Talanta 159 (2016) 401–411

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rapid and alternative fabrication method for microfluidic paper based analytical devices

Soheil Malekghasemi [a], Enver Kahveci [a,b], Memed Duman [a,*]

[a] Nanotechnology and Nanomedicine Division, Institute of Science, Hacettepe University, Ankara, Turkey
[b] UNAM-National Nanotechnology Research Center, Bilkent University, Ankara 06800, Turkey

ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form
15 June 2016
Accepted 20 June 2016
Available online 22 June 2016

Keywords:
Microfluidic paper based analytical device
Microwave irradiation
Point of care
Ink-jet printing
Silylation
Cellulose fibers

ABSTRACT

A major application of microfluidic paper-based analytical devices (μPADs) includes the field of point-of-care (POC) diagnostics. It is important for POC diagnostics to possess properties such as ease-of-use and low cost. However, μPADs need multiple instruments and fabrication steps. In this study, two different chemicals (Hexamethyldisilazane and Tetra-ethylorthosilicate) were used, and three different methods (heating, plasma treatment, and microwave irradiation) were compared to develop μPADs. Additionally, an inkjet-printing technique was used for generating a hydrophilic channel and printing certain chemical agents on different regions of a modified filter paper. A rapid and effective fabrication method to develop μPADs within 10 min was introduced using an inkjet-printing technique in conjunction with a microwave irradiation method. Environmental scanning electron microscope (ESEM) and x-ray photoelectron spectroscopy (XPS) were used for morphology characterization and determining the surface chemical compositions of the modified filter paper, respectively. Contact angle measurements were used to fulfill the hydrophobicity of the treated filter paper. The highest contact angle value ($141^\circ \pm 1$) was obtained using the microwave irradiation method over a period of 7 min, when the filter paper was modified by TEOS. Furthermore, by using this method, the XPS results of TEOS-modified filter paper revealed Si2p (23%) and Si-O bounds (81.55%) indicating the presence of Si–O–Si bridges and Si(OEt) groups, respectively. The ESEM results revealed changes in the porous structures of the papers and decreases in the pore sizes. Washburn assay measurements tested the efficiency of the generated hydrophilic channels in which similar water penetration rates were observed in the TEOS-modified filter paper and unmodified (plain) filter paper. The validation of the developed μPADs was performed by utilizing the rapid urease test as a model test system. The detection limit of the developed μPADs was measured as 1 unit ml^{-1} urease enzyme in detection zones within a period of 3 min. The study findings suggested that a combination of microwave irradiation with inkjet-printing technique could improve the fabrication method of μPADs, enabling faster production of μPADs that are easy to use and cost-effective with long shelf lives.

1. Introduction

Biosensors are used for early diagnosis, and they play a crucial role in preventing the progression of diseases [1]. Point-of-care (POC) diagnostics have attracted much attention as they improve health and treatment in resource-limited settings. POC diagnostics are preferred to other detection techniques owing to factors such as disposability, affordability, ease-of-use, and portability [2–4]. Recently, there is a widespread increase in the desire and willingness to use substances made from paper and paper-like (e.g., nitrocellulose membrane) materials for POC test systems, as paper and paper-like materials are inexpensive, plentiful, and degradable [5]. Among these systems, microfluidic paper-based analytical devices (μPADs) are extremely promising, as they are cost-effective, easy to operate, quick, precise, and sustainable over time and different environmental conditions [6,7].

The main fabrication concept of μPADs includes the separation of hydrophilic channels by hydrophobic walls. Several different approaches have been proposed in the literature for development of μPADs, including photolithography [8], wax dipping [9], flexographic printing [10], wax printing [11,12], plotting [13], plasma etching [14], wax screen-printing [15], and knife and laser cutting [16,17] technologies. However, these techniques have their own disadvantages. An obvious and common limitation of these methods is the difficulty regarding the deposition of biological and chemical reagents in the final form of the test system [18]. Additionally, photolithography and wax dipping methods require multiple processing steps, sophisticated and expensive

* Corresponding author.
E-mail address: memi@hacettepe.edu.tr (M. Duman).

http://dx.doi.org/10.1016/j.talanta.2016.06.040
0039-9140/

instruments, and are not suitable for mass production. Bruzewicz et al. [13] used the plotting technique and Polydimethylsiloxane (PDMS) as a hydrophobization agent to generate a microfluidic pattern. The main disadvantages of this method include the complex determination of the appropriate viscosity of dissolved PDMS in hexanes, the resolution quality of hydrophobic barrier, and the long curing period of PDMS. The drawback of wax printing involves multiple fabrications steps such as the printing of wax on paper by a wax printer, heating the paper in an oven, and printing reagents on a test zone by inkjet printing. Moreover, the wax is unevenly spread on the paper, thereby lowering the resolution quality. Another disadvantage of this technique is the lack of proper facilities for printed reagents in wax printer [11,12]. Li et al. [19] used Alkenyl ketene dimer and Alkyl ketene as hydrophobic agents and n-heptane as a solvent to prepare a solution. As n-Heptane is highly flammable, it can be explosive when its vapor mixes with air. Given these factors, it could be argued that n-Heptane is harmful for printer parts and especially for cartridges. Wang et al. also fabricated paper-based microfluidic device by using harmless inorganic solvent (1 M NaOH) as an etching agent. In this study NaOH etched the methylsilsesquioxane (MSQ) modified paper and the performances of this procedure was compared to wax and alkylketene dimer (AKD)-modified filter paper's procedure [20].

Among printing technologies, the ink-jet printing technique possesses several advantages over the previously discussed fabrication methods. The main advantages of this technology are high pattern precision and resolution, the rapid printing of multiple pages, low cost, reproducibility, and the ability to work with very small volumes of ink (picolitres) [21]. In addition, inkjet printers were modified to fabricate multianalyte chemical sensing paper [22], print DNA chips [23], and cell patterns [24].

The above-mentioned reasons clearly indicate that there is still a strong need for new fabrication approaches with high reproducibility, fast manufacturing time, cost effectiveness, safety, simplicity, and ease of use.

This study presents a cheap, rapid, and easy fabrication method for μPADs based on chemical pattering by microwave irradiation and inkjet-printing. Hydrophobization of paper was achieved and compared using two different chemicals (Hexamethyldisilazane (HMDS) and Tetraethylorthosilicate (TEOS)), which were irradiated by a kitchen type microwave device. Moreover, other common methods, such as normal heating and plasma treatment, were tested in order to compare the silanization efficiency in microwave irradiation. Furthermore, developed μPADs were adapted to the rapid urease test as a proof of concept by spotting related biomolecules into detection zones using an inkjet printer. This new and rapid fabricating method had several obvious advantages given the low number of process steps, cost-effectiveness, and fabrication of the μPADs within the shortest time span (~10 min).

2. Materials and methods

2.1. Chemicals and materials

Whatman® qualitative filter paper, Grade 4, was obtained from Sigma-Aldrich with a thickness and pore size of 205 μm and 20–24 μm, respectively. HMDS (98%) was obtained from Alfa Aesar. TEOS (99%), and hydrochloric acid (HCl 37%) were obtained from Merck. Distilled and deionized water was used for all aqueous sample and dilutions. 50 mM urea solution (pH 5.5) containing 1.5 g urea (Sigma-Aldrich), 6.5 mg phenolsulfonphthalein (PSP) as an indicator, 20 mg citric acid (Acros Organics N.V.), and 30 mg Sodium phosphate (Sigma-Aldrich) was used. Urease solution (pH 6.0) was prepared by using a mixture of urease (4000 units g^{-1}, Jack Bean Urease from Sigma-Aldrich) in 0.1 M potassium phosphate buffer.

2.2. Hydrophobization of filter paper

In order to hydrophobize the filter paper, two different silylating agents HMDS and TEOS were selected and their silylation efficiencies were compared by environmental scanning electron microscopy (ESEM), x-ray photoelectron spectroscopy (XPS), and water contact angle (WCA) measurements. Diluted hydrochloric acid (HCl) was also used as a catalyst when the HMDS was used as a silylating agent. The hydrophobization of filter paper was mainly achieved by microwave (kitchen type) irradiation. Moreover, two different common methods (heating and plasma treatment) were also selected and used to compare the efficiency of microwave irradiation.

2.2.1. Silylation of filter paper cellulose with HMDS

The HMDS solution was prepared by a mixture of HMDS and 1 M HCl in a mole-ratio of 9:1 to hydrophobize the filter paper (1 cm × 1 cm). Three different methods were used for the silylation of paper cellulose. In the heating method, filter paper was dipped in a glass beaker containing both HMDS and 1 M HCl mixture. The beaker was placed in a water bath at 80 °C for 2 h, 4 h, 8 h, 16 h, and 24 h. Plasma treatment (Vacuum Praha, Czech Republic with a 13.56 MHz radio frequency generator) was used to deposit HMDS-HCl (1 M) solution as a monomer on filter paper. This procedure was performed at RF power levels of 50 W and 100 W for 10 min and 15 min in each power level. In the microwave irradiation method, filter papers were soaked in HMDS-HCl (1 M) solution, and were then processed in a kitchen type microwave oven (0.65–0.7 kW) for 5 min and 10 min. The chemical analysis of the surface of hydrophobized paper was performed using x-ray photoelectron spectroscopy (XPS, Thermo-K-Alpha-Monochromated high-performance XPS Spectrometer) in which a Al K-Alpha source gun was operated at 1.4 kV focus voltage, 6 mA beam current, and 400 μm spot size. An environmental scanning electron microscope (ESEM-FEI Quanta 200 FEG) was used to examine the morphology of treated filter papers. The hydrophobicity of the treated filter paper was also performed using WCA measurements (DSA100, Krüss Gmbh) at room temperature.

2.2.2. Silylation of filter paper cellulose with TEOS

TEOS was selected as an alternative silylation agent instead of HMDS. In this case, the same three methods (heating, plasma treatment, and microwave irradiation) were used to create a hydrophobic layer on filter paper. In the heating method, filter paper was treated with a heated TEOS solution (80 °C) for 2 h, 4 h, 8 h, 16 h, and 24 h in a water bath. In the plasma treatment, the silylation of cellulose fibers by TEOS was achieved at RF power levels of 50 W and 100 W for 10 min, 15 min, and 30 min. In the microwave irradiation method, filter paper was immersed in a TEOS solution and irradiated by microwave at 0.65–0.7 kW for 5 min and 7 min. The silylation efficiencies of the cellulose fibers were also characterized using XPS, ESEM and WCA measurements.

2.3. Generation of microfluidic channels on filter paper

Hydrophilic channels on the modified filter paper were generated by two different approaches. In the case of the first one, the inkjet printer (HP- Deskjet 670C) was used to apply different concentrations of HCl solution (0.001–1 M) to etch a hydrophobic layer on the paper. Multiple etching cycles were performed (printing cycles are 3 times), and the regeneration of the native hydrophilic characteristics of cellulose fibers was compared by XPS, ESEM, WCA, and Washburn assay measurements.