## Electrospun nanofibers as solid phase extraction sorbents and support for alkylphenols colorimetric probes

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### Abstract

The thesis reports on fabricating alternative solid phase extraction (SPE) sorbents and colorimetric probes based on electrospun nanofibers for alkylphenols (APs). Hydroxyl methylated styrene [poly(co-styrene-CH<sub>3</sub>OH)] and 3-oxobutanoate styrene [poly(co-styrene-OCOCH<sub>3</sub>COCH<sub>3</sub>)] copolymers were synthesized and fabricated into sorbent materials by electro-spinning/spraying. The fabricated morphologies consisting of bead free fibers, beaded fibers and particles were evaluated as SPE sorbents using batch experiments. Electropun fibers proved to be better sorbents as they exhibited extraction efficiency that exceeded 95 % compared to 60 % for beaded fibers and 40 % for particles. In view to reduce sample and solvent volumes, smooth fibers were packed into pipette tips as SPE devices that yielded quantitative recoveries of APs from spiked wastewater samples. Recoveries ranged from 70 % to 125 % with LOD of 0.008, 0.01 and 0.1  $\mu$ g mL<sup>-1</sup> for 4-tert octylphenol (4-t-OP), 4octylphenol (4-OP) and 4-nonylphenol (4-NP) respectively, when using high performance liquid chromatography-fluorescence detector (HPLC-FLD). Furthermore. amino functionalised polydiacetylene polymers (PDAs), citrate capped gold (AuNPs) and silver nanoparticles (AgNPs) were evaluated as colorimetric probes for visual detection of APs. In colloidal studies, AuNPs probe showed a colour change from wine red to green upon introduction of analyte. UV-vis spectroscopy revealed the shifting of the surface plasmon resonance (SPR) peak from 525 nm to 729 nm induced by aggregation of AuNPs. For AgNPs probe, a colour change was observed from yellowish green to brown. Transmission electron microscopy (TEM) studies showed growth of AgNPs. A presumed oxidation of the analyte, forming an absorbing compound at 279 nm in both AgNPs and PDAs probes was also observed. For PDAs probe the colour change was from purple to pink. Concentrations as low as 30  $\mu g m L^{-1}$  were detectable in all colloidal based probes. Further colorimetric investigations were conducted with electrospun AuNPs-nylon 6 fiber mat. A colour change from purplish red to navy blue at concentrations of 1000  $\mu$ g mL<sup>-1</sup> was observed. Electrospun AgNPs –nylon 6 fiber mat did not show a distinct colour change. High resolution scanning electron microscopy (HRSEM) revealed the analyte inducing the assembly of AuNPs and AgNPs as they covered the surface of the nanofiber mat. Electrospun nanofibers are a platform for analysis and thus tuning their chemistry will lead to sensitive and selective methods.

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## List of Abbreviations

AgNPs	Silver nanoparticles
AIBN	Azobisisobutyronitrile
AP-MALDI	Atmospheric pressure matrix assisted laser desorption
APs	Alkylphenols
AuNPs	Gold nanoparticles
BET	Brunauer, Emmett and Teller analysis
ВНТ	Butylated hydroxytoluene
<sup>13</sup> C { <sup>1</sup> H} NMR	Carbon decoupled to proton nuclear magnetic resonance
CE	Capillary electrophoresis
CNFs	Carbon nanofibers
CNTs	Carbon nanotubes
Da	Dalton unit
DA	Diacetylene monomer
DBU	1,8-Diazabicyclo [5.4.0] undec-7-ene
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DPX	Disposable pipette tip extraction
DMF	N,N-dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
EDAX	Energy dispersive x-ray analysis
EDCs	Endocrine disrupting compounds
EE	Extraction efficiency
ESI	Electrospray ionization
ESI-MS	Electrospray ionization mass spectrometry
FLD	Fluorescence detector
FTIR	Fourier transform infrared
GPC	Gel permeation chromatography
GC	Gas Chromatography
GC-MS	Gas chromatography-mass spectrometry
HS-SPME	Headspace solid phase microextraction

<sup>1</sup> H NMR	Proton nuclear magnetic resonance
HPLC	High performance liquid chromatography
HRSEM	High resolution scanning electron microscope
ISs	Immunosorbents
LLE	Liquid-liquid extraction
LLME	Liquid-liquid microexraction
LSE	Liquid solid extraction
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MALDI-TOF	Matrix assisted laser desorption ionization-time of flight
MS/MS	Tandem mass spectrometry
MEPS	Micro extraction packed sorbent
MIPs	Molecularly imprinted polymers
MOFs	Metal organic frameworks
MWCNTs	Multiwalled carbon nanotubes
NHS	N-hydroxysuccinimide
РСВ	Polychlorinated biphenyl
PCDA	Pentacosadiynoic acid
PDA	Polydiacetylene
PDI	Polydispersive index
PNME	Packed needle microextraction
PPB	Parts per billion
PPCPs	Pharmaceutical and personal care products
PPM	Parts per million
PS	Polystyrene
PSU	Polysulfone
P&T	Purge and trap
RAMs	Restricted access materials
RSD	Relative standard deviation
SEM	Scanning electron microscope
SBSE	Stir bar sorptive extraction
SEC	Size exclusion chromatography
SME	Solid membrane extraction

SPDE	Solid phase dynamic extraction
SPE	Solid phase extraction
SPME	Solid phase microextraction
SWCNTs	Single walled carbon nanotubes
TBAB	Tertiary ammonium bromide
TEM	Transition electron microscopy
TFME	Thin film membrane extraction
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
TiO <sub>2</sub>	Titanium oxide
TLC	Thin layer chromatography
UV-vis	Ultraviolet-visible light
VBC	4-Vinylbenzyl chloride
VOCs	Volatile organic compounds

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# Chapter 1: Nanotechnology in analytical chemistry

#### **1** Introduction

Nanotechnology is a field of study that specifically involves the use of nanomaterials. It is a technology of designing, fabricating as well as application of nanostructured objects or nanomaterials. Nanomaterials have one dimension less than 100 nm, whereas nanostructured objects have two dimensions less than 100 nm and nanoparticles having three dimensions of less than 100 nm<sup>-1</sup>. Nanotechnology is applicable in multidisciplinary areas such as; chemistry<sup>2</sup>, engineering<sup>3</sup>, biology<sup>4</sup>, physics<sup>5</sup>, tissue engineering<sup>6</sup> as a result of the physical and chemical properties nanomaterials possess. The advantage of using such materials is that the smaller the size of the nanomaterial, the larger the surface areas and thus exposure of active sites. Further, the unique and tunable properties of nanomaterials compared to their bulk counterparts offers wide application in addressing environmental challenges.

#### 1.1. Classification of nanomaterial

Classifying nanomaterials present challenges due to complexity associated with accurately grouping them such that researchers have taken it as an area of interest <sup>7</sup>. Different properties used to classify nanomaterials were based on morphology, dimensionality, and composition but it has been found useful to include their size dependant properties and the number of dimensions of confinement as shown in Fig. 1.1 <sup>8</sup>:



Figure 1.1: Classification of nanostructed materials according to dimension, morphology and composition <sup>8</sup>.

Zero dimension (0D) nanomaterials are confined in all three dimensions *e.g.* quantum dots; nanoparticles and powders; whilst one dimension (1D) nanomaterials have two dimensions at the nm scale and no confinement along one dimension of the material *e.g.* quantum wires; fibers and ribbons; two dimension (2D) nanomaterials have one dimension at the nm scale and no confinement along two dimensions of the material *e.g.* quantum wells and films; and, three dimension (3D) nanostructured materials are organized with OD, 1D or 2D nanomaterials as building blocks. The size-dependent properties that can be found in 0D, 1D and 2D nanomaterials are either of surface effects and or quantum effects <sup>9</sup>.

#### **1.2** Methods or techniques for fabricating nanomaterials

There are a number of techniques available for fabricating nanomaterial that include the following:

- Electrospinning which makes use of a strong electric field that is applied to a droplet of a polymer melt or solution to form nanofibrous materials<sup>10</sup>.
- Phase separation; involves the dissolution of a polymer, gelation, extraction of the solvent it was dissolved in, then freezing and drying of the resulted crude that yields nanoscale porous foam <sup>11</sup>.
- Self assembly process; makes use of individual, pre-existing components that arrange themselves into a desired pattern <sup>12</sup>.
- Thin film deposition; thin film of materials are put onto a substrate or a previously deposited layer. The method allows one to control deposition of layers such that the end product is within the nanometer range <sup>13</sup>.
- Chemical vapour deposition occurs in a similar manner as thin film deposition. The difference is that the deposited material is in a gaseous form such that when it has deposited on a solid surface it changes its form into a solid layer <sup>14</sup>.
- Nanoimprinting; fabricates nanoscale patterns by mechanical deformation or molding of an imprint onto a substrate. The patterning process makes use of heat or UV light and pressure is applied. Upon completion of imprinting, disassembly of template from the substrate is carried out leaving specific nanoscale cavities <sup>15</sup>.

Amongst the techniques that have been mentioned some are have limitation in terms of controlling the diameter of the fiber formed, namely in phase separation whilst others such as self assembly and deposition methods requires perfect encoding of the patterns for an entire structure. Electrospinning is by far the most stable and easy method of fabricating nanofibers whether from a polymer melt or solution. Controlling of the diameter is also easily achieved.

#### 1.3 Nanomaterials in analytical chemistry

In analytical chemistry, nanomaterial can be applied as tools for analysis in order to enhance or minimise the duration of the analysis or as target analytes as shown in Fig. 1.2<sup>16</sup>. The comprehensive details of their use either as tools or analytes will be discussed in latter chapters. The work conducted in this thesis was specific to the technique for fabricating electrospun fibers and their application thereafter. The use of electrospun nanomaterials was recognized as part of analytical tools.



Figure 1.2: General overview of the areas nanomaterials can be applicable in analytical chemistry, where the interest of study could be in them as analytes or tools to carry out analysis.

#### 1.4 Aim

The aim of the studies in this thesis was to:-

- Evaluate polydiacetylene polymers, gold and silver nanoparticles as colorimetric probes.
- Employ the electrospinning technique to:
  - Fabricate SPE sorbents nanofibers from either synthesized or commercially available polymers for application in the sample preparation step as to separate, concentrate or clean-up of water matrices.
  - Fabricate nanofiber mats as supports for gold and silver nanoparticles to form composites as colorimetric probes for visual detection of the analytes of interest.

The class of target analytes which were studied for both applications were alkylphenols; 4-nonylphenol (4-NP), 4-octylphenol (4-OP) and 4-tert-octylphenols (4-t-OP).

#### 1.5 Scope of the thesis

The thesis is divided in two sections. Section 1 highlights the background of what is already in the public domain and addresses opportunities for further research that have necessitated the current studies. **Chapter 2**, reviews the trends in analytical chemistry and focuses on the use of nanomaterials in the analytical process. **Chapter 3**, reviews the electrospinning technique, as the method of choice for the fabrication of nanofiber materials. It also gives an outlook on a variety of applications of electrospun materials. It further addresses fundamental criteria that render the possibility of achieving desired morphologies with specific properties. **Chapter 4** gives the background on the monitoring of alkylphenols as they have been

realised as an environmental concern. This class of organic pollutants are semi polar and their chemistry is not fully understood. Applications conducted in this thesis centred on these analytes.

The second section consists of **Chapter 5** to **7**. In **Chapter 5** fabrication of electrospun styrene copolymers applied as SPE sorbents is reported. It describes the experimental protocols that were followed and the findings of the study. **Chapter 6** reports on the developments of polydiacetylene polymers, gold and silver nanoparticles colorimetric probes for alkylphenols. The results from the work carried out are discussed in detail. **Chapter 7** summarizes the research observations and highlights perspective work.

# Chapter 2: Use of nanomaterial in the analytical process

#### 2 Introduction

The analytical process encompasses several steps which include sampling, sample preparation, separation, detection and data processing. These are the determining steps that render the analytical method developed useful and meaningful. All the steps involved are equally important in order to obtain correct results that fulfil the analytical purpose <sup>17</sup>. Sampling and sample preparation are low resolution separation steps carried out prior to the use of high resolution separation and detection instrumentation. Low resolution steps account for about 60 % of the total analysis time <sup>18</sup>. Sampling is carried out from various matrices which could either be air, river water or sediments, wastewater, urine, plants and blood. Once the samples have been collected, the sample preparation step is carried out so as to isolate, purify and concentrate the analyte of interest from any matrix <sup>19</sup>.

Extraction is the most common sample preparation technique applicable to solids, liquids or gaseous samples. It aims to separate the analyte of interest from the sample matrix. Different methods of extraction are employed for specific group of analytes. In solid matrices, the analyte is extracted using either headspace solid phase microextraction (HS-SPME) or purge and trap (P&T) adsorptions typically followed by gas chromatography (GC) <sup>20</sup>. These techniques are frequently used for volatile or semi volatile organic compounds found in sediments. For non-volatile compounds, liquid-solid extraction, sonication and soxhlet extraction are employed and are usually followed by liquid chromatography (LC) <sup>21</sup>. When the analyte of interest is an inorganic compound, methods such as acid digestion, acid-base

leaching, complexation are used and normally quantified by ion chromatography or ion exchange through electrical conductivity as a commonly used detector <sup>22</sup>. Sample preparation for liquids and suspension matrices rely on; solid phase extraction (SPE), liquid-liquid extraction (LLE), dilution, evaporation, distillation, microdialysis/dialysis, lyophilization, filtration, centrifugation and sedimentation methods <sup>23</sup>.

Sorbent associated techniques namely; solid phase extraction (SPE), solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE) are more frequently used for sample preparation. The principle of extraction and separation is based on the partitioning of analyte molecules between a solid phase (sorbent) and a liquid phase of the sample. The widespread adoption of sorption techniques is due to the availability and diversity of sorbent materials that offer a broad range of properties. Other characteristics that make them prevalent are;

- Less or no consumption of organic solvents and therefore considered environmental friendly and economically viable.
- Ability to miniaturize the processes (maximise on time of operation by using minimum sample size and organic solvents).
- Applicability across different matrices and polarity of analytes.

Of particular interest is the development of enrichment techniques that extract polar pollutants, since these compounds are ubiquitous in aqueous samples and present particular difficulties in their extraction due to their polar characters <sup>24</sup>. Therefore much of the sorption techniques are applied for organic compounds.

#### **2.1 Sorption extraction techniques**

#### 2.1.1 Solid phase microextraction

SPME can be used for sampling and sample preparation, both in the laboratory and on site <sup>25</sup>. Pawliszyn and co-workers originated the development of SPME in 1989 and its subsequent commercialization by Supelco in 1993 <sup>26</sup>. Essentially there are two ways to perform the technique; (1) direct immersion of the fiber into an aqueous sample to extract analytes dispersed in the solution as shown in Fig. 2.1 [A]; (2) exposure of the fiber in the sample headspace to extract volatile compounds that partition between the gaseous and liquid phase. Both modes of extraction are assisted by a magnetic stirrer bar to reach maximum extraction. The process proceeds by the analytes having to separate from the sample matrix, which could either be liquid or headspace vapour to the fiber coating. Once equilibrium extraction is achieved, the concentrated analytes are desorbed either thermally or by the use of a solvent. Therefore the sorbent must be robust and be able to withstand high temperatures as thermal desorption is the popular way of eluting.



Figure 2.1: SPME device main components; a plunger for movement of the needle to introduce and remove the thin layer of fiber from the sample. A shows the direct immersion of the SPME into a sample  $^{27}$ .

SPME device is constructed in a syringe format that contains a steel microtube fused with a silica fiber coated with a stationary phase, typically a polymer as shown in Fig. 2.1. The mass of coated polymer is limited to the length of the silica fiber, which is believed to minimise extraction efficiency as complete enrichment may not be reached <sup>28</sup>. There is therefore a need to develop further the microextraction devices due to;

- Demands for more robust SPME systems
  - Shorter extraction times
  - o Higher extraction efficiency and sensitivity
  - Devices with larger coating capacity

Related microextraction techniques were developed such as the in tube/needle SPME formats namely; solid phase dynamic extraction (SPDE), thin-film microextraction (TFME), microextraction by packed sorbents (MEPS) and packed needle microextraction (PNME) as shown in Fig. 2.2 in order to maximise the application of SPME. Guan *et al.* reviewed their application and concluded that the different formats have a great advantage for on-site sampling and achieve high recoveries <sup>29</sup>.



Figure 2.2: Various types of solid phase microextraction, where SBSE is stir bar sorptive extraction, TFME is thin film membrane extraction, SPDE is solid phase dynamic extraction, PNME is packed needle microextraction and MEPS is microextraction by packed sorbents<sup>29</sup>.

#### 2.1.2 Stir bar sorptive extraction

SBSE is based on the same principle as discussed for SPME. It was developed by Baltussen *et al.* to overcome the limitation associated with the extraction capacity of SPME fibers <sup>30</sup>. In SBSE, a glass stirrer bar is coated with a thick layer of sorbent material as depicted in Fig. 2.2 B which is normally larger than the coating achieved in SPME. The larger sorbent coating gives SBSE the ability to achieve higher concentration factors <sup>31</sup>. The main disadvantage of SBSE is that it relies more on manual operation and on-site application is not yet feasible <sup>23c, 29</sup>. Currently the commercially available stir bar coating is with polydimethylsiloxane (PDMS) making it favourable for non-polar compounds. Developments to improve the application towards polar analytes are in place <sup>32</sup>.

#### 2.1.3 Solid phase extraction

SPE is by far the most widely used technique for the extraction and pre-concentration of several types of analytes from different matrices due to its efficiency and simplicity <sup>33</sup>. It is the oldest recognized method for sorption of different analytes with diverse volatility in simple and complex matrices. SPE was first introduced in the mid 1970s and became commercially available in 1978 <sup>34</sup>. Through development for a simple on-site, portable devices and alternative methods of sample preparation, SPME and SBSE were realised.

SPE is still recognised as the technique that affords better, simple, convenient and easily automated sample preparation methods. The use of solvents to drive the process is a pre-requisite but current methods make use of less volume of organic solvents. Simple manipulations within minimum time of analysis and high pre-concentration factors are achievable, not withstanding the fact that SPE cartridges can also be used for storage of analytes of interest <sup>35</sup>. Figure 2.3 shows the typical operations of SPE. It comprises of the 4 main steps namely; conditioning of the sorbent, loading of the sample, washing of interferences and lastly elution of the target analyte.



Figure 2.3: The principle of clean-up of matrix effects and concentrate of analytes *via* SPE  $^{36}$ .

The major limitation in packed SPE is achieving uniform packing of sorbent material to avoid poor efficiency. Generally for all the sorptive techniques discussed, the choice of the sorbent material still plays an enormous role amongst other factors in order to achieve high throughput from the sample preparation step. In all the techniques, the sorbent should address selectivity towards the analyte of interest by having an ability to retain it compared to the matrix it already exists in. Unfortunately there has not been a report where one sorbent material addresses all these factors. Therefore different classes of sorbent materials have been fabricated to address the complexity of the matrices as well as the wide range of analytes. Classical examples of sorbents are silica, graphitized carbon and cross-linked polystyrenedivinylbenzene (PS-DVB) polymeric sorbents, which are generally hydrophobic in nature. Of all the different types of sorbents available, polymeric sorbents have been shown to be the most suited for the determination of polar analytes <sup>37</sup>. Amongst sorbents that are known for selective adsorption are molecular imprinted polymers (MIPs)', restricted access media (RAM) and immunosorbents (ISs) which specifically address the challenge of selectivity <sup>28</sup>, <sup>38</sup>. MIPs are synthetic polymeric materials with specific binding sites at molecular level to selectively recognise a target analyte during re-binding. RAM and ISs are particularly suited for biological samples where the mode of selectivity is based on size exclusion in the case of RAM whilst for ISs, antigen-antibody interactions bring about selectivity.

Another feature that has been found to improve sorbent efficiency in SPE is its morphology. Generally classical SPE sorbents morphology has been in the form of particles with sizes within the micrometer scale <sup>39</sup>. While some of the sorbents work efficiently, exploration of alternative sorbent material with chemical stability and physico-chemical characteristics that are nanostructured are desirable as they could offer good sorbent performance <sup>16</sup>. Fabrication of nanostructured sorbent materials is an avenue that is currently being explored to improve the morphological properties such as the specific surface area and pore size of the sorbents.



Figure 2.4: Fractional part of nanoparticles involved in the analytical process.

#### 2.2 Types of nanomaterials in analytical processes

Nanomaterials are a promising resource for developments within the analytical discipline. A great deal of effort has been focussed towards developing nanomaterials as sorbent materials in the sample preparation step <sup>2, 16, 40</sup>. The numerous materials that have been applied thus far include, graphene, fullerenes, nanofibers, carbon nanotubes, silica nanoparticles, imprinted nanomaterials. Figure 2.4 gives proportional distribution of application of nanomaterials in the analytical process. It is clear that the application of nanostructured materials is wide such that a bigger portion of ~ 50% has been dedicated for use in the detection step. The unique size, shape and composition dependent optical properties exhibited by nanoparticles allows for further exploration on detection methods <sup>41</sup>. The optical properties are useful in the colorimetric (visual) detection of analytes as they help to identify trace pollutants in aqueous environment by a unique colour change. Discussions on different properties of nanomaterials will be presented, so as to reveal the growing research interest associated with their applications.

#### 2.2.1 Siliceous nanomaterials and its composites

Siliceous based nanomaterials include silica nanoparticles and porous silica materials synthesized from various silanes. Chemical modifications are possible to fine tune the surface so as to achieve good sorption capacity <sup>42</sup>. Generally, silica nanoparticles are spherical in nature with submicron diameters <sup>39</sup>. Siliceous nanomaterials are characterised by high surface area and low temperature modification ability <sup>16, 43</sup> and are widely available as commercial sorbents employed in sorptive extraction as well as HPLC columns. Their tunability results in a range of sorptive applications which include preconcentration of metals, pesticides and drugs <sup>44</sup>. They are the widest applied SPE sorbent materials and as stationary phase of the conventional LC, GC and electrophoresis columns <sup>45</sup>. The advances in synthesis of silica nanoparticles and its compatibility to other nanomaterials puts the nanocomposite synthesis at the peak of research as some of the materials are promising to remediate challenges across analytical applications. Figure 2.5 shows the typical morphologies of siliceous nanoparticles through HRSEM characterization labelled A and the TEM images of their gold (Au), silver (Ag) and platinum (Pt) nanoparticle composites at B, C and D respectively.



Figure 2.5: Typical HRSEM images of siliceous nanoparticles labelled A and TEM images of their metal nanoparticles composites namely;  $AuSiO_2$  at B,  $AgSiO_2$  at C and  $PtSiO_2$  at D <sup>46</sup>.

#### 2.2.2 Metallic nanoparticles

Metallic nanoparticles are inorganic/organic hybrid materials. These include gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), metal oxides *e.g.* TiO<sub>2</sub> and metal organic frameworks (MOFs). Over the past decade, researchers mainly focused on their selective catalysis, control of size, shape and reactivity of nanoparticles <sup>47</sup>. Currently, reviews have discussed their immobilization and analytical applications <sup>48</sup>. MOFs have had limited applications due to their character of being moisture sensitive. Formation of their composites was found to be ideally suited to develop for a wide range of applications.

Of great interest is the increasing application of AuNPs and AgNPs in various areas within the analytical processes. Studies relating to AuNPs applications are common across disciplines. AuNPs have been shown to improve recovery and the limits of detections for hydroxy-polycyclic aromatic hydrocarbons (OH-PAH) from sample preparation of urine samples <sup>49</sup>. Furthermore AuNPs have also been employed as modifiers of the stationary phases of LC, GC and CE where they showed improved robustness of columns and novel interactions with analytes studied, ranging from small organic compounds and complex biological compounds <sup>50</sup>. On the other hand AgNPs are gaining popularity due to their antimicrobial activity, although there are reports for applications in analytical chemistry. For an example, modified AgNPs were employed in LLME to separate and preconcentrate peptides and proteins in biological samples prior to analysis by atmospheric pressure matrix assisted laser desorption/ionization (AP-MALDI) ion trap mass spectrometry and matrix

Apart from the increased use in high resolution separation, AuNPs and AgNPs have been employed in their colloidal form as colorimetric detectors of DNA, proteins, many biological samples and polar organic pollutants in the environment <sup>41a, 52</sup>. Tunability of surface chemistry of nanoparticles allows for sensitivity and selectivity of a particular analyte to be achieved.

#### 2.2.3 Carbonaceous nanomaterials

Carbon nanomaterials include nanofibers, graphene, fullerenes, nanotubes, peapods, nanoonions, nanohorns as some are shown in Fig. 2.6. Graphene is considered the basic building block of graphitic forms that includes graphite, fullerene and carbon nanotubes (CNT). It possesses a single layer of carbon atoms in a closely packed honeycomb 2D lattice.

Fullerenes are closed cage carbon molecules containing pentagonal and hexagonal rings. Carbon nanotubes are in a shape of a cylinder capped fullerene like structure, formed by seamless roll up of single and multi-layers of graphene lamella, commonly known as single walled CNT and multi-walled CNT respectively. Carbon nanofibers are fabricated through a polymer melt/solution being subjected to electrostatic forces, *i.e.* through the electrospinning technique. A lot of work has focused on the fabrication and application of nanostructured carbon <sup>2, 53</sup>. A number of publications have reported on opportunities and challenges of carbonaceous nanomaterials given their wide applications for environmental monitoring <sup>54</sup>. Recent developments have shown carbonaceous nanomaterial gaining popularity as sorbents and as part of stationary phases for GC, CE and LC <sup>55</sup>.



Figure 2.6: Selected carbonaceous nanomaterials applicable in analytical chemistry<sup>2</sup>.

Characteristics such as the high surface to volume ratio, easy derivatization procedures, unique thermal, mechanical and electronic properties affords them wider applications across industries. Their structural characteristics facilitates the interactions with organic compounds *via* non covalent forces such as hydrogen bonding, electrostatic forces, van der Waals forces,  $\pi$ - $\pi$  stackings and hydrophobic interactions <sup>40</sup>. These unique and tunable properties of carbon nanomaterials enables a broad range of their applications in meeting the environmental challenges such as the requirements for sorbents, high flux membranes, depth filters, antimicrobial agents as well as for pollution prevention strategies <sup>56</sup>.

Amongst the class of nanomaterials, electrospun nanofibers are receiving a growing interest in various applications to address global issues. A review about their potential in addressing such environmental challenges, highlighting the advantages and research that needs to be done has been reported <sup>57</sup>.

In the analytical process, the major application of electrospun fibers is still on them being employed as good sorbent materials and as filter membranes. A recent review highlighted their use even on a broader spectrum that includes their potential use as LC stationary phase and their current use as colorimetric devices <sup>58</sup>. There are still limited reports on the application of electrospun nanofibers especially in high resolution separations as chromatographic columns or in electrophoresis and as part of detection devices. Therefore the studies constituting this thesis were dedicated to drive the infancy stage for the further application of nanofibers as sorbents for sample clean-up as well as platforms for colorimetric probes.
## Chapter 3: Electrospinning technique

## 3 Introduction

Recognition of electrospinning technology dates back to 1930s when filed patents on the fabrication process and influence of apparatus involved were available <sup>59</sup>. The electrospinning technology did not receive great interest at the time, despite the fact that the filter membrane industry was the initial sector that showed the applicability of electrospun nanofibers. In mid 1990's the technology was revived and researchers demonstrated the application of electrospun nanofibers to various industries <sup>60</sup>.

Electrospinning is a simple and inexpensive technique for fabricating fibers of submicrometer to nanometer diameters from polymeric materials <sup>61</sup>. Polymer solution or melts are a prerequisite in electrospinning as it depends on the polymer/melt entanglements and its physical properties for the process to take place. It is a technique that depends on electrical forces to change polymeric solution morphologies. An electric field is imposed on a polymer molten solution and depending on the viscosity of the solution, the resulting morphology can either be a nanofiber or fine droplets (polymer particles) <sup>62</sup>. When fine droplets are formed, the process is known as electrospraying (electrohydrodynamic spraying) and it yields particles of size varying between hundreds of micrometers down to nanometer <sup>63</sup>. Electrospinning set up comprises of three main components namely a voltage supply source, a collector and a syringe pump to control the flow of the polymer solution as shown in Fig. 3.1 <sup>64</sup>. Through invested research on the technique, intricate details have revealed how a polymer solution is processed to fibrous material. Many researchers have studied the process by experimentally observing the changes that result in the formation of desired materials whilst some have given comprehensive and detailed theoretical insights on electrospinning <sup>62, 65</sup>. Briefly, viscoelastic forces along the jet of a polymer melt and the surface tension tend to stabilize the charged jet. After the jet flows away from the droplet in a nearly straight line, it bends into a complex path and other changes in shape occur, during which electrical forces stretch the jet into a thin spray. As a result the solvent evaporates and nanofibers are formed. Simulating the electrospinning process using a mathematical model as shown in Fig 3.2 has given insights into how polymer entanglement results into electrospun fiber formation <sup>66</sup>.



Figure 3.1: A typical set-up of electrospinning showing the voltage source, syringe pump to control the flow of the polymer solution and the collecting plate <sup>64</sup>.



Figure 3.2: A numerical simulation of the electrospinning process <sup>66</sup>.

## 3.1 Electrospun polymer morphologies and their unique properties

Different morphologies result from electrospinning ranging from microspheres, beaded fiber and bead free fibers. Microsphere polymer particles are a result of low concentrations of the solution. The unique features from electrospraying are, the ease to control particle size, ease of coating the particle with desirable functionalities and the rapid rates at which the whole process occurs <sup>67</sup>. Within the different morphologies electrospinning yields are fibrous materials with diameter ranging between submicron to low nanometers. Various fiber arrangements that include random, aligned, core-shell, bundles and mats are achieved by simply altering the spinning parameters <sup>65a, 68</sup>. The primary characteristic of the nanofibers is its high specific surface area, high surface to mass ratio and even pore distribution <sup>69</sup>, that make them ideal materials in a wide range of applications. Experimental investigation of parameters governing efficiency of electrospinning polymer solutions has been conducted <sup>70</sup>, giving much insight to the technique.

## **3.2** Parameters affecting electrospinning technique

## 3.2.1 Suitable solvent

Not all polymers are spinnable especially highly cross-linked polymers as they are not readily soluble in most organic solvents. Therefore the selection of a suitable solvent is a fundamental step to address so as to appropriately dissolve the polymer. The solvent influences the critical concentration thereby allowing the transition from electrospraying to electrospinning. Therefore the spinnability of the polymer solution and the morphology is significantly affected by the choice of a solvent <sup>64</sup>. The selection of solvent is based on its vapour pressure, dielectric constant and polarity <sup>71</sup>. Evaporation of the solvent must be quick enough to maintain fiber integrity and yet not too rapid resulting in the hardening of fiber before reaching the nanometer range <sup>72</sup>. A combination of solvents has been found to address some of these challenges for a number of polymers.

## **3.2.2** Concentration of the polymer solution (viscosity)

Once the polymer solubility has been established in a selected solvent system, a minimum solution concentration is required for it to be spinnable. The molecular weight of the polymer directly affects its concentration, viscosity, conductivity and the dielectric property <sup>73</sup>. In a low viscous solution, mainly particles or microspheres are produced. Increasing the concentration of the polymer solution may result in beaded fibers which are considered a transitional state towards forming smooth fibers of submicron to nanometer diameter range. Optimization of the conditions has to be carried out when desiring smooth fibers such that the beads are eliminated and fabricated materials have reduced diameters. Viscosity and surface tension of the solution must neither be too high preventing the jet from forming nor be too low to eliminate dripping of the polymeric solution. The study and monitoring of the

influence of polymer solution concentration on fiber diameter have been conducted showing that all the parameters involved have to be optimized to form the desired diameter of the fiber <sup>74</sup>.

## 3.2.3 Technical parameters

The parameters considered as technical in electrospinning are namely; voltage applied, flow rate and tip to collector distance. An adequate power supply is essential to be optimised in order to overcome viscosity and surface tension of the polymeric solution. Depending on the type of polymer to be electrospun, high voltage may be required to achieve the desired nanofibers. It has been observed that the higher the voltage applied, the more likely the formed nanofibers are to exhibit larger diameters and beads<sup>73b, 75</sup>. It is not always the case though as some researchers reported that as applied voltage increases, electrostatic repulsion forces on the polymer also increases, and thus favours the narrowing of fiber diameter <sup>76</sup>. Whilst voltage influence may vary from one polymer to the other, the flow rate at which the polymer solution is fed significantly contributes to fiber size, shape and diameter distribution. Too few studies have reported the systematic monitoring of flow rate effects. Generally a lower flow rate is desirable to allow sufficient time for the solvent to evaporate resulting in nanofibers <sup>77</sup>.

## 3.3 Applications of electrospun nanomaterials

There is a growing need of research focussing on the fabrication of nanomaterials suitable for many applications as dictated by various disciplines. Electrospun nanofibers and polymeric nanoparticles have been applied in various fields that range from environmental monitoring for water or air matrices and in energy <sup>78</sup>as filters or membranes or even sorbents for matrix clean-up <sup>55b, 79</sup>, in biomedical applications <sup>80</sup>, tissue engineering <sup>81</sup>, protective clothing <sup>82</sup> fuel cells <sup>83</sup>. Figure 3.3 gives a pictorial view of the areas where electrospun materials have been applied.



Figure 3.3: Some areas where electrospun nanofibers are applied in order to address global issues <sup>57b</sup>.

The increasing demands for a convenient detecting method has opened an extensive research towards the construction of sensor based detectors and those based on electrospun nanomaterials are widely reported <sup>84</sup>. Nanofiber applicable in reinforcement employs mechanically stable nanofibers to improve the tensile strength and toughness of a material. In protective clothing, electrospun nanofibers are part of the fabric that make waterproof breathable fabrics to enhance moisture vapour transport and toxic chemical resistance. In

tissue engineering, nanofibers serve as matrices for cellular in-growth, proliferation, differentiation and formation of new tissues. For biological applications, fiber diameter optimization is critical as it has been reported that different cell type adhesion and migration depends on minimum fiber diameter <sup>85</sup>. Similarly to nanofibers, particles generated from electrospraying have found vast usefulness in biomedical application as drug delivery carriers <sup>86</sup>. All these applications depend on the chemistry or chemical components that make up the electrospun nanomaterial.

## 3.4 Summary

The electrospinning technique is undoubtedly a versatile approach for fabricating novel nanomaterials with fascinating surface characteristics. Having drawn attention to researchers, it opened an understanding of the fundamental structural control and reactivity associated with the diverse forms of morphologies produced. Currently there is a growing interest focused on many specific aspects of electrospun nanomaterials such as structure formation, functionalization and potential implementation in devices. These current trends make electrospinning one of the most powerful techniques for fabricating diverse nanostructured materials. Even on the work reported in this thesis, electrospinning was the centre technique to fabricate alternative sorbent materials for sample preparation and to devise electrospun fiber mats as solid state colorimetric probes.

# Chapter 4: Monitoring of alkylphenols in the environment

## 4 Introduction

The analytes of interest which were monitored were alkylphenols, classified as one of many ubiquitous endocrine disrupting compounds. Endrocrine disrupting compounds (EDCs) are known as a class of organic compounds that have xenobiotic and exogenous origins while mimicking or inhibiting the natural action of the endocrine system in animals and human<sup>87</sup>. The competing or inhibited actions can be synthetic pathways, secretion, transportation and binding of natural hormones that maintain the homeostasis, reproduction, metabolism, development, and/or behavior of living species<sup>88</sup>.

EDCs are broadly classified into several categories namely; hormones (natural and synthetic estrogen or steroids), pharmaceuticals and personal care products (PPCPs), industrial chemicals, pesticides, combustion byproducts, and surfactants<sup>89</sup>.Various types of natural and synthetic chemical compounds have been identified as EDCs. However, the definition and range of chemicals showing the behaviors of EDCs vary significantly; thus, a concern has been raised as to whether certain chemicals should be considered as EDCs. The primary effects of EDCs, as described earlier, are either the mimicking or inhibition of the behavior of natural hormones, such as estrogen, testosterone and/or thyroid. Although the disruption of the androgen and thyroid functions might exert greater or equal impacts on the environment, most research studies to date have focused on estrogenic EDCs.

## 4.1 **Properties of EDCs**

The molecular structures of several EDCs, with accompanying varying functionalities, are shown in Fig. 4.1. The EDCs shown in Fig. 4.1 have at least one aromatic group in their molecular structures. The hydrophobic properties are considered important characteristic for studying and controlling EDCs in the environment. Table 4.1 gives a summary of behaviour traits and sources of EDCs.



Figure 4.1: Selected EDCs that form different classes according to their disrupting behaviour.

Table 4.1: Summary of different EDCs and their typical effects on living organisms

EDCs	Main class	Common usage Example c	
			hormone target
DDT	Pesticide	Insecticide	Estrogen
PCBs	Chemical or	Industrial	Cortisol
	byproduct		
Estradiol	Pharmaceutical	Steroid	Estrogen
Diethylstilbestrol	Pharmaceutical	Drug	Estrogen
Bisphenol A	Industrial chemical	Plasticizer	Estrogen
Nonylphenol	Personal care product	Surfactant	Estrogen

Alkylphenols shown in Fig. 4.2, are phenolic molecules that are found to be persistent in the environment in water and soil matrices. The main source of these compounds in the environment is from industrial activities such as in production of drugs, textiles, dyes, pesticides and paper. They can naturally be released through the degradation of humic substances, tannins and lignins. The alkylphenols, 4-nonylphenol (4-NP) and-*tert*-octylphenol (4-t-OP) exist mainly as intermediates in the manufacturing industry. NP and OP are also degradation products of non-ionic surfactants alkylphenols ethoxylate used in industrial and institutional formulations <sup>90</sup>. Alkylphenols, OP and NP, have been shown to exist in the environment such as river water and sewage sludge, and in fish tissues <sup>89a, 91</sup>. In addition, the estrogenic activity of OP and NP has been extensively evaluated in a variety of assays <sup>92</sup>. Chemical properties of the studied APs are summarised in Table 4.2.



R = T-OP: C8 branched; OP: C8 linear; NP: C9 linear

Figure 4.2: AP standards that were studied, having a same phenolic head and different alkyl chain tail.

Table 4.2: Chemical properties of the studied APs

APs	MW (g/mol)	CAS No.	Water solubility	Log Kow
			$(\text{mg } \text{L}^{-1})$	
4-t-OP	206	140-66-9	4.82 (18)	5.28 (4.12)
4-OP	206	1806-26-4	3.11	5.50
4-NP	220	104-40-5	6.35	5.99 (5.79)

(experimental values), data from ref. 93

From the tabulated properties of APs, the logarithm of the partition coefficient (Log Kow) relatively serves as an indicator of the solubility of an organic compound. Generally it is inversely proportional to aqueous solubility and directly proportional to the molecular weight (MW). The trend of APs MW to Log Kow was expected whilst water solubility to Log Kow of 4-*t*-OP and 4-OP was not ideal. The behaviour is probably due to the similarities in their chemical structures. In the case of 4-NP it was clear that it is the less water soluble compound relative to the other two analytes. The higher the Log Kow, the more non-polar or less water soluble an organic compound is. These characteristics would play an enormous role in their mode of adsorption onto a sorbent material.

## 4.2 Environmental concerns

The presence and resulting adverse effects of EDCs are currently accepted in both the academic and public sectors. The on-going EDCs research trends can be classified into three major categories; the identification and determination of the effects of EDCs, the development and improvement of analytical methods, and the application and modification of water treatment options for the removal of EDCs. The occurrence of EDCs at low concentrations requires the development of a highly sensitive and specific analytical method for their detection and quantification. Below in Table 4.3 are reported methods developed for alkylphenols and other EDCs. As already discussed in Chapter 2, different types of extraction are employed to prepare ultimate samples to be analysed in different instrumentation.

Method	Matrix	Instrumentation	Reference
DLLME	Water	HPLC-FLD	2010 <sup>94</sup>
SPME	Water	GC-FID	$2010^{95}$
SBSE	Water	TG-GC-MS	2010 <sup>96</sup>
SPE	Wastewater	GC-MS-MS	2011 <sup>97</sup>
MEPS	Water	GC-MS	2011 <sup>88b</sup>
QuEChERS	Soil	GC-MS-MS	2010 <sup>98</sup>

Table 4.3: Recent method developments for analysis of APs

Researchers have tried to combine the categories of studies on EDCs and a great deal of work has been reported, addressing identification, isolation and determination of alkylphenols combined with method development. Reports on sample preparation of APs, evaluating different sorbent materials, mainly commercially available SPE sorbents, analysing with either GC or LC methods have been done <sup>97, 99</sup>. Difficulty to develop methods that are accurate due to matrix effects and unavailable wastewater blanks resulted in average recoveries. The data showed the great work done by various researchers in order to develop analytical methods through the use of various sorbents and techniques which will suit best the ubiquitous nature of these compounds.

Other researchers have embarked on the studies of the occurrence and fate as well as their effects on human health. Studies conducted on rats revealed that p-NP induced severe polycystic kidney disease <sup>100</sup>. Wang *et al.* studied the effect of APs in human milk and its relations to the dietery habits in Taiwan. They found that food patterns on cooking oil consumption and processed meat products from factor analysis was associated with OP concentration in human milk <sup>101</sup>. Other researchers have embarked on monitoring the sources

of APs in wastewater in order to advise on regulation and policies for industries that could contribute to the exposure of APs  $^{102}$ .

Though much work has been reported for these class of EDCs, APs are still very much of a great concern in the environment and their reactivity with other chemicals is yet to be fully understood. Thus development of simple but effective methods or tools for monitoring their presence in the environment is an area of research that is open for exploration.

# Chapter 5: Electrospun styrene copolymers for SPE of APs

## 5 Introduction

The development of styrene based hydrophilic copolymers as sorbents in SPE has been widely reported <sup>99b, 103</sup>. Some of the desirable features of such sorbents include ease of surface modification, chemical stability and physico-chemical characteristics <sup>103b, 104</sup>. Generally there are two ways of achieving surface modification of polymeric sorbents. One approach is to synthesize a hydrophilic copolymer using at least a monomer containing polar functional groups. An alternative route would be to modify a hydrophobic polymer so as to incorporate polar moieties into the polymer structure <sup>104b, 105</sup>. Styrene presents itself as a monomer whose functionalization and subsequent polymerization could be achieved in relative simple steps that help improve surface contact between the analyte and the sorbent. While some of the sorbent material with chemical stability and physico-chemical characteristics that are nanostructured are desirable as they have demonstrated potential to offer good sorbent performance <sup>16</sup>.

Fabrication of nanostructured sorbent materials is an avenue that needs to be greatly explored as the use of electrospun nanofibers has been suggested to be a possible way to improve morphological properties of the sorbent <sup>106</sup>. Current applications of electrospun fibers give a clear indication of the irreplaceable advantages in using the electrospinning technology. The fact that electrospinnable polymers range from natural to synthetic and ability to improve their applicability by introducing biological compounds, ligands and MIPs for selectivity, shows the vast impact electrospinning could have in research and development.

The application of electrospun nanofibers as sorbent materials in sample preparation dates back to 2007<sup>107</sup>. The work reported on the use of polystyrene nanofibers was adapted from a report by Shin et al. who showed polystyrene nanofibers as good filter media to separate water from a water-oil emulsion  $^{108}$ . Ever since then, researchers have embarked on packing nanofibers into different formats either as membranes, packed tips or columns as sorbents for sample preparation of organic compounds from biological to aqueous matrices <sup>109</sup>. Gu *et al.* have electrospun a number of polymers and applied them as sorbents, showing a great potential for industrial application of fiber packed tips or columns across environmental and pharmaceutical sectors <sup>110</sup>. Encapsulating imprinted molecules in electrospun fibers also demonstrated the flexibility of the fiber mats by combining the selectivity of extraction with robustness of sorbent material <sup>111</sup>. By 2010, electrospun nanofibers were employed as sorbent materials in SPME devices and as a thin layer chromatography (TLC) phase. The study was from the contributions by Olesik et al., who reported on the high surface to volume ratio advantages of electrospun fibers that facilitate the separation of organic compounds <sup>112</sup>. The current trends of applying electrospun fibers as sorbents have led to the construction of miniaturised devices that make use of minimum materials which results in reduced organic solvent consumption.

This part of the thesis addresses fundamental aspects on how the polarity and morphologies affects the efficiency of potential sorbent material. With the aid of electro-spinning/spraying, different morphologies from the same copolymer were fabricated. The sorbents were evaluated under batch mode for extraction efficiency and it was found that fibrous materials were better sorbent materials compared to microparticles. Recovery studies from fibers were also conducted for wastewater samples.

## 5.1 Experimental section

## 5.1.1 Reagents and Materials

All chemicals used in the study were of pure analytical grade unless otherwise specified. The solvents; tetrahydrofuran (THF), (98.0%), N,N-dimethyl formamide (DMF), (99.0%), methanol (MeOH), (99.8%), formic acid (90%), acetic acid (100%) were purchased from Merck Chemicals (Wadeville, Gauteng, South Africa). 4-Vinylbenzyl chloride, tertiary butylammonium bromide (TBAB), deuterated chloroform (CDCl<sub>3</sub>) and methyl acetoacetate were obtained from Sigma-Aldrich as well as the alkylphenol standards, 4-nonylphenol (4-NP), 4-octylphenol (4-OP), 4-tert-octyphenol (4-t-OP). All glassware was washed and rinsed thoroughly in ultra-pure water and then in methanol and dried in an oven at 120 °C. Stock solutions were freshly prepared for each standard compound at 10000  $\mu$ g L<sup>-1</sup> in methanol (LC-grade). Calibration mixtures at various concentration levels were obtained by appropriately diluting the stock solution with water generated from a MilliQ system, (Billerica, MA, USA). The calibration curves were obtained by performing a linear regression analysis, with analyte concentrations ranging from 20 to 120  $\mu$ g L<sup>-1</sup> and from 200 to 1000  $\mu$ g L<sup>-1</sup>.

## 5.1.2 Instrumentation

An Agilent 1200 series HPLC equipped with a fluorescence detector (G1321A) was employed for analysis at a flow rate of 0.8 mL min<sup>-1</sup> with a methanol:water (4: 1, v/v) mobile phase mixture. Chromatographic separation was achieved using a Zorbax SB Phenyl, 5  $\mu$ m; 4.6 - 250 mm analytical column, with subsequent detection at excitation of 220 nm and emission at 300 nm. The morphology of the nanofibers was studied with the aid of Vegan Tescan (TS5136ML) scanning electron microscope (SEM) from Brno, Czech Republic, operating at an accelerated voltage of 20 kV after gold sputter coating. The elemental contents of the surface modified nanofibers were determined by Environmental Scanning Electron Microscopy (SEM/ESEM-EDAX-QUANTA 200) at CSIR, (Port Elizabeth, South Africa). The instrument was operating at an accelerated voltage of 20 kV. The structures of all ligands were determined by <sup>1</sup>H NMR spectroscopy on a Bruker AMX 400 NMR MHz spectrometer and reported relative to tetramethylsilane (TMS)  $\delta$  0.00. The infrared spectra were recorded on a Perkin Elmer 100 FTIR (4000-650 cm<sup>-1</sup>). Thermal gravimetric analysis (TGA) was performed using a Perkin Elmer TGA. All TGA experiments were conducted under nitrogen gas flow at 10 mL min<sup>-1</sup>at heating cycle from 50 °C to 600 °C. Melting point apparatus 8889339 from Gallenkamp Co. in England was employed for checking the melting point of copolymers. Nitrogen adsorption/desorption isotherms were measured at 77 K using a Micromeritics ASAP 2020 Surface Area and Porosity Analyzer. Prior to each measurement, samples were degassed at room temperature for 24 h. The Brunuaer Emmett and Teller (BET) surface area, total pore volume and pore size distribution were calculated from the resulted isotherms. Analysis was carried out at Rhodes University, Nanotechnology Innovation Centre (NIC), (Grahamstown, South Africa).

Mass spectrometric analysis was conducted employing a Waters Synapt G2 using ESI positive source at cone voltage of 15 V with lock mass of leucine enkaphalin, at the Stellenbosch University Central Analytical Facility (Stellenbosch, South Africa). GPC analysis was achieved using a SEC instrument consisting of a Waters 1515 isocratic HPLC pump, a Waters 717 plus auto-sampler, Waters 600E system controller (run by Breeze Version 3.30 SPA) and a Waters in-line Degasser AF. A Waters 2414 differential refractometer was used at 30 °C in series with a Waters 2487 dual wavelength absorbance UV-vis detector operating at variable wavelengths. Tetrahydrofuran (THF, HPLC grade, stabilized with 0.125 % BHT) was used as eluent at flow rates of 1 mL min<sup>-1</sup>. The column

oven was kept at 30 °C and the injection volume was 100  $\mu$ L. Two PLgel (Polymer Laboratories) 5  $\mu$ m Mixed-C (300x7.5 mm) columns and a pre-column (PLgel 5  $\mu$ m Guard, 50x7.5 mm) were used. Calibration was done using narrow polystyrene standards ranging from 580 to 2x10<sup>6</sup> g mol<sup>-1</sup>. All molecular weights were reported as polystyrene equivalents. Samples were dissolved in BHT stabilized THF (2 mg mL<sup>-1</sup>). Sample solutions were filtered *via* syringe through 0.45  $\mu$ m nylon filters before subjected to analysis. Analysis was performed at Stellenbosch University, (Stellenbosch, South Africa).

## 5.1.3 Experimental procedures for synthesis 5.1.3.1 Synthesis of 4-vinylbenzyl methanol monomer

To NaOH (0.04 g, 1 mmol) and tertiary butyl ammonium bromide [Bu<sub>4</sub>NBr] (3.224 g, 10 mmol) in H<sub>2</sub>O (50 mL) was added 4-vinylbenzyl chloride (1.409 mL, 10 mmol) with vigorous stirring. The mixture was heated at 125 °C under reflux for 20 min and subsequently cooled in an ice/water bath. Upon cooling, the mixture was extracted with EtOAc (50 mL×3) and the combined organic layer was dried with MgSO<sub>4</sub>, filtered, concentrated and purified by column chromatography (EtOAc:hexane=1:5) to give **1** (4-vinylbenzyl methanol) as a colourless oil.<sup>1</sup>H NMR (CDCl<sub>3</sub>): d=7.45–7.34 (d, ArH, 4H), 6.79–6.72 (m, Ar CH=CH<sub>2</sub>, 1 H), 5.81–5.76 (d, CH=CH<sub>2</sub>, 1 H), 5.30–5.27 (d, CH=CH<sub>2</sub>, 1H), 4.70 (s, CH<sub>2</sub>, 2 H), 2.20 (s, OH, 1H); (M+); exact mass: found: 134.

#### 5.1.3.2 Synthesis of 4-vinylbenzyl 3-oxobutanoate monomer

In a three neck round bottom flask, methyl acetoacetate (1.1 mL, 10.188 mmol) and DBU (0.385 mL, 2.547 mmol) in toluene (30 mL) were mixed by stirring the solution. To this solution, 4-vinylbenzyl methanol monomer **1** (1.1393, 8.49 mmol) was added and the mixture

was stirred under reflux for 1h. After an hour, another portion of methyl acetoacetate (1.1 mL, 10.188 mmol) was added. The reaction mixture was further refluxed for another 1 h. Thereafter, the reaction was stopped and concentrated under rota vapour upon which an orange-red oil residue was formed. TLC was carried out giving the separation of three compounds in the product formed. The product was purified by column chromatography using Hex: EtOAc (5:1) ratio as the eluting solvent. The monomer was a yellowish oil, though complete purification was not achieved due to excess methyl acetoacetate. <sup>1</sup>H NMR (CDCl<sub>3</sub>): d=7.42–7.30 (d, ArH, 2H), 6.75–6.66(d, ArH, 2H), 6.73-6.69 (m, Ar CH=CH<sub>2</sub>, 1 H), 5.79–5.75 (d, CH=CH<sub>2</sub>, 1 H), 5.28–5.25 (d, CH=CH<sub>2</sub>, 1H), 5.17 (s, ArCH<sub>2</sub>, 2 H), 3.49 (s, COOCH<sub>2</sub>CO), 2.24 (s, COCH<sub>3</sub>).

## 5.1.3.3 Synthesis of hydroxyl methylated styrene copolymer

To 4-vinylbenzyl methanol monomer **1** (1.8615 g, 14 mmol) and styrene (15.89 mL, 15.9 mol) in toluene (37 mL) was added AIBN (0.175 g, 0.1066 mol). The mixture was purged with N<sub>2</sub> at room temperature for 0.5 h and then heated at 85 °C for 24 h under N<sub>2</sub>. Thereafter the solution was concentrated and the resulting residue was dissolved in THF (5 mL) and added slowly into vigorously stirred cold MeOH (0 °C, 50 mL). The resulting suspension was filtered by suction filtration to afford polymer **3** as a white powder. <sup>1</sup>H NMR: (CDCl<sub>3</sub>): d=7.06–6.51 (m, ArH,), 4.58 (s, ArCH<sub>2</sub>, 2H), 1.87 (s, OH), 1.6–1.3 (m, CH<sub>2</sub>): IR: n=3455.85, 3025.46, 2970.25, 1738.19, 1600.85, 1492.11, 1448.93, 1365.73, 1091.34, 1013.79, 792.95, 696.05 cm<sup>-1</sup>. Mp= 108- 110 °C

## 5.1.3.4 Synthesis of 3-oxobutanoate styrene copolymer

To 4-vinylbenzyl 3-butanoate monomer (0.8778 g, 4.1 mmol) and styrene (36.9 mmol, 3.84) in toluene (27 mL) was added AIBN (0.06 g, 0.266 mmol). The mixture was purged with N<sub>2</sub> at room temperature for 0.5 h and then heated at 85 °C for 24 h under N<sub>2</sub>. Thereafter the solution was concentrated and the resulting residue was dissolved in THF (5 mL) and added slowly into vigorously stirred cold MeOH (0 °C, 50 mL). The resulting suspension was filtered by suction filtration to afford polymer **4**. <sup>1</sup>H NMR: (CDCl<sub>3</sub>): d=7.06–6.51 (m, ArH,), 4.58 (s, ArCH<sub>2</sub>, 2H), 1.87 (s, OH), 1.6–1.3 (m, CH<sub>2</sub>): IR: n=3455.85, 3025.46, 2970.25, 1738.19, 1600.85, 1492.11, 1448.93, 1365.73, 1091.34, 1013.79, 792.95, 696.05 cm<sup>-1</sup>. Mp: around 98- 100 °C the polymer shrinks before it melt at 125-128 °C.

## 5.1.4 Electrospinning of the copolymers

The set-up for both electrospinning and electrospraying was the same. It comprised of a high voltage power supply (0-40 kV), syringe pump and a collector. Different polymer solution concentrations were prepared for the two copolymers. The aim was to generate different morphologies which would in turn be evaluated as sorbents for SPE. A combination of THF and DMF solvent was used in the preparation of the solutions. The polymer solution in a 20 ml plastic syringe was placed horizontally on the syringe pump at a specific flow rate for each spinning solution. Voltage was applied *via* an alligator clip connected to the syringe needle tip. A grounded aluminium foil collector was positioned below the needle. The distance between the needle tip and the collector was specific to each copolymer. All the electrospinning experiments were performed at room temperature and humidity. The conditions for sorbent fabrication are tabulated in Table 5.1. The fiber morphology was examined by SEM.

Co- polymer	Morphology	Concentration (w/v%)	Flow rate (mL h <sup>-1</sup> )	Voltage (kV)	Distance (cm)	Solvent (THF:DMF)
1	*	10 < 40	012	10.20	0.12	1.1 OP 1.4
1		10-<40	0.1-2	10-30	9-12	1.1 OK 1.4
	**	40	0.5-1.5	15-20	10-15	1:4
	***	$\geq 40$	0.1	20-30	10-18	1:1
2	*	≤40	0.5-2	10-30	9-15	1:1
	**	50	0.5-1	12-18	11-18	1:1 OR 1:4
	***	$\geq 50$	1	13-20	11-18	1:4

Table 5.1: Electrospinning and electrospraying conditions

<sup>1</sup> hydroxyl methylated styrene copolymer

<sup>2</sup> 3-oxobutanoate styrene copolymer

\* Microparticles, \*\* Beaded fibers, \*\*\* Bead free fibers.

## 5.1.5 Wastewater sampling

Samples from the wastewater treatment plant at Belmont Valley, (Grahamstown, South Africa) were collected in pre-washed plastic bottles from non-treated stream and from the pre-treated stream. After sampling, wastewater effluent samples were filtered initially using Whatman filter paper (125 mm diameter) and further using 47 mm  $\times$  0.2 µm pore size filter (polypropylene membrane filters). The effluents showed pH values between 7.75 and 8.16. Subsequently the pH of the filtrates was adjusted to 2.7-3 with HCl (2M) to facilitate adsorption.

## 5.1.6 SPE

#### **5.1.6.1 Extraction efficiency**

The evaluation of all the electrospun and electrosprayed sorbent materials was conducted using batch experiments. The comparison of extraction efficiency of the polymer microparticles, beaded fibers and bead free fibers was carried out at a constant mass of 10 mg for each material. The sample volume was also kept constant at 10 mL. The working sample concentration was 200  $\mu$ g L<sup>-1</sup> for the mixed standards in MilliQ water. The time for extraction was optimised to 30 min. The sorbents were individually weighed in a size 30 vials, aliquots of 10 mL from the mixed standard solutions were added. Within time interval of 5 min. the vials were inversely shaken for 30 min. The sorbents were filtered using a glass sintered filter and the collected eluent was subsequently analysed for the concentration of the analyte adsorbed. The sorbents were also collected and air dried after which preconcentration of the analytes was achieved by MeOH:formic acid mixture.

## **5.1.6.2 Relative recovery**

The sorbent mass was maintained at 10 mg packed in a pipette tip. The sorbents were initially conditioned with 100  $\mu$ L MeOH followed by equilibrating with 100  $\mu$ L of water. Aliquots of 1 mL of the wastewater sample spiked at three levels with 0.1  $\mu$ g mL<sup>-1</sup>, 10  $\mu$ g mL<sup>-1</sup> and 20  $\mu$ g mL<sup>-1</sup> mixed standards were loaded. The pipette column was then air dried followed by washing with 100  $\mu$ L MeOH then finally eluted with 200  $\mu$ L acidified MeOH.

## 5.2 Results and discussion

## 5.2.1 Synthesis of the functionalised styrene copolymers

The synthesis of the functionalized monomers and copolymers was carried out according to a method by Rongjun He *et al.*<sup>113</sup> illustrated in Scheme 5.1. The success in forming both monomers and copolymers was confirmed by the use of Fourier transform infrared (FTIR) and proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectroscopy.



Scheme 5.1: Synthetic pathway for the formation of the functionalised styrene copolymers.

The whole process of forming the desired copolymers was initiated by the modification of 4vinylbenzylchloride (4-VBC) reacting with sodium hydroxide (NaOH) in water catalysed by tertiary butylammonium bromide (TBAB). After the completion of the reaction, the crude product was separated by LLE. The desired monomers were separated by column chromatography. The successful formation of product **1** (4-vinylbenzyl methanol) and product **2** (4-vinylbenzyl 3-oxobutanoate) was evident from spectroscopic analysis. Deuterated chloroform was used as the NMR solvent for all the products analysed.

## **5.2.1.1** Characterization of monomers

Figure 5.1 shows the FTIR spectra of the monomers. The disappearance of C-Cl stretch at  $680 \text{ cm}^{-1}$  from the starting monomer and appearance of the O-H band at 3326.58 cm<sup>-1</sup>, confirmed the formation of the 4-vinylbenzyl methanol monomer. The (4-vinylbenzyl methanol) monomer (1) was further modified to (4-vinylbenzyl 3-oxobutanoate) by substituting the hydroxy group with acetoacetate functionality. The IR spectrum showed the two new C=O bands at 1741 cm<sup>-1</sup> and 1715 cm<sup>-1</sup> respectively as well as the disappearance of the OH band.



Figure 5.1: FTIR spectra of the monomers, from the starting monomer to the functionalised monomers.

## 5.2.1.2 Characterization of styrene copolymers

The functionalised monomers were then copolymerised with styrene using 2,2'-azobis (2methylpropionitrile) commonly known as azobisisobutyronitrile (AIBN) as the initiator. The purity of the polymers was confirmed with <sup>1</sup>H NMR and FTIR spectroscopy. The <sup>1</sup>H NMR spectrum confirmed that polymerisation had taken place. The insert in Fig. 5.2 shows the <sup>1</sup>H NMR of the 4-vinylbenzyl methanol monomer with typical olefinic protons around 5-6 ppm labelled 10 and 9 respectively. These protons were due to the doublets of the terminal olefinic protons and the multiplet proton at the junction of the olefinic functionality and the benzyl ring. Once polymerisation was achieved, the olefinic protons were not present in the

<sup>1</sup>H NMR of the copolymer as shown in Fig 5.2. The proton peaks of the aromatic ring of the copolymer were broad which was characteristic of a polymeric material. The polymers were formed as white powders.



Figure 5.2: <sup>1</sup>H NMR spectrum for the hydroxyl methylated styrene copolymer with an insert showing the spectrum of its monomer.

Other characteristic peaks for the hydroxyl methylated styrene copolymer [poly(co-styrene-OH)] were the singlet signal at 4.6 ppm due to the methylene protons and the hydroxy proton at 2.12 ppm shielded by the methylene protons of the bridging alkylchains on the benzyl groups. In the case of the 3-oxobutanoate styrene copolymer, new proton resonance peaks of the methylene groups bridging the C=O groups at 2.15 ppm and 3.53 ppm due to the 3-oxobutanoate functionality were observed in <sup>1</sup>H NMR shown in Fig. 5.3. Also, there was a shifting of the methylene group attached to the benzyl to 5.02 ppm from around 4 ppm in the monomer.



Figure 5.3: <sup>1</sup>H NMR of 3-oxobutanoate styrene copolymer.

The copolymers were further characterised for their molecular weight distribution. The results from gel permeation chromatography (GPC) and ESI-MS analysis confirmed that the copolymers were of lower molecular weight. For 3-oxobutanoate styrene copolymer the molecular weight (Mw) was 1770 Da with a relative good polydispersive index (PDI) of 1.409 whilst ESI-MS results showed the molecular ion at 1533.91108 m/z as depicted in Fig. 5.4 revealing the approximate mass of the copolymer which is believed to be within the mass range of GPC and ESI-MS results. In the case of hydroxy methylated styrene copolymer, unfortunately GPC analysis of the copolymer was not achieved as it eluted earlier, falling short or outside the calibration of the column used. ESI-MS results in Fig. 5.5, revealed an

ion at 1370.3781 m/z which was believed to be the molecular ion peak though not the highest fragmented ion observed. The speculation is based on undetectable mass observed from the GPC analysis. The spectrum showed a unique fragmentation pattern that had ion peaks that differed by a mass of 74 m/z. The fragmented ions were from 1370-1296 m/z, 1072-948 m/z, 998-924 m/z, 924-850 m/z, 850-776 m/z and 684-536 m/z ions. Due to the complexity of charged ions that may result from ESI-MS, at the current stage one can only speculate that the fragmented ions were due to reactions like retro Diels-Alder rearrangement which are plausible for these copolymers.



Figure 5.4: ES-MS chromatogram for 3-oxobutanoate styrene copolymer.

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Figure 5.5: ES-MS chromatogram for hydroxyl methylated styrene copolymer.

## 5.2.2 Electro-spinning/spraying of copolymers 5.2.2.1 SEM characterization

As already discussed in Chapter 3, there are a number of parameters that influence the type of morphology to be fabricated. Electrospinning and electrospraying processes are both generated by application of a relatively high voltage to a precursor solution. In electrospinning the jet does not break up due to the viscous nature of the polymeric solution and thus travels to the collector as fibers whilst in the case of electrospraying, break up of the jet to droplets is observed due to low viscosity of the solution. Figure 5.6 shows the difference of the end product from either process. In this part of investigation, a number of organic solvents namely; toluene, chloroform, THF, DCM and DMF were evaluated. The combination of THF:DMF were the solvents of choice to dissolve the copolymers.



Figure 5.6: Pictorial view of electrospinning and electrospraying process that may results in formation of different nanosized morphologies <sup>75a</sup>.

Figure 5.7 shows the SEM images of the fabricated materials from hydroxyl methylated styrene copolymer. Before it was electrospun, the morphology of the powder is irregular as shown in Fig. 5.7 **A**. Below the concentration of 40 % (w:v) ratio, solid particles were formed regardless of the solvent used **B**. It was observed that at 40 % in DMF: THF (4:1) ratio beaded fibres **C** were formed regardless of the variation of the electrospinning parameters *i. e.* voltage supply, flow rate and the distance between the spinneret and the collector. At conditions of the feed flow of 0.1 mL h<sup>-1</sup> applying 20 kV voltage at the distance range of 15 -17 cm in (1:1) DMF: THF the bead-free fibers were formed as shown in Fig. 5.7 **D**.

The 3-oxobutanoate styrene copolymer [poly(co-styrene-OCOCH<sub>3</sub>COCH<sub>3</sub>)] before electrospinning showed to be more crystalline as depicted in Fig. 5.8 **A** than the former copolymer. The morphologies formed from electrospinning and electrospraying ranged from red blood cells-like particles as seen in SEM image labelled **B** to spherical particles labelled **C** and partially beaded fibers as shown in Fig. 5.8 **D**. Particles formed from polymer concentrations ranging from 20 to 40 % w/v, whilst the fibers were fabricated through the following conditions; the feed flow was 1 mL h<sup>-1</sup> at 12 kV with the distance range of 11 cm-18 cm. The spinnable concentration of the copolymer was 55 % and above in a solvent combination of DMF:THF in a ration of 4:1.



Figure 5.7: Different morphologies produced under various experimental conditions. (A) is the hydroxyl methylated styrene copolymer before spinning, (B) are the particles or microspheres produced from using a low concentrated polymer solution, (C) are bead free fibers and (D) are beaded fibers.



Figure 5.8: The morphologies of the 3-oxobutanoate styrene copolymer **A** native conditions **B** & **C** are particles or microspheres at different conditions and concentrations, **D** partially beaded fibers.

The observations from the fabrication of electrospun materials were in agreement with similar work that has reported on the use of electrospinning technique <sup>114</sup>. Unique

morphologies from electropinning were directly affected by parameters such as molecular weight of the polymer, concentration of the polymer solution and solvents thus developing optimum conditions to form electrospun nanofibers was essential <sup>114b, c, 115</sup>. The use of DMF, THF and or their combination in preparation of polystyrene based polymers have been reported to be good solvent systems as they influence the dielectric constant of the polymers which is essential for good nanofiber formation <sup>114c, 116</sup>. Electrospraying does not require much manipulation of the parameters unless specific diameters of particles are desired whilst electrospinning takes into consideration a number of factors with viscosity or polymer solution concentration being the primary determinant factors in fabricating bead free fibers with smaller diameter <sup>114b</sup>. The choices of solvent, flow rate, voltage and distance between needle tip and collector are the secondary parameters to be optimized. In general the diameter of particles or fibers proved to be dependent on the concentration of the polymer solution as depicted for particle in Fig 5.9.



Figure 5.9: Electrosprayed particles generated from 40 % (**A**) and 20 % (**B**) polymer solutions respectively.

For the purposes of application, the SEM images in Fig. 5.10 and 5.11 are for the different morphologies evaluated as sorbent materials. In the case of hydroxyl methylated styrene copolymer, the bead free fiber had a diameter within an average of 800-961 nm. The particle size distribution varied with 25 %  $\leq$  0.5 µm, another 25 %  $\geq$ 3.5 µm and 50 % of material having particles size of 2.5 µm.



Figure 5.10: SEM images of morphologies from the electrospinning and electrospraying of hydroxy methylated styrene copolymer [poly(co-styrene-CH<sub>3</sub>OH)] into (A) Bead free nanofibers (B) Beaded fibers (C) Solid particles.

In the case of 3-oxobutanoate styrene copolymer, the average diameter ranged from 1-2.3  $\mu$ m for the bead free fibers. The solid particles were formed at a concentration of 40 % w/v with size distribution within the ranges of 48 %  $\leq$  4.5  $\mu$ m, another 47 %  $\geq$ 7.2  $\mu$ m and 5 % having particle size of ~ 10.5  $\mu$ m.



Figure 5.11: SEM images (A) bead free fibers, (B) solid particles, and (C) beaded fibers respectively formed from 3-oxobutanoate styrene copolymer [poly(co-styrene-OCOCH<sub>3</sub>COCH<sub>3</sub>)].

## 5.2.2.2 TGA characterization

Thermogravimetric analysis (TGA) for poly(co-styrene-CH<sub>3</sub>OH) and poly(co-styrene-OCOCH<sub>3</sub>COCH<sub>3</sub>) were carried out to check their thermal stability. Their thermograms are shown in Fig. 5.12. Two distinct losses were observed for the poly(co-styrene-OCOCH<sub>3</sub>COCH<sub>3</sub>) thermogram. A weight loss of ~30 % was observed around 320 °C due to the oxobutanoate functionality on the polymer. The second loss just after 350 °C was due to the styrene component of the polymer. The fact that only one loss was observed for the poly(co-styrene-CH<sub>3</sub>OH) thermogram at 400 °C served to confirm the successful incorporation of the hydroxy monomer on the chain of polystyrene. The phase change was attributed to the decomposition of the hydrocarbon component of the copolymers as a result of the polystyrene backbone.


Figure 5.12: TGA of the electrospun fibers.

## 5.2.2.3 EDAX characterization

The fabricated materials were also studied with the aid of energy dispersive X-ray analysis (EDAX) to confirm the relative abundance of elemental composition. From EDAX results one could confirm the presence of the expected elements on the surface of the sorbent material. Since EDAX gives relative quantities of each element on the surface, analysis was carried out to ascertain whether morphological properties affected the surface chemical properties of the sorbent material for particles, beaded or non-beaded fibers. The results were in agreement with the report by Jaworek *et al.* <sup>63</sup> amongst other researchers, who indicated that electrospraying had no significant change in chemical composition and physical properties for the formed fine powder. From Fig 5.13, EDAX spectra confirmed that modification had taken place. The spectra showed C and O as being the only elements found on the surface of the sorbent material. From the elemental map, both O and C atoms are

evenly distributed. The results obtained were similar for both copolymers in the form of nanofibers, beaded and microparticles.



Figure 5.13: An example of Energy dispersive X-ray mapping image for electrospun fibers of poly(co-styrene-CH<sub>3</sub>OH).

#### 5.2.2.4 BET analysis

Morphological properties are believed to possess specific surface characteristics. In order to study the surface properties brought about these unique morphologies within the same copolymer material, BET analysis was carried out. Figure 5.14 shows the typical  $N_2$  adsorption/desorption plot. The example depicted is that of hydroxyl methylated copolymer showing the type IV isotherm for the fibrous materials in Fig 5.14 **A** and **C**, according to IUPAC classification <sup>117</sup>, indicative of mesoporous material. The isotherm for the particles was similar though of macroporous material. BET surface area and pore size data for both copolymers of different morphologies are summarized in Table 5.2. The bead free fiber of hydroxyl methylated styrene copolymer showed the highest surface area of 43.7918 m<sup>2</sup> g<sup>-1</sup>

with pore size value of 275.9 Å, whilst particles had the highest pore size at 1091.5509 Å. The results confirmed the mesoporous nature of fibrous material with particles being macroporous.



Figure 5.14: BET nitrogen adsorption/ desorption plots for morphologies fabricated from hydroxyl methylated styrene copolymer where;  $\mathbf{A}$  is that of the bead free materials,  $\mathbf{B}$  electrosprayed particles and  $\mathbf{C}$  a plot of beaded fibers.

The 3-oxobutanoate styrene copolymer fiber showed a surface area of 8.1139 m<sup>2</sup> g<sup>-1</sup> which was lower than the former copolymer while the surface area of the particles were comparable to the previous copolymer. It is believed that the irregularity on the surface of the beaded adsorbent was a result of unusual characteristic of fibrous material, but previous reports claim

that a network with micrometre size fibers have larger pore sizes than those with submicron fibers <sup>118</sup>.

Copolymer	Morphology	Surface area $(m^2 g^{-1})$	Pore size (Å)
1	Fiber	43.7918	275.9208
	Beaded	4.2587	241.7670
	Particles	6.9427	1091.5509
2	Fiber	8.1139	348.0481
	Beaded	0.5582	311.0496
	Particles	6.4876	1357.7271

Table 5.2: Summary of the surface area of the different morphologies from BET analysis

Interpretation of adsorption studies data presents some challenges that makes it difficult to directly compare the copolymers as the chemistry on the surface of the adsorbent may lead to no apparent trend. Fiber diameter, mechanical properties of the fibers, chemistry and morphologies are known to influence the surface area and pore size of the adsorbent <sup>119</sup>. In this case the BET results confirmed the mesoporous nature of the fibers whilst paticles fabricated proved to be macroporous.

# 5.3 SPE studies

#### 5.3.1 Optimization studies

Electrospun fiber of hydoxyl-methylated copolymer with average diameter of 926 nm was used to optimize the method of analysis for the extraction of alkylphenols (AP's) in aqueous media. The extraction efficiency studies were monitored using HPLC-FLD. Some of the parameters that needed to be optimized included a feasible working concentration, sorbent mass as well as equilibrium time of extraction and the influence of pH. These were done in triplicate standards (n=3). Extraction efficiency (EE) was evaluated as the ratio of the peak area using equation 1 (eqn. 1):

$$\% EE = 100 \frac{Peak area (initial concentration) - Peak area (eluted concentration)}{Peak area (initial concentration)} \quad eqn. (1)$$

## 5.3.1.1 Optimization of concentration and time

The first step was to establish an appropriate concentration range applicable for the studies. Three concentrations were employed, namely: 50  $\mu$ g L<sup>-1</sup>, 100  $\mu$ g L<sup>-1</sup> and 150  $\mu$ g L<sup>-1</sup>. In these experiments the sorbent mass and sample volume were kept constant at 10 mg and 10 mL respectively. The extraction time was maintained at different intervals *viz* 30, 60 and 90 min. Figure 5.15 **A** shows a plot of the percentage of EE under different concentration. Under these conditions, the minimum concentration (50  $\mu$ g L<sup>-1</sup>) showed good EE as two of the analytes were undetectable within 30 min. As the concentration was increased so was the competition in adsorption among the three analytes observed with 4-t-OP being the least analyte to adsorb on the fiber under all the concentrations tested. In order to avoid ambiguity of the results from the least concentrated to the highly concentrated, 100  $\mu$ g L<sup>-1</sup> was used as the working standard to monitor other parameters.



Figure 5.15: Systematic approach of developing an efficient sample preparation method for APs by optimizing time, analyte concentration **A**, sorbent mass **B** and pH **C**.

# 5.3.1.2 Optimization of sorbent mass

On optimizing the sorbent mass; concentration, sample volume and reaction time were kept constant at 100  $\mu$ g L<sup>-1</sup>, 10 mL and 30 min, respectively. The reaction time was found to be significant as it seemed the longer the reaction time the less or insignificant was the difference in EE. The sorbent mass was varied at 10, 30 and 50 mg. Figure 5.15 **B** clearly shows a plausible trend as the increase in sorbent mass allowed more of the analytes to be extracted. The % EE was directly proportional to the increase in sorbent mass. Close examination of the results, shows that the adsorption of 4-t-OP was the lowest compared to

the other two analytes. The observation somehow confirmed the presumed weak interaction of 4-t-OP with the sorbent material.

# 5.3.1.3 Optimization of pH

The nature of the alkylphenol analytes is such that they are slightly acidic and therefore are expected to be found in their molecular form at low pH  $^{120}$ . Fig. 5.15 **C** gives a picture of how the analytes behave under acidic or basic medium. At low pH to neutral conditions, EE of APs on the fiber was relatively unchanged except in the case of 4-OP which showed a sharp drop of adsorption around pH 2. The optimum EE was between pH 8-10 which was a good range to work under as they exist in their neutral form. Beyond pH 10 a decrease in EE was observed. Alkylphenols are expected to be in their deprotonated form  $^{121}$  making them more hydrophilic and in the process rendering the fiber inefficient. pH adjustments to higher values was achieved through the addition of 1 M KOH whilst HCl was used to adjust to lower values.

## **5.3.1.4 Evaluation of eluting solvents**

Another area to address when fabricating a new material with potential use as an SPE sorbent, is finding a suitable solvent or an appropriate method to desorb the analytes by selectively overcoming the sorbent-analyte interaction. The solvents evaluated to elute the analytes from the sorbent included MeOH and acidified MeOH with either acetic acid and/or formic acid. The desorption data is presented in accordance with detector response as depicted in Fig. 5.16.



Figure 5.16: Effects of eluting solvents on analyte recovery.

Upon the use of MeOH as the eluting solvent, very low recoveries were obtained for all the analytes. It was evident that MeOH was not strong enough to break the interactions between the analytes and the sorbents. The interactions assumed to be involved were of the mixed mode, *i.e.*, the hydrogen bonding or hydrophilic interaction as well as the  $\pi$ - $\pi$  interactions or hydrophobic interactions. This meant that a solvent which could be capable to break interactions must exhibit a highly hydrophilic character. MeOH acidified with formic acid was chosen as the solvent mixture, and maintained at pH 3. Polarity of the solvents was the determining factor in the movement of the analytes towards the organic phase. The evident nature of 4-t-OP to be more polar than the straight chained APs was also proven as it showed to be easily desorbed to the organic media.

## **5.3.2** Comparative studies of the copolymers

Extraction efficiency (EE) in batch mode experiments was evaluated for all the sorbent materials to establish retention characteristics of the sorbents with alkylphenols in aqueous media. The errors associated with the experiments were based on the RSD from the averaged calculations (n>6).

The general trend for both copolymers indicated fibrous material to be better sorbents than particles as shown in Fig. 5.17 and Fig. 5.18 for poly (co-styrene-OCOCH<sub>3</sub>COCH<sub>3</sub>) and poly (co-styrene-CH<sub>3</sub>OH), respectively. The observed difference in their adsorption capacity was attributed to their morphological properties rather than their surface chemistries. Although the beaded fibers equally showed to be efficient in adsorbing the analytes, 4-nonylphenol (4-NP)  $\geq$  70 % and 4-octylphenol (4-OP)  $\geq$  60 % for poly (co-styrene-OH), the challenge they present is that the results may not always be consistent as the quantity of beads could vary, making the transition of beaded fibers a major drawback for them to be applicable. The general observation on beaded fibers has been that, it is a transition state to be optimised to form bead free fibers that are desirable to work with. <sup>114c, 115-116</sup>

Considering the bead free fibers, the EE of  $\geq 80$  % was achievable. Electrospun poly (costyrene-OCOCH<sub>3</sub>COCH<sub>3</sub>) fiber adsorbed the analytes better than poly (co-styrene-CH<sub>3</sub>OH). Both sorbents showed to be selective to linear chain alkylphenols. Such a trend could be due to the structural behaviour of the target analytes that impact on the nature of their interaction with the sorbents. The two sorbents exhibited a hydrogen bond donor/acceptor character. It would seem that the alkyl chain length was the main contributor towards selectivity amongst this class of organic compounds which directly translate to its polarity. The steric hindrance could be the limiting factor on the interaction of 4-t-OP with the sorbent materials.



Figure 5.17: Effects of morphology on extraction of 4-t-OP, 4-OP and 4-NP from aqueous media by poly(co-styrene-CH<sub>3</sub>OH).



Figure 5.18: Extraction efficiency and morphological effects of poly(co-styrene-OCOCH<sub>3</sub>COCH<sub>3</sub>) on 4-t-OP, 4-OP and 4-NP.

In as much as functionalization of polymers is desirable, the method used in the study to synthesize and subsequently electrospin the styrene copolymers had its own limitations. The sorbents proved to be fragile. It is believed that the molecular weight of the polymer is an initial contributor to its behaviour in solution as well as after tuning the surface or morphology. From the extent to which other researchers have been keen to arrest these characteristics in the electrospinning step,<sup>114b</sup> it is evident that it has to be taken into consideration. As the results from the experiments showed limitations from particles and beaded fibers, there was therefore no need to further apply them as SPE sorbents in the packed format. The bead free electrospun fibers superiority was further tested by applying them as SPE sorbents as they had been proposed to be ideal in packed devices<sup>55b, 122</sup>.

#### 5.3.3 Breakthrough and recoveries studies

To ascertain the ability of the fabricated material to be good SPE sorbents, recovery efficiency and breakthrough volumes studies were carried out. The sorbents were evaluated as SPE pipette packed columns that resulted in quantitative recoveries. The set-up for the studies was carried out under semi-automated conditions as shown in Fig. 5.19. The sample was loaded in a polypropylene syringe which was mounted in a syringe pump and connected to the pipette packed column *via* a Teflon tube. The fibers were packed on top of a frit to minimise their transfer into the collected sample. The vials were fitted with 200  $\mu$ L ampoules and fractions of 100  $\mu$ L were collected.



Figure 5.19: Experimental set-up for the semi automation of the breakthrough curve, where A is the pipette packed with the fiber, B is the syringe pump that controls flow rate, C is the syringe with the sample and D the Teflon tube.

Breakthrough curves were employed to evaluate the suitability of the sorbent for extracting AP's by breakthrough volume ( $V_B$ ) as it gives the loading capacity of the sorbent. A typical breakthrough curve forms a sigmoidal shape that gives an indication of the analyte mass transfer kinetics as a function of the sorbent retention characteristics <sup>123</sup>. There are two parameters that are obtained; hold-up volume ( $V_M$ ) and retention volume ( $V_R$ ). From these two parameters, chromatographic characteristics of the sorbent bed can be evaluated.

The breakthrough curves were plotted with the vertical axis representing the ratio of the eluted concentration of the analyte ( $C_e$ ) over the initial concentration of the analyte ( $C_i$ ) as a function of fractional volumes collected. After optimization of conditions, *i.e.* the working concentration and the flow rate, 4-t-OP was used as the model compound as other APs were found not to elute at concentrations between 0.5 - 10 µg mL<sup>-1</sup> at varied flow rates (0.05 - 0.1 mL min<sup>-1</sup>). The working conditions were; aqueous solution of 4-t-OP

(0.5  $\mu$ g mL<sup>-1</sup>) as a sample, 10 mg sorbent mass and the rate was 0.1 mL min<sup>-1</sup> as backpressure was observed at higher flow rates. As observed in Fig 5.20, the typical sigmoidal curves were not achieved and equations such as Boltzmann could not fit. Experimentally, V<sub>B</sub> are assumed to be the volume at which quantifiable concentration of analyte is observed and in this case at 200  $\mu$ L the analyte was eluting from both copolymer fibers. Without comparative data from theoretical calculations, the results could not confirm the validity or reproducibility of the proposed method. Thus a best line fit was found using Hill plot which assumes that no equilibrium is reached. The experiments were then treated as a continuous reaction. The results implied that the interaction of the analyte with the sorbents was too weak such that the analyte came into contact with the sorbents and was released through without proper interaction. Possible pore voids on the packed fiber will also allow the flow of analytes thus requiring a proper packing method of the sorbents. Therefore the automated method was not envisaged to result to quantifiable recoveries. The recovery studies from wastewater samples were then performed manually.



Figure 5.20: Breakthrough curves of the sorbents using 4-t-OP as the model compound.

#### 5.3.3.1 Real water analysis

# 5.3.3.1.1 Relative recoveries and analytical parameters

The efficiency of the electrospun sorbents was further tested with real samples, *i.e.* waste water samples spiked with 4-t-OP, 4-OP and 4-NP. Recovery efficiency (RE) is a ratio of the detector response of an analyte from an extracted sample to the detector response of the analyte from an extracted sample containing the same amount of analyte that was added to the extracted sample <sup>124</sup>. Recovery was obtained from the results of the spiked wastewater samples that underwent SPE. Wastewater samples were initially analysed after having been filtered prior to spiking so as to verify the presence or absence of the targeted analytes. Trace concentration of 4-t-OP was detected but below quantifiable levels whilst the other two analytes were not detected. To compare SPE studies, both polymers were applied under same

conditions. The detector was more sensitive to 4-t-OP having the lowest LOD of 0.001  $\mu$ g mL<sup>-1</sup>. Instrumentation detection limits were calculated using ANOVA. The analytical performance of the method summarized in Table 5.3 was obtained from the water sample (MilliQ) to minimize false positive or negative results because wastewater samples have compounds that may potentially coelute with the analytes which can cause signal suppression or elevation. The recoveries obtained are shown in Table 5.4. The results showed that both fibers varied from 70.01 % to 125 % for spiked level at 0.1  $\mu$ g mL<sup>-1</sup> whilst for higher concentration at 50  $\mu$ g mL<sup>-1</sup> recoveries ranged between 85.4 % to 120.1 %. Both fibers showed comparative results. Relative recovery (RR) was calculate using:

$$\% RR = 100 \frac{C_{found}}{C_{initial}} \qquad eq. (2)$$

The challenge was validation of the method as it relied on repeatability and sensitivity of the detector without the use of certified reference materials as they are non-existent for the wastewater matrix.

Table 5.3: Analytical parameters of the developed method.

Parameters	4-t-OP	4-OP	4-NP		
Equation regression line	y=11.691x -1.9956	y= 10.318x -2.4956	y= 11.321x - 9.5444		
Correlation coefficient	0.998	0.999	0.997		
LOD ( $\mu g m L^{-1}$ )	0.001	0.008	0.01		
$LOQ (\mu g mL^{-1})$	0.005	0.01	0.02		

Table 5.4: Relative recoveries of APs from spiked waste water samples.

Sorbent	Sample		% Recovery (% RSD)								
			4-t-OP			4-OP			4-NP		
		Concentration	0.1	10	50	0.1	10	50	0.1	10	50
		(µg ml <sup>-1</sup> )									
Poly(co-styrene-OH)	WW-		108	80.2	109.3	108	115	100.4	119.2	99.9	101.3
	pretreated		(5.02)	(0.7)	(3.3)	(5.5)	(3.5)	(5)	(9.8)	(2.8)	(3.1)
	WW-non		125	106	107.5	95.3	108	115.2	75.1	91.5	120.1
	treated		(8.7)	(0.7)	(4.5)	(6.2)	(4.4)	(4.8)	(7.01)	(5.02)	(4.2)
Poly(co-styrene-	WW-		100.3	95	99.4	70.03	122	85.4	70.7	105	98.2
OCOCOCH <sub>3</sub> )	pretreated		(7.01)	(2)	(3.6)	(9.2)	(5.8)	(5.03)	(8.03)	(7.6)	(3.3)
	WW-non-		113 (10)	98.9	89.3	79.03	147	90.02	72.1	109	100.1
	treated			(2.1)	(4)	(15.5)	(5.3)	(6)	(12.4)	(3.3)	(5.04)

# 5.4 Conclusion

Fabrication of functionalised styrene copolymers into beads, particles and fibers was successfully achieved through the electro-spinning/spraying technique. Electrospraying was demonstrated as a viable process for generating micro to nanometer particles. Electrospinning on the other hand produced submicron fibers for the synthesized styrene copolymers. Application of the fabricated materials as SPE sorbents was realised. EDAX results suggested that chemical conformation at the surface of the sorbents had little or no impact on their extraction efficiency. BET results on the other hand clearly showed the unique surface characteristics the sorbents possessed. Porosity proved to be the main contributor rather than the specific surface area on the morphological properties that affected the efficiencies of the sorbents. Thus electrospun fibers proved to be better sorbent materials as high extraction efficiency coupled with low detection limits were observed. A miniaturised manual operated method gave good recoveries and demonstrated the applicability of electrospun fibers. Automization of SPE method was not achieved from the breakthrough studies and thus requires further investigation or manipulations of the parameters that will enhance the performance of the packed SPE devise.

# Chapter 6: Colorimetric detection of alkylphenols

# 6 Introduction

Colorimetric detection is a field of study that is gaining a lot of interest due to the convenience of recognition signals being transformed into colour change and involves less use of complicated instrumentation <sup>125</sup>. Conjugated polymers *e.g.* polydiacetylene (PDAs), gold (Au) and silver (Ag) nanoparticles (NPs) have unique optical properties that make them suitable materials for colorimetry applications to evolve <sup>126</sup>. There are a number of synthetic procedures to form these colorimetric inducers which are reported in literature <sup>127</sup>. Modifications of these procedures seem to be able to open-up opportunities for the applications of PDAs, AuNPs and AgNPs into a wide range of areas such as catalysis, biological assays and analytical chemistry.

Polydiacetylenes (PDAs) are a family of polymers within conjugated polymers. They are made up of diacetylene (DA) monomers and are prepared *via* a number of ways but mainly by; self-assemble of DA monomers or using UV or  $\gamma$ -irradiation on self-assembled DA. The former method involves the use of chemical modifiers and has a tendency of not yielding efficient PDAs whilst the latter allows formation of pure PDAs as no initiators or catalysts are involved. Due to their water soluble or comparative behaviour, PDAs can also be prepared in aqueous solutions in the form of nanostructured liposomes, vesicles and wires <sup>128</sup>. The water compatibility behaviour PDAs possess is important and advantageous when designing colorimetric probes especially for biological molecules. A procedure showing the typical self-assembly of a monomer that is photoinduced to form PDA is shown in Scheme 6.1. The monomer; 10,12-pentacosadiynoic acid (10,12-PCDA) was dissolved in a small

volume of DMF or DMSO and then injected in water resulting in spontaneous formation of DA supramolecules (DA vesicle). Polymerization of the DA vesicle was achieved by irradiation with a 254 nm hand held UV lamp into the formation of PDA vesicle. PCDA monomer produces blue colour PDA vesicle <sup>126a</sup>.



Scheme 6.1: Self assemble of 10, 12-pentacosadiynoic acid monomer and subsequent photoinduced formation of polydiacetylene (PDA) vesicle as an example of the conjugated polymer  $^{126a}$ .

Owing to their fluorescence turn-on and turn-off behaviour, the blue or red shift that is induced by response to an external stimulus, PDAs are applicable as colorimetric probes. The color change from the interaction with the stimuli is blue to red or vice-versa. The maximum absorption wavelength of the blue colour is around 640 nm whilst the red phase absorbs around 550 nm <sup>129</sup>. A variety of PDA based colorimetric probes for volatile organic compounds (VOCs) mainly organic solvents, ions, antibodies, enzymes, proteins and DNA have been reported <sup>130</sup>. Not only have PDAs been applied in solution based studies but they have also been encapsulated into a solid matrix such as silica and polymeric material <sup>131</sup>. Colorimetric sensors based on PDAs embedded on electrospun fibers have been fabricated for organic solvents and gases <sup>128-129, 132</sup>. Modification of the polar side chain functionality in DA monomer stills allows for more PDAs based colorimetric probes to be fabricated.

Nanoparticles on the other hand have also been applied as colorimetric probes due to their surface plasmon resonance behaviour, which is a result of collective oscillation of electrons induced by incident light. Reports on the use of nanoparticles as colorimetric probes for visualization of phenolic compounds are available <sup>52, 133</sup> and give an idea of how nanoparticles interact with phenolic compounds. In some cases the color change is induced either by aggregation or further reduction of metal ions such that the growth of nanoparticles takes place through hydrogen bonding depending on what the nanoparticles have been functionalised with and or by a combination of interactions including the catalytic behaviour of nanoparticles <sup>134</sup>. Reports have indicated that the colorimetric probes for phenolics were solution or colloidal based. Solution based colorimetric probes may be tedious in terms of their preparation and are prone to contamination, but once the method has been mastered, they are with no doubt significant and will continue to receive attention. The major limitations are their stability and portability. Of current interest is to address the limitations

associated with colloidal studies by encapsulating nanoparticles into solid matrices for colorimetric detections. As it was already discussed in Chapter 2 section 2.2, that nanoparticles embedded on solid matrices *e.g.* silica or polymers have been reported and vary in their application. Ideally polymeric nanocomposites are attractive advanced materials due to improved physicochemical and optical properties that make them suitable for wide applications <sup>135</sup>. Depending on the method of synthesis of the composite, nanoparticles can be incorporated more on the surface than within a fiber. Polymers play an important role in the synthesis and application of metal nanoparticles. The major roles are that they can act as stabilizers to prevent agglomeration, oxidation and precipitation <sup>136</sup>. The systematic approach on developing a probe based on the composite where size, shape, distribution of nanoparticles and functionalities of polymers, is envisaged to result in selective detection of analytes. Thus numerous developments on colorimetric probes are continuously reported in literature.

In addition to PDA- electrospun nanofibers based colorimetric probes; recent colorimetric probes based on electrospun nanofiber/net have been reported <sup>137</sup>. A chip sensor based on polyaniline- polyamide electrospun composite for detections of Cu (II) ions is one example. The sensing mechanism was attributed to the oxidative forms of polyaniline such that when the Cu<sup>2+</sup> ions are introduced, a colour change from white to blue was visually detected at concentration levels as low as 1 ppb <sup>137a</sup>. Another development was for the detection of gaseous formaldehyde based on methyl yellow impregnated nylon 6 nanofiber net sensor strips. Upon exposure of formaldehyde on the strips, a colour changed from yellow to red was detected owing to the reaction between the dye (methyl yellow) and formaldehyde. The detection limits were as low as 50 ppb <sup>137b</sup>. Interest for studies in this thesis was towards fabrication of nanofiber-nanoparticle composites as colorimetric probes.

This part of the thesis reports on the work which was set out to evaluate analyte interactions with solution based PDAs, AgNPs and AuNPs colorimetric probe. Furthermore, the polymer nanoparticle composites were fabricated *via* an in-situ reduction step synthesis and electrospun into nanofibers. The in-situ reduction method was employed for the dispersion of nanoparticles embedded within the fiber. A certain concentration of the analyte allowed for the aggregation or movement of nanoparticles to the surface of the fiber resulting in a colour change. In the initial colloidal studies all AP standards were tested and showed a similar behaviour. It was then decided to choose one of the standards for all the colorimetric work and 4-t-OP was employed as the model compound.

#### **6.1 Experimental section**

# 6.1.1 Instrumentation

Absorbance measurements were performed using a Lambda 25 Perkin-Elmer UV spectrophotometer (Santa Clara, CA, USA) in 1 cm quartz cuvettes. High resolution scanning electron microscopy (HRSEM) analysis was conducted using JOEL JSM-700IF high resolution SEM. Samples were carbon coated prior to analysis and the analysis was done at Nelson Mandela Metropolitan University (NMMU) in Port Elizabeth, South Africa. Transmission electron microscope (TEM) images were acquired using a Zeiss Libra® 120 Plus energy filter transmission electron microscope (Germany) with a megaview iTEM camera. TEM samples were prepared by placing a drop of nanoparticles solution on the sample grid and allowing it to dry overnight before measurements.

#### 6.1.2 Reagents and materials

N-hydroxysuccinimide (NHS), ethylene diamine (99.5 %) oxalyl chloride (96 %), 10,12 PCDA (98 %), sodium sulfate, lithium aluminium hydride (LiAlH<sub>4</sub>), Gold (III) chloride hydrate (99.999 %) (HAuCl<sub>4</sub>•xH<sub>2</sub>O), sodium borohydride (NaBH<sub>4</sub>) (98.0 %), silver nitrate (AgNO<sub>3</sub>) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Acetic acid (99.5 %) hydrochloric acid (32.0 %) and nitric acid (55.0 %) methylene chloride were from B & M Scientific CC (Western Cape, SA). Formic acid (85.0 %) was purchased from Minema (Gauteng, SA) and ammonia (25 %) purchased from Merck (Wadeville, Gauteng, SA). All chemicals were of analytical grade and used as received. All the glassware and magnetic stirrer bar used were washed with freshly prepared aqua regia (1:3 HNO<sub>3</sub>: HCl) and then rinsed thoroughly with water and air dried before use. Ultrapure water obtained from a Millipore RiOS<sup>TM</sup> 16 and Milli-Q Academic<sup>®</sup> A10 system (Milford, MA, USA) was used throughout this study.

# 6.1.3 Functionalization of DA monomers

In a three neck round bottom flask containing a solution of 10,12-pentacosadiynoic acid (763 mg, 2 mmol) in DCM (5 mL) purged with argon, was added dropwise a solution of oxalylchloride (529 mg, 4 mmol) in DCM (3 mL). Furthermore, NHS (147.77 mg, 1.28 mmol) dissolved in DMF (2 mL) was also added into the same reaction vessel. After 1 h of stirring, another portion of NHS was added and the reaction was further stirred for 1 h at room temperature. TLC was used to monitor the reaction. The acylated PCDA intermediate was separated by evaporating the solvents and excess oxalylchloride by a rotary evaporator. The resulted residue was orange oil which was re-dissolved in DCM and divided into two

portions for reaction 1 and 2 as shown in Scheme 6.2 to form the amido and amino functionalised Das respectively.



Scheme 6.2: Synthetic procedure for the modification of PDCA monomer into amino conjugates labelled **A** and **B**.

**Reaction 1**: Ethylenediamine (400 mg) was dissolved in 2 mL of DCM and to it the acylated PCDA was added dropwise and the reaction mixture was stirred at room temperature for 4 h. Then the crude was extracted with DCM and water. The organic phase was dried with sodium sulfate and rotary evaporated to yield the crude product as a whitish powder. Purification was accomplished by column chromatography on a silica gel eluted with a mixture of ethyl acetate and methanol (70:30) to give diacetylene monomer **A** (252 mg, 80%) as a white powder; mp 111–114 °C. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): dd (ppm): 0.88 (t, CH<sub>3</sub>, 3H), 1.40(m, 16-CH<sub>2</sub>-, 32H), (2.18, t,-CH<sub>2</sub>-, 2H), (2.22, t, -CH<sub>2</sub>-, 4H), (2.83, t, -CH<sub>2</sub>-, 2H), (2.45, d, NH<sub>2</sub>) (3.31, q, -CH<sub>2</sub>-, 2H) (5.98, NHCO, 1H). <sup>13</sup>C {<sup>1</sup>H} NMR (400 MHz,CDCl<sub>3</sub>) : d (ppm) : 173.5, 77.6, 77.4, 65.3, 65.2, 41.6, 41.6, 41.3, 36.8, 31.9, 29.6, 29.6, 29.4, 29.3, 29.2, 29.1, 29.1, 28.9, 28.8, 28.7, 28.3, 25.7, 22.6, 19.2, 19.2, 14.1.

**Reaction 2**; Alternatively acylated chlorinated DA monomer was modified by dissolving it in DCM and was added to a cooled aqueous ammonia (25 %) solution which resulted in a white precipitate which was stirred for 2 h. As the reaction progressed, two immiscible layers were formed, where the desired product was extracted by DCM and separated by column chromatography to yield a cream white fluffy powder. The amido intermediated (600 mg, 1.6 mmol) was dissolved in dry ether and stirred on ice under argon. LiAlH<sub>4</sub> (615 mg, 16 mmol) was added dropwise to the solution and the reaction was continuously stirred under argon gas for 1 h. All the preparation procedures of DA monomers are depicted in Scheme 6.2 **B** <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): dd (ppm): 0.88 (t, CH<sub>3</sub>, 3H), 1.42(m, 16-CH<sub>2</sub>-, 32H), (2.18, t, -CH<sub>2</sub>-, 2H), (2.23, t, -CH<sub>2</sub>-, 4H), (2.83, t, -CH<sub>2</sub>-, 2H), (3.31, q, -CH<sub>2</sub>-, 2H) <sup>13</sup>C {<sup>1</sup>H}NMR (400 MHz, CDCl<sub>3</sub>) : d (ppm) : 75.6, 75.4, 63.3, 63.2, 42.1, 41.6, 41.6, 41.3, 36.8, 31.9, 29.6, 29.6, 29.4, 29.3, 29.2, 29.1, 29.1, 28.7, 28.6, 28.5, 28.1, 25.1, 22.6, 17.2, 17.5, 14.0.

## 6.1.4 Formation of PDA vesicle

Quantities of **A** 8.1 mg (1 mL) and **B** 7.2 mg (4 mL) were dissolved in 10 mL DCM and mixed. The solution was dried using a rotary vaporator. To the supernatant 10 ml of water was added and then sonicated for 15 min at 80 °C, after which it was cooled at 4 °C overnight before exposure to UV light (254 nm). Total conc. was 0.02 mmol  $L^{-1}$ . The formed vesicle was purple blue. The vesicle was covered with aluminium foil and kept in the fridge to minimize degradation.

#### 6.1.5 Synthesis of colloidal AuNPs

AuNPs were synthesized with the following procedure. HAuCl<sub>4</sub>•xH<sub>2</sub>O (5 mL, 0.001 M) solution was boiled and then 200 µL of trisodium citrate (0.1M) was added in the boiling solution under vigorous stirring. An immediate colour change of the solution from pale yellow to clear then blackish to a final colour, wine-red, indicating the formation of AuNPs. The colloidal solution was stirred for 30 min at room temperature. The average size of the AuNPs was determined by TEM. The wine red solution of AuNPs was stored at 4 °C before use and could maintain relative stability in aqueous solution against aggregation for at least a week.

## 6.1.6 Synthesis of colloidal AgNPs

Stock solutions of AgNO<sub>3</sub> (0.002 M), citrate (0.1 M) and NaBH<sub>4</sub> (0.05 M) were prepared. Silver nitrate and citrate solutions were stored in the fridge when in excess but for borohydride, fresh solution was prepared regularly due to its reactive behaviour in water. Aliquots of 5 ml of AgNO<sub>3</sub> were boiled in a reaction flask when reacting with citrate (200  $\mu$ l) whilst for borohydride (100  $\mu$ l) the reaction was done at room temperature. The uncapped AgNPs showed to be unstable, though citrate reduced seemed to be better than borohydride reduced AgNPs in terms of stability. To minimize the instability, polyethylene glycol (PEG) was used as the capping agent. Aliquots of 1 ml were added in the 5 ml solution of AgNO<sub>3</sub> and stirred prior the addition of the reducing agent.

#### 6.1.7 In-situ reduction of AuNPs in Polymers

An in-situ reduction technique was followed. About 39 mg of HAuCl<sub>4</sub>•xH<sub>2</sub>O was dissolved in a solution of 1.080 g of nylon 6 dissolved in 5 mL acetic acid and formic acid (1:1) and vigorously stirred at room temperature until a homogeneous yellow solution was formed. On addition of 0.3 mg of NaBH<sub>4</sub> as the reducing agent, the solution turned black for a few seconds then wine red signalling the formation of AuNPs. After stirring for sometime the solution became purplish red. The electrospinning of the purple solution was conducted at 25 kV and a flow rate of 0.5 mL h<sup>-1</sup> was controlled using a syringe pump. The nylon 6 composite fibres were collected on a stationary collector covered with an aluminium foil. The distance from the tip of the spinneret to the collector was 10 cm.

In a polysulfone PSU (20 % w/v) and polystyrene PS (25 % w/v) dissolved in a mixture of THF:DMF (1:4) solution, 20 mg and 25 mg of the gold salt were added respectively in preparation of PS and PSU AuNPs composites. Following the same method of preparing, NaBH<sub>4</sub> was used as a reducing agent with polymer to gold salt ratio being 1:1. The electrospinning conditions were 25 kV, 12 cm at the feed rate of 2 mL h<sup>-1</sup>.

# 6.1.8 In-situ reduction of AgNPs in nylon 6

The procedure followed was similar to the one employed for forming the AuNPs nylon 6 composite. Nylon 6 was dissolved in a combination of formic acid: acetic acid solvents (1:1) at a concentration of (1.08 g or 16 wt %) and was stirred until the solution was homogeneous. A measured amount of  $AgNO_3$  (0.1 g or 0.25 wt% in solution) was added. The solution was stirred under room temperature and monitored for 24 h to ensure the complete reduction of

AgNO<sub>3</sub>. Alternatively NaBH<sub>4</sub> was added as a reducing agent. The electrospinning conditions were the same as described for AuNPs-nylon 6 composite.

#### 6.2 Results and discussions

## 6.2.1 Solution based colorimetric probes

# 6.2.1.1 Polydiacetylene colorimetric probes

Through modification of the polar head group of 10, 12-Pentacosadiynoic acid (PCDA), two monomers were synthesized namely; amido ethylene amine 10, 12-PCDA conjugate and PCDA ethylene amine conjugate. The synthetic procedures followed methods reported in literature with minor modifications <sup>138</sup>. Both monomers **A** and **B** were formed through a two step synthesis. PCDA was firstly activated by NHS to form PCDA-NHS derivative which reacted with chlorine group from oxalyl chloride to form CI-PCDA derivative. The CI-PCDA derivative underwent modification with ethylenediamine or alternatively with ammonia and subsequent reduction of the carbonyl group, forming the two DA monomers **A** and **B** respectively. Figure 6.2 shows the FTIR spectrum for the overlayed monomers where P02 is the intermediate product with NH<sub>2</sub> band at 3394.95 and 3182.07 cm<sup>-1</sup> which are due to primary and secondary amine, and carbonyl (C=O) stretch at 1641.95 cm<sup>-1</sup>. Upon reduction of the intermediate product, there was a clear disappearance of the C=O band as shown in P021 spectrum (B-DA monomer). DA monomer **A** labelled P0121 showed the typical CONH stretch at 1641.395 cm<sup>-1</sup> and NH<sub>2</sub> stretch at 3288.51 cm<sup>-1</sup>, confirming the successful formation of the monomers.



Figure 6.1: FTIR spectra of the DA monomers after modification from 10,12-PCDA. Where P02 is the amido PCDA conjugate intermediate; P021 is the spectra of the primary amine monomer **B**; P0121 is the amido-amine monomer **A** and P012 is the NHS-PCDA intermediate.

The DA monomers were transformed into PDA vesicle solutions by sonication followed by UV irradiation. The amido ethylene amine-PDA was blue after UV irradiation and absorbed at 645 nm whilst the ethylene amine-PDA was pink with maximum absorption around 543 nm with a shoulder peak at 583 nm, shown in Fig 6. The PDAs did not show any colour change upon introduction of the analytes. Amongst possible reasons for the behaviour, poor polymerization could be one of them and the practicality of red phase PDA not recognizable for sensor applications <sup>126a, 138</sup>. A further step was to combine the monomers to form one

PDA vesicle. DA monomers **A** and **B** were mixed in 4: 1 mol ratio prior to hydration and polymerization steps. The colour transitions of PDAs were reported in literature and the results obtained were consistent with them  $^{139}$ .





Initially the colorimetric behaviour of the prepared PDA polymer was evaluated in aqueous media containing the analyte. At concentrations as high as 30 ppm of APs in water, there was no colour change observed. The standard matrix was then changed to methanol since solubility became a challenge in water as the concentration of APs increased. Unfortunately methanol interfered with the signal as it changed colour from a purplish vesicle to pink. Microscopically the change in colour from methanol as a blank was not found to have an effect on shifting or formation of new absorption peaks as it was observed in the case of the

analyte. When 4-t-OP was introduced, a new peak at 279 nm was observed with slight decrease in intensity of the polymer absorption peaks and an intensifying peak at 510 nm. Figure 6.3 shows the UV-vis results where blank methanol in PDA was overlayed with original aqueous PDA without analyte and 4-t-OP in methanol within the PDA vesicle.

It is well known that multiple mechanisms are responsible for chromic change although the mechanisms are not fully understood. There is a general view that the colour change is induced as a result of disturbance of the eyne-ene conjugation backbone <sup>129, 139-140</sup>. In this case it is believed that the PDA backbone perturbation was possible through the analyte ability to donate a proton on the eyne-ene bond whilst itself being oxidised to what was supposed to be a new compound that absorbed UV light around 279 nm. This work was not further investigated because it meant finding a better method to address selectivity due to interfering of the solvent. Therefore efforts were focused on nanoparticle work.



Figure 6.3: UV-vis spectrum of the PDAs overlayed with the analyte containing PDA showing a colour change from purple to pink.

# 6.2.1.2 Colloidal AuNPs as probes for alkylphenols

Reduction of gold (III) derivatives follows the most popular route of using citrate as a reducing agent in boiling water. The method which was introduced by Turkevitch *et al.* in 1951 <sup>141</sup> led to the formation of AuNPs of 20 nm. Furthermore Frens *et al.* <sup>142</sup> followed the same procedure but varied the ratio between the reducing/stabilizing agents (trisodium citrate) and gold salt and this resulted in AuNPs of different sizes (between 16 and 147 nm). The role of citrate has been recognized and was also found to be easily displaced by other organic compounds which have a higher affinity for gold ion. The phenomena has led to a number of methods of synthesizing AuNPs *via* the citrate route and on these bases, synthesis of citrate capped nanoparticles was achieved as depicted in Scheme 6.3 **A**, resulting in typical wine red citrate-AuNPs shown in Scheme 6.3 **B**. Characterization by UV-vis spectroscopy showed the typical surface plasmon resonance absorption peak of well dispersed AuNPs at 525 nm as shown in Fig. 6.4 **A**. TEM further confirmed the dispersed AuNPs with average diameter of 13. 26 nm as shown in Fig.6.4 **B** image.



Scheme 6.3: Synthetic pathway for wine red citrate capped AuNPs.



Figure 6.4: UV-vis spectrum **A** and TEM image **B** of the well dispersed colloidal citrate-AuNPs.

Evaluation of the AuNPs as probes for APs was conducted in methanol media, 4-t-OP was used as the model as it was observed that all the analytes were behaving in a similar manner. Different concentrations of 4-t-OP were prepared in methanol. This was after the realization that at trace concentrations there was no visible colour change though UV-vis studies showed broadening of the peak at 525 nm when the analyte was introduced. It was observed that the colour change was dependent on the concentration of the analyte. C-AuNPs at a constant volume of 200 µl were added in samples of equi-volume of the 4-t-OP at different concentration. The response was found not to be immediate for a 20 ppm analyte solution shown in Fig. 6.5 **b** unlike in higher concentrated samples ( $\geq$ 30 ppm) which showed an immediate colour change upon introduction of nanoparticles. As the concentration of the analyte was increased the wine red colour of the original AuNPs changed towards purple (Fig 6.5 c) and between 50 and 60 ppm the complete colour change to greenish was observable as depicted in Fig.6.5 d. This phenomenon was monitored with the aid of UV-vis and TEM. Figure 6.5 A-D showed clearly the decrease of intensity and red shifting of the absorption maxima of the C-AuNPs from 529 nm to 662 nm and further to 729 nm for a complete colour change. The TEM results in Fig. 6.6 A-D further confirmed that the dispersed nanoparticles of the original sample prior to the addition of the analytes, aggregates gradually until complete aggregation was reached such that small residues within the sample were changing morphology into cylindrically shaped nanoparticles.



Figure 6.5: UV-vis spectra showing the spectral shift of AuNPs at different concentrations of 4-t-OP signalling the colour change. As the colour changed from wine red to greenish (**a-d**), the SPR absorption peak decreases around 525 nm until it shifts to higher wavelength at 729 nm.



Figure 6.6: TEM images showed sequential aggregation of AuNPs as the analyte concentration was increased. The blank **A**, before addition of the analyte showing well dispersed AuNPs. At 20 ppm the AuNPs starts to aggregate shown in image **B**. Image **C** and **D** show complete aggregation and change in morphology of AuNPs respectively.

The observed behaviour was typical of AuNPs, where colour change was induced by interaction between NPs , capping agent and the analyte of interest such that the AuNPs aggregated <sup>52a, 143</sup>. Studies on citrate displayed by compounds having affinity for NPs have been reported <sup>144</sup> and it was found that the polarity of a target analyte was crucial in disturbing the environment of the nanoparticles to induce colour change. The results confirmed that the interaction between AuNPs and the target analyte was possible through the phenolic moiety of the analyte. Depending on the concentration of 4-t-OP, the colour change
could be immediate. Therefore the response of the probe to higher concentration of the analyte was indicative of the sensitivity of AuNPs.

#### 6.2.1.3 Colloidal based AgNPs colorimetric probe

Colloidal AgNPs were also synthesized for evaluation as a colorimetric probe. Both citrate and borohydride were used as reducing agents. The reaction conditions are summarized in Table 6.1. From the different conditions employed, AgNPs were formed having different colours as depicted in Fig 6.7 (**a**-**d**). Borohydride reduced AgNPs without the capping agent which were orange in colour (**c**), showed to be very unstable as decolourization was observed. Microscopic analysis confirmed the observations as TEM images in Fig.6.7 **C** showed aggregated spherical AgNPs. Upon using PEG as a stabilizer, brown AgNPs (**b**) were formed. Its TEM image in Fig. 6.7 **B** revealed spherical AgNPs having what looked like grains within the dispersed nanoparticles. The UV-vis spectrum of the orange AgNPs in Fig. 6.8 **c** showed broadening of the SPR absorption peak at 410 nm having a shoulder peak at 476 nm signifying the instability caused by aggregation. However the capped AgNPs showed a UV-vis spectrum with an unusual SPR absorption peak at 302 nm which could be a result of incomplete reduction as depicted in Fig. 6.8 **b**.

Citrate capped AgNPs showed the typical SPR absorption peak around 425 - 440 nm for both stabilised and unstabilised AgNPs revealed by the UV-vis spectra in Fig 6.8 **a** & **d** which was in agreement to what has been reported previously <sup>145</sup>. Characterization by TEM confirmed the formation of well dispersed spherical AgNPs with average diameters ranging from 3 to 6 nm as shown in Fig. 6.7 **A** and **D**.

The different colours observed for the AgNPs solutions have been previously reported to be influenced by the type of reducing and capping agent at different ratios <sup>146</sup>. The capped citrated-AgNPs were more stable although in terms of reactivity, both exhibited a similar behaviour. Upon testing the brown AgNPs on 4-t-OP, no colour change was observed. Therefore the citrate capped AgNPs were evaluated as the colorimetric probe.

AgNPs	Reducing	Stabilizer	Au: reducing agent :	Conditions	Colour
	agent		capping agent		
			(ml:µl:ml)		
1	Citrate	-	5:200	100 °C	Yellow
2	Borohydride	PEG	5:100:1	r.t	Brown
3	Borohydride	-	5:100	r.t	Orange
4	Citrate	PEG	5:100:1	100 °C	Green

Table 6.1: Various reaction conditions used to form colloidal AgNPs

r.t: room temperature



Figure 6.7: TEM characterization of the synthesized colloidal AgNPs formed from using borohydride for images **B** and **C** and citrate for images **A** and **D** as reducing agents. The AgNPs were generally spherical shaped though borohydride capped (**C**) were susceptible to aggregation.



Figure 6.8: UV-vis spectra of the synthesized colloidal AgNPs formed from using borohydride for **b** and **c** and citrate for **a** and **d** as reducing agents with an insert of unstabilised borohydride and citrate capped AgNPs.

On evaluating the colorimetric potential of AgNPs, it was found that a colour change was possible. Unlike AuNPs solution, colloidal AgNPs behaved differently in that the colour change was not induced by aggregation. Results from the UV-vis analysis (Fig. 6.9 A) showed an absorption peak around 276 nm region signalling the conversion of the analyte of interest into a new compound. The phenomenon is believed to occur as a result of the analyte interacting with unreacted Ag ions such that it is oxidised into a quinone like compound. A similar observation was reported where gallic acid reduced excess Ag ions to form AgNPs and inturn got oxidized. The conclusion was that AgNPs exhibit catalytic behaviours <sup>52b</sup>. Catalytic behaviours of nanoparticles mostly depend on their diameter, though shape and the physicochemical environment may also influence <sup>41b, 48a</sup>. Furthermore, TEM image in Fig. 6.10 **B** showed growth of AgNPs upon addition of the analyte. Growth of the AgNPs is

generally induced by the further reduction of unreacted Ag ions that surround the NPs such that their size increases until a different shape is formed. This behaviour depends on the reducing ability an analyte possess. Therefore, due to the interactions between the analyte and the AgNPs, colour change from greenish to brownish was observed as depicted in Fig. 6.9 B. The SPR peak of the nanoparticle around 450 nm did not change as the concentration of the analyte was increased. This was expected because the catalyst takes part in a reaction but is not in itself changed.



Figure 6.9: UV-vis spectra (**A**) showed the formation of an absorbing compound at 276 nm. Image (**B**) are samples in increasing concentration of the analytes which resulted in a colour change from green to brown upon addition of AgNPs. The stock concentration was 1000  $\mu$ g mL<sup>-1</sup> and the control is neat solution (without analyte).



Figure 6.10: TEM image **B** revealed growth of AgNPs upon addition of 4-t-OP with an insert at **A** showing clearly unreacted AgNPs at the lower magnification.

## 6.2.2 Fabrication of solid state probes and their application

#### 6.2.2.1 Gold polymer composite probes

On fabricating the solid state colorimetric probes based on AuNPs, the initial attempt was to evaluate a suitable polymer for the study. Polystyrene (PS), polysulfone (PSU) and polyamide (nylon 6) were all evaluated. Fabrication of AuNPs on electrospun polystyrene has been reported but the method employed was different from what is being reported in this thesis <sup>147</sup>. In this work in-situ reduction was employed for all the polymers which were evaluated. The composites of AuNPs with different electrospun fibers were successfully fabricated. Characterization was done by UV-vis spectroscopy and HRSEM. Figure 6.11 C shows the UV-vis spectrum of the PSU-AuNPs solution prior electrospinning. The SPR absorption peak at 568 nm confirmed in-situ reduction of Au ions. The electrospun PS and

PSU AuNPs composites did not show any response to trace concentrations of the analytes in aqueous media. It was observed that the hydrophobic nature of the polymers did not allow enough interactions. The matrix was then changed to methanol and the concentration of the analyte was also increased. The PS-AuNPs fiber composite did not show any colour change whilst from PSU-AuNPs fiber mat, paling of fiber mat from ruby red was observed though it was not too distinct. The fibers also showed fragility as they shrunk in methanol with no distinct colour change being observed. The HRSEM images of PSU-AuNPs composite before and after analyte addition are shown in Fig. 6.11 **A** and **B** respectively. Before the analyte was introduced the composite fibers had a rough surface without the AuNPs being visible but after analyte exposure, the embedded nanoparticles appeared at the lining of the fiber surface as indicated by the arrows in Fig 6.11 **B**, giving some sort of idea that perturbation of AuNPs might have occurred but did not lead to a colour change on the fiber mat.



Figure 6.11: **A** and **B** are HRSEM images of electrospun PSU AuNPs composite before and after analyte and **C** is the UV-vis spectrum of the polymer solution showing the typical SPR absorption maximum at 568 nm of AuNPs.

The nylon 6 composite was the better material for evaluating the solid state colorimetric probe as it showed a distinct colour change from a purplish fiber mat to a blue fiber mat upon exposure to the stock solution of the analytes. Figure 6.12 **a** & **b** shows the picture of the fiber mats before and after analyte exposure. HRSEM was used to characterize the fiber before and after analyte was introduced and their images shown in Fig. 6.12 **A**-**C**. It was observed that the AuNPs before addition of the analyte do not appear at the surface of the

fiber mat and thus confirming their embedment into the fibers as shown in Fig. 6.12 **A**. After the analyte has been introduced, a coming together of fibers and visibility of AuNPs on the surface was observed as depicted in Fig 6.12 **B**. Another change in morphology at the surface of the fiber was also visible to what look like a mop within the mat (Fig. 6.12 **C**). It was postulated that the unique morphology is suspected to be a result of agglomeration of AuNPs or a change from spherical nanoparticles to what looked like rods within the fiber. At the same time the colour change might be due to the combination of the two possibilities. Further studies are needed to confirm the course of the resulted morphology. The results thus far from both AuNPs solution based and solid state colorimetric probes clearly show that the movement or assembly of the nanoparticles induced by the introduction of the analyte accounts for the colour change observed.



Figure 6.12: Fiber mats showing a colour change from purplish red fiber to a blue fiber mat ( $\mathbf{a} \ \& \mathbf{b}$ ). Under microscope, the HRSEM images  $\mathbf{A}$  of electrospun nylon 6-AuNPs composite before while  $\mathbf{B}$  and  $\mathbf{C}$  are after analyte introduction, showed a change in the morphology of the fiber mat.

The major limitation observed from these studies was the high concentrations of the analytes that the probes respond to. The poor response of the probes to trace concentration can be explained because the interaction of nanoparticles depends on the polarity of the analyte of interest. In this study, the APs model compounds are semi polar and thus perturb the environment of the nanoparticles significantly at high concentrations. Figure 6.13 shows the effect of analyte concentration on the fiber mat. It became clear that organic compounds that have polar groups that possess much affinity towards AuNPs are highly likely to be visually detected through the use of the fabricated composite. In order to arrest the limitation associated with sensitivity of the probe, modifiers or ligands are to be used to enhance the response of the fiber mats.



Figure 6.13: Exposure of AuNPs-nylon 6 composite fiber mat to the sample, as concentration of the analyte increases, showed distinct colour change at  $1000 \ \mu g \ mL^{-1}$ .

#### 6.2.2.1.1 Silver Nylon 6 composite for colorimetric probes

Nylon 6-AgNPs solid probes were also fabricated and evaluated. Two methods of fabrications were followed. One method depended on the solvent combination of formic: acetic acid (1:1) used to prepare nylon 6 solution to reduce Ag ions. This type of method has been reported for forming AgNPs in nylon 6 but had used only formic acid <sup>148</sup>. An alternatively method made use of borohydride to reduce the Ag ion in-situ the polymer solutions. The solvent depended reduction with no use of borohydride resulted in orange and yellow fiber mats after spinning. Borohydride reduced AgNPs in nylon 6 solution after electrospinning were white fiber mat. The fiber mats are shown in Fig. 6.15. HRSEM images depicted in Fig. 6.14 show the nanofibers prior exposure to the analyte. There was no

significant difference on the surfaces of the nanofibers fabricated from both methods. AgNPs were rarely visible.



Figure 6.14: HRSEM images of electrospun nylon 6-AgNPs composite: **A** white fiber with borohydride added and **B** is without borohydride.



Figure 6.15: Images of nylon 6-AgNPs reduced with solvent combination of formic acid and acetic acid in the case A and B stirred for 3 h and 24 h respectively. C are borohydride reduced AgNPs. The fiber mats were introduced in increasing concentration of the analyte samples.

Upon introduction of different concentrations of the analyte, the white fiber mat became brownish (Fig 6.15 C) whilst from the other fiber mats the colour seemed to intensify to brown (Fig 6.15 A and B). HRSEM images of the fibers before and after the analyte was introduced are depicted in Fig 6.16. Before the analyte was introduced, the surface of the fiber showed rare occurrence of AgNPs. It was only after the analyte was introduced that the AgNPs appeared at the surface of fiber. The behaviour was similar to the nylon 6-AuNPs though nylon 6-AgNPs did not show any sign of agglomerated nanoparticles at the surface. The nylon 6-AgNPs showed the typical nanofiber net morphology which is believed to take part in influencing the reactivity of the material towards external stimuli <sup>57b</sup>. At this stage the results obtained for the AgNPs fiber based probe were not as convincing on the account of the colour change being distinct though from microscopic results a change of morphology was obvious.



Figure 6.16: HRSEM images: **A** is the fiber without the analyte; **B-D** are the fibers after analyte introduction showing AgNPs at the surface, the nano nets within the fibers and residual AgNPs respectively.

### 6.3 Conclusion

Through modification of polar chain functionalities on DA monomers, an aqueous PDA vesicle was developed from combining DA supramolecules which gave a colour change upon introduction of 4-t-OP. The work presented some challenges with regards to selectivity as interferences from organic solvent was observed. No further work was done to improve selectivity. It was interesting to see that both from PDA work and AgNP work the new absorption was due to an analogue structure of 4-t-OP, confirming the active site of the analyte to be through the polar side of the compound. Colorimetric probes based on colloidal

AuNPs and AgNPs for APs were prepared successfully. Colour change on AuNPs probe was induced by aggregation whilst for AgNPs growth of nanoparticles was observed. Depending on the concentration of the analyte, change of morphology from spherical to cylindrical shape was also observed. From solid state colorimetric probes, synthesis of nanoparticles in one pot was achieved and fabricated into electrospun nanofibers composite. Clear colour change from the AuNPs nylon 6 composite was observed whilst in the case of AgNPs intensifying shade from that of the original colour was observed. From HRSEM results the introduction of 4-t-OP on the fiber mat induced the assembly of nanoparticles to cover the surface of the fiber mats. Depending on the degree of the analyte to affect the movement of the nanoparticles, the colour change was observed. Based on the distinct colour changes observed, AuNPs based colorimetric probes outperformed the rest.

# Chapter 7: Summary and perspective work

Styrene copolymers were successfully synthesized and fabricated into sorbent materials for SPE of APs from aqueous media. Different morphologies were achieved through the use of electrospinning technique namely; beaded fibers, bead free fibers and particles. Upon evaluating their extraction efficiency, electrospun bead free fibers performed better as sorbent materials compared to particles and therefore were packed in pipette tips. The pipette packed fibers resulted in good recoveries of APs from spiked wastewater samples. The extraction efficiency was proven to be influenced by morphological properties as fibrous materials were mesoporous whilst particles were macroporous as shown by BET analysis results. Another limitation in their performance was attributed to their low molecular weight character which is believed to affect the mechanical properties of the copolymers. Solution polymerization synthesis employed did not result in mechanically stable copolymers. Results from the breakthrough experiments showed limitations associated with an automated set-up during construction of the device, packing of the sorbent was not well maintained such that voids within the fibers were not completely eliminated to allow enough interaction of the analyte with the sorbent. Therefore automatic manipulation to improve precision is an avenue to be better developed.

Further studies in this thesis focussed on developing colorimetric probes based on PDAs, AgNPs and AuNPs which demonstrated good naked eye detections. In both the liquid and solid phase studies, assembly of nanoparticles was a result of the interactions between the analyte and the probes translating into observable colour change. Though colorimetric detections were achieved, sensitivity was a challenge as the probes were responsive only to high concentrations ( $\geq 1000 \ \mu g \ mL^{-1}$ ) especially for electrospun based colorimetric probes. In-situ reduction and fabrication of the colorimetric probe composites had the advantage of having nanoparticles being embedded within the fiber, minimizing the probability of nanoparticles leaching out, but it presented a disadvantage as interactions between the nanoparticles and the analyte of interest were minimal leading to low sensitivity of the probe. These results showed the potential of fabricating electrospun hybrid materials for point of use naked eye detections that require no sample preparation step and thus minimizing the analysis time. Electrospun nanofibers and their hybrids have shown some potential to possibly be alternative materials with wide applicability for developing better analytical methods.

Analytical chemistry research would still rely much on developing new and improved materials and their evaluation thereafter. Further application of already existing materials is another way of making sure every fabricated material finds appropriate application in addressing relevant challenges. Improving sensitivity, selectivity and physico-chemical properties of the fabricated nanomaterials is still the crucial analytical parameter. Therefore a wide avenue for synthesizing, fabricating and characterization of nanomaterials is open for further developments.

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