TITLE: PROCESS FOR THE PREPARATION OF WOUND DRESSING SPONGE

ABSTRACT: The present invention relates to a process for the preparation of wound dressing sponge comprising hydrophilic polymer, acid and aqueous vehicle. The present invention also relates to efficient process for the preparation of wound dressing sponge, wherein process comprising steps of freeze-drying, annealing, packaging and sterilization.
PROCESS FOR THE PREPARATION OF WOUND DRESSING SPONGE

FIELD OF THE INVENTION

The present invention relates to a process for the preparation of wound dressing sponge comprising hydrophilic polymer, acid and aqueous vehicle.

The present invention also relates to efficient process for the preparation of wound dressing sponge, wherein process comprising steps of freeze-drying, annealing, packaging and sterilization.

BACKGROUND OF THE INVENTION

Ideally, wound dressing needs to provide some basic physical and biological function, such as avoiding fluid loss, human skin-like with flexibility, gas permeation, water vapour flux, absorbing wound exudate, mechanical strength, anti-bacterial or sterilization without causing wound aggravation, adherence to wound, non-toxicity, non-irritant and improvement of epithelization etc. Therefore, varieties of wound dressings have been developed to fulfil the aforementioned requirements in the form of non-woven, porous and membrane types are available for different wound conditions.

Wounds are often treated by covering them with products such as alginates, composites, contract layers, foams, hydrocolloids, hydrogels, impregnated gauzes, specialty absorptive, and transparent films. The theory behind the use of these products is that covering the wound decreases the risk of infection, keeps the wound from drying out, and decreases scarring.

Hydrophilic polymers include but not limited to polyacrylate, an alginate, chitosan, a hydrophilic polyamine, a chitosan derivative, polylysine, polyethylene imine, xanthan, carrageenan, quaternary ammonium polymer, chondroitin sulfate, a starch, a modified cellulosic polymer, a dextran, hyaluronan or combinations thereof.
The starch may be of amylase, amylopectin and a combination of amylopectin and amylase. Preferably, the hydrophilic polymer is chitosan.

Chitin and its derivatives has gained great interest in biomedical application including tissue engineering, drug delivery, wound healing, obesity treatment because of their versatile features like natural source, antimicrobial property, low immunogenicity, biodegradability and biocompatibility. Furthermore, these can be molded easily into various forms and shapes including sponges, films, gels, beads and fibres.

Chitin is a macromolecule commonly found in invertebrates, algae cell walls and yeast. Mostly extracted from crab or shrimp shells. Deacetylated form of chitin is called as chitosan, it should contain at least 60% of D-glucosamine residues. Prudden et al. has developed and commercialized wound dressing as medical product named "bas-Chitin W", manufactured by UNITIKA, Japan, and its clinical manifestation was good.

Chitosan is a linear, semi-crystalline polysaccharide composed of (1-4)-2-acetamido-2-deoxy-D-glucan (N-acetyl D-glucosamine) and (1-4)-2-amino-2-deoxyb-D-glucan (D-glucosamine) units.

\[
\text{HO} \quad \text{OH} \quad \text{OH} \\
\text{HO} \quad \text{NH}_2 \quad \text{NH}_2 \\
\text{HO} \quad \text{NH}_2 \quad \text{OH}
\]

Chitosan as wound dressing material is used in various types of conditions including haemostasis, pressure sores, diabetic ulcers, leg ulcer, donor sites and graft sites, surgical wounds, skin abrasions and lacerations, 1st and 2nd degree burns, trauma wounds.

The superior physical and mechanical properties including high surface area, porosity and tensile strength of wound dressing sponge depends on chitosan.
concentration, manufacturing process and freeze-drying parameters, further depends on the physico-chemical properties chitosan including solubility, molecular weight, degree of deacetylation and viscosity.

The antibacterial activity of chitosan is due to its cationic nature; hence, it can attach and penetrate the anionic bacterial cell wall, binds with DNA and inhibit transcription and protein synthesis. Further, chitosan can form impermeable layer and leads to bacterial cell death.

US 4,532,134 disclose the medical properties of chitosan which include hemostasis, hindered of growth of fibroblast, and improving tissue regeneration.

Japan Patent Publication Number 08-224293 mentions the medical properties of chitosan includes activating macrophage and leukocyte, anti-bacterial, and further preventing wound suppurred.

CN 1387922 A discloses the composition of Chitosan and collagen sponge composition and process for the preparation thereof. The sponge of this invention is prepared by 1. Chitosan and collagen are weighed. 2. Chitosan solution with glacial acetic acid formulated as a concentration of 0.5% to 3% of the solution. 3. Collagen with glacial acetic acid formulated as a solution concentration of 1% to 4% solution. 4. Mixing the two solutions, and then the mixture by vacuum freeze-dried to give quick-setting hemostatic sponge. Collagen is extra here which is also polymer.

US 2005/0147656 disclose the tissue dressing applied on a site of tissue injury to ameliorate bleeding, other forms of fluid loss and as protective covering. This patent discussed about the manufacture of tissue dressing pad assembly and tissue dressing sheet assembly. Further, indications and configurations for hydrophilic polymer sponge. The wound dressing sponge was prepared by freeze-drying technique and the duration to complete the process is about 42-48h.

US 2005/0203058 disclose stable compositions comprising α- and β-chitosan and derivatives thereof for controlled absorption and/or coagulation of fluids from a wound or bleeding site. This invention further discloses methods for preparing these
stable compositions and articles of manufacture comprising these compositions. The wound dressing pad was prepared by freeze-drying technique and the duration to complete the process is about 18h.

US 2009/0130186 disclose wound dressing composition comprising chitosan and silver nanoparticles. It also discloses the process for the preparation of chitosan, silver nanoparticles composition, wherein chitosan/water solution is processed by the addition of an acid to the chitosan/water solution, then silver nanoparticle solution is prepared and both the solution are mixed, this solution is further poured and freeze dried.

Freeze-drying process is well-established technique to fabricate porous materials for a wide range of applications. Freezing of solutions, emulsions or dispersions causes solute to be excluded by ice front into the interstitial spaces between ice crystals. Further sublimation leads to formation of porous structure (Jie Wu and J. Carson Meredith, 2014). Freeze-drying is a feasible strategy to improve physical, chemical and mechanical stability of the product.

All the prior art references shows use of hydrophilic polymers as wound dressing material in haemostasis, pressure sores, diabetic ulcers, leg ulcer, donor sites and graft sites, surgical wounds, skin abrasions and lacerations, 1st and 2nd degree burns, trauma wounds and process for preparing wound dressing sponges comprising hydrophilic polymers. However, the inventors of present invention provide the process for the preparation of wound dressing sponge by freeze-drying technique. The total duration to complete the process is about 12-15 hours, which is more time saving and cost effective.

**OBJECTIVE OF INVENTION**

The objective of the present invention is to provide a process for preparing wound dressing sponge comprising hydrophilic polymer, acid and water.
Another objective of the present invention is to provide an effective process for the preparation of wound dressing sponge, wherein process comprising freeze-drying, annealing, packaging and sterilization.

Still another objective of the present invention is to provide a process for preparing wound dressing sponge comprising chitosan, acetic acid and water.

Still another objective of the present invention is to provide an effective process for the preparation of chitosan wound dressing sponge, wherein process comprising freeze-drying, annealing, packaging and sterilization.

Still another objective of the present invention is to provide an effective process for the preparing wound dressing sponge, wherein the processing time is reduced, there by increasing the productivity.

Yet another objective of the present invention is to provide an effective process for preparing wound dressing sponge, where the process involves freeze-drying the product for 12-15 h, annealing at 60-80°C for 15-30 min, packaging in pouch, purged with nitrogen, heat sealed and sterilization by ⁶⁰Co source at the doses of 4 kGy to 25 kGy.

**SUMMARY OF INVENTION**

One embodiment of the present invention provides a process for the preparation of wound dressing sponge comprising hydrophilic polymer, acid and water, wherein the processing time in freeze drying is reduced.

Another embodiment of the present invention provides a process for preparing wound dressing sponge using freeze-drying technique for 12-15 h, annealing at 60-80°C for 15-30 min, packaging in pouch, purged with nitrogen, heat sealed and sterilization by ⁶⁰Co source at the doses of 4 kGy to 25 kGy.
Another embodiment of the present invention provides a process for preparing wound dressing sponge, the process comprising steps of:

1. Adding hydrophilic polymer to water and stirring at room temperature to obtain hydrophilic polymer dispersion,
2. Adding acid to the above polymer dispersion under stirring to cause dissolution of hydrophilic polymer,
3. Transferring above homogeneous viscous solution into trays,
4. Subjecting trays for freeze-drying process for about 12-15 h,
5. Heating the formed sponge at 60 - 80°C for 20 min in an oven for annealing,
6. Placing the obtained Sponge in pouch which is made up of triple laminated aluminium pouch (TLAP) or low-density polyethylene (LDPE), purged with nitrogen and heat sealed and
7. Irradiating the packaged sponge by using gamma irradiation $^{60}$Co source at the doses of 4 kGy to 25 kGy.

Yet another embodiment of the present invention provides a process for the preparation of wound dressing sponge comprising chitosan, acetic acid and water, wherein the processing time in freeze drying is reduced.

Another embodiment of the present invention provides a process for preparing chitosan wound dressing sponge using freeze-drying technique for 12-15 h, annealing at 60-80°C for 15-30 min, packaging in pouch, purged with nitrogen, heat sealed and sterilization by $^{60}$Co source at the doses of 4 kGy to 25 kGy.

Yet another embodiment of the present invention provides a process for preparing chitosan wound dressing sponge, the process comprising steps of:

1. Adding chitosan powder or flakes to water and stirring at room temperature to obtain chitosan dispersion,
2. Adding acetic acid to the above chitosan dispersion under stirring to cause dissolution of chitosan powder or flakes,
3. Transferring above homogeneous viscous solution into trays,
d) subjecting trays for freeze-drying process for about 12-15 h,
e) heating the formed sponge at 60 - 80°C for 20 min in an oven for annealing,
f) placing the obtained Sponge in pouch which is made up of triple laminated aluminium pouch (TLAP) or low-density polyethylene (LDPE), purged with nitrogen and heat sealed and

g) irradiating the packaged sponge by using gamma irradiation ⁶⁰Co source at the doses of 4 kGy to 25 kGy.

**DETAILED DESCRIPTION OF THE INVENTION**

A dressing is a sterile sponge or compress applied to a wound to promote healing and protect the wound from further harm. A dressing is designed to be in direct contact with the wound, as distinguished from a bandage, which is most often used to hold a dressing in place. Many modern dressings are self-adhesive.

Wound Dressing is categorised as follows: Absorptives, Alginates, Antimicrobial Dressings, Biophysical agents, Collagens, Composites, Contact layers, Elastic Bandages, Foams, Gauzes & Non-wovens, Honey (Active Leptospermum), Hydrocolloids, Hydrogels: Amorphous, Hydrogels: Impregnated, Hydrogels: Sheets, Impregnated Dressings, Silicone Gel Sheets, Transparent Films and Wound fillers. Sponges come under the categories of Antimicrobial Dressings.

Wound dressing may comprise a hemostatic sponge and a backing. Sponge may be placed in contact with a bleeding wound to accelerate and/or promote clotting of blood around the wound. Sponge may be used to promote clotting for blood flows that arise from trauma, medical procedures, nose bleeds, dental procedures, and/or other causes. Wound dressing may be made at least in part by freeze-drying a hemostatic solution to form.

Sponge used in the present invention is based on the fact that sponge 1) should not be reactive with the product 2) should be stable to gamma irradiation dose 3) should not show leaching property 4) should maintain its physical property after gamma irradiation 5) should maintain its integrity.
Hydrophilic polymers include but not limited to polyacrylate, an alginate, chitosan, chitin, salts of chitosan, a hydrophilic polyamine, a chitosan derivative, polylysine, polyethylene imine, xanthan, carrageenan, quaternary ammonium polymer, chondroitin sulfate, a starch, a modified cellulosic polymer, a dextran, hyaluronan or combinations thereof. The starch may be of amylase, amylopectin and a combination of amylopectin and amylase. Preferably, the hydrophilic polymer is chitosan. Hydrophilic polymer used in the present invention is a medium molecular weight ranging from about 1,90,000 to about 3,10,000 g/mol.

The concentration of hydrophilic polymer used in the present invention is 0.5% - 3.0%, preferably 1.0% to 2.0% and most preferably 1.75%.

Acids includes but not limited to acetic acid, glutamic acid, lactic acid, formic acid, hydrochloric acid, glycolic acid, succinic acid. Preferably the acid used is acetic acid. The concentration of the acid used in the present invention is about 0.5% to 2%, preferably 1% w/w to 2% w/w, most preferably 1.5%.

The acetic acid is added and mixed through the dispersion to cause dissolution of the chitosan solid in order to get homogeneous viscous solution. The rate of dissolution will depend on the temperature of the solution, the molecular weight of the chitosan. Preferably the chitosan solution percentage (w/w) is greater than 0.5% chitosan and less than 3.0% chitosan, preferably 1.0% to 2.0% and most preferably 1.75%. Preferably the acetic acid is added to the solution to provide for an acetic acid solution percentage (w/w) at more than 0.5% and less than 2%, preferably 1% w/w to 2% w/w, most preferably 1.5%.

Most preferably used aqueous vehicle is water percentage (w/w) is greater than 95% and less than 99%.

Manufacturing process for the preparation of chitosan wound dressing sponge which involves the following steps
Preparation of chitosan solution

The chitosan used to prepare the chitosan solution preferably has a degree of deacetylation in the range of 75-85%. The chitosan solution preferably prepared at room temperature by addition of chitosan powder or flakes to water under stirring. To the chitosan dispersion, acetic acid is added under stirring to facilitate dissolution of chitosan solid. Preferably, the concentration of chitosan should be in the range of 0.5-3% (w/w) hydrophilic polymer. Preferably, the acid should be acetic acid in the range of 1-2% (w/w) and the balance (about 95% - 97% w/w) being milli-q-water.

Freeze-drying of aqueous chitosan solution

Chitosan has greater affinity to form hydrogen bond with water. The water absorption depends on the initial moisture present and storage conditions, like temperature and humidity. The presence of water plays an important role especially in chitosan based solid formulations, affect flow properties, compressibility of powders and tensile strength. In addition, at higher amounts of water in chitosan structure leads to faster and significant damage or deterioration to the polymer by hydrolysis.

The prepared chitosan solution is subjected for freeze-drying step. The chitosan solution is transferred into tray or mold should have good thermal conductivity and made of iron, aluminium, silver. The mold may also be coated with thin inert metallic coating including nickel, gold, platinum or polytetrafluoroethylene (Teflon) or fluorinated ethylene polymer (FEP). Freezing of the chitosan, solution in this way improves the quality of product.

In the present invention, the time taken to complete freezing and drying steps is within 12 - 15 h, which significantly reduces the process time and cost to fabricate chitosan sponge with superior physical and mechanical properties.

The freezing temperature is preferably in the range of -10°C to -40°C, most preferably at -20°C. When frozen at -30°C and -40°C sponge is more closed and intact with smaller pore size, at -10°C the formed sponge is very porous in structure with
bigger pore size. At -20°C the formed sponge is microporous, intact and with optimum mechanical properties.

A freezing temperature of about -20°C with preferably ramp rate of -0.2°C/min to -0.6°C/min, most preferably -0.4°C/min for a preferable holding period of about 2h to 6h, most preferably for a period of 3h to forms a chitosan sponge with superior quality.

The frozen chitosan sample undergoes water removal, in drying step without damaging chitosan structure and integrity. In drying process, primary drying temperature is preferably in the range of 15°C to 30°C, most preferably 25°C with ramp rate of 0.05°C/min to 0.1°C/min, most preferably 0.08°C/min and preferable vacuum of 200 mTorr for a preferable holding period of 12 h to 15 h, most preferably, for a period of 12.5 h.

In secondary drying process, temperature is preferably in the range of 25°C to 50°C, most preferably 35°C with ramp rate of 0.2°C/min to 0.8°C/min, most preferably 0.5°C/min and preferable vacuum of 150 mTorr for a preferable holding period of 2h to 5h, most preferably, for a period of 3h.

Primary and secondary drying steps were carried out in presence of air. If nitrogen is used for primary and secondary drying, the formed sponge lost its water absorption capacity and swelling properties. Water content at the end of lyophilisation is NMT 25%, preferably NMT 20%.

Annealing

After freeze-drying, the prepared sponge is exposed to 60-80°C in an oven for 15 - 30 min for annealing to remove free water and residual acetic acid. Annealing at optimal or moderate temperatures can enhance tensile strength without affecting water absorption, swelling and other physical properties of sponge. Samples without annealing showed excess amounts of loose water, residual acetic acid and poor tensile properties.
Exposure to elevated temperature may change several polymer properties, including aqueous solubility, viscosity and appearance. Decomposition of chitosan increases as increase in temperature and duration of exposure. Exposure to higher temperature causes significant loss of moisture (dehydration), which results poor physical and mechanical properties. Dark brown colouration of sponge with poor tensile strength and no swelling properties.

**Packaging in pouch**

Finally, the sponge is placed in a pouch and evacuated, then it is desirably purged with inert gas such as argon or nitrogen gas and heat-sealed. The pouch is usually made of triple laminated aluminium pouch (TLAP) or low-density polyethylene (LDPE) pouch. The pouch should act as barrier to air and moisture to extend the shelf life of the product. When polypropylene (PP) was used as primary packaging material, lost its integrity and become brittle.

Selection of primary packaging material for sponge is based on 1) should not be reactive with the product 2) should be stable to gamma irradiation dose 3) should not show leaching property 4) should maintain its physical property after gamma irradiation 5) should maintain its integrity.

**Sterilization**

Chitosan based formulations especially applicable for wounds have to be sterilized. Commonly used sterilization methods include steam sterilization, exposure to dry heat and ethylene oxide or gamma irradiation. All these methods may cause irreversible alteration of physical, chemical and functional properties of chitosan.

Sterilization by saturated steam may cause chain scission of the polymer results in reduced viscosity. Similarly, sterilization by autoclave is not suitable for chitosan films and sponges results in reduced tensile strength. In addition, chitosan heated at 160°C for 2 h showed poor or no swelling and become insoluble in acidic solutions, which may be related to inter chain crosslinking of polymer functional groups.
Sterilization by ethylene oxide has to be quarantined prior to use in order to remove gas residue.

Gamma irradiation is a potential sterilization technique for chitosan-based formulations. Gamma irradiation also may cause chain scission, significant decrease in molecular weight and increase degree of deacetylation in a dose dependent manner. At higher gamma doses, sponges showed lower water sorption capacity and higher tensile strength due to polymer chain rearrangements.

Irradiation conditions are very important determinants in extent of radiation induced reactions in chitosan polymer network structure. Radiation induced scission of chitosan chains results in lower glass transition temperature. Irradiation in presence of air can alter its physical and mechanical properties of chitosan sponge including loss of water sorption capability, swelling and improved tensile strength, probably due to changes in chain interaction and rearrangement. Irradiation in anoxia showed poor water absorption and swelling properties. Sponge packaged in presence of inert gas like nitrogen did not affect sponge physical and mechanical properties.

Packaged pouch is sterilized by gamma irradiation at the dose in the range of 4kGy to 25 kGy.

**Advantages**

- Better water and blood absorbency & adhesion.
- Quicker hemostasis than zeolite/kaolin-based dressings due to inherent cationic charge of reengineered chitosan
- Quicker hemostasis than chitosan impregnated gauze (celox) due to higher porosity, blood absorbency and adhesion.
- Chitosan is obtained from purer sources compared to other chitosan from exoskeletons that competition use.
Formulations were developed using different concentrations of chitosan and various freeze-drying conditions. Further, the final product is sterilized by gamma irradiation at different doses. The formulations prepared with different variations were evaluated for their description, thickness, moisture content, acetic acid content, water absorption capacity, disintegration test, tensile strength, bacterial endotoxin test and sterility.

The following examples describes the nature of the invention and are given only for the purpose of illustrating the present invention in more detail and are not limitative and relate to solutions which have been particularly effective on a bench scale.

**Example 1**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Ingredient</th>
<th>Concentration (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chitosan</td>
<td>1.75</td>
</tr>
<tr>
<td>2</td>
<td>Acetic acid, glacial</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>Purified water</td>
<td>96.75</td>
</tr>
</tbody>
</table>

**Manufacturing Process**

Required quantity of chitosan was added to batch quantity of purified water under stirring at room temperature to obtain homogenous dispersion.

Required quantity of acetic acid, glacial was added to chitosan dispersion under stirring at room temperature to obtain homogenous viscous solution.

The obtained homogenous solution was transferred into trays and trays were subjected for freeze-drying process for 12 - 15 h.

After freeze-drying, obtained sponge was subjected for annealing in hot air oven at 80°C/20 min.
Sponges were cut into predetermined sizes and placed in triple laminated aluminium pouch, purged with nitrogen and heat-sealed.

Such packaged sponge was irradiated with gamma irradiation by $^{60}$Co source at the doses of 4 kGy for sterilization.

**Evaluation tests for wound dressing sponge:**

**Description:** Description of the product was evaluated by visual observation.

**Size:** Size of the sponge was measured using digital Vernier calliper

**Thickness:** Thickness of the sponge was measured using digital Vernier calliper

**Loss on drying:** Weigh the LOD bottle (W1). Weigh the sample in grams along with bottle and note the reading as W2. Dry the sample at 105°C for 2 hours and place in a desiccator to cool. Weigh the sample after drying (W3). Record the observations.

$$W2 - W3$$

$$\text{LOD} = \frac{W2 - W1}{W2} \times 100$$

**Water uptake capacity:** Water absorption capacity of sponge was measured by taking known weight of sponge sample was immersed in excessive distilled water at room temperature for 10 minutes. Then the swollen samples were collected carefully and blotted with tissue until no free water remained. The water absorption of the sponge was derived from the mass change before and after swelling.

$$\text{Water absorption} = \frac{\text{Weight after immersion}}{\text{Initial weight of sponge}}$$

**Acetic acid content:** Acetic acid content was measured using HPLC.

**Tensile strength:** Tensile strength of sponge was measured using universal testing machine.
Sterility test: Sterility testing was performed according to USP general chapter < 71>.

Antimicrobial effectiveness testing: Antimicrobial effectiveness testing was performed according to USP General Chapter (51).

Bacterial Endotoxin Test: Bacterial Endotoxin Test was performed for optimized formulation. The testing was performed according to USP General Chapter < 85 >.

Table 1. Specifications for wound dressing sponge:

<table>
<thead>
<tr>
<th>S.No</th>
<th>TEST</th>
<th>SPECIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Description</td>
<td>White, off-white to light brown Soft, flexible, sponge having characteristic odour of acetic acid packed in triple laminated aluminum pouch</td>
</tr>
<tr>
<td>2</td>
<td>Size (inch)</td>
<td>2 x 2</td>
</tr>
<tr>
<td>3</td>
<td>Thickness (mm)</td>
<td>5 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>Loss on drying (% w/w)</td>
<td>NMT 10</td>
</tr>
<tr>
<td>5</td>
<td>Water uptake capacity</td>
<td>NLT 37 times weight of chitosan sponge</td>
</tr>
<tr>
<td>6</td>
<td>Acetic acid content (ppm)</td>
<td>NMT 5000</td>
</tr>
<tr>
<td>7</td>
<td>Tensile strength (kgf/cm²)</td>
<td>NLT 1.0</td>
</tr>
<tr>
<td>8</td>
<td>Sterility</td>
<td>To pass the test as per USP</td>
</tr>
<tr>
<td>9</td>
<td>Anti microbial</td>
<td>To pass the test as per USP</td>
</tr>
</tbody>
</table>
The wound dressing sponge prepared as per Example 1 of the present invention is evaluated for the above characters at different stability conditions and the data is given below tables;

**Table 2**

<table>
<thead>
<tr>
<th>Stability Condition: 25°C/60% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
</tr>
<tr>
<td>White, off-white to light brown</td>
</tr>
<tr>
<td>Soft, flexible, sponge</td>
</tr>
<tr>
<td>having characteristic odour of</td>
</tr>
<tr>
<td>acetic acid packed in triple</td>
</tr>
<tr>
<td>laminated aluminum pouch</td>
</tr>
<tr>
<td>Off white soft, flexible sponge</td>
</tr>
<tr>
<td>having characteristic odor of</td>
</tr>
<tr>
<td>acetic acid packed in triple</td>
</tr>
<tr>
<td>laminated aluminum pouch</td>
</tr>
<tr>
<td>Off white soft, flexible sponge</td>
</tr>
<tr>
<td>having characteristic odor of</td>
</tr>
<tr>
<td>acetic acid packed in triple</td>
</tr>
<tr>
<td>laminated aluminum pouch</td>
</tr>
<tr>
<td><strong>Size</strong></td>
</tr>
<tr>
<td>2 inch x 2 inch</td>
</tr>
<tr>
<td>2 inch x 2 inch</td>
</tr>
<tr>
<td>2 inch x 2 inch</td>
</tr>
<tr>
<td><strong>Thickness</strong></td>
</tr>
<tr>
<td>5 mm ± 1.5 mm</td>
</tr>
<tr>
<td>5.21 mm</td>
</tr>
<tr>
<td>4.65 mm</td>
</tr>
<tr>
<td><strong>Loss on drying</strong></td>
</tr>
<tr>
<td>NMT 10 % w/w</td>
</tr>
<tr>
<td>7.55 %</td>
</tr>
<tr>
<td>7.89 %</td>
</tr>
<tr>
<td><strong>Water uptake capacity</strong></td>
</tr>
<tr>
<td>NLT 37 times weight of chitosan</td>
</tr>
<tr>
<td>43.7 times</td>
</tr>
<tr>
<td>52.45 times</td>
</tr>
<tr>
<td><strong>Acetic acid</strong></td>
</tr>
<tr>
<td>NMT 5000 ppm</td>
</tr>
<tr>
<td>156 ppm</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Sterility</td>
</tr>
<tr>
<td>Anti-microbial</td>
</tr>
<tr>
<td>performance</td>
</tr>
<tr>
<td>Bacterial Endotoxin test</td>
</tr>
</tbody>
</table>

**Table 3**

**Stability Condition: 40°C/75% RH**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Specification</th>
<th>Initial</th>
<th>3 Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>White, off-white to light brown Soft, flexible, sponge having characteristic odor of acetic acid packed in triple laminated aluminum pouch</td>
<td>Off white soft, flexible sponge having characteristic odor of acetic acid packed in triple laminated aluminum pouch</td>
<td>Off white soft, flexible sponge having characteristic odor of acetic acid packed in triple laminated aluminum pouch</td>
</tr>
<tr>
<td>Size</td>
<td>2 inch x 2 inch</td>
<td>2 inch x 2 inch</td>
<td>2 inch x 2 inch</td>
</tr>
<tr>
<td>Thickness</td>
<td>5 mm ± 1.5 mm</td>
<td>5.21 mm</td>
<td>5.02 mm</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>NMT 10 % w/w</td>
<td>7.55 %</td>
<td>8.02 %</td>
</tr>
<tr>
<td>Water uptake</td>
<td>NLT 37 times weight of chitosan</td>
<td>43.7 times</td>
<td>58.35 times</td>
</tr>
<tr>
<td></td>
<td>Sponge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid content</td>
<td>NMT 5000 ppm 156 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tensile strength</td>
<td>NLT 1.0 kgf/cm² 2.089 kgf/cm² 1.893 kgf/cm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterility</td>
<td>To pass the test as per USP Pass -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-microbial performance</td>
<td>To pass the test as per USP Pass -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial Endotoxin test</td>
<td>NMT 300 IU/g of chitosan Complies -</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
We Claim:

1. A process for the preparation of wound dressing sponge comprising hydrophilic polymer, acid and water, wherein the processing time in freeze drying is reduced.

2. A process for preparing wound dressing sponge, the process comprising steps of:
   a) adding hydrophilic polymer to water and stirring at room temperature to obtain hydrophilic polymer dispersion,
   b) adding acid to the above polymer dispersion under stirring to cause dissolution of hydrophilic polymer,
   c) transferring above homogeneous viscous solution into trays,
   d) subjecting trays for freeze-drying process for about 12-15 h,
   e) heating the formed sponge at 60 - 80°C for 20 min in an oven for annealing,
   f) placing the obtained Sponge in pouch which is made up of triple laminated aluminium pouch (TLAP) or low-density polyethylene (LDPE), purged with nitrogen and heat sealed and
   g) irradiating the packaged sponge by using gamma irradiation 60 Co source at the doses of 4 kGy to 25 kGy.

3. The process of claim 1, wherein the hydrophilic polymer is selected from the group of chitosan derivatives consisting of chitosan, chitin and salts of chitosan.

4. The process of claim 1, wherein the acid is selected from acetic acid, glutamic acid, lactic acid, formic acid, hydrochloric acid, glycolic acid, succinic acid, preferably acetic acid.

5. A process for the preparation of wound dressing sponge comprising chitosan, acetic acid and water, wherein the processing time in freeze drying is reduced.

6. A process for preparing chitosan wound dressing sponge, the process comprising steps of:
   a) adding chitosan powder or flakes to water and stirring at room temperature to obtain chitosan dispersion,
b) adding acetic acid to the above chitosan dispersion under stirring to cause
dissolution of chitosan powder or flakes,
c) transferring above homogeneous viscous solution into trays,
d) subjecting trays for freeze-drying process for about 12-15 h,
e) heating the formed sponge at 60 - 80°C for 20 min in an oven for
annealing,
f) placing the obtained Sponge in pouch which is made up of triple laminated
aluminium pouch (TLAP) or low-density polyethylene (LDPE), purged
with nitrogen and heat sealed and
g) irradiating the packaged sponge by using gamma irradiation $^{60}$Co source at
the doses of 4 kGy to 25 kGy.

7. The process of claim 4, where the composition comprises about 0.5 % to about
3% w/w of the chitosan, wherein said chitosan is deacetylated in the range of
75 - 85 %.

8. The process of claim 4, wherein the water concentration is ranges from 95 -
99% w/w.

9. The process of claim 4, wherein the composition comprises about 0.5 % to
about 2% w/w of the glacial acetic acid.

10. The process of claim 4, wherein the composition is subjected to freeze-drying
about 12 - 15 hrs, preferably 12.5 hours.
### INTERNATIONAL SEARCH REPORT

**International application No.**
PCT/IB2018/051585

### A. CLASSIFICATION OF SUBJECT MATTER

**C08F2 20/12, A61F13/02 Version=2018 .01**

According to International Patent Classification (IPC) or to both national classification and IPC.

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**C08F, A61F**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Total Patent One, IPO Internal Database

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
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Date of the actual completion of the international search

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Date of mailing of the international search report

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