

# Designing a Molecularly Imprinted Polymer-Based Nanomembrane for the Selective Removal of *Staphylococcus aureus* from Aqueous Media

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## Abstract

**Background and Objective:** Conventional applied techniques used for the detection of pathogenic microorganisms that are generally based on plating, serological and biochemical assays are unreliable and expensive while lacking sensitivity and specificity, compared to novel analytical methods. Investigation of reliable, rapid, analytical diagnosis methods seems necessary nowadays. In the present study, a highly accurate method was developed aiming to pre-concentrate and improve identification of *Staphylococcus aureus* as a major bacterial human pathogen using molecular imprinted polymer based membrane.

**Material and Methods:** Cellulose acetate was used as the basic membrane with methacrylic acid as the functional monomer, ethylene glycol dimethacrylate as the cross-linking monomer, antibody buffer media as the template molecule and 2, 2'-Azobis (2-methylpropionitrile) as the initiator agent. After selecting the best membrane composition based on the optimum ratio of antibody to imprinted monomer, electron microscopy was used to assess characterization and stabilization of the molecular imprinting of the templates on the membrane.

**Results and Conclusion:** Based on the results, suspension of *Staphylococcus aureus* with a dilution of  $3 \times 10^5$  included the highest bacterial mass absorption in no. 4 membrane filters, decreasing to  $1.3 \times 10^4$  after exposing to molecular imprinted polymer modified membranes. The manufactured nanomembrane could significantly be developed in quality control of food industries, compared to traditional methods due to the shorter necessary times of bacterial mass diagnosis with higher accuracies.

**Conflict of interest:** The authors declare no conflict of interest.

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## 1. Introduction

Pathogenic bacterial detection is definitely important in various aspects, including food hygiene and medicine [1]. Rapid, portable and reliable analytical diagnosis methods are critical in fast, timely accurate detection [2]. Common methods used for the detection of pathogenic bacteria mostly include culturing, serological and immunological assays as well as biochemical and genotypic analyses [1-3]. These methods include problems since they are usually difficult, expensive and complex. Furthermore, they lack the necessary sensitivity and specificity, compared to analytical methods, which are cost-effective and rapid [1,4-6]. Molecular imprinting polymer (MIP) is a potent method to provide synthetic diagnostic sites for various materials. In

this method, a monomer molecule with appropriate functional groups is first interacted with the template molecule and then the radical initiator agent is added to the solution to form the polymer. Eventually, removal of template molecule from the polymeric grid occurs using appropriate solvents. Then, the resulting template is emptied, which is quite appropriate for the target molecule in shapes and sites and can be used for the next isolation of template molecules and thus used instead of biological materials [7, 8].

Many research have been carried out on MIP nanomaterial composites in the last decades, covering various uses [9-19]. MansouriBoroujeni [20] developed a

protocol for polymers, which could detect *Saccharomyces* (*S.*) *cerevisiae* specifically, analyzing various strains of methicillin resistant *S. aureus* and *Escherichia* (*E.*) *coli*. Okan et al. [21] developed a MIP, which was based on micromechanical cantilever sensor systems and included high specificity, rapid response time (2 min) and easy use (reusable up to 4 times) to detect ciprofloxacin molecules in water. Zheng et al. [22] used an electrodeposition MIP on nanoscale multilayer modified electrodes to design electrochemical sensors to detect 4-nonylphenol in milk packing samples. The developed imprinted electrochemical sensor exhibited higher sensitivity and better selectivity, compared to conventional sensors. As recent studies showed, chemical recognition (e.g. manifold interactions of non-covalent nature between the MIP and the cell membrane) and physical recognition (e.g. shape and size-dependency) are greatly important for the bacterial detection [23]. Studies demonstrated that use of appropriate functional groups or monomers to reach desired chemical recognition between the MIP and the cell membrane was a crucial step for bacteria-imprinting. Considering importance of chemical recognition in bacteria recognition, selective functional polymers and monomers are mostly interested.

The MIP matrices, which are most commonly used to imprint bacteria, include polydimethylsiloxane, polyurethane, silica, polyacrylate and other crosslinked polymers. Since imprinting processes were carried out using combination of pre-polymers or monomers with specific functional groups such as polyamine, polyvinylpyrrolidone, methacrylic acid and dimethylamino ethyl methacrylate, it is noteworthy to achieve enhancements in the affinity of bacteria [23]. In fact, *S. aureus* is known as the agent of severe intoxications, including serious threats to public health [24-26]. In a study by Ahari et al. [27], *S. aureus* exotoxin was detected by a potentiometric MIP method; in which, designed biosensor in the molecular framework polymer was successfully capable of detecting exotoxins in densities up to  $10^{-3}$  M at 68 nm of the synthesized MIP during the first 32 days of the experiment. Potential differences did not alter and were constant at an optimum pH range of 5.0-8.5 and at an optimum temperature range of 15°C-25°C. Based on their results, pH and temperature could affect the precision of a potentiometric nanobiosensor for the detection of *S. aureus* exotoxin. In another recent study, a sensitive method for the isolation and detection of *S. aureus* was used using non-covalent molecular imprinting technique and dopamine as functional monomer. In their study, a layer of MIP was created on the surface of magnetic particles that were used due to their ability to pre-concentrate bacteria from large sample volumes; thus, resulting extremely sensitive detection rates. The researchers compared sensitivities of the two detection methods, including fluorescence microscopy and capillary electrophoresis, with that of laser-induced fluorescence

(CE-LIF) and found that the second method was more sensitive to detect the isolated bacteria [28]. The aim of the present study was to design a molecularly imprinted polymer-based nanomembrane for the selective removal of *S. aureus* from aqueous media.

### 1.1 The innovation of the study

A very few studies are available on the use of MIP filters and food microbiology; of which, the two highlighted studies previously addressed used the MIP method. In the current innovative study, MIP filters were used in food microbiology for the first time.

## 2. Materials and Methods

### 2.1 Materials

*Staphylococcus aureus* PTCC 1431 was provided by the Iranian Research Organization for Science and Research, Iran. Cellulose acetate membrane disc filters with mesh sizes of 1.2  $\mu\text{m}$  were purchased from Sartorius, Switzerland; *S. aureus* antibody from Abcam, USA; methacrylic acid from Sigma-Aldrich, USA; and ethylene glycol dimethacrylic acid, azobisisobutyronitrile, acetic acid, methanol, phosphate-buffered saline (PBS) solution (pH 6.9) from Merck, Germany. Baird-Parker Agar (BPA) culture medium was supplied by Hardy Diagnostics, USA. All chemicals used in this study included analytical grade and Milli-Q water (Le Montsur-Lausanne, Switzerland) was used to prepare solutions [23-26].

### 2.2. Preparation of the microbial cultures

First, *Staphylococcus aureus* PTCC 1431 was transferred to lactose broth and incubated at 37 °C overnight. Then, bacteria were inoculated into BPA containing potassium tellurite, egg and egg yolk and stored at 37 °C overnight for up to half its primary McFarland concentration using spectrophotometer (Cecil Instruments, UK), repressing  $10^8$  cell  $\text{ml}^{-1}$ . Liquid medium containing the bacterial mass was centrifuged at  $1220 \times g$  for 9 min. After discarding the supernatant, bacterial mass was rinsed with physiological serum and stirred well. Washing was carried out trice and then the mass was transferred into a warm water bath (36 °C) for 20 min to deactivate capsules and avoid possible interferences in recognition [23,29-32].

### 2.3. Preparation of the microbial concentrations

For the preparation of staphylococcal suspension/culture density, three various suspensions were prepared using spectrophotometer. Tubes containing the bacterial mass were vortexed and the measurement was carried out at 620 nm. A suspension of  $10^5$  cell  $\text{ml}^{-1}$  was used as the pathogenic dose for the assessment using MIP membrane [17].

### 2.4. Preparation of the molecular imprinted polymer-based membranes



In the present study, cellulose acetate membranes with a pore size of 1.2 and a diameter of 47 cm were provided by Macherey-Nagel, Germany, and used as the base of the membrane filters. Cellulose acetate was cut to the proper size and rinsed with deionized water. Then, it was transferred into a medium containing methacrylic acid as functional monomer, ethylene glycol dimethacrylate as cross-linking monomer, antibody buffer medium as template molecule and 2,2'-Azobis(2-methylpropionitrile) as initiator agent. By preparing a combination of various ratios of the functional monomers (e.g. methacrylic acid to bacterial antibody), ratio with the best function was selected as the best synthetic template for the entrapment of antibody in further assessments. In this study, two sets of MIP (coated with antibody) and NIP (molecularly non-imprinted polymer, no coating) membrane filters were synthesized based on various dilutions of antibody. The NIP particles were prepared and rinsed using a similar procedure with no template to use as control for the assessment of nonspecific adsorptions. Solutions were transferred into a sonicator (Fritsch, Germany) for 10 min at 25 °C and soluble gases were removed. Each solution was transferred into the nitrogen gas injection chamber (Roham Gas, N<sub>2</sub>, 99.999%) for 3 min and then exposed to UV using UV cabinet (CAMAG, Germany) for the start of radical reaction. Irradiation time was 20 h at 254 nm. Radiation initiated the radical reaction in presence of azobis isobutyronitrile initiator and the polymer particles were formed on cellulose acetate fibers [12,20]. To prepare MIPs, type of the template is highly important since the molecular detection depends on the extent of interactions that occur between the template and the functional monomer to form a stable complex and create applicable connection sites in the structure of molecular imprinted polymers. The greater the freedom of movement of the molecular structures (branches and connected functional groups), the less likely the coherent molecular template sites to form. In addition, the template molecule must be conformed to be able to polymerize free radicals, not participating in polymerization reactions. Cellulose acetate membrane was used in several studies, showing good imprinting functionality. In a recent report, a straightforward fast extraction method was developed based on MIPs photo grafted into the cellulose acetate membranes as selective recognition sites for improving control of melamine in dry milk samples [29,30]. Table 1 shows proportion of the components using various combination ratios of the functional monomer (MAA) to microbial antibody (e.g. 2:1, 3:1, 4:1, 6:1 and 8:1) to produce the best synthetic template as model for a better detection of *S. aureus*. It is described that the molar ratio of the functional monomer to template is greatly important and plays significant roles in specific affinity and number of MIP recognition sites in the membrane. High ratios of the functional monomer to template result in high nonspecific

affinity, while low ratios produce fewer complexations due to insufficient functional groups [31]. In this study, quantity of EGDMA and AIBN were considered based on the similar studies [29,30, 32].

**Table 1.** Composition of the membrane filters specified for *Staphylococcus aureus*

Polymer	Methacrylic acid (MAA) $\mu$ l	Ethylene glycol dimethacrylate (EGDMA) $\mu$ l	2,2'-Azobis(2-methylpropionitrile) (AIBN) mg
MIP* <sub>1</sub>	86	260	3.219
MIP <sub>2</sub>	129	260	3.219
MP <sub>3</sub>	172	260	3.219
MIP <sub>4</sub>	258	260	3.219
MIP <sub>5</sub>	344	260	3.219
NIP* <sub>1</sub>	86	260	3.219
NIP <sub>2</sub>	129	260	3.219
NIP <sub>3</sub>	172	260	3.219
NIP <sub>4</sub>	258	260	3.219
NIP <sub>5</sub>	344	260	3.219

\*MIP: Imprinted polymer, \*NIP non imprinted polymer

## 2.5. Calculation of the nanomembrane efficiency

The formation efficiency of MIPs on cellulose acetate membrane fibers was assessed using antibody assay. For each membrane, certain doses of the antibody were passed through the membrane using Millipore filtration set equipped with vacuum funnel (with a certain pressure). Entrapment of antibody was carried out followed by its release from the membrane using diluted acetic acid media, which included a 95:5 ratio of acetic acid to methanol. Optimizing pH and choosing the best buffer (normally phosphate and acetate buffers) media for washing of the composite membrane and removing the antibody are greatly important. In this study, a pH range of 5.5-8.5 was selected and measured using pH-meter (GLP, GLP 21, Crison, Barcelona) based on the literature [27, 30]. At each stage, the antibody washing solution was assessed by the detection kit and the highest antibody recovery rate was reported as optimal pH and buffer. The proportion of recovery could be calculated using the following equation:

$$\text{Recovery percentage} = \frac{a-b}{c} \quad \text{Eq. 1}$$

Where,  $a$  was the initial concentration of antibody in contact with the membrane,  $b$  was the concentration of antibody in the washing solution in contact with the membrane and  $c$  was the initial concentration of antibody.

## 2.6. Characterization of the nanomembranes

### 2.6.1. Infrared spectroscopy

Infrared spectroscopy (FTIR) spectra of the optimized MIP and NIP membranes were recorded using Shimadzu FTIR 4300 Spectrometer (Shimadzu, Japan) in the range of 400–4000 cm<sup>-1</sup> through preparing KBr disks from MIP and NIP membranes, respectively. [20-24]

### 2.6.2. Scanning electron microscopy

The membrane samples were first prepared through immersing in acetonitrile solvent and pressing between two

glass plates to remove the solvent and exposing to nitrogen gas to evaporate the solvent remainder. Then, samples were transferred into a sputter coater device (Philips, the Netherlands) containing argon gas to stabilize the gold-water coating. The gold-coated samples were transferred to the electron microscope chamber after 10 min and images were recorded at  $1.5 \times 10^3$  magnification [20,23,24].

## 2.7. Membrane lifetime

Lifetime of the designed membrane over a two-month period was assessed. This value shows the duration; through which, the polymer-based nanoparticle membrane can preserve its molecular shape for trapping bacteria. In other words, lifetime is the duration that the membrane is capable of doing mass detection with conventional systems. The assay was carried out every 4 days for 2 months. Results were plotted by recording the signals [24,23].

## 2.8. Statistical analysis

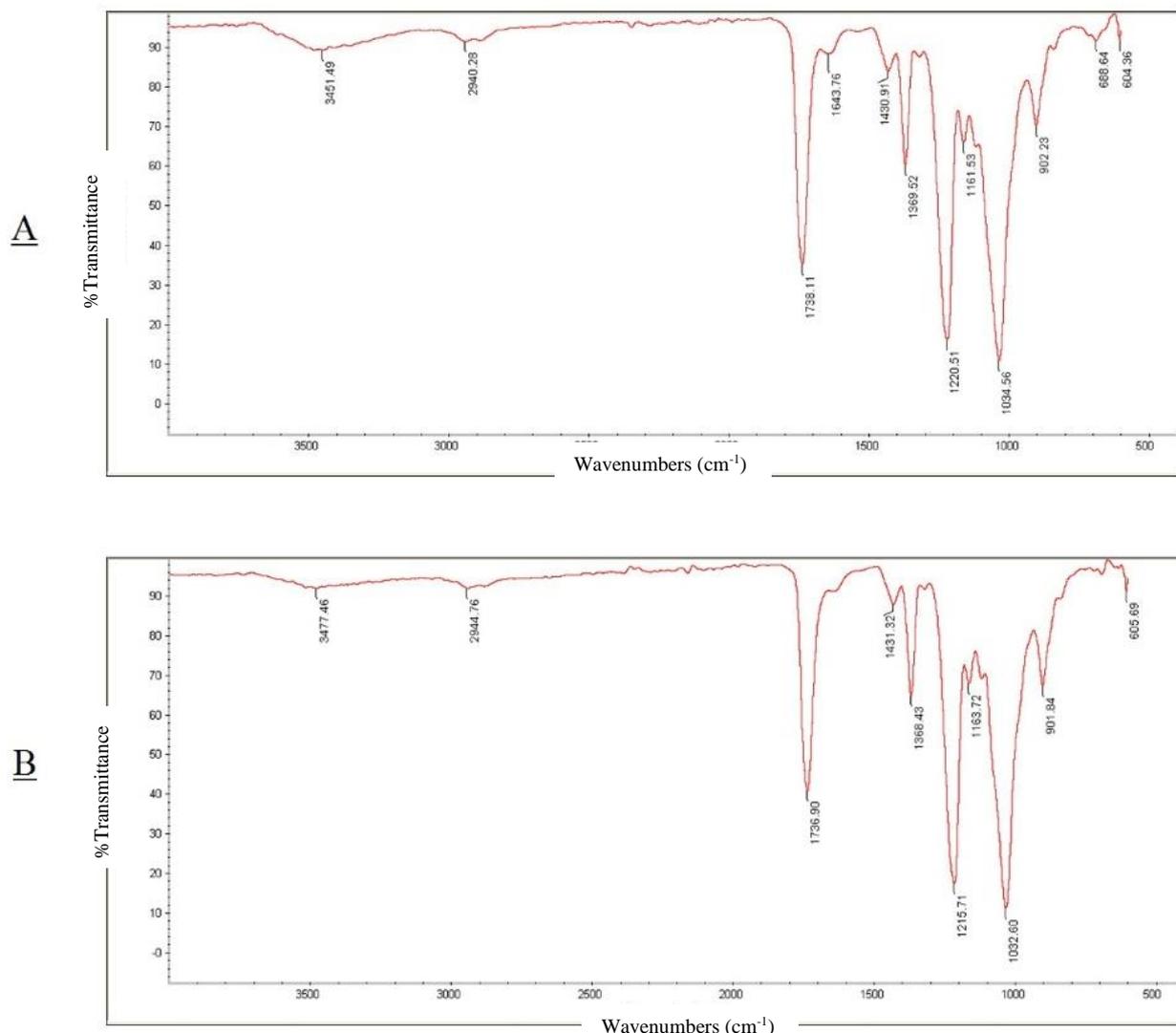
In this study, measurements were repeated three times and data were reported as mean  $\pm$ SD (standard deviation). Microsoft Excel 2013 (Microsoft, USA) was used for all analyses.

## 3. Results and Discussion

### 3.1. Characterization of the nanomembranes

#### 3.1.1. Infrared spectroscopy

Use of FTIR is essential to investigate molecular structure of cellulose acetate. The FTIR spectra of the pure cellulose acetate and modified membrane with antibody (*S. aureus*) are shown in Figure 1.

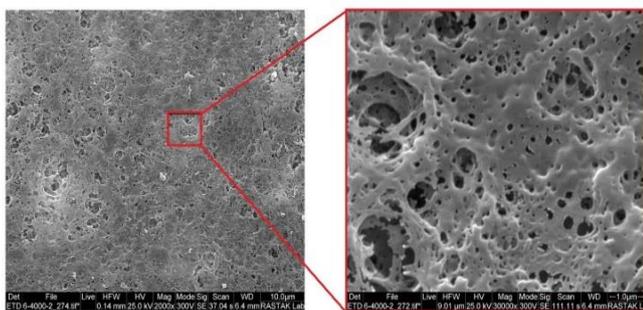


**Figure 1.** FTIR spectra of A: Blank cellulose acetate membrane B: Antibody imprinted cellulose acetate membrane

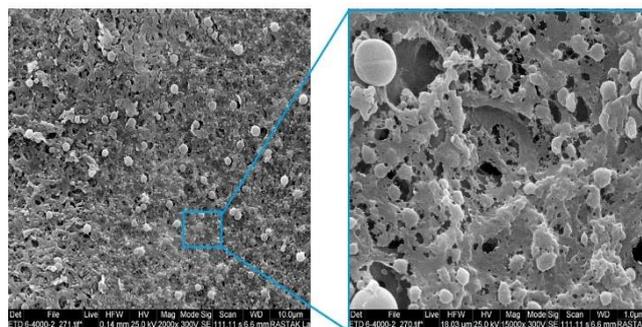
The major bands of cellulose acetate structure were assigned as follows: the –OH group of stretching and bending vibration included specific peaks at  $3451\text{ cm}^{-1}$  and  $1430\text{ cm}^{-1}$ . Furthermore, peak at  $1034\text{ cm}^{-1}$  of the C-O-C group in the ring, peak at  $1161\text{ cm}^{-1}$  of the C-O-C bridge group, peak at  $1369\text{ cm}^{-1}$  of the C-CH<sub>3</sub> bond of acetate group, peak at  $1735\text{ cm}^{-1}$  of the C=O carboxylic acid bond and peak at  $1215\text{ cm}^{-1}$  of the C-O bond of acetate group are shown in the figure. Peaks at  $2980$  and  $2940\text{ cm}^{-1}$  belonged to symmetric and asymmetric of the C-H bond from aliphatic CH<sub>2</sub>. Peaks at  $901$  and  $600\text{ cm}^{-1}$  belonged to the CH bond of ring and OH for out of plane, respectively. Comparison of spectroscopy of the two samples of pure cellulose acetate membrane and cellulose acetate membrane with antibody (*S. aureus*) showed that positions of the peaks were similar in the two spectra and the peak corresponding to the OH group in cellulose acetate membrane with antibody decreased by 5%, compared to that in pure cellulose acetate membrane did.

### 3.1.2. Scanning electron microscopy

Morphology of the cellulose acetate membrane was studied using scanning electron microscopy (SEM) in two steps with and without antibody (*S. aureus*). In general, the NIP included a regular smooth structure, whereas significant changes were seen in its morphology after membrane interaction and transplantation using *S. aureus*. The antibody agents (*S. aureus*) were introduced into the cellulose acetate membrane network as small white spots of approximately uniform sizes. Experiment conditions such as the quantity of the materials and gentle mixing during polymerization resulted in a uniform antibody distribution. Surface of the cellulose acetate membrane with antibody agent was different from that of cellulose acetate membrane due to the presence of *S. aureus* in polymeric cellulose acetate tissue. As shown in Figures 2 and 3, the cellulose acetate membrane held *S. aureus* well. Good dispersion of *S. aureus* in the membrane increased the free volume. Free volume refers to the area of polymer tissue that lacks polymer chains. Addition of antibody led to changes in the tissue structure and surface, which apparently became rough while the modified membrane was flexible.



**Figure 2.** Scanning Electron Microscopy Images of Cellulose Acetate Membrane



**Figure 3.** Scanning electron microscopy images of cellulose acetate membrane containing antibody

### 3.2. Efficiency of the molecular imprinted polymer-based membranes

To assess formation efficiency of the MIPs on cellulose acetate membrane fibers, an antibody assay was carried out; in which, Millipore filtration set equipped with vacuum funnel was used for each membrane. After entrapment, antibody was released from the membrane using diluted acetic acid. In this process, filtration was not possible without the use of vacuum funnel and thus a fixed pressure of 25 mm Hg was used for all the samples. The membrane could act as an antibody-based cavity and hence be capable of antigen-antibody binding. Therefore, various dilution ratios of *S. aureus* were filtrated through the membrane with a  $0.5\text{ ml min}^{-1}$  flow rate in an aqueous medium in this phase (Table 2).

**Table 2.** Experimental data for the filtration of various dilution ratios of *S. aureus* using the synthesized membrane

Run	Initial concentration	Reduction level	% Efficacy
1	$3 \times 10^5\text{ CFU ml}^{-1}$	$1.3 \times 10^4\text{ CFU ml}^{-1}$	4.5
2	$10^5\text{ CFU ml}^{-1}$	$5 \times 10^4\text{ CFU ml}^{-1}$	50
3	$10^4\text{ CFU ml}^{-1}$	$8 \times 10^3\text{ CFU ml}^{-1}$	80
4	$10^3\text{ CFU ml}^{-1}$	$9.9 \times 10^2\text{ CFU ml}^{-1}$	99
5	$10^2\text{ CFU ml}^{-1}$	$10^2\text{ CFU ml}^{-1}$	100

**Table 3.** The count of *S. aureus* after filtration by molecular imprinted and non-imprinted polymer membranes

Filter no.	Count ( <i>S. aureus</i> ) Baird Parker Agar	Filtration time (Minutes)
NIP <sub>1</sub>	$1.5 \times 10^5 \pm 0.1 \times 10^5$	07:00
NIP <sub>2</sub>	$1.2 \times 10^5 \pm 0.2 \times 10^5$	07:50
NIP <sub>3</sub>	$2.4 \times 10^5 \pm 0.2 \times 10^5$	04:00
NIP <sub>4</sub>	$1.3 \times 10^5 \pm 0.2 \times 10^5$	07:26
NIP <sub>5</sub>	$2.5 \times 10^5 \pm 0.2 \times 10^5$	08:10
MIP <sub>1</sub>	$8.0 \times 10^4 \pm 0.2 \times 10^5$	06:45
MIP <sub>2</sub>	$1.5 \times 10^5 \pm 0.2 \times 10^5$	04:46
MIP <sub>3</sub>	$4.3 \times 10^4 \pm 0.1 \times 10^5$	13:55
MIP <sub>4</sub>	$1.3 \times 10^4 \pm 0.2 \times 10^5$	03:49
MIP <sub>5</sub>	$4.6 \times 10^4 \pm 0.1 \times 10^6$	05:17
Blank	$2.1 \times 10^4 \pm 0.2 \times 10^5$	01:24

Initial concentration of suspension solution was  $3 \times 10^5$ . Data were written as mean  $\pm$  standard deviation ( $n = 3$ ).

The maximum and the minimum concentrations that could decrease its concentration effectively were  $3 \times 10^5$  and  $10^3$  CFU ml<sup>-1</sup>, respectively. These dilutions were used at a pathogenic dose range and the desorbed bacteria to the membrane in an acetate buffer solution were investigated [9]. Table 3 shows data recorded after carrying out the antibody assay.

Volume of the *S. aureus* solution and pressure of the vacuum funnel were respectively fixed at 15 ml and 25 mm Hg for all the samples. As a result, membrane filter no. 4 (MIP<sub>4</sub>) showed the best capacity to bind with the antibody of *S. aureus* and therefore was considered as the most usable membrane. Based on the results and Table 4, it can be claimed that this technique includes significant accuracy and sensitivity, compared to those the conventional methods do.

### 3.3. Membrane lifetime

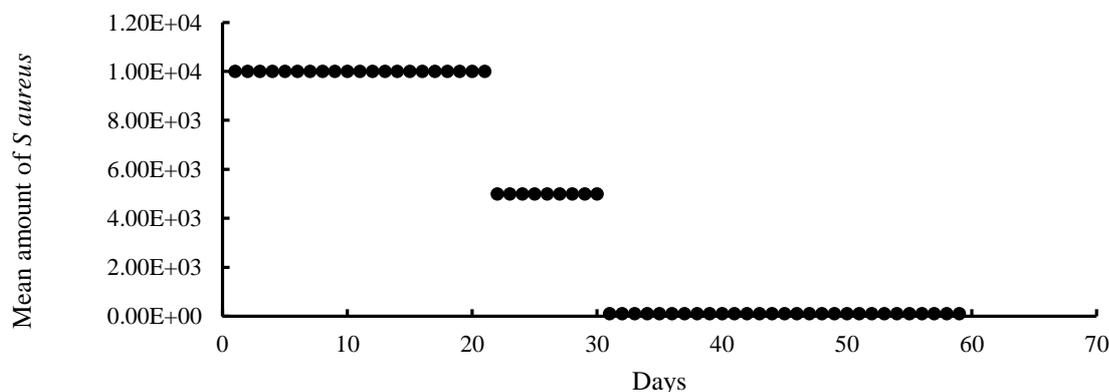
The biocompatible cellulose acetate modified membrane for the removal of *S. aureus* was assessed over two months and its efficiency was actively responsive until Day 21. Then, performance showed a gradual decline (Figure 4). Removal of pathogenic bacteria from the aqueous media is important.

Regarding various organic, biological and mineral constituents in aqueous media, selective removal of pathogenic bacteria from existing materials needs to design modern systems to preserve quality of the media. Design of cellulose acetate membrane using MIP method can be effective in ensuring health of the consumed water as well as selective removal of the pathogenic bacteria. Results of this study demonstrated that the polymer derived from methacrylic acid could effectively bind the *S. aureus* bacterial antibody template to the membrane tissue. Despite environmental instability of the antibody, it could be entrapped in the membrane. The duration of 21 days is valuable for *S. aureus* removal by the antibody-antigen mechanism. Zhou et al. created melamine imprinted polymers that showed high affinities to melamine and were

successfully used as solid phase extraction sorbents for the selective extraction of melamine from dairy products [17]. Zhang et al. [19] prepared cells, which were imprinted with artificial antibodies to produce a sandwich enzyme-linked immunosorbent assay (ELISA) for the detection of pathogenic microorganisms. The constructed ELISA was capable of target pathogen detection with high sensitivity and selectivity. Boroujeni et al. developed a protocol for polymers, which was capable of detecting *Saccaromyces cerevisiae* and analysis of various strains of *S. aureus* and *E. coli* [20]. However, the current study demonstrated successful production of these selective polymers in food microbiology and the membrane could act as an antibody-based cavity, capable of antigen-antibody binding to filtrate *S. aureus*.

## 4. Conclusion

Detecting very low concentrations of microorganisms in complex matrices may be difficult and challenging, especially when dealing with pathogens. As a result, higher sensitivity and selectivity of the assays are critical, which need innovative synthesize of superior MIPs for microorganisms with high specificity. In this study, the created nanomembrane was capable of removing *S. aureus* within a short time with relatively high sensitivity, which could be used for the improved inhibition of bacteria in biocompatible membranes. This could lead to a significant development in quality control of aqueous media in food industries, compared to traditional removal and detection methods. However, further studies are necessary for the accurate removal sensitivity assessment of the detection system. In fact, designing novel nanomembranes needs simulated aqueous media and sensitivity and specificity assessments using microbial dilutions. Therefore, further potential assessments of the MIP based nanomembranes in various food models are needed after detection and removal of interference factors in foods, including fats and proteins.



**Figure.4** Lifetime for CA molecularly imprinted membrane in removing *Staphylococcus aureus*



**Table 4.** Comparison of the common methods for the efficient selective removal of *Staphylococcus aureus* from the aqueous media

No.	Method	Efficiency	Sensitivity	Cost estimation	Ref.
1	Colony Counting and Culturing	gold standard	$10^{-10}$ CFU ml <sup>-1</sup>	It takes 24 -48 h energy	33
2	serological	The efficiency of serological diagnosis can be reduced by a variety of factors, including age and immunodeficiency. Serology is only useful for diagnosis when there is a clear relationship between antibody concentrations and infection. It is less useful for infections where antibodies may persist but do not provide protection against repeat infection or reactivation, such as herpes simplex, cytomegalovirus and varicella zoster virus, or for infections caused by commensal organisms.	$10^5$ CFU ml <sup>-1</sup>	It takes two weeks or more + expensive antibody	34
3	biochemical assays	It could not distinguish different strain	$10^{-13}$ CFU ml <sup>-1</sup>	The cost of materials varies depending on which molecule is selected for identification. However, identification is usually done within minutes to hours	35-38
4	MIP	Depending on the type of antibody used in the molecular format, only the specific strain of that antibody is detected. Here, only <i>Staphylococcus aureus</i> was detected	$10^3$ CFU ml <sup>-1</sup>	The process is done within 1 hour. So energy and personnel costs have dropped dramatically. The cost of the material is only related to the chemical, which is very small compared to the antibody	this article



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## 6. Conflict of Interest

The authors report no conflicts of interest.

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## طراحی نانوغشایی بر پایه پلیمر قالب مولکولی برای حذف انتخابی باکتری استافیلوکوکوس اورئوس از محیط مایع

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### چکیده

**سابقه و هدف:** روش‌های متداول تشخیص میکروارگانیسم‌های بیماری‌زا عموماً بر مبنای روش‌های کشت بر روی پلیت، سرولوژیکی و بیوشیمیایی هستند، که غیرقابل اعتماد و علی‌رغم گران بودن نسبت به روش‌های جدید، غیراختصاصی و غیرحساس می‌باشند. امروزه، تحقیق و بررسی روش‌های تشخیصی سریع و قابل اعتماد، یک ضرورت محسوب می‌شوند. در مطالعه حاضر، به منظور پیش‌تغلیظ و بهبود شناسایی استافیلوکوکوس اورئوس، میکروارگانیسم باکتریایی بیماری‌زا، روشی بسیار دقیق با استفاده از پلیمر قالب مولکولی (MIP) مورد بررسی قرار گرفت.

**مواد و روش‌ها:** استات سلولز به عنوان غشای پایه، متاکریلیک اسید به عنوان مونومر عملکردی، اتیلن گلیکول دی-متیل آکریلات به عنوان مونومر پیوند عرضی، محیط بافر پادتن به عنوان مولکول الگو و ۲ و ۲ آزوبیس (۲-متیل پروپیونیتریل) به عنوان عامل آغازگر مورد استفاده قرار گرفتند. پس از انتخاب بهترین ترکیب غشا، براساس نسبت بهینه پادتن به مونومر نقش بسته<sup>۱</sup>، برای ارزیابی خصوصیات و پایداری نقش بسته مولکولی الگوها بر روی غشا، از میکروسکوپ الکترونی استفاده شد.

**یافته‌ها و نتیجه‌گیری:** براساس نتایج به دست آمده، سوسپانسیون استافیلوکوکوس اورئوس با رقت  $10^5 \times 3$  بیشترین جذب باکتریایی بر روی فیلتر غشایی شماره ۴ داشت و پس از تماس با غشاهای مولکولی اصلاح شده پلیمر نقش بسته بتا میزان  $10^4 \times 1/3$  کاهش یافت. در مقایسه با روش‌های متداول، نانوغشای تولیدی به علت مدت زمان کوتاهتر لازم برای تشخیص توده باکتریایی و با دقت بالاتر، می‌تواند به بهبود قابل توجهی در کنترل کیفیت صنایع غذایی منجر شود.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافی مرتبط با انتشار این مقاله ندارند.

<sup>۱</sup> imprinted monomer