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BIOACTIVE ENZYMATIC PAPERS

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ABSTRACT

The thermal stability of Alkaline phosphatase enzyme (ALP) adsorbed on paper was measured using a colorimetric technique quantifying the intensity of the product complex. ALP adsorbed on paper retains its functionality and selectivity. Adsorption of ALP on paper increased the enzyme thermal stability by 2 to 4 orders of magnitude compared to the same enzyme in solution. Complex patterns of enzyme were also printed using a thermal inkjet printer on paper. Microfluidic channels were printed on paper to demonstrate the concept of paper-based bioassays as diagnostic devices. Paper is an ideal material for functional materials for functional bioactive surfaces.

INTRODUCTION

The benefits of many breakthroughs in biotechnology, medicine and environmental science have been restricted by the high cost and the limited availability of tests and materials. Most methods developed in the laboratory are difficult to scale up for mass production manufacturing. There is a need for low cost bioassays, in health and environmental diagnostics. Disposable materials for routine bioassays for the early detection of cancers and genetic conditions, for daily tests to monitor diabetes, and for instant water analysis

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of heavy metals and microbial activities are potential applications. Successful commercialization requires bioassays to be low cost, best achieved through high volume manufacturing and with commodity materials. Paper and printing are two technologies meeting these criteria. Paper, a non-woven made of cellulosic fibres, is highly wettable when untreated, easy to functionalise, biodegradable, sterilisable, biocompatible and cheap. It has long being used for analysis in chromatography [1, 2]. Printing is a high speed technology, highly adaptive and able to deliver patterns of materials such as functional fluids at an exceptional accuracy. Liquid flow in paper proceeds through capillarity action which is affected by paper structure and chemical treatment. Also, thin coatings of polymers and inorganics can be achieved through wet-end addition, surface sizing, coating and other surface treatments. This suggests that paper is the natural material of choice for the production of disposable bioassay devices. However, the biotechnology industry has a limited understanding of the effect of paper structure and chemical composition on fluid transport or in the biomolecule functionalisation of paper.

The concept of paper bioassays is to rely on paper for the transport of fluids which is achieved through capillary flow of the analyte within the paper structure. Hydrophobic barriers can be printed onto paper to control the directionality of the flow. Microfluidic systems for paper diagnostics have been pioneered by Whitesides at Harvard University [3, 4]. A first generation system was achieved by flexographic printing of a rigid photoresist polymer onto filter paper to provide channels. A second generation was made by plotting circuits with a pen filled with a polydimethylsiloxane/hexane solution [5]. Low resolution, low manufacturing speed, and poor resolution are serious limiting issues for these systems.

Peptides, enzymes and cells have already been printed under laboratory conditions using a variety of technologies including inkjet printing [6–16]. However, most trials have been limited to low volumes/areas and to very simple patterns. No strong fundamental understanding has been developed of the effect of the surface on biofunctionality. Very few studies have investigated paper as a substrate for bio-printing and bioactive paper.

Bioactive papers can also have industrial applications. Peptides and Enzyme immobilized on paper for the catalytic production of biofuels (diesel and ethanol), antibody immobilized on paper for the high selectivity separation of antigen in blood or fermentation streams, and antimicrobial papers are a few potential applications. The chemical stability, ability to be sterilized, easy functionalization, porosity, strength and light weight of paper also make it an ideal composite for biosurface engineering. Few scientific studies have described the immobilization of enzyme on paper. Among those are paper strips to test fish freshness using 2 enzymes (xantine oxidase and nucleoside

phosorylase) [17], and bioactive paper to monitor alcohol content in the breath using alkaline oxidase (AOD) [18].

Enzymes are model biomolecules of choice for a fundamental study because of their stereospecificity, there wide range and availability. While enzyme engineering and enzyme immobilization are well understood [19], there is little knowledge on the effect of the biomolecule spatial orientation, surface property and interaction biomolecule-surface on the functionality of immobilized enzymes and peptides. Voss et al. (2007) reported that the stability of HRP immobilized within porous silica nanoparticle increases by over an order of magnitude compared to the molecule in solution [20].

The first requirement of a bioactive paper is to retain the functionality, reaction rate and selectivity of its immobilized biomolecule. The second is to provide good biomolecule stability. The objective of this study is to evaluate the potential of paper for enzymatic bioactive paper for catalytic and diagnostic application. Alkaline phosphatase (ALP) was selected as model enzyme.

In the first part of the study, the thermal stability of Alkaline Phosphatase adsorbed on paper is measured and modeled. In the second, microfluidic systems and enzymatic patterns are printed by inkjet on paper. Last, the requirements and potentials of bioactive paper for diagnostic and industrial surfaces are analyzed.

THEORY

The kinetics of enzyme-substrate reaction can be expressed with the two-steps Michaelis-Menten model [19]:

$$E_a + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_aS \tag{1}$$

$$E_aS \xrightarrow{k_2} P + E_a \tag{2}$$

where E_a, S, P and ES represent the enzyme, substrate, product and enzyme complex concentration, respectively. However, enzyme activity is known to decrease as a function of time as some enzyme molecules become inactive when removed from their native biological surroundings [19]. The simplest enzymatic deactivation model consists of an active enzyme (E_a) molecule undergoing an irreversible structural or chemical change to some inactive form (E_i^*) [19].

$$E_a \xrightarrow{k_d} E_{i_1}{}^* \tag{3}$$

This model assumes that only the free enzyme can become inactive as complexation with the substrate stabilizes the integrity of the active site. By operating under excess of substrate, we can assume that at any time there is only a negligible concentration of free enzyme. With these assumptions and at equilibrium, the enzyme deactivation rate (r_d) can be written as been proportional to the active enzyme concentration:

$$r_d = k_d[E_a] \tag{4}$$

where, k_d is the deactivation rate constant. For a closed system the rate reaction (Eqn. 4) can be expressed as a function of time as:

$$\frac{d[E_a]}{dt} = -k_d[E_a] \tag{5}$$

$$\frac{d[E_a]}{[E_a]} = -k_d dt \tag{6}$$

From integration,

$$\ln \frac{[E_a]_{t=t}}{[E_a]_{t=0}} = -k_d t \tag{7}$$

$$\log \frac{[E_a]_{t=t}}{[E_a]_{t=0}} = -\frac{1}{2.30} k_d t \tag{8}$$

Enzyme deactivation is expected to follow a first order kinetics.

EXPERIMENTALS

Materials

Horseradish peroxidase (HRP) and Alkaline phosphatase (ALP) enzymes were both purchased from Aldrich and used as received. HRP was used to formulate bio-ink and ALP to be immobilized on paper to study deactivation of bioactive papers. HRP was dissolved in 100 mM sodium-phosphate buffer solution (pH 6.0) to a concentration of 1.0 mg/mL to print on paper. A liquid substrate system, 3,3′-Diaminobenzidine (DAB), from Aldrich, was used to identify the enzymatic activity of the printed HRP. ALP was dissolved to a concentration of 0.1 mg/mL in 1.0M diethanolamine buffer with 0.50 mM magnesium chloride and 5 M HCl to maintain pH at 9.7. Water (Millipore,

18 mΩ) was used for making all dilutions. The 5-bromo-4chloro-3indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) liquid substrate system, purchased from Aldrich, was used to quantify the enzymatic activity of ALP on paper. The biochemical reaction of ALP with BCIP/NBT results in a blue-purple complex; its intense colour can be observed visually, is very stable and will not fade upon exposure to light [21]. A filter paper (Whatman #4) was used as paper substrate to print on and immobilize biomolecules. Alkyl ketene dimer (Wax 88 konz, BASF) and alkenyl ketene dimer (Precis 900, Hercules Australia Pty Ltd) were used as cellulose hydrophobization agents. Both dimers have two hydrocarbon chains of C16–C20. Alkenyl ketene dimer, however, has one –C = C– in each of its two hydrocarbon chains. Analytical grade n-heptane (Aldrich) was selected as the solvent to make solutions of both dimers.

Methods

Printing HR-enzyme and microfluidic channels on paper

A basic Canon inkjet printer (Pixma ip4500) and ink cartridges (CLI, Y-M-C-BK, PGBK model) were reconstructed to print the hydrophobization solution and the HRP-enzyme solution on paper. This bubble jet printer offers a resolution of 9600 × 2400 dpi and is controlled by a personal computer which supplies page-data using MS Office 2003 software.

Microfluidic channels were printed on filter paper using an inkjet printer with electronically generated patterns of an alkenyl ketene dimer – heptane solution (6%, v/v). AKD printing left no visible mark on paper which retained its original flexibility. The printed filter paper samples were then heated in an oven at 100°C for 45 minutes to cure alkenyl ketene dimer on cellulose fibres.

Immobilization of enzyme on paper

Enzyme aqueous solutions were homogeneously applied onto paper following TAPPI standard (T 205 sp-95). Basically, circular paper samples (16 cm) were immersed into the enzymatic solution contained in a large Petri-dish. To ensure uniform enzyme concentration on paper, the samples were carefully kept horizontal while being removed from the stock solution. The well-soaked paper samples were then blotted using one set of standard blotting papers (Drink Coster Blotting, 280 GSM) to remove any extra solution. A polyethylene sheet (3M PP2500) (d = 16 cm) was placed underneath each blotted sample. Paper and polyethylene sheet were placed into a drying ring

assembled so that each sample bioactive paper-sheet is uppermost and in contact with the rubber seat of the next ring. The samples were left to dry in a dark chamber at 23°C and 50% relative humidity for 24 hours. The bioactive paper samples were then used for further deactivation experiment and this time was set as t = 0.

To visualize the uniformity of enzyme distribution on paper, a diluted ink-jet ink (cyan) was applied on the paper surface using the same technique. Analysis of the surface profiles (Figure 1a) and histogram distribution of grey values (Figure 1b) of five replicates of enzyme papers revealed a uniform distribution of ink on paper. The statistics of theses tests are summarized in Table I.

Thermal stability of bioactive papers

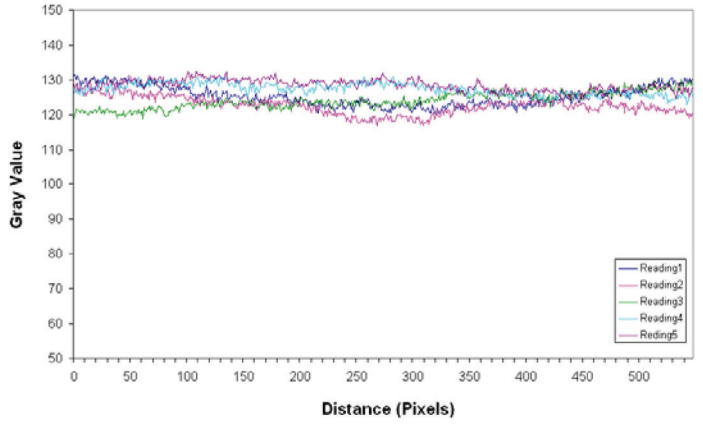
The bioactive papers were cut into small samples (6 cm × 2 cm) and aged at different temperatures in an oven. The ALP-paper was treated at 23°C, 60°C or 90°C. Bioactive papers were exposed to its liquid substrate after the aging treatment. This was done as follow: small droplets of freshly prepared liquid substrates were applied onto the aged bioactive papers using a 1.0 mL syringe equipped with a stainless steel flat-tipped needle (0.21 mm outer diameter). The enzyme-substrate reaction was allowed to proceed in a dark chamber for 2 hours at 23°C and 50% relative humidity; this treatment insures complete enzymatic reaction [22, 23]. From the colour intensity of enzyme-substrate reaction, the relative activity of the bioactive paper was measured. Each measurement reported results from the average of 6 to 8 full replicates.

Image analysis and activity measurement

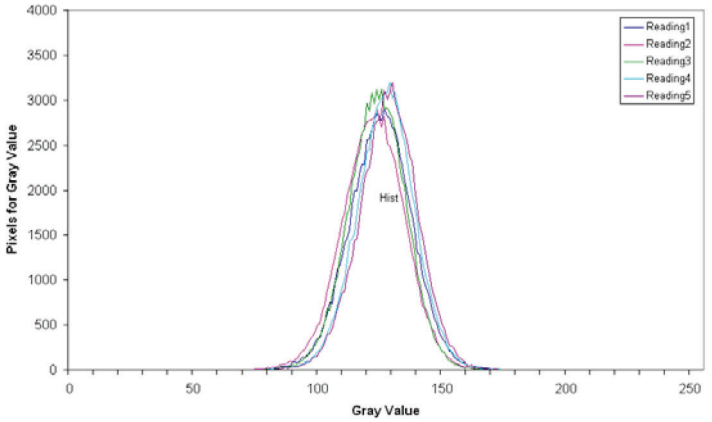
The colour intensity resulting from the enzyme-substrate reaction of bio-active papers was measured at 1200 dpi using a standard scanner (EPSON

Table I. Concentration distribution parameters of grey values.

Reading	*Area (pixels)*	*Mean*	*Standard Deviation*	*Relative Variability*	*Mode*	*Median*
1	87132	125.4	12.5	9.9	127	126
2	87132	122.6	12.4	10.2	124	123
3	87132	124.1	11.4	9.2	126	124
4	87132	127.3	11.6	9.1	129	128
5	87132	128.4	11.8	9.2	130	129



(a)



(b)

Figure 1. Concentration distribution on paper surface using ImageJ 1.41o. (a) Surface profile, (b) Histogram of the distribution of grey values.

PERFECTION 2450 PHOTO). The scanned images were analyzed using ImageJ software (ImageJ 1.41o). ImageJ calculates the grey values of RGB images. RGB pixels are converted to grey values using the built in formulas (grey = *(red + green + blue)/3*, or *grey = 0.299red + 0.587green + 0.114blue*).

For any selected area, the ImageJ software calculates the weighted average grey value within the selection, which can be related to the enzymatic activity of bioactive paper. Thus the average grey value is the sum of the grey values of all the pixels in the selection divided by the number of pixels. From the grey value analysis, the relative activity of bioactive papers was measured. Activity corresponding to the grey value at t = 0hr was considered as 100%. The relative activity at different time intervals was measured and normalized by the activity at t = 0hr, i.e. relative activity, $[Ea]_t/[Ea]_0 = I/I_0$. The *log* value of relative activity is defined as the residual activity, $log(I*100/I_0) = log([Ea]_t*100/[Ea]_0)$.

Calibration curve

Calibration curve for ALP (Figure 2) was built to qualify the extent of enzymatic reaction on paper from the intensity colorimetric sample produced. The colour intensity increases non-linearly with enzyme concentration on paper. Colour results from the product of the enzyme-substrate reaction on papers using different enzyme concentrations.

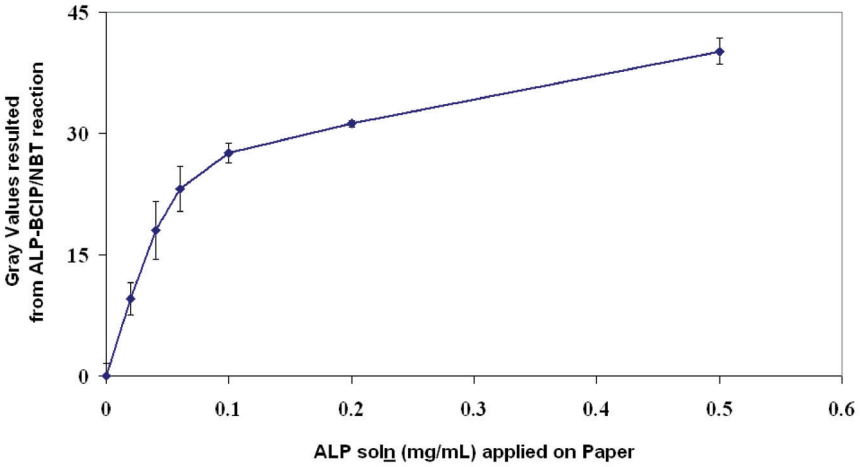


Figure 2. Calibration curve of gray values of the enzymatic products resulting from enzyme-substrate reaction on ALP enzymatic paper.

RESULTS

Thermal stability of ALP active papers

Bioactive paper samples were aged at different temperatures for various periods. After applying the liquid substrate to the aged enzyme paper and letting the enzyme-substrate reaction proceed to completion, the paper samples were scanned. The relative activities of bioactive papers were calculated from the weighted mean grey value of the scan images. The colorimetric evolution of the ALP-paper aged at 60°C and 90°C is shown as a function of time on Figure 3. Paper yellowing is becoming visible for papers treated at the higher temperature (90°C) and longer periods (4h and longer). The effect of ageing temperature and time on the relative activity of ALP-paper is illustrated on Figure 4 for treatments at 23°C, 60°C and 90°C. The Enzymatic

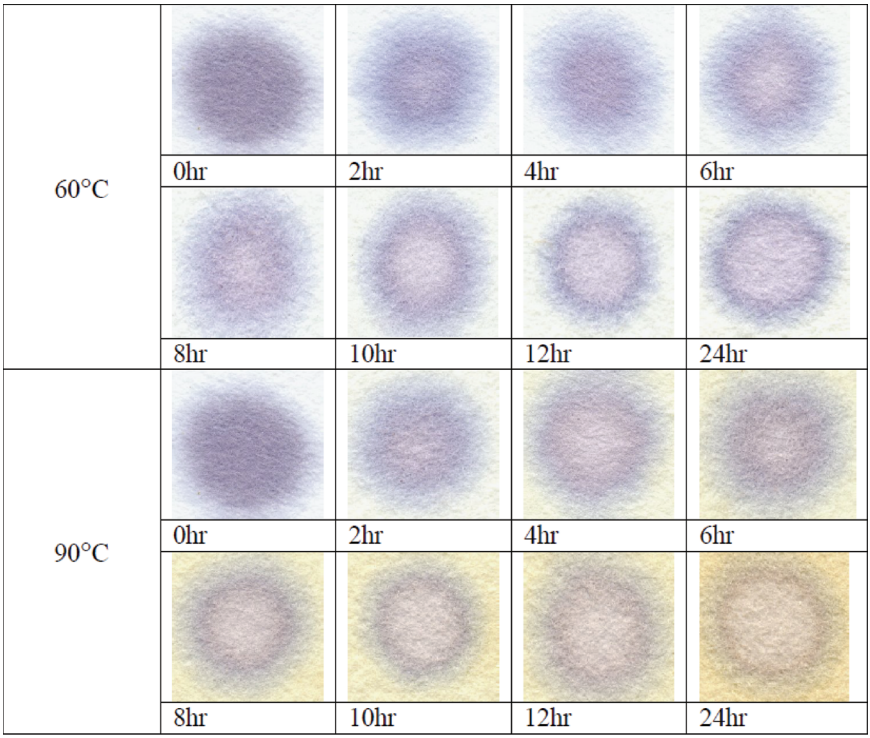


Figure 3. Aging of bioactive paper treated at 60°C and 90°C for various periods. The blue purple colour reveals the enzyme (ALP) – substrates (BCIP/NBT) reaction.

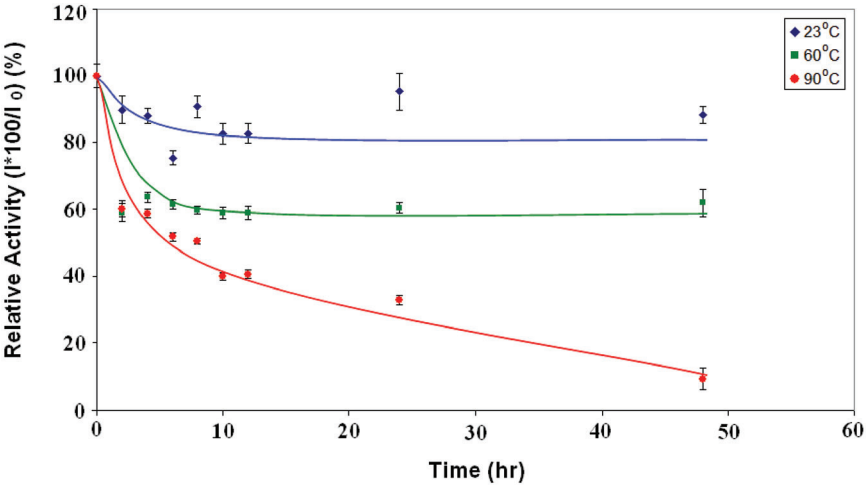


Figure 4. Effect of time and temperature on the activity of enzyme paper: ALP-paper; (I_0 = grey value at 0hr and I = grey value at 't' hr).

activity strongly decreased for ALP-papers heated at 90°C. For all three temperatures, the enzymatic activity quickly falls within the initial hours of thermal treatment and then gradually decreased at a slower rate. ALP-papers nicely retain their activity at 23°C temperature and exhibit only a moderate loss of activity when exposed at 60°C.

Enzyme affinity on paper surface

The ALP enzymatic paper was rinsed into water at t = 0 hr. After drying the rinsed sample at 23°C and 50% relative humidity for 24hrs the relative activity was found 58% (±1.66, n = 8). Figure 3 shows the relative activity of ALP enzymatic paper is about 81% at t = 24 hrs @ 23°C. A fraction of the enzyme molecules desorbed from the enzymatic paper while rinsed in water. Physisorption does not provide permanent fixation of enzyme on paper.

BioPrinting

The HRP enzyme was printed on paper and exposed, under standard conditions (23°C, 50% relative humidity), to a solution containing its specific liquid substrate 3,3′-Diaminobenzidine (DAB) resulting in a brownish colour of intensity proportional to the concentration of the product resulting from the enzymatic activity. Figure 5 is a picture of our research group obtained by inkjet printing the HRD enzyme followed by reaction with DAB.

Figure 5. APPI Surface Engineering Portrait (14.5 cm × 10.5 cm) realized by inkjet printing an enzyme (Horseradish peroxidase) and exposing the picture to its substrate (DAB).

Paper microfluidic systems

Intelligent paper-based, microfluidic systems were made using a series of techniques which combined surface modification and printing. Paper-based microfluidic switches and reactors were also produced and tested. Figure 6 illustrates a printed micro-fluidic system and the system filled with various fluids. Inkjet printing can precisely deposit biomolecules inside the microfluidic channels to fabricate paper based microfluidic bioassays and diagnostic

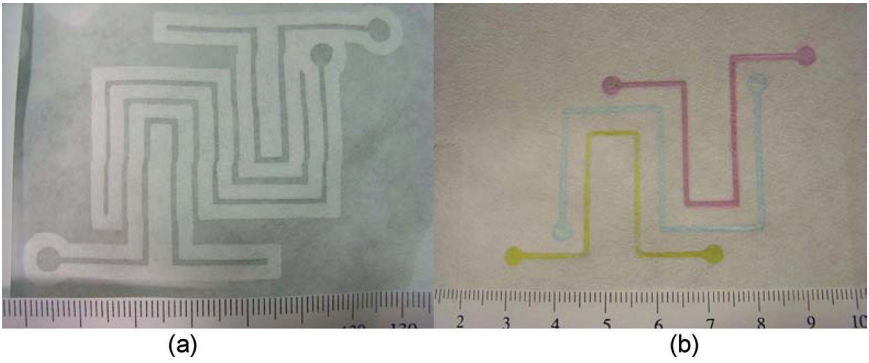


Figure 6. Paper printed microfluidic system as printed (a) and filled with three coloured liquids (b).

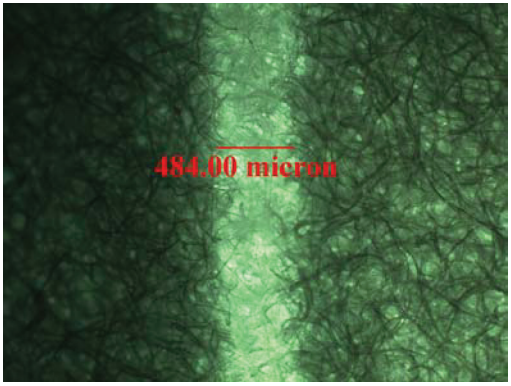


Figure 7. Resolution of paper microfluidic system.

devices [24]. Figure 7 shows the resolution of a liquid filled micro-fluidic channel. The thickness of the channel is 480 μm.

DISCUSSION

Immobilized enzymes on paper

The alkaline phosphotase (ALP) is responsible for removing phosphate groups from different types of molecules including proteins and alkaloids. It is most effective in an alkaline environment. The reaction can be shown as [25]:

$$\mathrm{p-Nitrophenyl\,Phosphate} + \mathrm{H_2O} \xrightarrow{\text{Alkaline Phosphatase}} \mathrm{p-Nitrophenol} + \mathrm{P_i} \quad (9)$$

where P_i = inorganic phosphate.

ALP randomly adsorbed on paper was shown to have retained its activity and selectivity upon immobilization on paper. This means that the active sites retained their structural integrity and remained available to the substrate. This was expected because of the low molecular weight of the ALP substrate: phosphatase (Mw = 95 D). The reaction rate of these adsorbed enzymes was not measured in this study and can not be compared to that of the enzymes in solution. It is therefore not possible to quantify the effect of mass transfer limitations, nor potential steric hindrance due to the orientation of the immobilized enzyme.

The experimental technique might still be further refined to prevent or limit the extend of paper yellowing induced by heating and enzyme buffer. Since bioactive papers are expected to be stored dry, the thermal stability of dry enzyme-paper needs to be measured and modelled.

Image analysis and activity measurement

The initial paper selected is white and its background does not contribute any grey value to the enzymatic activity measurement. After prolong exposure to high temperatures, paper can yellow which adds extra grey to the image analysis intensity measurement. This is especially catalysed by the alkaline conditions required for enzymes such as ALP. To normalize the yellowing effect of paper from its enzymatic activity, the grey value of the paper control was simply measured and subtracted from the grey value of enzyme-substrate paper.

Deactivation of biomolecules adsorbed on paper

Protein and enzyme deactivation are known to denature and become deactivated once removed from their native biological surroundings and exposed to perturbations such as temperature, mechanical forces, radiation, chemicals and heavy metals [19]. It is of interest to quantify and understand the effect of enzyme immobilization on paper on its stability.

Yang and Kim (1994) quantified the aging of ALP enzyme in aqueous medium. At 50°C the activity of ALP reduced to around 5% of its initial rate within 30 mins (Figure 8). Our experimental results suggest ALP immobilized on paper to be significantly more stable to heat than the same enzyme in aqueous solution. ALP adsorbed on paper retained 60% of its initial activity after 48 hours exposure to 60°C (Figure 4). Pelton *et al.* also reported that the stability of HRP immobilized within porous silica nanoparticle increases by over an order of magnitude compared to the molecule in solution [20]. OH containing molecules, such as alcohol, were also reported to stabilize HRP in solution [13].

Heat deactivates and denatures enzymes by modifying their conformation due to increased thermal movement and decreased solvent stabilization [19, 26]. Proteins can also aggregate when heated which can restrict accessibility to some of their active sites. Immobilization of enzyme on paper prevents protein aggregation and retards the conformation disorder by stabilizing the secondary and tertiary structures of enzyme through the establishment of a network of hydrogen bonds protein-cellulose.

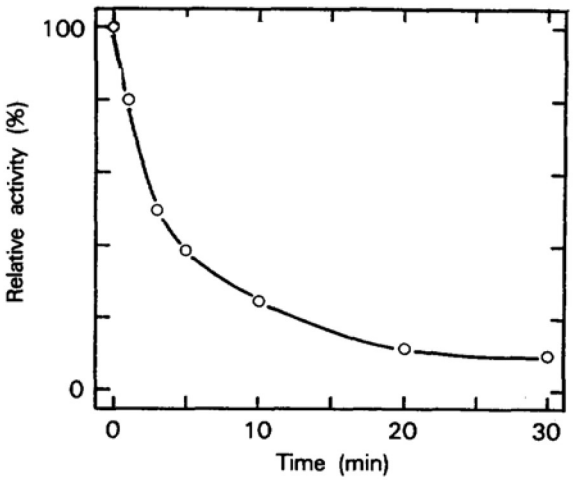


Figure 8. Relative activity curve of alkaline phosphatase heated at 50°C for various periods (Reproduced without permission from Yang and Kim, 1994 [23]).

Kinetic modelling of biomolecular deactivation on paper

In its simplest form, enzyme deactivation is expected to follow a first order kinetics described by equation (7). Enzyme activity plotted on a logarithmic scale is expected to vary linearly with time. That was not the case for ALP immobilized on paper (Figure 9), where two kinetic regimes are observed. Bailey and Ollis (1986) reported that the decay of enzyme activity with time does not always follow the first-order model; kinetics with two distinct rates is sometimes observed. Our experimental results (Figure 9) show two distinct linear regions with different rate constants for a first order kinetics (Table II).

Table II. Deactivation rate constants for ALP active papers.

	Deactivation rate constant of ALP active paper	
Temperature	k_{d_1} (hr^{-1})	k_{d_2} (hr^{-1})
23°C	62.8×10^{-3}	1.3×10^{-3}
60°C	102.1×10^{-3}	10.7×10^{-3}
90°C	229.5×10^{-3}	32.2×10^{-3}

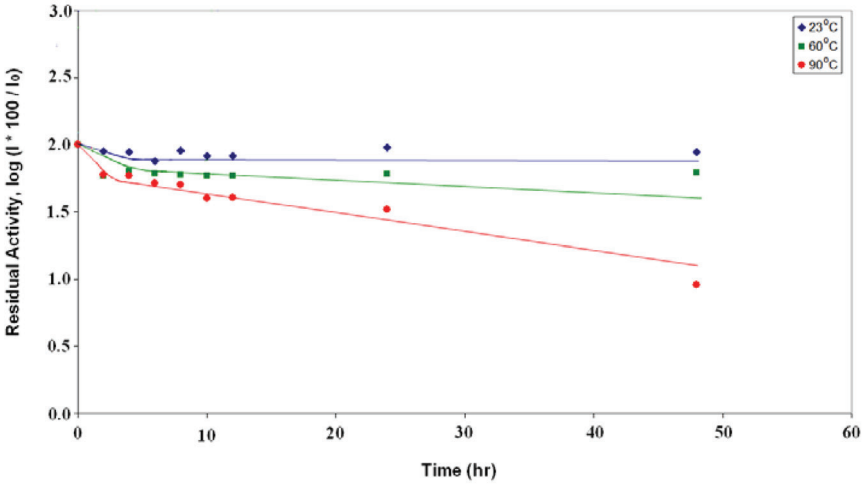


Figure 9. Residual activity curves of ALP active papers at different temperature. (I_0 = grey value at 0 hr and I = grey value at 't' hr).

Subscript 1 refers to the deactivation rate constant of the steeper region and subscript 2 describes the pseudo-plateau.

Redrawing Figure 8 into logarithmic scale shows that deactivation of ALP in aqueous medium also projects two different linear regions (Figure 10). From Figure 9, at 50°C, the deactivation rate constants for two different regions can be calculated to be 12.9 hr^{-1} and 3.4 hr^{-1}, respectively. In contrast, on paper, the deactivation rate constants of ALP vary from 229.5×10^{-3} hr^{-1} (phase-1, at 90°C) to 1.3×10^{-3} hr^{-1} (phase-2, at 23°C), which are 2–4 orders of magnitude smaller than that in aqueous medium.

CONCLUSION

The activity and the thermal stability of enzyme bioactive paper were quantified using a colorimetric technique by measuring the colour intensity formed by the product complex. Alkaline phosphatase (ALP) adsorbed from solution onto paper and dried remains functional and exhibits strong activity and selectivity. Adsorption of ALP onto paper by adsorption was found to increase the enzyme thermal stability by over two orders of magnitude compared to the enzyme in solution in its buffer. As an example, the ALP active paper retained 60% of its initial activity after 48 hours heating at 60°C; this

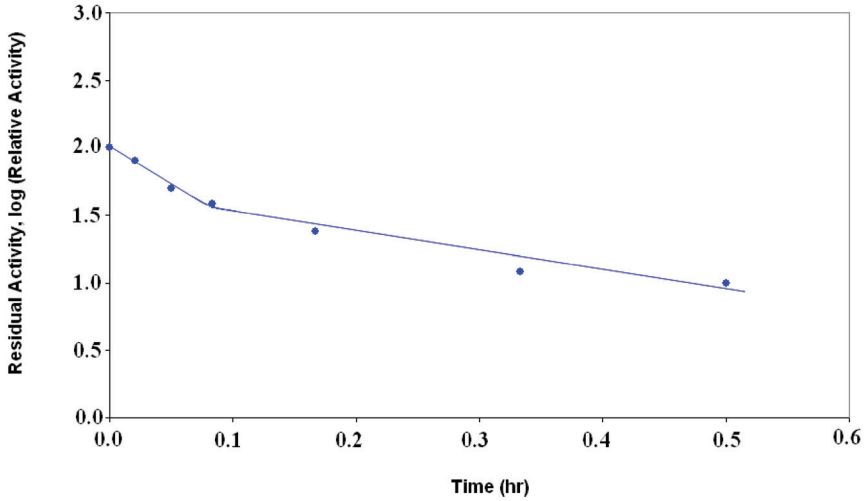


Figure 10. Residual activity curves of alkaline phosphatase heated at 50°C for various periods (Results from from Yang and Kim, 1994 [23]).

compares to less than 20% retained activity after 30 minutes heating at 50°C for the ALP enzyme in its buffer. The enzyme thermal deactivation was modelled and found not to follow the expected first order kinetics with respect to enzyme concentration but rather to form a two-step process.

Complex patterns of HRP-enzyme were printed on paper by modifying a common thermal inkjet printer. Microfluidic channels on paper were printed to demonstrate the concept of paper based bioassays as diagnostic devices. These accomplishments validate paper as a bioactive support exceptional for its flexibility and performance for diagnostic and industrial process applications.

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Transcription of Discussion

BIOACTIVE ENZYMATIC PAPERS

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Wolfgang Bauer Graz University of Technology

When we saw your ageing series (figure 3 in the proceedings paper, ed.), I noticed, in each case, some kind of ring formation. Do you have any explanation for that?

Gil Garnier

Yes, I do. Basically, what it means is that the absorption is fairly weak. The liquid substrate initially forms a kind of crown that is then absorbed and you get this ring. You do not get this ring with the polymer because you have a better absorption, so that means the affinity of the substrate and the dye is not too strong, and that gives us a few challenges for the analysis of the pictures.

Ilya Vadeiko FPInnovations

In your stability test, you chose three temperatures – room temperature, 60 °C and 90 °C. I was wondering what is the practical reason for studying the 90 °C temperature for such a long time?

Gil Garnier

We used horseradish peroxidase (HRP) because it is known to be the most robust enzyme. We started first at 40 °C – we started very quietly and carefully – but this enzyme is very robust, it did not degrade. You saw that at 23 °C it takes weeks to see any change. So we had to be a little bit harsher because we

wanted to better understand the effect. We used these two enzymes because they are so well known, so well characterised, and you can get a reactive content for everything, so we wanted to keep them. In order to really produce degradation, we had to increase the severity of the treatment. Another thing that convinced us that it is extremely robust, was that we used a Komora ink-jet printer and one of the concerns we were given is that the heat would denature the enzyme. So, first, it is not true because the enzyme is very robust and, second, the retention time of the printer is so low that it does not "see" the temperature. In order to convince ourselves, we wanted to study the thermal resistance of the strongest enzyme.

Ilya Vadeiko

Thank you.

John Roberts University of Manchester (from the chair)

While we're on that subject, could I ask you if you have an explanation for this dramatic increase in thermal stability of the alkaline phosphatase. Also is it a general feature of enzymes on immobilization?

Gil Garnier

Yes, and that was also reported by Bob Pelton. I think Bob's study was a horseradish peroxidase, the same enzyme but on silica, and Bob reported that thermal stability increases. I do not want to quote him, but it was at least an order of magnitude that was seen. So the way I look at it, my enzyme is an egg. This morning a few of you had an egg. If you take the egg and you boil it, it is 3 dimensional, and it is a gel and it has very little structure. It is held together by a very weak force, so if you heat it, you make a boiled egg very fast. But if you instead put egg on some toast, that means you are going to have some more bonding, you are going to have some OH groups that will form hydrogen bonds, and it could stabilise. I think if you put the egg on the toast in the oven, it will sustain more temperature before it starts to cook and change its structure. So what we are doing here is, I believe, to develop a network of bonds with the OH groups of cellulose that stabilise the enzyme. We are trying to characterise that work on the synchrotron using small angle X-ray scattering (SAXS). We saw a difference but it is a bit too complex, and that is why we realised we need more expertise.

John Roberts

So you see it as the immobilization inhibiting the denaturation of the protein in some way?

Gil Garnier

Yes, because we stabilise the structure, we provide other bonds which provide consolidation of the structure.

Roger Gaudreault Cascades R&D

Dealing with the PEO, did you try with and without co-factor and did you see an effect on the activity half-life?

Gil Garnier

Great question! No we did not try with co-factors, so the fact that we have low retention is maybe because it was not in a proper configuration. However, PEO is widely used in this industry to prevent the deposition of proteins, and it is known that some type of interaction occurs. We are not too sure exactly what type of interaction, but PEO is absolutely special.