INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
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www.vmsindia.org

ANTIMICROBIAL PROPERTIES OF CALCIUM CHLORIDE LOADED POLYANILINE

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Abstract:
In situ polymerised polyaniline (PANI) with calcium chloride produced by chemical method and tested fortheir antibacterial activity with respect to Escherichia coli. PANI with various concentration of calcium chloride showed a significant antibacterial effect against bacteria strains whilethe efficacy of neat PANI base was only marginal. After the calcium chloride impreganation, the PANI base exhibited different levels of antibacterial effect depending on the type of the bacterial strain. The results show that calcium chloride impregenation on PANI can be a suitable method for the preparationof PANI base material with improved antibacterial properties.

Introduction:

Outbreaks of food borne illness associated with consuming raw vegetables occur more frequently in recent years. Fresh-cut vegetables are highly susceptible to microbial contamination because of microbial cross-contamination through the shredders and slicers and the exposure of inner tissues to microbial attachment and growth after cutting. The pathogenic bacteria of the most concern in fresh vegetables include Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella Typhimurium. Chemical agents such as sodium hypochlorite, chlorine dioxide, sodium bisulfite, sulfur dioxide, organic acids, calcium chloride, trisodium phosphate, ozone, and cetyl pyridinium chloride have been studied as potential disinfectants.

Besides all its advantages, application of polymers in medicine brings also a problem related to the occurrence of nosocomial infections. Therefore, a considerable effort has been exerted to develop polymers or composites with efficient antibacterial properties. In addition to polymer materials which possess an intrinsic antibacterial activity, these properties can be also achieved through coating or adsorption of an antibacterial agent on to the polymer surface; immobilisation of an antibacterial agent in the polymer via ionic or covalent bonding or by direct incorporation of an antibacterial agent in to the polymer during its synthesis [1-5]. Recently published studies indicate that among the polymers inherently showing antibacterial properties are also conducting polymers such as polyaniline (PANI)[6-8], which has been the subject of considerable attention due to its potential in biomedical and other promising applications.Its efficacy against gram- positive and gram-negative bacteria and against fungi was first reported by Seshadri and Bhat[9].. Seshadri and Bhat (2005) prepared cotton fabrics coated with an insitu polymerised PANI salt. They observed significant reduction of the colony forming units(CFU) of gram-positive Staphylococcus aureus (S.aureus,95%), gram-negative Escherichia coli (E.coli,85%)and Candida albicans fungi(92%), which was explained by the activity of ions contained in PANI against the bacterial cell-wall. The following study reported total reduction of E.coli and S.aureus after 24h of incubation on composite PANI films. In this case, possible explanation of the observed antibacterial effect was based on the FTIR measurements revealing the possibility of the change in molecular structure of the PANI composite after its interaction with the bacterial species. These papers offered also two different explanations of the mechanism of the PANI antibacterial effect, namely possible reaction of acidic dopants on the polymer chains with the bacteria and electrostatic adherence between the PANI macromolecules and the bacteria. Antibacterial properties of functionalised and standard PANIs were subject of interest in the thorough work. Though all tested substances showed antibacterial activity, functionalised polymers were more efficient compared to the standard ones. Not only the PANI polymer alone but also its acetone extracted oligomers were reported to possess antibacterial properties . Also Humpo-liceketal. (2012) reported a notable cytotoxicity of the PANI emerlaldine salt on human cells. Though the last mentioned study was conducted using eukaryotic, not bacterial cells, it also confirms the negative influence of PANI on cellular viability.

In the present paper we prepared a material containing polyaniline and various conctration of calcium chloride and its microbial study against E. Coli were tested

Materials and Methods:

Aniline monomer was purified by distillation before use. Ammonium persulfate, and other chemicals were used as received.

Polymerization: 0.2M aniline and specific molar concentrations of calcium chloride were mixed with stirring at room temperature for 30 min. The stirring was then stopped, 50ml aqueous solution of 0.2M APS was added and the reaction was left for 12 hrs. The resulting PANI precipitate was washed with deionized water, methanol and ether several times. Finally the product was dried in vacuum at 80^0C temperature for 24 hrs.

Antibacterial Study:

Prior to testing, the samples were disinfected by an exposure to an UV-radiation source (258nm) emitted from a low-pressure Hg lamp UV-C Long Life 30W/G30TB, Phillips, The Netherlands).Polyaniline is stable under such treatment. As model microorganisms, the gram-negative E.coli strains were used. The test was performed according to ISO22196 with a modification. Nutrient broth with 1%peptone (M244) and nutrient agar No. 2(M1269) were used in the test (HiMedia Laboratories, India).

Results and Discussion:

Although PANI is considered as a promising conducting polymer for the application in biomedicine or more generally in biotechnology, only a few studies dealing with the antibacterial properties of this polymer have been published. Moreover, the majority of the studies is focused on the conducting PANI salt and only limited attention has been devoted to non-conducting PANI base. The published papers dealing with antibacterial activity of PANI and PANI based composites can be, for the purpose of the present study, divided into two groups: those concerning the activity of bare PANI and those concerning the properties of PANI containing calcium chloride. In the current study, calcium chloride are impregranated on the PANI surface by the immersion of naked PANI in a calcium chloride solution. The Electric conductivity behavior of calcium chloride impregranted PANI is shown in figure 1.

The results shows that, as the concentration was increases the electrical conductivity was increases up to the concentration of 0.4 mol. Further increase in the concnetraton no change of electrical conductivity observed.

The key problem in the evaluation of the studies dealing with the antibacterial effect of PANI combined with calcium chloride is their inconsistency in terms of different methods of PANI preparation, results evaluation or the lack of information about the concentration and form of Ag. A pioneering study published by Prabhakar et al[10] presented the reduction of biofilm formation (P. aeruginosa and B. subtilis) on polyurethane coated with a PANI–Ag nanoparticle composite. Tamboli et al[11] found that in situ polymerised powder of PANI–Ag possesses antibacterial activity against B. subtilis superior to that of Ag nanoparticles as such. Using the disc diffusion method, which cannot be directly correlated with our test procedure, the minimum bactericidal concentration of PANI–calcium chloride was determined to be 68 μg mL−1. An improvement of the antibacterial activity of PANI after the calcium chloride impregranation onto the polymer surface was observed also in the current work. The results were represented in table 1.

In conclusion the PANI –calcium chloride material prepared by chemical method has a potential materials towards E. Coli and will be a material for the various biomadeical applications.

PANI type	Antibacterial activity(CFU)
Pure PANI	20
Pure PANI+ 0.1 $CaCl_2$	10
Pure PANI+ 0.2 $CaCl_2$	6
Pure PANI+ 0.3 $CaCl_2$	0
Pure PANI+ 0.4 $CaCl_2$	0
Pure PANI+ 0.5 $CaCl_2$	0
Pure PANI+ 0.6 $CaCl_2$	0
Pure PANI+ 0.7 $CaCl_2$	0
Pure PANI+ 0.8 $CaCl_2$	0
Pure PANI+ 0.9 $CaCl_2$	0
Pure PANI+ 1.0 $CaCl_2$	0

Table.1- Antibacterial properties of PANI-calcium chloride material (CFU: Colony

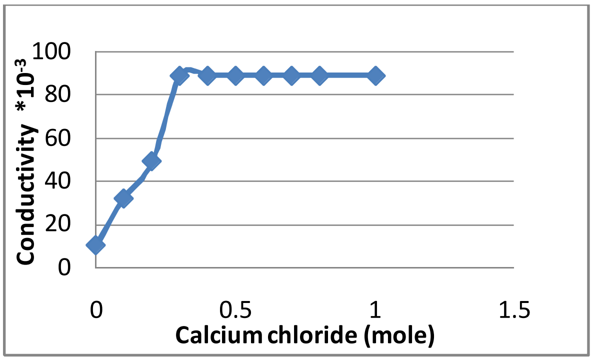

Figure 1: Effect of Calcium chloride concentration on electrical conductivity of PANI Forming Units)

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