(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 November 2016 (03.11.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/175727 A1

(51) International Patent Classification:
A61L 2/00 (2006.01) *A01N 59/12* (2006.01)
A61K 33/18 (2006.01) *A01P 1/00* (2006.01)

(21) International Application Number:
PCT/TR2016/050127

(22) International Filing Date:
27 April 2016 (27.04.2016)

(25) Filing Language: Turkish

(26) Publication Language: English

(30) Priority Data:
2015/05114 27 April 2015 (27.04.2015) TR

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) **Title**: PRODUCTION METHOD OF IODOPHOR BASED ANTIMICROBIAL HYDROGEL

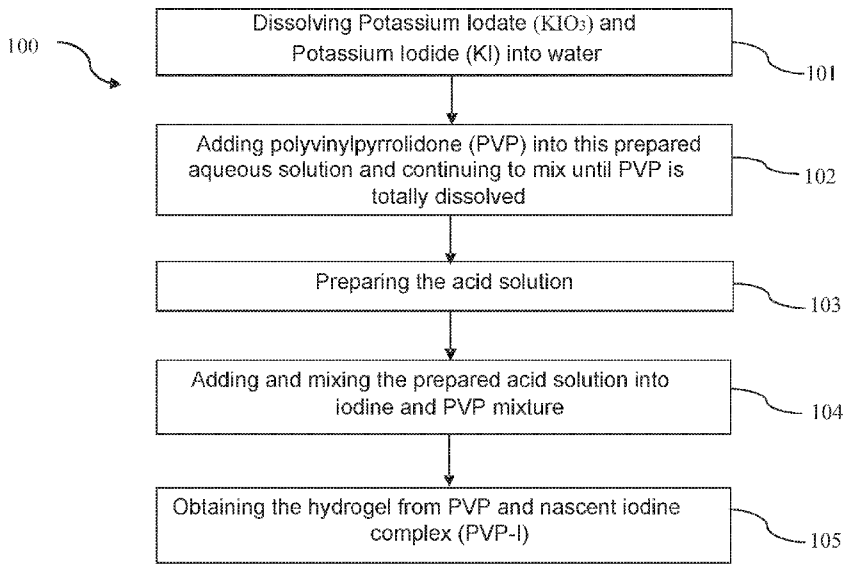

Figure 1

(57) **Abstract**: This invention is related to a hydrogel production method where antimicrobial activity is enhanced with the help of formation of polyvnylprolidone-iodine (PVP- I) complex containing nascent iodine in spite of reduced ratio of polyvnylprolidone (PVP) as compared to equivalent products containing PVP-I with the following steps: dissolving potassium iodate (KI03) and potassium iodide (KI) in water (101), adding polyvnylprolidone (PVP) into the aqueous solution so prepared and continuing to mix it until PVP is fully dissolved (102), preparing acid solution (103) and adding this acid solution into the mixture containing iodine and PVP and mixing it (104) and obtaining the hydrogel formed of PVP and nascent iodine complex (PVP-I) (105).

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PRODUCTION METHOD OF IODOPHOR BASED ANTIMICROBIAL HYDROGEL

Technical Area

This invention is related to production method of a hydrogel in which antimicrobial effect is enhanced thanks to formation of polyvnylprolydone-iodine (PVP-I) containing nascent iodine in spite of reduced polyvnylprolydone (PVP) ratio as compared to its equivalents containing PVP-I.

Previous Technique

It was determined that iodine was a valuable medicament and superior antimicrobial with its extraordinary effective range. Human body needs this element and iodine deficiency results in goiter disease. Since a long time, iodine solutions are used as wide-spectrum bactericide agents having effect on a wide range of microorganisms such as gram positive and negative bacteria, yeast, viruses and fungi.

Iodine forms in certain oxidation situations including wholly degraded iodide (I^-), oxidation diatomic free element (I_2), and certain high oxidation conditions combined with oxygen (for example hypo-iodate (IO^-), iodate (IO_3^-) and periodate (IO_4^-)). In case of water based solutions, iodide forms the efficiently dissolving tri-iodide (I_3^-) balance complex in the form of elemental iodine and in bonded iodine form with no antimicrobial activity. Different studies showed that iodine in free form exhibited microbial activity.

It is difficult to process iodine in its free form as it enters into chemical reaction with certain substances in and out of the body. It is also volatile and it sublimates in the atmosphere. Iodine dissolves in pure water only slightly and but if water contains iodide or iodine salt composed of poly-iodine and iodine, iodine will

dissolve easier. Iodine must be in dissolved state in order that it can be used for forming useable solutions. There are many dissolution approaches and some of them are given below.

First of all, Lugol's solution made in 1829 was a water based solution containing 1-5% iodine and 5-10% potassium iodine. Alcoholic solution of elemental iodine in the form of iodine tincture containing 2-7% molecular iodine was accepted as the most effective bactericide combination for a very long time. Lugol's solution and iodine tincture fell out of favor in the recent years due to the fact that they tend to cause chemical burns and tissue colorations and because of their relative instabilities, short term effects and characteristic odors. These solutions may cause swelling and bleeding of mucous membrane and they will be highly toxic when they contact with the bruises on the body. When only 0, 2ppm of iodine would be sufficient as antimicrobial on the surrounding tissue, one 1% iodine tincture solution might release extra 10000 ppm iodine powder. For that reason diluted concentrations are recommended.

Methods catching iodine in semi-stable form are based on complex formation of iodophor. Iodophors are known as products forming through complex formation of iodine with polymers. Complex agents used for that purpose are polyvnylprolydone (povidone or PVP), polyvinyl alcohol, polyurethane, cadexomers (recycled carbohydrate polymer complexes mixed with elemental iodine formulated in polyethylene glycol) and polydextrase (non-digestible polysaccarite. These iodine complexes will be referred to hereinafter as e PVP-I, PVA-I, PU-I, C-I, and PD-I respectively.

Iodophors are free complexes of elemental iodine and tri-iodide. They process iodine to add a continuous oscillation to iodine and serve as a polymeric carrier increasing solubility of iodine.

In the known state of technique, neutral and water soluble principal commercial polymers containing PVP were used as carrier. PVP is an organic polymer characterized with its colloidal features and physical immobility and it is devoid of natural antimicrobial activity, exhibits high molecular change, and it is non-ionic, non-detergent, water soluble, with extraordinary complex ability. Carriers may also exhibit variable degrees of effect increasing surface activity or dampen the solution in which they are used. These iodophor complexes are in the form of groped mycelium which is dispersed by water or bodily fluids. Until the iodine gets free to form antimicrobial concentrations, the bonding of iodine with polymer gradually weakens.

PVP-I water based solutions that are commercially well known are iso-Betadine and Braunol products containing 10% PVP-I and 7,5% PVP-I. Betadine is available in Swiss market. Contrary to iso-Betadine, Braunol and Betadine are stabilized with $NaIO_3$. For that reason, hereinafter Betadine will be referred to as standardized Betadine. And on the contrary, iso-Betadine will be referred to as non-standardized iso-Betadine. Lugol's solution contains 170 mg/L free iodine and non-standardized iso-Betadine is concentrated 85,17 and 8,5 times more then standardized Betadine and Braunol respectively.

Since these iodine complexes in aqueous solution is less concentrated and can mix better with bodily fluids and disperse relatively faster and as they highly reduce tissue irritation, bad odor, tissue coloring and corrosion on metal surfaces of medical materials, they are preferred to Lugol's solution and iodine tincture. While Braunol contains 7,5% PVP and 0.75% iodine, Betadine and iso-Betadine contains approximately 90% water, 8,5-9% PVP and 1% iodine. Since it I not possible with the available production methods to dissolve more elementary iodine iside PVP, PVP/iodine ratio in all of these products remained fixed as 1/10. Severe toxicological restrictions caused by PVP-I complexes when they are used widely on the body can be attributed to high iodine content in their combination

since they are absorbed by the body and result in concentrations in the thyroid glands.

When compared to iodine tincture and Lugol's solution, advanced oscillation properties of PVP-I iodophors increased use of iodine in skin preparations before surgery, surgery brushes, washes, syringes, lotions and ointments. However limited iodine reserves restrict efficiency times of these iodophors used as disinfectant. For that reason microorganisms surviving after the initial application may behave as seed allowing pathogen population in order to increase the population to its beginning level. Most of wide spectrum antimicrobial s that can be mixed with water exhibit this defect. Most important disadvantage of PVP-I complexes is their restricted use of their safe and efficient antimicrobial action on the skin and sound mucosa under certain circumstances. This restriction arises from their solubility. When the complex interacts with the injured area, results will be like above due to excessive oscillation of free iodine.

Traditional antimicrobial solutions exhibit a good anti-bacterial effect in the beginning but they are lacking the active anti-bacterial that is more effective in killing the microbes. Although other traditional antiseptic solutions have durably active anti-bacterial properties, in the beginning they don't have a good anti-bacterial effect. They have low viscosity and they don't form a film layer or a long term water proof bandage whey they are dried. For producing these solutions with the known state of technique, minimum 8,5-10% PVP is needed to dissolve 1-1,2% elementary iodine. And this makes the production process relatively non-economic. For that reason, it is necessary to produce a PVP-I combination containing less PVP but at the same time having same or better antimicrobial effect with very few or no side effects.

Quantities of iodine and PVP in iodine containing commercial bactericides are a necessary parameter to ensure legal compliance and effect and acceptance by the consumers. Primary element in the cost of iodine containing commercial

bactericides is costs of iodine and PVP. Since iodine based antiseptics are special chemicals, the cost is critical in terms of product acceptability.

Nascent iodine production has been investigated in order to resolve the problems inherent in PVP-I complexes. The patent document published in 2009 describes a method in which some current was applied between two electrodes placed in a glass container put into an electrolyte bath by using a certain amount of iodine. This effect allowing iodine atom to receive stimulation energy from the magnetic area caused a diatomic bond destroying the iodine molecules. According to this document, the simplest method to produce iodine by nascent reaction is to prepare iodine tincture. Accordingly, 1% iodine solution in weight inside ethyl alcohol is immersed in an electrolyte bath composed of copper sulfate, sulfuric acid and zinc chips and copper and nickel alloy anode on which AC current is connected is placed at both surfaces of the glass container. However in this method, iodine cannot be maintained in nascent form for a long time and it can only be used as additional iodine for the body.

In another patent document dating back to 2010, nano-iodine synthesis was investigated by using chemical design in back-bone structure that can dissolve in nature and allows production of minimum two populations of nano-iodine containing chemical compounds. First population of the particles might contain one pH buffer, additional compounds containing iodate (IO^{3-}) and iodide (I^-). Second population of particles might contain those components that would create a micro-frame in the range of $5<pH<6$ which surrounds the first population of particles immediately. A and B particles populations are maintained in dry condition. Particles are dispersed evenly and they do not contact with each other in dry state. In tile antimicrobial medium is achieved while I_2 is created. Most important disadvantage of this production method is the need for a very dry medium because even a small loss of humidity might start iodine release reaction before the application.

In another patent document application of which was made in 2010, glucose is used for nascent iodine production to be used as wound dressing. Glucose penetrated into the wound dressing and was derived in the wounded area and then was transformed into gluconic acid (proton source) inside the wound dressing. Acid disintegration constant (pKa) for gluconic acid is approximately 4. Quantity of gluconic acid that allows obtaining of the protons carrying out oxidation and transformation of iodide into free iodine was sufficiently low. Iodate is an oxidizing agent. Protons created in this fashion penetrate along and into the dressing in order to create elementary iodine on those areas where oxygen stress could be almost zero. Protons produced by bacteria or lactic acid under no-air conditions start creation by penetrating into the dressing. This dressing product could be applied only to single or double layered stupe and it activated when it start to touch wound fluid. For that reason, this process may be applied only on antibacterial stupe and cannot be produced in liquid or gel form which restricts its application.

Among the applications representing known state of technique, there is no study attempting to reduce the cost by decreasing PVP ratio in the complex while increasing antibacterial character with the help of nascent iodine.

Short Description of the Invention

Purpose of the invention is to find a production method for iodophor based antimicrobial hydrogel where polyvinylprolydone (povidone) and iodine complex in nascent and/or nano form.

Another purpose of the invention is to find a production method for an iodophor based antimicrobial hydrogel that reduces skin irritation thanks to its lower iodine and PVP content as compared to the traditional antimicrobial iodophors.

Another purpose of the invention is to find a production method for an iodophor based antimicrobial hydrogel that exhibits better and longer antimicrobial effect

as compared to the traditional PVP-I complexes thanks to the use of iodine in nascent form in spite of its low PVP and iodine content.

Another purpose of the invention is to find a production method for an iodophor based antimicrobial hydrogel that that could be used also as a solution with the addition of alcohol in it.

Detailed Description of the Invention

Following figures are provided for the production method for an iodophor based antimicrobial gel as the subject of this invention.

Figure- 1: Flow chart of the invented method

Figure-2: The table indicating the ratio of the components of the samples prepared by the invented method.

Figure-3: The table comparing antibacterial efficiencies of the main samples prepared by the invented method and control samples.

Comprising these steps,

(1) A production method of iodophor-based antimicrobial hydrogel performed to achieve the purpose of the invention (100)

- Dissolving in water of potassium iodate (KIO3) and potassium iodide (KI) (101),
- Adding polyvinylpyrrolidone (PVP) into this aqueous solution prepared and to continue mixing until the PVP is completely dissolved (102),
- Preparing acid solution (103),
- Adding and stirring acid solution prepared into iodine and PVP mixture (104),
- Obtaining the hydrogel from PVP and nascent iodine complex (PVP-I) (105)

In the invented method (100), it is aimed to obtain nascent iodine in aqueous medium. For that purpose, KIO_3 and KI are dissolved in water (101). After that, PVP is added into the solution and it is mixed until it is fully dissolved (102).

At another place, citric acid solution is prepared in water (103). Maximum 2,5 grams of citric acid is used for 10 grams of water. In one application of the invention, H_2O_2 is also used as oxidizer together with citric acid in acidic solution and in this manner, antimicrobial effect of the end product is enhanced. In this application of the invention, maximum 2,5 grams of citric acid and 5 grams of H_2O_2 is used for 10 grams of water.

The acid solution prepared in this manner is added preferably drop by drop into the PVP and iodine containing mixture and is mixed at the same time (104). Mixing process is done by using magnetic mixer and mixing process is continued until viscosity of the solution increases to such level that it would be difficult to mix it. Reactions occurring one after the other in this step are as follows.

$$KIO_3 + 5KI + 6\,H^+ \rightarrow 3I_2 + 3H_2O \quad \text{(Reaction A)}$$

$$I_2 + PVP + I^- \rightarrow PVP\text{-}I_3^- \quad \text{(Reaction B)}$$

It is though that iodine in nascent form was created in A reaction and resulting viscous hydrogel helped to retain iodine in nascent form and prevented it from proceeding to B step of the reaction. As a result, products with antimicrobial activity could be obtained.

With continuous mixing process and occurring of A and B reactions, hydrogel composed of PVP and nascent iodine complex (PVP-I) is obtained (105). Optimum components of the antimicrobial hydrogel produced by the invented production method (100) will be 1,2% iodine and 4.4% PVP.

Low pH medium, i.e. abundance of H^+ ions in the medium has great importance for continuous movement of A reaction toward right. For that reason, preferred pH is between 4 and 6. If pH gets higher in the medium, H^+ ions are consumed faster and as a result, the reaction is slowed down. In order to provide necessary pH medium, acid quantity in step 104 might be used in excess up to 40% of stoichiometric need. Acid quantity must be kept optimum because making the medium more acidic would not greatly enhance antimicrobial activity of the end product and besides, excessively acidic medium would cause skin irritation.

A and B reactions stay in balance during the production phase and during the shelf life of the product produced by the invented method (100). In order to shift A reaction towards right throughout the production and storage phases and retain the balance between the reactions, quantity of KIO_3 used in step 101 must exceed stoichiometric need by minimum 10% and PVP quantity must be as low as possible so that it could cover all surface of iodine that will be released during the reaction. In this manner, the compound can be produced economically and retains its consistency as viscous gel.

It was found that ratios of stoichiometric component required so that antimicrobial hydrogel prepared by the invented method (100) would contain 1.2% nascent iodine would be 0.33% KIO_3, 1,3% KI and 1,8% acid in weight. In the preferred application of the invention, maximum 2.5% citric acid and 0,36% KIO_3 in weight are used in order to ensure continuity of A and B reactions.

Hydrogel product obtained by the invented method (100) could be diluted by alcohol in 1:1 ratio and used as a solution containing 2.2% PVP and 0.6% iodine. The tests run showed that even if the invented hydrogel is diluted with alcohol and used as a solution, it would exhibit better and longer antimicrobial activity as compared to the equivalent products.

Affect of using solely boric acid instead of citric acid or using a mixture of citric acid and boric acid on the antimicrobial character of the invented hydrogel was also investigated. However analyses showed that using solely citric acid would yield the strongest antimicrobial effect. Therefore using citric acid solution is preferred in the invented method (100).

Analyses were performed to compare the antimicrobial characteristics of the hydrogels created by varied applications of the invented method (100). For that purpose, the samples were prepared in the manner described in the following examples.

Sample 1

At an application of the inventive method (100); 0,36 g of KIO_3 and 1,3 g of KI were dissolved in 81,9 g of water (101) and later 4,4 g of PVP was added in small amounts and mixed until totally dissolved and being homogeneous (102). An acid solution was prepared by dissolving 2,5 g of citric acid in 10 g of water (103) and added to the solution drop by drop (104). The hydrogel formation is completed when the viscosity of the mixture increase and the mixer can no longer mix the solution after 2-3 minute mixing by magnetic mixer (105).

Sample 2

In another embodiment of the method (100), the same procedure is started dissolving citric acid by water and later added PVP to this solution. Finally, the solution contains 0,36 g of KIO_3, 1,3 g of KI and 10 g of water added to the PVP, citric acid mixture. Mixing is continued by magnetic mixer until the increasing of viscosity of hydrogel has stopped.

Sample 3

In another embodiment; the same process was applied as Sample 1, however, H_2O_2 also dissolved with citric acid by water.

Sample 4

In another embodiment; the same process was applied as Sample 2, however H_2O_2 also dissolved with citric acid by water.

Sample 5

In another embodiment; the same process was applied as Sample 3, however the amount of PVP was changed as 8,8 g.

Sample 6

In another embodiment; the same process was applied as Sample 3, however the amount of PVP was changed as 2,2 g.

Sample 7

In another embodiment; the same process was applied as Sample 3, however 2,5 g of boric acid was used instead of citric acid as acid.

Sample 8

In another embodiment; the same process was applied as Sample 3, however mixture of 1,25 g of citric acid and 1,25 g of boric acid was used instead of citric acid as acid.

Quantities of the components in these 8 examples are given on a table in Figure 2.

In order to compare antimicrobial activity of main samples prepared with the ratios given in Figure 2 by using the steps of the invented method (100) and of the control samples prepared by the known state of technique, the samples were tried on different microorganisms. The table indicating that comparison is given in Figure 3. Two different combinations were used in the control samples.

Control sample 1

In the current usage of commercial PVP-I complex (Batticon®) containing 8-9% PVP and 1-1.2% molecular iodine (I2) is used directly without any changes.

Control sample 2

In the current usage of iodine tincture solution containing 2-7% molecular iodine is used directly without any changes.

In order to emphasize the importance of citric acid and PVP quantities in achieving antimicrobial effect, control samples were also compared with another group of samples prepared by molecular iodine and PVP dissolved in water in ratios given in Figure 4 (A1 and A7) in terms of antimicrobial activity. The results of this comparison are given on a table in Figure 5.

Since the samples produced by the invented method (100) were in hydrogel form, no measurement could be performed before they were diluted. For that reason, they were added onto the microorganisms after they were diluted by ethyl alcohol or water to make a solution

Agar diffusion method was used to determine antimicrobial activities. In this study, *Aeromonas hydrophila* ATCC 7965, *Bacillus cereus* RSKK 863, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* FMC 5, *Listeria monocytogenes* 1/2B, *Morganella morganii*, *Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC 3624, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* NRRLE 4463, *Staphylococcus aureus* ATCC 25923 (B), *Yersinia enterocolitica* ATCC 1501 bacteria and *Candida albicans* ATCC 1223 yeast was used. Each bacterium was infused to nutrient liquid media and yeast was infused to malt extract media and then they were diluted to contain 10^6-10^7 kob/ml (unit producing colony per millimeter) microorganisms. Each bacterium was infused 250 ml into the medium in Erlenmeyer with a temperature of 43-45°C which contained 25 ml of sterile medium (for bacteria, Mueller Hinton

agar and for yeast, malt extract agar). Agars that were infused with microorganism culture were poured into Petri dishes of 9 cm and after solidification, they were waited for 1 hour in 4°C. 4 small holes with a diameter of 4 cm were opened in Petri dishes and 50 ml of each main sample and control sample was added 50 ml into these holes. *Y. enterocolitica* and *C. albicans* was left to incubate in 25°C for 24-48 hours, whereas other microorganisms were left to incubate in 35°C for 18-24 hours. All Petri dishes were examined after incubation and diameters of forming inhibition zones were measured in millimeters.

Upon evaluation of the analyses, it was determined that PVP ratios from 8.8% up to 10% were not sufficient to produce PVP-I complexes. A PVP ratio of 4.4% would be sufficient and even optimally to produce PVP-I complexes with high antimicrobial activity. It was further observed that sequence of the steps applied in the invented method (100) played a great role in the antimicrobial activity of the end product. Initially, it was observed antimicrobial effect achieved in the end the product when an acid solution was prepared by citric acid and PVP, KI and KIO_3 were added onto it (like in Example 3) was much lower than the antimicrobial effect achieved in the end product when the sequence of steps in the invented method were followed (100).

When the table given in Figure 3 was examined, it was seen that invented PVP-I complex prepared by a more economic method (100) by creating nascent iodine instead of the molecular iodine used in PVP-I complexes in the known state of technique (such as Batticon®) exhibited much better antimicrobial activity.

And when the table given in Figure 5 was examined, it was seen that antimicrobial activity was much lower in A1, A2 and A3 samples than the control samples. Lowest antimicrobial activity was determined in A1 sample and by adding EDTA or citric acid to the samples, antimicrobial activity was achieved almost at the same level. However this increase was not found to be meaningful and sufficient. Besides that, it was found out that citric acid exhibited

antimicrobial characteristics and was helpful in quick cleaning of infected wounds or surfaces. This antiseptic feature was due to creation of a medium not convenient for the survival of bacteria thanks to the citric acid. Since no antimicrobial activity was seen in the samples containing only 0.1% EDTA or citric acid with no mixture of chemicals, these samples were not indicated in the figures. However when these samples were mixed with iodine and PVP containing complexes, an increase was observed in antimicrobial activity.

As may be seen in Figure 5 again, antimicrobial activity of A4 was much higher than that of A1. This fact showed that samples dissolving in alcohol were more effective than the one dissolving in water. It was thought that this was due to the iodine form and quantity of the released free molecules. In A6 and A7 samples, increases were observed in the antimicrobial activity when EDTA and citric acid was added and it was determined that these increases did not depend on the type of dissolving agent used.

It was shown that no important decrease was seen in antimicrobial activity when PVP ratio in A4 sample was reduced from 8.8% to 4.4%. Although antimicrobial activity of A5, 6 and A7 samples was much higher than that of the control sample 1, it was lower than control sample 2 which was thought to arise from the ratio of iodine used.

When the table given in Figure 3 was examined, PVP ratio of 4.4% was found to be highly efficient and determined to be the optimum value for the production of antimicrobial hydrogels having sufficient viscosity. When PVP ratio was increased to 8.8% or reduced to 2.2%, antimicrobial activity decreased. These findings could be explained as increased I_3^- production with high PVP ratio and resulting decrease in antimicrobial activity. And in the contrary scenario, reducing PVP ratio to 2.2% caused a reduction in viscosity. This reduction in viscosity prevented sufficient retaining of nascent iodine arising in the hydrogel production process and caused free movement of iodine from one from to the other in

hydrogel lamella which resulted in its transformation into form with lower antimicrobial activity.

Another observation was that when samples containing 4.4% PVP were diluted even with alcohol, they would exhibit better antimicrobial activity as compared to the control samples in the known state of technique (Batticon® and iodine tincture). If it is not intended to use then invented hydrogel on open wounds, it could be diluted with alcohol and used on all skin a disinfectant. If it is intended to use on open wounds, we recommend that it is used in hydrogel form without any dilution so that it would not cause irritation.

CLAIMS

1. Production method of iodophor based antimicrobial hydrogel **characterized by** the following steps (100)

– Dissolving by water of potassium iodate (KIO3) and potassium iodide (KI) (101):

- Adding polyvinylpyrrolidone (PVP) into this prepared solution and continuing to mix until PVP is totally dissolved (102) ,

- Preparing the acid solution (103),

- Adding and mixing the prepared acid solution into iodine and PVP mixture (104),

- Obtaining the hydrogel formed by PVP and nascent iodine complex (105)

2. Production method of iodophor based antimicrobial hydrogel like in Claim 1 **characterized by** the following step (100): preparing the acid solution contains water and citric acid (103).

3. Production method of iodophor based antimicrobial hydrogel like in Claim 2 **characterized by** the following step (100): preparing the acid solution contains maximum 2,5 g of citric acid in 10 g of water (103).

4. Production method of iodophor based antimicrobial hydrogel like in Claim 1 **characterized by** the following step (100): preparing the acid solution contains H_2O_2 as well as water and citric acid (103).

5. Production method of iodophor based antimicrobial hydrogel like in Claim 4 **characterized by** the following step (100): preparing the acid solution contains maximum 2,5 g of citric acid 5 g of H_2O_2 in 10 g of water (103).

6. Production method of iodophor based antimicrobial hydrogel like in Claim 1 **characterized by** the following step (100): adding the prepared acid solution into PVP and iodine mixture drop by drop and continuing to mix until the viscosity increase like to do mixing difficult (104).

7. Production method of iodophor based antimicrobial hydrogel like in Claim 1 **characterized by** the following reactions (100):
Consecutively;
$KIO_3 + 5KI + 6\ H^+ \rightarrow 3I_2 + 3H_2O$ (Reaction A) and

$I_2 + PVP + I^- \rightarrow PVP\text{-}I_3^-$ (Reaction B)

8. Production method of iodophor based antimicrobial hydrogel like in Claim 7 **characterized by** the following step (100): to ensure the suitable pH environment to form the nascent iodine by adding the prepared acid solution into iodine and PVP mixture and mixing; the amount of acid used is more than 40% of stoichiometric needs (104).

9. Production method of iodophor based antimicrobial hydrogel like in Claim 7 **characterized by** the following step (100): dissolving potassium iodate (KIO3) and potassium iodide (KI) in water, the amount of KIO3 used exceeds minimum 10% of stoichiometric needs, to maintain the balance between reactions and to shift the reaction A to right during the production and storage (101).

10. Production method of iodophor based antimicrobial hydrogel like in Claim 2 **characterized by** the following step (100): using 0,36% KIO_3, 1,3% KI, 4,4% PVP, 91,9% water and 2,5% of citric acid as maximum by weight.

11. Production method of iodophor based antimicrobial hydrogel like in Claim 4 **characterized by** the following step (100): using 0,36% KIO_3,

1,3% KI, 4,4% PVP, 86,9% water and 2,5% of citric acid as maximum and 5% H_2O_2 by weight.

12. Production method of iodophor based antimicrobial hydrogel like in Claim 10 or 11 **characterized by** the following step (100): obtaining hydrogel from 4,4% PVP and 1,2% nascent iodine PVP-I complex by weight.

13. With a method like in Claim 12 (100): using as a disinfectant including open wounds on skin of produced antimicrobial hydrogel.

14. With a method like in Claim 12 (100): using as a disinfectant on skin by diluted with ethyl alcohol of produced antimicrobial hydrogel.

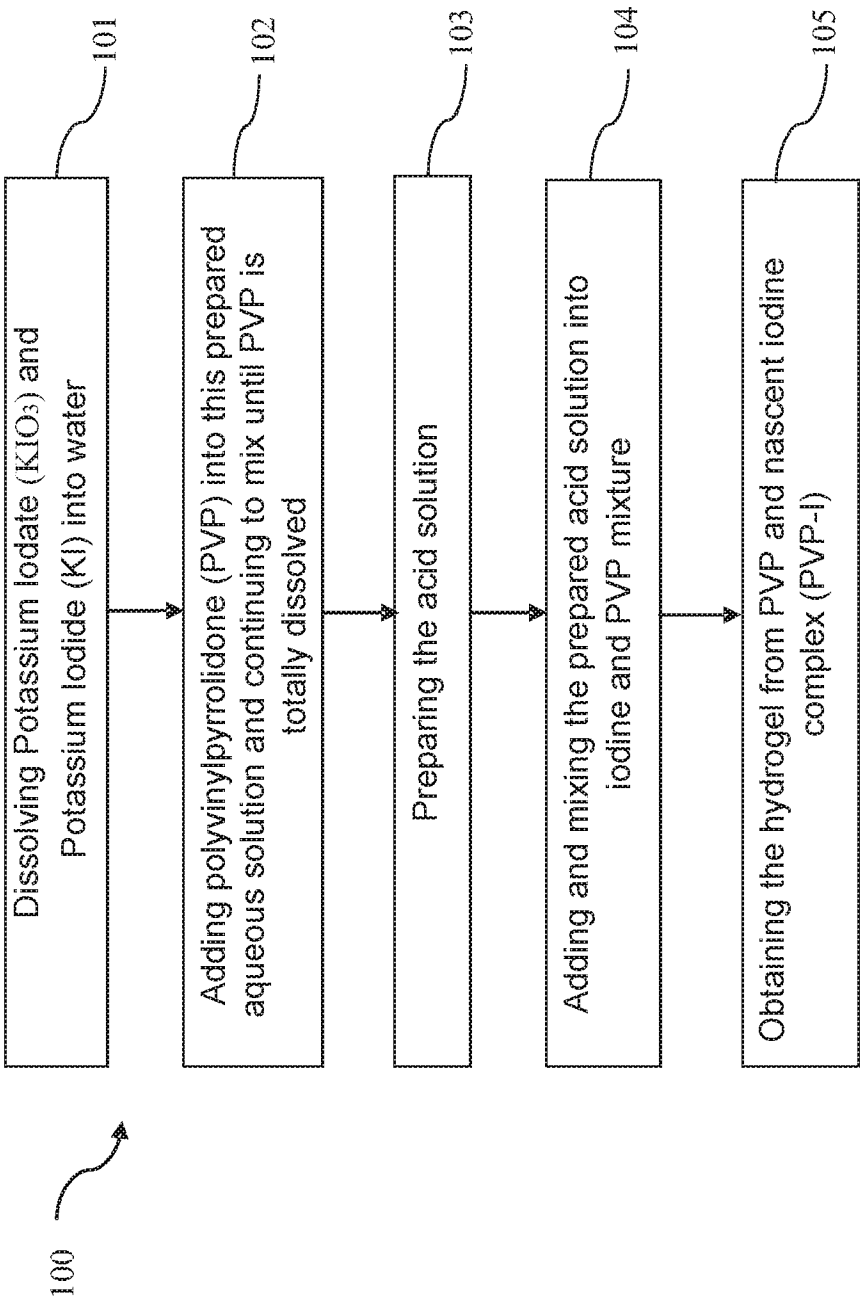
100
101
Dissolving Potassium Iodate (KIO_3) and Potassium Iodide (KI) into water
102
Adding polyvinylpyrrolidone (PVP) into this prepared aqueous solution and continuing to mix until PVP is totally dissolved
103
Preparing the acid solution
104
Adding and mixing the prepared acid solution into iodine and PVP mixture
105
Obtaining the hydrogel from PVP and nascent iodine complex (PVP-I)

Figure 1

Sample	KIO_3(g)	KI(g)	PVP(g)	Solvent (g)		Acid (g)				H_2O_2 (g)	Total (g)
1	0,36	1,3	4,4	Water	91,9	Citric	2,5			-	100
2	0,36	1,3	4,4	Water	91,9	Citric	2,5			-	100
3	0,36	1,3	4,4	Water	86,9	Citric	2,5			5	100
4	0,36	1,3	4,4	Water	86,9	Citric	2,5			5	100
5	0,36	1,3	8,8	Water	82,5	Citric	2,5			5	100
6	0,36	1,3	2,2	Water	89,1	Citric	2,5			5	100
7	0,36	1,3	4,4	Water	86,9	Boric	2,5			5	100
8	0,36	1,3	4,4	Water	86,9	Boric	1,25	Citric	1,25	5	100

Figure 2

Microorganism/ Sample	Diluted with alcohol (Sample1: alcohol ratio)	Diluted with alcohol (Sample1: alcohol ratio)	Diluted with water (Sample1: water ratio)	Diluted with alcohol (Sample2: alcohol ratio)	Diluted with alcohol (Sample3: alcohol ratio)	Diluted with alcohol (Sample4: alcohol ratio)	Diluted with alcohol (Sample5: alcohol ratio)	Diluted with alcohol (Sample6: alcohol ratio)	Diluted with alcohol (Sample7: alcohol ratio)	Diluted with alcohol (Sample8: alcohol ratio)	Control sample	Control sample
	1:1	1:3	1:3	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1	2
Aeromonas (A.Hydrophila)	19.2±0.3	11.0±0.0	12.0±0.0	16.0±0.0	25.2±0.3	22.0±0.0	22.0±0.0	21.0±0.0	9.0±0.0	15.0±0.0	14.0±0.0	22.3±0.3
Ecoli	13.2±0.3	8.0±0.0	10.0±0.0	11.2±0.3	19.0±0.0	17.0±0.0	17.0±0.0	20.5±0.5	8.0±0.0	11.2±0.3	13.3±0.3	21.3±0.5
Klebsiella (Kpneumoniae)	17.0±0.0	12.0±0.0	12.3±0.3	15.0±0.0	21.0±0.0	21.0±0.0	20.2±0.3	21.0±0.0	10.0±0.0	13.0±0.0	13.2±0.3	21.7±0.3
Morganella (M.Morganii)	20.0±0.0	9.0±0.0	13.5±0.0	15.0±0.0	22.0±0.0	21.2±0.3	20.0±0.0	21.2±0.3	10.0±0.0	13.2±0.3	19.2±0.3	29.8±0.3
Proteus (Pmirabilis)	20.0±0.0	11.0±0.0	8.0±0.0	22.5±0.5	17.0±0.0	17.0±0.0	12.0±0.0	17.0±0.0	10.0±0.0	20.0±0.0	19.8±0.3	27.2±0.3
Pseudomonas (Paeruginosa)	16.0±0.0	10.0±0.0	12.5±0.5	12.0±0.0	21.2±0.3	20.2±0.3	19.2±0.3	21.2±0.3	9.0±0.0	15.0±0.0	13.5±0.0	21.0±0.0
Salmonella (Styphimurium)	16.5±0.5	9.0±0.0	7.0±0.0	14.0±0.0	20.5±0.5	13.2±0.3	18.0±0.0	20.2±0.3	9.0±0.0	12.0±0.0	15.2±0.3	21.7±0.3
Yersinia (Yenterocolitica)	12.3±0.3	8.6±0.3	10.0±0.0	21.2±0.3	19.2±0.3	16.0±0.0	13.2±0.3	19.2±0.3	8.0±0.0	16.2±0.3	20.3±0.5	24.0±0.0
Bcereus	17.0±0.0	9.0±0.0	12.0±0.0	17.0±0.0	21.2±0.3	21.0±0.0	20.0±0.0	21.0±0.0	10.0±0.0	14.0±0.0	11.2±0.3	24.2±0.3
Bsubtilis	19.0±0.0	11.0±0.0	11.0±0.0	20.2±0.3	21.2±0.3	21.2±0.3	19.0±0.0	22.5±0.5	11.2±0.3	17.0±0.0	19.5±0.0	21.2±0.3
Listeria (Lmonocytogenes)	22.0±0.0	11.2±0.3	14.0±0.0	22.3±0.3	30.0±0.0	21.2±0.3	27.0±0.0	29.0±0.0	14.0±0.3	17.0±0.0	19.0±0.0	26.5±0.0
Mycobacterium (Msmegmatis)	12.0±0.0	8.0±0.0	11.2±0.3	12.3±0.3	20.2±0.3	16.0±0.0	17.2±0.3	20.0±0.0	8.0±0.0	11.2±0.3	15.0±0.5	21.2±0.3
Staphylococcus (Saureus)	15.0±0.0	10.2±0.3	11.0±0.0	12.3±0.3	24.0±0.0	18.3±0.3	20.5±0.5	24.0±0.0	9.2±0.3	12.2±0.3	12.5±0.0	24.2±0.3
Candida (Calbicans)	11.2±0.3	7.0±0.0	8.3±0.3	10.2±0.3	20.5±0.5	19.2±0.3	17.2±0.3	20.0±0.0	8.2±0.3	10.2±0.3	11.2±0.3	14.3±0.5

Figure 3

Sample	I_2(%)	PVP (%)	Solvent (%)		Citric acid (%)	EDTA (%)	Total
A1	1,2	8,8	Water	90	-	-	100
A2	1,2	8,8	Water	89,9	-	0,1	100
A3	1,2	8,8	Water	89,9	0,1	-	100
A4	1,2	8,8	Ethanol	90	-	-	100
A5	1,2	4,4	Ethanol	94,4	-	-	100
A6	1,2	4,4	Ethanol	94,3	-	0,1	100
A7	1,2	4,4	Ethanol	94,3	0,1	-	100

Figure 4

	A1	A2	A3	A4	A5	A6	A7	Control sample 1	Control sample 2
Aeromonas (A. Hydrophila)	7.5±0.0	10.3±0.3	11.0±0.0	15.2±0.3	19.0±0.0	20.0±0.0	21.0±0.0	14.0±0.0	22.3±0.3
E.coli	0.0±0.0	0.0±0.0	0.0±0.0	16.2±0.3	11.0±0.0	12.2±0.3	10.0±0.0	13.3±0.3	21.3±0.5
Klebsiella (K. pneumoniae)	8.0±0.5	10.2±0.3	9.2±0.3	15.2±0.3	16.2±0.3	17.0±0.0	16.0±0.0	13.2±0.3	21.7±0.3
Morganella (M. Morganii)	10.3±0.3	10.7±0.3	10.2±0.3	22.2±0.3	17.3±0.3	20.0±0.0	17.0±0.0	19.2±0.3	29.8±0.3
Proteus (P.mirabilis)	9.5±0.0	11.3±0.3	10.2±0.3	23.2±0.2	20.8±0.3	24.0±0.0	23.0±0.0	19.8±0.3	27.2±0.3
Pseudomonas (P.aeruginosa)	9.0±0.0	9.3±0.3	9.2±0.3	19.7±0.3	16.2±0.3	19.0±0.0	17.3±0.3	13.5±0.0	21.0±0.0
Salmonella (S.typhimurium)	7.3±0.3	8.7±0.3	8.0±0.0	16.7±0.3	14.0±0.0	16.0±0.0	17.2±0.3	15.2±0.3	21.7±0.3
Yersinia (Y.enterocolitica)	0.0±0.0	8.2±0.3	8.0±0.0	24.2±0.3	16.7±0.3	18.0±0.0	18.2±0.3	20.3±0.5	24.0±0.0
B.cereus	0.0±0.0	9.2±0.3	10.2±0.3	18.5±0.5	15.0±0.0	17.2±0.3	16.0±0.0	11.2±0.3	24.2±0.3
B.subtilis	0.0±0.0	10.8±0.3	11.2±0.3	12.7±0.3	15.2±0.3	18.0±0.0	15.0±0.0	19.5±0.0	21.2±0.3
Listeria (L.monocytogenes)	7.2±0.3	11.0±0.0	8.2±0.3	22.2±0.3	19.2±0.3	22.0±0.0	22.0±0.0	19.0±0.0	26.5±0.0
Mycobacterium (M.smegmatis)	0.0±0.0	7.7±0.3	8.2±0.3	16.2±0.3	15.0±0.0	16.0±0.0	14.0±0.0	15.0±0.5	21.2±0.3
Staphylococcus (S.aureus)	6.8±0.3	8.7±0.3	9.2±0.3	16.0±0.0	13.0±0.0	18.0±0.0	15.2±0.3	12.5±0.0	24.2±0.3
Candida (C.albicans)	8.3±0.3	8.0±0.0	8.2±0.3	14.2±0.3	13.0±0.0	13.0±0.0	13.2±0.3	11.2±0.3	14.3±0.5

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/TR2016/050127

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L2/00 A61K33/18 A01N59/12 A01P1/00
ADD.

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)
A61L A61K A01N

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)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
6 July 2016	14/07/2016

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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