Printed Paper based Glucose Sensor Manufactured in Pilot Scale

Liisa Hakola, Kaisa Lehtinen; VTT Technical Research Centre of Finland; Espoo, Finland

Abstract

VTT has long experience in development of inkjet printable functional inks including printable enzymes [1]. Also a method for controlling liquid flow on paper has been developed by VTT [2]. This method is based on printed channel boundaries that guide flow of liquids such as water, blood, urine or other suitable liquid analyte. This technology can be used to manufacture inexpensive and simple-to-use diagnostic devices and tests that can provide results by visual colour change. This provides possibility for point-of-care or home testing. Use of paper based diagnostics has been proven to work for e.g. glucose indication in laboratory scale [3]. Furthermore, VTT has studied and developed enzymes suitable for use in diagnostic devices [4, 5]. Many of the enzymes can be applied by using different printing technologies. The objective of this study was to demonstrate the feasibility of paper based diagnostics in pilot scale by utilizing roll-to-roll flexography printing and industrial scale inkjet printheads. A successful glucose sensor demonstrator was realized.

Introduction

Nowadays many people require constant health monitoring such as diabetic patients who test their blood glucose level regularly. Global prevalence of diabetes is currently 285 million and is forecast to increase to 439 million in 2030 [6]. Also many people are keen to monitor their health condition for weight management, for healthy life styles or just for fun. Also inexpensive health monitoring and onsite analysis of, for example, water quality in developing countries is a large market. This means that low-cost, disposable, easy-to-use diagnostic tests for point-of-care, home or onsite use are required. The benefits of paper based diagnostics include [7]:

- They have simple design and low-cost
- They are portable, flexible, disposable and bio-compatible
- High throughput in manufacturing can be achieved
- They are reproducible with high sensitivity and accuracy
- There is no need for professional medical personnel or complicated instruments
- There is potential for integration of high-density detection systems into a small device

VTT has developed a printable technology for manufacturing paper based sensors. This technology is based on printing fluidic channels or more precisely printing hydrophobic channel boundaries that guide the liquid flow in porous substrates such as paper. The fluidic channels guide the liquid analyte, such as blood or urine, into reaction spots where visual colour change shows the result. The technology can be used for both qualitative and quantitative testing depending on the composition of the reaction spots.

For example, different amounts of the reagent can be printed into different reaction spots, and reaction speed or resulting colour can be used for quantitative analyses. Figure 1 shows one example of a sensor that has been studied in this paper. In this case different amounts of enzyme ink could be printed into the round reaction spots. When an analyte liquid is introduced into the center of the structure, the analyte propagates towards all the reaction spots and quantitative analysis could be achieved. The colour change would then occur only in those reaction spots that have enough reagent to react to a certain analyte concentration.

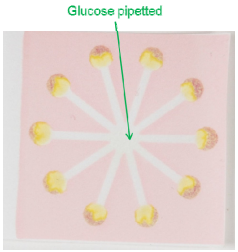

Figure 1. Fluidic channel structure used for demonstrating the glucose sensor. Light pink areas are the printed fluidic channel boundaries. Round areas that are partically red and partially yellow are the reaction spots. In this picture the colour change from red to yellow is not yet complete. The analyte is pipetted at the center of the structure.

The glucose sensor demonstrated in this paper consists of flexography printed liquid channel boundaries surrounding the depicted structure of Figure 1. Inkjet printing is used to print a colour changing ink, in this case a pH ink, and an enzyme ink to the reaction spots. The analyte liquid, in this case glucose, causes a reaction in the enzyme ink that triggers a pH reaction in the pH ink thus causing a visual colour change.

Materials and methods

Inks and substrates

The glucose sensor demonstrated here consisted of four printed layers, namely:

1. Flexography printed polystyrene layer for liquid guiding
2. Flexography printed PEI (polyethylene imine) layer for pH modification
3. Inkjet printed pH ink layer for visual colour change
4. Inkjet printed enzyme ink (glucose oxidase) for reaction with glucose

Figure 2 shows the printing layouts for layers 1, 3 and 4. The width of the flexography layout is 409 mm and of the inkjet layout is 32 mm.

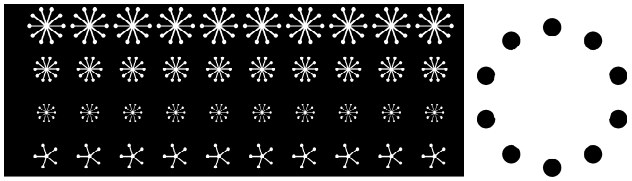

Figure 2. Printing layout for the glucose sensor: fluidic channel boundaries for flexography printing (on left) and reaction spots for inkjet printing (on right).

Tesorb paper substrate with 80 g/m^2 grammage from Tervakoski was used as the substrate.

Polystyrene with 192 k Mw was purchased from Sigma-Aldrich. For making a flexography printable ink 5 wt-% mixture was made with xylene. Also 1 g/10 g RED 40 UB 00 colorant from Sun Chemical was included in the ink in order to make liquid channel boundaries visible. This was required for proper registration of the inkjet printed layers.

PEI was purchased from Sigma-Aldrich. For making a flexography printable ink PEI was mixed with water (5wt-% PEI in water). PEI made the initially basic paper substrate alkaline which was a pre-condition for the pH ink to work for the purposes of the glucose sensor.

Inkjet printable pH and enzyme ink both contained the same ink base: PVP (polyvinylpyrrolidone), water, 1,2-propandiol and 0.05 % surfactant (Dynol 604). The pH ink contained approximately 2 wt % Phenol red dye (from Fluka) and the enzyme ink 5 mg/ml glucose oxidase. The pH ink resulted in colour change from red to yellow in the final demonstrator. The ink base was first mixed and then the dye or enzyme added. Before printing, the inks were filtered with 1 µm filter and left to stabilize overnight.

Printing equipment

Polystyrene was printed with VTT's pilot scale Maxi printing line and with laboratory scale RK Flexiproof as a reference (Figure 3). The polystyrene ink was printed on both sides of the substrate. For top side a channel layout was printed and for back side a compact area was printed. With Maxi two layers were printed on both sides with 30 m/min printing speed. Curing was done in 100 ^{o}C oven. Anilox size 15 cm^3/m^2 was used for the top side and 25 cm^3/m^2 for the back side. With RK Flexiproof three layers of the channel layout were printed on the top side and one layer of the compact are was printed on the back side. For both sides 18 cm^3/m^2 anilox and 60 m/min printing speed were used. No curing was needed.

Figure 3. Flexography printing equipment used: pilot scale Maxi printing line at VTT (on left) and laboratory scale RK Flexiproof printer (on right).

PEI was printed with RK Flexiproof printer. PEI layer could be eliminated by adding an alkaline component into the pH ink, such as NaOH (sodium hybdroxide).

Inkjet inks were printed with both laboratory scale inkjet printer (Dimatix Materials Printer DMP-2800 from Fujifilm Dimatix) and printer with industrial type printheads (Spectra S-Class printheads with Apollo II control unit both from Fujifilm Dimatix and XY-MDS2.0 precision table from iTi). The inkjet printers are in Figure 4. Both inks were printed only into the reaction spots of the biggest channel structure (in Figure 2 the uppermost channel layout). With DMP-2800 printer 10 pl drop size with 1270 dpi resolution was used. With S-Class printheads 30 pl drop size, 600 dpi resolution and 150 mm/s printing speed was used. One ink layer of the pH ink was printed. Enzyme loading was modified by printing several ink layers and 1, 2, 3, 5 and 10 layers were tested.

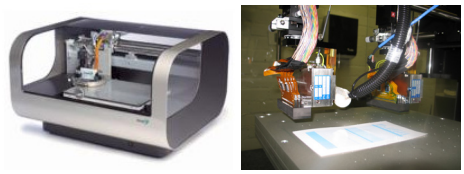

Figure 4. Inkjet printing equipment used: DMP-2800 (on left) and industrial scale printheads (on right).

Analysis of sensor performance

The performance of the glucose sensor was analyzed visually by manually pipetting 50 mM glucose into the fluidic channel. The colour change was monitored for duration of 10 minutes and photographs were taken 0, 5 and 10 minutes after glucose pipetting.

Results

Printability

The channel boundaries were successfully flexography printed with the laboratory scale printer since there is already earlier experience from that as described by Olkkonen et al [3].

With pilot scale flexography printing the channels with the smallest channel width were choked due to ink spreading and could not be used for making the final demonstrators (Figure 5). The sensor structures with the largest channel width performed well after printing and they were used for the final demonstrator. However, there was some leaking from top side of the paper substrate to the back side when introducing water drops into the channel. This might be eliminated by fine-tuning the pilot scale printing process and adding more polystyrene ink layer into the back side. From the laboratory scale samples also the ones with the largest channel width were used for the final demonstrator in order to have comparable results.

Figure 5. Pilot scale flexography printing of the bottom side of the paper (on left) and final flexography printed top side of the paper that shows how the smallest channels have choked (on right).

The pH ink was successfully inkjet printed and it provided good printability properties. The enzyme ink also provided good printability, but there was some gel formation during ink manufacturing. This was, however, eliminated by proper filtering before printing. With the laboratory scale inkjet printer at least five enzyme ink layers were required to produce the visual colour change. With the industrial type printheads even one ink layer was enough. This is due to the different drop sizes used which resulted in different enzyme loading on paper with one ink layer when using different printers.

Performance of the sensor

The idea of the sensor is that the glucose would be pipetted into the center of the structure and the liquid would flow to all reaction spots of a single structure. In analyzing the performance of the sensor the glucose was pipetted into a single channel in order not to waste printed samples. The demonstrators with the laboratory scale flexography printed channel boundaries were easy to demonstrate, but the demonstrators with pilot scale printed channel boundaries required careful fine-tuning of the inkjet printing and pipetting process. This is mainly due to channel leaking and choking challenges described.

Figure 6 shows a glucose sensor with laboratory scale flexography printed channel boundaries and laboratory scale inkjet printed reaction spots. The samples have five or ten ink layers of the enzyme ink thus corresponding to 0.0625 mg/cm^2 and 0.125 mg/cm^2 enzyme loading, respectively. The colour change is clearly visible after 10 minutes in both cases. With the higher enzyme loading some colour change can be already seen after 5 minutes.

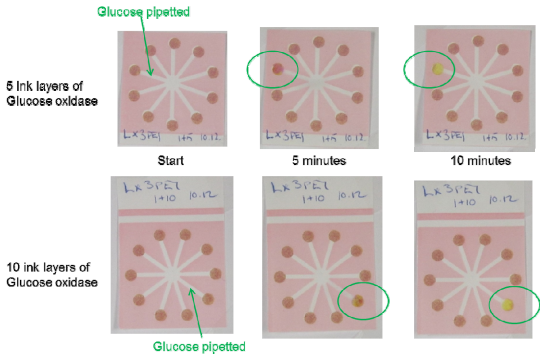

Figure 6. Laboratory scale flexography and inkjet printed samples with different enzyme loading. The glucose is pipetted into one channel and visual colour change can be observed in the marked reaction spot.

Figure 7 shows a glucose sensor with laboratory scale flexography printed channel boundaries and industrial scale inkjet printed reaction spots. The samples have one, two or three enzyme ink layers thus corresponding to 0.08 mg/cm^2, 0.16 mg/cm^2 and 0.24 mg/cm^2 enzyme loading, respectively. In this case all the samples needed 10 minutes to show visual colour change. With the one enzyme ink layer the colour change is weaker than with two or three enzyme ink layers.

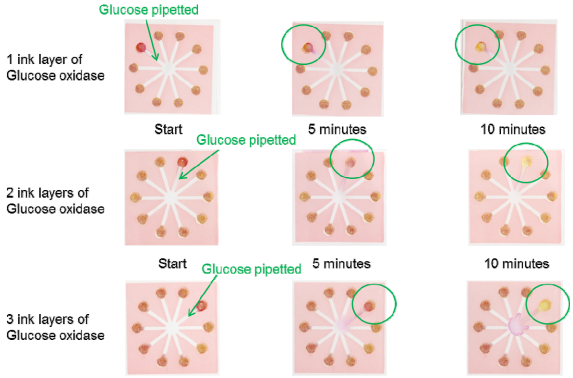

Figure 7. Laboratory scale flexography and industrial scale inkjet printed samples with different enzyme loading. The glucose is pipetted into one channel and visual colour change can be observed in the marked reaction spot.

Figure 8 shows a glucose sensor with pilot scale flexography printed channel boundaries and laboratory scale inkjet printed reaction spots. The enzyme loading is 0.0625 mg/cm^2 with five enzyme ink layers.

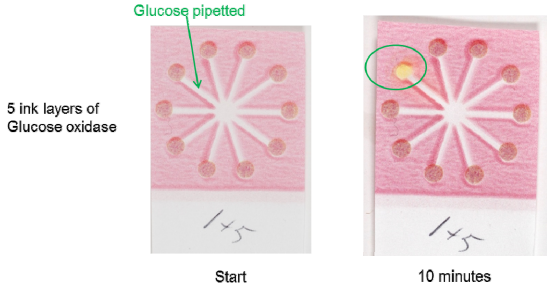

Figure 8. Pilot scale flexography and laboratory scale inkjet printed samples with 5 enzyme ink layers. The glucose is pipetted into one channel and visual colour change can be observed in the marked reaction spot.

Figure 9 shows a glucose sensor with pilot scale flexography printed channel boundaries and industrial scale inkjet printed reaction spots. The enzyme loading is 0.08 mg/cm^2 with one enzyme ink layer. This was the most difficult demonstrator version and many non-working demonstrators were observed.

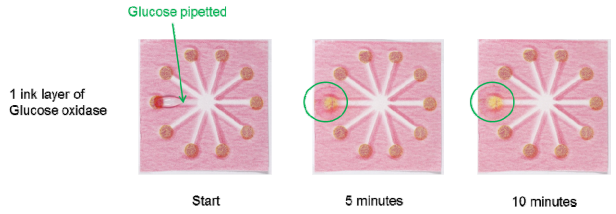

Figure 9. Pilot scale flexography and industrial scale inkjet printed samples with 1 enzyme ink layer. The glucose is pipetted into one channel and visual colour change can be observed in the marked reaction spot.

Conclusions

Successful demonstration of a pilot scale printable glucose sensor was realized with liquid channels manufactured in both pilot scale and laboratory scale flexography. The glucose concentration used in this study corresponds to those typical in blood thus making it sensitive enough for real applications. Inkjet printable pH and enzyme inks were developed. The inks were evaluated to be suitable for both laboratory scale and industrial scale inkjet printing. It was also realized that modification of enzyme ink loading can be used for modification of reaction speed of the glucose sensor demonstrator. This provides potential also for quantitative testing. Fine tuning of the pilot scale printed channels, of the thickness of PEI layer and of the enzyme loading in inkjet printing could further improve the performance of the demonstrator. The manufacturing process could be further simplified by adding an alkaline ink component into the pH ink thus eliminating PEI coating step.

To summarize, VTT is now able to tune printed fluidic channels to be manufactured in pilot scale and eventually in production scale in order to develop disposable paper based sensors.

Acknowledgements

The authors would like to thank research assistant Pirjo Hakkarainen for carrying out inkjet printing trials, and research assistants Jenni Tomperi and Pekka Ontero for carrying out the pilot scale printing trial.

References

[1] Liisa Hakola, Jonas Hartman, Kaisu Honkapää, Petri Laakso, Raimo Penttilä, Marja Pitkänen, Elina Rusko, Thea Sipiläinen-Malm, Ilkka Vanttaja. New methods for improving food product safety and communication. Proceedings of 29th International conference on Digital Printing Technologies & Digital Fabrication2013, NIP29, 29.9.-3.10.2013, Seattle, USA. p. 498–502.

[2] Patent application US2013004665. Inventors: Kaisa Lehtinen, Juuso Olkkonen. Assignee: VTT. Method of manufacturing liquid flow guiding structures to porous substrates. Priority date 15.12.2009, published 3.1.2013. 8 pages.

[3] Juuso Olkkonen, Kaisa Lehtinen, Tomi Erho. Flexographically Printed Fluidic Structures in Paper. Anal. Chem. vol. 82 (2010) p. 10246–10250.

[4] Heini Virtanen, Hannes Orelma, Tomi Erho, Maria Smolander. Development of printable bioactive paper containing laccase. Process Biochemistry, vol 47 (2012) 1496 – 1502.

[5] Heini Virtanen, Maria Smolander, Harry Boer. Development of printable indicators and sensors containing oxidoreductase enzymes. OxiZymes in Marseille 16-19 September 2012, poster.

[6] Anon. The Diabetes Market Outlook to 2016. Business Insights, Reference Code: BI00042-006, Publication Date: May 2011, 132 pages

[7] Andres W. Martinez, Scott T. Phillips, and George M. Whitesides. Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices. Anal. Chem. vol. 82 (2010) p. 3–10.

Author Biography

Liisa Hakola graduated as Master of Science for Graphic Arts from Helsinki University of Technology in 2002. Since her graduation Liisa has worked at VTT as Senior Scientist in the field of printed functional solutions. Her research work focuses on new indicator concepts, industrial inkjet printing, as well as printed electronics and diagnostics. She has presented several international scientific papers in many conferences.