The application of ESCA and SIMS to the study of saccharides, protein and the C. albicans cell wall
by Yong He

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University
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Abstract:
Polymers for permanent or temporal implantation in the human body are widely used in modern medicine. Infections associated with these polymers are frequently encountered. Candida albicans is one of the organisms associated with these infections. The attachment of C. albicans to polymer surfaces is an important step in the initiation of both superficial and deep-seated candidasis. Different methods have been used to examine the adhesion, but employing modern surface analytical instrument (ESCA, SIMS) for adhesion study has not been done previously and is employed in this study for the first time.

In order to use ESCA and static SIMS as surface analytical techniques to study the adhesion, a protocol for the interpretation of the resulting spectra must be developed. The objective of this study is to collect and interpret ESCA and SIMS spectra of model saccharides, protein and C. albicans cell wall, compare those spectra, and attempt to identify saccharides and protein constituent that contribute to SIMS spectra from the C. albicans cell wall.

Glucose, mannose, galactose, glucuronic acid, methyl α-D-mannopyranoside, cellobiose, maltose, mannan, glucan, glutamic acid and albumin were used as model saccharides and protein. ESCA analysis was performed on a Surface Science Instruments X-Probe ESCA instrument. SIMS analysis was performed on a PHI model 3700 static SIMS subsystem with a Balzers 511 quadrupole mass analyzer.

Stereoisomers looked identical in ESCA analysis, while a difference was seen in SIMS analysis. The positive ion SIMS spectra of C. albicans cell wall looked like the positive ion SIMS spectrum of mannose, while their negative ion SIMS spectra clearly indicated the. involvement of amino acids. The ESCA and SIMS spectra shown in this experiment were the first report of saccharides and C. albicans. The protocol for spectral interpretation described . in this study should be generally useful in ESCA and static SIMS saccharides, polysaccharides, saccharide acids and protein interpretation. This experiment contributed the fundamental work for further adhesion study.

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Yong He

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Chemical Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 1995

N378
H341

APPROVAL

of a thesis submitted by

Yong He

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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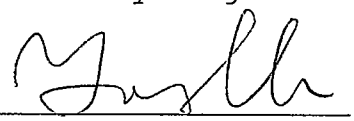
Signature 

Date 4/20/1995

ACKNOWLEDGMENTS

The author wishes to thank the faculty and staff of the Chemical Engineering Department at Montana State University and Chemical Engineering Department at University of Washington for their guidance and assistance.

Thanks are extended to my advisor Dr. Bonnie Tyler, Dr. John Sears and Dr. Diane Brawner for their advice, encouragement, and support throughout the course of this research and thesis preparation.

Thanks are also extended to Pat Schamberger, Kevin Siedlecki, Ace Baty, Wayne Marchwick and Erika Johnston for their assistance and support.

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ABSTRACT

Polymers for permanent or temporal implantation in the human body are widely used in modern medicine. Infections associated with these polymers are frequently encountered. *Candida albicans* is one of the organisms associated with these infections. The attachment of *C. albicans* to polymer surfaces is an important step in the initiation of both superficial and deep-seated candidasis. Different methods have been used to examine the adhesion, but employing modern surface analytical instrument (ESCA, SIMS) for adhesion study has not been done previously and is employed in this study for the first time.

In order to use ESCA and static SIMS as surface analytical techniques to study the adhesion, a protocol for the interpretation of the resulting spectra must be developed. The objective of this study is to collect and interpret ESCA and SIMS spectra of model saccharides, protein and *C. albicans* cell wall, compare those spectra, and attempt to identify saccharides and protein constituent that contribute to SIMS spectra from the *C. albicans* cell wall.

Glucose, mannose, galactose, glucuronic acid, methyl α-D-mannopyranoside, cellobiose, maltose, mannan, glucan, glutamic acid and albumin were used as model saccharides and protein. ESCA analysis was performed on a Surface Science Instruments X-Probe ESCA instrument. SIMS analysis was performed on a PHI model 3700 static SIMS subsystem with a Balzers 511 quadrupole mass analyzer.

Stereoisomers looked identical in ESCA analysis, while a difference was seen in SIMS analysis. The positive ion SIMS spectra of *C. albicans* cell wall looked like the positive ion SIMS spectrum of mannose, while their negative ion SIMS spectra clearly indicated the involvement of amino acids. The ESCA and SIMS spectra shown in this experiment were the first report of saccharides and *C. albicans*. The protocol for spectral interpretation described in this study should be generally useful in ESCA and static SIMS saccharides, polysaccharides, saccharide acids and protein interpretation. This experiment contributed the fundamental work for further adhesion study.

INTRODUCTION

Polymers for permanent or temporal implantation in the human body have become more widely used in modern medicine. Infections associated with these polymers are frequently encountered, particularly in skin-penetrating devices [1]. These infections have proved very difficult to eradicate without the removal of the device. *Candida albicans* is one of the organisms associated with these infections [2,3]. The attachment of *Candida albicans* to various polymer surfaces is an important step in the initiation of both superficial and deep-seated candidiasis.

Problems In This Area

Although polymers are being used for tissue substitution and intravascular devices with increasing frequency, few studies on the adhesion of *C. albicans* to polymer surfaces have been reported [4]. The few studies previously reported primarily used macroscopic techniques such as microscopy to study cell adhesion. In one study, the adhesion of *C. albicans* was examined microscopically after colonized catheters were removed from patients to obtain information on the morphology of the attached fungi [5]. In another study, attached Candida species were examined quantitatively and were

shown to adhere to polyvinyl chloride and Teflon catheters in large numbers *in vitro* [6]. However, there have been few studies employing modern surface analytical methods to study cell adhesion.

Because adhesion is governed by the molecular structure of the outermost region of a solid surface, it is not enough to obtain elemental information [7]. A surface analysis technique with high sensitivity and lateral resolution that can provide detailed molecular information about surface structures is required. Concerning adhesion, there are several analytical questions that need to be answered: What is the nature, concentration, and location of all atomic and molecular species present in the surface region? Knowing the answers to these questions is an indispensable prerequisite for the controlled modification of the "molecular architecture" of the surface.

To answer these questions, researchers must consider the unambiguous identification of unknown surface species (atoms as well as molecules or molecular clusters); the quantification of these surface species (i.e., information on the relative or absolute coverage by these species), and the location of these surface species (i.e., information on their lateral and depth distributions). The identification should be universal; it should be possible to identify all types of elements and molecular species. Quantification should be possible, even for very low surface concentrations, and

locating species should be possible with high lateral and depth resolution. In addition, all types of materials and all sample shapes should be accessible.

Because most materials involved in fungal adhesion are organic in origin, the following techniques are important in this regard: X-ray photoelectron spectroscopy (XPS), also known as electron spectroscopy for chemical analysis (ESCA), surface mass spectrometry (MS), and scanning tunneling microscopy (STM) and its related counterpart, atomic force microscopy (AFM) [8].

In ESCA, information on the elemental composition of the uppermost atomic layers is obtained with sensitivities down to 0.1% of a monolayer [7]. Important information on the chemical environment of the identified elements is supplied by the chemical shift (i.e., the influence of the chemical environment on the exact energy of the emitted photoelectrons). However, identification of unknown complex molecules by this chemical shift is not possible, and the achievable lateral resolution is limited to a few micrometers. STM and AFM allow lateral resolution in the sub-nanometer range so that single atoms can be probed. However, these techniques cannot be used to identify unknown surface species [7].

Motivation of This Work

The most important feature of surface Mass Spectrometry

(MS), in addition to high sensitivity, is its ability to provide detailed molecular information and information at shallow depths. It allows the identification and quantification of all elements, isotopes, and molecular species [9]. Therefore, surface MS is an excellent technique for surface analysis. As in any MS technique, the quality and reproducibility of the data require that several criteria be met: controlled desorption of atoms and molecular species, efficient ionization of these desorbed particles, and unambiguous identification of the generated ions by their charge/mass ratios. A considerable fraction of molecular surface species should survive these processes without fragmentation. It has been shown that, static secondary ion mass spectrometry (static SIMS) meets these criteria and is well suited for elemental and molecular applications [10].

Static SIMS has been widely applied to structural characterization of polymers by using fingerprint spectra or fragments characteristic of the backbone and pendant groups. This includes the characterization of the surface chemistry of polymers of biomedical importance [11,12]. Using this powerful technique for the study of the interaction of polymers with biological molecules and systems has also been studied by Mantus and co-workers. They demonstrated that static SIMS could be used to analyze proteins adsorbed to biomaterial surfaces, and established a spectral interpretation protocol by examining homopolymers of 16 amino

acids [13].

In order to use static SIMS and ESCA as a surface analysis technique to study the adhesion of *C. albicans* to protein coated polymer surfaces, a system consisting of large biomolecules, such as proteins and polysaccharides, a protocol for the interpretation of the resulting spectra must be developed.

Objectives

The main purpose of this experiment is to demonstrate that static SIMS and ESCA can be used to identify saccharides and proteins in fungal films. The objectives are:

(1) Collect and interpret ESCA and static SIMS spectra of model saccharides and proteins. In this experiment, glucose, mannose, galactose, glucuronic acid and methyl α-D-mannopyranoside were used as model monosaccharides. Cellobiose, maltose, mannan and glucan were used as model disaccharides and polysaccharides. Bovine serum albumin was used as the model protein.

(2) Collect and interpret ESCA and static SIMS spectra of *C. albicans* cell wall purified antigens and crude antigen.

(3) Compare the ESCA and static SIMS spectra of model compounds and *Candida* cell wall antigens, and attempt to identify saccharides and protein constituents that contribute to SIMS spectra from the *C. albicans* cell wall antigens.

LITERATURE SURVEY

Static SIMS

SIMS is the mass spectrometry of ionized particles which are emitted as secondary ions when a surface is bombarded by energetic primary ions with energies on the order of several KeV. These primary ions result in the emission of secondary particles characteristic of the chemical composition and structure in the uppermost layer. Most secondary particles are emitted as neutrals, whereas only a fraction 10^{-6} - 10^{-1} of the total are positively or negatively charged secondary ions. The secondary ions consist of molecular ions, atomic ions and cluster ions. It is the secondary ions which are detected and analyzed by a mass spectrometer, either a quadrupole or time of flight analyzer.

In static SIMS the primary ions are kept at a sufficiently low energy (5-25 KeV) to allow secondary ion emission from only the top few atomic layers of a surface. It is the mass spectrum of these secondary ions that provide a detailed chemical analysis of the surface.

Principles of Sputtering And Ion Formation

Figure 1 [7] schematically outlines the mechanism of SIMS according to the current understanding of this method. In the

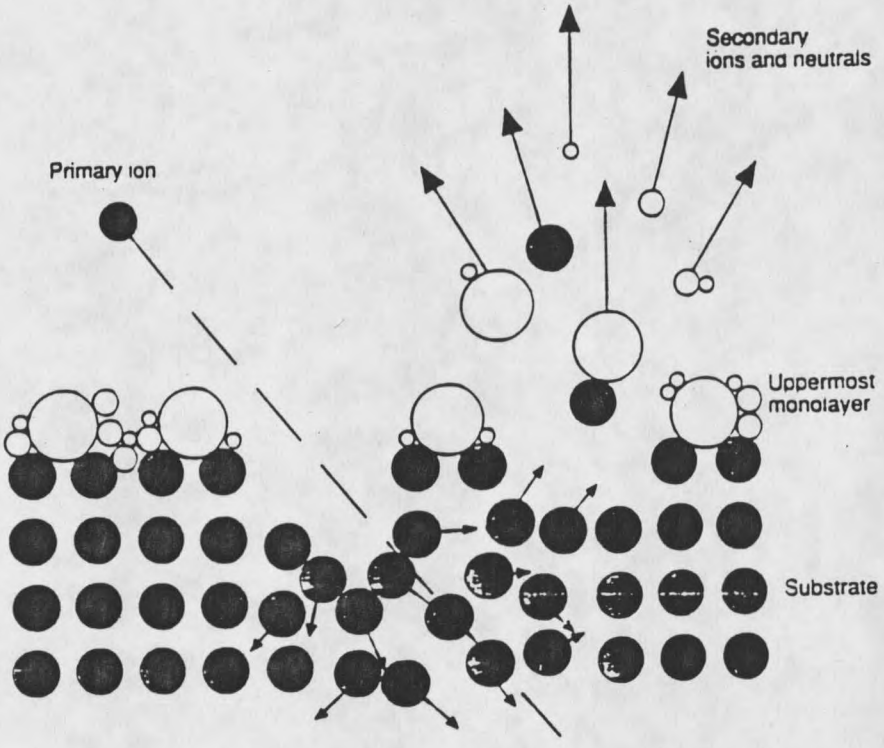

Figure 1. Particle emission from a surface after excitation with primary ions of KeV energy

case of ion bombardment, the energy of the primary beam, usually Xe^+ or Ar^+ ion beams, is transferred to the target by a collision, a billiard-ball-type process, also called "collision cascade", consisting of a cloud of target particles set in motion by the primary ions or by target particles. These primary ions and target particles are already moving. The dimension of the collision cascade depends on the energy and mass of the primary ion beam as well as the density and structure of the target material. For the bombardment of organic materials with 10-KeV Xe^+, typical values are 3 nm for the diameter of the collision cascade, and 15 nm for the depth

of the collision cascade, according to model calculations [10].

Most recoil particles have low energies, and only cascade particles from near-surface regions - with momenta directed toward the surface - can overcome the surface binding energy and thus leave the target (sputtered particles). SIMS, therefore, is a very surface-sensitive analytical technique (information depth is < 3 monolayers). The charge state of the sputtered particles depends largely on the chemical environment in the uppermost monolayer (matrix effect), which in general, prevents the direct quantification of SIMS results.

Ions originating from an elemental matrix can be positively or negatively charged, depending on their electron configurations in the outermost shell. The highest secondary ion yields (i.e., number of secondary ions X^q emitted from a surface species M per number of primary ions) of molecular ions are achieved from monolayers on noble metal substrates. Typical secondary ions are Me^+ or Me^-, $(M + H)^+$, $(M - H)^-$, $(M + Sa)^+$, and $(M + Me)^+$, where Me is a metal, M is a molecule, H is hydrogen, and Sa is either sodium or potassium. From bulk materials and thick layers, typically $(M + H)^+$ and $(M - H)^-$ and larger fragments can be obtained. To elucidate the fragmentation process, fragmentation rules that are known from electron impact mass spectrometry can be applied such as α-,β-cleavages; rearrangement processes.

Application of Static SIMS

The importance of static SIMS for surface analysis lies in the possibility of studying not only the elemental composition but also the chemical structure of surfaces. This is because the surface mass spectrum includes cluster ions as well as elemental ions. These ions directly reflect the surface chemistry of the sample [14,15].

There are two general areas of application of the static SIMS technique. The first application as a spectrometric method and the second as a surface analysis method. As a mass spectrometric method, static SIMS can be used to desorb and ionize biomolecules from specially prepared surfaces. The surface sensitivity of the static SIMS technique is not fully exploited in the purely mass spectrometric applications. As a surface analytical method, static SIMS is unrivaled in its molecular selectivity because of the groundwork provided by mass spectrometry. Static SIMS has been used to analyze a wide variety of "real" surfaces, ranging from semiconductor materials to complex copolymers. The spectra produced reflect the surface chemistry of the material but, in general, do not contain large ($m/z > 500$) molecular ions or fragments. However, useful information is readily extracted from the fragment ions in lower mass range. The ability of static SIMS to produce a surface-sensitive mass spectrum gives it great potential as a probe of proteins on surfaces.

SIMS Instrumentation

Vacuum Systems SIMS experiments are performed in high vacuum for two reasons: first, to avoid scattering of the primary and secondary beams; and second, to prevent interfering adsorption of gases on the surface under investigation.

Ion Gun For static SIMS, a broad beam source is used, with a diameter of 1 mm - 1 cm. Beam currents of 10^{-10} - 10^{-8}A are used, giving monolayer lifetimes > 100s. The beam energy lies between 500 eV and 5 KeV. The lower ion beam energies cause less disruption in the surface and sub-surface of the sample. Less disruption gives more confidence that the analysis is representative of the original surface, but also means a low sputter rate. High beam accelerating voltages give higher sputter rates, and also give higher beam currents from a given ion source. Higher values of beam energy therefore contribute to a more rapid analysis. The beam species must also be sufficiently heavy (m > 30), to produce efficient sputtering, and the inert gases argon and xenon are often chosen to preclude chemical modification of the surface.

Mass Spectrometers There are several common types of mass spectrometer: quadrupole mass spectrometers, magnetic sector mass spectrometers, and time-of-flight mass spectrometers. The one used for this thesis is the quadrupole

mass spectrometer.

A quadrupole mass spectrometer employs a combination of direct current (DC) and radio frequency (RF) potentials as a mass "filter". Mechanically, the quadrupole consists of four parallel rods arranged symmetrically. Ideally, these four rods should have the shape of a hyperbola in cross section so that idealized hyperbolic fields can be produced according to quadrupole theory. However, in practice, cylindrical rods are often used to approximate the hyperbolic-field requirements. Opposite rods (i.e., those diagonally opposite) are connected together electrically and to RF and DC voltage generators.

Ions are formed in ion source and expelled by a small electric (repeller) field. The ion current is continuous. The ions are accelerated but only to a rather low kinetic energy, about 5-15 KeV. They are then ejected along the center line between the four stainless steel rods. The ions would pass straight through to the detector at the far end if it had not been for the fact that the two pairs of opposite rods are connected, in parallel, to both a DC and an RF source. The DC voltage is kept at a constant fraction, about 16%, of the peak RF voltage. A given RF peak voltage then allows ions of only one integral mass to pass through the quadrupole - hence the name mass filter. A typical RF voltage range is a few hundred to a few thousand volts. The RF power supply has to be extremely stable while being able to deliver about 1 KW of power [9].

ESCA

Of all the contemporary surface characterization methods, electron spectroscopy for chemical analysis (ESCA) is the most widely used. ESCA is also called X-ray photoelectron spectroscopy (XPS), and these two names can be used interchangeably. The popularity of ESCA as a surface analysis technique is attributed to its high information content, its flexibility in addressing a wide variety of samples, and its sound theoretical basis [16].

Surface analysis by ESCA involves irradiation of the solid *in vacuo* with monoenergetic soft x-rays and sorting the emitted electrons by energy. Figure 2 [16] illustrates the ESCA experiment. A sample is irradiated with X-rays of known energy hν. This causes the sample to emit photoelectrons. These electrons are collected, passed through a hemispherical energy analyzer and counted. Some of these electrons will escape from the surface with no loss of kinetic energy and contribute to sharp peaks in the electron spectra. The spectrum obtained is a plot of the number of emitted electrons versus their binding energy. Each element has a unique elemental spectrum, and the spectral peaks from a mixture are approximately the sum of the elemental peaks from the individual constituents. The energies of these emitted electrons are characteristic of the compounds contained in the sample. The elements and the binding environment of elements in a sample are identified by the kinetic energy of these

photoelectrons. The intensity of the peaks can be used as a quantitative measure of the concentration of elements present in the sample.

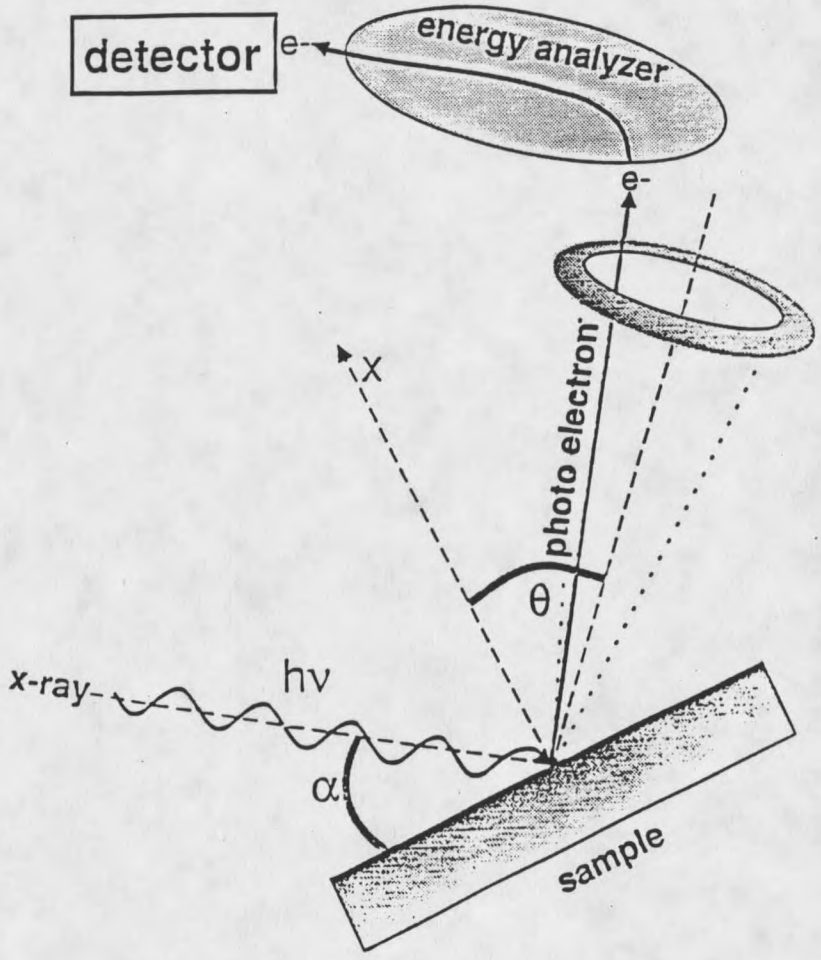

Figure 2. Cartoon illustration the principle of x-ray photoelectron spectroscopy. The sample is irradiated with x-rays and electrons are emitted from the sample. The energy of the electrons is then analyzed and electrons are counted by the detector.

Because the photoelectrons can only travel a short distance through the sample without undergoing an inelastic collision, ESCA is highly surface sensitive. Under typical

conditions, only the upper 50 Å to 100 Å of the sample is analyzed [16].

Principles of The Technique

The Photoelectric Effect Photoemission When a photon impinges upon an atom, one of three events may occur: (1) the photon can pass through with no interaction, (2) the photon can be scattered by an atomic orbital electron leading to partial energy loss, and (3) the photon may interact with an atomic orbital electron with total transfer of the photon energy to the electron, leading to electron emission from the atom. The third process accurately describes the photoemission that is the basis of ESCA. Total transfer of the photon energy to the electron is the essential element of photoemission.

No electrons will be ejected from an atom regardless of the illumination intensity unless the frequency of excitation is greater than or equal to a threshold level characteristic of each element. Thus, if the frequency (energy) of the excitation photon is too low, no photoemission will be observed. As the energy of this photon is gradually increased, at some value, the photoemission of electrons from the atom will be observed. Once the threshold frequency is exceeded, the number of electrons emitted will be proportional to the intensity of the illumination (i.e., once photon of sufficient energy were used to stimulate electron emission,

the more the photons bombarding the sample, the more photoelectrons will be produced). The kinetic energy of the emitted electrons is linearly proportional to the frequency of the exciting photons. If the photons have energy higher than the threshold value, the excess energy will be transmitted to the emitted electrons. The photoemission process from excitation to emission is extremely rapid (10^{-16} sec). The basic physics of this process can be described by the Einstein equation:

$$E_B = h\nu - KE \qquad (1)$$

where E_B is the binding energy of the electron in the atom (a function of the type of atom and its environment), $h\nu$ is the energy of the X-ray source (a known value) and KE is the kinetic energy of the emitted electron that is measured in the ESCA spectrometer. Thus, E_B, the quantity that provides valuable information about the photoemitting atom is easily obtained from $h\nu$ (known) and KE (measured).

Binding Energy Binding energies are commonly expressed in electron volts (eV; 1 eV = 1.6 x 10^{-19} joules). A negatively charged electron is bound to the atom by the positively charged nucleus. The closer the electron is to the nucleus, the more tightly bound. Binding energy will vary with the type of atom (i.e., a change in nuclear charge) and the addition of other atoms bound to that atom (bound atoms will

alter the electron distribution on the atom of interest). Different isotopes of a given element have different numbers of neutrons in the nucleus, but the same nuclear charge. Changing the isotope will not appreciably affect the binding energy. Weak interactions between atoms such as those associated with crystallization or hydrogen bonding do not alter the electron distribution sufficiently to change the binding energy. Therefore, the variations in the binding energy which provide the chemical information content of ESCA are associated with covalent or ionic bonds between atoms. These changes in binding energy are called "binding energy shifts" or "chemical shifts".

For gases, the binding energy of an electron in a given orbital is identical to the first ionization potential of that electron. In solids, the influence of the surface is present and additional energy must be accounted for removing an electron from the surface. This extra energy is called the work function ϕ_s and the Einstein equation now becomes:

$$E_B = h\nu - KE - \phi_s \qquad (2)$$

In this equation ϕ_s is the difference between the work function of the sample and the work function of the spectrometer. For a conducting sample, the sample can be grounded to the spectrometer and the ϕ_s will be sample independent and equal to the work function of the spectrometer. For an insulating sample, however, ϕ_s will be

sample dependent and an exact calculation of binding energy from the ESCA data is not possible. The contribution to the work function from the sample is typically only a few eV which is small enough that peaks can still be readily assigned. Absolute differences in binding energies for elements can be obtained, even for insulating samples, because ϕ_s is constant across the spectrum.

Binding energies for ESCA spectra of some polymers are commonly determined by shifting the spectra by a constant value based on a predefined binding energy for a reference peak. Some typical peak assignments are listed in Table 1 [16].

Table 1. Common ESCA Peak Assignments

C1s		O1s	
Binding Energy (eV)	Functional group	Binding Energy (eV)	Functional group
285.0	hydrocarbon **C**-H, **C**-C	532.2	carbonyl C=**O**, O-C=**O**
286.0	amine **C**-N	532.8	alcohol, ether C-**O**-H, C-**O**-C
286.5	alcohol, ether **C**-O-H, **C**-O-C	533.7	ester C-**O**-C=O
288.0	carbonyl **C**=O		
288.2	amide N-**C**=O		
289.0	acid, ester O-**C**=O		

Auger Electrons In addition to the photoelectrons emitted in the photoelectric process, Auger electrons are