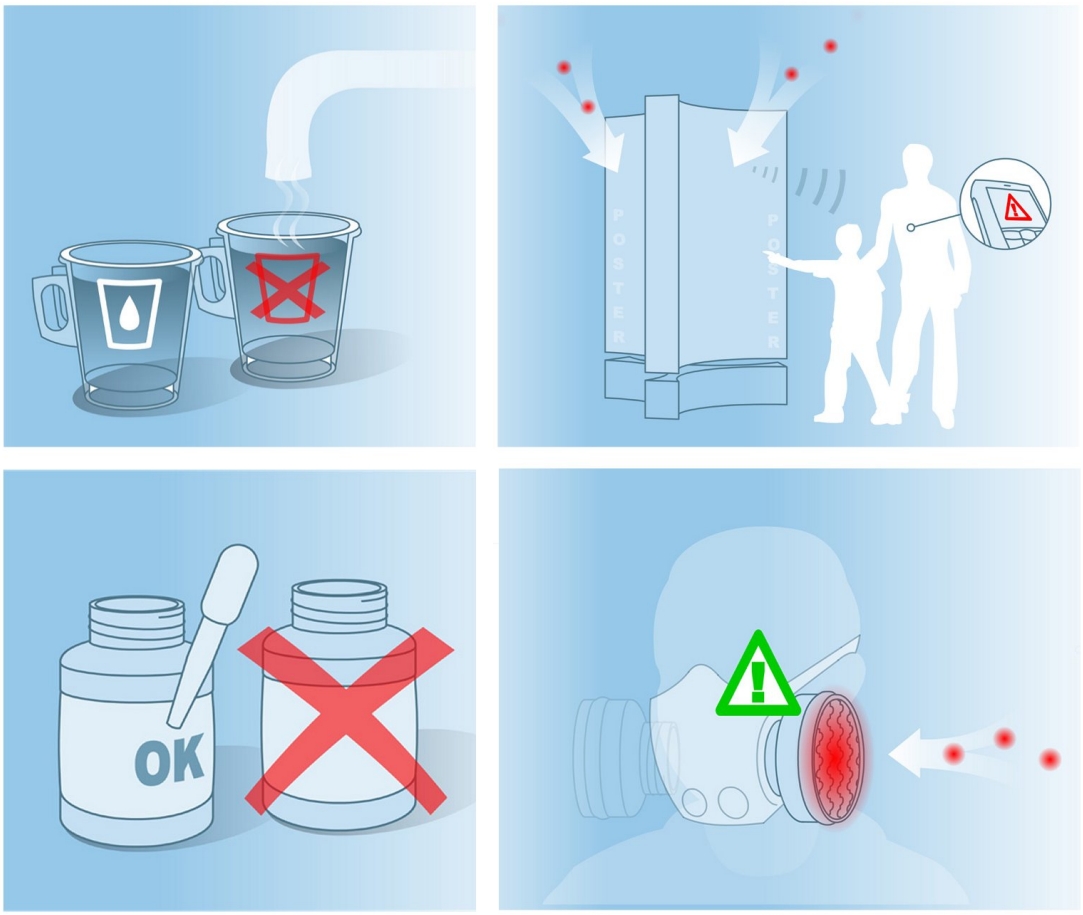

Bioactive paper and fibre products

Patent and literary survey

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ISBN 951–38–6603–3 (URL: http://www.vtt.fi/publications/index.jsp)
ISSN 1459–7683 (URL: http://www.vtt.fi/publications/index.jsp)

JULKAISIJA – UTGIVARE – PUBLISHER

VTT, Vuorimiehentie 3, PL 1000, 02044 VTT
puh. vaihde 020 722 111, faksi 020 722 4374

VTT, Bergsmansvägen 3, PB 1000, 02044 VTT
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Technical editing Anni Kääriäinen

Published by

Series title, number and report code of publication

VTT Working Papers 51
VTT–WORK–51

Author(s)
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Title

Bioactive paper and fibre products
Patent and literary survey

Abstract

In this patent and literary survey bioactive papers and fibre products, i.e. paper-like products, cardboard, fabrics and their combinations, etc., with active recognition and/or functional material capabilities are reviewed. The focus is on materials that specifically react with the target entity or environmental condition, and this reaction initializes different events, like a discharge of molecules or signalling function, in the product. The aim of this publication is to review and provide basic information on biomolecules and their potential utilisation for functional purposes, embedded signalling – concentrating especially on conducting polymers and optics – low-cost manufacturing on a large scale, modification of fibres and grafting or immobilisation of bioactive components. In addition, some application scenarios for bioactive paper products are presented.

Keywords
bioactive, functional, paper, cardboard, fabric, woven, non-woven, biomolecule, embedded signalling, conducting polymers, biosensors, optical signalling, low-cost manufacturing

ISBN
951–38–6603–3 (URL: http://www.vtt.fi/publications/index.jsp)

Series title and ISSN		Project number
VTT Working Papers 1459–7683 (URL: http://www.vtt.fi/publications/index.jsp)		534-E5SU00763
Date May 2006	Language English	Pages 83 p.
Name of project Bioactive Paper	Commissioned by Tekes (Finnish Funding Agency for Technology and Innovation), VTT	

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1. Background

This patent and literary survey was written in the Bioactive Paper project jointly funded by Tekes, the Finnish Funding Agency for Technology and Innovation, and VTT, the Technical Research Centre of Finland. The results of this survey were presented in the industrial seminar "Bioactive Paper and Fibre Products" held on February 6, 2006, in Espoo, Finland.

Here we are concerned with bioactive papers and fibre products, i.e. paper-like products, cardboard, fabrics and their combinations, etc., with active recognition and/or functional material capabilities. We are concentrating on materials that are formed from elementary units, like woven fabric formed from thread and paper from cellulose fibres, and thus have a three-dimensional structure in contrast to plastic films.

In this publication the focus is on materials that specifically react with the target entity or environmental condition, and this reaction initializes different events, like a discharge of molecules or signalling function, in the product. Applications in which non-biomolecule-based antimicrobials, antivirals, etc., are passively embedded into the product and are passively destroying or inhibiting the growth of micro-organisms are not included in this survey because they have been studied extensively and a great number of such commercial products exist.

The aim of this publication is to review and provide basic information on biomolecules and their potential utilisation for functional purposes, embedded signalling – concentrating especially on conducting polymers and optics – low-cost manufacturing on a large scale, modification of fibres and grafting or immobilisation of bioactive components. In addition, some application scenarios for bioactive paper products are presented.

Due to the multidisciplinary nature of bioactive paper products, this survey was written by an interdisciplinary research group with expertise in biochemistry, physics, chemistry, electrical engineering, detection methods and mass manufacturing methods. In addition to national collaboration, international collaboration with research organizations like "SENTINEL – The Canadian Network for the Development and Use of Bioactive Paper" (http://www.papersci.mcmaster.ca), is needed in the future to bring alive the promises of bioactive paper.

2. Introduction

Bioactive paper and Ambient Intelligence share the vision that technology will become invisible, embedded in our natural surroundings, present whenever we need it, enabled by simple and effortless interactions, attuned to all our senses, adaptive to users and context, and autonomously acting with a flavour of biomolecular activity (Lindwer *et al.*, 2003). Embedded systems for Bioactive paper are going to impact on the way we live, if they will be present without constraining our ordinary lives and making them safer. Applications ranging from safe environment, smart buildings and home security, smart fabrics or e-textiles, and food safety to manufacturing systems are poised to become part of society and human lives. Due to BSE, SARS, bird flu, and the 9/11 events, a lot of research have accelerated recently, driven by fears of health, terrorism and biowarfare. Therefore, diagnostics is one of the key application areas of "Bioactive paper". Diagnostic applications require biomolecular recognition and transduction of the recognition to an electric or visual signal. Biosensors are very descriptive products within this category.

Biosensors (chemical and biosensors) are defined as measurement devices that utilize chemical or biological reactions to detect and quantify a specific analyte or event (Schultz & Taylor, 1996). Biosensors consist of an analyte-specific receptor and a transducer, which converts the change in the receptor to a detectable signal (Figure 2-1).

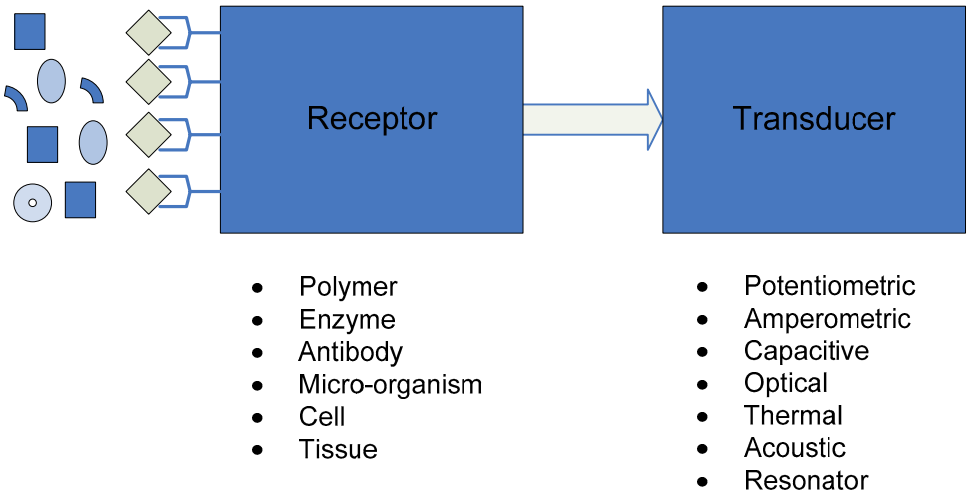

Figure 2-1. Basic building blocks of a biosensor.

The ideal properties of a biosensor are the following: high sensitivity, high specificity, easy calibration, high linearity and dynamic range, no background signal, no non-specific binding, good stability, no errors from environmental variables, good dynamic response (low measurement time), and biocompatibility.

As illustrated in Figure 2-1, there are various ways in which biomolecular recognition can be realized. They have differences in many critical attributes, but in general the major issue in reliable use of biosensors is the so-called background signal (false alarm), which may be due to many reasons, e.g. non-specific binding. This background signal can be reduced by sampling protocols and sample preparation. A lot of effort has been put into the development of surfaces and surface modifications to prevent non-specific binding (Pavlickova *et al.*, 2004).

In 1976 Clemens *et al.* incorporated an electrochemical glucose biosensor in a bedside artificial pancreas and this was later marketed by Miles (Elkhart) as the Biostator. Since then, blood glucose self-testing devices and strips have become a major application of biosensors. The specificity of the blood glucose test strips is based on enzymatic reactions (glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide, using molecular oxygen as the electron acceptor). Another common receptor group is antibodies. Enzyme-linked immunosorbent assay (ELISA) is the most conventional way of realizing biomolecular recognition based on antigen-antibody binding. Below is a brief description of some potential technologies available for biomolecular recognition.

In an electro(bio)chemical sensor a biological process is transduced to an electrical signal – such an example is an enzyme electrode. Thermal biosensors are based on measuring the heat formed or absorbed during a chemical reaction. An optical biosensor uses light to transduce the biomolecular binding event to a detectable signal. The most commonly used optical biosensor is a surface plasmon resonance (SPR) sensor, where a receptor is immobilized onto a gold surface and the binding of analyte to the receptor changes the refractive index near the surface. This change in refractive index is detected as a change in the angular resonance conditions of the incident light. An acoustic sensor is based on the mass increase (binding) influence on the resonant frequency of a quartz crystal or cantilever.

In this publication we review the various biomolecular recognition elements, e.g., enzymes and antibodies, and their immobilization in Chapter 3. Various detection and signalling methods are reviewed in Chapter 4, where our focus has been on optical methods and the use of conductive polymers. We complete our technology review of Bioactive paper in Chapter 5, where we review various methods from paper manufacturing to printing to make bioactive paper products. Finally, in Chapter 6, we give a brief overview of possible applications of bioactive paper.

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3. The role of biocatalysts in functional materials

Various biomolecules, particularly enzymes, antibodies and nucleic acids, have been widely harnessed for analytical as well as other functional purposes. These biomolecules can be incorporated into paper or paper-like materials, enabling the mass-production of functional materials for various purposes.

In this chapter the different types of biomolecules, as well as examples of their potential utilisation for functional purposes in different application fields, are reviewed (see Figure 3-1). Potential application areas include, e.g., diagnostics and packaging. An example of the various possibilities enabled by the introduction of biomolecules into materials in the area of food packaging is illustrated in a patent application by the Procter & Gamble Company. A multifunctional food wrap comprising a material web, an adhesive and at least one secondary function is presented in the PCT publication (Hamilton *et al.*, 2004). The material web described in this patent application is selected from the group consisting of paper, polymeric films, plastic films, cloths, fabrics, wovens, non-wovens, laminates, metal foils and coated papers. The secondary function can be antimicrobial protection (by using bacteriocins, enzymes), food preservation, atmosphere modification (by using oxygen-absorbing enzymes), odour elimination (by using surfactant-treated chitosan), product spoilage indication (by using detector antibodies), temperature indication, flavour enhancement and moisture absorption.

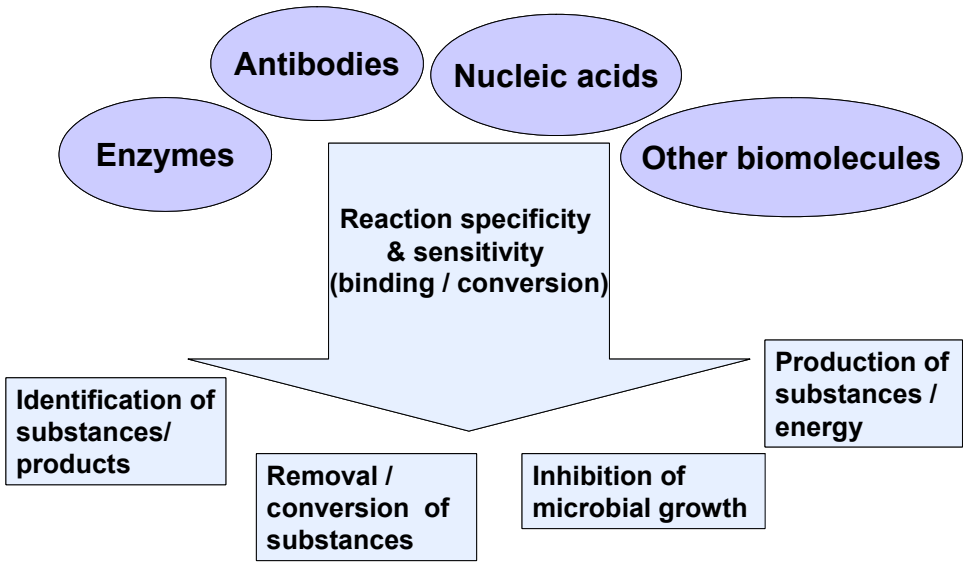

Figure 3-1. Possibilities of introducing material functionalities with the aid of biocatalysts.

3.1 Enzymatic reactions and enzyme-based functional devices and materials

The utilisation of enzymes in functional materials can be based on different properties related to enzymatic reactions:

- substrate specificity (identification of metabolites, removal of substances, antimicrobial action)
- sensitivity of the reaction to external conditions like temperature or humidity (indicators for relative humidity and time-temperature history)
- participation of the enzymes in electron transfer processes (energy production in biofuel cells).

3.1.1 Systems based on the substrate specificity of enzymes

The substrate specificity of enzymes can be utilised in functional materials in several different ways. The enzymes can

- detect the presence of specific compounds
- detect the presence of specific enzymes (particular substrate introduced into the functional material)
- catalyse specific reactions aiming at the removal of specific compounds from the environment (oxygen removal by oxidases) or cell disruption (by lysozyme)
- produce specific reaction products with antimicrobial properties.

In addition to the above-mentioned approaches, enzymes are widely used for labelling purposes in the immunochemical detection systems described in Chapter 3.2.

3.1.1.1 Detection of the presence of specific compounds

Enzymes have been used for analytical purposes for more than 100 years. The analytical reactions are often carried out in solution and then followed by spectrophotometrical measurement.

Enzymes have also been widely utilised in diagnostic test strips – evaluation of the glucose level of blood in the control of diabetes with glucose oxidase-based test strips probably being the most important application of this type of enzyme-containing material – and several test strips are commercially available.

In addition to glucose, other compounds can be detected with the aid of enzyme-containing, paper-like materials. For example, ATP degradation products indicating the quality deterioration of fish can be analysed by enzymatic test strips manufactured by Transia. Similar strips are available e.g. for lactose and galactose (Figure 3-2).

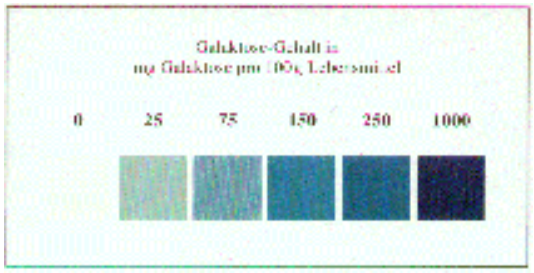

Figure 3-2. Colour scale of enzymatic test strip for lactose (Sension).

Barzana *et al.* (1986) patented a method using enzymes in conjunction with a colour indicator such as a redox dye or pH indicator to detect the presence of a compound (e.g. ethanol) in the gaseous phase. The enzymes (e.g. alcohol oxidase) are immobilized on an inorganic or organic substrate and dehydrated. Elsewhere, the performance of a commercial ALCO-SCREEN colorimetric saliva dipstick assay of serum ethanol concentration (patented by Adams, 1985) was estimated by Rodenberg *et al.* (1990). The assay consists of a small plastic strip with a reactive test pad on the end, which contains alcohol oxidase, peroxidase and leucodye buffer complexed with hydrogen. However, the performance of the saliva dipstick assay was poor in determining alcohol use among emergency department patients. On the other hand, Smyth *et al.* (1999) reported that wetted ALCO-SCREEN test strips could be used to measure ethanol (10 µl/l) in the gas phase and were suitable for detecting low-oxygen injury in modified atmosphere (MA) packages containing lightly processed lettuce, cauliflower, broccoli and cabbage. Utilisation of enzymes as diagnostic tools incorporated in packaging materials has been suggested e.g. by Cameron and Talasila (1995). They explored the potential of detecting the unacceptability of packaged, respiring products by measuring ethanol in the package headspace with the aid of alcohol oxidase, peroxidase and a chromogenic substrate.

The ability of the enzymes to specifically catalyse numerous different reactions has been utilised to a vast extent in different analytical devices. Analogous to enzymatic test strips, glucose oxidase-based biosensors for blood glucose are by far the most established devices based on the biosensor technology. Compared with enzymatic test strips, which involve pricking the finger, it would be beneficial to have a continuous monitoring system that could potentially be realised as an implantable electrochemical sensor that continuously monitors the glucose levels.

Other examples of biosensors have been presented by Guiseppi-Elie (1994), who described chemical and biological sensors having electroactive polymer thin films attached to microfabricated devices and possessing an immobilized indicator molecule, which may be a bioactive molecule such as an enzyme or member of a specific binding pair of biological origin. The binding of an analyte or member of the specific binding pair reagent may result in a change in the electrical impedance of the highly conducting layer. The above-mentioned biosensor is designed to work in an aqueous environment, not in air. Stanford *et al.* (2003) patented a sensor capable of detecting chemical and/or biological agents in both aqueous and gaseous environments. The sensor comprises metal interdigitized electrodes coated with hybrid polymer-based conducting film and an instrument for applying electrical voltage to the electrodes and registering the change in the electrical current. The hybrid film comprises indicator biomolecules such as acetylcholinesterase. These types of biosensors based on conductive polymers will be discussed in detail in Chapter 4.

3.1.1.2 Detection of the presence of specific enzymes or micro-organisms

In addition to the direct determination of the substrates of enzymatic reactions, the specific reactions between the enzymes and their substrates can be utilised to detect the presence of the enzyme itself.

Van Veen (2004) patented a method for non-invasive detection of contamination with a micro-organism in a closed (sterile) container, which is based on detecting an extracellular enzyme (or its activity) of the micro-organism. A substrate (indicator) is provided to either the contents (growth medium) of the container or alternatively in a coating of the inner side of a package, and the conversion of this substrate by the enzyme is detected either visually or by means of an optical measuring device.

DeCicco and Keeven (1995) described an indicator based on the colour change in the chromogenic substrates of enzymes produced by contaminating microbes. The indicator can be applied to contamination detection in liquid health care products. Hydrolytic enzymes were also reported to indicate fungal spoilage in bakery products (Marin *et al*., 2003). Colorimetric detection of the enzymatic activity of peroxidase has been proposed for use as a tool for the evaluation of the freshness of rice grains (Chen & Chen, 2003).

The detection of micro-organisms as such was proposed by Namiki (1996). The principle of the indicator is the degradation of the lipid membrane by micro-organisms and the subsequent diffusion of a coloured compound. Colpas *et al.* (2004) developed a method of detecting the presence or absence of micro-organisms, for example wound pathogens, by contacting a sample (e.g. a wound) with a detectably labelled substrate

(e.g. a bandage conjugated with a chromogenic dye). The presence of an enzyme (protease) produced and/or secreted by the micro-organism will result in modification of the substrate (e.g. colour change).

3.1.1.3 Introduction of specific compounds by enzymatic reactions

The functionality of materials can also be based on the production of various substances in the enzymatic reaction, e.g. in antimicrobial materials.

Antimicrobial packaging systems combine food packaging materials with antimicrobial substances to control undesirable microbial surface contamination of foods. As microbial spoilage occurs mainly on the surface of the food product, antimicrobial packaging could be useful in reducing the microbial contamination level on the surface of the packaged product. The use of preservative food packaging films can offer advantages over the direct addition of preservatives to the food product. Fewer additives are present in the total food product and the production process can be simplified by combining the packaging step with the addition of preservatives (Heirlings, 2003). However, care has to be taken not to use antimicrobial packaging for masking high initial contamination.

Antimicrobial properties can be added to packaging materials in several ways, including sachets or pads containing antimicrobial volatiles, incorporation of antimicrobials into a matrix of packaging material, coating or adsorbing antimicrobials onto the packaging surface, using inherently antimicrobial substances as packaging raw materials or immobilization of antimicrobial substances onto the packaging surface (Appendini & Hotchkiss, 2002). From the safety aspect, the natural and non-leachable antimicrobials look most promising as incontrollable migration of chemicals to the food product may cause a potential safety hazard (Koert, 2005).

Naturally occurring antimicrobials (basil, oregano, allyl-isothiocyanate, etc.) and traditional food preservatives (potassium sorbate, nisin, sodium benzoate, etc.) are recognised as safe antimicrobials that may be incorporated into food contact inks and coated or printed onto the surface of the packaging using existing production techniques (spray coating, flexo printing, etc.). Chitosan, the second most abundant natural biopolymer after cellulose, is an edible and biodegradable material having antimicrobial activity against bacteria, yeasts and moulds (Yalpani *et al*., 1992). Due to its good film-forming properties, chitosan may be successfully used in food packaging materials, coated papers and surface treated films (Muzzarelli, 1986; Vartiainen *et al*., 2004; Gällstedt *et al.*, 2001). Long-chain active biomolecules and enzymes having suitable functional groups can be immobilized onto a pre-activated packaging surface in various

ways, such as ionic and covalent immobilization, cross-linking, graft copolymerization and entrapment (Tiller *et al*., 2002).

Hydrogen peroxide, a substance with antimicrobial activity, is produced in the reaction catalysed by glucose oxidase, which can be covalently immobilized onto amino or carboxyl plasma-activated bi-oriented polypropylene films via suitable coupling agents (Vartiainen *et al*., 2005b). In the work by Vartiainen *et al.* the biomolecules attached to the packaging surface retained the necessary enzymatic activity level for complete inhibition of food spoiling bacteria in various conditions for a storage period of one month.

A different "release-on-demand" approach to the use of antimicrobial substances is presented by Thijssen *et al.* (2003), who patented a preservative-releasing packaging system that only releases its preservative when bacterial growth occurs. An antimicrobial substance is provided in or on the packaging material in a covering (also called a capsule) of carbohydrates and/or proteins, which can be decomposed by a micro-organism. Thus the antimicrobial substance only comes into contact with the packaged goods at the moment when there is microbial activity. In addition to packaging applications, the antimicrobial coating can also be used in systems vulnerable to infection by micro-organisms. A suitable antimicrobial substance can be selected from bacteriocins, metals or derived metals, antibiotics, vegetable toxins such as defensins, lectins and anti-fungal proteins, hydrogen peroxide producing enzymes such as oxidases, organic acids, sodium diacetate, sodium nitrite, lysozyms and antimicrobial substances from spices.

The catalytic activity of thrombin together with the antibiotic Cipro has been utilised in a bioactive wound dressing composed of polyester and polyurethane. The enzyme initiates the blood clotting procedure and the antibiotic simultaneously prevents the infection (Anon., 2005b).

3.1.1.4 Removal of specific compounds by enzymatic reactions

Enzymes can also be used to remove or inactivate certain unwanted compounds from the environment. For antimicrobial purposes, e.g. lysozyme, an enzyme attacking the cell wall of bacteria has been proposed as an antimicrobial agent in packaging materials (Buonocore *et al*., 2004; Park *et al*., 2004). The antimicrobial activity is primarily directed towards Gram -positive bacteria, but some effects on Gram-negative bacteria have also been observed.

In addition to antimicrobial activity, other functions can also be added to packaging materials. Hotchkiss (2005) presented an example of in-situ processing of milk by removal of lactose by attaching a lactase enzyme to PE. An analogous example is the removal of naringin, the bitter compound of citrus juice by naringinase enzyme immobilised into a polyvinyl alcohol matrix by glutaraldehyde (Del Nobile *et al*., 2003).

Enzymes immobilised onto flexible support materials have also been proposed for use in specific applications like the removal of lipid and protein films covering documents and works of art. Proteases are also used in the restoration processes of damaged documents. In these applications the immobilisation of the bioactive molecule is followed by reduced hazards both to the document and the person carrying out the work. Procedures for protease immobilisation in polyamide or polyester have been proposed by Moeschel *et al.* (2003) and Nouaimi *et al.* (2001).

Lehtonen *et al.* (1991) patented a packaging material that removes oxygen from a package. The oxygen-removing layer comprises an enzyme (preferably glucose oxidase) in a liquid phase sandwiched between plastic films. The enzyme solution also contains glucose and, preferably, catalase to decompose hydrogen peroxide formed in the reaction between the oxygen and glucose catalyzed by glucose oxidase. Glucose oxidase-based oxygen-removal and shelf-life extension of foodstuffs was first presented by Lehtonen and co-workers in another patent publication (Aaltonen *et al*., 1990). The glucose oxidase, glucose and catalase-containing composition was either added to the food product itself or the composition was packed in a separate perforated or highly oxygen-permeable bag. Alcohol oxidase has also been utilized in oxygen scavengers (either placed into the food container itself or encapsulated with a gas permeable membrane constructed from fibres, paper, etc.). A suitable alcohol substrate (e.g. ethanol) was introduced into the food container as a vapour, either alone or in combination with the flush of inert gas (Hopkins *et al.*, 1988).

3.1.2 Systems based on the sensitivity of enzymes towards external conditions

3.1.2.1 Temperature

The reaction rate of enzymatic reactions is to a great extent dependent on ambient temperature. The correlation between the temperature and the reaction rate can be harnessed as time-temperature indicators (TTIs) to indicate the exposure of a temperature-sensitive product to the storage conditions the product and the enzyme-based TTI have been exposed to.

The CheckPoint® time-temperature indicator (TTI) labels manufactured by Vitsab Inc. are based on an enzymatic lipid hydrolysis reaction causing a pH change in the reaction mixture (http://www.vitsab.com/) (Figure 3-3). CheckPoint® TTIs are activated by breaking the seal between a lipolytic enzyme-containing solution and its lipid substrate. The reaction is visualized with a pH dye, the colour of which changes from green to yellow as the pH changes during the reaction. The indicators are produced in two versions: one for consumer packages, showing the difference between acceptable and inedible, and another intended for retailers to be attached to the transport package to indicate the different phases of ageing. VITSAB TTI can also be included directly into the package in such a way that the indicator cannot be removed or replaced at a later stage (Laursen *et al*., 1998). The latest versions of CheckPoint are "semi-dry" paper labels that are moistened with the biochemical reagents.

Figure 3-3. Enzymatic time-temperature indicator (VITSAB).

Another enzymatic (or chemical) time-temperature indicator is the Food Sentinel System™ by Sira Technologies Inc. (US) (Woodaman, 2002). This is a time and temperature trans-informative barcode directly printed onto standard "full data" food package labels, shipping cartons and polymer bags. It prevents a product that has been exposed to excessive temperatures from being sold by making the universal product code (UPC) unscannable (http://www.siratechnologies.com/fspem/ttm.html).

Bioett® is a system that originates from a label with an enzyme-based biosensor incorporated in a passive radio frequency circuit. After activation at source the enzyme affects the substrate so that its conductivity changes as a function of time and temperature. When the status of the product is to be checked, an electric and/or magnetic field is applied over the electric circuit and the signal is collected by a handheld scanner and translated into graphs to display the temperature history (www.bioett.com; Sjöholm & Erlandsson, 2001).

In addition to isolated enzymes, the temperature-dependent growth of whole micro-organisms has been utilised in temperature abuse indicating systems by Crylog (http://cryolog.free.fr/). Cryolog's eO and Traceo labels are based on food grade micro-

organisms, which behave in the same way as the micro-organisms responsible for the deterioration of the product (Figure 3-4). As the food product deteriorates, the colour of the labels changes (Vaillant, 2003; Louvet *et al*., 2005).

Figure 3-4. Time-temperature integrating eO label (Cryolog).

3.1.2.2 Humidity

Some examples of the utilisation of enzymatic reactions in humidity sensors and indicators can also be found in the patent literature. Enzymatic reactions have been utilised in humidity and moisture indicators in two basic ways: the water molecules present can participate in the enzymatic reaction as such, or the presence of moisture can be followed by dissolution of other reagents participating in the reaction.

In the system described by Sahlberg *et al*. (2003) water participates in the enzymatic hydrolysis reaction catalysed by urease as a reagent. The accumulation of the reaction products is followed by an increase in conductivity, which can be read electrically. This label-type moisture-sensitive system could be applicable to diapers and evaluation of the moisture exposure of fibre materials and transported goods.

In another indicator system described by Powell (1982) the enzymatic reaction catalysed by glucose oxidase is activated by the dissolution of the substrate (glucose) in the presence of moisture. In the enzymatic reaction the chromogen added to the system undergoes a visual colour change due to the hydrogen peroxide produced. The main application of this layered disc or strip-type system could be in the moisture indication in diapers.

The use of enzymes in a moisture indicator for a wound dressing is also mentioned by Eakin (2003). The indicator described is based on a colour change taking place as moisture enables the mixing of the indicator compounds. An enzyme (e.g. glucose oxidase) can form part of the colour changing reaction mixture.

3.1.3 Systems based on the ability of enzymes to produce energy

Biofuel cells can convert chemical energy to electricity with high overall efficiency using biocatalysts – i.e. enzymes. In comparison with traditional fuel cells, the introduction of enzymes enables the operation of the cell under mild conditions and the utilisation of various, renewable chemicals as fuels (Figure 3-5). Biofuel cells can be utilised in various applications, including miniaturised electronic devices, self-powered sensors and portable electronics. It is also anticipated that implanted biofuel cells could utilise body fluids, particularly blood, as the fuel source for the generation of electrical power, which may then be used to activate pacemakers, insulin pumps, prosthetic elements, or biosensing systems. Biofuel cells have also been suggested for use in the military or security fields for the detection of explosives.

A wide variety of different oxidoreductases are found in nature and many of these enzymes have been applied as catalysts for the anodic and cathodic half-cell reactions of biofuel cells e.g. by Pizzariello *et al.* (2002), Katz and Willner (2003), Kim *et al*. (2003) and Mano *et al.* (2003). At VTT a Tekes-funded project "Printable miniature power source" is currently being carried out in collaboration with Helsinki University of Technology and Åbo Akademi University. This project is focused on fungal laccases as biocatalysts for the cathode half-cell. Fungal laccases are copper-containing enzymes with a relatively high oxidation potential for phenolic compounds, and use molecular oxygen as their electron acceptor by reducing it into water.

The work has focused on the construction of printable enzyme electrodes, which is a challenging area since most conductive inks are based on various solvents, which is contradictory to the fact that most enzymes need aqueous solutions for their stability and catalytic activity (Boer *et al*., 2005). The results have shown that the enzymatic activity can be retained and maintained for months in different conductive inks, depending on the storage conditions. The performance of the ink layers measured both electrochemically and as the expressed activity was also heavily dependent on the ink composition. Under optimised conditions, a fuel cell containing a laccase-based cathode maintained its capacity to generate power for several days.

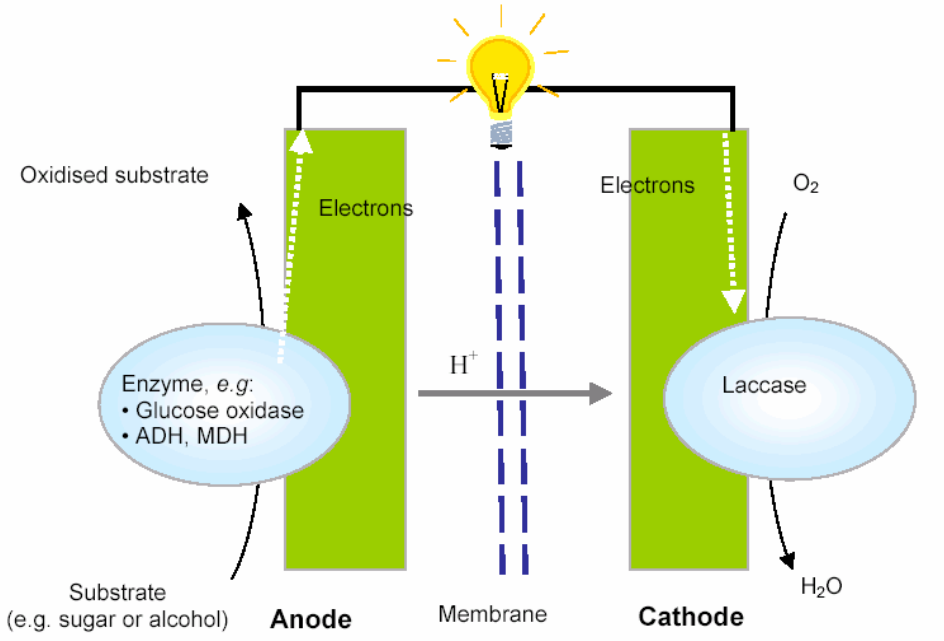

Figure 3-5. Schematic figure of a biofuel cell.

3.2 Immunochemical reactions

Immunoassay is a technology for identifying and quantifying organic and inorganic compounds. Immunoassay utilises antibodies that have been developed to bind with a target compound or class of compounds. These antibodies selectively bind to a target analyte having a specific physical structure that is present in a sample matrix. The binding sites on an antibody attach to their corresponding target analyte (antigen).

Immunochemical detection methods have been utilised in clinical chemistry, environmental analysis and evaluation of food safety. Immunochemical test cards have been developed for *E. coli* O157 and *Salmonella* (Transia). For example, the *E.coli* O157 test is based on one-step immunoassay. A dye pad is impregnated with anti-*E.coli* O157 antibody-dye conjugate. If E.coli O157 antigens are present in the sample, they will react with the conjugate dye and bind to anti-*E.coli* O157 immobilised in the test window, thus generating a coloured band. Typically for pathogen-detecting immunoassays, the enrichment of bacteria is often recommended prior to analysis, hence complicating the direct adaptation of this system to material-integrated functionality.

However, some examples of material-integrated pathogen-detecting systems based on immunochemical reactions have been presented. The commercially marketed Toxin Guard™ by Toxin Alert Inc. (Ontario, Canada, http://www.toxinalert.com/) is a system of building polyethylene-based packaging material that is able to detect the presence of pathogenic bacteria *(Salmonella, Campylobacter, Escherichia coli* O157 and *Listeria)*

with the aid of immobilised antibodies. As the analyte (toxin, micro-organism) is in contact with the material, it will first be bound to a specific, labelled antibody and then to a capturing antibody printed as a certain pattern (Bodenhamer, 2000). The method could also be applied to the detection of pesticide residues or proteins resulting from genetic modifications. This coating is effective on commercial printing equipment running plastic film at 200 feet per minute with 144 tests per square foot (Anon., 2003 / 9th June).

Another commercial system for the detection of specific micro-organisms like *Salmonella sp., Listeria sp.* and *E.coli* is the Food Sentinel System™ by Sira Technologies Inc. (US) (Figure 3-6). This system is also based on immunochemical reaction and the reaction takes place in a bar code (Goldsmith, 1994; Woodaman, 2002). If the particular micro-organism is present, the bar code is converted to unreadable. This commercial system based on the enzymatic or immunochemical detection of pathogens is patented in the US and 17 different venues worldwide (http://www.siratechnologies.com/fspem/tb.html).

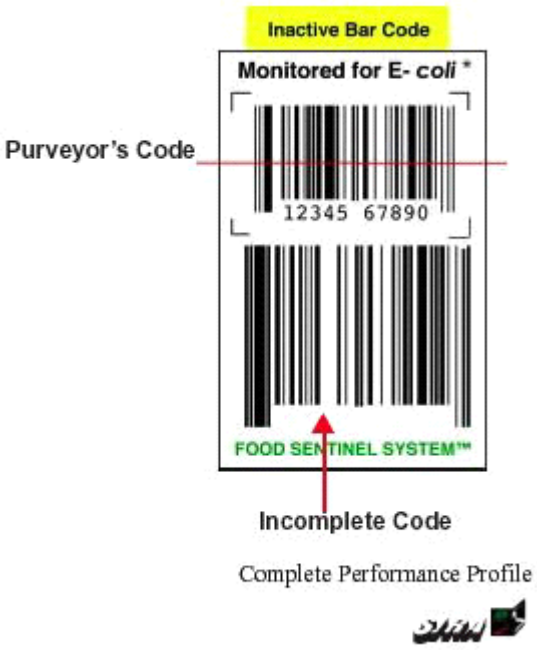

Figure 3-6. Bar code-incorporated pathogen detection system (Sira Technologies Inc.).

Hartman *et al.* (2004) presented a system for detecting gaseous and/or liquid form analytes in packaged materials (e.g. perishable food). The indicator label consists of a film formed from paper or a polymer, an adhesive layer and a detecting system based on an immobilized (capture) antibody layer and a detector antibody layer. The presence of an analyte (micro-organism, enzyme, biogenic amine, etc.) can be detected by visual or non-visual (e.g. UV responsive) means.

In addition to analytical purposes, immunochemical reactions can be harnessed to react and bind pathological effectors or to harvest other specific components from body fluids. For example, McCain and Jarret (1989) proposed the immobilisation of biologically active compounds for polymer support for therapeutic purposes.

3.3 Nucleic acids

The harnessing of nucleic acids for diagnostic purposes has been widely studied in recent years. The basic principles and recent developments of nucleic acid-based biosensors have been reviewed by Junhui *et al.* (1997) and Hahn *et al.* (2005) respectively. The basic principle common to nucleic acid biosensors relies on nucleic acid hybridisation. Different detection methods, such as optical, electrochemical and piezoelectric, have been used to detect the hybridisation.

Nucleic acids have also been widely utilised in microarray-based systems emerging from traditional biochemical assays. For instance, Affymetrix, in collaboration with Institut Pasteur, is developing a microarray-based pathogen detection method that could be used to type multiple pathogens in a single experiment, with high sensitivity and specificity. In addition, this technology will be used to identify genetically manipulated strains through the detection of antibiotic resistance and toxin genes.

In addition to detection systems based on nucleic acid hybridisation, other ways of utilising nucleic acids have also been proposed. The use of DNA as a nanomaterial has been reviewed by Ito and Fukusaki (2004). The use of DNA as conductive nanowire is described. The conductivity of DNA can be utilised in coordinated doping by metals, detection of DNA mismatch and the measurement of biomolecules. Other ways of utilising DNA can be based on nanostructure formation based on self-assembling macromolecules or molecular recognition by synthetic oligonucleotides (aptamers) having an affinity towards target molecules.

Anon. (2005a) reported on carbon nanotubes coated with strands of DNA that were developed at the University of Pennsylvania and Monell Chemical Sciences Centre. They could be used to create sensors that screen packages for explosive gases. The nanosensor consists of single-stranded DNA, which serves as the “detector” and a carbon nanotube, that functions as a “transmitter”. However, a significant number of problems must be solved before these carbon nanotube sensors can be commercialised. The affinity of aptamers towards several compounds, e.g. dinitrotoluene, carcinogenic aromatic amines and ethanolamine, has been studied (Bruno, 1997; Brockstedt *et al.*, 2004; Mann *et al.*, 2005).

Electro-optical nucleic acid-based sensors developed for detecting volatile compounds in air have been presented by White and Kauer (2004). Nucleic acids (RNA, DNA and modified nucleic acids) with attached fluorophores are dispersed on a substrate, which can be paper, fiberglass, silk or fabrics made of synthetic materials. The sensor provides a characteristic optical response when subjected to excitation light energy in the presence of a volatile compound. The inventors mentioned that their invention is distinctly different from other nucleic acid-based sensing materials that work only when both the analytes and nucleic acid materials are dissolved in an aqueous solution. For example, Krull *et al*. (1998) presented a biosensor for the direct analysis of nucleic acid hybridization by the use of an optical fibre functionalized with nucleic acid molecules and fluorescent compounds.

The UK-based Stanelco company is launching a new intelligent PulsLine label that protects foods and high-value goods from counterfeiting and tampering. PulsLine identifies products as fake or genuine and incorporates uncopyable biocodes, DNA, radio frequency identification tags and holograms to meet each customer's specification (Anon., 2005b). DNA-based anti-counterfeiting labels are also provided by Applied DNA Sciences Inc. (Figure 3-7).

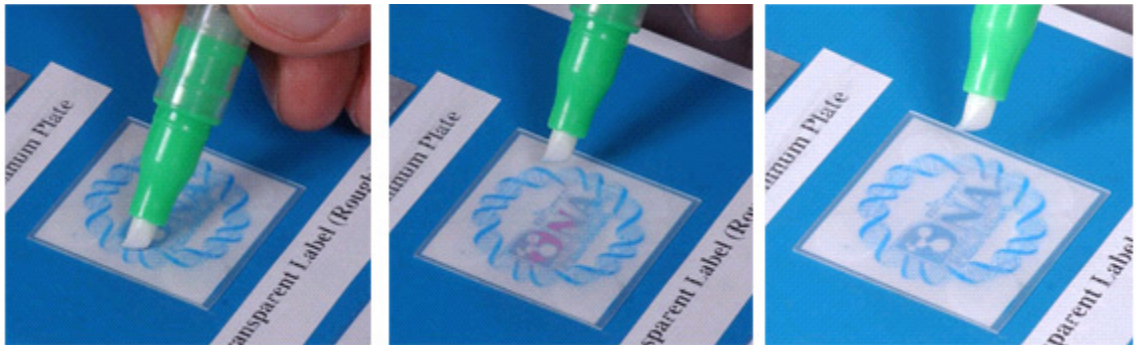

Figure 3-7. DNA-based anti-counterfeit system (Applied DNA Sciences, Inc.).

3.4 Examples of functional devices based on other biomolecules

In addition to the biomolecules described above, various biomolecules and bioactive molecules have been proposed for utilisation in functional materials.

For diagnostic purposes for instance, researchers at the University of Surrey (England) and at Texas A&M University are developing protein-detecting nanosensors that can be incorporated as a strip or patch in packaging in contact with food to detect whether proteins in the food are going off. A polymer imprinting process is used to create nano-sized pores, shaped to either bind or block proteins. Once a physical change takes place

in the polymer, a sliver of quartz crystal can detect this through changes in resonance (Anon., 2005c).

Solvatochromatic dyes, which cannot be truly categorised as bioactive molecules or biomolecules, have been used to indicate the presence of bacteria by incorporating them into various substrates, such as films, woven and non-woven fabrics, paper towels, coform and airlaid materials and bonded carded webs. Solvatochromatic dyes change colour in response to a change in the polarity of the environment, which, in turn, has been found to be dependent upon the presence or absence of bacteria due to their bi-lipid cell membranes (Lye *et al.*, 2005).

A specific indicator material for the detection of *Escherichia coli* O157 enterotoxin has been developed at the Lawrence Berkeley National Laboratory (Kahn, 1996; Stevens & Quan, 1998). This sensor material, which can be incorporated in the packaging material, is composed of cross-polymerised polydiacetylene molecules and has a deep blue colour. The molecules specifically binding the toxin are trapped in this polydiacetylene matrix and as the toxin is bound to the film the colour of the film changes from blue to red. According to PIRA (Anon., 2005a–c), a similar approach has been taken at Ohio State University, where nanorods have been coated with E. coli-sensitive, colour-changing material.

Ahvenainen *et al*. (1997) and Smolander *et al*. (2002) utilised a reaction between hydrogen sulphide and myoglobin in a freshness indicator for the quality control of modified-atmosphere-packed poultry meat. The freshness indication is based on the colour change in myoglobin by hydrogen sulphide (H_2S), which is produced in considerable amounts during the ageing of packaged poultry during storage. The indicators were prepared by applying commercial myoglobin dissolved in a sodium phosphate buffer on small squares of agarose. Neary (1978) patented a colour-changing food spoilage indicator essentially consisting of liquid crystal cholesteric compositions (e.g. cholesteryl or cholestanyl chloride) disposed in a carrier of plastic film semi-permeable to the gases generated in food spoilage (e.g. ammonia, hydrogen and carbon dioxide). Naturally occurring betalain or flavonoid-based molecules have been used as colour-changing pH-sensitive dyes for detecting amines in packaged foodstuff (Williams & Myers, 2005).

A sensor array based on biological sensing elements like bacterial periplasmic binding proteins, membrane proteins, odorant binding proteins and DNA binding proteins whose characteristics change when they are bound to different ligands has been proposed by Cass (2001).

Several examples can also be found in the development of active materials. For instance, chitosan, the second most abundant natural biopolymer after cellulose, which is an edible and biodegradable material also having antimicrobial activity against bacteria, yeasts and moulds (Yalpani *et al.*, 1992), has been widely harnessed. Due to its good film-forming properties, chitosan may be successfully used in food packaging materials, coated papers and surface treated films (Muzzarelli, 1986; Vartiainen *et al.*, 2004; Gällstedt *et al.*, 2001). In the EU-funded Solplas project the combination of tailored chemical surface activation by cold atmospheric plasma treatment and subsequent wet-chemical coating using a bio-based antimicrobial solution resulted in coated plastic films with good antimicrobial activity and improved barrier properties against oxygen transmission (Vartiainen *et al.*, 2005a). The anti-microbial activity of chitosan can also be utilised in hygienic products manufactured from polypropylene non-woven coated with chitosan (Anon., 2001). Chitosan has also been used to treat cotton fabrics to impart anti-microbial activity (Zhang *et al.*, 2003).

Infection-resistant biomaterials like wool and silk have also been produced by sorption of antibiotics into the material (Choi *et al.*, 2004a–b). Synthetic materials like polyester have been modified with antibiotics (Buchenska *et al.*, 2003; Anon., 2005b). Bioactive peptides as antimicrobial substances have been proposed by McDaniel (2005), who mention wood, paint, adhesive, glue, paper, textile, leather, plastic and cardboard as potential materials for protection.

3.5 Immobilisation of biomolecules

The leading techniques in the immobilisation of biomolecules onto solid supports are physical adsorption, entrapment and covalent attachment (Shriver-Lake, 1998). A suitable method can usually be found for any biomolecule. However, immobilisation may introduce unwanted changes in the structure of a biomolecule, which reduces its activity. For example, many proteins lose their biological activity due to denaturation, dehydration or oxidation.

3.5.1 Physical adsorption

Physical adsorption is a simple method for coating surfaces. It takes advantage of non-covalent interactions such as van der Waals forces, hydrogen bonding, or hydrophobic interactions. Molecules attached through adsorption tend to slowly leach from the surface. The adsorbed molecules form a randomly-oriented, heterogeneous surface (see Figure 3-8), which may lead to reduced activity, and the surface density is not always very high.

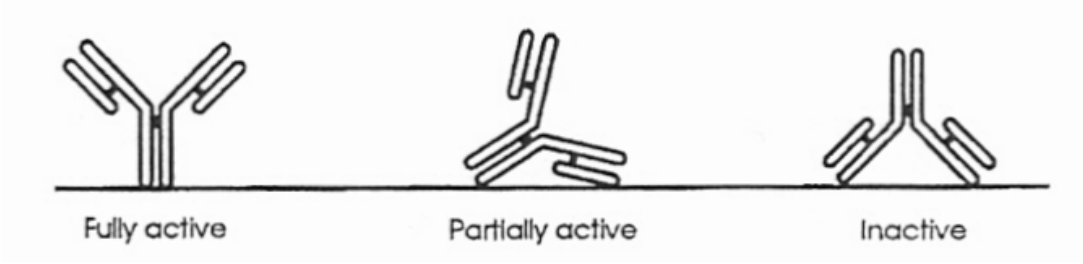

Figure 3-8. Ideal representation of an IgG antibody immobilized by a random coupling procedure (Lu et al., 1996).

3.5.2 Entrapment

Immobilisation through entrapment has been used with polymeric materials. The biomolecules are mixed with the reactants before the polymerisation is complete, polymerisation is allowed to occur, and the biomolecules become entrapped in the polymer matrix. Studies have shown that entrapment may protect the proteins against proteolytic deactivation, enhance their thermal stability and improve their reusability (e.g. Sunil *et al.*, 2001; Ortega *et al.*, 1998). Chen *et al.* (2002) entrapped lipase enzymes in organic-inorganic hybrid sol-gel polymers. By forming the gels within the pores of a non-woven polyester fabric they obtained a novel immobilized biocatalyst in sheet configuration. They also observed increased specific activity and thermal stability compared with a free enzyme.

In addition to acting as a carrier for embedded functional additives like biological or organic compounds, there are many other reasons for the use of such sol-gel coatings to functionalise textiles. Nano-sized oxide sol-gel particles form well-adhering transparent oxide layers on textiles to provide stability against light, heat, chemical and microbial attacks. Such layers may improve the mechanical properties of the textiles, e.g. high mechanical strength, wear and abrasion resistance, and offer possible ways of varying the material properties like hydrophobicity, electric conductivity, or even optical properties. Compounds can be non-diffusibly embedded into sol-gel coatings and the controlled release of e.g. biocides is also possible (Mahltig *et al.*, 2005).

However, there are some problems with entrapment as an immobilisation method. The structure of the polymer network may reduce or completely block the diffusion of a large analyte. There might also be problems with leaching if the entrapped molecule is small, and the reactive prepolymers or the conditions during the polymerisation may be harmful to the biomolecule.

3.5.3 Covalent immobilisation

Most stable and uniform bioactive surfaces are usually achieved by covalent immobilisation. In order to covalently link biomolecules onto solid supports, both need to contain functional chemical groups through which the immobilisation occurs. Quite commonly, the surface or the biomolecule is derivatised with a homo- or hetero-bifunctional chemical linker that acts as a bridge between the surface and the biomolecule. A homo-bifunctional linker contains two identical reactive groups, whereas hetero-bifuncional cross-linkers contain two different reactive groups. As an example of a homo-bifunctional cross-linker, glutaraldehyde contains two aldehyde groups that are reactive towards primary amines. If both the surface and the biomolecule are known to contain primary amines, glutaraldehyde can be used to link these together. Photoreactive cross-linkers bind non-specifically to any reactive group when exposed to light. The cross-linkers may be available in different lengths – longer ones to reduce steric hindrances – and may include cleaving sites.

In addition to primary amines, sulhydryls, carbonyls, carboxylic acids and carbohydrates of biomolecules can also be used in immobilization. Carbohydrate moieties of proteins (e.g. antibodies) can be chemically or enzymatically oxidized into aldehydes that form covalent bonds with hydrazide-activated surfaces (Lu *et al.*, 1996). Antibody Fab -fragments can be covalently attached onto gold surfaces by disulfide anchors (Vikholm-Lundin, 2005). Gold-thiol self-assembly can also be exploited in the immobilisation of other sulfhydryl-containing molecules.

Inorganic surfaces such as quartz, glass or silica can be derivatised using organofunctional silanes. These coupling agents react with surface hydroxyl groups and provide a new reactive residue to bind biomolecules or cross-linkers (Shriver-Lake, 1998).

3.5.4 Affinity immobilisation

The natural affinity between certain molecules can be applied to biomolecule immobilisation. For example, different sugar binding molecules, such as maltose-binding protein, cellulose-binding domain (CBD) and chitin-binding domain, are used as immobilisation agents. In this case the protein to be immobilised is produced as a fusion protein with a suitable affinity tag and is easily attached to appropriate surfaces. There are many different affinity fusion tag systems; some have been well reviewed by e.g. Terpe (2003) and Nilsson *et al.* (1997).

As cellulose, being the most abundant biopolymer on earth, is a major constituent of many commercial products, CBD has been exploited in the functionalising of many

materials. CBD has been used for enzyme targeting to get the stone-washed look for denim, and CBD-conjugated fragrance particles have been added to washing powders. Anti-microbial agents have been targeted at cellulose-containing surfaces through CBD, and even whole cells have been immobilised using CBD. Cellulosic filters can be functionalised with CBD. CBD can also be utilised in the modification of paper properties, e.g. to increase tensile strength (Levy & Shoseyov, 2002).

The affinity between gold and thiol-containing molecules has also been exploited in various applications.

3.5.5 Examples of materials used in immobilization

According to Chen and Gorski (2001), several different strategies have been used in the immobilization of enzymes for electro-chemical biosensor applications such as covalent bonding, bioaffinity attachment and entrapment in various polymers, redox gels, sol-gel derived glasses, carbon pastes and carbon-polymer electrodes. Some of these different material groups are reviewed in this chapter.

Redox gels

In the work by Bu and co-workers (1996) enzyme electrodes were fabricated by entrapping glucose oxidase (Gox) in charged ferrocene-containing redox gels formed by the copolymerization of a vinyl ferrocene-hydroxypropyl-beta-cyclodextrin inclusion complex, acrylamide and N,N'-methylenebis(acrylamide) together with a positively or negatively charged monomer at pH 7 in the presence of Gox in an aqueous solution. They observed that both 2-acrylamido glycolic acid monohydrate (AGA) and acrylic acid (ACA) containing gels and their sensors possess much better storage and operational stabilities than the neutral gels and sensors, indicating that entrapped Gox is more stable in the negatively charged gels.

Sol-gel derived glasses

The article by Sampath and Lev (1997) gives an example of a method by which enzymes have been entrapped in sol-gel derived glasses. They have developed a rhodium-modified amperometric biosensor based on a ceramic-carbon composite electrode. The sensors are comprised of rhodium (Rh) metal and glucose oxidase-modified graphite particles embedded in a porous, organically-modified silicate network. The continuous supply of oxygen from the top of the electrode through the porous structure facilitates operation, even in oxygen-free solutions, in a gas diffusion/biosensing mode of operation.

Rigid conducting carbon-polymer composites

According to Cespedes and Alegret (2000), carbon-polymer composites are ideal for the construction of electrochemical sensors. The plastic nature of these materials makes them modifiable, permitting the incorporation of catalysts, mediators and cofactors that improve the response of the biosensor. The proximity of the redox centres of the biological material and the conducting sites on the sensing surface also favours the transfer of electrons between electroactive species. Furthermore, the sensing surface can be renewed by a polishing procedure.

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4. Embedded sensing and signalling

4.1 Introduction

Biosensors are devices that combine the selectivity and specificity of a biological sensing element such as an enzyme or an antibody with a suitable transducer. The integration of these enzymes or other biomaterials with electrical and optical transducers is an important consideration in the development of biosensors since it affects the electrical communication between the enzyme redox centres and electrodes, and the interaction of light with the biomolecules.

A signal is only produced in electrical biosensors if the electrons can be transferred from the redox centre of an immobilized enzyme to the electrode. In non-conductive polymers electron transfer can be accomplished in several ways. For example, electron transfer mediators such as molecular oxygen can be used in "electron shuttles" between the enzyme and the electrode. These mediators transfer electrons by diffusion, which can be a rather slow process. In addition, electron transfer mediators have to be recycled for the current to be generated. By using direct electron transfer between the enzyme and the transducer, the signal transduction and detection are faster. This can be achieved with self-assembled monolayers (SAMs), in which protein molecules in the first monolayer on an electrode surface can be oriented towards the electrode.

Chen and Gorski (2001) have reported various strategies for the immobilization of enzymes to the electro-chemical sensors, e.g. covalent bonding, bioaffinity attachment and entrapment in various polymers, sol-gel derived glasses, carbon pastes and carbon-polymer electrodes (see Chapter 3.5, p. 25). The electrical communication between the enzymes and electrodes has been accomplished either by using redox mediators or by direct electron transfer between the enzyme and the electrode surface.

In optical biosensors, either the light intensity or the light wavelength, i.e. colour, is changed due to binding of the analyte molecules to the receptor (see Figure 2-1, p. 7), and this change is detected. The binding event can be sensed directly via the small changes in the refractive index in the proximity of the transducer surface, or via the changes in the physical structure of the sensor. In indirect sensing, labels that induce the optical activity of the sample by tagging them with optically active molecules such as fluorophores are used. In addition, chromophores that are molecules that change their colour due to the analyte are used for optical signalling.

Various electrical and optical transduction methods for biosensors are reported in this chapter. The main emphasis is on the use of conducting polymers and optics for sensing and signalling analyte molecules.

4.2 Conducting polymer-based biosensors

4.2.1 General information

Conducting polymers are a class of polymers that have a conjugated backbone structure, i.e. they have alternating single and double bonds in their main chain. This is the basic reason behind their electronic conductivity. In addition, a dopant like a strong acid is needed to achieve high enough conductivity. Conducting polymers are very useful tools for communication with biological systems. These materials have high electrical conductivity and can be switched between different oxidation states so they can conveniently uptake and release anions like biomolecules. In the case of polyaniline, pH switching provides another means for anion uptake (Wallace & Kane-Maguire, 2002). Signal transduction is based on electron movement in the polymer lattice with different, highly complicated mechanisms, which are still relatively poorly understood.

The simplest way to use conducting polymers in sensing is to use an undoped polymer, which is allowed to react with some dopant-like molecule. Thus the existence of this molecule can be detected because the conductivity of the polymer changes after being in contact with the dopant. Another method is to use a doped polymer, which can sense the presence of a dedoping agent.

Some commercial applications of conducting polymer arrays have been developed. AromaScan, "an artificial nose", was developed at the University of Manchester Institute of Science and Technology (UMIST) in the UK. It is composed of 32 slightly different conducting polymers, whose resistance varies depending on the binding species, giving a unique fingerprint from which the analyte can be identified. (www.Aromascan.com.) AlphaMOS has a commercial sensor that combines different sensor technologies in which conducting polymers are the sensing elements for polar molecules (www.alpha-mos.com). Zellweger Analytics has developed a portable electronic nose for the detection of toxic gases in the environment (www.zelana.com).

United States Patent Application No. 20050062486 ("Multifunctional conducting polymer structures" by Qi and Mattes, 2005) includes the use of conducting polymers as sensors in distributed sensing systems, as sensors and operating elements in multifunctional devices, and for conducting polymer-based multifunctional sensing fabrics suitable for monitoring humidity, breath, heart rate, blood (location of wounds), blood pressure, skin temperature, weight and movement in a wearable, electronic-embedded sensor system. The fabric comprising conducting polyaniline fibres can be used to distribute energy for resistive heating as well as for sensing the fabric temperature.

4.2.2 Advantages in using conducting polymers

There are several advantages in using conductive polymers. Direct electron transfer is obviously one of the main advantages, providing faster sensor response times and simplicity because no extra additives are needed. Unfortunately this is not always possible and mediators have to be used. Secondly, depositioning of biomolecules in biosensors has been achieved in many different ways, for example covalent cross-linking and gel encapsulating. With conducting polymers, electropolymerizing provides a reproducible, non-manual method for producing polymer coatings with electrochemically-controlled thickness and the opportunity to immobilize different biomolecules with spatial location. The reagentless and simple synthesis ensures superior biocompatibility (Cosnier, 2003). In addition, conductive polymers are relatively inexpensive to produce and are suitable for high-volume production. Finally, conductive polymers provide a label-free method of detecting many different biomolecules, so conductive polymers could replace routine clinical analyses like radioisotope labels in clinical applications so the problems in handling and disposing radioactive residues could be avoided (Vidal *et al.*, 2003).

In biosensor applications, conducting polymers enable direct electron transfer from the biomolecule to the electrode without the need for mediators, providing fast and straightforward communication with biological systems at the molecular level. Previously, the enzyme had to be immobilized close to the electrode surface for direct electron transfer but with conductive polymers the enzyme can be located a considerable distance from the electrode surface (Habermuller *et al.*, 2000). Most redox enzymes, however, particularly large ones, do not interact directly with the electrode because their redox centres are located far from the enzyme surface. Therefore, electron carriers can be added to the conducting polymers. With time, these carriers tend to leak out of the polymer matrix. Therefore, direct electron transfer via the polymer matrix is a clear advantage, giving the sensor longer life and more stability. Direct electron transfer is easier with smaller enzymes (Heller, 1990). Large multi-enzyme complexes with several redox-cofactors can transfer electrons between the sub-units of the enzyme, bringing the electrons closer to the surface and, finally, via conducting polymers chains, to the electrode (Habermuller *et al.*, 2000). Figure 4-1 shows a simplified suggested mechanism for electron movement with the aid of a conducting polymer matrix.

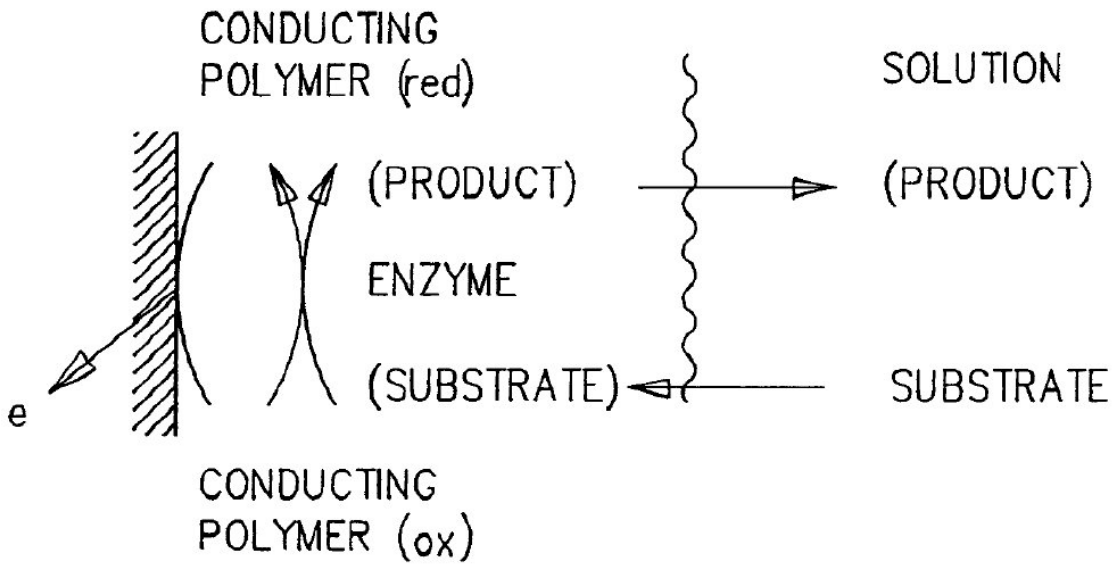

Figure 4-1. A simplified pathway suggested for electron movement with the aid of a conducting polymer matrix (Gerard et al., 2002).

4.2.3 Electrical measurement methods of entsymatic conducting polymer biosensors

According to the review article by Gerard *et al.* (2002), different types of biosensors having some kind of conducting polymer as a component have been developed. The types of biosensors that are dealt with in the article are the following: amperometric, potentiometric, conductometric, optical, calorimetric and piezoelectric. All of these, except optical and calorimetric biosensors, involve some sort of electrical response. Table 4-1 shows several examples of enzyme/conducting polymer combinations suitable for amperometry and potentiometry.

Table 4-1. Biosensors based on conducting polymers (Gerard et al., 2002).

Substrates or species to be determined	**Enzyme**	**Polymer**	**Detection**
Glucose	Glucose oxidase	Polypyrrole	Amperometry Potentiometry
		Poly(N-methylpyrrole)	Amperometry
		Polyaniline	Amperometry
		Polyindole	Amperometry
	Glucose dehydrogenase	Polypyrrole	Amperometry
D-Alanine	D-amino acid oxidase	Polypyrrole	Amperometry
Atrazine	Tyrosinase	Polypyrrole	Amperometry
Cholesterol	Cholesterol oxidase and Cholesterol esterase	Polypyrrole	Amperometry
Choline	Choline oxidase	Substituted polypyrrole	Amperometry
Glutamate	Glutamate dehydrogenase	Polypyrrole	Amperometry
Fructose	Fructose dehydrogenase	Polypyrrole	Amperometry
Hemoglobin	Pepsin	Polyaniline	Conductometry
L-Lactate	Lactate oxidase Lactate dehydrogenase	Polyphenyline di-amine Polyaniline Polypyrrole-polyvinyl sulphonate	Amperometry Amperometry Amperometry
Lipids	Lipase	Polyaniline	Conductometry
Phenols	Tyrosinase	Substituted polypyrrole	Amperometry
Urea	Urease	Polypyrrole	Amperometry Potentiometry Conductometry Capacitance measurement Admittance measurement
Uric acid	Uricase	Polyaniline	Amperometry
Triglycerides	Lipase	Polyaniline	Conductometry

4.2.3.1 Amperometric biosensors

Amperometric detection is the most commonly used technique. In amperometric biosensors the current produced during the oxidation or reduction of a product or reactant is usually measured at a constant applied potential. The role of conducting polymers is to immobilize enzymes on an electrode surface and to promote the electron transfer between the redox catalyst and the electrode. Electrical parameters (conductivity, capacitance, electrochemical potential) associated with the redox state of the conducting polymers may change or be modulated by the interaction with biomolecules, providing direct biosensor response. Some examples of conducting polymer-based amperometric biosensors are briefly described in the following chapters.

Foulds and Lowe (1988) studied the interaction of glucose oxidase with ferrocene-containing polymers of pyrrole and the use of this system in the construction of a reagentless glucose sensor. Reagentless glucose electrodes were generated by the synthesis of N-substituted pyrrole monomers containing redox-active side chains designed to accept electrons from the reduced form of the enzyme.

Skinner and Hall (1997) investigated the origin of the glucose response in a glucose oxidase polyaniline system. The origin of the response signal was explored using immittance spectroscopy, and it was shown that an RC sub-circuit in the equivalent circuit model was sensitive to peroxide concentration. The interaction of peroxide with polyaniline at potentials where it either oxidised or reduced polyaniline was also discussed in the article.

Vidal *et al.* (2003) have written an extensive review article about electropolymerized conducting polymers in amperometric biosensors. According to them, the measurement of the amperometric current is a simple and sensitive transduction that relates to the analyte concentration, even if the analyte itself is not electroactive.

4.2.3.2 Potentiometric biosensors

Potentiometric sensors measure potential changes in the polymer matrix during the binding of an analyte to the sensor. Potentiometry is a less common detection method than amperometry in biosensors having enzymes immobilized in an electrodeposited polymer layer. According to Gerard *et al.* (2002), the rate of potential change rather than steady state potential values should be considered in the case of biosensors having a very slow response.

According to the article by Trojanowicz (2003), the sensitivity of polypyrrole to pH changes has been utilized in the design of potentiometric biosensors for glucose, urea creatinine and acetylcholine.

In the work of Yamato *et al.* (1995) an enzyme electrode was made by co-immobilization of three enzymes (creatininase, creatinase, sarcosine oxidase) in an active polypyrrole (PPy) matrix to detect creatinine enzyme.

4.2.3.3 Conductometric biosensors

Conductometric biosensors measure the changes in the conductive properties of the polymer, which can arise from a change in the redox potential or pH of the polymer. In practice, conductivity/resistance is measured before and after exposure to the analyte (Gerard *et al.*, 2002). Conductometric biosensors based on conducting polymers have been developed for penicillin (Nishizawa *et al.*, 1992) and for glucose, urea, lipids and hemoglobin (Contractor *et al.*, 1994).

In their work, Nishizawa *et al.* (1992) reported on a novel enzyme sensor consisting of an interdigitated microarray electrode coated with a polypyrrole and penicillinase membrane (Figure 4-2). The enzyme reaction acidifies polypyrrole, resulting in an increase in its electrical conductivity. The conductivity changes were detected as an increase in the current between the two arrays at a constant voltage.

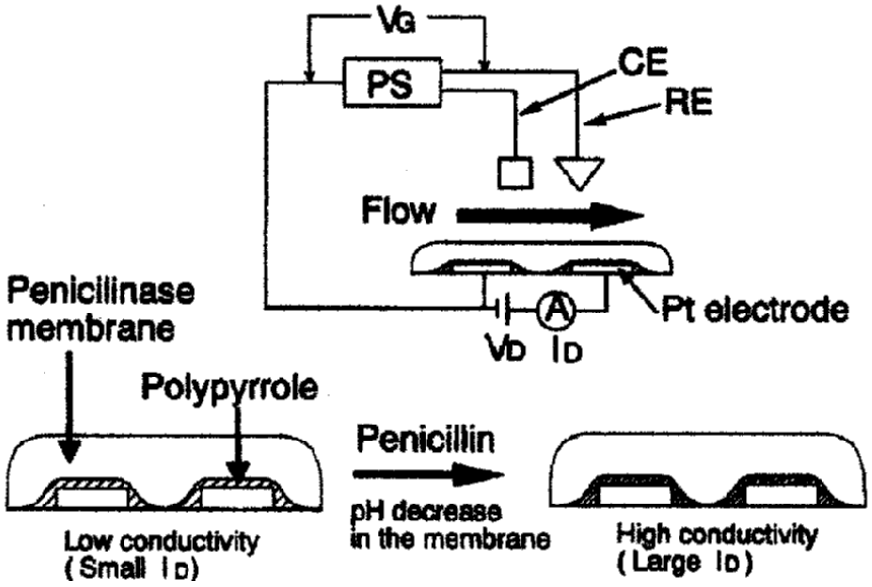

Figure 4-2. Principle of the penicillin sensor and the configuration of the electrochemical apparatus for in-situ conductivity measurement: PS, potentiostat; CE, Pt counter electrode; RE. Reference electrode (SCE); V_G, gate voltage; V_D and I_D, drain voltage and current (Nishizawa et al., 1992).

4.2.3.4 Some examples of conducting polymers with enzymes

The most obvious way to combine conducting polymers with enzymes is to use the enzyme as part of a dopant. Sung and Bae (2000) studied glucose oxidase (GOD) conjugated with a polyanion (poly2-acrylamido-2-methylpropane sulfonic acid) (poly-AMPSA), via a (polyethylene oxide) spacer and this compound was used as a dopant for electrochemical polymerisation of pyrrole. They chose to use the conjugate with a known dopant, AMPSA, instead of just the enzyme as such. The results of the study showed that the bioactivity of GOD was well preserved after conjugation with AMPSA and polymerisation in the presence of the conjugate was successful even though the dopant was large. The GOD-AMPSA-PPy film had a homogeneous and closely packed structure but plain GOD didn't form good films with PPy due to a weak negative charge. The current response vs. glucose concentration was studied and the enzyme-electrode had a rapid response time and high sensitivity to glucose. The response was virtually linear up to a 20 mM glucose concentration.

Brahim *et al.* have incorporated both polyaniline and polypyrrole in hydrogel networks (Brahim *et al.*, 2002; Brahim *et al.*, 2003). The hydrogel they used was based on hydroxyethyl methacrylate (HEMA) and a composite of the conducting polymer and the gel could be used as a matrix for several oxidase enzymes. This kind of system could be used to sense glucose. The usage of hydrogel as the supporting medium gives additional opportunities because it responds to specific environmental stimuli like pH, temperature and ionic strength, and may also possess high levels of hydration, which might be important in bio-related applications.

4.2.4 Utilization of conducting polymers with biomolecules

This chapter discusses examples of how conducting polymers can be utilised when they are used together with biological components other than enzymes. The utilization of conducting polymers with enzymes was discussed in the previous chapter (Chapter 4.2.3, p. 40).

4.2.4.1 Conducting polymers and nucleic acids

Conducting polymers have good surface confinement capability and can be used to immobilise DNA on their surfaces. They have the ability to induce electrical signals accrued from DNA interactions. One example is basepair recognition for switching the electronic properties of the conducting polymer. If oligonucleotide (ODN) is grafted onto a polypyrrole (PPy) chain, a reduced current response is seen during the duplex

formation. Wang and Jiang (2000) have studied the use of nucleic acids as counter-ions (dopants) for polypyrrole, and they showed that it really is possible to use these ions as dopants. The incorporation of ODN-dopants was mainly demonstrated with an electrochemical quartz crystal microbalance (EQCM), which is a dynamic method that shows the growth patterns of polypyrrole.

Polymerisation of pyrrole was done in the presence of different lengths of oligonucleotides and followed with EQCM, which showed the time-frequency recordings during the polymerisation reaction. There were successive changes in the resonance frequency after each potential cycle during electropolymerisation. Since each frequency reduction reflects the increased mass, the reaction was said to follow normal conducting polymer growth pattern. Cyclic voltammetry showed results that corresponded to the EQCM-results and oligomer with 20 repeat units; oligo(dG20) showed the largest growth current (and frequency change) and was thus chosen as the best candidate to function as a dopant. Short oligonucleotides (reason not understood) or chromosomal DNA (probably too big to diffuse) couldn't function as dopants.

Applications for conducting polymer-DNA composites could be genoelectric devices, composite materials, bioactive interfaces, genetic analysis and probing of DNA charge transfer. Of particular interest is the distinctly opposite current peaks observed in the presence of complementary and non-complementary oligonucleotides.

4.2.4.2 Conducting polymers and antibodies

There are different methods that can be used to bind biological molecules to conducting polymers. If physical entrapment is used, the biological component can be attached easily but it is usually randomly oriented and thus inaccessible to the target analyte. Immobilisation of a secondary counter-ion with the biological component produces problems like reproducibility and accessibility. Adsorption techniques are used to attach the bioactive molecule to the surface, and this kind of outer layer immobilisation overcomes the problem of burial. However, there are problems with the loss of the biological component due to diffusion into the sample solution. Covalent attachment produces no problems with reproducibility or orientation but it is time consuming and expensive.

Minett *et al.* (2002) have compared the differences between direct incorporation versus covalent attachment when the antibody to *Listeria* has been attached to polypyrrole. The study of the physical entrapment method ("doping method") described a PPy-a*Lis* polymer film using the antibody as counter-ion. A modified ELISA test kit for *Listeria* was used to study the response to *Listeria* micro-organisms (antibody-antigen binding).

Positive and negative controls (polymers having anti-*salmonella* or NO_3^- anion instead of anti-*Listeria*) were used in order to get reliable results. A clear difference between the positive and negative control was seen, which proved that the dopant was active, but there were problems with reproducibility: the antibody was sometimes buried in the polymer matrix and couldn't function reliably.

Another important study described the detection of polychlorinated biphenyls (PCBs). The existing methods for detecting PCBs (gas chromatography, mass-selective detectors, mass spectrometers) are accurate but tedious and too expensive for large-scale screening purposes. Bender and Sadik (1998) studied a novel and rapid immunosensing method for PCBs using conducting polymer electrodes. The method is based on the measurement of changes in current due to the specific binding of the analyte to an improved antibody-immobilised conducting polymer matrix, which was synthesised by a unique electrochemical assembly of pyrrole in the presence of a polyclonal anti-PCB antibody. The results were promising: antibodies were not only incorporated but still retained their bioactivities when immobilised into the conducting polymer matrix. The linear dynamic range of detection varied between 0.3 and 100 ng/mL with a correlation coefficient of 0.997. High selectivity for PCBs in the presence of potential interference such as chlorinated anisoles, benzenes and phenols was also achieved. Thus the detection method could be applied to continuous monitoring of effluent such as waste streams and groundwater.

Redox reactions are typically utilized in biosensors based on enzymatic reactions, but there are no electroactive species in antibody-antigen interactions. Currently, the antibody-antigen interactions are supposed to affect the capacitive properties of the polymer layer. At the molecular level this arises from a conformational change in the polymer and a change in the polymer redox state as a result of the antibody binding to antigen. The potential shift has been proved to be proportional to the antigen concentration (Sargent *et al.*, 1999). Li *et al.* (2005) have developed a highly sensitive electrochemical immunosensor using impedance detection with a detection limit of 10 pg/ml. With biotin-avidin linking, an impedimetric immunosensor with a detection limit of 10 pg/ml had been developed earlier (Ouerghi *et al.*, 2002).

4.2.4.3 Conducting polymers in drug delivery

There is a request for new drug release systems in which bioactive molecules contained in a reservoir can be supplied to a host system while controlling the rate and period of the delivery. Optimally, the drug is delivered to the precise region of the body where it is physiologically required. The system should also be able to sense some chemical species (pH, concentration of an analyte) or it could be controlled using an external

trigger. Conducting polymers have been used in drug delivery; they work because the electrochemical switching of the polymer is accompanied by the movement of counter-ions in and out of the membrane for charge balance. A variety of anions, including salicylate and ferrocyanide, glutamate and ATP, have been electrostatically entrapped into the conducting polymer matrix and released during reduction. The polymer should be able to sense some external impulse, and the simplest sensors use an undoped polymer that is exposed to the dopant vapour. Gas sensors (NH_3, SO_2, etc.), ion sensors (Cl^-, pH, etc.) and biosensors (glucose, proteins, DNA, etc.) like this have been presented but they cannot yet function as a drug delivery system.

Pernaut and Reynolds (2000) present a conducting polymer that senses a chemical species in solution and subsequently releases another chemical substance (drug) as a function of the concentration of the sensed species. Polypyrrole/adenosine triphosphate (ATP) was chosen as the conducting polymer/anionic drug model and they used a conducting polymer membrane on a conducting substrate in contact with a solution or liquid electrolyte. The release properties were the following: an electrochemically-triggered release of ATP followed a reaction in which the anionic drug was incorporated during electrosynthesis and remained electrostatically bound to the polymer inside the membrane. When a negative potential neutralised the positive charges on the polymer the drug was allowed to diffuse out of the membrane. Control of the delivery rate by synthesis conditions was possible since the low polymerisation rate produced dense membranes and ATP was trapped and released slowly. The high polymerisation rate produced porous membranes where ATP was not trapped and was released faster. The results showed that the PPy/ATP membrane could be used to sense chemical species using a number of parameters, including electrical conductivity, electrode potential and optical absorption.

4.2.4.4 Conducting polymers as biofunctionalised surfaces

In some applications it is important to use conducting polymers in connection with living tissue and find ways of modifying the surface properties of the polymer so that it is biocompatible but the original properties are still retained. Here are shown two examples of how polypyrrole can be surface functionalised. Cen *et al.* (2004) used hyaluronic acid (HA) to improve the surface biocompatibility of polypyrrole and the surface was readily functionalised through a cross-linker. The immobilised HA retained its biological activity on PPy and the surface exhibited a high cell attachment ratio of 80%, which is desirable for a biomaterial designed specifically for the application of nerve generation and nerve repair. Blood compatibility was also improved in comparison with pristine PPy.

Sanghvi *et al.* (2005) used an M13 bacteriophage library to screen 109 different peptides to find which one could be attached to chlorine-doped PPy. The best one, called T59, was used to promote cell adhesion on PPyCl.

4.3 Optical sensing and signalling

This chapter discusses optical signalling and sensing. There are only two properties of light that can be used for signalling; light wavelength, i.e. colour, and light intensity and thus optical signals can only be based on colour change or intensity change, or their combination.

Optical biosensors can be based on either direct or indirect detection methods. In direct detection sensing is based on small changes in the refractive index or changes in the physical structure caused by the binding of the analyte, which changes the light intensity or wavelength. In indirect detection the optical signal is generated by attaching an optically active label, e.g. fluorophore, to the analyte, and the signal emitted by the label is detected. Chromophores that change their colour due to an analyte are also widely used for optical signalling. Chromophores and labels are discussed in Chapter 4.3.1 and some simple optical sensors are introduced in Chapter 4.3.2.

4.3.1 Chromophores and labels

There are various methods for implementing optical signalling: the pattern can be changed, for example, due to an enzyme activity (see Figure 3-6, p. 21); chromophores can react to the analyte by changing their colour (see Figure 3-2, p. 12); fluorescent molecules (Haugland *et al.*, 2005) and quantum dots (Medintz *et al.*, 2005) that are excited by using shorter wavelengths and emit longer wavelengths when the excited state relaxes are used as labels to mark analytes; the colour of the light scattered by nanoparticles (Seydack, 2005) changes due to the binding of the analyte (see Figure 4-3) and molecular beacons (Broude, 2002) fluorescence in the presence of the target DNA sequence (see Figure 4-4).

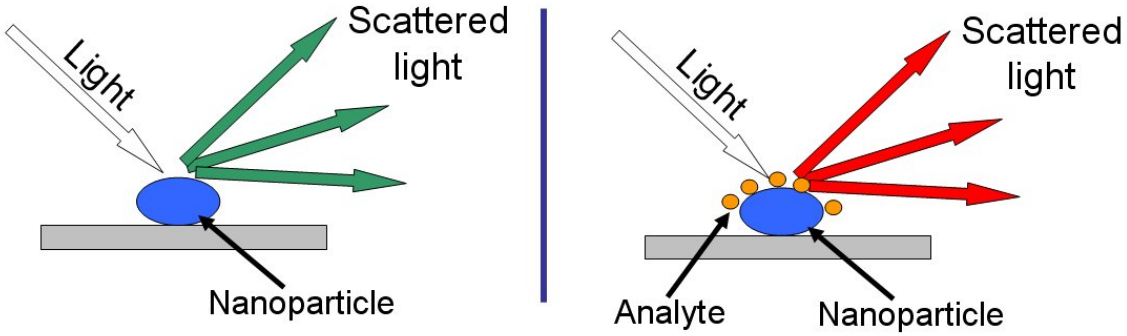

Figure 4-3. The colour of the light scattered by a nanoparticle changes due to the binding of the analyte molecules.

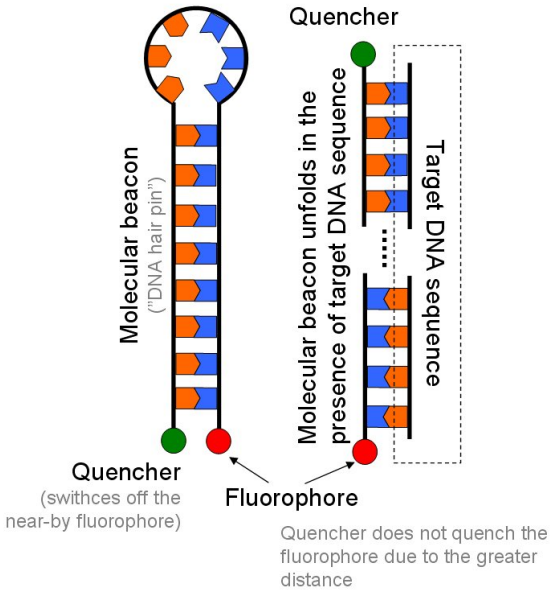

Figure 4-4. Molecular beacon. The quencher switches off the near fluorophore when the molecular beacon is folded. Fluorophore starts to fluorescence when molecular beacon unfolds due to the target DNA sequence.

The colour change of a chromophore due to a chemical reaction caused by an analyte is widely used in commercial test strips and kits for example for pH, proteins, glucose and ketones. The tests are usually done from liquid samples but Rakow and Suslick (2000) have developed a colorimetric vapour-sensing device that is based on the colour change caused by the binding of the odiferous compounds to the metal ions. The advantages of the colour-based signalling are – simple visual readout and ease of use – because the chromophores can be readily attached to the paper or fibre product and there is no need for additional chemical processing. The disadvantages of the colorimetric signalling are that it does not provide quantitative measurements without additional readout devices,

and the chromophore and its surface chemistry has to be chosen and tailored individually for each signalling application.

Fluorophores, nanoparticles and molecular beacons are labels, i.e. tags or marks, that are used to mark the analytes and induce the optical activity of the sample. Excluding the molecular beacons, they are not intrinsically specific towards analytes and have to be immobilized with some bioactive molecules, such as antibodies, in order to specifically react with the analyte.

The use of fluorophores in the bioactive paper products is compromised by the fact that, in most cases, complex sample handling is required: first the analyte has to be immobilised to the surface by using *e.g.* antibodies, then fluorescent molecules are specifically bound to the analytes using antibody-fluorophore conjugates, excess fluorophores are washed away, and, finally, the signal from the fluorophores attached to the surface is measured using a special reader device. This kind of complex sample handling does not seem feasible in bioactive paper products.

On the other hand, labels that directly change their colour or signalling due to the binding event, such as nanoparticles and molecular beacons, seem more suitable for use in bioactive paper products because they can be readily attached to the product and do not need complex sample handling, washing, etc. Anyhow, a special reader device or signalling structure is still most likely needed with these labels.

4.3.2 Simple optical sensors

Optical biosensors are based on the measurement of absorbed or emitted light or change in the wavelength of the light as a consequence of a biochemical reaction. Most of the optical biosensors are based on precisely aligned components with small manufacturing tolerances and require controlled operation environment in order to operate properly. These kinds of sensors are, for example, Young and Mach-Zehnder interferometers, conventional surface plasmon resonance sensors and ring resonators. Low-cost manufacturing of these kinds of sensors on large areas on fibre substrates does not seem feasible and thus they are not discussed here.

However, there are some simple optical biosensors that seem more suitable for mass manufacturing: 1) sensor holograms that are based on reflection gratings manufactured into a smart polymer material, 2) crystalline colloidal arrays (CCA) of polymer spheres, and 3) functionalized optical fibres. The sensor holograms and CCAs form complete sensors without the need for additional components, whereas the fibre optical sensors require an external light source and a detector.

Sensor holograms (see Figure 4-5) are reflection gratings that are manufactured into a smart polymer and reflect light with a certain wavelength (colour) when illuminated with white light – i.e. light from ordinary light bulbs or fluorescent lamps. They are volume holograms that are recorded, exposed, within a smart polymer, *e.g.* hydrogel, that reacts to the wanted external stimuli by swelling or contracting. The swelling or contraction of the smart polymer changes the spacing between the grating lines, which changes the colour of the light diffracted by the grating. Sensor holograms are potentially mass-manufacturable (Marshall *et al.*, 2003). The advantage of the sensor holograms is that they provide a direct visual signal under normal interior lighting without the need for additional optical components. The disadvantage is that the smart polymer has to be tailored for each individual application. These kinds of sensors have been used to detect, for example, divalent metal ions (González *et al.*, 2005) that are important in biological processes, pH (Marshall *et al.*, 2003), water content, solvents in water (Blyth *et al.*, 1996), proteases (Millington *et al.*, 1995) and glucose (Kabilan *et al.*, 2005; Domschke *et al.*, 2004). No publications on the integration of sensor holograms with fibre or paper-like products were found in this study.

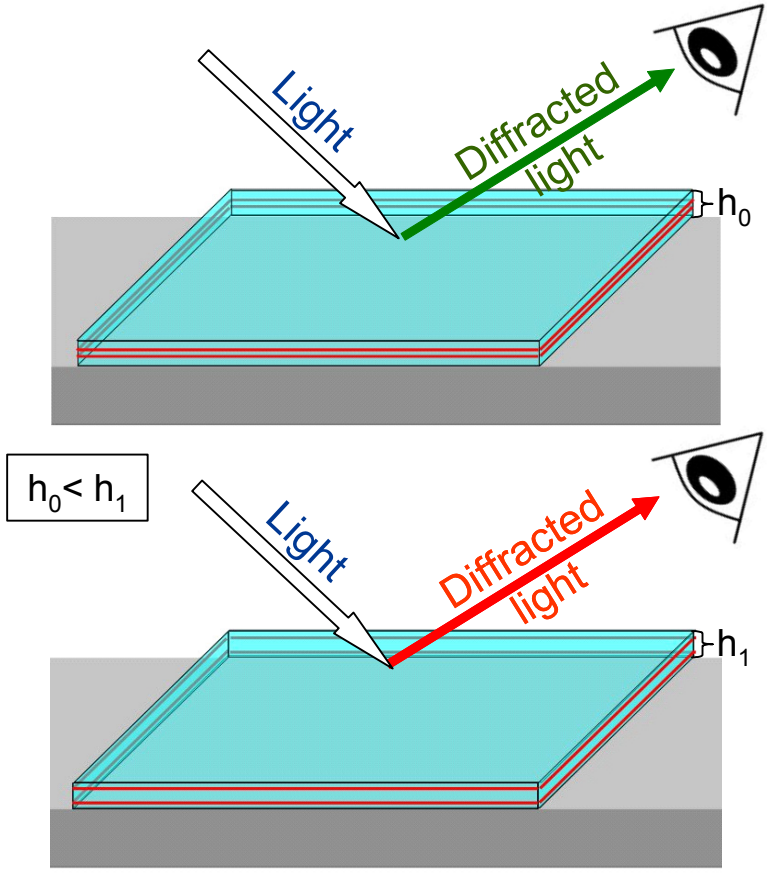

Figure 4-5. Operating principle of sensor holograms. A smart polymer swells in the presence of analyte molecules. The swelling increases the spacing between the grating lines recorded within the polymer substrate and this changes the colour of the diffracted light.

Sensors based on crystalline colloidal arrays (CCA) of polymer spheres (see Figure 4-6) are similar to sensor holograms in the sense that they are also based on gratings and hydrogels (Holtz & Asher, 1997). In CCAs the grating is formed by a colloidal array of spheres within a hydrogel that is solidified, *i.e.* polymerized. The hydrogel contains a bioactive recognition group or agent that reacts to the desired stimulus and changes the volume of the hydrogel. This volume change changes the properties of the grating formed by the spheres, and thus the colour of the light reflected or transmitted from it. The advantages and disadvantages of CCA sensors are same as with the sensor holograms. CCA sensors have been developed, for example, for temperature, Pb^{2+}, Ba^{2+}, K^{+} (Holtz & Asher, 1997), urea (Zeng *et al.*, 2001), glucose and galactose (Holtz *et al.*, 1998). CCA sensors integrated with fibre products were not found in this study.

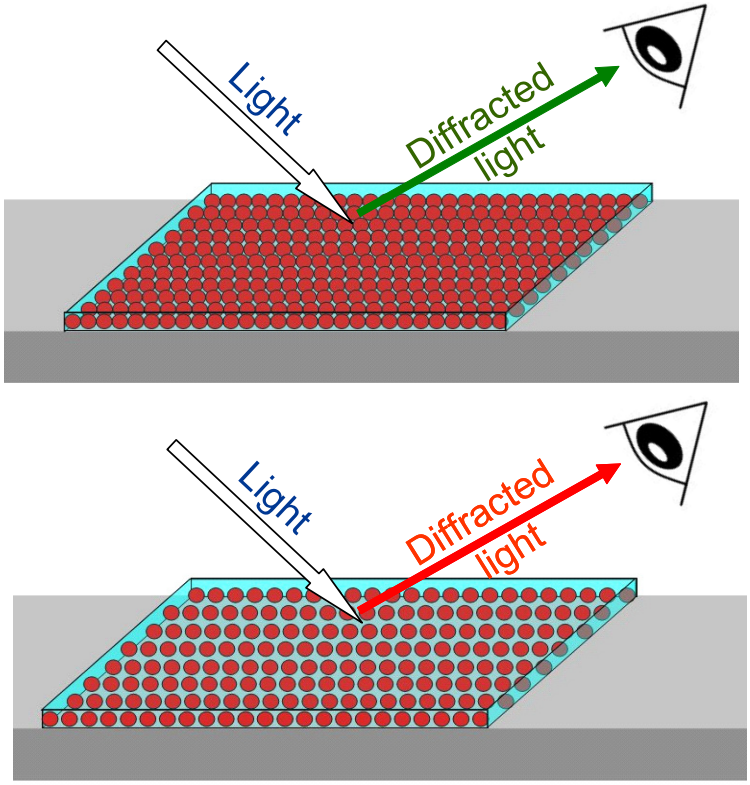

Figure 4-6. The operating principle of the CCA sensor. A smart polymer swells in the presence of analyte molecules. The swelling increases the spacing between the spheres forming the grating structure and this changes the colour of the diffracted light.

Optical fibres are thin threads made from glass or polymer that guide light from point to point in a similar fashion to electric wires conducting electricity. The basic structure of an optical fibre is shown in Figure 4-7. In the centre is a core, which is surrounded by a cladding and a jacket. The light propagates along the core but a small part of the light wave, called the evanescent wave, travels outside the core region. In optical fibre sensors the jacket and the cladding are removed from the small part of the fibre, and at that part of the fibre the evanescent wave travels in the sample volume.

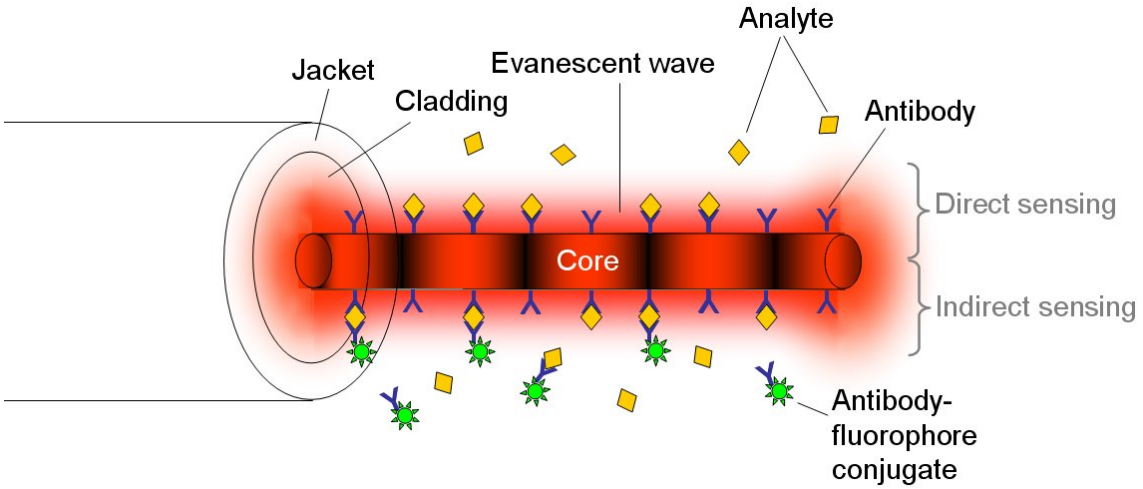

Figure 4-7. The basic structure of the optical fibre and the operating principle of the fibre optical biosensor. Light propagates along the core but the evanescent wave extends to the surrounding medium. The jacket and the cladding are removed in the sensing region and the evanescent wave propagates in the sample media. In direct sensing the evanescent wave is used to sense small changes near the core due to the binding of the analyte molecules. In indirect sensing the evanescent wave is used to excite the labels (e.g. fluorophores) attached to the analyte molecules. Note that direct and indirect sensing methods are not mixed in fibre sensors as depicted in this schematic figure - only one sensing method is implemented in one sensor.

Fibre optical sensors may be based on either direct or indirect sensing. In direct sensing (shown in the upper part of the core in Figure 4-7) the evanescent wave is sensing small changes in the refractive index at the near proximity of the fibre core, caused by the binding of the analyte molecules, and this changes the intensity of the light propagating in the fibre. It is also possible to utilize surface plasmon resonance (Wang *et al.*, 2005) and Bragg gratings (Du *et al.*, 1998) in fibre sensors. In indirect sensing the evanescent wave is used to excite labels, *e.g.* fluorophores, as shown at the lower part of the core in Figure 4-7, and the light emitted by the labels is detected. In indirect sensing the sample handling is more complicated because the excess labels have to be washed away before measurement.

A biological recognition element has to be incorporated into the fibre in order to have a fibre sensor that specifically reacts to the analyte. This recognition element can be an enzyme, antibody, antigen, nucleic acid, whole cell or some biomimetic material (Monk & Walt, 2004).

Optical fibres are widely used in optical networks and they are low-cost components. Due to their flexibility, sensing, signalling and lighting capability, their integration into textiles and composites is of great interest, and some patents and publications related to their integration into woven fabrics and textiles (Jayaraman *et al.*, 1999; Guy, 2005;

Burr *et al.*, 2005) and composites (Bogdanovich & Wigent, 2005; Du *et al.*, 1998) have been published. Lumitex, Inc. (www.lumitex.com) has developed a commercial woven fibre optic backlighting technology for thin flat panels. Jayaraman *et al.* (1999) have patented an idea about the use of optical fibres as penetration sensors in a shirt, and Khalil *et al.* (2003) have studied the integration of chemical fibre optical sensors with knitted, woven and non-woven materials. No publications on the integration of optical fibres with papers and cardboards were found in this study.

4.4 Other measurement techniques

4.4.1 Gravimetric biosensors

Gravimetric biosensors generally use thin piezoelectric quartz crystals, which function either as resonating crystals (quartz crystal microbalance, QCM) or as bulk/surface acoustic wave (SAW) devices. In most of these sensors the mass response is inversely proportional to the crystal thickness, which, at a limit of about 150 microns, gives inadequate sensitivity (Walton *et al.*, 1993). Walton *et al.* describe a new system in which acoustic waves are launched in very thin (10 microns) tensioned polymer films to produce an oscillatory device. Because the polymer films are so thin, a 30-fold increase in sensitivity is predicted and verified by adding known surface masses. The results show the real-time binding of protein (IgG).

4.4.2 Calorimetric biosensors

The basic principle of calorimetric biosensors is that all biochemical reactions involve a change in enthalpy, and this change can be detected. Mosbach and Danielsson (1981) developed an enzyme thermister where the temperature change in an enzyme reaction was measured by a thermister device.

Xie *et al.* (1993) studied a ferrocene mediated thermal biosensor that combines electrochemistry and calorimetry. In their work a thermal signal generated by the redox reaction was measured instead of the electrochemical signal.

4.5 Conclusions

In many bioactive paper and fibre applications the information on the presence of analyte molecules has to be signalled to the users by some means. This chapter presented various sensing and signalling methods, focusing on the use of conducting polymers and optics.

In practice, a strict chemical or biochemical polymer sensor that is highly sensitive to a given analyte is relatively hard to develop because most sensors suffer from interference with similar compounds. This property can be used in designing sensor arrays of numerous different sensor elements. Each sensor responds to a different signal and hence produces a unique fingerprint that allows the identification of an analyte (Albert *et al.*, 2000).

In optical sensing and signalling the intensity or wavelength of the light is used to measure and signal the presence of analyte molecules. Specificity towards the analyte is obtained by integrating a bioactive molecule, such as an antibody or enzyme, into the structure. Optical signalling is flexible and can be adapted to a vast variety of signalling needs, from simple colour changing tests to complex quantitative measurements.

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5. Mass manufacturing of bioactive paper

5.1 Introduction to mass manufacturing of bioactive paper products

Mass manufacturing of bioactive paper is a relatively unexplored territory. It seems as though the bioactive paper solutions are typically niche products, and their mass manufacture is still in its infancy. An extensive patent and technology survey was made for this publication in an attempt to map the technologies currently being used in the production of bioactive paper products and the technologies that have commercial potential for the mass production of bioactive paper and bioactive paper products. Only a few actual cases in which mass production techniques are used in the manufacturing of bioactive paper were found. These cases were almost totally limited to manufacturing passive bioactive paper products, such as anti-microbial papers, clothing and packaging materials.

However, bioactive paper products need to be manufactured with mass production methods to make their price affordable. A low price is essential for the adoption of these products in everyday life. The methods and technologies involved in paper manufacturing and printing are mature and the interactions covering their performance are well understood. These factors should make it relatively easy to adapt these methods to the production of bioactive paper products. Figure 5-1 shows an illustration of modern tissue paper machine.

Paper making and printing processes are true mass production methods. For the production of bioactive products, this can be both a weakness and a strength. For example, if a paper-like test strip for the detection of a harmful virus or bacteria were to be manufactured in a typical modern high-speed paper machine by grafting a virus detecting biomolecule to the pulp fibres, over six billion test strips could be made in less than four hours – assuming that a test strip area is 5 cm^2 and the paper machine is ten meters wide and runs at a speed of 2000 m/min. This calculation shows that bioactive paper products could be manufactured in large amounts very quickly. On the other hand, modern paper machines are typically designed to manufacture products in large batches, and to make products that have as little variability in the product quality as possible. Therefore, they are not cost efficient if they are just used for a short time – i.e. for four hours as in the example above.

Figure 5-1. Advantage DCT100 tissue machine (© Metso Paper).

It is also difficult to make products that have variable properties with a paper machine, which means that it would be very difficult to make one billion test strips that have detection properties for a different virus than the rest of the test strips. However, different mass production methods, such as ink jet printing or coating, can be used to make products with more variable properties, but this reduces the manufacturing capacity. Figure 5-2 illustrates the relationship between the manufacturing capacity and the achievable product variability. Some suitable applications for each manufacturing category are also indicated.

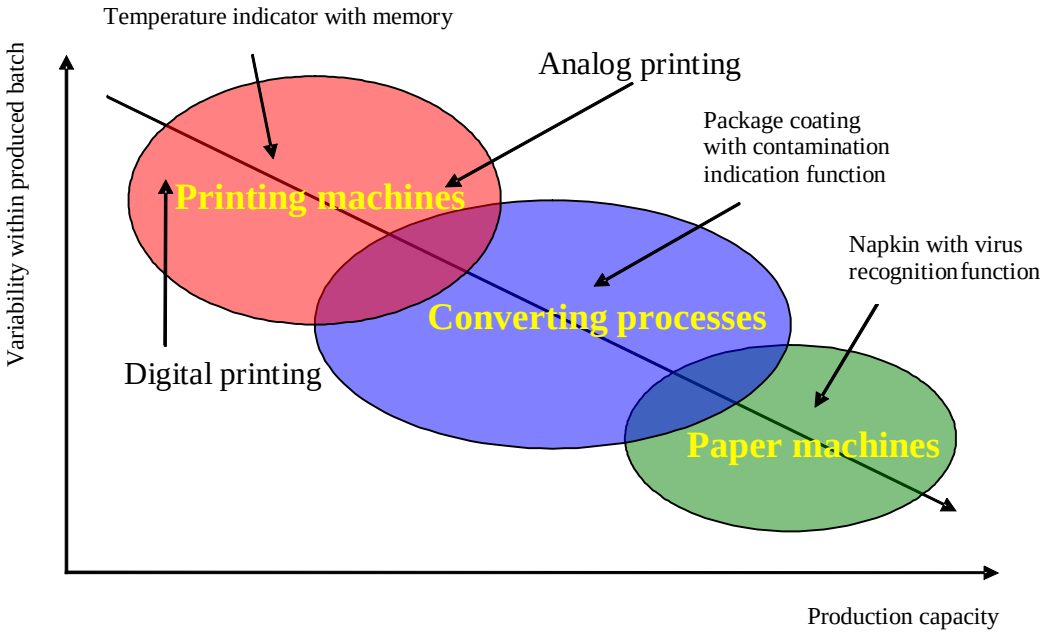

Figure 5-2. Schematic illustration of relationship between product variability and manufacturing capacity.

It should be noted that though most of the text in this chapter concerns the manufacture of paper, these processes and treatment methods are mostly applicable to different types of natural fibres and could also be used in the textile industry.

5.2 Activation of paper components in web forming (paper machine)

Paper can be functionalized at different levels of manufacture: the paper components can be tailored to meet certain required quality demands, functional chemicals can be added to the paper during and after the web forming, and functional components can be added to the finished paper product during converting processes or be printed on the paper surface. This chapter concentrates on the modification of raw materials for paper.

Modification of raw materials has several advantages. These include cost-effective and fast manufacturing of large amounts of materials and the ability to change the bulk properties of the material manufactured. However, modification of raw materials also has some obvious disadvantages and limitations. For example, the conditions in a paper machine are very harsh for certain types of additives, such as enzymes, that usually cannot tolerate the high temperatures that are typical of modern paper machines with a high degree of closure of water systems. High temperatures in the drying section of the paper machine can be another even more serious problem.

The types of modification that can be made at the paper machine level can be divided into grafting of active components onto the paper's components, immobilization of components in the paper web and embedding of the functional components inside the web.

5.2.1 Introduction to chemical activation of lignocellulosic pulps

Functionalization of fibres has several advantages: bioactive material can be evenly distributed throughout the whole paper structure and bioactive agents are efficiently immobilized. Theoretically, all components found in pulps (cellulose, lignin, hemicellulose) can be activated by chemical means for further functionalization.

Papermaking fibres can be functionalized in a multitude of ways. Active components can be added direct to the fibre surfaces, the fibres can be activated or radicalised by chemical treatments so that they become more suitable for the addition of bioactive molecules, or different materials can be added so that they form layers that have different properties on the fibre surface. The bonds between the fibres and the active substances can be chemical or electrostatic bonds. Functionalization of fibres can also be carried out with the aid of enzymatic treatment.

5.2.2 Grafting of fibres

A broad variety of cellulosic materials have been employed as substrates in grafting. Cellulose grafting is a process aimed at introducing some branches of polymers or functional groups into the main polysaccharide chain in order to confer specific additional properties on the cellulose itself, without destroying its intrinsic characteristics.

The chemical principles of cellulose grafting are free radical polymerisation, anionic polymerisation, cationic polymerisation, ring opening polymerisation, polyaddition, polycondensation and coupling of macromolecules onto cellulose. An extensive review of the methods can be found in "Comprehensive Cellulose Chemistry". Important radical generating systems are Ce(III)/Ce(IV), Mn(II)/Mn(III) and Fe(II)/H_2O_2. In addition to these, the ceric ion method has been used to graft a number of monomers onto cotton-based filter paper.

The review found that some products and patents related to functionalization of fibres already exist, especially in the clothing industry. For example, Weibel (2003) has patented a method by which active substances can be directly attached to fibres by electric charge. One method for producing multilayer coatings on different surfaces (such as fibres), where different coating layers are bound together with covalent bonds, is covered with Wischerhoff's (2002) patent. A similar approach to making surfaces suitable for the addition of bioactive materials is described in the patent by Klesse *et al.* (1992). The patent by Busch *et al*. (2002) describes a specific method for fixing active substances to fibres by activating them; the invention originally related to fixing substances to hair.

Mochizuki *et al*. (1985) have patented a method of rendering regenerated cellulose-based fibres more suitable for the addition of bioactive groups. In addition to the methods presented above, Swedish researchers have demonstrated a method of coating fibres with a multitude of materials, including bacteria-killing bioactive substances, by using charged polymer solutions. An article was published in Ny Teknik / Tekniska Tidskrift in March 2004.

Papermaking fibres typically carry an anionic charge. However, they can be cationized by different techniques. The obtained cationic sites can then be used as fixing points for functional anionic additives. For example, the cationic cellulose fibre can be obtained by oxidation of the fibre to introduce aldehyde groups, followed by reaction of part of the aldehyde groups with a nitrogen-containing reagent such as betaine hydrazide hydrochloride. This method is patented by Besemer *et al*. (2005a). Lignin-rich fibres can also be functionalised with cationic groups. For example, the chemical bonding of

glycidyl trimethyl ammonium chloride (GTAC) was studied in the jointly funded FIBREFUN project. The alkali catalysed bonding was carried out in both organic solvent (80% 2-propanol) and aqueous reaction media at pH 10. The results based on nitrogen analysis show that addition of the GTAC reagent onto fibre was successful both in the organic solvent and the aqueous reaction medium.

Aldehyde and carboxy groups can also be used as starting functionalities to bind bioactive or other components to fibre. The preparation of oxidized cellulose containing fibrous materials is described in several patents. For example, Jaschinski and Gunnars (2002) describe a 4-hydroxy TEMPO-assisted oxidation of cellulose containing fibrous material. The primary hydroxy groups at C(6) of the glucose units of cellulose are oxidized into aldehyde and/or carboxy groups.

A process for oxidizing cellulose is one in which a nitroxyl compound such as TEMPO is oxidized using an oxidizing agent in the presence of a complex of a transition metal such as Mn, Fe, Cu, and a complexing agent such as a polyamine, or an oxidative enzyme, and the resulting nitrosonium ion is used to selectively oxidize cellulose 6-hydroxy-methylene groups to carbaldehyde groups and carboxylic acid groups. Jetten and van den Dool (2004) have a patent on the area.

It is also possible to graft cyclodextrins to fibres. Besemer *et al*. (2005b) have patented a technique for coupling cyclodextrins and other encapsulating oligosaccharides to fibrous and/or polysaccharidic carriers by ionic bonds. The ionic bonds can be produced by introducing cationic or anionic groups into the cyclodextrins, and, where appropriate, by introducing oppositely charged groups in the carrier material. The products can be used for odour control in the fibrous material. The carrier to which the cyclodextrin and other encapsulating agents, with or without inclusion compounds, can be coupled has a fibrous structure and is substantially water-insoluble. It is usually a polymer having a partial or full polysaccharide nature, such as in cellulose, hemicellulose or synthetic carbohydrates. The molecular weight of the carrier is preferably above 5 kDa. Lower molecular weights are also feasible as long as the polysaccharide is water-insoluble. The polymer may be natural or synthetic and may be a mixture of various polymer types, such as cellulose-acrylate, or starch-acrylate, or starch-protein and the like. The carrier can be positively charged (cationic derivatisation) by amino- or azido-alkylation, or oxidation to introduce aldehyde functions followed by reaction with amines (reductive amination) or other nitrogen-containing reagenats. Cationisation of the carrier can also be achieved by applying a cationic additive such as PAE (poly(amide)amine-epichlorohydrin) to the carrier. The anionic or cationic derivatisation is performed to an extent that allows sufficient coupling of oppositely charged components. In general, a degree of ionisation of 0.1–50 ionic charges per 100 monomer units of the carrier is used.

5.2.3 Chemo-enzymatic fibre modification

A novel, targeted chemo-enzymatic method of introducing new functionalities into fibres has recently been suggested by VTT. The fibre modification method exploiting the power of enzymes capable of directly introducing new substituents to fibres by covalently bonding can be considered as a more specific and targeted modification method than the traditionally used methods, such as sorption or chemical coupling of functionalized components to the fibres. Due to their surface specificity, the benefit of enzyme-based methods over purely chemical methods is in the retention of important chemical and physical fibre properties, such as strength.

Various ways of modifying lignocellulosic fibre materials by enzymatic treatments have also been presented. A process for altering the surface charge of lignocellulosic fibres by reacting the material with an oxidase in the presence of an added phenolic carboxylic acid in order to increase the negative charge of the material has been described by Pedersen *et al.* (2001). In another patent publication Pedersen and Felby (1998) describe a process for the manufacture of a lignocellulose-based product, produced by treating lignocellulosic material and a phenolic polysaccharide with an enzyme capable of catalyzing the oxidation of phenolic groups in the presence of an oxidizing agent. It is stated that the phenolic polysaccharide functions as an adhesive/binder and provides boards having good strength properties. These documents basically describe processes in which the functional groups originally present in the lignocellulosic material are enhanced. However, even when new functionalities (e.g. carbonyl or carboxyl groups) are produced, such groups are directly derived from the corresponding hydroxyl and carbonyl groups already present on the fibres.

The method developed at VTT allows the direct introduction of novel functional properties into fibres via enzymatic lignin activation. These functionalities can be explored both for improving the processability (for improved process performance or saving of resources) and for designing completely novel properties in fibres. The latter option is especially tempting with regard to extending the uses of paper for novel areas, such as carriers for printed electronic circuits or novel consumer products.

Several patent applications related to the chemo-enzymatic modification of fibres have been filed. E.g. fibre products with improved strength properties and enhanced adhesion, altered hydrophobic hydrophilic characters and fibre conductivity have been described (Buchert *et al*., 2005a–c). Additionally fibre modification by the chemo-enzymatic method has been proposed as a way to product identification or tracing (Grönqvist *et al.*, 2005). This method of fibre modification could also be a potential tool to couple bioactive molecules into fibre based products (Figure 5-3).

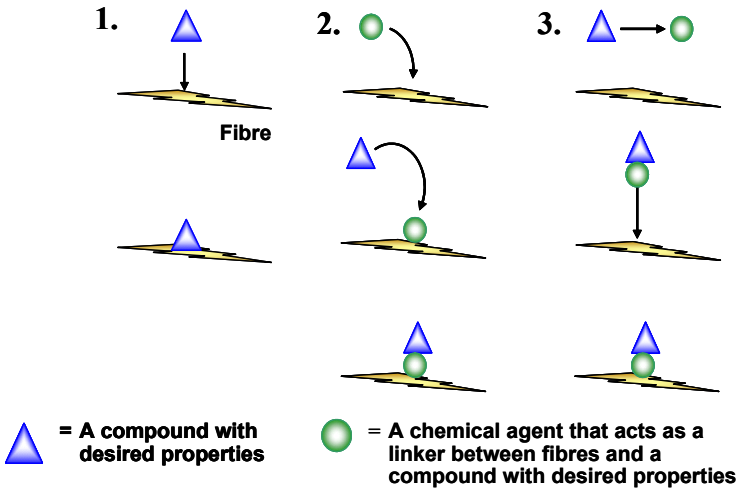

Figure 5-3. Three methods for functionalizing fibres.

5.2.4 Immobilisation of active components in the paper web

The structure of paper is relatively open compared with the structure of typical plastics, and it can be modified quite easily. Two types of pores are present in paper: pores between the paper's components and pores inside the raw materials. Examples of the former are voids between the fibres and of the latter, the micropores in the fibre cell wall, hollow lumen inside the fibre, or pores in the surface of scalenohedral precipitated calcium carbonate particles used as fillers in papermaking. These voids in the paper can probably be utilized as points where bioactive agents can be stored and released later. Scmitt *et al*. (1972) patented a method of immobilizing enzymes in the pores of paper in 1972, in which enzymes are added to the paper making pulp together with carbonyl polymers and immobilised in the pores of the paper. Allan *et al*. (1991) have demonstrated a method of using the micropores of the cellulose fibre cell wall as storage and release bases of bioactive material. Figure 5-4 shows the different kinds of pores in the structure of paper.

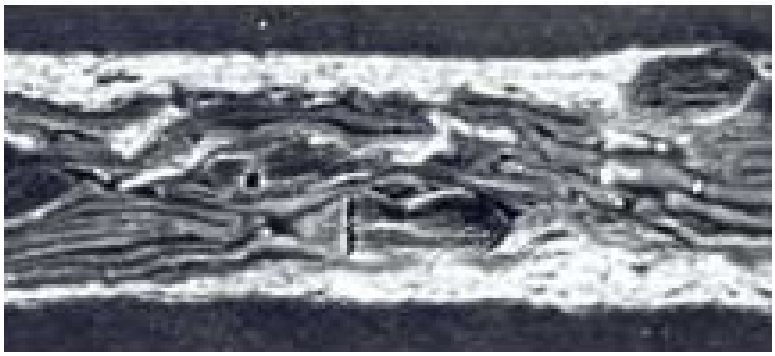

Figure 5-4. Cross-section of coated paper illustrating the different kinds of pores in paper (Knowpap).

It is also possible to manufacture artificial fibres that could be used as a substrate for bioactive materials or, being bioactive by nature, using them together with conventional fibres in the paper machine. Patents related to these types of inventions include a patent by Aston *et al*. (2003) which claims to include the use of silicon-based fibres in bioactive applications, and Akita *et al*. (2000), which makes claims on several types of fibres designed for the immobilisation and release of active substances.

5.2.5 Embedding of active components

Active components can also be embedded inside the paper and, especially, paperboard structure. The Japanese companies Oji Paper and Toppan Forms have reportedly (Anon., 2004) created a chip that can be embedded in a paper sheet less than 200 micrometers thick. This kind of device can be used to transmit information on the changes created by bioactive substances present in paper web. Embedding of more sophisticated electronics in the paper structure goes beyond the scope of this study.

5.3 Addition of active materials by coating and/or converting

Paper converting processes are usually used when the surface properties of paper or carton board are modified. It is typically difficult to alter the bulk properties of paper with unit operations of converting.

5.3.1 Surface sizing

In paper converting terms, surface sizing refers to a unit operation in which (typically, natural water-soluble) polymeric materials such as starch are added to the paper surface in an attempt to alter the porosity of the paper surface and by this influence the adsorption properties of the paper. Typically, starch is transferred to the paper surface in a size press, in which the starch is first applied to a transfer drum that is then pressed against the paper.

The literature study did not reveal any current applications where a process similar to surface sizing is used to add bioactive materials to paper. However, surface sizing could probably be utilized in adding active components to paper. This method has several advantages: the materials are deposited on the surface of the paper, the transferred amount can be varied from a couple of grams per square meter to even hundreds of grams per square meter, and the size formulation (pH, solids content, components…) can be varied to great extent.

5.3.2 Spraying

In paper converting terms, spraying refers to a multitude of techniques in which material is added to the paper surface as liquid droplets or mist. Compared with surface sizing by spraying, it is possible to add very little material to the surface; spraying of dilute starch solutions has been used to alter the surface properties of paper.

In this literature study no applications were found in which spraying is used to add bioactive components to paper. Still, spraying appears to be a potential technique for adding bioactive components to paper; it is a non-contact surface treatment method and it is possible to treat large areas of substrate material with relatively thin layers of coating.

5.3.3 Coating

In papermaking terms, coating refers to a process in which the fibre network (base paper) is covered with material that contains inorganic pigments, binders and a wide variety of materials such as optical brighteners, dispersing agents and other special materials that serve to fulfil different kinds of specific purposes. The main component of any coating is the pigment. There are many coating methods, such us blade coating and curtain coating, in regular use in paper making.

It is quite likely that any bioactive substance that can be added to the paper machine can be added as part of the coating as well. For example, if a bioactive molecule can be grafted onto a component of a paper web, it can probably be grafted onto a coating component. Tailoring coating formulations to meet the required properties, such as pH, solids content and coating thickness needed for different end uses and coating methods, can be done relatively easily. Figure 5-5 illustrates coated cardboard.

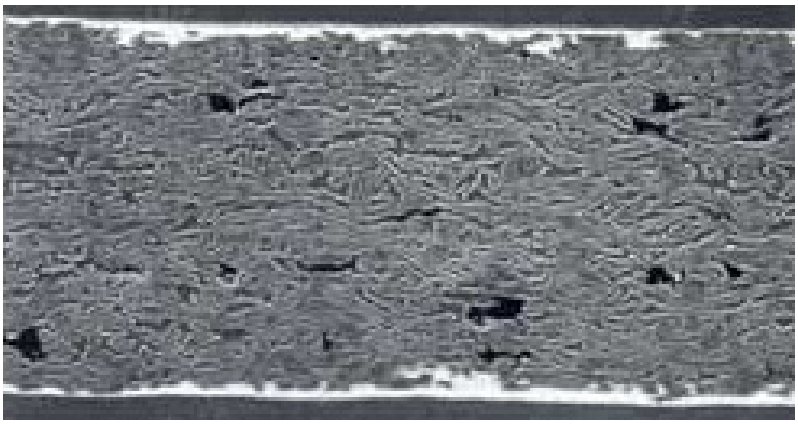

Figure 5-5. Coated cardboard (Knowpap).

5.3.4 Plasma treatment

In plasma treatment plasma is typically generated by leading a carrier gas through a high voltage electric field. This results in the formation of "mist", which consists of ions and electrons and excitated and radicalized molecules. This plasma can then be led to the surface of virtually any substrate. This results in collisions and chemical reactions between the surface and the reactive species of the plasma. Plasma treatment is a very powerful technique and the properties of the surface can be changed drastically.

Hot plasmas are typically used for the modification of inorganic materials, such as metals. Unlike hot plasmas, the temperature of cold plasma treatments ranges from the ambient temperature up to 100 °C and are, therefore, applicable to organic materials, such as paper. However, cold plasmas are less ionized compared with the highly ionized hot plasmas.

Plasma treatments of material surfaces can be categorized into two main types: plasma treatments with 1) surface-activating gases, e.g. argon, helium, nitrogen, oxygen or ammonia, and 2) polymer-forming molecules. The former types of plasma treatments are employed when the introduction of different functional groups onto the material surface is aimed at. In plasma polymerization the (monomer) vapour fed into the plasma chamber is deposited and polymerized onto the substrate. These two types of plasma procedures have been used for the modification of wood, paper or cardboard surfaces in order to enhance the adhesion properties of the surfaces with polymeric materials or to create hydrophobic water barrier layers on the cellulosic materials. Monomers that have been deposited on lignocellulosic surfaces include hydroxyethyl methacrylate, n-butyl methacrylate, tetrafluoromethane and hexamethyldisiloxane (Denes *et al.*, 1999; Denes & Young, 1995; Kwok *et al.*, 1997; Setoyama, 1996).

Treatments of different substrates with monomers or surface-activating gases are normally carried out using vacuum equipment. However, recent development work on plasma sources has focused on devices operating at atmospheric pressure. Atmospheric plasma sources provide continuous and quite inexpensive processing of material surfaces. In addition, after-treatments of wet or pre-cured coatings are enabled by atmospheric plasma procedures. Atmospheric plasma treatments have been shown to be especially beneficial in the curing of sol-gel coatings.

It seems obvious that plasma treatment can be used in the manufacture of bioactive paper products in two ways. It can be used to treat the surface to have optimal adhesion properties for the addition of bioactive molecules (activation) or cold temperature plasma modification can be used to add the bioactive molecules directly onto the surface by leading them into contact with the surface together with the carrier gas. For

example, in the EU-funded Solplas project the combination of tailored chemical surface activation by cold atmospheric plasma treatment and subsequent wet-chemical coating using a bio-based anti-microbial solution resulted in coated plastic films with good anti-microbial activity and improved barrier properties against oxygen transmission (Vartiainen *et al*., 2005).

5.3.5 Hybrid sol-gel coatings

Sol-gel hybrid coatings are promising new materials consisting of organic-inorganic components with a nanoscale structure. Sol-gel coatings with a thickness of only several microns consist of different parent substances that will build a nanoscale network through hydrolysis and condensation reactions. Alkoxides are common precursors in sol-gel concepts and organic epoxides are widely used as modifiers in sol-gel materials. By using different precursors the coatings have various combinations of properties, depending on the structure and chemical composition of the coating (Brinker & Scherer, 1990). Ceramic components provide hardness and scratch/abrasion resistance, whereas polymer components provide flexibility and repelling properties for the tough and durable coatings. By tailoring and optimizing the composition the properties of a sol-gel thin coating can be directed in a certain direction and the integration of active nanoparticles such as photocatalytic TiO_2 or anti-microbial particles within the sol-gel network offers many possibilities to create smart functionality in the coatings.

One of the major advantages in using these coatings is their transparency to visible light; thus they do not remarkably change the appearance of the substrate. Sol-gel thin coatings can be tailored to have good adhesion to many substrate materials, such as metals, ceramics, glass, polymers, concrete, even on wood and paper. In view of lignocellulosic surfaces, adhesion of sol-gel coatings to these substrates is not considered to be a problem since sol-gel networks have been reported to bond covalently with the functional groups of lignin and cellulose (Figure 5-6). Application of sol-gel coatings to different substrates can be carried out by spin, spray, dip, roll or drain coating; spray or roll coating could be the most optimal treatment types for paper surfaces.

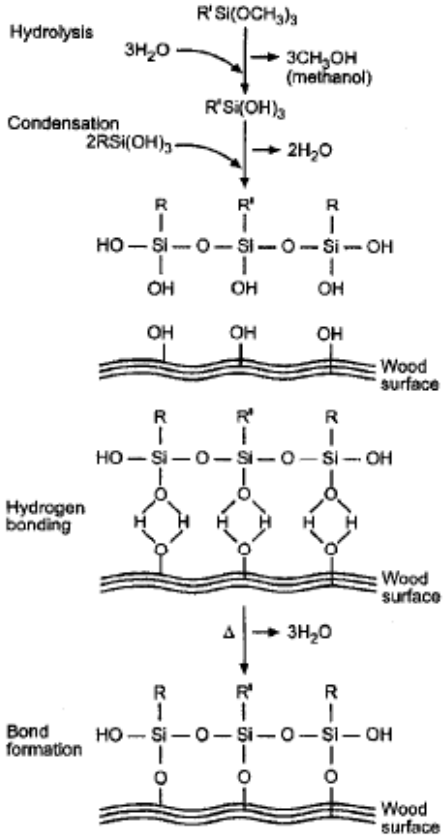

Figure 5-6. Bond formation of a sol-gel network with a lignocellulosic substrate according to Denes et al. (1999).

Sol-gel technology has been utilized in some healthcare products that consist of silica glass beads with entrapped organic and bioactive molecules (Figure 5-7). Contrary to the high temperatures used in traditional glass-making processes, sol-gel glass is prepared at room temperature. This enables doping of sol-gel glass particles with numerous organic compounds. One of the reported applications for doped sol-gel glass is a sunscreen lotion concept based on sol-gel glass doped with a high concentration of UV light absorbing molecules.

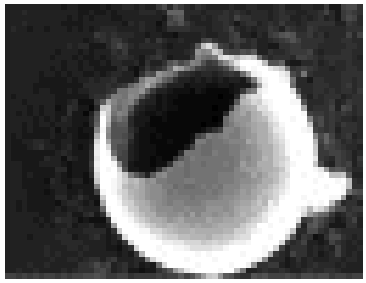

Figure 5-7. Sol-gel sphere with 85% free space (diameter 1 micron) (http://www.solgel.com/biz/featcom/solgel.asp).

5.3.6 Lacquering

Lacquering is usually used to increase the gloss of printed paper and to protect printed products from wetting and mechanical damage. It can also act as a barrier coating. Lacquers can be applied on top of prints in printing machines or with separate machines.

This review found no applications in which lacquering is used in production of bioactive products.

5.3.7 Laminating

Laminating is used to protect the laminated products from environmental influences. No bioactive paper products in which laminating is used were found. Laminating seems to be a process that, by its nature, is not compatible with bioactive products: laminating is used to isolate the product from its surroundings and bioactive papers should be in touch with their surroundings. This means that laminating can only be used in applications where bioactive products need to be well protected from all external influences.

5.3.8 Dispersion coating

Dispersion coating is a process in which polymer dispersions, such as latexes, are applied, metered and dried so that they form a uniform, non-porous film on the surface of paper. The purpose of dispersion coating is to enhance the paper's barrier properties. Dispersion coating is usually made using separate converting machines; it can also be applied in printing press.

No applications in which dispersion coating is used together with bioactive materials were found in this review. The applicability of dispersion coating in the manufacture of bioactive paper products seems to be similar to the way in which dispersion coatings are used today: as a protection layer for bioactive material towards possibly hazardous substances. Dispersion coating can also be used to seal the active material from one side so that it only comes into contact with activating influences from the desired direction – for example, only gases from the inside of a box, not from the atmosphere.

5.4 Printing of functional substances

Printing techniques enable easy disposition of different kinds of active and functional substances in patterns having a specified size and shape. Properties such as accessibility

and dissolution rate of these bioactive patterns can be modified by changing these factors. For example, the patent by Figueroa *et al*. (2004) describes how ink-jet printing can be utilized to create bioactive patterns that represent certain dissolution rates. This means that the dissolution of printed substances from the substrate can be controlled.

Each printing technique has its own characteristics with regard to how printing ink is transferred. Figure 5-8 illustrates the difference between rotogravure and flexography printing.

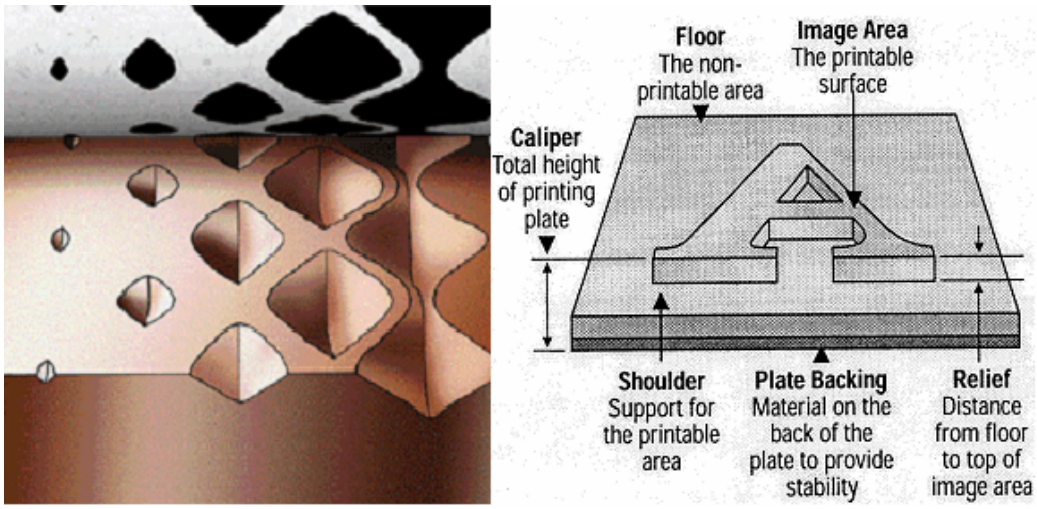

Figure 5-8. In rotogravure printing ink is transferred with cups on the printing cylinder, and in flexography by the elevated areas of the printing cylinder.

The quality and characteristics of the image printed by different printing methods also differ drastically. Figure 5-9 illustrates the difference between rotogravure, flexography and ink jet printing.

Figure 5-9. Rotogravure, flexography and ink jet printed letters.

The printing methods with the most potential for mass production of bioactive paper products are described in this chapter.

5.4.1 Gravure printing

Gravure printing is typically used in the production of high-quality publications. Compared with other printing methods it has high resolution and a very high production capacity. Based on these factors, gravure printing has been widely considered to be one of the most promising mass production techniques for disposable active components, such as the components in cheap and disposable printed electronics. However, a drawback to gravure printing is that it is very demanding on all of the materials involved in the printing process – it places the highest demands of all printing processes on the quality of the printing substrate – and the printing inks used in gravure printing need to be high quality and represent very low viscosity.

No cases were found in which gravure printing is utilized in the production of bioactive paper products.

5.4.2 Flexography printing

Flexography is typically used in the printing of packages and packaging materials. This method is easier to use than gravure printing. For example, the inks can be more viscous and the demands on the substrate quality are not so strict as in gravure printing. This also means that it is easier to transfer relatively large amounts of materials with flexography than it is with gravure printing.

Flexography is widely used in the printing of conductive materials, but no examples of the printing of bioactive materials, or patents related to it, were found in this review.

5.4.3 Screen printing

Screen printing is a method that is typically used in sheet fed printing rather than roll to roll. It has been even disputed whether screen printing can be referred to as a “mass production method”. However, screen printing has several advantages over other printing methods: the inks used are extremely viscous and can have a high solids content, their composition is relatively simple and the material amounts transferred can be at least one or two decades higher than in any other printing method.

Because of the simplicity of the method, screen printing is already utilized in the printing of functional materials or functional components, even on an industrial scale. A well-known application is the printing of glucose indicators. A literature search for biosensors manufactured with screen printing reveals almost 200 patents and articles

related to the printing of biosensors or biosensor parts. As one example of these numerous articles, a patent by Bilitewski *et al*. (1993) describes a method of producing a biosensor containing enzymes.

A company called Ercon Inc. is known to manufacture "biocompatible" inks for silk screen printing.

5.4.4 Ink jet printing

Ink jet printing has been seen as one of the most promising methods of transferring bioactive materials to carrier substrates such us paper. It has several advantages, such us the possibility to exactly control the amount of transferred material, and it is a non-contact printing method – meaning that the printing equipment does not come into contact with the printing substrate. Therefore, ink jet printing does not make any harsh demands on the quality of the printing substrate and all kinds of substrates can be used, ranging from smooth to very rough and even 3D surfaces. Inkjet printing is also very material efficient, because it doesn't waste valuable ink material during set-up or printing. The most significant drawback to ink jet printing is that the inks need to be very homogenous and stable, and have a low viscosity. This means that to achieve the high quality needed from the ink jet inks these inks usually need to have numerous chemicals, which all serve some dedicated purposes and may harm the bioactive substances present in the inks.

Ink jet printing or ink jet printing-like processes have been used to make patterns of bioactive materials and some patents related to ink jet printing of these materials exist. For example, ink jet-like fluid disposition methods for the transfer of bioactive materials to different kinds of substrates have been patented, as the extensive patent from Childers *et al*. (2004) demonstrates. Tabata (2004) has patented a method of printing stimulating bioactive substances on textiles. This patent is not limited to ink jet printing.

Ink jet printing can be utilized in several applications in diagnostics, genetics and medical science. Kido *et al*. (2000) from the University of California have demonstrated ink jet printing of immunological reagents. Hammock and Kido (2002) also have a patent for fabricating microarrays by means of ink jet printing.

In the field of genetics, ink jet printing can be utilized for printing and synthesizing DNA, dosing proteins and synthesizing peptides. For example, Allain *et al*. (2004) have ink jet printed DNA dissolved in water and Goldmann and Gonzales (2000) have managed to transfer over a million genes in an area of 10 cm × 10 cm by ink jet printing. The challenge in dosing proteins is their sensitivity to chemicals and

mechanical stress, as well as their surface-active nature, which results in nozzle clogging. However, Bae *et al*. (2004) have managed to ink jet print proteins from a solution containing 0.1 mg/ml protein, 10 mM phosphate buffer and glycerol.

In medical science, ink jet printing has been used for making tissues, in laser surgery and in drug dosing. Tissue making is based on ink jet printing bio-polymers and cells. Xu *et al*. (2005) from Clemson University have ink jet printed cells in order to fabricate tissues; the printing fluid contained 5 million cells/ml and only 8% of the cells dissolved during printing.

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6. Application scenarios

6.1 A day as Jack

Jack is just an ordinary guy who just woke up on a sunny Monday morning in April. And, once again, typical of him, Jack had been celebrating too much the previous weekend. Well, he is single and not responsible to anyone. Or maybe to his employer, but he really doesn't care too much. "Oh, s**t. I feel awful. I have to check my blood sugar level", Jack thinks. Jack really is an ordinary guy, he is one of those hundreds of millions whose body does not produce enough insulin, or the cells ignore the insulin – i.e., those who have type II diabetes. Jack is really lucky to live in the 21st century, when blood glucose level testing is so easy. His blood glucose tester has 25 integrated bioactive paper test strips and an automatic lancet that draws less than a microliter of blood, and the lancetting is practically painless. The result is ready in five seconds and it is automatically downloaded to data management software running in his mobile phone. Jack is not sure if he is happy or not when he sees that his blood glucose level is normal. He can only think: "Please let me have some painkillers."

Luckily, Jack finds a few painkillers and once again he is ready for a boring day. He is a team leader in an expert group specializing in renovating buildings with moisture and fungus damage. Although Jack is bored, he is also very aware of the fact that without his really profitable job he couldn't afford to have such an expensive lifestyle. Nearly every day he remembers to thank the builders of the 20th century who really didn't have a clue about the consequences of the techniques and materials they used. "Lucky me", he thinks. Jack has a nice, rather new, three-bedroom detached house just outside the downtown area. Before he moved in, he totally renovated the house. He assembled moisture and fungus sensors, which was actually really easy. It was integrated in the wallpaper and he just had to disperse a little bit of adhesive and hang it on the wall (see Figure 6-1). Even Jack was able to do it. Unfortunately, he didn't have enough cash to buy the full house security network solution. So he just has to rely on the visual signal on the wallpaper. But Jack has already decided to invest in this security network, and he really wants to integrate his fridge with the network too. A headache alone would have been bad enough in the morning, but the smell of the spoiled foodstuffs in the fridge is something Jack really doesn't want to think about. He had read in VTT's research news that VTT had developed new freshness sensors for food packages. These sensors were not only able to show the freshness status but were also able to transmit the freshness information to an intelligent fridge. Earlier this year, Jack was forced to buy a new fridge because the old one suddenly decided not to cool down anymore. Even though Jack is careful, or maybe because of that, he bought a brand new, expensive fridge with network capabilities and integrated RFID reader. It was important to Jack that he could integrate his new fridge with the home monitoring network he was planning to buy. Then he could finally get rid of smelly surprises on Monday mornings.

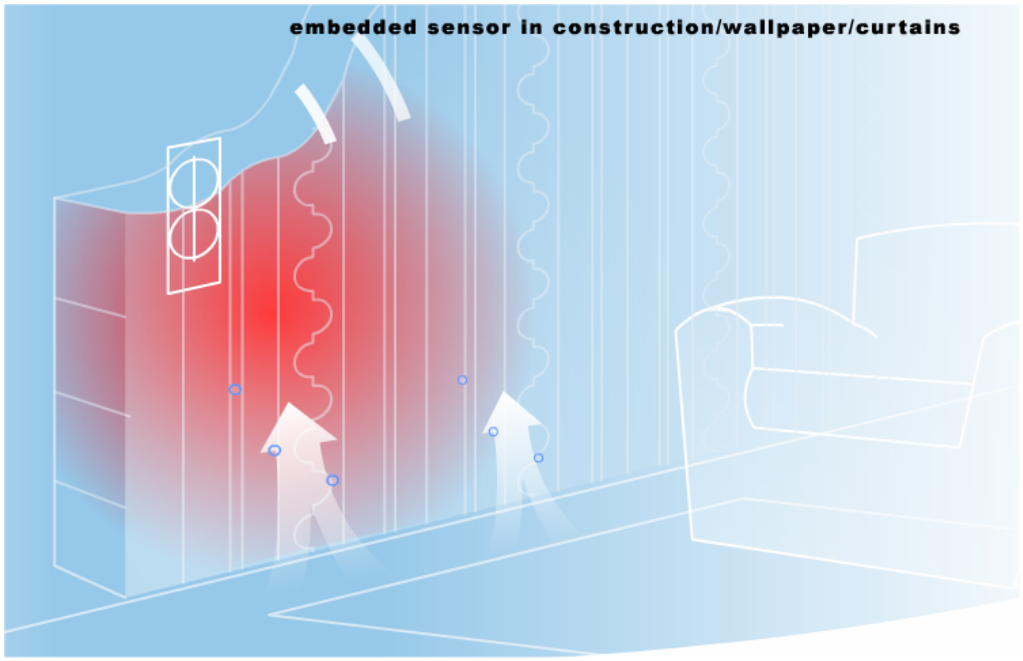

Figure 6-1. Embedded moisture and fungus wallpaper sensor on Jack's living room wall.

By lunchtime Jack's headache was gone and his team went to a local Trattoria to have lunch. They had never been disappointed about the food quality there, or the service. Jack bought a tabloid to read the latest celebrity gossip. As he was going back to the table, he noticed a poster advertising the tabloid he had just bought. "Try our new allergenic test on the front page." "Hey, what's this", Jack thought. "Another science fiction thing, they can't be serious about it." Jack removed the protective strip and below it found allergen test spots for various pollens (various trees), house dust mites and many other common allergens (see Figure 6-2). While Jack was eating he checked the test spots to see any changes. After couple of minutes the birch pollen test spot started to turn yellow, indicating an increased birch pollen count. "I knew it", Jack thought. For the past two years he had been relying on an allergen sensor network in which allergen alerts were automatically sent to registered mobile phone users. The sensor network was really extensive as regards both geography and allergen count. After finishing his lunch Jack was feeling a bit odd. He suddenly sneezed and had a runny nose, typical symptoms of his allergy. Jack immediately checked his mobile phone to see if any allergen alerts had arrived (see Figure 6-2). But he had had a really lousy morning: after testing his blood glucose level he had left his phone at home. Was the sensor in the tabloid really correct? Jack rushed to the bus stop where he knew there was a poster in which node of the sensor network was integrated. The OLED display on the poster clearly showed that the birch pollen count had gone up. Jack was wary of using medication (except painkillers), but now he took two non-sedating antihistamine pills to relieve the symptoms.

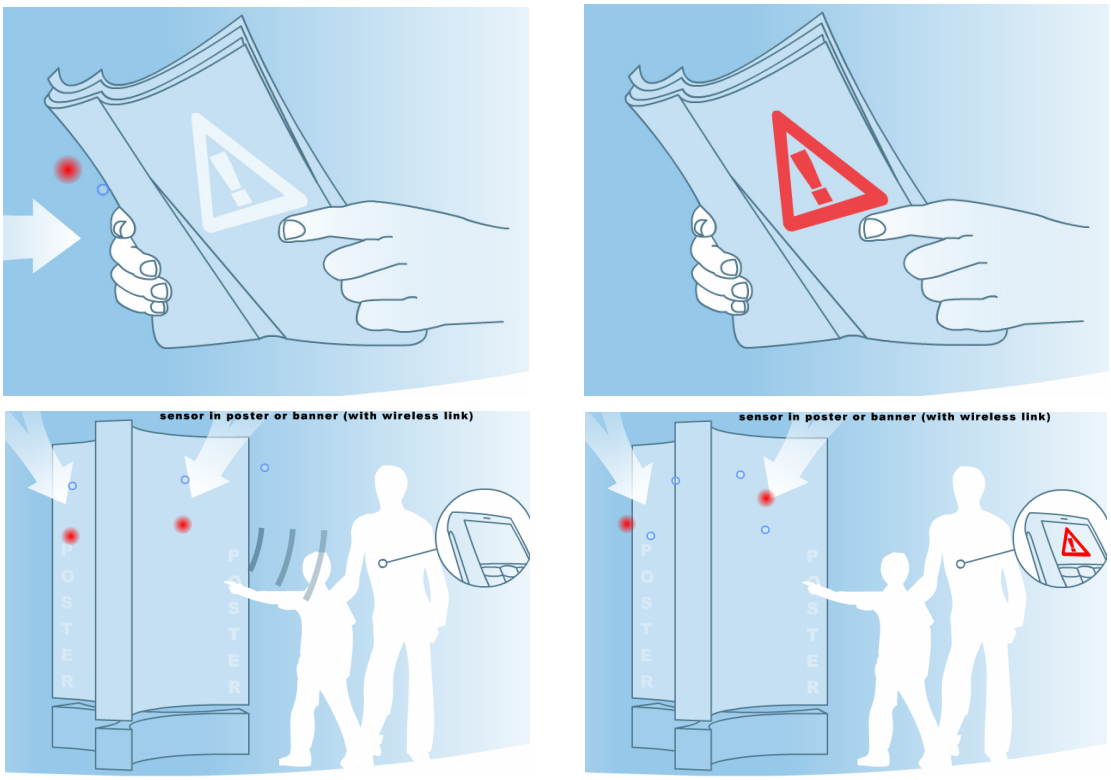

Figure 6-2. Allergen diagnostic sensor integrated with a newspaper (above) and a poster (below).

6.2 Bioactive paper innovations

In the previous chapter we presented a fictional story about Jack and how bioactive paper innovations had changed his life. We anticipate that bioactive paper innovations will become invisible helpers in our ordinary lives, will be embedded in our natural surroundings, will be present whenever we need them, will be enabled by simple and effortless interactions attuned to all our senses, and will act autonomously with a flavour of biomolecular activity. Embedded systems of bioactive paper are going to impact on the way we live, without constraining our ordinary lives but instead making them easier and safer. Applications ranging from protection and safety (clothing, purification of water and air, indoor air, personal protective equipment, homeland security against biological and chemical terrorism), diagnostics of health, food safety and quality, environment, testing of microbial contamination, and counterfeit prevention (brand protection, authentication) are poised to become part of society and human life. Due to bird flu, SARS, mad cow disease and the 9/11 events, a lot of research has accelerated recently, driven by fears of health, terrorism and bio-warfare.

Innovative products like bioactive paper require multidisciplinary know-how in many areas – low-cost manufacturing methods for integration of bioactive components, printing, coating, grafting, spraying, manufacturing of nano-scale structures on large surfaces and their integration with micro and macro devices, bio-compatible materials development, bio-material immobilisation into and onto paper and non-wovens and other substrates, modification of substrate properties, new detection methods, integration of electronics and optics onto large area substrates – and there is also a lack of visionaries who can make the future. Although individual technologies are already available as building blocks for bioactive paper innovations, there are still huge challenges in how to integrate them with functional components and devices, how to manufacture them at low cost, and how to take usability into account.

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ISBN 951–38–6603–3 (URL: http://www.vtt.fi/publications/index.jsp)
ISSN 1459–7683 (URL: http://www.vtt.fi/publications/index.jsp)