KINETICS STUDY OF THE ADSORPTION OF MUCIN ONTO TITANIUM SURFACE

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M.Sc. /SCIEN/04208/2006-07

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

IN PARTIAL FULFILMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ANALYTICAL CHEMISTRY

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA
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JANUARY, 2009

DECLARATION

I hereby declare that the work in this thesis entitled 'THE KINETICS STUDY OF THE ADSORPTION OF MUCIN ONTO TITANIUM SURFACE' has been performed and written by me in the Department of Chemistry, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for a degree at any university.

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CERTIFICATION

This thesis entitled **The Kinetics Study of the Adsorption of Mucin onto Titanium Surface** by AYENI, Modupe Bukola meets the regulations governing the award of the degree of Master of Science in Analytical Chemistry of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to all the people that have helped me during the course of this work and to all I will ever help in life.

ACKNOWLEDGEMENTS

I am forever grateful to the Great I am, the Almighty God, without whom I am nothing but with whom I have been made to tell a success story in this programme.

My sincere thanks go to my supervisors, Prof. J. A. Lori and Prof. E. J. Ekanem for their support and guidance throughout this research work. It is a great privilege and opportunity to have been schooled via them.

My profound gratitude goes to several people who have contributed to this project. I cannot but mention the Ayodeles, for their support and encouragement; Estella, who assisted with useful scientific discussions; my class mates for their co-operation, Mr. A. F. Smith and all other staff of Chemistry Department, for their assistance.

Lastly but very dear to my heart, I thank my darling mum, my relatives, friends and pastors for their prayers and support. Once again thank you very much, your children will equally tell success stories in their entire endeavours in life in Jesus Name (amen). God bless you all.

ABSTRACT

The kinetics of adsorption of mucin onto titanium surface was investigated. The amount of titanium, concentration of mucin solution, reaction temperature and pH were optimised by preliminary experiments as 0.02 mg, 0.1 mg/ml, 35 °C and 6.4, respectively. The amount of mucin adsorbed increased with time until equilibrium was attained after about 115 minutes with the maximum concentration of mucin adsorbed being 0.0603 mg/ml for Ca-Ti followed by that of unto K-Ti implying that the adsorption is not influenced by the presence of Ca^{2+} ions. Analysis of kinetic data obtained with Lagergren, Bhattacharya/Venkobacharya and Flick's models revealed that the adsorption is first order with respect to mucin molecules and that diffusion is the predominant mechanism for the adsorption.

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ABBREVATIONS

BSA	bovine serum albumin
Ca-Ti	calcium treated titanium
Ce	equilibrium concentration of mucin
Co	initial mucin concentration
Ct	concentration of mucin at time t
EDTA	ethyl diamine tetra acetic acid
et al	et alia; and others
g	gramme
g	relative centrifugal force
K-Ti	potassium treated titanium
K_p	intra-particle diffusion rate constant
K_{ad}	adsorption rate constant
ln	natural logarithm
Log	logarithm
M	molarity
mg	milligram
Min	minute
ml	milliliter
PBS	phosphate buffered saline
q_e	concentration of mucin at equilibrium
q_t	concentration of mucin at time t
R^2	coefficient of determination
$t^{1/2}$	square root of time t
Un-Ti	untreated titanium (control)

CHAPTER ONE

INTRODUCTION

1.1 Adsorption of Protein on Solid Substrates

The adsorption of dissolved proteins on solid substrates is a widespread phenomenon (Kasemo, 1998). For many applications, such as protein chromatography, the use of medical implants or preparation of solid- phase immunoassays for medical diagnostic tests, it is important to understand the driving forces for the protein adsorption process and the effect of adsorption on the protein conformation and activity. This may help to develop new biocompatible materials or coatings which prevent or favour protein adsorption (Horbett and Brash 1995).

Surface-protein adsorption is being studied with regard to the total amount and composition of adsorbed protein, the thickness and uniformity of the surface coverage, the kinetics of the protein- adsorption process, the preferential adsorption of certain protein species from complex mixtures such as desorption, organisation and arrangement of adsorbed protein and the denaturation of adsorbed proteins by interfacial forces (Brash, 1981). However, the relationship of a surface toward protein adsorption and the significance of the adsorbed protein layer in a material's thrombogenic nature still remain ill-defined and need to be studied (Andrade and Hlady, 2004).

It is now widely recognised that biocompatibility in a number of situations is controlled to a large extent by the initial interfacial reactions that occur when the biomaterial comes into contact with tissues, especially on the protein adsorption process that takes place at an interface. This is more significantly demonstrated with the thrombogenesis that occurs when a foreign material comes in contact with blood (Lee and Kim, 1974). A considerable amount of data is available concerning the role of protein adsorption in the initiation of clotting on

polymer surfaces (Brash, 1981). Adsorption is an important factor in the context of response of soft tissues to implanted polymers (Bagnash, 1977).

The adsorption of proteins onto titanium is important in a variety of oral biological events. Salivary proteins (e.g.mucin) are selectively adsorbed onto tooth surfaces forming the acquired enamel pellicle (Mayhall, 1970). The pellicle influences the initial attachment of microorganisms to the tooth surface and remains interposed between the enamel and dental (Wang *et al*., 1997). In research, proteins are receiving increased interest as potential inhibitors of enamel or dentine demineralisation (Diana *et al*., 1995).

1.2 Biomaterials' Application

Biomaterials are used to repair, restore or replace a damaged or diseased tissue. Biomaterials may be of natural origin, such as purified collagen, protein fibres, polysaccharides and treated tissues. They could also be synthetic biomaterials, such as metals, ceramics or polymers which are often called biomedical biomaterials to differentiate them from natural biomaterials (Yannas and Burke, 1980).

The earliest recorded use of biomaterial was nearly 4000 years ago, as sutures' for wound repair. Ancient Indians of about 2,500 years ago used hair, leather, cotton, animal sinew and tree bark as suture materials. The use of gold wire suture in 1550 was the first recorded application of a biomedical material and was followed by increasingly wide use of metal plates and pin to repair broken bones (Lyman *et al*., 1974).

Recent advances in materials science and surgery now make it possible to rebuild many parts of the human body. The synthetic polymer industry has expanded and polymeric materials with vast properties are available. Some of these polymers have mechanical properties that resemble those of natural tissues, making them suitable as biomedical materials with many applications such as intracorporeal (implanted) materials and extracorporeal materials.

Biomaterial science encompasses aspects of physical sciences, engineering, biology and medicine (Black, 1998).

1.3 Biocompatibility of Implants

Traditionally, the primary focus of the search for biomedical materials has been on finding biocompatible materials. A biocompatible biomaterial is one that is inert toward the physiological environment (Black, 2006). This has been modified to include materials having minimal interaction with the environment (Hench, 1991). A biomaterial may be compatible in one application for a specific purpose but not in a similar application at a different site (Kamimski *et al.*, 1981). Therefore, successful application of a biomaterial as a medical device requires both an acceptable biological response to the implanted materials and absence of physiologically induced damage. This is the measure of the overall interaction between biomaterials and living systems (Black, 1984).

Adverse biological responses to an implanted biomedical material including excessive-foreign body response, thrombogenicity, and immunogenic, antileukotactic (predisposition towards infection) and mutagenic complications have seen reported (kambie, 1993.)

The defence mechanisms of the body must be considered in the application of implant materials. This is because physiological responses do not exhibit uniquely differentiable stages. The responses are continuous and often integrated among various defence mechanisms against injury or invasion of foreign materials (Williams and Bagnall, 1981). The correlation between biomaterial surface and blood compatibility has been much studied. A review by Andrade (1985 a) indicates that no foreign material can ever be truly blood compatible, but that differences in the extent of the response and its relationship to proper device function could be exploited.

Performances in an implant or extracorporeal devices are the ultimate tests of a biomaterial. In addition to performance, it is the evaluation that should determine any adverse reactions are short- and long-term effects of the physiological environment on the implant. The reactions of implants to their host depend on the chemical and physico- chemical properties of the material as well as on the site ions and type of implantation. The biocompatibilities of biomaterials also include the entire field of bio-safety and bio-functionality (Williams and Bagnall, 1981).

The international standard organisation (ISO), in directive 10993 (ISO, 1992), described the methods for biological evaluation of medical devices as the bases for assessment of biocompatibility. Also, the guidelines for physicochemical characterisation of biomaterials, in directive 80-216 (NIH, 1980), explained the level –II surface characterisation test. This is the technique that provides information on surface chemistry morphology and interfacial properties of implants to be used to screen biomedical applications.

1.4 Titanium: Occurrence, Chemistry and Biomaterial Application

Titanium (Ti) with atomic number 22 and atomic weight 47.90 is a member of the first series of transition elements of group 4A of the periodic table. It is the ninth most plentiful in the lithosphere and the fourth most common structural metal following aluminium, iron and magnesium**.** It constitutes 0.63% of the earth's crust and has been detected in sea water, most plants and in all forms of animal life.

Titanium was discovered by Williams Gregory, an English mineralogist in 1790. Five years later Martins Heinrich Klaproth found that a Hungarian mineral, rutile, was the oxide of a new element which proved to be identical to that of Gregory. The name, titanium, from the Titans of mythology, was assigned to the new element of Klaproth (Noe, 1972). More than one hundred years passed before any use was made of the new element. It was first employed

in the United States as an additive to iron and steel; titanium (IV) oxide pigments became available in 1918.

Titanium has five naturally occurring isotopes and four artificial radioactive ones. It is widely distributed in rocks and minerals; the common commercial sources of minerals are rutile and ilment. Titanium occurs as titanium (IV) oxide (TiO_2) in nature as the mineral rutile, brookite and anatase which have the same formula TiO_2, but vary in crystal habit. Titanium also occurs in the form of titanates, the most extensive deposits being ferrous titanate, (Fe $Ti0_3$), ilment or calcium titanate (perovskite) ($CaTiO_3$) and calcium titanium silicate (CaO. TiO_2.SiO) (Bamberger, 1980; Deeson, 1973).

Titanium, despite its common occurrence, proved difficult to prepare in a pure state. This is because, though it is electrochemically active (Barksdale, 1980), it cannot be electrodeposited from aqueous solution. The high strength to weight ratio and good corrosion resistance make it attractive for aeroplanes and chemical application and technology have advanced rapidly in the last decade (Scheffer, 1981). It has good resistance to air at about 650 $^{\circ}$C; at higher temperatures, it is contaminated by oxygen and becomes a brittle metal. It does not react with nitrogen.

Powerful oxidants such as hydrogen per-oxide (70-90%) attack titanium. It is resistant to aqueous solutions of oxidizing agents like nitric acid, chromic and hypochlorous acids. Titanium resists most aqueous salt solutions like chlorides, hypochlorite, sulphides, sulphates, nitrates, phosphates and others. Resistance is limited to fluorides, hot concentrated solutions of aluminium, calcium, magnesium and zinc chlorides (Jaffee, 1973). These chlorides hydrolyse and become acidic and since they are none oxidising, do not favour the formation of a protective film. Although, it resists most neutral and oxidising environments, it has little resistance to non-oxidising acids. Reducing conditions can impair the protective films and break them down with a resultant corrosion if hydrogen ions are reduced on the surface.

Many materials act as inhibitors against corrosive attack and several titanium alloys have better resistance to specific corrodents than the pure metals (Noe, 1972).

Metallic titanium is readily soluble in hydrofluoric acid but violent if the acid added is much. With fluoroboric acid the reaction is complete and less violent if the acid is cold. Sulphuric acid reacts with it slowly at room temperature and faster at elevated temperatures. Hydrochloric acid dissolves titanium slowly at room temperature and more rapidly at elevated temperature. Titanium and its inorganic compounds are non –toxic, unless the associated radicals are toxic or the compounds are strongly acidic or basic (Scheffer, 1981).

According to Byan (2006), the high strength, low weight outstanding corrosion resistance possessed by titanium and titanium alloys have led to a wide range of successful applications which demand high levels of reliable performance in surgery and medicines as well as in aerospace, automotive, chemical plant, power generation, oil and gas extraction, sports and other industries.

More than 1,000 tonnes (2.2 million pounds) of titanium devices of every description and function are implanted in patients worldwide every year. Requirements for joints replacement continue to grow as people live longer or damage themselves more through hard sports or are seriously injured in road traffic and other accidents. By being light, strong and totally biocompatible, titanium is one of few materials that naturally match the requirement for implantation in the human body.

'Fit and forget' is an essential requirement where equipment in critical applications, once installed, cannot readily be maintained or replaced. There is no more challenging use in this respect than implants in human body. The effectiveness and reliability of the implants, and medical and surgical instruments and devices are essential factors in saving lives and in the long term, relief suffering and pain. Titanium is judged to be completely inert and immune to corrosion by all body fluids and tissue, and is, thus, wholly – bio-compatible.

The natural selection of titanium for implantation is determined by a combination of favourable characteristics which include immunity to corrosion, bio-compatibility, strength, low modulus and density and the capacity for joining with bone and other tissue (osseointegration). The chemical and physical properties of titanium alloys combine to provide implants which are highly damage-tolerant.

Forms and materials specifications are detailed in a number of international and domestic specifications, including ASTM and BS7252/ISO05832. Other applications of titanium implants are in bone and joint replacement, dental implants, Maxillofacial and craniofacial treatments, cardio-vascular devices, external prosthesis and surgical instruments (Byan, 2006).

1.5 Mucin

Mucin is a glycoprotein with two major characteristics:

(1) A high content of O-linked oligosaccharides (the carbohydrate content of mucin is generally more than 50%), and (2) the presence of repeated amino acid sequences (tandem repeats) in the centre of their polypeptide backbones, to which the o-glycan chains are attached in clusters. These sequences are rich in serine, threonine and proline (Brockhausen and Kuhns, 1997).

Both secretory and membrane –bound mucin occur. The former is found in the mucus present in the secretion of the gastrointestinal (pancreatic, small intestine and salivary), respiratory and reproductive tracts (Rosalind and Stuart, 1980). Mucus consists of about 94% water and 5% mucin, the remainder being a mixture of various cell molecules, electrolytes and remnants of cells. Secretory mucin generally has an oligometric structure and often has a very high molecular mass. The oligomers are composed of monomers linked by disulphide bonds. Mucus exhibits a high viscosity and, often is a gel. These qualities are a function of its mucin

content. The conformations of mucin often become those of rigid rods. The high content of sulphate residues found in many mucins confers a negative charge on them. With regards to function, mucins help to lubricate and form a protective physical barrier on epithelial surfaces.

Membrane- bound mucin participates in various cell–cell interactions. The density of oligosaccharide chains makes it difficult for proteases to approach their polypeptide backbones, making mucin to be resistant to their action. Mucin tends to “mask” certain surface antigens. Many cancer cells form excessive amounts of mucin; perhaps the mucin masks certain surface antigens on such cells and thus protects the cells from immune surveillance. Mucin also carries cancer-specific peptides and carbohydrate epitopes (an epitope is a site on an antigen recognised by an antibody, also called antigenic determinant). Some of these epitopes have been used to stimulate an immune response against cancer cells (Robert and Murray, 2000).

1.6 Research Objective

It is well known that serum proteins adsorb to inorganic surfaces almost immediately on contact with blood following surgical implantation and that the original surfaces are therefore no longer in contact with the host tissue (Albrektsson and Sennerby, 1990). However, little is known about the specificity of this process and still less is known about the proteins that adsorb on the titanium surfaces. In terms of the host tissue-biomaterial interaction, the role of adsorbed proteins is an important one since subsequent events depend on the composition and conformation of the protein layer and its tendency to change over time (Kotharis, 1995).

Lori and Hanawa (2004) monitored the adsorption of the simplest amino acid, glycine, on gold and titanium surfaces in Hank’s solution using electrochemical quartz crystal microbalance (EQCM) and observed adsorption of more glycine on titanium than on gold.

Adsorption of protein has been thoroughly studied by the use of different techniques (Ivarsson and Lundstrom, 1986). Bentaleb *et al*. (1998) studied the kinetics of the homogeneous, exchange of alpha-lactalbumine protein adsorbed on titanium oxide surface with radio-labelling technique and summarised that the protein release rates were higher than the pure desorption rates.

It has already been demonstrated that proteins are able to influence the rate of metal ion release (Clark and Williams, 1982). There are many ways in which the chemistry of the metal could control the biological responses, for example, by the influence of released metal ions on the immune system (McNamara and Williams, 1982), on chemotaxis (McNamara *et al*., 1983), on the integrity of cell membranes (Rae, 1975) and on the activity of intracellular enzymes (Rae, 1978). One further possibility is that the proteins present in the exudates that accumulate around the implant could be adsorbed onto the metal surface and thereby influence the subsequent development of the biological response (Williams *et al*., 1985). It is a reasonable hypothesis that protein- metal interactions are important in the determination of the tissue response. Although several studies have been made on the Ti-protein interactions for the development of body implants, for example, studies of the mechanism of adsorption of mucin on Ti surface reported by Lori and Nok (2004); Kinetic study of the adsorption of lactalbumine on TiO_2 was reported by Bentaleb *et al*. (1998). Kotharis (1995) investigated the adsorption of several serum proteins on TiO_2. There has not been any report however, on the kinetics of adsorption of mucin on Ti metal surface. Since mucin is a glycoprotein present in the saliva and damaged parts where titanium metal is mostly used as implant, it is very necessary to study the kinetics of mucin adsorption onto Ti so as to know the correlation between it and the Ti in terms of the kinetic parameters. Due of the dearth of published work on this to confirm or deny the above hypothesis; this research work is carried out with the

aim of kinetically studying the adsorption of mucin onto the Ti surface. The following objectives will achieve the above aim through investigation of

1) rate constants,
2) rates of adsorption,
3) the effect of pH on rates and rate constants,
4) the effect of Ca^{2+} ion on rates and rate constants, and
5) the effect of K ion on rates and rate constants.

Scientific data generated will help in understanding the adsorptive rates of mucin on titanium metal as a biomedical material.

CHAPTER TWO

LITERATURE REVIEW

Surface properties such as amount and conformation influence biolayer formation that is produced almost immediately after a material is implanted in a biological environment (Kasemo, 2002). Upon adsorption, proteins may change conformation (due to their relatively low structural stability) to reveal new bonding sites or restrict access to previously available sites (Lampin *et al*., 1999; Denis *et al*., 2002; Horbett, 1994). The adsorbed protein layer mediates interactions between the underlying material and cells (Cacciafesta *et al*., 2001). Hence, cell-surface interactions are modulated by the concentration, composition and conformation of adsorbed proteins (Nath *et al*., 2004; Underwood and Steele, 1991).

2.1 Protein Adsorption and Interfaces

Protein-surface interactions are the result of various interactions within the protein and between the protein and the surface. Protein adsorption at the interfaces involves diffusion, collision adhesion and exchange of proteins (Chan *et al*., 1981; Norde, 2003; Vroman and Adams, 1969). Proteins are composed of up to twenty different amino acids with the primary structure generally specific to each protein (Horbett, 2003). Hydrogen bonding, ionic interactions, disulfide bonds and a variety of other interactions control the secondary and tertiary structures of the polypeptide chains and give proteins their structural properties (Norde, 1986; Norde and Lkyleman, 1991). When a protein is in solution, there is a balance of forces to stabilise the protein. However, when this balance is disturbed by interaction with an interface, protein adsorption may occur. Differences in surface and protein charges, hydrophilicity and polarity, all influence the interaction between an adsorbed protein and the surface. The interplay between these forces together with the possible structural changes and

unfolding of protein molecules have made it difficult to understand the general characteristics of protein adsorption at interfaces (Hersel, 2003).

The size and shape of proteins play important roles in adsorption, number of binding sites increasing with increase in protein size. The vroman effect (Vroman and Adams, 1969; Jung *et al*., 2003), describes the competive adsorption of protein mixtures where smaller proteins adsorb first and may then be replaced by larger ones which have a greater affinity for a particular surface.

2.2 Surface Charge

Most proteins and surfaces carry charge. However, the charge is dependent on the pH and ionic strength of the surrounding solution. Attractive forces arise if the protein and interface have opposite charges (Norde, 1986). Most charged amino acid residues in a protein are located in their aqueous periphery (Norde, 2003). If the charges are balanced over the surface of the protein molecule, as the surrounding solution reaches the protein's isoelectric point, intramolecular electrostatic interactions favour a compact structure. The amount of protein adsorbed is at its maximum around its isoelectric point, IP (Norde, 2003). When there is an excess of charge, either positive or negative, intramolecular forces will lead to repulsion and expansion of the molecule as well as protein unfolding and a less stable protein (Norde, 1986). There is a tendency for adsorption characteristics of less stable protein to be governed by charges in a specific region of a protein. Hence if a region of a protein carries a charge which is oppositely charged with respect to the interface, this portion of the protein may adsorb to the interface (Haynes and Norde, 1994).

2.3 Hydrophobic Effects

Hydrophobic effects occur when spontaneous dehydration and subsequent aggregation of non-polar components occur in an aqueous environment. Hydrophobic interactions arise due to the tendency of water to exclude non-polar groups which result in a large increase in entropy of the water molecules released and hence a reduction in the Gibbs energy of the system. If either a protein or the contacting surface is hydrophobic, the entropic gain due to dehydration acts as a driving force for protein adsorption. Conversely, if the protein and interface are hydrophilic, hydration of the surface is energetically favourable and removal of water from the surface via an adsorption of the protein, is not favoured ($\Delta G > O$) (Norde, 2003).

The heterogeneous and dynamic natures of proteins make it difficult to define a protein as either hydrophobic or hydrophilic. Proteins generally contain both polar and non-polar regions (Andrade *et al.*, 1992). Non-polar regions tend to orient themselves towards the centre of the molecules to avoid water contact but geometrical, structural and functional constraints imply that this is not always possible (Norde, 2003; Andrade, 1985 b). The degree of hydrophobicity can also affect the extent of denaturation that occurs upon adsorption: if a protein adsorbs on a hydrophobic surface, it will rearrange its structure to expose the hydrophobic region to the surface (Andrade, 1985 b) for a hydrophobic protein. This may involve little or no-denaturation but for a hydrophilic protein, the internal polar regions can be exposed resulting in denaturation of the protein.

2.4 Protein Structure and Stability

Proteins are composed of a linear chain of amino acids which have a highly ordered structure derived from x-helices and B-sheets and stabilised by hydrogen bonds. Intermolecular hydrogen bonds reduce the rotational mobility of bonds in the polypeptide chain and hence

conformational entropy. Structural rearrangements affect protein-surface interaction and, hence, biological function (Van Tassel *et al*., 1997).

2.5 Adsorption Measurement

There are several methods available to study protein interactions at surfaces. The interaction of protein with surfaces has been examined for single protein solutions (Malmsten, 1995; Castillo *et al*., 1985) as well as mixtures of proteins (Bohnert et al., 1988; Kamiya *et al*., 1994). Analysis of protein deposition at mono-and sometimes sub monolayer levels is challenging in terms of sensitivity and resolution as well as retaining analyte during sample preparation techniques such as X-ray photoelectron spectroscopy (xps) (Kamiya *et al*., 1994; Ichijima *et al*., 2002), total internal reflection fluorescence (TIRF) (Lowe *et al*., 1986), surface matrix assistance laser desorption ionisation time-of- flight mass spectrometry (surface-MALDI-MS) (McArthur *et al*., 2001), time-of - flight static secondary ion mass spectrometry (ToF SSIMS) (McArthur *et al*., 2004), enzyme -linked immunosorbed assay (ELISA) (Laprestle, 1988), SDS-polyacrylamide gel electrophoresis SDS –PAGE) (Minarik and Rapp, 1989; Yan *et al*., 1993), western blotting and rediaolabeling (Garrett *et al*., 1999; Hyon *et al*., 1994; Ramsden, 1994), allow only static measurements. Surface MALDI-MS, SDS -PAGE and western blotting require extraction of the entire protein, which may be difficult as some proteins are thought to be permanently bound (Kiaei *et al*., 1992). The molecular weight resolution and sensitivity of SDS-PAGE is such that low molecular weight bands are poorly resolved (Kuizenga *et al*., 1996). SSIMS and XPS measure adsorbed proteins under vaccum, while XPS does not identify protein species. Protein labeling techniques such as radiolabelling, TIRF and AFM may disturb protein adsorption characteristics.

Techniques such as ellipsometry (Malmstein, 1994), surface plasmon resonanace (SPR) (Su and Zhang, 2004), optical wavegaide lightmode spectroscopy (OWLS) (Hook *et al*., 2002), quartz crystal microbalance (QCM) (Lori and Hanawa, 2004) and dual polarisation interferometry (DPI) (Cross *et al*., 2003) allow kinetics of the protein adsorption to be monitored. Ellipsometry is model dependent and it is necessary to know the refractive index of adsorbing layers. The QCM technique is sensitive to water associated with adsorbed protein; while OWLS and SPR are only sensitive to the bound protein.

The conformation and orientation of adsorbed protein is also of interest in biomaterials research. Overall changes in x–helix content of adsorbed proteins may be obtained from circular dichroism (CD) measurements (Ishihara *et al*., 1998) or from infrared spectra such as attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FIIR) (Chittur, 1983). Antibodies are commonly used to detect and quantify bound protein and monoclonal antibodies that bind to known sites on protein molecules can also be used to infer protein conformation from altered platelet binding responses.

2.6 Protein-Dye Binding

Laboratory practice in protein purification often requires a rapid and sensitive method for the quantitation of protein. Methods presently available partially fulfill the requirement for this type of quantitation. The standard Lowry procedure (Lowry et al., 1951) is subject to interference by compounds such as K (Vallejo and Lagunas, 1970), Mg^{2} (Kuno and Kihara, 1967), Tris ((Kuno and Kihara, 1967), thiol reagent (Vallejo and Lagunas, 1970) and carbohydrates (Lo and Stelson,1972). The relatively insensitive biuret reaction (Mokrasch and McGilvery, 1956) is subject to interference by Tris (Robson *et al.*, 1968), ammonia (Gornall *et al*., 1949) and glycerol (Zishka and Nishimura, 1970). Even the modified procedure for eliminating problems with the Lowry and Biuret assays (Bennett, 1967) present

problems since more complications and time are involved in the modified procedures. The dye binding techniques in the literature are for the most part insensitive assays involving the binding of Orange G to protein. The exception to this rule is the Amidoschwarz 10-B binding assay (Scaffner and Weissmann, 1973). This procedure too, has its drawbacks since the precipitation of the protein by trichloroacetic acid followed by filtration on millipore membranes is required.

The protein assay which involves the binding of Coomassie Brilliant Blue G-250 eliminates most of the problems involved in the procedures described above, and is easily utilised for processing large numbers of samples, as well as adaptablility to automation. It is based on the observation that Coomassie Brilliant Blue G-250 exists in two different color forms, red and blue (Reisner *et al.*, 1975). The red form is converted to the blue upon binding of the dye to the protein (Reisner *et al*., 1975). The protein–dye complex has a high extinction coefficient thus leading to great sensitivity in measurement of the protein. The binding of the dye to the protein is a very rapid process (approximately 2 min), and the protein-dye complex remains dispersed in solution for a relatively long time (approximately 1 hr), thus making the procedure very rapid and yet not requiring critical timing for the assay.

2.7 Adsorption of Proteins on Surfaces

Ghada *et al.* (1994) used enzyme linked immunosorbent assay (ELISA) for the estimation of protein adsorption from blood plasma at some model solid surfaces. The majority of these surfaces were made in the wells of microinterplates of polystyrene commonly used for ELISA purposes.

Bergerstroem and Hoffman (1994) studied the fibrinogen adsorption of a series of linear and branched, low molecular weight polyethylene oxide, PEO, bound to solid polystyrene surfaces. The results revealed a dependence of molecular weight below 1500

$gmo1^{-1}$ for linear PEOs. The branched PEOs have smaller exclusion volume (from GPC) than the corresponding linear PEOs, consistent with restriction in conformation freedom for the branched compounds.

Fibrinogen was adsorbed from a 1% plasma solution in a buffer on common polymeric materials and the amount adsorbed after 24 hours was determined. After 5 days of residence time in buffer, the adsorbed fibrinogen was eluted with a 1% solution of surfactant sodium dodecyl sulphate (SDS). The percent of fibrinogen that remained on the surfaces after elution is referred to as fibrinogen retention. Correlations between surface properties and the amount of fibrinogen adsorbed or fibrinogen retention were established. These models also revealed the most important variables that are related to protein behavior on these surfaces (Perez Luna *et al.*, 1994).

Lori and Nok (2004) investigated the role of electrostatic interactions in the adsorption of mucin to titanium *in vitro*. The building profile of mucin to titanium was analysed according to an adsorption isotherm. Mucin was dissolved and the solution suspended in commercial pure titanium powder treated with calcium, magnesium or potassium at pH 3.0 and 7.0. The amount of unadsorbed protein in the supernatant fluid was measured. The maximum amount of adsorbed mucin was 0.13 mg/1.0 g of Ti. The mucin-Ti association constant was then estimated. Pretreatment of titanium with calcium or magnesium resulted in increased adsorption of mucin to titanium. No increase in adsorption was recorded following pre-treatment of titanium with potassium. The results imply the involvement of electrostatic interactions in the adsorption of mucin to titanium.

The adsorption of bovine serum albumin (BSA) onto titanium powder was studied as a function of protein concentration and pH and in the presence of calcium and phosphate ions. Isotherm data revealed that the adsorption process did not follow the Langmuir model

(inflection points) (Elligsen, 1991; Veerman *et al.,* 1987; Williams *et al.,* 1985; Williams and Williams, 1988, Williams and Williams, 1989 and Sunny and Sharma, 1991).

Soderquist and Walton (1980) and Van and Norde (1983) observed maximum adsorption of BSA onto titanium at 10 min and 15 min for 0.1 and 0.5gl^{-1} BSA respectively, after which there was a decrease in adsorption to a steady state. Previous studies of albumin adsorption onto other surfaces also recorded kinetic 'overshoot'. These studies, indicated that desorption after sometime may be the result of rearrangements in the structure of already adsorbed molecules. Conformational changes in the molecules lead to some unfolding which results in an increased number of protein sites contacting the surface. Some protein molecules may become detached in favour of the 'spreading' of other adsorbed molecules.

The adsorption of BSA onto titanium surface has been found to increase in the presence of calcium acetate, although this effect decreased with increasing calcium acetate concentrations. Calcium is known to adsorb to BSA hence the zeta-potential values become less negative. The presence of Na_2HPO_4 decreased adsorption of BSA onto titanium with the effect increasing with increasing phosphate concentration (Healy and Ducheyne, 1992; Hughes *et al.,* 1995 and Shimabayashi *et al.,* 1991).

Lori and Hanawa (2004) studied the adsorption of BSA on gold and titanium metals in Hanks solution using electrochemical quartz crystal micro balance (EQCM).

Hughes *et al*. (1995) showed that the adsorption of BSA onto HAP increased with decreasing pH, but the isotherms were continuous, being described by the Langmuir model.

Dynamic contact angle (DCA) analysis was used on the basis of the multiloop Whilelmy-technique to study the interfering effects between protein adsorption /desorption and changes in wettability parameters such as advancing and receding contact angles and wetting tension hysteresis. Using DCA, different experimental dental plague-reducing biomaterial surface modifications were investigated. Another investigation dealt with the effects of sand-blasting

and acid-etching of titanium surfaces on their dynamic wettability behaviours (Rupp *et al.*, 2000).

2.8 Rate Expression and Order of Reaction

From experimental results one needs to know how the speed of a reaction varies with respect to individual reactant concentrations. Only then is it possible to derive a rate expression which summarises what controls the speed of a particular reaction in terms of the relevant concentrations, which is not necessarily all the reactants. The rate at which a reaction depends on the concentrations of the reactant(s) can be presented in the form of a rate expression. For the reaction: fA + gB + hC etc. ==> products

Rate = $k [A]^a [B]^b [C]^c$ etc.

k = the rate constant, which is constant at a constant temperature.

[ ] = the concentrations of reactants A, B or C etc.

a, b, c represent the individual order of reaction with respect to each reactant etc.

An individual order of reaction is the power to which the concentration term is raised in the rate expression. Powers of 1 are not shown by mathematical convention. The overall order of the reaction is a + b + c etc. Orders of reaction can only be determined by rate experiments, never from a balanced equation. The order of a reaction may or may not be the same as coefficients of the reactants in the balanced equation. The order of reaction is a consequence of the mechanism of the reaction and can only be found from rate experiments and cannot be predicted from the balanced equation depending on the coefficients of reactants in the balanced equations; i.e. the orders of reaction a/b/c etc may or may not coincide with the molar ratios f/g/h etc in the balanced equation and 1 is not shown by mathematical convention in either the equation or rate expression. For a single reactant we can examine

theoretically the effect of changing concentration on the rate of reaction by using a simplified rate expression of the form

$Rate(r) = k [A]^a$.

The constant k will include the rate constant k and any other constant reactant concentration terms.

[A] = the variable concentration of a reactant A,

a = the order with respect to reactant A.

For first order, the rate doubles if concentration doubles or the rate is reduced to 1/3rd if the concentration is reduced by a factor of 3, etc. That is, the rate is proportional to concentration. In rate investigations, each reactant concentration should be individually investigated to determine its individual order and ultimately to derive the full rate expression for the reaction. Any other reactant/catalyst concentration must be kept constant. The mathematics of first order rate equations is given as

Rate = $-d [A]/dt = k_1 [A]$, on integration we get: $k_1 t = \ln [A_o] - \ln [A]$

k_1 = 1st order rate constant (s^{-1})

$[A_o]$ = initial concentration of A at t=0 (mol dm^{-3})

[A] = concentration of A at time t (mol dm^{-3})

A graph of ln [A] versus t will have a gradient of $-k_1$

and an intercept of $\ln [A_o]$

The above equations can be expressed in amounts of A e.g. in number of moles of A.

If a = initial moles of A, x = moles of A reacted,

So (a-x) = number of moles A remaining at time t

Rate = $-d (a-x)/dt = k (a-x)$

On integration we get: kt = ln (a) - ln (a-x)

A plot of ln (a-x) versus t has a gradient of -k,

It is also possible from the type of graph of concentration versus time, to measure the initial gradient, which gives the initial rate of reaction and applies to any order of reaction (Atkins and Paula, 2002).

2.9 Kinetics Rate Constant

Rate constant is an extremely useful quantitative characteristic of a chemical or physical process. It gives the relationship between reaction rate and concentration of reactants. When the rate constant equation is considered by assuming the adsorption of reactants as a first-order and reversible reaction, the rate constant can be calculated by Lagergren equation (Gaid *et al*., 1994). Lagergren first order kinetics expression was used in literature mostly to show adsorption capacity of different adsorbents (Ho and Mckay, 1998). The linear form of the first order equation of Lagergren is generally expressed as follows:

$\text{Log}\ (q_e - q_t) = \log (q_e) - (k_{ad}/2.303)\ t$

q_e and q_t = the adsorption concentration at equilibrium and at time t in (mg/ml) respectively.

k_{ad} = the adsorption rate constant of the first order adsorption

t = the adsorption time (minute)

The plot of log (qe - qt) versus t should give a linear relationship from which the order parameters can be determined using the slope and intercept of the adsorption graph; if the intercept is equal to the log of qe, then it is first order.

The adsorption rate constant of the first order adsorption, K_{ad}, for the different surfaces using Bhattacharya/Venkobacharya equation (Bereket *et al*., 1997) is generally expressed as $\ln (1-u_t) = -(K_{ad})\ t$

$u_t = C_o - C_t / C_o - C_e$

K_{ad} = rate constant of the first order adsorption in (mg/ml min), t is the time (minute).

C_o, C_e and C_t = concentrations of mucin in solution initially, at equilibrium and time t (mg/ml) respectively

The plots of ln (1-u ($_t$) versus t should give straight lines for the first order equations.

The structure of the solid and its interaction with the diffusing substances influence the rate of transport. The adsorption process on a porous adsorbent generally involves three stages: (i) extra-particle; (ii) internal diffusion (or intra-particle diffusion) and (iii) actual adsorption (Chang et al., 2003). The adsorption step is usually very fast for the adsorption of organic compounds on porous adsorbents compared to the external or internal diffusion step (Sarkar et al., 2003) and it is known that the adsorption equilibrium is reached within several minutes in the absence of internal diffusion (Mak and Chen, 2004). Adsorbents may be a barrier to the other by virtue of a concentration gradient (Treybal and Robert, 1981). To provide definite information on the rate-limiting step, an internal diffusion model based on Fick's second law is used to test if the internal diffusion step is the rate-limiting step (Bhattacharyya and Sharma, 2004). The intra-particle model equation is given as:

$q_t = k_p t^{½} + C$

k_p = the intra-particle diffusion rate constant in (mg/ml min),

C = the boundary layer thickness

q_t = the amount of mucin adsorbed (mg/ml) at time t.

t = the time (minute).

According to internal diffusion model, a plot of q_t versus $t^{½}$ should give a straight line with a slope, k_p, and an intercept zero if the adsorption is limited by the internal diffusion process (Bhattacharyya and Sharma, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Reagents: All reagents were of analytical grade unless otherwise stated. Also deionised water was used in the preparation of solutions unless otherwise stated.

3.1.1 Phosphate Buffer Saline Solutions (0.02 M, pH 3 - pH 8)

The phosphate buffer saline was prepared from 0.02 M anhydrous sodium dihydrogen orthophosphate, 0.015M sodium chloride and 0.02 M disodium hydrogen orthophosphate dihydrate in de-ionised water and then mixed to make solutions of pH 3, 3.4, 4.0, 4.4, 5.0 5.4, 6, 6.4 7, 7.4 and 8 using a pH meter to monitor the desired pH.

3.1.2 Mucin Solutions

Mucin solutions with concentrations ranging from 0.02 to 1.0 mg/ml were made using Eppendorf micropipette for respective volumes of stock mucin solution (4 mg/ml), and transferring each into 20 ml volumetric flask, making it up to the mark with water and storing in the refrigerator at 4 °C.

3.1.3 Bovine Serum Albumin Solution

Bovine serum albumin (BSA) solutions with concentrations ranging from 0.2 to 0.8 mg/ml were made using Eppendorf micropipette for measuring respective volumes of stock bovine serum albumin solution (4 mg/ml), transferring each into a 10 ml volumetric flask, making it up to mark with water and storing in the refrigerator at 4 °C.

3.1.4 Calcium Chloride Solution

Calcium chloride (0.1M) was made by dissolving 1.4699 g of the salt, in water in a 10 ml volumetric flask and making up to the mark with more water.

3.1.5 Potassium Chloride Solution

Potassium chloride solution (0.1M) was made by dissolving 0.7456 g of the salt, in water in a 10 ml volumetric flask and making up to the mark with more water.

3.2 Standard Calibration Curve

A standard calibration curve was generated using varying concentrations of bovine serum albumin (BSA) ranging from 0.2 to 0.8 mg/ml prepared in section 3.1.3 and measuring absorbance at 595 nm against the blank. The results are presented in Appendix I (a) and the calibration curve in Appendix I (b)

3.3 Optimisation of Variables Used In the Adsorption Studies

3.3.1 Optimisation of Mass of Ti: Titanium metal (crushed sponge) powder (BDH Chemicals Ltd., Poole, England) was used. Titanium masses ranging from 0.02 to 0.10 g were weighed into centrifuge tubes, 10 ml of 0.1 mg/ml mucin solution at pH 4.7 (the isoelectric pH of mucin) was poured into each of the tubes and allowed to incubate at 25 ±1 □C in a thermostated bath (Grant J. B. Series, England) for 2 hours with constant stirring. The tubes were centrifuged at 656 g for about 5 minutes. Then the free protein estimation (mucin not bound to Ti) was performed in duplicates using the method of Bradford (1976). The supernatant solution (0.1 ml) was placed in a test tube containing 5.0 ml Bradford reagent. The absorbance was measured at 595 nm with a uv-visible spectrophotometer (spectronic 20, Bausch and Lomb, N Y) against a reagent blank (prepared from 0.1 ml water

and 5.0 ml of Bradford reagent). The calibration curve Appendix I (b) generated using bovine serum albumin in section 3.2.1 was used to determine the concentration of free mucin corresponding to the absorbance measured. The amount of bound mucin to Ti was then calculated by subtracting the amount of unadsorbed mucin in the supernatant from the amount of mucin in the control (see Appendix II). Respective amounts of bound mucin were then plotted against respective masses of Ti. The results are presented in Fig 1.

3.3.2 Optimisation of the Concentration of Mucin Solution

Mucin solutions with concentrations ranging from 0.02 to 1.0 mg/ml were made according to section 3.1.2. The optimum mass of titanium (0.02 g) obtained in previous section 3.3.1 was weighed into centrifuge tubes. 10 ml of a respective mucin solution at pH 4.7 (the isoelectric pH of mucin) with concentrations ranging from 0.02 to 1.0 mg/ml was poured into each tube. While allowing the adsorption process to go on, the tubes were incubated at 25 ± 1 °C in the thermostated bath (Grant J. B. Series, England) for 2 hours with constant stirring. The tubes were then centrifuged at 656 g for about 5 minutes. Protein estimation and the concentrations of bound mucin were calculated according to section 3.3.1. Respective amounts of bound mucin were then plotted against respective initial amount of mucin (mg/ml). 0.1 mg/ml. The results are presented in Figure 2 while details are given in Appendix III.

3.3.3 Optimisation of Temperature for the Adsorption of Mucin onto Ti

Titanium (0.02 g) obtained in section 3.3.1 was weighed into each of centrifuge tubes. A 10 ml aliquot of 0.01 mg/ml (optimum concentration of mucin solution obtained in section 3.3.2) was poured into each tube, incubated at a respective temperature ranging from 25 °C to 45 °C in the thermostat bath. The adsorption at a particular temperature was determined after for 2 hours with constant stirring. The tubes were then centrifuged at 656 g for about 5 minutes.

Protein estimation and the concentration of mucin were calculated according to section 3.3.1. Respective amounts of bound mucin were then plotted against respective temperatures. The results are presented in Figure 3 and details are given in Appendix IV.

3.3.4 Optimisation of pH for the Adsorption of Mucin

The determined mass of titanium metal (0.02 g) was weighed into each of nine centrifuge tubes. Mucin solution (0.1 mg/m)l obtained in section 3.3.2 was made by placing certain weighed amounts of mucin into 20 ml volumetric flasks and making up with the phosphate buffer saline prepared in section 3.1.2 to give 0.1 mg/ml mucin solution at the pH of 5.0, 5.5, 6.0, 6.4, 7.0, 7.4 and 8.0. Mucin solutions (10 ml in each case) at the pH 5 to 8 were measured and poured into tubes containing the 0.02 g optimum mass of titanium metal. The tubes were incubated at 35 °C in the thermostated bath for 2 hours with constant shaking. They were then centrifuged at 656 g for about 5 minutes. Protein estimation and the concentration of mucin were calculated according to section 3.3.1 except that the blank used was prepared from 0.1 ml phosphate buffer saline at a particular pH for the corresponding pH and 5 ml Bradford reagent. For all the optimisation processes carried out, control experiments were equally set up. Respective amounts of bound mucin were then plotted against respective pH values. The results are presented in Figure 4 and Appendix V.

3.4 Pre-Treatment of Ti

Titanium metal (1 g each) was weighed into two different test tubes and suspended for 24 hours at 25 °C in 1 ml of 0.1 M calcium chloride prepared in section 3.1.4 and 0.1M potassium chloride prepared in section 3.1.5. Titanium metal suspended in de-ionised water served as control. The titanium metal from each tube was then washed thrice with water and left to dry at room temperature.

3.5 Kinetic Studies: Effect of Contact Time on Rates of Adsorption of Mucin onto Treated and Untreated Ti

The effect of contact time was determined by the ‘’limited bath technique’’. The optimum masses of titanium metal (0.02 g) was weighed for each of treated (Ca – Ti and K –Ti) and control (Un –Ti) and added to 10 ml of 0.1 mg/ml mucin solution prepared in phosphate buffer saline (PBS) pH 6.4 under shaking. The temperature of the solution was held constant at 35 °C in a thermostated bath. After 5 minutes intervals in the range 0 - 130 minutes, 0.1 ml of the supernatant was taken for spectrophotometric measurements in duplicate using the method of Bradford (1976). Protein estimation and concentration of mucin were calculated according to section 3.3.1 except that the blank used was prepared from 0.1 ml of the phosphate buffer saline (PBS) of pH 6.4 and 5 ml of Bradford reagent.

3.6 Adsorption Rate Constants

The time- concentration profile characteristics of the adsorption of mucin onto surfaces were tested with Lagergren first – order (Lagergren, 1898), Bhattacharya/Venkobacharya (Ho and McKay, 1998) and the intra-particle diffusion

(Weber and Morris, 1963) models. The linear form of the first order equation of Lagergren is generally expressed as follows:

$\text{Log}\ (q_e - q_t) = \log (q_e) - (k/2.303)\ t$

where q_e and q_t are the adsorption concentrations (mg/ml) at equilibrium and time t, respectively, t is the adsorption time (minute), K_{ad}, (ml/ml min) is the adsorption rate constant of the first order adsorption. If first order kinetics is applicable, the plot of log (qe - qt) versus t should give a linear relationship from which the order parameters can be determined from the slope and intercept of the adsorption plot. If the intercept is equal to log qe, then the adsorptions first order.

The adsorption rate constant K_{ad}, of the first order adsorption for the different surfaces using Bhattacharya/Venkobacharya equation (Bereket *et al.*, 1997) is generally expressed as follows:

$\ln (1-u_t) = -\square (K_{ad})\, t$

$u_t = C_o - C_t / C_o - C_e$

Where K_{ad} is rate constant of the first order adsorption in (mg/ml min) and C_o, C_e and C_t are concentrations (mg/ml) of mucin in solution at initial, equilibrium and time t (min), respectively. The plots of $\ln (1-u_t)$ versus t should give straight lines for the different surfaces for first order equations.

Adsorption of sorbate on sorbent proceed in several steps involving transport of the solute molecules from the aqueous phase to the surface of the solid particulates and diffusion of the solute molecules into the interior of the pores. The linear driving force concept was equally used to analyse the kinetic data by a diffusion-controlled adsorption model. The intra-particle diffusion rate constant is given by the equation

$q_t = k_p t^{½} + C$

where k_p is the intra-particle diffusion rate constant in (mg/ml min), C is the boundary layer thickness and q_t is the amount of mucin adsorbed (mg/ml) at time t (t is the time in minutes). If the plot of q_t versus $t^{½}$ results in a linear relationship with zero intercept, the adsorption process is particle- diffusion controlled.

CHAPTER FOUR

RESULTS AND DICUSSION

4.1 Standard Calibration Curve

A standard calibration curve (Appendix I (a)) was generated according to section 3.2.1 for use as a reference instrument in the determination of free mucin in the respective test mucin solutions.

4.2 Optimisation of Variables Used in the Kinetic Studies

4.2.1 Optimisation of Mass of Ti

The results for the amount of mucin bound to different mass samples of Ti are given in Appendix II. The plot of the respective amounts of mucin bound to Ti as a function of the mass of Ti is presented in Fig. 1 from which it was observed that 0.02 g was the optimum mass of Ti for use in further experiments. The amount of mucin adsorbed decreased as the mass of Ti increased. The decrease in the amount adsorbed with increasing mass of Ti is due to the concentration gradient between solute concentration in solution and the solute in the surface of the Ti. This can be explained by the fact that a fixed mass of Ti can only adsorb a certain amount of protein. Therefore, the higher the adsorbent dose, the larger the amount of protein that the fixed dose can adsorb (Vandivelan and Kumar, 2005). The main objective in an optimisation process is the economic profit. However, this is very hard to estimate. A good practical objective function is the minimisation of adsorbent use and maximisation of the reaction speed for the kinetic studies of the adsorption of mucin onto titanium.

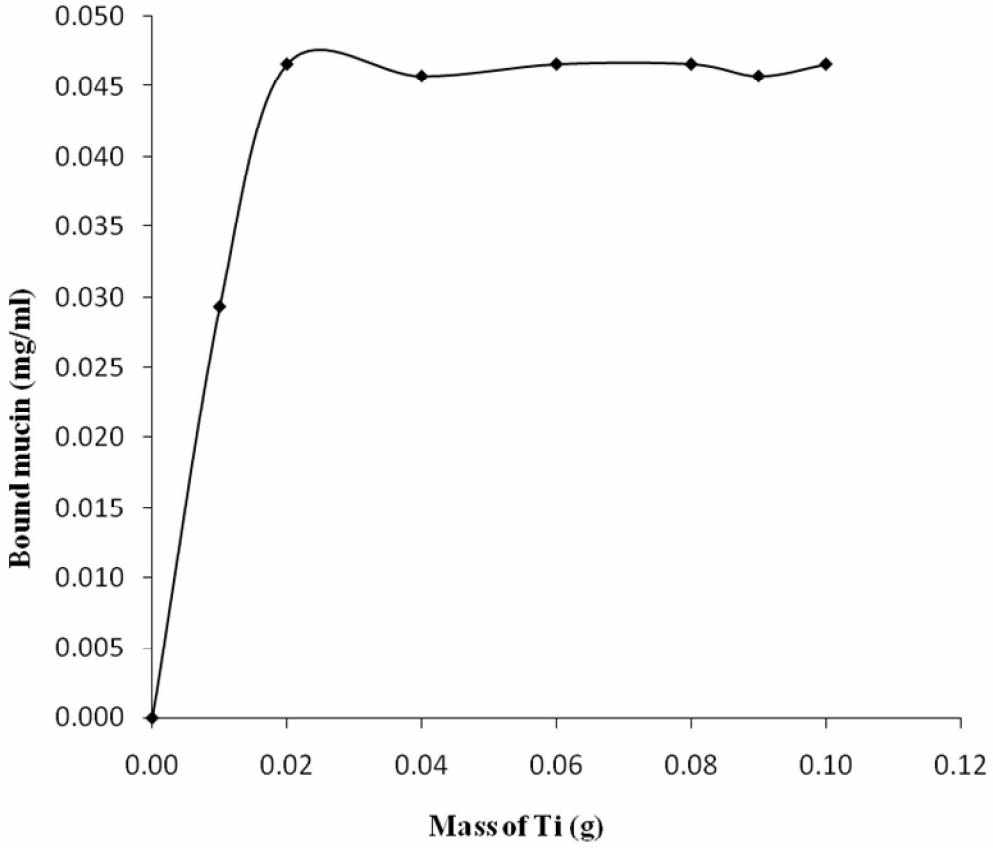


Figure1: Optimisation of mass of Ti

4.2.2 Optimisation of the Concentration of Mucin in the Adsorption of Mucin onto Ti

The results of the optimisation of mucin bound to 0.02 g of Ti from the stated amounts of mucin are presented in Appendix III. The plot of the concentration of mucin adsorbed (bound mucin) as a function of initial concentrations of mucin is presented in Figure 2 and from which it was evident that 0.1 mg/ml of mucin was the optimum concentration adsorbed and was used in further experiments.

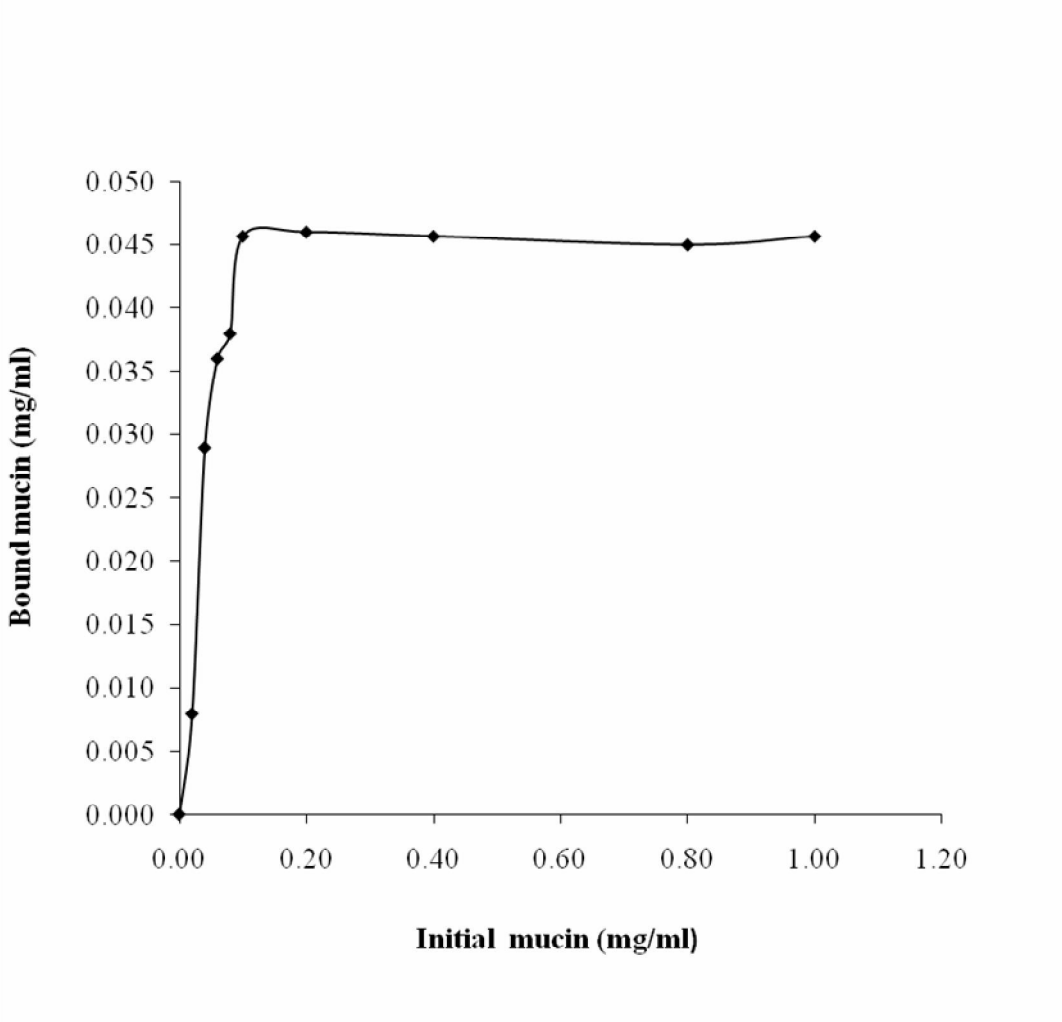


Figure 2: Optimisation of the concentration of mucin in the adsorption of mucin onto Ti

4.2.3 Optimisation of Temperature for the Adsorption of Mucin onto Ti

The results of the effect of temperature on the adsorption of mucin onto titanium metal are shown in Figure 3 (details Appendix IV) from which it is evident that the optimum temperature of the adsorption process is 35 °C which is consistent with the physiological temperature of 37 °C.

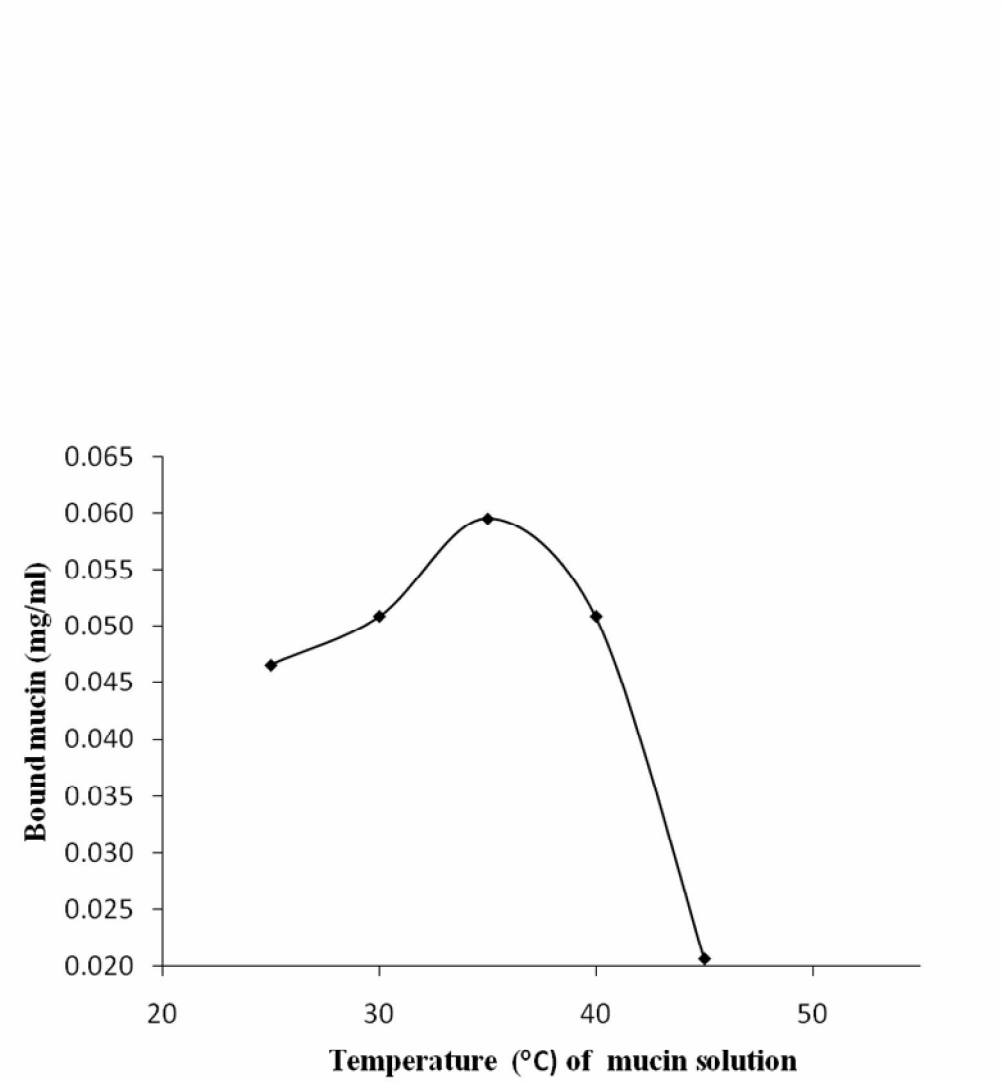


Figure 3: Optimisation of temperature for the adsorption of mucin onto Ti

4.2.4 Optimisation of pH for the Adsorption of Mucin onto Ti

The pH optimisation profile as shown in Figure 4 (details Appendix V) reveals that the optimum pH for the adsorption process was pH 6.4. The net charge of a given protein depends on its isoelectric pH and the pH of the environment. The isoelectric pH of mucin is 4.7. At higher pH values mucin undergoes a neutral – acid transition and becomes negatively charged; while at lower pH values mucin undergoes a neutral-basic transition and becomes positively charged. At pH 6.4 the protein and the titanium (whose surface is in oxide form) are both negatively charged; so the electrostatic repulsion barrier should be overcome mainly by hydrophobic interaction. The nature of the hydrophobic interaction is however not apparent since titanium oxide surface is hydrophilic. The maximum amount of mucin was adsorbed at pH 5 because of its closeness to its isoelectric pH. The amount of mucin adsorbed decreased beyond pH 7.4 meaning that the protein began to denature at higher pH values.

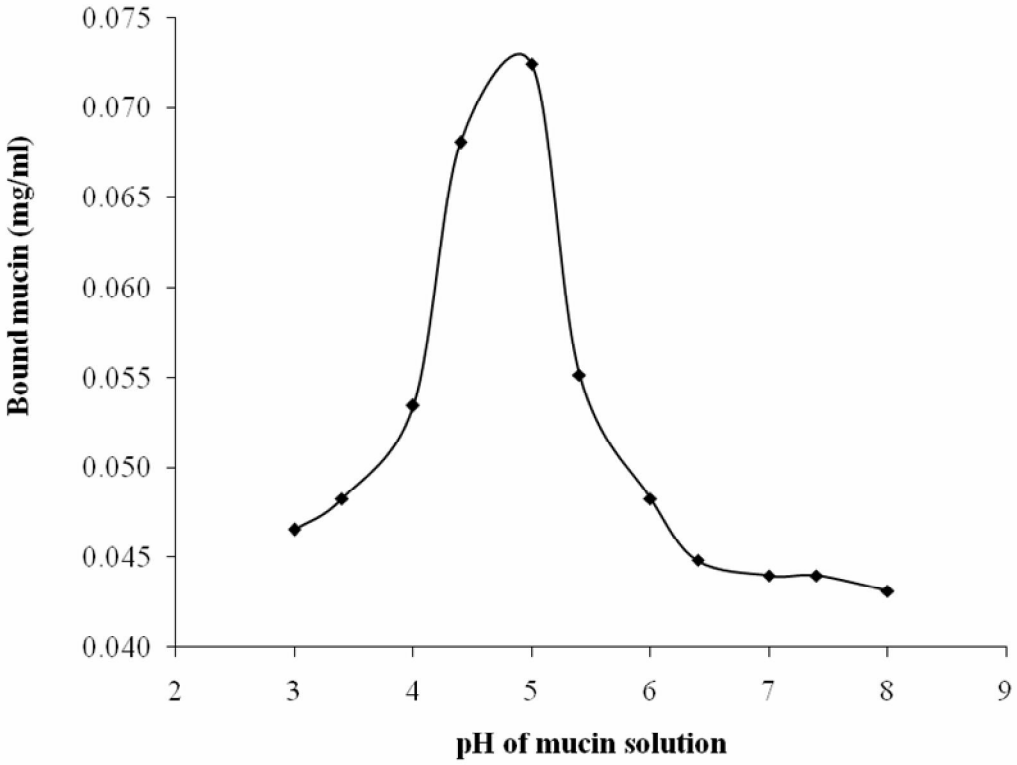


Figure 4: Optimisation of pH for the adsorption of mucin onto Ti

4.3 Kinetics Study of Adsorption of Mucin onto Ti

4.3.1 Effect of Incubation Time and pH on the Rates of Adsorption of Mucin onto Un-Ti, Ca–Ti and K–Ti

The rates of adsorption profiles are shown in Figure 5 and results obtained are presented in Appendices VI-IX. Adsorption processes attained equilibrium at 115 minutes. There were rapid increases in the rates of adsorption initially from 0 to 60 minutes but which began to slow down till equilibrium was attained for the three types of titanium surfaces. The initial rapid increases in rates could be attributed to the concentration gradient existing at the start of the adsorption process between solute concentration in solution and that at the Ti surface. As the surface coverage increased the protein concentration gradient reduced and gave way to slower adsorption. The kinetic regimes described by protein adsorption curves reversed transport-limited and adsorption-limited regimes. During the initial stage of the adsorption, diffusion is the rate-limiting step at the short times. Following the sufficient adsorption to the surface, an adsorption- limited became prominent. The adsorption-limited regime is divided into two regions, one in which there is a linear decrease in adsorption rate and a second region in which there is a non-linear approach to saturation is seen.

The amount of mucin adsorbed at equilibrium (q_e) for Un-Ti, Ca-Ti and K-Ti were 0.0209, 0.0603 and 0.0249 mg/ml, respectively. These were equivalent to 20 %, 60 % and 24 % of the initial mucin solution 0.1 mg/ml used. Amounts adsorbed at 1 hour of the adsorption process were 0.0150, 0.0509 and 0.0192 mg/ml for Un-Ti, Ca-Ti and K-Ti, respectively. These correspond to 71.8 %, 84.4 % and 77.1% of the total protein adsorbed at equilibrium. This means that much of the adsorption occurred at 1 hour of the adsorption process.

Mucin is negatively charge at the pH of the experiment. Though the amount of protein adsorbed at equilibrium onto Ca-Ti was up to 3 times that for untreated titanium (Un-Ti), equilibrium of the adsorption process is not influenced by the presence of the calcium ions.

The amount of mucin adsorbed by the potassium treated titanium (K-Ti) simply suggests that calcium ions only open up more binding sites for protein adsorption onto Ti surface.

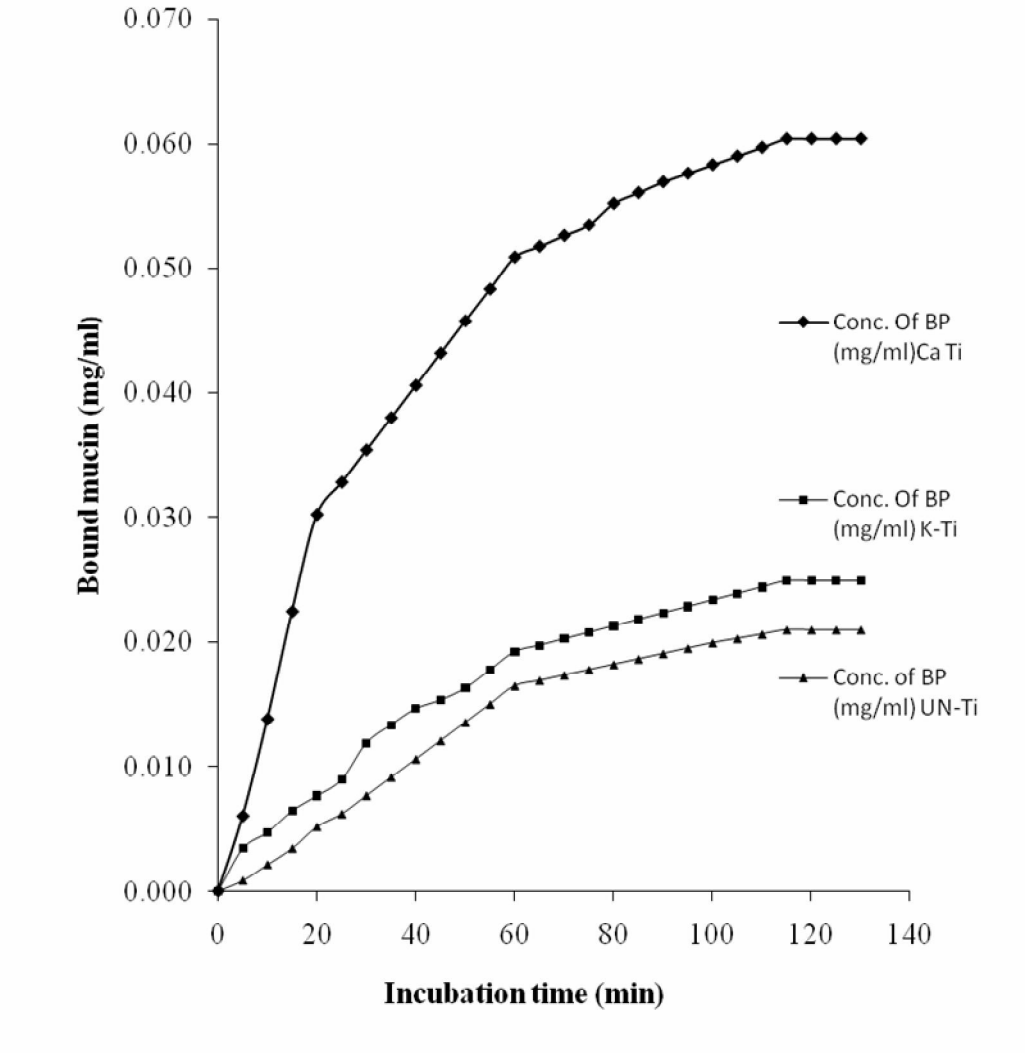


Figure 5: Effect of incubation time on the rates of adsorption of mucin onto Ca –Ti, K-Ti and Un –Ti

4.3.2 Adsorption Rate Constant

An error function is required to evaluate the fitness of each kinetic obtained from the optimisation process employed. In the present study, the linear coefficient of determination, R^2, was used. The values for rate constants, log of the concentrations at equilibrium (qe), intercepts and R^2 for each equation, were fitted to the experimental data and results are presented in Appendices X-XVI. The highest values of R^2 were obtained when the experimental data were fitted into the intra particle diffusion equation (R^2= 0.955-0.980).

The results presented in Appendices X-XII and the adsorption rate constants profiles shown in Figures 6-9 were obtained when the data from adsorption experiments were analysed using Lagergren equation for the first- order reaction. When the values of log (qe – qt) were plotted against time, the linearity of the plots were high, rate constants were calculated from the slopes and intercepts were almost the same as the log of the concentration at equilibrium (qe), meaning first – order reaction (Lagergren, 1898). The first order rate constants were 3.45 x 10^{-2}, 2.99 x10^{-2} and 2.99 x10^{-2} (mg/ml min) for Ca-Ti, K-Ti and Un-Ti, respectively. The k_{ad} value for Ca-Ti was the highest, followed by K-Ti and was least for Un-Ti. The adsorption rate constant profiles shown in Figures 10-13 were obtained when the data were analysed using Bhattacharya/Venkobacharya equation. When ln (1-u_t) was plotted against time (min), straight line plots were obtained for the three types of Ti surfaces. The range of the linearity of the plots were given as R^2 = 0.930-0.947 and the first order rate constant obtained as 4.606 x10^{-3}, 1.612 x10^{-2} and 4.606 x10^{-3} (mg/ml min) for Ca-Ti, K-Ti and Un-Ti, with details given in Appendices XIII-XV.

The results presented in Appendix XVI and the intra- particle diffusion rate constant profiles shown in Figures 14-16 were obtained when the data were analysed using Flick's intra-particle diffusion equation. These gave straight lines for the treated and untreated Ti and the

intercept almost zero meaning that the adsorption process is limited by the internal diffusion process (Bhattacharya and Sharma, 2004). This is an indication that the particle diffusion mechanism was predominant. The rate constants were calculated from the slopes, intercepts gave the boundary layer thickness and the range of R^2 was 0.955-0.980. The intra-particle diffusion rate constants were obtained as 0.005, 0.001 and 0.001 (mg/ml min) for Ca-Ti, K-Ti and Un-Ti, respectively with details given in Appendix XV.

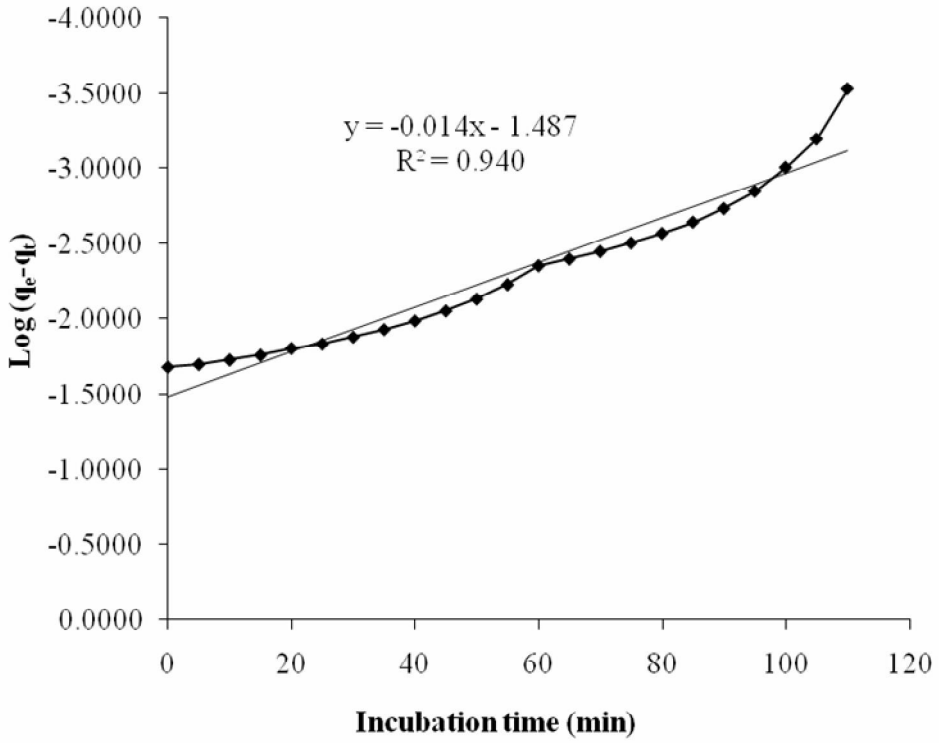


Figure 6: Lagergren plot for determining the effect of incubation time and pH on rate constant for the adsorption of mucin onto Un- Ti

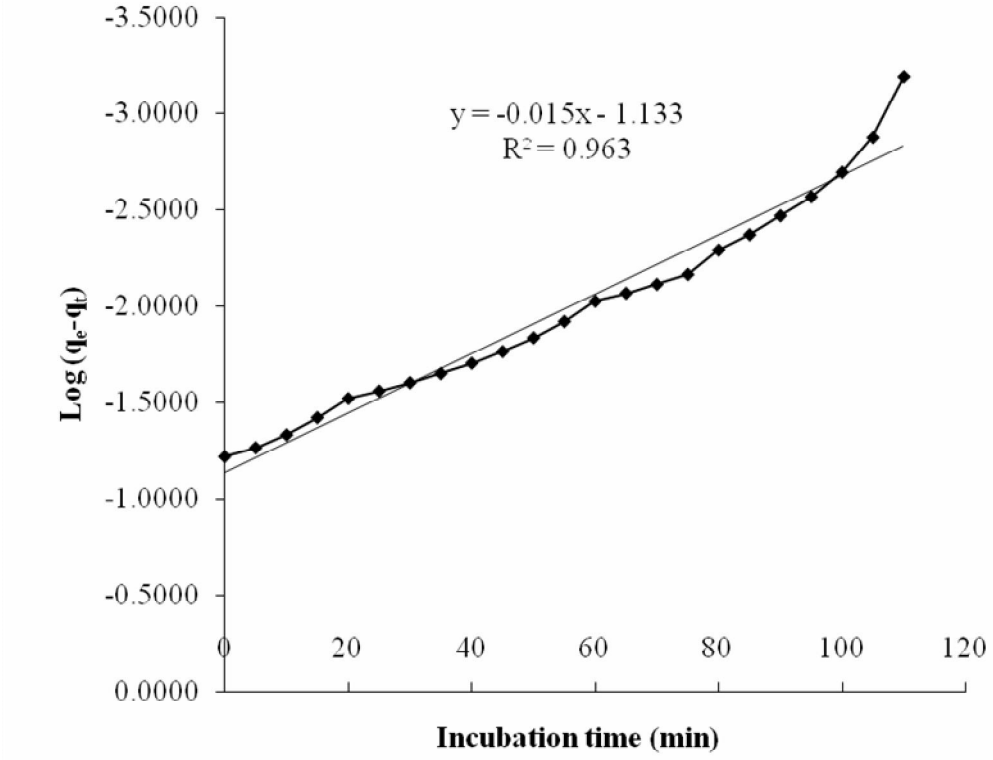


Figure 7: Lagergren plot for determining the effect of incubation time and pH on rate constant for the adsorption of mucin onto Ca-Ti

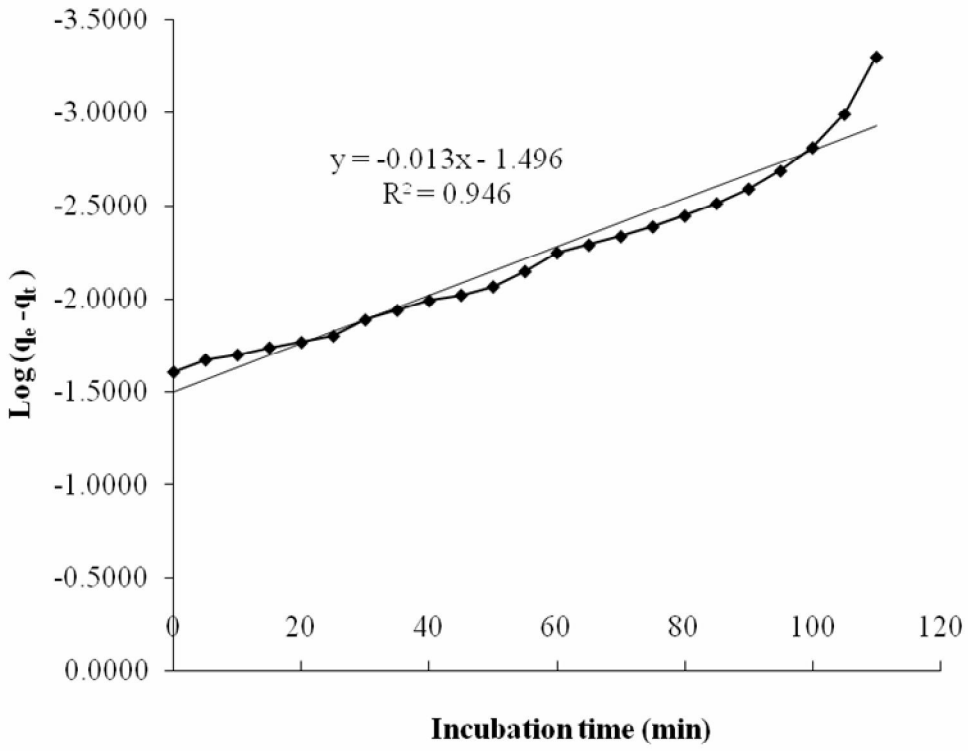


Figure 8: Lagergren plot for determining the effect of incubation time and pH on rate constant for the adsorption of mucin onto K-Ti

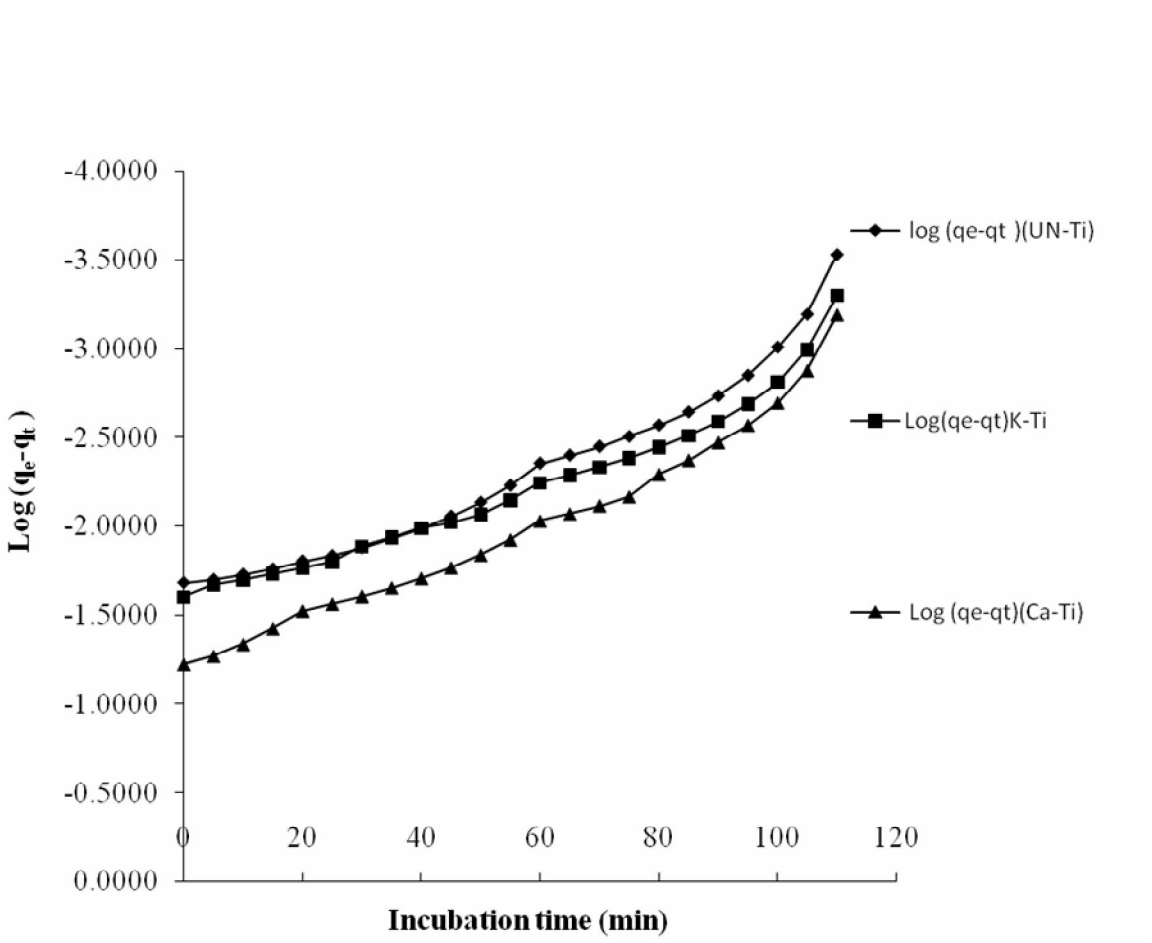


Figure 9: Lagergren plots for determining the effect of incubation time and pH on rate constants for the adsorption of mucin onto Un-Ti, Ca-Ti and K-Ti

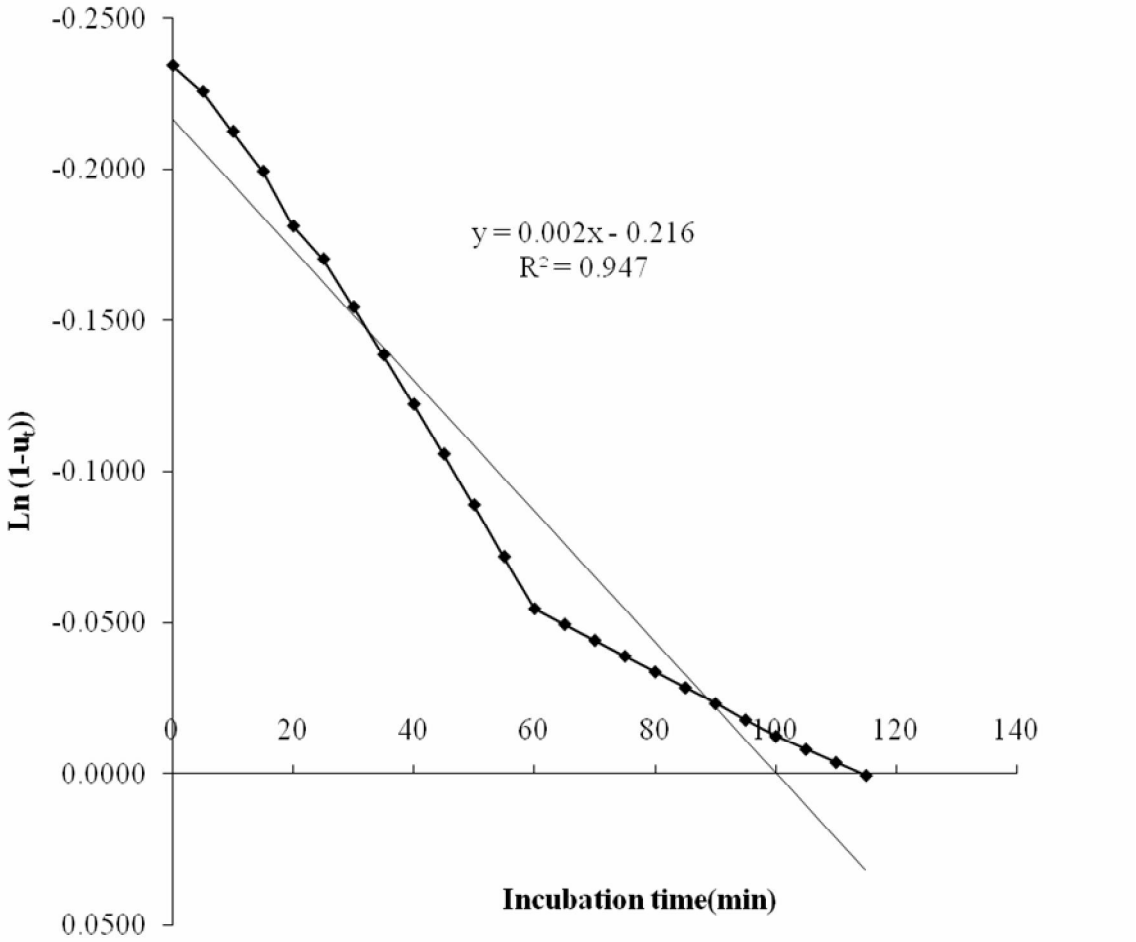


Figure 10: Bhattacharya/Venkobacharya plot for determining effect of incubation time and pH on rate constant for the adsorption of mucin onto Un-Ti

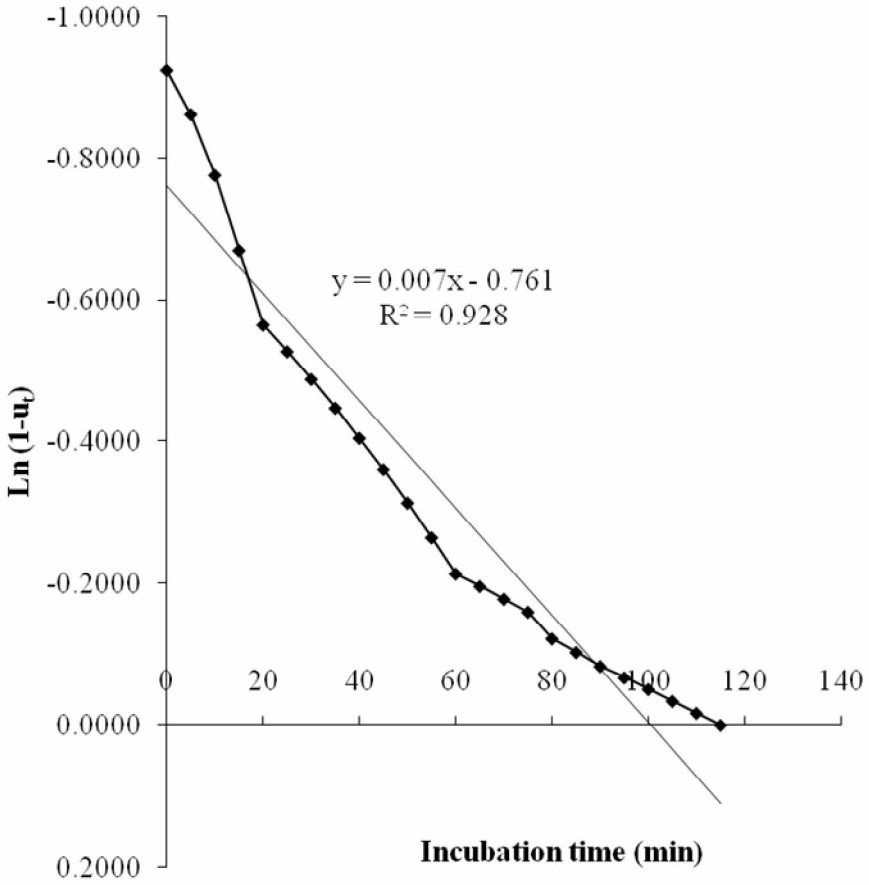


Figure 11: Bhattacharya/Venkobacharya plot for determining effect of incubation time and pH on rate constant for the adsorption of mucin onto Ca-Ti

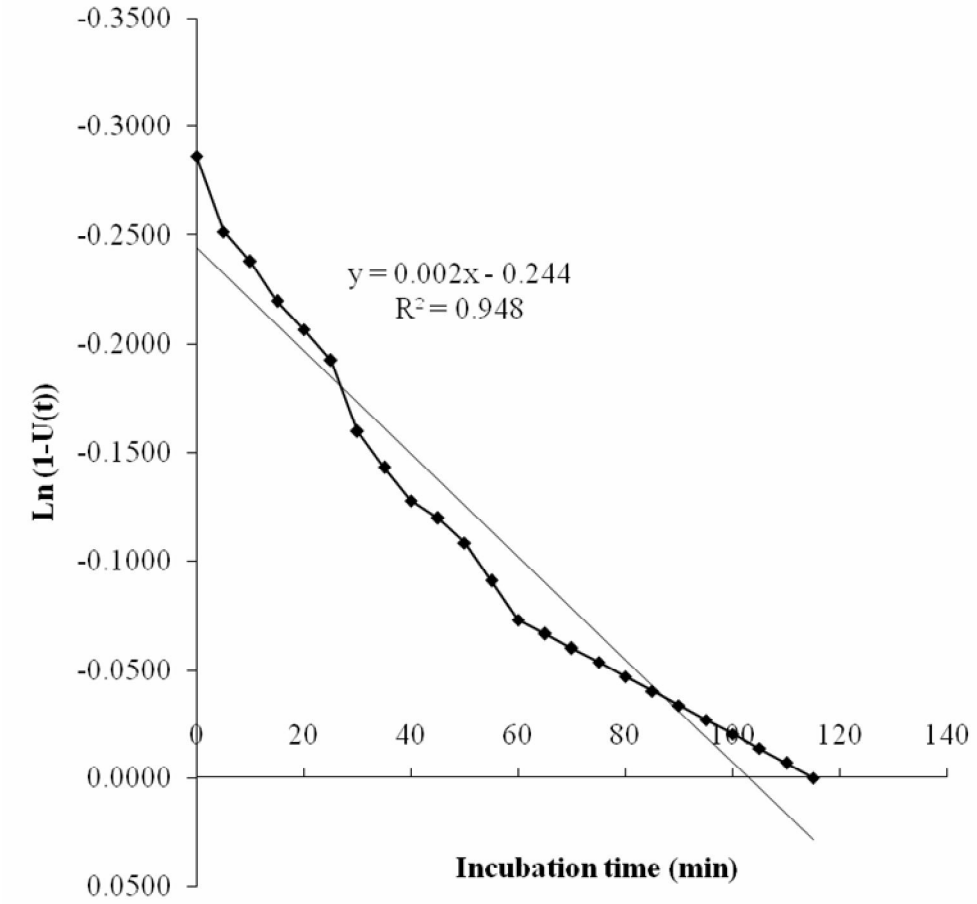


Figure 12: Bhattacharya/Venkobacharya plot for determining effect of incubation time and pH on rate constant for the adsorption of mucin onto K-Ti

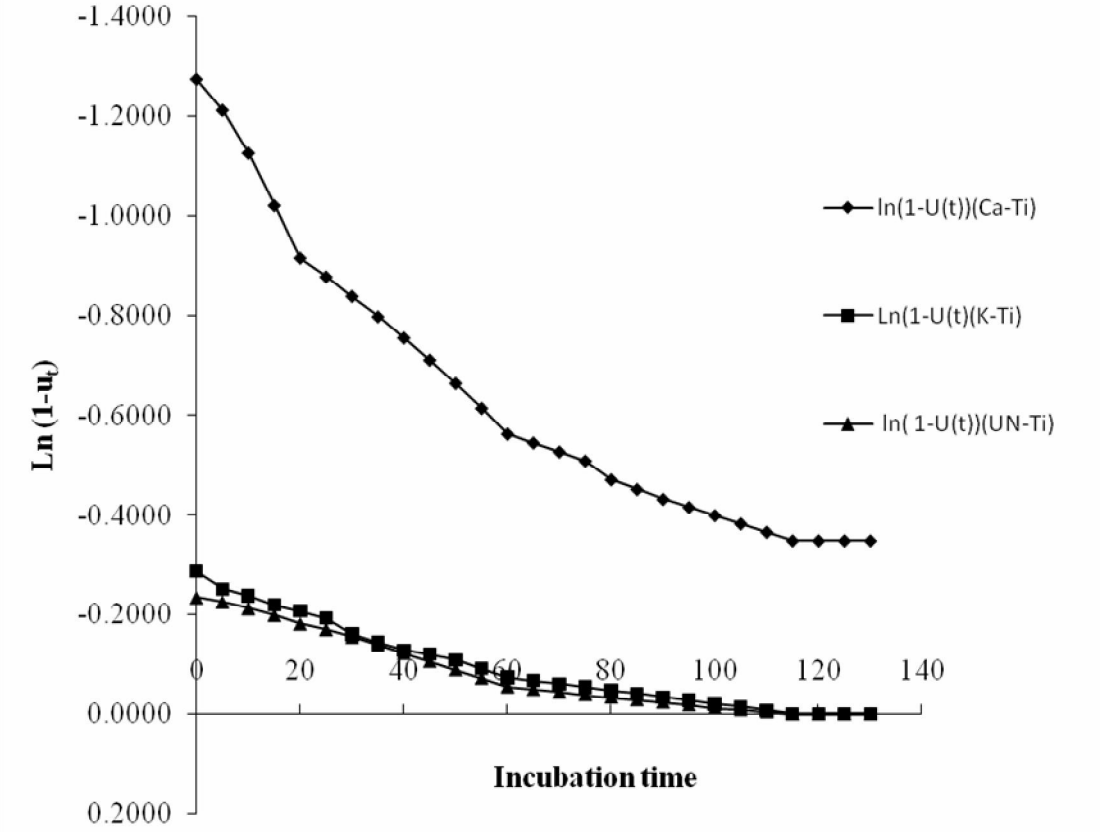


Figure 13: Bhattacharya /Venkobacharya plots for determining the effects of incubation time and pH on rate constants for the adsorption of mucin onto Un-Ti, Ca-Ti and K-Ti

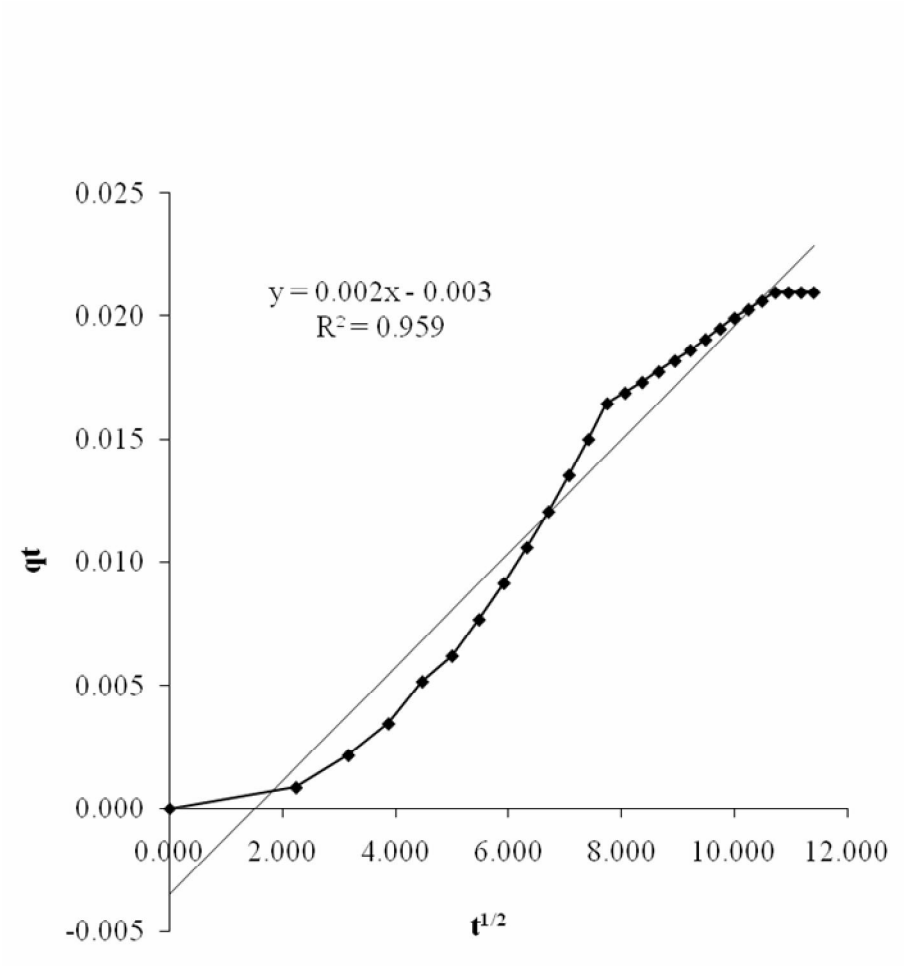


Figure14: Flick's plot for determining intra-particle diffusion rate constant for the adsorption of mucin onto Un-Ti

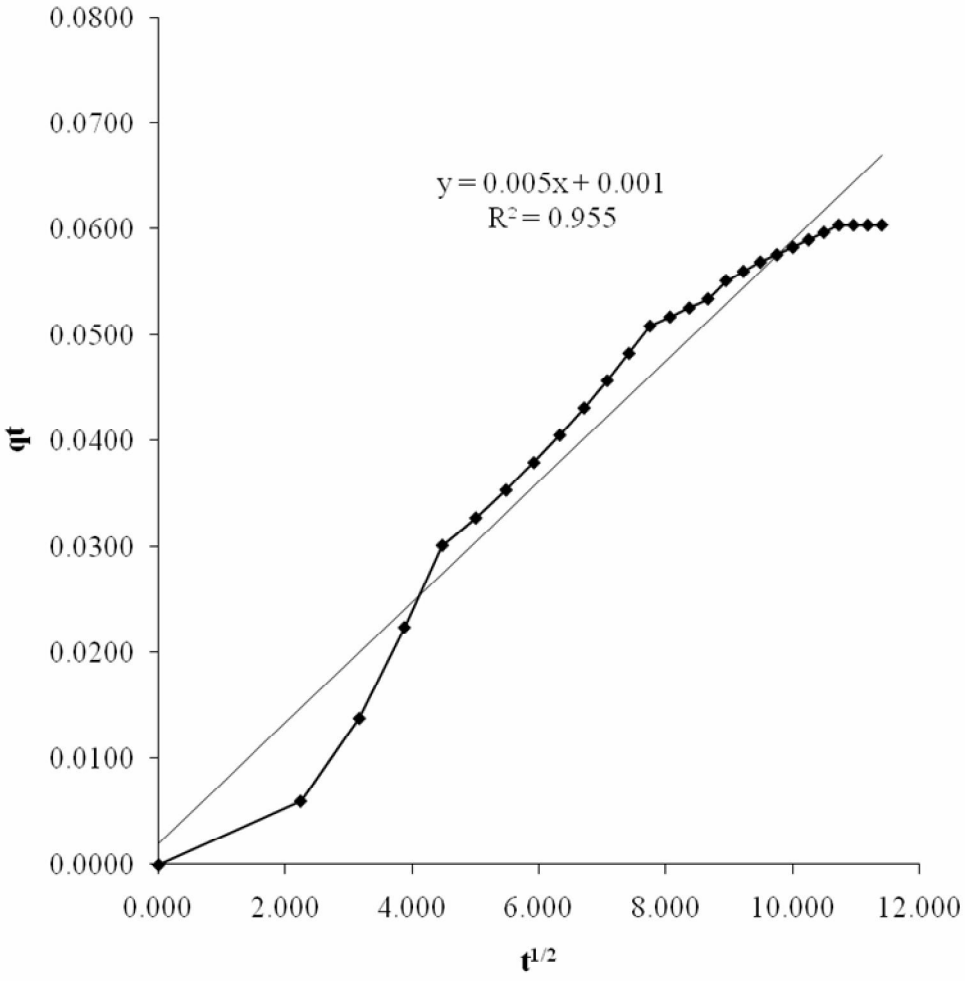


Figure 15: Flick's plot for determining intra-particle diffusion rate constant for the adsorption of mucin onto Ca-Ti

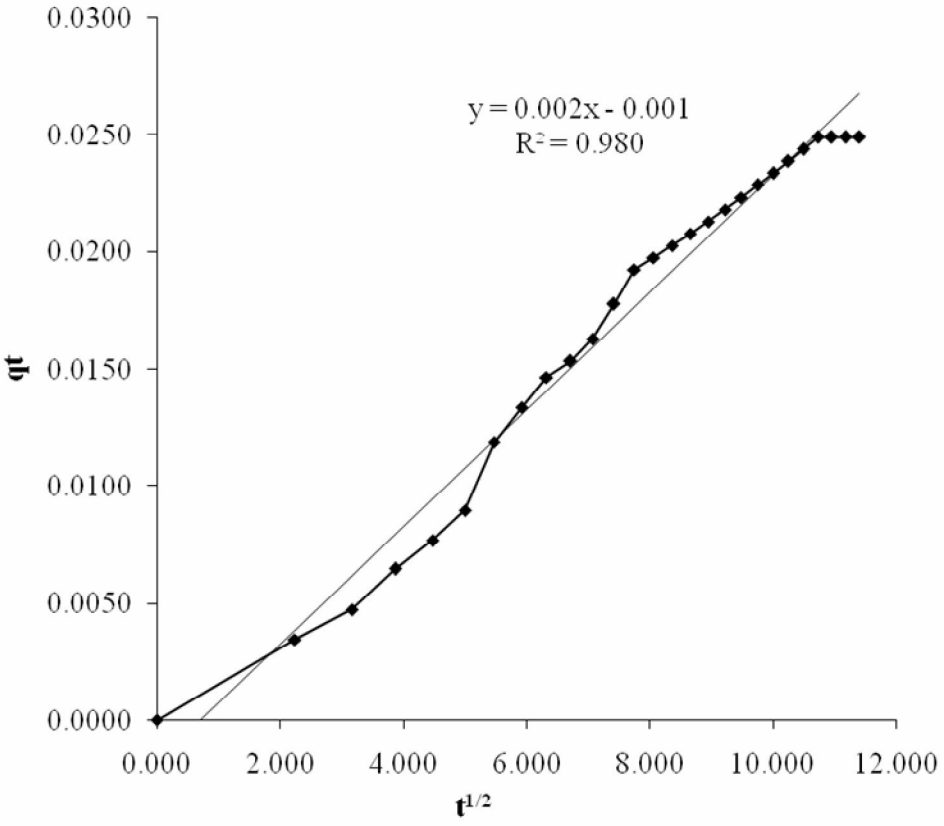


Figure 16: Flick's plot for determining intra-particle diffusion rate constant for the adsorption of mucin onto K-Ti

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

Lori and Nok (2004) had already observed that mucin adsorbed onto Ti surface. The aim of this work was to kinetically study the adsorption process of mucin onto the Ti surface. In order to arrive at this, amount of titanium, concentration of mucin solution, reaction temperature and pH were optimised by preliminary experiments.

The rates of adsorption were investigated for calcium treated titanium (Ca-Ti), potassium treated titanium (K-Ti) and untreated titanium (Un-Ti) metals.

The rate constants for calcium treated, potassium treated and untreated titanium metals were investigated using three models.

The results revealed that the adsorption is first order with respect to mucin molecules and that diffusion is the predominant mechanism for the adsorption process.

5.2 Conclusion

Based on the data obtained from these studies, the optimum values of reaction parameters including mass of Ti metal, concentration of mucin solution, pH and temperature respectively were obtained as 0.02 g, 0.1 mg/ml, 6.4 and 35 °C. The amount of mucin adsorbed at equilibrium from the initial mucin solution (mg/ml) onto Ca-Ti, K-Ti and Un-Ti surfaces were found to be 0.045±0.006, 0.017±0.003 and 0.014±0.00 (mg/ml), these being equivalent to 60 %, 24 % and 20 %, respectively. The rates of adsorption increased within the 1 hour of the adsorption process and thereafter began to decrease in a steady pattern till equilibrium was attained. The results showed that 84 %, 77 % and 70 % of total protein adsorbed at

equilibrium occurred at 1 hour of the adsorption process for Ca-Ti, K-Ti and Un-Ti respectively.

Adsorption rate constants values obtained using Lagergren first order rate equation were 3.45 x 10^{-2}, 2.99 x10^{-2} and 2.99 x10^{-2} (mg/ml min) for Ca-Ti, K-Ti and Un-Ti respectively.

Using Bhattacharya/Venkobacharya first order rate equation, adsorption rate constants values obtained were 4.606 x10^{-3}, 1.612 x10^{-2} and 4.606 x10^{-3} (mg/ml min).

Intra-particle diffusion rate constants values obtained using Flicks diffusion equation were 0.005, 0.001 and 0.001 mg/ml min for Ca-Ti, K-Ti and Un-Ti respectively.

These results revealed that the adsorption reaction is a first –order reaction with respect to the mucin molecules in solution and that the kinetic regimes described by protein adsorption curves shown the transport-limited and adsorption-limited regimes. During the initial stage of the adsorption, diffusion was the rate-limiting step at the short times. Following the sufficient adsorption to the surface, an adsorption limited process was the event. The amount of mucin adsorbed on Ca-Ti was 3 times that absorbed by the Un-Ti and that large amount of protein adsorbed occurred within 1 hour of the adsorption process. From these it can be concluded that much mucin is adsorbed onto calcium treated titanium but the adsorption is due to diffusion.

5.3 Recommendation

Titanium coated with hydroxyapatite and hydroxyapatite ceramics are important in a variety of oral biological events. Salivary proteins are selectively adsorbed on these implants and tooth. Since biological apatite is a major constituent of the hard tissues of bone and teeth, it is recommended that the optimised method be used to study the kinetics of adsorption of mucin *in vitro* on these materials.

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APPENDIX I (a)

BSA Calibration Curve

BSA (mg/ml)	Absorbance (595nm)
0	0.00
0.2	0.24
0.4	0.48
0.6	0.72
0.8	0.92

APPENDIX II

Optimisation of Mass of Ti Metal

Mass of Ti (g)	Bound mucin (mg/ml)
0.00	0.000
0.01	0.029
0.02	0.047
0.04	0.046
0.06	0.047
0.08	0.047
0.09	0.046
0.1	0.047

APPENDIX III

Optimisation of Concentration of Mucin Solution

Initial mucin (mg/ml)	Bound mucin (B) (mg/ml)
0.000	0.000
0.020	0.025
0.040	0.029
0.060	0.034
0.080	0.038
0.100	0.046
0.200	0.046
0.400	0.046
0.800	0.045
1	0.046

APPENDIX IV

Optimisation of Temperature of Mucin Solution

Temperature (°C)	Bound mucin (B) (mg/ml)
25	0.047
30	0.051
35	0.059
40	0.051
45	0.021

APPENDIX V

Optimisation of pH for the Adsorption of Mucin onto Ti

pH	Bound Mucin (mg/ml)
3	0.047
3.4	0.048
4	0.053
4.4	0.068
5	0.072
5.4	0.055
6	0.048
6.4	0.045
7	0.044
7.4	0.044
8	0.043

APPENDIX VI

Effect of Incubation Time and pH on Adsorption of Mucin onto Un-Ti (Rate of adsorption)

Time(min)	Bound mucin (mg/ml)	Rates =δ[C]/ δt (min□¹)
0	0.000	0.00000
5	0.0009	0.00017
10	0.0022	0.00022
15	0.0034	0.00023
20	0.0052	0.00026
25	0.0062	0.00025
30	0.0077	0.00026
35	0.0091	0.00026
40	0.0106	0.00027
45	0.0121	0.00027
50	0.0135	0.00027
55	0.0150	0.00027
60	0.0165	0.00027
65	0.0169	0.00026
70	0.0173	0.00025
75	0.0178	0.00024
80	0.0182	0.00023
85	0.0186	0.00022
90	0.0191	0.00021
95	0.0195	0.00021
100	0.0199	0.00020
105	0.0203	0.00019
110	0.0206	0.00019
115	0.0209	0.00018
120	0.0209	0.00017
125	0.0209	0.00017
130	0.0209	0.00016

APPENDIX VII

Effect of Incubation Time and pH on Adsorption of Mucin onto Ca-Ti (Rate of adsorption)

Incubation time (min)	Bound mucin (B) (mg/ml)	Rates = $\delta[C]/\delta t$ (min^{-1})
0	0.0000	0.0000
5	0.0060	0.0012
10	0.0138	0.0014
15	0.0224	0.0015
20	0.0302	0.0015
25	0.0328	0.0013
30	0.0353	0.0012
35	0.0379	0.0011
40	0.0405	0.0010
45	0.0431	0.0010
50	0.0457	0.0009
55	0.0483	0.0009
60	0.0509	0.0008
65	0.0517	0.0008
70	0.0526	0.0008
75	0.0534	0.0007
80	0.0552	0.0007
85	0.0560	0.0007
90	0.0569	0.0006
95	0.0576	0.0006
100	0.0583	0.0006
105	0.0590	0.0006
110	0.0597	0.0005
115	0.0603	0.0005
120	0.0603	0.0005
125	0.0603	0.0005
130	0.0603	0.0005

APPENDIX VIII

Effect of Incubation Time and pH on the Adsorption of Mucin onto the K-Ti

Incubation time (min)	Bound mucin (mg/ml)	Rate == δ[C]/ δt (min^{-1})
0	0.0000	0.00000
5	0.0034	0.00069
10	0.0047	0.00047
15	0.0065	0.00043
20	0.0077	0.00038
25	0.0090	0.00036
30	0.0119	0.00040
35	0.0134	0.00038
40	0.0147	0.00037
45	0.0153	0.00034
50	0.0163	0.00033
55	0.0178	0.00032
60	0.0192	0.00032
65	0.0197	0.00030
70	0.0203	0.00029
75	0.0208	0.00028
80	0.0213	0.00027
85	0.0218	0.00026
90	0.0223	0.00025
95	0.0228	0.00024
100	0.0234	0.00023
105	0.0239	0.00023
110	0.0244	0.00022
115	0.0249	0.00022
120	0.0249	0.00021
125	0.0249	0.00020
130	0.0249	0.00019

APPENDIX IX

Efficiency of 0.1 mg/ml mucin solution adsorbed on 0.02 g Ti

Type	qe(mg/ml)	% of total adsorbed at qe	Amount adsorbed at 1hr	% of the amount adsorbed at 1 hr
Un-Ti	0.0209	20.9	0.0150	71.8
Ca-Ti	0.0609	60.3	0.0509	84.4
K-Ti	0.0249	24.9	0.0192	77.1

APPENDIX X

Data Using Lagergren Equation for Determining Rate Constant for the Adsorption of Mucin onto Un-Ti

Incubation time (min)	Bound mucin (mg/ml)	qe -qt	log (qe-qt)
0	0.000	0.0209	-1.6799
5	0.0009	0.0200	-1.6981
10	0.0022	0.0187	-1.7271
15	0.0034	0.0175	-1.7582
20	0.0052	0.0157	-1.8033
25	0.0062	0.0147	-1.8329
30	0.0077	0.0132	-1.8785
35	0.0091	0.0118	-1.9295
40	0.0106	0.0103	-1.9873
45	0.0121	0.0088	-2.0540
50	0.0135	0.0074	-2.1328
55	0.0150	0.0059	-2.2291
60	0.0165	0.0044	-2.3532
65	0.0169	0.0040	-2.3976
70	0.0173	0.0036	-2.4470
75	0.0178	0.0031	-2.5029
80	0.0182	0.0027	-2.5670
85	0.0186	0.0023	-2.6422
90	0.0191	0.0018	-2.7332
95	0.0195	0.0014	-2.8486
100	0.0199	0.0010	-3.0060
105	0.0203	0.0006	-3.1929
110	0.0206	0.0003	-3.5279
115	0.0209	0.0000	0.0000
120	0.0209	0.0000	0.0000
125	0.0209	0.0000	0.0000
130	0.0209	0.0000	0.0000

Slope	Rate constant K_{ad} (mg/ml min)	R^2	Log of qe	Intercept
0.014	0.032242	0.940	-1.686	-1.487

APPENDIX XI

Data Using Lagergren Equation for Determining Rate Constant for the Adsorption of Mucin onto Ca-Ti

T incubation time(min)	Bound mucin (B mg/ml)	qe-qt	Log (qe-qt)
0	0.0000	0.0603	-1.2197
5	0.0060	0.0543	-1.2655
10	0.0138	0.0465	-1.3325
15	0.0224	0.0379	-1.4215
20	0.0302	0.0301	-1.5210
25	0.0328	0.0275	-1.5600
30	0.0353	0.0250	-1.6028
35	0.0379	0.0224	-1.6504
40	0.0405	0.0198	-1.7037
45	0.0431	0.0172	-1.7646
50	0.0457	0.0146	-1.8353
55	0.0483	0.0120	-1.9199
60	0.0509	0.0094	-2.0251
65	0.0517	0.0086	-2.0667
70	0.0526	0.0077	-2.1127
75	0.0534	0.0069	-2.1642
80	0.0552	0.0051	-2.2901
85	0.0560	0.0043	-2.3700
90	0.0569	0.0034	-2.4681
95	0.0576	0.0027	-2.5664
100	0.0583	0.0020	-2.6938
105	0.0590	0.0013	-2.8747
110	0.0597	0.0006	-3.1906
115	0.0603	0.0000	0.0000
120	0.0603	0.0000	0.0000
125	0.0603	0.0000	0.0000
130	0.0603	0.0000	0.0000

Slope	Rate constant K_{ad} (mg/ml min)	R^2	Log of qe	Intercept
0.015	0.034545	0.963	-1.122	-1.133

APPENDIX XII

Data Using Lagergren Equation for Determining Rate Constant for the Adsorption of Mucin onto K-Ti

Incubation time (min)	Bound mucin (mg/ml)	qe-qt	Log(qe-qt)
0	0.0000	0.0249	-1.6038
5	0.0034	0.0215	-1.6685
10	0.0047	0.0202	-1.6955
15	0.0065	0.0184	-1.7344
20	0.0077	0.0172	-1.7638
25	0.0090	0.0159	-1.7977
30	0.0119	0.0130	-1.8859
35	0.0134	0.0115	-1.9379
40	0.0147	0.0102	-1.9895
45	0.0153	0.0096	-2.0198
50	0.0163	0.0086	-2.0652
55	0.0178	0.0071	-2.1462
60	0.0192	0.0057	-2.2460
65	0.0197	0.0052	-2.2875
70	0.0203	0.0046	-2.3334
75	0.0208	0.0041	-2.3847
80	0.0213	0.0036	-2.4429
85	0.0218	0.0031	-2.5101
90	0.0223	0.0026	-2.5897
95	0.0228	0.0021	-2.6872
100	0.0234	0.0015	-2.8131
105	0.0239	0.0010	-2.9911
110	0.0244	0.0005	-3.2980
115	0.0249	0.0000	0.0000
120	0.0249	0.0000	0.0000
125	0.0249	0.0000	0.0000
130	0.0249	0.0000	0.0000

Slope	Rate constant K_{ad} (mg/ml min)	R^2	Log of qe	Intercept
0.013	0.0299	0.949	-1.604	-1.496

APPENDIX XIII

Data Using Bhattacharya/Venkobacharya Equation for Determining Rate Constant for the Adsorption of Mucin onto Un-Ti

Time(min)	Bound mucin (B) (mg/ml)	Ci-Ct	Ci-Ce	Ci-Ct/Ci-Ce	In (1-U(t)
0	0.000	0.1000	0 0.0791	1.2642	-0.2345
5	0.0009	0.0991	0.0791	1.2533	-0.2258
10	0.0022	0.0978	0.0791	1.2370	-0.2127
15	0.0034	0.0966	0.0791	1.2206	-0.1994
20	0.0052	0.0948	0.0791	1.1988	-0.1813
25	0.0062	0.0938	0.0791	1.1858	-0.1704
30	0.0077	0.0923	0.0791	1.1672	-0.1546
35	0.0091	0.0909	0.0791	1.1487	-0.1386
40	0.0106	0.0894	0.0791	1.1302	-0.1224
45	0.0121	0.0879	0.0791	1.1116	-0.1058
50	0.0135	0.0865	0.0791	1.0931	-0.0890
55	0.0150	0.0850	0.0791	1.0746	-0.0719
60	0.0165	0.0835	0.0791	1.0561	-0.0545
65	0.0169	0.0831	0.0791	1.0506	-0.0494
70	0.0173	0.0827	0.0791	1.0452	-0.0442
75	0.0178	0.0822	0.0791	1.0397	-0.0389
80	0.0182	0.0818	0.0791	1.0343	-0.0337
85	0.0186	0.0814	0.0791	1.0288	-0.0284
90	0.0191	0.0809	0.0791	1.0234	-0.0231
95	0.0195	0.0805	0.0791	1.0179	-0.0178
100	0.0199	0.0801	0.0791	1.0125	-0.0124
105	0.0203	0.0797	0.0791	1.0081	-0.0081
110	0.0206	0.0794	0.0791	1.0037	-0.0037
115	0.0209	0.0791	0.0791	0.9994	0.0006
120	0.0209	0.0791	0.0791	0.9994	0.0006
125	0.0209	0.0791	0.0791	0.9994	0.0006
130	0.0209	0.0791	0.0791	0.9994	0.0006

Slope	Rate constant K_{ad} mg/ml min	R^2	Intercept
0.002	0.004606	0.947	0.216

APPENDIX XIV

Data Using Bhattacharya/Venkobacharya Equation for Determining Rate Constant for the Adsorption of Mucin onto Ca-Ti

Incubation time (min)	Bound mucin (mg/ml)	Ci-Ct	Ci-Ce	Ci-Ct/Ci-Ce	In (1-U(t))
0	0.0000	0.1000	0.0397	2.5189	-0.9238
5	0.0060	0.0940	0.0397	2.3669	-0.8616
10	0.0138	0.0862	0.0397	2.1715	-0.7754
15	0.0224	0.0776	0.0397	1.9543	-0.6700
20	0.0302	0.0698	0.0397	1.7589	-0.5647
25	0.0328	0.0672	0.0397	1.6937	-0.5269
30	0.0353	0.0647	0.0397	1.6286	-0.4877
35	0.0379	0.0621	0.0397	1.5635	-0.4469
40	0.0405	0.0595	0.0397	1.4983	-0.4043
45	0.0431	0.0569	0.0397	1.4332	-0.3599
50	0.0457	0.0543	0.0397	1.3680	-0.3134
55	0.0483	0.0517	0.0397	1.3029	-0.2646
60	0.0509	0.0491	0.0397	1.2377	-0.2133
65	0.0517	0.0483	0.0397	1.2160	-0.1956
70	0.0526	0.0474	0.0397	1.1943	-0.1776
75	0.0534	0.0466	0.0397	1.1726	-0.1592
80	0.0552	0.0448	0.0397	1.1292	-0.1215
85	0.0560	0.0440	0.0397	1.1074	-0.1021
90	0.0569	0.0431	0.0397	1.0857	-0.0823
95	0.0576	0.0424	0.0397	1.0684	-0.0661
100	0.0583	0.0417	0.0397	1.0510	-0.0497
105	0.0590	0.0410	0.0397	1.0336	-0.0331
110	0.0597	0.0403	0.0397	1.0162	-0.0161
115	0.0603	0.0397	0.0397	0.9989	0.0011
120	0.0603	0.0397	0.0397	0.9989	0.0011
125	0.0603	0.0397	0.0397	0.9989	0.0011
130	0.0603	0.0397	0.0397	0.9989	0.0011

Slope	Rate constant K_{ad} (mg/ml min)	R^2	Intercept
0.007	0.016121	0.932	0.716

APPENDIX XV

Data Using Bhattacharya/Venkobacharya Equation for Determining Rate Constant for the Adsorption of Mucin onto K-Ti

Incubation time (min)	Bound mucin (B mg/ml)	Ci-Ct	Ci-Ce	Ci-Ct/Ci-Ce	Ln(1-U(t)
0	0.0000	0.1000	0.0751	1.3316	-0.2863
5	0.0034	0.0966	0.0751	1.2856	-0.2513
10	0.0047	0.0953	0.0751	1.2684	-0.2378
15	0.0065	0.0935	0.0751	1.2455	-0.2195
20	0.0077	0.0923	0.0751	1.2294	-0.2065
25	0.0090	0.0910	0.0751	1.2122	-0.1924
30	0.0119	0.0881	0.0751	1.1731	-0.1597
35	0.0134	0.0866	0.0751	1.1536	-0.1429
40	0.0147	0.0853	0.0751	1.1364	-0.1279
45	0.0153	0.0847	0.0751	1.1272	-0.1198
50	0.0163	0.0837	0.0751	1.1146	-0.1085
55	0.0178	0.0822	0.0751	1.0951	-0.0908
60	0.0192	0.0808	0.0751	1.0756	-0.0729
65	0.0197	0.0803	0.0751	1.0687	-0.0664
70	0.0203	0.0797	0.0751	1.0618	-0.0600
75	0.0208	0.0792	0.0751	1.0549	-0.0535
80	0.0213	0.0787	0.0751	1.0480	-0.0469
85	0.0218	0.0782	0.0751	1.0411	-0.0403
90	0.0223	0.0777	0.0751	1.0343	-0.0337
95	0.0228	0.0772	0.0751	1.0274	-0.0270
100	0.0234	0.0766	0.0751	1.0205	-0.0203
105	0.0239	0.0761	0.0751	1.0136	-0.0135
110	0.0244	0.0756	0.0751	1.0067	-0.0067
115	0.0249	0.0751	0.0751	0.9998	0.0002
120	0.0249	0.0751	0.0751	0.9998	0.0002
125	0.0249	0.0751	0.0751	0.9998	0.0002
130	0.0249	0.0751	0.0751	0.9998	0.0002

Slope	Rate constant K_{ad} (mg/ml min)	R^2	Intercept
0.002	0.004606	0.930	0.244

APPENDIX XVI

Data using Flick's Equation for Determining Rate Constant for the Adsorption of Mucin onto Un-Ti, Ca-Ti and K-Ti
Bound mucin (mg/ml)

$t^{1/2}$	Un-Ti	Ca-Ti	K-Ti
0.000	0.0000	0.0000	0.0000
2.236	0.0009	0.0060	0.003448
3.162	0.0022	0.0138	0.004741
3.873	0.0034	0.0224	0.0065
4.472	0.0052	0.0302	0.0077
5.000	0.0062	0.0328	0.0090
5.477	0.0077	0.0353	0.0119
5.916	0.0091	0.0379	0.0134
6.325	0.0106	0.0405	0.0147
6.708	0.0121	0.0431	0.0153
7.071	0.0135	0.0457	0.0163
7.416	0.0150	0.0483	0.0178
7.746	0.0165	0.0509	0.0192
8.062	0.0169	0.0517	0.0197
8.367	0.0173	0.0526	0.0203
8.660	0.0178	0.0534	0.0208
8.944	0.0182	0.0552	0.0213
9.220	0.0186	0.0560	0.0218
9.487	0.0191	0.0569	0.0223
9.747	0.0195	0.0576	0.0228
10.000	0.0199	0.0583	0.0234
10.247	0.0203	0.0590	0.0239
10.488	0.0206	0.0597	0.0244
10.724	0.0209	0.0603	0.0249
10.954	0.0209	0.0603	0.0249
11.180	0.0209	0.0603	0.0249
11.402	0.0209	0.0603	0.0249

APPENDIX XVII

Flick's intra-particle diffusion Kp (mg/ml), intercept and coefficient of determination, R^2 for the Adsorption of Mucin onto Un-Ti, Ca-Ti and K-Ti

Type	Kp (mg/ml min)	Intercept	R^2
Un-Ti	0.002	0.003	0.956
Ca-Ti	0.005	0.001	0.955
K-Ti	0.002	0.003	0.980

APPENDIX I (b)

BSA Calibration Curve

