USE OF SILICA-BASED SORBENTS FOR SEPARATION AND DETERMINATION OF V(IV) AND V(V)

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ABSTRACT

USE OF SILICA-BASED SORBENTS
FOR SEPARATION AND DETERMINATION OF
V(IV) AND V(V)

Vanadium is one of the essential trace elements for plants and animals. While it is beneficial for normal cell growth, it becomes toxic at high concentrations. In the present study, silica-based sorbents were prepared and used for sorption/speciation of vanadium from waters prior to inductively coupled plasma mass spectrometric (ICP-MS) determination. Among the sorbents developed, 3-APTES-modified silica, has been shown to be an efficient material for the speciation of vanadium. Solution pH of 2.0 and 3.0 can be used for the respective sorption of V(V) and the total vanadium, namely, V(IV) and V(V). The concentration of V(IV) can then be calculated from the difference. Desorption from the sorbent was realized with 0.5 M thiourea prepared in 0.2 M HCl. The validity of the method was checked via spike recovery experiments with four different types of water; namely, ultra pure, bottled drinking, tap, and sea water. The method works efficiently (>85% recovery) for ultra pure, bottled drinking, and tap water; and a relatively high recovery (>70%) was obtained even for sea water which has a very heavy matrix. For trypsin-immobilized silica, the sorption percentage towards V(IV) and V(V) is almost constant (>90%) within the pH range 4.0-8.0 which demonstrates the possibility of using this sorbent for the sorption of both vanadium species. The sorption of vanadate ion was investigated both from a kinetic perspective and also in terms of Freundlich, Dubinin-Radushkevich and Langmuir isotherm models; and Langmuir model was found to describe the sorption better for both of the functionalized silicas.

ÖZET

SİLİKA BAZLI SORBENTLERİN V(IV) VE V(V)'İN AYRILMASI VE TAYİNİNDE KULLANILMASI

Vanadyum bitki ve hayvanlar için gerekli eser elementlerden biridir. Normal hücre büyümesi için yararlı olsa da yüksek derişimlerde zehirli hale gelir. Bu çalışmada, sulardaki V(IV) ve V(V)'in sorpsiyonu/türlemesi için silika bazlı sorbentler hazırlanmış ve indüktif eşleşmiş plazma kütle spektrometri (ICP-MS) ile tayini öncesinde kullanılmıştır. Geliştirilen sorbentler içinde 3-APTES ile modifiye edilmiş silikanın vanadyum türlemesi için etkin bir malzeme olduğu bulunmuştur. Çözelti pH'sı olarak 2.0 ve 3.0'ün, sırasıyla V(V) ve toplam vanadyum sorpsiyonunda kullanılabileceği, vanadyum(IV) derişiminin ise farktan bulunabileceği belirlenmiştir. Vanadyum, 3-APTES ile modifiye edilmiş silikadan, 0.2 M HCl içinde hazırlanmış 0.5 M tiyoüre ile geri alınmıştır. Metodun validasyonu, çeşitli sulara (saf su, şişelenmiş içme suyu, musluk suyu ve deniz suyu) eklenen V(V)'in geri kazanım testleri ile gösterilmiştir. Metot, saf su, şişelenmiş içme suyu ve musluk suyunda iyi çalışmakta (>85% geri kazanım), oldukça ağır bir matrikse sahip deniz suyu içinde bile %70'in üzerinde geri kazanım sağlamaktadır. pH 4.0-8.0 aralığında her iki vanadyum türü için %90'ın üzerinde sorpsiyon gösteren tripsin ile immobilize edilmiş silikanın ise toplam vanadyum tayininde kullanılabileceği gösterilmiştir. Vanadat iyonu sorpsiyonu hem kinetik hem de Freundlich, Dubinin-Radushkevich ve Langmuir modelleri açısından incelenmiş ve incelenen derişim aralığında her iki sorbent için de sorpsiyonun Langmuir izoterm modeline uyduğu belirlenmiştir.

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CHAPTER 1

INTRODUCTION

1.1. Environmental Aspects

Toxic heavy metal pollution in air, soil, and water are global problems that are a growing danger to the environment. In addition to natural sources, there are hundreds of sources including coal, natural gas, paper, and several industries etc. (Bailey et al. 1999; Gupta et al. 2000) for heavy metal pollution. Depending on the chemical form and exposure level, heavy metals can potentially be very harmful to humans and have a negative effect on the environment. Thus the detection and quantification of heavy and/or essential metals at relatively high-to-trace levels in environmental, geological, biological etc. matrices have become necessary for the global systems.

The simplest way for removing heavy metals is filtration or precipitation. These two techniques can be helpful in removing a significant concentration of the metals but may not be sufficient to reduce the concentration of the contaminant to the legal threshold limit. Therefore more sensitive analytical methodologies are required. Currently, the most common methods of metal removal include solid phase extraction, ion exchange or chelation. In solid phase extraction the components of the sample are distributed between the sample phase and a sorbent material. The sorbent material is a solid in which the sample components are bound by a variety of mechanisms, including adsorption, ion exchange, and complex formation etc. The method is simple; it enables the complete isolation of the analytes of interest from a complex matrix with a preconcentration factor of several orders of magnitude to be achieved. The drawback associated with the above mentioned methods is the lack of the efficiency with which the analyte binds to the sorbent being often low because of equilibrium effects (e.g. may exhibit slow release kinetics). In addition, harsh chemicals are used which may be toxic and bring the problem of contamination.

A long time ago, due to inadequate analytical techniques, concentrations around mg L^{-1} levels were called “trace”. As the analytical performance of the instruments improved, concentrations from μg L^{-1} to ng L^{-1} levels (or even lower) are now detected

and considered as trace. In the second edition of IUPAC Compendium of Chemical Terminology, (McNaught and Wilkinson 1997), "Any element having an average concentration of 100 parts per million atoms or less than 100 μg g^{-1}" is considered as "trace". However, it is very difficult to specify a specific element as trace. One element which is at high concentration in one sample can be in trace amount in another one.

Trace analysis, or analysis in general, can be considered to include several steps. These are sample collection (if the analysis is not being carried out *in situ*), storage and transportation of sample to the laboratory, preparation for the measurement, calibration of the instrument (if an instrumental determination is necessary), measurement, data analysis and reporting.

Depending on the sensitivity required in the quantitation step, generally two approaches are followed in trace element determinations. The first strategy is to use or develop special measurement systems which are capable of detecting the low concentrations of certain species directly in the sample. Instrumental developments have produced a variety of methods suitable for trace element determinations without an enrichment step. For example, graphite furnace atomic absorption spectrometry (GFAAS) (Zih-Perenyi et al. 2000), hydride generation atomic absorption spectrometry (HGAAS) (De la Calle Guntinas et al. 1991) are still widely applied in the determination of metals at low concentrations. On the other hand, inductively coupled plasma mass spectrometry (ICP-MS) (Mandal and Suzuki 2003) has been extensively used in the determination of trace metals in many samples either directly or after a matrix separation step. The second strategy in trace analysis is to use some preconcentration methods to increase the concentrations of the analytes to a measurable level using available techniques. Meanwhile, the analyte is usually separated from the interfering components.

In addition to the determination of the amount of trace elements in environmental and biological matrices it is also very important to evaluate their chemical form(s). As different chemical forms of an element may exhibit different reactivity, toxicity and bioavailability, determination of the individual chemical form is the main concept of the speciation analysis.

One of the key issues of speciation analysis is to preserve the composition of the sample and the species of interest during sampling, storage and pretreatment, such as dissolution, extraction and preconcentration. Any treatment that would result in a shift

of equilibria or in a destruction or transformation of one species into another must carefully be avoided.

1.2. Vanadium Species in the Environment

Vanadium is widely distributed in the earth's crust and has been recognized as a potentially dangerous pollutant (Lazaridis et al. 2003). It originates from primary sources such as ores, concentrates, metallurgical slags, and petroleum residues. In addition, it is widely used in the production of special steels, temperature-resistant alloys, pigments and paints. Making up about 0.014% of the Earth's crust, it is the fifth most abundant transition metal (Moskalyk and Alfantazi 2003). It is also found at rather high concentrations in some freshwaters and is listed as a metal of concern by the United States Environmental Protection Agency (USEPA). Its concentration may vary region-to-region and found about 30 nmol/L levels (Tracey et al. 2007) in ocean waters.

Vanadium has oxidation states from −1 to +5. In nature and biological systems, it is most commonly found in the +4 and +5 states as tetravalent (IV) vanadyl (VO^{2+}) and pentavalent (V) vanadate (HVO_4^-,VO_3^- and/or $H_2VO_4^-$) species respectively. A number of monomeric and polymeric tetravalent [V(IV)] and pentavalent [V(V)] vanadium species can be present in aqueous solutions and the composition of each depends upon pH and species concentrations. In presence of oxidizing agents the vanadium ion is present as the hydrated monomer of vanadate (HVO_4^{2-} or $H_2VO_4^-$) at micromolar concentrations near neutral pH, whereas in the presence of reducing agents, the anion is reduced to the vanadyl cation (VO^{2+}), within a few minutes. If the conditions are strongly reducing, vanadium may also exist as trivalent [V(III)] and divalent [V(II)] ions (Angelos 2002).

The stability and dominance of specific anions depends on the pH and redox potential (Eh) of solution. Thus the behavior and the forms of vanadium in waters can be predicted from its Eh-pH diagram. As can be seen from Figure 1.1, redox conditions and the acidity/alkalinity existing in the solution have a direct influence in the distribution of vanadium species (Peacock and Sherman 2004). Vanadium exists in cationic forms below pH 3.0; while the anionic form predominate between pH 4.0 and 11.0.

Although vanadium is toxic at high concentrations it is also important in biological systems and at low concentrations (μg L^{-1}). Indeed, it is essential to cell growth and found to possess anti-cancer, anti-diabetic, and anti-HIV properties. Although it is a nutritional element, vanadium is not accumulated by the plants and animals; the only organisms known to bioaccumulate vanadium to a significant degree are mushrooms, tunicates and sea squirts. The occurrence of vanadium in sea squirts is supposed to be one of the main sources of this metal in crude oil and oil shales which is also an indication of the environmental pollution. At mg L^{-1} concentrations, vanadium is toxic to plants, mice, freshwater organisms, and humans. Moreover, the V(V) species is more toxic than the V(IV) and V(III) oxidation states. Thus, it is important to both separate and quantify the vanadium species in order to assess their potential risk to the environment and to biological systems.

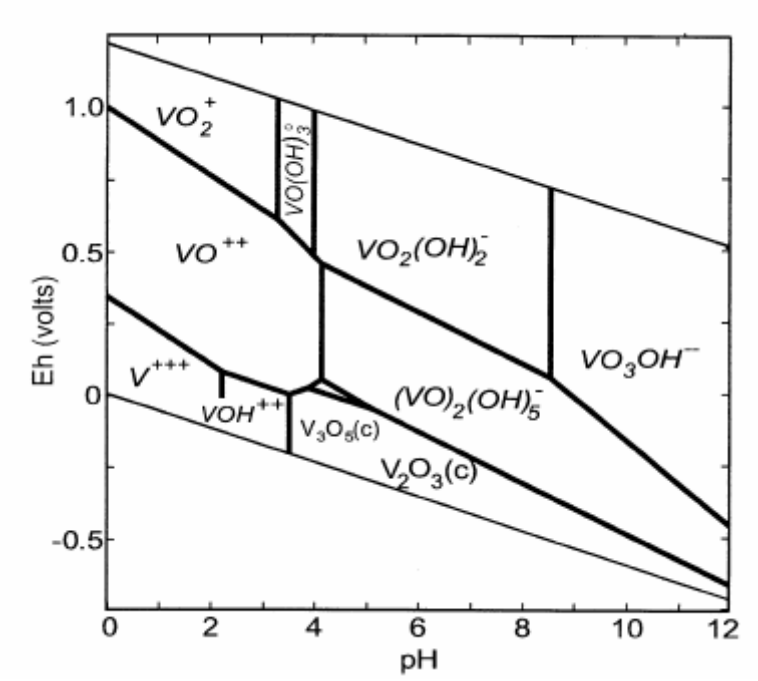

Figure 1.1. Eh-pH diagram for aqueous vanadium species in the system V-O-H) (Source: Peacock and Sherman 2004).

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1.3. Vanadium Determination Methods

There are many methods for vanadium determination in various matrices. The conventional methods, volumetry and gravimetry can also be used. For example, V(V) can be titrated potentiometrically with iron(II) in the presence of citrate, pyrophosphate and EDTA with zinc(II) in excess which allows the determination of vanadium(V) even in a weakly acidic media (Umetsu et al. 1991). In addition Nair and Cristine (2009) developed a new analytical reagent, 2-Hydroxy-4-*n*-propoxy-5-bromoacetophenone oxime, for the gravimetric determination of V(V) in the pH range 4.0-6.0 which gave a brown colored precipitate with V(V).

Since vanadium content in natural samples is very low (in the range of μg L^{-1}) more powerful analytical methods are required for the determination. Only a few of them show sufficient sensitivity, such as neutron activation analysis (NAA), electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) as well as some UV–vis spectrophotometric methods. In order to obtain high UV responses various chromogenic reagents as chelating ligands must have been used which forms a single distinct complex with V(IV) or V(V) and the ligand with a large UV absorptivity rapidly. Kumar et al. (2007) developed a sensitive method for the determination of V(V) based on the interactions of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) with *N*-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) where a blue colored derivative occurred in acidic medium. Amin et al. (2009) also developed a sensitive, selective and rapid method for the determination of vanadium ion based on the rapid reaction of V(V) with 2,3-dichloro-6-(2,7-dihydroxy-1-naphthylazo) quinoxaline (DCDHNAQ) in citric acid–sodium hydroxide buffer solution (pH 3.3) forming a violet complex which absorbs at 573 nm. Spectrophotometric methods for vanadium determination are useful due to their simplicity and low cost instrumentation which makes the technique available to a wide variety of users. However, detection limits for these methods ranged from only 0.1 to 0.5 mg L^{-1} and therefore did not meet the lower detection requirements typical of environmental samples. Therefore, without sample pretreatment/preconcentration direct spectrophotometric determination of vanadium is unlikely to be suitable for environmental and biological samples.

Separation techniques such as liquid chromatography (LC) and capillary electrophoresis (CE) can be used to determine vanadium species. Cowan et al. (2000) dynamically modified neutral polystyrene resin with dipicolinic acid for the separation of V(V) over the pH range 0–3. In addition Huang et al. (2002) applied two kind of synthesized chelating stationary phases, bis(2-aminoethylthio) methylated resin and aminobutyro hydroxamate resin and used a hyphenated method-chelation ion chromatography (CIC) coupled on-line detection with inductively coupled plasma mass spectrometry (ICP-MS) for the determination of vanadium species spiked in artificial seawater samples.

As mentioned previously, capillary electrophoresis (CE) is an attractive approach for the determination and separation of vanadium species. The main advantages of CE include experimental simplicity, higher efficiency, rapid separations and lower cost of analysis. Chen et al. (2007) used capillary zone electrophoresis (CZE) with UV detection to determine vanadium species. Nitrilotriacetic acid (NTA), hydroxyethylethylenediaminetriacetic acid (HEDTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), ethylene glycol-bis(2-aminoethylether)-tetraacetic acid (EGTA) and 2,6-pyridinedicarboxylic acid (PDCA) were investigated to determine whether these ligands formed stable anionic complexes with vanadium.

A variety of atomic spectrometric techniques have been also used for the determination of vanadium, among which are atomic absorption spectrometry (AAS) with flame and graphite tube atomizers, inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS) and X-ray fluorescence spectrometry (XRF). Vanadium determination by FAAS requires the use of a nitrous oxide–acetylene flame. A direct method for the determination of vanadium in fuel oils by FAAS has been developed by Bettinelli and Tittarelli (1994). Fernandes et al. (2007) described an analytical procedure for vanadium determination in human hair slurries by ETAAS using longitudinal heating (LHGA) and transversal heating (THGA) graphite furnace atomizers. Additionally, a new method for the ETAAS determination of vanadium in milk and infant formulas using suspensions is described by López-García et al. (2009). In addition, de Souza et al. (2006) optimized a procedure to prepare crude oil samples as detergentless micro-emulsions and applied it for the determination of vanadium and 10 additional elements using ICP-OES. Propan-1-ol was used as a co-solvent allowing the formation of a homogeneous and stable

system containing crude oil and water. Compared to ICP-OES, ICP-MS is more often used to determine vanadium species because of its multi-isotope detection capability with higher sensitivity. Aureli et al. (2008) used HPLC with on-line ICP-MS for the determination of V(IV) and V(V) in natural mineral water. Cationic and anionic tetravalent and pentavalent vanadium species were converted into V–EDTA complexes, i.e. $[VO(EDTA)]^{2-}$ and $[VO_2(EDTA)]^{3-}$, which could be effectively separated on a short anion exchange column.

1.4. Vanadium Speciation

It is nowadays well recognized that understanding of biogeochemical processes depends upon the knowledge of the chemical forms, or species, that are present in the natural environment. Despite this well-known requirement, speciation of many elements in the natural environments is not adequately known and vanadium is not an exception. As mentioned in the previous sections, vanadium occurs in several oxidation states in natural waters and, thus, its behavior can be affected by changes in the redox status of the aquatic environment. In natural water speciation studies, a rapid and reliable separation technique coupled with a suitable detection system is required. Samples should be analyzed as soon as possible after collection without use of sample preservation techniques such as acidification, which may modify the natural equilibria of species present.

For vanadium, most speciation studies in natural waters deal with the determination of the total amount of dissolved V(IV) and V(V), owing to the different interactions of these two forms with living organisms. Soldi et al. (1996) studied the distribution of V(IV) and V(V) in aqueous solution and used Chelex 100, a chelating resin with immunodiacetic acid functional groups, on the basis of the Gibbs-Donnan model. The method consists of sorbing both V(IV) and V(V) at a pH of about 4.5 and stripping first V(V) at basic (pH 10) and then V(IV) at acidic conditions (pH 0.8). In another study, a simple and rapid two-step method for the separation and determination of V(IV) and V(V) using Sephadex DEAE A-25 with Eriochrome Cyanide R (ECR) was proposed by Bosque-Sendra et al. (1998). In the first step, V(IV) was retained on the solid support as V(IV)-ECR complex and then determined by solid phase spectrometry. Subsequently, in the second step, ascorbic acid and ECR were added to

the resulting solution and the content of V(V), thus transformed into V(IV)-ECR complex, was measured.

Minelli et al. (2000) developed a sensitive method for the monitoring and speciation of V(IV) and V(V) in Italian waters at trace levels. A strong anion xchange column, SAX loaded with disodium ethylendiaminetetraacetic acid (Na_2EDTA) was used to trap both vanadium species at pH 3. The vanadyl ion (VO^{2+}) was selectively eluted by means of an aqueous solution containing Na_2EDTA, tetrabutylammonium hydroxide and isopropanol. Subsequently determination was performed with ETAAS. The concentration of vanadate ion was calculated by subtracting the vanadyl ion concentration from the total concentration of vanadium.

Wu et al. (2005) developed a flow injection (FI) system using a micro-column packed with quinine modified resin as solid phase extractant for preconcentration and separation of trace amount of V(V) and V(IV) in water samples. The determination was performed with fluorination assisted electrothermal vaporization (FETV) inductively coupled plasma optical emission spectrometry (ICP-OES). At pH 3~3.8, the modified resin is selective to V(V) and almost not to V(IV), while, V(IV) could be quantitatively retained by the modified resin at pH 5~7. The two vanadium species adsorbed by the modified resin could be readily desorbed quantitatively with 0.5 M HCl.

In another study, a flow injection system coupled to ICP-OES was used for the on-line preconcentration and subsequent determination of vanadium by Muyano et al. (2006). Trace amounts of vanadium were preconcentrated at pH 7.0 by sorption on a conical minicolumn packed with immobilized yeast cells in the absence of complexing reagent. Vanadium was removed from the minicolumn with a 50% HCl solution. A similar study utilizing microcolumns packed with l-methionine immobilized on controlled pore glass (CPG) as solid phase extractant was proposed by Pacheco et al. (2008). At pH 5.0, l-methionine is selective only towards V(V) while, total vanadium was quantitatively adsorbed by the solid phase at pH 9.0 (as V(V)) due to oxidation of V(IV) in alkali media. Vanadium species retained by l-methionine were quantitatively eluted from the column with 10% HCl. Effects of acidity, sample flow rate, concentration of eluent and interfering ions on the recovery of the analytes have also been investigated.

Amberlite IRA-904 resin modified with tetrakis (*p* carboxyphenyl) porphyrin (TCPP) was used to preconcentrate vanadium species (Pyrzynska and Wierzbicki 2005). Several parameters, such as sorption capacity of the chelating resin, pH for retention of

V(IV) and V(V) and volume of sample and eluent were evaluated. Both vanadium species sorbed on TCPP-modified resin were eluted by use of 2.0 M nitric acid and determined by AAS. For speciation studies, CDTA was added to the sample for complexing V(IV) which was not retained on the microcolumn. The proposed method was applied to a reference standard material (TM-25.2) and river water sample.

1.5. Silica: A Support in Metal Ion Sorption

Solid surfaces have been an area for extracting trace metals from solutions for years. In recent years, modification of these surfaces with a specific functional group to make the surface available to form chelates with the metal ions has gained attraction in scientific research. The donor atoms in forming chelates usually include oxygen, sulfur, nitrogen, phosphorus, and the functional groups such as hydroxyl, ether, phosphoryl, carbonyl, carboxylic, amine, nitro, nitroso, azo, diazo, nitrile, amide, thiol, thioether, thiocarbamate, bisulphite, etc.

A variety of solid supports are available for use in laboratories and industrial applications such as organic polymers (e.g. agarose, dextran, etc.), ion exchange resins, and inorganic substrates (silica, alumina, etc.). However, selectivity of the surface with the immobilized functional groups towards metal ion(s) depends on the factors like the size of the modifier, the activity of the loaded group and the characteristics of hard–soft acid–base. The insertion of suitable specific functional groups into the polymeric matrices makes them capable of reacting with metal species under certain favorable conditions to form metal complexes. Slow kinetics, irreversible adsorption of organic, substances swelling, different sensitivity towards many chemical environments and loss of mechanical stability in operation are the main disadvantages exhibited by polymeric resins. These problems suggest the use of inorganic supports in place of polymeric resins.

Among various adsorbents, silica gel can be used very successfully as it does not swell or strain, has good mechanical strength and can undergo heat treatment. In addition, chelating agents can be easily loaded on silica gel with high stability, or be bound chemically to the support, affording a higher stability.

The surface of silica gel is characterized by the presence of silanol groups (Kvitek et al. 1982) and in particular, silica gel presents high sorption capacity for metal

ions, such as Cu, Ni, Co, Zn or Fe (Sarkar et al. 1996). Retention is highly dependent on sample pH with quantitative retention requiring pH values over 7.5–8, as under acidic conditions silanol groups are neutral and the ion-exchange capacity of the silica gel is greatly reduced or even decreased to zero at low pHs. In addition, this sorbent has a very low selectivity, and is prone to hydrolysis at basic pH values. Consequently, modification of the silica gel surface has been performed to obtain solid sorbents with greater selectivity.

In most of the methods for preparation of immobilized silica gel, a two-step procedure has been used for loading the surface with specific organic compounds, physical adsorption and chemical immobilization. In the first method, the organic compound is directly adsorbed on the silanol group of silica gel surface (impregnated or loaded sorbent), either by passing the reagent solution through a column packed with the adsorbent (Liang et al. 2005), or by shaking the adsorbent in the reagent solution (Lorena et al. 1998). In the second approach, a covalent bond is formed between the silica gel surface groups and those of the organic compound. Chemisorption of chelating molecules on silica surface provides immobility, mechanical stability and water insolubility, thereby increasing the efficiency, sensitivity and selectivity of the analytical applications (Jal et al. 2004). It is proposed that chemical modification of silica surface by organic chelating group provides greater selectivity for the analyte than that offered by traditional ion-exchanger. The most convenient way to develop a chemically modified surface is by simple immobilization (or fixing) of the group on the surface by adsorption or electrostatic interaction or hydrogen bond formation of other type of interaction. Simple impregnation of the solution of modifiers or covalent binding, the so-called covalent grafting of the chelating molecule to silica matrix via silanization, is the common practice of developing a functionalized silica surface.

1.6. Proteins

Proteins are linear polymers of amino acids, and are found in all living organisms. They are macromolecules of relative molecular weight (MW) ranging from several hundreds to many thousands of Da. About 20 amino acids are commonly found in plant and animal proteins, and these are combined in countless ways to form a great

variety of protein molecules. This diversity is needed because of the enormous number of different functions that proteins perform in living organisms.

Protein molecules consist of a "backbone", formed from a large number of peptide bonds. The nature of the component amino acid side-chains is responsible for the varied individual properties of different proteins. An individual amino acid has both carboxylic acid and amino groups, but in protein molecules these are used in the formation of the peptide bonds, with the exception of those at the C terminal and N-terminal of the protein (Figure 1.2).

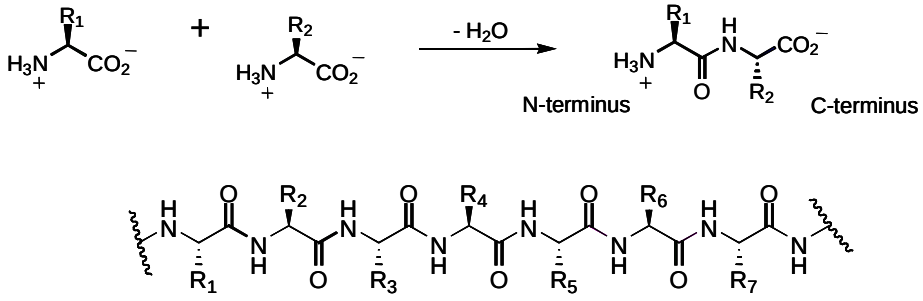

Figure 1.2. A typical protein: peptide bonds, N-terminal and C-terminal diagram.

1.7. Immobilization of Proteins

The concept of binding proteins to several substrates or their modification by means of polymers has gained great attraction of the scientists in recent years (Shtilman 1993). In general, the immobilization procedure consists of three steps. First, the solid matrix is activated to make it reactive toward the functional group of the ligand is carried out. Next, the ligand is coupled to the activated matrix either directly or by a one step procedure with the use of a condensation agent. Finally, remaining active groups are deactivated by a large excess of a suitable low molecular weight substance. A successful immobilization of the target protein to a solid support depends on various factors. (Bisswanger 2004). Ligand type (protein, sugar, DNA, or a low molecular weight substance) and size, the analyte to be used, amino acid composition, pH stability can be listed among the important factors that should be considered during the choice of the suitable immobilization technique Type of the connection between matrix and the

protein and the type of the matrix to which the protein is bound has a crucial role before starting immobilization.

Methods used for the immobilization of enzymes fall into four main categories (Tischer and Kasche 1999):

1. Physical adsorption onto an inert carrier,
2. Inclusion in the lattices of a polymerized gel,
3. Cross-linking of the protein with a bifunctional reagent, and
4. Covalent binding to a reactive insoluble support.

Physical adsorption of an enzyme onto a solid is stated as the simplest way of preparing immobilized proteins (Tischer and Kasche 1999). The method is based on the physical interaction between the protein and the surface of the matrix which is achieved by mixing a concentrated solution of protein with the solid. A major advantage of adsorption is that usually no additional reagent is required and only a minimum of activation steps are required. As a result, adsorption is cheap and easily carried out.

Enclosing (entrapping) proteins within the lattices of polymers is another method for immobilization. The method is based on the polymerization of the matrix in an aqueous solution of the protein. As there is no bond formation between the protein and the polymer matrix, the protein molecules are not disrupted which is the main advantage of this method.

Another method for immobilization of proteins is achieved by cross-linking of the protein, either to other protein molecules or to functional groups on an insoluble support matrix. Cross-linking of a protein can be both expensive and insufficient, as some of the protein material may act as a support which can lower the activity of the protein. Generally, cross-linking is best used in conjunction with one of the other methods.

The most widely studied immobilization technique is the formation of covalent bonds between the protein and the support matrix. There are several methods that can be used for the formation of covalently binded proteins to support matrices where the choice is limited by the fact that the binding reaction must be performed under conditions that do not cause loss of protein activity, and the active site of the protein must be unaffected by the reagents used.

The functional groups of proteins suitable for covalent binding include (i) the amino groups of the of lysine and arginine, (ii) the carboxyl groups of aspartic and glutamic acids, (iii) the phenol ring of tyrosine and phenyalanine, (iv) the thiol group of

cysteine, (v) the hydroxyl groups of serine and threonine, (vi) the imidazile group of histidine, (vii) the indole group of tryptophan, (viii) methylmercapto group of methionine and (ix) amido group of asparagines and glutamine.

As mentioned above, the choice of the proper immobilization technique is very important as the active sites in the protein molecule should be protected so that the protein activity is not disrupted. Ligand type (protein, sugar, DNA, or a low molecular weight substance) and size, the analyte to be used, amino acid composition, pH stability can be listed among the important factors that should be considered during the choice of the suitable immobilization technique (Tischer and Wedekind 1999).

1.8. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively coupled plasma mass spectroscopy (ICP-MS) was developed in the late 1980's to combine the easy sample introduction and quick analysis of ICP technology with the accurate and low detection limits of a mass spectrometer. The resulting instrument is capable of trace multielement analysis, often at the part per trillion levels. ICP-MS has been used widely over the years, finding applications in a number of different fields including drinking water, wastewater, natural water systems/hydrogeology, geology and soil science, mining/metallurgy, food sciences, and medicine.

Plasma is collection of electrons, ions and neutral atoms that is electrically neutral. It is an ionized gas, usually argon, which is sustained by a radio frequency (RF) generator. The RF generator applies an electric force through a copper coil that as consequence ionizes the argon gas onto Ar^+ ions and electrons. The argon gas is selected among others because is the noble gas found in more abundance in the atmosphere and has a low ionization energy (15.68 eV) relative with other noble gases with less atomic mass. As consequence, it is cheaper and easier to ionize than others noble gases. In the plasma, the temperature can reach 10,000 K and the high temperature of the ICP ensures almost complete decomposition of the sample into its constituent atoms, and the ionization conditions within ICP result in highly efficient ionization of the most elements in the periodic table.

The sample is introduced into the ICP as a liquid which usually contain less than 0.1 % dissolved solids to prevent salt build-up on the nickel cones. The sample is

converted to an aerosol by means of a pneumatic nebulizer and the droplets pass through a spray chamber, into the injector tube of the quartz torch and then into the central channel of the ICP (Figure1.3).

Since atomization/ionization occurs at atmospheric pressure, the interface between the ICP and MS components becomes crucial in creating a vacuum environment for the MS system. Ions flow through a small orifice, approximately 1 millimeter in diameter, into a pumped vacuum system. Here a supersonic jet forms and the sample ions are passed into the MS system at high speeds, expanding in the vacuum system (Jarvis et al. 1992). The entire mass spectrometer must be kept in a vacuum so that the ions are free to move without collisions with air molecules. Since the ICP is maintained at atmospheric pressure, a pumping system is needed to continuously pull a vacuum inside the spectrometer. In order to most efficiently reduce the pressure several pumps are typically used to gradually reduce pressure to 10-5 mbar before the ion stream reaches the quadrupole. If only one pump were used, its size would be excessive to reduce the pressure immediately upon entering the mass spectrometer.

In the first stage of the mass spectrometer ions are removed from the plasma by a pumped extraction system. An ion beam is produced and focused further into the actual unit. There are several different types of mass analyzers which can be employed to separate isotopes based on their mass to charge ratio. Quadrupole analyzers are compact and easy to use but offer lower resolution when dealing with ions of the same mass to charge (m/z) ratio. Double focusing sector analyzers offer better resolution but are larger and have higher capital cost.

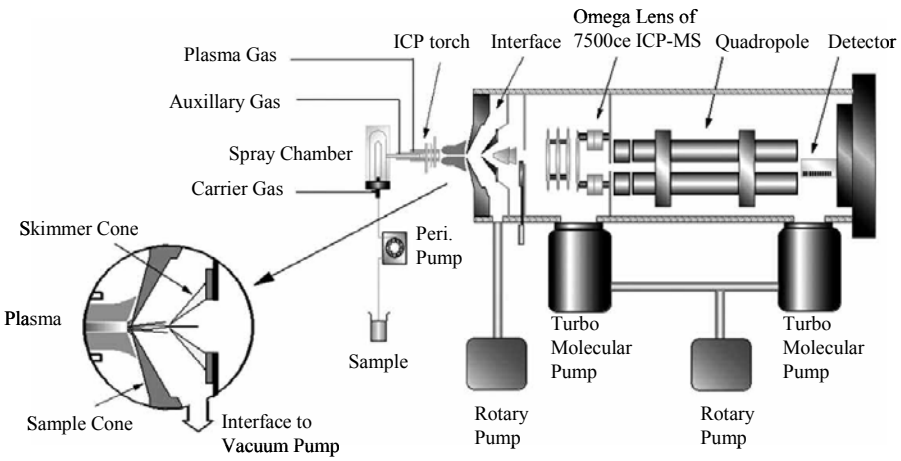

Figure 1.3. Schematic diagram of an Agilent 7500 Series ICP-MS instrument.

The quadrupole mass filter is made up of four metal rods aligned in a parallel diamond pattern. A combined DC and AC electrical potential is applied to the rods with opposite rods having a net negative or positive potential. Ions enter into the path between all of the rods. When the DC and AC voltages are set to certain values only one particular ion is able to continue on a path between the rods and the others are forced out of this path. This ion will have a specific m/z ratio. Many combinations of voltages are chosen which allows an array of different m/z ratio ions to be detected.

The most common type of ion detector found in an ICP-MS system is the channeltron electron multiplier. This cone or horn shaped tube has a high voltage applied to it opposite in charge to that of the ions being detected. Ions leaving the quadrupole are attracted to the interior cone surface. When they strike the surface additional secondary electrons are emitted which move farther into the tube emitting additional secondary electrons. As the process continues even more electrons are formed, resulting in as many as 108 electrons at the other end of the tube after one ion strikes at the entrance of the cone (Jarvis et al. 1992).

1.9. Characterization Methods

Characterization of the sorbents was carried out using scanning electron microscope (SEM), elemental analysis, elemental analysis, thermo gravimetric analysis (TGA), Brunauer-Emmett-Teller (BET) surface area analysis, Fourier transform infrared spectroscopy (FTIR) and zeta meter.

1.9.1. Scanning Electron Microscopy (SEM)

SEM was invented in the early 1960's and it is now far from being only a specialist laboratory instrument (Lawes and James 1987). The principle of SEM is the scanning of the surface of a solid material in a raster pattern with a beam of electrons as source. The energy of the electron beam can range from a few hundred eV to 100 keV. This beam is focused by one or more condenser lenses into a spot sized 0.4 nm to 5 nm. By the help of scanning coils or pairs of deflector plates in the electron optical column beam is deflected horizontally and vertically so that scanning occurs in a raster fashion over a rectangular area of the sample surface. The contact of the source electrons with

the sample results in the production of backscattered, secondary and Auger electrons as well as X-ray fluorescence photons and various other photons. Basically, backscattered and secondary electron signals are used to study the surfaces (Skoog, et al. 1998, Strobel and Heineman 1989). Backscattered electrons are elastically reflected source electrons and secondary electrons are the emitted electrons from the surface atoms. Those are detected by photomultiplier tube and finally, the 2D or 3D image is displayed on the CRT monitor. Since electrons interact with air molecules both by being scattered and absorbed, a high vacuum media is essential for the quality of analysis (Figure1.4).

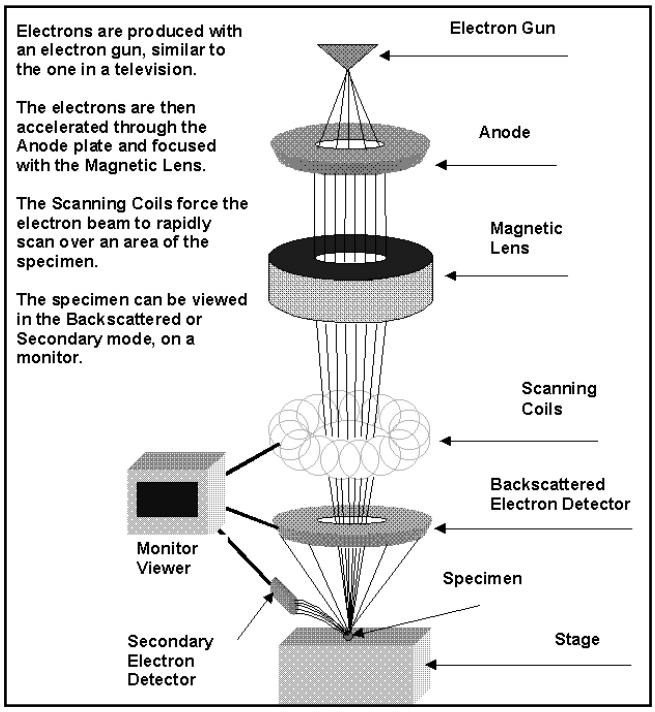

Figure 1.4. Schematic of Scanning Electron Microscopy.
(Source: Renssealer Polytechnic Institute 2007)

Throughout the study, SEM/EDS characterizations were performed by a Philips XL-30S FEG model instrument. The powder samples were attached onto adhesive carbon tapes supported on metallic disks. Sample surfaces were then observed at different magnifications and the images were recorded. Elemental EDS analysis was performed at randomly selected areas on the solid surfaces each being approximately 20 μm×20 μm in dimension. EDS mapping was carried out at 1000x magnification with a voltage applied at 18 kV under vacuum conditions of $3.5x10^{-5}$ mbar.

1.9.2. Elemental Analysis

Elemental analysis is a process where a sample of some material (e.g., soil, waste or drinking water, bodily fluids, minerals, chemical compounds) is analyzed for its elemental and sometimes isotopic composition. Elemental analysis can be qualitative and quantitative. The elemental analysis refers to CHNX analysis, the determination of the percentage weights of carbon, hydrogen, nitrogen, and heteroatoms (X) (halogens, sulfur) of a sample. This information is important to help determine the structure of an unknown compound, as well as to help ascertain the structure and purity of a synthesized compound. The most common form of elemental analysis, CHN analysis, is accomplished by combustion analysis. In this technique, a sample is burned in an excess of oxygen, and various traps collect the combustion products, carbon dioxide, water, and nitric oxide. The weights of these combustion products can be used to calculate the composition of the unknown sample. In this study, carbon, nitrogen, sulfur and hydrogen contents were determined using a Leco 932 CHNS analyzer**.**

1.9.3. Thermo Gravimetric Analysis (TGA)

Thermal stability of the sorbents is investigated using TGA in which the mass loss of the sample is monitored as a function of temperature. Thus it is performed in order to see the effect of heat on the structure of synthesised sorbents The solid samples are heated from 25ºC to 650ºC in 10 minute intervals in N_2 atmosphere. TGA was performed using a Perkin Elmer Diamond TG/DTA instrument.

1.9.4. Brunauer-Emmett-Teller (BET) Surface Area Analysis

Gas sorption (both adsorption and desorption) at the surface of dry solid powders is the mostly used method for determining the surface area of these powders. In a gas sorption method, firstly the material is heated and degassed to remove previously adsorbed molecules. Then known doses of an inert gas, such as nitrogen, are introduced and the gas is adsorbed (or desorbed). The sample material is placed in a vacuum chamber at a constant and very low temperature, and the pressure upon is

varied in a wide range to obtain adsorption and desorption isotherms. Various amounts of gas molecules will be adsorbed or desorbed at different doses of the gas. Since the area occupied by one gas molecule is known, an appropriate adsorption model can be used to determine the total surface area of the sample. The most well known isotherm equation for multilayer adsorption is the equation derived by Brunauer, Emmett, and Teller in 1938. So the surface area analysis technique based on gas adsorption/desorption and this equation is named as BET Surface Area Analysis (Becman Coulter Inc. 2006).

1.9.5. Zeta Potential Measurements

Each colloidal microscopic particle in a suspension, having a surface charge, produces a difference in electrical potential between its surface and the bulk of the suspension. This difference, in millivolts, is called the zeta potential. Zeta potential, ξ, can be easily determined because when the suspension is subjected to a direct current (DC) between two ends, charged colloid will move with a velocity proportional to its zeta potential. This phenomenon, movement of charged particles through two ends of a voltage, is called "electrophoresis". Determination of zeta potential is important to more fully understand the bulk properties of many suspensions because the key for many bulk-scale processes is again the control of individual colloids.

The zeta potential was measured using a Zeta Meter 3.0 (Zeta Meter Inc.) equipped with a microprocessor unit having molybdenum anode and platinum cathode. The unit automatically calculates the electrophoretic mobility of the particles and converts it to the zeta potential using the Smoluchowski equation.

$$\zeta = \frac{4\Pi\eta}{\varepsilon} \text{ x } U \qquad (1.1)$$

The Smoluchowski equation, the most elementary expression for zeta potential, gives a direct relation between zeta potential and electrophoretic mobility, where U is electrophoretic mobility at actual temperature, η is viscosity of the suspending liquid, ε is dielectric constant, π is constant and ζ is zeta potential.

1.9.6. Particle Size Analysis

Particle size analysis is an analytical technique by which the distribution of sizes in a sample of particulate material is measured. Laser Particle Size Analysis consists in measuring the size of particles (powders, suspensions and emulsions) using the diffraction and diffusion of a laser beam. During the laser diffraction measurement, particles are passed through a focused laser beam. These particles scatter light at an angle that is inversely proportional to their size. The angular intensity of the scattered light is then measured by a series of photosensitive detectors. The map of scattering intensity versus angle is the primary source of information used to calculate the particle size. In this study, Malvern Particle Size Analyzer (MS2000) was used.

1.9.7. Ultraviolet/Visible Spectrometry (UV/VIS)

Ultraviolet and visible (UV/VIS) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range. Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels, and UV/VIS spectroscopy is usually applied to molecules or inorganic complexes in solution. The UV/VIS spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert Law.

The UV/VIS spectral range is approximately 190 to 750 nm, as defined by the working range of typical commercial UV/VIS spectrophotometers. Since the UV/VIS range spans the range of human visual acuity of approximately 400 - 750 nm, UV/VIS spectroscopy is useful to characterize the absorption, transmission, and reflectivity of a variety of technologically important materials, such as pigments, coatings, windows, and filters. This more qualitative application usually requires recording at least a portion of the UV/VIS spectrum for characterization of the optical or electronic properties of materials.

The light source is usually a deuterium discharge lamp for UV measurements and a tungsten-halogen lamp for visible and NIR measurements. The instruments

automatically swap lamps when scanning between the UV and visible regions. The wavelengths of these continuous light sources are typically dispersed by a holographic grating in a single or double monochromator or spectrograph. The spectral bandpass is then determined by the monochromator slit width or by the array-element width in array-detector spectrometers. Spectrometer designs and optical components are optimized to reject stray light, which is one of the limiting factors in quantitative absorbance measurements. The detector in single-detector instruments is a photodiode, phototube, or photomultiplier tube (Skoog et al. 1998).

1.10. Aim of the Study

The purpose of this study is to find out a novel solid support for the determination of vanadium in environmental, biological and geological samples. Among the various adsorbents, silica was used as the solid support material. After obtaining the newly synthesized material, the experiments concentrated on the examination of the sorption behavior of this substance towards several essential or/and trace elements. Meanwhile, the sorbents were characterized using several techniques such as scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM/EDS), elemental analysis, thermo gravimetric analysis (TGA), and Brunauer-Emmett-Teller (BET) surface area analysis. In addition, particle size distribution and zeta potential of the proposed sorbents were also elucidated

CHAPTER 2

EXPERIMENTAL

2.1. Instrumentation and Apparatus

An Agilent 7500ce quadrupole (Tokyo, Japan) inductively coupled plasma mass spectrometer (ICP-MS) with a high solid nebulizer was used in the measurements. The vanadium signal was measured at m/z=51 (natural abundance of 99.75%). The operating conditions of the instrument are given in Table 2.1.

Table 2.1. ICP-MS operating conditions.

Forward power	1500 W
Reflected power	1W
Coolant gas flow rate	15 L min^{-1}
Auxilary flow rate	0.90 L min^{-1}
Sample uptake time	25 sec
Integration time	100 msec

In batch sorption studies, GFL 1083 water bath shaker (Burgwedel, Germany) equipped with microprocessor-controlled thermostat was used to provide efficient mixing. The pH measurements were performed by using a Denver pH/ion meter (Colorado, USA) with a pH/ATC plastic-body electrode. The elemental composition of the newly synthesized sorbents was determined by LECO-CHNS-932 elemental analyzer (Mönchengladbach, Germany). Images of sorbents and polystyrene were taken with Philips XL-30S FEG scanning electron microscop (Eindhoven, The Netherlands). Thermal properties of sorbents were analysed with Perkin Elmer Pyris Diamond TG/DTA (Boston, MA, USA). Mastersizer 2000, Hydro 2000S (Malvern Worcs, U.K.) was used for determination of size distribution of polystyrene nanoparticles. Point of

zero charge of the sorbents was determined with Zeta-Meter System 3.0+ (Staunton, USA).

2.2. Reagents and Solutions

All reagents and chemicals were analytical grade. Ultra pure water (18.2 MΩ) was used throughout the study. Glassware and plasticware were cleaned by soaking them in dilute nitric acid (10%) and rinsed with distilled water prior to use.

1. Standard V(IV) stock solution (1000.0 mg L^{-1}): Prepared by dissolving 0.496 g of vanadyl sulfate pentahydrate ($VOSO_4.5H_2O$) in 5 mL of concentrated H_2SO_4 and diluting to 100.0 mL with ultra pure water.
2. Standard V(V) stock solution (1000.0 mg L^{-1}): Prepared by dissolving 0.178 g of vanadium pentaoxide (V_2O_5) in 1.0 mL of concentrated HNO_3 and diluting to 100.0 mL with ultra pure water.
3. Standard Te(VI) stock solution (1000.0 mg L^{-1}): Prepared by dissolving 0.536 g of $Na_2TeO_4.2H_2O$ in ultrapure water and diluted to 250.0 mL with ultrapure water.
4. Standard Te(IV) stock solution (500.0 mg L^{-1}): Prepared by pre-reduction of Te(VI) by boiling with 6.0 M HCl.
5. Standard As(V) stock solution (2000.0 mg L^{-1}): Prepared by dissolving 0.766 g of As_2O_5 in 1.0% (v/v) HCl and diluted to 250.0 mL with ultrapure water.
6. Standard As(III) stock solution (2000.0 mg L^{-1}): Prepared by dissolving 0.659 g of As_2O_3 in 5.0 mL NaOH and 2.5 mL H_2SO_4 and diluted to 250.0 mL with ultrapure water.
7. Standard Se(VI) stock solution (1000.0 mg L^{-1}): Prepared by dissolving 0.598 g of Na_2SeO_4 in ultrpure water and diluted to 250.0 mL with ultrapure water.
8. Standard Se(IV) stock solution (1000.0 mg L^{-1}):prepared by dissolving 0.833 g of $Na_2SeO_3.5H_2O$ in 1.0% (v/v) HNO_3 and diluted to 250.0 mL with ultrapure water.
9. Standard Sb(III) stock solution (1000.0 mg L^{-1}): Prepared by dissolving 0.685 g of $C_8H_4K_2O_{12}Sb_2.3H_2O$ in ultrapure water and diluted to 250.0 mL with ultrapure water.
10. Standard Sb(V) stock solution (1000.0 mg L^{-1}): Prepared by dissolving 0.540 g of $KSb(OH)_6$, in ultrapure water and diluted to 250.0 mL with ultrapure water.

11. Calibration Standards: Lower concentration standards were prepared daily from their stock standard solutions.
12. pH buffers, ranging from 2.0 to 10.0 were prepared using various concentrations of KHP, NaOH, HCl and KH_2PO_4 (analytical grade).
13. pH adjustment: Various concentrations of HCl and NH_3 solutions were used.

Functional silane was 3-aminopropyl triethoxysilane, 3-APTES (Merck) and porous silica was of particle diameter 0.2-0.5 mm (Merck). Glutaraldehyde was purchased from Fluka and hydrogen peroxide (30%) was also obtained from Merck.

2.3. Aqueous Calibration Plot

Standard solutions from 0.010 mg L^{-1} to 1.0 mg L^{-1} were prepared from 1000.0 mg L^{-1} V(IV) and V(V) respectively with simple dilution. All standards contained 1.0% (v/v) HNO_3 corresponding to a HNO_3 concentration of 0.144 M and measured with ICP-MS. In addition the limit of detection (LOD) based on 3s (3 times the standard deviation above the blank value) were also evaluated.

2.4. Redox Behavior of V(IV) and V(V)

As mentioned in Introduction, vanadium speciation is very important in understanding the biological and physiological roles of the element. However, one of the problems which researches may encounter with is the possibility of redistribution of vanadium species when the matrix of the sample, such as its pH, salinity or redox potential changes. The common four oxidation states of vanadium are +5, +4, +3 and +2 and each of them can be distinguished by its color (Table 2.2). Since V(IV) and V(V) have different interactions in environmental matrices, most of the speciation studies have focused on the determination of these ions. Pentavalent vanadium exists as VO_2^+ in acidic and $H_2VO_4^-$, HVO_4^{2-} or VO_4^{3-} in alkaline solutions and it is the existing form in waters exposed to oxygen.

Table 2.2. The color of the vanadium solutions depending on the oxidation states. (Source: Haber 2009)

Oxidation State	Species	Color
+5	VO_2^+	Yellow
+4	VO^{2+}	Blue
+3	V^{3+}	Green
+2	V^{2+}	Violet

Tetravalent vanadium (IV), as vanadyl cation VO^{2+}, is present in reducing environment. It is stable in acidic solution below pH 2, but is oxidized to the pentavalent state by oxygen at higher pH. This complicates the determination of V(IV) in natural samples with pH > 2, since stabilization of this form is necessary.

In order to clarify the actual forms of vanadium in natural waters and determine the total vanadium concentration in environmental matrices an accurate and rapid oxidation and/or reduction process is required. For this purpose, a spectrophotometric method was used due to its simplicity and low cost instrumentation. Here, it has to be mentioned that the vanadium concentrations applied in the spectrometric method are relatively high when compared to the concentrations used in ICP-MS, still it is helpful to understand the species present in the solution.

The dual beam Varian Cary 50 spectrophotometer equipped with a Xe lamp, Czerny-Turner monochromator and a silicon diode detector was used in all determinations. Standards for V(IV) and V(V) were prepared in ultrapure water in the concentration range of 1.0 mg L^{-1} to 1000.0 mg L^{-1}. The absorption spectra were obtained with separate solutions of 100.0 mg L^{-1} V(IV) and V(V).

2.4.1. Reduction of V(V)

As a starting experiment, for understanding if several reducing agents namely $FeCl_2$, $FeSO_4$, L-cysteine and ascorbic acid were successful for the reduction of V(V), 10.0 mL of 1000.0 mg L^{-1} V(V) solution was prepared and added to react with 0.05 M

of the reducing agents. The color of the solutions was observed if there was any difference which was the indication of change in the oxidation state.

In order to find the optimum amount for the reduction of vanadate a detailed experiment was performed with various concentrations of $FeSO_4$, L-cysteine and ascorbic acid. The mole ratio method was employed by preparing a series of mixtures by using a constant volume of the 10.0 mg L^{-1} vanadate containing the varying amount of the candidate reducing agent. The applied reagent/analyte ratios were 1:5, 1:2, 1:1, 2:1, 5:1, 10:1, 20:1, 50:1, 100:1, 200:1. The pH of the solutions was adjusted to 2.0 and the volume was kept constant at 50.0 mL. Each solution was measured on UV-VIS spectrophotometer together with its respective blank. A mole ratio curve was plotted in order to find the stoichiometric amount that corresponds to the reduction of V(V) to V(IV).

2.4.2. Oxidation of V(IV)

For the oxidation of V(IV) to V(V), a similar procedure was applied as in the previous section but with the oxidizing agents, namely, KIO_3, $KBrO_3$, $K_2S_2O_8$ and $K_2Cr_2O_7$. As a starting experiment 1000.0 mg L^{-1} vanadyl solution was prepared and the oxidizing agents were added in a way that the concentration of each was 0.05 M. The color change was observed and a more detailed experiment was performed with $KBrO_3$.

In a series of solutions, the concentration of vanadyl ion was kept constant at 100.0 mg L^{-1} while the concentration of $KBrO_3$ was varied. The $KBrO_3$/vanadyl ratios examined were 1:10, 1:1, 2:1, 5:1, 10:1, 100:1. The pH of the solutions was held constant at 2.0. The absorption spectra of the appropriate blank solutions were also recorded. A mole ratio curve was plotted in order to find the stoichiometric amount of $KBrO_3$ that oxidizes V(IV) to V(V).

2.5. Synthesis of the Newly Functionalized Sorbents

The novel sorbents for the determination and the speciation of vanadium were synthesized as follows. Silica gel was chosen as the solid support matrix since it offers several advantages over other matrices (e.g. polymers). Its high specific surface area,

great resistance to organic solvents, high thermal resistance can be mentioned among its advantages over other organic/inorganic supports. It is the polymer of silicic acid consisting of SiO_4 units in tetrahedral geometry and has a stoichiometry of SiO_2 (Figure 2.1). Silica gel is a porous, granular form of silica synthetically manufactured from sodium silicate or silicon tetrachloride.

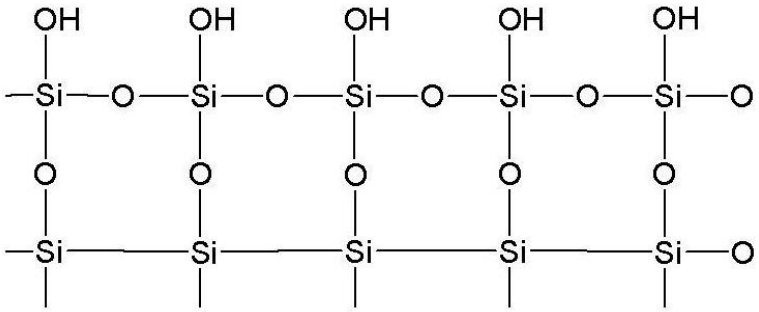

Figure 2.1. Silica structure.

2.5.1. Modification of Silica Gel Surface with 3-APTES

For the synthesis of amino-functionalized silica (3-aminopropyl) triethoxysilane (3-APTES) was used as the organic source. Prior to silanization, pretreatment of silica gel is carried out to remove the possible surface contaminants (e.g metal ions) and activate the silanol groups on the surface. In many instances activation is achieved by acids to break siloxane bonds and form silanol groups. In order to activate the silica gel surface, 20.0 grams of silica gel was mixed with 100.0 mL of 0.01 M acetic acid solution for 1.0 hour. Then it was filtered using a vacuum pump and washed with distilled water until the pH of the supernatant had risen to 6.0. Afterwards it was transferred to a round bottomed flask containing 50.0 mL of toluene and 12.0 mL of 3-APTES. The mixture was stirred for 24.0 hours under reflux conditions at 110°C under the N_2 atmosphere. The resulting product was filtered off and washed with ethanol. After removal of the traces of solvent, the resulting product was further dried at 50.0°C for 24.0 hours. The route of the functionalization of silica structure is illustrated in Figure 2.2.

2.5.2. Modification of Silica Gel Surface with 3-MPTMS

Synthesis of 3-MPTMS-modified silica gel was similar to the procedure described in Part 2.5.1; the only difference was the use of (3-mercaptopropyl) trimethoxysilane (3-MPTMS) instead of 3-APTES. The synthesis route is given in Figure 2.3.

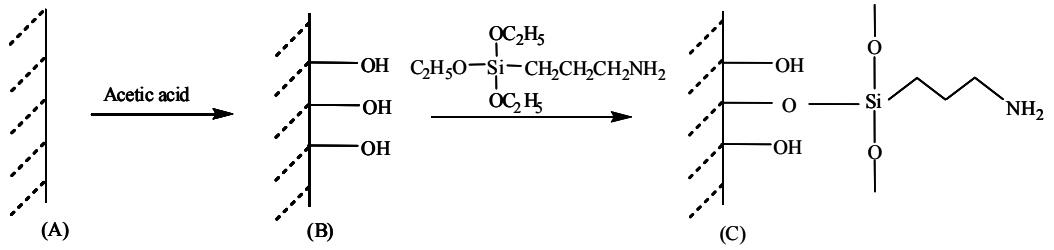

Figure 2.2. Amine modification of silica surface. (a) Silica surface, (b) Activated silica, (c) 3-APTES-modified silica.

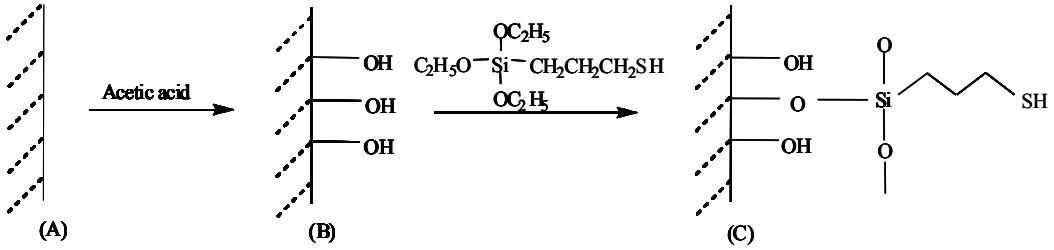

Figure 2.3. Mercapto modification of silica surface. (a) Silica surface, (b) Activated silica, (c) 3-MPTMS-modified silica.

2.5.3. Modification of Silica Gel Surface with 3-APTES and 3-MPTMS

A similar procedure was applied, as explained previously, for the synthesis of the bifunctional sorbent (3-APTES- and 3-MPTMS-modified silica). First the activation of silica was performed with acetic acid and then it was mixed with 9.0 mL of 3-APTES and 9.0 mL of 3-MPTMS in 30.0 mL toluene for 24.0 h under reflux at 110°C under N_2 atmosphere and the reaction product was, then, filtered and washed with 20.0 mL

acetone and 10.0 mL toluene. Finally, the modified sorbent was further dried at 50.0°C at for 24.0 hours.

2.5.4. Synthesis of Trypsin-Immobilized Silica

Trypsin is an enzyme with a molecular mass of 23.400 Da which belongs to the group of serine proteases and it is one of the three main digestive proteinases. It consists of a single chain polypeptide of 223 amino acid residues. It has an active site consisting of aspartic acid, histidine and serine residues and the surface of trypsin possesses S-S, amine and thiol groups. It attracts interest due to a variety of possible applications, such as peptide synthesis, semi-synthesis of human insulin and transesterification. In addition, it is widely used in large-scale processes by the detergent and dairy industries.

For the synthesis of trypsin-immobilized silica, surface activation was performed with acetic acid as mentioned above. The reaction scheme is outlined in Figure 2.4. One gram of dry silica gel was mixed with 15.0 % (v/v) 3-APTES in 20.0 mL acetone and incubated at 50.0^{o}C for 2.0 h with constant mixing. The treated silica gel was then washed with water, dried at 60.0^{o}C for 2.0 h and was suspended in 0.05 M phosphate buffer (pH=7.0). In trypsin immobilization, glutaraldehyde was used as the cross-linking agent which is a bifunctional reactive agent capable of reacting with the surface amino groups of enzymes and carriers. Glutaraldehyde was added to the silica suspension and the suspension was filtered after stirring at 20^{o}C for 2 h. The activated silica gel produced was incubated overnight at 4.0^{o}C with 50.0 mg trypsin in 10.0 mL 0.05 M TRIS/HCl buffer (pH 8.0) with 0.02 M $CaCl_2$ (to reduce enzyme auto-digestion). The carrier was then washed with TRIS buffer and vacuum filtered through a Buchner funnel.

2.6. Determination of the Amount of Trypsin Bound to Silica

The protein content in the effluents after immobilization was quantified using the Bradford method which is a popular protein assay because it is simple, rapid, sensitive and inexpensive (Bradford et al. 1976). The Bradford assay is based on the direct binding of Coomassie brilliant blue G-250 dye (CBB G-250) to proteins at arginine, tryptophan, tyrosine, histidine, and phenylalanine residues. The color change

produced when the dye binds to proteins provides a measure of the total protein content at 595 nm using UV-visible spectrometry. Bovine serum albumin (BSA) is often used as a calibration standard since it has a greater dye-binding capacity than most proteins.

The preparation of Coomassie Reagent was performed by dissolving 10.0 mg of CBB G-250 in 5.0 mL of 95 % (v/v) ethanol to which 10.0 mL of 85 % (v/v) phosphoric acid had been added and the whole was diluted to 100.0 mL with ultra pure water. The final solution was filtered through filter paper and stored in an amber bottle at 4.0ºC. The standard BSA solution (200.0 μg mL^{-1}) was prepared by weighing 0.020 g of BSA and dissolving it in water to a final volume of 1.0 mL.

Blank, BSA standards, and protein samples were prepared for absorbance measurement using a UV-visible spectrometer. The order of mixing these reagents is; water, either BSA or sample protein, and lastly Coomassie solution. Then each sample was allowed to incubate at room temperature for 5 minutes and absorbance of each sample was finally measured at 595 nm.

2.7. Determination of Trypsin Activity

Enzyme activity is a measure of the quantity of active enzyme present and is thus dependent on conditions, which should be specified (Klibanov 1997). It is equal to the moles of substrate converted per unit time (μmol min^{-1}=U). Specific activity is the amount of product formed by an enzyme in a given amount of time under given conditions per milligram of enzyme. It is equal to the activity of an enzyme per milligram of total protein (in μmol min^{-1} mg^{-1}).

Trypsin activity of immobilized enzyme was monitored by using p-toluene sulfonyl aginine methyl ester (TAME) as the substrate. One unit hydrolyzes 1 mmole of p-toluene sulfonyl-L-arginine methyl ester (TAME) per minute at 25°C in the presence of calcium ion.

The activity towards p-tosyl-L-arginine methyl ester (TAME) was determined as follows: 2.6 mL of 46 mM of Tris-HCl (pH 8.2) containing 11.5 mM $CaCl_2$ was added to a cuvette along with 0.3 mL of 10 mM TAME. Then 0.1 mL of enzyme solution was added at zero time and mixed immediately. One unit of TAME activity was defined as the amount of trypsin, which resulted in an increase of one absorbance unit at 247 nm per minute. Thus, the rate of hydrolysis was monitored at an absorbance of 247 nm and

the change in absorbance value was determined and unit mg^{-1} protein was calculated. In addition, specific activity was expressed as unit of enzymatic activity per mg protein (Worthington 1972).

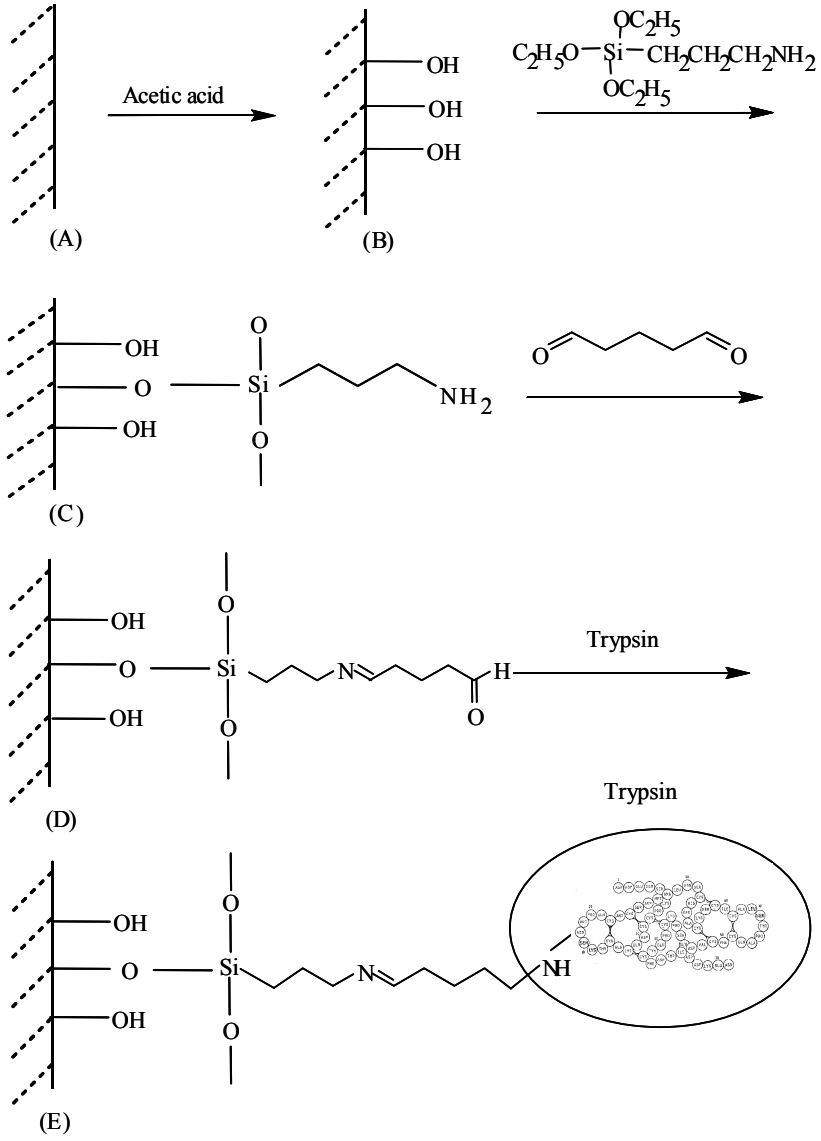

Figure 2.4. Trypsin immobilization to silica surface. (a) Silica surface, (b) Activated silica, (c) 3-APTES-modified silica (d) Glutaraldehyde-treated silica and (e) Trypsin-immobilized silica.

2.8. Characterization of the Synthesized Sorbents

A number of characterization experiments were performed to understand whether the functional groups were attached to the support material. Characterization of the sorbents was performed using techniques such as Fourier transform infrared spectroscopy (FTIR), Brunauer-Emmett-Teller (BET) surface area analysis and elemental analysis. Images of newly synthesized materials were taken with scanning electron microscopy (SEM) while their thermal degradation behaviors were investigated through TGA measurements.

2.9. Sorption Studies

In order to understand the chemical behavior of V(IV) and V(V) in aqueous solutions, several chelating or ion exchange resins, nano sized zero-valent iron (nZVI), chitosan, silica and the functionalized silicas were investigated. The properties of the commercially available sorbents used are given in Table 2.3.

Table 2.3. Properties of the commercially available sorbents investigated for vanadium speciation.

Sorbent	Property	Functional/Active Group	Form
Amberlite IR 410	Strongly basic cation exchanger	Benzyl methyl (2 hydroxyethyl) ammonium	-
Amberlite IR 120	Strongy acidic cation exchanger	Sulfonic acid	H
Duolite A-7	Weakly basic anion exchanger	Alkyl amine	-
Duolite C 467	Chelating resin	Amino phoshoric	-
Duolite GT-73	Chelating resin	Thiol	-
Chelex 100	Chelating resin	Iminodiacetic acid	Na
Duolite XAD-761	Adsorbent resin	Methyol	-

Chitosan flakes were synthesized by Boyacı (2008) from chitin under inert atmosphere by the method of Rigby and Wolfrom (1973). Briefly, 50.0 grams of chitin are treated with 40.0% (w/w) NaOH solution at 115^{o}C for 6.0 hours under inert atmosphere. After washing with water until neutralization, 85% removal of acetyl groups are obtained (Muzzarelli 1973 and references therein).

Nano sized zero-valent iron (nZVI) was synthesized using liquid-phase reduction, with the addition of sodium borohydride ($NaBH_4$) as the reducing agent to iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$) solution (Wang and Zhang 1997; Wang et al. 2006). The proposed reaction is:

$$Fe^{2+}(aq) + 2BH_4^-(aq) + 6H_2O \rightarrow Fe^o + 2B(OH)_3 + 7H_2 \uparrow$$

The preparation of nZVI was performed as follows; 5.34 g $FeCl_2.4H_2O$ was dissolved in a 4:1 (v/v) ethanol/water mixture (24.0 mL ethanol + 6.0 mL ultra pure water) and stirred on a magnetic stirrer. On the other hand, 3.05 g $NaBH_4$ was dissolved in 100.0 mL of ultra pure water. The final BH_4^- / Fe^{2+} ratio is adjusted to 3.0, since excess $NaBH_4$ is needed for better growth of nanoparticles. Sodium borohydride solution was added dropwise (from a burette) to the iron(II) solution which was being strirred on magnetic stirrer. Black solid particles immediately appeared after introducing the first drop of $NaBH_4$ solution. After adding the whole borohydride solution, the mixture was left stirred for further 10.0 minutes. To separate the black iron nanoparticles from the liquid phase, vacuum filtration was used. At this point, solid particles were washed at least three times with 25.0 mL portions of absolute ethanol to remove all of the water. This washing process is probably the key step of the synthesis since it prevents the rapid oxidation of zero-valent iron nanoparticles. Synthesized nanoparticles were finally dried in oven at 50.0^{o}C overnight. Drying in evacuated ovens must be avoided because this would cause iron nanoparticles to spontaneously ignite upon exposure to air.

In order to find the most suitable sorbent among the commercial materials, for vanadium speciation, 1.0 mg L^{-1} separate standard solutions of V(IV) and V(V) were prepared at different pHs adjusted with various concentrations of HNO_3 and NH_3. From each of these solutions 10.0 mL were taken into which 50.0 mg of the tested sorbent were added. The resulting mixture was shaken manually for 1-2 minutes and then for a further 30 minutes on a shaker at room temperature. Sorption studies with batch method

were carried out by using GFL 1083 water bath shaker equipped with a microprocessor thermostat. At the end of the shaking period, the mixture was filtered and the filtrate was analyzed for its vanadium content by ICP-MS.

The percentage of vanadium sorption was calculated using Equation 2.1, where C_i is the initial and C_f is the final concentration in the solution.

$$Sorption\ \% = \frac{C_i - C_f}{C_i} \times 100 \tag{2.1}$$

For the studies with 3-APTES-modified silica, the effect of the sorbent amount, shaking time, solution pH, reaction temperature, ionic strength, and successive loadings was also investigated. In all these experiments, batch sorption was followed by the filtration of the mixture through blue-band filter paper and analysis of the filtrate for the determination of the vanadium concentration by ICP-MS.

2.9.1. Studies Utilizing 3-APTES-Modified Silica

2.9.1.1. Effect of pH

In order to investigate the effect of pH on the sorption of V(IV) and V(V), 1.0 mg L^{-1} standard solutions were prepared at different pHs from 2.0 to 10.0 with various concentrations of HNO_3 and NH_3. From each of these solutions 10.0 mL were taken into which 50.0 mg of 3-APTES-modified silica were added. The mixture was first shaken for 1-2 minutes manually and then for 30.0 minutes on a shaker at room temperature. After the solid and the liquid phases were separated by filtration, the liquid was analyzed for vanadium with ICP-MS. The sorption experiments were also performed with both 3-APTES and 3-MPTMS functionalized resin for pH values 1.0 to 3.5 with 0.5 increments. In addition to the sorption experiments with the functionalized sorbents, sorption behavior of silica (activated with acetic acid) towards V(IV) and V(V) was also investigated as a function of pH and initial ion concentration to better understand the effect of functionalization.

2.9.1.2. Effect of Silica Gel Pretreatment

Acid leaching is a treatment that may allow an improvement in the properties of the natural materials (e.g. silica gel, clay materials etc.). For this reason, this method is usually called "acid activation". It is the treatment of the material with solutions of inorganic acids and starts with the de-aggregation of particles and removal of soluble mineral impurities. If the treatment is intense enough, silica gels with high surface area and high porosity are obtained which are more promising as sorbents and may be competitive in different applications (Schubert et al. 2008). For this purpose, a pretreatment step was added to the functionalization procedure before the attachment of the amino group to the silica gel surface. It was carried out through sonication with either acetic acid or hydrogen peroxide. After the pretreatment step, synthesis of the 3-APTES-modified silica was accomplished. As soon as the sorbent was synthesized, its sorption towards 1.0 mg L^{-1} V(V) was realized at pH 2.0 and the effect of the pretreatment on sorption before modification was investigated.

2.9.1.3. Effect of Sorbent Amount (Solid/Liquid Ratio)

For the quantitative sorption of the analyte from the solution, the determination of the reasonable sorbent amount is also an important factor. Sorbent amounts varying from 5.0 mg to 200.0 mg were added to 10.0 mL of 1.0 mg L^{-1} of V(V) solutions at pH 2.0 and shaken at 25.0^{o}C. After filtration, V(V) concentration in solution was determined with ICP-MS.

2.9.1.4. Effect of Shaking Time

In order to obtain efficient sorption, the optimum contact time was investigated. For this purpose, 10.0 mL of 1.0 mg L^{-1} V(V) solutions at pH 2.0 containing 50.0 mg of 3-APTES-modified silica gel were shaken at 25.0^{o}C for time intervals of 1.0, 2.0, 5.0, 10.0, 15.0, 30.0, 45.0, 60.0, 75.0 and 90.0 minutes. After filtration, the resulting solutions were analyzed by ICP-MS and percent sorption was calculated.

2.9.1.5. Effect of Initial Concentration

The sorption ability of 3-APTES-modified silica gel was also investigated for initial V(V) concentrations from 0.010, 0.10, 0.50, 1.0, 10.0 and 100.0 mg L^{-1} since the extent of removal of vanadium from the aqueous solution may depend on this concentration. For this purpose, 10.0 mL of these solutions (at pH 2) were taken and 50.0 mg of sorbent was added to each of them. Then the mixtures were shaken for 30.0 minutes on the shaker at 25.0°C. After filtration, the filtrates were analyzed with ICP-MS.

2.9.1.6. Effect of Reaction Temperature

The sorption efficiency of a sorbent towards a particular sorbate may change with the reaction temperature. Therefore the effect of temperature on the sorption of V(V) was studied at 25°C and 60°C. The V(V) ion concentration, solution volume, shaking time, solution pH and sorbent amount were 1.0 mg L^{-1}, 10.0 mL, 30.0 min., pH of 2.0, and 50.0 mg, respectively. The results of these experiments were also used to investigate the thermodynamic parameters of sorption (ΔG^o, ΔS^o and ΔH^o) utilizing the well-known equations 2.2, 2.3 and 2.4 (Atkins and de Paula 2002; Yersel et al. 2005):

$$\Delta G^o = -RT \ln R_d \tag{2.2}$$

$$\Delta H^o = R \ln \frac{R_d(T_2)}{R_d(T_1)} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)^{-1} \tag{2.3}$$

$$\Delta S^o = \frac{\Delta H^0 - \Delta G^o}{T} \tag{2.4}$$

R_d (mL g^{-1}) is the ratio of V(V) ion distributed between solid (sorbent) and liquid (aqueous solution of vanadate) phase at equilibrium and is defined by the Equation 2.5.

$$R_d = \frac{C_{solid}}{C_{liquid}} \tag{2.5}$$

where, C_{solid} is the concentration of V(V) in sorbent (mg g^{-1}) and C_{liquid} is the concentration of V(V) ion in solution after sorption (mg L^{-1}).

2.9.1.7. Effect of Ionic Strength

The presence of inorganic salts may have an influence on the sorption of metal ions. Accordingly, the effect of ionic strength on the sorption of V(V) by the 3-APTES-modified silica gel was investigated in 0.10, 0.010, 0.001 M NaCl solution. The sorption ability of the sorbents was compared with and without the addition of NaCl. The concentration of V(V), solution volume, shaking time, solution pH, sorbent amount and reaction temperature were 1.0 mg L^{-1}, 10.0 mL, 30 min., pH of 2.0, 50.0 mg, and 25.0^{o}C, respectively

2.9.1.8. Effect of Solution Volume

Especially for low analyte concentrations, it is very important to get satisfactory results from large volumes of samples. Therefore, the effect of sample volume on the sorption efficiency of the sorbents was investigated in two different strategies. In the first one, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mL of solutions containing an absolute amount of 10 μg V(V) were prepared and 100.0 mg of 3-APTES-modified silica were added into each. After the sorption (shaking and filtration) process with the same conditions, the percent sorption was determined by ICP-MS.

In the second strategy, 1.0 mg L^{-1} of V(V) solutions in different volumes from 10.0 to 1000.0 mL (at pH 2.0) were prepared and 100.0 mg of 3-APTES-modified silica were added to each. The prepared mixtures were shaken at 25.0^{o}C for 30.0 minutes, filtered and percent sorption was determined as before. As a complementary experiment, a series of experiments were performed for 1000.0 mL of 1.0 .0 mg L^{-1} of V(V) solutions with varying sorbent amounts (100.0, 250.0, 500.0, 1000.0 and 2000.0 mg) and the effect of sorbent on sorption efficiency was examined.

2.9.1.9. Desorption from the Sorbent

After the uptake of V(V) by 3-APTES-modified silica, its release was investigated using several eluents namely HNO_3, HCl, H_2SO_4, H_3PO_4 (0.10 M, 1.0 M and 6.0 M), EDTA (0.10 M and 0.30), KI, KSCN, thiourea, L-cysteine, citric acid, ascorbic acid, tartaric acid, thioglycolic acid, and KOH. For this purpose, firstly a usual sorption step was applied with a standard V(V) solution; 10.0 mL of 1.0 mg L^{-1} V(V) at pH 2.0 was prepared into which 50.0 mg of sorbent was added. After having been shaken for 30 minutes for sorption, the mixture was filtered and the sorbent was taken into the eluent for another 30 minutes for elution. At the end of this period, the solution was filtered and the eluate was analyzed for its vanadium concentration.

2.9.1.10. Sorption Isotherm Models

The whole sorption process can be described by the sorption isotherm which, at constant temperature, provides measured data relating to the amount sorbed and the equilibrium concentration measured in the bulk phase after the sorption equilibrium is completed. Thus, in addition to the previous experiments, the equilibrium sorption isotherm studies were conducted in order to reveal the concentration-dependence of the partitioning of vanadium species between the liquid solutions and the sorbent at a particular temperature. Many theoretical and empirical models have been developed to represent the various types of adsorption isotherms. The Langmuir, Freundlich and Dubinin-Radushkevich (D-R) models are among the most frequently used isotherm models for this purpose.

Langmuir nonlinear form is given in Equation 2.6.

$$Q_e = Q_{\max} \frac{bC_e}{1 + bC_e} \tag{2.6}$$

where Q_{max} (mmol g^{-1}) and b (L $mmol^{-1}$) are Langmuir constants; Q_{max} is the amount of vanadate ion sorption corresponding to monolayer coverage, b is the affinity of the species for the sorbent, C_e (mmol L^{-1}) is the amount of vanadate in liquid phase at equilibrium and Q_e is the amount of vanadate sorbed on the surface of the sorbent

(mmol g^{-1}) at equilibrium. The values of constants are evaluated from the linearized form of the equation which is defined in Equation 2.7.

$$\frac{1}{Q_e} = \frac{1}{Q_{\max}} + \frac{1}{Q_{\max} b C_e} \tag{2.7}$$

The intercept and slope of plot of $1/Q_e$ vs. $1/C_e$ are used for the evaluation of Q_{max} and b.

For heterogeneous surfaces, isotherm is described by Freundlich which is valid for low concentrations. The isotherm deviate as the saturation point is approached (Umplebay et al. 2001).

Freundlich nonlinear isotherm is described by Equation 2.8.

$$Q_e = K_F C_e^{1/n} \tag{2.8}$$

where K_F (mg g^{-1}) and n are constants for a given sorbent-sorbate system. These constants are evaluated from linearized form of the equation (Equation 2.9)

$$\log Q_e = \log K_F + \frac{1}{n} \log C_e \tag{2.9}$$

The intercept and slope of plot of $\log Q_e$ versus $\log C_e$ give K_F and 1/n, respectively.

D-R isotherm model assumes that the ionic species preferentially bind to most energetically favorable sites of sorbent associated with multilayer adsorption of ions (Guibal, et al. 1998). D-R isotherm is generally described by Equation 2.10 (Kavitha and Namasivayam 2007):

$$Q_e = q_s \exp\left(-B\varepsilon^2\right) \tag{2.10}$$

where

$$\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right) \tag{2.11}$$

D-R parameter, B (mol^2 kj^{-2}), gives information about the mean free energy of sorption per molecule of sorbate which requires to transfer it to the surface of the solid

from infinity in the solution; q_s (mg g^{-1}) corresponds to the sorption monolayer capacity (Şeker et al. 2008). Mean free energy of sorption can be calculated from D-R parameter B by Equation 2.12.

$$E = (2B)^{-\frac{1}{2}} \tag{2.12}$$

The constants q_s and B are calculated from the intercepts and the slope of the experimental plot, lnq versus ε^2.

These studies were also performed through batch process. The accurately weighed amount of 3-APTES-modified silica (50.0 mg) was added into 10.0 mL of solutions containing the specified concentrations of V(V) (ranging from 100.0 to 1000.0 mg L^{-1}) and the mixtures were shaken in a thermostated water bath at 25^{o}C for an hour. At the end of the shaking period, the solid and solution phases were separated through filtration and the concentrations of vanadium in the supernatant solutions were determined by ICP-MS.

With the use of the data obtained in these experiments the sorption capacity (maximum amount of the vanadium sorbed per g of silica) was determined. In addition to 3-APTES-modified silica, the sorption capacity experiments were also conducted with mercapto and amino modified silica, Duolite A-7 (a chelating resin with alkyl amine functional groups) and nZVI in the same manner to compare their vanadium sorption capacity with that of the novel sorbent (3-APTES-modified silica).

2.9.1.11. Interference Studies

Interference studies were performed with Mo(VI), V(V), Sb(III), Sb(V), Te(IV), Te(VI), Se(IV), Se(VI) ions. Firstly, the sorption of 3-APTES-modified silica towards Mo(VI), V(V), Se(IV) and Se(VI) was investigated. For this purpose, 10.0, 100.0 and 1000.0 μg L^{-1} concentrations of the above ions were prepared and mixed with 3-APTES-modified sorbent. In the sorption, conditions optimized for vanadate were applied (solution volume, shaking time, solution pH, sorbent amount and reaction temperature were 10.0 mL, 30 min, pH of 2.0, 50.0 mg, and 25^{o}C, respectively). The percent sorption of each ion was determined by ICP-MS after acidification with concentrated HNO_3 so as to have 1.0% (w/v) acid concentration in the final solutions.

In the second part of the interference study, 10.0 μg L^{-1}, 100.0 μg L^{-1}, 1000.0 μg L^{-1} of each species were added separately into 10.0 μg L^{-1}, 100.0 μg $L^{-1}$and 1000.0 μg L^{-1} V(V) solutions. The sorption experiments were performed under optimized conditions for vanadate described in previous paragraph, which is followed by a filtration step. After proper acidification and reduction steps ICP-MS was used for the analysis.

2.9.1.12. Method Validation and Spike Recovery Experiments

The efficiency of the proposed method was first checked via spike recovery tests. This was realized by spiking 10.0 mL aliquots of ultrapure, bottled drinking, tap, and sea water samples with 10.0 μg L^{-1}, 100.0 μg L^{-1} and 1000.0 μg L^{-1} of V(V) solutions separately and applying the batch process. After sorption, elution was performed using 0.5 M thiourea (in 0.2 M HCl) and shaking for 2 hours at 50.0^{o}C. The concentration of vanadium in the eluates was determined by ICP-MS and the percent recovery in each sample was calculated.

2.9.2. Studies Utilizing Trypsin-Immobilized Silica

2.9.2.1. Effect of pH

The pH of 10 mL portions of V(IV) and V(V) were adjusted to 2.0, 4.0, 6.0, 8.0 and 10.0 using HNO_3 or NH_3 at various concentrations. Then 10.0 mg trypsin-immobilized silica was added into these solutions in falcon tubes and the mixtures were shaken for 30 minutes. After separation of liquid and solid phases by filtration, the solutions were acidified and analyzed with ICP-MS for their vanadium concentrations.

2.9.2.2. Sorption Behavior of the Intermediate Products During the Immobilization Procedure

As mentioned in previous sections, trypsin was immobilized onto silica in four steps. The first step was to wash (activate) silica with H_2O_2 or CH_3COOH (or to use as it

is) followed by modification with 3-APTES. The modified product was then crosslinked with glutaraldeyhde and finally immobilized with trypsin. After each step, an appropriate amount of the product obtained was taken and its sorption behavior was tested towards V(IV) and (V) under the similar conditions as given previously.

2.9.2.3. Sorption Using Buffers

As mentioned in previous sections, solution pH is possibly the most important factor on sorption and, therefore, must be carefully controlled. Buffers offer an effective way to control pH; however, components of the buffers may interact with dissolved species or surfaces which may result in a change in sorption of the analyte. Hence, to understand the effect of buffers on sorption capacity solutions at desired pH (2.0-10.0) were prepared using various concentrations of KHP, NaOH, HCl, and H_2P.

2.9.2.4. Sorption Studies After Ascorbic Acid Reduction

Ascorbic acid was found to be the most effective reducing agent among the chemicals investigated and, therefore, the stoichiometric ratio of the vanadate ions to ascorbic acid was determined. Several experiments were performed to understand the effect of ascorbic acid on the sorption. At first, to facilitate whether ascorbic acid deactivates the possible binding sites of the immobilized solid, 10.0 mg of the newly synthesized solid was shaken in 1% (w/w) ascorbic acid for 30 minutes on an orbital shaker. The sorbent was filtered and dried in an oven at 50^oC for 30 minutes. Subsequently, this sorbent was used to determine percent sorption of 1.0 mg L^{-1} V(IV) and V(V) to understand if there is any significant change on the sorption.

As a subsequent experiment, trypsin-immobilized silica was utilized for the determination of percent sorption of 1.0 mg L^{-1} V(IV) and V(V). For this purpose, 10.0 mg sorbent, 10.0 mL sample volume and 30 minutes shaking time and were used at all times.

2.9.2.5. Sorption Studies After $KBrO_3$ Oxidation

After determining the stoichiometric amount of $KBrO_3$ that oxides vanadyl ion to vanadate, a sorption process was performed with the varying concentrations of ($4x10^{-3}$, $4x10^{-4}$ and $4x10^{-5}$ M) $KBrO_3$. For this purpose the sorption process was performed as explained in the previous sections.

2.9.2.6. Effect of Sorbent Amount (Solid/Liquid Ratio)

The dependence of V(IV) and V(V) sorption on the amount of trypsin-immobilized silica was studied for varying amounts of the sorbent from 5.0 mg to 200.0 mg. For this purpose, separate solutions of 10.0 mL of 1.0 mg L^{-1} of V(IV) and V(V) at pH 4.0 were shaken at 25.0^{o}C with the specified amounts of the sorbent for sorption. After usual filtration and then acidification steps vanadium concentrations in the filtrates were determined with ICP-MS.

2.9.2.7. Effect of Shaking Time

The sorption of V(IV) and V(V) on trypsin-immobilized silica was studied as a function of time for time intervals of 1.0, 2.0, 5.0, 10.0, 15.0, 30.0, 45.0, 60.0, 75.0 and 90.0 minutes at 25.0^{o}C using 10.0 mL of 1.0 mg L^{-1} V(IV) and V(V) solutions at pH 4.0 containing 10.0 mg of the sorbent. After filtration, the resulting solutions were analyzed by ICP-MS and percent sorption was calculated.

2.9.2.8. Effect of Initial Concentration

The effect of V(IV) and V(V) concentration on the sorption of trypsin-immobilized silica was tried by varying the vanadium concentrations from 0.010, 0.10, 0.50, 1.0, 10.0, 100.0 and 1000.0 mg L^{-1} while keeping the other parameters constant (10.0 mL of sample solutions, pH 4.0, 50.0 mg of sorbent and 30.0 minutes shaking time).

2.9.2.9. Effect of Reaction Temperature

The sorption efficiency of trypsin-immobilized silica towards V(IV) and V(V) was examined at two reaction temperatures (25^{o}C and 60^{o}C) by keeping the other optimum sorption conditions constant (10.0 mL of sample solutions, pH 4.0, 50.0 mg of sorbent and 30.0 minutes shaking time). The thermodynamic parameters relating the reaction conditions were evaluated.

2.9.2.10. Effect of Solution Volume

The effect of sample volume on the sorption efficiency of the sorbents was investigated in two different strategies as in the previous sections. In the first one, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mL of solutions containing an absolute amount of 10 μg V(IV) and V(V) were prepared and 50.0 mg of trypsin-immobilized silica were added into each. After the sorption (shaking and filtration) process with the same conditions, the percent sorption was determined by ICP-MS.

In the second strategy, V(IV) and 1.0 mg L^{-1} of V(V) solutions in different volumes from 10.0 to 1000.0 mL (at pH 2.0) were prepared and 100.0 mg of trypsin-immobilized silica were added into each. The prepared mixtures were shaken at 25.0^{o}C for 30.0 minutes and percent sorption was determined as before.

2.9.2.11. Sorption Isotherm Models

The sorption isotherms for vanadium sorption on trypsin-immobilized silica were obtained at 25^{o}C by varying the V(IV) and V(V) concentrations (ranging from 10.0 to 200.0 mg L^{-1}) in the solution while keeping other sorption parameters constant (10.0 mL of sample solutions, pH 4.0, 50.0 mg of sorbent and 30.0 minutes shaking time). The concentrations of vanadium in the supernatant solutions were determined by ICP-MS. The data obtained were used to evaluate Langmuir, Freundlich and D-R isotherms.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Characterization

3.1.1. Scanning Electron Microscopy (SEM)

Characterization of the silica-based sorbents was first performed by using scanning electron microscopy. Typical SEM images of the sorbents with the functional silanes and trypsin are shown in Figures 3.1 and 3.2 respectively.

From all the SEM images taken throughout the study (both the images given in Figure 3.1 and the images not shown in the thesis manuscript), it can be mentioned that surface morphology of the silica changes during modification reactions with functional silanes. Silica particles become more brittle and hence smaller due to magnetic stirring.

In addition to the above mentioned sorbents, the surface of the trypsin-immobilized silica was also examined with SEM. In order to understand the effect of amount of the trypsin concentration used to treat the silica surface, two trypsin concentrations, 5.0 mg mL^{-1} and 10.0 mg mL^{-1}, were used in the immobilization procedure. Similarity between the surface textures of silica obtained after the immobilization with the above mentioned concentrations indicates that increasing the trypsin concentration, at least twice, does no have a much degradation effect on the silica surface (Figure 3.2). Moreover SEM was used to see whether a pretreatment step (activation of surface silanol groups) with 30 % H_2O_2 instead of acetic acid caused degradation of the silica surface. No difference was observed on the surface morphology except having been obtained a smother surface.

3.1.2. Elemental Analysis

In addition to SEM, elemental analysis was performed to reveal the percentages of C, H, N and S in the synthesized sorbents (Table 3.1). Figure 2.4 indicates the

immobilization scheme of trypsin onto silica which can be helpful to comment on these percentages. As can be seen from the results, the appearance of N on the surface and the increase in the percentages of C and H indicate the binding of 3-APTES to the silica surface. Furthermore, the percentage of carbon increases in the second step which proves that the surface is modified with glutaraldehyde.

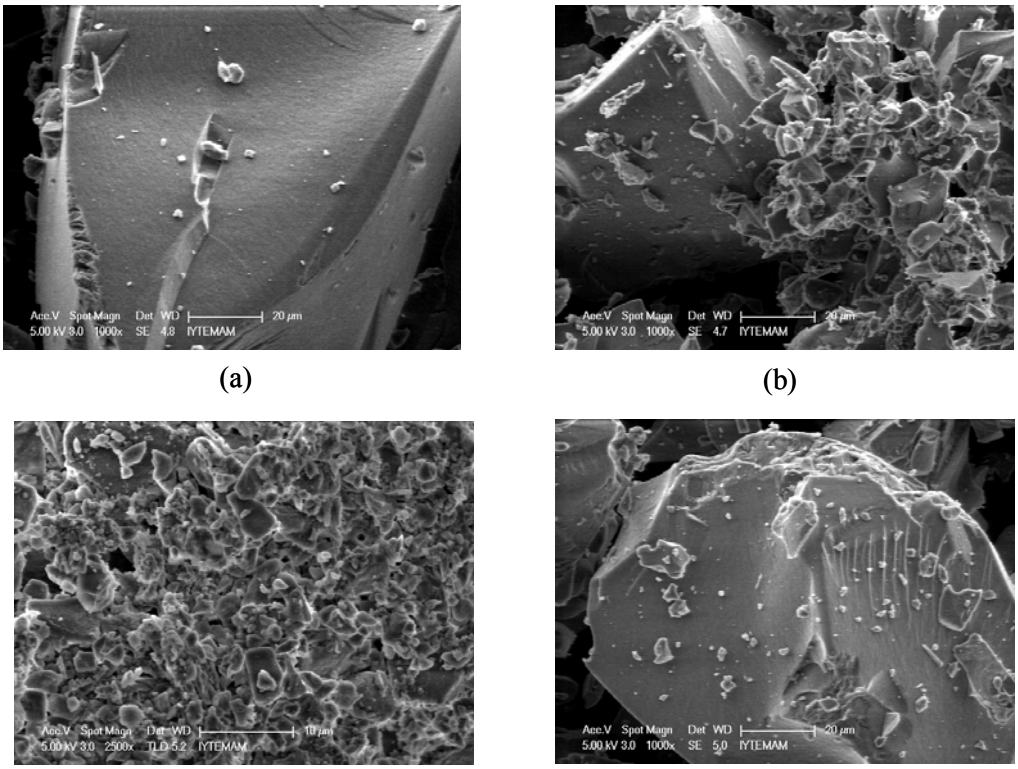

Figure 3.1. Typical SEM images of the unmodified and functional silane modified silicas. (a) unmodified silica, (b) 3-APTES-modified silica, (c) 3-MPTMS-modified silica, (d) 3-APTES and 3-MPTMS-modified silica (bifunctional).

3.1.3. Thermo Gravimetric Analysis (TGA)

Thermo gravimetric analysis allowed the determination of the degree of surface modification through comparison of the percent weight loss as a function of temperature. The thermographs are provided in Figures 3.3 and 3.4 for unmodified silica, 3-APTES-modified silica, 3-APTES and glutaraldehyde-modified silica 3-MPTMS-modified silica, both 3-MPTMS and 3-APTES-modified silica and trypsin-

immobilized silica. When the TGA curve of silica gel is considered, 3-4% weight loss observed at ~110 ^{o}C is attributed to the removal of adsorbed water from the silica structure. Increasing the temperature induces condensation between silanol groups, resulting in the release of water, which is then vaporized, and decreasing the sample weight. After 200^{o}C weight decreases gradually and ~7% weight losses is observed up to 620^{o}C. When the silica gel modified with functional silanes is heated to 620^{o}C, the total loss from the sample is about 10-13%. Approximately 8-10% difference in the weight loss compared to that of the unmodified silica is related to the modification of the surface.

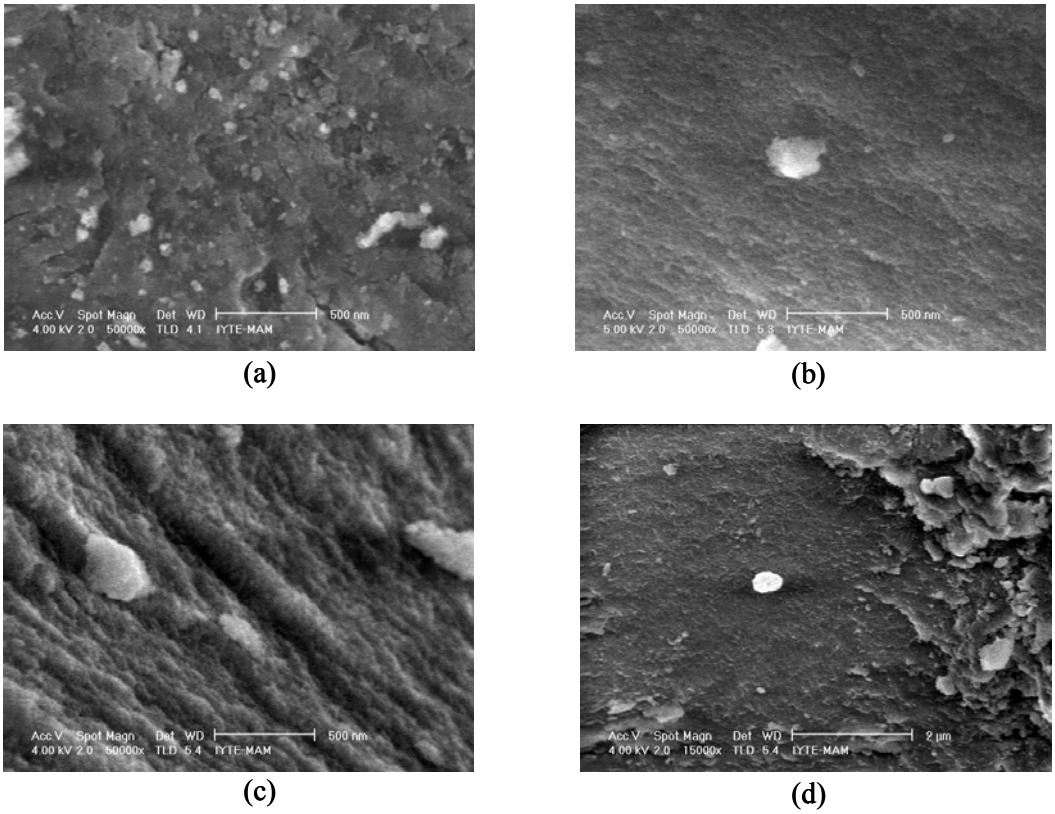

Figure 3.2. Typical SEM images of the unmodified and trypsin-immobilized silicas (a) unmodified silica, (b) trypsin-immobilized silica (5.0 mg L^{-1} trypsin) (c) trypsin-immobilized silica (10.0 mg L^{-1} trypsin) (d) trypsin-immobilized silica (10.0 mg L^{-1} trypsin, pretreatment with 30 % H_2O_2).

Table 3.1. Elemental analysis results of the unmodified and modified silicas.

Sorbent	C %	H %	N %	S %
Unmodified silica	0.16	0.88	ND	ND
3-MPTMS-modified silica	8.34	1.96	ND	7.60
3-MPTMS and 3 APTES-modified silica	4.62	1.38	0.81	1.83
3-APTES-modified silica	6.16	1.85	2.17	ND
3-APTES and glutaraldehyde-modified silica	13.22	2.48	2.56	ND
3-APTES, glutaraldehyde and trypsin-immobilized silica (Trypsin-immobilized silica)	11.73	2.45	2.39	ND

ND: Not detected

When the trypsin-immobilized silica is considered (Figure 3.4), as in the previous case, 3-4% weight loss at ~110^{o}C is again related to the removal of adsorbed water. After 300^{o}C weight decreases gradually and it can clearly be seen that in the second step of the reaction (crosslinking with glutaraldeyhde), a weight loss of 15-18 is observed. Additionally, a weight loss of 20-25% is observed with trypsin-immobilized silica which, again, indicates the modification of the silica surface. Thus, by considering TGA results, it can be concluded that additional weight loss in the functionalized sorbents compared to the unmodified silica is the indication of the success of the modification of the silica surface with functional silanes and trypsin.

3.1.4. Brunauer-Emmett-Teller (BET) Surface Area Analysis

Three fundamental parameters, (i) specific surface area (m^2 g^{-1}), (ii) specific pore volume; distribution of pore size or pore area, and (iii) particle size are sufficient for physical characterization of the silica surface. Nitrogen sorption isotherm is an efficient way for providing information about the pore system of materials. Exact knowledge of specific surface area of silica gel is essential to express concentration of reactive surface species. It provides an idea about surface loading, which is directly related to steric hindrance. Pore size distribution gives a deeper insight in availability of reactive silanol groups towards the reacting molecule. It also explains the sorption

characteristics of silica. As the organic fragments enter the channels, the isotherms are expected to have gradual changes at each stage of modification. As can be seen from Table 3.2, the pore volume and size were reduced apparently after each modification step. The table demonstrates also that the decrease of the pore volume from 0.101 to 0.053 $cm^3 g^{-1}$ is likely to be an indication of the organic groups having been successfully introduced into the inner channels. Accordingly, the decrease in specific surface area by nitrogen adsorption indicates that the silica surface is covered with the functional groups. A corresponding decrease in surface area and average pore diameter was also observed. These findings are in accordance with a previous study (Takei et al. 2000) in which it was reported that nitrogen molecules were adsorbed preferentially to silanols and a weak adsorption was observed on organic surfaces compared with bare silica surfaces.

3.1.5. Zeta Potential Measurements

Zeta potential measurements were carried out as a function of pH. A sample of 0.01 g trypsin-immobilized silica was prepared in 50 mL distilled water and put on a shaker for 30 minutes at room temperature. An aliquot taken from the supernatant was used in the measurements. The average of 15 measurements was taken to represent the zeta potential. The applied voltage during the measurements was generally varied in the range of 50–150 mV.

The magnitude of electrostatic interaction between the modified surfaces and the metal ions is a function of zeta potential. By knowing the isoelectric point (iep), the sign of surface charge is estimated, e.g., metal oxides are positively charged at $pH<pH_{iep}$ and negatively charged at $pH>pH_{iep}$, and this helps in understanding of sorption phenomena. Figures 3.5 and 3.6 indicate the zeta potentials of suspensions (100.0 mg sorbent in 50.0 mL sample) of the unmodified and modified sorbents as a function of pH.

It can clearly be seen that the three curves have different characteristics in terms of surface potential. In the case of silica, the zeta potential approaches zero at pH 2.3. This is due to surface silanol (Si-OH) groups losing a proton. The aqueous phase becomes slightly acidic (since it receives protons) whilst the silica surface becomes negative (due to the formation of $Si\text{-}O^-$).

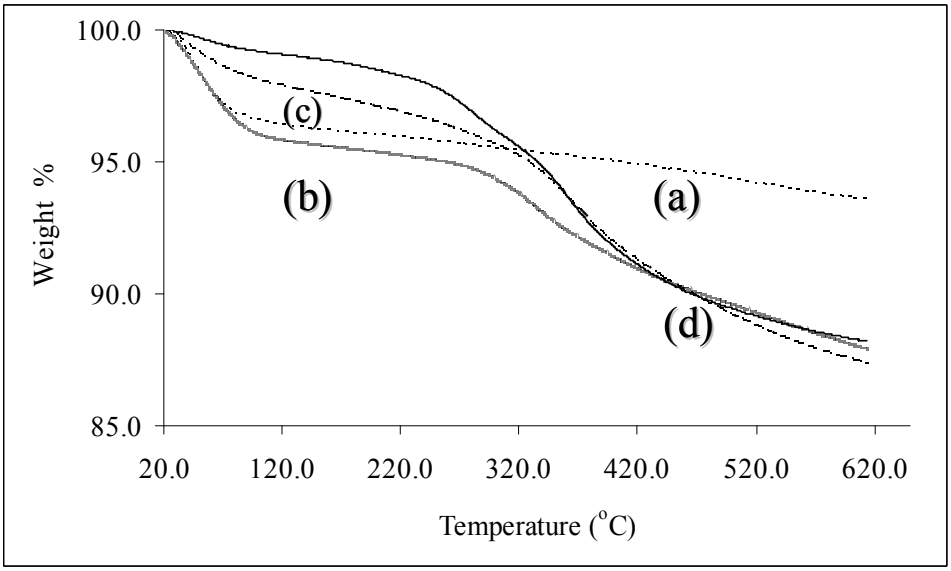

Figure 3.3. TGA curves of the unmodified and functional silane-modified silicas (a) unmodified silica (b) 3-APTES-modified silica (c) 3-APTES and 3-MPTMS-modified silica (d) 3-MPTMS-modified silica.

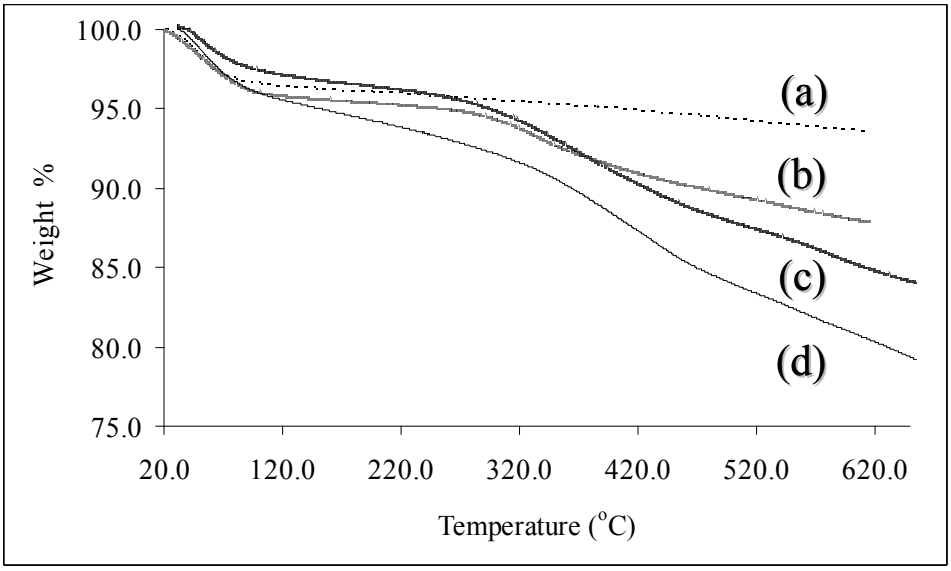

Figure 3.4. TGA curves of unmodified and modified silicas (a) unmodified silica (b) 3-APTES-modified silica (c) 3-APTES+glutaraldeyde-modified silica (d) 3-APTES+glutaraldeyde+trypsin-immobilized silica.

In the acidic range of pH, apparent differences in surface charge can be noted among the unmodified, 3-APTES-modified, and trypsin-immobilized silicas. Unmodified silica has a negatively charged surface at any pH greater than 2.5 (negative zeta potential) whereas completely different surface characteristics are observed for the modified silicas. The isoelectric points for 3-APTES-modified silica and trypsin-immobilized silica were 7.0 and 9.0 respectively. This means that both 3-APTES-modified silica and trypsin-immobilized silica display positive zeta potential in the entire acidic pH range. For trypsin-immobilized silica, this region extends even to 9.0. At higher pH values, zeta potential values become negative (negatively charged surfaces at pH values rather than 7.0 and 9.0 for 3-APTES-modified silica and trypsin-immobilized silicas, respectively).

Table 3.2. BET analysis results of the unmodified and modified silicas.

Sorbent	**Surface area[a] (m^2g^{-1})**	**Average pore width[b] (A^o)**	**Pore volume[c] ($cm^3\ g^{-1}$)**
Unmodified silica	232.5	88.8	0.101
3-MPTMS-modified silica	248.6	77.3	0.081
3-MPTMS and 3 APTES-modified silica	195.6	80.4	0.068
3-APTES-modified silica	195.1	85.0	0.065
3-APTES and glutaraldehyde-modified silica	182.9	71.2	0.075
3-APTES, glutaraldehyde and trypsin-immobilized silica (Trypsin-immobilized silica)	114.5	45.6	0.053

[a]BET surface area
[b]Pore diameter according to the maximum of the BJH pore size distribution
[c]Single point total pore volume

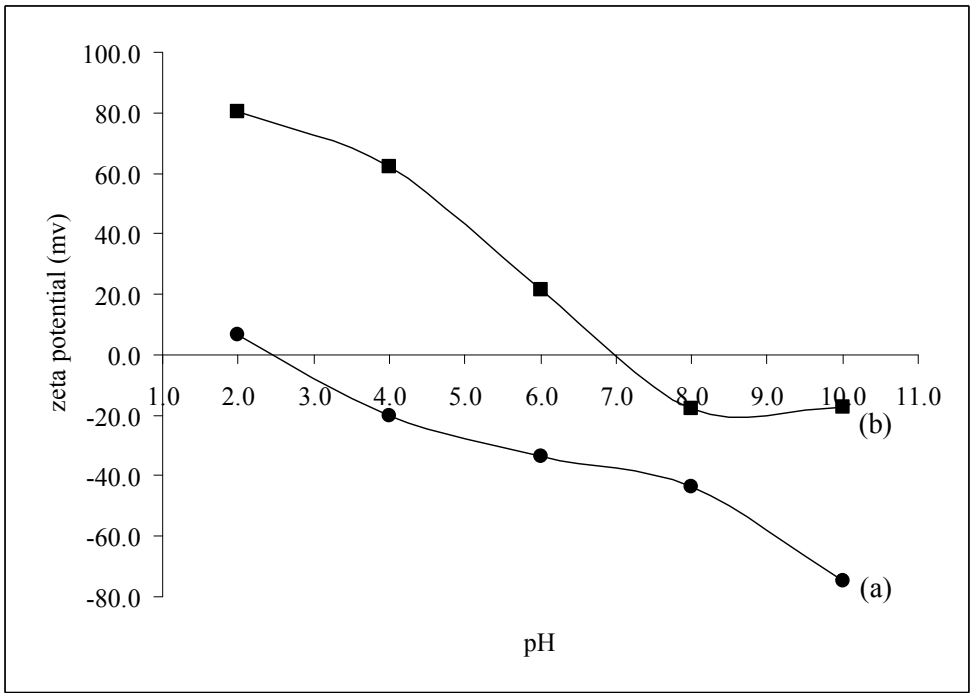

Figure 3.5. Effect of pH on zeta potential of the unmodified and modified silica, (a) unmodified silica (b) 3-APTES-modified silica (100.0 mg sorbent, 50.0 mL sample volume).

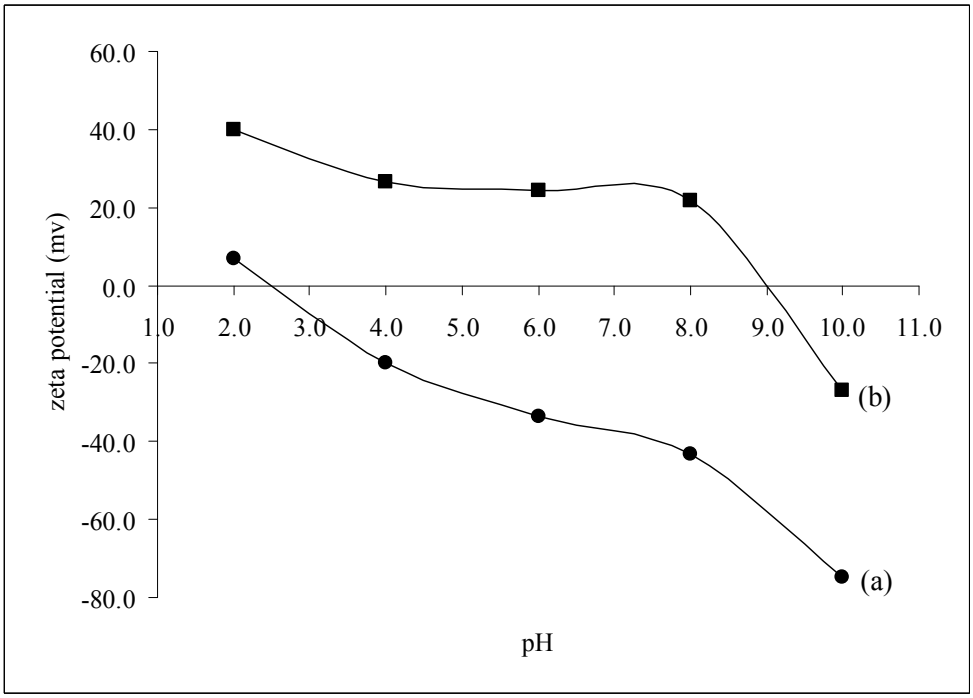

Figure 3.6. Effect of pH on zeta potential of the unmodified and modified silica, (a) unmodified silica (b) trypsin-immobilized silica (100.0 mg sorbent, 50.0 mL sample volume).

3.1.6. Particle Size Measurements

Particle size measurements are also required for physical characterization of the solids. Table 3.3 indicates the particle size distribution of the unmodified and modified silicas. The particle size distributions of these samples were measured using the Malvern particle size anaylzer. The reults are given in values of the volumetric diameters d_{10}, d_{50}, and d_{90}. These are defined as the points on the particle distribution where, respectively, 10%, 50%, and 90% by volume of the particles are smaller than the stated diameter. The d_{10} statistic is therefore an indicator of the proportion of fines in a particle size distribution, while d_{50} gives the mean volume and d_{90} a measure of the proportion of large particles present.

As can be seen from the table, as the surface is modified with functional silanes or other organic molecules, smaller silica particles are obtained. This is probably due to the effect of mechanical stirring applied throughout the modification steps. It has to be mentioned that the sorbents were not sieved before the sorption experiments and were used as they were.

3.1.7. Determination of the Amount of Trypsin Bound to Silica

As explained in Section 2.6, Bradford method was applied for the determination of the amount of trypsin bound to silica. Moreover, to check the effect of the starting trypsin concentration on the immobilization efficiency, two concentrations were tried; namely 5.0 mg L^{-1} and 10 mg L^{-1}. The results of the immobilization experiments are summarized in Table 3.4. As seen from the table, almost a quantitative (92-96%) immobilization of trypsin to silica was achieved regardless of the staring concentration. Also, the pretreatment of silica surface with H_2O_2 before the immobilization step did not have any effect on the results. Needless to mention, the amount of trypsin immobilized onto silica surface rather than the percentage is more important. With this in mind, as it is easily seen from the table, the amount of trypsin immobilized depends on the starting concentration; and in the sorption studies, this is more important than the percent immobilization. The results of the sorption studies will be discussed in the related sections.

Table 3.3. Particle size distribution of the unmodified and modified silicas.

Sorbent	d_{10}* (µm)	d_{50}* (µm)	d_{90}* (µm)
Unmodified silica	203	303	455
3-MPTMS-modified silica	87	146	236
3-MPTMS and 3-APTES-modified silica	33	92	175
3-APTES-modified silica	38	142	326
3-APTES and glutaraldehyde-modified silica	32	104	236
3-APTES, glutaraldehyde and trypsin-immobilized silica (Trypsin-immobilized silica)	38	117	242

*d_{10}, d_{50} and d_{90} cumulative undersize (µm).

3.1.8. Determination of Trypsin Activity

The activity of immobilized trypsin was determined using Worthington's assay. One unit of trypsin hydrolyzes 1 mmole of p-toluene sulfonyl-L-arginine methyl ester (TAME) per minute at 25°C in the presence of calcium ion to prevent autolysis of trypsin. The specific activity is calculated as units of enzyme activity per mg of protein.

Table 3.4. Immobilization efficiency as a function of trypsin concentration.

Trypsin Concentration (mg mL^{-1})	Silica Pretreatment*	Trypsin Immobilized (mg g^{-1})	% Immobilization
10.0	no	96	96
10.0	yes	96	96
5.0	no	46	92
5.0	yes	46	93

(*silica pretreatment: sonication with 30 % H_2O_2)

It was found that trypsin immobilized on silica retained a highest amount of activity (78%) which indicates the success of immobilization.

3.2. Vanadium Determination

Interest in the determination of vanadium in natural waters lies in the speciation of the two most frequently occurring oxidation states, namely, V(IV) and V(V) as they have different biological and physiological properties. So determination of both of these species is very important.

3.3. Calibration Curves for V(IV) and V(V) Using ICP-MS

Plots of signal versus concentration constructed for V(IV) and V(V) using ICP-MS can be seen in Figure 3.7. Similar responses were obtained with both species and the graphs were linear at least up to 1000.0 μg L^{-1}. The limit of detection (LOD) based on 3s (3 times the standard deviation above the blank value) for the above-mentioned calibration strategies was 0.041 μg L^{-1}

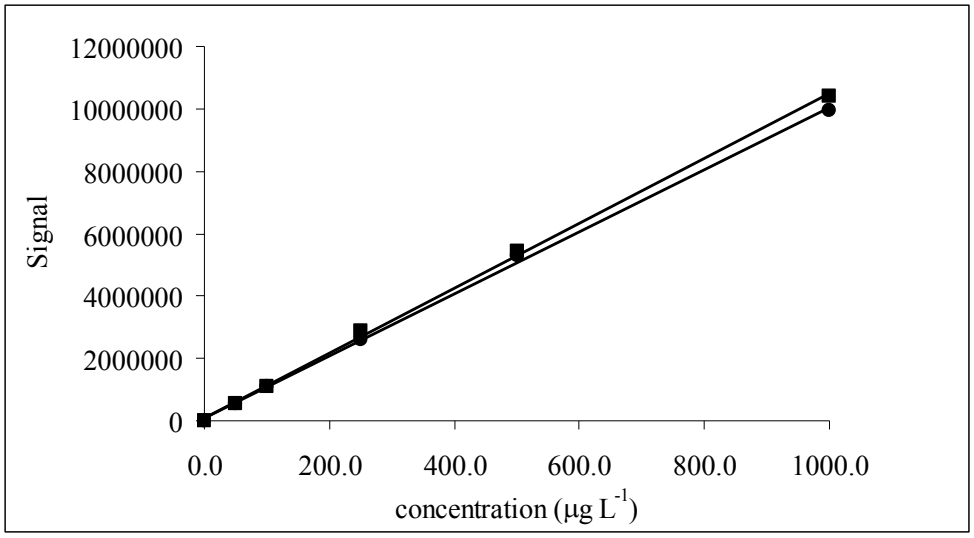

Figure 3.7. Calibration plots of V(IV) and V(V). (▪)V(IV) (y= 9963x - 91527, R^2=0.9990), (●) V(V) (y= 10411x + 95079, R^2=0.9992) (used in the calculations of low vanadium concentrations).

3.4. Calibration Plots of V(IV) and V(V) Using UV-VIS Spectrometry

Absorption spectra of V(IV) and V(V) species in 0.01 M HNO_3 were also recorded using a UV-VIS spectrophotometer as explained in section 2.4. Figure 3.8 shows that V(IV) has an absorption band which gives a maximum at 766 nm whereas no maximum is observed (only a shoulder is seen) in case of V(V). In both cases the spectra were obtained against a blank solution. The wavelengths 766 nm and 276 nm were used for V(IV) and V(V), respectively. Calibration plots obtained with the given conditions are demonstrated in Figures 3.9 and 3.10. Vanadyl, V(IV), graph was linear up to 1000.0 mg L^{-1} whereas that for vanadate,V(V), was up to 100.0 mg L^{-1}.

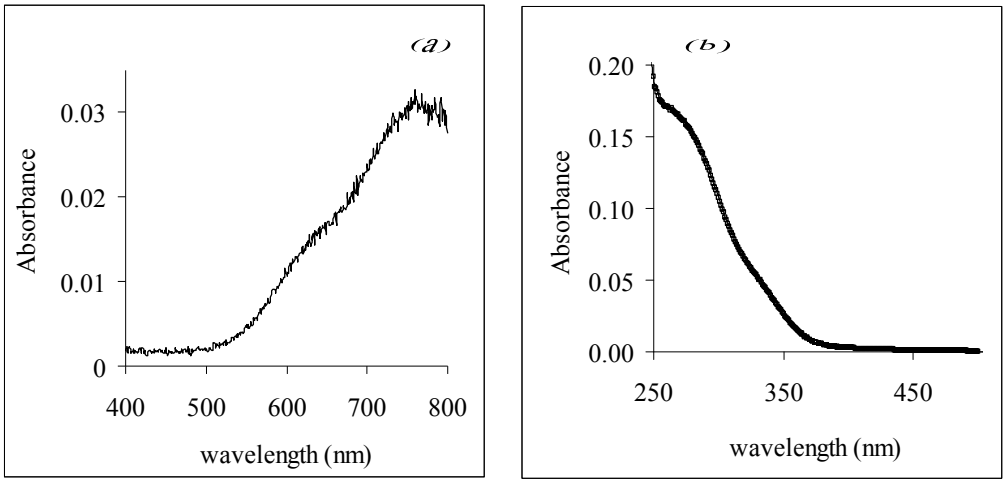

Figure 3.8. The absorption spectra of (a) vanadyl and (b) vanadate species.

3.5. Redox Chemistry of Vanadium

Chemistry of vanadium is complicated by the existence of a variety of forms, depending both on the species concentration and the pH of the solution. If both V(IV) and V(V) species are present in the solution and the total vanadium concentration is to be determined, a preliminary reduction or oxidation step is necessary.

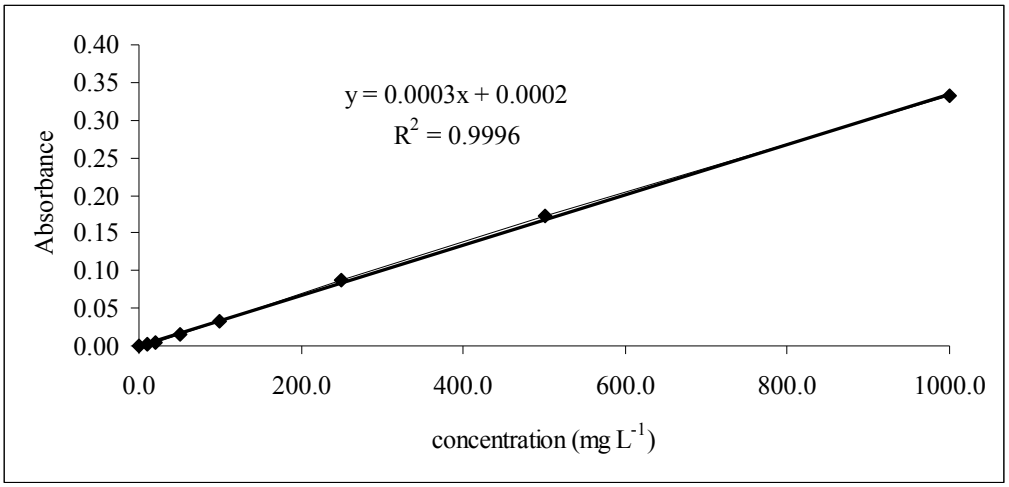

Figure 3.9. Calibration plot of V(IV) obtained at 766 nm with UV-VIS spectrophotometry (used in the calculations of mole ratio method).

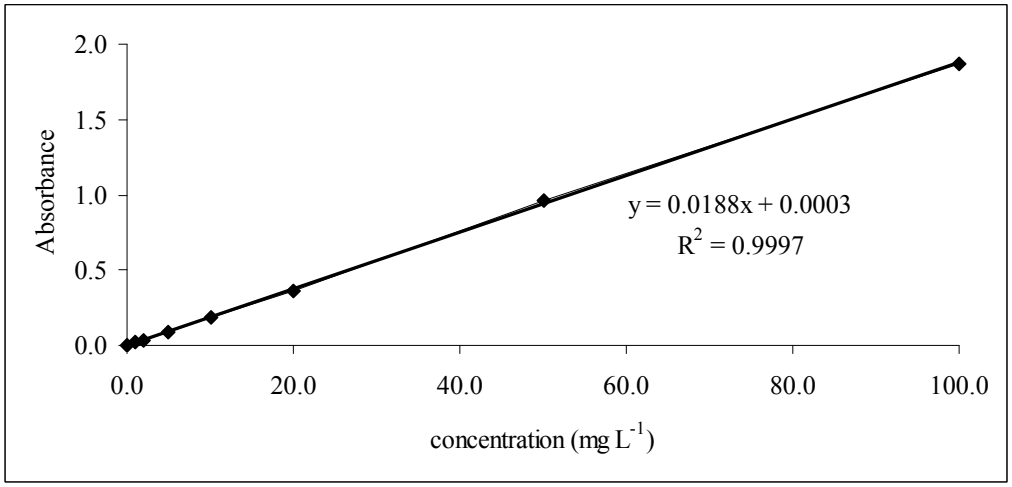

Figure 3.10. Calibration plot of V(V) obtained at 276 nm with UV-VIS spectrophotometry (used in the calculations of mole ratio method).

3.5.1. Reduction of V(V)

As mentioned in the previous sections, vanadium occurs in several oxidation states (+5, +4, +3, +2), each giving a different color to their respective concentrated solutions. So before the spectrophotometric trial, conversion of the yellow V(V) solutions to the blue V(IV) is sought in the concentrated solutions. As explained in 2.4.1, among the reductants investigated ($FeCl_2$, ascorbic acid $FeSO_4$, and L-cysteine),

ascorbic acid and $FeSO_4$ have changed the yellow color of V(V) to blue which was assumed to be due to the formation of V(IV) in the solution. Reduction using $FeSO_4$ was not preferred because it was suspected that the color change might also occur due to a ligand exchange reaction with sulphate ions to give $[V(H_2O)_5(SO_4)]$ (Rakib and Durand 1996). Therefore, the subsequent experiments were performed only with ascorbic acid.

L-Ascorbic acid reduces aqueous V(V) according to the following equation:

$$2H^+ (aq) + H_2A (aq) + 2VO_2^+(aq) \rightarrow A + 2VO^{2+}(aq) + 2H_2O$$
$$\text{(yellow)} \qquad\qquad \text{(blue)}$$

As explained in 2.4.1, in order to determine the stoichiometric amount for the reduction of vanadate (VO_2^+) to vanadyl (VO^{2+}) ion, different molar concentrations of ascorbic acid:vanadate (1:5, 1:2, 1:1, 2:1, 5:1, 10:1, 20:1, 50:1, 100:1, 200:1) were prepared for a fixed concentration at pH 2.0. The absorption spectra of a reference blank solution were also recorded for each of the prepared solutions. Blank-corrected absorbance spectra for vanadyl and vanadate ions are shown in Figure 3.11.

As can be seen from Figure 3.11, the similarity between the spectra of the two species has shown that the vanadate ion is successfully reduced to vanadyl ions in the presence of ascorbic acid. The absorption maximum, 766 nm, was used in the succeeding measurements. Although the reagent/analyte molar ratio of 1:1 was found to be sufficient for the reduction of vanadate to vanadyl, the ratio 2:1 was chosen in the subsequent experiments to guarantee the reduction in other possible sample matrices. (Figure 3.12). This value is also in accordance with a previous study (Wilkins et al. 2006).

3.5.2. Oxidation of V(IV)

As explained in section 2.4., the change of the color of the solution from blue to yellow was assumed due to the conversion of V(IV) to V(V) in the solution. Among the oxidants tested (KIO_3, $KBrO_3$, $K_2S_2O_8$ and $K_2Cr_2O_7$), only $KBrO_3$ offered this color change. For this reason, the subsequent experiments were performed only with this oxidizing agent.

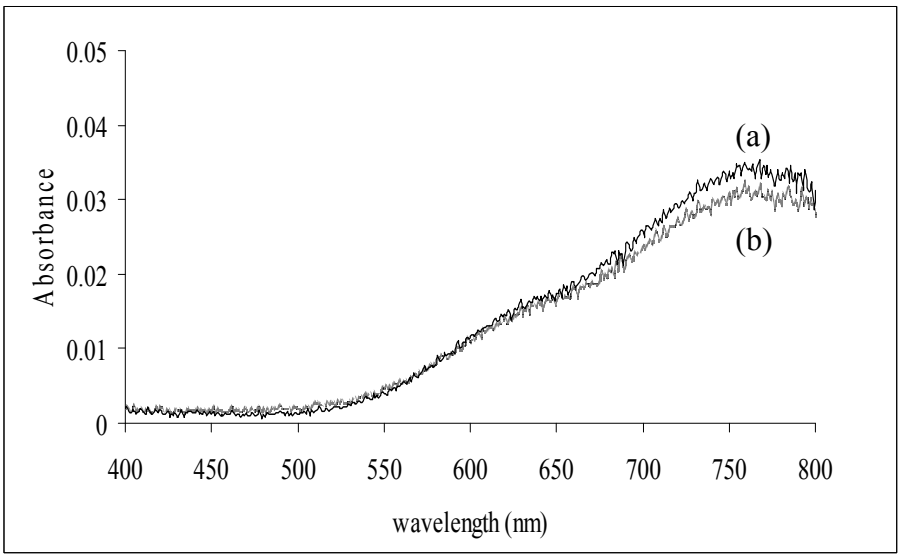

Figure 3.11. The absorption spectra of (a) vanadyl and (b) vanadate after reduction with ascorbic acid (species concentration: 100.0 mg L^{-1}).

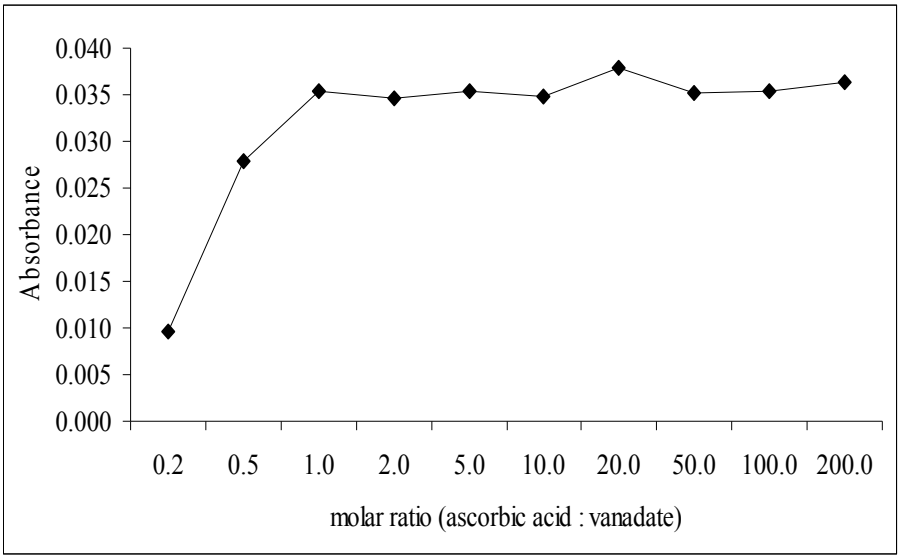

Figure 3.12. Molar ratio curve in the reaction betwen ascorbic acid and vanadate (λ=766 nm).

Potassium bromate ($KBrO_3$) is a stable solid and is used to oxidize alcohols to aldehydes, ketones, esters and carboxylic acids. Bromate itself is a very strong oxidizing agent and can easily be handled when compared with liquid bromine (Shawale et al. 2008). $KBrO_3$ oxidizes aqueous V(IV) according to the following equation:

$$BrO_3^-(aq) + 5\ VO^{2+}(aq) + 2H_2O \rightarrow \frac{1}{2}\ Br_2(l) + 4\ H^+(aq) + VO_2^+(aq)$$

The strong similarity between their respective absorbance spectra (Figure 3.13) indicated that the vanadyl ion is almost completely oxidized to vanadate ion by $KBrO_3$. The succeeding experiments were, therefore, concentrated on the determination of the stoichiometric amount by considering different reagent/analyte ratios.

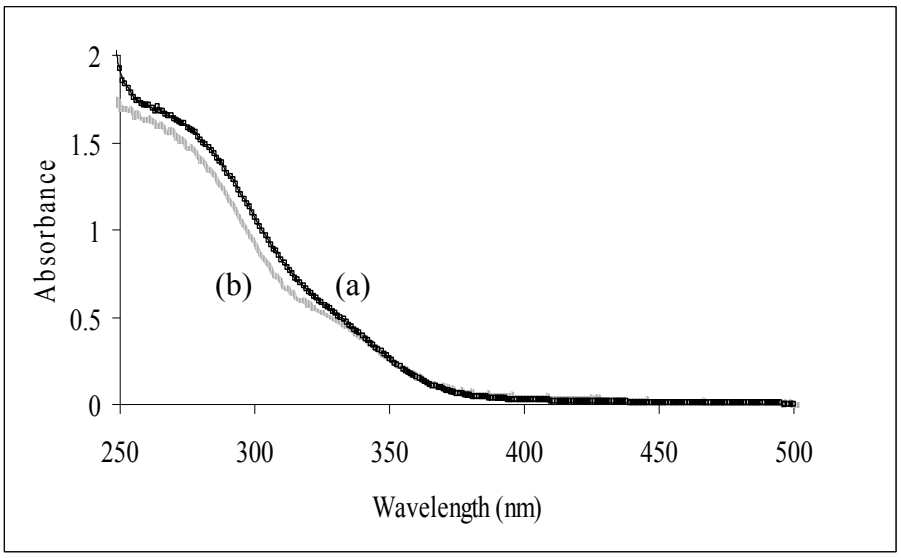

Figure 3.13. The absorption spectra of (a) vanadate and (b) vanadyl after oxidation with $KBrO_3$ (species concentration: 100.0 mg L^{-1}).

For this purpose, the solutions were prepared in several reagent/analyte proportions (1:10, 1:1, 2:1, 5:1, 10:1 and 100:1) to determine the optimum the molar ratio for the oxidation of vanadyl ions by $KBrO_3$. The pH of the solutions was 2.0 and a blank spectrum was also recorded for each reagent/analyte ratio. The measurements were made at 276 nm which is the wavelength of maximum absorbance for the vanadate ion. As seen from Figure 3.14, the reagent/analyte molar ratio of 1:1 was sufficient for the oxidation but 2:1 ratio was chosen for the subsequent experiments to be on the safe side.

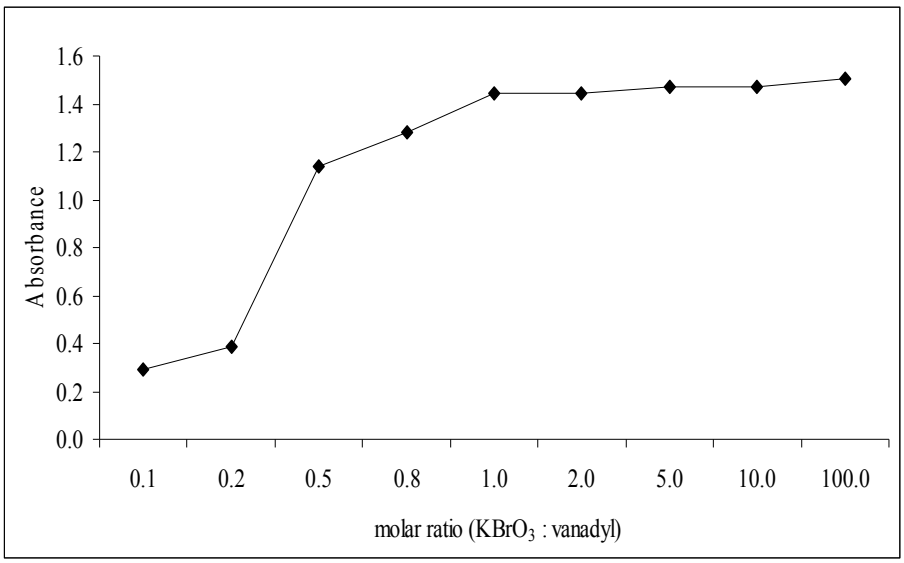

Figure 3.14. Molar ratio curve in the reaction betwen $KBrO_3$ and vanadyl (λ=276 nm).

3.6. Studies for Sorption and Speciation of V(IV) and V(V)

As mentioned throughout the text, vanadium is sensitive to redox conditions and the pH of the natural matrices hence an accurate determination of each species is important to evaluate the potential risk of the element. Figures 3.15 and 3.16 indicate the distribution of vanadium species obtained by MINTEQ program. The distribution diagrams have priority importance in order to clarify the sorption process.

As can be seen from Figure 1.1, V(IV), as the vanadyl cation VO^{2+}, may be present in reducing environment. It is stable in acidic solution below pH 2, but is oxidized to the pentavalent state by atmospheric oxygen at higher pH values. Pentavalent vanadium, the vanadate ion, which exists as VO_2^+ in acidic, as $H_2VO_4^-$ in neutral, and as $HV_2O_7^{3-}$ in alkaline solutions. Therefore it is very important to consider both the pH and the redox conditions of the sample solutions since these factors determine the form of the species.

3.6.1. Studies Utilizing Several Solid Sorbents

In this study, our purpose was to find a proper sorbent for the speciation and determination of vanadium. For this purpose, several ion-exchange or chelating resins, chitosan and nano sized zero-valent iron were tried and their selectivity towards V(IV) and V(V) were examined. Standard solutions of V(IV) and V(V) (1.0 mg L^{-1}, 10.0 mL) were prepared separately from their respective stock solutions. The pH of the solutions was adjusted to 2.0, 4.0, 6.0, 8.0 and 10.0 where percent sorption was determined using the batch process.

The details of the procedures were given in Experimental. The percentage sorption graphs for the selected sorbents as a function of pH are shown in Figures 3.17-3.25. Among the sorbents investigated, Amberlite IR-120, Duolite GT-73, Duolite A-7 and nZVI were able to absorb both V(IV) and V(V) at the pH values tried (2.0-8.0). In addition, it has to be mentioned that nZVI can not be used below pH 3.5 due to dissolution. Duolite XAD-761 and Duolite C-467 did not show much affinity towards any of the vanadium species for pH values 2.0, 6.0 and 8.0. Amberlite IR-410 becomes selective when the pH>6.0. For chitosan, 80-100% sorption was obtained at only pH 4.0. Chitosan was selective both for V(IV) and V(V) except pH 10.0. As can be seen from the figures, none of them gave promising results for the speciation of vanadium. Therefore, further experiments were carried out with functionalized silicas.

3.6.2. Studies with 3-APTES-Modified Silica

3.6.2.1. Effect of pH

Solution pH is one of the most important factors in controlling the sorption of vanadium species by the sorbents. Therefore, the ability of the modified silica to take up vanadium from aqueous solution was firstly investigated as a function of pH. These experiments were not only performed with 3-APTES-modified silica but also with 3-MPTMS-modified silica for comparison.

Figure 3.26 indicates the sorption behavior of 3-APTES-modified silica towards V(IV) and V(V) as a function of pH. As can be seen from the figure, the proposed

sorbent exhibits maximum sorption at pH 2.0 for V(V) while it does not show much affinity for V(IV) at this pH. When the pH value is higher than 2.0 both V(IV) and V(V) are taken up by the sorbent (40-80 % depending on pH). This indicates the importance of adjusting the pH of the solution to 2.0 if V(IV) and V(V) are to be determined separately.

When Figures 3.26 and 3.27 are considered, it can clearly be seen that the percent sorption graphs for the novel sorbent and the unmodified silica show strong similarity both for V(IV) and V(V) when the pH is > 3.0. In addition, Figure 3.27 points out that when a similar experiment is performed with the unmodified silica, it can clearly be seen that V(V) is not quantitatively taken up at pH 2.0 where the maximum sorption is observed with the 3-APTES-modified silica. At first glance, for pH values higher than 3.0, it can be observed that both of V(IV) and V(V) are taken up by the sorbent to a certain degree (40-80 % depending on pH) and that the sorbent does not show any selectivity to V(IV) or V(V). However, a healthier comment would be that a possible oxidation of V(IV) to V(V) at pH's higher than 3.0 is taking place, and is thus leading the sorbent to exhibit sorption only towards V(V). These results indicate the importance of pH adjustment prior to the sorption step. A solution pH of 3.0 can be used for the quantitative sorption of both V(IV) and V(V), whereas pH of 2.0 would allow only for the sorption of V(V). The concentration of V(IV) can be determined from the difference.This is an attractive feature of this study that with a simple modification of the silica surface, V(IV) and V(V) species can be determined independently without any need to determine the total vanadium amount and subtracting any species from that amount.

In the subsequent stages of this study a more detailed study on the effect of pH was performed for the modified silica namely 3-APTES-modified, 3-MPTMS-modified, bifunctional (both 3-APTES and 3-MPTMS-modified) and a physical mixture of the 3-APTES and 3-MPTMS-modified for the pH values of 1.0, 1.5, 2.0, 2.5, 3.0 (Figure 3.28). As can be seen from the figure, 3-MPTMS-modified silica does not serve as a suitable sorbent for either vanadium determination/speciation.

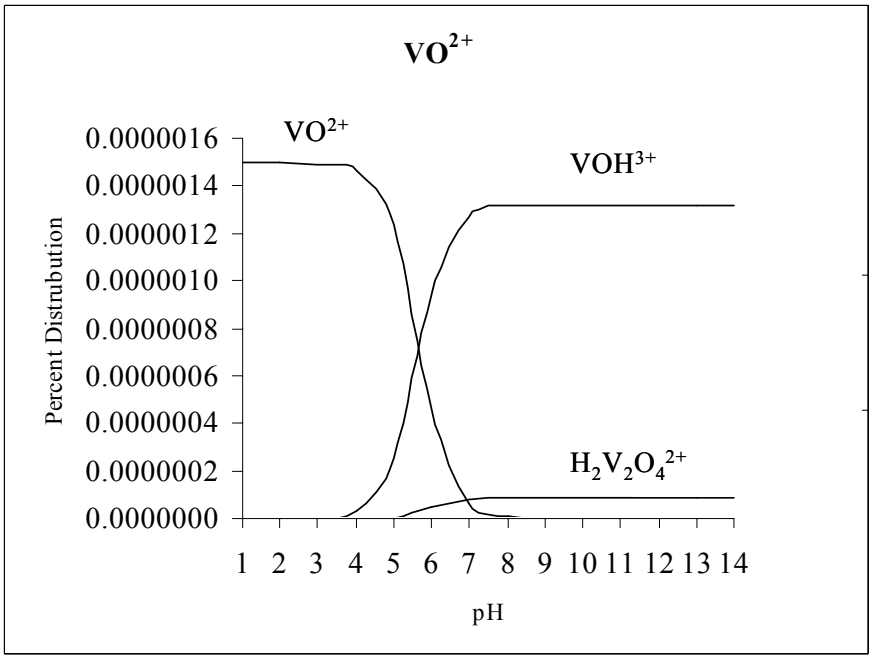

Figure 3.15. Distrubution diagram for V(IV) in aqueous solutions obtained by using the MINTEQ program.

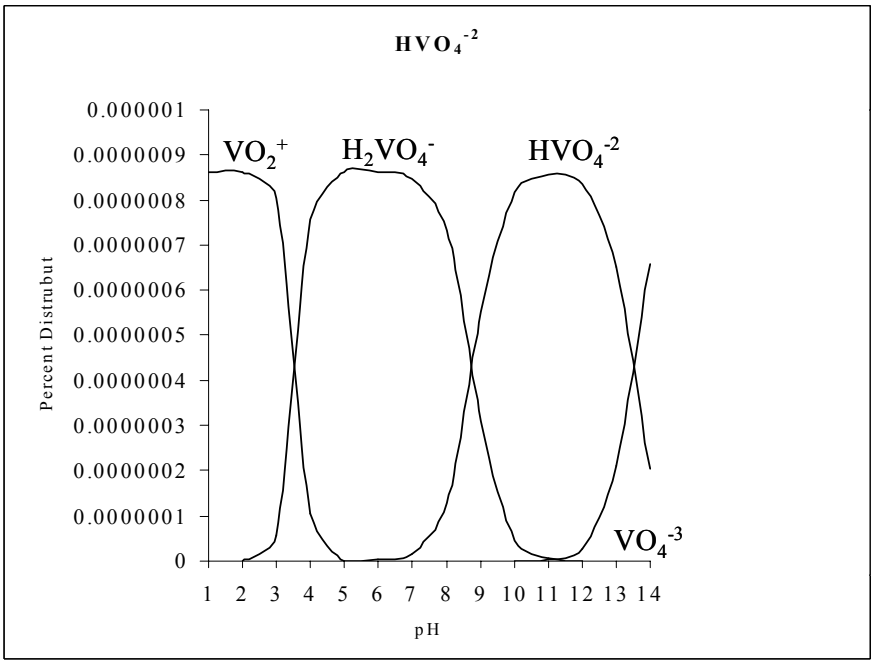

Figure 3.16. Distrubution diagram for V(V) in aqueous solutions obtained by using the MINTEQ program.

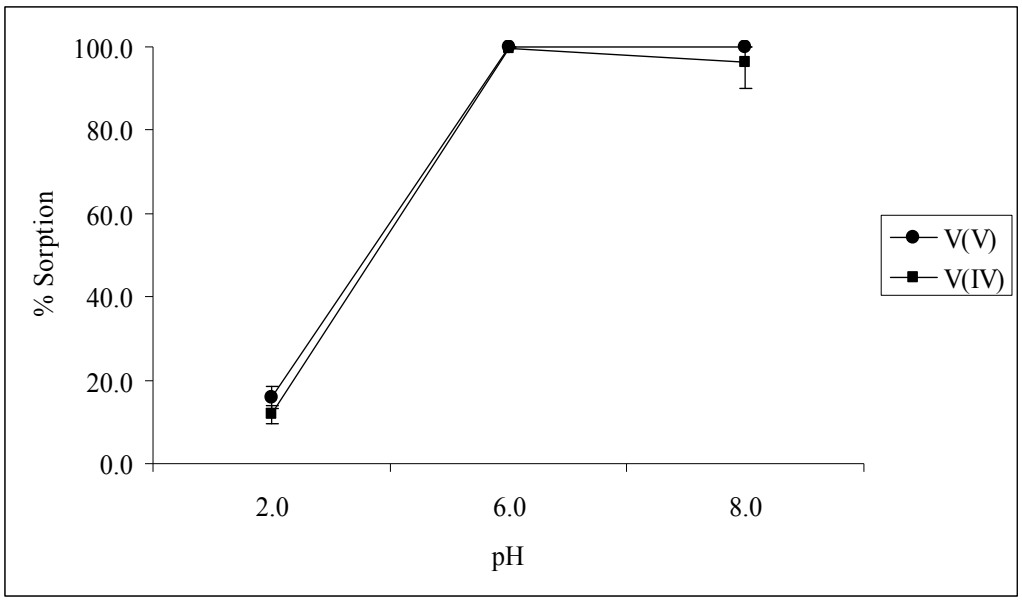

Figure 3.17. Effect of pH on the sorption of V(IV) and V(V) towards Amberlite IR-410 (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

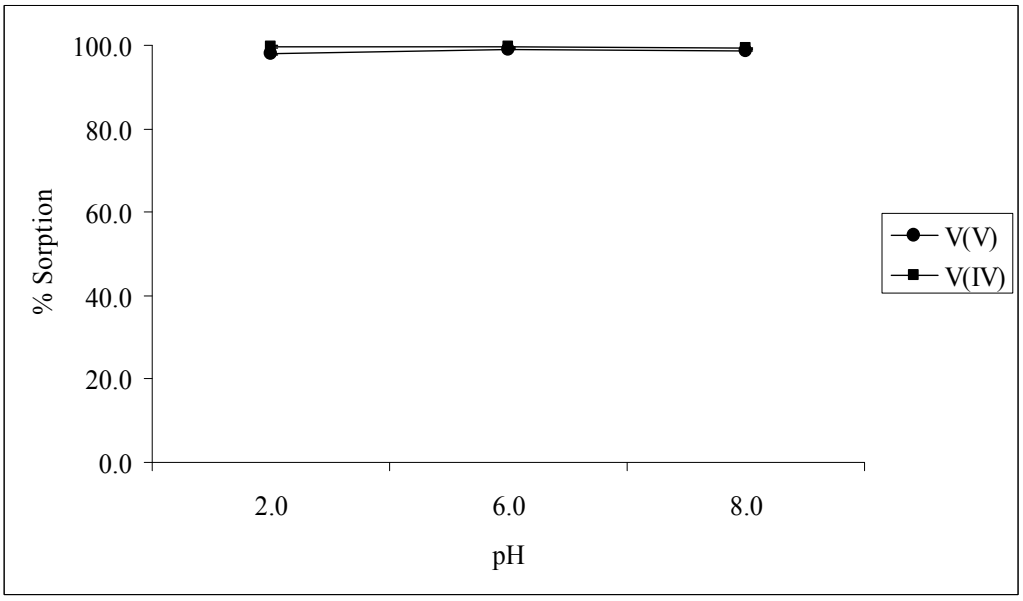

Figure 3.18. Effect of pH on the sorption of V(IV) and V(V) towards Amberlite IR-120 (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

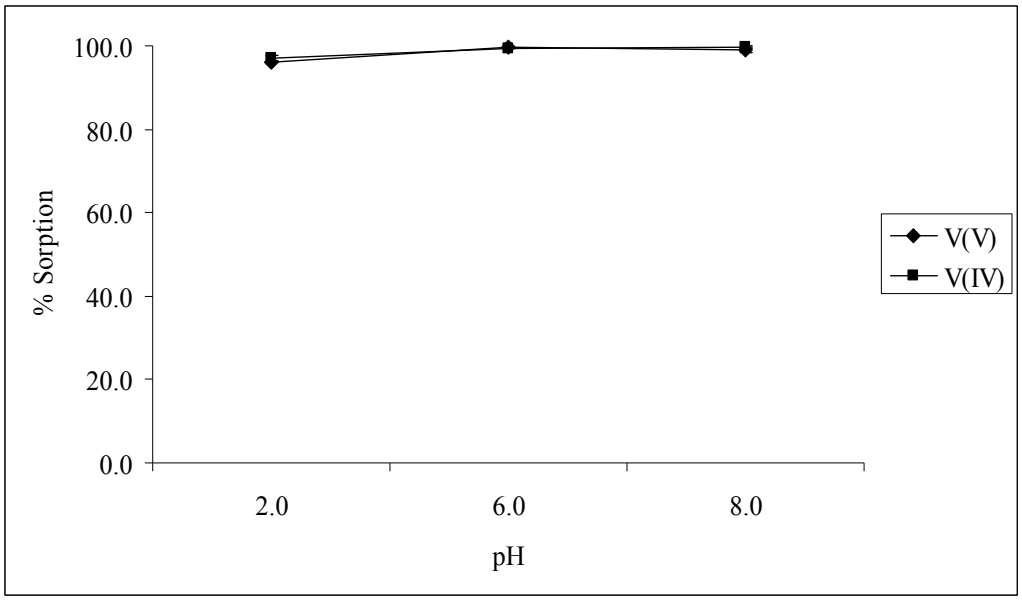

Figure 3.19. Effect of pH on the sorption of V(IV) and V(V) towards Duolite GT-73 (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

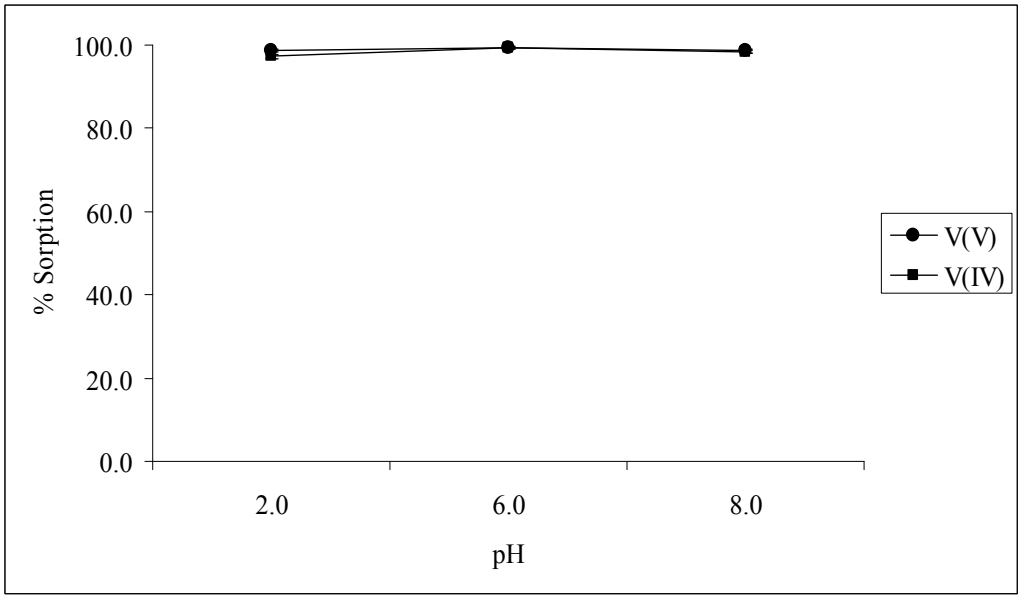

Figure 3.20. Effect of pH on the sorption of V(IV) and V(V) towards Duolite A-7 (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

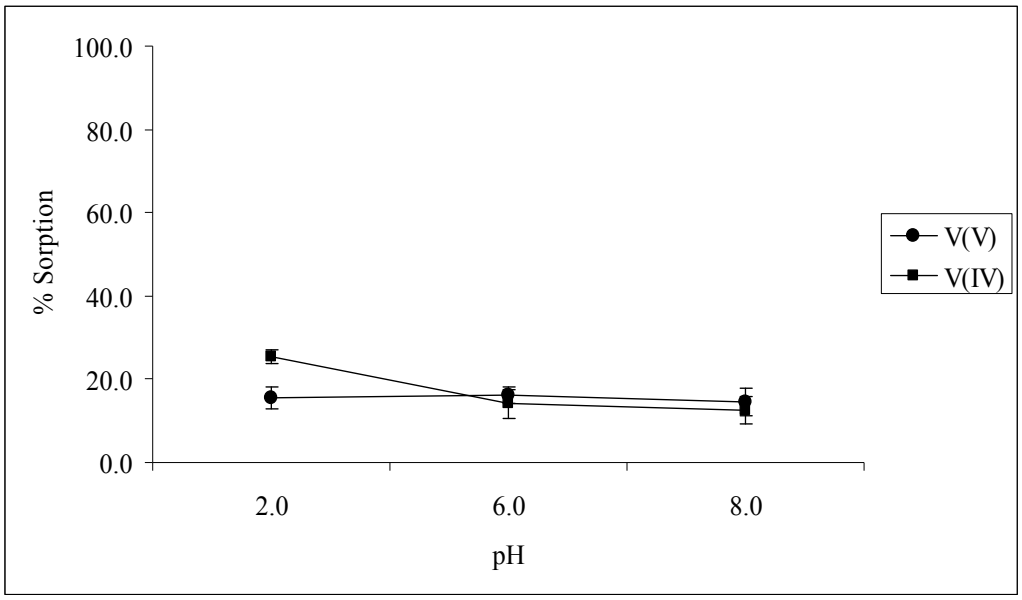

Figure 3.21 Effect of pH on the sorption of V(IV) and V(V) towards Duolite C-467 (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

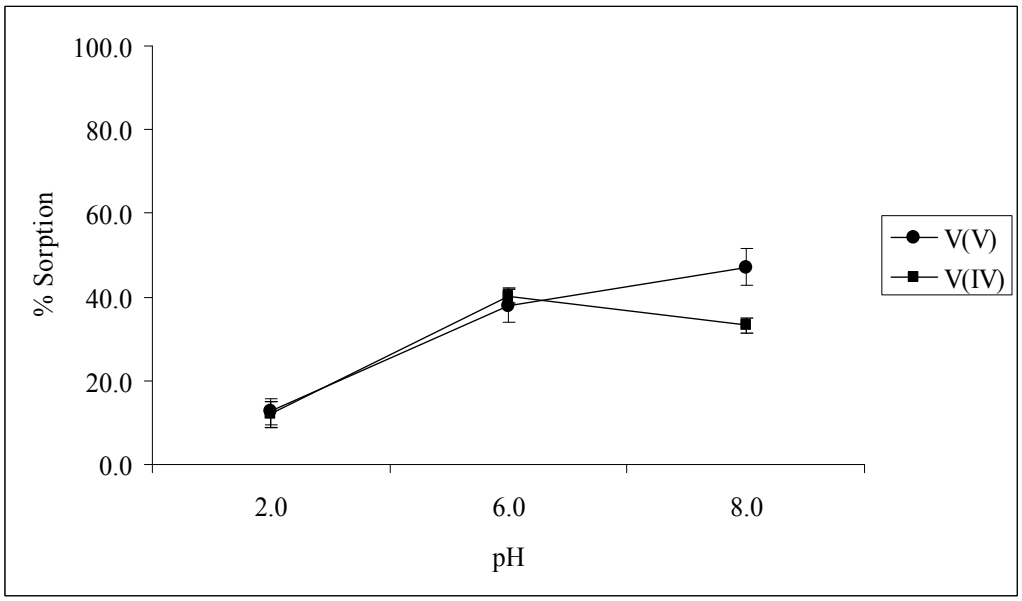

Figure 3.22. Effect of pH on the sorption of V(IV) and V(V) towards Duolite XAD-761 (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

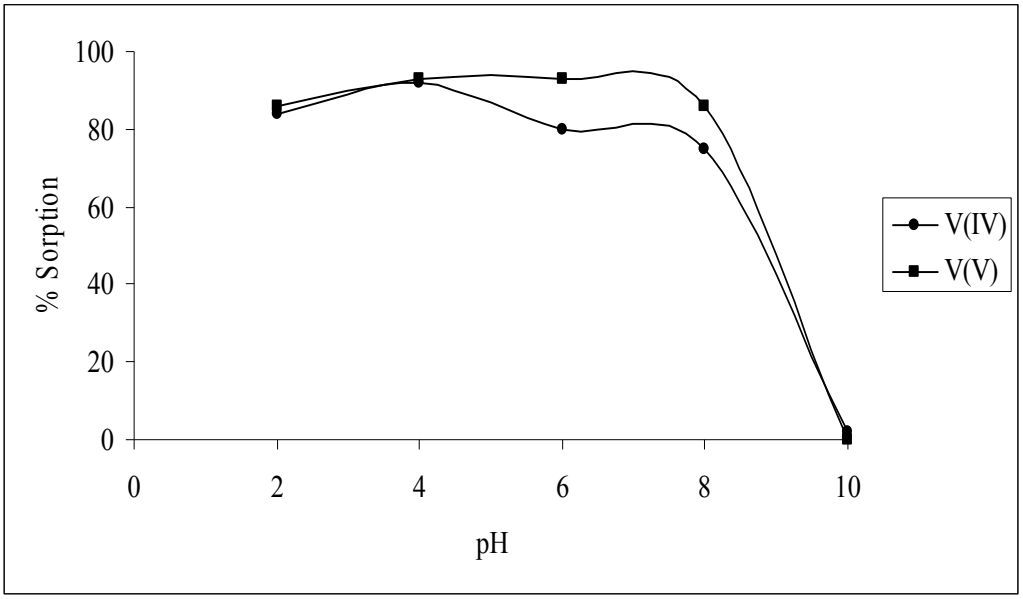

Figure 3.23. Effect of pH on the sorption of V(IV) and V(V) towards Chelex 100 (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

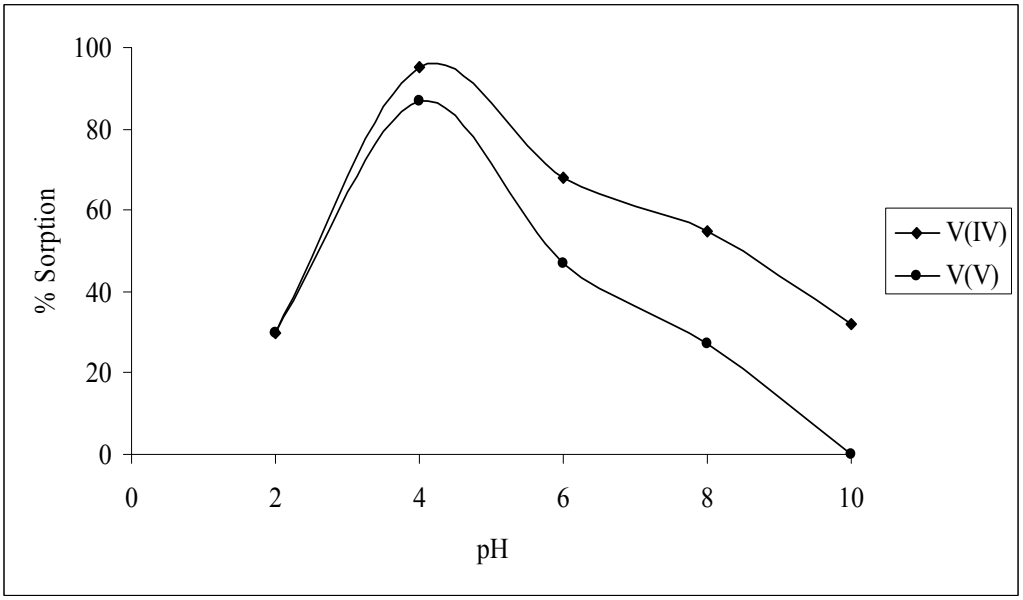

Figure 3.24. Effect of pH on the sorption of V(IV) and V(V) towards Chitosan (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

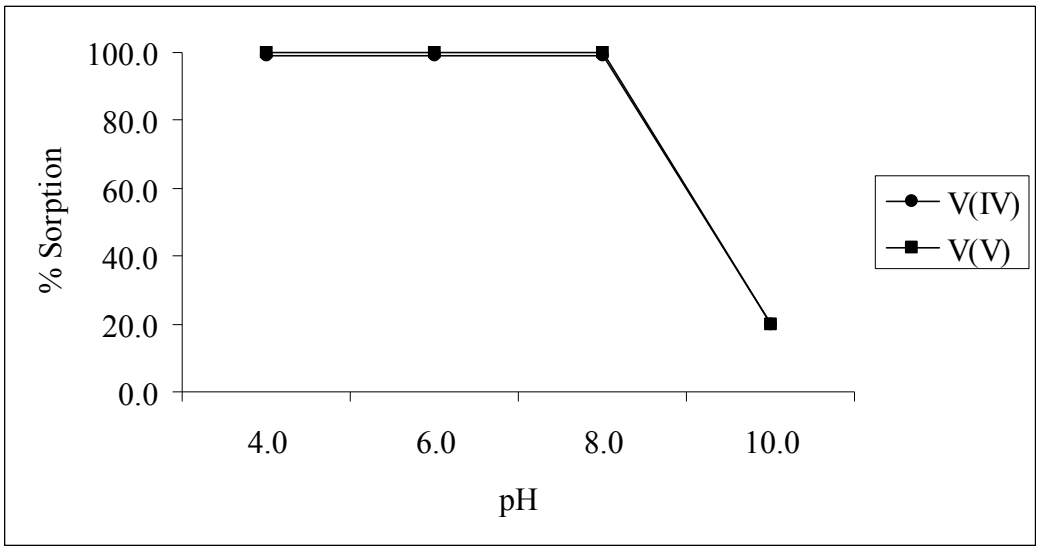

Figure 3.25. Effect of pH on the sorption of V(IV) and V(V) towards nano-sized zerovalent iron (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

For bifunctional silica (both 3-APTES and 3-MPTMS-modified), a significant difference is not observed in V(IV) or V(V) sorption. Nevertheless, pH 2.5 can be suitable for total vanadium determination if this sorbent is aimed to be used. When the case for sorption by adding separately equal amounts of 3-APTES and 3-MPTMS-modified silica to V(IV) and V(V) solutions is considered, it can be said that a similar trend is observed at pH 2.0 as with 3-APTES-modified silica. It is probably due to the amino (-NH_2) groups on the silica surface and not to mercapto (-SH) groups. Thus modification of the surface with 3-MPTMS does not have a diverse effect for vanadium speciation.

3.6.2.2. Effect of Silica Gel Pretreatment

Surface pretreatment via functional group attachment provides a unique opportunity to obtain silica gel having higher surface areas which have great importance in adsorption and ion-exchange studies. Moreover, impurities are removed with the acid pretreatment. Table 3.5 indicates the effect of silica gel pretreatment on sorption. As seen from the table, when silica gel is pretreated with acetic acid, more reliable results are obtained, thus in the subsequent stages acetic acid pretreatment was employed.

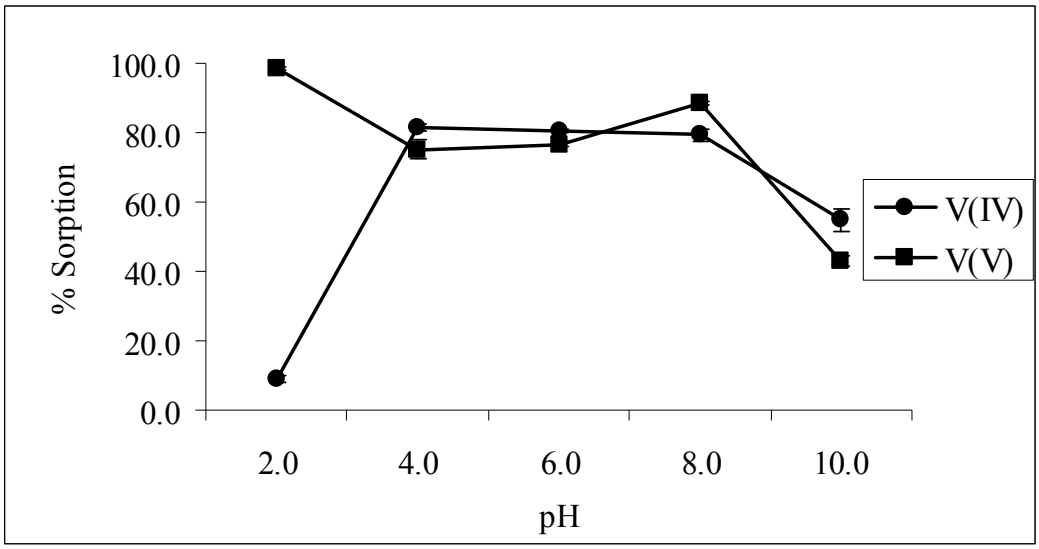

Figure 3.26. Effect of pH on the sorption of V(IV) and V(V) towards 3-APTES-modified silica (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

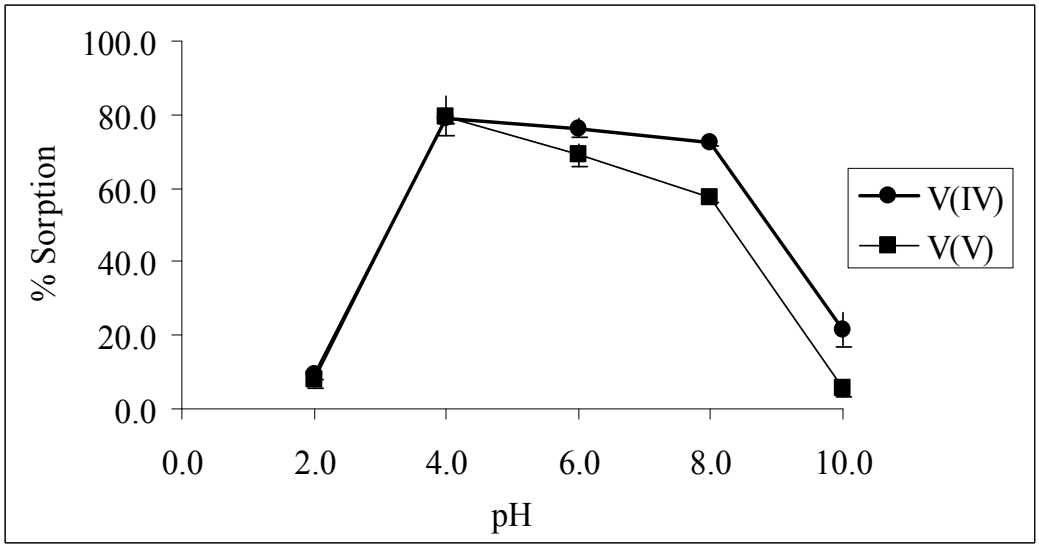

Figure 3.27. Effect of pH on the sorption of V(IV) and V(V) towards unmodified silica (1.0 mg L^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

Table 3.5. Effect of the silica gel pretreatment on sorption (50.0 mg of sorbent, sorption time of 30 min., solution pH of 2.0, 10.0 mL of solution and 1.0 mg L^{-1} V(IV) or V(V) at 25°C, n=3).

Silica Gel Pretreatment	% Sorption	
	V(IV)	V(V)
-	7.2 (±0.5)	91.3 (±4.5)
H_2O_2	24.1 (±10.5)	95.5 (±0.5)
CH_3COOH	12.8 (±1.8)	98.2 (±0.1)

3.6.2.3. Effect of Sorbent Amount

As explained in part 2.8.1.3, the optimum amount of the sorbent for maximum take up was determined by increasing the amount of 3-APTES-modified silica while keeping other parameters constant. Figure 3.29 indicates the vanadate sorption behavior with discrete amount of 3-APTES-modified silica. As can be seen from the figure, a maximum sorption at 50.0 mg is observed for the given concentration of V(V). Thus, this amount is assumed to be adequate for quantitative sorption of V(V) under the experimental conditions obtained.

3.6.2.4. Effect of Shaking Time

In order to find out the time required for the sorption equilibrium to be reached, the sorption experiments were carried out as a function of shaking time keeping the other parameters constant. A quantity of 50.0 mg of 3-APTES-modified silica was added to 10.0 mL of 1.0 mg L^{-1} V(V) solutions having a pH of 2.0 at 25.0ºC and the samples were shaken for 1.0, 2.0, 5.0, 15.0, 30.0, 45.0, 60.0, 75.0 and 90.0 minutes. The results given in Figure 3.30 demonstrate the very fast kinetics of sorption. Even a reaction time of 5 minutes is sufficient to achieve equilibrium (95 % sorption) for V(V). Still, a shaking time of 30.0 minutes was used to guarantee the quantitative sorption.

3.6.2.5. Effect of Initial Concentration

The extent of removal of heavy metals from aqueous solution depends strongly on the initial metal ion concentration. The variation in the percent sorption of 3-APTES-modified silica for different initial concentrations of V(V) were studied at the conditions described in section 2.8.1.5. The results are given in Table 3.6. In order to demonstrate the efficiency of the modification similar experiments were also conducted with the unmodified silica. As can be seen from the table, V(V) is not quantitatively absorbed by the bare silica. When the case for 3-APTES-modified silica is considered, it is seen that the sorption percentage does not decrease under 93.5(±0.9) % until an initial concentration of 100.0 mg L^{-1}. The reason for the decrease for the initial concentration of 1000.0 mg L^{-1} is due to exceeding the maximum sorption capacity of the sorbent (detailed information will be given in the subsequent sections). It can be concluded that the decrease in the percentage sorption with an increase in the initial metal ion concentration is reasonable as the greater number of available functional groups are occupied by the analyte species at high concentrations.

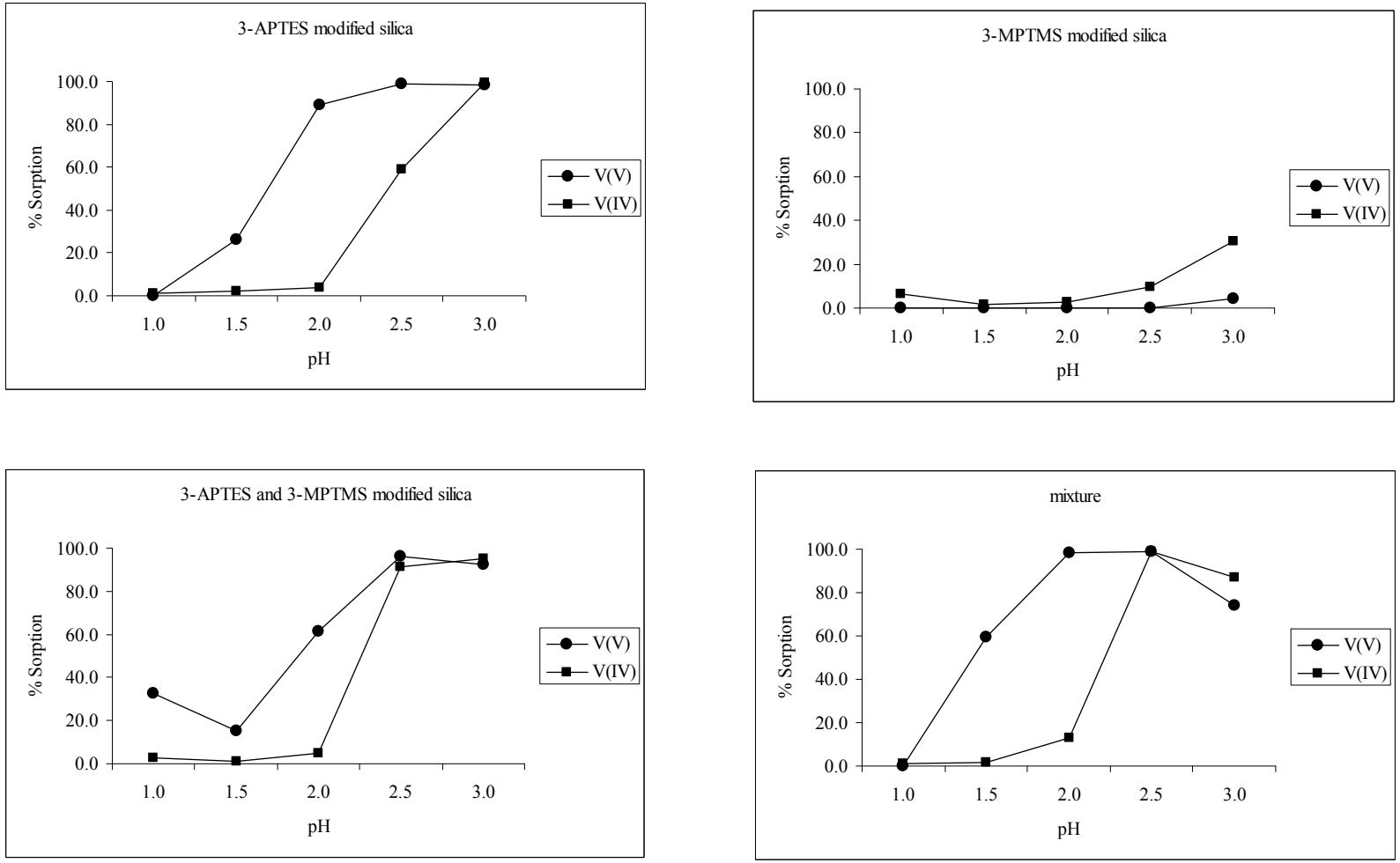

Figure 3.28. Effect of pH on the sorption of V(IV) and V(V) towards modified silicas (1.0 mgL^{-1}, 10.0 mL V(V) or V(IV) solution, 30 min. shaking time, 50.0 mg sorbent at 25^{o}C sorption temperature, n=3).

Figure 3.29. Effect of sorbent (3-APTES-modified silica) amount on sorption (1.0 mg L^{-1} V(V), pH 2.0, 10.0 mL solution volume, 30 min. shaking time, 25°C sorption temperature, n=3).

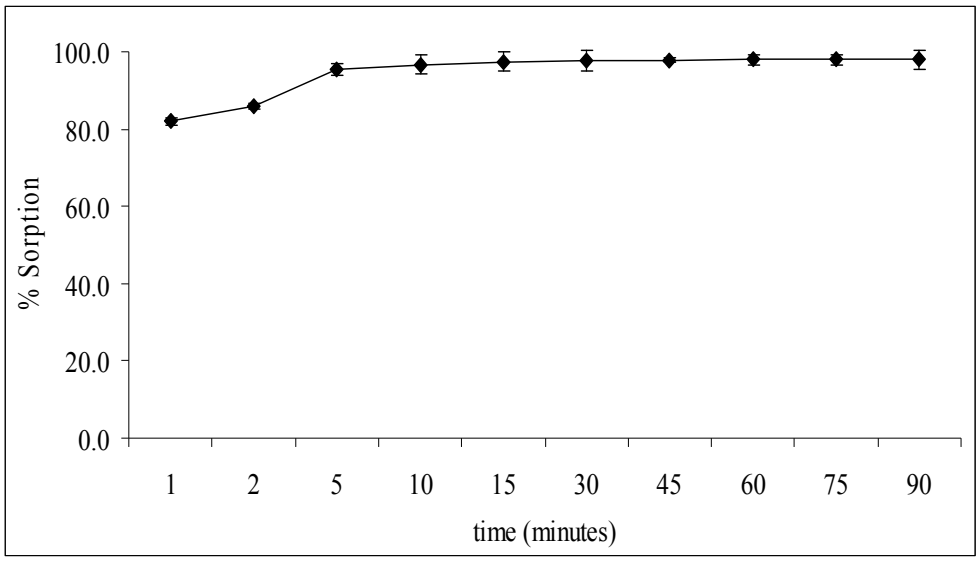

Figure 3.30. Effect of shaking time on sorption (1.0 mg L^{-1} V(V), pH 2.0, 10.0 mL solution volume, 50.0 mg sorbent, 25°C sorption temperature, n=3).

Table 3.6. Effect of initial V(V) concentration on the sorption by unmodified and 3-APTES-modified silica (pH 2.0, 10.0 mL solution volume, 50.0 mg sorbent, sorption time of 30.0 min., 25°C sorption temperature, n=3).

Concentration (mg L^{-1})	% Sorption	
	Unmodified silica	3-APTES-modified silica
0.010	39.5 (± 5.9)	91.2 (± 0.2)
0.10	6.3 (± 1.2)	97.3 (± 0.3)
1.0	7.7 (± 2.2)	97.8 (± 0.7)
10.0	2.9 (± 2.0)	95.9 (± 0.6)
100.0	7.7 (± 3.3)	93.5 (± 0.9)
1000.0	7.9 (± 0.4)	60.6 (± 4.3)

3.6.2.6. Effect of Sorption Temperature

The effect of reaction temperature on the sorption of V(V) was studied at 25.0 and 60.0°C. The change in the percent sorption of 3-APTES-modified silica as a function of temperature is given in Figure 3.31 which shows a decreased sorption with an increase in the temperature. This exothermic behavior is associated with decrease in entropy indicating that the spontaneous sorption is enthalpy-driven. The summary of thermodynamic parameters is given in Table 3.7.

In order to understand the effect of temperature on the adsorption better, it is important to study the thermodynamic parameters such as standard Gibbs free energy change, ΔG^o, standard enthalpy change ΔH^o, and standard entropy change, ΔS^o.

The magnitude of the change in free energy can be used to determine the type of adsorption. In this study, ΔG^o values were found to be −8.7, and −11.5 kJ mol^{-1} for 298 K and 333 K, respectively. Since physisorption has a range of −20 and 0 kJ mol^{-1}; and chemisorption −80 to −400 kJ mol^{-1}, it can be concluded that physisorption contributes to the sorption mechanism (Yu et al. 2001). It is seen that when the temperature is increased from 298 to 333 K, ΔG^o is changed from −8.7 to −11.5 kJ mol^{-1}. It is also an evidence of physisorption.

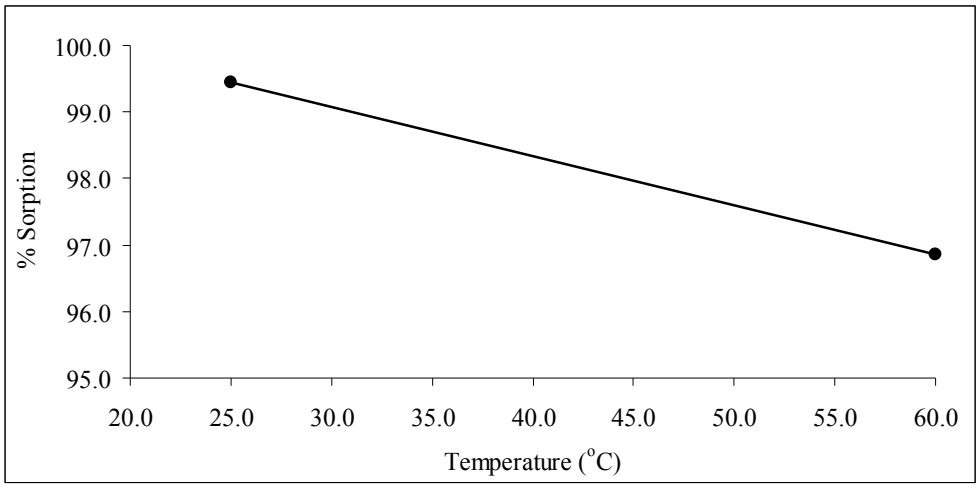

Figure 3.31. Effect of reaction temperature on sorption (1.0 mg L^{-1} V(V) solution, pH 2.0, 10.0 mL solution volume, 50.0 mg sorbent, sorption time of 30.0 min., n=3).

The negative values of ΔG^o at different temperatures indicate the spontaneous nature of the sorption process. If heat of an adsorption process is <40 kJ mol^{-1}, it is a physical process, and at the same time, the activation energy of a physical process is also generally low (Mc Bride 1994). In addition, negative values of ΔS (decrease in entropy) are a sign of the system becoming less random.

Table 3.7. Thermodynamic parameters for the sorption of V(V) by 3-APTES-modified silica

Temperature (K)	ΔG^o (kJ mol^{-1})	ΔS^o (J $mol^{-1}K^{-1}$)	ΔH^o (kJ mol^{-1})
298	-8.7	-281.6	-15.6
333	-11.5	-243.5	

3.6.2.7. Effect of Ionic Strength

The effect of ionic strength on the sorption capability of 3-APTES-modified silica was investigated by the addition of various concentration of NaCl into V(V) solution. The results are illustrated in Figure 3.32. According to the results obtained, even with the addition of a relatively high concentration of 0.1 M NaCl, no change in sorption was observed. These findings can be assumed to demonstrate the potential application of the novel sorbent to relatively heavy sample matrixes.

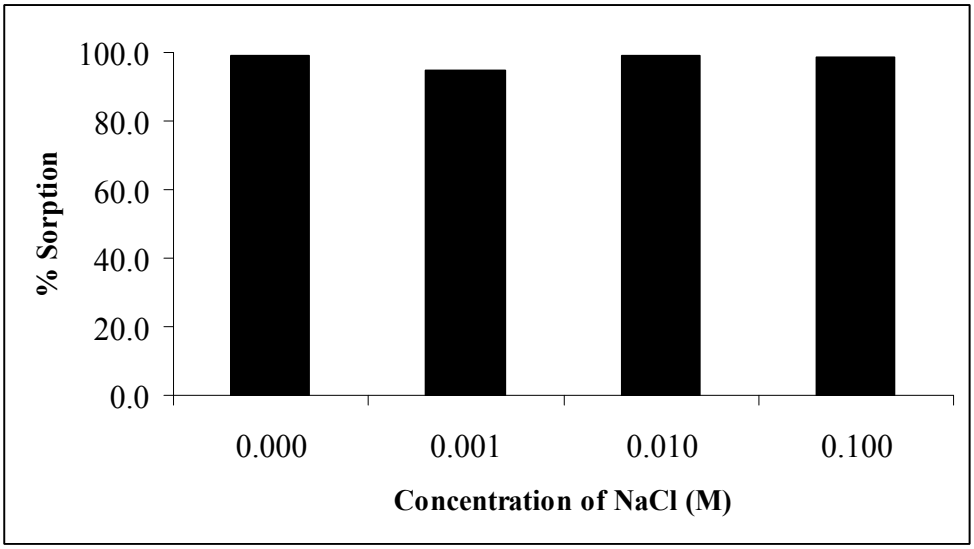

Figure 3.32. Sorption percentage of 3-APTES-modified-silica for V(V) at various NaCl concentrations (1.0 mg L^{-1} V(V) solution, pH 2.0, 10.0 mL solution volume, 50.0 mg sorbent, sorption time of 30.0 min., 25^{o}C n=3).

3.6.2.8. Effect of Sample Volume

Preconcentration and separation of metal ions are usually performed at lower concentrations. To avoid the possible inaccuracy for low analyte concentrations, use of relatively large sample volumes is usually suggested. For this purpose, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mL sample volumes at a constant absolute V(V) of 10 μg and being other parameters constant, were adopted to test the effect of sample volume. The results are outlined in Table 3.8. It can be seen that relatively acceptable recoveries (85-99%) were obtained with sample volumes less than 250.0 mL. This

provides the use of the proposed methodology with relatively large sample volumes. On the other hand, percent sorption decreased with increasing sample volumes (beyond 250.0 mL).

As mentioned in Experimental, in another set of experiments, sample volumes (10.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mL) were changed while keeping the V(V) concentration constant (absolute amount changes depending on the solution volume) at 1.0 mg L^{-1} (Table 3.9).

Table 3.8. Effect of sample volume on sorption of V(V) by 3-APTES-modified silica (10.0 μg V(V), 100.0 mg of sorbent, sorption time of 30.0 min., pH of 2.0, at 25^{o}C, n=3).

Sample Volume (mL)	V(V) concentration (mg L^{-1})	Absolute V(V) Amount (μg)	% Sorption
10.0	1.0	10	99.2 (± 0.2)
25.0	0.40	10	98.1 (± 0.6)
50.0	0.20	10	97.5 (± 0.6)
100.0	0.10	10	93.7(± 0.5)
250.0	0.04	10	87.1 (± 1.0)
500.0	0.02	10	63.1 (± 0.3)
1000.0	0.01	10	40.7 (± 2.2)

As a supplementary experiment, for 1000.0 mL, 1.0 mg L^{-1} V(V) solutions, the sorbent amount is increased gradually and percent sorption values are calculated. As can be seen from Table 3.10, quantitative sorption is obtained as the amount of sorbent is increased which enables to work with large sample volumes and preconcentration factors.

Table 3.9. Effect of sample volume on sorption of V(V) by 3-APTES-modified silica (1.0 mg L^{-1} V(V), 100.0 mg of sorbent, sorption time of 30.0 min., pH of 2.0, at 25°C, n=3)

Sample Volume (mL)	V(V) concentration (mg L^{-1})	% Sorption
10.0	1.0	99.2 (± 0.2)
25.0	1.0	99.4 (± 0.1)
50.0	1.0	95.7 (± 0.8)
100.0	1.0	88.5 (± 0.2)
250.0	1.0	66.4 (± 0.6)
500.0	1.0	40.0 (± 1.2)
1000.0	1.0	17.5 (± 3.5)

Table 3.10. Effect of sorbent amount on sorption of 1000 mL, 1.0 mg L^{-1} V(V) by 3-APTES-modified silica (sorption time of 30.0 min., pH of 2.0, 25°C, n=3).

Sample Volume (mL)	Sorbent Amount (g)	V(V) concentration (mg L^{-1})	% Sorption
1000.0	0.1	1.0	17.5 (±2.6)
1000.0	0.2	1.0	37.6 (±3.5)
1000.0	0.5	1.0	81.3 (±1.5)
1000.0	1.0	1.0	91.3 (±2.5)
1000.0	2.0	1.0	98.8 (±3.5)

3.6.2.9. Desorption from the Sorbent

The efficiency/feasibility of various eluents for the desorption of V(V) was investigated for as explained in section 2.8.1.9. At first, various solutions; namely, HCl, HNO_3, H_2SO_4, H_3PO_4, and $H_2C_2O_4$ were used for the elution of the previously sorbed vanadium ions from 3-APTES-modified silica gel by the batch method. As can be seen from Table 3.11, these eluents did not give a quantitative desorption. In fact, elution using high concentrations of acids and bases may not be suitable for organically modified silica gel due to the great possibility of protonation of active donor centers in the ligand. Indeed, acid or base treatment may increase the chance of hydrolysis of the bound complexing agent. Use of other strong complexing agents, ethylenediaminetetraacetic acid (EDTA) and 1,2-diaminocyclohexanetetraacetic acid (CDTA), to back extract the metal ion from the sorbent were also tried. Quantitative results were also not obtained. Subsequently, eluents namely KI, KSCN, thiourea, L-cysteine, citric acid, ascorbic acid, tartaric acid, thioglycolic acid, and KOH were tried and 0.5 M thiourea (in 0.2 M HCl) gave efficient desorption (93(±3)%) while the elution percentage for the others changed between 10-80%. Therefore, 0.5 M thiourea was used as the eluent in further studies.

3.6.2.10. Sorption Isotherm Models

A comparison of adsorption models namely Freundlich, Dubinin-Radushkevich and Langmuir was made for the sorption of V(V) by 3-APTES-modified silica at pH 2.0 at 25^{o}C. The Langmuir isotherm appeared to be linear within the whole concentration range with high correlation coefficient, which shows that the Langmuir isotherm could efficiently describe the monolayer adsorption process. Linear forms of isotherms are illustrated in Figure 3.33, Figure 3.34 and Figure 3.35 respectively. These linear equations were also used in the calculations of coefficients summarized in Table 3.12.

3.6.2.11. Interference Studies

In order to perform a detailed interference study, the initial experiments were planned to understand the sorption behavior of 3-APTES-modified silica towards several metal ions at pH 2.0 where V(IV) and V(V) can be separated. The percent sorption values of the chosen species by 3-APTES-modified silica at the optimized conditions for V(V) can be found in Table 3.13. The sorbent showed no significant selectivity towards any of the metal ions. Another interference study was conducted with the species namely, As(III), As(V), Fe(II), Fe(III), Mo(VI), Sb(III), Sb(V), Se(IV), Se(VI), Te(IV) and Te(VI) as described previously. The results given in Table 3.14 show that 3-APTES-modified silica exhibits significant sorption towards the species, especially at low ion concentrations, except for Fe(II), Sb(V), Te(IV) and As(V). The oxyanions of Mo(VI), Sb(III) and Se(VI) were almost completely removed from the solutions at all concentrations tested. This finding, i.e. the selectivity of the novel sorbent to Mo(VI), Sb(III) and Se(VI) can offer a new potential of the novel sorbent for the selective determination of these ions in addition to V(IV)/V(V) speciation. Based on the previous results, the interference effects of As(III), Fe(III), Mo(VI), Sb(III), Se(IV), Se(VI) and Te(IV) on V(V) sorption were investigated in such a way that various concentrations of the ions were prepared and spiked with V(V). Any effect causing at least 15% decrease in the sorption was considered as interference. Table 3.15 shows the effect of each species on sorption as a function of V(V) concentration. As given in the table, all three investigated concentrations of Te(IV), Se(IV), Se(VI) and Fe(III) showed interference by decreasing the percent sorption of V(V). A remarkable interference effect of all the ions tested (decrease in >50 percent sorption) was seen for low (10.0 μg L^{-1}) vanadate concentration. For higher (100.0 and 1000.0 μg L^{-1}) vanadate concentrations, Te(VI), Se(IV), Se(VI) and Fe(III) still showed interferences whereas no interference was observed from Sb(III), As(III) and Mo(VI) ions. Moreover, no enhancement of V(V) signal was observed.

Table 3.11. Percent recovery of the proposed methodology (initial and final volumes, 10.0 mL, 1.0 mgL^{-1} V(V) concentration, , 50.0 mg sorbent, pH 2.0, 25^{o}C solution temperature and 30.0 min. sorption/desorption time, n=3).

Eluent	**% Recovery**
0.1 M HCl	44 (± 2)
0.1 M HNO_3	35 (± 3)
0.1 M H_3PO_4	18 (± 1)
0.1 M H_2SO_4	51 (± 1)
1 M HCl	55 (± 5)
1 M HNO_3	46 (± 2)
1 M H_3PO_4	38 (± 3)
1 M H_2SO_4	48 (± 3)
6 M HCl	64 (± 3)
6 M HNO_3	62 (± 7)
6 M H_3PO_4	60 (± 4)
6 M H_2SO_4	41(± 3)
0.1 M EDTA (pH=8.0)	59 (± 3)
0.3 M EDTA (pH=8.0)	62 (±1)
0.5 M $H_2C_2O_4$	52 (±8)
1 M NaOH	25 (± 1)
1 % Ascorbic Acid	57 (± 6)
1 M Tartaric Acid	15 (± 2)
1 M Citric Acid	10 (± 1)
0.5 M L-cysteine	50 (± 5)
1 M TGA	53 (± 7)
3 M TGA	79 (± 8)
NH_3-NH_4 Buffer	62 (± 4)
6 M HCl	65 (± 10)
5% AA	53 (± 3)
3 M KOH	34 (± 1)
0.5 M KI	~ 0
2 M KSCN	~ 0
0.5 M Thiourea (in 0.2 M HCl)	93 (± 3)

Table 3.12. Summary of models coefficients.

Adsorption model	Parameter	
Langmuir	R^2	0.9896
	Q_{max} (mmol g^{-1})	3.02
	B (L $mmol^{-1}$)	0.88
Freundlich	R^2	0.884
	K_F (mg g^{-1})	1.32
	1/n	0.44
Dubinin-Radushkevich	R^2	0.933
	B (mol^2 kJ^{-2})	$5*10^{-9}$
	q_s	0.007
	E (kJ/mol)	10

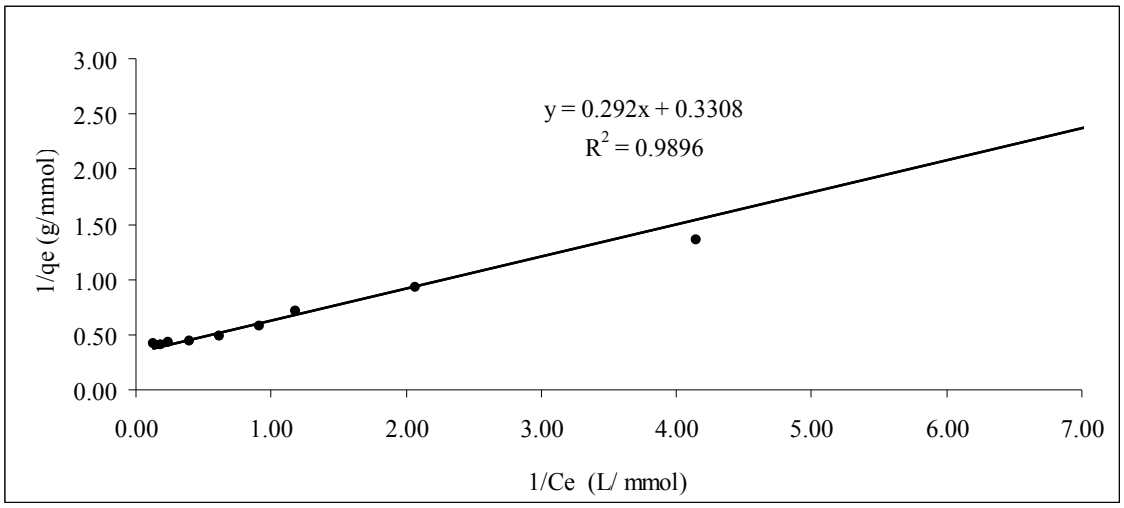

Figure 3.33. Linear fit of Langmuir model for vanadate sorption by 3-APTES-modified silica.

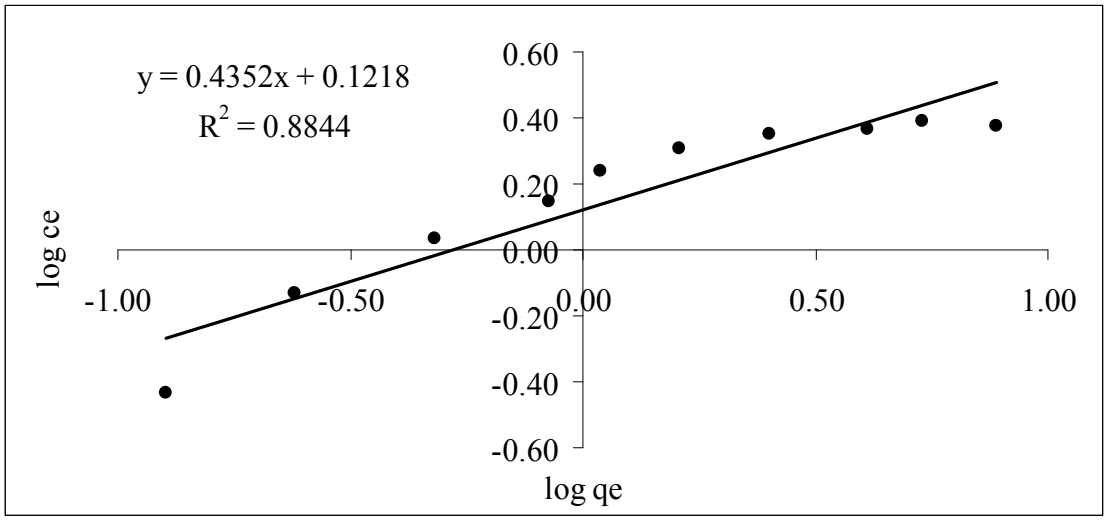

Figure 3.34. Linear fit of Freundlich model for vanadate sorption by 3-APTES-modified silica.

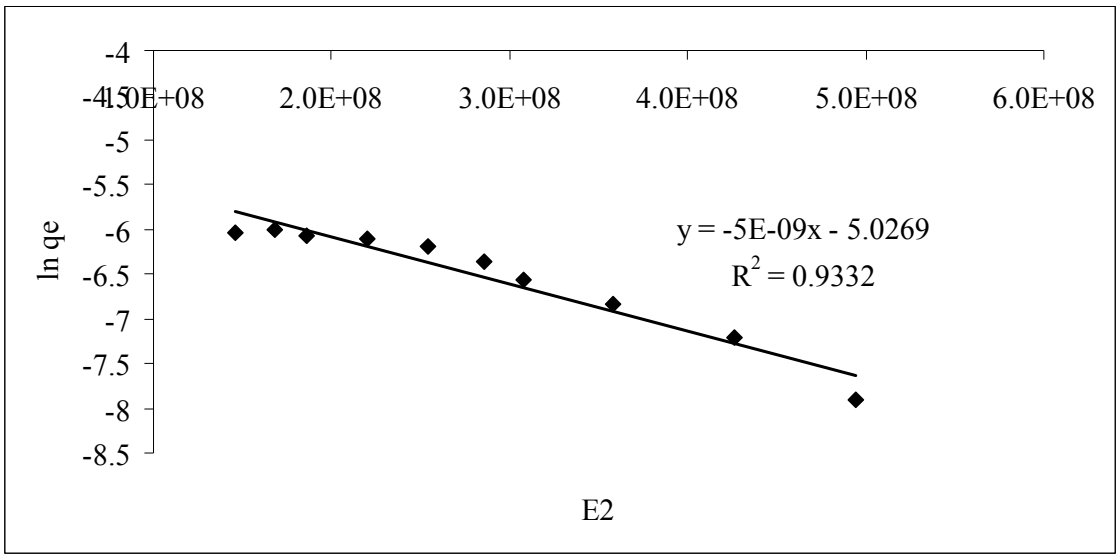

Figure 3.35. Linear fit of D-R model for vanadate sorption by 3-APTES-modified silica.

Table 3.13. Percent sorption of chosen species by 3-APTES-modified silica at the optimized conditions for V(V) (50.0 mg sorbent, pH 2.0, 25°C solution temperature and 30 min. sorption time, n=3).

Element	% Sorption
Al	< 1.0
B	< 5.0
Ba	< 1.0
Cd	< 1.0
Co	< 1.0
Cr	< 1.0
Cu	< 1.0
Li	< 1.0
Mg	< 1.0
Mn	< 1.0
Ni	< 1.0
Pb	< 1.0
Sr	< 1.0
Tl	< 1.0
Zn	< 1.0

Consequently, the interference effect of As(III), Fe(III), Mo(VI), Sb(III), Se(IV), Se(VI), Te(IV) on V(V) sorption was investigated in such a way that various concentrations of the ions were prepared and spiked with V(V). Table 3.15 demonstrated the effect of each species on sorption as a function of V(V) concentration.

Any effect causing at least 15 % decrease in the sorption was considered as interference. For all concentrations of Te(IV), Se(IV), Se(VI) and Fe(III) shows an interference effect by decreasing the percent sorption of V(V) in the solution. A remarkable interference effect (decrease in percent sorption > 50) is examined when lower concentrations of vanadate (10.0 μg L^{-1}) are considered. If the case for Sb(III), As(III) and Mo(VI) is investigated, a similar situation is observed for low vanadate concentrations where sorption is decreased >40 %. No interference effect was observed for 100.0 μg L^{-1} and 100.0 μg L^{-1} V(V) concentrations in higher Mo(VI), As (III) and Sb(III) concentrations. This may be due to the stabilization of vanadate species by these ions. Moreover, no enhancement of V(V) signal was observed.

Table 3.14. Percent sorption of chosen species by 3-APTES-modified silica at the optimized conditions for V(V) (50.0 mg sorbent, pH 2.0, 25.0°C solution temperature and 30.0 min. sorption time, n=3).

Element	% Sorption		
	10.0 μg L^{-1}	100.0 μg L^{-1}	1000.0 μg L^{-1}
As(III)	61.6 (±2.7)	20.7 (±3.9)	17.0 (±3.8)
As(V)	11.7(±2.7)	~ 0	~ 0
Fe(II)	~ 0	~ 0	~ 0
Fe(III)	88.3 (± 6.2)	58.4 (±5.3)	44.8 (±4.5)
Mo(VI)	100.0 (± 0.5)	100.0 (±0.2)	100.0 (±0.5)
Sb(III)	100.0 (± 0.5)	99.5 (±) 0.3	88.8 (±0.2)
Sb(V)	~ 0	~ 0	~ 0
Se(IV)	100.0 (±0.2)	62.0 (±6.8)	63.9 (± 5.9)
Se(VI)	90.1 (±0.2)	92.3 (±0.2)	95.5 (±0.1)
Te(IV)	~ 0	~ 0	~ 0
Te(VI)	82.0 (±4.9)	21.1 (±1.3)	7.8 (±1.4)

3.6.2.12 Method Validation and Spike Recovery Experiments

The proposed method was validated via spike recovery tests due to the unavailability of a proper certified reference material for vanadium speciation. Spiking was applied to four different types of water; namely, ultra pure, bottled-drinking, tap, and sea water. Spiked samples were subjected to the same sorption/elution cycles as previously. The results given in Table 3.16 show that the proposed methodology works efficiently for ultra pure, bottled-drinking and tap water samples with respective percent recovery values of (>87%). Although not quantitative, a relatively high recovery (>70%) obtained for sea water can also be considered as an indication of the efficiency of the proposed methodology even in a heavy matrix sample.

Table 3.15. Summary of interference study; N: no interference, I: interference (signal reduction >15%).

Element	10.0 μg L^{-1} V(V)			100.0 μg L^{-1} V(V)		1000.0 μg L^{-1} V(V)
	10.0 μg L^{-1}	100.0 μg L^{-1}	1000.0 μg L^{-1}	100.0 μg L^{-1}	1000.0 μg L^{-1}	1000.0 μg L^{-1}
Te(VI)	I	I	I	I	I	I
Se(IV)	I	I	I	I	I	I
Se(VI)	I	I	I	I	I	I
Sb(III)	I	I	I	N	N	N
As(III)	I	I	I	N	N	N
Mo(VI)	I	I	I	N	N	N
Fe(III)	I	I	I	I	I	I

3.6.3. Studies with Trypsin-Immobilized Silica

3.6.3.1. Sorption by the Intermediate Products During Immobilization Procedure

The sorption behavior of each of intermediate products in the synthesis procedure was investigated as explained in section 2.8.2.2. As can be seen from the graphs, (Figure 3.36 and 3.37) is noticeable that the sorption percentage of the sorbents (silica and H_2O_2 treated silica) increases gradually as the synthesis proceeds. It indicates the applicability of trypsin-immobilized silica for vanadium sorption as well as the intermediate products in the overall synthesis. As can be seen from the figures the sorption percentage decreases in the second step (glutaraldehyde addition after NH_2 modification to the silica surface). The reason for this situation is probably the steric effect of the surface which prevents the approach of the vanadyl or vanadate species to the surface. In addition, although the first step (NH_2 attachment to the silica surface) is sufficient for the speciation of V(IV) and V(V), if the removal of total vanadium is required, trypsin immobilization becomes necessary.

Table 3.16. Spike recovery results for vanadate ion with ultra-pure, bottled-drinking, tap and sea water samples after desorption from 3-APTES-modified silica (50.0 mg sorbent, pH 2.0, 25.0^{o}C solution temperature and 30.0 min. sorption time; eluent: 0.5 M thiourea, n=3).

Sample	V(V) spike ($\mu g\ L^{-1}$)	V(V) found ($\mu g\ L^{-1}$)	Recovery %
Ultrapure Water	10.0	10.8 (± 1.3)	108 (± 13)
	100.0	90.0 (± 4.5)	90 (± 5)
	1000.0	930 (± 3.0)	93 (± 0.3)
Drinking Water	10.0	10.8 (± 0.3)	108 (± 3)
	100.0	89.4 (± 3.1)	89 (± 3)
	1000.0	960 (± 5.0)	96 (± 0.5)
Tap Water	10.0	9.9 (± 1.2)	99 (± 1)
	100.0	91.8 (± 4.0)	92 (± 4)
	1000.0	870 (± 3)	87 (± 0.3)
Sea Water	10.0	9.9 (± 0.4)	99 (± 4)
	100.0	93.3 (± 1.1)	93 (± 1.1)
	1000.0	730 (± 3)	73 (± 0.3)

3.6.3.2. Effect of pH

Solution pH should be taken into account as one of the most important parameters that affect sorption since the speciation of the analytes may strongly depend on the pH of the solution. It also affects the chemistry of the binding site of the sorbent. Therefore the ability of the trypsin-immobilized silica to retain vanadium from aqueous solution was investigated as a function of pH. In addition to the individual sorption of V(IV) and V(V) species examined, sorption was also investigated for the solution that contains both of the species together. The results can be found in Figure 3.38 and Figure 3.39. As can be seen from the figures, for both of the sorbents synthesized, pH is one of

the key parameters for sorption of vanadium species from the solution. The sorption percentage of the sorbents is almost constant in the pH range 4.0 to 8.0. Under these conditions, V(V) is probably retained as $H_2VO_4^-$ as the predominant species in this pH region. Moreover, V(IV) is possibly oxidized to V(V) by atmospheric oxygen. As a result, the same trend for the sorption of V(IV) is observed as in the case of V(V). Therefore the consequent parts of this work will focus on the stabilization of V(IV) species under oxic conditions.

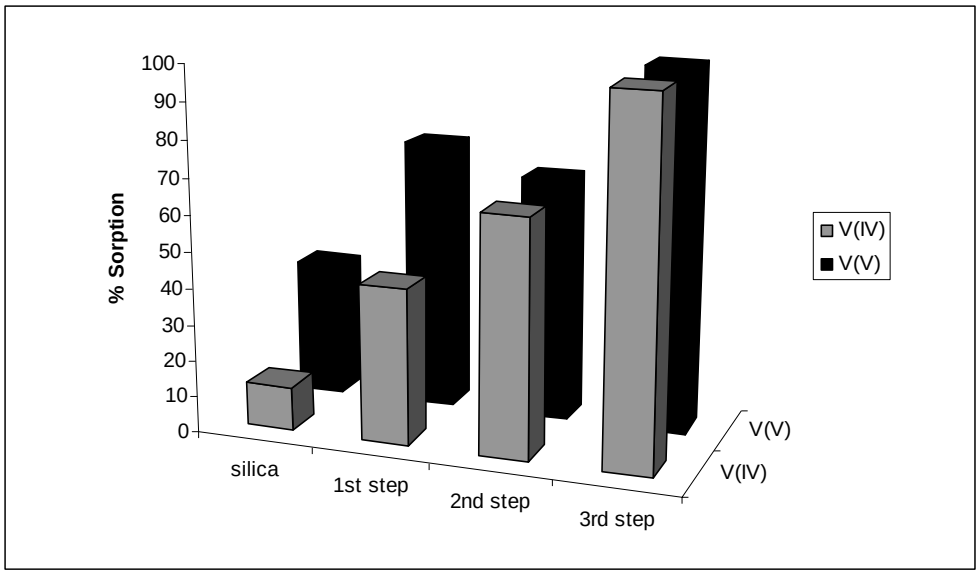

Figure 3.36. Sorption of V(IV) and V(V) towards trypsin-immobilized silica through steps of synthesis (1.0 mg L^{-1} V(IV) or V(V), 10.0 mg sorbent, pH 6.0, 25.0^oC solution temperature and 30.0 min. sorption time, n=3).

Under acidic conditions, V(IV) and V(V) are retained less by the sorbents depending on the distribution of the species present in the solution. By considering the speciation graphs (Figure 3.15 and 3.16) it can be concluded that V(IV) and V(V) ions are not retained much as VO^{2+} and VO_2^+ species, respectively. Then, as the pH increases beyond 8.0, sorption decreases considerably. This is possibly due to the change of the chemical form of the vanadium species to the anion HVO_4^{2-}. In the case of sorption study with both of the vanadium together, it is clearly seen that the percent sorption has decreased when compared with the individual sorption of V(IV) or V(V) species. This is probably because of the fact that both of the species are retained together on the sorbents which lowers the available responsible surface for sorption.

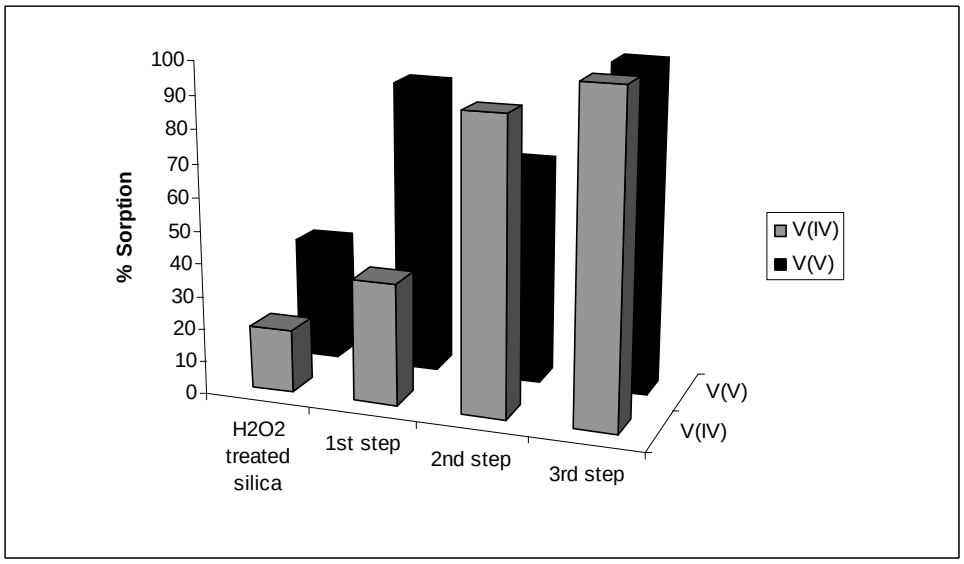

Figure 3.37. Sorption of V(IV) and V(V) towards trypsin-immobilized H_2O_2 treated silica through steps of synthesis (1.0 mg L^{-1} V(IV) or V(V), 10.0 mg sorbent, pH 6.0, 25.0^{o}C solution temperature and 30.0 min. sorption time, n=3).

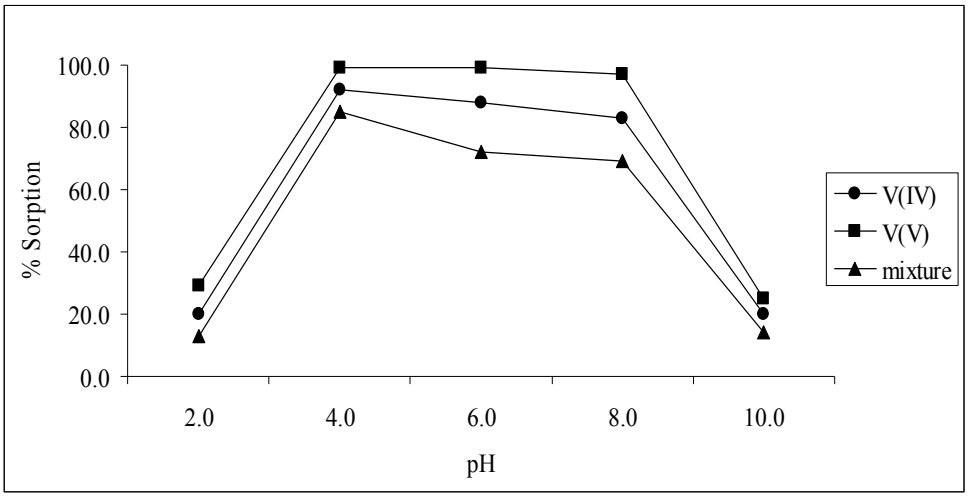

Figure 3.38. Effect of pH on the sorption of V(IV) and V(V) towards trypsin-immobilized silica (1.0 mg L^{-1} V(V) or V(IV) solution, 10.0 mL sample volume, 30 min. shaking time, 10.0 mg sorbent. at 25^{o}C sorption temperature, n=3).

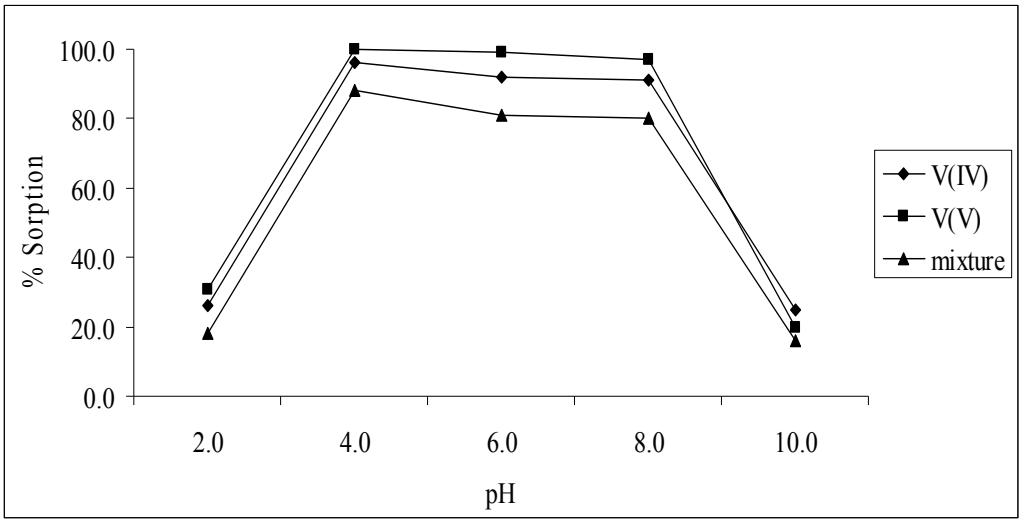

Figure 3.39. Effect of pH on the sorption of V(IV) and V(V) towards trypsin-immobilized H_2O_2 treated silica (1.0 mg L^{-1} V(V) or V(IV) solution, 10.0 mL sample volume, 30 min. shaking time, 10.0 mg sorbent. at 25^oC sorption temperature, n=3).

3.6.3.3. Sorption Using Buffers

Buffer solutions were used to control the pH during sorption keeping in mind that the ions constituting the buffers may interact with both the analyte species and the sorbent surfaces and thus affect sorption. In order to understand this effect, the pH adjustment was done by two ways and the sorption percentage of the sorbents towards V(IV) and V(V) was investigated. In the first case, 1.0 mg L^{-1} V(IV) or V(V) solutions were prepared and pH was adjusted with dilute NH_3 or HNO_3, whereas in the second, vanadium solutions were prepared in buffers. The results are indicated in Figures 3.40 and 3.41. A more detailed study is performed with buffers in 0.5 intervals to understand the exact behavior of vanadium species in buffered solutions (Figure 3.42).

When the sorption capacity of the sorbent towards vanadium species in buffers is compared with the vanadium solutions (pH adjusted but not buffered), it is observed that the sorption percentage decreases. This may be due to the reduction of capacity of the sorbent towards vanadium species in the presence of ions like Na^+, K^+, Cl^- and $H_2PO_4^-$.

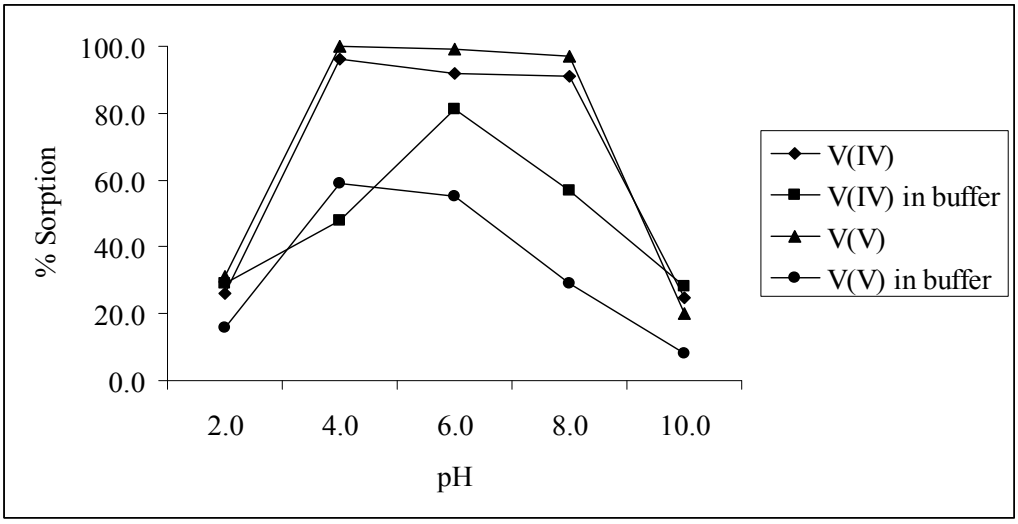

Figure 3.40. Effect of pH on the sorption of V(IV) and V(V) towards trypsin-immobilized silica (1.0 mg L^{-1} V(V) or V(IV) solution, 10.0 mL sample volume, 30 min. shaking time, 10.0 mg sorbent. at 25^{o}C sorption temperature, n=3).

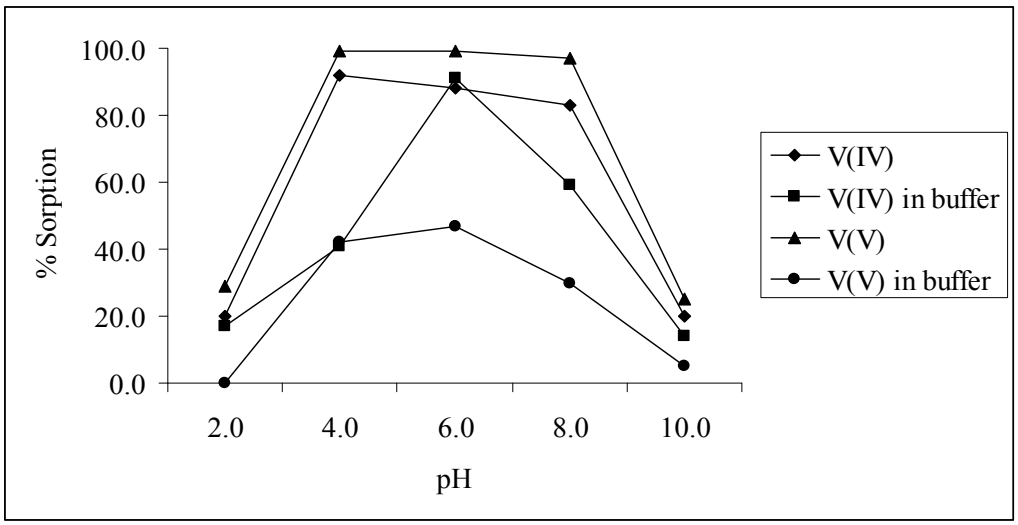

Figure 3.41. Effect of pH on the sorption of V(IV) and V(V) towards trypsin-immobilized H_2O_2 treated silica (1.0 mg L^{-1} V(V) or V(IV) solution, 10.0 mL sample volume, 30 min. shaking time, 10.0 mg sorbent. at 25^{o}C sorption temperature, n=3).

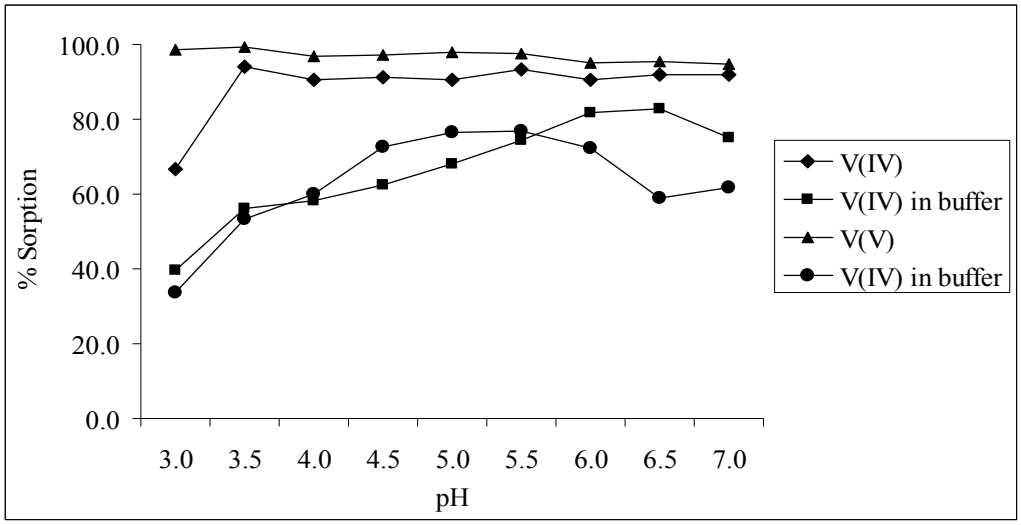

Figure 3.42. Effect of pH on the sorption of V(IV) and V(V) towards trypsin-immobilized silica (1.0 mgL^{-1} V(V) or V(IV) solution, 10.0 mL sample volume, 30 min. shaking time, 10.0 mg sorbent. at 25^{o}C sorption temperature, n=3).

3.6.3.4. Sorption Studies After Ascorbic Acid Reduction

In order to understand whether ascorbic acid disturbs the possible binding sites of the immobilized solid, 10.0 mg of the newly synthesized solid was shaken in ascorbic acid for 30.0 minutes in an orbital shaker. The sorbent is filtered and dried in an oven at 50.0 ^{o}C for 30.0 minutes. Subsequently, this sorbent is used to determine percent sorption of 1.0 mg L^{-1} V(IV) and V(V) to understand if there is any significant change on the sorption. Table 3.17 demonstrates the results for the ascorbic acid pretreatment on sorption. As seen from the results, percent sorption for the vanadium species is nearly the same for both of the sorbents. It has no considerable effect meaning that it does not damage the active sites of the newly synthesized sorbents.

As a subsequent experiment, trypsin-immobilized solid was utilized for the determination of percent sorption of 1.0 mg L^{-1} V(IV) and V(V). The reaction conditions were as follows; 10.0 mg sorbent, 10.0 mL sample volume and 30.0 minutes shaking time. As the results of this experiment given in Table 3.18 are considered, it can be said that sorption has decreased in the presence of ascorbic acid.

Table 3.17. Effect of ascorbic acid pretreatment on sorption (10.0 mg sorbent, 10.0 mL sample volume, 1.0 mg L^{-1} V(IV) or V(V), 1 % (w/w) ascorbic acid, pH=4.0, n=3).

Sorbent	Ascorbic Acid Pretreatment	% Sorption	
		V(IV)	V(V)
Trypsin-immobilized silica	no	83.3 ± 1.6	89.3 ± 0.3
Trypsin-immobilized silica	yes	93.6 ± 0.8	97.6 ± 0.8
Trypsin-immobilized H_2O_2 treated silica	no	94.7 ± 0.5	95.5 ± 0.6
Trypsin-immobilized H_2O_2 treated silica	yes	93.5 ± 4.3	96.6 ± 0.1

Table 3.18. Effect of ascorbic acid addition on V(IV) and V(V) sorption (1.0 mg L^{-1} V(IV) or V(V), 10.0 mL sample volume, 10.0 mg sorbent, pH=4.0, n=3).

Sorbent	Ascorbic Acid Addition	% Sorption	
		V(IV)	V(V)
Trypsin-immobilized silica	no	95.7 ± 0.1	88.8 ± 3.3
Trypsin-immobilized silica	yes	~0	~0
Trypsin-immobilized H_2O_2 treated silica	no	95.2 ± 0.7	95.4 ± 2.6
Trypsin-immobilized H_2O_2 treated silica	yes	~0	~0

3.6.3.5. Sorption Studies After $KBrO_3$ Oxidation

By using the stoichiometric amount that oxidizes vanadyl ions to vanadate by $KBrO_3$, the effect of oxidation on sorption was tried to be determined. For this purpose, 1.0 mg L^{-1} V(IV) was prepared at pH 2.0 into which an appropriate amount (4 $x10^{-3}$ M, 4 $x10^{-4}$ M, 4 $x10^{-5}$ M) of $KBrO_3$ is added. The sorption was performed both at pH 2.0 and 4.0. When Table 3.19 is examined, for pH 2.0, it can be said that $KBrO_3$ does not have a significant effect on sorption. Indeed, sorption is only enhanced approximately 10-25 % as $KBrO_3$ is added to the solutions. For pH 4.0, addition of $KBrO_3$ causes a small decrease in vanadium sorption.

When the case for V(V) is taken into account, $KBrO_3$ has again no significant effect on sorption (Table 3.20). If the results are compared for the ones for V(IV) it can be said that sorption has increased at pH 2.0. Normally, if V(IV) is oxidized to V(V) at this pH, it is expected to have approximately the same values for sorption. This may be due to having different species in the solution which indicates the importance of species determination within all pH values.

Table 3.19. Effect of addition different concentrations of $KBrO_3$ on V(IV) sorption (1.0 mg L^{-1} V(IV), 10.0 mL sample volume, 10.0 mg sorbent, n=3).

pH	$KBrO_3$ Concentration (M)	% Sorption
2.0	-	~0
	$4 x10^{-3}$	13.0 ± 4.0
	$4 x10^{-4}$	15.7 ± 2.3
	$4 x10^{-5}$	18.5 ± 8.6
4.0	-	98.9 ± 0.1
	$4 x10^{-3}$	93.6 ± 0.5
	$4 x10^{-4}$	94.9 ± 0.5
	$4 x10^{-5}$	94.5 ± 0.7

Table 3.20. Effect of addition of different concentrations of $KBrO_3$ on V(V) sorption (1.0 mg L^{-1} V(V), 10.0 mL sample volume , 10.0 mg sorbent, n=3).

pH	$KBrO_3$ Concentration (M)	% Sorption
2.0	-	33.7 ± 2.1
	4×10^{-3}	33.4 ± 0.4
4.0	-	97.9 ± 0.6
	4×10^{-3}	96.3 ± 0.7

3.6.3.6. Effect of Sorbent Amount

The effect of amount of trypsin-immobilized silica on the sorption of V(IV) and V(V) was studied at a pH of 4.0 with different sorbent doses varying from 5.0 to 200.0 mg at a fixed ion concentration of 1.0 mg L^{-1}. The results are depicted in Figures 3.43 and 3.44. It was observed that even with a very small amount of sorbent (5.0 mg) almost 100% sorption is obtained for both of the vanadium species. To be on the safe side, 10.0 mg of sorbent amount was selected in the subsequent studies.

3.6.3.7. Effect of Shaking Time

The sorption data for the uptake of 1.0 mg L^{-1} V(IV) and V(V) ions vs. shaking time at pH 4.0 for a fixed sorbent amount of 10.0 mg are shown in Figures 3.45 and 3.46. The results indicated that vanadium sorption is not much dependent on time since it was observed that a very rapid uptake of V(IV) and V(V) occurred even in 1.0 minute. To guarantee quantitative sorption, 30.0 minutes of shaking time was used in further studies.

3.6.3.8. Effect of Initial Concentration

To evaluate the effect of the initial metal ion concentration on the sorption behavior of V(IV) and V(V) on trypsin-immobilized silica with initial ion concentrations ranging from 0.01 to 1000.0 mg L^{-1} at pH 4.0 were prepared and into each of the solutions, a fixed amount of sorbent (10.0 mg) was added. Plots were prepared between the vanadium ion adsorbed versus the vanadium ion concentration (Figures 3.47 and 3.48). It is observed that for the same shaking time (30.0 minutes), the percentage sorption was higher for lower initial vanadium concentrations and decreased with increasing vanadium concentration possibly because the sorption capacity of the trypsin-immobilized silica is exceeded.

3.6.3.9. Effect of Sorption Temperature

The variation in temperature also affects the sorption of trace metal ions onto the solid surfaces. Therefore the sorption of V(IV) and V(V) ions onto trypsin-immobilized silica was undertaken of temperatures 25.0 and 60.0^{o}C under the optimized conditions. The change in the percent sorption of trypsin-immobilized silica as a function of temperature is given in Figures 3.49 and 3.50 which show a decreased sorption with an increase in temperature. This observation is associated with decrease in entropy indicating that the spontaneous sorption is enthalpy-driven. In addition, the summary of thermodynamic parameters is given in Table 3.21 and 3.22.

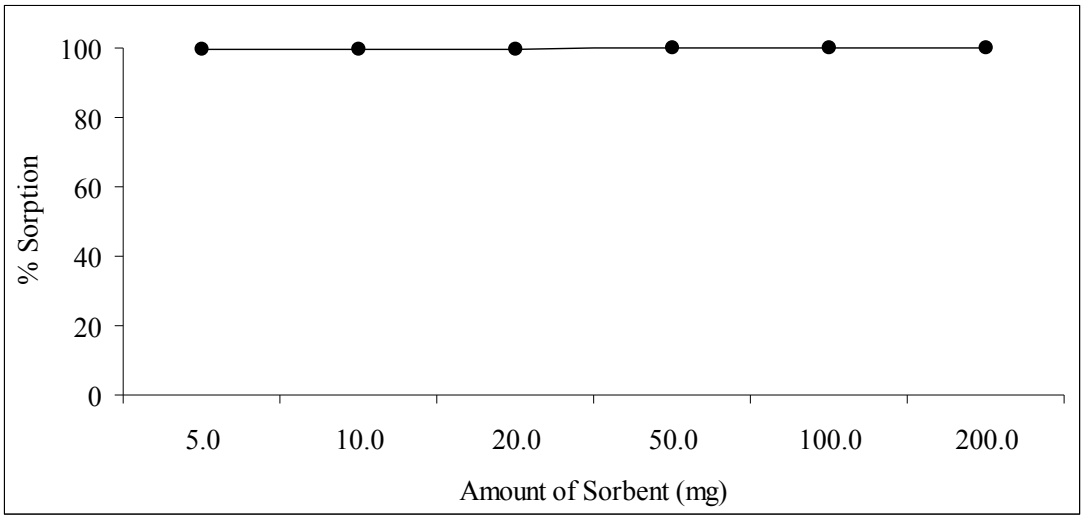

Figure 3.43. Effect of sorbent (trypsin-immobilized silica) amount on sorption (1.0 mg L^{-1} V(IV), pH 4.0, 10.0 mL solution volume, 30 min. shaking time, 25°C sorption temperature, n=2).

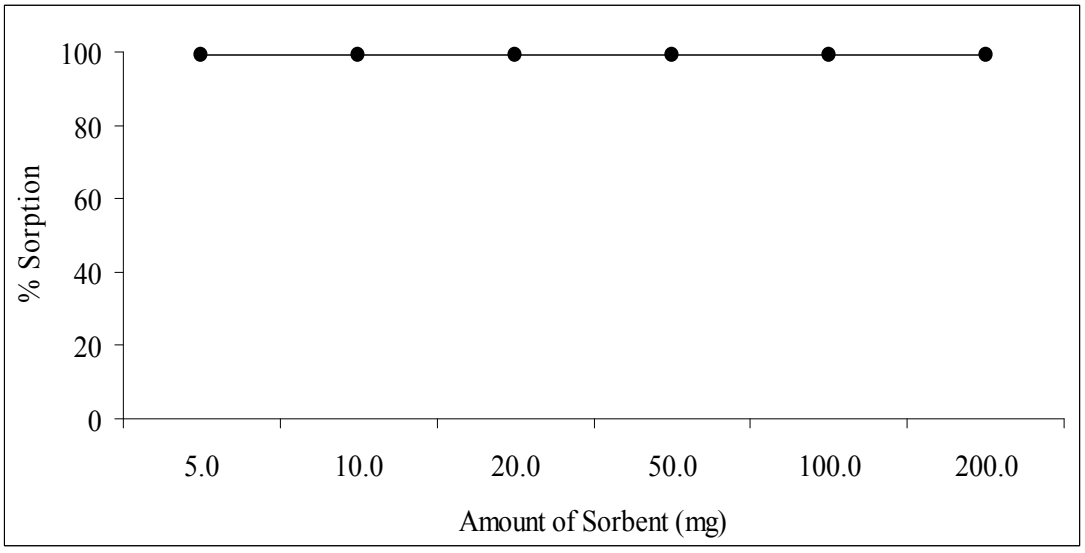

Figure 3.44. Effect of sorbent (trypsin-immobilized silica) amount on sorption (1.0 mg L^{-1} V(V), pH 4.0, 10.0 mL solution volume, 30 min. shaking time, 25°C sorption temperature, n=2).

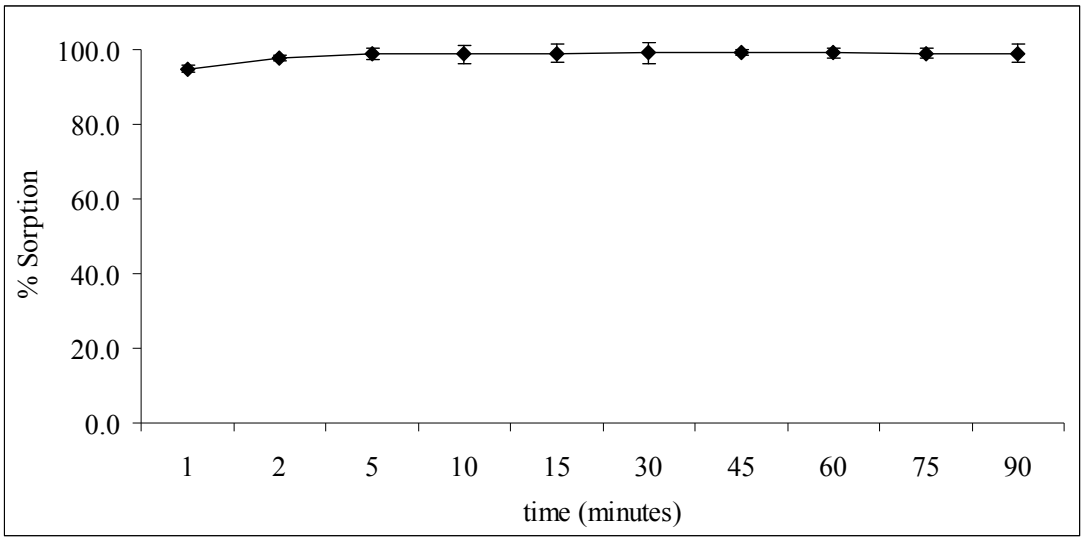

Figure 3.45. Effect of shaking time on sorption (1.0 mg L^{-1} V(IV), pH 4.0, 10.0 mL solution volume, 25°C sorption temperature, 10.0 mg sorbent, n=2).

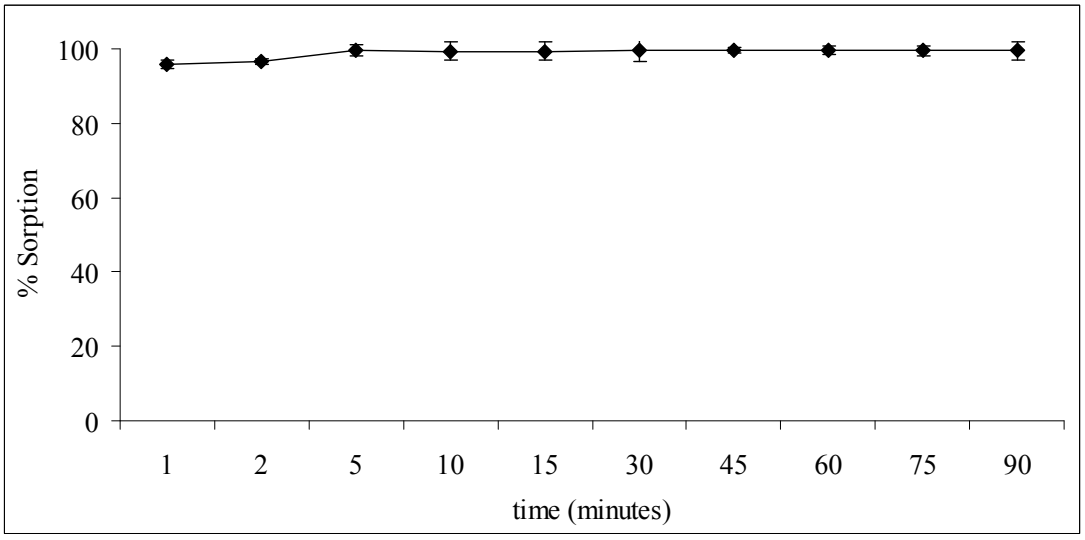

Figure 3.46. Effect of shaking time on sorption (1.0 mg L^{-1} V(V), pH 4.0, 10.0 mL solution volume, 25°C sorption temperature, 10.0 mg sorbent, n=2).

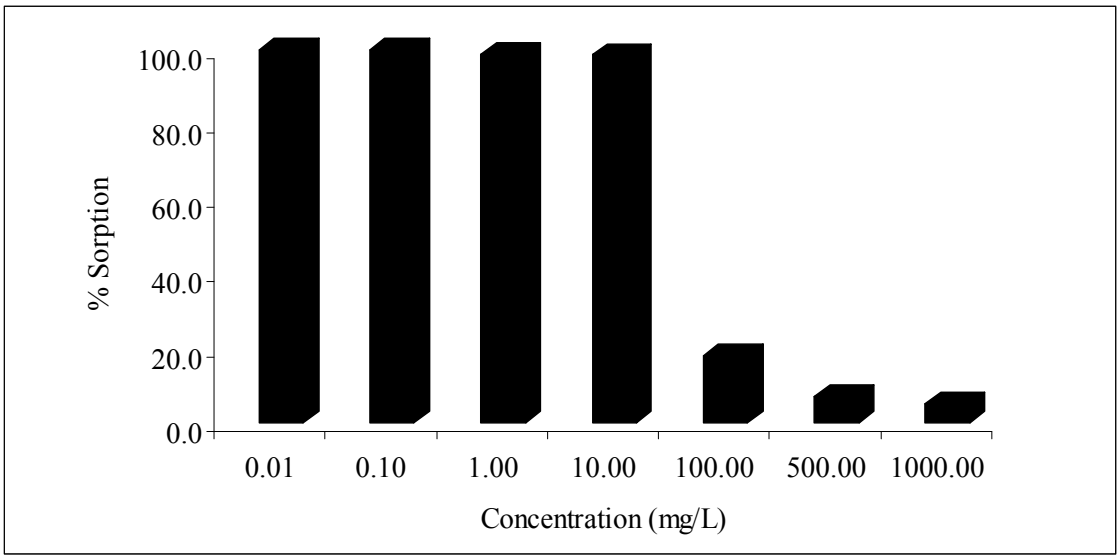

Figure 3.47. Effect of initial V(IV) concentration on the sorption of by trypsin-immobilized silica (10.0 mg sorbent, sorption time of 30.0 min., solution pH of 4.0, 10.0 mL of solution volume, n=2).

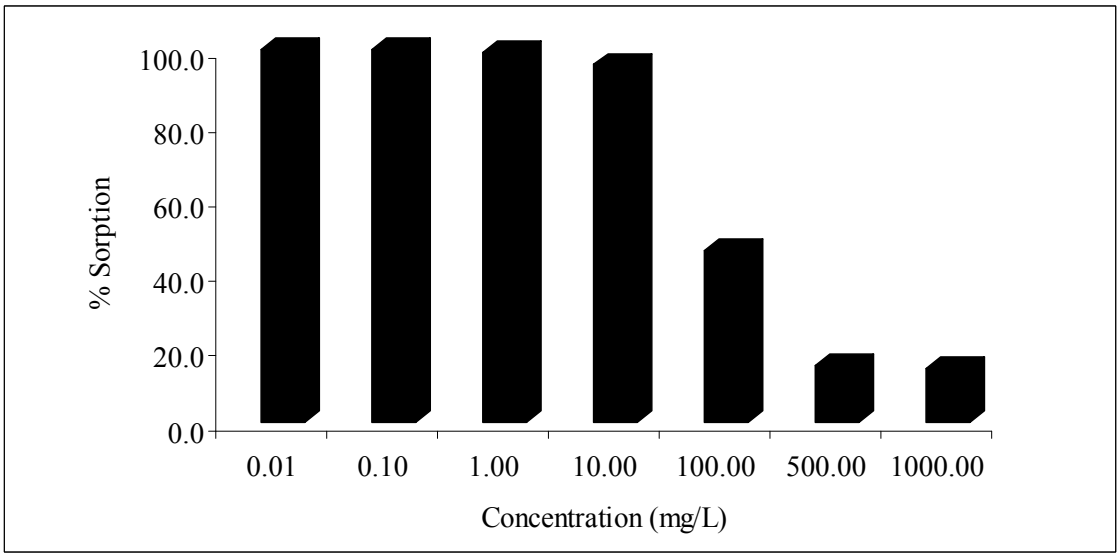

Figure 3.48. Effect of initial V(V) concentration on the sorption of by trypsin-immobilized silica (10.0 mg sorbent, sorption time of 30.0 min., solution pH of 4.0, 10.0 mL of solution volume, n=2).

Table 3.21. Thermodynamic parameters for the sorption of V(IV) by trypsin-immobilized silica.

Temperature (K)	ΔG^o (kJ mol^{-1})	ΔS^o (J $mol^{-1}K^{-1}$)	ΔH^o (kJ mol^{-1})
298	-13.8	-169	-64.4
333	-7.9	-169	

Table 3.22. Thermodynamic parameters for the sorption of V(V) by trypsin-immobilized silica.

Temperature (K)	ΔG^o (kJ mol^{-1})	ΔS^o (J $mol^{-1}K^{-1}$)	ΔH^o (kJ mol^{-1})
298	-12.4	-180	-66.2
333	-6.9	-178	

3.6.3.10. Effect of Sample Volume

In order to explore the possibility of concentrating low concentration of analytes from large volumes, the effect of sample volume on the retention of V(IV) and V(V) was also investigated. As mentioned in the section 3.6.2.8, two different routes was followed to understand the effect of sample volume. In the first route, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mL sample volumes at a constant absolute amounts of V(IV) and V(V) (10.0 μg) were prepared. All the other parameters were kept constant at their respective optima. As can be seen from Table 3.23 almost quantitative recoveries (93-99%) were obtained even with a sample volume of 500.0 mL. This provides the use of the proposed methodology with relatively large sample volumes. On the other hand, almost 75 % percent sorption was obtained both for V(IV) and V(V) for 1000.0 mL of sample volume.

In the second route, sample volumes (10.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mL) were changed while keeping the V(IV) and V(V) concentration constant (absolute amount changes depending on the solution volume) at 1.0 mg L^{-1} (Table 3.24). As in the previous section, acceptable recoveries (> 90%) were obtained up to 1000.0 mL of sample volume.

Table 3.23. Effect of sample volume on sorption of V(IV) and V(V) by trypsin-immobilized silica (10.0 μg V(IV) or V(V), 100.0 of sorbent, sorption time of 30.0 min., pH of 4.0).

Sample Volume (mL)	Vanadium Concentration (mg L^{-1})	% V(IV) Sorption	% V(V) Sorption
10.0	1.00	99.6	99.2
25.0	0.40	98.1	97.9
50.0	0.20	97.3	98.7
100.0	0.10	97.3	96.7
250.0	0.04	97.3	92.9
500.0	0.02	95.0	93.1
1000.0	0.01	74.8	80.7

Table 3.24. Effect of sample volume on sorption of V(IV) and V(V) by trypsin-immobilized silica (1.0 mg L^{-1} V(V), pH of 4.0, 100.0 mg sorbent, sorption time of 30.0 min.).

Sample Volume (mL)	Vanadium Concentration (mg L^{-1})	% V(IV) Sorption	% V(V) Sorption
10.0	1.0	99.6	99.2
25.0	1.0	99.1	98.7
50.0	1.0	98.5	98.2
100.0	1.0	97.6	98.5
250.0	1.0	95.3	94.6
500.0	1.0	89.3	89.8
1000.0	1.0	86.2	71.0

3.6.3.11. Sorption Isotherm Models

The sorption data at 25°C have been subjected to different sorption isotherms namely Langmuir, Freundlich and D–R to assess sorption capacity of V(IV) and V(V) ions. The results of the V(IV) and V(V) sorption experiments are shown in Figures 3.51-3.56. It was found that all data were fitted with the Langmuir isotherm which indicates that maximum sorption occurs when the surface is covered by a monolayer of the sorbate. In addition summary of model coefficients are depicted in Table 3.25.

With the use of the data obtained in sorption isotherm experiments, the sorption capacities (maximum amount of vanadium sorbed per g of silica) of Duolite A-7 (a chelating resin with alkyl amine functional groups), nZVI, 3-APTES-modified silica, trypsin-immobilized silica and bifunctional 3-MPTMS and 3-APTES-modified silica were calculated (Table 3.26). As can be seen from the table, all the newly synthesized sorbents except bifunctionalized silica have high sorption capacity.

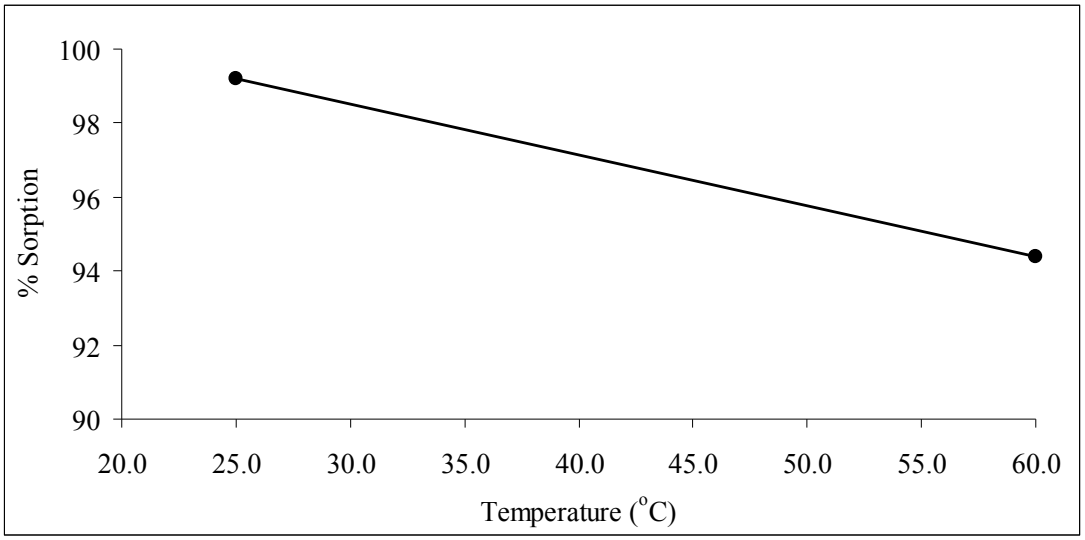

Figure 3.49. Effect of temperature on sorption for 10.0 mg trypsin-immobilized silica at pH 4.0 in 10.0 mL, 1.0 mg L^{-1} V(IV) solution (n=2).

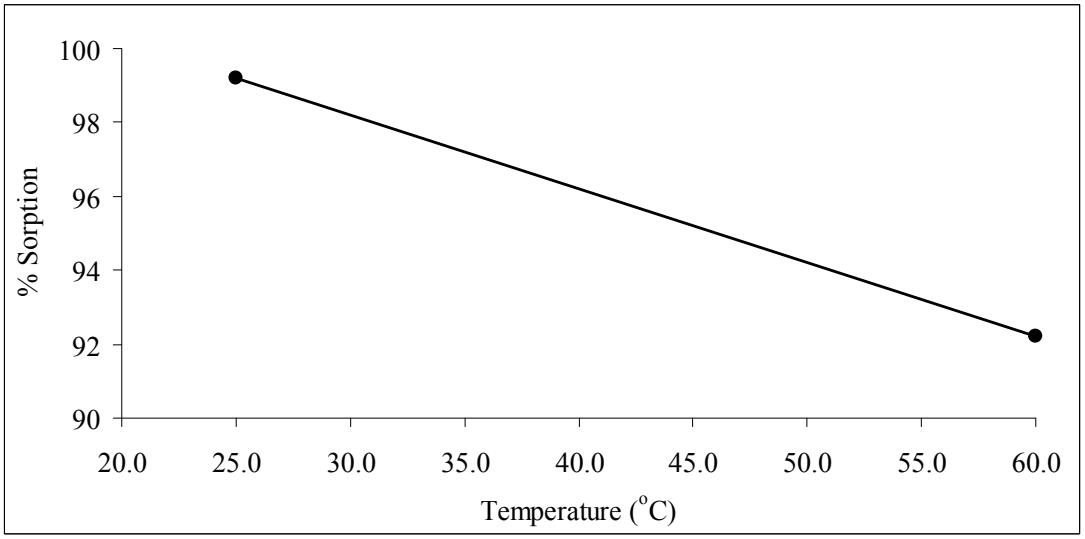

Figure 3.50. Effect of temperature on sorption for 10.0 mg trypsin-immobilized silica at pH 4.0 in 10.0 mL, 1.0 mg L^{-1} V(V) solution (n=2).

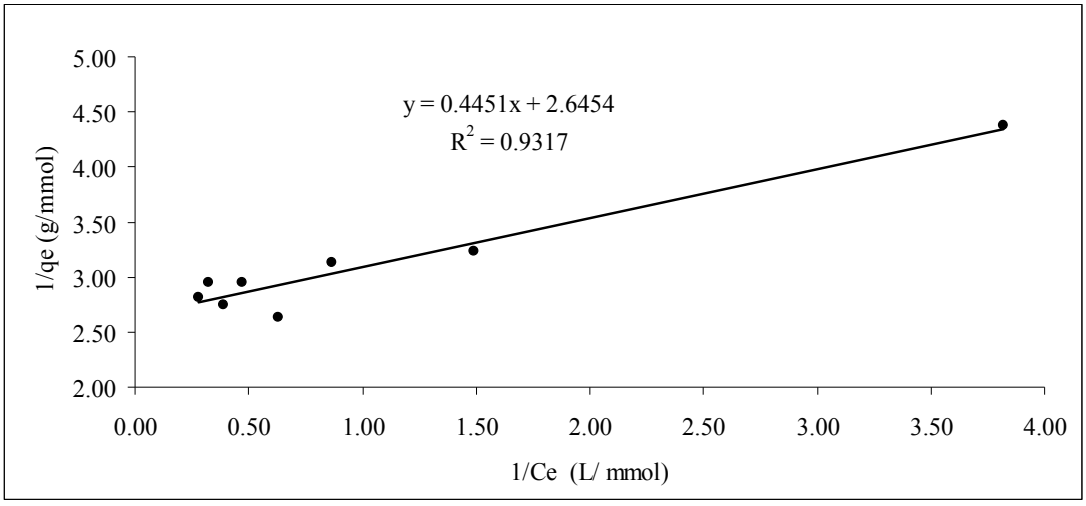

Figure 3.51. Linear fit of Langmuir model for vanadyl sorption by trypsin-immobilized silica.

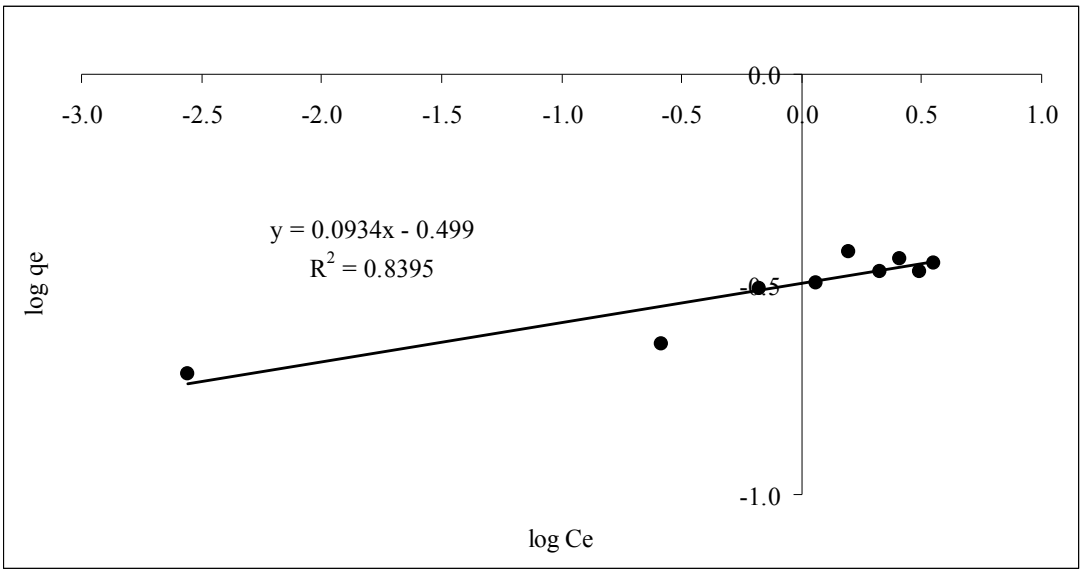

Figure 3.52. Linear fit of Freundlich model for vanadyl sorption by trypsin-immobilized silica.

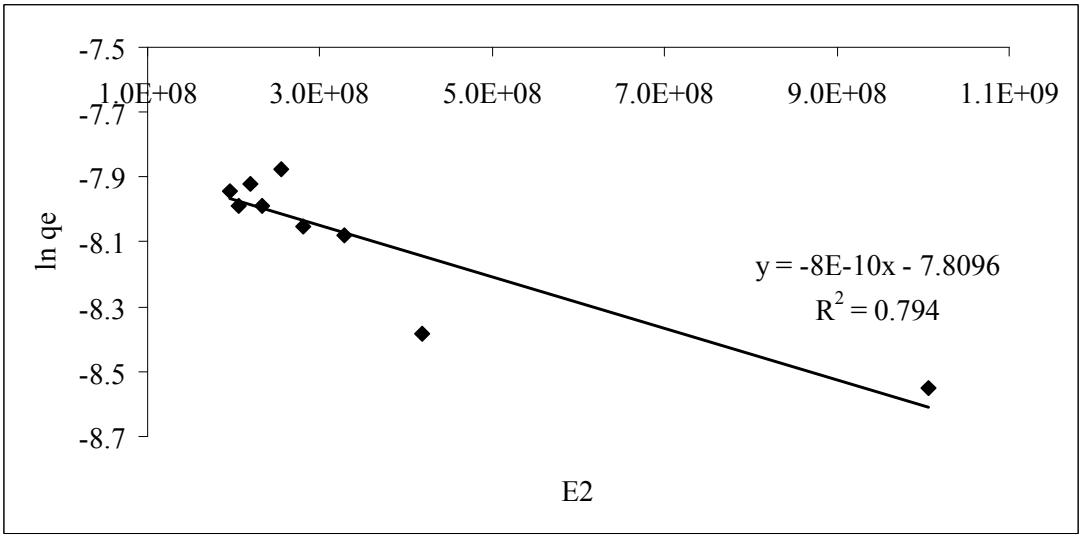

Figure 3.53. Linear fit of D-R model for vanadyl sorption by trypsin-immobilized silica.

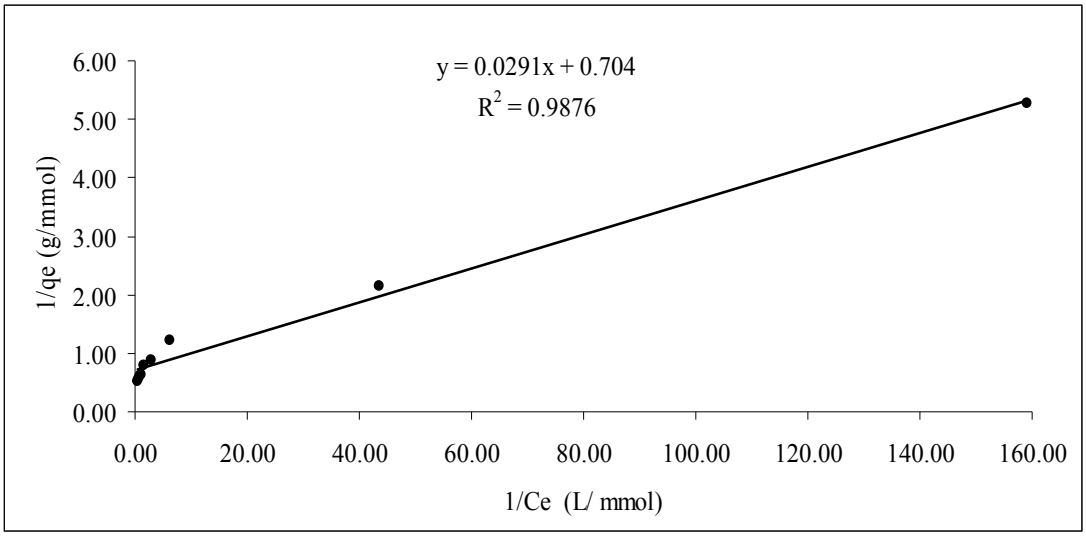

Figure 3.54. Linear fit of Langmuir model for vanadate sorption by trypsin-immobilized silica.

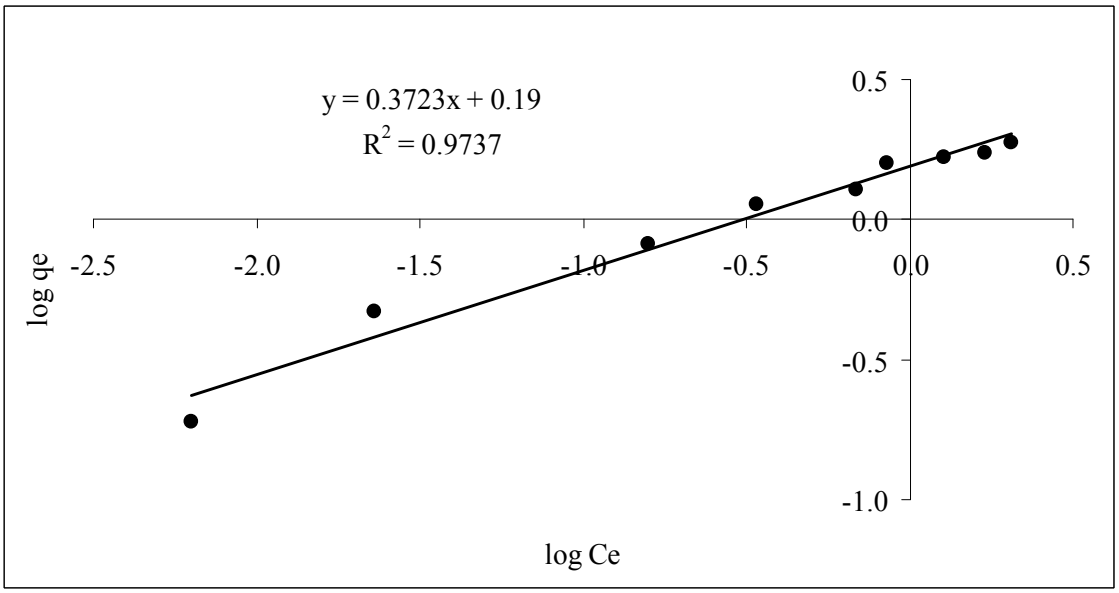

Figure 3.55. Linear fit of Freundlich model for vanadate sorption by trypsin-immobilized silica

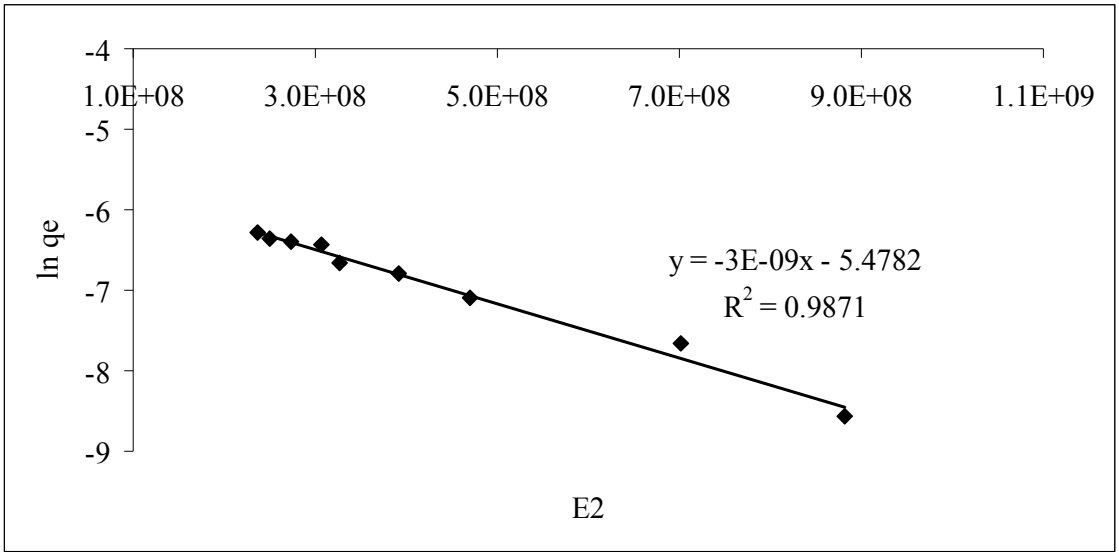

Figure 3.56. Linear fit of D-R model for vanadate sorption by trypsin-immobilized silica.

Table 3.25. Summary of models coefficients.

Adsorption model	Parameter	V(IV)	V(V)
Langmuir	R^2	0.9317	0.9876
	Q_{max}	0.38	1.42
	b	0.17	0.041
Freundlich	R^2	0.8395	0.9737
	K_F	0.32	1.55
	1/n	0.093	0.3723
Dubinin-Radushkevich	R^2	0.794	0.9871
	B	$8*10^{-10}$	$3*10^{-9}$
	q_s	0.0004	0.0042
	E (kJ/mol)	25	13

Table 3.26. Comparison of the sorption capacities of the sorbents.

Sorbent	Experimental	Langmuir	Freundlich
	mmol g^{-1}		
Duolite A-7	0.6	0.6	0.4
nZVI	3.2	2.7	1.4
3-MPTMS and 3-APTES-modified silica	0.5	0.8	0.2
3-APTES-modified silica	2.4	3.0	1.3
Trypsin-immobilized silica	1.8	1.4	1.6

CHAPTER 4

CONCLUSION

In this study, two novel sorbents, namely, trypsin-immobilized silica and 3-APTES-modified silica have been developed and used for the sorption of vanadium from waters. Initial studies were concentrated on the synthesis and characterization of the sorbents and the results have indicated the success of surface modification with the functional groups investigated. After characterization, the experiments were continued with the examination of the sorption performance of these substances towards vanadium species. In addition to the sorbents developed, sorption behavior of several commercial resins was also investigated for the same purpose.

Since the chemistry of vanadium is complicated by the existence of a variety of forms, depending both on the species concentration and the pH of the solution, an accurate and rapid oxidation and/or reduction process was investigated to clarify the actual forms of vanadium in natural waters and determine the total vanadium concentration in environmental matrices. L-Ascorbic acid was found to reduce aqueous V(V) to V(IV) and the oxidation of aqueous V(IV) was realized using $KBrO_3$.

In the case of trypsin-immobilized silica, the sorption percentage of the sorbents through V(IV) and V(V) is almost constant (>90%) within the pH range of 4.0 to 8.0 and under the experimental conditions applied. Quantitative uptake towards both vanadium species occurred even in 1 min which demonstrated the very fast kinetics of the sorption process. In addition, only 5 mg of the sorbent was sufficient for quantitative sorption of V(IV) and V(V). Almost quantitative recoveries (93-99%) were obtained even with a sample volume of 500.0 mL. The decrease in sorption at a high temperature is associated with the exothermic behavior and all data were fitted with the Langmuir isotherm. Although these results have demonstrated the possibility of using trypsin-immobilized silica for the sorption of both vanadium species, this sorbent was incapable of speciating V(IV) and V(V) in waters.

The sorption performance of various substances, namely, several sorbents prepared in our research group (nano sized zero-valent iron (nZVI), chitosan, bifunctionalized silica), and some commercial chelating and ion exchange resins

(Amberlite IR-120, Duolite GT-73, Duolite A-7, Duolite XAD-761 and Duolite C-467) were also investigated towards V(IV) and V(V) in aqueous solutions. It was shown that Amberlite IR-120, Duolite GT-73, Duolite A-7 and nZVI were able to retain both V(IV) and V(V) at the pH range 2.0-8.0. Duolite XAD-761 and Duolite C-467 did not show much affinity towards any of the vanadium species for pH values 2.0, 6.0 and 8.0. Amberlite IR-410 becomes selective when the pH>6.0. For chitosan, 80-100% sorption was obtained at only pH 4.0.

The other novel sorbent, 3-APTES-modified silica, has been shown to be an efficient material for the speciation of vanadium in waters. The optimum pH for the speciation of V(IV) and V(V) was found to be 2.0 where only V(V) is sorbed by the sorbent. A solution pH of 3.0 can be used if the total concentration of vanadium is to be determined. The concentration of V(IV) can then be calculated from the difference. Desorption from the sorbent was realized with 0.5 M thiourea in 0.2 M HCl. Three isotherm models, namely, Langmuir, Freundlich, and Dubinin-Radushkevich, were tested in order to reveal the concentration-dependence of the partitioning of vanadium species between the liquid and the solid phase (sorbent) at 25.0^{o}C. Langmuir model appeared to be the most appropriate within the whole concentration range with high correlation coefficient which indicates the monolayer sorption process. In addition, sorption of V(V) by 3-APTES-modified silica decreased with the increase in solution temperature and this exothermic behavior is associated with a decrease in system entropy. An interference effect is observed for Te(IV), Se(IV), Se(VI), and Fe(III) ions through decreasing the percent sorption of V(V). Additionally, Mo(VI), Sb(III) and Se(VI) were almost completely removed from the solutions at all tested concentrations. This promising sorption behavior of the novel sorbent towards the above-mentioned oxyanions has also boosted further studies in the authors' laboratory for selective determination of these ions. The validity of the method was checked via spike recovery experiments with four different types of water (ultra pure, bottled-drinking, tap, and sea water) and it was found that the method worked efficiently (>85% recovery) for ultra pure, bottled-drinking, and tap water samples. Although not being quantitative, a spike recovery of >70% obtained for sea water may also indicate the efficiency of the proposed methodology to samples having a relatively heavy matrix.

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