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Functionalized polymeric magnetic nanoconstructs for selective capturing and sensitive detection of *Salmonella typhimurium*

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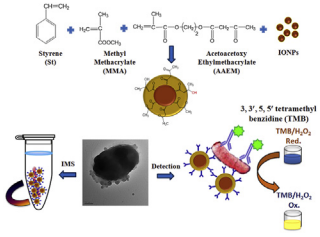
HIGHLIGHTS

- In-situ synthesis of novel functionalized polymeric magnetic nanoconstructs (FPMNCs).
- Covalent attachment of antibodies through surface acetoxy groups of nanoconstructs.
- FPMNCs-Ab can quickly sequester the targeted bacteria.
- FPMNCs-Ab also selectively capture and detect *Salmonella typhimurium.*
- FPMNCs based immunoassay is sensitive and specific and can be used in the field conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

Rapid detection and enumeration of pathogens is essential for monitoring contamination and spoilage of food products to ensure improved quality control management. Functionalized polymeric magnetic nanoconstructs (FPMNCs) were developed as an effective immunomagnetic separator and sensing platform for the selective capturing of *Salmonella typhimurium*. Novel FPMNCs were prepared in three stages involving synthesis of iron oxide (IO) dispersion, capping with sodium oleate and encapsulation of preformed IO nanoparticles by in-situ free radical emulsion polymerization of styrene (St), methyl methacrylate (MMA) and acetoacetoxy ethylmethacrylate (AAEM). PMMA improves the stability of FPMNCs by bridging extremely hydrophobic PS and hydrophilic PAAEM. Core-shell morphology of hydrophobic core of IO, PS & PMMA and hydrophilic shell of PAAEM was demonstrated by SEM, TEM and FTIR studies. FPMNCs with surface functionalized acetoacetoxy groups were covalently attached with polyclonal antibodies against *Salmonella* common structural antigen (CSA-1-Ab) without using any linker and catalyst. Colorimetric readout signal was acquired using CSA-1-Ab-HRP as secondary antibody after formation of sandwich immunocomplex with bacteria where the optical density of the samples were recorded using ELISA plate reader at 450 nm. The developed immunoassay was specific and selective which captures only targeted *S. typhimurium* with a detection limit of 10 cells/mL lower than infectious dose of salmonellosis infection. Minimal interference of food matrix with high signal to noise ratio was

Abbreviations: FPMNCs, Functionalized polymeric magnetic nanoconstructs; IO, Iron oxide; NPs, Nanoparticles; IMS, Immunomagnetic separation; Ab, Antibody; CSA-1-Ab, Antibody against common structural antigen of *Salmonella*.

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shown by various food samples. In addition, the performance of developed FPMNC based immunoassay was superior to commercially available immunomagnetic microbeads demonstrating undisputed advantage for capturing and detecting specific bacteria without any pre-enrichment of sample.

1. Introduction

Microbial contamination of food products is a grave predicament posing significant threat to public health as well as unbalancing socio-economic structure of nations, lost productivity, lawsuits and compromise branding of manufacturing or processing companies. *Salmonella*, a globally distributed food-borne pathogen, is often associated with raw, under cooked meat & chicken food products, unwashed fruits and vegetables. *Salmonella typhimurium*, gram negative rod like bacteria, are the most common serotypes of *Salmonella* spp. It is the predominant cause of human gastroenteritis, a major source of mortality and morbidity worldwide infecting adult and infant populations alike. 80.3 million cases of food borne illness are globally estimated accounting for nearly 1.55 million deaths globally [1]. Symptoms of non-typhoidal salmonellosis or gastroenteritis usually appear 12–72 h after consumption of contaminated food necessitating determination, identification and quantification of bacteria. Conventional method of plating is considered time consuming as it involves generic and selective enrichment processes, various biochemical as well as serological analysis for the identification of bacterial serotypes. These limitations necessitate development of new detection methods and procedures benefiting food and beverage industries and eventually consumers [2].

Immunomagnetic separation (IMS) techniques precede many routine and specialized sensing methods to overcome some of the challenges posed by complex nature of food while offering simplistic and sensitive approach of specific binding of antibody-antigen (Ab-Ag). Highly heterogeneous food matrices consist of various components including particulate matter, biochemical and inorganic food constituents, fats and non-target background flora. IMS process encompasses antibody coated magnetic beads which separate and enrich target analyte from solution without any culturing process. It offers added advantage of preserving microbial viability while eliminating effect of sample matrix. Magnetic particles of micro to nanometer scale have been widely studied for the detection, concentration, separation, purification and identification of bacteria. Over the past few years magnetic nanoparticles (NPs) have garnered considerable attention for IMS technique as small sized particles allow effective Ab-Ag interaction enabling improved accessibility to targets without strong mechanical agitation [3–6]. NPs on the other hand can be easily functionalized to generate surface groups to successfully attach specific antibodies and other biomolecules [7,8]. Chockalingam et al. [9] have functionalized magnetic NPs by gum arabic to crosslink with collagen on the surface of *Staphylococcus aureus*. Several functional polysaccharides, polymers and silane coated magnetic NPs have been synthesized to prevent self-oxidation of iron oxide core which results in their clustering [10]. However, in most of these reports further functionalization of iron oxide is pre-requisite for linking biomolecules. Protein A-conjugated iron oxide nanoparticles for separation of *Vibrio cholerae* from water samples was reported, where chitosan coated iron oxide NPs required additional functionalization with Protein A [11]. Wan et al. [12] have designed quaternized magnetic NPs-fluorescent polymer system for identification of different water related pathogens. Post separation cell-NP complexes are coupled with detection systems enabling readout signal. Fluorescence spectroscopy [13], surface enhanced Raman spectroscopy [14,15], flow cytometry and microfluidics based analysis have been employed for detecting bacterial antigen. However, these methods suffer from drawbacks of requiring sophisticated series of assays, are technically complex, cost intensive, need large analyzing equipment and skilled personals and are not operable in rural settings. Colorimetric methods relying on accuracy, precision, cost-effectiveness and simple management of enzyme linked immunosorbent assays (ELISAs) are a suitable alternate to complicated systems [16].

Pitch et al. [17] have studied the synthesis of PS/AAEM magnetic nanoparticles for the immobilization of enzyme *Laccase* but no report, using acetoxy functionality for the detection of pathogen, is available till today. Moreover, we observed that poly(St-AAEM) based system has poor dispersion and storage stability. In the present work we report the sensitive detection of bacteria using acetoacetoxy functionalized magnetic nanoconstructs (FPMNCs). Combination of three different monomers (St, MMA and AAEM) of gradual hydrophobicity to hydrophilicity were chosen in order to address the dispersion and long term storage stability of the developed FPMNCs. Immunoassay parameters were investigated along with efficient antibody coupling and effective immunomagnetic separation of bacteria by nanoconstructs. We further evaluated the developed system with various real food samples spiked with different concentrations of *S. typhimurium*.

2. Experimental

2.1. Materials

Analytical grades of ferrous chloride ($FeCl_2 \cdot 4H_2O$), ferric chloride ($FeCl_3 \cdot 6H_2O$), ammonium hydroxide (25%), skimmed milk, ammonium persulphate (APS), sodium peroxydisulphate (SPDS), styrene (St) & methyl methacrylate (MMA) were purchased from CDH Chemicals (Mumbai, India). Sodium oleate (99% purity) and acetoacetoxy ethylmethacrylate (AAEM) were from Sigma Aldrich (NJ, USA). Anti-*Salmonella* antibody coated magnetic microbeads (Dynabeads® anti-*Salmonella)* were purchased from Invitrogen Inc. (New Delhi, India). Styrene was purified by conventional method using (0.1 M) sodium hydroxide to remove hydroquinone.

Heat killed bacteria *Salmonella typhimurium* (10^9 CFU/mL), affinity purified polyclonal antibody (Ab) against common structural antigen of *Salmonella* (1 mg/mL) and horse raddish peroxidase labeled antibody conjugates (Ab-HRP, 0.1 mg/mL with 4:1 M HRP to Ab ratio) were obtained from Kirkegaard-Perry Laboratories (KPL), Inc. (Gaithersburg, USA). ELISA substrate 3, 3′, 5, 5′tetramethyl benzidine (TMB) and Pierce® BCA Protein Assay kit were obtained from Bangalore Genie (Bangalore, India) and Thermo Scientific (Mumbai, India) respectively. Different cross-reactants of *Salmonella typhimurium* including *Pseudomonas aeruginosa*, *Shigella sonnei*, *Escheria coli* and *Staphylococcus aureus* were obtained from Religare Ranbaxy Pharmaceuticals (Gurgaon, India). The bacteria were isolated under strictly maintained conditions and used after proper heat inactivation. The detailed method is as follows: 100 μL of bacterial solution were platted on sterilized agar plate and laid