Study of the Preparation of Mesoporous Magnetic Microspheres and Their Applications

Mårten Ericson

Master of Science Thesis
Stockholm 2009
Mårten Ericson

Study of the Preparation of Mesoporous Magnetic Microspheres and Their Applications

Examiner: Per Olof Persson, Industrial Ecology

Master of Science Thesis

Stockholm 2009

Presented at

Industrial Ecology
Royal Institute of Technology

www.ima.kth.se
Abstract

Treatment of wastewater using magnetic technology is a rising field. In this thesis, the latest research on the subject is reviewed and several adsorbents with different coatings, which impart them unique properties, are discussed. Separation of particles from aqueous solution using magnetic technology is more convenient compared to conventional techniques, such as filtration and centrifugation. The adsorbents described in this thesis are effective for adsorption of several types of contaminants, such as heavy metals and different types of dyes.

Magnetic microspheres were synthesised using porous polystyrene microspheres as template. The microspheres were first sulfonated using chlorosulfonic acid followed by stirring in the presence of ferrous chloride which then was oxidised and magnetic nanoparticles were formed on the surface.

The sulfonated microspheres had a surface area of 420 m²/g and the magnetic 175 m²/g, indicative of Fe₃O₄ nanoparticles were successfully formed in the pores. The weight fraction of the Fe₃O₄ nanoparticles in the magnetic microspheres was 33 %.

Adsorption and desorption studies of the cationic dye, methylene blue, using mesoporous magnetic microspheres were performed. The results show that the mesoporous magnetic microspheres have good ability to adsorb methylene blue at low concentrations. In a cycle study the adsorption efficiency were nearly 100 % throughout the study. Using a 6/4 EtOH/H₂O with saturated KCl solution the desorption efficiency in the cycle study were about 95 %.

The microspheres were used as carriers for TiO₂ in order to overcome the problem with the separation of TiO₂ from solution. The TGA results show that the microspheres contained about 12 % of TiO₂. The TiO₂ coated microspheres were used for the photocatalytic degradation of phenol. However, the TiO₂ microspheres did not work. This was a result from that the phenol had too little contact with the TiO₂. A possible way of solving this problem could be to decrease the size of the microspheres, thus increase the surface area.

Lysozyme was adsorbed and separated using the porous microspheres. The lysozyme adsorption worked best at pH 9.6, which is the pI for lysozyme. The lysozyme could be extracted from the microspheres by using a pH 13 buffer. Also, by using MeOH/H₂O and EtOH/H₂O solutions with saturated KCl the lysozyme could be desorbed. An adsorption and desorption mechanism was also presented.

Keywords: Adsorption, bioseparation, magnetic adsorbents, magnetic microspheres, magnetic separation, photocatalyst, wastewater
Sammanfattning

Vattenrening med magnetisk teknologi är en ny och alltmer uppmärksammad teknik. Magnetisk separation är ett enkelt och snabbt sätt att separera något från en lösning. Magnetisk separation är mer lätthanterligt jämfört med traditionell separationsteknik såsom centrifugering och filtrering.

Med porösa polystyren mikrosfärer som mall, syntetiserades magnetiska mikrosfärer. Först så sulfoneras mikrosfärerna med klorosulfonisk syra, följt av att de rördes om i en järnkloridlösning. Magnetiska nanopartiklar bildades i porerna och på ytan av mikrosfärerna.

Sulfoneras mikrosfärerna hade en specifik ytarea på 420 m²/g och de magnetiska 175 m²/g, detta indikerar att Fe₃O₄-nanopartiklar bildades på ytan och i porerna. Massfraktionen av Fe₃O₄ var 33 %.

Adsorption- och desorptionsstudier på de magnetiska mikrosfärerna utfördes. Färgämnet metylblått användes i studien. Resultaten visade att magnetiska mikrosfärerna hade en bra adsorptionsförmåga vid låga koncentrationer av metylblått. Cykelstudier visade att adsorptionsverkningsgraden var nära 100 % under flera adsorptionscykler. Desorptionsförsök med olika lösningsmedel visade att en mättad KCl 6/4 EtOH/H₂O lösning gav en desorptionsverkningsgrad på ca 95 %.


Nyckelord: Adsorption, magnetiska adsorbenter, bioseparation, magnetiska mikrosfärer, fotokatalys
Table of Contents

Chapter 1: Introduction .............................................................................................................. 1
  1.1 Aims and Objectives ........................................................................................................ 2
  1.2 Methods ............................................................................................................................ 2
  1.3 Limitations ....................................................................................................................... 2

Chapter 2: Background ............................................................................................................... 3
  2.1 Updated Research Progress in Magnetic Adsorbents ...................................................... 3
    2.1.1 Mesoporous Magnetic Adsorbents ............................................................................ 3
    2.1.2 Coated Magnetic Nano-Adsorbents .......................................................................... 6
    2.1.3 Magnetic Carbon Adsorbents .................................................................................. 10
    2.1.4 Other Magnetic Adsorbents .................................................................................... 11
    2.1.5 Conclusions ............................................................................................................. 12
  2.2 Updated Research Progress in Magnetic Photoocatalyst ............................................... 14
    2.2.1 Different Preparation Techniques and Magnetic Carriers ....................................... 14
    2.2.2 Degradation of Organic Pollutants by Magnetic Photocatalysts ............................. 16
    2.2.3 Conclusions ............................................................................................................. 18
  2.3 Updated Research Progress in Bioseparation Using Magnetic Technology .................. 19
    2.3.1 Bioseparation ........................................................................................................... 19
    2.3.2 Conclusions ............................................................................................................. 20

Chapter 3: Magnetic Microspheres for Removal of Methylene Blue From Wastewater ......... 21
  3.1 Experimental .................................................................................................................. 21
    3.1.1 Materials .................................................................................................................. 21
    3.1.2 Preparation .............................................................................................................. 21
    3.1.3 Characterisation ....................................................................................................... 22
    3.1.4 Adsorption and Desorption Optimisation ............................................................... 22
  3.2 Results and Discussion ................................................................................................... 23
    3.2.1 Preparation of Magnetic Microspheres ................................................................... 23
    3.2.2 Characterisation ....................................................................................................... 24
    3.2.3 Adsorption and Desorption Study of Methylene Blue ............................................ 25
    3.2.4 Adsorption and Desorption Mechanism .................................................................. 27
  3.3 Conclusions .................................................................................................................... 30

Chapter 4: Mesoporous Microspheres with Photocatalytic Ability ......................................... 33
  4.1 Experimental .................................................................................................................. 33
    4.1.1 Materials .................................................................................................................. 33
    4.1.2 Preparation of Photocatalytic Microspheres ........................................................... 33
    4.1.3 Characterisation ....................................................................................................... 33
    4.1.4 Photocatalytic Degradation of Phenol ..................................................................... 33
  4.2 Results & Discussion ..................................................................................................... 34
    4.2.1 Preparation of Photocatalytic Microspheres ........................................................... 34
    4.2.2 Characterisation ....................................................................................................... 34
    4.2.3 Photocatalytic Degradation of Phenol ..................................................................... 35
  4.3 Conclusions .................................................................................................................... 37
Chapter 5: Mesoporous Microspheres for Bioseparation .......................................................... 39
  5.1 Experimental .................................................................................................................. 39
    5.1.1 Material .................................................................................................................. 39
    5.1.2 Separation of Lysozyme ......................................................................................... 39
  5.2 Results & Discussion ...................................................................................................... 40
    5.2.1 Separation of Lysozyme ......................................................................................... 40
    5.2.2 Adsorption and Desorption Mechanism ............................................................... 42
  5.3 Conclusions ................................................................................................................... 43

Chapter 6: Conclusions ............................................................................................................ 45

References .............................................................................................................................. 47
  Chapter 1 ............................................................................................................................ 47
  Chapter 2 ............................................................................................................................ 48
  Chapter 3 ............................................................................................................................ 55
  Chapter 4 ............................................................................................................................ 56
  Chapter 5 ............................................................................................................................ 56
  Chapter 6 ............................................................................................................................ 56

Publications ............................................................................................................................ 57

Acknowledgment .................................................................................................................... 57

End Note .................................................................................................................................. 57
CHAPTER 1: INTRODUCTION

It is important to develop new techniques for the treatment of wastewater but also to improve the existing ones. In most of all industry there are non-environmentally friendly chemicals in the effluents. There is a need to remove these substances but also to recycle and reuse them, which is beneficial from an economical point of view.

Today there are several techniques available for removal of pollutants from effluents. A few worth to mention are chemical oxidation, biological treatment, nanofiltration, and adsorption [1.1-1.4]. There has been a lot of research in the field of adsorption. Prominent among recently studied adsorbents are zeolites, hydrogels, nanotubes, waste materials, and active carbon [1.5-1.11]. Active carbon is the most commonly used adsorbent because it adsorbs a variety of substances and possesses a great adsorption capacity [1.12]. However, active carbon is generally difficult to separate from aqueous solutions. One approach to solve this problem has been to incorporate magnetic particles [1.13], a technique which has drawn much attention lately.

One of the main applications for photocatalysts is the degradation of pollutants from wastewater. This technique is becoming increasingly important, especially for removing low trace contaminants but also for bacteria and viruses [1.14, 1.15]. Some of the advantages with photocatalytic decomposition are, low costs, complete mineralisation thus, no waste disposal problem [1.16]. The photocatalysts have the same problems as many adsorbents, due to the size they are difficult to separate from aqueous solutions. Also within this field magnetic particles have been incorporated with the photocatalysts, thus making the separation easy and convenient [1.17].

Bioseparation is a growing field and with the increasing research proteomics, new and better separation techniques is required in order to meet the demands of high selectivity, efficiency etc. [1.18]. One technique that has recently got much attention is to use magnetically separable particles with tailored coatings, which can adsorb different types of proteins [1.19]. This technique fulfils the high demands that are required for the separation of proteins.

The applications for the porous polymer microspheres are numerous, for example, ion exchange, liquid chromatography, adsorption, purification, and catalyst [1.20-1.24]. Some of the reasons for their extensive applications are that the microspheres show high physical endurances, temperature durability, inertness, and low degradability. Hence, integrating magnetic particles in the microspheres opens for applications in new areas, such as environmental remediation.

In this thesis, we focus on the latest research progress of magnetic adsorbents for wastewater treatment. Different coatings of the magnetic particles and their applications, including their adsorption abilities are discussed. Further, adsorption, desorption, and separation studies using porous magnetic microspheres were performed. Then, TiO₂ coated
microspheres were synthesised and used for the photocatalytic degradation of phenol. Finally, the porous microspheres were used for separation of protein.

This was the final degree project work in the education of Master of Science in Chemical Engineering at The Royal Institute of Technology (KTH). The work has been executed at the State Key Laboratory of Chemical Engineering, Department of Chemical Engineering and Biological Engineering, Zhejiang University, China.

1.1 AIMS AND OBJECTIVES

The aims of this thesis were to review and increase the knowledge about the recent studies in wastewater treatment using magnetic technology. Following, implement the knowledge gained from the literature study in similar cases and develop new ideas for future advances in the waste water treatment. The objectives were to study the methods in the treatment of wastewater using magnetic technology and to improve/develop strategies for separation and removal of pollutants from wastewater. Further, to explore the possibility of using the microspheres as carriers for photocatalysts. Finally, by using the porous microspheres perform a bioseparation.

1.2 METHODS

The thesis started with an extensive literature study in the recent research progress on the applications of magnetic adsorbents in the treatment of wastewater, which resulted in an article later to be published in a scientific journal.

Weekly seminars where the research group presented recent published articles in contiguous fields. This was to keep everyone updated on the recent research in various fields and to gain new ideas to implement in the research.

The experimental work was done at the State Key Laboratory of Chemical Engineering, Zhejiang University. The work included: repeating previous work done by others, this to familiarise with the methods and techniques. Then to design, develop, improve, and evaluate applications for magnetic microspheres based on the previous work.

UV/Vis-spectrophotometry, XRD, TGA, and N₂-sorption have been used to the evaluate results from different experiments that were carried out.

1.3 LIMITATIONS

The research was performed under a short period of time, therefore the limitations were the time frame. However, new inspiring ideas have been formed during this work which could be fundamental for further research.
CHAPTER 2: BACKGROUND

2.1 UPDATED RESEARCH PROGRESS IN MAGNETIC ADSORBENTS

The areas of application for magnetic particles are numerous, for example data-storage, cancer treatment, drug release, nuclear waste, and magnetic resonance imaging [2.1-2.5]. Adsorbents with incorporated magnetic particles have proved to be useful for removal of different contaminants, such as heavy metals and dyes [2.6, 2.7] and the magnetic adsorbents show great ability for regeneration and reuse [2.8].

Separating and removing particles using magnetic technology have shown to be more efficient and selective compared to other methods such as centrifugation and filtration. Magnetic adsorbents have also shown to be efficient for rapid separation of large volume samples and in suspended systems [2.9-2.11].

There are some differences between nano- and micro-sized magnetic adsorbents. For one, the nano-sized adsorbents have no internal diffusion resistance. The internal diffusion resistance may cause slow adsorption and desorption rates. Also, the nano-sized adsorbents have greater specific surface area compared to the micro-sized. This reduces the amount of adsorbent needed to achieve satisfactory results [2.12, 2.13]. The magnetic force acting on particles in a field gradient is direct proportional to the particle volume. If the magnetic adsorbent is too small no separation will occur when applying a magnetic force. This is due to the force will not be strong enough to overcome the Brownian motion [2.10].

Fe₃O₄ is the most commonly used material in the field for synthesising magnetic adsorbents. The nano-sized Fe₃O₄-particles show superparamagnetic properties in room temperature and can be attracted by a magnet. However, the particles do not retain the magnetism after the magnet is removed. This is due to their superparamagnetic properties. Also, the magnetic nano-particles do not cluster and an easy separation using an external magnetic field can be achieved [2.6].

2.1.1 MESOPOROUS MAGNETIC ADSORBENTS

Mesoporous materials have applications in many areas, such as separation, catalysis, and adsorption [2.14]. Incorporating Fe₃O₄-particles in mesoporous materials is of great interest for the development of high quality adsorbents combined with convenient separation using an external magnetic field. However, poor magnetic response in the magnetic adsorbents has been a problem. The underlying cause has been found to be structure irregularities and low Fe₃O₄ mass fraction [2.15-2.17].

Recently Liu et al. [2.6] have prepared mesoporous magnetic microspheres using macroporous polydivinylbenzene (PDVB) microspheres with a diameter of 350-450 µm as a template. The PDVB microspheres were first sulfonated with chlorosulfonic acid. Subsequently, the sulfonated microspheres were stirred in the presence of ferrous chloride and
magnetic Fe₃O₄ nano-particles (MNPs) were formed and distributed all over the pore wall. A scheme of the process is given in Figure 2.1.

The synthesised mesoporous magnetic microspheres were used for removal of the dyes methyl violet (MV) and basic fuchsin (BF) (Figure 2.2) from an aqueous solution. The sulfonic groups distributed over the pore walls are advantageous for adsorption of cationic dyes. The results showed that the microspheres had good adsorption abilities, and 99.1 % of BF and 92.8 % of MV were adsorbed from the solution. The mesoporous magnetic microspheres had a specific surface area of 239 m²/g and a Fe₃O₄ weight-fraction of 25 wt%. It was stated that the Fe₃O₄ weight fraction could be improved by additional cycles of stirring in the presence of ferrous chloride. Noteworthy, the magnetisation curves showed that the magnetic microspheres had ferromagnetic properties at 5 K. However at 300 K, the microspheres have superparamagnetic properties. As desorbent, anhydrous ethanol was used, which made recycling and reusage of the microspheres possible. After the fourth cycle, 91.8 % BF and 86.3 % MV were removed [2.6]. Maintaining good adsorption abilities is important from an economical point of view.
Mesoporous silica-coated MNPs were synthesised by Wu et al. [2.18]. MNPs with a diameter of <5 µm were coated with a thin layer of silica followed by addition of cetyltrimethyl-ammonium chloride (CTAC). The magnetic particles were collected by separation using a magnet and then mixed with tetraethyl orthosilicate (TEOS) [2.18]. The products were then calcinated in presence of N₂ and to improve the silanation reaction with mercapto-propyl-trimethoxy-silane (MPTS) the products were steamed [2.19]. A detailed scheme of the synthesis is presented in Figure 2.3. Dong et al. [2.19] demonstrates that the silica-coated MNPs are effective for adsorbing mercury. With a mercury loading capacity of 14 mg/g, the silica-coated magnetic particles show great potential for removing mercury and can be used in industries with high mercury concentrations in the wastewater discharge. A high loading capacity reduces the amount of adsorbent needed and also the amount of waste.

![Figure 2.3. Scheme for preparation of mesoporous silica-coated magnetic particles [2.19]](image)

The mesoporous magnetic microspheres synthesised by Liu et al. were discovered to only be able to adsorb cationic species. To solve this problem, Zubieta et al. treated a silica lamellar mesoporous material with chitosan using a similar synthesis route as Wu et al. [2.18], and succeeded to remove an anionic- and a cationic dye, Tectilon Blue and Rhodamine B [2.20]. However, Zubieta et al. did not utilise magnetic separation. Nevertheless, it is likely that the technique could be adapted on the mesoporous magnetic microspheres. This might be of great interest for further research.

Deng et al. [2.21] have prepared mesoporous magnetic microspheres (~500 nm) with a Fe₃O₄@SiO₂ core. Initially, Fe₃O₄-particles were coated with a thin layer of silica followed by templating with cetyltrimethylammonium bromide (CTAB) and finally, the templates were removed with acetone. The procedure is shown in Figure 2.4. Deng et al. [2.21] have removed different species of microcystins using the mesoporous magnetic microspheres. Microcystins are toxic molecules with high molecular weight (about 1000 Daltons) which are produced in cyano-bacterial blooms [2.22]. Three different species of microcystins were used in the experiment; MC-RR, MC-YR, and MC-LR. Within one minute, 95.4 % MC-RR, 97.2 % MC-YR, and 97.5 % MC-LR had been adsorbed from the aqueous solution. Worth to mention is that only 0.05 mg/µg-microcystins of the microspheres were needed for removal of the microcystins. Acetonitril was used as desorbent and after eight cycles the removal efficiency was 90 % [2.21]. These results indicate that the method could have a high commercial value.
2.1.2 COATED MAGNETIC NANO-ADSORBENTS

The preparation of polyacrylic acid (PAA)-coated MNPs has been developed by Liao et al [2.23]. The binding of PAA onto MNPs were performed by initially sonicate MNP in a pH 6 buffer followed by addition of carbodiimide. PAA-solution was added and bound to the MNPs which then were recovered from the solution using a magnet. Figure 2.5 step I shows a scheme of the synthesis of PAA-coated MNPs. From this point, there were two existing synthesis routes for functionalising the PAA-coated MNPs. Mak et al. [2.24] have treated the PAA-coated MNPs with sulfanilic acid as shown in Figure 2.5 step II. Huang et al [2.25] have prepared amino-functionalised PAA-coated MNPs using diethylenetriamine via activation with carbodiimide as shown in the scheme in Figure 2.5 step III. By altering the surface of the PAA-coated MNPs, different types of contaminants could be adsorbed. The three different adsorbents described above have been proved to be useful for adsorption of cationic dyes, multivalent metal ions, and enzymes [2.8, 2.24-2.26].

Mak et al. [2.8] have studied adsorption of the cationic dye methylene blue (MB) (Figure 2.6) using PAA-coated MNPs. The results showed that the PAA-coated MNPs were an effective adsorbent for this particular dye and the adsorption was performed in approximately 2 minutes. By using acetic acid in methanol solution, 80 % of the dye was desorbed and after 3 desorption steps all of the MB was desorbed from the adsorbent. Chen et al. [2.26] have performed a separation of the enzyme bromelain via adsorption by magnetic PAA-coated
MNPs. Also here the adsorption process was rapid. In approximately 1 minute the bromelain present in the solution was adsorbed. The adsorptions were quick due to the absence of internal diffusion. [2.8, 2.26]

![Methylene blue](image)

Figure 2.6. Molecular structure of methylene blue

As demonstrated by Mak et al., [2.8] PAA-coated MNPs can be very useful for removal of cationic dyes. Unfortunately, it is not as easy for anionic dyes to be adsorbed onto PAA-coated MNPs and they also show a low ability for adsorption of heavy metal ions [2.27]. Mak et al. [2.24] have used the sulfonated PAA-coated MNPs for adsorption of multivalent cation metal ions from aqueous solutions. The result and a comparison of adsorption of multivalent cation metal ions from aqueous solutions are shown in Table 2.1. The results were found to be satisfying. The sulfonated PAA-coated MNPs adsorbed nearly 100 % of the multivalent metal ions, in comparison with the PAA-coated MNPs which only adsorbed approximately 30-40 % of the metal ions in the study [2.24].

<table>
<thead>
<tr>
<th>Cation</th>
<th>Recovery (%)</th>
<th>Sulfonated magnetic nano-particles</th>
<th>PAA-coated magnetic nano-particle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co²⁺</td>
<td>~100</td>
<td></td>
<td>30.5</td>
</tr>
<tr>
<td>Al³⁺</td>
<td>~100</td>
<td></td>
<td>34.5</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>~100</td>
<td></td>
<td>37.1</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>97.2</td>
<td></td>
<td>38.3</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>~100</td>
<td></td>
<td>78.5</td>
</tr>
</tbody>
</table>

Sulfonated PAA-coated MNPs do not adsorb anionic metals or substances. To circumvent this problem and to get a magnetic nano-adsorbent for both anionic and cationic species, Huang et al. [2.25] have used the amino-functionalised PAA-coated MNPs. The results in Table 2.2 confirm that amino-functionalised MNPs have good adsorption abilities, both for cationic and anionic species. The recovery was nearly 100 % for all the different metal ions. This demonstrates the high feasibility of the method and also its superiority compared to sulfonated PAA-coated MNPs and PAA-coated MNPs. The adsorption rate was fast, and equilibrium was achieved in only a few minutes [2.25].

Humic acid (HA) coated MNPs have recently been synthesised by Liu et al. [2.28]. Ferric chloride and ferrous sulphate were dissolved in water followed by the addition of ammonium hydroxide and HA-sodium salt. The precipitate was the HA-coated MNPs and without further
treatment they could be used for removal of Cd(II), Cu(II), Hg(II), and Pb(II) from the aqueous solution. Both HA and Fe₃O₄ are naturally abundant [2.29, 2.30], so they possess a minimal treat for the environment. Also, for the preparation of HA-coated MNPs environmentally friendly salts and solvents were used [2.28]. The feasibility of the HA-coated MNPs for removal of heavy metals from water is shown in Table 2.3.

Table 2.2. Comparison of recovery of metal ions using either amino-functionalised magnetic nano-particles, or PAA-coated magnetic nano-particles, with initial concentration of 100 mg/L [2.25]

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Amino-functionalised magnetic nano-particles</th>
<th>PAA-coated magnetic nano-particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe³⁺</td>
<td>100</td>
<td>82.3</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>100</td>
<td>41.0</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>~100</td>
<td>34.7</td>
</tr>
<tr>
<td>Co²⁺</td>
<td>~100</td>
<td>32.3</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>98.7</td>
<td>30.1</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>100</td>
<td>18.6</td>
</tr>
<tr>
<td>Cd²⁺</td>
<td>100</td>
<td>8.5</td>
</tr>
<tr>
<td>AuCl₄⁻</td>
<td>~100</td>
<td>-</td>
</tr>
<tr>
<td>PdCl₄⁻</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>HCrO₄⁻</td>
<td>96</td>
<td>-</td>
</tr>
</tbody>
</table>

The removal efficiency is pH-dependent, and as a result the adsorption efficiency can differ up to 20 % for Cu and about 7 % for Cd in unfavourable conditions. The study showed that there was no interference in adsorption of the four metal species related to adsorption of other natural abundant metals in water, such as calcium. Further, the influence of dissolved organic matter on adsorption of heavy metal onto HA-coated MNPs was minimal. This concludes that the HA-coated MNPs selectively adsorb heavy metals.

Table 2.3. Comparison of removal (%) of heavy metals from water from different sources, with initial concentration C in mg/L [2.28]

<table>
<thead>
<tr>
<th>Matrix</th>
<th>pH</th>
<th>Hg</th>
<th>Pb</th>
<th>Cd</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>7.8</td>
<td>1.011</td>
<td>99.8</td>
<td>1.006</td>
<td>99.4</td>
</tr>
<tr>
<td>Ground water</td>
<td>6.4</td>
<td>1.012</td>
<td>99.8</td>
<td>1.005</td>
<td>99.5</td>
</tr>
<tr>
<td>River water</td>
<td>7.6</td>
<td>1.011</td>
<td>99.9</td>
<td>1.005</td>
<td>99.4</td>
</tr>
<tr>
<td>Lake water</td>
<td>7.2</td>
<td>1.011</td>
<td>99.2</td>
<td>1.006</td>
<td>99.4</td>
</tr>
<tr>
<td>Sea water</td>
<td>7.7</td>
<td>1.013</td>
<td>99.9</td>
<td>1.20</td>
<td>99.0</td>
</tr>
</tbody>
</table>

Chang et al. [2.27] have coated MNPs with carboxymethyl chitosan, via activation by carbodiimide followed by addition of a carboxymethyl chitosan solution. Chitosan have many
amino-groups that can serve as chelation sites, and this makes it prominent to adsorb several metal cations, [2.31-2.33] fluoride and numerous types of dyes [2.27, 2.34-2.36]. Chitosan is a natural polysaccharide which is a non-toxic and biodegradable polymer product produced by deacetylation of chitin. There are many applications for chitosan in various fields, such as biomedicine, drug delivery, and paper industry [2.37-2.39]. In a study by Chang et al. [2.34] Co(II) was adsorbed from aqueous solution using the chitosan MNPs. The adsorption rate proved to be fast, and equilibrium was reached in approximately 1 minute. In previous reports, Chang et al. have used the chitosan MNPs for removal of Cu(II) ions. The results showed that the maximum adsorption capacity was 21.5 mg/g and that the adsorption rate was fast. Also here the equilibrium was achieved in approximately 1 minute [2.35]. Recovering metals from effluents is not only important from an environmental aspect, but also from an economical point of view, due to high raw material costs.

Two acid dyes, acid green 25 and crocein orange g (Figure 2.7) have been used in an adsorption study by Chang et al. [2.27]. The adsorption abilities were high for both of the dyes, 1883 mg/g for crocein orange g and 1471 mg/g for acid green 25. This was due to the large specific surface area of the MNPs. However, acid dyes have a strong affinity to chitosan which causes problems in the desorption step. The maximum desorption achieved for crocein orange g was 83.6 % and for acid green 66.1 %. Desorption efficiency is as important as adsorption efficiency.

With low desorption percentage the economy of the method becomes lower. It has been suggested that multiple desorption steps could improve desorption percentage, but no further research has been done. Figure 2.8 shows the adsorption mechanism of the acid dyes onto the amino-groups of chitosan [2.27]. Chitosan MNPs can be used not merely for adsorption of cationic species, but also for anionic species as shown in this case.
Low concentrations of fluoride in drinking water have beneficial effects on dental health, however higher concentrations have negative effects on human health [2.40, 2.41]. Several methods for removing fluoride from water have been developed, such as reverse osmosis, and nanofiltration [2.42, 2.43]. The drawbacks of the methods have often been high operational costs. Wei et al. [2.36] have studied the adsorption of fluoride on chitosan MNPs, and also the surface structure. Groups of NH, NH₂, NH₃, and Fe-O, create an amorphous surface on the chitosan MNPs. As mentioned earlier these groups work as chelation sites, on which the fluoride ions can be disposed. The results showed that the initial adsorption rate was 2.08 mg/g min. The high adsorption efficiency of fluoride using chitosan MNPs gives the method great commercial value.

2.1.3 MAGNETIC CARBON ADSORBENTS

Active carbon is a good adsorbent with a high specific surface area. Due to the good adsorption ability, active carbon has often been used over the years for removal of all types of pollutants [2.44-2.47]. It has been reported by Long et al. [2.48] that carbon nanotubes (CNTs) are more efficient in adsorbing dioxins in comparison with active carbon. Active carbon is generally very difficult to separate from aqueous solution [1.13]. Incorporating the CNTs with MNPs makes it easy to separate adsorbent from solution by utilising the magnetic properties. Hence, overcoming the problem caused by their size which occurs when using conventional separation techniques, such as filtration or centrifugation for the separation of nano-sized particles [2.34].

Peng et al. [2.49] have prepared the CNTs by catalytic pyrolysis of a mixture containing propylene and hydrogen with Ni as catalyst. The MNPs were incorporated by initially dissolving the catalyst particles in nitric acid and hydrofluoric acid. Finally, ferric chloride and ferrous sulphate were mixed with the CNTs under nitrogen, and NaOH was added. The CNTs with incorporated MNPs can be used for adsorption of heavy metals and dyes from aqueous solution [2.49-2.51]. By chemical oxidation, the CNTs improve their metal adsorption due to progression of the number of oxygen-containing groups on the surface. In the study by Peng et al., [2.49] the adsorption of Pb(II) and Cu(II) using the magnetic CNTs were nearly 100 %, with a CNT recovery rate of approximately 98 %. Attempts of removing dyes from water using magnetic CNTs have been executed by Qu et al. [2.50] and Gong et al. [2.51]. Three different dyes were examined in these studies, MB, neutral red (NR), and brilliant cresyl blue (BCB) (Figure 2.6, 2.9). The results showed that the CNTs possess high adsorption abilities for these specific dyes, 99.2% MB, 98.3 % NR, and 98.8% BCB were adsorbed [2.51].

From rice husk based activated carbon (RHC) Yang et al. [1.13] have synthesised mesoporous MNPs. The MNPs were used for adsorption of MB from aqueous solution. A scheme of the preparation and adsorption of the MNPs is shown in Figure 2.10. The RHC-
Fe$_3$O$_4$ particles have a specific surface area of 770 m$^2$/g and an adsorption capacity of 321 mg/g for MB in aqueous solution. Yang et al. show an environmentally friendly way of removing contaminants.

![Molecular structure of neutral red and brilliant cresyl blue](image)

Figure 2.9. Molecular structure of neutral red and brilliant cresyl blue

As mentioned earlier, Fe$_3$O$_4$ is the most commonly used material for magnetic adsorbents. However, in one study Gorria et al [2.52] have synthesised active carbon with incorporated nickel nano-particles. To prevent leakage of Ni, sucrose was used in the synthesis which proved to protect the Ni-particles from acid corrosion in a low pH-environment. It was discovered that the synthesis of active carbon with integrated Ni was cost-effective and easy to produce due to inexpensive materials, easily available chemicals, and a simple synthesis procedure.

![Scheme for the preparation of magnetic rice husk based activated carbon and the adsorption of MB](image)

Figure 2.10. Scheme for the preparation of magnetic rice husk based activated carbon and the adsorption of MB

2.1.4 OTHER MAGNETIC ADSORBENTS

A more original method of adsorbing heavy metals from aqueous solution has been presented by Wang et al [2.53]. Gellan gel beads were prepared with MNPs, thus giving them magnetic properties. The magnetic gellan gel beads were then used for adsorbing Pb$^{2+}$, Cr$^{3+}$, and Mn$^{2+}$ from aqueous solution. Parameters such as temperature, pH, adsorbent dosage, and initial concentration were investigated. Interestingly, it was noticed that with increasing temperature, starting from 10 °C to 30 °C the Pb$^{2+}$ adsorption decreased approximately 10 % and adsorption of Cr$^{3+}$ and Mn$^{2+}$ were almost constant. When raising the temperature to 60 °C, the adsorption of Pb$^{2+}$ decreased with nearly 40 % and Mn$^{2+}$ with 10 %. However, adsorption of Cr$^{3+}$ increased with approximately 35%. This shows the importance of controlling the physical parameters when adsorbing contaminants from aqueous solution.

Rocher et al. [2.54] have prepared alginate beads containing active carbon and MNPs for removal of the cationic and anionic dyes, MB and methyl orange (MO) (Figure 2.6, 2.11). The adsorption to magnetic alginate beads containing active carbon was compared with active carbon without alginate beads and the results showed that the magnetic alginate beads were more efficient in removing MB. The maximum adsorption capacity for alginate beads was 38.9 mmol/g active carbon compared to 0.62 mmol/g active carbon for the active carbon
without alginate beads. The adsorption capacity for MO proved to be quite similar in both cases, 1.32 mmol/g active carbon for alginate beads and respectively 0.86 mmol/g active carbon for the active carbon without alginate beads. These results show that it is possible to use environmental friendly and also cost-effective methods for removing organic compounds from aqueous solutions [2.54]. Lim et al. have synthesised a magnetic calcium-alginate adsorbent and experiments were performed for adsorption of arsenic and copper ions. The maximum adsorption capacity for arsenic and copper respectively, was found to be 6.75 mg/g and 60.24 mg/g, which is higher than the commercial adsorbents presently available [2.55].

![Methyl orange](image)

**Figure 2.11. Molecular structure of methyl orange**

Due to the functional groups of bismuthiol, -SH- and -NH-, it serves as a good complex binder for heavy metals, such as Pb, Cu, and Cd [2.56, 2.57]. Suleiman et al. [2.58] have prepared bismuthiol-silica coated MNPs for adsorption of heavy metals. The adsorption abilities were good. ~98% Pb, ~99% Cu, and ~99% Cr were adsorbed from the aqueous solution. It was found that this method was useful for rapid adsorption of the metals in the study and also good for adsorbing metals from large volumes.

An important environmental application has been reported by Machado et al. [2.59]. A magnetic adsorbent based on polymer coated vermiculite, used for adsorbing contaminants spilled on water. The adsorbent is hydrophilic and floats on water, thus making it useful for adsorption of spilled oil.

### 2.1.5 CONCLUSIONS

In this chapter, a number of different adsorption techniques have been discussed. One difficulty, but possibly also an advantage, is that the adsorbents are specific and do not adsorb several kinds of contaminants. This can be advantageous in separation processes. The progress in this field has been very remarkable over the last few years. New types of adsorbents have been successfully synthesised when it proved that the previous ones were not sufficient. It is very likely that adsorption and separation using magnetic technology will improve further, generating a powerful tool for removal of hazardous matter and also for recycling waste.

To the best of my knowledge, there are no full scale attempts of implementing magnetic adsorbents. One of the reasons for this is that the industry tends to rely on existing technique and not try new methods. However, it is most likely that this technique will be in operation in the future. A possible full scale setup for treatment of waste water using magnetic adsorbents is presented in Figure 2.12. In step 1. unprocessed water is introduced in the process, for example dyeing of textiles, the waste water is transferred to a balance tank (4.) that works as a
buffer tank and from there the waste water is brought to the adsorption and desorption tank (5.) and mixed with the magnetic adsorbents via mechanical stirring. The concentration pollutant in the waste water can be measured via online monitoring with UV/Vis spectroscopy, this to control that the sufficient adsorption is achieved and then a magnetic separation of adsorbent and waste water using an electromagnet can be performed. After several adsorption cycles the adsorbent must be regenerated, a desorption solution is added in step 6 with the same procedure as with the adsorption. After the desorption, the product can be reused in process to minimise material costs and waste (8.). There are no fundamental differences in the structure layout of the complete process compared to conventional techniques.

Figure 2.12. Flow chart for a possible setup for the treatment of waste water using magnetic adsorbents

The textile industry is one of the major big industries that affects both economics and environment and consumes high quantity of water and chemicals, sometimes hazardous chemicals, in the process of dyeing textile. Therefore, strategies of reducing emissions from this industry and also reducing raw material are of high importance, both in economically and environmentally terms. The magnetic adsorbents can provide the strategy for reducing the economics and environmental effects.

Several of the commercial adsorbents use stationary packed beds to perform the adsorption, also moving beds are sometime used. However there are some disadvantages with packed beds. One is the pressure drop that occurs when pumping a fluid through a packed bed. If the flow is high then it would require a big pump which is environmentally and economically disadvantageous. Further, packed beds do not provide the fast adsorption and separation of adsorbent. Therefore, a fluidised bed could be a more proper method. Also fluidised beds have different kinetics than the fixed bed which could be more advantageous for the magnetic adsorbents. However, fluidised beds also require a high load of energy input which affects the economy. Conventional stirring may be applied for the adsorption process. The exact method for every individual magnetic adsorbent must be evaluated and tested in small and big scale.
2.2 UPDATED RESEARCH PROGRESS IN MAGNETIC PHOTOCATALYST

Since the discovery of the photocatalytic properties of TiO\textsubscript{2} in 1972 by Fujishima and Honda [2.60], many new types of applications for TiO\textsubscript{2} have been discovered, such as photovoltaics [2.61]. Photocatalytic reactions have recently drawn much attention due to the potential of producing hydrogen from water using solar light [2.62-2.64]. One of the main applications for photocatalysts is the degradation of pollutants from waste water. This technique is becoming increasingly important, especially for removing low trace contaminants but also for bacteria and viruses [1.14, 1.15, 2.65-2.68]. The photocatalysts have the same problems as many adsorbents, due to the size they are difficult to separate from aqueous solutions. [1.17].

The size of the TiO\textsubscript{2} particles have found to be important for the photocatalytic efficiency, when decreasing TiO\textsubscript{2} particle size the specific surface area increases, thus the contact area increases which results in an increase of efficiency [2.69]. However, separation and recovery of the TiO\textsubscript{2} particles is difficult due to the small particle size. Therefore, incorporating TiO\textsubscript{2} in magnetic carriers is of great interest due to the effective separation and recovery that can be achieved by magnetic separation. Further, the carriers can be fluidised by an external magnetic field [2.70]. Several different types of magnetic carriers have recently been investigated, such as barium ferrite [2.71], Fe\textsubscript{2}O\textsubscript{3}/SiO\textsubscript{2} [2.72], magnetite [2.73], and magnetic poly(methyl methacrylate) microspheres (mPMMA) [2.74].

In this chapter, several different types of magnetic carriers and the preparation of photocatalysts using these carriers are discussed. Also, the degradation of different organic contaminants using the magnetic photocatalysts is discussed. The sol-gel technique is the most commonly used technique for the preparation of semi-conducting photocatalysts. There are several articles and reviews addressing this technique. Therefore, in this chapter we focus on alternative synthesis route for the preparation of photocatalysts.

2.2.1 DIFFERENT PREPARATION TECHNIQUES AND MAGNETIC CARRIERS

Magnetic Fe\textsubscript{2}O\textsubscript{3}/SiO\textsubscript{2}/TiO\textsubscript{2} nanoparticles were synthesised by Wang et al. [2.72]. The synthesis route is presented in Figure 2.13. Fe\textsubscript{3}O\textsubscript{4} particles were dispersed in water followed by addition of tetraethyl orthosilicate (TEOS) at high pH. Subsequently, the Fe\textsubscript{3}O\textsubscript{4}/SiO\textsubscript{2} particles were transferred to an autoclave and dispersed in hexane, water, and TBOT solution and the autoclave was heated. The product was then calcined under high temperature and the Fe\textsubscript{3}O\textsubscript{4} particles were transferred into Fe\textsubscript{2}O\textsubscript{3}.

![Figure 2.13 Scheme for the preparation of Fe\textsubscript{2}O\textsubscript{3}/SiO\textsubscript{2}/TiO\textsubscript{2} magnetic photocatalyst catalyst [2.72]](image-url)
Xuan et al. [2.70] successfully synthesised hollow Fe$_3$O$_4$/TiO$_2$ spheres by using a poly(styrene-acrylic acid) (PSA) core. First, PSA-spheres with a diameter of 270 nm were dispersed in ethanol followed by addition of aqueous FeCl$_3$. Second, the pH was adjusted to 9-10 using aqueous NH$_3$ followed by addition of Na$_2$SO$_3$. Fe$_3$O$_4$ particles were formed on the PSA surface. The PSA/Fe$_3$O$_4$ particles were dispersed in a mixture of butanol, ethanol, and TBOT and stirred overnight to get a saturated adsorption of TBOT on the spheres. Subsequently, water was added to hydrolyse the TBOT into amorphous titania. The PSA/Fe$_3$O$_4$/TiO$_2$ particles were dispersed in THF, this to dissolve the PSA and then transferred to an autoclave. The amorphous titania was transformed to anastase form by solvothermal heating with hexane in the autoclave. A scheme for this procedure is presented in Figure 2.14.

The preparation of black sand based magnetic photocatalyst was developed by Luo et al. [2.74, 2.75]. Black sand (BS), a natural magnetic material, was used as magnetic core in the preparation of magnetic photocatalyst. BS was dispersed in water with TEOS and was stirred for a short while following by addition of ammonium hydroxide. The formed particles were dried and then the BS/SiO$_2$ was dispersed in a mixture of propanol and Titanium tetra-isopropoxide (TPOT) followed by stirring and heating. The product was then calcinated in order to transfer the TiO$_2$ into crystalline phase. The synthesis process of BS/SiO$_2$/TiO$_2$ is shown in Figure 2.15.

TiO$_2$ coated mPMMA microspheres were prepared by Chen et al. [2.76]. The magnetic particles were oleic acid coated (OMP) and introduced with the PMMA microspheres via modified suspension polymerisation. Described shortly, the OMP were mixed with divinylbenzene, methyl methacrylate, benzoyl peroxide, and hexane and was let reacted under heating. Then the formed mPMMA was magnetically separated from the solution. Subsequently, the mPMMA was mixed with TiO$_2$ powder and heated close to the glass transition temperature of PMMA which resulted in a soft surface that the TiO$_2$ could be adhered to. The process is shown in Figure 2.16.
2.2.2 DEGRADATION OF ORGANIC POLLUTANTS BY MAGNETIC PHOTOCATALYSTS

The general photocatalytic process is illustrated in Figure 2.17 and by equations 1-6. When the TiO$_2$ photocatalyst is irradiated with light, an electron in the valence band can be excited to the conduction band and a hole in the valence band is generated (equation 1). The back reaction (equation 1*) produces heat. Further, the free electron reacts with the oxygen and forms the super oxide anion (equation 2). The hole in the valence band take one electron from the water and $\text{H}^+$ and $\cdot\text{OH}$ is formed (equation 3). The super oxide anion combines with $\text{H}^+$ and forms the $\text{HO}_2$ (equation 4). The $\text{HO}_2$ reacts with $\text{H}^+$ and forms hydrogen peroxide which then dissociates into hydroxyl radicals (equation 5, 6). [2.77]

\[ \text{TiO}_2 \rightarrow \text{hv} \rightarrow e^- + h^+ \quad (1) \]
\[ e^- + h^+ \rightarrow \text{heat} \quad (1*) \]
\[ e^- + \text{O}_2 \rightarrow \cdot\text{O}_2^- \quad (2) \]
\[ h^+ + \text{H}_2\text{O} \rightarrow \text{H}^+ + \cdot\text{OH} \quad (3) \]
\[ \cdot\text{O}_2^- + \text{H}^+ \rightarrow \text{HO}_2 \quad (4) \]
\[ \text{HO}_2 \rightarrow \text{H}^+ \rightarrow \text{H}_2\text{O}_2 \quad (5) \]
\[ \text{H}_2\text{O}_2 + e^- \rightarrow \text{OH}^- + \cdot\text{OH} \quad (6) \]

Wang et al. [2.72] used the Fe$_2$O$_3$/SiO$_2$/TiO$_2$ for photocatalytic degradation of MB. After 80 min, 80% of the 50 mL 25 mg/L MB was degraded using 0.1 g photocatalyst. The results showed that the prepared photocatalyst had nearly the same photocatalytic ability compared with TiO$_2$ powder. This indicates that the SiO$_2$ layer prevented the electrons from transferring to the Fe$_2$O$_3$ that would reduce the photocatalytic ability.

Rhodamine B was photocatalytic degraded using hollow Fe$_3$O$_4$/TiO$_2$ spheres prepared by Xuan et al. [2.70]. In the experiment, 25 mg photocatalyst was used for the degradation of 60 mL 0.3 mg/L rhodamine B. After 80 min nearly 100% of the dye was degraded. This concludes that the hollow Fe$_3$O$_4$/TiO$_2$ spheres are an excellent photocatalyst.

Photocatalytic degradation of p-phenylenediamine using TiO$_2$-coated magnetic PMMA microspheres was performed by Chen et al. [2.76]. Experiments using different concentrations of p-phenylenediamine were performed. The experiments showed that the TiO$_2$-coated magnetic PMMA microspheres were excellent for removing low concentration contaminants.
After 90 min using 3.7 g photocatalyst, 100% of 3.7 L 3.5 mg/L of p-phenylenediamine were removed and in 240 min, 70% of 3.7 L 332 mg/L was removed. Also, the efficiencies were similar to what could be obtained with TiO₂ powder.

![Mechanism for the photocatalytic induced reactive groups and the degradation process of organic pollutants](image)

The TiO₂/CoFe₂O₄ prepared by Fu et al. [2.78] was used for photocatalytic degradation of the dye Procion Red MX-5B. The experiment was carried out using 4 mg of the photocatalyst in 50 mL 10mg/L Procion Red MX-5B solution. The prepared photocatalyst had different weight ratio of TiO₂, 10, 50, 70, and 90%. Obviously, with higher content follows higher efficiency. However, at very low concentrations of Procion Red MX-5B the difference between 70 and 90% was very little. In about 250 min nearly 100% of the dye was degraded using the photocatalyst with 70 and 90% content of TiO₂. In comparison, with TiO₂ powder the dye was completely degraded after 175 min.

Lee et al. [2.71] used TiO₂ with a BaFe₂O₄ core for the degradation of Procion Red MX-5B. The experiment was carried out using 30 mg of the photocatalyst in 50 mL 10mg/L Procion Red MX-5B solution. After 350 min 80% of the dye was decomposed. The results were not as good compared to the photocatalyst with CoFe₂O₄ core.

Luo et al. [2.74, 2.75] used black sand as magnetic core coated with TiO₂ for degradation of several different types of dyes and organic compounds. For the degradation of phenol, 0.2 g photocatalyst was dispersed in 75 mL phenol solution with the concentration of 20 mg/L. About 10% phenol was degraded after 120 min of irradiation as compared to the TiO₂ powder which degraded about 40% under the same conditions. For the degradation of different dyes, 0.3 g photocatalyst was dispersed in 200 mL dye with the concentration of 60 mg/L. About 8% of the dye rose bengal, 12% of orange II, and 10% of eosin b was degraded after 120 min of irradiation.

To enhance the photocatalytic performance Xu et al [2.77] dope the TiO₂/SiO₂/Fe₃O₄ photocatalyst doped with silver. It was found that this photocatalyst had better photocatalytic efficiency, about 30% higher efficiency, in the degradation of orange II as compared to TiO₂.
powder. After 20 min using 50 mg photocatalyst, 90 % of 0.1 L 17.5 mg/L orange II was removed. In comparison with TiO₂ powder were 70 % was removed after 20 min. The process can be described with equation 1-8. Compared to the un-doped photocatalyst reaction 7 and 8 do not take place. The formation of extra super oxide anion boosts the photocatalytic degradation.

\[
e^- + Ag \rightarrow Ag^+ \quad (7)
\]
\[
Ag^+ + O_2 \rightarrow O_2^- \quad (8)
\]

2.2.3 CONCLUSIONS

In this chapter, several different magnetic carriers for the TiO₂ photocatalyst have been reviewed. The magnetic carriers provide an effective way of the separation of TiO₂ which otherwise is very difficult and expensive to separate. However, the photocatalyst need to be more efficient and more research has to be put in the field before they will have industrial applications. The photocatalyst have high potential for providing a cheap and efficient way of removing pollutants from effluents.
2.3 Updated Research Progress in Bioseparation Using Magnetic Technology

The magnetic technology has many applications in several areas. Biotechnology is perhaps the most prominent field for the magnetic technology. The magnetic technology can be used for drug delivery, enzyme immobilisation, protein separation and isolation [2.79, 2.80].

In this chapter, a brief review of the recent progress on the magnetic technology for bioseparation is presented.

2.3.1 Bioseparation

Ding et al. [2.81] and Shamim et al. [2.82] used magnetic particles covered by thermosensitive polymer for adsorption of human serum albumin (HSA) and bovine serum albumin (BSA). The results showed that by increasing the temperature the magnetic particles decreased in size and could adsorb more protein. Also, it was found that with increasing pH the adsorption decreased. This was attributed to the electrostatic forces between the protein and adsorbent. The protein could be desorbed from the magnetic particles by decreasing the temperature. This was a result from the structure change on the surface. It was found that the protein could be deformed in the adsorption process due to interaction with the polymer. The deformed protein could not be easily desorbed by changing temperature and therefore the efficiency of the adsorbent decreased when it was reused. A scheme of the adsorption and desorption process is shown in Figure 2.18

![Figure 2.18 Scheme for the separation of HSA by magnetic particle coated with thermosensitive polymer](image)

BSA was separated from aqueous solution using surface functionalised magnetic poly(styrene-divinylbenzene-glycidyl methacrylate) microspheres [2.83]. The microspheres had a size of 6 µm. The adsorption capacity were high, about 80 mg/g and with a desorption efficiency of 94 % using a NaSCN solution.

Li et al. [2.84] use magnetic beads with Env protein covalently linked to magnetic beads in the process of separation of antibodies used in the neutralisation of HIV-1. This shows the usability and importance of the magnetic technology in the biotechnology field.

Lipase is an enzyme which generally breaks down fats and is used in food industry and pharmaceutical industry [2.85, 2.86]. Wu et al [2.87] use magnetic Fe₃O₄ nanoparticles coated with chitosan for the adsorption, immobilisation, and oxidation of lipase. It was found that by adding glutaraldehyde the enzyme maintain the activity even after desorption as to if not glutaraldehyde was added the enzyme lost up to 60 % of the activity. This phenomenon can
be used for controlling processes in the food industry and pharmaceutical industry more accurate.

Recently, Luo et al [2.88] prepared cellulose microspheres with fabricated Fe₃O₄ particles in the cellulose pores. The synthesised microspheres were used for adsorption with controlled release of BSA. The release of BSA from the microspheres could be controlled by changing pH. At pH 7.4, which is close to the pH in the human body, nearly 100% of the BSA could be released. However, the process was very slow, about 500 h. This could be exploited for slow drug delivery which releases the active substance slowly.

Lin et al. [2.89] use amino-functionalised magnetic nanoparticles with trypsin immobilised on the surface. These particles were used for digestion of BSA, myoglobin, and cytochrome c. After the digestion the microspheres could easily be removed by magnetic separation and the samples could be analysed. This is an interesting way of studying the digestion of proteins and to be able to investigate the process without interference of the enzyme in the analysis.

Chiang [2.90] use Fe₃O₄ particles coated with glycidyl methacrylate-iminodiacetic acid for extraction of protein from a specific fluorescent modified protein from E-coli bacteria. The result shows that the magnetic particles were specific and were effective in adsorbing and extracting this specific protein.

2.3.2 CONCLUSIONS

In this chapter, we have shown that the magnetic technology can be used not only for environmental purpose but also in the field of biotechnology. By altering the coatings of the magnetic particles different kinds of bio-molecules can be separated which can be exploited when there is a need to separate in a moiety of bio-molecules. Without doubt, the magnetic technology can provide the fast, specific, and efficient separation which is important in this field.
CHAPTER 3: MAGNETIC MICROSPHERES FOR REMOVAL OF METHYLENE BLUE FROM WASTEWATER

Most dyes used in industries are considered hazardous, so, it is necessary to remove these from effluents. Organic dyes show low biodegradability, hence, other methods than the biological treatment is required for removing dyes from effluents [3.4]. Also, other conventional techniques such as reverse osmosis and chemical coagulation have problems with removing the colour completely [3.5].

Methylene blue (MB) is a cationic dye and is used in many areas, such as medicine and biotechnology [3.6, 3.7]. Several materials have recently been studied to remove MB from wastewater such as carbon nano-tubes, fly ash, red mud, and vermiculite [3.8-3.12]. However, these methods do not provide an easy and quick separation process.

In this study, mesoporous magnetic microspheres have been synthesised and used for the adsorption of MB. MB is a model dye and been used extensively in adsorption studies, hence make a comparison between other methods convenient. The chemical structure of MB is shown in Figure 2.6. Liu et al. [3.13] reported that the mesoporous magnetic microspheres have good ability to adsorb the cationic dyes methyl violet and basic fuchsine. Further, magnetic separation makes the reusage of the adsorbent convenient.

3.1 EXPERIMENTAL

3.1.1 MATERIALS

Porous polystyrene microspheres with a diameter of ~200 µm which were prepared in house were used in this study. Chlorosulfonic acid and ferrous chloride were purchased from Jingshanting Chemical Corporation of Shanghai, China. Dichloromethane, sodium hydroxide, potassium chloride, anhydrous ethanol, and hydrogen peroxide were purchased from Lanling Chemical Corporation of Linan in Zhejiang, China. Methylene blue was purchased from Shanghai San’aiisi Chemical Company, China. All the chemicals were used without further treatment.

3.1.2 PREPARATION

3.1.2.1 PREPARATION OF SULFONATED MICROSPHERES

The porous magnetic microspheres were prepared according to the previous reported method by Liu et al [3.13]. Into a 250 ml 3-neck round bottom flask 75 ml dichloromethane and 5.78 g polystyrene microspheres were added. The solution was cooled using an ice bath, subsequently, 0.8 ml chlorosulfonic acid in 75 ml dichloromethane was added dropwise into the stirring solution. The solution was kept stirring for 24 h. The products were then filtrated and washed with dichloromethane followed by drying in a vacuum oven for 12 h. Finally, the microspheres were washed with deionised water and again dried in a vacuum oven.
3.1.2.2 Preparation of Magnetic Microspheres

Into a 250 ml 3-neck round bottom flask with a mechanical stirrer and argon inlet, 2.11 g of the sulfonated microspheres as synthesised, were added with 80 ml 1 M FeCl₂ solution. The solution was kept stirring for 24 h. The products were then filtrated and washed three times with deionised water and then transferred to another 3-neck round bottom flask with a mechanical stirrer and a condenser, 20 ml deionised water and 80 ml 2 M NaOH was added. The mixture was heated using an oil bath, when the temperature reached 75 °C, 20 ml 30 % H₂O₂ was added dropwise into the mixture and the solution was kept stirring at 75 °C for 2 h. The products were filtrated and washed three times with deionised water, one time with anhydrous ethanol, and then dried in a vacuum oven.

3.1.3 Characterisation

Optical microscope photos were taken using a NOVEL XS-2100 microscope instrument connected to a Canon powershot A630 with a Canon 640 52 mm objective. TGA were carried out on a Perkin Elmer TGA-7 apparatus with a heating rate of 10 °C/min, starting from room temperature to 700 °C. The surface areas were determined by nitrogen sorption porosimetry on a micromeritics ASIC-2 apparatus. A 5.2 × 5.2 × 2.8 cm Fe-SrBr magnet was used for the magnetic separation.

3.1.4 Adsorption and Desorption Optimisation

The adsorption optimisation was performed by varying pH and sonication time (contact time adsorbent/MB) as shown in Table 3.1. In principle, into a small vial 0.2 g magnetic microspheres were added with 4 mL of 1×10⁻⁶ M MB solution and then the solution was sonicated for 2 min. The pH value of the system was regulated by using a specific pH buffer. Subsequently, the magnetic microspheres were separated using a magnet and the solution was collected and the adsorption efficiency were determined using an UNICO 3802 UV/Vis spectrophotometer.

<table>
<thead>
<tr>
<th>Run (pH, 0.2 g, 2min)</th>
<th>A01</th>
<th>A02</th>
<th>A03</th>
<th>A04</th>
<th>A05</th>
</tr>
</thead>
<tbody>
<tr>
<td>A06</td>
<td>pH 6</td>
<td>pH 7</td>
<td>pH 8</td>
<td>pH 9</td>
<td>pH 10</td>
</tr>
<tr>
<td>A07</td>
<td>pH 11</td>
<td>pH 12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run (Time, 0.2 g, pH 7)</th>
<th>A11</th>
<th>A12</th>
<th>A13</th>
<th>A14</th>
<th>A15</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>2 min</td>
<td>3 min</td>
<td>4 min</td>
<td>5 min</td>
<td></td>
</tr>
</tbody>
</table>

The desorption optimisation was performed by varying the parameters of EtOH/H₂O volume ratio, salt type, salt concentration, and sonication time, as shown in Table 3.2. In principle, into a small vial 0.2 g magnetic microspheres were added with 4 mL of the
desorption solution and then the solution was sonicated for 2 min. Subsequently, the magnetic microspheres were separated using a magnet and the solution was collected and the adsorption optimisation efficiency were determined using a UV751GW UV/Vis spectrophotometer.

<table>
<thead>
<tr>
<th>Table 3.2. Different parameters for the optimisation of the desorption of MB from microspheres</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run (E/W, 0.2 g, 2min) E/W(v/v)</td>
</tr>
<tr>
<td>Run (1 M salt, 0.2 g, 2min) E/W (0/10) NaCl  KCl MnCl2</td>
</tr>
<tr>
<td>Run (1 M salt, 0.2 g, 2min) E/W (4/6) NaCl  KCl MnCl2</td>
</tr>
<tr>
<td>Run (1 M salt, 0.2 g, 2min) E/W (6/4) NaCl  KCl MnCl2</td>
</tr>
<tr>
<td>Run (KCl, 0.2 g, 2min) E/W (6/4)</td>
</tr>
<tr>
<td>Run (Time, 0.2 g, Sat. KCl) E/W (6/4)</td>
</tr>
</tbody>
</table>

Saturated KCl

3.2 RESULTS AND DISCUSSION

3.2.1 PREPARATION OF MAGNETIC MICROSPHERES

The porous polystyrene microspheres used in this study were prepared by free radical suspension polymerisation with toluene as porogen according to the routes proposed by Liu et al. [3.14]. The synthesised microspheres used for the preparation of the magnetic microspheres had a diameter of ~200 µm.

First, the polystyrene microspheres were sulfonated in the presence of chlorosulfonic acid and -SO3H were formed on the surface and on the pore walls. The sample was labelled as PS-SO3H. Subsequently, the sulfonated microspheres were stirred in the presence of ferrous chloride for 24 h to ensure full ion exchange. Finally, the ferrous ions were oxidised using hydrogen peroxide and Fe3O4 particles were formed on the surface and in the pores of the microspheres. A scheme of this process is shown in Figure 3.1. The weight fraction of Fe3O4 in the microspheres was very low, so the microspheres showed little magnetic response. Therefore, the process described above was repeated. After each cycle, a sample was collected and labelled as Mag 1 and Mag 2.
3.2.2 CHARACTERISATION

A magnetic response comparison between PS-SO$_3$H and Mag 2 is presented in Figure 3.2. The PS-SO$_3$H microspheres show no attraction toward the magnet. The Mag 2 microspheres show a strong attraction to the magnet. After the removal of the magnet, the microspheres did not agglomerate nor retained magnetic properties. This indicates that microspheres are superparamagnetic and that it is the Fe$_3$O$_4$-nanoparticles which are responsible for the attraction to the magnet.

Optical microscope images of the PS-SO$_3$H, Mag 1, and Mag 2 are presented in Figure 3.3. The sulfonated microspheres are a slightly transparent, however the magnetic microspheres are less transparent, and this is a result of the Fe$_3$O$_4$ nanoparticles on the surface and in the pore walls of the microspheres. The magnetic microspheres became almost completely opaque after two repetition of the process described in chapter 3.1.2. Also, the microspheres maintain the spherical shape after sulfonation and magnetisation and are of uniform size.
The results from the TGA are presented in Figure 3.4, and shows that the major decomposition region of the polystyrene microspheres is from 400 to 600 °C. The weigh-loss between room temperature and 100 °C was attributed to the microspheres containing a small amount of water. According to the results Mag 1 and Mag 2 had a weight fraction of Fe₃O₄-nanoparticles of 23 % and 33 % respectively. However, Liu et al. [3.13] reported a weight fraction Fe₃O₄-nanoparticles of Mag 1 to 9 % and Mag 4 to 25 %. The difference may be a result from insufficient washing of the microspheres in the preparation of the microspheres.

From the N₂-sorption analysis the specific surface area of the PS-SO₃H, Mag 1, and Mag 2 were calculated to 420 m²/g, 243 m²/g, and 175 m²/g respectively. Figure 3.4 b) shows the pore size distribution for PS-SO₃H, Mag 1, and Mag 2. The PS-SO₃H had some micropores, however, these pores were unavailable or closed in Mag 1 and 2, indicating that Fe₃O₄-nanoparticles were formed in these pores. The average pore diameter of PS-SO₃H was calculated to 4.9 nm, for Mag 1: 5.9 nm, and for Mag 2: 6 nm. Further the average pore volume decreases from 0.50 cm³/g to 0.36 cm³/g and 0.26 cm³/g for the magnetic microspheres compared with the PS-SO₃H microspheres. Indicating, Fe₃O₄-nanoparticles were successfully formed in all of the pores of the microspheres.

3.2.3 ADSORPTION AND DESORPTION STUDY OF METHYLENE BLUE

An adsorption and desorption study using the porous magnetic microspheres for the removal of MB were performed. This was to evaluate the performance of removing cationic dyes using magnetic microspheres. In this experiment, the microspheres were adsorbing 4 mL 1×10⁻⁶ M MB-solution under sonication at 80 % for 2 minutes at 22-25 °C. Liu et al. [3.13] reported that a 4/6 MeOH/H₂O with saturated KCl were effective as desorption solution for the desorption of methyl violet and basic fuchsin. The same desorption solution was used in this study. The adsorption and desorption efficiencies were evaluated by UV-spectrophotometer at
the wavelength 665 nm which was the most suitable peak as seen in Figure 3.6 a). A scheme of the adsorption, desorption, and separation process is presented in Figure 3.5.

![Figure 3.5. Scheme of the adsorption and desorption process of MB with the magnetic separation](image)

The result from the adsorption and desorption study is presented in Figure 3.7. The adsorption efficiency was 75-80 % throughout the study and the desorption efficiency was 80-90 %. The adsorption and desorption efficiency was not decreasing dramatically during the cycles, indicating that the microspheres is a robust method for removing cationic dyes. However, Liu et al reports an adsorption efficiency of 99.1 % for basic fuchsin and 92.7 % for methyl violet. This concludes that the adsorption and desorption efficiency for MB can be improved.

![Figure 3.6. a) UV-spectrum of methylene blue, b) standard curve for methylene blue](image)
3.2.4 ADSORPTION AND DESORPTION MECHANISM

The proposed adsorption and desorption mechanisms are presented in Figure 9 and 10. The affinity of MB to $-\text{SO}_3^-$ is higher than for $\text{Na}^+$. The MB has a $\text{Cl}^-$ group which interacts with the $\text{Na}^+$ and forms $\text{Na}^+\text{Cl}$, i.e. an ion exchange is achieved. In the case of the desorption, an ion exchange is also performed. However, using the co-solvent EtOH/H$_2$O/KCl the process can be speed up. The EtOH/H$_2$O/KCl interfere the electrostatic interaction between MB and $-\text{SO}_3^-$, thus the counterions are changed and the dye is desorbed from the microspheres.
3.2.5 Adsorption Optimisation and Desorption Optimisation

The result from the adsorption optimisation is presented in Figure 3.10. The adsorption efficiency is most likely pH-dependent. This is attributed to the change of charge and ionisation that appear with pH change. The pH was changed in the range of pH 6-12. The result from the study is shown in Figure 3.10 a). The result shows that there is no significant change in adsorption efficiency between pH 6-12. However, with increasing pH a slight faster adsorption rate was detected. The results show that the magnetic microspheres can be used in a broad pH range which is important for industrial use where the pH might fluctuate with time. Unfortunately, in acid environment, pH 1–3, the Fe₃O₄ particles start to dissolve. Therefore should acid environment be avoided. However, after short exposure of strong acid the microspheres retain the magnetic properties. It was found adsorption time is what affects the adsorption efficiency the most. After 5 minutes of adsorption nearly 100 % of the MB was adsorbed.

The results from the desorption optimisation is presented in Figure 3.11 a)-g). In the optimisation of the desorption, several types of salts, salt concentrations, and EtOH/H₂O-ratios were examined. As seen in Figure 3.11 a) the EtOH/H₂O 10/0 had the highest desorption efficiency. However, the efficiency was only about 40 % so further optimisation was required. The dye is adsorbed ion exchange between the dye and the -SO₃⁻ and there are two major ways of breaking this interaction. One is through polarity differences of the co-solvent and the other one is ion exchange. Therefore was salts introduced to the desorption solution. However, most salts are insoluble in EtOH so it was necessary to use an EtOH/H₂O ratio with H₂O ≠ 0 to dissolve the salts.
In the optimisation study many different salts were examined besides the ones mentioned above, NH$_4$Cl, LiCl, CaCl$_2$, NaSCN, NaNO$_3$, KI, NaHCO$_3$. However, it was found that these salts interacted and changed the structure of the dye or affected the following adsorption step. Nevertheless, it cannot be ruled out that some of these salts enhance the desorption rate, but due to the difficulties to measure this, and KCl show good ability to increase the desorption rate, no further research was made.

### 3.3 CONCLUSIONS

After the adsorption and desorption optimisation, another adsorption/desorption cycle study were performed. The result is presented in Figure 3.12. The adsorption efficiency of MB was approximately 100% throughout the study and the desorption was about 95%. Interesting noticing, after several desorption cycles the desorption efficiency was over 100%, this can be attributed to that some of the MB was still in the microspheres after every desorption cycle. This study confirms that the magnetic microspheres are an excellent adsorbent for cationic dyes.

A small deterioration of the microspheres was detected after some time, this due to the physical stress that occurs in the sonication. However, no noticeable reduction in performance was detected. This concludes that the magnetic microspheres prepared by this route are very robust and are suitable for reuse and regeneration, i.e. the microspheres have high potential for industrial use.

One of the drawbacks with the magnetic microspheres is that the Fe$_3$O$_4$ dissolves in harsh environment. Down to pH 4 there is no visual detection of the leakage, however UV results show a slight increment of dissolved iron. In strong acid environment, pH 0-1, there is a noticeable leakage of Fe$_3$O$_4$ from the microspheres and the magnetic response of
microspheres is affected. Magnetic microspheres that endure acid environment are of interest for further research.

Adsorption efficiency (%)

Adsorption cycle

Figure 3.12. Adsorption a) and desorption b) efficiencies of methylene blue \([1\times10^{-6} \text{ M}]\) over several cycles. Sonication time 5 min at 80 %, 27-30 °C. EtOH/H\(_2\)O 60/40 with saturated KCl was used as desorbing solution.

Adsorption via electrostatic interactions, as used in microspheres in this report, is a more selective adsorption method compared to the chemical adsorption of active carbon. This can be made use of in separation of a mixture of dyes, thus make the separation and recovery easier. Also, chemical adsorption is generally more difficult to desorb and regenerate compared to adsorption via electrostatic interactions.
CHAPTER 4: MESOPOROUS MICROSPHERES WITH PHOTOCATALYTIC ABILITY

In this chapter, TiO$_2$ coated microspheres for the photocatalytic degradation of phenol are studied. Phenol is a commonly used chemical in the industry and agriculture and is a common pollutant. Therefore, phenol was chosen as a model pollutant in order to evaluate the photocatalytic performance. To the best of my knowledge, this is the first time to use polystyrene microspheres as carriers for TiO$_2$.

4.1 EXPERIMENTAL

4.1.1 MATERIALS

Anhydrous ethanol, n-butanol, titanium butoxide (TBOT), and phenol were purchased from Sinopharm Chemical Reagent Co., Ltd. All the chemicals were used without further treatment.

4.1.2 PREPARATION OF PHOTOCATALYTIC MICROSPHERES

The TiO$_2$ coated microspheres were prepared according to the previous reported method by Xuan et al. [2.70]. Into a 100 mL 3-neck round bottom flask with a mechanical stirrer and argon inlet, 1 g PS-SO$_3$H was mixed with 50 mL n-butanol and 5 mL ethanol. In 10 mL ethanol, 2 mL of TBOT was dissolved and added to the mixture. The solution was stirred overnight. Following, 10 mL of aqueous ethanol was added and the mixture was aged for 12 h. The synthesised microspheres were washed three times with ethanol and three times with deionised water. One fraction of the prepared microspheres was heated to 300 °C and the other to 500 °C for 2 h.

4.1.3 CHARACTERISATION

TGA were carried out on a Perkin Elmer TGA-7 apparatus with a heating rate of 10 °C/min, starting from room temperature to 700 °C. XRD pattern was determined on a D/max-rA XRD instrument using a copper X-ray source. The surface areas were determined by nitrogen sorption porosimetry on a micromeritics ASIC-2 apparatus.

4.1.4 PHOTOCATALYTIC DEGRADATION OF PHENOL

First, 90 mL of phenol solution with a concentration of 60 mg/L was prepared. Then, in three 50 mL beakers 30 mL phenol was added. The photocatalytic degradation was performed according to Table 4.1. In one beaker, 0.5 g of heat treated TiO$_2$ coated microspheres was added and in the other, 0.5 g of the TiO$_2$ microspheres with no heat treatment was added and in the last beaker heat treated SO$_3$H microspheres was added. The solution was stirred in the dark for 2 h in order to ensure establishment of the adsorption equilibrium. Then the solution
was exposed to UV irradiation using three 50 W Hg lamps. Samples were collected every hour to measure the degradation efficiency by UV/Vis spectrophotometry.

<table>
<thead>
<tr>
<th>Run (type)</th>
<th>P01</th>
<th>P02</th>
<th>P03</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO(_2)H(^a)</td>
<td>TiO(_2)-SO(_2)H</td>
<td>TiO(_2)-SO(_2)H(^0)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Heat treated samples (300 °C)

4.2 RESULTS & DISCUSSION

4.2.1 PREPARATION OF PHOTOCATALYTIC MICROSPHERES

First, the PS-SO\(_2\)H microspheres were stirred with TBOT in order to get a full adsorption of TBOT on the surface. Subsequently, water was added which hydrolysed the TBOT into Ti(OH)\(_x\). Last, the prepared microspheres were heated in an oven, this to transfer the titanium from anastase to crystalline form. The sample that was heated to 500 °C was completely burned out. A scheme of the preparation of TiO\(_2\)-coated microspheres is shown in Figure 4.1.

![Figure 4.1. Scheme for the preparation of TiO\(_2\) coated microspheres](image)

4.2.2 CHARACTERISATION

Optical images of TiO\(_2\)-SO\(_2\)H and heat treated TiO\(_2\)-SO\(_2\)H are presented in Figure 4.2 a)-b). Figure 4.2 a) shows that the microspheres were successfully coated with TiO\(_2\) and b) that the microspheres were decomposed and carbon was formed on the surface hence the black surface.

The result from the TGA is presented in Figure 4.3 a) and shows a comparison between the thermal decomposition of SO\(_2\)H, TiO\(_2\)-SO\(_2\)H, and heat treated TiO\(_2\)-SO\(_2\)H microspheres. The results shows that the weight percent of TiO\(_2\) in the heat treated TiO\(_2\)-SO\(_2\)H microspheres was 12 % and without heat treatment 8 %. The difference of the TiO\(_2\) weight percent is attributed to decomposition of the polystyrene in the heat treatment.

The XRD results presented in Figure 4.3 b) shows that there is no crystalline form of TiO\(_2\) in the TiO\(_2\)-SO\(_2\)H microspheres. In the TiO\(_2\)-SO\(_2\)H microspheres that were heated to 300 °C little crystalline TiO\(_2\) can be discernible. The TiO\(_2\)-SO\(_2\)H microspheres that were heated to 500 °C the characteristic peaks for crystalline TiO\(_2\) are clearly visible. However, these microspheres were almost completely burned out thus giving a strong signal to noise ratio.

The pore size distribution results are shown in Figure 4.3 c). When the microspheres are heat treated the pore size distribution becomes narrower. The micro and the high end mesoporous are burned out.
4.2.3 PHOTOCATALYTIC DEGRADATION OF PHENOL

The photocatalytic degradation of phenol processes is explained by Figure 4.4 and 4.5. In Figure 4.4 a simplified scheme for the formation of the reactive species that reacts with the phenol is presented. In Figure 4.5 a possible and simplified mechanism for the photocatalytic degradation of phenol is presented. The complete and exact pathway for the degradation is
very complicated and has many steps and intermediates. However, the end products are water and carbon dioxide.

Figure 4.4. Simplified scheme for the photocatalytic induced formation of reactive species [2.76]

Figure 4.5. Simplified mechanism of the photocatalytic degradation of phenol [4.16]

A scheme for the setup used for the photocatalytic degradation of phenol is presented in Figure 4.6. The UV-lamps produce heat so the beaker was cooled down in a water bath to ensure constant temperature in the study.

Figure 4.6. Scheme of the setup used for the degradation of phenol using TiO₂ coated microspheres

The results from the study of the photocatalytic degradation of phenol are presented in Figure 4.7 a)-c). As shown in Figure 4.7 a) it is obvious that the microspheres adsorb the phenol. This phenomenon could be used for other purposes. Unfortunately, the photocatalytic degradation of phenol using the TiO₂ coated microspheres did not work. In Figure 4.7 b) there is a noticeable increase of absorbance in time. This was attributed to that TiO₂ was leaking
from the microspheres, which concludes that the interaction between the microspheres and TiO₂ were not strong. However, the heat treated microspheres showed no leakage of TiO₂ from the microspheres.

Figure 4.7. Results from the photocatalytic degradation of phenol by a) heat treated sulfonated microspheres, b) TiO₂ coated microspheres c) heat treated TiO₂ coated microspheres

4.3 CONCLUSIONS

Several different methods were examined for the preparation of photocatalytic magnetic microspheres. The methods were the following: sol-gel technique, physical deposition of TiO₂ by heat treatment, and the method described above. The methods were tried under different conditions such as temperature and molar ratio. However, neither of them worked. The magnetic microspheres could not be coated with TiO₂, however the sulfonated microspheres were successfully coated with TiO₂. The Fe₃O₄-particles should not interfere with the TiO₂-coating. According to the literature, Fe₃O₄-particles can be coated with TiO₂. The magnetic microspheres have R-SO₃Na groups on the surface and the sulfonated microspheres have R-SO₃H groups. This is probably the main reason why the magnetic microspheres could not be
coated with TiO$_2$. Several attempts of replacing the Na with H were executed. However, only strong acids at low pH (pH 0-2) were able to change the Na to H, at pH 3-4 there was no change. Unfortunately, the Fe$_3$O$_4$ dissolves in strong acid environment and the magnetic microspheres lose their magnetic ability. However, after treatment with acid the microspheres could be coated with TiO$_2$. This concludes that if the Fe$_3$O$_4$-particles can be protected from the acid, TiO$_2$ coated magnetic microspheres could be synthesised. There are several possible ways of protecting the Fe$_3$O$_4$, for example to coat the particles with oleic acid or SiO$_2$. Another possibility is to synthesise magnetic microspheres using another method that the Fe$_3$O$_4$-particles are on the inside of the microspheres and not on the surface as with the method used in this thesis.

There are several possible reasons why the photocatalytic degradation of phenol did not work. The microspheres were too large to disperse in the phenol solution and because of that there was too little contact between the TiO$_2$ and phenol. This could be solved by using nano-sized microspheres which easily disperse in solution and have much greater surface area. The setup used for the photocatalytic degradation was very simple. If a photo reactor would have been used, the results would perhaps been successful. Also, using a glass beaker was not optimal, a beaker made of quartz would have been better because then the solution could have been exposed to more UV-light thus having higher efficiency.

The heat treated TiO$_2$ microspheres were also used for photocatalytic degradation of the anionic dye creosol red. The dye was pH and temperature sensitive and it was difficult to stabilise these parameters so the dye was changes to phenol. However, the microspheres showed quite good ability to adsorb this anionic dye and it could be desorbed using EtOH/H$_2$O 6/4 with saturated KCl. One interesting idea is to heat treat the magnetic microspheres under N$_2$ atmosphere thus making magnetic microspheres with active carbon surface.
CHAPTER 5: MESOPOROUS MICROSPHERES FOR BIOSEPARATION

Recently, all the genes in the human DNA was determined and identified in the HUGO-project. The HUGO-project was completed ahead of the time schedule. This was a result of the technological advances which took place during this time [5.1]. At present, the human proteomes are being investigated and catalogued [5.2]. Again, the technological advances probably make this work go faster and easier than planned. Quick, selective, and effective separation techniques in the biotechnology field are of high importance in order to study and fully understand the proteomes and the different processes in which the proteomes take part.

Mass spectrometry is the most used instrument for the characterisation of proteins. However, many samples cannot be directly analysed and there is a need for purification and preconcentration. For example in the enzymatic digestion of proteins were the digested intermediates and product are in small quantities there is a need for selective adsorption in order to investigate the process [5.3].

One promising separation technique is to use magnetic adsorbents in the separation of proteomes [5.3-5.5].

Lysozyme (Figure 5.1) is used in this as a model protein for the separation of proteins using magnetic microspheres.

![Figure 5.1. Three dimensional structure of lysozyme [5.6]](image)

5.1 EXPERIMENTAL

5.1.1 MATERIAL

Lysozyme was purchased from Sigma-Aldrich Co. H$_3$BO$_3$, NaOH, KCl, ethanol, methanol, citric acid, were purchased from Sinopharm Chemical Reagent Co., Ltd. All chemicals were used as received.

5.1.2 SEPARATION OF LYSOZYME

A 50 mL lysozyme solution (0.7 g/L) was prepared in a pH 9.6 buffer, which is near the pI for lysozyme. Into a vial, 0.2 g microspheres and 4 mL of lysozyme solution were added. The mixture was shaken for 30 min and then separated. The lysozyme was extracted from the microspheres by adding a pH 13 buffer and again shaken for 30 min. The adsorption and extraction efficiencies were determined using a UNICO 3802 UV/Vis spectrophotometer.

The adsorption and desorption process was optimised according to Table 5.1 and 5.2. Parameters such as pH and amount of adsorbent were evaluated.
Table 5.1 Different parameters for the optimisation of the adsorption of lysozyme

<table>
<thead>
<tr>
<th>Run (type, pH 9.6, 30 min)</th>
<th>L01</th>
<th>L02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mag 2</td>
<td>L21</td>
<td>L22</td>
</tr>
<tr>
<td>PS-SO$_3$H</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run (pH, 30 min)</th>
<th>L23</th>
<th>L24</th>
<th>L25</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 9.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2 Different parameters for the optimisation of the desorption of lysozyme

<table>
<thead>
<tr>
<th>Run (pH, 30 min)</th>
<th>M01</th>
<th>M02</th>
<th>M03</th>
<th>M04</th>
<th>M05</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run (E/W 6/4, pH 7, 30 min)</th>
<th>M11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sat. KCl</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run (M/W 4/6, pH 7, 30 min)</th>
<th>M21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sat. KCl</td>
<td></td>
</tr>
</tbody>
</table>

5.2 RESULTS & DISCUSSION

5.2.1 SEPARATION OF LYSOZYME

The results from the adsorption and desorption study of lysozyme is presented in Figure 5.2 a)-g). The magnetic microspheres did not adsorb lysozyme and the sulfonated microspheres showed quite low ability to adsorb the lysozyme as shown in Figure 5.2 a). This may be due to the pore volume and pore size, the lysozyme is too big to enter the pores and therefore the low adsorption capacity. At pH 4 the lysozyme did not adsorb any UV light and also at pH 11 the spectrum looks different compared with the other (Figure 5.2 b) and e)). This can be attributed to structural changes in the protein or denaturation of the protein. The adsorption efficiencies at pH 6 and 8 the adsorption were very low. This concludes that it is mainly the electrostatic interaction between the SO$_3^-$ group in the microspheres and the positive charge density in the protein that is responsible for the adsorption. For the extraction of the protein several different solution were used. By varying the pH the protein could be extracted from the microspheres. However, it seems that only at pH 13 the protein could be extracted (Figure 5.2 f)). The MeOH/H$_2$O 4/6 with saturated KCl showed nearly the same extraction efficiency as the pH 13 buffer (Figure 5.2 g)).
a) Adsorption of lysozyme

b) pH 4

c) Adsorption at pH 6

d) Adsorption at pH 8

e) Adsorption at pH 11

f) Lysozyme desorption at different pH

- Original
- Magnetic microspheres
- Sulfonated microspheres
5.2.2 Adsorption and Desorption Mechanism

A proposed mechanism for the adsorption and desorption of lysozyme onto the microspheres are shown in Figure 5.3 and 5.4. Lysozyme has a positive charge density at pH 9.6 and this can be exploited in the separation of different proteins. By changing the pH in the solution the proteins can be extracted from the microspheres as shown in Figure 5.6.

Figure 5.3. Scheme for the adsorption of Lysozyme

Figure 5.4. Scheme for the extraction of Lysozyme
5.3 CONCLUSIONS

The approach presented herein for the separation of protein could be very useful for sample preconcentration and purification. However, the adsorption and desorption efficiency is quite low. However, the efficiency can most likely be improved by decreasing the size of the microspheres, thus increasing the surface area and optimise the pore size and volume. The size of lysozyme is \( 4.5 \times 3.5 \times 3.5 \) nm [5.7] and the pore diameter distribution of the microspheres is from 1-100 nm, however the majority of the pores lie in the region of 1-10 nm [3.14]. The adsorbed lysozyme probably blocks the pores thus the theoretical maximum efficiency decreases. However, by increasing the pore diameter this phenomenon could be avoided. Liu et al. [3.14] reports that by using 1-chlorodecane as a porogen instead of toluene, the pore size increases.
CHAPTER 6: CONCLUSIONS

In this thesis, we have shown several possible applications for the porous polystyrene microspheres, both environmental and biotechnological applications. The magnetic microspheres showed great capacity of adsorbing cationic dyes. Also, by using the magnetic microspheres the problem with separating adsorbent from the effluent was easily solved by applying an external magnetic field. The porous polystyrene microspheres were successfully coated with TiO₂. Unfortunately, the magnetic microspheres could not be coated with TiO₂, however, the non-magnetic microspheres provide a much easier separation compared with when TiO₂ is used without being embedded in a carrier. The TiO₂ coated microspheres were not able to be used for photocatalytic degradation. One possible reason for that was that there were no or very little contact between the TiO₂ and the pollutant. This could perhaps be solved by decreasing the size of the microspheres thus increasing the surface area and make the microspheres disperse more easily in the solution. Suzuki et al. [6.1] solves this problem by incorporating TiO₂ in DNA. The DNA interacts easily with many compounds thus giving a great contact between the TiO₂ and pollutant. Finally, the microspheres were used for separation of protein from aqueous solution. The results show that it is possible to use these microspheres for separation of proteins. However, it would require further and more extensive research in the mechanisms and how the proteins get affected by the adsorption process. This separation method could be interesting using in lab scale were the microspheres can be altered in order to be more specific and efficient. The applications for the microspheres will probably increase in the future thus extensive research is being put in this field.
REFERENCES

CHAPTER 1


CHAPTER 2


[2.40] Limeback H., A re-examination of the pre-eruptive and post-eruptive mechanism of the anti-caries effects of fluoride: is there any caries benefit from swallowing fluoride?, Community Dent Oral Epidemiol., 1999, 27, 62-71


[2.58] Suleiman J.S, Hu B., Peng H., Huang C., Separation/preconcentration of trace amounts of Cr, Cu and Pb in environmental samples by magnetic solid-phase extraction with Bismuthiol-II-immobilized magnetic nanoparticles and their determination by ICP-OES, Talanta, 2009, 77, 1579-1583


53


[2.89] Lin S., Yun D., Qi D., Deng C., Li Y., Zhang X., Novel microwave-assisted digestion by trypsin-immobilized magnetic nanoparticles for proteomic analysis, J. Proteome Res., 2008, 7, 1297-1307

CHAPTER 3


CHAPTER 4

CHAPTER 5


[5.3] Li Y., Xu X., Qi D., Deng C., Yang P., Zhang X., Novel Fe₃O₄@TiO₂ core-shell microspheres for selective enrichment of phosphopeptides in phosphoproteome analysis, J. Proteome Res., 2008, 7, 2526-2538


[5.6] Protein Data Bank, 2009, http://www.pdb.org, This work has been released into the public domain by the copyright holder. This applies worldwide, Assessed: 2009-06-15


CHAPTER 6
PUBLICATIONS

Ericson M., Wang L., Recent research progress on application of magnetic adsorbents in the treatment of wastewater, submitted to Adsortion


ACKNOWLEDGMENT

I would like to thank all those people who helped me with this work, without them, I could not have completed this project. In no specific order, Professor Wang Li, Liu Qingquan, Per-Olof Persson, He Yingfang. Also, I would like to thank the group members at Zhejiang University for the good working environment and helping me in the laboratory.

END NOTE

All cited text in this thesis has been credited to the original authors. All data is my original and have not been manipulated and can be repeated. All figures have been made by me.