CHEMISTRY JOURNAL OF MOLDOVA. General, Industrial and Ecological Chemistry. 2016, 11(2), 17-25
http://cjm.asm.md
http://dx.doi.org/10.19261/cjm.2016.11(2).03

RECENT TRENDS IN ALGINATE, CHITOSAN AND ALGINATE-CHITOSAN ANTIMICROBIAL SYSTEMS

Ivancic Albert

Faculty of Chemistry and Chemical Technology, Moldova State University, 60, Alexei Mateevici str., Chisinau MD2009, Republic of Moldova
Institute of Chemistry of Academy of Sciences of Moldova, 3, Academiei str., Chisinau MD2028, Republic of Moldova
e-mail: ivancicalbert@gmail.com; phone: (+373 68) 344 034

Abstract. Natural polysaccharides alginate and chitosan have been used extensively, separately or in mixtures (systems), in manufacturing of pharmaceutical products (antimicrobial) and not only. Alginates usually serve as basis for antimicrobial systems, while chitosan, in certain proportions, enhances their physicochemical and antimicrobial properties. Focusing on the recent literature (mostly since 2000), this review outlines the main synthetic approaches for the preparation of systems based on both polymers as well as identify potential areas of their application as antimicrobial agents. Various techniques used for systems preparation like microparticles, films, fibers, nanoparticles, sponges, applications and usefulness of these systems as carriers of antimicrobial compounds will also be discussed.

Keywords: alginate, chitosan, antimicrobial system, ionotropic gelation, drug.

Received: August 2016/ Revised final: October 2016/ Accepted: October 2016

Introduction

Nowadays, new systems with antimicrobial properties, that are more potent, less toxic to humans, with prolonged action and preferentially based on natural compounds, are of high interest. Consequently, antimicrobial systems based on natural polymers, such as alginates and chitosan, which fulfil the above mentioned properties and are relatively accessible and ecological as they can be obtained from various agro-industrial waste; are being studied increasingly. According to the available data, this review intends to consolidate for the first time the knowledge about the most popular alginate/chitosan antimicrobial systems, though more attention being paid to the alginate-chitosan systems with encapsulated antimicrobial compounds, namely the synthesis techniques, some physicochemical and bioactive properties as well as areas of use.

Alginates represent anionic polysaccharides, linear copolymers with homopolymeric blocks of 1,4-linked *β*-D-mannuronate and *α*-L-guluronate residues [1,2] (Figure 1), spread as a mixture of calcium, sodium and potassium salts of alginic acid in the cell walls of all brown algae (*Phaeophyceae*) [1,2,3] and several bacteria (*e.g. Azotobacter vinelandii*) [3].

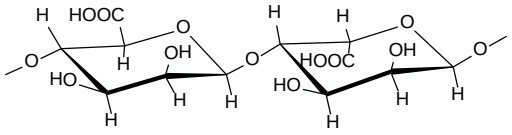

Figure 1. Alginic acid fragment (*β*-D-mannuronate and *α*-L-guluronate (1-4)-linked).

Alginates have the property to form gel in the presence of certain divalent (or multivalent) cations, particularly calcium ions, and entrap other materials in this gel [2]. The rheological properties of the formed gel depend on the ratio mannuronate:guluronate; rich in guluronate alginates, produce strong and brittle gels, while rich in mannuronate alginates produce weaker but more elastic gels [2,4]. Calcium alginate gels are generally non-toxic [5], biocompatible [5-7], likewise alginic acid and sodium alginate gels [5]. Also, calcium alginate systems show some antioxidant and antimicrobial properties [8], while sodium alginate systems demonstrated good antioxidant properties [9,10], but do not have antibiotic activity on the microorganisms studied in the paper [9]. Alginate gels have non-adhesive to cell properties [11].

Alginates in the form of various systems, like gels [2-5,12,13], water/oil soluble films [13-23], fibers [8,24-26] and others [13,18,27-29], have found application in the fields such as medicine and pharmaceuticals [5,8,12,14,16,22,24,26-29], food industry [4,15,17-21,23] and textiles [2,24-26].

Chitosan represents natural, cationic aminopolysaccharide copolymer [30] consisting of randomly distributed units of D-glucosamine and N-acetyl-D-glucosamine linked *β*-1,4 (Figure 2), obtained by partial deacetylation of chitin contained in the exoskeletons or cuticles of many invertebrates [30-32], cell walls of some fungi, mushroom [31] and yeasts [31,32].

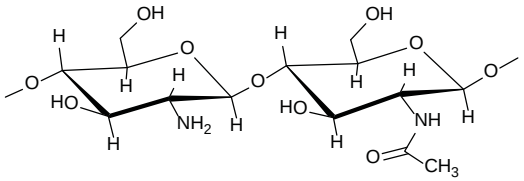

Figure 2. Chitosan fragment (D-glucosamine and N-acetyl-D-glucosamine (1-4)-linked).

Chitosan has multiple useful properties like non-toxicity [32], biocompatibility [13,32-34], antimicrobial activity [13,32-45], biodegradability [13,32-34], antioxidant activity [13,35,41,44], haemostatic properties [30,46,47] and others [13,30]. Chitosan easily can be moulded in diverse forms: hydrogel [13,32,33], powder [13,34], paste [13,34], film [13,32-34,40,41,45], fibers [32,34], sponge [13,32], scaffold [32-34] and others [13,30,33,38,42-44], which have found application, alone or in combination with other compounds, in the fields of medicine [13,30,32-34,36-43,46,47], textile [32], food industry [33,35,40,44,45], water treatment [33], etc.

Several papers were drafted in the field of development and analysis of alginate or chitosan based systems containing antibacterial substances. Compared to other works written in this field, the present paper highlights the possibility of obtaining alginate/chitosan or alginate-chitosan with antimicrobial substances systems, of different chemical nature. Based on alginate were obtained and analysed antimicrobial systems containing: silver ions [8,26], nanocrystalline silver [8], ethylenediaminetetraacetic acid [16], 3-(trimethoxysilyl)propyl-octadecyldimethylammonium chloride [28], lysozyme [12,16], lactoperoxidase [20], nisin [16-18], lysosomes [29], grape fruit seed extract [16,19], essential oils [21-23] and others. Like alginates, chitosan was used as matrix for various antimicrobial systems with: copper ions and nanoparticles [13], silver nanoparticles [13,44], silver oxide [45], lomefloxacin [42], ofloxacin [43], lysozyme [44], nisin, natamycin, peptide P34 [40], essential oils [41], etc. Obtained systems showed more pronounced antimicrobial activity than alginate/chitosan and antimicrobial compounds apart.

Due to the opposite charges, alginate (-) and chitosan (+) form polyelectrolyte complexes, that can be moulded in the form of various systems. Based on a literature review of the last fifteen years of research in this field, it was identified that the most studied alginate-chitosan systems are: a) microparticles; b) films; c) fibers; d) nanoparticles; e) sponges.

Microparticles

Alginate-chitosan microparticles are especially used in the pharmaceutical, for sustained-release of antimicrobial compounds. Microparticles provide stability, mask an unpleasant taste and odour, and reduce toxic side effects of encapsulated bioactive substances.

Microparticles can be obtained using one of the methods: ionotropic gelation of alginate with calcium ions in the presence of chitosan, tripolyphosphate cross-linking method or both of them. Using first method were obtained alginate-chitosan microsystems with antimicrobial compounds like: amoxicillin [48,49], nitrofurantoin [50], metronidazole [51], polymyxin B [52], cefaclor [53], rifampicin, isoniazid, pirazinamid [54,56] (Figure 3). By the use of the tripolyphosphate cross-linking method were obtained alginate-chitosan microcapsules with metronidazole [57]. Alginate-chitosan beads with sulfathiazole [58] were obtained by mixing elements of both methods.

Encapsulation of amoxicillin in alginate-chitosan mucoadhesive microcapsules as gastroretentive delivery system, resulted in enhanced stability [48] and controlled release of antimicrobial drug in the simulated gastric fluid, compared to amoxicillin plain drug. [48,49] Reducing approximately two times and more of concentration of used chitosan (0.5% w/v and lower) to obtain antimicrobial alginate-chitosan microparticles resulted in selective sustained-release of active principle (nitrofurantoin [50], polymyxin B [52], rifampicin, isoniazid and pyrazinamide [54,56]) in simulated intestinal fluid, while the release in simulated gastric fluid was very slow. Cefaclor release from alginate-chitosan microparticles in simulated gastric fluid is intensifying with increasing of alginate concentration up to 7% w/v (chitosan concentration - 0.5% w/v) [53]. Similar release profile was observed for systems containing antituberculosis drug rifampicin and for which obtaining was used 1% alginate and 1.5% chitosan [55]. Alginate-chitosan beads with metronidazole were obtained; due to high contents of chitosan (5% w/v) float on gastric juice and consequently are retained in the stomach where they gradually release antibiotic compound [51]. In vivo studies have shown that the encapsulation of rifampicin, isoniazid and pyrazinamide in alginate-chitosan microcapsules resulted in an increase of about 13-15 times in their biological half-life compared to non-encapsulated substances, leading to an increased duration of action of the antituberculous compounds [56].

Using tripolyphosphate cross-linking method were obtained metronidazole containing alginate-chitosan microcapsules, characterized by good mucoadhesive property and prolonged release in simulated intestinal fluid due to ionic cross-link between negatively charged tripolyphosphate and positively charged chitosan molecules [57].

Alginate-chitosan microsystems with sulfathiazole were obtained using the stages: 1. ionotropic gelation of alginate with calcium chloride in presence of chitosan and sulfathiazole; 2. treatment of obtained beads with sodium tripolyphosphate solution; 3. beads irradiation with microwave radiation. Obtained beads showed release-retarding properties in the simulated gastric fluid, due to alginate-chitosan complexation and alginate cross-linking, especially after microwave treatment [58].

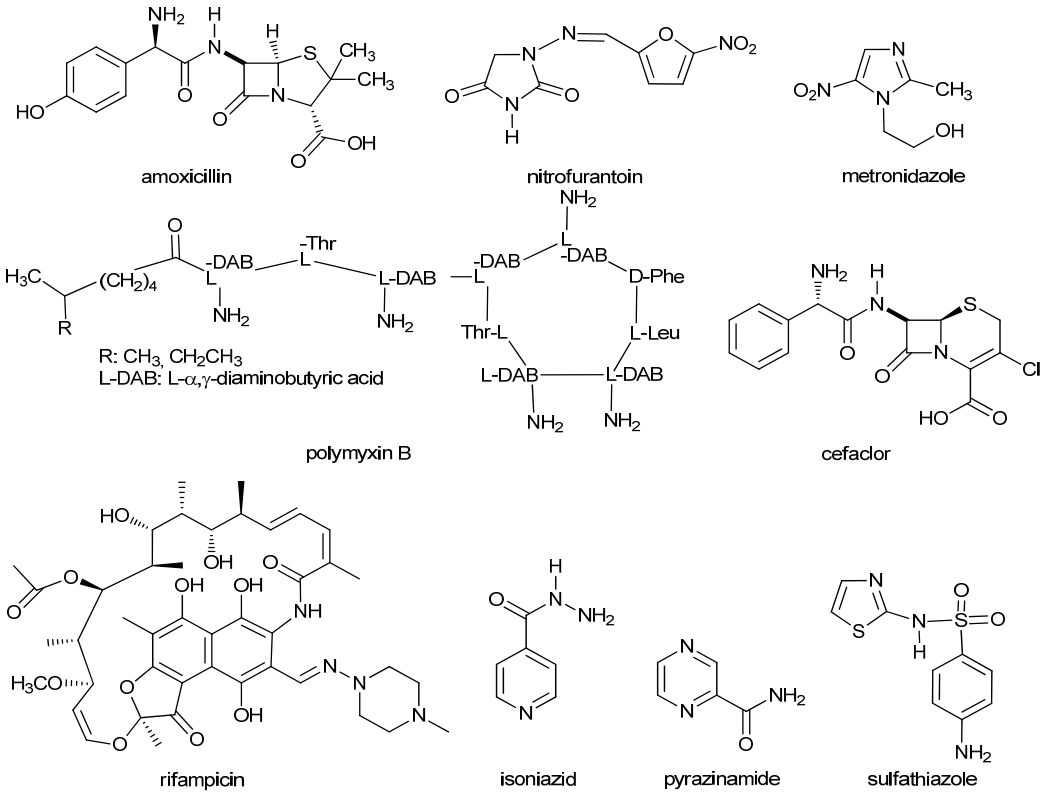

Figure 3. Structures of some antimicrobial compounds encapsulated in alginate-chitosan microparticles.

Films

As microsystems, alginate-chitosan films show some antimicrobial properties either due to mechanical blockage of microorganisms on the surface of films or due to antimicrobial effect of the chitosan itself [59,60]. Inclusion of antimicrobial substances like: silver nanoparticles [61], copper ions [62], natamycin [63,64], minocycline [65], ciprofloxacin [66], silver sulfadiazine [67] and chlorhexidine [68] (Figure 4) leads to increased antimicrobial properties of alginate-chitosan films which allows their use in various fields such as: wound dressings materials development [59-61,66,67], antibacterial functional coatings with controlled release [62,65], pharmaceutical forms with sustained release [67,68], scaffolds for tissue engineering [60], food packaging [61,63,64] and water treatment [62].

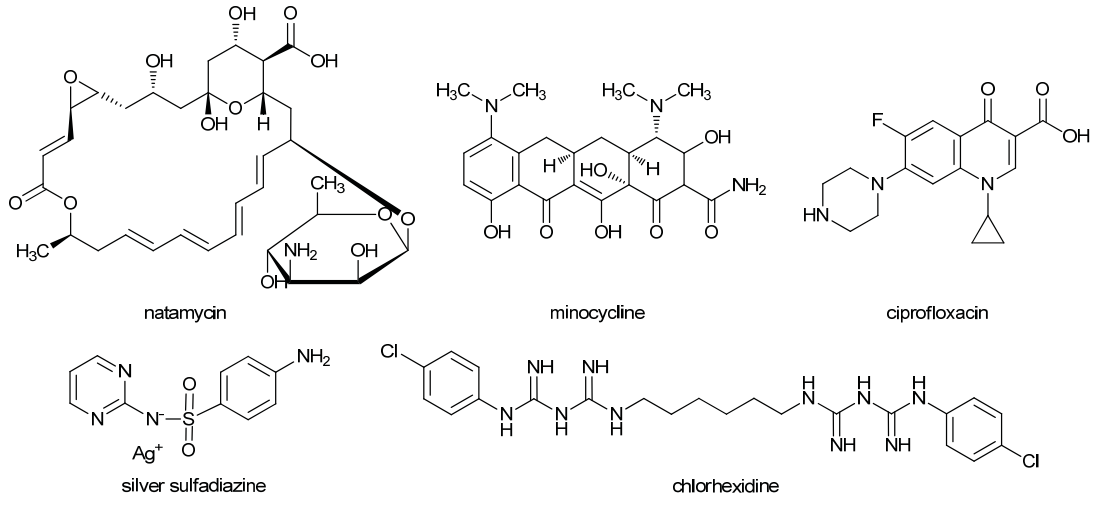

Figure 4. Structures of some antimicrobial compounds incorporated in alginate-chitosan films.

Films can be obtained without [61,62,67,68] or using calcium chloride [59,60,63-66] to cross-linking alginate. Structurally, antimicrobial membranes may consist of a homogenous alginate-chitosan mixture [59-64,67,68] or two and more individual layers of alginate and chitosan which adhere to each other [62,65,66].

Fibers

As it was already mentioned, alginate and chitosan can be moulded in form of fibers. Using alginate-chitosan mixtures can be obtained fibers with advanced useful properties, compared to individual polymers. Wet spinning technique [69-75] is the most common method for manufacturing these fibers. Using this method, it can be obtained fibers consisting entirely of alginate-chitosan mixture [70,75], alginate coated with chitosan [69,71-73] (Figure 5) or *vice versa* - chitosan coated with alginate [74] (Figure 6).

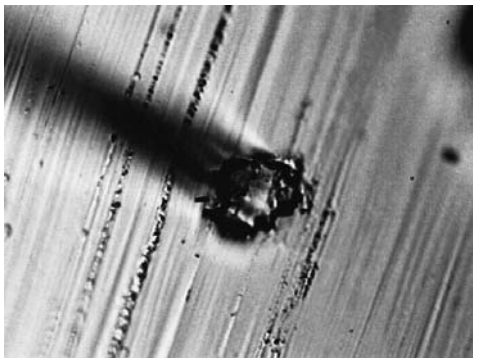

Figure 5. Microscopic cross view of chitosan-alginate fiber stained by ninhydrin (adapted from [73]).

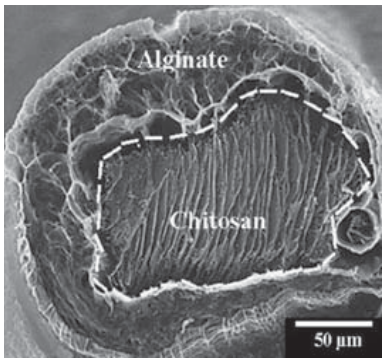

Figure 6. LV-SEM image of alginate-chitosan fiber cross section (adapted from [74]).

Due to chitosan and antimicrobial compounds (sulfathiazole [71]), alginate-chitosan fibers show antimicrobial activity - inhibit the growth of various bacteria that inhabit the skin of mammals, such as *Staphylococcus aureus* [69,70,72], *Escherichia coli* [70,71], *Micrococcus luteus*, *Staphylococcus epidermidis* [69]. This property together with the ability to carry cationic [74] and anionic [75] drugs, allow antimicrobial alginate-chitosan fibers to be a potential candidate for wound dressing materials.

Nanoparticles

A preliminary review of the literature showed the possibility of encapsulation into alginate-chitosan nanoparticles of various chemical structures of antimicrobial compounds like: benzoyl peroxide [76], gatifloxacin [77], levofloxacin [78], daptomycin [79], nisin [80] (Figure 7) and *Ocimum sanctum* methanolic extract [81].

Nanoparticles were prepared using ionotropic gelation (with or without some modifications) [76-81], freeze-drying and spray-drying methods [82]. In the majority of cases, the nanoparticles obtained by ionotropic gelation method are stables (zeta potential – $\zeta < -30$ mV or $\zeta > +30$ mV) [77,80,81], have a nearly spherical shape, with sizes ranging from < 100 nm [76,78,81] to 200-900 nm [77,79,80]. Freeze-drying and spray-drying methods allow obtaining of nanoparticles, together with microparticles, which have irregular shapes (fibrids-like) and sizes around 900 nm and larger, while spray-dried systems show higher antimicrobial activity than freeze-dried ones [82]. Pristine and drug loaded alginate-chitosan nanoparticles show antimicrobial activity against: *Staphylococcus aureus* [79-82], *Escherichia coli* [81,82], *Propionibacterium acnes* [76], *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus hominis*, *Staphylococcus lundunensis*, *Staphylococcus haemolyticus*, *Staphylococcus warneri* [79], *Bacillus cereus*, *Pseudomonas aeruginosa*, *Bacillus cereus* [81]. Drug loaded nanoparticles release antimicrobial compound over an extended period of time (4 hours and more) [77-80], keeping the antimicrobial activity even after 30 laundering cycles of fabrics treated with nanoparticles [81]. Due to this properties, alginate-chitosan antimicrobial nanoparticles can be potentially usable in medicine: as potential carrier systems with sustained release of antimicrobial drugs [76-79] and as components in developing of fabrics for medical use [81,82]; in food industry as preservatives [80].

Sponges

Alginate-chitosan sponge represent a tridimensional porous scaffold, prepared by freeze-drying technique of mixture [83] or consecutively deposited one above the other layers of alginate and chitosan [84-86]. Pore sizes range from <100 µm [85, 86], 200-400 µm [83] and approximately 500 µm [84], as a function of various factors such as cross-linking time with Ca^{2+} ions - with its increasing pore sizes being reduced [83] (Figure 8). In the same time with increasing of calcium ions concentration the release rate of antimicrobial compound from systems decreases, because of this, systems containing the lowest concentration of calcium ions (5%), in the conditions of the experiment, showed shortest time in reducing of number of viable bacteria cells to zero [85].

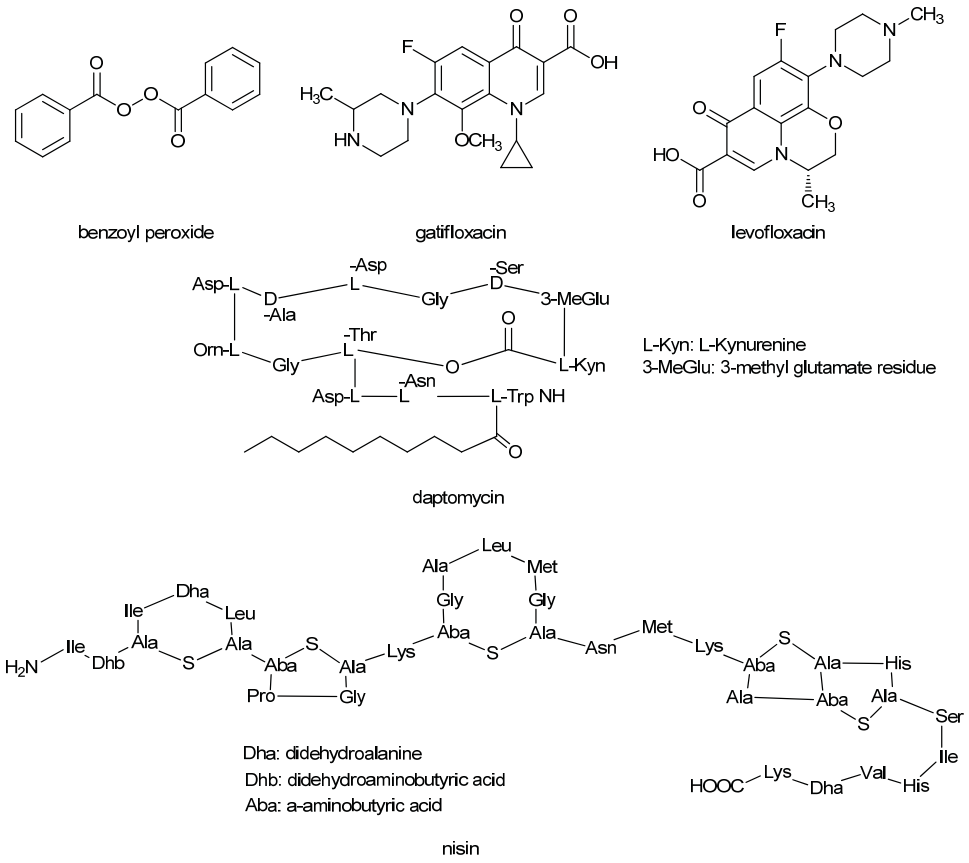

Figure 7. Structures of some antimicrobial compounds encapsulated in alginate-chitosan nanoparticles.

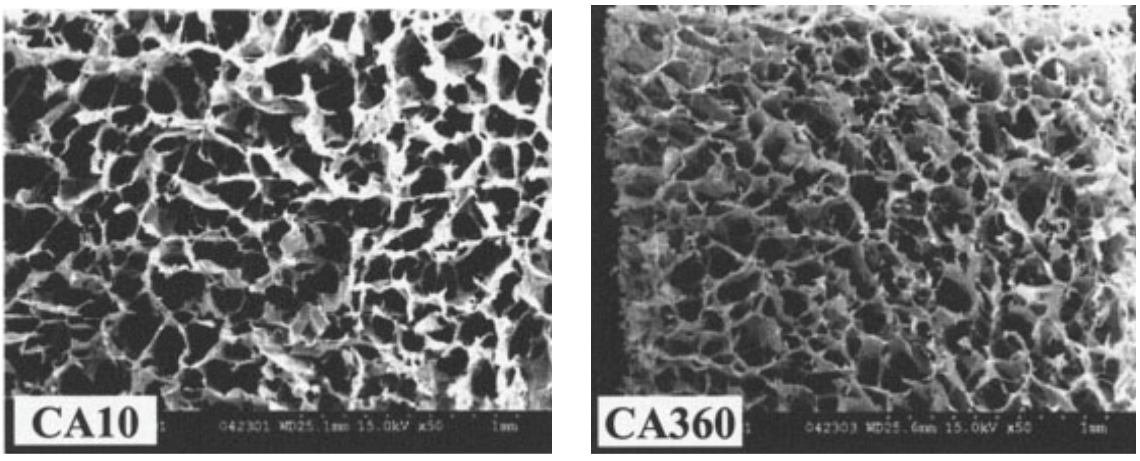

Figure 8. SEM images of alginate-chitosan sponges prepared with cross-linking time 10 min (left) and 360 min (right) (adapted from [83]).

In the alginate-chitosan sponges were encapsulated antimicrobial compounds like: silver sulfadiazine [83,86], gentamicin [84] and ciprofloxacin [85]; in result, increased the antimicrobial activity against: *Staphylococcus aureus* [83-86], *Pseudomonas aeruginosa* [83,86] and *Escherichia coli* [85], compared to pristine alginate-chitosan sponges.

All of the studied alginate-chitosan antimicrobial sponges demonstrated sustained release of antimicrobial drugs from 4 hrs [85] to 30 days [84]. Due to these properties, alginate-chitosan antimicrobial sponges can be potentially used in wound dressing manufacturing [83,85,86] and in tissue engineering applications [84].

Conclusions

The most studied alginate-chitosan antimicrobial systems in the literature: microparticles, films, fibers, nanoparticles and sponges, have been analyzed and classified. Based on their properties, areas of potential application like medicine, pharmaceutics, food industry, fabrics production, were identified. Although a considerable amount of research has been conducted in this field, further work is required because chemists are still faced with the challenge of developing a universal methodology that is compatible with a large variety of alginate-chitosan antimicrobial systems.

Acknowledgements

The author thanks the Moldavian State Program project (No. 16.00353.50.06A) for financial support and Dr. hab., Prof. Macaev Fliur for helpful discussions.

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