Journal of Multidisciplinary Engineering Science Studies (JMESS)
ISSN: 2912-1309
Vol. 1 Issue 2, December - 2015

Physical And Antimicrobial Properties Of Nanoemulsions Containing Molecular Iodine

Jens-Peter Krause,
Tassilo Seidler, Mont Kumpugdee-Vollrath*,
Daniel Lehmann, Maria Maier
Beuth Hochschule für Technik Berlin
Luxemburger Str. 10, 13353 Berlin, Germany, *email: vollrath@beuth-hochschule.de

Hans-Peter Welzel,
Peter Scholtyssek,
Karin Fichtner, Ernst-Josef Strätling
Hofmann & Sommer GmbH & Co. KG
Lindenstr. 11
07426 Königsee-Rottenbach, Germany

Stefan Lemke
A. BaurPharma GmbH & Co. KG
Bouchéstr. 12
12435 Berlin, Germany

***Abstract*—Nano-scaled oil-water-emulsions (mean droplet diameter 160 – 200 nm) were developed as carrier for elemental iodine. An iodine content of 0.1% w/w could be achieved at 10% w/w Miglyol emulsions by pre-dissolving iodine in Miglyol. The nanoemulsions were stable against phase separation and did not show any aggregation or coalescence over a storage period of at least six month. The content of iodine dissolved in Miglyol only slightly reduces during contact with a planar interface of water or aqueous polysorbate solution. At emulsions, a distinct decrease in iodine content was measured but a microbial effect could be proved.**

Keywords—nanoemulsion; elemental iodine; antimicrobial effects)

I. INTRODUCTION

Disinfectants are an essential part of infection control practices and aid in the prevention of disease outbreak [1]. A disinfectant is an agent that frees from infection, usually a chemical agent. The disinfectant adsorbs at and diffuses through the surface of microbes [2]. The agent binds to the vulnerable sites (e.g. plasma membrane, cytoplasmic proteins, nucleic acids etc.) and, finally, disrupts the vulnerable sites leading to the death of the microbes. Effective disinfectants are urgently needed [3,4,5]. Iodine has been in use for nearly 180 years as disinfectant because of its high reactivity. When iodine is added to water, I_2 molecules and water molecules react to substances such as hypoiodite and iodide (OI^-, I^-). Iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal [6,7]. It is also known, that the activity of iodine preparations is given over a broad pH-range and less impacted by wound secrets [8]. In aqueous solution, iodine forms at least seven iodine species in a complex equilibrium, with I_2, HOI, H_2O^+, I^- are responsible for antimicrobial efficacy [9]. I_2 and HOI are essentially responsible for biocidal action [10]. The mechanism of microbial activity of iodine is not fully understood. Iodine rapidly penetrates into microorganisms and attacks key groups of proteins (in particular the free-sulfur amino acids cysteine and methionine nucleotides), and fatty acids [11,12]. The antiviral action of iodine seems to be similar as in bacteria [13].

The insolubility and instability of iodine in aqueous solutions were overcome by the development of iodophores, which are complexes of iodine and a solubilizing agent or carrier. Lugol's solution, for example, is an aqueous solution of iodine and potassium iodide. Preparations containing polymeric iodophores like polyvinylpyrrolidone, polyethoxy-ethanol-derivatives or quaternary compounds usually used for disinfection. One of the commonly used preparations is povidone-iodine, a water-soluble compound, which is a complex of molecular iodine and polyvinylpyrrolidone. Some methods are described in literature to stabilize the iodine content of povidone-iodine (e.g anodic oxidation [14], thermal treatment of the polymer [15], addition of acid [16] and to improve the disinfection properties [17,18,19]. Gels or surfactants are also added to the polymer improved product properties [20,21,22,23].

Disadvantages of commercial preparations are allergic reactions [24], systemic adverse reactions [25], incomplete disinfection [26] and reduced activity. The reduction of disinfectable activity, up to ten times of molecular iodine, seems to be due to the drastically increased size of the complex and its changed hydrophilic-lipophilic balance. From pharmaceutical studies it is concluded, that free molecular iodine is almost entirely responsible for the actual microbiological activity [27]. The simplest formulation of molecular iodine is an “iodine tincture”, a solution of iodine in alcohol, which is obsolete because of its adverse reactions. Alternatives are solutions of iodine in sugars, glycerine, glycol or propylene glycol, for medication of mastitis and other applications [28,29,30]. A controlled release of low level molecular iodine should overcome this problem [31].

The aim of the project was to study antimicrobial and physical properties of nanoemulsions, which are important for a use as lipophilic carriers for free molecular iodine.

II. MATERIALS AND METHODS

Miglyol 810N (Sasol Germany), a medium chain triglyceride, iodine (purity 99.5%, Carl Roth Germany),

Polysorbate 20 (Caesar & Loretz Germany) and deionized water were used for emulsion preparation. Dichlormethane p.a. (Merck Darmstadt Germany), potassium iodide (purity 99%, Carl Roth, Germany), acetic acid p.a. (Th. Geyer, Germany), starch (Carl Roth, Germany) and sodium thiosulphate solution 0.1 m (Carl-Roth, Germany) were used for determination of iodine.

A. Preparation of emulsion and characterization

The preparation of emulsion samples was carried out by a three step procedure. In the first step 1.0 % w/w of iodine was solubilized in Miglyol 810N by a magnetic stirrer for 24 hours at room temperature to give a final concentration of 0.1% w/w iodine at a 10% w/w oil-water-emulsion. In the second step the iodine-Miglyol solution was mixed with water containing 2.0% w/w Polysorbate 20 and emulsified by an Ultra Turrax T18 basic (IKA Works Inc., Wilmington, USA) for 2 min. at 14,000 rpm to produce a pre-emulsion. Nanoemulsions were produced by homogenizing the pre-emulsion two or seven times at 1,000 bar at a high pressure homogenizer (Microfluidizer M-110P, Microfluidics, USA). Particle size distribution was determined by photon correlation spectroscopy (Zetasizer 3000, Malvern Instruments, UK). The mean average diameter and the polydispersity index (PI) of the particle distribution were calculated. The PI characterizes the width of the particle size distribution and is zero for ideally monodisperse particles. The same device was used to measure the Zeta Potential of diluted emulsion in destilled water at 20°C. Emulsion stability was evaluated by changes in the particle parameters and by observing the phase separation after storage. Studies on the stability of iodine in Miglyol were carried out at various interfaces. Aliquots of a 1% w/w iodine in Miglyol solution were poured at glass vessel containing water as well as a 2% w/w aqueous Polysorbate 20 solution. The interfacial area formed was calculated to 4,500 mm². The glass vessel was closed and the iodine content of Miglyol was measured in dependence of the storage time and compared with the stock solution.

B. Determination of iodine

The content of iodine dissolved in Miglyol was determined by a two phase titration [32]. Aliquots of 5.0 g were treated with potassium iodide (1.0 g) and 2 ml of water. After addition of acetic acid (1 ml, 12% w/w), the solution was diluted with 50.0 ml of water and titrated with sodium thiosulphate solution (0.1 m) in the presence of starch as indicator. The titration must be done slowly and carefully up to the absence of the dark blue iodine-starch reaction because of the two phase system. The recovery rate of dissolved iodine was about 95%.

The content of iodine in nanoemulsions could be determined only after extraction of the iodine with methylene chloride. Aliquots of 20.0 g of nanoemulsion were diluted with 300 ml of water by stirring (1 h, room temperature) and the oil-iodine-phase was extracted 10 times with 10 ml of methylene chloride. After the extraction process, the methylene chloride was dried and the volume supplemented to 100 ml ready for determination by an absorption measurement at 520 nm (UV-VIS-Spectrometer Hitachi U-2000, Japan). The linear range of the calibration curve was found to be between 0.05 and 0.15 mg iodine per ml methylene chloride. Iodine contents in methylene chloride determined by titration corresponded to results of absorption measurements within a relative failure of 5%.

C. Microbiological Studies

All microbiological studies were carried out with 10% w/w Miglyol nanoemulsions containing 0.1% w/w elemental iodine. Emulsions were produced by two cycles of high pressure homogenization at 1,000 bar. Basic european norm (DIN) EN 14885:2006 (chemical disinfectants and antiseptics - phase 1) was used for testing antimicrobial effects with the modified DIN norms mentioned below.

In a defined manner vegetative cells or endospores containing suspensions were co-incubated with the proving antiseptic formulation resulting in highest concentration of 80%. Tests were carried out at room temperature and (based on DIN protocol) considering obligate interacting time from 5 to 120 minutes followed by neutralization of similar amounts by using a defined neutralizing medium.

After finishing interacting time lethal effects on microorganisms were estimated by detecting potential survivors in plate surface methods according prescription norms by modifying some steps.

Bactericidal effects were tested on *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923, cultivated in standard agar (by SIFIN). Standardized amounts of viable cells were co-incubated as described in DIN EN 1040:2005.

Levurocidal and fungicidal effects were tested on *Candida albicans* ATCC 10231 and *Aspergillus niger* (laboratory strain), cultivated in Sabouraud agar with 2% Dextrose (by SIFIN). Standardized amounts of viable cells were co-incubated as described in DIN EN 1275:2005 similar to bacterial isolates but modified by using of 0.05% Tween 80 solution for harvesting and centrifugation of *Aspergillus niger* isolates to homogenize microbial material.

Sporicidal effects were tested on *Bacillus Subtilis* spore suspension (ready to use by Merck), cultivated in Standard Agar (by SIFIN) and Nutrient Broth I (by SIFIN). Standardized amounts of viable cells were co-incubated as described in DIN EN 14347:2005.

III. Results and Discussion

The aim of the study was to investigate properties of nanoemulsions as carrier for elemental iodine and their antimicrobial effects. To produce iodine-nanoemulsions, iodine was solved in Miglyol, because the solubility is much more higher than in water (about 0.03% weight [33]). A 1.0% w/w iodine-Miglyol-solution could be easily formed at room temperature without noticeable sublimation of iodine during processing.

Miglyol in water emulsions (10% w/w) were prepared as blanks. The particle parameters clearly

indicate that two cycles of high pressure homogenization are sufficient to prepare a stable emulsion with nano-scaled droplets. Droplet diameters of about 130 – 140 nm after seven homogenization cycles only slightly differed from 150 – 160 nm after two cycles. All nanoemulsions were stable against phase separation. The droplet size increased at about 200 nm after a storage time of more than seven month. The PI of both preparations was rather similar and decreased over the storage period from about 0.2 up to 0.1. A PI of approx. 0.001 – 0.050 is typically for monodisperse polystyrene standard particles [34]. A PI of 0.100 still indicates a relatively narrow distribution. PIs up to 0.250 are reported for parenteral fat emulsions, whereas values higher than 0.500 point to very broad particle distribution without any logarithmic normal distribution [35].

Beside costs, a further advantage of a two cycle homogenization process is the much lower energy input into the emulsion. Iodine has an appreciable vapor pressure even below its melting point (e.g. 0.28 mbar at 20°C) and could sublimate at heating due to the homogenization process. High pressure homogenization is based on cavitation. The static pressure of a fluid decreases below its vapor pressure, which could also contribute to the sublimation of iodine.

The particle size of nanoemulsions with iodine was in the same range as for the blanks (fig. 1). After 50 days of storage, the trend towards greater droplets slowed down for samples stored at a refrigerator as expected. After seven month of storage, the mean droplet size was approx. 20 – 30 nm smaller than for samples stored at room temperature. The values of PI point to a narrow distribution for all samples. A phase separation of iodine nanoemulsions was never observed.

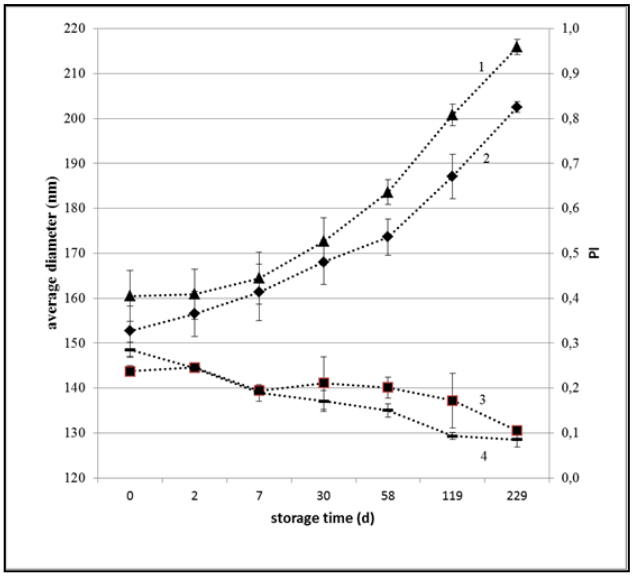

Fig. 1 Average Droplet Diameter of nanoemulsions (blank ◆ 2, 1% iodine ▲ 1) and PI (blank ■ 3, 1% iodine ▬ 4) with error bars

It is interesting to note, that an average zeta potential of –32.1 ± 0.6 mV and –36.8 ± 0.6 mV without and with iodine, respectively was measured after 13 days (fig. 2). The same difference was found at slightly increased level after 28 days of ripening (-35.2 ± 0.5 mV and -40.1 ± 0.6 mV). The higher zeta potential of emulsions with iodine could hint to an additional charge effect of iodine molecules adsorbed at the droplet surface.

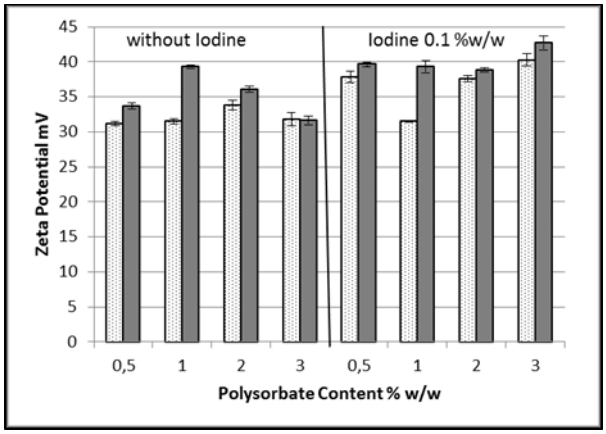

Fig. 2 Zeta potential of nanoemulsions with polysorbate content of 0.5 – 3.0% w/w, without and with 0.1% w/w iodine after ripening of 13 days (dotted bars) and 28 days (filled bars) with error bars

The impact of different interfaces at the behavior of iodine dissolved in Miglyol was studied at planar interfaces. Aliquots of an 1% w/w iodine–Miglyol solution were poured to water and to a water-polysorbate solution (2% w/w) and stored in closed glass bottles. The recovery rate of iodine for the blank was 91% and was set as the start value (fig. 3).

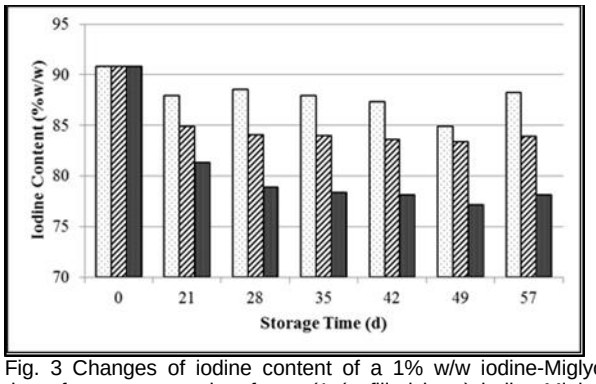

Fig. 3 Changes of iodine content of a 1% w/w iodine-Miglyol solution after storage at interfaces (1 (unfilled bars) iodine-Miglyol solution, 2 (shaded bars) water interface, 3 (filled bars) polysorbate interface) and of a 0.1% iodine–Miglyol-Nanoemulsion [4]

The iodine content of the blank did not changed significantly and leveled at 88% ± 2% after three weeks of storage. Only a small part of iodine seems to sublimate until a saturated atmosphere was reached. In comparison to the blank the iodine content at the water interface slightly decreased and reached a constant value of 84% ± 0.6% after 21 days. The polysorbate solution changed the iodine content of Miglyol to a constant value of 78.9% ± 0.8% after 4 weeks of storage. Despite the low distribution coefficient of iodine between water and Miglyol, the concentration gradient is responsible for a small decrease of the iodine content. In the presence of polysorbate, micelles are formed and could serve as a solubilizer for elemental iodine. The relatively low interfacial area of only 4,500 mm², compared to those of emulsions (about 106 fold area for an average diameter of 200 nm), restricts the transport process of iodine into the water and explains the overall low

decrease of iodine content at planar interfaces. The iodine content of a nanoemulsions amounted only to 36% of the original content of 0.1% directly after formation.

The microbial efficiency of a product can be only measured correctly if toxic effects of the neutralizing agent („control B“) can be excluded. Furthermore, the testing procedure has to be validated to ensure a complete neutralization of the product („validating method C”). Three different neutralizing agents were tested. Only the combination of egg yolk and polysorbate 80 delivered valid results, whereas two different phosphate buffer solutions (K_2HPO_4 and KH_2PO_4) provided insufficient neutralization. Hence, the obligate interacting time could not be guaranteed. Since the DIN protocol for the evaluation of sporicidal effects differed in validation procedures, all neutralizing agents were suitable. The product showed bactericidal effects for both *Pseudomonas aeruginosa* and *Staphylococcus aureus* during the whole survey (tab. 1).

table 1: Results of the examinations for bactericidal activity (CFU – colony forming units)

	Pseudomonas aeruginosa			*Staphylococcus aureus*		
	0 weeks	8 weeks	12 weeks	0 weeks	8 weeks	12 weeks
CFU/ml in the test suspension	$5{,}5 \times 10^7$	$7{,}4 \times 10^7$	$1{,}6 \times 10^8$	$1{,}0 \times 10^8$	$1{,}5 \times 10^8$	$>6{,}6 \times 10^7$
CFU/ml after testing procedures	$<1{,}0 \times 10^1$	$<1{,}0 \times 10^1$	$<1{,}0 \times 10^1$	$<1{,}0 \times 10^1$	$<1{,}0 \times 10^1$	$2{,}0 \times 10^2$
microbial reduction	>lg 5	>lg 5	>lg 5	>lg 5	>lg 5	>lg 5

It should be noted that the results are limited since the procedure was successfully validated during the first iteration only. The product also showed levurocidal effects over the whole survey. A fungicidal as well as sporicidal effect could not be fully demonstrated because of inconsistent results between different trials. Moreover, a reduced activity of the product over time was concluded for both species (tab. 2).

table 2: Results of the examinations for levurocidal and fungicidal activity (CFU – colony forming units)

	Candida albicans			*Aspergillus niger*		
	0 weeks	8 weeks	12 weeks	0 weeks	8 weeks	12 weeks
CFU/ml in the test suspension	$6{,}3 \times 10^6$	$5{,}1 \times 10^6$	$4{,}7 \times 10^6$	$3{,}0 \times 10^5$	$5{,}4 \times 10^5$	$1{,}8 \times 10^6$
CFU/ml after testing procedures	$<1{,}0 \times 10^1$	$<1{,}0 \times 10^1$	$<1{,}0 \times 10^1$	$1{,}5 \times 10^1$	$<1{,}0 \times 10^1$	$>6{,}6 \times 10^3$
microbial reduction	>lg 4	>lg 4	>lg 4	>lg 4	>lg 4	<lg 4

IV. Conclusion

The formation of stable nano-scaled emulsions as carrier for elemental iodine is possible. The great interfacial area of the emulsions as well as the solubilizing effect of the emulsifier distinctly, reduces the iodine content over the storage time. However, a bactericidal, a yeasticidal (levurocidal) as well as a restrictedly fungicidal effect of the emulsion could be detected.

In order to authorize the product for a specific purpose, it has to be evaluated under further testing standards according to its intended use.

Acknowledgment

The Authors would like to thank the Federal Ministry of Economics and Technology, Germany (BMWi) for the financial support (grant no. KF 2971601SK2).

References

[1] C. P. Gerba, “Chapter 29 - Disinfection, In Environmental Microbiology (Third edition)”, edited by Ian L. Pepper, Charles P. Gerba and Terry J. Gentry, Academic Press, San Diego, 2015, pp. 645-662

[2] W. Gottardi “Iodine and disinfection: theoretical study on mode of action, efficiency, stability, and analytical aspects in the aqueous system” Arch Pharm (Weinheim). 1999;332(5):pp. 151-7.

[3] H. Wisplinghoff, R. Schmitt, A. Wöhrmann, D. Stefanik, H. Seifert, “Resistance to disinfectants in epidemiologically defined clinical isolates of Acinetobacter baumannii”, Journal of Hospital Infection, Volume 66, Issue 2, 2007, pp. 174-181, ISSN 0195-6701, http://dx.doi.org/10.1016/j.jhin.2007.02.016.

[4] S. Harbarth, “What can we learn from each other in infection control? Experience in Europe compared with the USA”, Journal of Hospital Infection, Volume 83, Issue 3, 2013, pp. 173-184,

[5] C. Dettenkofer, A. Ammon, P. Astagneau, S.J. Dancer, P. Gastmeier, S. Harbarth, H. Humphreys, W.V. Kern, O. Lyytikäinen, H. Sax, A. Voss, A.F. Widmer, “Infection control – a European research perspective for the next decade”, Journal of Hospital Infection, Volume 77, Issue 1, 2011, pp. 7-10

[6] “Guideline for Disinfection and Sterilization in Healthcare Facilities”, 2008, Healthcare Infection Control Practices Advisory Committee (HICPAC), Atlanta

[7] W. Gottardi “Iodine and iodine compounds. In: Block S S, editor. Disinfection, sterilization, and preservation”. 4th ed. Philadelphia, Pa: Lea & Febiger; 1991, pp. 152–166.

[8] R.W. Lacey, “Antibacterial activity of povidone iodine towards non-sporing bacteria”, J. Appl. Microbiol. 46, 1979, pp. 443-449

[9] G. McDonnell, A. Denver Russell, “Antiseptics and Disinfectants: Activity, Action, and Resistance”, Clin Microbiol Revv.12(1); 1999

[10] W. Gottardi, “Iodine and desinfection: theoretical study on mode of action, efficiency, stability, and analytical aspects in the aqueous system”, Arch. Pharm. Pharm. Med. Chem.1999, 332, pp. 151-156

[11] S. L. Chang, “Modern concept of disinfection”, J Sanit Eng Div Proc ASCE. 1971;97: p. 689

[12] K. Apostolov, “The effects of iodine on the biological activities of myxoviruses”, J Hyg (Lond). 1980 Jun; 84(3): pp. 381-388.

[13] V. S. Springthorpe, S. A. Satter, “Chemical disinfection of virus-contaminated surfaces”, Crit Rev Environ Control. 1990;20: pp. 169–229

[14] W. Gottardi, „Pharmazeutische Iodophorpräparate mit kontrolliertem Iod/Iodid Verhältnis und Verfahren zu ihrer Herstellung“ AT 45 467

[15] H. Beller, W.A. Hosmer, 1953 DE 10 37 075 H. Beller, W.A. Hosmer „Verfahren zur Herstellung eines Desinfektionsmittels aus Polyvinylpyrrolidon und Jod“

[16] I. R. Buxton, S. T. Leslie, S. T. A. Malkowska, A. J. Miller, R. B. Miller, D. A. Prater „Jod enthaltende pharmazeutische Zusammensetzung“ EP 0 448 288 B1

[17] R. Yanai, N. Yamada, K. Ueda, M. Tajiri, T. Matsumoto, K. Kido, S. Nakamura, F. Saito, T. Nishida, “Evaluation of povidone-iodine as a disinfectant solution for contact lenses: Antimicrobial activity and cytotoxicity for corneal epithelial cells, Contact Lens and Anterior Eye”, Volume 29, Issue 2, May 2006, pp. 85-91

[18] P. Durani, D. Leaper, “Povidone-iodine: use in hand disinfection, skin preparation and antiseptic irrigation”, Int. Wound J. 2008 Jun;5(3): pp. 376-87.

[19] M.M. Coogan, M Patel, D Mladenova, “Efficacy of three surface disinfectants for dental radiographic films and gloves”, Journal of Dentistry, Volume 32, Issue 5, July 2004, pp. 385-389

[20] S. Moulay, (2013). “Molecular iodine/polymer complexes”, Journal of Polymer Engineering, 33(5), pp. 389-443. Retrieved 24 Apr. 2015, from doi:10.1515/polyeng-2012-0122

[21] S. I. Ahmad, N. Mazumdar, S. Kumar, “Functionalization of natural gum: An effective method to prepare iodine complex”, Carbohydrate Polymers, Volume 92, Issue 1, 30 January 2013, pp. 497-502

[22] D. K. Jeng, “Povidone-iodine (PVP-I) alcohol gel antimicrobial pre-operative skin preparation” WO 98/44930

[23] W. M. Winicov, „Germizide Detergenz-Iod-Zusammensetzungen, die Polyvinylpyrrolidone und kompatible nichtionische Tensidkomplexe enthalten“ DE 695 27 643 T2

[24] A. Weinstabl, P. M. Amann, G. Wurpts, H. F. Merk. „Jodallergie. [Iodine allergy]” Hautarzt 2012;63(5): pp. 360–363.

[25] A. Trinavarat, L. O. Atchaneeyasakul, “Treatment of epidemic keratoconjunctivitis with 2% povidone-iodine: a pilot study” J Ocul Pharmacol Ther 2012;28(1): pp. 53–8

[26] M. Nishimura, N. Kariya, U. Hulan, C. Y. Duan, T. Shimono, “Comparison of the hand disinfectant effects between super hypochlorous water and 7.5% povidone-iodine”, Pediatric Dental Journal, Volume 14, Issue 1, 2004; pp. 1-3

[27] S. Punyani, P. Narayama, H. Singh, P. Vasudevan, 2006, “Iodine based water disinfection: A review”, J. Sci. INd. Res 65, pp. 116-120

[28] R. McConn-Stern, T. Walsh, „Präparate für die Wundheilung, die Iod und einen Zucker enthalten“ DE 694 26 385 T2, EP 0 683 670 B1

[29] M. T. Scholz “Liquid antiseptic composition containing iodine and a sugar and/or sugar alcohol” EP 2 249 805 A1 WO 2009/088826

[30] C. D. Gradle, A. Roselle, “Iod-Propylenglycol-Zitzentauchbad” DE 601 21 432 T2

[31] Y. Duan, K. Dinehart, J. Hickey, R. Panicucci, J. Kessler, W. Gottardi, “Properties of an enzyme-based low-level iodine disinfectant”, Journal of Hospital Infection, Volume 43, Issue 3, November 1999, pp. 219-229

[32] G. Jander, K. F. Jahr, „Maßanalyse: Theorie und Praxis der Titrationen mit chemischen und physikalischen Indikationen“, Walter de Gruyter, 2002

[33] F. C. Kracek, “Solubilities in the System water-iodine to 200°”, J. Phys. Chem., 35 (2) 1931, pp 417–422

[34] C. K. Ober, K. P. Lok, M. L. Hair, “Monodispersed, micron-sized polystyrene particles by dispersion polymerization”, J. Polym. Sci. B Polym. Lett. Ed., 23: 1985, pp. 103–108

[35] S. Benita, H. Bernhard L. Böhm, “Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs”, CRC Press, 1998