

# *Synthesis of novel magnetic Mesoporous adsorbent for isolation of sugar*

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**CERTIFICATE**

This is to certify that the dissertation entitled “**Synthesis of novel magnetic mesoporous adsorbent for isolation of sugars**” being submitted by **Vijaylakshmi Tirkey** to the Department of Chemistry, National Institute of Technology, Rourkela, Orissa, for the award of the degree of Master of Science is a record of bonafide research carried out by her under my supervision and guidance. To the best of my knowledge, the matter embodied in the dissertation has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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## 1. Introduction

The accurate detection of sugars is of great interest for improving long term health care. Boronate affinity chromatography has been proved as a promising method to purify sugars/glycoproteins from a long time [1-3]. Based on the unique property of boronate ligand to reversibly form cyclic esters producing a five or six membered ring with a 1, 2 diol in cis-coplaner geometry boronate affinity chromatography has been used as an general extraction module for carbohydrates/glycoconjugates [4-6]. The complexation of aryl boronic acid with 1,2-diols or 1,3-diols have been largely used for separation [7-8], detection[9-10] and transport of sugars [11-12]. For practical applications aryl boronic acid is immobilized on insoluble solid supports to promote solid phase extraction and reusability of aryl boronic acid as well as development of new efficient, selective and cheap sensors for bioseparation.

The 3- amino phenyl boronic acid [APBA] is the most common ligand used in boronate affinity chromatography [13-14].The aniline group on this ligand is used to couple it to solids supports and the meta amino substitution also lowers this ligand's pKa which is 8.8 and in this case the pH is above 8 or else the analyte can lose their biological activity at such high pH but their pH stability has also to be considered.

In the primary interactions esterification occurs between a boronate ligand and a cis -diol compound. This esterification requires that the hydroxyl groups of the diol be on the adjacent carbon atoms and in an approximately coplanar configuration (i.e. they should occur as a 1,2-cis diol or 1,3-cis diols) as they give stronger ester bonds stronger ester bond with 1,2 cis diols. The mechanism of interaction between boronic acids and cis diols is not fully understood. In aqueous solution under basic conditions, the boronate is hydroxylated and goes from a trigonal coplaner form to a tetrahedral boronate anion, which can then form esters with cis-diols. This resulting cyclic diester can be hydrolysed under acidic conditions, thus reversing the reaction. The covalent bonds form and it is quite reversible in aqueous solution.



Conventionally, MPBA carrying agarose or acrylamide-based polymeric beads have been used in chromatographic studies involving separation [15]. Recently Shimomura et al have synthesized a solid magnetic support by modifying magnetite particles with graft polymerization of acrylic acid [16 -18]. However, these polymer or magnetic polymer based supports suffer from severe drawbacks owing to its less surface area which ultimately results in less binding efficiency.

Development of a simpler and more versatile system for purification of sugars and sugar containing biomolecules has always been of great interest in this field. In recent years, magnetic adsorbents have been emerged as a new type of matrices for immobilization of proteins and enzymes. In the present work we have prepared magnetic mesoporous silica with surface active Boronate ligands which can be used as a binder and carrier for sugars.

## **2. Experimental:**

### **2.1 Chemicals**

All chemicals used here are of reagent grade and were used without further purification. FeCl<sub>3</sub>, FeSO<sub>4</sub> and Tetraethyl orthosilicate (TEOS) were obtained from Merck, Germany. m-aminophenyl boronic acid was supplied from Sigma-Aldrich, USA.

### **2.2. Preparation of boronic acid functionalized magnetic mesoporous silica**

The synthetic strategy for these surface-functionalized magnetic mesoporous silica has been outlined in scheme I.

#### **Step 1**

FeSO<sub>4</sub>.6H<sub>2</sub>O + FeCl<sub>3</sub> 90°C / N<sub>2</sub> atm Black ppt trisodium citrate Stabilised particle/ferrofluids

#### **Step 2**

TEOS

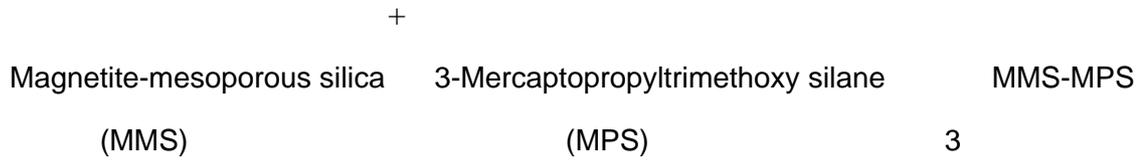
Ferrofluid

silica

Magnetite impregnated mesoporous

2

**Step 3** Synthesis of 3-Mercaptopropyltrimethoxysilane modified magnetite-Mesoporous Silica



**Step 4** Polygrafting of acrylic acid on MMS-MPS



4

**Step 5** attachment of 3-Aminophenyl boronic acid on PAA-MMS

PAA-MMS

APBA

MMS-APBA

4

For the preparation of magnetite First 50 ml of deoxygenated water was deaerated and degassed with nitrogen gas before use. 0.2978 gm of  $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$  and 0.3467 gm of  $\text{FeCl}_3$  in molar ratio 1:2 was dissolved in deionised water, 2.5 ml of 25% aqueous ammonia was added by vigorous stirring and nitrogen gas protection for an hour. The molar ratio of  $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_3$ , aqueous  $\text{NH}_3$  was found to be 0.00107 : 0.00214 : 0.1469. The pH of the suspension was maintained around 9 and the temperature was maintained around 70°C. After the reaction a black precipitate was obtained, 0.1 gm of  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$  was added to stabilise the particles. Then the suspension was filtered and washed with sufficient deionised water. The black precipitate obtained was found to be 0.25 gm. To make a concentration of 0.75 gm/l, 0.25 gram of the magnetite nanoparticles were resuspended in 330 ml of deionised water under continuous stirring at room temperature.

0.95 gm of tetraethylorthosilicate (TEOS) was slowly added to a solution containing 45 ml of 0.661 M and 0.0122 M cetyltrimethylammonium bromide (CTAB). The pH of the resultant mixture was measured to be around 11 and at room temperature under vigorous stirring. The resultant mixture was then poured into 330 ml of magnetite suspension (the suspension density was 0.75 gm/l) in deionised water while stirring on a magnetic stirrer. The whole mixture was stirred for 1 hour with 2 to 3 drops of HCl added and subsequently left unstirred for 2 hours. The final mixture was filtered and washed with deionised water till the surfactant was completely removed and the pH of the supernatant was measured to be around 7. All materials were subsequently washed with 1:1 mixture of ethanol and water to remove the surfactant CTAB from the mesopores. The nanocomposites so obtained are dried at room temperature in vacuum.

The magnetite-Mesoporous particles were treated with MPS in order to introduce mercapto groups onto their surfaces: a mixture of 0.5 g of magnetite, 0.5 g of MPS and 5 ml of dried toluene was refluxed under nitrogen for 20 h. The treated magnetite was filtered off, washed on a filter with dried toluene and then with methanol, and dried at 60 °C in vacuo.

Graft polymerization of acrylic acid was done by taking 0.25 g of MPS-magnetite, 1.78 g of acrylic acid and 5 ml of distilled water were charged. After deaeration of the mixture, a solution of 0.1833 g of ceric diammonium nitrate in 0.315 ml of 1 N nitric acid was added. The polymerization was carried out at 25°C with stirring under nitrogen. After a given time, the polymerization was stopped by the addition of hydroquinone. The reaction mixture was diluted with distilled water and centrifuged at 105 m/s<sup>2</sup> until the magnetite particles were precipitated completely. The precipitated magnetite particles were dispersed in distilled water and centrifuged once more. This procedure was repeated several times and the precipitated particles were dried at a temperature below 60 °C in vacuo. Although the magnetite particles can be separated with a magnet, centrifugal separation is favorable for experimental convenience and reproducibility. The amount of grafted poly(acrylic acid) was determined from the weight increase of the magnetite i.e. 0.3171 g. Attaching of 3-amino phenylboronic acid to magnetite particles were done by attaching it to the poly(acrylic acid)-grafted magnetite (PAA-magnetite), as shown in Scheme 4, by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid). EDC was used as a water-soluble condensing agent. A mixture of 0.005 g of PAA-magnetite, 0.08 g of 3-aminophenylboronic acid per mole of carboxyl groups of the grafted poly(acrylic acid) and 1.25 ml of distilled water was placed into a flask and stirred for 10 min. Subsequently, the pH value of the mixture was adjusted to 6.0 with 1 N sodium hydroxide solution, and the same molar amount of EDC as 3-aminophenylboronic acid was added. Then the mixture was stirred at 25 °C, the pH value being maintained at 6.0 by the addition of 1 N hydrochloric acid. After 20 h of stirring, the magnetite particles were completely precipitated. The

precipitated magnetite particles, i. e. the magnetite modified with 3-aminophenylboronic acid (DHBP-magnetite), were dispersed in distilled water, filtered off, and washed on a filter with distilled water. This washing procedure was repeated several times and the washed DHBP-magnetite was dried at a temperature below 60 °C in vacuo. The synthesized materials were characterized through XRD, SEM, SEM-EDX and FTIR analyses.

## **Result and Discussion**

### ***Synthesis***

The magnetite is prepared by a base to an aqueous mixture of Fe<sup>+2</sup> and Fe<sup>+3</sup> chloride at a 1:2 molar ratio. The precipitated magnetite is black in colour. The overall reaction may be written as following:



A complete precipitation occurs in the range of pH-9 to pH 14 and a molar ratio of Fe<sup>+3</sup> : Fe<sup>+2</sup> is 2:1 under a nitrogen atmosphere. The bubbling of nitrogen gas through the solution not only protects oxidant of the magnetite but also reduces the particle size. The Ferrofluid are colloidal suspensions of magnetic particles Fe<sub>3</sub>O<sub>4</sub>, forming magnetisable fluids that remain liquid in the most intense magnetic fields and finds widespread application. Magnetite-mesoporous silica was prepared by a co-precipitation approach using CTAB as a template. The as prepared Mesoporous silica Fe<sub>3</sub>O<sub>4</sub> material is expected to have high surface area and relatively high saturation magnetisation

The surface of MMS was coated with acrylic acid through radical graft polymerization. The radical graft polymerisation is a redox system consisting of ceric ion (Ce<sup>+4</sup>) and the Mesoporous silica Fe<sub>3</sub>O<sub>4</sub> particle carrying the reducing group like the mercapto group capable of initiating the radical polymerisation of acrylic acid. The introduction of mercapto groups onto the surface by the reactions of the surface silanol groups with 3-mercaptopropyltrimethoxy silane. In this redox system or polymerisation, polyacrylic acid was grafted on the surface of MPS- magnetite based on the propagation of the polymer from the radicals formed by the reaction of Ce<sup>+4</sup> with mercapto groups. The particle obtained from the redox graft polymerisation gave a stable colloidal dispersion in water. DHBP groups were attached to the poly(acrylic acid) grafted magnetite (PAA-magnetite), as shown in Scheme 4, by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid). EDC was used as a water-soluble condensing agent.

### ***XRD***

**Figure 1** a) XRD pattern of the black precipitate prepared in step1 2) XRD pattern of magnetite silica (MMS)

Figure 1a illustrates the XRD patterns of black particles. The position and relative intensity of all diffraction peaks match well with those of the magnetite (JCPDS 89-4319) and broad peaks indicates nanocrystalline nature of the particles. Figure 1b shows XRD pattern of magnetite-porous silica, the broad peak are due to amorphous silica matrix.

### ***SEM and SEM-EDX***

The morphology of the products MMS, MMS-MPS, MMS-PAA was investigated by scanning electron microscopy (SEM, Model JEOL JSM- 6480LV). Figure 2a, 2b and 2c shows SEM images of the above samples respectively. The composition of surfaces were investigated by energy dispersive X-ray spectroscopy.

















**Figure 2** SEM images of a) MMS, b) MMS-MPS, c) MMS-PAA

**Table 1** EDX data for MMS

Element	App Conc	Intens ity Corrn.	Weight%	Weight%	Atomic%
				Sigma	
C K	30.4	0.5678	37.75	0.70	48.89
O K	50.1	0.8480	41.59	0.64	40.44
Si K	25.3	0.9979	17.88	0.31	9.90
Fe K	3.05	0.7737	2.78	0.23	0.77
Totals			100.00		
Element	App Conc	Intens ity Corrn.	Weight%	Weight%	Atomic%
				Sigma	
C K	19.1	0.3943	36.62	1.10	50.05
O K	34.5	0.7694	33.76	0.91	34.64
Si K	21.0	1.0165	15.58	0.41	9.11
S K	11.6	0.9207	9.53	0.32	4.88
Fe K	4.68	0.7823	4.50	0.41	1.32
Totals			100.00		

**Table 2** EDX data for MMS-MPS

Element	App Conc	Intens ity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	12.86	0.3795	28.74	1.23	43.03
O K	35.02	0.8780	33.83	0.93	38.03
Si K	15.82	0.9895	13.57	0.39	8.69
S K	11.76	0.9311	10.71	0.37	6.01
Fe K	12.44	0.8021	13.16	0.64	4.24
Totals			100.00		

Table 3 SEM-EDX values for MMS-PAA

***FTIR***

[pic]

Figure 3 FTIR spectrum of MMS

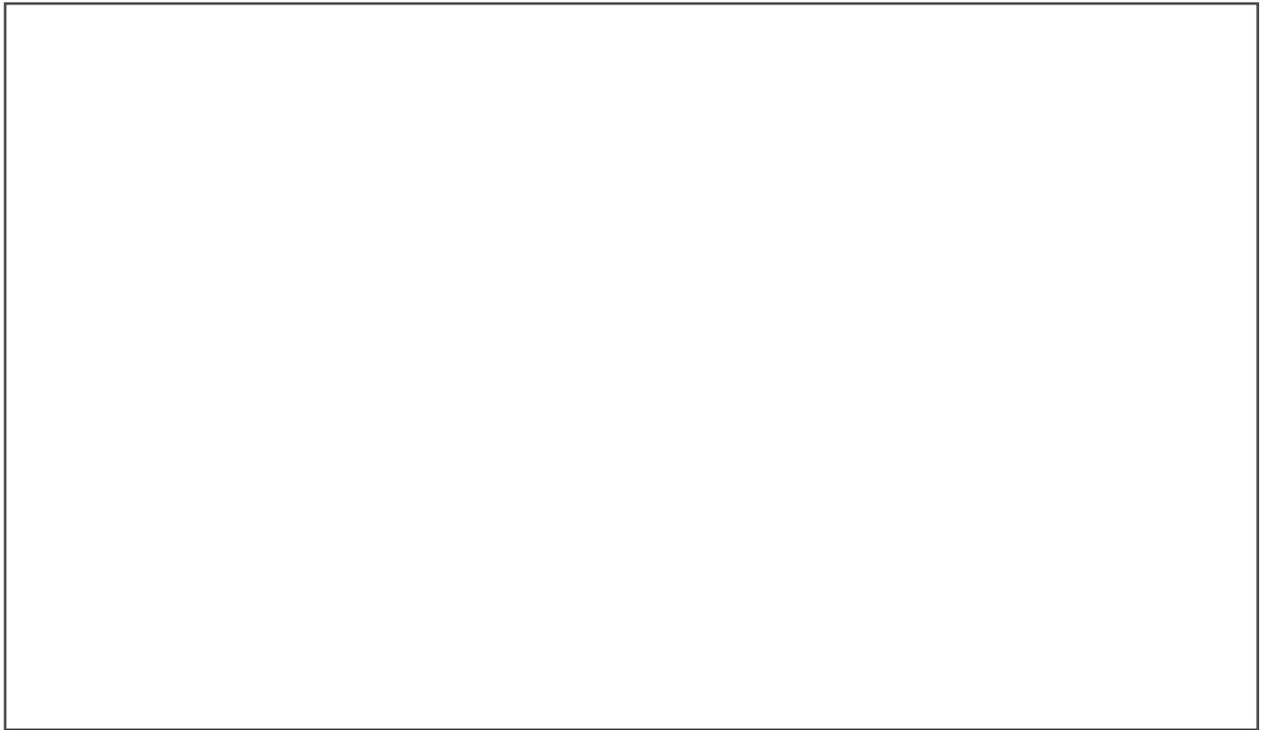


Figure 3 b FTIR spectrum of MMS-MPS

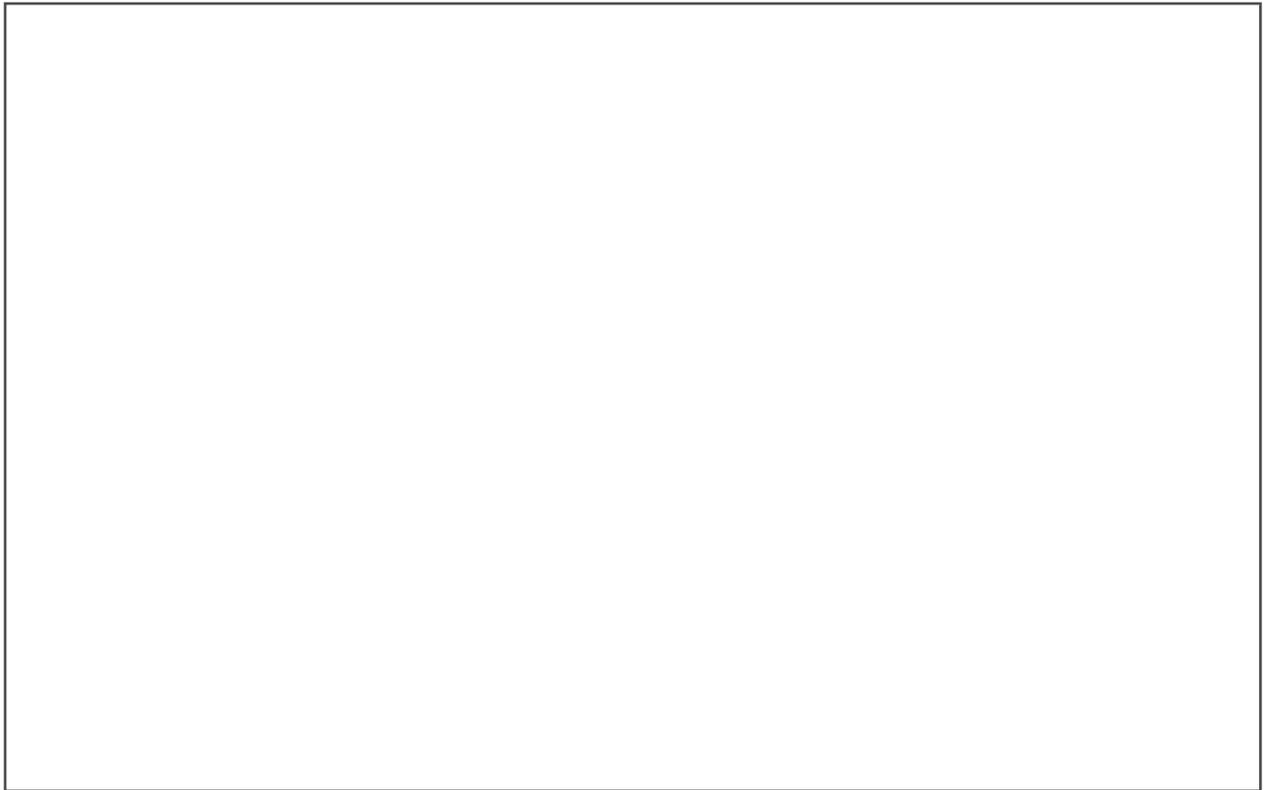


Figure 3c FTIR spectrum of MMS-PAA

Fig. 3a illustrates the IR spectra of the magnetite -mesoporous , three peaks at  $2852\text{ cm}^{-1}$  for C-H stretching , $2821\text{ cm}^{-1}$  for C-H stretching and  $3650\text{cm}^{-1}$ -  $3200\text{cm}^{-1}$  for O-H. fig 3b illustrates the IR spectra of the 3-Mercaptopropyltrimethoxysilane coated on magnetite - Mesoporous silica surface, the  $2927.47\text{ cm}^{-1}$  for C-H stretching (asymmetric), $2855.13\text{ cm}^{-1}$  for C-H stretching (symmetric),Around  $2550\text{ cm}^{-1}$  for SH group and  $1455.63\text{ cm}^{-1}$  for Methylene group (bend). Fig 3c illustrates the IR spectra of the polygrafted grafted poly (acrylic acid) on MPS-Magnetite surface.  $2927.47\text{ cm}^{-1}$  for C-H stretching (asymmetric), $2855.13\text{ cm}^{-1}$  for C-H stretching (symmetric), $1735.66\text{ cm}^{-1}$  for C=O , $1409.55\text{ cm}^{-1}$  for methylene groups and  $1237.89\text{ cm}^{-1}$  for C – O.

## Conclusion

The magnetite nanoparticles were synthesised Successfully using Ferrous sulphate and ferric chloride in 1:2 molar ratio using the co-precipitation method in an inert atmosphere and later impregnated into mesoporous silica matrix. The magnetite nanoparticles readily responds to the external magnetic field and nanoparticles were well dispersed. Poly(acrylic acid) was grafted onto the surface of magnetic by the redox polymerization of acrylic acid initiated from the surface which is indicated by SEM-EDX and FTIR. DHBP groups were attached through amide linkages by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid) shown by the FTIR.

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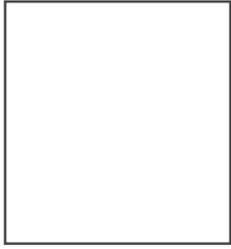
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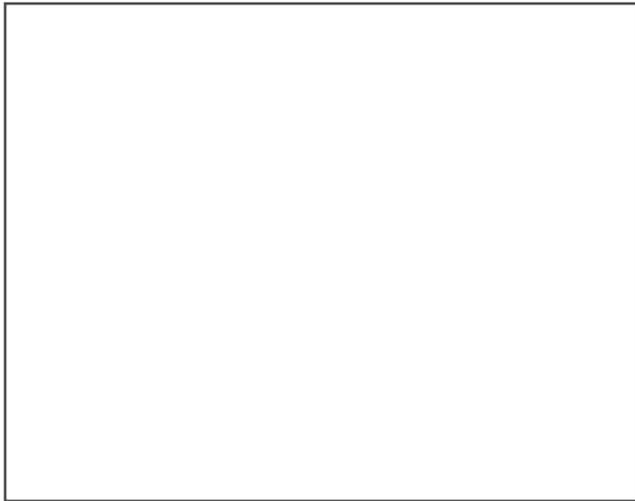
Ethanol , NH<sub>3</sub>





20 h 600C





b

a

b

a

c

4400.0

4000

3600

3200

2800

2400

2000

1800

1600

1400

1200

1000

800

600

450.0

-5.6

0

5

10

15

20

25

30

35

40

45

50

55

60

65

70

75

80

85

90

95

100

105

111.4

cm-1

%T

3853.91

3838.57

3801.84

3748.90

3673.48

3649.05

3615.02

3566.15

3445.70

2921.93

2852.30

2358.34

1747.66

1699.21

1683.87

1650.00

1557.69

1541.46

1521.39

1489.35

1474.04

1419.61

1396.62

1067.83

962.17

794.81

576.72