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Research Article

Adsorption of DNA on Coated Magnetic Beads: Comparison of Physical and Chemical Adsorption

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Abstract
Based on their unique properties various iron magnetic nanoparticles have proved to be excellent nanomaterials for applications in separation and concentration process. Immobilization of deoxyribonucleic acid (DNA) molecules on the magnetic beads are acutely important and have potential uses in many techniques such as DNA extraction, concentration, biosensors, microarrays and next generation sequencing. In this study the adsorption of single stranded DNA (ssDNA) via poly-l-lysine coated iron oxide magnetic beads was performed under varying conditions of poly-l-lysine amount, initial DNA concentration, ionic strength, bonding type and length of DNA. The adsorption process was examined via Langmuir and Freundlich isotherm models. The ionic interaction between negatively charged DNA and positively charged surface of magnetic beads showed multilayer adsorption with Freundlich adsorption isotherm, covalent bonding between modified DNA and surface by crosslinking provided higher adsorption efficiency with Langmuir adsorption isotherm. Both adsorption methods provided magnetic beads with favorable adsorption of ssDNA.

Keywords: ssDNA, magnetic beads, adsorption, Langmuir, Freundlich

Kaplanmış Manyetik Kürelere DNA Adsorpsiyonu: Fiziksel ve Kimyasal Adsorpsiyonun Karşılaştırılması

Öz
Kendilerine has özellikleri nedeniyle çeşitli demir manyetik nanopartiküllerin ayırma ve yoğunlaştırma uygulamalarında mükemmel nanomateryal oldukları kanıtlanmıştır. Deoksiribonükleik asit (DNA) moleküllerinin manyetik kürelere immobilizasyonu son derece önemli olmakla birlikte DNA ekstraksiyonu, yoğunlaştırılması, biyosensörler, mikrodiziler ve yeni nesil dizileme gibi birçok teknikte potansiyel kullanım alanına sahiptir. Bu çalışmada, poli-l-lizin miktarı, başlangıç DNA konsantrasyonu, iyonik kuvveti, bağlanma tipi ve DNA uzunluğunun değişen koşulları altında poli-l-lizin kaplı demir oksit manyetik küreler aracılığıyla tek iplik DNA'nın (ssDNA) adsorpsiyonu, gerçekleştirilmiştir. Adsorpsiyon işlemi Langmuir ve Freundlich izoterm modelleri ile incelenmiştir. Negatif yüklü DNA ile pozitif yüklü manyetik küreler arasındaki iyonik etkileşimi Freundlich adsorpsiyon izotermiyle çok katmanlı adsorpsiyon, modeli gösterirken, DNA ve yüzey arasındaki kovalent bağlanma Langmuir adsorpsiyon izoterminde daha yüksek adsorpsiyon verimi göstermiştir. Her iki adsorpsiyon yöntemi, manyetik kürelere uygun ssDNA adsorpsiyonu sağlamıştır.

Anahtar Kelimeler:ssDNA, manyetik küreler, adsorpsiyon, Langmuir, Freundlich

1. Introduction

Magnetic beads are micro- or nano- sized particles consisting of magnetic materials like iron or nickel (Rocha-Santos, 2014). In addition to being manipulated under magnetic field they have specific properties that make them preferable for different purposes of use. They remain suspended in solution and therefore show colloidal stability by two mechanisms; electrostatic stabilization and steric stabilization. Their size distributions are homogenous and all particles show high and uniform magnetic content (Modh et al., 2018). They can be modified with different active groups such as amine, carboxyl, sulfhydryl, aldehyde, and hydrogen and also with ligands such as biotin, streptavidin, proteins, enzymes, antibodies and even cells (Aguilar-Arteaga et al., 2010). Having different surface

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functional groups ensures magnetic beads to play important roles in biomedical applications (Llandro et al., 2010; Colombo et al., 2012), separation and concentration process (Zhang et al., 2008; Ruffert et al., 2014), biosensing applications (Takamura et al., 2015) and contrast agents in imaging applications (Ruffert, 2016). Under magnetic field, drug loaded magnetic particles are targeted towards specific tissues or cells to release it at the right location, therefore, the use of magnetic beads decreases the toxic side effects of drug on the healthy cells and improves the efficiencies of the therapies. Gallo et al. (1993) targeted the anticancer drug oxantrazole adsorbed on magnetic beads to the brain of rats who suffer from the rat glioma-2 (RG-2) brain tumor. They showed that the drug targeted to the brain by magnetic beads was 100-400 times higher than the drug solution administered into the blood circulation. Alexiou et al. (2000) presented a study showing the treatment of the rabbits bearing the VX-2 squamous cell carcinoma by the drug mitoxantrone loaded magnetic beads. The number of the studies presenting the magnetic bead-based drug delivery systems has been increased in the last years (Kakar et al., 2013). In addition to medical applications, magnetic beads have been used in the construction of certain biosensors to detect different agents (Xu, 2012; Rocha-Santos, 2014). By immobilizing biomolecules onto their surface, they provide higher sensitivity and specificity. In the separation and concentration process, magnetic beads were also used due to their ease of use, higher specificity and easy manipulation under magnetic field (Ruffert, 2016). In the literature, magnetic bead separation processes have been studied for soil clean up (Funada, 2018), wastewater treatment (Lee et al., 2015; Lei et al., 2016; Fuks et al., 2018; Pal et al,. 2018), concentrating pathogenic bacteria (Almand et al., 2016; Lim et al., 2016), viruses (Satoh et al., 2003; Almand et al., 2016; Sakudo et al., 2016), and DNA (Satoh et al., 2008; Bordelon et al., 2013). DNA adsorption to the surface of magnetic beads has been mainly used to extract DNA from the sample matrix with higher concentration and lower impurities (Berensmeier, 2006). The current methods used in the DNA extraction methods have consecutive steps that require more time and great effort. Generally, initial step to extract DNA from complex matrix is the lysis of tissue or cell by physical forces (such as sound, pressure or temperature) or by chemical agents (such as detergents, SDS or CTAB). This initial step is followed by centrifugation, separation (by organic solvent such as phenol/chloroform) and precipitation (by alcohol) steps (Tan and Yiap, 2009). Due to being time consuming, increasing the risk of cross-contamination and increasing the risk of degradation, current methods have been replaced by new techniques for effective DNA extraction (Berensmeier, 2006). The present work was aimed to adsorb DNA molecules on coated magnetic beads with high efficiency. In this study, the surface of iron oxide beads were coated with poly-l-lysine solution and DNA molecules were adsorbed on coated surface by electrostatic interactions and also by covalent bonding. The amount of poly-l-lysine and magnetic beads were optimized for effective adsorption. Adsorption of DNA by electrostatic interaction between surface and DNA and covalent bonding with a crosslinker between surface group and modified DNA were evaluated for their effects on the adsorption of DNA on magnetic beads. Also, the effect of the DNA length was evaluated for the adsorption capacity of the magnetic beads. The results of this study provided a better understanding of single stranded DNA adsorption on poly-l-lysine coated magnetic beads via two adsorption methods; ionic interactions and covalent bonding via crosslinking

2. Materials ve Methods

Iron oxide (Fe_3O_4) magnetic beads were purchased from Nanografi Nano Technology (Ankara, TURKEY) and nanoparticles were in 18-28 nm diameters (with 98.45% purity). Poly-l-lysine solution (0.1% in water) was purchased from Sigma Chemical Co. (St.

Louis, Mo, USA). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and N-ε-maleimidocaproyl-oxysulfosuccinimide ester (Sulfo-EMCS) were supplied by Thermo Fisher Scientific. DNA molecules were single stranded DNA (ssDNA) used in previous study (Bayrac et al., 2017). They were supplied by Integrated DNA Technologies, Inc. (Iowa, USA) and their sequences were given at Table 1.

Table 1. ssDNA used in the study

ssDNA	**Sequence***	**Length (bases)**
Thiol modified short ssDNA	5'-AAGGGCTGGCTGGGATGGAA-3'	20
ssDNA	5'-AAGGGCTGGCTGGGATGGATGGATGGCATTACCTATGCGGTAGATTGCCGACGACCACGACTCACTCCACGGACCCCACT-3'	80
Thiol modified ssDNA	5'-/5ThioMC6-D/AAGGGCTGGCTGGGATGGATGGATGGCATTACCTATGCGGTAGATTGCCGACGACCACGACTCACTCCACGGACCCCACT-3'	80
Thiol modified long ssDNA	5'-AAGGGCTGGCTGGGATGGA$\mathbf{N_{42}}$TCACTCCACGGACCCCACT-3'	100

2.1 Poly-l-lysine coating of magnetic beads

Magnetic nanoparticles were suspended in poly-l-lysine solution and incubated at room temperature with shaking rate of 400 rpm. The magnetic beads were removed and washed twice with 1 X PBS (pH 7.2). Different concentrations of magnetic nanoparticles (0.5, 1, 5, 10, 50, and 100 µg mL^{-1}) were coated with 0.01% poly-l-lysine solution at room temperature for 2 hours. The amount of poly-l-lysine before incubation with magnetic beads and remained in solution after the removal of magnetic beads were measured spectrophotometrically at 200 nm (Optizen Pop, Korea) and coating efficiency was calculated for each concentration of the magnetic beads with the following equation.

Coating efficiency (%) (Co-Cf)/Co x 100 (1)

where Co was the initial poly-l-lysine amount, Cf was the poly-l-lysine amount remained after incubation.

The effect of initial poly-l-lysine amount on coating efficiency was evaluated. Magnetic beads (10 µg mL^{-1}) were coated with coating solutions (0.00156, 0.00312, 0.00625, 0.0125, 0.025 and 0.05% poly-l-lysine solution) at room temperature for 2 hours. The coating efficiencieswere calculated for each poly-l-lysine solution with the equation.

2.2 DNA adsorption on coated magnetic beads

DNA adsorption on magnetic beads by electrostatic attraction was performed in 1X PBS (pH 7.2) containing 1 µM of ssDNA (unmodified, 80-mer long). Briefly, 10 µg mL^{-1} of magnetic bead coated with 0.0125% poly-l-lysine solution was washed with 1X PBS and 1 µM DNA solution was added onto the magnetic beads. Upon 1 hour incubation

at room temperature, the magnetic beads were removed. The concentration of DNA before incubation with the magnetic beads and remained in the solution after the removal of the magnetic beads were measured spectrophotometrically at 260 nm. The adsorption efficiency of the DNA onto the coated magnetic beads was calculated by the following equation.

$$\text{Adsorption efficiency } (\%) \frac{\text{Co}-\text{Cf}}{\text{Co}} \text{ x } 100 \quad (2)$$

where Co and Cf were the initial and final DNA concentrations, respectively, V was the reaction volume and m was the amount of magnetic beads used in the experiment.

DNA adsorption on magnetic beads through a covalent bond was performed with the use of Sulfo EMCS. DNA molecules used in this study were 80-mer long molecules with same sequence as the DNA previously used but were modified with –SS groups at 5' ends. Firstly, the DNA solution was prepared in 1X PBS with the final concentration of 0.1 mM of TCEP and 1 μM of DNA and incubated for 30 minutes at room temperature. TCEP was used to reduce disulfide bonds (-SS) attached on 5' end of ssDNA to –SH groups. After incubation, Sulfo EMCS solution was added to have final concentration of 2 mM and mixed by pipetting. Previously coated magnetic beads (10 μg mL^{-1}) were then incubated with this DNA solution for 1 hour at room temperature. The adsorption efficiency of DNA onto the coated magnetic beads was calculated by the Equation 2.

2.3 Adsorption isotherm modeling

Two adsorption models were tested for adsorption of DNA molecules onto coated magnetic beads. According to Langmuir isotherm model;

$$\frac{\text{Ce}}{\text{qe}} = \frac{1}{\text{qsKb}} + \frac{\text{Ce}}{\text{qs}} \quad (3)$$

Freundlich isotherm model expressed adsorption as;

$$\text{qe} = \text{KfCe}^{1/\text{n}} \quad (4)$$

where qe was the adsorption capacity of magnetic beads (μg g^{-1}) at equilibrium; Ce was the concentration of ssDNA at equilibrium (μg L^{-1}); qs was the Langmuir monolayer adsorption capacity (μg g^{-1}); Kb was the Langmuir adsorption equilibrium constant (L μg^{-1}), Kf was the Freundlich adsorption capacity constant and n was a parameter of adsorption intensity. From different series of adsorption experiment Ce and qe values were calculated and plots of Ce/qevs Ce and lnqe vs lnCe were drawn to test Langmuir and Freundlich adsorption isotherm models, respectively.

2.4 Effect of DNA length on adsorption capacity of coated magnetic beads

Modified ssDNA molecules of different lengths (20, 80 and 100-mer long DNA with –SS groups at 5' ends) were suspended in 1X PBS (pH 7.2) and DNA solutions with crosslinker were prepared as previously mentioned. After incubating coated magnetic beads (10 μg mL^{-1}) with DNA solutions at different concentrations (0.01-10 μM), magnetic beads with immobilized DNA molecules were removed from supernatant. The adsorption efficiencies of different DNA molecules onto the coated magnetic beads were calculated by the Equation 2.

2.5 Statistical analysis

The statistical analysis was performed using analysis of variance (ANOVA). For each set of data, the mean values and standard deviations of three measurements were calculated and error bars represent the standard deviation of these three measurements. P-values less than 0.5 were considered statistical significant. Adsorption models were evaluated using a determination coefficient (R^2).

3. Results and Discussion

3.1 Poly-l-lysine adsorption

In this study, the aim was to immobilize DNA molecules onto poly-l-lysine coated magnetic beads. The adsorption of poly-l-lysine onto magnetic beads was evaluated for effective coating. As shown in Figure 1, with the increase in poly-l-lysine concentration up to 0.0125%, adsorption capacity of magnetic beads increased gradually. However, further increase in poly-l-lysine concentration caused decreases in the adsorption capacity. The concentration of poly-l-lysine resulting in maximum adsorption efficiency of magnetic beads (approximately 93%) was determined as 0.0125%.

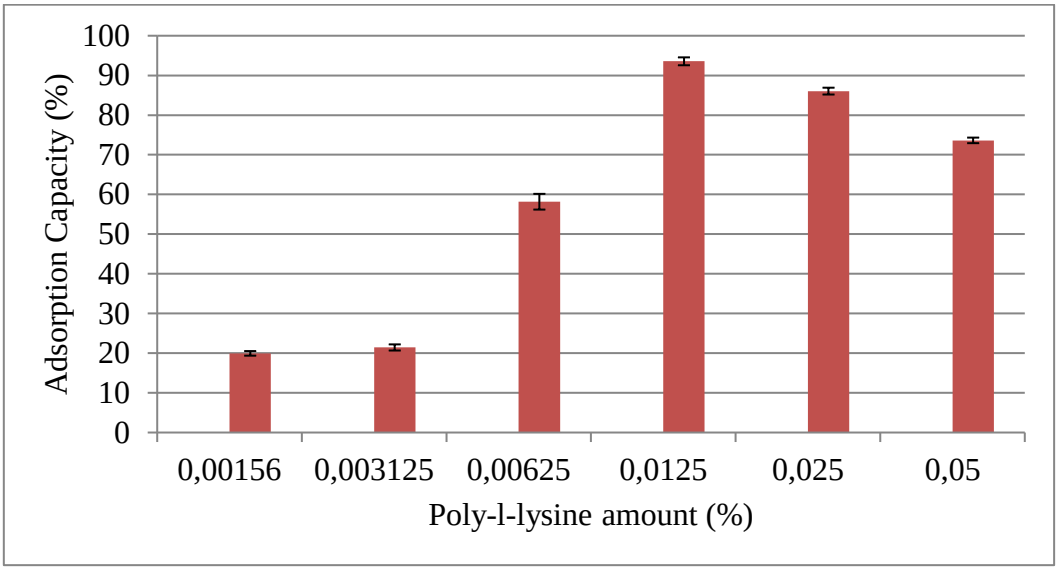

Figure 1. The effect of poly-l-lysine concentration on adsorption capacity of coated magnetic beads**;** 10 µg mL^{-1} of magnetic beads were coated with different concentration of poly-l-lysine solutions at room temperature (The error bars represent standard deviations (n=3) ($p < 0.05$))

3.2 DNA immobilization via electrostatic interactions

Poly-l-lysine coating provides with magnetic beads positively charged groups homogenously covering their surface. The negatively charged phosphate groups of DNA molecules, therefore, are attracted towards the surface due to electrostatic interactions. In order to evaluate the effect of unmodified ssDNA concentration on adsorption capacity of the coated magnetic beads, DNA solutions at different concentrations (in 1 x PBS) were adsorbed onto the magnetic beads, as previously mentioned. Figure 2A shows the change in adsorption efficiency of magnetic beads with the change in DNA concentration. While 0.1 µM of DNA was adsorbed on the magnetic beads with approximately 60% of efficiency, adsorption with ten times higher DNA concentration resulted in 78% adsorption efficiency. Through physical adsorption the maximum adsorption efficiency (app. 80%) was achieved with 10 µM of DNA. The effect of ionic strength on physical adsorption of unmodified ssDNA was also investigated in this study. The adsorption studies of DNA were performed with DNA solutions (10 µM) at different concentration of phosphate buffer (0,1X-10X, pH 7.2). As observed from Figure 2B, when the phosphate concentration in DNA solution was increased, adsorption efficiency was also increased gradually. These results can be explained by the fact that the repulsion between DNA molecules itself decreased at higher phosphate concentrations which leaded to higher surface density of DNA on coated magnetic beads.

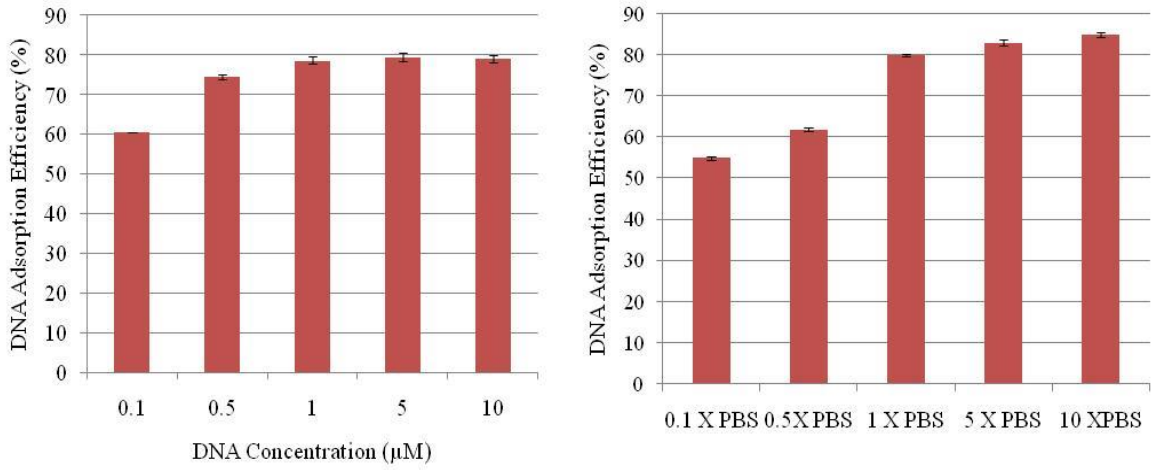

Figure 2. The effect of ssDNA concentration (A) and ionic strength (B) on physical adsorption capacity of coated magnetic beads**;** different concentration of DNA solutions were adsorbed on 10 µg mL^{-1} of magnetic beads at room temperature (The error bars represent standard deviations (n=3) ($p < 0.05$))

3.3 DNA immobilization via covalent bonding

The ssDNA molecules were attached to poly-l-lysine coated magnetic beads via covalent bonding. The attachment of DNA was achieved via using a crosslinker, N-[ε-maleimidocaproyloxy] sulfosuccinimide ester (Sulfo EMCS), which has two functional groups. N-hydroxysuccinimide (NHS) ester groups formed an amide bond with N terminus of lysine on the surface of magnetic beads while maleimide groups formed thioether bond with reduced 5' end of DNA. Since covalent bonding between modified ssDNA and poly-l-lysine on the surface of magnetic beads were much stronger than electrostatic attraction between negatively charged phosphate groups of DNA and positively charged surface, higher adsorption efficiency was expected for lower concentration of DNA. The results shown in Figure 3 confirmed the expected results. With the increase in DNA concentration, higher adsorption efficiency was achieved. As compared with adsorption efficiency of DNA onto magnetic beads via electrostatic attraction, covalent bonding resulted in higher DNA density on the surface of coated magnetic beads. The highest adsorption efficiency was approximately 99% for 10 µM DNA which was only 79% when same amount of DNA was adsorbed physically. Also, DNA molecules at 0.1 µM concentration were adsorbed onto magnetic beads by electrostatic attraction with 60% efficiency while ten times lower concentration of DNA leaded to same adsorption efficiency due to covalent bonding.

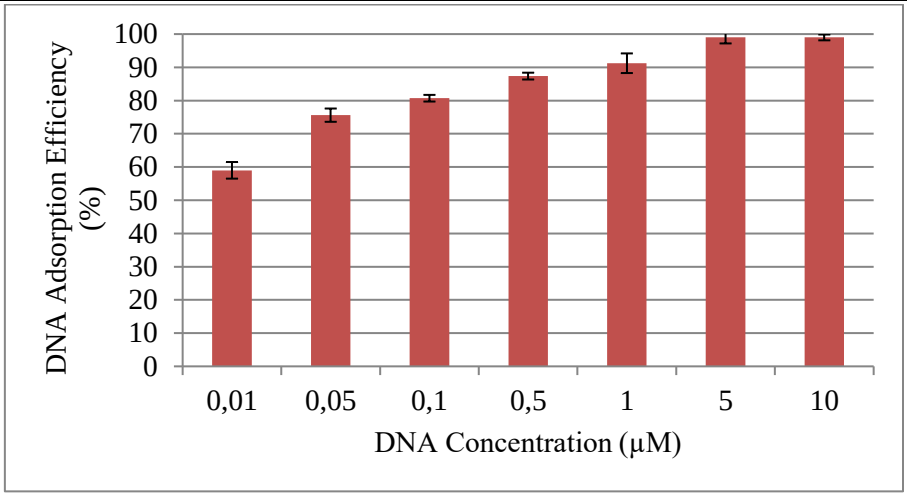

Figure 3. The effect of modified ssDNA concentration on covalent adsorption capacity of coated magnetic beads**;** different concentration of DNA in crosslinker solutions were adsorbed on 10 µg mL^{-1} of magnetic beads at room temperature (The error bars represent standard deviations (n=3) ($p < 0.05$))

3.4 Adsorption isotherm modeling

In order to evaluate the adsorption mechanisms of unmodified and modified DNA onto coated magnetic beads via electrostatic attractions and covalent bonding, respectively, two adsorption models were tested in this study. The experimental data showing adsorption process at different concentrations of DNA were applied to adsorption equations of Langmuir and Freundlich isotherm models (Equation 3 and 4), and the best fitted models with highest linear regression (R^2) were determined for two adsorption processes for DNA. Table 2 summarized model parameters for adsorption of ssDNA on poly-l-lysine coated magnetic beads. Physical adsorption result of unmodified DNA was fitted to Freundlich adsorption model rather than Langmuir model. Freundlich isotherm model described multi-layer adsorption process. However, according to covalent adsorption result, best fitted model was Langmuir adsorption model with monolayer process.

The adsorption characteristics were also evaluated based on adsorption parameters. The RL value of Langmuir adsorption isotherm model is a factor related with nature of adsorption process. The values between 0 and 1 (0<RL<1) show favorable adsorption processes between the adsorbate and surface. The value of 1/n in Freundlich model is the Freundlich exponent which should be higher than 1 (1/n<1) to indicate more favorable adsorption. The 1/n value of Freundlich adsorption model for physical adsorptionbetween unmodified DNA and surface group of magnetic beads via was 0.377 which indicated a favorable process. Moreover, adsorption by covalent bonding between thiol-modified DNA and surface groups with crosslinker was also favorable adsorption explained by RL value (0.016) of Langmuir adsorption model.

Table 2. Adsorption isotherm model parameters for ssDNA on poly-l-lysine coated magnetic beads

Adsorption	Langmuir isotherm			Freundlich isotherm		
	R^2	Kb	RL	R^2	Kf	1/n
Physical adsorption (via electrostatic attraction)	0.883	-	-	0.929	1.525	0.377
Covalent adsorption (via crosslinker)	0.960	4.261	0.016	0.936	-	-

3.5 Effect of DNA length on adsorption capacity

The effect of DNA length on the adsorption efficiency in covalent adsorption process was evaluated with the adsorption of 20-,80-, and100-mer long DNA on poly-l-lysine coated magnetic beads. As shown in Figure 4, there was not a significant difference between the adsorption efficiencies of 20-,80-, and100-mer long DNA at same concentration. Although the adsorption efficiencies increased gradually with the increase in DNA concentration, there was no noticeable change in efficiencies with theincreasein the length of DNA. As the length of DNA increased, the negative charge of phosphate groups on backbones also increased for longer DNA. More negatively charged DNA molecules may result in more adsorption efficiency in physical adsorption process between positively charged surface groups and negatively charged DNA. However, for covalent adsorption of modified DNA onto magnetic beads by crosslinker, change in length and thus change in negative charge did not show any significant differences in this study.

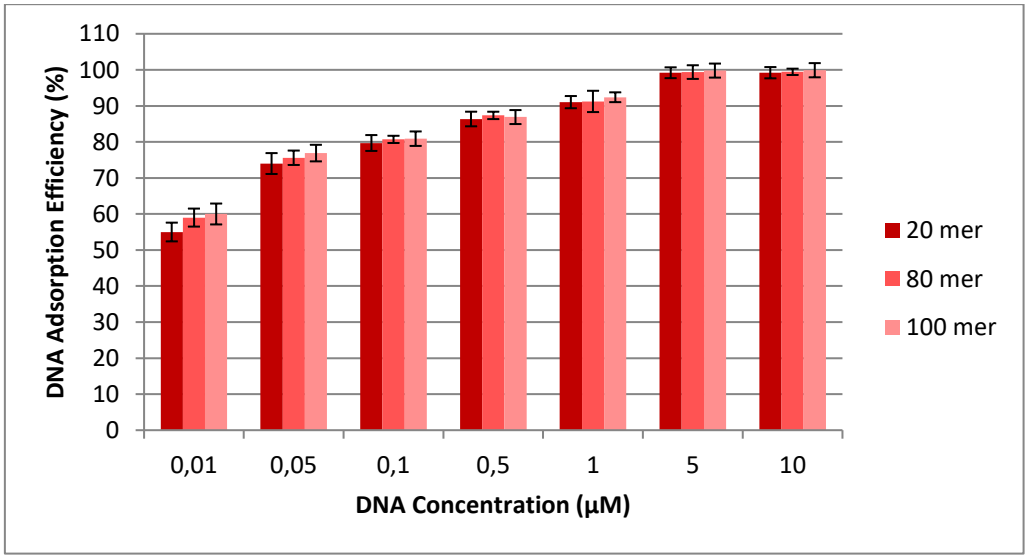

Figure 4. The effect of modified ssDNA lenght on covalent adsorption capacity of coated magnetic beads**;** different concentration of 20-, 80-, and 100- mer DNA in crosslinker solutions were adsorbed on 10 µg mL^{-1} of magnetic beads at room temperature (The error bars represent standard deviations (n=3) ($p < 0.05$))

4. Conclusion

In this study, the two adsorption methods were evaluated for effective DNA adsorption onto poly-l-lysine coated iron oxide magnetic beads. While ionic interaction between negatively charged DNA and positively charged surface of magnetic beads showed multilayer adsorption with Freundlich adsorption isotherm, covalent bonding by crosslinking provided higher adsorption efficiency with Langmuir adsorption isotherm. Both adsorption methods provided magnetic beads with favorable adsorption of ssDNA. These results provided further insights about DNA adsorption onto poly-l-lysine coated magnetic beads and thus they will have potential to be used as carriers in molecular techniques such as DNA extraction, concentration, microarrays and next generation sequencing.

5. Acknowledgments

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6. References

Aguilar-Arteaga, K., Rodriguez, J.A., Barrado, E. 2010. Magnetic solids in analytical chemistry: A review, Anal. Chim. Acta, 674, 157–165.

Alexiou, C., Arnold, W., Klein, R.J., Parak,. F.G., Hullin, P., Bergemann, C., Erhardt, W., Wagenpfeil, S., Lübbe, AS. 2000. Locoregional cancer treatment with magnetic drug targeting,*Cancer Research*, 60 (23), 6641-8.

Almand, E.A., Goulter, R.M., Jaykus, L.A. 2016. Capture and concentration of viral and bacterial foodborne pathogens using apolipoprotein H, Journal of Microbiological Methods, 128, 88-95.

Bayrac, C., Eyidoğan, F., Öktem, H.A. 2017. DNA aptamer-based colorimetric detection platform for *Salmonella* Enteritidis, Biosensors and Bioelectronics, 98, 22-28.

Berensmeier, S. 2006. Magnetic particles for the separation and purification of nucleic acids, Applied Microbiology and Biotechnology, 73 (3), 495–504.

Bordelon, H., Russ, P.K., Wright, D.W., Haselton, F.R. 2013. A Magnetic Bead-Based Method for Concentrating DNA from Human Urine for Downstream Detection, Plos ONE, 8(7), e68369.

Colombo, M., Romero, S.C., Casula, M.F., Gutiérrez, L., Morales, M.P., Böhm, I.B., Heverhagen, J.T., Prosperi, D., Parak, W.J. 2012. Biological applications of magnetic nanoparticles, Chem. Soc. Rev., 41, 4306–4334.

Fuks, L., Herdzik-Koniecko, I., Polkowska-Motrenko, H., Oszczak, A. 2018. Novel procedure for removal of the radioactive metals from aqueous wastes by the magnetic calcium alginate, International Journal of Environmental Science and Technology, 1-12.

Funada, M., Nakano, T., Moriwaki, H. 2018. Removal of polycyclic aromatic hydrocarbons from soil using a composite material containing iron and activated carbon in the freeze-dried calcium alginate matrix: Novel soil cleanup technique,Journal of Hazardous Materials, 351, 232-239.

Gallo, J.M., Varkonyi, P., Hassan, E.E., Groothius, D.R. 1993. Targeting anticancer drugs to the brain: II. Physiological pharmacokinetic model of oxantrazole following intraarterial administration to rat glioma-2 (RG-2) bearing rats,Journal of pharmacokinetics and biopharmaceutics, 21(5), 575-92.

Kakar, S., Batra, D., Singh, R., Nautiyal, U. 2013. Magnetic microspheres as magical novel drug delivery system: A review, Journal of Acute Disease, 1-12.

Lee, K.H., Baek, M.K., Cho, E.S., Park, I.H. 2015. Magnetic Separation and Transfer of Wastewater Contaminants Using Magnetic Traveling Wave and Micro Magnetic Bead, IEEE Transactions on Magnetics, 11-15 May 2015,Beijing, China.

Lei, Z.M., An, Q.D., Fan, Y., Lv, J.L., Gao, C., Zhai, S.R., Xiao, Z.Y. 2016. Monolithic magnetic carbonaceous beads for efficient Cr(vi) removal from water, New Journal of Chemistry, 40(2), 1195-1204.

Lim, M.C., Lee, G.H., Huynh, D.T.N., Hong, C.E., Park, S.Y., Jung, J.Y., Park, C.S., Ko, S., Kim, Y.R. 2016. Biological preparation of highly effective immunomagnetic beads for the separation, concentration, and detection of pathogenic bacteria in milk, Colloids and Surfaces B: Biointerfaces, 145, 854-861.

Llandro, J., Palfreyman, J.J., Ionescu, A., Barnes, C.H.W. 2010. Magnetic biosensor technologies for medical

applications: A review, Med. Biol. Eng. Comput., 48, 977–998.

Modh, H., Scheper, T., Walter, J.G. 2018. Aptamer-Modified Magnetic Beads in Biosensing, Sensors, 18 (1041), 1-22.

Pal, P., Edathil, A.A., Chaurasia, L., Rambabu, K., Banat, F. 2018. Removal of sulfide from aqueous solutions using novel alginate–iron oxide magnetic hydrogel composites, Polymer Bulletin, 1-21.

Rocha-Santos, T.A.P. 2014. Sensors and biosensors based on magnetic nanoparticles, TRAC Trend Anal. Chem., 62, 28–36.

Ruffert, C., Bigall, N.C., Feldhoff, A., Rissing, L. 2014. Investigations on the separation of platinum nanoparticles with magnetic beads, IEEE Trans. Mag., 50, 5200804.

Ruffert, C. 2016. Magnetic bead-Magnetic bullet, Micromachines, 7 (21), 1-17.

Sakudo, A., Baba, K., Ikuta, K. 2016. Capturing and concentrating adenovirus using magnetic anionic nanobeads,International Journal of Nanomedicine, 11 (9), 1847-1857.

Satoh, K., Iwata, A., Murata, M., Hikata, M., Hayakawa, T., Yamaguchi, T. 2003. Virus concentration using polyethyleneimine-conjugated magnetic beads for improving the sensitivity of nucleic acid amplification tests,Journal of Virological Methods, 114(1), 11-19.

Satoh, K., Iwata-Takakura, A., Yoshikawa, A., Gotanda, Y., Tanaka, T., Yamaguchi, T., Mizoguchi, H. 2008. A new method of concentrating hepatitis B virus (HBV) DNA and HBV surface antigen: An application of the method to the detection of occult HBV infection,Vox Sanguinis, 95(3), 174-180.

Takamura, T., Ko, P.J., Sharma, J., Yukino, R., Ishizawa, Sh., Sandhu, A. 2015. Review: Magnetic-particle-sensing based diagnostic protocols and applications, Sensors, 15, 12983–12998.

Tan, S.C., Yiap, B.C. 2009. DNA, RNA, and Protein Extraction: The Past and The Present, Journal of Biomedicine and Biotechnology, 574398, 1-10.

Xu, Y., Wang, E. 2012. Electrochemical biosensors based on magnetic micro/ nanoparticles, Electrochim. Acta, 84, 62–73.

Zhang, L., Qiao, S., Jin, Y., Yang, H., Budihartono, S., Stahr, F., Yan, Z., Wang, X., Hao, Z.; Lu, G.Q. 2008. Fabrication and size-selective bioseparation of magnetic silica nanospheres with highly ordered periodic mesostructure, Adv. Funct. Mater., 18, 3203–3212.