# MOLECULARLY IMPRINTED POLYMERS ON THE SURFACE OF MAGNETIC NANOPARTICLES FOR SELECTIVE SEPARATION AND DETECTION OF NUCLEOBASES

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**Abstract**

Nucleobases are the main building blocks in DNA and RNA playing very important roles in cell metabolism. Changes of bases in DNA sequence may affect function of products of gene expression - proteins, and this can lead to inherited diseases and most human cancer types. Pharmacological studies, clinical diagnosis and others areas need a quick, inexpensive and exact method for determination of nucleobases. In this work, we explore the possibility of improving detection of nucleobases by using capillary electrophoresis in combination with isolation by magnetic particles modified by molecularly imprinted polymer.

**Keywords:** Capillary electrophoresis, nucleobases, magnetic particles, molecularly imprinted polymers

## 1. INTRODUCTION

Deoxyribonucleic acid (DNA) is probably the most important biomolecule in living organisms, which encodes genetic information *via* combination of four bases: guanine, adenine, thymine and cytosine [1]. The changes in the concentration of each nucleobase may lead to mutation and may also indicate the presence of various diseases [2]. For example, abnormal changes of thymine concentration or a deficiency of thymine in DNA my result in symptoms of mental retardation, renal failure cardiovascular disease or cancer [1]. Therefore, the determination of such changes in biological samples has a great significance for clinical diagnostics [3]. Up to now, various analytical methods including gas chromatography [4], liquid chromatography [5], fluorescence spectroscopy [6] and electrochemical methods [7] have been used to determine the DNA bases. However, these techniques are expensive and demanding for sample preparation techniques [1].

Molecular imprinting is a method of solid phase extraction based on creating of selective binding sites for targeted analytes [8]. Molecularly imprinted polymers (MIPs) have attracted attention due to their advantages of predetermination, low cost, easy preparation, physical and chemical stability and great specificity [9]. So far, MIPs have been used for selective separation of proteins, drugs [10], pesticides [11] and others substances.

The use of nanomaterials - magnetic nanoparticles - in combination with MIPs provides large surface-to-volume ratio and well defined shape, moreover possibility of rapid separation of magnetic particles by the aid of an external magnet is favorable [1,9].

Dopamine (DA) is a small molecule which can self-polymerize to generate polydopamine at alkaline pH and spontaneously adhere to different kinds of materials form a conformal layers [12]. Polydopamine has number of advantages such as stability and durability; it is practical, eco-friendly and contains a lot of functional groups. Therefore, DA can serve as suitable functional monomer facilitating the synthesis of MIPs [9].

In this work, the conditions of formation of molecularly imprinted polymers on the surface of magnetic nanoparticles for separation and detection of nucleobases were investigated. Furthermore, the resultant polymers were used for detection of thymine in combination with capillary electrophoresis (CE) in real samples.

## 2. MATERIALS AND METHODS

### 2.1. Materials and reagents

Dopamine hydrochloride, Trizma® base, sodium dodecyl sulfate and thymine were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity. Dynabeads™ MyOne™ Silane magnetic particles and sodium tetraborate decahydrate were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

### 2.2. Preparation of magnetic MIPs

Briefly, 50 µl of magnetic nanoparticles (MPs) (40 mg·ml$^{-1}$) were washed by 200 µl of 20 mM TRIS (pH 8.5) for three times. Thymine (0.5 mg·ml$^{-1}$) was dissolved in 20 mM TRIS (pH 8.5) and 500 µl of solution was added to the washed MPs for preparation of MIPs. To prepare non-imprinted polymers (NIPs) used as a control, only 500 µl of 20 mM TRIS (pH 8.5) was added to the washed MPs. The mixtures were stirred for 1 hour and then 100 µl of dopamine (17.5 mg·ml$^{-1}$) disolved in 20 mM TRIS (pH 8.5) was added and the reaction was continued overnight at room temperature. The product was collected with a magnet, supernatant was removed and discarded. The template was washed out three times by 200 µl of 20 mM TRIS (pH 8.5).

### 2.3. Sample preparation

After washing of MIPs and NIPs by 20 mM TRIS (pH 8.5), 200 µl of sample was added and mixture was left shaking for 2 hours. After supernatant removal, MIPs and NIPs were washed three times with 200 µl of 20 mM TRIS (pH 8.5) and samples were analyzed by CE.

### 2.4. Detection by capillary electrophoresis (CE)

Quantification of thymine was performed by CE instrument 7100 (Agilent Technologies, Germany) with absorbance detection at wavelength of 260 nm. Fused silica capillary with an internal diameter of 75 µm, with the total length of 64.5 cm and an effective length of 56 cm was used. The sample was introduced hydrodynamically by 40 mbar for 5 s and a separation voltage of 15 kV was applied. A background electrolyte (BGE) was composed of 40 mM sodium borate buffer, 60 mM sodium dodecyl sulfate (SDS) at pH 9.8. Prior to the analysis, the capillary was washed for 100 seconds using BGE.

## 3. RESULTS AND DISCUSSION

### 3.1. Preparation of molecularly imprinted magnetic nanoparticles

The MIP-based separation procedure using MPs is schematically shown in **Figure 1.**

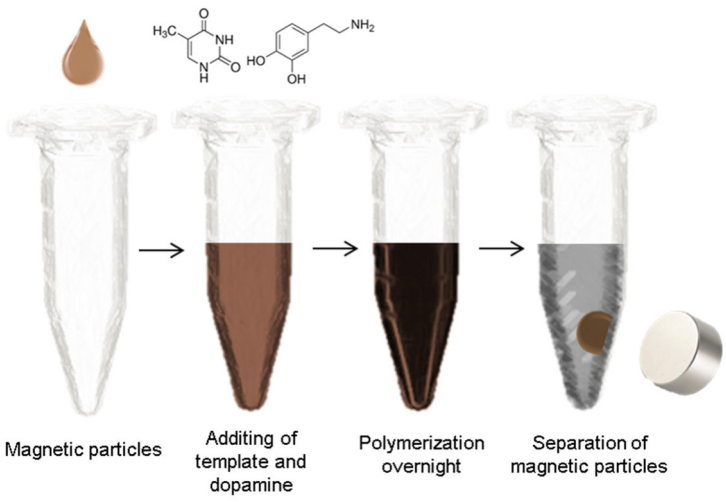


**Figure 1** Separation of magnetic particles by the aid of an external magnet

Before the created imprinted material can be used for chosen application, it is necessary to optimize several crucial steps. Briefly, the template molecules have to be washed out by an appropriate solution, because washing can cause changes and damages of binding sites. Five different approaches were used for template washing (**Figure 2A**). Interesting result was obtained for 6 M ethanol however this solvent caused the damage of thymine specific cavities and polydomamine layer. From this reason, 20 mM TRIS was chosen at the most effective approach.

As can be seen from **Figure 2B**, the surface of magnetic MIPs has to be washed several times to remove almost all template molecules from cavities. The first column represents the signal of thymine (0.5 mg·ml$^{-1}$) and after third washing of MIPs, the peak area of eluted template decreased to 1 % of its original value.

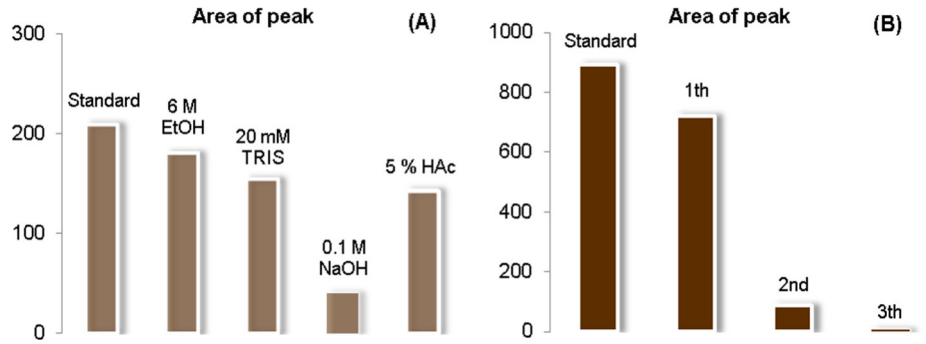


**Figure 2** Optimization of MIP properties. (A) MIPs washing by five different solvents - 6 M ethanol (EtOH), 20 mM TRIS (pH 8.5), 0.1 M NaOH and 5 % acetic acid (HAc) *Standard = CE analysis of 0.1 mg·ml$^{-1}$ thymine. (B) Optimization of number of washing steps for perfect removing of template *Standard = CE analysis of 0.5 mg·ml$^{-1}$ thymine without MIP process

### 3.2. Selectivity verification

Detection of nucleobases is important not only for pharmacological studies and clinical diagnostics, but also for other areas of science. However, the selectivity of extraction/isolation and subsequent detection is essential. Therefore, in this study, all canonical nucleobases were tested using the magnetic MIPs developed for thymine and thymine was by far the most efficiently isolated nucleobase, which proofs that the selectivity was more than satisfactory compared to even structurally very similar analytes (e.g. thymine *vs.* uracil). Moreover, as shown in **Figure 3,** the nonspecific sorption on polymeric layer without presence of cavities was reasonably low and this background signal can be subtracted for quantification purposes.

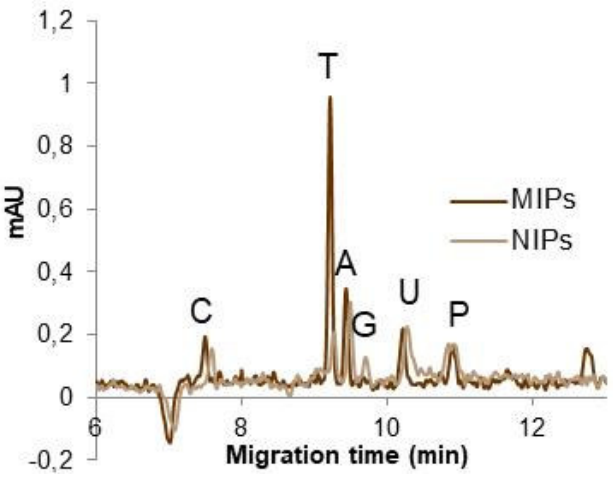


**Figure 3** Electropherograms nucleobase mixture applied on the polymeric layer with (MIPs) and without (NIPs) cavities selective for thymine. CE separation parameters: BGE - 40 mM sodium borate buffer, 60 mM SDS, pH 9.8; detection - 260 nm; hydrodynamic injection - 40 mbar for 5 s; separation voltage - 15 kV

However, when analyzing complex biological samples, appropriate sample preparation is critical, because these samples, such as tissues, blood, urine and saliva, are complex matrices with moderate-to-high levels of proteins and other interferents. Traditional methods of sample pretreatment are time-consuming, require various consecutive steps and often expensive material [13]. Thus, the isolation of the thymine by magnetic MIPs is currently being evaluated in the environment of more complex biological samples to eliminate the adsorption of proteins and improve the sample washing procedure.

## 4. CONCLUSION

Power and variability of molecular imprinting technology in combination with high surface area of nanomaterials and magnetic properties of iron oxide creates an extremely effective tool for bioanalytical applications. As demonstrated in this study, the reasonable selection suitable polymer and low sample volume analytical technique (CE) enables to improve the determination of small molecules which are even structurally very similar.

## ACKNOWLEDGEMENTS

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