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# Achieving high antimicrobial activity: Composite alginate hydrogel beads releasing activated charcoal with an immobilized active agent

Andrea Osmokrovic[a], Ivan Jancic[b], Jovana Vunduk[c], Predrag Petrovic[d], Marina Milenkovic[b], Bojana Obradovic[a,*]

[a] Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia
[b] Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia
[c] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11000 Belgrade, Serbia
[d] Innovation Center of the Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia

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ABSTRACT

New composites based on Ca-alginate hydrogels were produced that release activated charcoal (AC) particles with adsorbed povidone iodine (PVP-I) as a model antimicrobial substance in a physiological-like environment. Composite beads with different alginate (0.5–1.5%w/w) and AC (1–20%w/w) concentrations were analyzed by FE-SEM and characterized regarding textural parameters, swelling, and AC release kinetics. PVP-I was easily adsorbed onto AC particles within the optimized beads (0.5%w/w alginate, 20%w/w AC) as indicated by UV–vis spectroscopy, EDX and FT-IR analyses. The obtained beads have shown strong bactericidal effects against two standard bacterial strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and clinical multi-resistant wound isolates (*MRSA, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis* and *Proteus mirabilis*) and, at the same time, exhibited negligible PVP-I desorption in physiological saline solution. Thus, the obtained composites could provide utilization of potent antiseptics such as iodine, in wound dressings, without the concern of systemic absorption.

## 1. Introduction

Throughout the history and up to date, chronic wounds have always represented a huge medical problem. These wounds often produce large amounts of exudates, which may adversely affect healing (e.g. chronic venous ulcers exudate in average 0.5 ml/cm$^2$/24 h; Thomas, Fear, Humphreys, Disley, & Waring, 1996). Also, all chronic wounds are colonized with microorganisms and the only question remaining is if clinical consequences are exhibited, locally as infection or systemically as sepsis. Thus, efficient treatment of chronic wounds today includes exudate management and prevention/reduction of wound infection. However, bacterial resistance to topical and systemic antibiotics is rapidly increasing due to their misuse and overuse. In addition, bacterial resistance and cross-resistance data have been reported for many antiseptics (Kunisada, Yamada, Oda, & Hara, 1997; Lachapelle, Castel, Casado, Leroy, & Micali, 2013; Lanjri, Uwingabiye, Frikh, Abdellatifi, & Kasouati, 2017; Leaper & Durani, 2008; Yasuda, Yoshimura, Katsuno, Takada, & Ito, 1993) as well as evidences of cross-resistance between antibiotics and antiseptics (Chuanchuen, Beinlich, Hoang, Becher, & Karkhoff-Schweizer, 2001; Leaper & Durani, 2008). Povidone iodine (PVP-I) presents an exception, since, up to date, instances of acquired or cross-resistance for iodine have not been documented (Fleischer & Reimer, 1997; Kunisada et al., 1997; Lachapelle et al., 2013; Leaper & Durani, 2008; Yasuda et al., 1993). Still there are concerns regarding possible systemic absorption of iodine since it is significantly permitted through the skin in a time dependent manner (Nesvadbova, Crosera, Maina, & Larese Filon, 2015). Many clinical reports suggest that absorption is enhanced in cases when PVP-I is applied to injured skin, mucosal surface, large areas of intact skin and to a thinner skin such as in infants (Dela Cruz, Harper Brown, Leikin, Franklin, & Hryhorczuk, 1987; Erdogan, Tatar, Unluturk, Cin, & Uysal, 2013; Findik, Gezer, Aydogdu, Oz, & Kucukbayrak, 2010). In burn patients, with second and third degree burns occupying over 25% of the entire skin surface, total iodine levels in serum were reported to noticeably increase, which may lead to possible systemic complications as well (Nesvadbova et al., 2015). In fact, Below et al. (2006) emphasized that, depending on the PVP-I concentration and the wound area, 0.3–4.5% of the iodine is absorbed through the skin. Furthermore, in patients with normal renal

* Corresponding author.
*E-mail addresses:* aosmokrovic@tmf.bg.ac.rs (A. Osmokrovic), ijancic@pharmacy.bg.ac.rs (I. Jancic), vunduk@agrif.bg.ac.rs (J. Vunduk), ppetrovic@tmf.bg.ac.rs (P. Petrovic), marinama@pharmacy.bg.ac.rs (M. Milenkovic), bojana@tmf.bg.ac.rs (B. Obradovic).

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**Table 1**
Results of AST of wild microbial strains used in the present work.

| Bacteria isolated from wounds | Resistance information |
|---|---|
| Methicillin-resistant *Staphylococcus aureus (MRSA)*[*] | Benzylpenicillin, Oxacillin, Imipenem, Gentamicin, Ciprofloxacin, Moxifloxacin, Erythromycin, Clyndamicin, Tetracycline, Fusidic Acis, Rifampicin |
| *Escherichia coli* (ESBL-EC[**]) | Cephalosporins, Ampicillin, Amoxicillin/clavulanic acid, Piperacillin, Ciprofloxacin, Sulfamethoxazole/Trimethoprim |
| *Pseudomonas aeruginosa* | Ceftazidime, Piperacillin, Gentamicin, Ciprofloxacin |
| *Enterococcus faecalis* | Ampicillin |
| *Proteus mirabilis* | Cefalexin, Ceftazidime, Ceftriaxsone, Ampicillin, Amoxicillin/clavulanic acid, Piperacillin, Amikacin, Gentamicin, Ciprofloxacin, Sulfamethoxazole/Trimethoprim |

* mecA gene confirmed.
** Extended-spectrum β-lactamase-producing *Escherichia coli*.

function, the serum iodine levels rose during PVP-I administration and it took more than 1 week after the last application to return to normal levels (Steen, 1993).

In this work, we have hypothesized that the problem of transdermal iodine absorption could be solved by its immobilization. Adsorption was chosen as the simplest immobilization method and activated charcoal (AC) as one of the most effective and widely used adsorbents due to a large specific surface area. In addition, AC has been in medical use as a nonspecific antidote in treatments of poisonings. Different studies have shown that AC adsorbs bacteria, viruses and other biochemical substances *in vitro* and *in vivo* (Cole, 2002; Drucker, Goldhar, Ogra, & Neter, 1977; Howell, Sandeman, Phillips, Lloyd, & Davies, 2006; Kerihuel, 2010; Naka, Watarai, Tana, Inoue, & Kodama, 2001; Nolan, McDevitt, & Goldmann, 1975; Sandeman, Howell, Mikhalovsky, Phillips, & Lloyd, 2008; Xiang-Nan, Zhen, Guo-Zheng, & Zong-Ming, 1987). Today many modern wound dressings contain a layer of activated charcoal typically in the form of activated carbonized cloth (*e.g. Actisorb*, Johnson & Johnson Medical Ltd., USA; *Sorbsan plus carbon*, Aspen Medical Europe Ltd., UK; *Clinisorb*, Clinimed Ltd., UK; *Carbonet*, Smith & Nephew, Canada). This cloth is obtained by carbonization and activation of viscose rayon resulting in predominantly microporous structure (Starek, Zukal, & Rathousky, 1994), so that it is effective and mostly used for adsorption of small molecules (< 2 nm in diameter) such as odor molecules. On the other hand, structure of activated charcoal can be tailored to be meso- and macroporous by varying the method of preparation, thus offering the advantage in potential adsorption of larger molecules and even bacterial cells (Ryoo, Joo, Kruk, & Jaroniec, 2001).

Still, AC layer or particles cannot be used as a wound dressing alone but in conjunction with an absorption pad for moisture regulation, which is usually made of a polymer hydrogel such as alginate. Alginate is a natural polysaccharide, composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues, in different proportions depending on the extraction source. Soluble Na-alginate can form hydrogels in the presence of divalent and trivalent cations, via ionic interactions between carboxylate groups and chelating ions. Commonly used cations to crosslink Na-alginate are $Ca^{2+}$, which bind preferentially to G units resulting in the well-known “egg-box” structure (Grant, Morris, Rees, Smith, & Thom, 1973). However, the cation exchange is a reversible process so that in a media containing monovalent cations such as $Na^{+}$, Ca-alginate gels undergo swelling and subsequently degradation (Mitrovic, Stojkovska, & Obradovic, 2010). Thus, encapsulation of agents within the alginate matrix may provide their sustained release when exposed to biological fluids, which normally contain NaCl. Ca-alginate hydrogels in wound dressings were shown to absorb excess exudates and at the same time, maintain a moist physiological microenvironment, and thus promote rapid granulation and reepithelisation (Lee & Mooney, 2012; Qin, 2007).

The aim of this work was to utilize advantageous properties of AC to strongly adsorb PVP-I as a model antimicrobial substance, in conjunction with alginate hydrogels that can serve for controlled AC delivery as well as moisture regulation in wounds. The function of AC particles, directly released in the wound area, would be to *in situ* adsorb microorganisms and products of metabolism, and in the same time provide the action of the adsorbed substance to potentially reduce the bacterial load in open wounds. Thus, in the first phase, the goal was to produce and characterize composite alginate hydrogels with different alginate and AC concentrations and to select the optimal formulation regarding AC release kinetics in physiological saline solution as a model for biological fluids. In the second phase of this work, the aim was to investigate the possibility to adsorb PVP-I on AC particles within the selected alginate/activated charcoal (A/AC) composite beads followed by PVP-I desorption and antimicrobial studies in order to evaluate stability and retained activity of the adsorbed PVP-I.

## 2. Materials and methods

### 2.1. Materials

Medium viscosity sodium alginate (A2033, molecular weight = 80,000–120,000, mannuronic (M) to guluronic (G) residue ratio M/G = 1.56) and calcium chloride dihydrate were supplied from Sigma (St. Louis, MO). Activated charcoal (AC) in the form of fine powder (MEKS 95) was purchased from Trayal (Krusevac, Serbia), sodium citrate from Himedia (Mumbai, India), and NaCl from Centrohem (Stara Pazova, Serbia). Povidone iodine powder was a kind gift from Hemofarm A.D. (Vrsac, Serbia). Tryptic Soy broth was supplied from Himedia (Mumbai, India) while *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 27853) originate from American Type Culture Collection (Rockville, Maryland). Wild microbial strains (*MRSA, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Proteus mirabilis* and *Candida albicans*) were supplied by the City Institute of Public Health (Belgrade, Serbia) and the results of their antimicrobial susceptibility tests (AST) are shown in Table 1.

### 2.2. Preparation of A/AC composite beads

Fine AC powder was dispersed in aqueous solutions of sodium alginate by vigorous mixing using the mechanical stirrer Ultra-Turrax® T25 (Janke and Kunkel Ika-Labortechnik, Staufen, Germany) at 20000 rpm for 5 min. A series of suspensions were prepared with final concentrations of alginate in the range 0.5–1.5% w/w and AC in the range 1–20% w/w. The obtained suspensions were extruded using a syringe or a peristaltic pump through a blunt edge stainless steel needle (16G) and the resulting droplets were collected in a gelling bath (0.9% w/w $CaCl_2{\cdot}2H_2O$) forming insoluble beads. The beads were left in the bath for additional 30 min to complete gelling, followed by washing in distilled water for several times. The obtained A/AC beads were further used for impregnation with the antimicrobial substance or dried in air at room temperature until the constant weight.

The A/AC beads were labeled according to the alginate concentration (% w/w) in starting suspensions as 0.5, 1 and 1.5 as well as according to the AC concentration in suspensions, as low (5% w/w), medium (10% w/w) and high (20% w/w). There was one exception,