CHAPTER 4

Molecular imprinting of *Staphylococcus aureus* in polyacrylamide gel slab for detecting the presence of the bacteria as a contaminant

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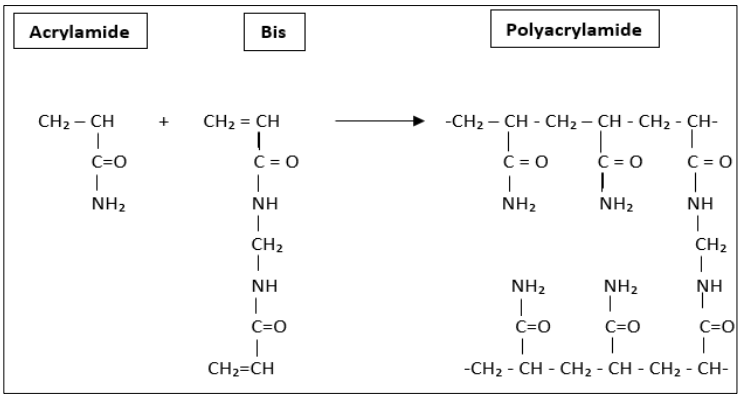
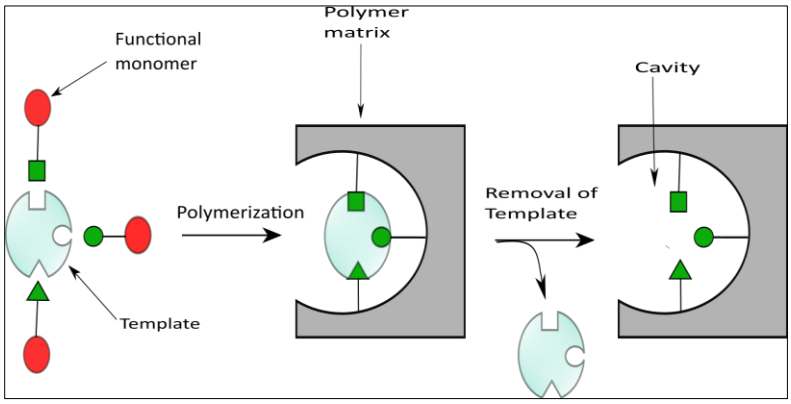
Research highlights:

1. Whole bacterial cells of *Staphylococcus aureus* were entrapped in 4%, 6% and 8% of polyacrylamide gel slabs (1×1×0.2 cm) for molecular imprinting.
2. Template *Staphylococcus aureus* bacteria were removed by ultrasonicating the gels for 9 hr. in 0.2N NaOH and 1% SDS solution to imprint.
3. All the three concentrations of the gels 4%, 6% and 8% were allowed to adsorb *Staphylococcus aureus* over a period of 180 min. Only 4% of the gel showed highest adsorption at 75 min of incubation for *Staphylococcus aureus*.
4. Recognition specificity of 4% imprinted gel was tested against three bacteria *Staphylococcus aureus*, *Salmonella typhi and Escherichia coli* at 75 min. of incubation. The 4% imprinted gel showed no recognition specificity by adsorbing 5.763×10^6 CFU/ml, 5.13×10^6 CFU/ml and 11.42×10^6 CFU/ml for *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* respectively.





4.1. Introduction:

From the past two decades, analytical chemistry has been focusing on the identification methods of complex organic and biological systems, laying much more emphasis towards organisms such as bacteria and viruses (Avnir, Lev, & Livage, 2006; Besanger & Brennan, 2006; Lukowiak & Strek, 2009). Traditional techniques for identification and detection are majorly antigen-antibody systems, colony count method followed by genotyping or polymerase chain reactions (PCR). Though these techniques have good specificity, process of fabrication or detection strategies are lengthy or complicated. Antibodies are fragile and susceptible to irreversible conformational changes. They tend to restrict the conditions of the system. For applying bacterial colony count method, culturing on specific media may require several hours. Though, several sophisticated methods have been tried to out, the issue of identification of selective species and its concentration determination still persists (Avnir et al., 2006; Besanger & Brennan, 2006). Owing to these reasons, efforts have been made to advance the synthetic materials for their specificity and selectivity. Such detection and removal methods are useful in many fields like food industry, environmental areas, microbiological areas, water supply etc. Molecular imprinting generates materials with specific molecular recognition ability using a target template molecule cross-linked with polymer around it (Mosbach, 1994). When the target is removed, imprint of the target molecule is left behind in cross linked polymer network. Usually, in literature specific protein is used as a template to imprint the polymer, but protein as a template also confronts many problems such as fragility of the protein and low mass transport ability. In case of large proteins, it is difficult to remove the imprinting molecule from the cross-linked polymer. Rebinding of the small proteins into the imprinted cavities itself is a major task to achieve. Proteins being fragile can also undergo denaturation during fabrication process and can lead to low recognition and high nonspecific adsorption (Avnir et al., 2006; Besanger & Brennan, 2006). Pang et al., in 2005 imprinted polyacrylamide gel using surface protein A of *Staphylococcus aureus* with low degree of cross-linking functional monomers (Pang, Cheng, Li, Lu, & Zhang, 2005). The gel allowed the proteins to pass in and out with good binding specificity but has not been applied to any practical uses yet (Pang et al., 2005). Though, several attempts for imprinting of polymers using whole bacteria, viruses and other cells as templates have been carried out but satisfactory results have not been achieved so far. The reasons behind less success of imprinting polymers with whole cells could be large molecular sizes, complexity and fragility of the molecules. Although,

several surface components such as peptides, proteins and other small organic molecules can be attributed for the specific recognition when whole cells are used as templates.

Previous studies have explored the use of cell surface components or proteins as templates to imprint the polymers. Cai *et al.,* in 2018 used an imprinting technique in which bacterial surface was complexed with exogenous target protein and imprinted silica films with exogenous protein (Cai, Li, Lu, & Collinson, 2018). Moreover, Xue *et al.,* in 2009 has imprinted polyacrylamide gel beads using Protein-A of *Staphylococcus aureus* as template. But the method could only be used as a technique for recognition (Xue, Pan, Xie, Wang, & Zhang, 2009). The bacteria are very common pathogens causing fever, headache, skin and joint infections, bloody diarrhoea and bacteremia leading to death. Further, the imprinted gel beads are difficult to handle while testing the samples due to their shape. The beads applied more for the separation or removal of the bacteria from the given sample. Instead, gel slabs of particular sizes in combination with spectrophotometry make it convenient to handle and test against the target bacteria when it comes to its practical application. This study focuses on the development of an optical biosensor for the detection of *Staphylococcus aureus* using whole cell molecularly imprinted polyacrylamide gel slabs (1×1×0.2 cm). The purpose of the present research study is to prepare polyacrylamide gel imprinted with surface features using whole bacterial cells, in the present case *Staphylococcus aureus*, so that it will be possible to detect *Staphylococcus aureus* specifically if they are present in any given sample. The basis for discriminating *S. aureus* from other bacteria is based on the surface morphology and spatial arrangements of surface proteins. To our knowledge, so far no polyacrylamide gel slabs (1×1×0.2 cm) imprinted with whole bacterial cells in combination with spectrophotometry has been reported for the recognition of *Staphylococcus aureus*. The study describes successful imprinting of the gel slabs with the bacteria followed by its characterization, adsorption and specificity tests.

4.2. Materials and methods:

4.2.1. Instruments:

Spectrophotometer (Agilent Technologies, Cary 60 UV-Vis), Ultrasonicator bath (Life-Care 3KHz), Laminar air flow, Incubator shaker (REMI, CIS-24 Plus), Centrifuge (Sigma), Bench top shaker (REMI, RM-12), SDS PAGE set up (Banglore Genie), pH meter (AnaLab).

4.2.2. Materials:

Staphylococcus aureus, *Salmonella typhi* and *Escherichia Coli* (DH5α) were used in the study. Acrylamide (AM) (A8887) and N-N- methylene Bis-acrylamide (Bis-AM) (2610-OP) were purchased from Sigma-Aldrich, India. Sodium dodecyl sulphate (SDS) (54468), Sodium hydroxide (NaOH) (1949181), Ethanol, Crystal violet (28376), Gram's iodine (10436), Safranin (16281), Acetone (66951), Disodium hydrogen phosphate (Na_2HPO_4) (59443) and Sodium dihydrogen phosphate (NaH_2PO_4) (22249) were purchased from Sisco research laboratories Pvt. Ltd. (SRL), India. APS (Ammonium persulfate) (248614) and TEMED (N,N,N',N'-tetramethylethane-1,2-diamine) (T9281) were purchased from Sigma-Aldrich, India. Luria broth (LB) (M575) and Luria Agar (LA) (M557) were purchased from Himedia. All other reagents used were of analytical grade and were used without further purification. Autoclaved doubled distilled water was used throughout the experiments.

4.2.3. Methods:

4.2.3.1. Preparation of *Staphylococcus aureus* whole cell imprinted polyacrylamide gel slabs:

Staphylococcus aureus bacteria were incubated overnight in Luria broth in incubator shaker at 37 ˚C, 150 rpm. The bacterial cells were re-inoculated in 25 ml of Luria broth for 4 hours at 37 ˚C, 150 rpm. Spectrophotometer was used to take the O.D. at 600 nm. When $O.D._{600}$ reached the value 1.0, the cells were collected by centrifugation at 10000 rpm for 15 min at 4 ˚C. The pellet was washed and re-suspended in 3 ml of phosphate buffer (pH 8.0) solution.

SDS PAGE set up was washed and cleaned with autoclaved double distill water. The set up was sealed with 0.2 cm wide spacers and 1% agarose gel at it borders. Then, the pre-mixed solution of acrylamide and bis-acrylamide (30%) was mixed with phosphate buffer (pH 8.0) as stated in the Table 1 to prepare different concentrations of the gels. Then 100 µL of the suspended cells were mixed with the reaction mixture for each concentration of the gel separately (2, 4, 6, 8 and 10%). Following it, 80 µL of TEMED and 10 µL of 10% APS were added to initiate the polymerization. The mixture was then poured into the sealed PAGE set up and was allowed to polymerize for 30 min **(Table 4.1)**. After it, the gel was removed from the set up and was cut into (1×1×0.2 cm) slabs. The gel slabs were then checked for the presence of entrapped *Staphylococcus aureus* by Gram's staining.

GEL PERCENTAGE	2%	4%	6%	8%	10%
30% Acrylamide & Bis-acrylamide (ml)	0.54	1.07	1.6	2.13	2.67
Phosphate buffer (pH=8) (ml)	7.32	6.82	6.22	5.78	5.24
TEMED(µl)	80	80	80	80	80
APS(µl) 10%	8	8	8	8	8

Table 4.1. Preparation of Acrylamide gels of various concentrations

2.3.2. Removal of entrapped bacteria:

The polyacrylamide gel slabs were treated in two solution mixtures separately to remove the entrapped bacteria. 10% Acetic acid and 1% SDS were mixed together to treat polyacrylamide gel slabs having entrapped bacteria. The gel slabs were kept inside test tubes in 50% Acetic acid and 1% SDS solution. The tubes were then kept inside ultrasonicator bath for 9 hr. and were washed thoroughly with double distilled water. The gel slabs were checked for complete removal of bacteria by Gram's staining. Instead of 10% Acetic acid and 1% SDS solution for the removal of bacteria from the gel slab 0.2 N NaOH and 1% SDS solution was used. The same procedure was carried out separately even with 0.2 N NaOH and 1% SDS solution **(Table 4.2).**

Sr. No.	Washing solution concentration
1	10% Acetic acid:1% SDS (1:1)
2	NaOH + SDS (0.2 N NaOH, 1% SDS)

Table 4.2. Two different washing-lysis solutions used for the removal of entrapped bacteria

4.2.3.2. Gram's staining of polyacrylamide gel slabs:

(1×1×0.2 cm) slabs of polyacrylamide gel were processed for Gram's staining to check the presence or removal of entrapped *Staphylococcus aureus* bacteria. The slabs were dipped into ready to use crystal violet stain for 5 min. Then the slabs were thoroughly rinsed in autoclaved

double distilled water. Subsequently, the slabs were dipped into Gram's Iodine solution for 5 min. and were then rinsed in autoclaved double distilled water thoroughly. The gel slabs were further dipped into 95% ethanol for 30 min. on rotary shaker at 50 rpm. Then, the gel slabs were washed thoroughly with water. The slabs were then allowed to get dried. Following it, the slabs were dipped into safranin solution for 5 min. The gel slabs were then rinsed in autoclaved doubled distilled water, dried and observed in compound microscope at 100X magnification in oil immersion **(Figure 4.1) (Table 4.3)**.

4.2.3.3. Adsorption experiments:

In adsorption experiments, dynamic adsorption of bacteria onto the imprinted gel slab surfaces (1×1×0.2 cm) was carried out by incubating with bacterial cultures for varying time periods from 0 to 180 min with 15 min. intervals. Non-imprinted gel slabs were used as negative controls in this study. *S. aureus* culture was inoculated with 1% inoculum in 500 ml of Luria Broth and allowed to grow until the OD_{600} became 1.0. The cells were pelleted down at 9503 g, 4˚C for 15 min. The pellet was dispersed in 500 ml of phosphate buffer (pH 8.0). The OD_{600} of the cell suspension was adjusted to 0.11and it was divided into test tubes (3 ml/test tube). Further as a procedure, both imprinted and non-imprinted gel slab surfaces (1×1×0.2 cm) were placed separately in different test tubes with cell suspension for the different time points. Thus, two sets of the test tubes were prepared separately for imprinted and non-imprinted gels of 2, 4 and 6% of the polyacrylamide gel slabs. Bacterial cell suspension without any gel was considered as a control throughout the experiment. All the test tubes were sealed and were kept on shaker at 50 rpm at room temperature. During the incubation period, the supernatants were taken out and change in concentration of bacteria was recorded by taking O.D. at 600 nm with a spectrophotometer at 15 min. intervals.

4.2.3.4. *Staphylococcus aureus* specific recognition test and plate count:

Microbial strains *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia Coli* were cultured overnight in Luria Broth (LB) at 37˚C and 150 rpm. The bacterial cells were reinoculated and grown at 37˚C and 150 rpm for 4 hr. till the O.D. at 600 nm became 1.0. The cells were then pelleted down by centrifugation at 9503 g, 4˚C for 15 min. The bacterial cells were then suspended into phosphate buffer (pH 8.0) by gentle vortex mixture. For different bacterial concentrations, optical densities were measured at 600 nm with UV spectrophotometer. A linear relationship was established between the optical densities and bacterial counts using plate count method. For that,

different optical densities measured for bacterial cells were cultured on Luria Agar (LA) plates by incubating at 37˚C for 48 hr. to count the colonies for respective O.D.

Three sets of test tubes were prepared for three different bacterial species. Each of the sets had 2 subsets for imprinted and non-imprinted gel slabs for 4% of acrylamide gel. Each of the test tubes had 3 ml of the respective bacterial suspensions, in phosphate buffer pH 8.0. The suspensions were incubated for 180 min. at 50 rpm and room temperature. The optical density of the initial suspension OD_{600} = 0.11 was recorded as a reference. The supernatant from each tube was withdrawn at 15 min. intervals to measure changes in the optical density. Then, this change in the optical density was related to change in bacterial concentration. Thus, resulting change in bacterial concentration was calculated from the linear relationship between the optical density and the bacterial count.

4.2.3.5. DSC measurements:

DSC measurements were carried out by NETZSCH DSC 200 F3 analyzer from 0 to 150˚C for 4% non-imprinted, imprinted gel slabs.

4.3. Results and discussion:

4.3.1. Confirmation and removal of entrapped bacteria in polyacrylamide gel:

The polyacrylamide gel is a biocompatible polymer and when it gets cross-linked, contains macroporous structure which allows large molecules to pass through. Further, the polymer has amide functional groups in its polymeric chain forming hydrogen bonds with polar solvents. In this study, various concentration of polyacrylamide gels (2, 4, 6, 8 and 10%) were prepared with *Staphylococcus aureus.* During Gram's destaining, the polyacryamide gel tends to shrink due to dehydration. The gels were washed and stored in autoclved double distilled water at 4˚C after the destaing procedure. Water was removed just before observing under compound microscope.

No.	Concentration of gel	Entrapment of Bacteria
1	2% Acrylamide gel	Not Observed
2	4% Acrylamide gel	Observed
3	6% Acrylamide gel	Observed
4	8% Acrylamide gel	Observed
5	10% Acrylamide gel	Not Observed

Table 4.3. Entrapment of *Staphylococcus aureus* observed in different concentrations of polyacrylamide gel

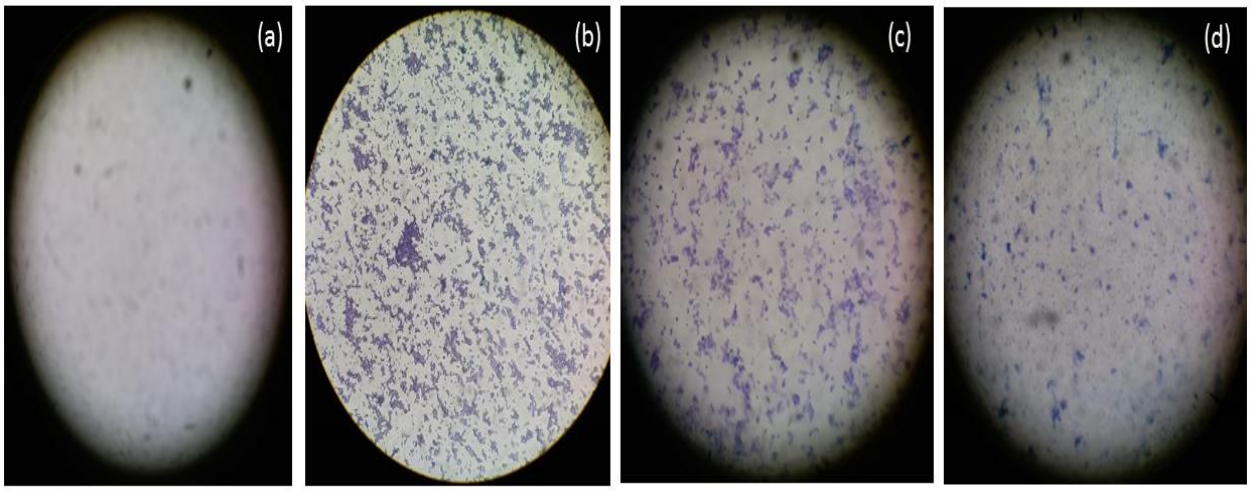


Figure 4.1: Gram's staining of various concentrations of polyacrylamide (PAM) gels stating entrapment of *Staphylococcus aureus*: (a) Blank, (b) 4% PAM, (c) 6% PAM, (d) 8% PAM.

Figure 4.1 shows that *Staphylococcus aureus* got entrapped in only the three concentrations of the polyacrylamide gels of 4, 6 and 8%. Looking at the picture, it is evident that as the concentration of the ployacrylamide gel increases the density of the gel increases. Thus, increased concrentration of polyacrylamide adds more planes to the gel and due to it, entrapped bacteria could not have been observed clearly by Gram's staining at 100X in compound microscope. In case of 4% of Gram's stained polyacrylamide gel, the entrapped *Staphylococcus aureus* can be visualized clearly more so on the upper planar surface. Where as in 6 and 8% of the Gram's stained polyacrylamide gel, entrapped bacteria could not be seen with that much clarity due to entrapment more different planes of the gel. This may be attributed to the decreased pore sizes of the gel because of increased concetrations. Thus, it can be expected that, the gel concentration has major impact on pore size, surface area, adsoption and imprintng capacity. All these factors altogather can influence rebinding, adsorption capacity of the *Staphylococcus aures*. Apart from these, it can also impact specificity of adsorption as well.

As a procedure of imprinting, the next step was to remove entrapped bacteria. Two different solutions were tried to remove the entrapped bacteria from these all three gels: (a) 10% acetic acid: 1% SDS (1:1) (b) 0.2N NaOH, 1% SDS. After sonication for several hours, gels treated with both the solutions were stained using Gram's staining. Gels treated with 10% acetic acid: 1% SDS (1:1) solution showed no removal or difference even after the treatment where as gels treated with 0.2N NaOH, 1% SDS got broken during destainig procedure into very small peices. This breakage of the gel after the treatment explains the removal of entrapped bacteria from it. SDS is

a detergent, which denatures membrane proteins and dissolves lipids resulting in lysis of the cells. NaOH stabilizes the pH during the procedure and proivides the suitable environment. Thus, 1% SDS and 0.2N NaOH were optimzed as lysis-removal solution with 9 hr of sonication for the entrapped bacteria.

4.3.2. Adsoprtion and recognition specificity of the imprinted gel slabs:

Figure 4.2, **Figure 4.3(a)** and **Figure 4.3(b)** describes the dynamic adsorption by 4% imprinted and non-imprinted polyacrylamide (1×1×0.2 cm) gel slab in *Staphylococcus aureus* suspension. Initially, up to 75 min. of incubation into the *staphylococcus aureus* suspension, both the imprinted and non-imprinted PAM showed increased adsorption and almost reached the maximum value at 75 min. When even after 75 min. of the incubation, it was allowed to continue, it showed decrease in adsorption. Though, adsorption capacities of 4% imprinted polyacrylamide was higher but both imprinted and nonimprinted exhibited almost same pattern of adsorption. This same pattern of adsorption suggests nonspecific adsorption. Yet, specificity needed to be checked further. The same experiment was performed with 6% and 8% of the imprinted polyacrylamide gel slabs of the same size. Where both of the concentrations of the imprinted gels appeared to exhibit nonspecificity. In the case of 6% of imprinted polyacrylamide gel, it showed saturation of cavities in just 30 min., but when the incubation was continued, non-imprinted 6% polyacrylamide gel showed more adsorption than imprinted one **(Figure 4.4(a) & 4.4(b)).** This behavior established that 6% of imprinted polyacrylamide does not work. 8% of imprinted polyacrylamide also exhibited same kind of behavior when kept in *Staphylococcus aureus* suspension. After 150 min. of incubation, non-imprinted gel slab showed more adsorption than imprinted one. This kind of adsorption behavior pattern proved its non-specificity **(Figure 4.5(a) & (b))**.

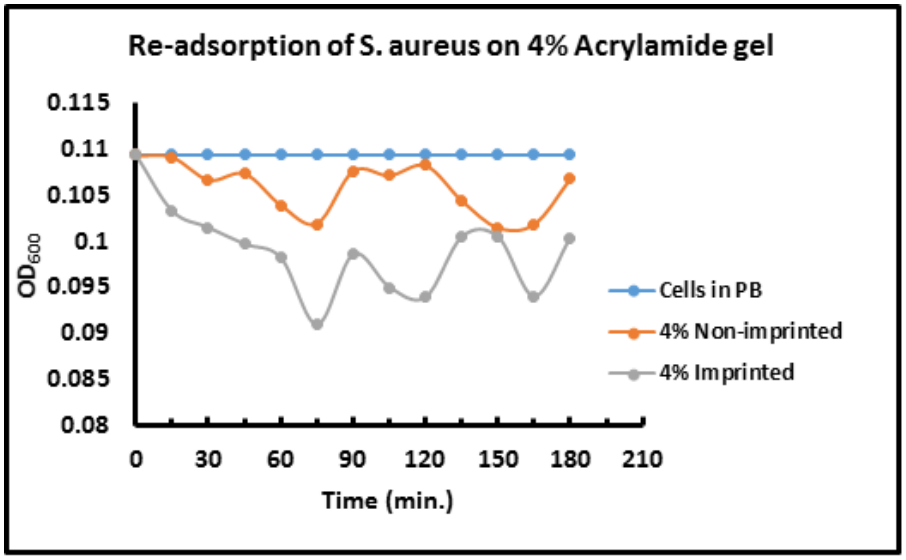


Figure 4.2. Dynamic adsorption of *Staphylococcus aureus* onto 4% imprinted and non-imprinted polyacrylamide gel slabs.

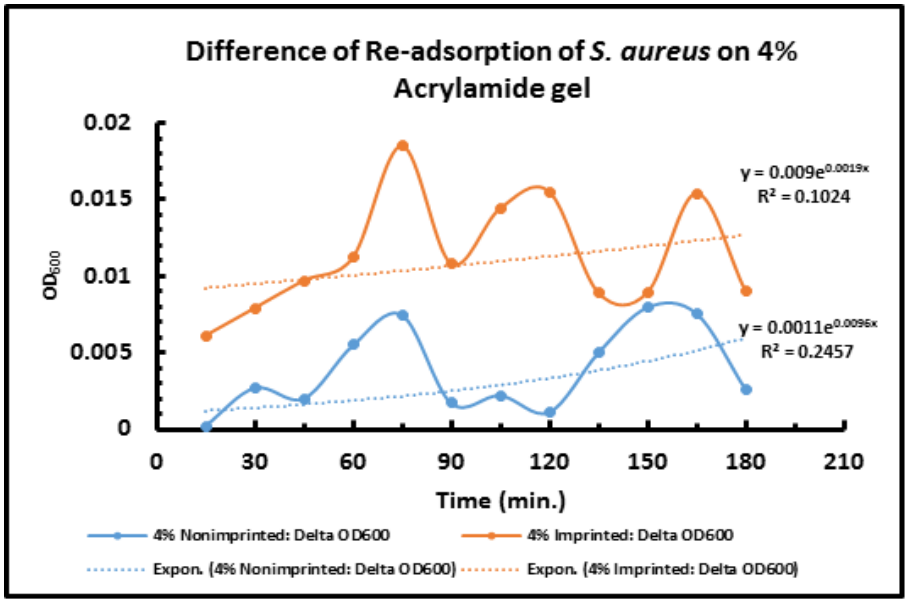


Figure 4.3(a). Difference in adsorption of *Staphylococcus aureus* on 4% imprinted and nonimprinted polyacrylamide gel over a period of 180 min.

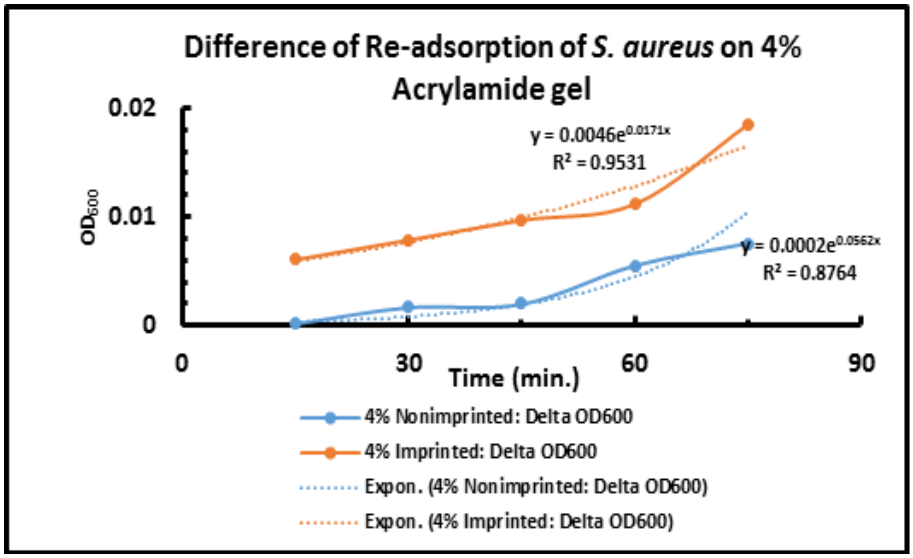


Figure 4.3(b). Difference in adsorption of *Staphylococcus aureus* on 4% imprinted and non-imprinted polyacrylamide gel over a period of 75 min.

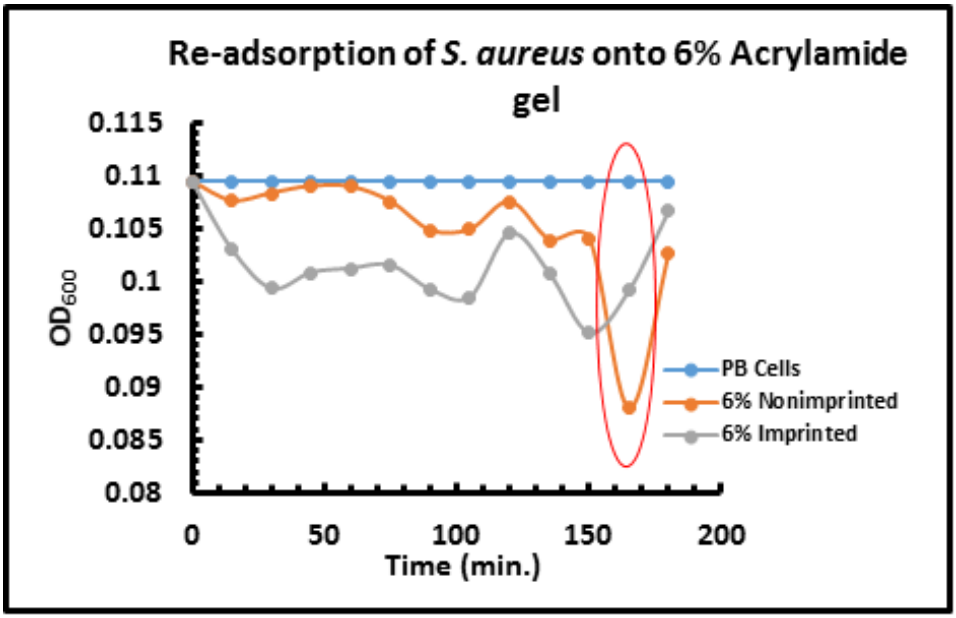


Figure 4.4(a). Dynamic adsorption of *Staphylococcus aureus* onto 6% imprinted and non-imprinted polyacrylamide gel slabs.

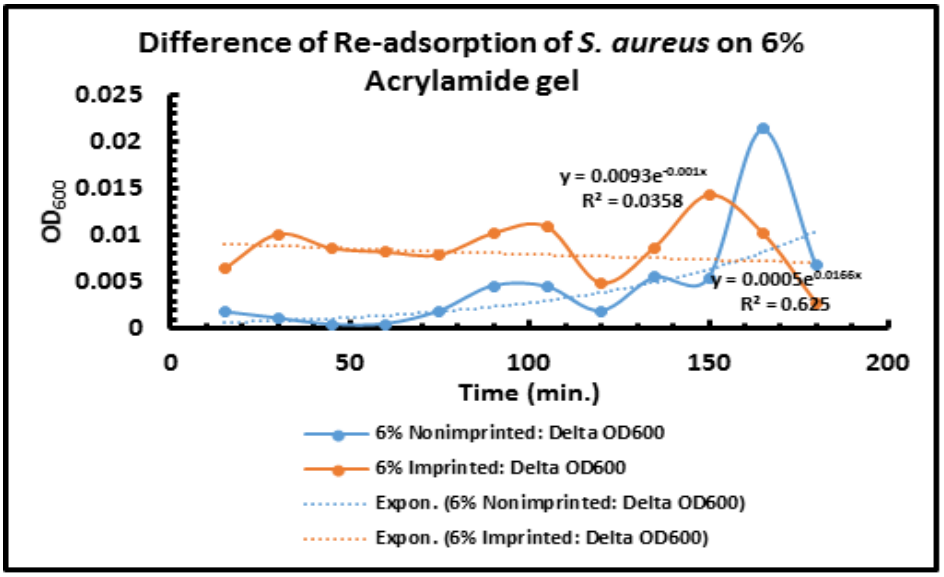


Figure 4.4(b). Difference in adsorption of *Staphylococcus aureus* on 6% imprinted and nonimprinted polyacrylamide gel slabs over a period of 180 min.

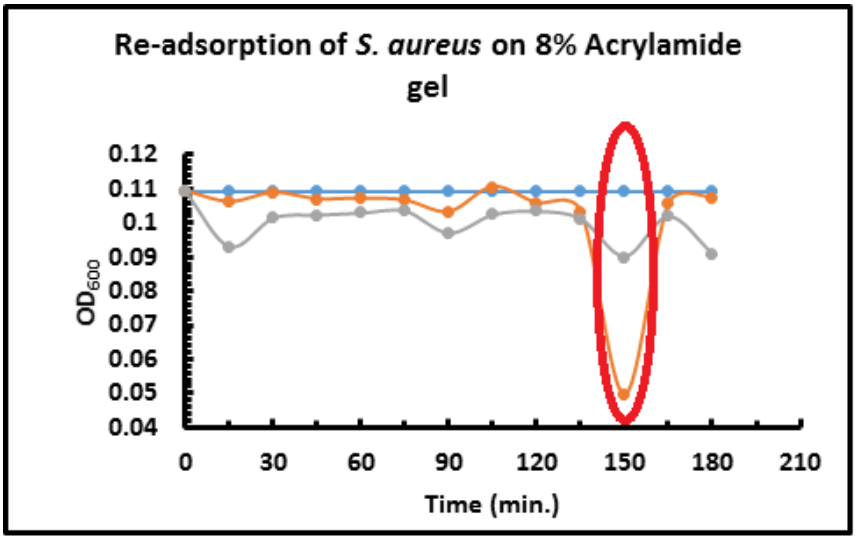


Figure 4.5(a). Dynamic adsorption of *Staphylococcus aureus* onto 8% imprinted and non-imprinted polyacrylamide gel slabs.

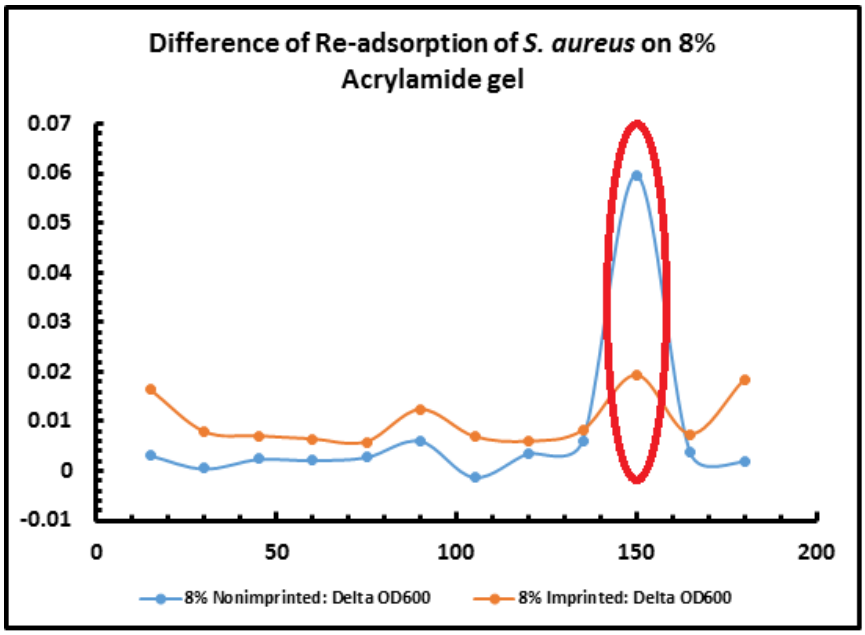


Figure 4.5(b). Difference in adsorption of *Staphylococcus aureus* on 8% imprinted and non-imprinted polyacrylamide gel slabs over a period of 180 min.

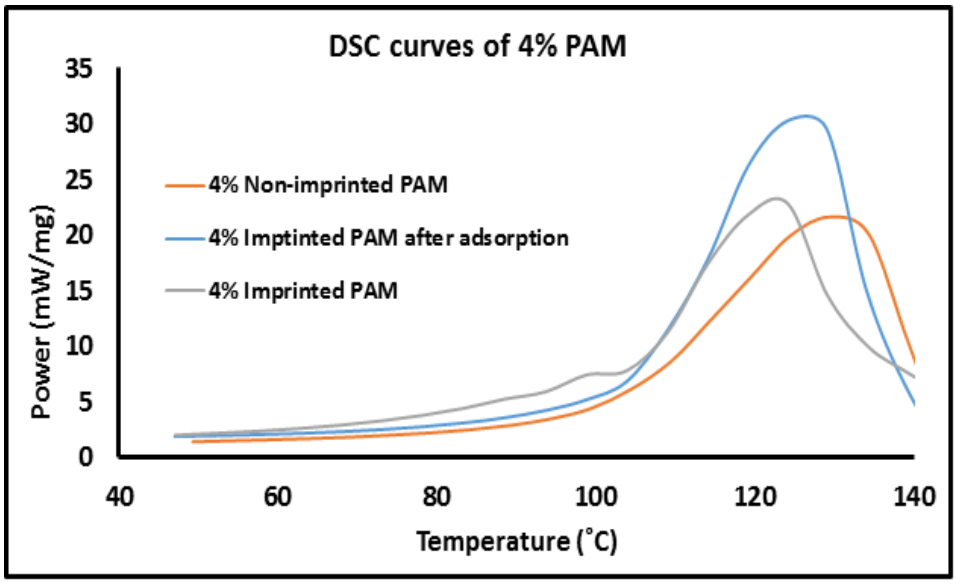


Figure 4.6. Overlay of DSC curves of 4% nonimprinted and imprinted PAMs before adsorption, and imprinted PAM after adsorption

Figure 4.6 exhibits the overlay of different DSC curves. Non-imprinted 4% polyacrylamide gel showed smaller thermogram values when compared to 4% imprinted

polyacrylamide gel before and after adsorption of *Staphylococcus aureus*. The reason behind it may be hydrogen-bonding between the reabsorbed *Staphylococcus aureus* and polyacrylamide gel network (Xue et al., 2009). It also explains that *Staphylococcus aureus* cells were effectively adsorbed onto the imprinted gel surface. This also leads its higher thermal stability. Imprinted gel showed higher thermo values compared to non-imprinted as not all of the templates get completely removed from the prepared gel and some of the bacterial components still remained embedded in the gel. During imprinting process, cavities are formed on the sites which are left behind by the bacterial entities. During adsorption process, these sites are again selectively occupied by the bacterial entities. *Staphylococcus aureus* bacterial surface offers many hydrogen-bonding sites through its various surface proteins and lipids. Thus, increased binding of the bacteria to the gel surface suggests imprinted gel showing high adsorption (Pang et al., 2005; Parmpi & Kofinas, 2004). In contrast, nonimprinted polyacrylamide gel showed nonspecific adsorption due to lack of imprinted cavities.

4.3.3. Recognition specificity of *Staphylococcus aureus* bacteria towards 4% imprinted gel:

The selective recognition and adsorption test for *Staphylococcus aureus* was carried out at room temperature, 50 rpm and at 75 min. of incubation. 2 different bacteria other than *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* were tested against the 4% imprinted gel separately for its specific binding pattern **(Figure 4.7(a) and (b))**. Both the bacteria carry different surface proteins with different shapes. Dynamic adsorption pattern onto 4% imprinted polyacrylamide gel can be observed over a period of 75 min. Till 60 min. of adsorption pattern, both *Salmonella typhi* and *Escherichia coli* showed adsorption. The results with both bacteria showed that the recognition capacity expected from imprinting is not present. But, after 75 min. of incubation period the gel showed a little specificity towards *Staphylococcus aureus* leading to its saturation for cavities. Looking at the adsorption pattern, *Salmonella typhi* and *Escherichia coli* both seem to interfere with the specific recognition of *Staphylococcus aureus*. Only the template *Staphylococcus aureus* should diffuse and bind into the cavities. Even after the absence of cavities complementary to *Salmonella typhi* and *Escherichia coli*, both tend to be adsorbed on the gel surface. Thus, the specificity expected to be attained by imprinting the polymer with *Staphylococcus aureus* could not be realized when it came to differentiating the bacteria depending on the surface features.

Moreover, concentrations of all the three adsorbed bacteria were calculated based on change in optical densities of their respective suspensions after 75 min. **(Table 4.4 & Figure 4.9)**. Plate count method for all the three bacteria namely, *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* was carried out for a range of optical densities. Then, the graph showing linearity between bacterial count against optical densities was plotted for each. Thus, adsorption capacity of 4% imprinted gel against all the bacteria was calculated in terms of bacterial count (CFU/ml) **(Figure 4.8(a), (b) and (c))**. Adsorption capacity of the 4% imprinted gel for *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* was found to be 5.763×10^6 CFU/ml, 5.13×10^6 CFU/ml and 11.42×10^6 CFU/ml respectively **(Table 4.4, Figure 4.9)**. These results suggest that 4% imprinted gel has no specificity for *Staphylococcus aureus.* This explains that the strategy does not generate the complementary cavities for the whole cell template bacteria. The gel could not offer the cavities which are complementary to size, shape and orientation of the functional groups of *Staphylococcus aureus*. This observation rejects the possibility of whole cell molecular imprinting of *Staphylococcus aureus* in polyacrylamide gel by this strategy. The results also raise a question towards successful implementation of molecularly imprinted polymers reported earlier.

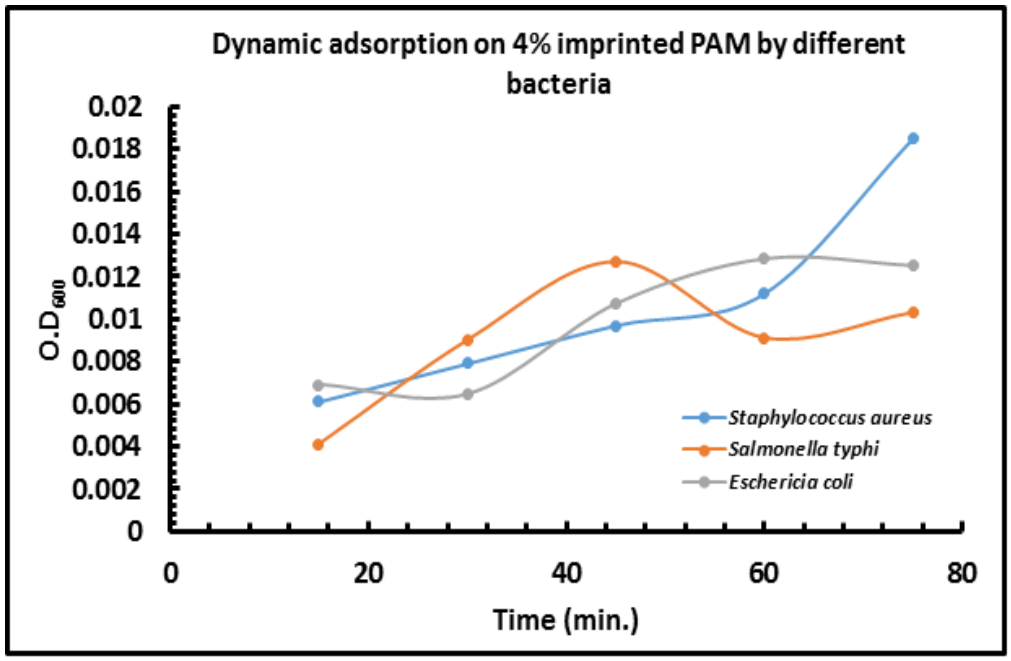


Figure 4.7(a). Dynamic adsorption on 4% imprinted polyacrylamide gel by different bacteria over a period of 75 min.

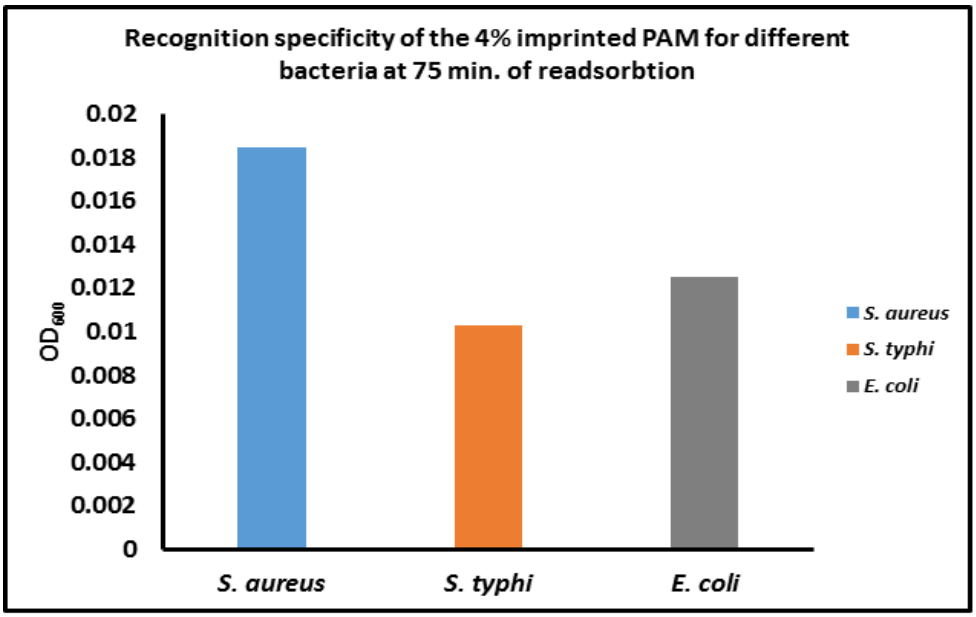


Figure 4.7(b). Recognition specificity of the 4% imprinted PAM for different bacteria at 75 min. of adsorption period in terms of change in optical density

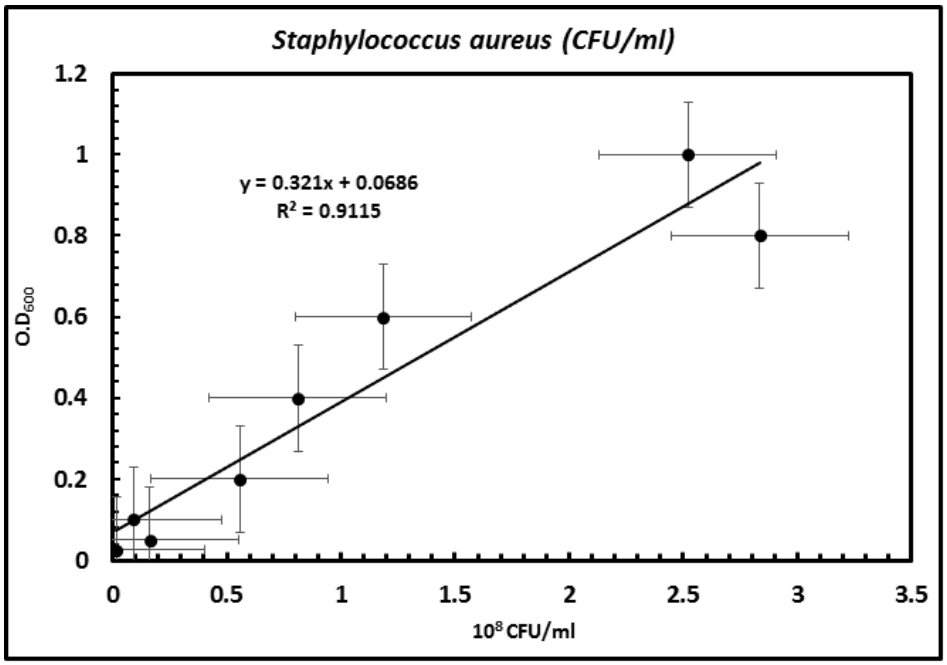


Figure 4.8(a). Calibration curves relating OD_{600} measurements versus viable cell count in CFU/ml for *Staphylococcus aureus* obtained by plate count method

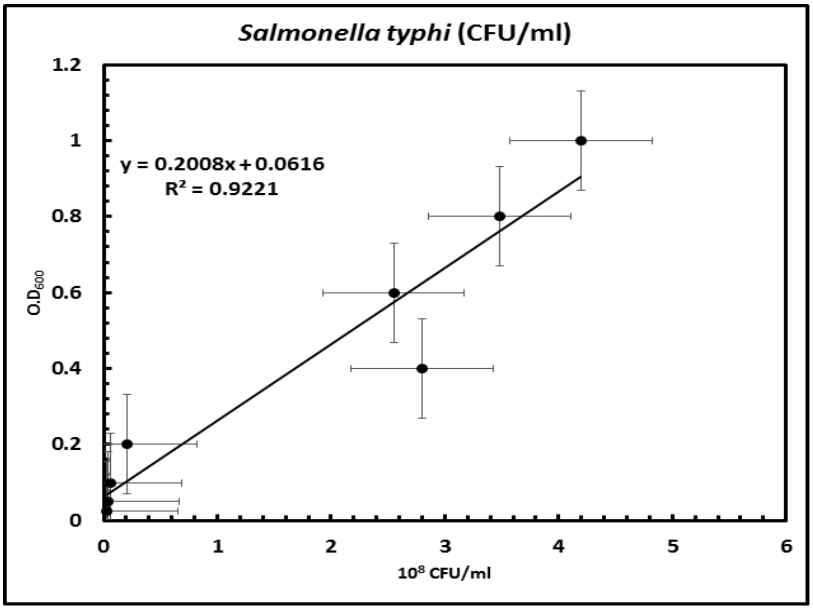


Figure 4.8(b). Calibration curves relating OD_{600} measurements versus viable cell count in CFU/ml for *Salmonella typhi* obtained by plate count method

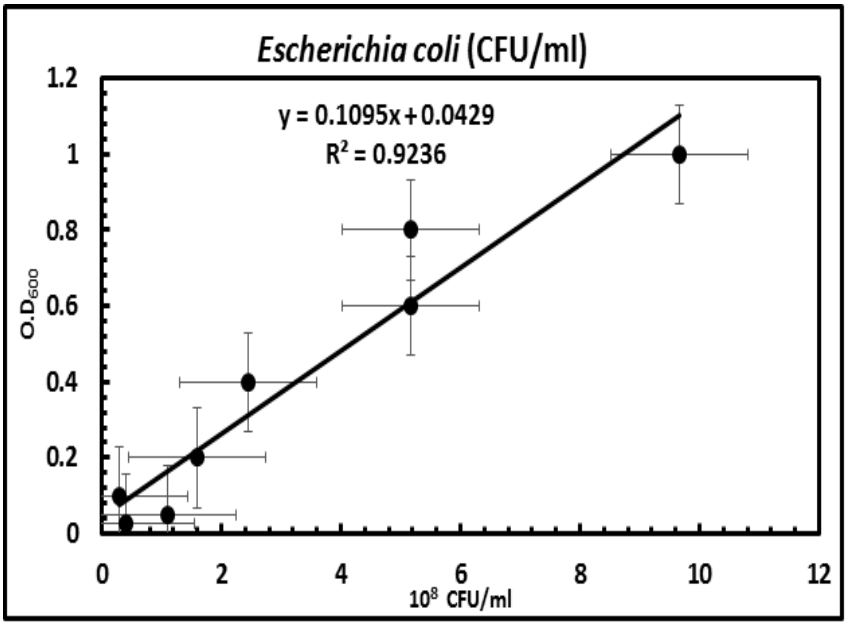


Figure 4.8(c). Calibration curves relating OD_{600} measurements versus viable cell count in CFU/ml for *Escherichia coli* (CFU/ml) obtained by plate count method

Bacterial species	OD_{600} before adsorption by 4% Imprinted PAM at 75 min.		OD_{600} after adsorption by 4% Imprinted PAM at 75 min.		Absolute adsorption
	OD_{600}	Relative CFU/ml (10^8)	OD_{600}	Relative CFU/ml (10^8)	**Absolute relative CFU/ml (10^6)**
Staphylococcus aureus	0.1094	0.127110	0.0909	0.06947	**5.763**
Salmonella typhi	0.1094	0.238047	0.0991	0.1867	**5.134**
Escherichia coli	0.1094	0.60730	0.0969	0.4931	**11.42**

Table 4.4. Change in the optical density of the bacterial suspensions before and after adsorption on 4% imprinted PAM

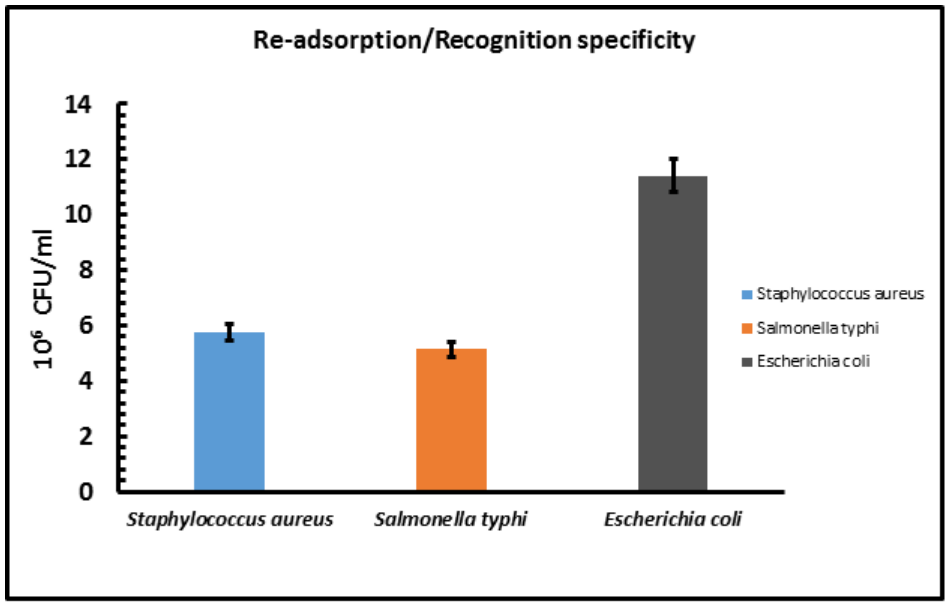


Figure 4.9. Recognition specificity of 4% imprinted polyacrylamide gel for *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* after 75 min. of incubation.

4.4. Conclusion:

Gel slabs of (1×1×0.2 cm) of 4%, 6% and 8% polyacrylamide having macroporous structure were prepared with *Staphylococcus aureus* for the purpose of imprinting. Amongst all these three concentrations, only 4% could show adsorption of *Staphylococcus aureus*. The 4%

imprinted gel was tested first against *Staphylococcus aureus* over a period of 180 min of incubation for its adsorption. After 75 min. of incubation with *Staphylococcus aureus* bacterial suspension, the gel slab showed highest adsorption. 4% nonimprinted control polymer also showed same pattern of absorption of *Staphylococcus aureus*. Further, nonspecific adsorption with other bacteria was checked with *Salmonella typhi* and *Escherichia coli* on 4% imprinted polyacrylamide gel. Over a period of 75 min. of incubation, the adsorbed *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* bacteria were calculated as 5.763×10^6 CFU/ml, 5.13×10^6 CFU/ml and 11.42×10^6 CFU/ml respectively. The results demonstrated that surface molecular imprinting is not effective for detecting the presence of specific bacteria in samples. As a concluding remark it can be said that idea of whole cell molecular imprinting to detect target bacterial cells seems to be far from success.