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Inkjet Printed Polyelectrolytes for Microfluidic Paper-based Analytical Devices

Risto Koivunen[1], Eveliina Jutila[1], Roger Bollström[2], Patrick Gane[1,2]
[1] Aalto University; School of Chemical Technology, Department of Forest Products Technology; Espoo, Finland
[2] Omya International AG; Oftringen, Switzerland

Abstract

Paper-based microfluidic devices can provide practical analytics platforms for applications such as point-of-care medical diagnostics. So far immobilisation or separation of analytes on such devices has received limited attention. This study introduces inkjet printed polyelectrolyte patterns as possible platforms for immobilisation of cationic and anionic compounds through surface charge interaction. Both cationic (polydiallyldimethylammonium chloride) and anionic (sodium polyacrylate) polyelectrolytes were inkjet printed on a custom designed porous pigment coating, having fine particle internal pore structure to ensure high surface contact between the analytical sample and modified pore walls. Printed polyelectrolyte patterns were themselves largely invisible, including under UV light. In a proof of principle test, a controllable degree of separation of anionic Uranine and Tartrazine dyes from aqueous solution passing through a printed cationic polyelectrolyte region could be observed. However, weakly cationic Rhodamine B could not be captured on anionic regions.

Introduction and background

Paper-based analytical devices provide possibilities for easily transportable, inexpensive and disposable sensors in various fields of analytics, including in-field environmental monitoring [1] and point-of-care medical diagnostics. One major research area of such analytical devices is paperfluidics or paper-based microfluidics. On paperfluidic devices, aqueous liquid is transported in porous hydrophilic medium by capillary and/or surface wicking without external pumping, while hydrophobic patterns are used to direct the flow.

Liquid travelling on a paperfluidic device may pass through a number of reaction areas, where analytes of interest may react with pre-applied reagents, before arriving at a detection zone where the final result is analysed. Some examples of demonstrated reactions are detection of nitrite levels from saliva [2], detection of liver enzyme alanine aminotransferase from blood [3] and detection of glucose or ketones from urine [4]. Detection methods can vary from colorimetric analysis, either with the unaided eye or by external reader, to more sophisticated instrumental methods such as fluorescence microscopy or electrochemical detection [5]. Sensitivity of detection can be improved by concentrating samples by means of evaporation [6].

Despite the wide interest in paperfluidic devices, separation or immobilisation of analytes in such naturally porous matrix has received limited attention. With the ability to separate, components that could interfere with detection can be removed from the sample. An example of such is size-based separation of agglutinated red blood cells from blood plasma, required for colorimetric analysis of plasma content [7]. By employing an immobilisation mechanism, the analyte of interest is captured on a small test region for analysis. Immobilisation is common in lateral flow tests, such as the commercial early pregnancy test based on lateral flow and detection of human chorionic gonadotropin in urine [8].

This study investigates the application of a well-known phenomenon in colloidal chemistry in a possible new environment for the immobilisation and separation of analytes from liquid samples travelling on a paperfluidic device, namely the charge or hydrophobic destabilisation in aqueous dispersion of colloidal particulates or macromolecules, and/or the adsorption thereof, by local modification of pore surface chemistry. Two polyelectrolytes, cationic polydiallyldimethylammonium chloride (polyDADMAC) and anionic sodium polyacrylate (SPA), were inkjet printed in aqueous solution on a speciality designed porous coating material in order to adjust the surface charge of the pore walls selectively. These polyelectrolytes are commercially used in industrial applications, for example as additives in paper making and paper coatings to provide dispersion, or in wastewater purification as thickeners to provide flocculation. Presence of cationic polyelectrolyte as additive in paper has been discovered to improve deinkability of papers printed with dye-based inkjet inks [9].

Besides their use as paper-making additives, polyelectrolytes have also been applied experimentally to papers as surface treatments. Multi-layer polyelectrolyte treatment has been found to affect spreading of inkjet ink on paper, with the specific effects depending on the number of layers and the nature of the top layer [10].

In the field of paper-based diagnostics, the effect of two cationic polyelectrolytes, used as paper-making additives to improve retention and wet strength, has been studied in relation to paper-based blood typing sensors, with high molecular weight cationic polyacrylamide discovered to increase retention of red blood cells on paper, presumably due to electrostatic interaction possibly assisted by some amount of cell flocculation [11].

Inkjet deposition of alternating cationic and anionic polyelectrolyte solutions has been studied for formation of multilayer polyelectrolyte structures, such as self-assembling hydrogels [12], micro-arrays encapsulating fluorescent Rhodamine 6G dye [13] and surface-functionalised nano-mechanical cantilevers [14]. Multilayer structures have also been formed out of alternatingly printed layers of anionic nano-gold particle and cationic polyelectrolyte solutions [15].

Inkjet printed polyelectrolytes have also been applied into patterning top-layers of dip-coated polyelectrolyte multilayers. Compounds of interest can then be selectively adsorbed onto the patterned areas by exposing the surface to a suitable solution. This method has been used to form conductive circuitry by dipping the surface into catalyst and metal plating solutions to adsorb metal ions [16], and to form arrays of biosilica frustules [17].

In addition to jetting on its own, anionic polyelectrolyte has also been applied as a component in the synthesis of stable inkjettable silver nanosol dispersion for printed electronics [18]. When a polyelectrolyte is used as a stabilising agent, room-temperature sintering by silver particle coalescence can be triggered by exposing the stabilised particles to oppositely charged polyelectrolyte, either by pre-applying the polyelectrolyte to the substrate before printing, or by printing it separately afterwards [19].

As is the case for polymers in general, polyelectrolyte solutions are affected by the high shear rates present in inkjet printheads, which limit jettable polymer content within an ink as increasing polymer molecular weight increases the viscoelastic behaviour. However, in some cases these limitations can be circumvented by jetting a solution of monomers or oligomers and only polymerising it into a polyelectrolyte on the actual substrate [20].

In order to form a working printed polyelectrolyte separation or immobilisation zone, as investigated in this study, the following requirements should be met:

1. polyelectrolyte ink has to be reliably inkjettable with sufficient dry solids content,
2. polyelectrolyte ink has to penetrate the full depth of the porous substrate,
3. deposited polyelectrolyte has to remain immobile on the surfaces of the porous substrate when re-wetted – a renowned problem in some cases if adsorption is not supported by charge (Coulombic) attraction with the surface or by van der Waals forces,
4. deposited polyelectrolyte has to be able to capture analytes of interest by surface charge, and
5. polyelectrolyte surfaces should not become fully saturated by the adsorbed analytes from a sample.

In order to ensure close interaction between the modified pore walls and liquid samples, a custom pigment coating consisting of functionalised calcium carbonate (FCC) and micro-fibrillated cellulose (MFC), inspired by earlier studies on functional coatings by the authors [21], was utilised as the porous base material. This custom material has finer pore dimensions than the cellulose filter and chromatography papers most commonly used in paperfluidic studies. Although MFC itself is swellable in water, its fine particle nature, with little to no long range structure, together with the particulate inorganic pigment avoids problems with swelling and warping of a fibre-based sheet, which is likely to happen to cellulose papers when subjected to the large volumes of aqueous ink required for full-depth ink coverage of the porous substrate, as well ultimately due to the wetting of the sample itself.

While printing of hydrophobic barriers, demonstrated also by the authors for cellulosic papers [22] and functional pigment coatings [23], is the most common way of patterning paperfluidic devices, this study uses cutting, a method capable of producing quite complex paperfluidic patterns [24], to form simple channels for the initial testing. However, in the longer term, polyelectrolyte patterns are intended to be integrated into the same devices having hydrophobic barriers. Figure 1 demonstrates a potential device design that could be achieved by combining hydrophobic and polyelectrolyte patterns.

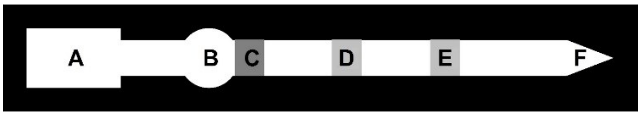

Figure 1. Potential device design displaying (A) elution zone, (B) reaction zone, (C) valve mechanism, (D) separation zone, (E) immobilising zone and (F) concentration zone. Black walls indicate printed hydrophobic barriers, while white area indicates native hydrophilic substrate.

The device is operated by placing analytes of interest on the reaction zone (B), where they react with pre-applied reagents, such as enzymes, and catalysts. This zone is kept wet by water transported from the elution zone (A), connected to a liquid supersource by capillary action, thus compensating for evaporation. The valve mechanism (C) initially prevents the sample from travelling further, but after a pre-defined period of time it is activated, and the excess liquid from the elution zone transports the reaction products and catalysts to the channel beyond. The anionic reaction catalysts, which might interfere with detection, are captured on a cationic separation zone (D) while the cationic reaction products are captured at an anionic immobilising zone (E), with possible non-ionic compounds present travelling freely to the concentration zone (F) where they become concentrated at the tip of the triangular configuration by a combination of evaporation and capillary action, providing a qualitative Marangoni effect.

Materials and Methods

Functional pigment coating

The speciality coating consists of:

1. porous functionalised calcium carbonate (FCC) pigment (Omya International AG, Oftringen, Switzerland), formed to contain 11.9 % calcium carbonate and 88.1 % hydroxylapatite with a specific surface area of 160 m2g^{-1}, and
2. micro-fibrillated cellulose (MFC) Arbocel MF-40-7 (J. Rettenmaier & Söhne GmbH + Co KG, Rosenberg, Germany), added at a level of 20 pph, based on 100 parts by weight of pigment, as a binder.

The coating slurry was prepared to a dry solids content of 13.8 w/w% and coated on impermeable SuperYUPO® pigmented polypropylene film (Yupo Corporation, Tokyo, Japan), formerly known as Synteape®, with a mechanical drawdown K202 Control Coater (RK PrintCoat Instruments Ltd., Herts, UK) using the orange labelled wire-wound rod, applying a 60 µm thick wet film onto the substrate, with a speed setting of 6 m·min^{-1}. Coated substrates were allowed to dry at room temperature overnight. Dried coating layers had a basis weight of 7.8 ± 1.3 g·m^{-2} and thickness of 24 ± 6 µm.

Functional inks and printing

The two inks, referred to from here on as polyDADMAC ink and SPA ink, consisted of 1.0 w/w% polyelectrolyte, either polyDADMAC with average molecular weight < 100 kDa (Sigma-Aldrich, St. Louis, USA, product code 522376, supplied at 35 % dry solids content) or SPA with minimum molecular weight > 1 kDa, available under the commercial tradename Topsperse G XN (Coatex, Genay, France, supplied at 70.2 % dry solids content), respectively, in aqueous solution with 25 w/w% ethanol to decrease surface tension. Easily evaporating organic solvent was preferred over surfactants for reducing surface tension, since the latter can remain on the printed device and be dissolved when the coating is re-wetted. On a paperfluidic device with hydrophobic barriers, such free surfactant molecules could subsequently adsorb onto the hydrophobised surfaces and cause leaks.

The ink surface tension was measured with a CAM200 contact angle measurement system (KSV, Espoo, Finland) employing the pendant drop method, in a laboratory room maintained at 22 °C. Viscosity was measured with an MCR-300 rheometer (Paar-Physica, Graz, Austria) at a shear rate of 100 s^{-1} at 30 °C.

Jettability of inks was studied and patterns printed with a DMP-2831 inkjet printer (Fujifilm Dimatix, Santa Clara, USA) employing DMC-11610 ink cartridges with 10 pl nominal drop volume. The printhead was maintained at 30 °C while the printer mounting plate was heated to 40 °C to promote drying of the inks. Printing was performed with 15 out of 16 available nozzles.

For the pattern testing, rectangles of nominally 4 x 180 mm^2 were printed with both polyelectrolyte inks, adopting a 10 µm drop space setting and applying 1, 3 or 5 layers of ink on top of each other. When applying multiple layers, the previously printed layer was allowed to dry for 1 min before printing the following new layer.

Separation tests

Printed substrates were cut with scissors into approximately 3 x 50 mm^2 strips, so that a polyelectrolyte printed region was to be found between 18 and 22 mm from the bottom of the strip. These strips were then attached onto further 90 x 85 mm^2 sheets of SuperYupo, marked at 10 mm intervals, using double-sided tape so that the bottoms of the strips matched with the bottom of the sheet. Each sheet contained seven strips, one with native coating, three with SPA ink (1, 3 and 5 layers) and three with polyDADMAC ink (1, 3 and 5 layers). The mounted sheets were then attached with masking tape to a 100 x 100 mm^2 glass slide to provide solid backing.

The following water-soluble dyes were chosen as model compounds for separation tests, with each dyestuff dissolved in deionised water at 0.1 w/w%:

1. Crystal violet (Merck KGaA, Darmstadt, Germany, product number 1.15940.0025)
2. Rhodamine B (Kremer Pigmente GmbH & Co. KG, Aichstetten, Germany, product number 94900)
3. Uranine (Kremer Pigmente GmbH & Co. KG, Aichstetten, Germany, product number 94236)
4. Tartrazine 85 (Kremer Pigmente GmbH & Co. KG, Aichstetten, Germany, product number 94175)

Crystal violet is a cationic colourant, Rhodamine B is very weakly cationic, while Uranine and Tartrazine 85 are anionic. Single 0.5 µl drops of the chosen solution were applied with Microcaps micropipettes (Drummond Scientific Company, Broomall, USA) to the coating strips, centred approximately 10 mm from the bottom of the strip. Further 0.5 µl drops of non-ionic 1 w/w % Ferroin solution (Merck KGaA, Darmstadt, Germany, product number 1.09193.0100), for highlighting the water wicking front, were applied approximately 30 mm from the bottom of the strip. The drops were allowed to dry in air for at least 30 min.

Passage and separation of the colourants on the strips were observed in a thin layer chromatography chamber (Camag, Muttenz, Switzerland). Approximately 5 mm of deionised water was placed in the bottom of the chamber, with the atmosphere inside the closed chamber being allowed to saturate for approximately 15 min before placing the first set of samples inside. The samples were positioned inside the chamber so that the bottom of each of the coating strips was in contact with the water, allowing it to absorb into the highly wicking speciality coating. Water then travelled along the coating, dissolved the dye from the coating at its position of application and carried it to the polyelectrolyte treated region. Figure 1 shows a schematic of the experimental design to identify the elution and trapping of the dyestuff whilst recording the position of the advancing water front. The samples were observed for 8 min inside the chamber.

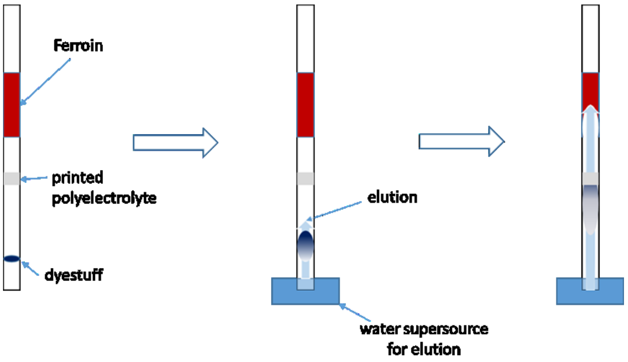

Figure 2. Schematic of the elution and water front identifying technique.

Results and discussion

Ink jettability

Both the anionic and cationic polyelectrolyte inks were found to be jettable with the printer used. Experimentally determined jetting settings and custom waveforms for the two polyelectrolyte inks are listed in Tables 1 and 2.

Table 1: Ink characteristics and jetting settings.

Ink	PolyDADMAC ink	SPA ink
Surface tension / mN·m^{-1}	36.0 ± 0.8	35.9 ± 1.6
Viscosity / mPa s	2.5 ± 0.1	1.6 ± 0.1
Jetting frequency / kHz	6	6
Jetting voltage / V	18	18
Jetting speed / m·s^{-1}	10	5

Table 2: Custom waveforms for the inks.

Ink	Segment	Level / %	Slew	Duration / µs
PolyDADMAC	Jetting 1	0	0.65	3.392
	Jetting 2	100	1.20	4.672
	Jetting 3	67	2.00	2.944
	Jetting 4	13	2.00	0.512
	Non-jetting 1	40	1.00	3.712
	Non-jetting 2	27	1.00	6.976
	Non-jetting 3	13	1.00	0.832
SPA	Jetting 1	100	0.35	5.632
	Jetting 2	0	0.06	21.248
	Non-jetting 1	27	0.35	5.632
	Non-jetting 2	0	0.06	21.248

In preliminary tests, SPA ink proved to be moderately reliable with the given print settings, being repeatedly able to jet for at least 30 min in one go with all nozzles still jetting in the end. PolyDADMAC ink on the other hand displayed poor reliability, with nozzles frequently becoming non-jetting, though not actually clogged, presumably due to the high molecular weight and resulting viscoelastic effects at printhead.

Detection of printed patterns

Polyelectrolyte rectangular areas printed on the coating were difficult to detect with the unaided eye. When applied at 10 µm drop spacing, a single layer of either ink could be detected when the substrate was observed against a light table, but not under ordinary office lighting. When 3 or 5 layers of either ink were applied, the pattern could also be detected with care under office lighting. The nominally 4 mm wide rectangle was measured as actually being approximately 5 mm wide. This amount of line spreading suggests that the deposited inks likely covered also the whole depth of the coating, though this does not automatically guarantee that the contained polyelectrolyte also penetrated the whole depth.

To evaluate the visual detection of the inks, single layer test rectangles were also printed with both inks at drop spacing settings of 12, 15 and 20 µm, resulting in lower applied ink volumes per unit surface area. When printed with SPA ink, none of these patterns were visible when examined against a light table. With polyDADMAC ink, a pattern printed at 12 µm drop spacing was faintly visible against a light table, while patterns printed at 15 and 20 µm drop spacing were not.

The patterns were also observed under 365 nm wavelength UV light. In this case, 3 or 5 layers of either ink applied at 10 µm drop spacing could be faintly detected, while the others were not observable. Fluorescence of both the native and printed coating will be studied more quantitatively in future work, as low background fluorescence from the coating and polyelectrolytes is beneficial when a detection method based on fluorescence, such as fluorescence microscopy, is used for detection of analytes. However, cationic polyelectrolytes are known to sometimes quench fluorescence of other materials, which might in some cases result in weaker limit of detection. However, fluorescence quenching itself can also be used as an analytical tool, as demonstrated with a paper-based explosive detector [25].

Separation of cationic compounds

Figure 3(a) displays the results of pilot tests conducted with strongly cationic Crystal Violet, on samples featuring either no polyelectrolyte region or 1, 3 or 5 layers of SPA or polyDADMAC ink, with the images taken after 8 minutes of elution. Figure 3(b) displays the same for the very weakly cationic Rhodamine B.

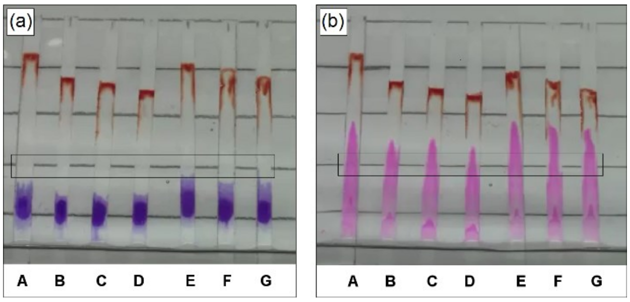

Figure 3. *(a) Crystal Violet dye (violet) after 8 min of elution on samples featuring either plain coating (A), 1/3/5 layers of SPA ink (B-D) or 1/3/5 layers of PolyDADMAC ink (E-G), with the approximate location of the polyelectrolyte printed areas marked with the black rectangle. Ferroin colourant (red) on top indicates the water front. (b) Rhodamine B dye (magenta) after 8 min of elution using the same patterning as in (a).*

Crystal Violet dye transported very slowly on the coating compared to the water front, and never reached the printed polyelectrolyte zones within the observed period. This is presumably due to interaction between the cationic dye and anionic charge on the partly zwitterionic FCC and anionic MFC binder in the coating. Rhodamine B, being only very weakly cationic, transported faster but did not exhibit immobilisation on the polyelectrolyte regions, presumably due to low charge-to-mass ratio.

Separation of anionic compounds

Figure 4(a) displays the results of tests conducted with anionic Uranine, on samples featuring either no polyelectrolyte region or 1, 3 or 5 layers of SPA or polyDADMAC ink, with the image taken after 8 minutes of elution. Figure 4(b) displays the same for anionic Tartrazine 85.

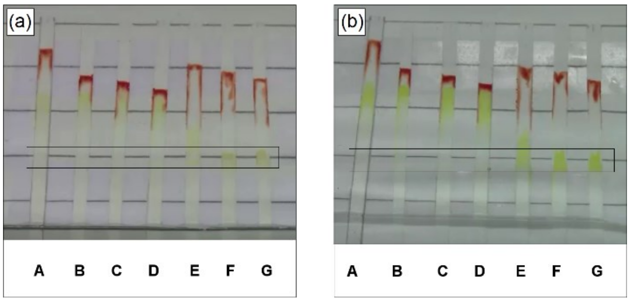

Figure 4. *(a) Uranine dye (yellow) after 8 min of elution, with UV illumination applied to enhance visibility, on samples featuring either plain coating (A), 1/3/5 layers of SPA ink (B-D) or 1/3/5 layers of PolyDADMAC ink (E-G), with the approximate location of the polyelectrolyte printed areas marked with the black rectangle. Ferroin colourant (red) on top indicates the water front. (b) Tartrazine 85 dye (yellow) after 8 min of elution using the same patterning as in (a).*

Uranine dye is light yellow in colour, and thus not very strongly visible in Figure 4(a), even though additional 365 nm UV illumination has been applied to take advantage of its fluorescence. Imaging of Uranine was also attempted under UV light only, for stronger contrast, but this did not produce pictures of sufficient quality. Nonetheless, careful examination of B-D shows that there is little separation of Uranine from the water front when the sample has passed through the anionic SPA region. On E, featuring a single layer of printed cationic polyDADMAC, part of Uranine has passed through the printed region, though significantly slowed down in the process. On F and G, featuring 3 and 5 printed layers of polyDADMAC respectively, the Uranine dye has been completely captured by the polyelectrolyte region.

As shown in Figure 4(b), Tartrazine 85 has a stronger yellow colour and is thus more visible on the coating. Furthermore, it can be seen to behave in a very similar fashion to Uranine, confirming the ability of the printed cationic polyDADMAC region to slow down or capture anionic molecules.

Conclusions

This first stage study demonstrates that polyelectrolyte solutions can be inkjet printed on pigment coating. Furthermore, it shows that printed cationic polyelectrolyte regions impart a chromatographic effect on anionic analytes passing through them, with the magnitude of effect depending on the number of printed polyelectrolyte layers. This immobilisation also indicates that the cationic polyelectrolyte has formed a water-fast layer on the coating, and so has adsorbed onto the partial zwitterionic anionic sites of the FCC, and/or on the anionic MFC binder.

With printed anionic polyelectrolytes, results were limited. Cationic Crystal Violet dye transported so slowly on this coating that it never reached the printed region, due to the strong anionic charge of the components in the coating. Thus, anionic polyelectrolyte regions on this coating design might be redundant, due to strong capture of the dye by the coating, but on less anionic coatings they should still be useful. No clearly observable effect was achieved in the case of Rhodamine B, displaying the limitations of the method when applied to only weakly charged or charge-hidden molecules.

The concept is demonstrated here for simple colourants, but the principles should apply to any compounds exhibiting surface charge in solution, if they do not undergo size exclusion.

Future work

The separation concept will be explored with further sample analytes. Furthermore, the permanence of polyelectrolyte immobilisation in the coating will be investigated. In the longer term the study will be extended to cover other polyelectrolytes, especially such that could be reliably jetted at higher concentrations than the 1 w/w% illustrated in this study.

The effect of different quantities of analyte reaching the polyelectrolyte regions may be studied to characterise possible saturation of the polyelectrolyte surfaces by the analyte. To this purpose, the pipetting method of colourant application lacks accuracy, and inkjet printing could be employed as an alternative method for applying the test analyte on the samples. Combining polyelectrolyte patterns with printed hydrophobic barriers will provide opportunities for more complex test patterns.

Acknowledgements

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Author Biography

Risto Koivunen received his M.Sc. (Tech) degree in printing technology from Aalto University. Currently he is pursuing doctoral studies at Aalto University, specialising in functional inkjet printing for paperfluidic applications.