IMMOBILIZATION STUDIES UTILIZING SOLID SUPPORTS FOR THE DETERMINATION OF FRUCTOSE BY DANSYLAMINOPHENYL BORONIC ACID (DAPB ACID) AND CHROMATE BY DIPHENYLCARBAZIDE (DPC)

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IN
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Approval of the Graduate School of Natural and Applied Sciences.

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ABSTRACT

IMMOBILIZATION STUDIES UTILIZING SOLID SUPPORTS FOR THE DETERMINATION OF FRUCTOSE BY DANSYLAMINOPHENYL BORONIC ACID (DAPB ACID) AND CHROMATE BY DIPHENYLCARBAZIDE (DPC)

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Immobilization of fluorescent chemosensors and chromogenic reagents on solid supports for developing optical sensors result in improved analytical performance characteristics such as continuous read-out, increased sensitivity, lower reagent consumption and possibility of using the sensor in solvents where the free molecule displays low solubility.

The aim of this study is to immobilize dansylaminophenyl boronic acid (DAPB acid) and diphenylcarbazide (DPC) into various solid supports for the determination of fructose and hexavalent chromium, respectively.

DAPB acid reacts with diol containing molecules to produce electron transfer resulting fluorescence quenching. Whereas DPC reacts specifically with chromate to produce a magenta colored complex having absorption maximum at 540 nm.

Utilizing sol-gel technology, inorganic polymer matrices which enabled to observe fluorescence and absorbance signal in VIS region has been constructed. Also methylmethacrylate (MMA) and methacrylic acid (MAA), which are known to give transparent organic co-polymers, are chosen as monomers in the synthesis of organic co-polymer. Hydrogels such as polyvinyl alcohol and Ca-alginate gel have been utilized for their good optical characteristics in the working range.

Several considerations in the construction of host matrix were taken into account, such as the porosity of the polymers, functionalization of the matrix and use of additives for increasing the affinity of the medium toward the dopant molecule and swelling properties of organic polymers.

The performances of the immobilizations were evaluated in terms of the transmittance and leaching properties of the host matrix, optical properties of dopant and optical response characteristic of the dopant for the analyte. The sensor applications of the immobilized probe molecule DPC were investigated.

Studies regarding the enhancement of the performance of the flow injection analysis method for fructose determination, previously carried out in our laboratory, based on the fluorescence quenching of DAPB acid probe in solution were stated.

Key Words: Immobilization, Sol-gel, Dansylaminophenylboronic acid (DAPB acid), diphenylcarbazide (DPC)

ÖZ

DANSİLAMİNOFENİLBORONİK ASİT İLE FRUKTOZ TAYİNİ VE DİFENİLKARBAZİD İLE KROMAT TAYİNİ İÇİN KATI DESTEK MADDELERE TUTUKLAMA ÇALIŞMALARI

BULUT, Mukadder
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Temmuz 2006, 114 sayfa

Floresans özellik gösteren kimyasal probların, optik sensör tasarımı için, katı destek maddelere tutuklanması, tutuklanacak olan kimyasalın çözünürlüğünün düşük olduğu durumlarda çözücü içerisinde kullanımına yardımcı olur. Ayrıca sürekli okuma, duyarlılık artışı ve daha az reaktif tüketimi gibi avatajları da beraberinde getirir.
Bu çalışmanın ana amacı sırasıyla fruktoz ve altıdeğerlikli kromun tayininde kullanılmak üzere dansilaminofenilboronik asidin ve difenilkarbazitin katı destek maddelere tutuklanmasıdır. Dansilaminofenilboronik asit diol içerikli moleküllerle reaksiyona girerek sönümlenme ile sonuçlanan elektron transferine neden olur. Difenilkarbazit ise kromat ile neredeyse spesifik bir reaksiyon vererek absorbsiyon maksimumu 540 nm olan majenta renkli bir kompleks oluşumuna neden olur.

Sol-gel teknolojisi kullanılarak, floresans ve görünür bölgedeki absorbans sinyallarini gözlemleyebilme olanağı sunan inorganik polimer materyali geliştirildi.
Ayrıca ko-polimeri ışığı geçirgen olarak bilinen metakrilik asit ve metilmetakrilat kullanılarak organik ko-polimer sentezlendi. Polivinil alkol ve kalsiyum aljinat jel gibi çalışma bölgemizde iyi optik özelliklere sahip hidrojeller de kullanıldı.

Katı destek maddenin oluşturulmasında, polimerin porozitesi, ortamın dopant moleküle karşı afinitesini arttırmayı sağlayacak özellikte katkı maddelerinin kullanılması ve organik polimerlerin şişme özellikleri gibi birçok durum göz önünde bulunduruldu

Tutuklama çalışmalarının performansı, matriksin optik geçirgenliği, dopantın matriksten sızması, dopant molekülün optik özellikleri ve analite karşı dopantın gösterdiği karakteristik optik duyarlılığı yönünden değerlendirildi. Sensör uygulamaları için, tutuklanmış prob molekül difenilkarbazid incelendi.

Daha önce, çalışma gurubumuzun fruktoz molekülü tayini için,sulu ortamdaki DAPB asit prob molekülünün flloresans sönümlenmesine bağlı olarak geliştirmiş olduğu geliştirdiği akışa enjeksiyon sisteminin duyarlılığının geliştirilmesi üzerine çalışmalar yapıldı.

Anahtar Kelimeler: Tutuklama, Sol-jel, Dansilaminofenilboronik asit(DAPB asit), Difenilkarbazit (DPC)

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TO MY FAMILY

AND MY HUSBAND

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INTRODUCTION

1.1 Immobilization

Immobilization is a technique used for the physical or chemical fixation of cells, organelles, proteins or any type of molecule onto a solid support, into a solid matrix, in order to increase their stability and make their repeated or continued use possible [1].

1.1.1 Benefits of Immobilization

Immobilization offers the following advantages [2-5]:

- Multiple or repetitive usage of a single batch of sensing molecule.
- The ability to stop reaction rapidly by removing the sensor from the reaction solution.
- Stabilization of sensing molecule by bonding.
- Product and/or reaction solution are not contaminated with the sensing molecule (especially useful in the food and pharmaceutical industries)
- Long shelf life, predictable decay rates, elimination of reagent preparation.
- Economical analytical systems

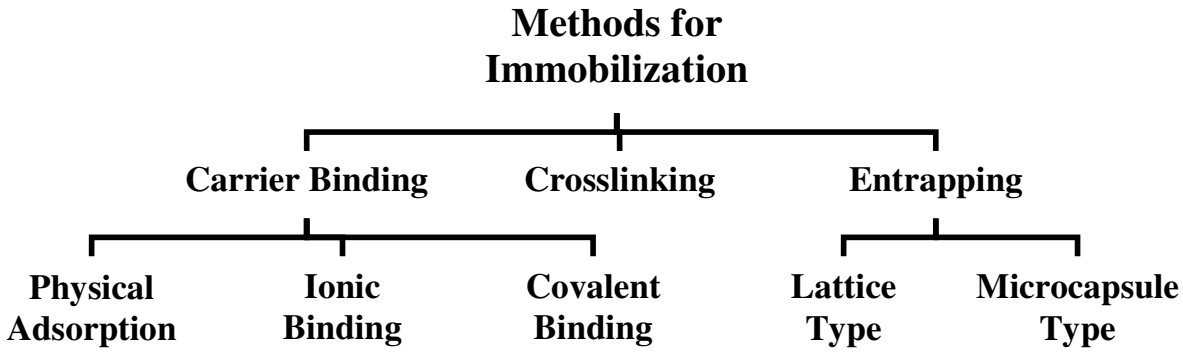
1.1.2 Methods for Immobilization

Immobilization of the analyte-sensitive fluorophore in the porous support matrix is a critical part in the fabrication process of a fluorescence based sensor. The immobilization method chosen for a particular sensor is generally based on the following considerations:

i. Compatibility of the fluorophore with the immobilization technique,
ii. Minimization of leaching, particularly for solution-based sensing,
iii. Influence of the immobilization method on the spectral or sensing properties of the fluorophore,
iv. Complexity and reproducibility of the method,
v. Support matrix considerations; for example, the requirement of hydrophobicity or high permeability,
vi. The ease of transfer of method to mass-production.

The methods for immobilization are shown in Table 1.1 [6].

Table 1.1 Methods for Immobilization



1.1.2.1 Carrier Binding

The carrier binding method is the oldest immobilization technique for dopant molecules such as sensors and enzymes. In this method, the amount of dopant molecule bound to the carrier and the activity after immobilization depend on the nature of the carrier [7].

The selection of the carrier depends on the nature of the dopant molecule itself, as well as its following properties

- Particle size,
- Specific surface area,
- Molar ratio of hydrophilic to hydrophobic groups,
- Chemical composition, of the dopant molecule.

According to the binding mode, the carrier binding method can be sub-classified into three groups.

i) Physical adsorption

ii) Ionic binding

iii) Covalent binding

i) Physical Adsorption

This method is based on the physical adsorption of dopant molecule on the surface of water-insoluble carriers. Adsorption tends to be less disruptive than chemical means of attachment because binding is mainly by hydrogen bonds, multiple salt linkages and Van der Waal's forces. Hence, the method cause little or no conformational change of the molecule or destruction of its active center.

This method is simple and cheap but it has the disadvantage that the adsorbed molecule may leak from the carrier due to weak binding forces between the dopant molecule and the carrier. Another disadvantage is the non-specific adsorption of foreign molecules that are present in the matrix [8].

ii) Ionic Binding Mode

Ionic binding method is based on the electrostatic attraction between oppositely charged groups of the carrier material and the dopant molecule.

Polysaccharides and synthetic polymers having ion-exchange centers are usually used as carriers. The ionic binding method cause little changes in the conformation and the activities of the binding side of the sensing molecule.

Leakage of the dopant molecule from the carrier may occur in reaction solutions of high ionic strength or upon variation of pH due to the weaker binding forces compared to covalent binding [8].

iii) Covalent Binding Mode

In covalent binding, the atoms are linked by means of shared electron pairs. This method is the most intensely studied immobilization technique between the sensing molecule and the support matrix [9-11].

1.1.2.2 Cross-Linking

Immobilization of sensing molecule has been achieved by intermolecular cross-linking of dopant molecule, either to other dopant molecules or to functional groups on an insoluble support matrix. This method is both expensive and insufficient, as some of the sensing molecule will inevitably be acting mainly as a support and the reactions are carried out under relatively harsh conditions and so may lead to significant loss of activity [12, 13].

1.1.2.3 Entrapping

Entrapping is based on incorporating sensing molecules into the lattices of a semi-permeable gel or enclosing the sensing molecules in a semi-permeable polymer membrane. It is performed in such a way to retain the sensing molecule while allowing penetration of analyte. This method can be classified into lattice and microcapsule types.
Lattice type involves the entrapment of sensing molecules within the interstitial spaces of a cross-linked water-insoluble polymer.

In microcapsule type the entrapment takes place within a semi-permeable polymer membrane [14-16].

Entrapment differs from covalent binding and cross-linking that the sensing molecule itself does not bind to the gel matrix or the membrane. This characteristic allows the wide applicability of this method.

1.2 Solid Supports Used for Entrapment

1.2.1 Hydrogels

Hydrogels are cross-linked 3-D network structures composed of hydrophilic polymers, which do not dissolve in water at physiological temperature and pH, but absorb a great deal of water. Hydrogels have increasingly attracted attention in sensor designing because of their biocompatibility, flexibility and optical transparency [17,18].

1.2.1.1 Polyvinylalcohol (PVA)

Polyvinyl alcohol (PVA) is a water-soluble, biocompatible and degradable polymer. PVA-based hydrogels have been prepared by chemical and physical cross-linking methods (Figure 1.1) [19-21].

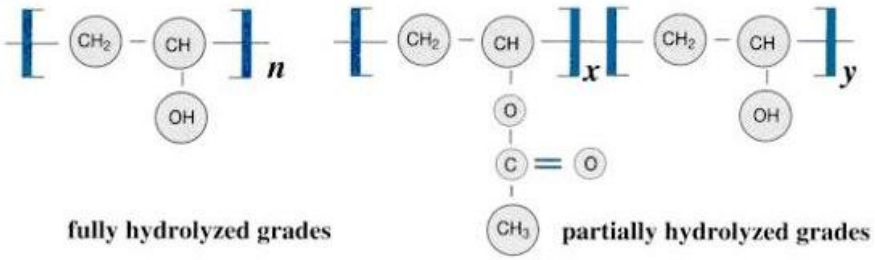


Figure 1.1 Structure of Polyvinyl alcohol

Poly(vinyl alcohol) can be readily crosslinked for improved water resistance [22]. The most practical means of crosslinking PVA is with chemical additives, e.g., glyoxal, urea-formaldehyde, glutaraldehyde. Boric acid, borax and succinic acid also reacts strongly with PVA and are widely used as gelling agent. PVA is extremely sensitive to borax, which causes gelation by forming a bisdiol complex.

PVA offers many advantages such as excellent optical property, hydrophilicity, biocompatibility, tough and clear film forming property and ease of handling [23].

1.2.1.2 Calcium Alginate Gel

Alginic acid is a linear polysaccharide normally isolated from many strains of marine brown seaweed and algea. It consists of two uronic acids: D-mannuronic acid (M) and L-guluronic acid (G) [24].

In contrast to most other polysaccharide gels, alginate gels can be developed and set at constant temperature. This unique property is particularly useful in applications involving fragile materials like cells or tissues with low tolerance for higher temperatures [25].

An alginate gel can be developed instantaneously in the presence of divalent cations like Ca^{2+} , Ba^{2+} or Sr^{2+}, at low pH. Gelling occurs when the divalent cations take part in the interchain ionic binding between guluronic acids blocks (G-blocks) in the polymer chain giving rise to a three dimensional network [26].

Positively-charged calcium ions, $Ca^{2+,}$ are attracted to the negatively-charged carboxylic acid groups, COO^- , of the alginate polysaccharide chains. Ca^{2+} ions fit into guluronate structures like eggs in an egg-box. (Figure 1.2)

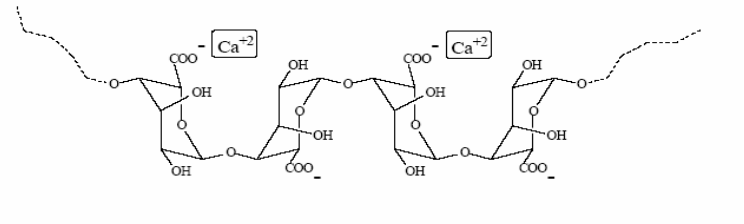


Figure 1.2 Representation of Ca-Alginate Gel Structure

1.2.2 Co-polymers

When two different types of monomers are joined in the same polymer chain, the polymer is called a copolymer.

Methacrylic acid interacts ionically with the amine functional group via hydrogen bonding with a variety of polar functionalities such as hydroxyl groups, carbamates, and carboxylic esters (Figure 1.3) [27]. Hence it is one of the most widely used monomers in Molecular Imprinting Technology and sensor designing.

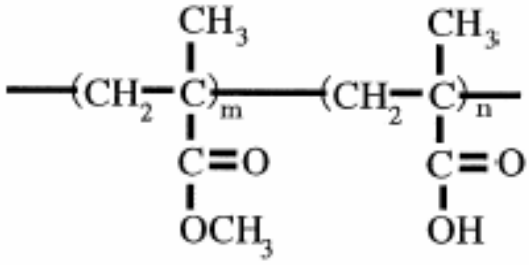


Figure 1.3 Poly(methylmethacrylate-co-methacrylic acid) Structure

1.2.3 Sol-gel

The basic process involves hydrolysis and condensation polymerization of the appropriate metal (mainly silicon) alkoxide solution.

This process is followed by a temperature program which controls the densification process. As the polymerization takes place in solution, the monomer solution gradually thickens and becomes a gel. On drying, the gel slowly shrinks and when dry, becomes a rigid, amorphous, porous mass called a xerogel. The rate at which the gel dries out and collapses on the substrate has a major effect on the porosity, which in turn affects the mechanical, optical and electronic properties of these amorphous oxide glasses [28].

The characteristics of the xerogel of a particular composition are related to the factors that affect the rate of hydrolysis and polycondensation reactions. So far, pH, gelation temperature, aging time and temperature and concentration and molar ratio of water and

metal alkoxide have been identified as the most important parameters [29-32].

The hydrolysis that initiates the sol-gel process may be acid- or base-catalyzed. The pH value of the sol has significant influence on the microstructural properties of the final material.

Acid catalysis is associated with fast hydrolysis rates and relatively long gelation times, whereas, under basic conditions, hydrolysis is slow and condensation rates are faster, giving rise to shorter gelation times. Consequently, acid catalysis generally results in weakly branched structures with small pores (<2nm), whereas base catalysis results in a particulate gel with large pores [33].

Organic modification of ceramic sol-gel materials is a convenient way of controlling the material properties through the rich library of metal alkoxide monomers bearing a nonhydrolizable substituent. The introduction of organic groups covalently attached to the silica network has provided a way of controlling the polarity of the pore surface. In this way, organically modified matrices exhibited remarkably lower polarity compared with those prepared without organic substituents. This reduction in polarity is due to the lower polarities of the R groups as compared with the strongly polar silanol (Si–OH) groups on the surface [34].

Sol-gel immobilization of sensor reagents is generally achieved by addition of the reagent to the precursor solution, either at the start of the process or a later stage before coating. In both cases,

immobilization is achieved by physical entrapment in the nanometer-scale cage-like network, which grows around the dopant.

It is immediately evident that the main feature of sol-gel entrapment is its simplicity. Sol-gel entrapment offers the advantages of a chemically inert matrix which is both photochemically and thermally stable and has good optical transmission properties in the visible and near infrared regions.

1.3 Coating Methods

The general prerequisites for obtaining wet chemical coatings with high optical qualities on glass can be stated as the coating step has to be carried out under cleanroom conditions, the coating liquid has to be filtered and the glass has to be cleaned properly. There are various coating techniques such as; dip coating, spray coating, flow coating, spin coating, capillary coating and roll coating [35].

Dip and Spin coating techniques are further explained in the following sections.

1.3.1 Dip Coating Technique

Dip coating technique can be described as a process where the substrate to be coated is immersed in a liquid and then withdrawn with a well-defined withdrawal speed under controlled temperature and atmospheric conditions (Figure 1.4) [35].

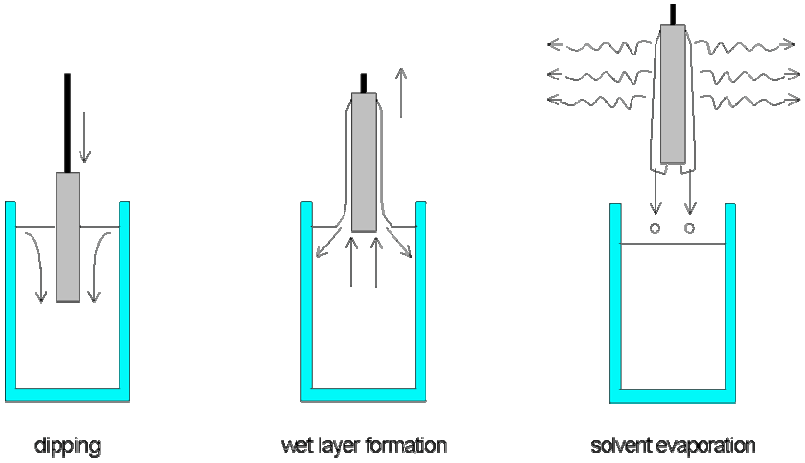


Figure 1.4 Stages of Dip Coating Process

1.3.2 Spin Coating Technique

In the spin coating process, the substrate spins around an axis which should be perpendicular to the coating area. The spin-on process has been developed for the so-called spin-on glasses in microelectronics and substrates with a rotational symmetry, e.g. optical lenses or eye glass lenses (Figure 1.5) [35].

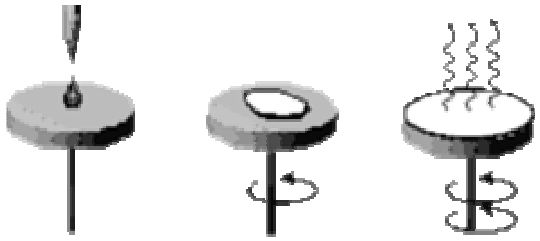


Figure 1.5 Stages of Spin Coating Process

1.4 Determination of Fructose

The most common analytical methods are based on GC [36] or HPLC [37,38] determinations with either UV–VIS spectrophotometric [39,40] or refractive index [37] detection but often involve tedious sample preparation and derivation. Alternatively, enzyme biosensors are commonly used for the determination of glucose or fructose, based on the reaction of glucose or fructose with enzymes [41]. The enzymatic method has the disadvantage that it requires maintaining a strict physiological condition for enzyme activity, in spite of its comparatively good selectivity [42].

1.4.1 Determination of Fructose with Boronic Acids

Boronic acids [R-B$(OH)_2$] have the unique properties of forming reversible complexes with diol-containing compounds to give boronate esters [43-45]. All saccharides and polysaccharides have a number of cis- and trans-diols and therefore rapidly form diol-boronic acid complexes when dissolved in basic aqueous media [46]. Such tight binding allows boronic acids to be used as the recognition moeity in the construction of PET (Photoinduced Electron Transfer) molecules specific for saccharides [47].

M.Volkan et al. reported a flow injection procedure for a fluorometric determination of fructose, glucose, dopamine, and epinephrine based on quenching of the fluorescence of m-dansylaminophenyl boronic acid (DAPB acid) [48].

1.4.2 Photo Induced Electron Transfer Systems and DAPB Acid (m-dansylaminophenylboronic acid)

Generally, PET model compounds are composed of three parts: a fluorophore module is usually based on a polycyclic aromatic system and is the site of both photonic transactions of excitation and emission, a spacer module holds the fluorophore and receptor close to, but separate from each other, a receptor module responsible for guest complexation. Dansylaminophenylboronic acid,DAPB acid, used in our studies, is a PET molecule.

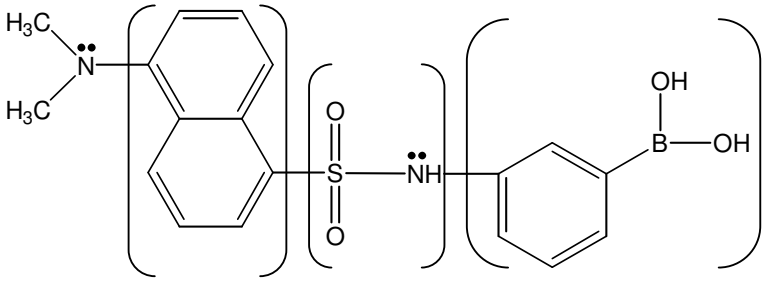


Figure 1.6 Chemical Structure of m-Dansylaminophenyl Boronic Acid, a PET molecule.

The response which signals an interaction between analyte and receptor is usually communicated by changes in fluorescence intensity either through chelation enhanced quenching or chelation enhanced fluorescence. Charge-separating process, especially those separating a full electronic charge, is highly sensitive to environmental stimuli. Pet is ideal process fitting this description, even though charge shift can

be seen intrinsically charged systems. This environmental sensitivity shows up in fluorescence (quantum yield and life time but not wavelength) since it competes with electron transfer to deactivate the photoproduced excited (normal PET) state or to activate the photoproduced excited state (reverse PET). [46-51]

1.4.3 Organized Media

Organized media (OM) are amphiphile aggregates or polymers which form anisotropic microstructures in solution. Examples of widely utilized OM include surfactant micelles, crown ethers and cyclodextrins [52]. OM assemblies have a great potential in many areas of analytical chemistry. These systems have been used in separation science as mobile and stationary phase modifiers [53, 54], in spectrometry to enhance luminescence [55].

Molecules which posses both hydrophilic and hydrophobic structures may associate in aqueous media to form dynamic aggregates commonly called micelles, that are able to include or to organize solutes in their interior or in their colloidal surface. When a solute passes from the aqueous medium to the micellar medium, some changes are usually observed in several properties like reactivity, solubility or spectroscopic characteristics [56-59]; thus in fluorescence there have been important increases in the sensitivity [60-62]. Also the relative high viscosity of these micellar microenvironments can inhibit quenching by molecular oxygen [63].

1.5 Preconcentration and Speciation of Chromium

Chromium is an essential trace element in the human body playing a role in the metabolism of glucose and certain lipids, mainly cholesterol [64-65].

In anything other than trace amounts, chromium compounds should be regarded as highly toxic.

Chromium can exist in oxidation states ranging from -2 to +6 but is most frequently found in the environment in the trivalent (Cr^{3+}) and hexavalent (CrO_4^{2-},$Cr_2O_7^{2-}$) oxidation states.

Hexavalent chromium is more toxic than the trivalent form because its oxidizing potential is high and it easily penetrates biological membranes [66].

Chromium salts are used extensively in industrial processes and may enter a water supply through the discharge of wastes. Effluents from industrial facilities dealing with chromium are frequently monitored to ensure proper chromium removal prior to release into the environment [67, 68]. Therefore, accurate determination of chromium is important for monitoring environmental pollution and for quality control of industrial products.

Methods available for measuring chromium in water samples include spectrophotometry [69-76], titrimetry [77], colorimetry [78], atomic absorption spectrometry [79, 80] and ion chromatography [81, 82].

According to Castillo et al., although Cr(VI) shows a characteristic spectrum in the visible range, the use of a chemical reaction for

chromium derivatization offers advantages, such as improving selectivity. In the field of optical sensors, different systems have been proposed to immobilise chromogenic reagents onto membranes or onto fiber optic surfaces [83].

O.S.Wolfbeis et al. have introduced an optical probe for determination of chromate ion, based on a sol-gel film containing diphenylcarbazide (DPC) [84].

1.5.1 Diphenylcarbazide (DPC)

DPC (diphenylcarbazide) (Figure 1.7) is a;

- White crystalline solid which gradually turns pink on standing.
- Its melting point is suitable for the sol-gel process (172-173 ºC)
- Slightly soluble in water and soluble in alcohol, acetone and glacial acetic acid.
- Gives sensitive color reactions with many ions particularly with those of the second group of the periodic system (Some of the colored compounds are oxidation products, while others are chelate salts)[85].
- Forms a red-violet colored complex with hexavalent chromium in acidic medium.
- Molar absorptivity based on chromium is 35,000 M^{-1} cm^{-1} at the wavelength of 540 nm [86].

- Does not bind Cr(III)
- Reaction of Cr(VI) with DPC (Figure 12) is considered near specific if interferences by Mo(VI) (> 200 mg L^{-1}), mercury salts (>200 mg L^{-1}) vanadium concentrations (>10 times of Cr(VI)) and ferric iron (> 1 mg L^{-1}) are avoided [87].

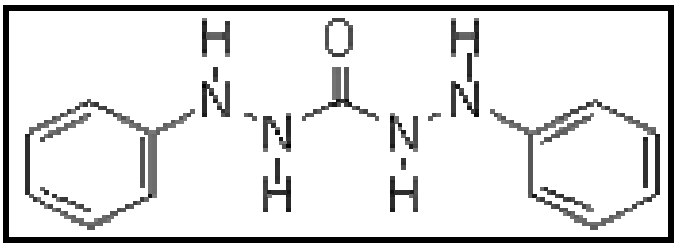


Figure 1.7 Structure of DPC

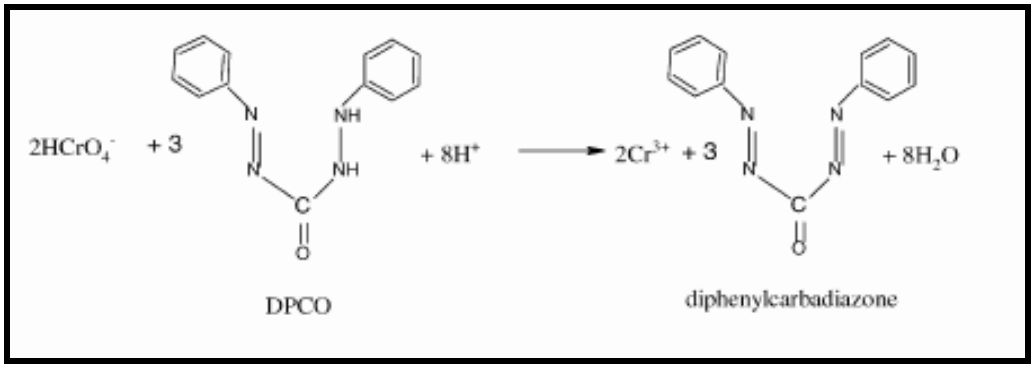


Figure1.8 Reaction between DPC and Hexavalent Chromium

1.6 The Aim of This Work

In previous studies carried out in our laboratory, flow injection analysis methods (FIA) based on the fluorescence quenching of a dansylaminophenylboronic acid DAPB acid probe in solution, which was successfully applied to fructose determination in commercial fructose syrups and wine [48] and absorbance measurement of diphenylcarbazide (DPC) chromophore in solution which was applied to the determination and speciation of chromium in natural waters have been developed. In view of the analytical performance characteristics of these systems, DAPB acid and DPC seemed a good starting point to develop a solid phase sensors for fructose and chromate respectively. Therefore the aim of this study can be stated as follows:

In part I we first outline the immobilization of DAPB acid to inorganic and organic polymeric matrices. Secondly the studies regarding to the increase in sensitivity of the FIA method for fructose determination, mentioned above, in the presence of organized media was investigated.

In part II the immobilization of DPC to inorganic and organic polymeric matrices was examined.

The performance of the immobilizations will be evaluated in terms of the transmittance and leaching properties of the host matrix, optical properties of dopant and optical response characteristic of the dopant for the analyte.

CHAPTER 2

EXPERIMENTAL

PART I

Immobilization Studies on DAPB Acid for the Determination of Fructose

2.1 Chemicals and Reagents

i. Alginic acid sodium salt ($C_6H_9NaO_7$), Fluka
ii. Calcium chloride ($CaCl_2$), Riedel de Haen
iii. Polyvinyl alcohol (PVA), Aldrich
iv. Sodium tetraborate ($Na_2B_4O_7$)(Borax)
v. Boric Acid (H_3BO_3), Fluka
vi. Succinic Acid ($C_4H_6O_4$), Fluka
vii. Glutaraldehyde ($C_5H_8O_2$), Fluka
viii. Tetramethoxysilane (TMOS) 98% ,[$Si(OCH_3)_4$], Aldrich
ix. Tetraethoxysilane (TEOS) 98% , [$Si(OC_2H_5)_4$] Aldrich
x. Hexadecyltrimethoxysilane,(HDTMOS), [$CH_3(CH_2)_{15}Si(OCH_3)_3$], Aldrich
xi. Phenyl-tetraethoxysilane (Ph-TEOS), [$C_6H_5Si(OC_2H_5)_3$],Aldrich
xii. Methanol , extra pure,(CH_3OH), Merck
xiii. Ethanol , HPLC grade,(C_2H_5OH), Aldrich
xiv. Brij 30, [$C_{12}H_{25}(OCH_2CH_2)_4OH$], Aldrich
xv. TritonX-114 (Octylphenoxypolyethanol), ($C_{28}H_{50}O_8$), Sigma
xvi. Igepal,[4-(C_9H_{19})C_6H_4-(OCH_2CH_2)$_5$OH], Aldrich

xvii. Cetyltrimethylammonium bromide (CTAB), [$CH_3(CH_2)_{15}N(CH_3)_3Br$], Merck

xviii. Sodium dodecyl sulphate (SDS), [$CH_3(CH_2)_{11}OSO_3Na$], Aldrich

xix. Nitric Acid 65%, extra pure ,(HNO_3), Merck

xx. Hydrochloric Acid 35% extra pure, (HCl), Merck

xxi. Methylmethacrylate (MMA) 99%, ($C_5H_5O_2$), Merck

xxii. Methacrylic acid (MAA) 99%, ($C_4H_6O_2$), Merck

xxiii. Ethylene glycol dimethacrylate (EDGMA) 98%, ($C_{10}H_{14}O_4$), Merck

xxiv. Benzoylperoxide (BP), ($C_{14}H_{10}O_4$), Merck

xxv. Acetone 99%, (C_3H_6O), J.T.Baker

xxvi. m-Dansylaminophenyl boronic acid (DAPB acid),($C_{18}H_{19}O_4N_2BS$), solution (10^{-3} M): Prepared by dissolving 3.70 mg DAPB acid (Fluka) in 1 mL dimethylsulphoxide (DMSO, Labscan) and diluting with deionized water to 10 mL.

xxvii. Sodium Phosphate Buffer (pH 9): Prepared by dissolving 2.627 g Sodium Phosphate Salt (dibasic),(Na_2HPO_4), Fisher, and 0.0209 g Sodium Phosphate Salt (monobasic),(NaH_2PO_4), Fisher, in 100.0 mL deionized water.

xxviii. Fructose stock solution (1 M): Prepared by dissolving 4.5 g fructose, ($C_6H_{12}O_6$), (Fluka) in 25.00 mL phosphate buffer (pH 9.00).

xxix. Sodium dodecyl sulphate stock solution (0.1 M): Prepared by dissolving appropriate amount of SDS (Aldrich) in phosphate buffer at pH 9.00.

xxx. Cetyltrimethylammonium bromide stock solution (0.1 M): Prepared by dissolving appropriate amount of CTAB (Merck) in phosphate buffer at pH 9.00.

xxxi. PVA (10% w/v) stock solution: 10.0 g PVA was dissolved in 100.0 mL phosphate buffer at pH 9.00.

xxxii. Na-alginate (10% w/v) stock solution: 10.0 g alginic acid sodium salt was dissolved in 100.0 mL phosphate buffer at pH 9.00.

All other reagents were of analytical reagent grade. De-ionized water obtained from a Milli-Q water system was used for sample and standard preparations. All the glass and plasticware were soaked in 10% HNO_3 for at least 24 hours and then rinsed with de-ionized water.

2.2 Apparatus

Fluorescence (emission and excitation) spectra of doped samples were recorded at room temperature using a Perkin Elmer LS-50B Luminescence Spectrometer by 90 ° reflection with excitation emission slits of 15 nm. Best excitation and emission wavelengths were 324 nm and 529 nm, respectively. For enhanced fluorescence measurements the emission and excitation slits were adjusted to 5.0 nm and 7.5 nm, respectively.For absorption studies, Shimadzu UV-VIS 160 Spectrometer was used.

All spin coating procedures onto glass substrates were performed with the Chemat Technology Spin Coater KW-4A.

2.3 Preparation of Glass Supports

Microscopic glass slides (Sail Brand, unground edges 25.4x76.2 mm; 1mm-1.2mm thickness) were cut into 25.4 mm x 13.0 mm sized

pieces in order to place them in the cuvettes. These glass supports were first treated with 1% non-ionic detergent solution (TritonX-114) for 15 min in an ultrasonic bath, then washed with deionized water and kept in piranha solution ($1H_2O_2$:$5H_2SO_4$, v/v) overnight. Finally, the slides were rinsed with deionized water, dried in an oven at 110 ° C and stored in a dessicator before use.

2.4 Preparation of Solid-Supports

2.4.1 Preparation of PVA films

Pre-cleaned glass supports were dipped into $5.00x10^{-4}$M DAPB acid containing %3(w/v) PVA solution for 5 seconds and kept at 100ºC for 2 hours. The coated supports were then treated with different types of crosslinkers shown in Table 2.1. Crosslinker solutions were spread over the glass slides by using a sprayer and the slides were dried at 100 ºC for hardening the film.

Table 2.1 Types and concentrations of crosslinkers

Type	Crosslinker	Concentration of crosslinker
1	Borax	1%
2	Boric acid	3%
3	Succinic acid	5%
4	Glutaraldehyde	8%

2.4.2 Preparation of Ca-Alginate Gel Films

Pre-cleaned glass supports were dipped into $5.00x10^{-4}$M DAPB acid containing Na-Alginate (3% w/v) solution for 5 seconds and kept at room temperature for 15 minutes. 3% (w/v) $CaCl_2$ solution was spread over the glass slides by using a sprayer and the slides were dried at room temperature for five minutes. The prepared slides were kept in $CaCl_2$ solution for further hardening of films.

2.4.3 Preparation of Co-Polymer Films

4.0 mL of methyl methacrylate (MMA), 1.0 mL of methacrylic acid (MAA), 0.05 g of benzoyl peroxide (BP), 10.0 mL of acetone and 3.7 mg of DAPB acid were mixed using magnetic stirring at 60 °C for 4 hours. After polymerization, glass substrates were coated with the co-polymer formed.

Coated substrates were maintained at room temperature overnight for the evaporation of acetone.

2.4.4 Preparation of Polymethylmethacrylate (PMMA) Films

5.0 mL of MMA, 0.05 g of benzoyl peroxide, 10.0 mL of acetone and 3.7 mg of DAPB acid were mixed using magnetic stirring at 60° C for 4 hours. After polymerization, glass substrates were coated with the polymer formed. Coated substrates were maintained at room temperature overnight for the evaporation of acetone.

Also, a polymer blend was prepared using the hydrophilic polymer polyethyleneimine and the hydrophobic polymer polymethyl methacrylate. They were mixed at a ratio of 1:5 (v/v) in acetone used as co-solvent.

2.4.5 Preparation of Sol-Gel Films

Type 1

300 µL of precursor mixture was prepared using different molar ratios of Me-TriMOS (Methyl Tri-Methoxysilane), TMOS (Trimethoxysilane), 1:1, 1:2, 1:3). 400 µL of methanol were added to these precursor mixtures and sonicated for 3 minutes. Then, 100 µL of 1.0 mM HCl were added to this mixture to start hydrolysis and condensation reaction. After 5 minutes of sonication, 40 µL of dye solution of DAPB acid ($1.0x10^{-3}$ M in methanol) were added to the sol-gel solution and the mixture was further sonicated for 5 minutes for homogeneity. The sol-gel solution was left to stand several hours at room temperature for gelation to take place. The support materials were spin-coated with this sol-gel at 3000 rpm for 60 seconds and then, recovered slowly and steadily to provide an even coating that was allowed to dry for 24 hour at ambient temperature.

Type 2

4.0 mL of TEOS, 3.8 mL of EtOH , 1.92 mL of deionized water were mixed immediately. 100 µL of 0.1 M HCl and 1.0 mL of DAPB acid solution (10^{-3} M in EtOH) were added to this mixture respectively followed by 30 minutes mixing.

The support materials were spin-coated with this sol-gel solution at 3000 rpm for 60 seconds and then, recovered slowly and steadily to provide an even coating that was allowed to dry for 48 hour at ambient temperature.

Type 3

2.0 mL of TEOS, 2.0 mL of EtOH and 175 µL of Brij 30 solution (35 µL Brij 30/mL MeOH, non-ionic surfactant) were mixed immediately. Then, 1.0 mL of 0.1 M HCl was added dropwise to start hydrolysis and condensation reaction and the mixture was stirred for 5 hours. Under constant magnetic stirring, 2.0 mL of stock DAPB acid solution ($1.0x10^{-3}$ M in EtOH) was added to the above mixture and it was further stirred for 1 hour at ambient temperature. The support materials were spin-coated with the sol-gel at 3000 rpm for 60 seconds and then recovered slowly and steadily to provide an even coating that was allowed to dry for 48 hour at ambient temperature for 1 hour at 50° C.

Type 4

1.0 mL of TEOS, 1.0 mL of EtOH and 400 µL of deionized water were mixed and sonicated for 5 minutes**.** After that, 10 µL of 1.0 M HCl was added to the mixture to initiate hydrolysis and condensation reaction. After the termination of this reaction, 400 µL of DAPB acid solution ($1.0x10^{-3}$ M in EtOH) was added and left to stand in an ultrasonic bath for 3 minutes for obtaining homogeneity.

The support materials were spin-coated with this sol-gel solution at 3000 rpm for 60 seconds and then recovered slowly and steadily to provide an even coating that was allowed to dry for 48 hour at ambient temperature.

Type 5

4.5 mL of TEOS, 2.0 of mL deionized water and the 3.7 mg of DAPB acid were mixed under constant magnetic stirring. Then, 60 µL 0.1 M HCl was added to initiate the hydrolysis and condensation reaction. The mixture was refluxed for 4 hours at 60 °C . After the termination of the reaction, pre-cleaned glass supports were spin-coated at 3000 rpm for 60 seconds and then recovered slowly and steadily to provide an even coating that was allowed to dry for several hours at ambient temperature.

After the drying process, all the films prepared as explained in the procedures above, were washed under flowing water in order to get rid of any molecules adsorbed on the surface of the film.

Type 6

0.0018 g of dopant molecule (DAPB acid) was dissolved in ethanol/water solution (2.5 mL ethanol/ 675 µL water) with ultrasonic treatment and 125 µL HDTMOS and 1.625 mL TMOS were added as precursors. Under ultrasonic mixing, 75 µL 3% (v/v) HCl was added to initiate the hydrolysis and condensation process. After 3 minutes of sonication, the sol was allowed to stand for gelation and drying for 5 minutes.

2.5 Spectroscopic Studies on Solid Supports

2.5.1 Examination of the Optical Transmittance of the Solid Supports

In this study, optical transmittance of the solid supports was examined in the wavelength range of 200-800 nm by a Shimadzu UV-VIS 160 spectrophotometer. The glass substrates coated by dip and spin coating methods were fixed in UV-VIS cuvettes and placed in the light path. The glass substrates coated with these materials in the absence of the analyte were used as reference.

2.5.2 Leaching Tests

The coated substrates were tested in order to investigate whether the dopant molecule DAPB acid leached out of the matrix. The emission signal of the investigated molecule was monitored by placing the substrates into a fluorescence cuvette which has been filled with phosphate buffer (pH 9.00). This test was repeated for ten times and the coated substrates were kept in buffer solution for 5 minutes. At the and of the period of 5 minutes the emission signal was recorded.

2.5.3 Examination of the Spectral Activity of DAPB Acid

2.5.3.1 Fluorescence Measurements on Coated Substrates

The success of immobilization was checked in terms of activity. The coated substrates prepared were examined by fluorescence measurements in the absence and presence of analyte. Concentration of the analyte solution was varied in the range of 10^{-2}-10^{-4} M (at pH 9.00).

2.5.3.2 Nuclear Magnetic Resonance (NMR) Studies

NMR studies were performed in order to examine whether the monomers (MMA and MAA) react with DAPB acid.
First, the NMR spectrums of DAPB acid, MAA and MMA alone in acetone were taken in order to have an idea about the blank solutions. These spectrums were taken as reference and the spectrum of DAPB acid in these monomers were taken.

2.5.3.3 Fluorescence Studies on the Effect of Methacrylic Acid and Polyethyleneimine on the Quenching of DAPB Acid

Two DAPB acid solutions at the same pH (9.00) were prepared in 4.0 mL of 6.7 M ammonia, one containing as well 1.50 mL of methacrylic acid. The emission signals of these two solutions were recorded at 508 and 529 nm and then quenching of the emission signals were monitored by adding 25 μL of 10^{-2} M fructose solution to these mixtures.

Concentrated ammonia solution was added to the above solutions dropwise to adjust the pH of the medium at 9.00; since methacrylic acid has a pKa of 4.66, it was affecting the pH seriously. DAPB acid fluorescence was examined whether it was working in ammonia and MAA.

A study similar to the above procedure was performed in order to examine the effect of polyethyleneimine on fluorescence quenching of DAPB acid. 3.50×10^{-5} M of DAPB acid solution at pH 9.00 was monitored as blank solution and then DAPB acid solution (pH 9.00) was prepared containing 300 µL polyethyleneimine (total concentration of PEI 3% (v/v)) and the fluorescence emission signal of this solution was recorded. Finally, quenching of the emission signals were monitored by adding 25 µL analyte solution (1.0×10^{-2} M fructose) to these mixtures.

2.6 Spectroscopic Studies on Enhanced Fluorescence for Fructose Determination with DAPB Acid (Organized Media)

Perkin-Elmer LS-50B Luminescence Spectrometer was used for all the fluorescence measurements. Maximum excitation and emission wavelengths used were 324 nm and 529 nm as stated in literature [89]. The emission and excitation slits were adjusted to 5.0 nm and 7.5 nm, respectively.

2.6.1 Fluorescence Spectra of DAPB Acid in Buffered Medium

$3.5x10^{-5}$ M DAPB acid solution was prepared in phosphate buffer solution (pH 9.00). The emission spectrum of this solution was examined in order to observe the enhancement of the signal.

2.6.2 Fluorescence Spectra of DAPB Acid in Different Surfactant Solutions and PVA

10.0 mL of $3.5x10^{-5}$ M DAPB acid solutions at pH 9.00 were prepared in:

1. 0.025 % (w/v) PVA solution, in pH 9.00 buffer
2. $1.0x10^{-3}$ M Brij 30 solution, in pH 9.00 buffer
3. $1.0x10^{-2}$ M SDS solution, in pH 9.00 buffer
4. $1.0x10^{-3}$ M CTAB solution, in pH 9.00 buffer

The fluorescence spectra of the above solutions were examined and compared with the emission spectrum of DAPB acid mentioned in section 2.6.1. ($\lambda_{exc.}$= 324 nm, exc. slit= 5.0 nm and em. slit= 7.5 nm)

2.6.3 Effect of PVA and Surfactants on the Determination of Fructose

Effect of polyvinyl alcohol (PVA) on the determination of fructose was studied by examining $3.5x10^{-5}$ M DAPB acid solution containing different concentrations of PVA in the range of 0.01%-0.55% (w/v). The fluorescence intensities were recorded and the suitable PVA concentration was selected for further calibration studies.

Effect of Brij 30 (non-ionic surfactant) in the concentration range of $3.0x10^{-4}$-$1.0x10^{-2}$M, $1.0x10^{-2}$ M SDS (anionic surfactant) and $1.0x10^{-3}$ M CTAB (cationic surfactant) at pH 9.00 were also examined as stated above.

2.6.4 Determination of Fructose

Calibration plots for fructose in the range of $2.50x10^{-5}$ – $1.0x10^{-4}$M were obtained by using $3.5x10^{-5}$M DAPB acid, 0.01 % (w/v) PVA , $1.0x10^{-3}$ M Brij 30 and $1.0x10^{-2}$ M SDS, respectively. Emission signal of DAPB acid was measured at 508 nm (λexc=324 nm, excitation slit=5.0 nm and emission slit=7.5 nm).

PART II

Immobilization Studies on Diphenylcarbazide for the Determination of Cr (VI)

2.7 Chemicals and Reagents

i) s-Diphenylcarbazide solution ($1.65x10^{-3}$M): Prepared by dissolving 200 mg of s-diphenycarbazide (1,5-diphenylcarbohydrazide) (Fluka) in 100.00 mL of 95% ethyl alcohol and diluted to 500.00 mL by an acid solution prepared by 40 mL of H_2SO_4 and 360 mL of deionized water.

ii) Chromium (VI) stock solution (50.0 mg/L): Prepared by dissolving 141.4 mg anhydrous potassium dichromate ($K_2Cr_2O_7$, Merck) in 1.0 mL de-ionized water.

iii) Chromium (VI) Standard Solution (1000 mg/L) in approx. M nitric acid, Fisher Scientific

iv) Mercury Standard Solution (1000 mg/L) in approx. M nitric acid, Fisher Scientific

v) Vanadium Standard Solution (100 μg/mL) in %2 (v/v) nitric acid, Leeman Labs.Inc.

vi) Molydenum Standard Solution (100 μg/mL) in %2 (v/v) nitric acid, Leeman Labs.Inc.

vii) Iron Standard Solution (1000 mg/L) in 0.5 mol L^{-1} nitric acid, Merck

2.8 Apparatus

The batch type studies were performed by a Shimadzu UV-VIS 160 spectrophotometer.

2.9 Preparation of Diphenylcarbazide (DPC) Doped Sol-Gel Films and Resins

Type 1

First, dopant molecule was dissolved in an ethanol/water solution (5.3 mL methanol/0.8 mL de-ionized water) with ultrasonic treatment and then 1.3 mL of TMOS and 0.3 mL of Me-TMOS were added as precursors. Under constant stirring, 30 µL of concentrated nitric acid was added to initiate the hydrolysis and condensation reaction. After 1 hour mixing, films were prepared by spin coating technique.

Type 2

TMOS:Me-TMOS:de-ionized water:MeOH were mixed in molar ratios of 1:0.25:5.25:15.5 with the addition of CTAB as surfactant. The dopant to surfactant (CTAB) molar ratio was 0.21. The rest of the procedure was as explained in Type 1.

Type 3

0.06 g of dopant molecule (DPC) and 0.43 g surfactant (CTAB) was dissolved in ethanol/water solution (2.5 mL ethanol/ 675 µL water) with ultrasonic treatment and 125 µL HDTMOS and 1.625 mL TMOS were added as precursors. Using ultrasonic mixing, 75 µL of 3% (v/v) HCl was added to initiate the hydrolysis and condensation process. After 3 minutes of sonication, the sol was allowed to stand for gelation and drying for 5 minutes.

Blank for Type 3

0.43 g surfactant (CTAB) was dissolved in ethanol/water solution (2.5 mL ethanol/ 675 µL water) with ultrasonic treatment and 125 µL HDTMOS and 1,625 mL TMOS were added as precursors. Under ultrasonic mixing, 75 µL 3% (v/v) HCl was added to initiate the hydrolysis and condensation process. After 3 minutes of sonication, the sol was allowed to stand for gelation and drying for 5 minutes.

2.10 Spectrophotometric Studies of Aqueous Solutions of DPC (1,5-diphenylcarbohydrazide) and DPC-Cr(VI) complex

The calibration line of the Cr(VI)-DPC complex was derived using absorbance at 540 nm, at pH 2.00. Cr(VI) standard solutions in the range of 10 µg/L to 1.0 mg/L were prepared in de-ionized water and mixed with the same volume of 200 mg /L DPC solution. Absorbance

measurements were performed by Shimadzu 160 UV-VIS spectrophotometer.

2.10.1 Effect of pH on the Complexation Reaction of Cr(VI) and DPC

The effect of pH on the complex formation of Cr (VI) with diphenylcarbazide (DPC) was studied in the pH range of 1-7 without using a buffer solution. 400 µg/L Cr (VI) and 200 mg/L DPC solutions were prepared in varying concentrations of H_2SO_4 solutions (pH 1-7) by appropriate dilutions from 1.0 M H_2SO_4. The measurements were performed by using the Shimadzu UV-VIS 160 spectrophotometer at 540 nm.

2.11 Spectrophotometric Studies on DPC-Doped Sol-Gel Resins

2.11.1 Uptake Efficiency of DPC -Doped Sol-Gel Resins for Cr(VI)

Uptake studies were performed for Type 3 and Type 4 sol-gels prepared in resin form as explained in Section 2.9. The other resins were not examined due to the leaching problem.

Micro-columns (3 cm-length) were prepared from PEEK (polyether etherketone) tubing (od 3.2 mm, id 2.0 mm). 0.05 g resin (100-150 µm) was packed in the tubing and both ends of the tubing were plugged with small pieces of sponge in order to hold the resin.

10.0 mL of 5.0 mg/L Cr(VI) solution was loaded to the column using Gilson Minipuls 3 peristaltic pump with the flow rate of 0.5 mL min^{-1}. The effluent was analyzed for its Cr (VI) content using the method given in section 2.10.

2.11.2 Recovery of Cr (VI) by Using DPC-Doped Sol-Gel Resins

Recovery studies were performed for sol-gel type 3 and type 4 prepared in resin form as explained in section 2.9.
10.0 mL of 6M HCl , 500µL of 1.0 M NaOH, 10.0 mL of 0.1 M NaOH, 10.0 mL of 0.2 M NaOH and 0.5 M 500 µL of NaOH were used as eluents alternately (0.5 ml min^{-1}) from the resins used in the recovery studies as explained in Section 2.12.1. The Cr (VI) concentration in the effluents was analyzed by the spectrophotometric method that was explained in section 2.10.

2.11.3 Leaching Studies on DPC-Doped Sol-Gel Resins

The DPC-doped sol-gel resin was tested for leaching of the dopant molecule (diphenylcarbazide). 0.1 g resin was kept in 5 mL de-ionized water and for 24 hour. Then, the resin was removed by centrifuge and the effluent was analyzed for its diphenylcarbazide content.

2.12 Preparation of DPC-Doped Sol-Gel Films

Pre-cleaned glass supports were spin coated with Type 3 DPC-doped sol-gel at 2000 rpm for 30 seconds and dried at room temperature for 7 days.

2.12.1 Spectrophotometric Studies on DPC-Doped Sol-Gel Films

DPC-doped sol-gels were dipped into Cr(VI) solutions of the concentration range of 1 to 10 mg/L at pH 2.00, for 5 minutes.
Then, they were dried at room temperature and absorbance values were recorded in order to obtain a calibration graph.

2.13 Preparation of DPC Doped-Sol-Gel Impregnated Filter Papers

DPC-doped sol-gel Type 3 was embedded into filter papers and dried for 7 days. Then, they were saturated within a hydrophilic sol-gel [(30 µL surfactant (Brij 30) was dissolved in ethanol/water solution (2.5 mL ethanol/ 675 µL water) with ultrasonic treatment and 1.625 mL TMOS were added as precursors. Under ultrasonic mixing, 75 µL of 3% (v/v) HCl was added to initiate the hydrolysis and condensation process.] Samples (or papers) were cut into 1cm^2 pieces with a scissor and soaked into deionized water for 24 hours to wash out the undoped DPC.

2.13.1 Spot Test Studies with DPC-Doped Sol-Gel Impregnated Filter Papers

Saturated filter papers were dipped into varying concentrations of Cr(VI) solutions ranging between 1 to 5 mg/L at pH 2.00 for 10 minutes.In order to test the repeatability, 4 parallel sets were examined.

2.13.2 Effect of Interfering Ions to Spot Test

To determine the effect of interfering ions, same test as explained in Section 2.11.1 was performed in the presence of 1.0 mg/L iron(II), 10.0 mg/L vanadium(V), 10.0 mg/L molybdenum(VI) and 10.0 mg/L mercury (II) in chromium solutions separately.

CHAPTER 3

RESULTS AND DISCUSSION

PART I

Immobilization Studies on DAPB Acid for the Determination of Fructose

In a general approach, immobilization of chemosensors on solid supports for developing optical sensors should result in improved analytical performance characteristics such as continuous readout, increased sensitivity, lower reagent consumption and possibility of using the sensor in solvents where the free molecule displays low solubility.

There are several important parameters in the immobilization process such as the optical transparency of the solid support within the working wavelength range of the dopant, preservation of active sites of chemosensor molecule while and after the immobilization, constitution of a suitable media without a leaching problem and nature of binding forces between the chemosensor and the host matrix.

When immobilizing a chemosensor molecule in a solid support, interactions between the host matrix and the dopant is important in view of the fact that the dopant molecule has to be held in the host matrix without loss of its activity. It seems more likely that physical attractions would cause little damage to active sites. This situation was

taken into consideration that solid support (or monomer) selection was carried out carefully.

In this part of the study, three different kinds of polymeric solid supports; hydrogels, sol-gels and organic polymers were synthesized to immobilize DAPB acid for fructose determination with PET (Photoinduced Electron Transfer) reaction. The polymer solid supports transparent within the wavelength range of 200 to 800 nm were selected and further examined after the immobilization of the dopant molecule for the excitation and emission wavelengths which were expected to be the same as before immobilization. The most important point was the leaching problem of the dopant molecule so additional care was taken by controlling the process at every single step.

3.1 Spectroscopic Studies on Solid Supports

3.1.1 Optical Transmittance

Since optical transmittance of the matrix is important for the fluorescence measurements of DAPB acid (λ_{exc}324 nm, λ_{em} 529 nm), the solid supports were examined in the wavelength range of 200 to 800 nm with the Shimadzu UV-VIS 160 spectrophotometer for their optical transparency. All the solid supports resulted in good optical transparency (See Appendix 1).

The most important factor in the immobilization studies is protecting the active sites and the spectroscopic characteristics of the dopant molecule. In this respect, after the immobilization of DAPB acid, the excitation and emission wavelengths of this molecule were examined by using the LS50B Luminescence Spectrometer. The results obtained were the same as stated in the literature and the measurements performed before immobilization indicated that the spectroscopic characteristics of DAPB acid were not affected by the immobilization process (See Appendix 2).

3.1.2 Leaching Studies

3.1.2.1 Hydrogels

3.1.2.1.1 Polyvinyl Alcohol (PVA)

PVA was cross-linked by gluteraldehyde, succinic acid, boric acid and borax to increase the rigidity of the structure (Table 3.1).

Table 3.1 Leaching Test Results for DAPB Acid Doped-PVA that is Crosslinked by Different Crosslinkers

Type	Crosslinker	Crosslinker (w/v) %	Leaching
1	Glutaraldehyde	8	√
2	Succinic acid	5	√
3	Boric acid	3	√
4	Borax	1	√

The fluorescence studies on these cross-linked hydrogels showed that fluorescence signal of the immobilized DAPB acid decreased after each contact with the phosphate buffer (pH 9.00) indicating that in each case dopant molecule leached out the polymeric matrix (Figure 3.1). As a consequence, it was decided that PVA was not suitable support matrices for the entrapment of DAPB acid.

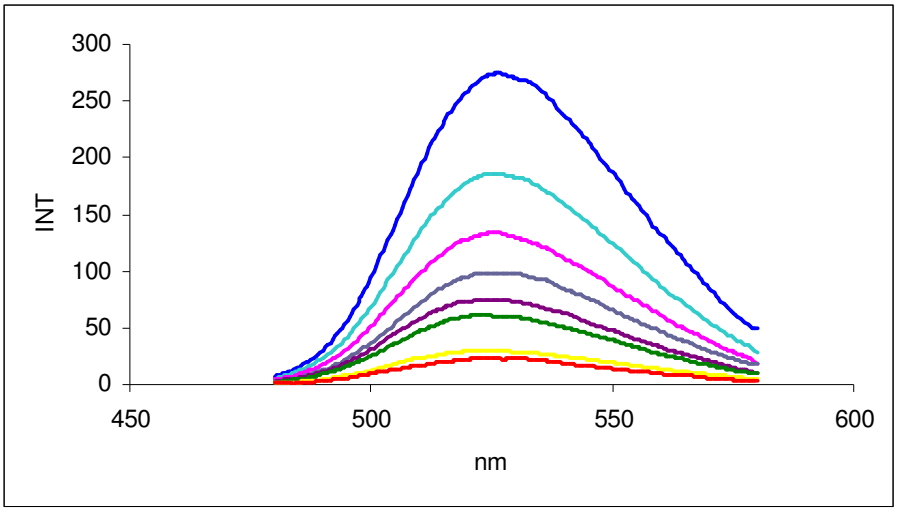


Figure 3.1 Fluorescence signals of DAPB acid-doped PVA film after each contact with the buffer. Blue line corresponds to the first run and red line corresponds to the last run.

3.1.2.1.2 Calcium Alginate Gel

The fluorescence studies performed with the Ca-Alginate Gel film resulted in a continuous decrease of fluorescence signal of immobilized DAPB acid after each contact with buffer. This decrease was observed due to leaching related to the loss of rigidity of the gel in the buffer solution.

This may be due to the instability of the gel in common buffer solutions with high concentration of phosphate and citrate ions that can extract Ca^{2+} from the alginate and liquefy the system [45].

Although, alginate is by far the most widely used polymer for immobilization and microencapsulation technologies because of its biocompatibility, low cost and good optical characteristics, it was decided that it is an unsuitable support for DAPB acid because of the leakage of the dopant molecules.

3.1.2.2 Organic Polymers

3.1.2.2.1 Co-Polymer of MMA and MAA

In the immobilization or molecular imprinting studies, it is expected that there will be a non-covalent interaction between the entrapped chemical and the functional group of the host matrix.

MAA that interacts with the amine functional group via hydrogen bonding through carboxylic acid and carbamate as shown in Figure 3.2 was a good candidate for this purpose.

However, PMAA is a water-soluble polymer which requires further treatment in order to be a more suitable matrix for immobilization. Thus, a copolymer of MMA with MAA was prepared resulting in a less swollen and more rigid structure.

In the direction of our expectations, glass supports coated with DAPB acid containing MAA-MMA copolymer showed no leaching after several washing cycles.

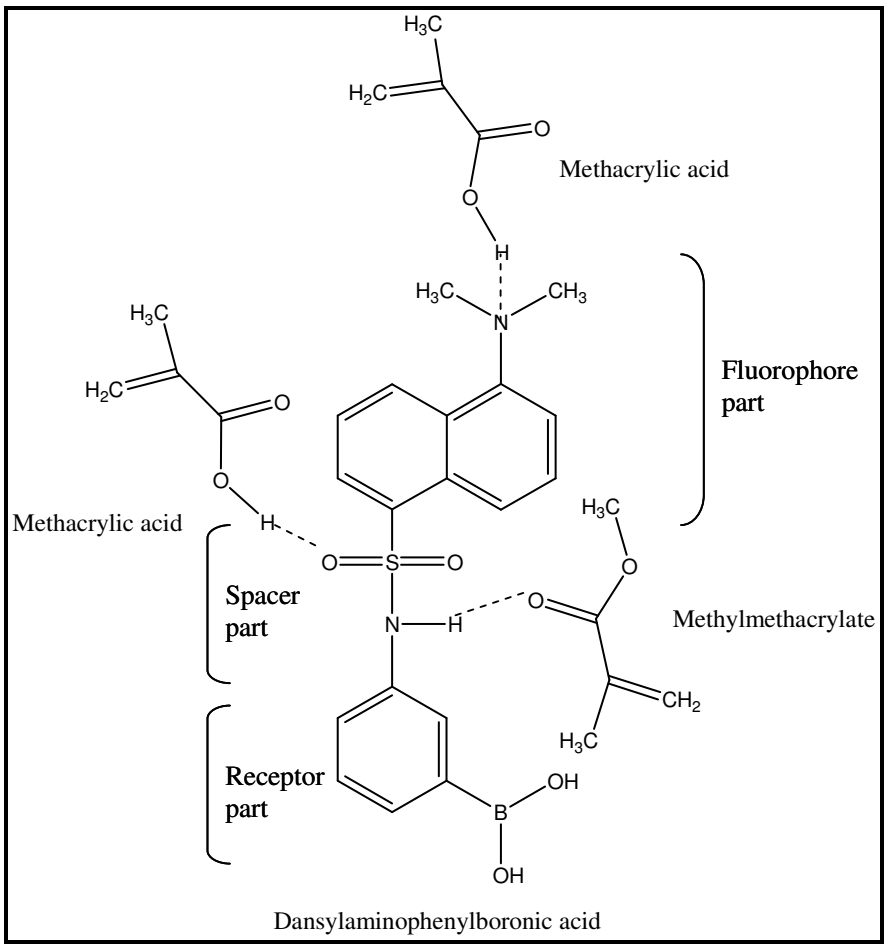


Figure 3.2 Potential binding points of DAPB acid and co-polymer of Methacrylic Acid and Methylmethacrylate

3.1.2.3 Sol-Gel

As stated previously, sol-gel material is a quite suitable solid support for physical entrapment [35].

Furthermore, arrangement of the pore size by the use of an acid or base catalyst and control of the hydrophobicity of the medium by the organically modified silanes guided us in this study.

DAPB acid molecule does not have a large radius like enzymes and proteins. That is why the leaching problem that is not faced in enzyme immobilization was of great importance to us. It was aimed to reduce the leaching problem by using the acid catalyzed sol-gel process in order to obtain a more compact structure.

It was also taken into consideration that the short range forces should be effective in the entrapment of DAPB acid in the sol-gel matrix. Hence, organically modified silanes, methyltrimethoxysilane and hexadecyl trimethoxysilane, were used to replace hydrophilic –OH groups with alkyl chains that allow the accommodation of naphthalene group in a more hydrophobic environment.

All of the DAPB acid -doped sol-gel coatings had a leaching problem but it was removed by 5 minutes of washing with de-ionized water; this treatment was repeated for 15 to 20 times. As shown in Figure 3.3-i, a fluorescence signal was observed in the supernatant solution which was decided to be the indication of leaching. But after 20 washing cycles, the fluorescence signal became steady and no signal was observed for the supernatant (Figure 3.3-ii). This behaviour can be explained as the washing off the reagent that could not be entrapped properly.

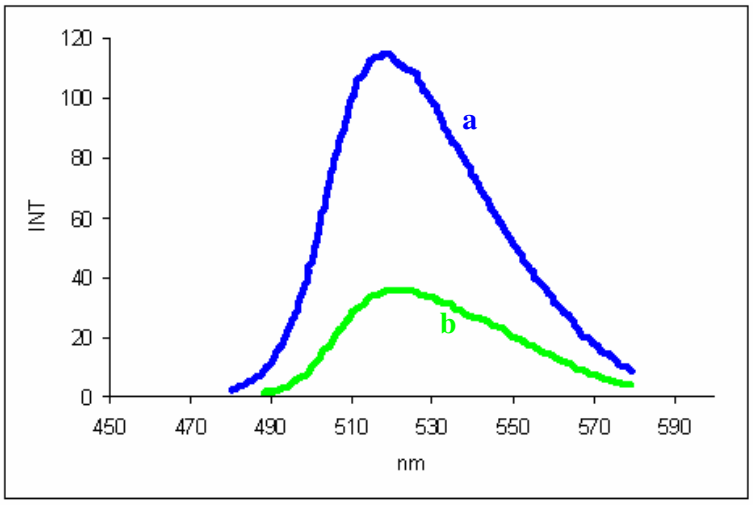


Figure 3.3-i a) Emission signal of immobilized -DAPB acid in sol-gel matrix and b) DAPB acid in supernatant (in phosphate buffer)(λ_{ex}=324 nm λ_{em}=520 nm exc. slit= 5.0 nm and em. slit= 7.5 nm)

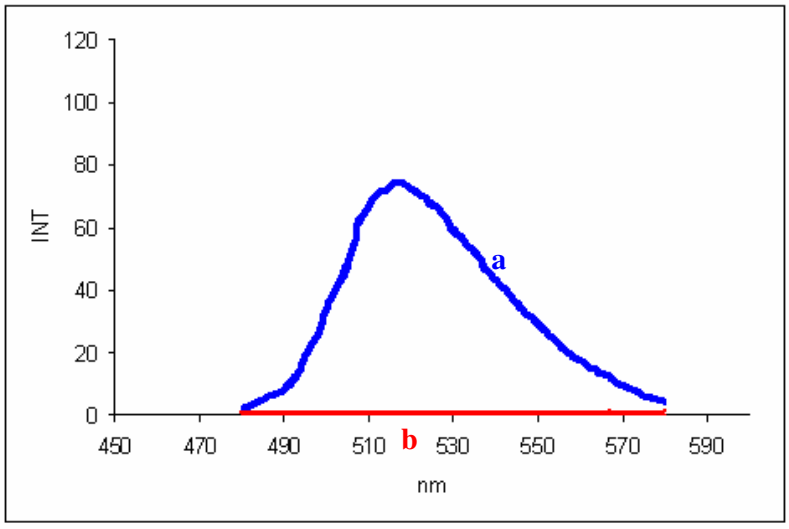


Figure3.3-ii a)Emission signal of immobilized -DAPB acid in sol-gel matrix after washing cycles b) Emission profile of DAPB in supernatant (in phosphate buffer)(no signal) λ_{ex}=324 nm λ_{em}=520 nm exc. slit= 5.0 nm and em. slit= 7.5 nm)

3.1.3 Examination of the Spectral Activity of DAPB Acid

3.1.3.1 Fluorescence Measurements

Fluorescence studies were not performed with the hydrogels because of the leaching problem. Hence, the fluorescence emission of DAPB acid in sol-gel and in MAA-MMA co-polymer was examined in the presence of the analyte.

Despite the fact that the DAPB acid molecules entrapped were contacted with the diols, the expected decrease of the emission signal due to the condensation reaction between the boronic acid groups and 1,2 - 1,3 diols was not obtained.

One possibility was the blockage of boronic acid site as Si-O-B due to the condensation reaction of DAPB acid with silanol groups. The other one was the possible alteration in the electron flow mechanism responsible for quenching.

In the same way, the DAPB acid emission signal was recorded in the methacrylic acid and methylmethacrylate co-polymer matrix but the expected decrease for the emission signal due to the presence of the analyte molecule could not be observed. For this reason, NMR and fluorescence studies were further performed in order to investigate whether MAA had an effect on DAPB acid emission.

Appropriate conditions for the NMR studies of MAA were obtained on the other hand NMR studies for the sol-gel could not be performed due to the presence of water in the sol-gel structure.

3.1.3.2 NMR Studies

NMR studies were performed to examine the effect of MAA and MMA on DAPB acid. NMR spectrum of DAPB acid in acetone was compared with the NMR spectra of DAPB acid in MAA and DAPB acid in MMA.

According to the NMR results, hydrogen of N---H group of the spacer could not be observed when MAA was mixed with DAPB acid. On the other hand, there was no change in the NMR spectrum of DAPB acid when it was mixed with MMA (See Appendix 3).

These observations indicated that, MAA was reacting with the N-H bond of the spacer group of the DAPB acid molecule which might influence the PET mechanism. In this respect, fluorescence studies were performed to investigate the behavior of DAPB acid in the presence of MAA.

3.1.3.3 Fluorescence Studies with DAPB Acid in the Presence of MAA Monomer Solutions

Fluorescence studies with MAA reagent were performed in order to support the results of the NMR studies. As can be seen from Figure 3.4-a, emission signal of DAPB acid was significantly suppressed in the wavelength range of 470 to 550 nm in the presence of MAA when compared to the spectrum of DAPB acid alone (Figure 3.4-b)

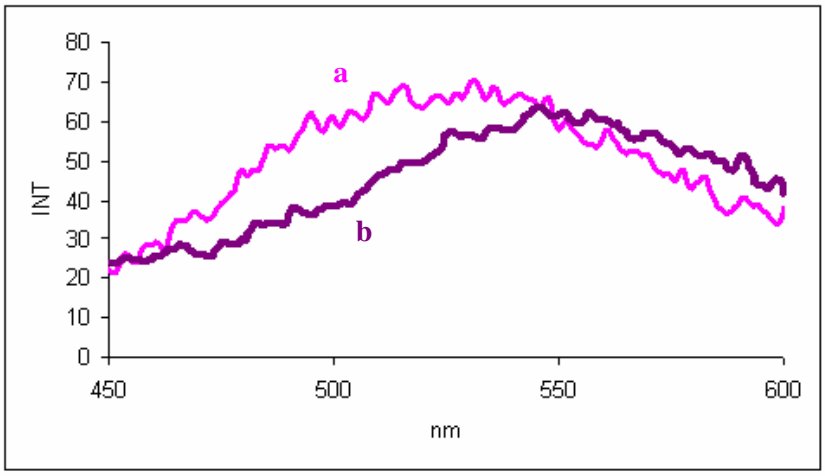


Figure 3.4 a) Pink line represents the fluorescence spectrum of $3.5x10^{-5}$ M DAPB acid (pH 9.00) **b)** Violet line represents the fluorescence signal of $3.5x10^{-5}$ M DAPB acid in the presence of 27% (v/v) MAA (pH 9.00) (λ_{ex}=324 nm, λ_{em}=529 nm exc. slit= 5.0 nm and em. slit= 7.5 nm)

Fluorescence measurements were further performed with fructose addition. Normally, after the addition of fructose, the fluorescence intensity was quenched in basic medium at pH 9.00 (Figure 3.5). However, as can be seen from Figure 3.6, no change in fluorescence intensity was observed with the addition of analyte to the DAPB acid in MAA media. According to these results, it was decided that MAA suppressed the activity of DAPB acid and therefore it is not a suitable monomer for DAPB acid immobilization.

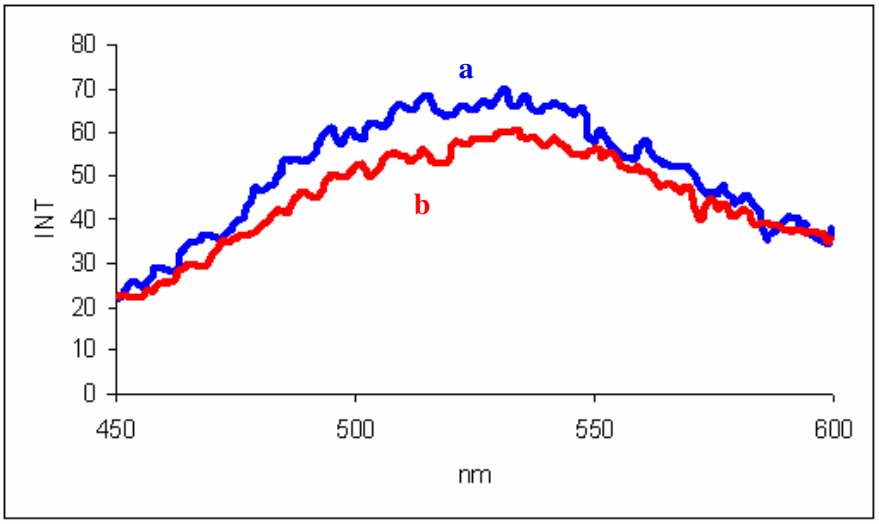


Figure 3.5 a) Blue line represents the fluorescence spectrum of $3.5x10^{-5}$ M DAPB acid (pH 9.00) **b)** Red line represents the spectrum of $3.5x10^{-5}$ M DAPB acid after the addition of fructose ($1.0x10^{-4}$ M final concentration of fructose) (λ_{ex}=324 nm, λ_{em}=529 nm exc. slit= 5.00 nm and em. slit= 7.5 nm)

Therefore, a polymer solid support was prepared with only MMA monomer which was quite hydrophobic. As it is known [28], porogens influence the polymer morphology such as inner surface area and average pore size. It was thought that, a solid support having a porous surface would facilitate the diffusion of fructose into a hydrophobic solid matrix and therefore plentiful amount of acetone was used as porogen during the preparation of this polymer. No reaction between DAPB acid and the fructose was observed.

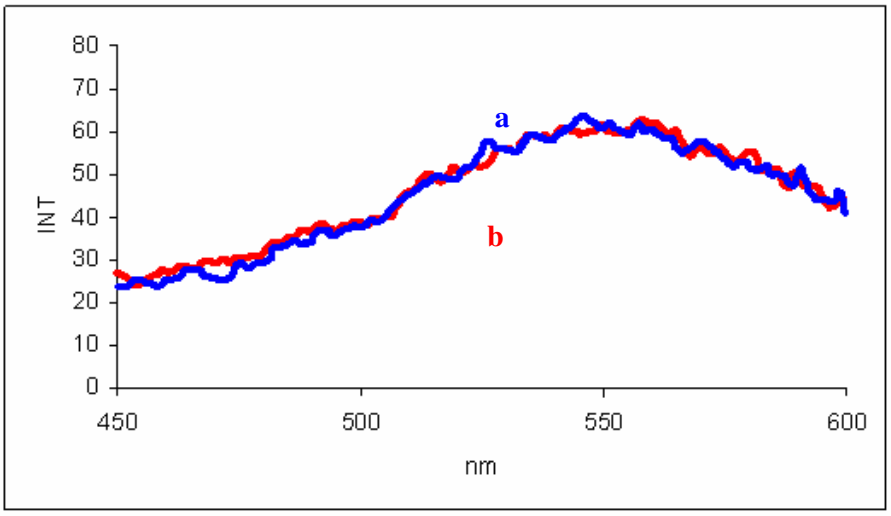


Figure 3.6 a) Blue line represents the fluorescence spectrum of $3.5x10^{-5}$ M DAPB acid (at pH 9.00)in the presence of 27.0%(v/v) MAA(pH 9.00) **b)** Red line represents the fluorescence spectrum of $3.5x10^{-5}$ M DAPB acid after the addition of fructose in the presence of 27.0%(v/v) MAA (pH 9.00) ($1.0x10^{-4}$ M final concentration of fructose) (λ_{ex}=324 nm, λ_{em}=545 nm exc. slit= 5.0 nm and em. slit= 7.5 nm)

So a relatively small analyte, hydronium ion, instead of fructose was selected to investigate whether the hydrophilic analyte could be diffused into this porous and hydrophobic solid matrix. As can be seen from Figure 3.7 the fluorescence intensity of DAPB acid was totally quenched at acidic pH values.

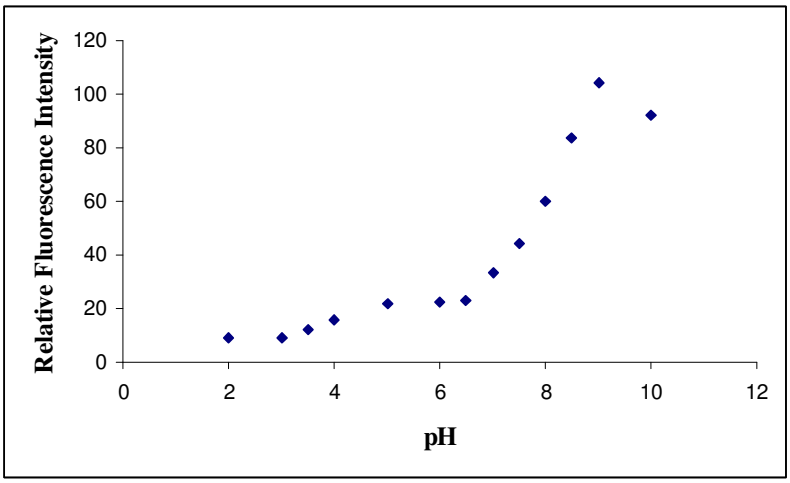


Figure 3.7 Influence of pH on the emission intensities of 3.5×10^{-5} M DAPB acid in 1.0% (v/v) Dimethylsulfoxide (DMSO). (λ_{exc}= 324 nm, λ_{em}= 529 nm exc. slit= 5.00 nm and em. slit= 7.5 nm)

So, the fluorescence measurements of DAPB acid entrapped in PMMA were carried out at pH 2.0 buffer instead of pH 9.0 buffer. Although a sharp decrease in the fluorescence signal was expected, no change was observed.

In order to increase the diffusibility of the analyte into the matrix, a relatively hydrophobic solvent, ethanol, was used. But the polymer became opaque which did no allow to carry out fluorescence measurements. Due to the high solubility of PMMA in most of the organic solvents, they were not tried further.

Thereafter, a polymer blend was prepared by mixing a hydrophilic polymer; polyethyleneimine (PEI) and a hydrophobic polymer;

PMMA. PEI was expected to facilitate the diffusion of the hydrophilic analyte and buffer into the solid matrix.

The fluorescence studies performed for MAA were repeated for PEI. The emission signal measured for $1.0x10^{-4}$M DAPB acid in the presence of 3.0% (v/v) PEI was 40 units lower than expected as shown in Figure 3.8 a and b, respectively.

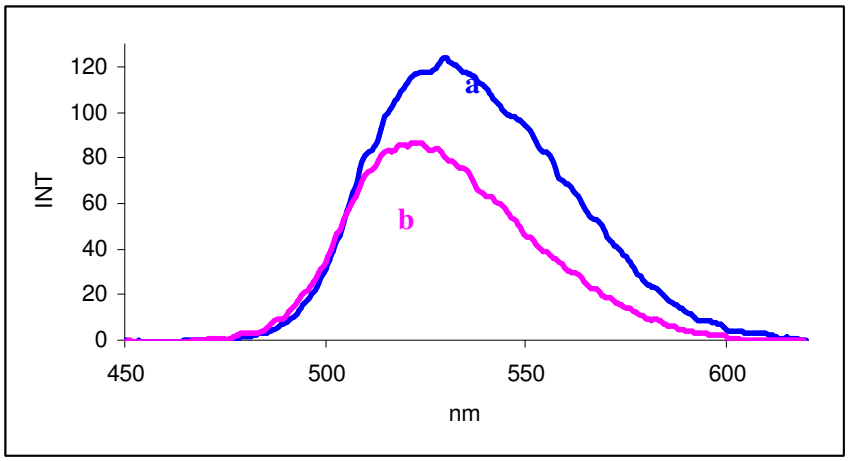


Figure 3.8 a)Blue line represents the emission profile of $1.0x10^{-4}$ M DAPB acid b)Pink line represents the emission profile of $1.0x10^{-4}$ M DAPB acid in 3.0% (w/v) PEI at pH 9.00.

Furthermore, the results were also the same upon the addition of fructose as shown in Figure 3.9

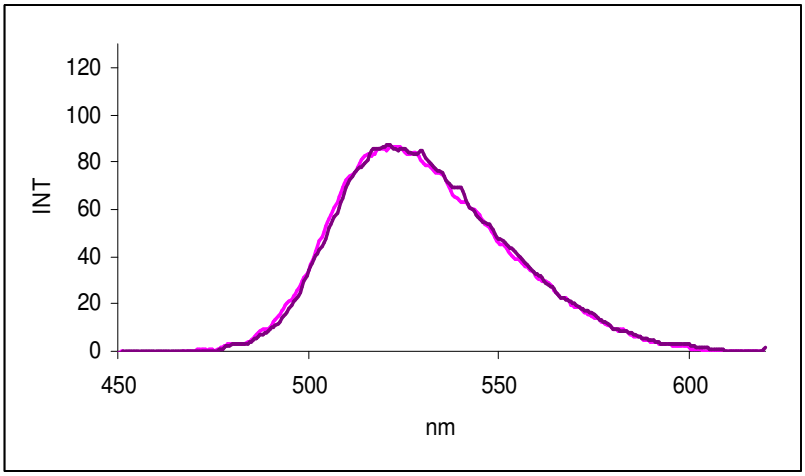


Figure 3.9 Pink line represents the emission profile of $1.0x10^{-4}$ M DAPB acid in 3.0% (w/v) PEI at pH 9.00 and purple line represents the emission profile of $1.0x10^{-4}$ M DAPB acid in 3.0% (w/v) PEI after contact with $1.0x10^{-2}$ M fructose at pH 9.00.

The problems encountered during the study with these polymers are summarized in Table 3.2. As a consequence, it can be assumed that quenching is controlled by the polymeric matrices investigated rather than the analyte.

Table 3.2 Summary of the Results of Immobilization of DAPB acid into a Solid Matrix

Matrix	Leaching	Reaction with dopant	Diffusion Problems
Hydrogels			
1) Ca-Alginate	+		
2)Polyvinylalcohol	+		
Sol-Gels			
1)Hydrophilic (with co-solvent)	+		
2)Hydrophilic (withoutco-solvent)		+	
3) Hydrophobic		+	
Organic polymers			
1) Co-polymer of MAA and MMA		+	
2) PMMA			+
3)PMMA-PEI blend		+	+

3.2 Fluorescence Studies of DAPB Acid in Organized Media

Previously, a study was performed about the batch type determination of fructose by DAPB acid in our laboratory that resulted in a detection limit of 1.0×10^{-4} M fructose.

During the immobilization studies, it was realized that PVA enhanced the emission signal of DAPB acid which might be due to the decrease of external conversion related to the high viscosity of PVA solutions.

As stated, external conversion refers to nonradiative processes in which excited states transfer their excess energy to other species, such as solvent or solute molecules. In some cases luminescence efficiencies increase with solvent viscosity due to the reduced rate of bimolecular collisions and rate of dynamic quenching [88].

Considering this advantage of PVA, fluorescence studies were performed to investigate the enhancement effect of organized media on fructose determination. Besides PVA, three surface active reagents; Brij 30, SDS and CTAB were tried.

As can be seen from Figure 3.10, an enhancement in the fluorescence intensity of DAPB acid was observed for PVA, Brij 30 and SDS as compared to the results obtained in aqueous medium under identical conditions. On the contrary, CTAB did not produce a notable enhancement on the fluorescence.

During enhanced fluorescence calibration studies for fructose determination, reproducible results could not be obtained for the surface active reagents. Hence, only the effect of PVA was investigated for signal enhancement.

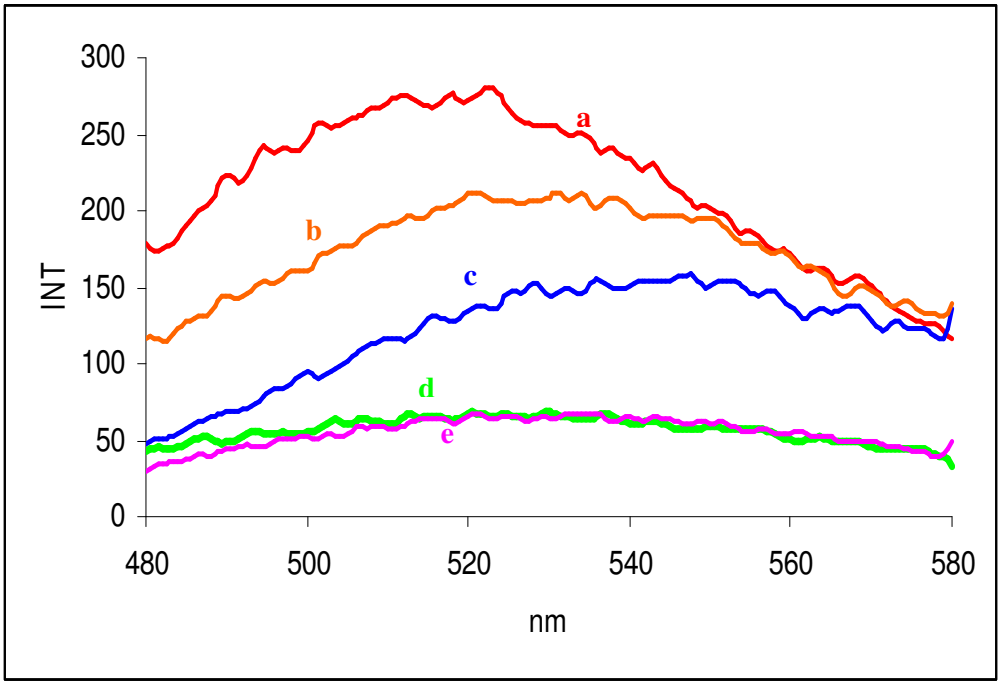


Figure 3.10 Fluorescence spectra of $3.5x10^{-5}$ M DAPB acid in different surfactant and polymers (pH 9.0) [a]0.025% (w/v) PVA solution [b]$1.0x10^{-3}$ M Brij 30 solution [c]$1.0x10^{-2}$ M SDS solution [d]DAPB acid alone [e]$1.0x10^{-3}$ M CTAB solution ($\lambda_{exc.}$= 324 nm, exc. slit= 5.0 nm and em. slit= 7.5 nm)

3.2.1 Effect of Polyvinyl Alcohol

Effect of PVA on emission signal of DAPB acid was studied at PVA concentrations in the range of 0.01- 0.55 % (w/v) and it was concluded that the optimum working concentration was 0.0125% for PVA.

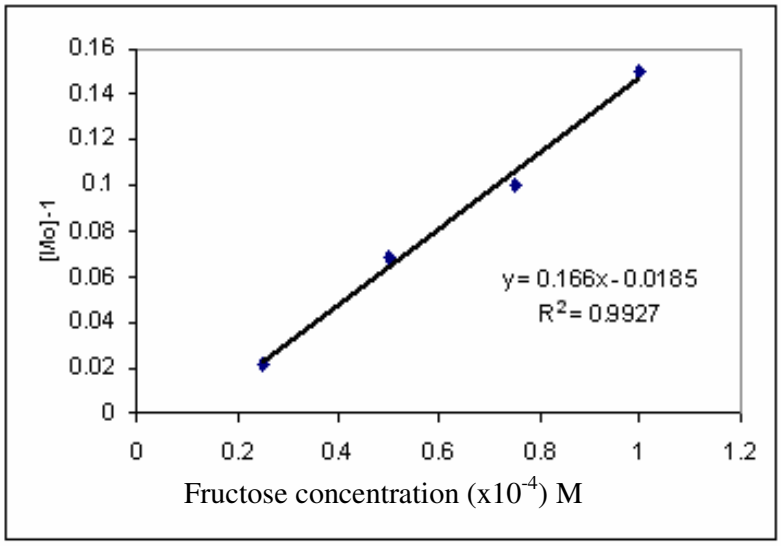


Figure 3.11 Calibration line for fructose ($2.50x10^{-5}$ M-$1.0x10^{-4}$ M) in 0.0125% (w/v) PVA, Io=Fluorescence intensity of DAPB acid, I=Fluorescence signal of DAPB acid in the presence of fructose as quencher. ($\lambda_{exc.}$= 324 nm, $\lambda_{em.}$= 508 nm, exc. slit= 5.0 nm and em. slit= 7.5 nm)

The calibration line for fructose in the presence of 0.0125% (w/v) PVA is given in Figure 3.11. As can be seen from the figure, the lowest concentration limit was extended up to $2.5x10^{-5}$ M.

PART II

Immobilization Studies on DPC for the Determination of Cr (VI)

Chromium speciation and determination studies have been carried out in our laboratory using flow injection analysis technique. Both chemiluminescence and spectrophotometric methods were applied. DPC was used as the selective complexing reagent for chromium. In order to prepare a chemical sensor for chromium, immobilization studies for DPC were carried out.

All the solid support matrices examined in Part I (summarized in Table 3.2) were also tried for DPC immobilization. The results of the immobilization study are summarized in Table 3.3.

Among them, the most promising results were obtained for the hydrophobic sol-gels (Table 3.4). Sol-gel Type 1 was modified with the addition of a surface-active reagent, CTAB, used as an extracting reagent which was known to facilitate the diffusion of Cr(VI) inside the structure.

Consequently, a more hydrophobic sol-gel was obtained by using a precursor with longer carbon chains (sol-gel Type 3) and it was selected for further studies due to its higher hydrophobicity.

Table 3.3 Summary of the Results of Immobilization of DPC into a Solid Matrix

Matrix	Leaching	Reaction with dopant
Hydrogels		
1) Ca-Alginate	+	
2) Polyvinylalcohol	+	
Sol-Gels		
1) Hydrophilic (with co-solvent)	+	
2) Hydrophobic		
Organic polymer		
Co-polymer of MAA and MMA		+

Table 3.4 Compositions of DPC-doped Sol-Gels

Type	Precursor	Co-solvent	Acidity	Surfactant	Hydrophilicity
1	TMOS/Me-TriMOS	Methanol	Acidic	No	Hydrophobic
2	TMOS/Me-TriMOS	Methanol	Acidic	CTAB	Hydrophobic
3	TMOS/HDTMOS	Ethanol	Acidic	CTAB	Hydrophobic

This sol-gel was prepared in various forms like glass beads, thin films coated on glass slides and sol-gel impregnated filter papers in the manner of different applications.

3.3 Spectrophotometric Studies on Aqueous Solutions of Cr(VI) and DPC Complex and DPC Alone

The wavelength yielding maximum absorbance was identified by scanning the Cr(VI)-DPC complex solution over the wavelength range of 200-800 nm with the Shimadzu UV-VIS 160 spectrometer. The maximum absorbance value obtained was 540 nm for the Cr(VI)-DPC complex as can be seen from Figure 3.12.

DPC alone also had a characteristic spectrum in the UV range (Figure 3.12). The maximum absorbance value was obtained at 230 nm but at higher concentrations the signal at this wavelength became over-range. So 279 nm was chosen as the working wavelength for further studies.

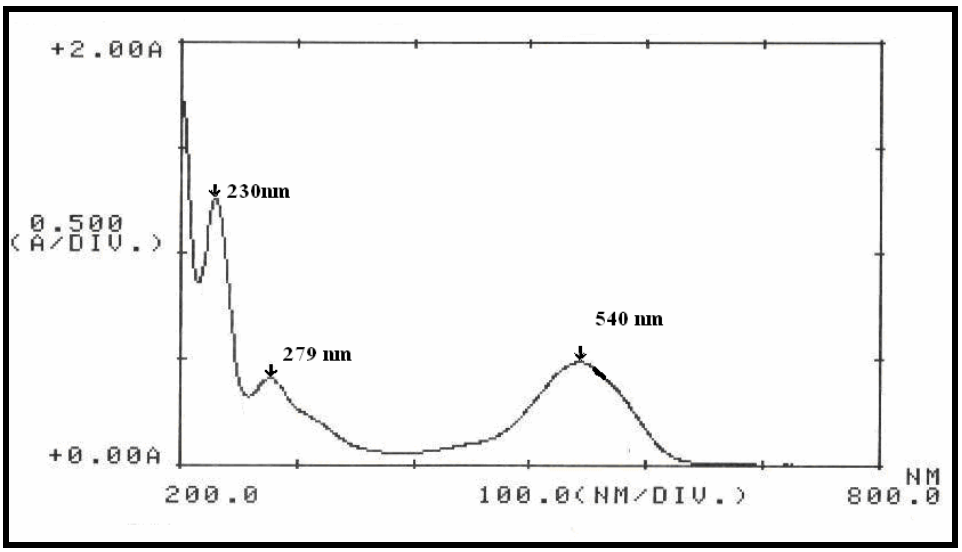


Figure 3.12 Absorbance Spectrum of 1.0 mg/ L Cr (VI) + 10.0 mg L DPC solution at pH 2.00

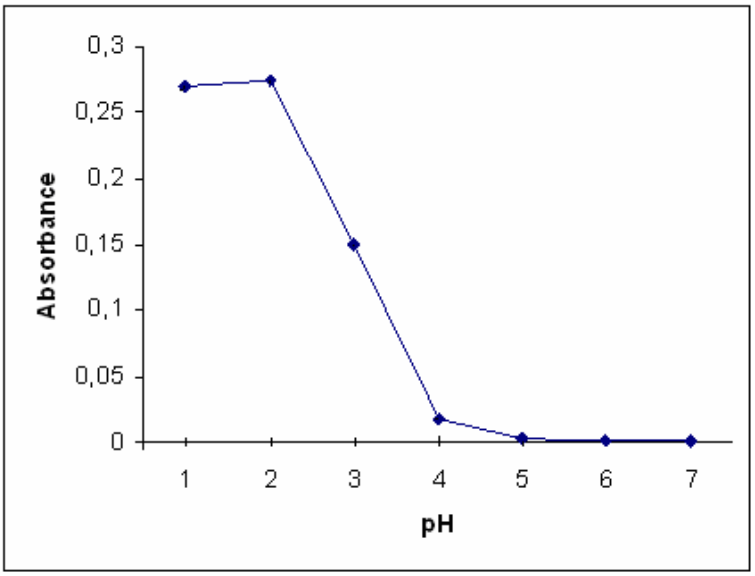


Figure 3.13 Effect of pH on the Reaction Between the Cr(VI) and DPC (λ_{abs} =540 nm)

Effect of pH on the reaction between Cr(VI) and DPC was examined in order to obtain the suitable pH for stable complex formation and uptake studies. The results showed that the optimum pH was 2.0 and no complex formation was observed at pH $\geq$ 5.0 (Figure 3.13).

The calibration graph of the Cr(VI)-DPC complex solution is shown in Figure 3.14. As can be seen, the increase in absorbance of the complex formed was linear up to 1.0 mg/ L Cr(VI) in the presence of 200 mg/ L DPC. The detection limit (3s) was 10 µg/ L. This calibration graph was further used in take-up studies in order to determine the concentration of Cr(VI) which was not retained on the column.

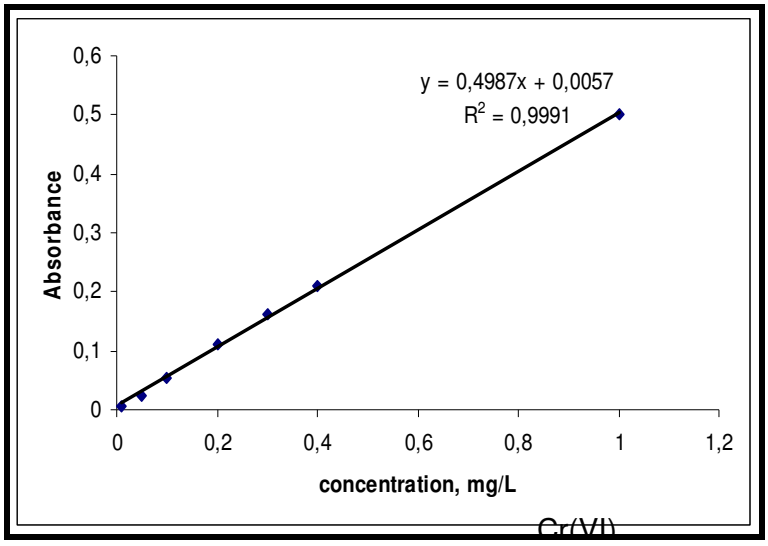


Figure 3.14 Calibration Graph of Cr(VI)-DPC Complex in Aqueous Medium (λ_{abs} =540 nm, 200 mg/ L DPC)(pH 2.0)

Calibration studies were also performed for aqueous solutions of DPC and the linear region is shown in Figure 3.15. This calibration graph was further used to estimate the leached amount of DPC from the DPC doped sol-gel resin.

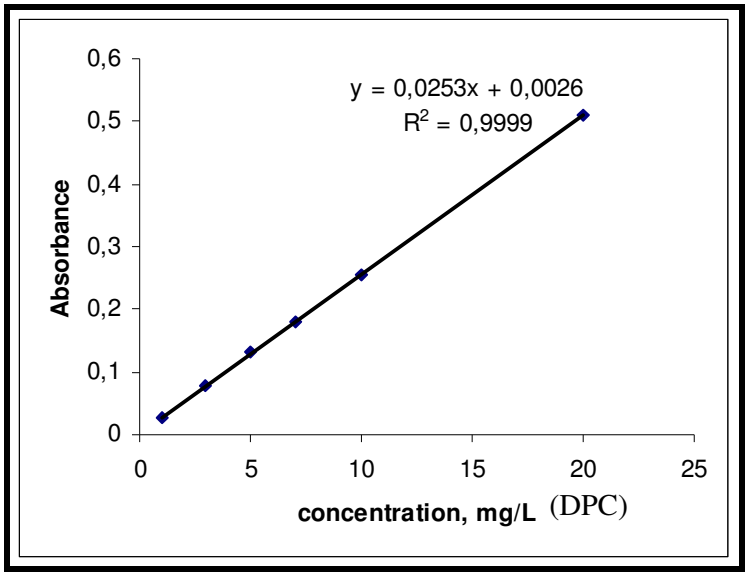


Figure 3.15 Calibration Graph of DPC in Aqueous Medium (λ_{abs} =279 nm)

3.4 Leaching Studies of DPC-Doped Sol-Gel Resin

Possibility of losing the active sites of DPC was checked by the absorbance measurements of the complexation product of DPC and Cr (VI) (λ_{abs}540 nm). The magenta color observed was the proof of the protection of the active sites. Besides, there was no spectral shift for the absorbance wavelength of the product.

The resins prepared were dried to constant weight. The final weight of the resins obtained was 2.5 g. This value was used to calculate the DPC amount in the resin.

DPC-Doped sol-gel resin was tested for leaching of the dopant molecule. As can be seen from Figure 3.16, leaching of DPC was observed to a certain extent. Utilizing the calibration study performed for DPC, the leached amount was calculated to be 14%. In this respect, it was decided to wash the resin with appropriate amount of de-ionized water before use.

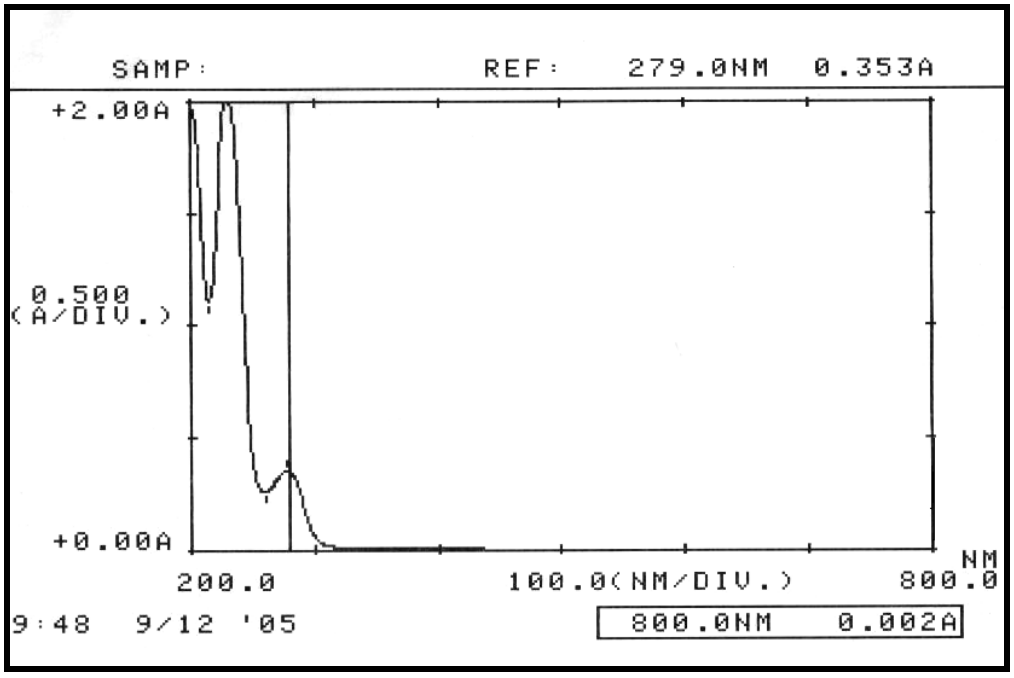


Figure 3.16 UV-VIS spectrum of supernatant of resin kept in 5 ml of deionized water for 24 hour

3.5 Take-up and Recovery Studies of CTAB and DPC-CTAB-Doped Sol-Gels for Cr(VI)

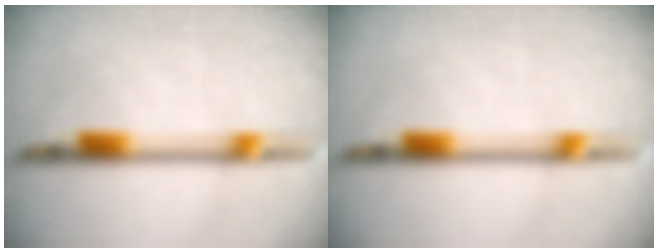
DPC doped sol-gel beads were planned to be used as renewable surfaces in flow type sensors [89]. In these systems, the injected beads are usually discarded following the measurement. Hence, the irreversibility of the complex formation between Cr(VI) and DPC was not a problem In any case, the resin was designed as a "Renewable Surface"; in other words the resin prepared was disposable.

Uptake studies were performed for CTAB and DPC-CTAB-doped sol-gel resins utilizing UV-VIS spectrophotometry. Both resins showed high uptake efficiencies indicating that CTAB played the main role in the diffusion and retaining of Cr(VI) in the sol-gel structure (Table 3.5).

Table 3.5 % Take-up Values of Sol-Gel Beads for Cr(VI), particle size: 100-150 µm, pH 2.0, n=3

Type of resin	Flow (mL/min)	Conc. of Cr(VI) (mg/L)	Cr(VI) capacity (mmol Cr(VI)/ g resin)	Sample Volume(mL)	%Take -up
DPC-CTAB-doped sol-gel	0.5	5.0	------	10.0	97±3
CTAB-doped sol-gel	0.5	5.0	0.02	10.0	99±1

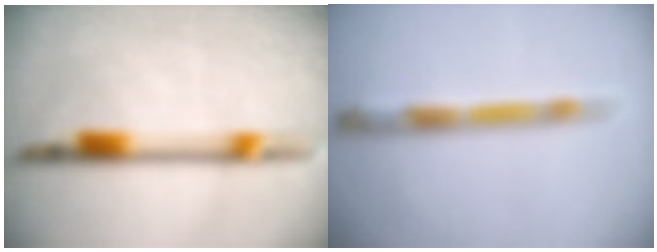
CTAB-doped sol-gel resin was also examined for its selectivity to Cr(VI). As can be seen from Figure 3.17, Cr(III) which is a green colored solution was not retained on the resin. So it was decided that, the resin was not selective to Cr(III).



(a) **(b)**

Figure 3.17 CTAB-Doped Sol-Gel Resin **(a)** before contact with 10.0 mL of 5.0 mg/ L Cr(III) and **(b)** after contact with 10.0 mL of 5.0 mg/ L Cr(III) solution

Same study was performed for Cr(VI) and it was observed that the yellow colored CR(VI) solution was retained on the resin (Figure 3.18).



(a) **(b)**

Figure 3.18 CTAB-Doped Sol-Gel Resin **(a)** before and **(b)** after contact with 10.0 mL of 5.0 mg/ L Cr(VI) solution

Recovery studies were carried out for CTAB-doped sol-gel resins however the irreversibility of the reaction between the hexavalent chromium and DPC did not allow the recovery studies to be performed for DPC-CTAB doped sol-gel.

Table 3.6 % Recovery Values of CTAB-doped Sol-Gel Beads for Cr(VI), particle size: 100-150 µm, pH 2.0, n=3

NaOH Conc. (M)	Conc. Of Cr(VI) (mg/L)	Sample volume (mL)	Flow Rate mL/min	Conc. Factor	Recovery (%)
0.1	**5**	**10**	**0.5**	**-----**	**73±2**
0.2	**5**	**10**	**0.5**	**-----**	**100±1**
0.5	**5**	**10**	**0.5**	**20 fold**	**99±1**

100% recovery was obtained when 0.2 M NaOH was used as an eluent. 20-fold preconcentration was achieved for 0.5 M NaOH however higher concentration of NaOH lead to the decomposition of the resin.

3.6 DPC-doped Sol-Gel Films

So as to get whether the DPC-doped sol-gel resin was proper for the spectrophotometric purposes, the resin was tested for its transparency. A piece of glass was taken as reference and the T% was measured. The results indicated that the DPC-doped sol-gel resin has high transparency (Figure 3.19) 93.5% at 540 nm.

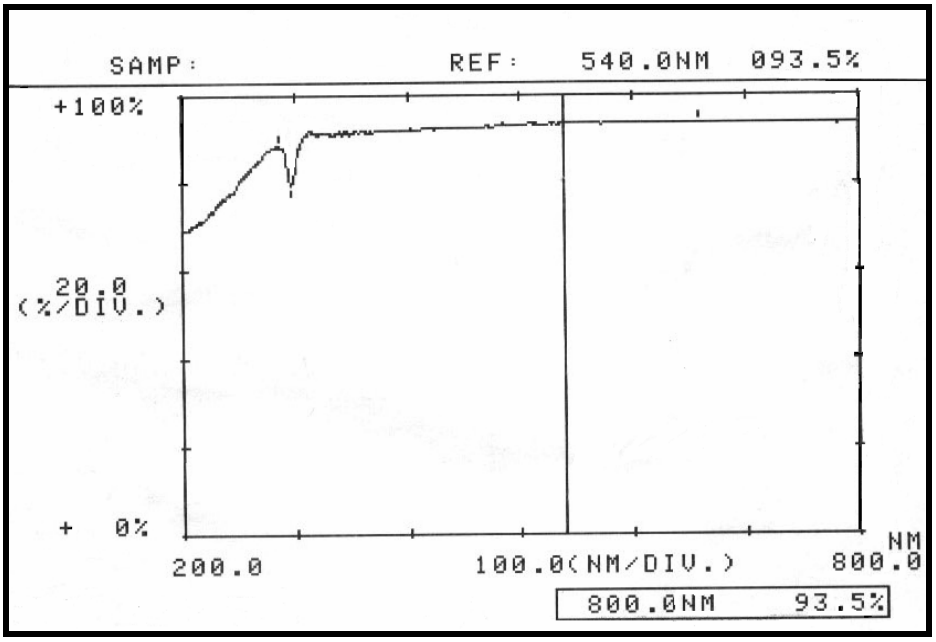


Figure 3.19 UV-VIS Spectrum for DPC-Doped Sol-Gel Film

Sol-gels bi-coated on glass substrates were more suitable for the spectrophotometric purposes. These were tested in order to derive a calibration line for Cr(VI) in the concentration range of 1.0 to 10.0 mg/ L (Figure 3.20). It was observed that the calibration line deviated from linearity for concentrations above 5.0 mg /L which might be due to the insufficient amount of DPC doped.

Glass substrates used for the spin coating of DPC-doped sol-gels were not coated homogeneously due to the geometric shapes of the glass substrates and also the insufficient cleaning of glass surface resulting in the stripping of the film from the surface.

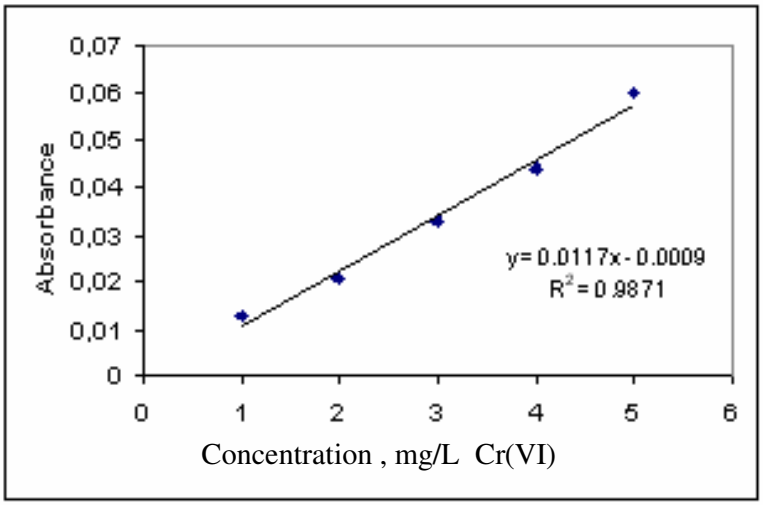
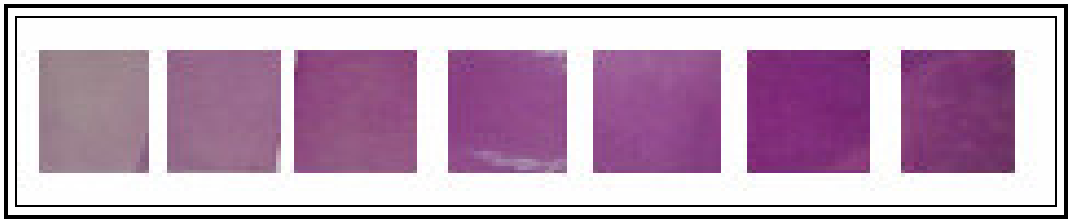


Figure 3.20 Calibration line for Cr(VI)-DPC complex (λ_{abs} =540 nm)

3.7 DPC-doped Sol-gel Impregnated Filter Papers

A practical semi-quantitative Cr(VI) determination method similar to pH studies was improved by the impregnation of DPC-doped sol-gel on filter papers.

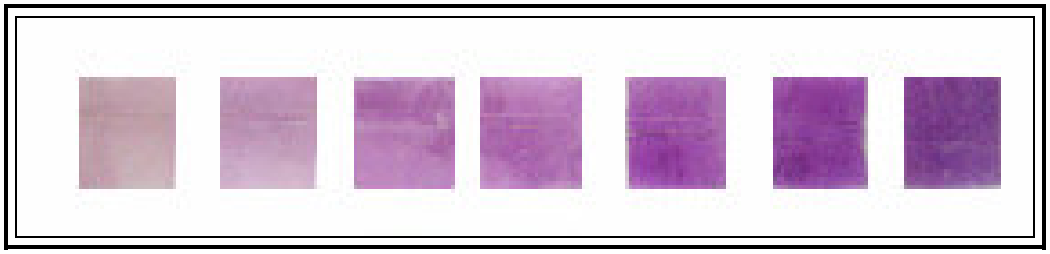
The hydrophobic character of DPC-doped sol-gel impregnated on the filter papers limited their use with water containing matrices. Therefore, previously impregnated and dried papers were dipped into a hydrophilic sol-gel to obtain a hydrophilic surface. After contact with the Cr (VI) solutions, meaningful color developments were obtained by using these filter papers (Figure 3.21).



(a) **(b)** **(c)** **(d)** **(e)** **(f)** **(g)**

Figure 3.21 Color developments of the filter papers for **(a)**1.0 mg/ L Cr(VI) **(b)** 2.0 mg/ L Cr(VI) **(c)** 3.0 mg/ L Cr(VI) **(d)** 4.0 mg/ L Cr(VI) **(e)** 5.0 mg/ L Cr(VI) **(f)** 7.0 mg/ L Cr(VI) **(g)** 10.0 mg/ L Cr(VI) solutions at pH 2.0. (for 10 minutes)

Interference studies were performed for Cr(VI) in the presence of 1.0 mg/ L Fe (II), 10.0 mg/ L V(V), 10.0 mg/ L Mo(VI) and 10.0 mg/ L Hg(II). Iron formed a yellow colored complex but this had no effect on the observation of the magenta Cr (VI)-DPC complex as shown in Figure 3.22. However, vanadium and molybdenum formed pink colored complexes with DPC, so the magenta color could not be observed properly. In the presence of mercury, color change of the Cr (VI)-DPC complex was observed weakly.



(a) **(b)** **(c)** **(d)** **(e)** **(f)** **(g)**

Figure 3.22 Color developments of the filter papers for **(a)**1.0 mg/ L Cr(VI) **(b)** 2.0 mg/ L Cr(VI) **(c)** 3.0 mg/ L Cr(VI) **(d)** 4.0 mg/ L Cr(VI) **(e)** 5.0 mg/ L Cr(VI) **(f)** 7.0 mg/ L Cr(VI) **(g)** 10.0 mg/ L Cr(VI) solutions at pH 2.0 (in the presence of 1.0 mg/ L Fe(II)) (for 10 minutes)

CONCLUSION

In this study, the immobilization of m-dansylaminophenylboronic acid (DAPB acid) molecule into various polymer matrices was attempted. DAPB acid reacts with 1,2 and 1,3 diols via its boronic acid end by the photoinduced electron transfer (PET) mechanism.

Five different matrix types were investigated for the immobilization studies namely hydrogels, methacrylic acid methyl methacrylate co-polymer, Polymethylmethacrylate (PMMA), PMMA and polyethyleneimine(PEI) blend and sol-gel. Hydrogels like polyvinylalcohol and Ca-alginate gel were eliminated due to the leaching problem, polymethylmethacrylate and polyethyleneimine for having diffusion problems were not suitable matrices for DAPB acid.
For the methyl methacrylate co-polymer and sol gel matrices it was demonstrated that upon immobilization, the DAPB acid probe was kept tightly inside the matrix, did not leach out even after excessive washing steps and DAPB acid probe did not experience any significant spectral change. However the immobilization of the PET probe on a solid support is a difficult task as the sensing properties can be drastically changed upon immobilization. DAPB acid is constituted by a benzylboronic moiety with the function of binding and the dansyl fluorophore with the role of signaling. In our studies the emission of the fluorophore was quenched due to the monomers or precursors or the formed matrix and no further quenching was observed even in the presence of high analyte concentration.

The possible explanation is the change in DAPB acid's acid–base properties and / or electron transfer mechanism due to the matrix microenvironment experienced by the probe. Hence it was concluded that none of these immobilization strategies were suitable for sensor application of DAPB acid in fructose determination.

During these studies, it was realized that polyvinylalcohol (PVA) had a positive effect on the emission signal of DAPB acid related to its high viscosity that possibly reduce the external quenching. Relatedly it was aimed to decrease the detection limit ($1.0x10^{-4}$ M) of a flow injection analysis method for fructose determination, previously carried out in our laboratory, based on the fluorescence quenching of a dansylaminophenylboronic acid (DAPB acid) probe in solution. In this study, a detection limit of 2.5x $10.^{-5}$M was reached in the presence of PVA.

In the second part of this study different probe designs; sol-gel beads, sol-gel coated glass slides and sol-gel impregnated papers, were developed by physical immobilization of chromophore DPC in a hydrophobic sol gel matrix containing an additive CTAB. CTAB was found to facilitate the sorption of Cr(VI) into the sol-gel matrix which resulted in the occurrence of the reactions in the host matrix itself. DPC -chromate complex having an absorbance maxima at 540 nm was retained in the sol-gel matrix. Column work, spot tests and sensor studies utilizing the prepared probes were investigated.

High take up values for aqueous chromate solutions obtained with perfectly transparent DPC doped sol- gel beads demonstrated the possibility of using this resin as a renewable surface in flow type sensor applications. As a future work this possibility will be investigated for the on-line determination and speciation of chromium via bead injection technique using our automated flow system equipped with jet ring cell attachment.

In the sensor studies, the immobilization can be performed directly on the surface of optical fibers (intrinsic sensors), or on a suitable substrate, which serve as the interface between the sample and the fiber-optic system (extrinsic sensors). The reagents immobilized into the sensor are responsible for the extraction of the analyte into the sensing material and generating an optical signal proportional to the change in the concentration of the analyte. The glass slide probes were found to be effective for Cr(VI) preconcentration and determination from aqueous solutions in the concentration range of 1.0-5.0 mg/ L. Thus, the present work demonstrates the possibility of developing a chemical sensor for determination of Cr(VI) in aqueous samples.

Filter papers impregnated with DPC doped sol-gel were prepared for a rapid screening test to detect chromate, the toxic form of chromium, in liquids. For spot test applications, suggested probes should be simple to use, easy to interpret, and provide results in minutes. Filter papers simply immersed into the solution and color change was observed in 10 minutes which indicates the presence of chromate.

A color scale was established in the range of 1.0- 7.0 mg/ L chromate concentration. Since no instruments are needed, the probes are convenient and can be used anywhere. Unfortunately in ambient air the shelf life of the probes are 3 weeks.

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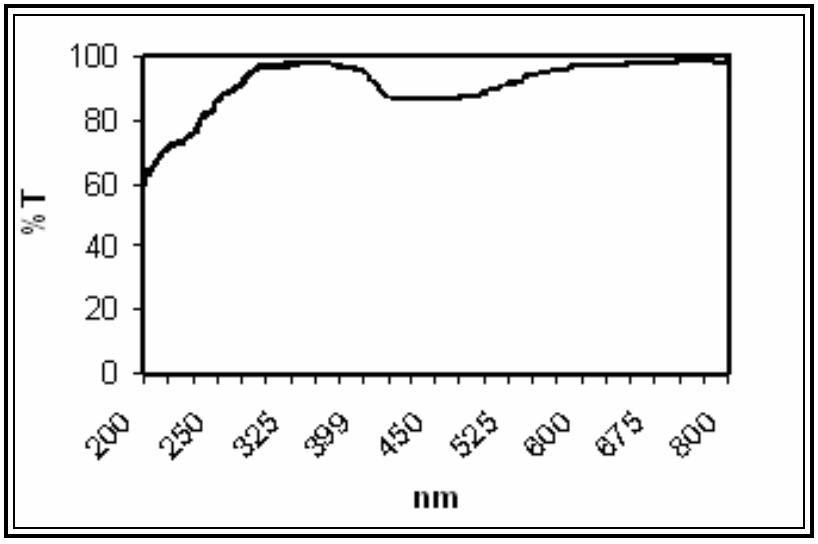
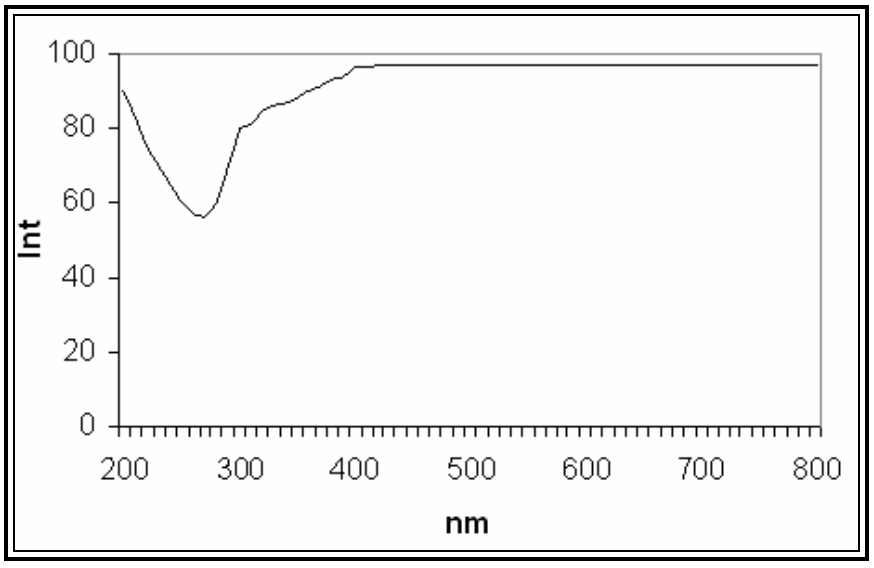
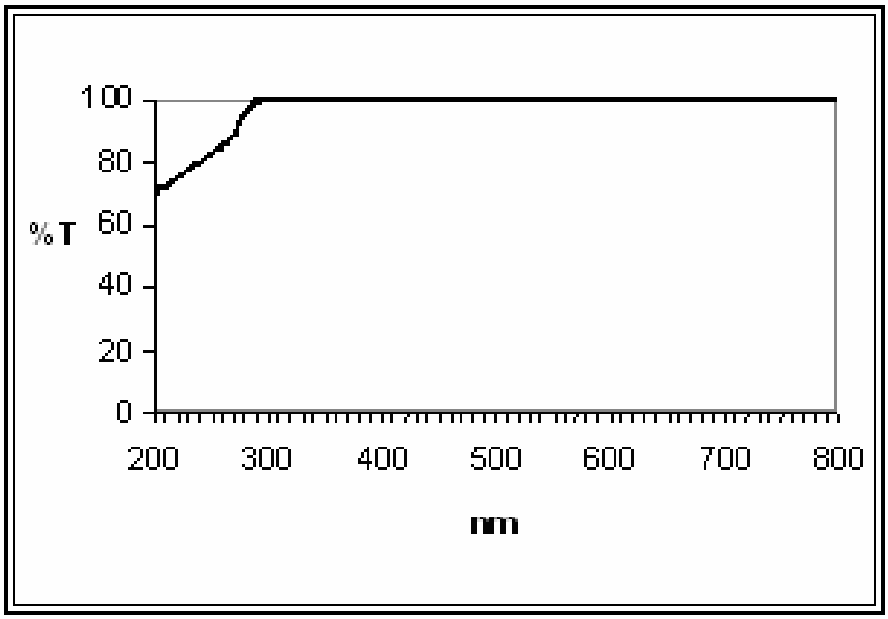
APPENDIX 1
(TRANSMITTANCE SPECTRUMS OF STUDIED POLYMERS DOPED WITH DAPB Acid and DPC)



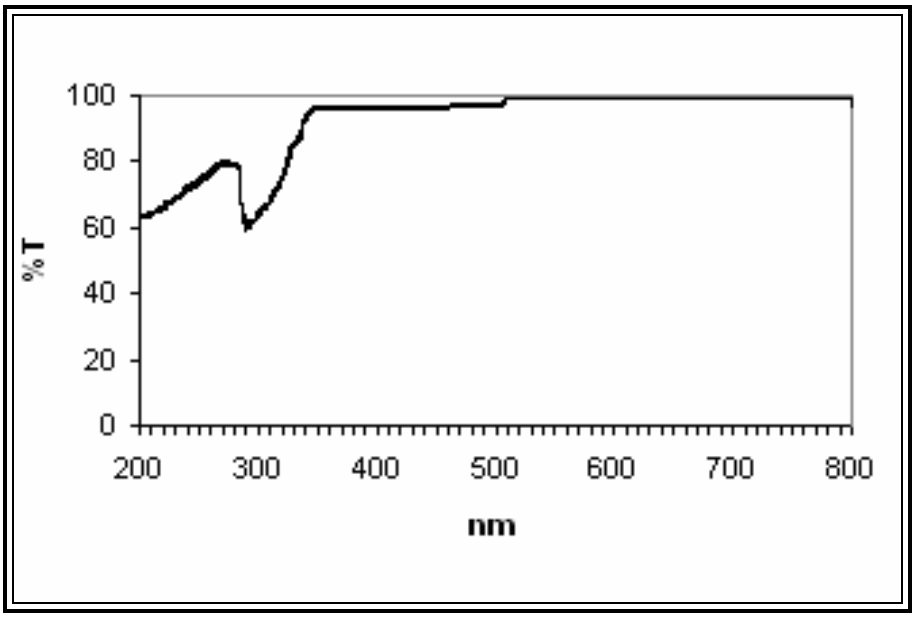
Transmittance spectrum of DAPB acid doped-Ca-alginate gel in the UV-VIS region (200-800 nm)



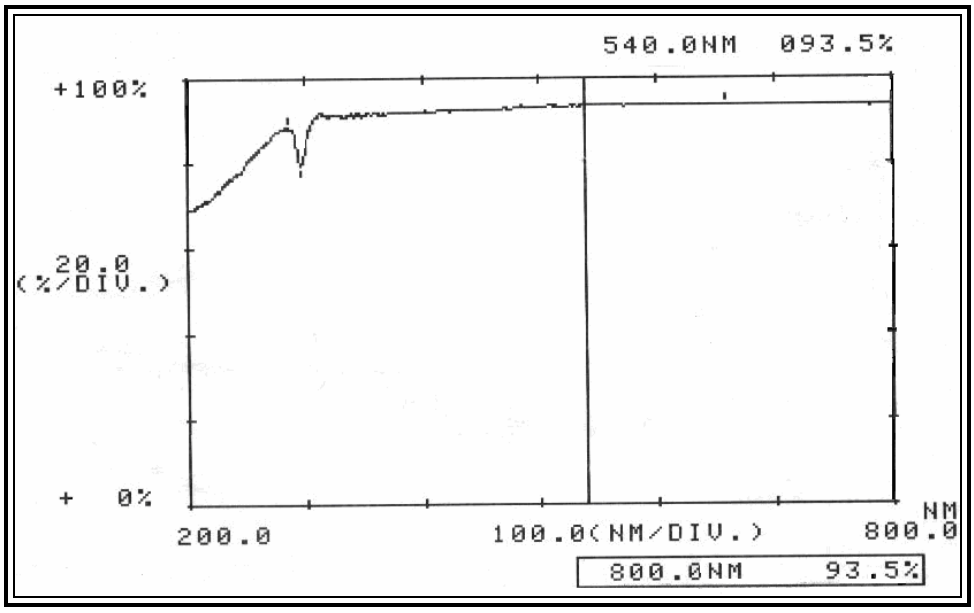
Trasmittance spectrum of DAPB acid-doped PVA Film in the UV-VIS region (200-800 nm)



Transmittance spectrum of DAPB acid-doped sol-gel in the UV-VIS region (200-800 nm)

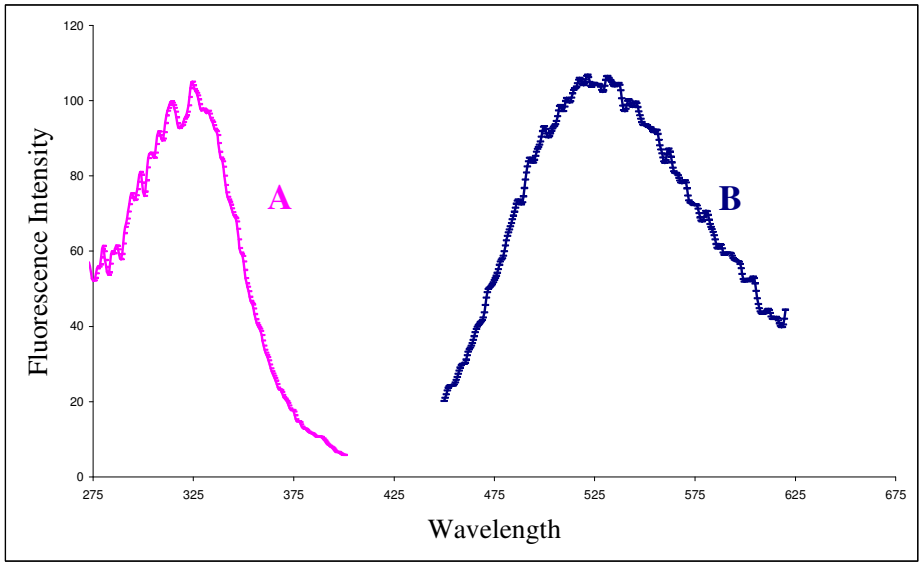


Transmittance spectrum of DPBA-doped P(MMA-MAA)co-polymerin the UV-VIS region (200-800 nm)

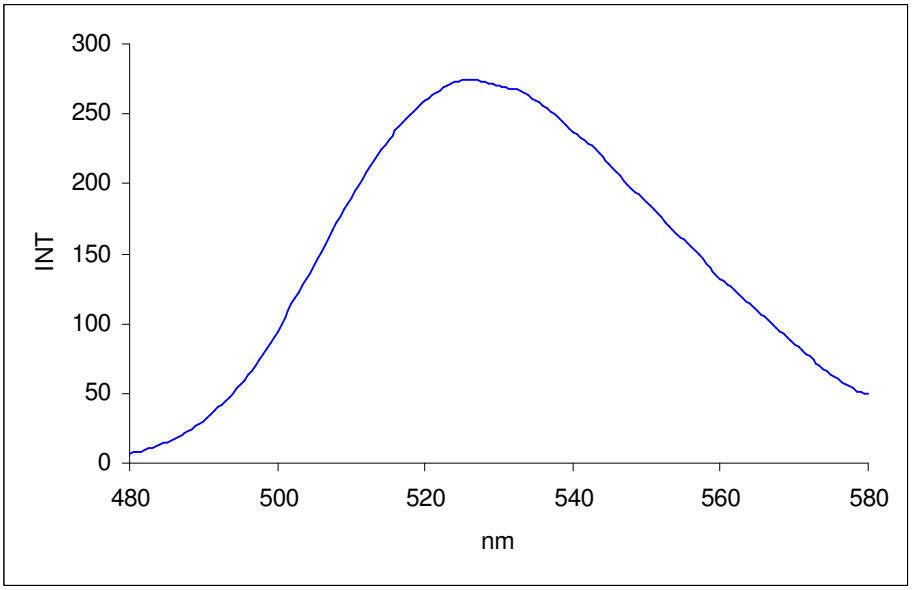


Transmittance spectrum of DPC-doped sol-gel in the UV-VIS region (200-800 nm)

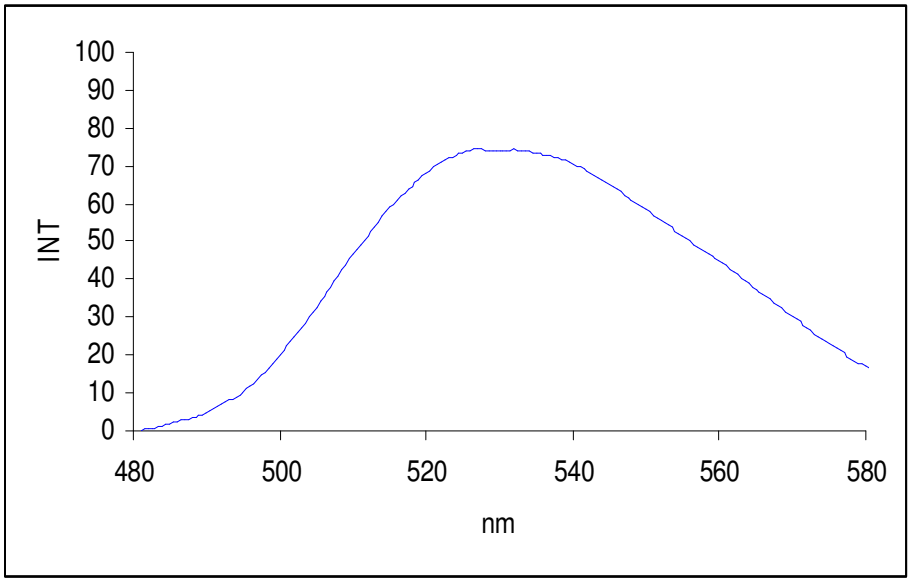
APPENDIX 2



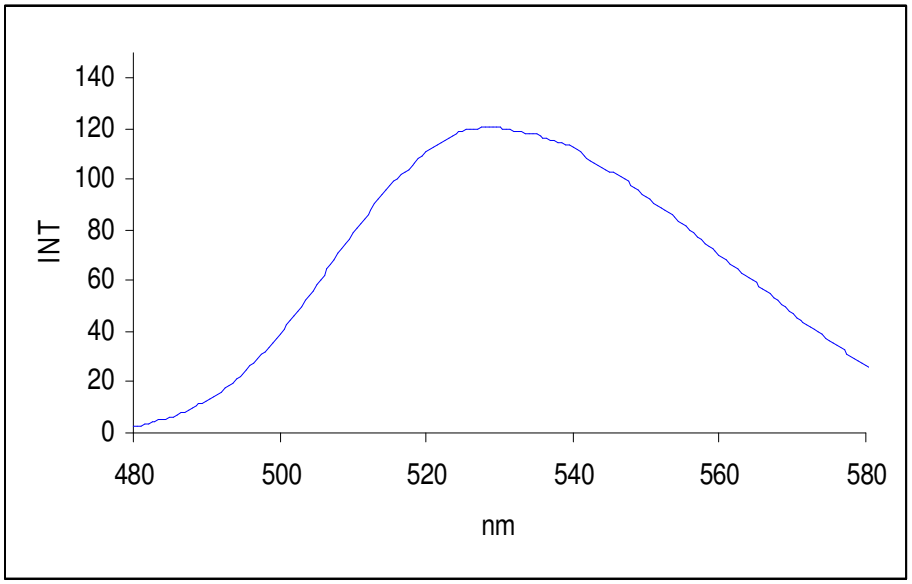
Excitation (A) and Emission (B) Profile of DAPB acid ($3.5x10^{-5}$ M), using 529 nm for emission and 324 nm for excitation.



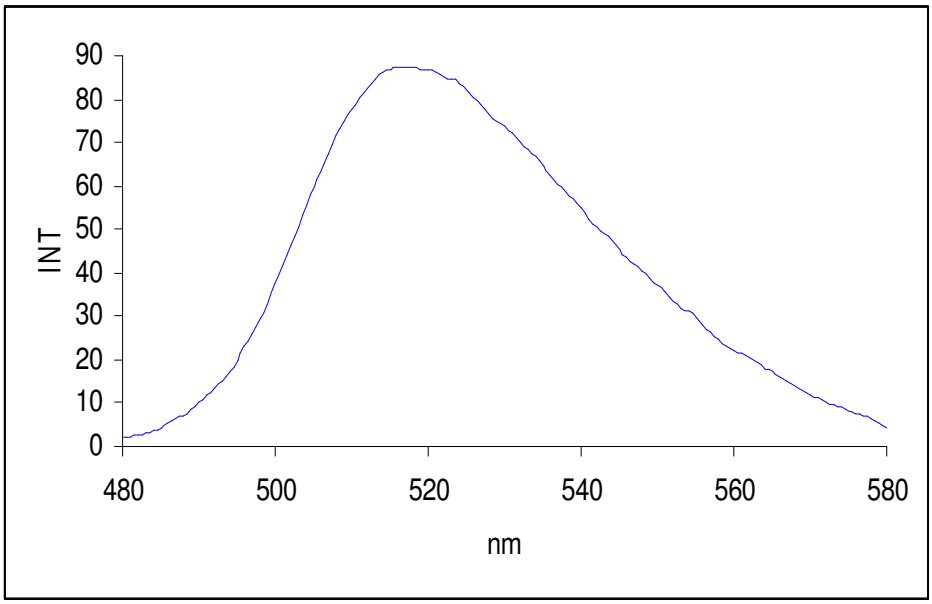
Emission profile of $1.0x10^{-4}$ M DAPB acid doped- PVA Film
$\lambda_{exc.}$=324 nm



Emission profile of $1.0x10^{-4}$ M DAPB acid doped -Ca-Alginate Film
$\lambda_{exc.}$=324 nm

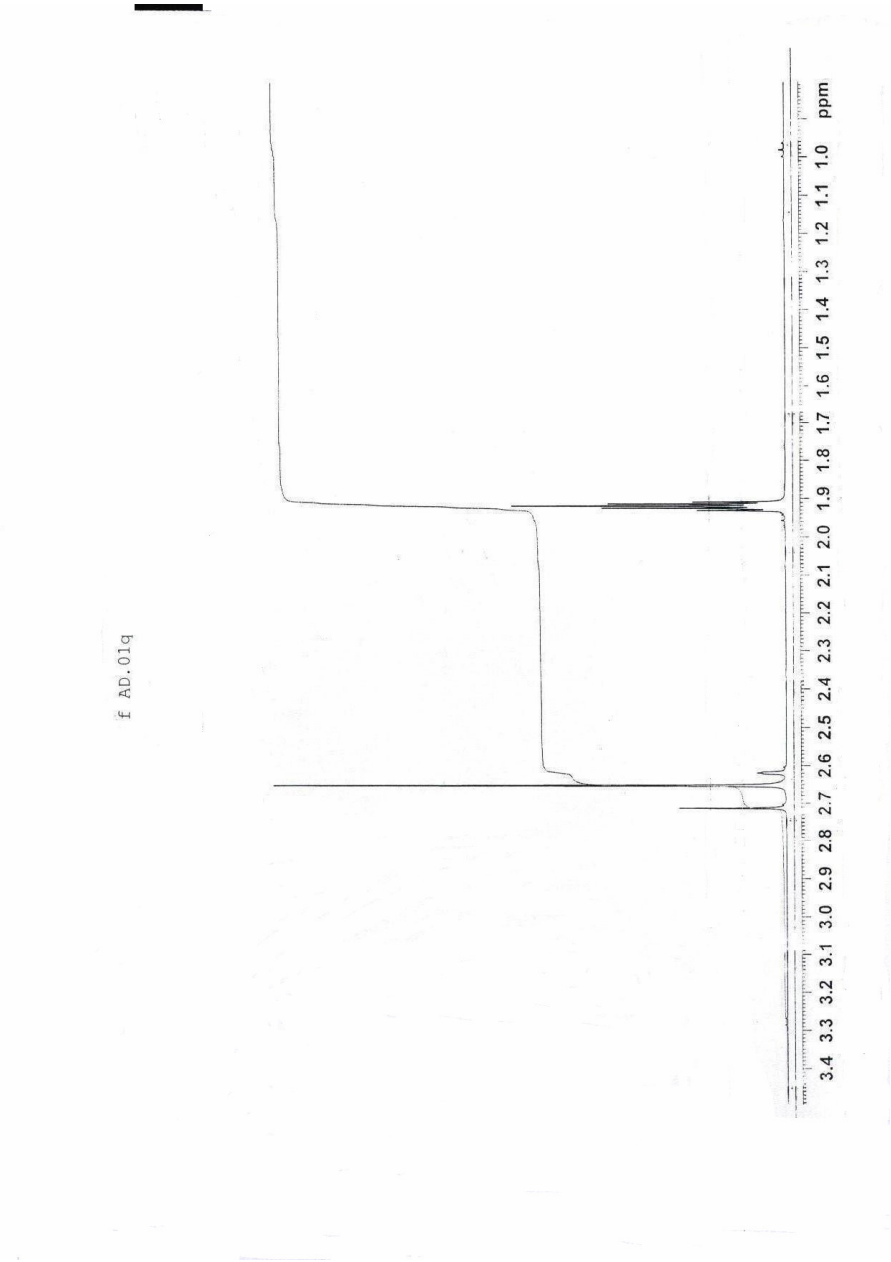


Emission profile of
$1.0x10^{-4}$ M DAPB acid doped -Polymer (MAA-MMA) $\lambda_{exc.}$=324 nm

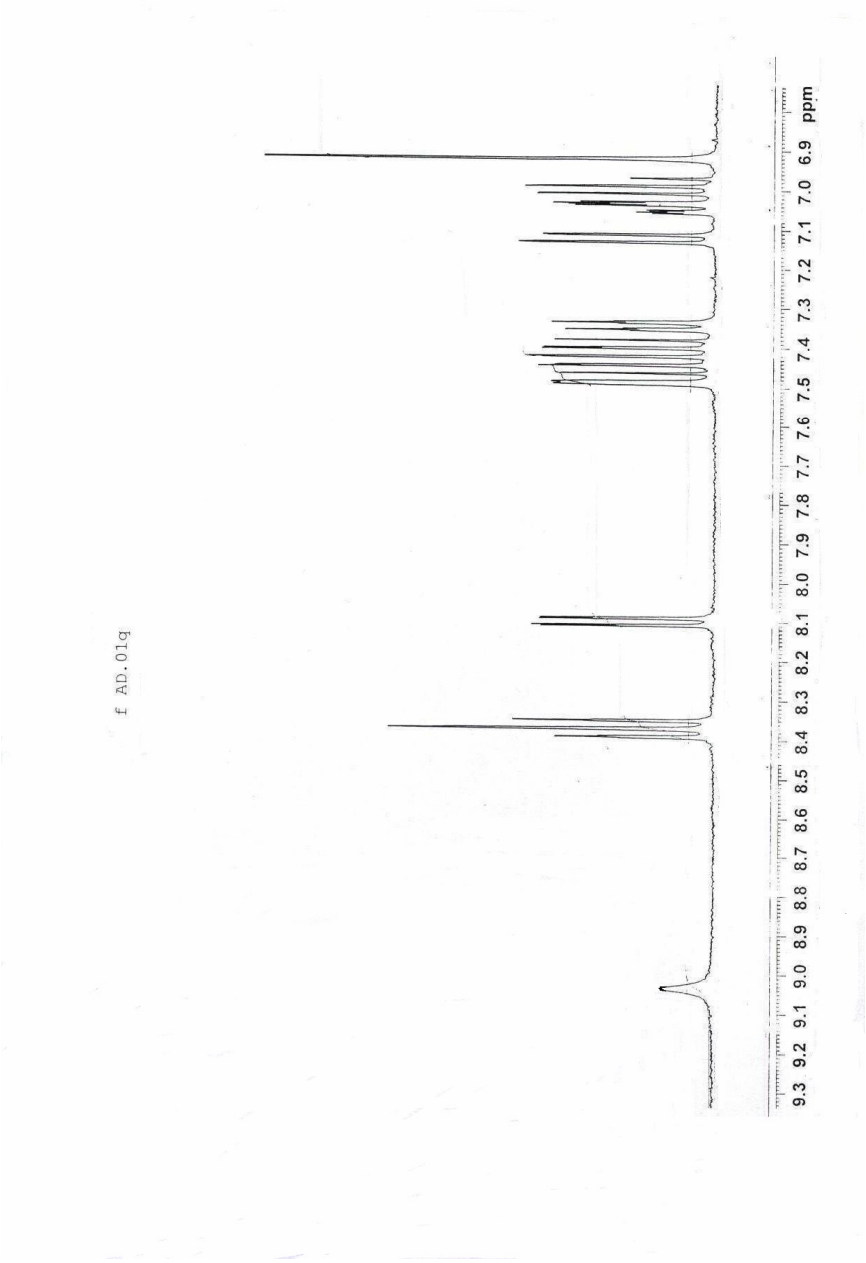


Emission profile of 1.0×10^{-4} M DAPB acid doped -Sol-Gel Film
$\lambda_{exc.}$=324 nm

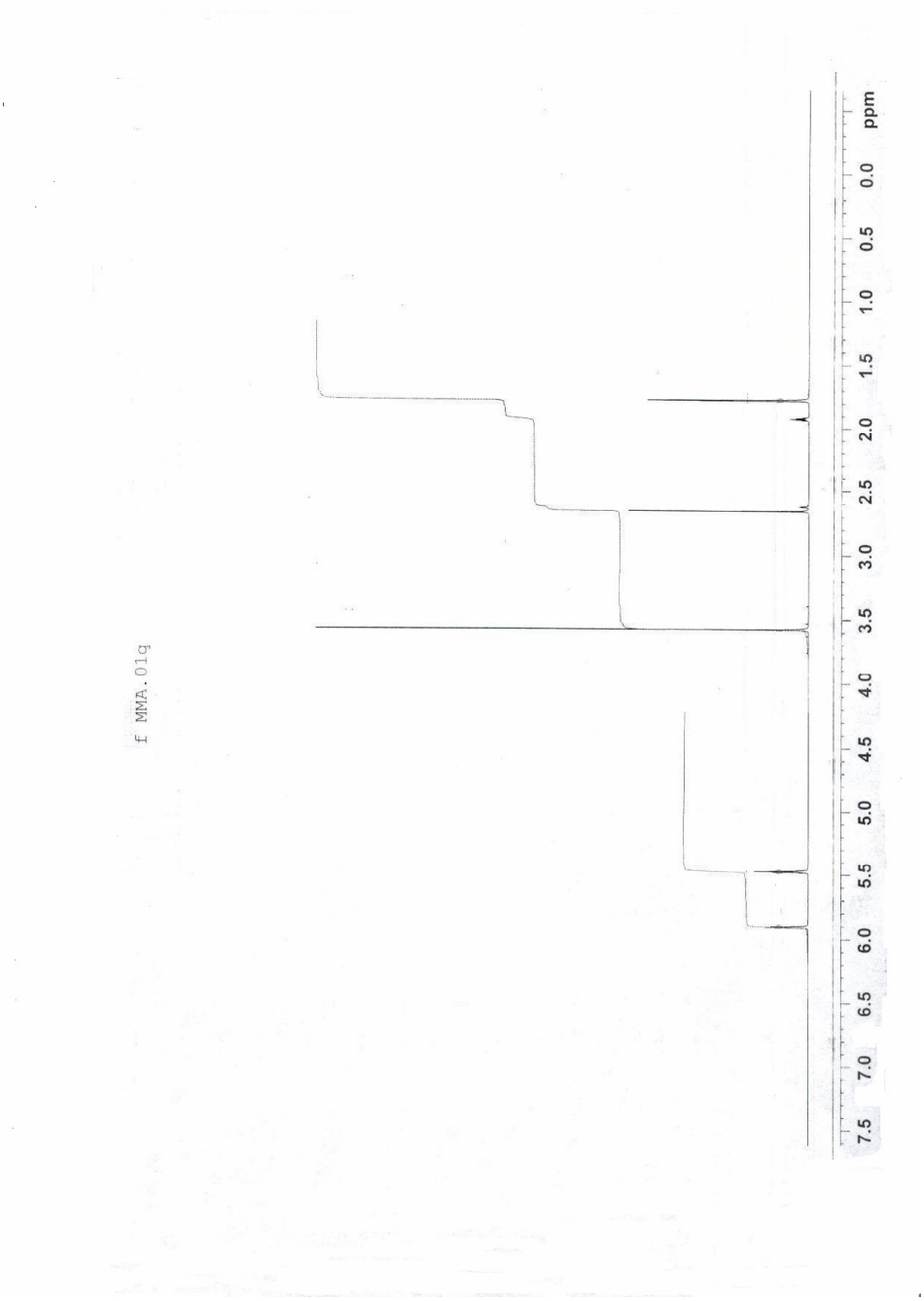
APPENDIX 3



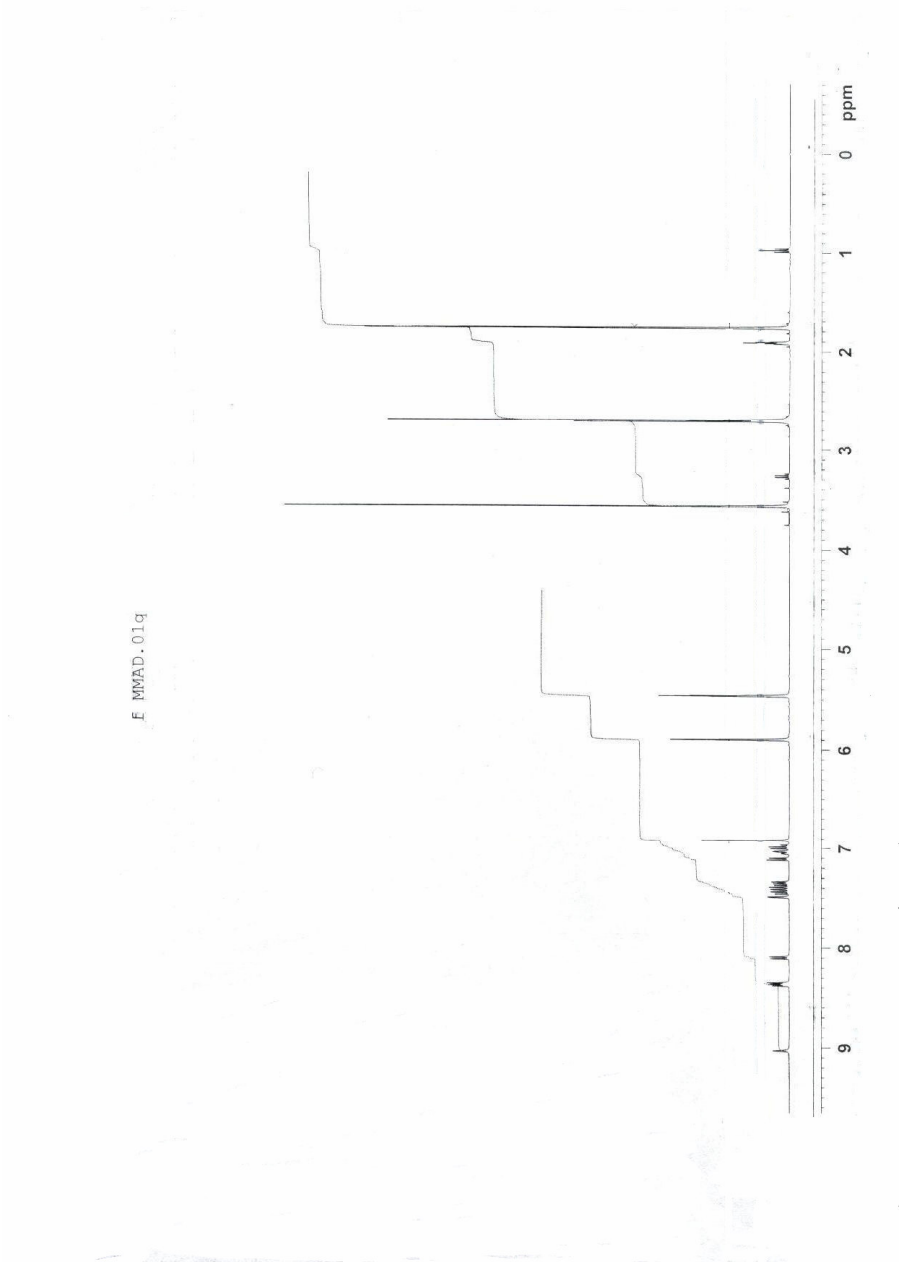
NMR Spectrum of DAPB acid in acetone



NMR Spectrum of DAPB acid in acetone

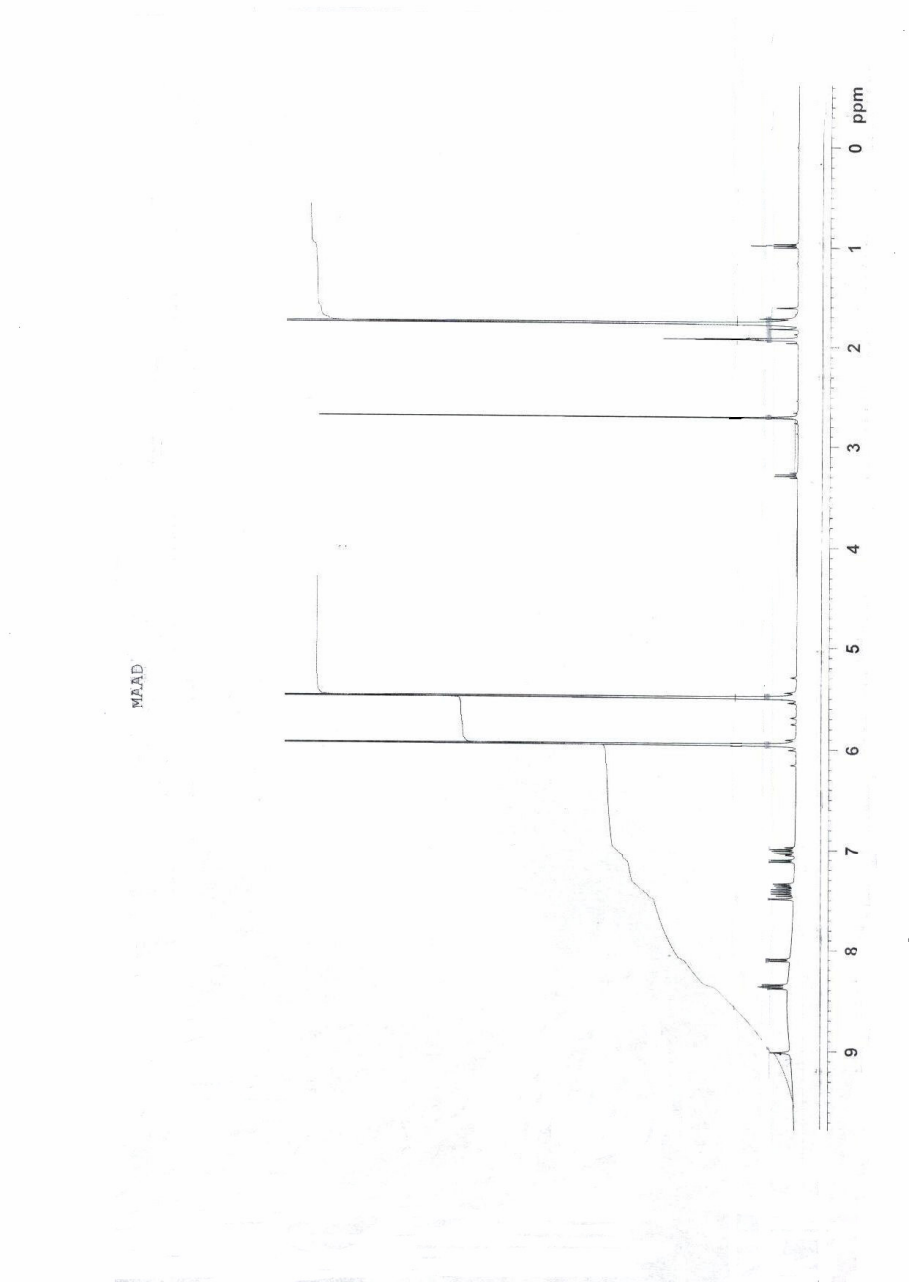


NMR spectrum of methylmethacrylate in acetone



NMR Spectrum of DAPB acid in methylmethacrylate and acetone

NMR Spectrum of methacrylic acid in acetone



NMR Spectrum of DAPB acid in methacrylic acid and acetone