLE OF THE INVENTION: COMPOSITIONS AND METHODS FOR AGRI-INPUTS AND USES THEREOF							
6. AUTHORIZED REGISTERED PATENT AGENT(S)			IN/PA No.	1325			
			Name	Tarun Khurana			
			Mobile No.	9810617992			
			IN/PA No.	1609			
			Name	Meenakshi			
			Mobile No.	9910307992			
			IN/PA No.	1722			
			Name	Abhishek Pandurangi			
			Mobile No.	9920866540			
			IN/PA No.	2066			
			Name	Antony David			
			Mobile No.	9884411536			
IN/PA No.	2553						

	Name	Tapan Shah
	Mobile No.	8130473066
	IN/PA No.	2194
	Name	Mita N Sheikh
	Mobile No.	9920659185
	IN/PA No.	2660
	Name	Dhakshina Moorthy C
	Mobile No.	7093004694
	IN/PA No.	3609
	Name	Shashi Kant Verma
	Mobile No.	9560024888
	IN/PA No.	3617
	Name	Swapnil Mahadeo Sanap
	Mobile No.	8149497652
	IN/PA No.	3616
	Name	Shubhangi Shivnath Dhakane
	Mobile No.	8149497652
	IN/PA No.	2230
	Name	Dr. Padmapriya V.
	Mobile No.	9789076871
	IN/PA No.	3567
	Name	Ghag Sneha Vijay Hema
	Mobile No.	9619932948
	IN/PA No.	2773
	Name	Kuldeep Singh
	Mobile No.	9891199936
	IN/PA No.	2275
	Name	Monomayee Mukherjee
	Mobile No.	8910700172
	IN/PA No.	2967
	Name	S. Govindhaswamy @ Manikandan
	Mobile No.	9962167221
	IN/PA No.	3431
	Name	Priya Chauhan
	Mobile No.	9410997524
7. ADDRESS FOR SERVICE OF APPLICANT IN INDIA	Name	KHURANA & KHURANA, Advocates and IP Attorneys
	Postal Address	E-13, UPSIDC, Site-IV, Behind Grand Venice, Kasna Road, Greater Noida 201310, UP, National Capital Region, India.
	Telephone No.	0120- 4296878/4909201
	Mobile No.	9810617992
	Fax No.	0120- 4516201
	E-mail ID	info@khuranaandkhurana.com docket@khuranaandkhurana.com
8. IN CASE OF APPLICATION CLAIMING PRIORITY OF APPLICATION FILED IN CONVENTION COUNTRY, PARTICULARS OF CONVENTION APPLICATION		

Country	Application No.	Filing Date	Name of Applicant	Title of Invention	IPC (as classified in the convention country)
-	-	-	-	-	-

9. IN CASE OF PCT NATIONAL PHASE APPLICATION, PARTICULARS OF INTERNATIONAL APPLICATION FILED UNDER PATENT CO-OPERATION TREATY (PCT)

International Application No.	International filing date as allotted by the receiving office
-	-

10. IN CASE OF DIVISIONAL APPLICATION FILED UNDER SECTION 16, PARTICULARS OF ORIGINAL (FIRST) APPLICATION

Original (First) Application No.	Date of filing of Original (First) Application
Nil	Nil

11. IN CASE OF PATENT OF ADDITION FILED UNDER SECTION 54, PARTICULARS OF MAIN APPLICATION OR PATENT

Main Application/Patent No.	Date of filing of Main Application
Nil	Nil

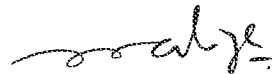
12. DECLARATIONS

(i) Declarations by Inventor(s)

We, the above named inventor(s) is/are the true & first inventor(s) for this invention and declare that the applicant(s) herein is/are my/our assignee.

Signature

Name(s)



RALIYA, Ramesh

(ii) Declaration by the Applicant(s) in the convention country

We, the Applicant(s) in the convention country declare that the applicant (s) herein is/are my/our assignee or legal representative.

Date:

Signature:

Name(s)

(iii) Declaration by the Applicant(s)

I/We hereby declare(s) that

- ✓ I am /We are in possession of the above mentioned invention.
- ✓ Provisional Specification relating to the invention is filed with this application.
- ☒ The invention as disclosed in the specification uses the biological material from India and the necessary permission from the competent authority shall be submitted by me/us before the grant of patent to me/us.
- ✓ There is no lawful ground of objection to the grant of patent to me/us.
- ✓ I am/we are the true & first inventor(s).
- ✓ I am / we are the assignee or legal representative of true & first Inventors.
- ☒ The application or each of applications, particulars of which are given in para 8, was the first application in

convention country / countries in respect of my/our invention.

☒ We claim priority from the above mentioned application(s) filed in convention country/countries and state that no application for protection in respect of the invention has been made in a convention country before that date by me/us or by any person from which I/We derive the title.

☒ Our application is based on International application under Patent Cooperation Treaty as mentioned in para 9.

☒ The application is divided out of my/our application particulars of which are given in para -10 and pray that this application may be treated as deemed to have been filed on N/A under section 16 of the Act.

☒ The said invention is an improvement in or modification of the invention particulars of which are given in para 11.

13. Following are the attachments with the application: (General Requirements)

a. Form 2

Item	Details	Fee	Remarks
Provisional Specification	No. of pages: 25		
No. of Claim(s)	No. of claims: 17 and No. of pages: 3		
Abstract	No. of pages: 1		
No. of Drawing(s)	No. of pages: 12 No. of drawings: 13		

b. Provisional specification (in conformation with the international application)/as amended before the International Preliminary Examination Authority (IPEA), as applicable (2 copies).

c. Sequence listing in electronic form

d. Drawings (in conformation with the international application)/as amended before the International Preliminary Examination Authority (IPEA), as applicable (2 copies).

e. Priority document(s) or a request to retrieve the priority document(s) from DAS (Digital Access Service) if the applicant had already requested the office of first filing to make the priority document(s) available to DAS.

f. Translation of priority document/Specification/International Search Report/International Preliminary Report on Patentability.

g. Statement and Undertaking on Form 3

h. Declaration of Inventorship on Form 5

i. Power of Authority

j. Official fee Rs. _____ Cheque No. _____ Bank _____

I/We declare that to the best of my/our knowledge, information and belief the fact and matters stated herein are correct and I/We request that a patent may be granted to me/us for the said invention.

Dated this: 08th day of May, 2023

Signature



Name of the signatory **TARUN KHURANA[IN/PA-1325]**
AGENT FOR THE APPLICANT
Khurana & Khurana, Advocates and IP Attorneys

To,
The Controller of Patents,
The Patent Office, at Delhi

FORM 2 THE PATENTS ACT, 1970 (39 of 1970) & The Patent Rules, 2003 PROVISIONAL SPECIFICATION (See sections 10 & rule 13)		
1. TITLE OF THE INVENTION COMPOSITIONS AND METHODS FOR AGRI-INPUTS AND USES THEREOF		
2. APPLICANT (S)		
NAME	NATIONALITY	ADDRESS
Tidal Vision Products	US	3710 Iron Gate Rd, Bellingham, WA 98226, United States of America.
3. PREAMBLE TO THE DESCRIPTION		
PROVISIONAL SPECIFICATION The following specification describes the invention.		

TECHNICAL FIELD

[0001] The invention relates to plant nutrition and specifically to encapsulation of nutrients.

5 BACKGROUND

[0002] Agricultural production across the globe is facing unprecedented challenges due to climate change, extreme weather, soil erosion pathogen resistance and environmental pollution. Global population is projected to reach about 10 billion by 2050, thus the demand for food is expected to increase by 60%. The rate of increase of yield and fertilizer (an agri-input) to yield ratio achieved by the green revolution has been declining and detrimental climate change is expected to further limit crop productivity and nutritional quality. To increase yield and improve nutritional quality, plants require balanced nutrition in addition to water and sunlight. However, conventional agri-inputs and their delivery are highly inefficient due to low use efficiency and environmental release. Therefore, more-effective formulations and compositions which can stimulate plant growth, alleviate the negative effect of abiotic/biotic stress, deliver active ingredient efficiently to the plant at the right time in right quantity and release in response to plant's signal are required.

[0003] Biomass-based renewable materials are being explored globally due to their importance for a circular green economy and sustainability. Chitin is the most abundant natural amino polysaccharide. It is present most abundantly in crustaceans, insects, and fungi. Chitin and its derivatives are being considered as a new functional biomaterial of high potential in interdisciplinary applications. For instance, chitosan, a deacetylated derivative of chitin, is being used in industries/sectors such as medicine, textile, food, energy, and water. To date, translational application of chitosan in agriculture is under exploited.

SUMMARY

[0004] In a first aspect, the disclosure provides methods of modifying chitosan for the formulation of chitosan-based nanoparticles.

[0005] In a second aspect, the disclosure provides chitosan-based nanoparticles. The chitosan based nanoparticles may encapsulate nitrogen and/or nitrogen rich compound and/or mixture of compounds such as but not limited to potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia
5 nitrate, ammonium nitrate, ammonium sulfate, Urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine, and alanine.

[0006] Further aspects and embodiments are provided in the foregoing
10 drawings, detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The following drawings are provided to illustrate certain embodiments described herein. The drawings are merely illustrative, and are not
15 intended to limit the scope of claimed inventions and are not intended to show every potential feature or embodiment of the claimed inventions. The drawings are not necessarily drawn to scale; in some instances, certain elements of the drawing may be enlarged with respect to other elements of the drawing for purposes of illustration.

20 [0008] Figure 1 is a depiction of Morphological characterizations of the liquid chitosan formulation (TgA-TgE) obtained by Electron Microscopy.

[0009] Figure 2 is a depiction of Morphological characterizations of the liquid chitosan formulation (CTgA-CTgE) obtained 2 by Electron Microscopy.

[00010] Figure 3 is a depiction of Colloidal characterization with reference
25 to hydrodynamic size of the liquid chitosan formulation (TgA- E and CTgA-CTgE) obtained by Dynamic Light Scattering.

[00011] Figure 4 is a depiction of Colloidal characterization with reference to surface zeta potential of the liquid chitosan formulation (TgA- E and CTgA-CTgE) obtained by Dynamic Light Scattering.

30 [00012] Figure 5 is a table depicting the pH and TKN of Align N Batches 1-7.

[00013] Figure 6 is the UV wavelength analysis of Align N Batches 1-7.

[00014] Figure 7 is the FTIR analysis of Align N 1.

[00015] Figure 8 is the FTIR analysis of Align N 2.

[00016] Figure 9 is the FTIR analysis of Align N 3.

5 [00017] Figure 10 is the FTIR analysis of Align N 4.

[00018] Figure 11 is the FTIR analysis of Align N 5.

[00019] Figure 12 is the FTIR analysis of Align N 6.

[00020] Figure 13 is the FTIR analysis of Align N 7.

10 DETAILED DESCRIPTION

[00021] The following description recites various aspects and embodiments of the inventions disclosed herein. No particular embodiment is intended to define the scope of the invention. Rather, the embodiments provide non-limiting examples of various compositions, and methods that are included within the scope of the claimed inventions. The description is to be read from the perspective of one of ordinary skill in the art. Therefore, information that is well known to the ordinarily skilled artisan is not necessarily included.

Definitions

[00022] The following terms and phrases have the meanings indicated below, unless otherwise provided herein. This disclosure may employ other terms and phrases not expressly defined herein. Such other terms and phrases shall have the meanings that they would possess within the context of this disclosure to those of ordinary skill in the art. In some instances, a term or phrase may be defined in the singular or plural. In such instances, it is understood that any term in the singular may include its plural counterpart and vice versa, unless expressly indicated to the contrary.

[00023] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, reference to "a substituent" encompasses a single substituent as well as two or more substituents, and the like.

[00024] As used herein, “for example,” “for instance,” “such as,” or “including” are meant to introduce examples that further clarify more general subject matter. Unless otherwise expressly indicated, such examples are provided only as an aid for understanding embodiments illustrated in the present disclosure, and are not meant to be limiting in any fashion. Nor do these phrases indicate any kind of preference for the disclosed embodiment.

[00025] Encapsulation and slow release of nutrients is one method for balancing the nutritional needs of plants. One such nutritional need is nitrogen in the form of amine, nitrate and ammonia. Encapsulation of nitrogen compounds having chemical functional groups as amine, nitrate, ammonia, or any one or all of these such as but not limited to potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, Urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine and alanine is a way of releasing nitrogen in effective amounts for plant growth utilization. There are several benefits of the use of chitosan as a urea-encapsulating/embedding agent. The first benefit is that the chitosan polymer is bio-safe and biodegradable. The chitosan polymer has a biological origin, and has been used for immobilization of enzymes for food, and in biosensing applications. Chitosan can be harvested from fungus, shrimp, crab, lobster, or any combination of these sources. A second benefit compared to inorganic coatings such as sulfur coatings is the absence of cracks. Cracks in the coating cause an immediate release of the coated nutrient upon contact with water. Immediate release of the nutrient defeats the purpose of the encapsulation, which is to slowly release the nutrient. A third benefit of the chitosan polymer coating is the overall health of the soil. The chitosan polymer results in lowered acidification of the soil. Acidification can of the soil can be detrimental to specific plant species. Coatings such as sulfur lower the pH of the soil. Additionally, the soil microbes are an important part of soil health. Synthetic polymers cannot be metabolized by soil microbes.

[00026] Embodiments include chitosan-based nanoparticles. In certain embodiments the chitosan-based nanoparticles comprise nitrogen in the form of amine and/or nitrate and/or ammonia. The nitrogen may be encapsulated by/in the chitosan-based nanoparticles. In particular embodiments, the nitrogen in the form of amine, nitrate, and/or ammonia may be selected potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine, alanine, and combination thereof.

[00027] In certain embodiments, the chitosan-based nanoparticles may comprise at least 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 85, 90, or 95% (w/v) nitrogen. In particular embodiments wherein at least 50, 55, 60, 65, 70, 75, 85, 90, or 95% of the nitrogen is in the chemical form of the amine and/or nitrate and/or ammonia.

[00028] In one embodiment, the encapsulation process begins with the chitosan polymer. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. In certain embodiments the chitosan has a Degree of DeAcetylation (DDA): >80%, MW: <110 KDa. In particular embodiments the DDA is > 85%. The DDA may be 90%. The DDA may be > 95%. In particular embodiments the DDA is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

[00029] Prior to usage for the formulation of nanoparticles, the chitosan must be dissolved in a solution. Dissolution medium: acidic water having pH ranges between 1 and 5.5, or having a pH < 1, < 2, < 3, < 4, < 5, < 5.5. The acid used may be any acid. In particular embodiments the acid used is one of or a combination of the acids including nitric acid and/or acetic acid and/or hydrochloric acid and/or sulfuric acid. To dissolve the chitosan, the aqueous acidic solution may be kept at temperature between about 68 and about 212 degrees F. In particular embodiments, the temperatures is kept at about 68, 72, 82, 92, 101, 112, 122, 132, 142, 152, 162, 172, 182, 192, 202, or 212 degrees F.

During the entire reaction period the solution may be kept stirring at rpms between about 50 and about 2500 rpm. After completion of the reaction, typically between 0.3 and 4 hours, the solution was filtered through 200 mesh (74 micron) followed by more than 635 mesh (<20 micron). In the filtered solution, antimicrobial agents such as but not limited to potassium sorbate are added (0.1% v/v). In certain cases, as described below a linker such as sodium tripolyphosphate and/or ammonium sulfate is added in the solution before the addition of antimicrobial agents.

[00030] In one embodiment, the reaction is carried out with specific solutions prepared in accordance with one and/or all of the procedures in Example 1 at the temperature between 68 and 250 degrees F. During the entire reaction period the solution was kept stirring at 400 rpm for uniformity of the substances. After completion of the reaction, typically between 0.3 and 4 hours, the solution was filtered through 200 mesh (74 micron) followed by more than 635 mesh (<20 micron). The nitrogen % in the final solution was maintained between 0.1% and 100%, specifically between 18-24% using the compound and/or mixture of compounds described above.

[00031] In certain embodiments, the chitosan nanoparticles may be formed in the presence of and or loaded with nitrogen in the form of amine and/or nitrate and/or ammonia. The nitrogen may be encapsulated by/in the chitosan-based nanoparticles. In particular embodiments, the nitrogen in the form of amine, nitrate, and/or ammonia may be selected potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine, alanine, and combination thereof.

[00032] > 95% particles were in the spherical, nearly spherical shape or small linear fragments having size between 0.1 nm and 8 microns. (By TEM measurement). 0.1 nm and 10 microns with polydispersity index between 0.01 to 0.99 (by DLS measurement). Surface zeta potential ranged between +5 and 70

mV (measured by DLS). N%: 18-24 Application onto the seed surface, mixing in the soil, exposing root and/or exposing leaves. The application rate ranged between 0.01% to 50% v/w or v/v.

[00033] Embodiments include methods of treating a plant or part with any of the compositions described herein. The plant part may be any plant part including, but not limited to a seed, leaf, stem, flower, or root of a plant. Examples species of plant parts include, but are not limited to cereals such as wheat, barley, rye, oat; canola, cotton, eggplant, lettuce, sorghum, soybean, rice, oil seed rape, sugar beet, sugarcane, grapes, lentils, sunflowers, alfalfa, pome 5 fruits; stone fruits; peanuts; coffee; tea; strawberries; turf; vegetables, such as tomatoes, potatoes, cucurbits and lettuce.

[00034] Further embodiments include methods of treating a plant growth medium with any of the compositions described herein. Plant growth medium may comprise one or more of soil, peat, moss, wood residue, leaf mold, sawdust, bark, bagasse, rice hull, sand, perlite, vermiculite, calcinated clay, polystyrene, 15 urea formaldehyde resins, and hydroponic liquids.

Example 1

Preparation of chitosan using various acids

[00035] [Acetic Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 20 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid between 0.3% and 1.9% (v/v) was added. The temperature 25 and agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 30 -1% v/v) and mixed well using agitation.

[00036] [Acetic Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be
5 fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and
10 agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00037] [Acetic Acid + Ammonium Sulphate] A germ free or autoclaved
15 water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the
20 soaking of the flakes with water, acetic acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of Ammonium Sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The
25 solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00038] [Nitric Acid + Acetic Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80%
30 DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan

variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid and nitric acid having a ratio of 1:1, 1:2, 2:1 and a total v/v acid strength between 0.3% and 1.9% was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4
5 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cool down to 77 degree F, and a solution of potassium sorbate was added (1% v/v) and mixed well using agitation.

[00039] [Nitric Acid + Acetic Acid + NaTPP] A germ free or autoclaved
10 water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the
15 soaking of the flakes with water, acetic acid and nitric acid having a ratio of 1:1, 1:2, 2:1 and a total v/v acid strength between 0.3% and 1.9% was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the
20 solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00040] [Nitric Acid + Acetic Acid + Ammonium Sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm.
25 When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid and nitric acid
30 having a ratio of 1:1, 1:2, 2:1 and a total v/v acid strength between 0.3% and 1.9% was added. When the flakes became invisible to the solution, a solution of

ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

5 [00041] [Nitric Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be
10 fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, nitric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and
15 agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00042] [Nitric Acid + Ammonium sulphate] A germ free or autoclaved
20 water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the
25 soaking of the flakes with water, nitric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The
30 solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00043] [Nitric Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, nitric acid between 0.3% and 1.9% (v/v) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 -1% v/v) and mixed well using agitation.

[00044] [Hydrochloric Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, hydrochloric acid between 0.3% and 1.9% (v/v) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 -1% v/v) and mixed well using agitation.

[00045] [Hydrochloric Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, hydrochloric acid between 0.3% and 1.9% (v/v) was

added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00046] [Hydrochloric Acid + Ammonium sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, hydrochloric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00047] [Sulfuric Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, sulfuric acid between 0.3% and 1.9% (v/v) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution

temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 -1% v/v) and mixed well using agitation.

[00048] [Sulfuric Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, sulfuric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00049] [Sulfuric Acid + Ammonium Sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, sulfuric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00050] [Nitric Acid + Acetic Acid + Hydrochloric Acid + Sulfuric Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan

flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid, nitric acid, hydrochloric acid, sulfuric acid having a ratio of 1:1:0:0, 1:0:1:0, 1:0:0:1, 1:1:1:0, 1:1:2:0, 1:1:1:1, 1:1:1:2 and a total v/v acid strength between 0.3% and 1.9% was added. The temperature and agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cool down to 77 degree F, and a solution of potassium sorbate was added (1% v/v) and mixed well using agitation.

[00051] [Nitric Acid + Acetic Acid + Hydrochloric Acid + Sulfuric Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid, nitric acid, hydrochloric acid, sulfuric acid having a ratio of 1:1:0:0, 1:0:1:0, 1:0:0:1, 1:1:1:0, 1:1:2:0, 1:1:1:1, 1:1:1:2 and a total v/v acid strength between 0.3% and 1.9% was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00052] [Nitric Acid + Acetic Acid + Hydrochloric Acid + Sulfuric Acid + Ammonium Sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or

lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid, nitric acid, hydrochloric acid, sulfuric acid having a ratio of 1:1:0:0, 1:0:1:0, 1:0:0:1, 1:1:1:0, 1:1:2:0, 1:1:1:1, 1:1:1:2 and a total v/v acid strength
5 between 0.3% and 1.9% was added. When the flakes became invisible to the solution, a solution of ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree
10 F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

EXAMPLE 2

[00053] Various of the above chitosan solutions were prepared using chitosan from two different sources. These sources are designated Tg and CTg.
15 Tg and CTg were used to create chitosan-based nanoparticles using the above identified procedures to yield the following products

TgA = Tg source chitosan-based nanoparticles prepared with acetic acid.

TgB = Tg source chitosan-based nanoparticles prepared with acetic acid and sodium tripolyphosphate.

20 TgC = Tg source chitosan-based nanoparticles prepared with acetic acid and nitric acid.

TgD = Tg source chitosan-based nanoparticles prepared with acetic acid, nitric acid, and sodium tripolyphosphate.

25 TgE = Tg source chitosan-based nanoparticles prepared with nitric acid and sodium tripolyphosphate.

CTgA = CTg source chitosan-based nanoparticles prepared with acetic acid.

CTgB = CTg source chitosan-based nanoparticles prepared with acetic acid and sodium tripolyphosphate.

30 CTgC = CTg source chitosan-based nanoparticles prepared with acetic acid and nitric acid.

CTgD = CTg source chitosan-based nanoparticles prepared with acetic acid, nitric acid, and sodium tripolyphosphate.

CTgE = CTg source chitosan-based nanoparticles prepared with nitric acid and sodium tripolyphosphate.

5 [00054] The formulation and intermediates were characterized for the physicochemical characterizations. The physical morphology (shape and size) was characterized using the transmission or scanning electron microscope (Figures 1 and 2). Resultant solution/s obtained for the Tg sourced chitosan had > 95% particles were in the spherical, nearly spherical shape or small linear fragments
10 having size between 0.1 nm and 5 microns. More specifically, the desired size obtained and utilized for further formulation development was less than 0.5 micron was used for further formulation development and/or other agricultural use for crop growth, stimulation, germination, and trait improvement purpose. Similarly, resultant solution obtained for the CTg sourced chitosan had the
15 physical structure as spherical shape and size ranges between 0.1 nm and 8 microns. More specifically the size obtained between 0.1 nm and 200 nm was used as a selection parameter for the delivery to the crop for growth, stimulation, germination, and trait improvement purposes.

The dynamic stability and hydrodynamic size in the solution was characterized
20 using the dynamic light scattering or DLS the nanoparticles were subjected to dynamic light scattering (DLS) and analyzed for Z average, Intensity, Number, and Polydispersity (PDI) (Figure 3). Resultant solutions had ranges between 0.1 nm and 10 microns with polydispersity index between 0.01 to 0.99. Furthermore, the surface zeta potential of the resultant solutions characterized by DLS (Figure
25 4) and it ranged between +5 and 70 mV. The major determinant factor for zeta potential was chitosan concentration and pH of the solution. Higher chitosan concentration and lower pH yielded higher zeta potential.

EXAMPLE 3

The ability of the various chitosan-based nanoparticles generated in Examples 1
30 and 2 to load nitrogen and/or nitrogen rich compound and/or mixture of compounds such as but not limited to potassium nitrate, calcium nitrate,

magnesium nitrate, urea nitrate, urea, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, Urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine and alanine was tested. Encapsulation and slow release of nutrients is one method for balancing the nutritional needs of plants. Several solutions were prepared and tested.

Preparation of chitosan solutions

[00055] Preparation for solution A: Chitosan solution in Acetic Acid

10 [00056] Step 1. Take 150 ml water in a 250 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 300 RPM. The stirrer was continuously ON through the process until it says stirrer STOP. Step 3. After 3 minutes of step 2, Add 8 gram chitosan flake. Step 4. After 7 minutes of step 3, Add 8 mL of 99.7 % Glacial Acetic Acid. Step 5. After complete dissolution of the flake (after 17 minutes after step 4), add 50 mL water. Step 6. After 10 minutes of step 5, The stirrer stopped. Step 7. Filter the solution with 200 mesh size filter. The total volume of the solution was 210 mL and 104.335 g/100 mL. The calculated % of the Chitosan of the solution is 3.8% (w/v). The pH of the solution is 3.88.

[00057] Preparation for solution B: Chitosan solution in HCL

20 [00058] Step 1. Take 150 ml water in 250 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 300 RPM. The stirrer was continuously ON through the process until it says stirrer STOP. Step 3 After 3 minutes of step 2, Add 8 gram chitosan flake. Step 4. After 7 minutes of the step 3, Add 8 mL of 35.4 % HCL. Step 5. After complete dissolution of the flake (after 85 minutes after step 4), added 50 mL water. Step 6. After 26 minutes of the step 5, The stirrer stopped. Step 7. Filter the solution with 200 mesh size filter. The total volume of the solution in mL was 222.096 mL at 105.37 Gram/100 mL. The calculated % CS of the solution was 3.6 % (w/v) with a pH of the solution at 0.66.

30

[00059] Preparation for solution C: Chitosan solution in Nitric Acid

[00060] Step 1. Take 800 ml water in 2000 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 600 RPM. The stirrer was continuously ON through the process until it says stirrer STOP. Step 3. After 2 minutes of step 2,
5 Add 20 gram chitosan flake. Step 4. After 5 minutes of the step 3, Add 20 mL of 69.71 % Nitric Acid. Step 5. After complete dissolution of the flake (after 110 minutes after step 4), added 100 mL water. Step 6. After 14 minutes of the step 5, The stirrer stopped. Step 7. After 10 minutes of step 6, add 80 ml of water to make up the solution to 1000 mL. Step 8. Filter the solution with 200 mesh size filter.
10 The total volume of the solution in mL was 1000 mL at 100.686 Gram/100 mL. The calculated % CS of the solution is 2 % (w/v) with a pH of the solution at 0.84.

[00061] Preparation for solution D: Chitosan solution in Acetic Acid

[00062] Step 1. Take 800 ml water in 2000 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 600 RPM. The stirrer was continuously ON
15 through the process until it says stirrer STOP. Step 3. After 4 minutes of step 2, Add 20 gram chitosan flake. Step 4. After 10 minutes of the step 3, Add 20 mL of 99.7 % Glacial Acetic Acid. Step 5. After complete dissolution of the flake (after 10 minutes after step 4). Step 6. After 17 minutes of the step 5, The stirrer was stopped. Step 7. After 10 minutes of the step 6, Add water 180 ml to make up the
20 solution to 1000 mL. Step 8. Filter the solution with 200 mesh size filter. The total volume of the solution in mL was 1000 ML at 100.472 Gram/100 mL. The calculated % CS of the solution was 2 % (w/v) with a pH of the solution at 3.91.

[00063] Preparation for solution E: Chitosan solution in H_2SO_4

[00064] The H_2SO_4 solution did not work, as the chitosan did not dissolve
25 in solution, this effectively terminated the experiment, and the H_2SO_4 solution was not used in any encapsulation production.

[00065] Preparation for solution F: Chitosan solution in HCL

[00066] Step 1. Take 800 ml water in 2000 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 600 RPM. The stirrer was continuously ON
30 through the process until it says stirrer STOP. Step 3. After 2 minutes of step 2, Add 20 gram chitosan flake. Step 4. After 6 minutes of the step 3, Add 20 mL of

35.4 % HCL. Step 5. After complete dissolution of the flake (after 44 minutes after step 4), added 100 mL water. Step 6. After 12 minutes of the step 5, The stirrer stopped. Step 7. After 10 minutes of the step 6, Added 80 mL water to make up to 1000 mL and mixed thoroughly. Step 8. Filter the solution with 200
5 mesh size filter. The total volume of the solution in mL was 1000 mL at 100.328 Gram/100 mL. The calculated % CS of the solution was 2% (w/v) with a pH of the solution at 1.09.

[00067] Preparation of Linker - Ammonium sulphate (AS) solution

[00068] Step 1. Take 0.200 gram ammonium sulphate in 50 mL capacity
10 volumetric flask. Step 2. Add 30 mL water. Step 3. Vortex the solution – resultant solution becomes clear. Step 4. Make up the volume to 50 mL using 20 mL water. The total volume of the solution in mL was 50 mL; 49.764 g. The calculated % AS of the solution was 0.4 % w/v. The calculated N % of the solution was 0.0856% w/v. The calculated the S % of the solution was 0.0980% w/v. The pH
15 of the solution was 5.75.

[00069] Preparation of Linker - Tri sodium Polyphosphate (TPP) solution

[00070] Step 1. Take 0.200 gram in 50 mL capacity volumetric flask. Step
2. Add 30 mL water. Step 3. Vortex the solution – resultant solution becomes
20 clear. Step 4. Make up the volume to 50 mL using 20 mL water. The total volume of the solution in mL was 50 mL; 49.821 g. The calculated % TP of the solution was 0.4 % w/v. The calculated P% of the solution was 0.101%. The pH of the solution was 9.76.

[00071] Formulation of the Encapsulated Urea

25 **[00072] Align N # 1**

[00073] Step 1. Take 2000 mL beaker. Step 2. Add 175 mL solution A.
Step 3. Start over head stirrer, it was continued through the process until it says stop. Step 4. After 5 minutes of the step 3, add 100 mL water. Step 5. After 6 minutes of the step 4, add 100 gram urea. Step 6. After 13 minutes of the step 5,
30 add 100 gram urea. Step 7. After 1 minutes of the step 6, add 50 mL water. Step 8. After 8 minutes of the step 7, add 50 mL water. Step 9. After 10 minutes of the

step 8, add 135 gram urea. Step 10. After 9 minutes of the step 9, add 175 solution B. Step 11. After 18 minutes of step 10, add 25 mL water. Step 12. After 52 minutes of the step 11, add 10 mL Ammonium sulphate solution (0.1 gram in 10 mL or 1% w/v). Step 12. After 15 minutes of the step 12, stirrer was stopped. The solution makes up to 1000 mL using 125 mL water. Step 13. Filter the solution with 200 mesh size filter. Step 14. Solution is ready for further use/ QC testing analyses, stored in 1 L bottle. The method resulted in chitosan encapsulation of the nutrients, with the solution having Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 18.24% (analysed by TKN), Ammonium sulphate: 0.01 (w/v), Chitosan: 1.29 % (w/v), Acetic Acid: 0.7 % (v/v), HCl: 0.7 % (v/v), pH: 3.67. Weight (gram/100 mL): 110.832 gram

[00074] **Align N # 2**

[00075] Step 1. Take 2000 mL beaker. Step 2. Add 200 mL solution D. Step 3. Started over head stirrer, it was continued through the process until it says stop. Step 4. After 3 minutes of the step 3, add 200 mL distil water. Step 5. After 5 minutes of the step 4, add 100 gram urea. Step 6. After 7 minutes of the step 5, add 100 gram urea. Step 7. After 5 minutes of the step 6, add 100gram urea. Step 8. After 8 minutes of the step 7, add 135 gram urea. Step 9. After 10 minutes of the step 8, add 100 solution F. Step 10. After 13 minutes of the step 9, add 100 solution F. Step 11. After 19 minutes of step 10, add 75 mL water. Step 12. After 5 minutes of the step 11, add 25 mL Ammonium sulphate solution (0.1 gram in 25 mL water or 0.4% w/v). Step 13. After 15 minutes of the step 12, stirrer was stopped. The solution was 1 L. Step 14. Filter the solution with 200 mesh size filter. Step 15. Solution is ready for further use/ QC testing analyses, stored in 1 L bottle. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having, Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 18.05 (analysed by TKN), Ammonium sulphate: 0.01 (w/v), Chitosan: 0.8 % (w/v), Acetic Acid: 0.4 % (v/v), HCl Acid: 0.4 % (v/v), pH: 4, and a Weight (gram/100 mL): 110.622 gram.

[00076] **Align N # 3**

[00077] **Step 1.** Take 2000 mL beaker. Step 2. Add 200 mL water. Step 3. Started over head stirrer, it was continued through the process until it says stop. Step 4. After 2 minutes of the step 3, add 200 mL solution D. Step 5. After 5 minutes of the step 4, add 100 gram urea. Step 6. After 3 minutes of the step 5, add 100 gram urea. Step 7. After 6 minutes of the step 6, add 100 gram urea. Step 8. After 11 minutes of the step 7, add 135 gram urea. Step 9. After 10 minutes of the step 8, add 200 solution C. Step 10. After 50 minutes of the step 9, add Ammonium sulphate solution (0.1 gram in 25 mL water or 0.4% w/v). Step 11. After 35 minutes of the step 10, stirrer was stopped. The solution was made up to 1000 mL, by adding 60 mL water. Step 12. Filter the solution with 200 mesh size filter. Step 13. Solution is ready for further use/ QC testing analyses, stored in 1 L bottle. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v: 20.1 (calculated); 18.17 (analysed by TKN), Ammonium sulphate: 0.01% (w/v), Chitosan: 0.8 % (w/v), Acetic Acid: 0.4 (v/v), Nitric Acid: 0.4 (v/v), a pH:3.22, and a Weight (gram/100 mL): 110.622 gram.

[00078] **Align N # 4**

[00079] **Step 1.** Take 1000 mL beaker. Step 2 Add 300 mL water. Step 3. Start magnetic stirrer, it was continued through the process until it says stop. Step 4. After 07 minutes of the step 3, add 100 gram urea. Step 5. After 4 minutes of the step 4, add 100 gram urea. Step 6. After 2 minutes of the step 5, add 100 gram urea. Step 7. After 4 minutes of the step 6, add 135 gram urea. Step 8. After 15 minutes of the step 7 (solution was not completely dissolved like semi crystals appearing bottom of the beaker), and stirrer stop. Step 9. After step 8, Take another separate 2 L beaker and added 200 mL solution C and started overhead stirrer. Step 10. After 2 minutes of step 9, add semi dissolve solution of urea (prepared in the step 7) transfer into solution C. Step 11. After 3 minutes of step 10, add 100 mL water. Step 12. After 17 minutes of step 11, add 50 mL water. Step 13. After 8 minutes of the step 12, added ammonium sulphate solution (0.1 gram in 25 mL water or 0.4% w/v). Step 14. After 25 minutes of the step 13, stirrer was stopped. The solution was make up to 1000 mL for that 25 mL water

was added. Step 15. Filter the solution with 200 mesh size filter. Step 16. Solution is ready for further use/ QC testing analyses, stored in 1 L bottle. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 18.42 (analysed by TKN), Ammonium sulphate: 0.01 (w/v), Chitosan: 0.4 % (w/v) Nitric Acid: 0.4% (v/v), pH: 2.87, and Weight (gram/100 mL): 110.586 gram.

[00080] Align N # 5

[00081] Step 1. Take 2000 mL beaker. Step 2. Add 200 mL solution D. Step 3. Started over head stirrer, it was continued through the process until it says stop. Step 4. After 2 minutes of the step 3, add 200 mL water. Step 5. After 4 minutes of the step 4, add 100 gram urea. Step 6. After 4 minutes of the step 5, add 100 gram urea. Step 7. After 12 minutes of the step 6, add 100gram urea. Step 8. After 11 minutes of the step 7, add 135 gram urea. Step 9. After 13 minutes of the step 8, add 200 solution C. Step 10. After 25 minutes of the step 9, add TPP solution (0.1 gram TPP in 25 mL water or 0.4% w/v). Step 11. After 20 minutes of the step 10, stirrer was stopped. The solution was made up to 1000 mL by adding 80 mL water. Step 12. Filter the solution with 200 mesh size filter. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 18.06 (analysed by TKN), TPP: 0.01% (w/v), Chitosan: 0.8 % (w/v), Acetic Acid: 0.4 % (v/v), Nitric Acid: 0.4 % (v/v), pH: 3.14, and a Weight (gram/100 mL): 110.726 gram.

[00082] Align N # 6

[00083] Step 1. Take 1000 mL beaker. Step 2. Add 400 mL water. Step 3. Started magnetic (bottom head) stirrer, it was continued through the process until it says stop. Step 4. After 5 minutes of the step 3, add 100 gram urea. Step 5. After 9 minutes of the step 4, add 100 gram urea. Step 6. After 4 minutes of the step 5, add 100 gram urea. Step 7. After 12 minutes of the step 6, add 135 gram urea. Step 8. After 30 minutes of the step 7 (solution was not completely dissolved, crystals were appearing at bottom of the beaker), and stirrer stop. Step 9. Take another separate 2 L volume beaker and added 200 mL solution C. The overhead

stirrer was started. Step 10. After 4 minutes of step 9, added urea semi dissolve solution (prepared in step 7) transfer into solution C. Step 11. Step 12. After 1 minutes of step 10, add 50 mL water. Step 13. After 15 minutes of the step 11, add TPP solution (0.1 gram in 25 mL water or 0.4% w/v). Step 14. After 20 minutes of the step 12, stirrer was stopped. Step 15. The solution was made up to 1000 mL by adding 25 mL water. Step 16. Filter the solution with 200 mesh size filter. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 17.9 (analysed by TKN), TPP: 0.01 % (w/v), Chitosan: 0.4% (w/v), Nitric Acid: 0.4% (v/v), pH 2.88, Weight (gram/100 mL): 110.506 gram.

[00084] Align N # 7

[00085] Step 1. Take 1000 mL beaker. Step 2. Add 400 mL water. Step 3. Start magnetic stirrer, it was continued through the process until it says stop. Step 4. After 5 minutes of the step 3, add 100 gram urea. Step 5. After 10 minutes of the step 4, add 100 gram urea. Step 6. After 20 minutes of the step 5, add 100 gram urea. Step 7. After 10 minutes of the step 6, add 135 gram urea. Step 8. After 305 minutes of the step 7 (solution was not completely dissolved like semi crystals appearing bottom of the beaker), and stirrer stop. Step 9. Take another separate 2L beaker and added 200 mL solution D, and started overhead stirrer. Step 10. After 2 minutes of step 9, add urea semi dissolve solution (prepared in the step 8) transfer into solution D. Step 11. After 3 minutes of step 10, add 25 mL water. Step 12. After 11 minutes of the step 11, add ammonium sulphate solution (0.1 gram in 25 mL water or 0.4% w/v). Step 13. After 26 minutes of the step 12, stirrer was stopped and the solution was made up to 1000 mL by adding 50 mL water. Step 14. Filter the solution with 200 mesh size filter. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v : 20.01 (calculated); 18.19 (analysed by TKN), Ammonium sulphate: 0.01 % (w/v), Chitosan: 0.4 % (w/v), Acetic Acid: 0.4 % (v/v), pH : 4.76, and Weight (gram/100 mL): 110.29 gram.

[00086] pH analysis (Figure 5) was performed according to standard protocols for drinking water as described by the United States EPA. Total

Kjendahl Nitrogen (TKN) analysis (Figure 5) was performed in accordance with method 1688 of the U. S. EPA Office of Water. The UV-Vis analysis (Figure 6) was performed according to OPPTS 830.7050. The FTIR analysis (Figures 7-13) was performed on and according to the manual for the Agilent-Cary 630.

5 **Example 4: Application and uses of the chitosan and/or chitosan/nitrogen**

nanoparticles The resultant chitosan and/or chitosan loaded nitrogen nanoparticle products or mixtures of products obtained as a result of the above Examples were used for the multidimensional effects on plants such as growth, protection, and nutrition. The multidimensional effects on plants were obtained by applying the
10 resultant product of the above Examples onto the seed surface, mixing in the soil, exposing root and/or exposing leaves. The application rate ranged between 0.01% to 50% v/w or v/v. Furthermore, the resultant products of the above Examples were used to mix with other agrochemicals to act as growth promoter, symbiont, nutrient, protection agent against pathogens or non-desired plants for synergistic
15 effects.

[00087] The invention has been described with reference to various specific and preferred embodiments and techniques. Nevertheless, it is understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

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We Claim:

1. A composition comprising: chitosan and at least 1% (w/v) nitrogen.
- 5 2. The composition of claim 1, wherein at least 50% of the nitrogen is in the chemical form of amine and/or nitrate and/or ammonia.
3. The composition of claim 2, wherein the composition comprises nanoparticle of chitosan and a nitrogen rich compound and/or mixture of
10 compounds selected from the group consisting of potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine and
15 alanine.
4. The composition of claim 1, wherein the chitosan is present at between about 0.01% and about 1.5% (w/v).
- 20 5. The composition of claim 1, further comprising a linker.
6. The composition of claim 5, where the linker is sodium tripolyphosphate and/or ammonium sulfate.
- 25 7. A method of producing chitosan nanoparticles, the method comprising:
 dissolving chitosan in acidic water, where the water is made acidic by an acid selected from the group of acetic acid, nitric acid, sulfuric acid, hydrochloric acid, and combinations thereof; and
 forming nanoparticles from the dissolved chitosan.

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8. The method according to claim 7, wherein a linker is added to the dissolved chitosan.

9. The method according to claim 8, wherein the linker is sodium
5 tripolyphosphate and/or ammonium sulfate.

10. The method according to claim 7, wherein nitrogen rich compound and/or mixture of compounds selected from the group consisting of potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium
10 nitrate, ammonium sulfate, urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine and alanine is/are added the dissolved chitosan.

11. The method according to claim 10, wherein the nanoparticles comprise
15 chitosan and nitrogen rich compound and/or mixture of compounds.

12. The method according to claim 11, wherein the nanoparticles comprise at
least 1% (w/v) nitrogen.

20 13. The method according to claim 11, wherein the chitosan is present at between about 0.01% and about 1.5% (w/v).

14. A method of treating a plant or a plant part, the method comprising
25 treating the plant or plant part with a composition according to any of claims 1-7.

15. The method according to claim 14, wherein the plant part is selected from the group consisting of a seed, leaf, stem, flower, or root of a plant.

30 16. A method of preparing a plant growth medium, the method comprising treating the growth medium with a composition according to any of claims 1-7.

17. The method according to claim 16, wherein the growth medium is soil, peat, moss, wood residue, leaf mold, sawdust, bark, bagasse, rice hull, sand, perlite, vermiculite, calcinated clay, polystyrene, urea formaldehyde resins, and hydroponic liquids, and combinations thereof.

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For Tidal Vision Products



Tarun Khurana

Regd. Patent Agent [IN/PA-1325]

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Dated: 08th May, 2023

ABSTRACT
**COMPOSITIONS AND METHODS FOR AGRI-INPUTS AND USES
THEREOF**

5 Disclosed herein are compositions comprising: chitosan and at least 1% (w/v)
nitrogen. In certain cases, at least 50% of the nitrogen is in chemical form of
amine and/or nitrate and/or ammonia. Also disclosed herein are methods of
producing chitosan nanoparticles by dissolving chitosan in acidic water, where the
water is made acidic by an acid selected from the group of acetic acid, nitric acid,
10 sulfuric acid, hydrochloric acid, and combinations thereof; and forming
nanoparticles from the dissolved chitosan. Further disclosed is the treatment of
plants or plant parts with the compositions disclosed herein.

For Tidal Vision Products



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Tarun Khurana

Regd. Patent Agent [IN/PA-1325]

Dated: 08th May, 2023

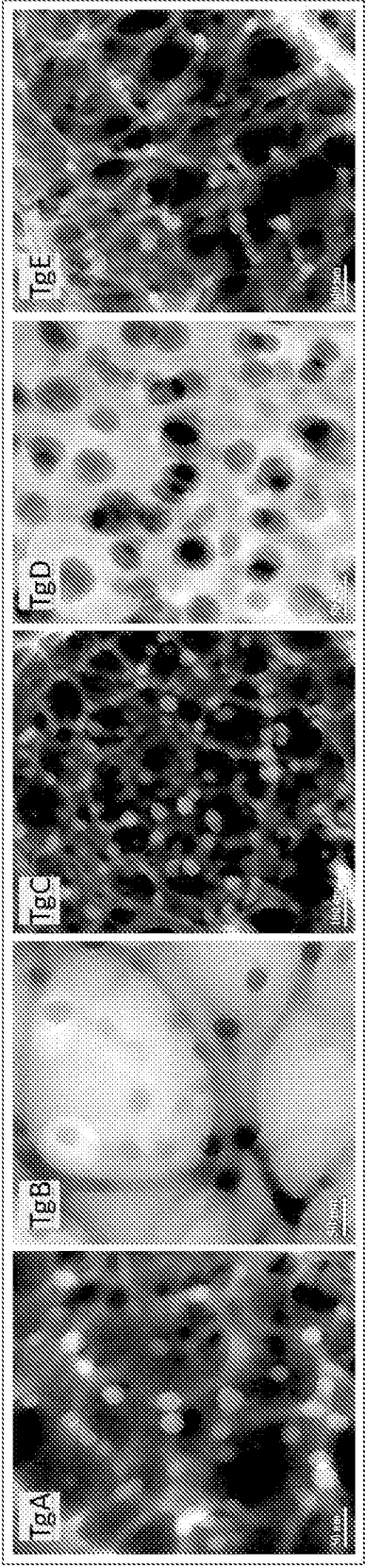


Fig. 1

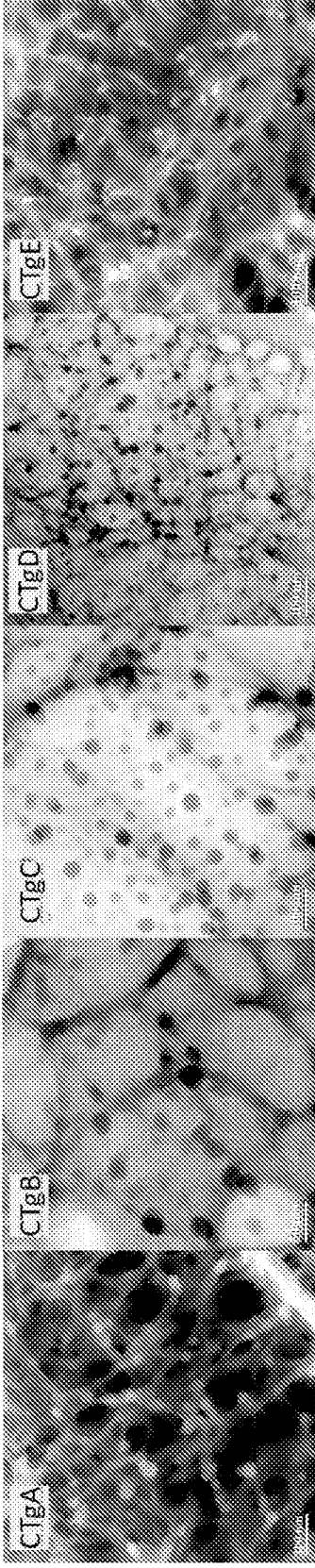


Fig. 2

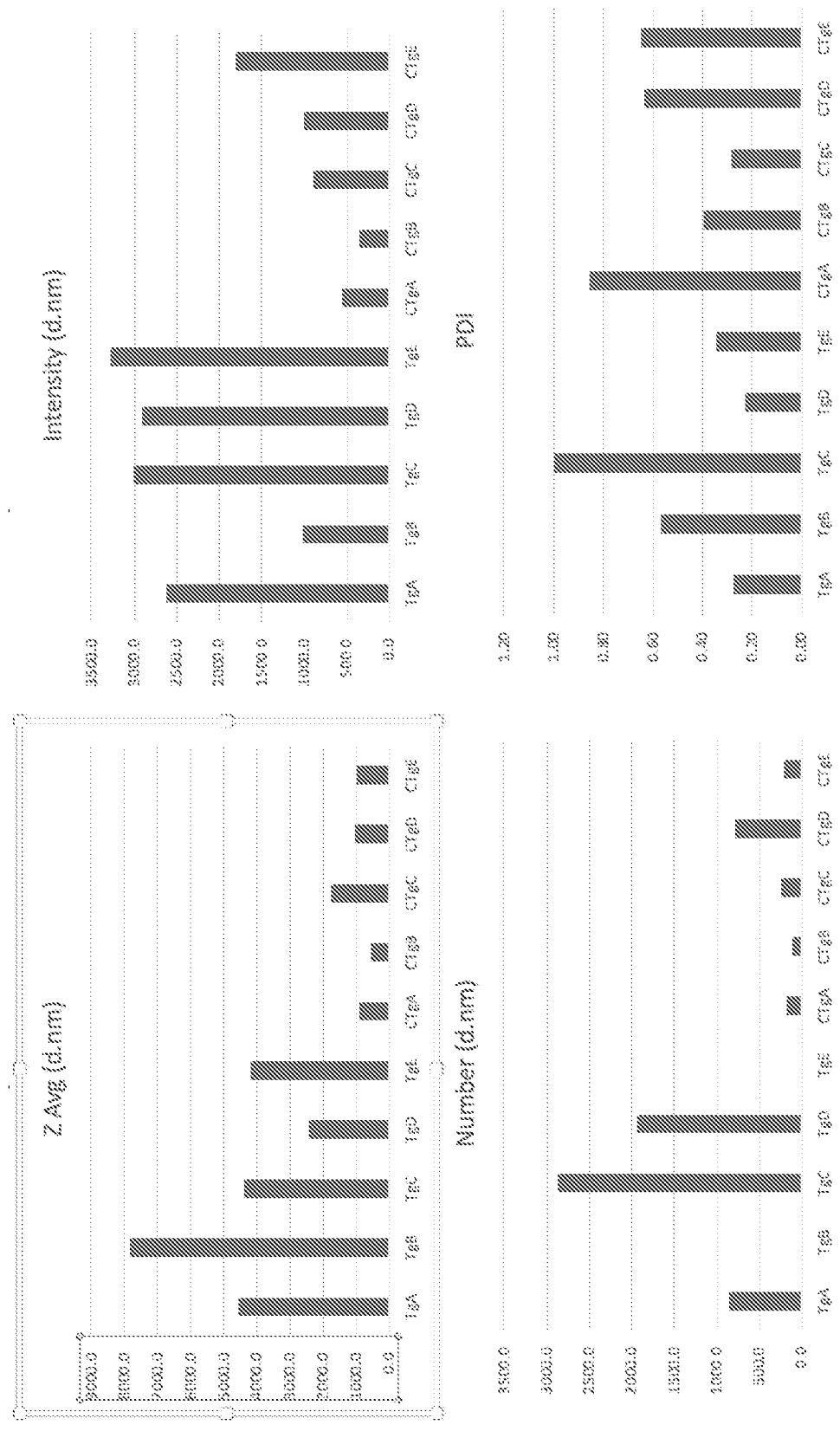


Fig. 3

[Signature]

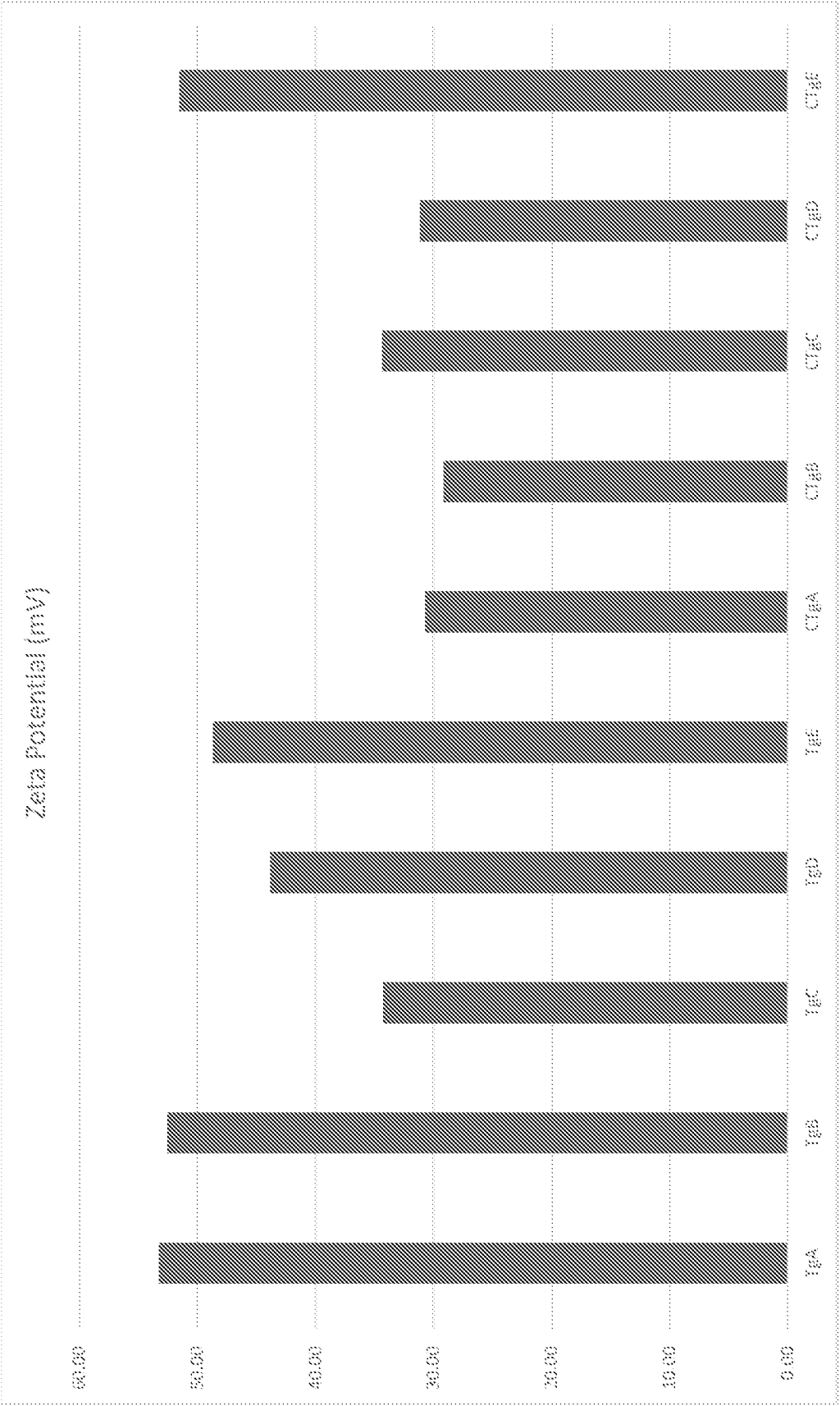


Fig. 4

Align Batch #	pH	TKN% (w/v)
1	3.67	18.24
2	4	18.05
3	3.22	18.17
4	2.87	18.42
5	3.14	18.06
6	2.88	17.9
7	4.76	18.19
CS (soln D)	4.05	0.31
N source	8.75	18.41
TBA = To be analysed		
N source = Urea		

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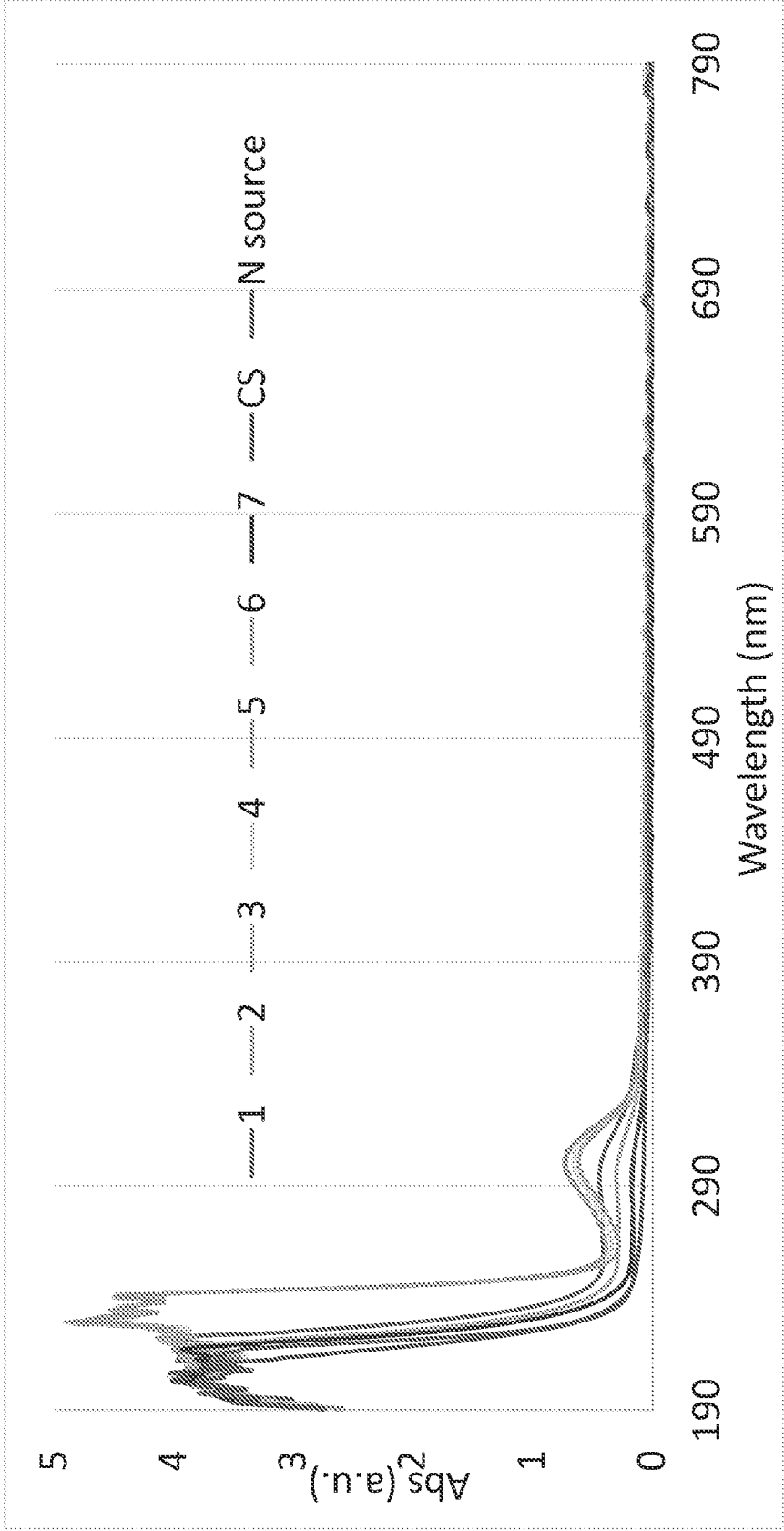


Fig. 6

[Signature]

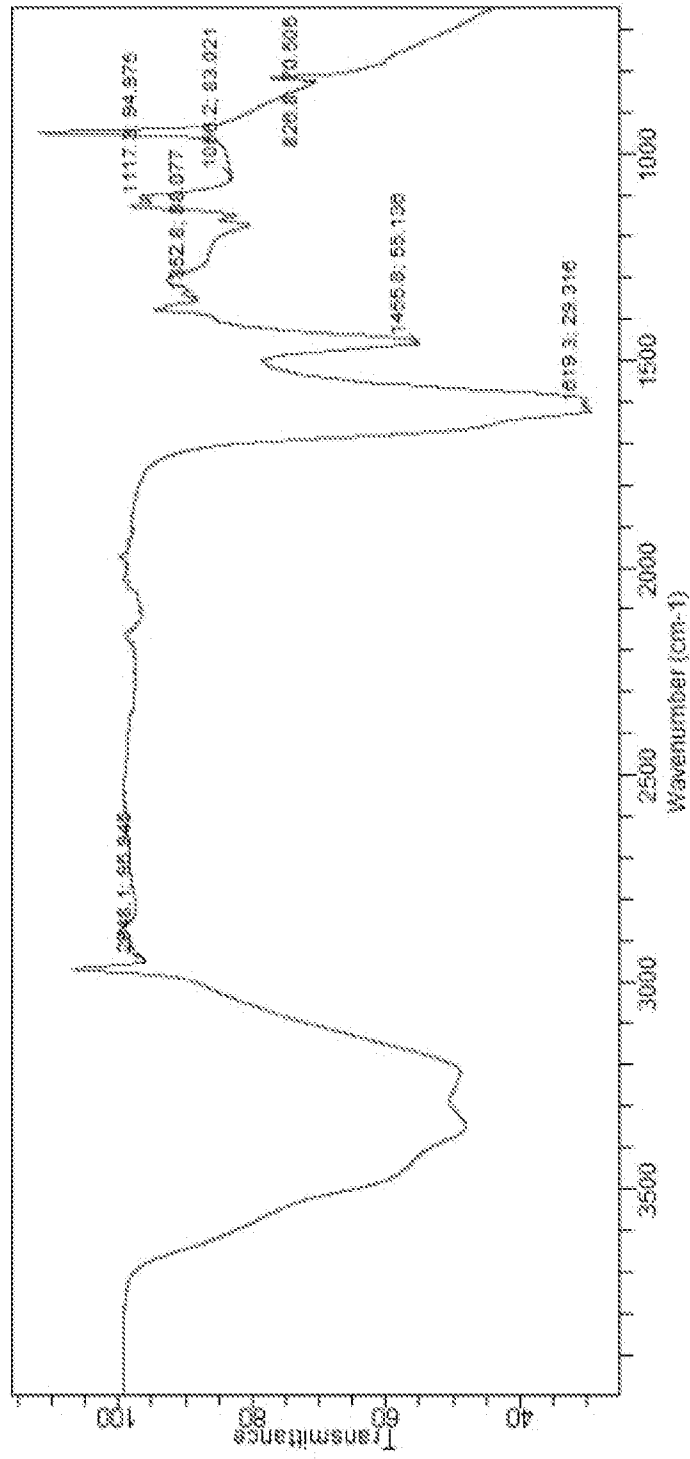


Fig. 7

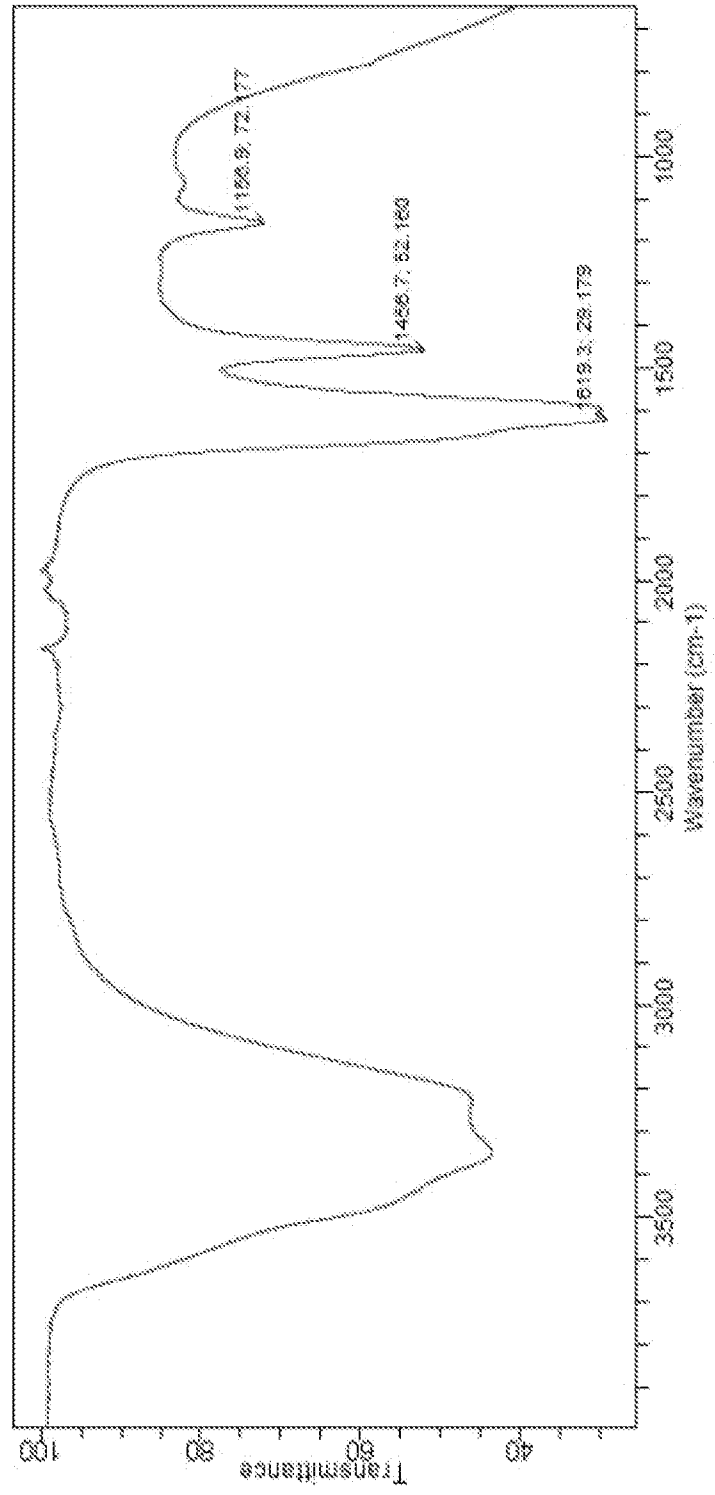


Fig. 8

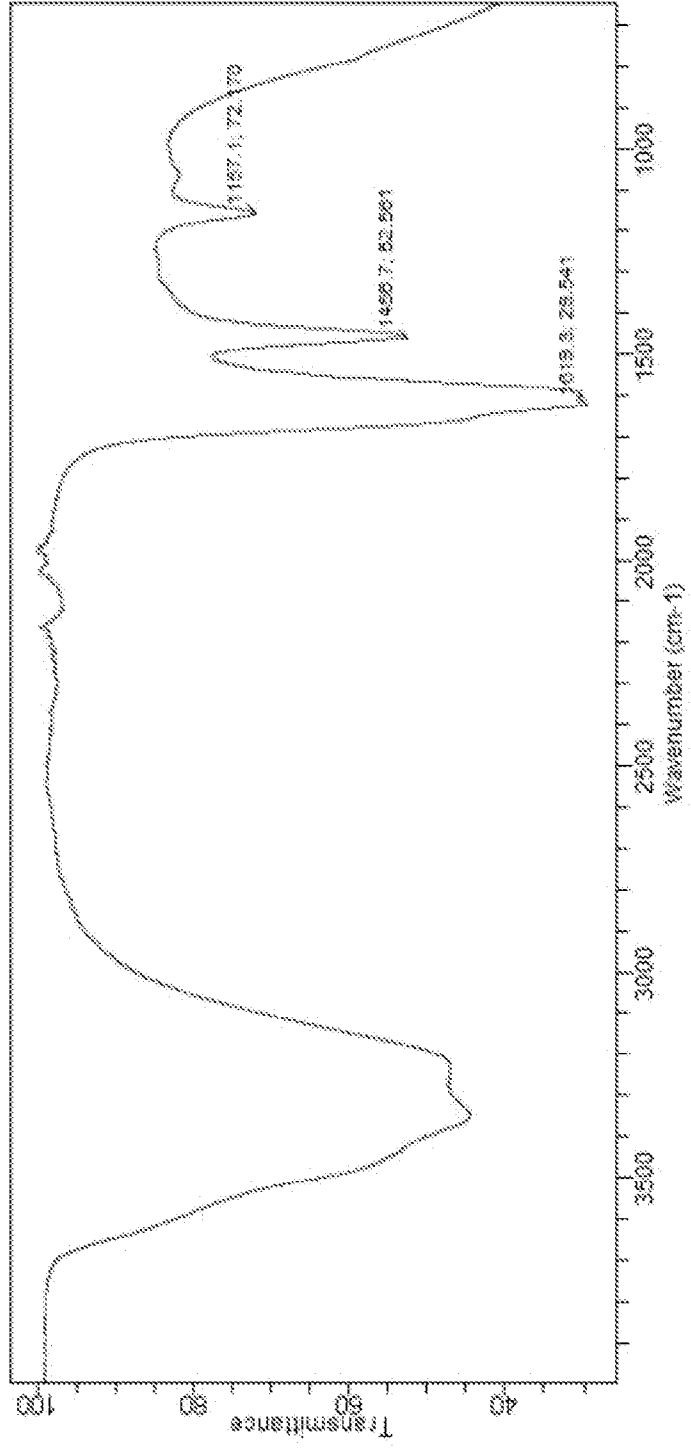


Fig. 9

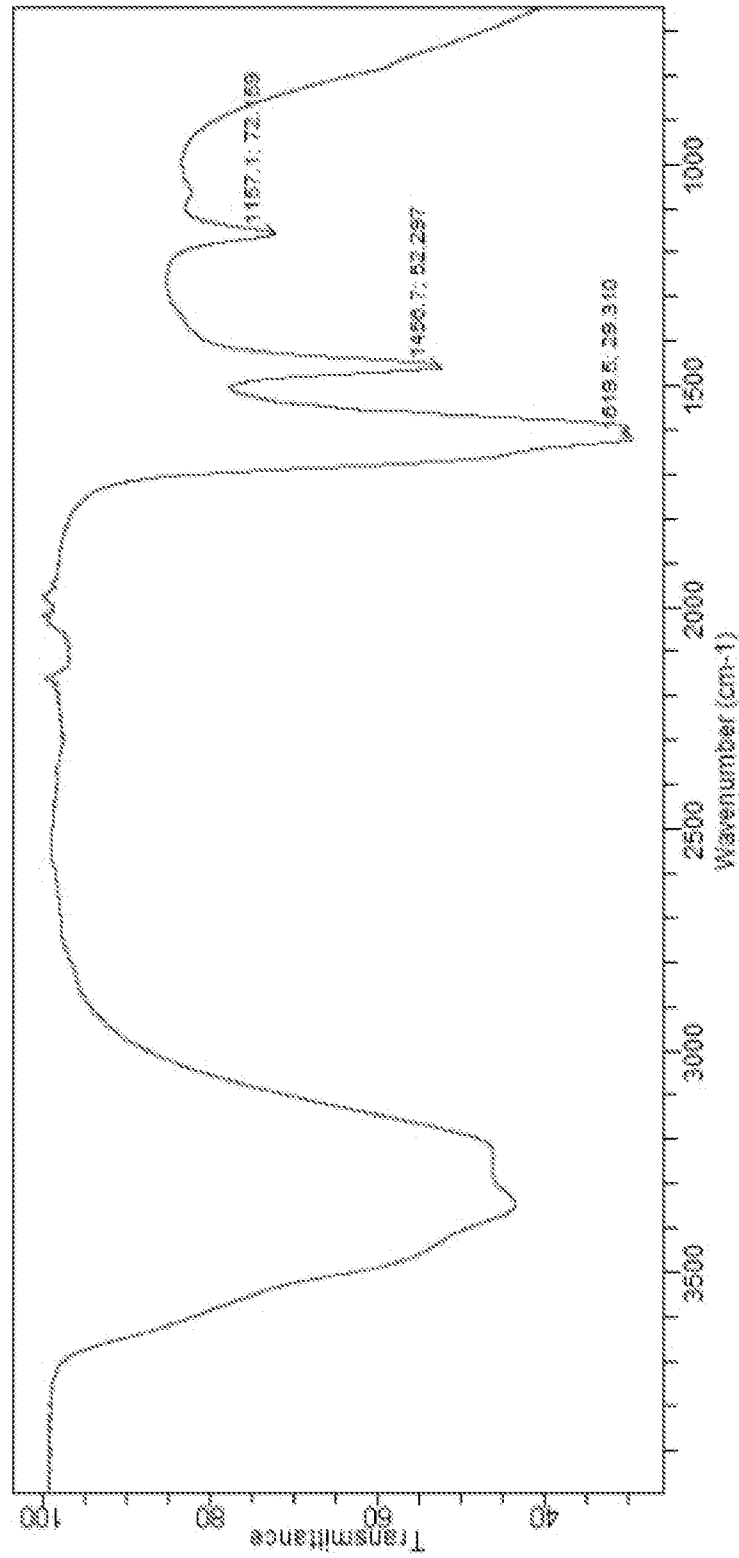


Fig. 10

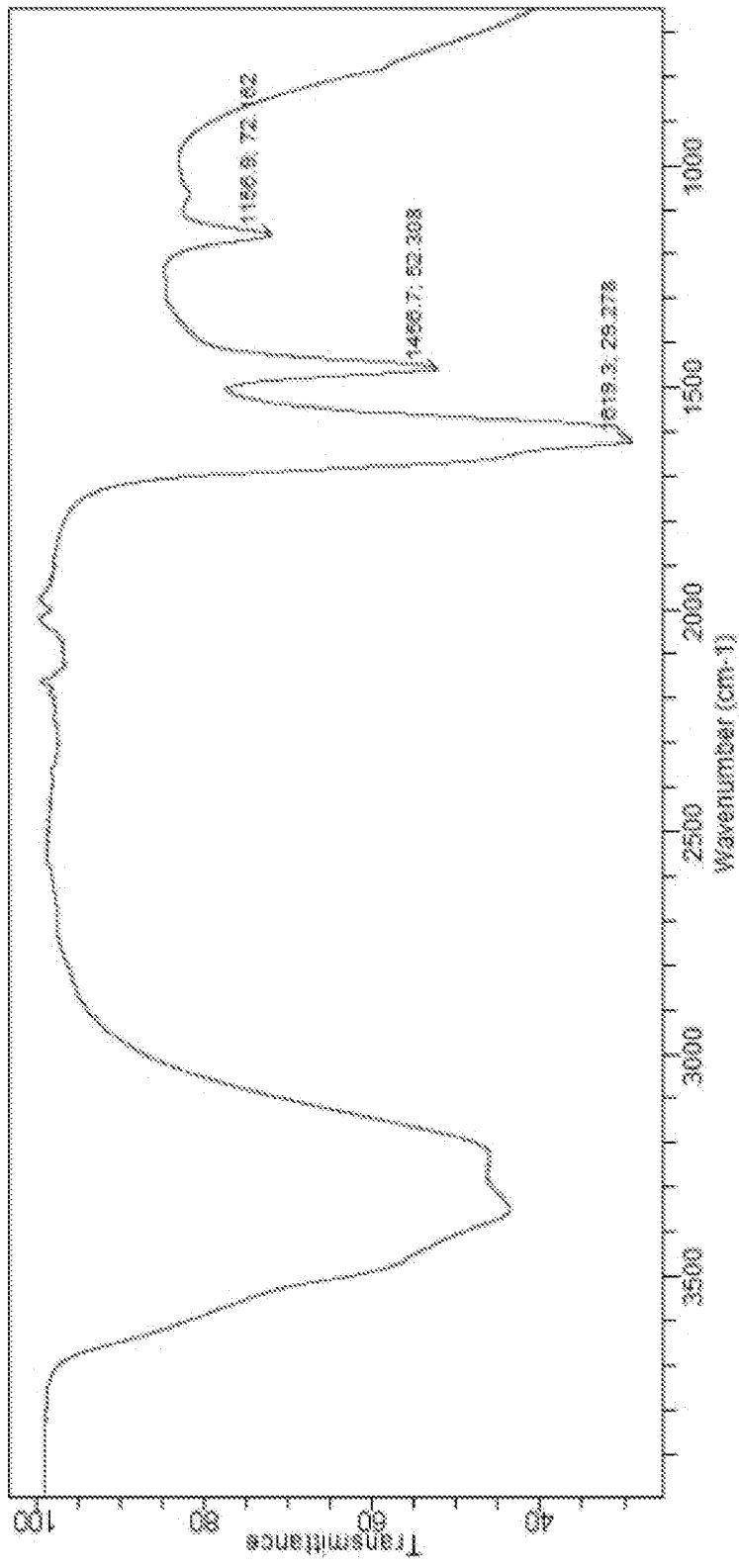


Fig. 11

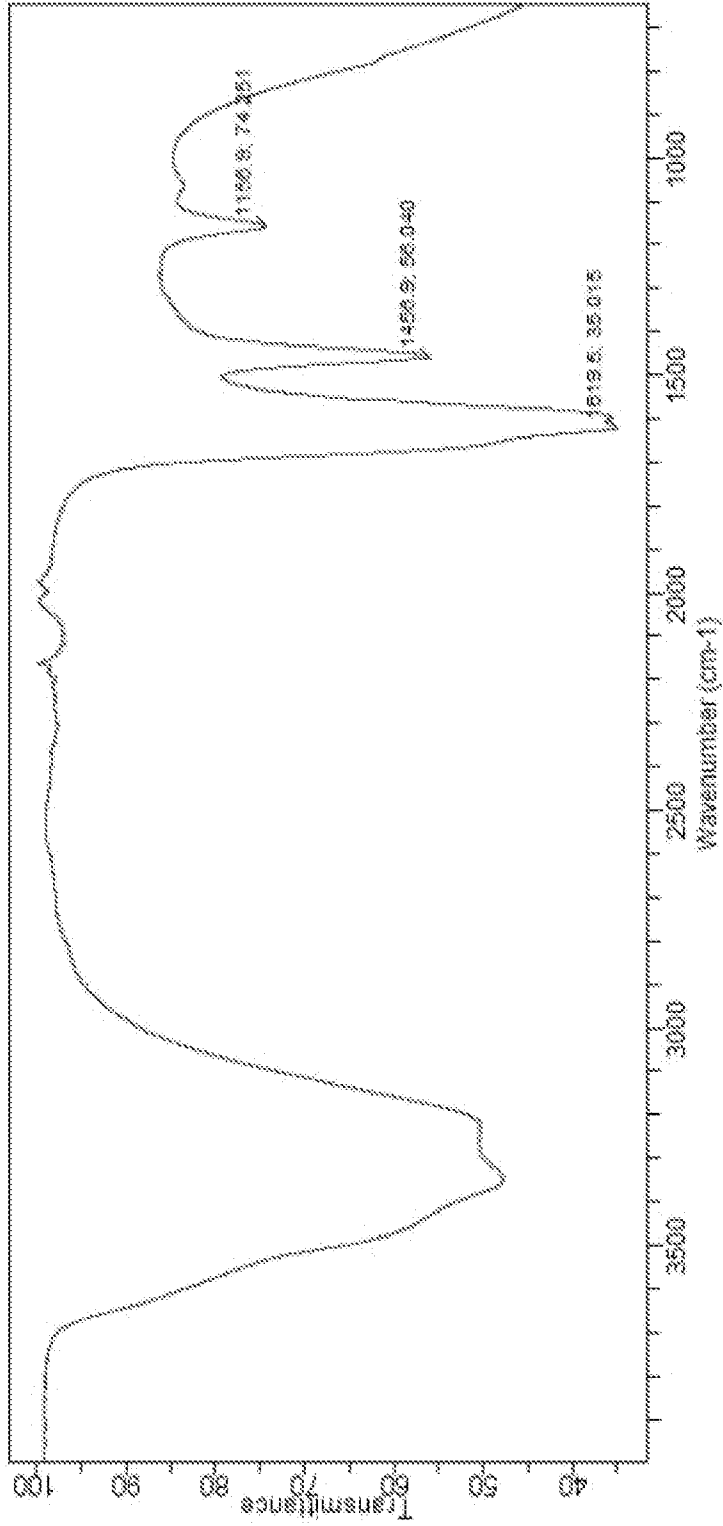


Fig. 12

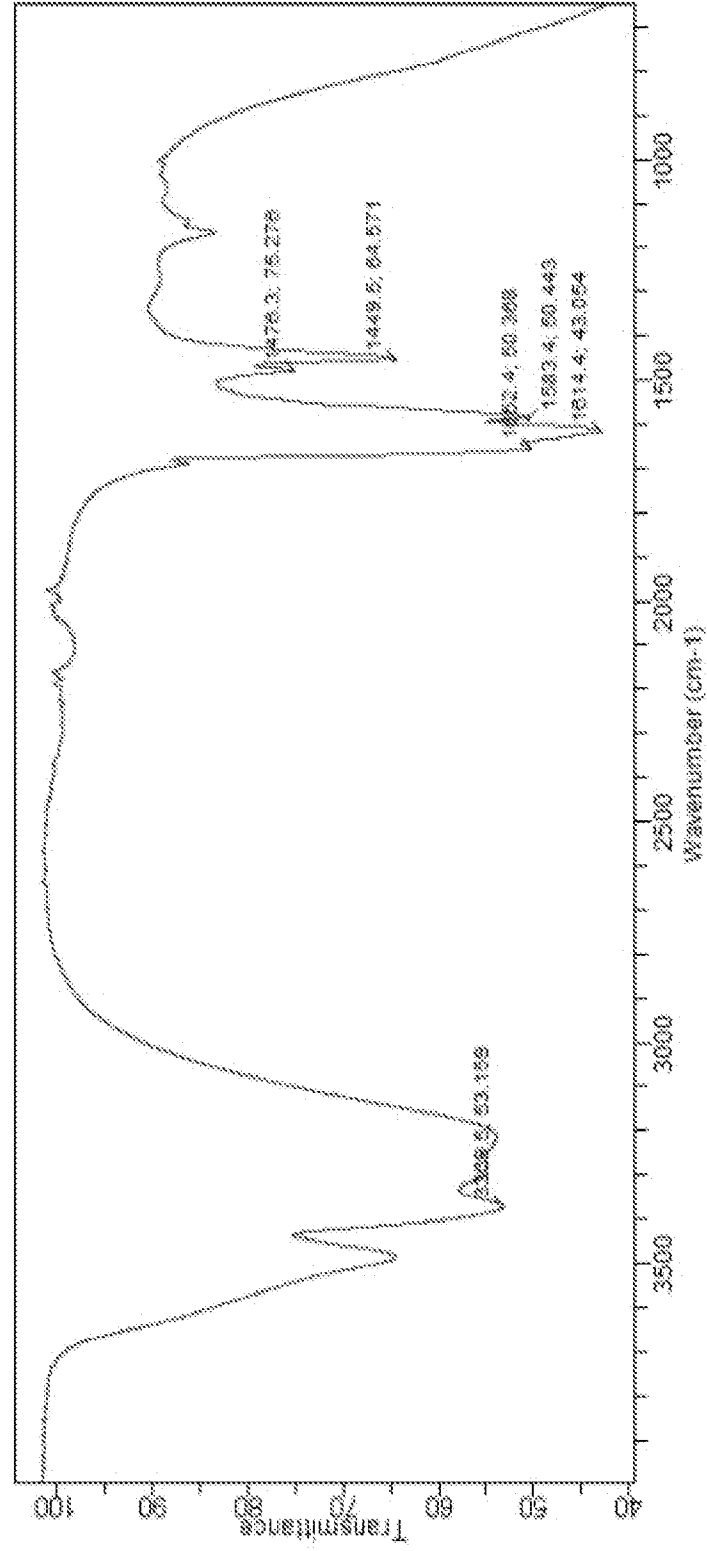


Fig. 13