Investigation of interaction between magnetic silica particles and lambda phage DNA fragment

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ABSTRACT

Nucleic acids belong to the most important molecules and therefore the understanding of their properties, function and behavior is crucial. Even though a range of analytical and biochemical methods have been developed for this purpose, one common step is essential for all of them – isolation of the nucleic acid from the from complex sample matrix. The use of magnetic particles for the separation of nucleic acids has many advantages over other isolation methods. In this study, an isolation procedure for extraction of DNA was optimized. Each step of the isolation process including washing, immobilization and elution was optimized and therefore the efficiency was increased from 1.7% to 28.7% and the total time was shortened from 75 to 30 min comparing to the previously described method. Quantification of the particular parameter influence was performed by square-wave voltammetry using hanging drop mercury electrode. Further, we compared the optimized method with standard chloroform extraction and applied on isolation of DNA from *Staphylococcus aureus* and *Escherichia coli*.

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1. Introduction

Adsorption of biological macromolecules to solid surfaces has important applications among others in DNA microarrays [1], DNA-based sensors [2], high-quality DNA purification by chromatographic techniques [3] and DNA hybridization studies [4]. Nucleic acids have to be isolated, purified, and concentrated from complex samples for both research and clinical diagnostic applications. Such sample preparation is commonly accomplished through solid phase extraction, relying on the reversible interactions between nucleic acids and a solid support, such as silica. The silica support required for these methods is implemented either in the form of a filter membrane [5] or as silica-coated magnetic particles [6].

Therefore detail understanding the nature of DNA interactions with silica surfaces is important. The binding between DNA and silica is based on intermolecular electrostatic interactions, dehydration of the silica surface and DNA and intermolecular hydrogen bond between DNA and silica contact layer [7]. The mechanism of nucleic acid adsorption on the silica surface is widely discussed emphasizing the importance of presence of monovalent or divalent ions in the media [8–10]. Also the role of external parameters such as temperature, pH and/or ionic strength as driving forces should be well described [11]. Therefore, the influences of all these parameters on the DNA interactions with silica surface were investigated in this study. All key steps including DNA adsorption and desorption environment were characterized using sensitive and efficient electrochemical detection. Based on obtained results, a DNA isolation method using magnetic silica particles was suggested and successfully applied to

samples of bacterial cells of *Escherichia coli* and *Staphylococcus aureus*.

2. Material and methods

2.1. Chemicals

All chemicals of ACS purity were obtained from Sigma–Aldrich (USA) unless otherwise stated. Acetate buffer for electrochemical analysis was: $0.2\,M\,CH_3COOH + 0.2\,M\,CH_3COONa$, pH 5. The solutions used for washing MPs were: (a) phosphate buffer I: $0.1\,M\,NaCl + 0.05\,M\,Na_2HPO_4 + 0.05\,M\,NaH_2PO_4$; (b) $1\times$ *Taq* buffer: $0.01\,M\,Tris–HCl$, $0.05\,M\,KCl$, $0.015\,M\,MgCl_2$, pH 8.3 (BioLabs,USA); (c) H_2O; (d) 5 M NaCl.

The immobilization solution was: $0.1\,M\,Na_2HPO_4 + 0.1\,M\,NaH_2PO_4$; 0.6 M guanidinium thiocyanate (Amresco, USA); 0.15 M Tris–HCl (pH 7.5); NaCl (0.5; 1.25; 2.5; 3; 4; 5 M); 2.5 M LiCl; 2.5 M CsCl; 2.5 M KCl; $2.5\,M\,SrCl_2$; $2.5\,M\,CaCl_2$.

The elution solutions were: (a) phosphate buffer II: $0.2\,M\,NaCl + 0.1\,M\,Na_2HPO_4 + 0.1\,M\,NaH_2PO_4$; (b) Tris–EDTA (pH 6.5, 7.5, 8.0, 9.0, 9.1 and 9.6): $0.01\,M\,Tris + 1 \times 10^{-4}\,M\,EDTA$; (c) 0.01 M Tris–HCl (pH 9).

2.2. Polymerase chain reaction (PCR)

Taq PCR kit and DNA isolated from bacteriophage λ (48 502 bp) were purchased from New England BioLabs (USA). Primers for part of gene *xis* were synthesized by Sigma–Aldrich (USA). The sequence of a forward primer was 5′-CCTGCTCTGCCGCTTCACGC-3′ and the sequence of a reverse primer was 5′-TCCGGATAAAAACGTCGATGACATTTGC-3′. The 50 μl reaction mixture composed of 5 μl $1\times$ standard *Taq* reaction buffer, 1 μl of 200 μM deoxynucleotide solution mix, 1 μl of each primer (0.2 μM), 0.25 μl of 1.25 U *Taq* DNA polymerase and 1 μl of 0.5 μg/μl of λ DNA. The PCR tubes with mixture were placed into the Mastercycler ep *realplex* (Eppendorf, Germany) and cycling conditions were: initial denaturation at 95 °C for 120 s followed by 10–60 cycles of denaturation at 95 °C for 15 s, annealing at 64 °C for 15 s, extension at 72 °C for 45 s and a final extension at 72 °C for 5 min. The amplicons were analyzed by agarose gel electrophoresis and chip-based capillary electrophoresis.

2.3. Agarose gel electrophoresis

1.2% agarose gel (Mercury, USA) was prepared by boiling of $1\times$ TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM ethylenedi-aminetetraacetic acid, Bio-Rad, USA) for 2 min in microwave reactor (Anton-Paar GmbH, Austria). Then the gel was cooled on 60 °C and ethidium bromide was added (5 μl per 100 ml of the gel). The gel was transferred into electrophoretic bath containing TAE buffer. Samples prepared with 5% (v/v) bromophenol blue and 3% (v/v) glycerol were loaded into a gel in 5 μl aliquots. DNA ladder (New England BioLabs, USA) in the size range 0.5–1.5 kbp was used to monitor the size of analyzed fragment. The electrophoresis (Bio-Rad, USA) was running at 100 V and 6 °C for 45 min. The bands were visualized using gel projection system at 312 nm (Vilber-Lourmant, France).

2.4. Scanning electron microscopy

A scanning electron microscope (SEM) with motorized stage, full software control and image acquisition was recognized as a relatively easy way for automated high resolution documentation of particles. For each experiment, 3 independent samples of particles on different tablet sections (glass, pure Si and Millipore syringe filters) were documented. FEG-SEM TESCAN MIRA 3 LM (Czech

Republic) was used for documentation. This model is equipped with a high brightness Schottky field emitter for low noise imaging at fast scanning rates. The SEM was fitted with Everhart–Thronley type of SE detector, high speed YAG scintillator based BSE detector and panchromatic CL Detector.

2.5. Staphylococcus aureus and Escherichia coli cells preparation

The *S. aureus* or *E. coli* cells were centrifuged (5000 rpm, 15 min, Centrifuge 5417R, Eppendorf, Germany). To the pellet 75 μl of the lysate solution (6 M guanidine hydrochloride, 0.1 M sodium acetate) was added. The lysis was carried out at 20 °C, 1 h at 600 rpm (Thermomixer 5355 Comfort/Compact, Eppendorf, Germany). From the solution, 10 μl was taken away for comparison of different kinds of magnetic separations (amount of lysed cells was 20×10^6) and for comparison of optimized magnetic separation and non-phenolic extraction the final amount of lysed cells were: 75×10^3, 15×10^4, 20×10^6, 40×10^7, 80×10^7.

2.6. DNA isolation using magnetic microparticles

For DNA isolation from PCR mixture the magnetic microparticles Dynabeads MyOne Silane (Life Technologies, Invitrogen, Norway) and magnetic stand Magnetic Particle Concentrator-S (Life Technologies, Invitrogen, Norway) were used. Experiments with magnetic particles were performed in RNA/DNA UV cleaner box UVT-S-AR (Biosan, Latvia). Multi-spin MSC-6000 centrifuge (Biosan, Latvia) placed in UV cleaner box was used for centrifuging and vortexing of a sample. Thermomixer 5355 Comfort/Compact (Eppendorf, Germany) was used for heating.

10 μl of the MPs in the storage solution was transferred to a microcentrifuge tube and placed to a magnetic stand. The MPs were separated from solution due to an external magnetic field and the storage solution was removed. The microcentrifuge tube was then removed from magnetic stand, 20 μl of a washing solution was added to MPs and the suspension was mixed with the pipette to reach equal dispersion of the MPs in the washing solution. The microcentrifuge tube was placed to a magnetic stand and the washing solution was removed. This washing step was repeated. The composition of washing solution and the number of washing steps were optimized.

Subsequently, 10 μl of the sample solution and 10 μl of the immobilization solution were added to clean MPs. The immobilization took place in the multi-spin MSC-6000 or Thermomixer 5355 Comfort/Compact depending on the need to heat the microcentrifuge tube. The settings on multi-spin MSC-6000 were as follows: 20 s of shaking and centrifugation at 2400 rpm for 1 s. Other immobilization parameters such as immobilization solution composition, presence of alkali metal chloride in immobilization solution, level of shaking, immobilization temperature and time were optimized.

After the immobilization, the washing step was repeated, rinsing out the immobilization solution and all potential impurities. Subsequently, 30 μl of elution solution was added to the washed MPs with adsorbed nucleic acid and the elution was carried out in the Thermomixer 5355 Comfort/Compact. Due to the heating the nucleic acids were released from the particles. The composition of elution solution and its pH, level of shaking, elution temperature and time were optimized. Using magnetic stand, the solution containing only nucleic acids was transferred into clean microcentrifuge tube and electrochemically analyzed.

2.7. DNA isolation using commercially available kit

Dynabeads DNA DIRECT Universal kit (Life Technologies) was applied for DNA isolation Cells of *S. aureus* and *E. coli* (20×10^6) were centrifuged (5000 rpm, 15 min, Centrifuge 5417R, Eppendorf,

Germany) and the pellet was resuspended in 200 μl of Dynabeads magnetic particles in lysis solution. The reaction occurred during 5 min in 20 °C. The vial was placed on magnetic stand (2 min) and the supernatant was removed. The beads were washed by 200 μl of washing buffer and the particles were resuspended in 30 μl of provided buffer. The elution was carried out at 65 °C for 5 minutes and particles were removed from the isolated DNA using magnetic stand.

2.8. Non-phenolic extraction (NaCl/chloroform/isopropyl alcohol)

Lysed cells were mixed with 0.9% solution of NaCl at 4 °C and the samples were centrifuged (5000 rpm/15 min/4 °C). After the supernatant removal the procedure was repeated twice more. Subsequently, 500 μl of NaCl solution (0.9%) and 3 ml of Fassano solution (5 M NaCl, 0.5 M EDTA, 1 M Tris, 10% SDS) and 35 μl of proteinkinase K (−20 °C, 20 mg/ml) were added. This solution was incubated for 18 hours at 37 °C. Subsequently, the 1.4 ml of 5 M NaCl and 5 ml of chloroform was added and the mixture was shaken for 2 h. After centrifugation (8000 rpm/15 min/4 °C) the top layer was removed to clean vials and the addition of chloroform was repeated until the white ring of precipitated proteins disappeared. The isoamylalcohol (−20 °C) was added and the mixture was shaken for 10 min and centrifuged (8000 rpm/15 min/4 °C). After the supernatant removal the pellet was dried out and dissolved in TE buffer (0.5 M EDTA, 1 M Tris, pH 8).

2.9. Chip-based capillary electrophoresis

Analyses on an automated microfluidic Experion electrophoresis system (Bio-Rad, USA) were carried out according to the manufacturer's instructions with supplied chemicals (Experion DNA 1 K analysis kit, Bio-Rad). After priming the chip, 4 μl of the loading buffer were pipetted to the each sample wells and 1 μl of the sample was added. After chip vortexing, the electrophoresis was carried out. For operation and standard data analysis Experion software V. 3.10 (Bio-Rad, USA) was used. The DNA 1K Ladder (25–1000 bp) was used as a standard.

2.10. Electrochemical analysis

Electrochemical measurements were performed with AUTOLAB PGS30 Analyzer (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland) using a standard cell with three electrodes. A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was employed as the working electrode. An Ag/AgCl/3 M KCl electrode served as the reference electrode. Pt electrode was used as the auxiliary electrode.

Adsorptive transfer technique was used for the electrochemical determination of DNA [12]. The adsorptive transfer technique is based on the sample accumulation (120 s) onto the working electrode surface and consequently on the electrode washing and square wave voltammetric (SWV) measurement. All experiments were carried out at room temperature (21 °C). SWV measurements were carried out in the presence of acetate buffer pH 5.0. SWV parameters: start potential 0 V, end potential, potential step 5 mV, frequency 280 Hz, and amplitude 25.05 mV [13,14]. For smoothing and baseline correction the software GPES 4.9 supplied by Eco-Chemie was employed.

3. Results and discussion

Bacteriophage λ, a virus whose host is the cell of *E. coli*, is one of the best characterized phages and therefore it is an excellent model sample for development of methods for various molecular biology
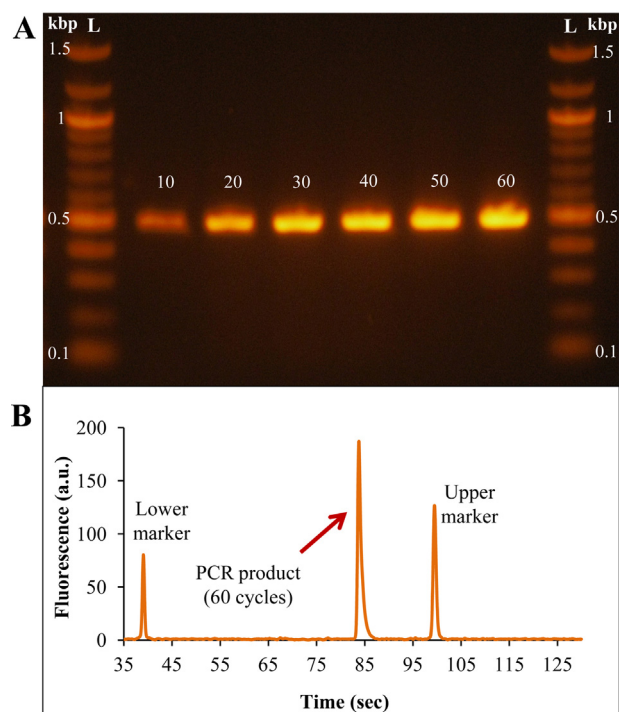

Fig. 1. (A) Gel electrophoresis of PCR product in 1.2% agarose gel and TAE buffer, the experimental parameters: 45 min, 100 V at 6 °C. L ladder, 10, 20, 30, 40, 50, 60 cycles of PCR. (B) Chip electrophoresis electropherogram of 60× cycled PCR product.

methods including those aimed at DNA isolation. Phage λ DNA fragment (498 bp) was amplified in 10–60 cycles and the PCR products are shown in Fig. 1A. In lanes L the DNA ladder was injected in the size range from 0.1 to 1.5 kbp. Other lanes contain PCR amplified DNA fragment differing in the number of amplification cycles. As expected, the signal intensity increased with the number of PCR cycles. For subsequent experiments DNA fragment amplified by 60 PCR cycles was chosen. Since the high number of cycles may result in unspecific amplification the chip-based capillary electrophoretic analysis (chip CE) was used to verify the purity of the obtained product. As shown in Fig. 1B, only one peak is present in the electropherogram. Based on the assumption that chip CE is providing better resolution (5 bp) than conventional gel electrophoresis, it can be concluded that no (or negligible amount) side products are present in the PCR product. Moreover, the Sanger sequencing analysis reviled that no significant increase in number of sequencing errors was observed. This result supported also the purity verification.

3.1. Monitoring of silica surface–DNA interaction

As mentioned above, the understanding of processes taking place between the solid silica surface and DNA molecule play a key role in almost all areas of DNA. Commonly used methods for monitoring these interactions are spectrophotometry, dynamic light scattering, quartz crystal microbalance, and/or atomic force microscopy. In this study, we demonstrate that electrochemical analysis is a sensitive and powerful tool for this type of investigations.

Magnetic particles used in this study are silica coated magnetic particle with the diameter of 1 μm. The beads are composed of highly cross-linked polystyrene with evenly distributed magnetic material. The beads are further coated, enclosing the iron oxide inside the beads and presenting a bead surface with silica-like chemistry. The micrograph of magnetic particles used for DNA
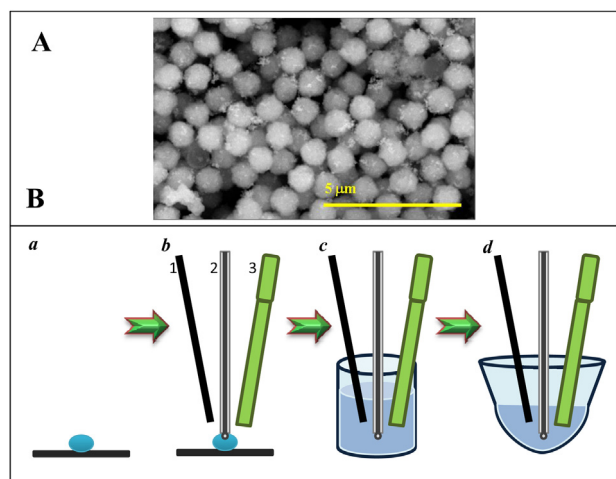
Fig. 2. (A) SEM micrograph of Dynabeads MyOne Silane. (B) Scheme of adsorptive transfer technique: (1) auxiliary electrode, (2) working electrode, (3) reference electrode, (a) sample drop on the parafilm. (b) DNA adsorption on surface of working electrode. (c) Washing of working electrode. (d) DNA detection in electrolyte.

isolation is shown in Fig. 2A. To quantify the isolation efficiency the SWV measurement was performed by adsorptive transfer technique, which is optimal for sensitive and effective DNA determination [15–17]. Nucleic acids can be easily accumulated onto surface of HMDE [18–20]. Therefore, it is possible to use adsorptive transfer technique for their detection. The working electrode is immersed into the very low volume (5 μl) of sample and nucleic acid is adsorbed for the desired time of accumulation [21]. The scheme of this technique consisting of four key steps (a – sampling, b – accumulation of the analyte on the electrode surface, c – washing of the excess of the analyte and d – SWV determination of the analyte) is shown in Fig. 2B.
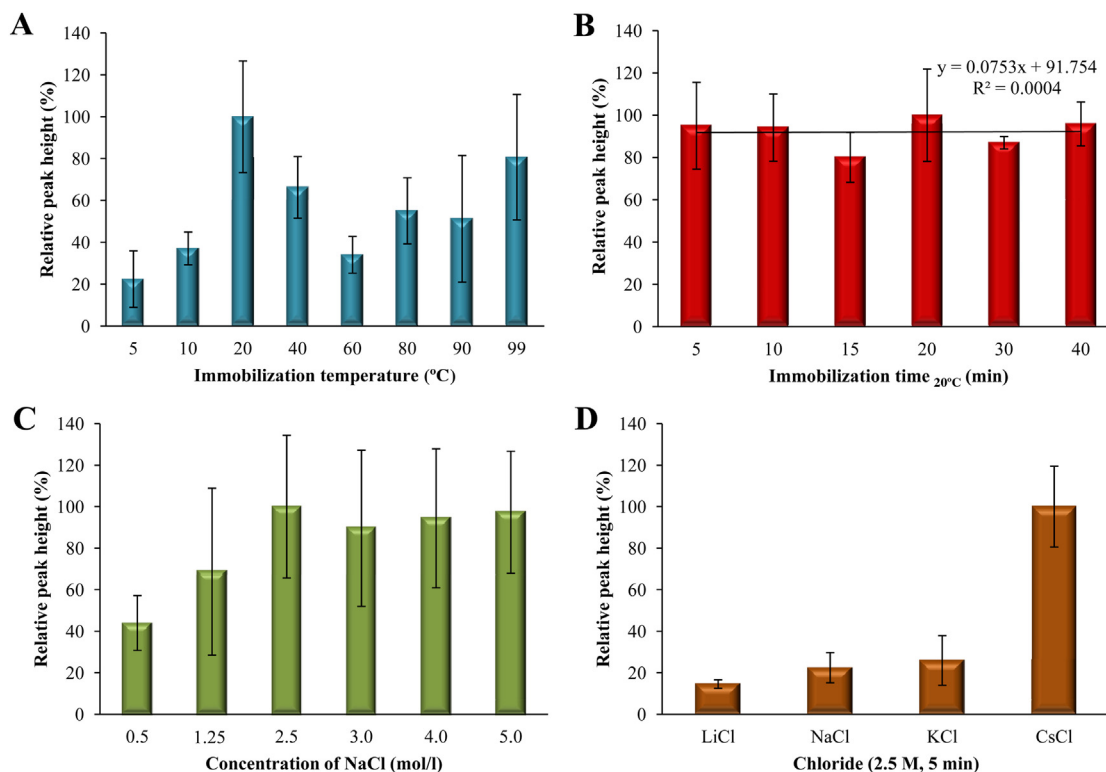
3.1.1. Immobilization

The immobilization step (adsorption) is the most important process of the whole magnetic separation. During the immobilization DNA adsorbs to the MPs surface. Generally, DNA isolation by MNPs may be performed either manually or automatically [21,22]. Both of these approaches have its advantages, however in this case the manual procedure was carried out due to the relatively low number of samples analyzed.

Immobilization temperature. The effect of temperature within the range from 5 to 99 °C on DNA yield was investigated (Fig. 3A). The highest DNA yield was obtained at 20 °C. RSD for measurement at this temperature for 5 measurements was 26.7%. Smaller DNA yields at lower temperature than 20 °C is probably caused by retarding the reaction kinetics of DNA adsorption onto the MPs surface.

Immobilization time. The kinetics of the DNA adsorption was investigated. It was found that the immobilization time has no impact on the isolation yield, which is shown in Fig. 3B. The kinetics of DNA coupling to MPs surface is rapid and therefore the shortest time of immobilization (5 min) was used for the following experiments.

Composition of immobilization solution. The immobilization solution was composed of four components (sodium phosphate, guanidinium thiocyanate, Tris–HCl and alkali metal chloride). It is well known that the presence of metal ions influence significantly the interaction between silica surface and DNA molecule [8,9]. Due to the negatively charged silica surface and the negative charge of the DNA phosphate backbone a strong electrostatic repulsion is taking place. It has been shown that water molecules, even located at long distances from the solute (>10 Å), are structurally important and dehydratation of the DNA molecule by the ions leads to the change of the DNA conformation (from B-DNA to A-DNA) [7,23,24].

The effect of alkali metal chloride concentration and type of alkali metal chloride to isolated DNA amount was investigated. Ramanowski et al. demonstrated 4-fold increase in the adsorbed

Fig. 3. Effect of immobilization solutions and conditions on the peak height: (A) Effect of immobilization temperature. (B) Effect of immobilization time. (C) Effect of concentration NaCl in the immobilization solution. (D) Effect of alkali metals in the immobilization solution.
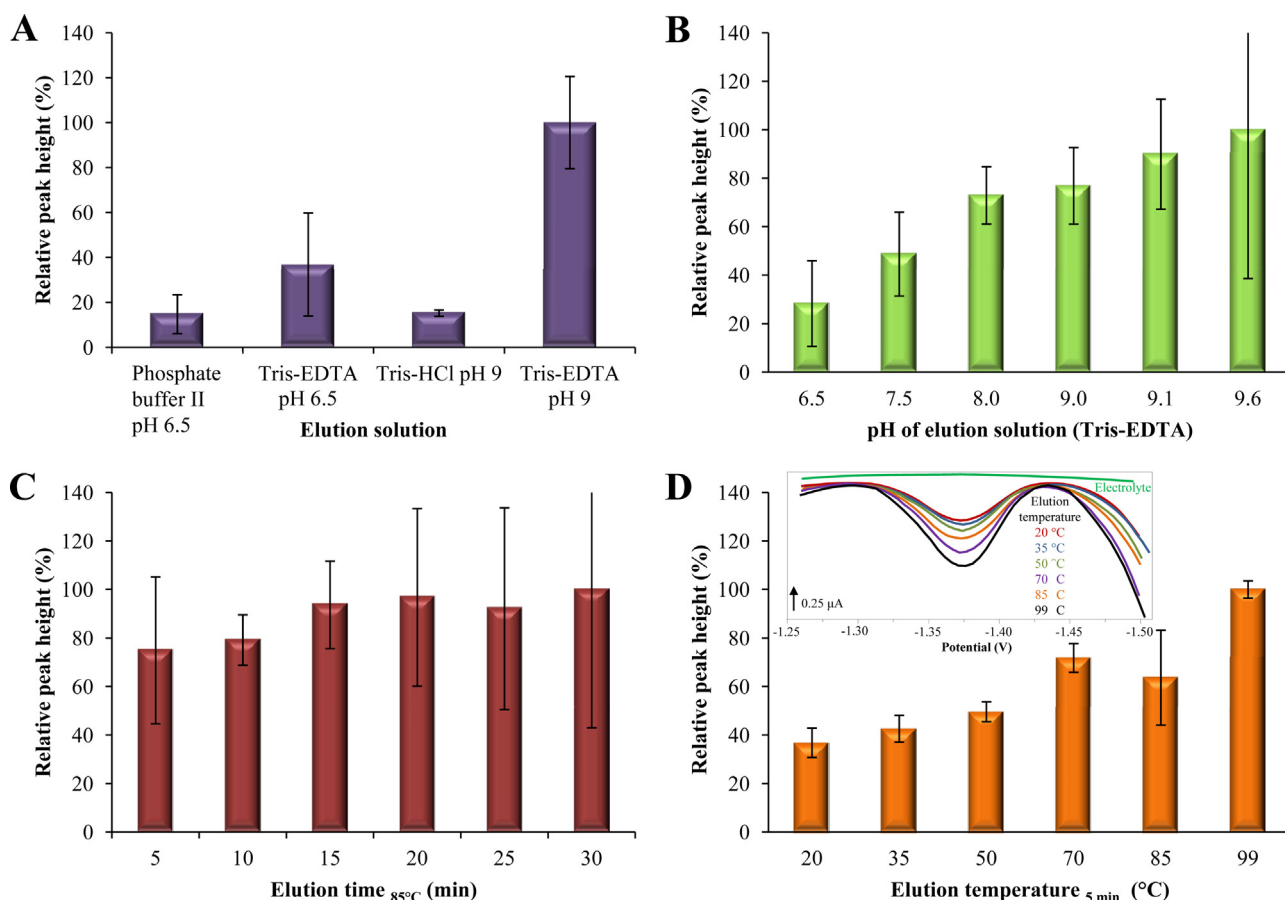
Fig. 4. Effect of elution solutions and conditions on the peak height: (A) Effect of elution solution composition: (1) Phosphate buffer II (pH 6.5), (2) Tris–EDTA (pH 6.5), (3) Tris–HCl (pH 9), (4) Tris–EDTA (pH 9). (B) The effect of elution solution pH. (C) The effect of elution time. (D) The effect of elution temperature. Inset: Typical SWV voltammograms. SWV parameters were as follows: time of accumulation 120 s, start potential 0 V, end potential −1.8 V, potential step 5 mV, frequency 280 Hz, amplitude 25.05 mV.

DNA in presence of Na^+ ions in the concentration range from 0.05 to 0.2 M [25]. In our work the concentration range of applied ions was increased (0.5–5 M). It was found that the signal of DNA increased linearly depending on the type of alkali metal ion concentration within the range from 0.5 M to 2.5 M. Maximum signal intensity was obtained using 2.5 M of alkali metal ion, however further increase of the concentration did not provide improved results. RSD for 5 measurements at 2.5 M concentration was 34.4% (Fig. 3C). Sufficient yield (90–97%) was achieved also at higher concentrations (RSD 29–38%), however 2.5 M concentration was chosen as optimal.

It is known from the literature that the ionic strength influences DNA adsorption to silica surface [7]. For binding DNA on MPs silica surface the high concentration of metal ions is necessary due to the neutralization of the electrostatic repulsion. On the contrary, the elution of DNA from MPs proceeds better without the presence of metal ions [26]. Therefore the influence of ionic strength was further investigated. The effect of different types of alkali metals solutions on the DNA yield is shown in Fig. 3D. With rising of the relative atomic mass the ionic strength increases and amount of isolated DNA also increases. The highest DNA yield was determined by using CsCl (RSD 19.5%). This is also supported by the fact the Cs^+ ions exhibit the highest electrolytic mobility. Even though the lithium ions are the smallest ones in the group, their electrolytic mobility is the smallest due to the strongest hydratation [27].

3.1.2. Elution (desorption) of DNA from silica surface

Elution solution composition. During this step DNA adsorbed onto MPs surface is released from the surface. Thereby solution of purified nucleic acid is obtained. For investigation of the influence of elution solution, four solutions were compared. It is shown in Fig. 4A, that the Tris–EDTA solutions achieved higher DNA yield. This is caused by the increase in electrostatic repulsion between DNA and silica surface caused by complexation of the metal ions from the solution by the EDTA addition. The highest DNA yield was determined by using Tris–EDTA pH 9 (RSD 20.5%). The second, Tris–EDTA solution pH 6.5 had isolation efficiency 36.9% (RSD 22.9%). Because of that, the pH of elution solution Tris–EDTA was further investigated. The pKa of silanol groups is in the range from 5 to 7 [7]. As expected, the desorption efficiency increased with increasing pH of the elution solution (Fig. 4B) due to the increasing number of deprotonized silanol groups and increased silica-DNA electrostatic repulsion. The highest isolation efficiency was determined at pH 9.6 but the reproducibility was very unsatisfactory (RSD 61.4%). The pH 9.1 was determined as optimal, the yield was 89.9% and RSD of 22.7% was reached. The lowest RSD (11.8%) was obtained at solution pH 8 but the isolation yield was low (76.9%). With the increase of elution solution pH the eluted DNA amount increased significantly.

Elution time. With the rising elution time the amount of eluted DNA increased (Fig. 4C). The highest isolation efficiency was determined at elution time 30 min. However, the reproducibility was very poor (RSD 57%). For this reason as well as to the time-frame purposes the optimal desorption time of 15 min was proposed. The DNA yield was 93.7% and RSD 18%.

Elution temperature. Finally, the elution temperature was explored. With the rising elution temperature, DNA yield increased

Table 1
Parameters of DNA isolation procedure (solution composition and pH, temperature, time, shaking and number of washing steps for previously described method and here presented optimized method.

Step	Parameter	Optimized method
Immobilization	Alkali metal chloride	2.5 M CsCl
	Time (min)	5
	Temperature (°C)	20
	Shaking	hard
Elution	Elution solution	Tris–EDTA
	pH of elution solution	9.1
	Time (min)	15
	Temperature (°C)	99
	Shaking	hard
Recovery (%)	28.7%	
Method duration (min)	30	

Fig. 5. (A) Electrochemical detection of isolated PCR product depending on the number of PCR cycles. Typical SWV voltammograms. SWV parameters were as follows: time of accumulation 120 s, start potential 0 V, end potential −1.8 V, potential step 5 mV, frequency 280 Hz, amplitude 25.05 mV. Inset: Dependence of the isolated DNA yield on the PCR cycles number.

(Fig. 4D). The highest amount of eluted DNA was obtained at 99 °C. A very good RSD value of 3.5% was reached. At temperature 99 °C the DNA denaturation is taking place, so the single stranded DNA is released to the elution solution. Moreover we believe that elevated temperature leads to the interruption of interactions between DNA chain and silica surface by the Brownian motion.

3.2. DNA isolation procedure

The interaction between DNA and silica surface was intentionally monitored using silica particles which are not part of any commercial kit for DNA isolation. This fact enabled us to use solutions with precisely controlled composition laboratory made solutions and offered the summary of the optimized method and its comparison with previously used procedure [21] is given in Table 1. It clearly follows from the obtained results that the optimized method is significantly shorter (30 min) compared to the previous method (75 min) and the recovery is extensively increased from 1.7% to 28.7%. PCR can be considered as the most commonly
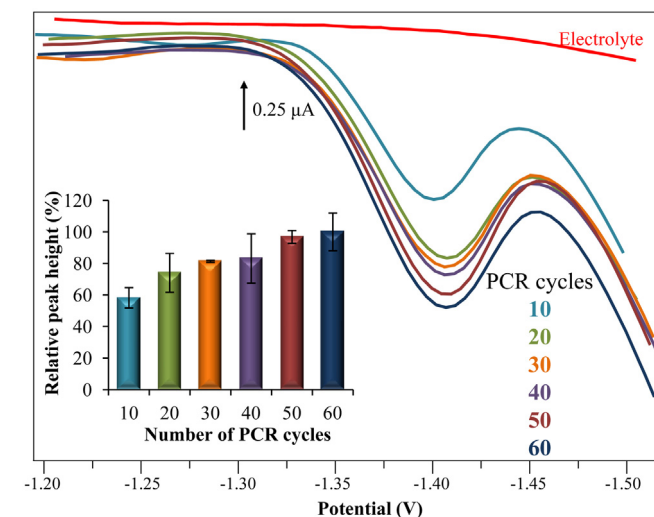
used method for detection of nucleic acids [28]. Approaches based on amplification of nucleic acids by PCR are used for numerous applications including environmental and clinical studies. From the environmental point of view, a signaling pathway induced by stress triggers specific cascades leading to activation of transcription factors. An activated transcription factor is bounded into promoter of a stress gene to start transcription. To investigate the stress cell response, nucleic acids and/or translated protein detection is needed [29]. However, prior to detection of nucleic acids by PCR, an isolation of sufficient amount of nucleic acids without contamination by other nucleic acids is unavoidable [30–37]. In this step, our optimized method can be easily applied and used for
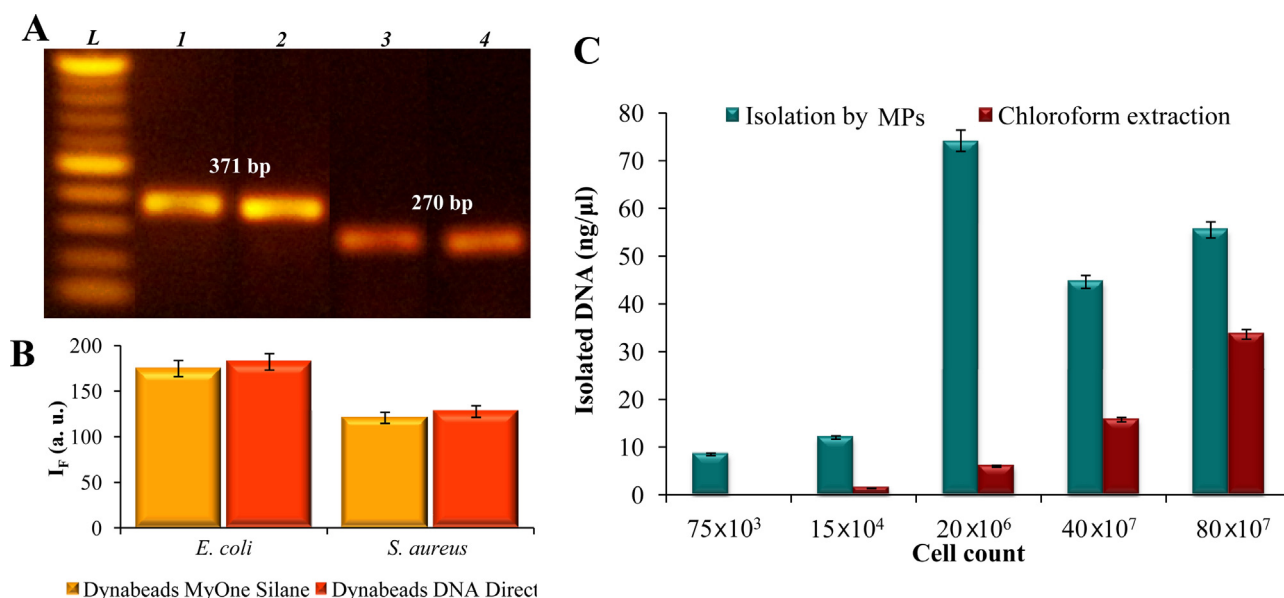

Fig. 6. (A) Gel electrophoresis of PCR product in 1% agarose gel, the experimental parameters: 60 min, 100 V. Ladder (L) contains the following DNA fragments (from the bottom): 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 kb. (1) PCR product (371 bp) amplified from the isolated E. coli DNA by Dynabeads MyOne Silane. (2) PCR product (371 bp) amplified from the isolated E. coli DNA by Dynabeads DNA Direct. (3) PCR product (270 bp) amplified from the isolated S. aureus DNA by Dynabeads MyOne Silane. (4) PCR product (270 bp) amplified from the isolated S. aureus DNA by Dynabeads DNA Direct. (B) The fluorescence intensity difference between several bands of isolated DNA samples after PCR. (C) DNA yield isolated from different count of S. aureus cells by optimized magnetic separation and chloroform extraction.

easy-to-use isolation of DNA, which can be further analyzed. This advantage of easy-to-usage without contamination by operators is also advantageous for clinical sample analyses. To test our method, the electrochemical quantification of DNA amplified by various number of PCR cycles and isolated by here presented optimized method is shown in shown in Fig. 5. The typical voltammograms of the MPs isolated DNA samples are shown in the same figure and in the inset the relative change of the electrochemical signal dependent on number of PCR cycles is expressed. It can be assumed that the optimized method is appropriate and concentration dependent approach for DNA isolation.

3.3. Isolation of bacterial DNA

MPs-based isolation was applied to real samples of bacterial cells of *E. coli* and *S. aureus*. Two procedures of isolation (1) in this work described method, (2) isolation kit (Dynabeads DNA Direct) were compared. Genomic DNA was isolated from bacterial cells and PCR was used for amplification of fragments specific for each bacteria. By gel electrophoresis (Fig. 6A), products with the size of 371 bp and 270 bp were obtained by both MPs-based procedures for *E. coli* and *S. aureus*, respectively. The fluorescence intensity provided by ethidium bromide visualization was quantified and the DNA yield obtained by both methods was compared. As shown in Fig. 6B, both methods of MPs-based isolation provided comparable results.

Finally, MPs-based extraction was compared to the golden standard of DNA isolation – chloroform extraction. As follows from results shown in Fig. 6C, the MPs-based extraction is extremely suitable for isolation from low sample amount. For instance, in case of 75,000 cells, 8.5 ng/μl of DNA was obtained by MPs-based isolation; however the amount isolated by chloroform extraction was undetectable. Generally, as expected increasing amount of cells led to the increasing amount of isolated DNA, however the MPs-based extraction provided significantly higher yields. Extremely better results were obtained for 20×10^6 cells. This is caused by the fact that 20×10^6 cells is an optimal amount of cells for MPs-based isolation procedures (both methods tested in this work).

4. Conclusion

In this study, the optimized DNA isolation method using MPs was suggested. It can be concluded that this method is providing certain improvements compared to previously published procedure such as increased efficiency and shorter time. Due to the current trend in analytical and biochemical method development focusing on miniaturized devices and the lab-on-chip concept, the application of rapid and efficient procedures employing nanomaterials is in the center of attention.

Acknowledgements

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