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(54) Abstract Title
**Removing pathogens from water**

(57) A method for the removal of pathogens from water comprising treating the water with an aminated polysaccharide and a citrate or a polyphosphate. Also claimed is a kit for the removal of pathogens from water comprising an aminated polysaccharide and a citrate or a polyphosphate, wherein the additives are in a water-soluble form. The aminated polysaccharide may be chitosan in the form of a salt with a mono-basic anion. The salt may be an hydrochloride. The citrate or polyphosphate may be in the form of a sodium salt. The water may be treated with the aminated polysaccharide before treating with the citrate or a polyphosphate. The water may be additionally treated with an ionic polymer, which may also be provided in the kit.

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# WATER TREATMENT METHOD AND PRODUCT

This invention relates to a method of treating water, particularly to make it potable, and to products and mixtures for use in such treatment.

## Background of the invention

Access to potable water is important, but often not easy, for people and farm animals in developing countries, for travellers to such countries, for people in disaster situations, e.g. flooding, and also for military personnel. The raw water available in such situations may be contaminated *inter alia* with microorganisms, algae, humus, clay, organic and inorganic particulates and unwanted chemicals, e.g. heavy metals or chlorinated organic compounds such as pesticides. Many of the existing means of purifying water in such conditions require the use of sterilising agents, e.g. halogen containing agents which are potentially toxic or have undesirable environmental effects.

The development of emergency kits and methods for water purification has largely been driven by military interest, and chlorine producing tablets were first used by the US forces during World War I. During World War II iodine tablets were developed and have become widely used for emergency, military and commercial purposes. However such tablets can produce a water of offensive smell and taste and do not clarify the water. There is therefore a considerable hesitancy in drinking water treated in this way.

Subsequently a product CHLOROFLOC sold by Control Chemical of Alexandria which is based on a disinfection flocculation technique was developed and evaluated in a number of studies conducted between 1988 and 1992. This product has been compared to a more recent effervescent tablet comprising a complex seven component mixture (US Patent 5,681,475)

comprising a number of undesirable ingredients, e.g. organo chlorine compounds and aluminium sulphate.

An alternative form of water purification has been suggested by Kawabata *Prog. Polym. Sci. V 7, 1-34, 1992* in which microorganisms and viruses are captured and coagulated by the use of a solid cross-linked poly (N-benzyl-4-vinylpyridinium halide) used alone. However this concept is difficult to scale up, and also the use of the one agent in the solid phase means that if all the microorganisms are to be removed an excess of the comparatively expensive, non biodegradable and potentially toxic and environmentally unfriendly polymer must be used. To try to overcome some of these disadvantages Kawabata describes an attempt to impart biodegradability by the incorporation of N-benzyl-4-pyridinium groups into the biodegradable poly(methylmethacrylate).

The importance of using materials which are non toxic, and which do not degrade into toxic species has been recognised by the strict controls placed on the use of conventional polyelectrolytes by certain countries, e.g. Japan, Germany and France, for fear that they or their degradation products, may contaminate the water (Bolto Prog. Polym. Sci. Vol. 20, 1995, 987-1041 at page 1020)

Chitosan is a linear polysaccharide which is generally prepared by the alkaline deacetylation of chitin. Chitin itself is a naturally occurring polysaccharide which can be obtained from a number of sources, but is generally obtained on an industrial scale from crustacean , e.g. crab or shrimp, shells. Chitosan is composed of 1,4-beta-linked D-glucosamine and N-acetyl-D-glucosamine residues. Chitosans in their base form, and in particular those of high molecular weight and/or high degrees of N-acetylation, are practically insoluble in

water. Typically an octamer is soluble in water whatever the degree of N-deacetylation. The average pKa of the glucosamine residues in chitosan is about 6.8 and chitosan forms salts with acids, e.g. HCl and acetic acid. The salts with monobasic acids tend to be water soluble. Chitosan also has the considerable advantage that it degrades *in vivo* to non-toxic components and is readily and cheaply available.

Chitosan has been widely used in a number of water treatment processes, e.g. in swimming pools, in the treatment of waste water and in the preparation of drinking water. The addition of chitosan to waste water initiates a flocculation process in which the microorganism impurities are removed by sedimentation and/or filtration. Additionally the chitosan binds strongly to toxic and environmentally undesirable (heavy) metals, e.g. Hg, Cd and Pb, and transition metals, e.g. Cr, Ni, Mn and Cu. Chitosan also has the ability to remove polychlorinated compounds, such as PCBs, from water. However water soluble chitosan (i.e. chitosan in its salt form) on its own does not produce a full precipitate and cannot therefore be used to remove these contaminants by filtration.

Water soluble chitosan has also been suggested in combination with bentonite for the removal of lipids and oils from waste water (US Patent 5,269,939). However the chitosan is added after the bentonite. Very low concentrations of chitosan in combination with bentonite have been suggested for the removal of particulates and colour from drinking water on a large scale (US Patent 5,543,056). These processes have to be taylored *inter alia* to the quality of the incoming raw water, pH, mixing speed and the quality of the bentonite and are thus not easy to apply to water of unknown quality. In US Patent 5,433,865 a highly viscous form of chitosan is described for use in combination with an anionic polysaccharide optionally additionally with bentonite for the purification of waste water. US Patent

6,071,417 describes a method for removing water soluble macromolecular compounds from aqueous fermentation products to which the macromolecular compounds have been added as flocculants. This method comprises adding a polyphosphate and an alkaline earth metal salt to the fermentation product, then adding kaolin and filtering.

Chitosan has been used in combination with, for exmple carboxymethyl cellulose (CMC), to increase flocculation and sedimentation in water purification, but we have found that chitosan in combination with a variety of CMCs or with a number of other polymeric anionic species, e.g. alginates and dextran sulphates, does not give quantitative or near quantitative precipitation of the chitosan or of its complexes with the added polymers. Furthermore such precipitates as were obtained from the combinations with these polymers were not removed easily by filtration or sedimentation. We have also found that a large number of small anionic species, e.g. tartrates, oxalates, sulphates etc. do not provide satisfactory results in that they either give no precipitate or only a partial precipitate which is difficult to remove by filtration when admixed with an aqueous solution of chitosan

Recently Shu et. Al. *International Journal of Pharmaceutics 212 (2001) 19-28 and Ibid. 201 (2000) 51-58* have disclosed the formation of citrate cross-linked chitosan films and polyphosphate chitosan complexes in the form of beads for use in the controlled release of drugs, but have not considered the use of chitosan, and citrates or polyphosphates, admixed as separate entities for use in water treatment.

It will thus be seen that there is a very considerable literature relating to the use of chitosan to purify water on a large scale, and also that there have been many more or less successfull efforts to produce small scale water treatment products which do not contain chitosan.

Despite this there is still a considerable demand for a simple and effective means of purifying water, (particularly in emergency and military conditions), which is cheap, effective, stable, environmentally friendly and can be easily transported. Such means should also preferably leave an acceptable taste in the purified water.

We have now found a simple, specific and effective means of removing impurities, and in particular pathogenic impurities, from water. Our means uses non toxic, cheap, stable (hence giving prolonged shelf life), and in the preferred aspects of the invention edible, components, which are readily biodegradable to other non toxic components. Furthermore our means avoids the use of halogen containing sterilising agents. The means also involves the formation of precipitates which can readily be removed by filtration using, for example an ordinary coffee filter paper or a simple cloth filter of suitable pore size. The means is also simple to use and can give results which are equal to, or better than other existing means.

According to the invention we provide a method for the removal of pathogenic materials from water which comprises treatment of the water with an aminated polysaccharide and either a citrate or a polyphosphate both the aminated polysaccharide and the citrate or polyphosphate being in water soluble form.

According to the invention we also provide a kit for the purification of water which comprises an aminated polysaccharide and a citrate or a polyphosphate both the aminated polysaccharide and the citrate or polyphosphate being in water soluble form.

The aminated polysaccharide may be an aminated derivative of starch (e.g. such as can be obtained by treating starch with N-(3-chloro-2-hydroxypropyl) trimethylammonium chloride)

or of cellulose, or a partially deacetylated homopolymer of N-acetyl glucosamine, e.g. those derived from chitin. The aminated polysaccharide is preferably a chitosan.

The water solubility of the aminated polysaccharide depends on molecular weight, the pH of the solution (i.e. whether the polysaccharide is in salt form) and, in the case of chitosan, on the degree of acetylation,. The form of aminated polysaccharide, e.g. chitosan, used should be such as to have sufficient water solubility for the present purposes. We prefer the aminated polysaccharide to be such that a concentration of at least 0.1, and preferably at least 0.2% w/w can be readily obtained in water at 20°C.

We prefer the aminated polysaccharide to be present in solution at from 0.01 to 4.0% w/w, and preferably from 0.05 to 2.0% w/w. We particularly prefer to use a relatively high concentration of aminated polysaccharide, e.g. above about 0.25g, preferably above about 0.5 g, per litre and most preferably above about 1 g per litre. The upper limit of the aminated polysaccharide concentration will be set by the solubility of the particular aminated polysaccharide used.

Citrates and polyphosphates, particularly the sodium salts, tend to have high solubilities in water.

The aminated polysaccharide may be in the form of a salt with a mono basic anion, e.g. an organic anion such as acetate or propionate or preferably a glutamate or asparaginate. However we prefer to use a salt, e.g. a chitosan salt, with an inorganic anion, e.g. a halo anion, especially the chloride anion which latter imparts relatively little smell or taste to the resulting water.

We prefer the chitosan to have a degree of deacetylation of from about 40-100%, preferably from about 75-95%. We also prefer the chitosan to have a molecular weight in the range 3,000 to 2,000,000D, and preferably 10,000 to 2,000,000D. We further prefer the aminated polysaccharide to be of a reasonable commercial purity, e.g. not to contain excessive levels of heavy metals or other potentially toxic contaminants.

The citrate is preferably in the form of a salt, e.g. a salt with the ammonium or, preferably, an alkali metal cation, e.g. the sodium cation. We particularly prefer sodium citrate. We prefer not to use salts of citric acid with alkaline earth metals. We also prefer all the acid functions of the citrate to be in salt form.

When a citrate is used we prefer to use from 1 to 5:1, and more preferably from 1.5 to 2.5:1 equivalents of citrate to aminated polysaccharide calculated on the free amino functions in the aminated polysaccharide and the anionic groups of the citrate. In the case of chitosan the number of free amino functions will depend on its degree of deacetylation.

We prefer the polyphosphate to be of low molecular weight and preferably to be a tri-, tetra- or penta-polyphosphate. The polyphosphate may be in the form of a salt with the ammonium cation, or more preferably and giving a better taste in the final product, a salt with an alkali metal, e.g. the sodium, cation. We prefer all the acid functions of the polyphosphate to be in salt form.

When a polyphosphate is used we prefer to use from 0.5 to 5.0:1, preferably from 0.5 to 1.5:1 equivalents of polyphosphate to aminated polysaccharide calculated on the free amino functions in the aminated polysaccharide and the anionic groups of the polyphosphate.

Both polyphosphates and citrates have good toxicity profiles and small amounts remaining in the water will be acceptable from a safety point of view. Indeed small amounts of citrate may impart a palatable citric taste to the water.

We provide the use of a combination of both a citrate and a polyphosphate in the kit and method of the invention.

We have found that the use of a water soluble, non toxic anionic polymer, e.g. an alginate or CMC, in addition to the citrate and/or polyphosphate can help in the formation of an easily filterable precipitate. The proportion of anionic polymer used can be much less than would neutralise the amino groups on the aminated polysaccharide. These low proportions of anionic polymer are such that in the absence of the citrate and/or polyphosphate no, or only an incomplete, precipitate would normally be formed. The anionic polymer should be in readily soluble solid form, or, if in a liquid, e.g. solution, form should be of such a viscosity that it is easily, and preferably quickly, soluble in water. The anionic polymer is preferably in salt form, e.g. an alkali metal salt such as the sodium salt. The proportion of the anionic polymer should be such that it corresponds to no more than 0.5, preferably no more than 0.2, and more preferably no more than 0.1, equivalents of the aminated polysaccharide calculated on the free amino functions in the aminated polysaccharide and the anionic groups of the anionic polymer. When an anionic polymer is used the amount of citrate and/or polyphosphate may reduced correspondingly. We have found that the use of an anionic

polymer is particularly useful when the aminated polysaccharide is of high water solubility, e.g. when a chitosan of low molecular weight and/or high degree of deacetylation, is used.

We have found that a suitable CMC is one having a molecular weight of about 90 KD and an average of about 0.75 carboxymethyl groups per glucose residue.

For hard raw water containing relatively high proportions of magnesium and/or calcium, it is desirable to adjust the pH before, or during the application of the method, or the using of the kit of the invention. However the addition of a relatively high concentration of the aminated polysaccharide salt, e.g. the hydrochloride (which if necessary can be achieved by the addition of more, e.g. two or more units, of the salt) will lower the pH of most waters to within the desired range. We also contemplate adding a buffering agent, e.g. citric acid or mono sodium citrate, to the water before, or together with, the aminated polysaccharide. For reasons of the taste we prefer to avoid or minimise the use of buffering agents. Any such buffering agents must, of course, be non toxic.

We prefer the pH of the product water to be within the range of palatably potable water.

The method of the invention may be carried out continuously, e.g. by mixing the aminated polysaccharide and the citrate and/or polyphosphate with the water to be treated on a continuous basis followed by settling or filtration to remove the precipitate. The exact conditions used will depend on the raw water concerned, the particular aminated polysaccharide used, whether a citrate or a polyphosphate is used and whether an additional flocculating agent or anionic polymer such as CMC is used, but can readily be ascertained by simple experiment. Continuous, or semi-continuous, use of the method is most likely to be

appropriate for the purification of large quantities of water in fixed purification plants, but may also be used in relatively large field operations, e.g. in a disaster or to supply a large body of troops. Appropriate, and relatively large kits, according to the invention are contemplated for use in such continuous, or semi-continuous, operations.

We also provide small scale kits according to the invention which can be easily carried by a soldier, or which can be supplied to individuals in the case of an emergency. Such kits would contain one or more, say up to 10, units, each unit comprising the desired dosage of at least the aminated polysaccharide, e.g. chitosan, and citrate or polyphosphate and optionally also comprising the anionic polymer, e.g. CMC, and/or a buffering agent. The components of the kit may be packaged as separate sub-units. Each such unit of the kit is designed to be suitable for the treatment of from about 1 to 25 litres of water. The kit can also suitably comprise a container in which to mix the water and the ingredients, although any suitable container will serve, e.g. a soldier's billy can, and also means of filtering the treated water, e.g. a funnel made of plastics material and a supply of conventional filter paper such as is used in coffee making or in laboratories.

The units or sub-units may be put up as sachets in foil or plastics material, the sachets preferably being water impervious. The units may be arranged so as to facilitate the addition of the aminated polysaccharide and the citrate/polyphosphate in the order desired, e.g. the aminated polysaccharide before the citrate/polyphosphate.

We have found that it is desirable to have the aminated polysaccharide fully dissolved in the water before, the citrate or polyphosphate is dissolved in the water. When solid aminated

polysaccharide is used it is therefore desirable to add the aminated polysaccharide to the water before the addition of the citrate or polyphosphate.

The kit is also desirably provided with instructions (written and/or pictorial) as to how it should be used, such instructions preferably being printed on the container, e.g. the sachet. The instructions should, when solid aminated polysaccharide is used, indicate that the aminated polysaccharide be added to the water before the citrate/polyphosphate.

The components for use in the method and kit according to the invention may be in liquid, e.g. solution or suspension, form, but are preferably in solid, e.g. dry form.

According to the invention we also provide a mixture, and preferably a dry mixture of citrate and/or polyphosphate with an anionic polymer, e.g. CMC in its sodium salt form.

The particle size of the solid components should be such that they will dissolve readily in water. We prefer the components to be such that they will dissolve within a few minutes of addition to well stirred water. Thus we prefer finely divided, but easily flowable dry forms of the components.

We prefer the aminated polysaccharide, optionally together with any buffering agent, to be added to and dissolved in the water before the addition of the citrate and/or polyphosphate. We prefer the citrate and/or polyphosphate to be dissolved in the water before any anionic polymer. This may be achieved either by adding these latter components separately in the correct order or by adding them together. If they are added together the citrate/polyphosphate

will tend to dissolve more rapidly than the anionic polymer thus providing the desired sequence of dissolution.

The time required after the aminated polysaccharide and the citrate and/or polyphosphate have been added to the water before the water can be used will vary depending on the quality of the raw water, the specific nature and ratio of the active ingredients, and the volume and degree of mixing of water. This time can easily be ascertained by simple observation, e.g. of the formation of a precipitate and/or a clear supernatant.

The method and kit according to the invention may be used to remove bacteria, e.g. *E. coli*, *salmonella* and *ETEC. coli* from the raw water. They may also be used to remove other pathogenic microorganisms, e.g. viruses, such as HSV, hepatitis C, HIV, polio and rota viruses, and parasites, e.g.*Giardia lamblia* and *Cryptosporidium.* Additionally they may be used to remove heavy and/or radioactive metals. The method and kit may be used to extract such metals from waters, e.g. effluent or mining waters, containing them. The method and kit may also be used to remove colour and other contaminants, e.g. particulates, dyes, turbidity and polychlorinated compounds such as PCB from the water.

Without being bound by theory we believe that the invention works *inter alia* by the aminated polysaccharide, e.g. chitosan absorbing negatively charges species, such as bacteria. We therefore prefer all the aminated polysaccharide in the solution to be precipitated carrying such negatively charged species with it. We also believe that the precipitated aminated polysaccharide complex forms a three dimensional structure or net that entraps and removes the various contaminants, e.g. bacteria, viruses, parasites, clay, humus, and organic matter.

We particularly prefer to use relatively high concentrations of aminated polysaccharide and citrate and/or polyphosphate as such high concentrations tend to make the mesh size of the net formed by the precipitate small and thus more effective in the removal of contaminants.

Especially preferred processes and kits according to the invention involve chitosans, preferably in the form of their hydrochloride salts, citrate, especially sodium citrate, and CMC.

The invention is illustrated, but in no way limited, by the following Examples; Examples 3 and 4 are included for comparative purposes and do not illustrate the invention:

Examples

In all Examples unless otherwise stated, the following materials have been used.

| **Materials** | **Manufacturer** | **Batch no.** |
|---|---|---|
| *Chitosan, hydrochloride | Pronova | FP-806-01 |
| Sodium citrate | Merck | A870648 |
| Hydrochloric acid | Merck | K26840217 |
| Sodium hydroxide | Merck | B553298 |
| Disodium tartrate | Fluka | 393248/1 |
| Sodium oxalate | Fluka | 407624/1 |
| Sodium ascorbate | Fluka | 397402/1 |
| Sodium sulphate | Merck | TA381949 |
| Sodium dihydrogenphosphate | Fluka | 264352 |
| Disodium hydrogenphosphate | Riedel de Haen | 32210 |
| Trisodium phosphate | Merck | A277178 |
| Tripolyphoshate, pentasodium salt | Sigma | 46H1055 |
| ‡CMC, sodium salt | Sigma | 34H0310 |
| #Dextran sulphate | Pharmacia | 284301 |
| †Alginic acid, sodium salt | Fluka | 285044 |
| Uricult sticks | Orion diagnostica | BK007, CH010 |
| Filter paper | Munktell | No 8 and no 3 |
| Water | Medicarb | 18.2 M Omega |

*Deacetylation degree: 84%. Molecular weight: approx. 200KD.
†Molecular weight approx 150KD
#2.3 Sulphate groups per glucose residue. Molecular weight approx. 500KD

‡Degree of substitution 0.65-0.9. Molecular weight approx 90KD

Example 1 Precipitation of chitosan with sodium citrate

250 Mg chitosan hydrochloride is dissolved in 250 ml of the water to be purified with agitation until complete dissolution, giving a pH of 4.2. Sodium citrate (approx. 250 mg) is added to the solution giving a charge ratio between the chitosan and the citrate of approx. 1:2. Precipitation starts almost immediately and after a short period the precipitate settles to provide a clear water phase having a pH of about 6.4. The pH of the product can be adjusted by conventional means if desired.

Example 2 Precipitation of chitosan with tripolyphosphate

250 Mg chitosan hydrochloride is dissolved in 250 ml of the water to be purified with agitation, the resulting solution having a pH of 4.2. Pentasodium tripolyphosphate (approx. 100 mg) is added to the solution giving a charge ratio between the chitosan and the pentasodium tripolyphosphate of approx. 1:1. Precipitation starts almost immediately and after a short period the precipitate settles to provide a clear water phase having a pH of about 6.4. The pH of the product can be adjusted by conventional means if desired.

Example 3 Use of other polyanions to try to form filterable complexes with chitosan

Initial experiments with the sodium salt of alginic acid, carboxymethylcellulose sodium salt (CMC) or dextran sulphate sodium salt were carried out. The dextran sulphate only provided turbidity to the solution with no filterable precipitate. Both CMC and alginic acid gave somewhat more hopeful results and we therefore carried out 6 experiments with different

quantities of chitosan and CMC using chitosan:CMC ratios (w/w) of approx. 1:0.3, 1:0.5, 1:1, 1:2.5 and 1:10.

In the last 3 experiments where there was a weight excess of CMC the water became turbid, but the turbidity was not removable by filtration. In the remaining experiments precipitates together with clear water phases were produced, but when further CMC was added to the filtrate further precipitates were produced indicating that not all of the chitosan had been removed in the initial precipitate.

Example 4 Use of other salts in order to form filterable precipitates with chitosan

A. In this experiment sodium tartrate, sodium oxalate and sodium ascorbate were used.

250 Mg of chitosan hydrochloride was dissolved in 250 ml of the water to be purified with agitation until complete dissolution giving a pH of 4.2. Amounts of the comparison salts were added corresponding to the amount of citrate used in Example 1. None of these salts produced filterable precipitates.

B. In this experiment we used sodium sulphate and each of the three sodium phosphates. 250 Mg of chitosan hydrochloride was dissolved in 250 ml of the water to be purified with agitation until complete dissolution giving a pH of 4.2. 250 Mg of the chosen salt were than added. None of these salts produced filterable precipitates.

Example 5 Analysis of chitosan remaining in water after precipitation of the complex

750 Mg chitosan hydrochloride was dissolved with shaking in a flask containing 750 ml of the water to be purified, and a small sample (sample 1) withdrawn. 750 Mg of sodium citrate was then added to the flask with shaking. 30 Min. after the addition of the citrate the precipitated complex was filtered off and a second sample (sample 2) was withdrawn from the water phase.

The two samples were analysed for their chitosan content. The result showed that sample 1 contained 1 mg/ml of chitosan (the expected value), whereas in sample 2, even after concentration 20 times, no chitosan could be found. The detection limit of the analytical method is 3.0 µg/ml.

Analytical method: Solutions of chitosan in dilute acetic acid are shown to induce a metachromatic effect in the visible spectrum of the anionic dye sodium 2′-hydroxy-1,1′-azo-naphtalene-4-sulfonate. Quantitative use of the metachromatic effect enables the determination of the concentration of chitosan. (Gummow and Roberts, Makromol. Chem. 186, 1239-1244, 1985)

Example 6 Treatment of different kinds of tap water with chitosan/sodium citrate

Tap water from 3 different areas in Sweden (Gotland, Uppsala and Stockholm) and with different hardness (Uppsala: 16 °dH and Stockholm: 6 °dH) was treated with chitosan/ sodium citrate using the following procedure:
500 Mg of chitosan hydrochloride was dissolved in 250 ml of each of the tap waters and the pH tested and adjusted if necessary to be in the range 4-5 by addition of hydrochloric acid.

500 Mg of sodium citrate was added and within a few minutes precipitation occurred in each of the different tap waters.

The treated water (Uppsala) was after filtration freeze dried and the residue was analysed by NMR. The result showed small amounts of sodium citrate but no detectable amounts of chitosan.

The considerable difference in hardness of the waters, each of which produced a satisfactory precipitate, demonstrates that the process is applicable to different types of water.

Example 7 Treatment of dirty river water

500 Mg of chitosan hydrochloride is dissolved in 250 g of water from a dirty river (Bällstaån, Bromma, Sweden; turbidity: 38.1 FNU) and the pH adjusted to 4.5 with dilute hydrochloric acid. 500 Mg of sodium citrate was added and within a few minutes precipitation occurred giving a water of the appearance of normal tap water (turbidity: 0.46 FNU), all the colour having been removed. Turbidity was analysed at the same time on a sample of water for injection (WFI, turbidity: 0.10).

River water 1) before the above treatment and 2) after the above treatment were tested by dipping Uricult sticks (a culture based system for detection of bacteria in urine) in the water samples for 10 secs., draining off excess water and incubation at a temperature of 36±2°C for 24-48 hours.

The results were as follows:

1) The stick was covered by approx. 10% bacterial colonies or other impurities
2) The stick had no bacterial colonies

The experiment above was repeated with sodium tripolyphosphate instead of sodium citrate. 230 Mg of sodium tripolyphosphate was used in this experiment. The pH after addition of tripolyphosphate was 7.3 and dilute HCl was added to give a pH of 6.5.
Turbidity after treatment: 9.2 FNU. The Uricult stick had no bacterial colonies after the treatment.

Example 8 Purification of water containing *E.Coli*

*E.Coli* (ATCC 25922) was added to 250 ml of water to a concentration of about $10^8$ cfu/ml. compared to a McFarland suspension (a suspension of barium sulphate in water where the optical density corresponds approx. to a bacterial suspension. Different concentration of barium sulphate corresponds to different concentrations of bacteria). 250 Mg of chitosan hydrochloride were then dissolved in the water, 250 mg of sodium citrate were then added and the resulting solution left to stand for 30 mins. and then filtered. The water before and after treatment was tested using Uricult sticks as in Example 7 above. The water before treatment caused the stick to be 80-90% covered with bacterial colonies: the water after treatment produced no colonies on the stick.

In control experiments we:

1) treated the *E.Coli* containing water with chitosan hydrochloride only,
2) treated the *E.Coli* containing water with sodium citrate only, and
3) filtered the *E.Coli* containing water

For all of these control treatments the stick was 80-90% covered with bacterial colonies.

Example 9 Purification of water containing *ETEC Coli* and *Salmonella*

*ETEC Coli* (E55:1) was added to 250 ml of water to a concentration of about $3x10^3$ cfu/ml. 250 Mg of chitosan hydrochloride was then dissolved in the water and after about 30 mins. 250 mg of sodium citrate was added. The resulting precipitate was filtered off after 20 mins. The concentration of the bacteria in the water before and after treatment was measured by culturing on agar dishes.

The same procedure was carried out, but using, in place of the *ETEC coli, salmonella* (M206) at an initial concentration of about $2x10^5$ cfu/ml.

The treatment yielded water containing no *ETEC Coli* (100% removal) and 80 cfu/ml of *salmonella* (99.96% removal) respectively.

Example 10 Treatment of water with chitosan and sodium citrate in combination with CMC sodium salt

250 Mg chitosan hydrochloride was dissolved in 250 ml of the water to be purified with agitation giving a solution of pH 4.2. To this solution sodium citrate was added in 4 different concentrations alone or in combination with CMC sodium salt. The CMC sodium salt had 0.65-0.90 carboxymethyl groups per glucose residue. For calculation purposes 0.75 carboxymethyl groups per glucose residue has been used. Thus addition of:

1) 120 Mg sodium citrate

2) 90 Mg of sodium citrate and, after dissolution of the citrate, 9g of a 1% aqueous solution of CMC sodium salt, corresponding to 90 mg of CMC sodium salt. 90 Mg of this CMC sodium salt is equivalent to 30 mg of citrate with respect to negative charges.

3) 60 Mg of sodium citrate and, after dissolution of the citrate (approx. 10 secs.), 18 g of a 1% aqueous solution of CMC sodium salt, corresponding to 180 mg of CMC sodium salt. 180 Mg of this CMC sodium salt is equivalent to 60 mg of citrate with respect to negative charges.

4) 105 Mg of sodium citrate and, after dissolution of the citrate (approx. 10 secs.) 4.5 g of a 1% aqueous solution of CMC sodium salt, corresponding to 45 mg of CMC sodium salt. 45 Mg of this CMC sodium salt is equivalent to 15 mg of citrate with respect to negative charges.

Experiments 2 and 3 gave the fastest precipitations and the clearest water. After standing overnight experiments 1 and 4 gave almost clear water phases.

In these experiments we found it preferable to dissolve the sodium citrate before addition of the CMC sodium salt.

Example 11 Treatment of water with chitosan and sodium citrate in combination with CMC sodium salt

250 Mg chitosan hydrochloride was dissolved in 250 ml of tap water (Stockholm) with agitation giving a solution of pH approx. 5.4. To this solution 200 mg sodium citrate was added.

1) After dissolution of citrate, 2 ml of 1% CMC sodium salt solution was added (corresponds to 20 mg of CMC sodium salt) and a precipitate was obtained within 10 min. The water phase was still turbid but became clear after filtration.

2) After dissolution of citrate, 5 ml of 1% CMC sodium salt solution was added (corresponds to 50 mg of CMC sodium salt). Precipitates within 1 min. Clear water phase. Fast filtering speed about 2 min.

3) After dissolution of citrate 10 ml of 1% CMC sodium salt solution was added (corresponds to 100 mg of CMC sodium salt). Precipitates within 1 min. Turbid water phase; turbidity removed on filtration. Fast filtering speed about 2 min.

4) To the chitosan solution was added a dry mixture of 200 mg sodium citrate and 50 mg CMC sodium salt,. The result was comparable to that in experiment 2).

Example 12 Treatment of water with chitosan and sodium citrate in combination with alginic acid sodium salt

250 Mg chitosan hydrochloride was dissolved in 250 ml of the water to be purified with agitation to give a solution of pH of 4.2. To this solution 200 mg sodium citrate was added and after dissolution 5 ml of 1% sodium alginate was added (corresponds to 50 mg of sodium alginate). A precipitate formed within 1 min. giving a clear water phase and a fast filtering speed of about 5 mins.

Example 13 Low molecular weight chitosan in water treatment

200 Mg chitosan (free amine, Mw 22.000 Dalton) was added to 250 ml of water with the

addition of 1M hydrochloric acid. When all chitosan was dissolved the pH was adjusted to 4.5 with 1M HCl. Sodium citrate, 200 mg, was added to the chitosan solution. The water became turbid and CMC, 40 mg, was added and a filterable precipitate was obtained within approx. 1 min.

Example 14 Water treatment kit

The kit compromises 10 each of separate sachets containing 1) 2.0 g of chitosan hydrochloride and 2) 1.6 g of sodium citrate in admixture with 0.4 g CMC sodium salt with instructions to dissolve the contents of one chitosan sachet in 1 L of the water to be purified followed by one citrate/CMC sodium salt sachet; to await the formation of a precipitate and the clearing of the supernatant water and then either to decant the supernatant water ready for use, or to filter it using the filters provided. These filters are no. 3 filter papers or similar. The kit also comprises a funnel to support the filter papers.

Optionally the kit may include vessels/flasks to contain the water to be treated and the treated product water.

Claims

1. A method for the for the removal of pathogenic materials from water, which comprises treatment of the water with an aminated polysaccharide and a citrate or a polyphosphate, the aminated polysaccharide and the citrate or polyphosphate being in water soluble form.

2. A kit suitable for the removal of pathogenic materials from water, comprising an aminated polysaccharide and a citrate or polyphosphate, the aminated polysaccharide, the citrate or polyphosphate being in water soluble form.

3. A method or kit according to claim 1 or 2 respectively, wherein the aminated polysaccharide is chitosan in the form of a salt with a mono basic anion.

4. A method or kit according to claim 3, wherein the salt is an hydrochloride.

5. A method or kit according to any one of the preceding claims, wherein the citrate or the polyphosphate is in salt form.

6. A method or kit according to claim 5, wherein the citrate or the polyphosphate is a salt with an alkali metal cation.

7. A method or kit according to claim 6, wherein the salt is a sodium salt.

8. A method or kit according to any one of the preceding claims, wherein from 1 to 5 :1 equivalents of citrate to aminated polysaccharide, or from 0.5 to 5.0:1 equivalents of polyphosphate to aminated polysaccharide, are incorporated calculated on the free amino functions in the aminated polysaccharide and the anionic groups of the citrate or polyphosphate respectively.

9. A method according to any one of the preceding claims, wherein the water is treated with the aminated polysaccharide before it is treated with the citrate or polyphosphate.

10. A method according to any one of the preceding claims, wherein the water is additionally treated with an anionic polymer.

11. A kit according to any one of claims 2 to 9, wherein the kit additionally comprises an anionic polymer.

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**Claims searched:** 1-11 **Date of search:** 15 July 2002

# Patents Act 1977
# Search Report under Section 17

## Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.T): C1C

Int Cl (Ed.7): C02F (1/50)

Other: ONLINE: WPI, EPODOC, JAPIO; CAS ONLINE

## Documents considered to be relevant:

| Category | Identity of document and relevant passage | | Relevant to claims |
|---|---|---|---|
| A | GB 2364047 A | (PROCTOR & GAMBLE) see whole document | |
| A | US 6217780 | (ZODIAC POOL CARE) see examples | |
| A | US 6045785 | (WACHTER) see examples | |

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