Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.982961

55 (2015) 2996–3002
September

Taylor & Francis
Taylor & Francis Group

Laccase-catalysed modification of PES membranes using amine-bearing modifiers

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Received 26 February 2014; Accepted 21 October 2014

ABSTRACT

Surface modification of membranes has been known as an effective tool to minimize (or even eliminate) undesired interactions between the membrane surface and the components of a flowing solution (e.g. adsorption or adhesion) leading to fouling. In this research, laccase-catalysed modification of poly(ethersulfone) (PES) membranes using aminophenols has been investigated. 4-Aminophenol modifier and laccase from *Trametes versicolor* biocatalyst were used to develop and establish an innovative antifouling modification layers that show reduction of protein adsorption (as a first step to suppress the biofilm growth). The modified PES membranes were evaluated based on e.g. water flux, protein repellence and SEM. The clean water flux of the most modified membranes was improved relative to the unmodified membrane and significant reduction in protein adsorption was obtained.

Keywords: Laccase-catalysed modification; Poly(ethersulphone) membrane; Aminophenol; Antifouling surface; Biocatalysis

1. Introduction

Aminophenols are interesting electrochemical materials, because they have two groups (–NH_2 and –OH), which can be oxidized [1]. Therefore, they can show electrochemical behaviour resembling both anilines and phenols. In polymerization of aminophenol, the relative position of amino and hydroxyl group is important. The reported electrochemical properties of the three positional isomers (ortho, meta and para) are strongly different [2,3]. The electrochemical oxidation of 4-aminophenol (4-AP) on a mercury electrode in aqueous medium indicated that the electrooxidation of 4-AP occurs by the loss of two proton and two electron through two sequence steps to yield firstly p-quinoneimine that is hydrolysed to 4-benzoquinone. The formation of polymer film on carbon past electrode [1], platinum [4], graphite [5] gold [6], etc. using 4-AP has been presented. Also, the ability of chemical oxidativly polymerized 4-AP and its nanocompound on growth inhibition of both *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram −ve) bacterial strains at different levels has been reported [7].

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Presented at the Conference on Desalination for the Environment: Clean Water and Energy 11–15 May 2014, Limassol, Cyprus

In our previous researches, we investigated the modification of poly(ethersulphone) (PES) [8,9] membranes using laccase [10–12] from *Trametes versicolor* that able to oxidize phenolic compounds to yield reactive radicals that can covalently bind to each other (polymerization) and/or to a PES membrane (biografting), mainly via their OH-groups [13,14]. In the current research, we use 4-AP as modifier (substrate for laccase), and the modified PES membranes have been evaluated based on e.g. their flux and protein repellence (first step to reduce biofouling). This substrate of laccase was chosen because they carry amino group that could add unique properties to the PES membranes under eco-friendly conditions. Two different concentrations of the modifier were used to modify the PES membranes at various modification times using 0.5 U ml^{-1} laccase in 0.1 M sodium acetate buffer (pH 5.5). A variety of techniques was used to characterize both the blank and the modified membranes including quantified colour changes and gravimetrically determined grafting yields. Scanning electron microscope (SEM) was used to further characterize the modified membranes. The combination of these data allows an outlook on laccase-catalysed modification of membranes using 4-AP modifier.

2. Experimental

2.1. Chemicals

Chemicals were purchased from different sources as following: 4-AP (99%): Sigma–Aldrich, Catechol (>98%): Oxford Laboratory Reagent (India), Sodium acetate (anhydrous, ≥99%): Polskie Odczynniki Chemiczne S.A. (Poland) and Acetic acid (99.9%): Laboratory Chemicals (Egypt). Flat sheet commercial PES membrane: Sartorius (symmetric, 0.2 μm pore size, 50 mm diameter, 150 μm thickness, water flow rate $> 28\ ml\ min^{-1}\ cm^{-2}$ at $\Delta p = 1$ bar). Laccase from *T. versicolor* (>10.4 U mg^{-1}, Fluka). All chemicals were used as received. Deionized water was used in all experiments. All used solutions were prepared freshly before using.

2.2. Laccase assay

The laccase activity was determined with catechol as substrate. The assay mixture contained 0.33 ml of 10 mM catechol, 2.67 ml of 0.1 M sodium acetate buffer (pH 5), with 0.025 U ml^{-1} laccase. Oxidation of catechol is monitored by following the increase in absorbance at 400 nm ($\varepsilon = 26{,}000\ M^{-1}\ cm^{-1}$) [15], with a reaction time of 20 min. One unit of laccase activity is defined as the amount of enzyme required to oxidize 1 μmol of catechol per min at 25˚C.

2.3. Membrane modification

The flat sheet membranes were immersed in 40 ml sodium acetate buffer containing different concentrations of 4-AP and enzyme laccase. Air was supplied as O_2 source and was used for gentle continuous mixing to ensure a homogenous reaction medium. After a specific modification time (30, 60 or 120 min), the membranes were removed from the reaction medium and washed by spraying deionized water, followed by three times dipping and shacking in freshly boiled deionized water (100˚C). The modified membranes were dried in glass dishes placed in desiccators supplied with self-indicating blue silica gel for 48 h before evaluation.

2.4. Colour measurements

The CIELAB coordinates for the modified membranes were measured with an X-Rite (SP62 Sphere Spectrophotometer, CIE $L^*a^*b^*$ and ΔE^* at D 65/10º). The colour values L^* (lightness), a^* (red–green axes), b^* (yellow–blue axes) and E^* (the degree of total colour change) were determined relative to the unmodified membrane as standard (compare mode; ΔL^*, Δa^*, etc.). Aperture size was 8 mm diameter. Three readings were taken from three different places on each sample and the average value was calculated. The membranes were washed by filtration with at least 200 ml deionized water and then dried for 48 h before the actual colour change was measured.

2.5. Pure water flux

A dead-end stirred filtration cell (Millipore, Model 8050, active transport area 13.4 cm^2) was used to measure pure water flux of unmodified and modified membranes at a constant transmembrane pressure of 1 bar at 24 ± 1˚C and 200 rpm. The pure water flux was calculated with Eq. (1), in which J_w = water flux ($m^3\ m^{-2}\ s^{-1}$), Q is the volume of permeate collected (m^3), Δt is the sampling time (s) and A is the membrane area (m^2).

$$J_w = \frac{Q}{\Delta t \cdot A} \quad (1)$$

2.6. Grafting yield

The amount of material grafted onto the membrane surface was calculated from the difference in weight of the membrane, before and after grafting process relative to the membrane unit area. Before grafting, all the membranes were kept for 48 h in glass-covered dishes in desiccators supplied with self-indicating blue silica gel to remove any moisture. To remove any enclosed or loosely bound material, the weight of the membrane after grafting was measured after washing the membrane by filtration of at least 300 ml deionized water.

2.7. Bovine serum albumin (BSA) adsorption

BSA was used as a model compound to evaluate protein adsorption on unmodified and modified membranes as described in our previous research [14]. Briefly, the membranes were immersed in 50 ml BSA solution (1 g l^{-1} BSA solution at pH 7 was prepared using 0.1 M sodium acetate buffer) and gently shaken (200 rpm) at 25˚C for 24 h. BSA concentration in the solution was measured using a UV–Vis spectrometer (280 nm), and from this the adsorbed amount was calculated.

2.8. Scanning electron microscope

Unmodified and modified membranes were imaged using a SEM (Jeol Jsm 6360LA, Japan). The membrane samples were cut using a very sharp shaving blade and were then coated with Au, and imaged at a voltage of 30 kV, and a resolution of 1,280 × 960 pixels.

2.9. Mechanical properties

Samples were cut in a dog-bone-like shape. The total length of each sample was 37 mm, the gauge length of the samples was about 16 mm; the width was 13 mm at the top and 7.2 mm (narrowest) at the middle of the sample, to force a fracture in the middle of the sample. Tensile testing of the films was performed with the Texture Analyser T2 (Stable Micro Systems, Ltd, Surrey, United Kingdom), at a constant crosshead speed of 5 mm/min until breaking. Stress–strain curves were calculated from load–elongation curves measured for two samples from each film. The tensile strength was calculated from the stress–strain curves.

3. Results and discussion

PES membranes were modified with 4-AP using laccase biocatalyst as illustrated in the experimental part. The addition of 4-AP (monomer) onto the PES membrane and the reaction of the monomers to form homopolymer in solution are competitive [15]. The samples were analysed after extensive washing using boiled water (100˚C) until clear washing water is obtained (no absorption signal was detected by spectrophotometer). The total colour change (ΔE^*) as function of grafting yield is illustrated in Fig. 1. Different modification conditions are used: two concentrations of 4-AP (5 and 15 mM), two modification temperatures (25 and 40˚C) and different modification times (30, 60 and 120 min) using 0.5 U ml^{-1} laccase and 0.1 M sodium acetate buffer; pH 5.5.

As observed, For membranes modified using 4-AP, the total change of colour increased gradually with increasing the grafting yield up to 168.1 μg cm^{-2} (ΔE^* = 25.77 at 15 mM 4-AP, 2 h, 40˚C; this point not shown in Fig. 1). The lower colouration at high concentration and longer modification time for modification at 25˚C is possibly due to lower enzyme activity and/or poor aqueous solubility of the 4-AP at reaction temperature 25˚C. As noticed in Fig. 1, three different colour changes at the same grating yield 15 μg cm^{-2} (At 40˚C: 5 and 15 mM 4-AP with 60 and 30 min modification time, respectively; At 25˚C: 5 mM 4-AP at 120 min

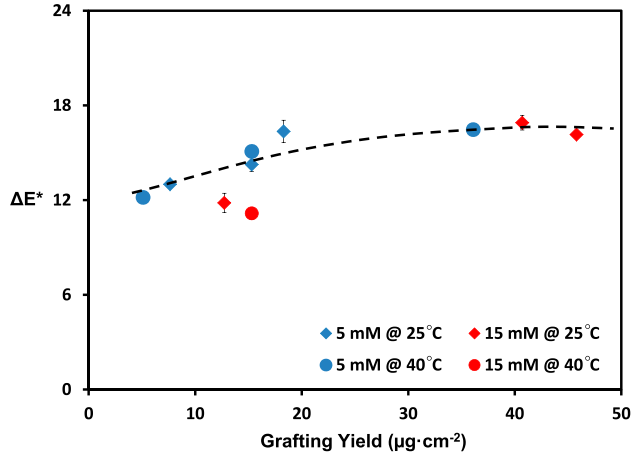

Fig. 1. Total colour change (ΔE^*) as a function of grafting yield; the reference reaction condition is 0.5 U ml^{-1} laccase, pH 5.5 and 0.1 M sodium acetate buffer. Two concentrations of 4-AP modifier are used (5 mM; blue and 15 mM; red). The membranes are modified at two modification temperatures (25˚C; diamond and 40˚C; circular) with different modification times (30, 60 and 120 min). Dashed black line is a guide for general performance of the modified membranes.

modification time), thereby the formed layers most probably differ in shape/structure (see their protein repellence in the following section). Generally, the change in ΔE^* values is very slightly at grafting yield greater than 20–25 μg cm^{-2} at different modification conditions. This is most probably due to formation of more layers with significant increases in the grafting yield that accompanied with difference in colour saturation (not measured here) instead of change in the total colour.

The clean water flux of the most modified membranes (as shown in Fig. 2) increases up to 17.7% (5 mM 4-AP, 60 min modification time, 25˚C reaction temperature) relative to the unmodified membrane. The highest clean water flux reduction at the highest grafting yield (168.1 μg cm^{-2}) was found to be 9.1% for 15 mM 4-AP in combination with 120 min modification and 40˚C reaction temperature (this point is not shown in Fig. 2). This is could be related to formation of homogenous layer without plugging the pores as show in our previous study using phenolic acids [14] (see SEM in the following section).

The adsorbed amount of bovine serum albumin (see Fig. 3) decreases with modification, but there is no relationship between the grafting yield and the extent of protein repellence of the modified membranes. However, it well observed that the best protein repellence obtained with modified membranes using low modifier concentration; 5 mM 4-AP modified for 120 min at 25˚C (up to 90.8% reduction in protein adsorption was obtained). This may be explained based on our previous propose about the effect of the

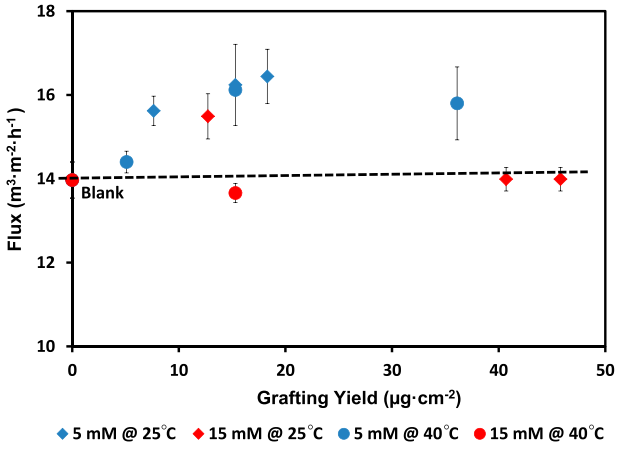

Fig. 2. Clean water flux as a function of grafting yield; the reference reaction condition is 0.5 U ml^{-1} laccase, pH 5.5 and 0.1 M sodium acetate buffer. 4-AP (5 mM; blue and 15 mM; red) modifier is used at two reaction temperature (25˚C; diamond and 40˚C; circular) with different modification times (30, 60 and 120 min).

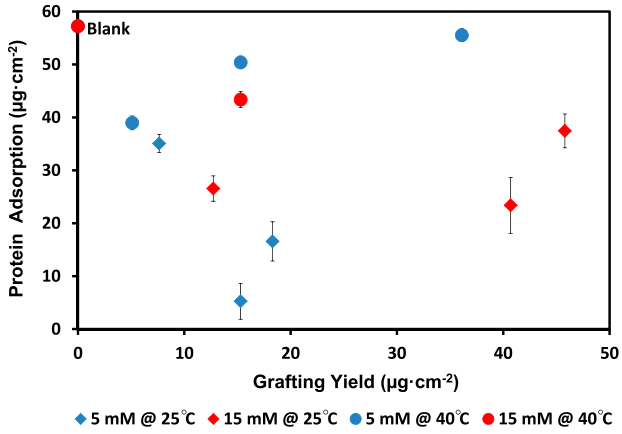

Fig. 3. Protein adsorption as a function of grafting yield; the reference reaction condition is 0.5 U ml^{-1} laccase, pH 5.5 and 0.1 M sodium acetate buffer. 4-AP (5 mM; blue and 15 mM; red) modifier is used at two reaction temperature (25˚C; diamond and 40˚C; circular) with different modification times (30, 60 and 120 min).

structure of the modifier [14,16]. Modifier with two legs (active groups) produces brush-like polymer chain. At high monomer concentration, this brush-like polymer chains will grow (grafting density will be increased and/or the chain length will be increased). At both high monomer concentration and long modification time, the formed polymer chains tend to cross-linking or interfere by adsorption to each other. The interfered chains may entrap protein molecules that resulted in increasing in the protein adsorption at high grafting yields (tertiary adsorption). We can notice this behaviour with each studied individual parameter in which the protein adsorption first significantly decreased at low grafting yield and then the adsorption re-increased with increases the grafting yield.

This significant reduction in protein adsorption resulted in remarkable improvement in residual flux after protein adsorption test and back and forward washing. Whereas unmodified membranes flux reduced by 15.96% than its original flux, all the modified membranes showed residual fluxes much better than the unmodified one. For example, the modified membrane showed the best protein repellence lost only 0.5% of its original flux (after modification); keep in your mind that its flux after modification improved by 3.97% than its flux before modification. This is resulted in final flux of the modified membrane better than the flux of unmodified membrane (fluxes after protein adsorption are 11.74 and 16.32 m^3 m^{-2} h^{-1} for unmodified and modified membranes, respectively). Moreover, the final flux of the modified membrane that acquired the highest grafting yield (168.1 μg cm^{-2})

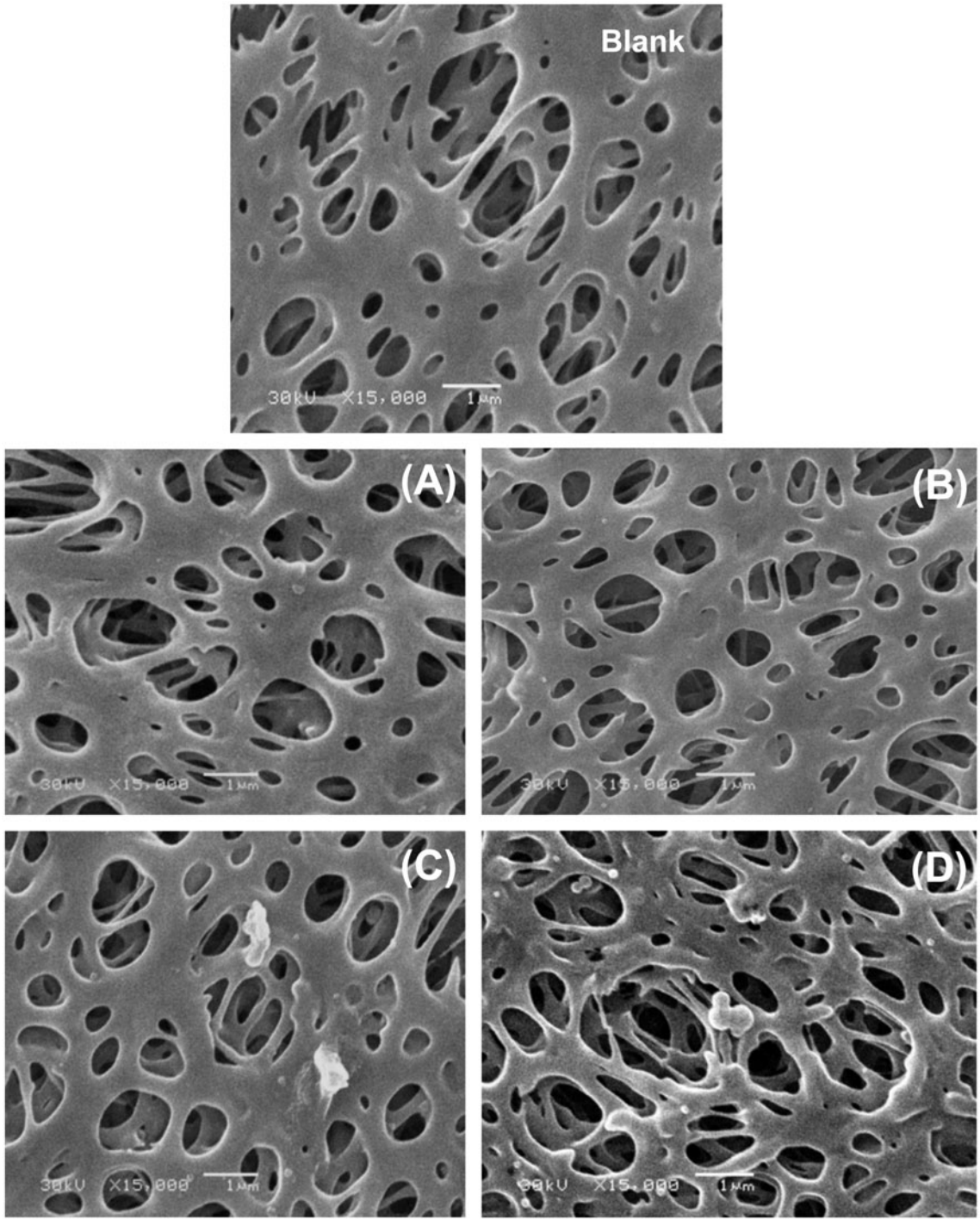

Fig. 4. SEM images (15,000× magnification, scale bar is 1 μm) for blank membrane, and 4-AP modified membranes: images (A) and (B) show modified membrane at 25°C using 5 and 15 mM 4-AP, respectively. Images (C) and (D) show modified membrane at 40°C using 5 and 15 mM 4-AP, respectively. The reference reaction condition is 0.5 U ml^{-1} enzyme, 120 min modification time, pH 5.5 and 0.1 M sodium acetate buffer.

and showed the highest flux reduction due to modification (9.1%) is still better than the flux of unmodified membrane after protein adsorption (11.74 and 12.36 $m^3 m^{-2} h^{-1}$ for unmodified and modified membranes, respectively). So, the final flux (after modification and protein adsorption) of the modified

Table 1
Tensile strength of unmodified and modified membranes using two concentrations of 4-AP modifier (5 and 15 mM) at different reaction temperatures (25 and 40°C) and different modification times (30, 60 and 120 min)

	Reaction temperature 25°C				Reaction temperature 40°C			
	5 mM 4-AP		15 mM 4-AP		5 mM 4-AP		15 mM 4-AP	
Modification time (min)	GY ($\mu g\,cm^{-2}$)	Tensile strength ($N\,mm^{-2}$)	GY ($\mu g\,cm^{-2}$)	Tensile strength ($N\,mm^{-2}$)	GY ($\mu g\,cm^{-2}$)	Tensile strength ($N\,mm^{-2}$)	GY ($\mu g\,cm^{-2}$)	Tensile strength ($N\,mm^{-2}$)
Blank	0	3.14 ± 0.70	0	3.14 ± 0.70	0	3.14 ± 0.70	0	3.14 ± 0.70
30	7.64	3.48 ± 0.36	12.73	3.14 ± 0.15	5.1	2.30 ± 0.51	15.30	2.97 ± 0.44
60	18.3	2.91 ± 0.80	40.70	2.75 ± 0.07	15.3	3.19 ± 0.83	101.86	4.21 ± 0.19
120	15.3	3.29 ± 0.40	45.80	2.60 ± 0.82	36.1	4.17 ± 0.34	168.10	4.76 ± 0.30

membranes is still much better than the flux of the unmodified membrane after protein adsorption.

SEM images of the grafted PES membranes shown in Fig. 4 do not show the homopolymer lumps that were observed in our previous study with 4-hydroxybenzoic acid (see SEM and AFM images in Ref. [14]) at any of the chosen conditions. This is because the reaction rate of aminophenols is very fast and the modification process seems to be initiated across the entire membrane surface at the same time as in case of gallic acid [14] although the structure of the aminophenols analogues 4-hydroxybenzoic acid (both of them carries two legs). Some very tiny lumps appeared at higher temperatures and under conditions at which homopolymer are most likely to be formed (higher substrate concentrations with long modification time).

From Table 1, it seems the modification does not affect harmfully on the mechanical properties of the modified membranes using 4-AP at different modification conditions used in this study. Actually, slightly improvement in the membrane strength at high grafting yield was recorded. At this point, we can see successful modification of PES membranes using 4-AP under very mild conditions. This modification shows good repellence for protein adsorption as a first step to reduce the bio(fouling) phenomena that is currently tested in our lab.

4. Conclusions

The results presented in this work show that PES membranes can be successfully modified with 4-AP using the enzyme laccase under very mild conditions. The modified membranes showed a considerably reduced protein adsorption and the mechanical properties were not significantly influenced. The clean water flux improved after modification, and the residual flux after protein adsorption is still very good. This very mild modification opens a broad area of interesting applications for PES membranes especially in water desalination.

Acknowledgement

This work was supported by The Science and Technology Development Fund (STDF), [Project ID 6070].

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