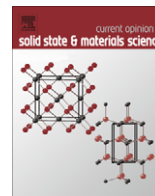




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Chemical labeling for quantitative characterization of surface chemistry

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ABSTRACT

The development and recent achievements of chemical labeling based surface characterization techniques are reviewed. Chemical labeling utilizes the specificity of chemical reactions to attach labeling molecules to surface functionalities, in order to facilitate the detection, identification and quantification of surface species. In this report, different chemical labeling based techniques are compared in terms of sensitivity, specificity and ease of operation. The two most widely used techniques, XPS derivatization and fluorescence labeling, are discussed in detail.

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1. Introduction

The identification of chemical composition on surfaces is of great interest for a variety of applications, including polymer surface characterization [*1,2,*3,4–11,*12], self-assembled monolayer (SAM) characterization [13–17,*18], surface wetting [19,20], protein adsorption [21,22], cell adsorption [21,23], and bio-sensors [24]. Traditional surface spectroscopic techniques such as infrared (IR) spectroscopy and XPS can detect concentrations as low as a few percent of a monolayer in favorable circumstances. However, the density of surface functional groups can be much less than a monolayer. In addition, IR and XPS do not detect functional group directly. For example, these techniques have difficulty distinguishing a C–O bond in an ester from a C–O bond in an alcohol. Moreover, IR also is not an intrinsically surface sensitive technique. It collects signal from a region many molecules thick close to the surface.

To overcome the drawbacks of traditional surface characterization techniques, chemical labeling based techniques have been widely used to identify and quantify surface functional groups [5,6]. In general, chemical labeling involves covalent attachment of labeling molecules to functional groups and characterization by techniques sensitive to the label molecules. Commonly employed characterization techniques include X-ray photoelectron spectroscopy (XPS) [4,25,26,*27,28,*29,*30], IR [31], ultraviolet-visible spectroscopy (UV–Vis) [*18,32], time-of-flight secondary ion mass spectrometry (ToF-SIMS) [33,34], electron spin resonance (ESR) [35–37], and fluorescence spectroscopy [8–11,38,*39]. Chemical labeling based techniques are more specific than tradi-

tional methods. Usually, a labeling molecule is chosen to only bond to one kind of functional group. These chemical labels are easier to detect than surface functional groups. Therefore, chemical label based techniques are more sensitive.

In this article, we will review these chemical labeling based surface characterization techniques and briefly discuss the pros and cons of different techniques in terms of sensitivity, specificity, accuracy and ease of operation.

Sensitivity is one of the most important factors in chemical labeling based techniques for surface characterization. Even a full monolayer, at most about 10^{15} species/cm², represents a small number of functional groups (~ 1 nano mole/cm²). However, in practice, the actual density to be detected could be much less than a monolayer. Sensitive chemical labeling based techniques, for example FLOSS (fluorescence labeling of surface species) [13,*18,38], can detect surface functional groups with densities as low as 10^9 /cm² [*18]. ToF-SIMS is a sensitive surface characterization technique but the application of ToF-SIMS for the detection of surface functional groups has a reported detection limit on the order of 10^{13} /cm² [33,34], similar to the reported sensitivity of XPS [9]. The sensitivity of chemical labeling based IR or UV–Vis depends on the cross-section of the chemical label. Usually, the detection limit will not be better than 10^{12} /cm² [*18,31]. Fluorescence spectroscopy is an extremely sensitive technique because it measures a signal on what is, in principle, a zero background. Other spectroscopic techniques like IR and UV–Vis measure a signal decrease on a large background signal, limiting the sensitivity. In principle, single molecule detection is possible for FLOSS.

The ease of operation is also an important factor for chemical labeling based techniques. Surface sensitive techniques, e.g. XPS

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and SIMS, need to be operated in UHV environment and the sample may be destroyed as a result of the measurement. Spectroscopic based techniques (fluorescence, IR and UV-Vis) do not require the vacuum environment necessary for XPS and SIMS and are in principle non-destructive. These features are critical when analyzing biological samples. In addition, by choosing a reversible labeling reaction, the initial surface can be recovered after chemical labeling.

The specificity and accuracy of chemical labeling are of critical importance. An ideal labeling reaction should have two characteristics. The first is that the labeling molecules should bond to only one kind of functional group, and not bond to other groups. Second, the reaction should be complete in a short time, leaving very few groups unlabeled. The degree of completion of labeling reactions may affect the accuracy of chemical labeling based techniques.

One of the challenges for chemical labeling based techniques is to translate the relative signal measured from the instrument to an absolute number of surface functional groups. The convenience and accuracy of the calibration process are critical for quantifying the functional group density.

It should be kept in mind that these chemical labeling based techniques are not suitable for analyzing high concentrations of surface functional groups, i.e. close to a full monolayer. The labeling molecule usually occupies more space than a small functional group. Therefore, functional groups that are close to each other will not be labeled completely. In these cases, chemical labeling based techniques may underestimate the concentration of surface functional groups. This effect is more critical for fluorescence labeling, since the fluorescence signal may be quenched if the fluorescent dyes interact with each other. The upper detection limit of chemical labeling based techniques depends on the size of labeling molecules.

There are two kinds of chemical labeling techniques. The first is direct characterization, which detects the chemical label bound to the surface directly. The second one, indirect characterization, is useful for situations where direct surface detection is not applicable. In such cases, for example, the chemical label is released from the surface to a solution and solution phase detection is performed. This procedure requires reversible chemical labeling reactions. Reversible reactions are also of interest for applications that require the recovery of the original surface after surface characterization.

In this review, we will focus on the two most widely used chemical labeling based surface characterization techniques: XPS derivatization and FLOSS.

2. XPS derivatization

X-ray photoelectron spectroscopy (XPS) has been widely used to determine the nature of functionalities on the surface of polymers [4,25,26,30,40–44], carbon fibers [26,45,46], and carbon blacks [*27–29]. While XPS mostly provides information about the atomic composition of the surfaces, it can also provide some details about the oxidation states of the elements. XPS is sensitive to functional groups located within the mean free path (~1 nm for electron energies in the range 10–500 eV) of the surface, typically much shorter than the X-ray penetration depth (>microns) [47]. The theory and practice of XPS is discussed in detail in a number of reviews [48] and textbooks [47,49]. However, obtaining quantitative information typically requires a deconvolution of peaks in XPS spectra, which decreases the reliability of the results [28,*29]. In addition, XPS cannot unambiguously identify functional groups. For example, while a peak might be interpreted as arising from a C atom bound by a single covalent bond to O, XPS data alone cannot distinguish whether this C–O is associated with an ether or an alcohol.

To overcome the ambiguity that might result from deconvolution, and to identify functional groups rather than chromophores, selective chemical derivatization (CD) of functional groups (Fig. 1) can be performed prior to XPS measurements [25,26,28,*29,44]. The determination of functional groups in this case is based on the detection of atoms present in the CD agent that are not present in the surface prior to CD [25,26,28,*29,44]. XPS was used to determine and quantify functional groups on polytetrafluoroethylene (PTFE) surfaces after radio frequency glow discharge (RFGD) treatment in ammonia, supposedly to improve hemocompatibility of PTFE vascular prostheses [41]. Benzaldehyde derivatives (chlorobenzaldehyde and bromosalicylaldehyde) and molecular bromine were used to label amino groups and carbon–carbon double bonds, respectively [41]. CD-XPS revealed increased concentration of amine surface groups as a result of the RFGD treatment [41].

Black carbons (BC) (wood chars and soot, diesel soot, hexane soot), which have found applications as sorbents due to their high specific surface area, are believed to contain many different kinds of oxygen functionalities (Fig. 1) [28,*29]. CD-XPS analysis of was performed on BC [28]. Carboxyls, carbonyls and hydroxyls were identified and their concentrations were determined [28]. Trifluoroacetic anhydride (TFAA), trifluoroethanol (TFE), and trifluoroethyl hydrazine (TFH) were used to label hydroxyl, carboxylic, and carbonyl groups, respectively (Fig. 2) [28]. The XPS signal from fluorine, present in the CD derivatives but not in the original black

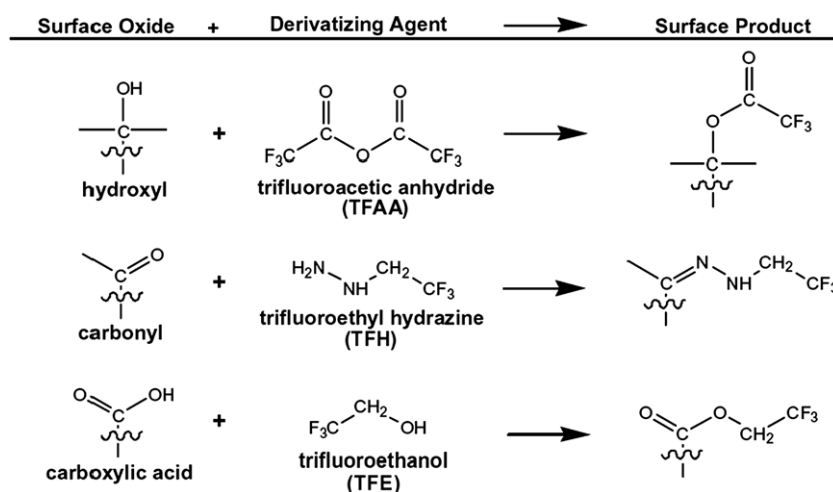


Fig. 1. Examples of chemical derivatization of hydroxyl, carbonyl, and carboxyl functional groups by fluorine containing derivatizing agents. Reprinted with permission from [29]. Copyright (2006) Elsevier.

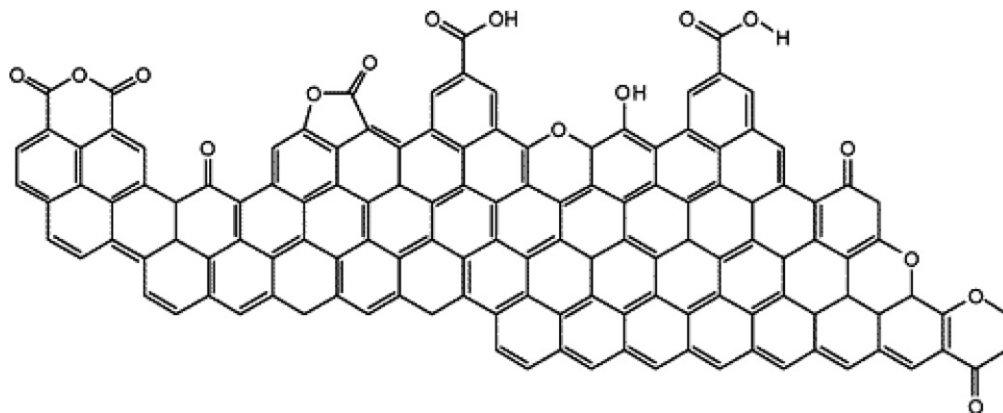


Fig. 2. Possible oxygen containing functionalities on the graphene sheet structure of black carbons. Reprinted with permission from [28]. Copyright (2006) American Chemical Society.

carbons, was used to determine the concentration of each functionality [28]. It was found that carboxyls are not the most abundant oxides, compared to hydroxyls and carbonyls. In addition, the results suggested that oxides, other than hydroxyls, carboxyls, and carbonyls, were present [28]. A comparison of XPS and energy dispersive spectroscopy, a bulk sensitive technique, showed that the concentration of oxides on the surface of black carbons was several times bigger (~ 5 times) than in the bulk [28].

A similar XPS labeling strategy was recently used to investigate the distribution of oxides on natural BC after oxidation treatments with hydrogen peroxide, ozone, nitric acid, and ammonium persulfate [29]. As-produced black carbons had significant oxide content ($\sim 12\%$) and ozone was the most aggressive oxidant compared to H_2O_2 , or HNO_3 . Interestingly, persulfate was found to be a highly selective oxidant, selectively increasing the concentration of carboxylic groups [29]. The detection limit of CD-XPS technique is estimated to be $\sim 10^{13}$ groups/cm² (10^{-10} mol/cm²) [9].

XPS CD has been used, together with voltammetric techniques, for quantification of carboxylic groups on the surface of SWCNTs and Multi Walled Carbon Nanotubes (MWCNTs), via emission of N1s electrons from nitrogen in the 4-Nitrophenol (4-NP) labels [50]. The same research group used XPS CD for the relative quantification of *ortho*- and *para*-quinone functionality on graphite, glassy carbon, and carbon nanotubes [51].

3. Fluorescence labeling of surface species (FLOSS)

Fluorescence labeling is a promising method for the detection and quantification of surface functional groups because of the high sensitivity of fluorescence spectroscopy. In addition, fluorescence spectroscopy is easy to operate, and can be applied *in situ*. It has been widely used in biological applications [52–60], polymer chemistry [2,3,5–10,39] and the study of self-assembled monolayers [13–17]. It has been demonstrated that the chemical labeling based fluorescence techniques are able to identify and quantify surface functionalities down to $\sim 10^9$ groups/cm² [18], which makes fluorescence based detection $\sim 10,000$ times more sensitive than the CD XPS methods [9,18]. The idea of using fluorescence labeling to study polymer surfaces was introduced in the 1980s by Whitesides [11]. Dansyl cadaverine was used to label carboxylic acid groups at the surface of acid-treated polyethylene (PE) and characterized by fluorescence spectroscopy [11].

The first paper, to our knowledge, that systematically described fluorescence labeling as a surface characterization technique was published in 1996, where the authors described four reaction schemes for the labeling of carboxylic acid, alcohol, ketone and amine groups on polyethylene (PE) surfaces [9]. The calibration

of fluorescence was done by a so called ‘depletion method’. Non-modified PE films were immersed in fluorescence dye solutions of different concentrations, allowing adsorption of dye molecules onto the films. Afterwards the PE films were washed in ethanol to remove the dye molecules on the surface. Fluorescence signals were collected from these samples before and after washing dye off the films and were compared to the fluorescence signals of the dyes washed off and measured in ethanol, the latter providing an absolute measure of the number of dye molecules removed [9]. However, it is difficult to obtain an accurate calibration curve by the ‘surface depletion method’ when the concentration transferred into solution is small.

In 2004, McArthur et al. extended fluorescent labeling for quantitative surface analysis to self-assembled monolayers (SAM) in their analysis of the photoreaction of octadecyltrichlorosilane (OTS) SAMs on silicon dioxide (Fig. 3) [38]. They employed a simple calibration method involving the deposition of known numbers of fluorescent dye molecules on an OTS SAM surface by a ‘drop and dry’ method. A known volume of diluted dye solution was dropped onto a defined area of the SAM surface and fluorescence measurements were taken. The fluorescence signals were plotted versus total volume of drops deposited on the surface (i.e. number of dye molecules), and show a linear behavior (inset of Fig. 3) suggesting that saturation is not an issue in the range explored. This calibration procedure is fast, accurate and easy to perform. It provides a simple relation between the fluorescence signal detected from the surface and the density of surface functional groups. Using this

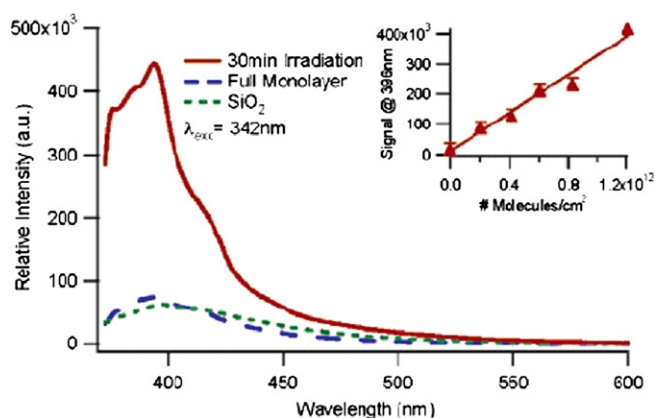


Fig. 3. Emission spectra of 1-pyrenemethylamine reacted with 30 min UV-irradiated ODS SAM (solid), unirradiated ODS SAM (dashed), and SiO₂ (dotted). Inset: calibration plot. Reprinted with permission from [38]. Copyright (2004) American Chemical Society.

technique, the concentrations of oxygen containing functionalities on the OTS SAM surface were measured as a function of UV irradiation time (Fig. 3) [38]. It was observed that number of oxygen containing functionalities increased as a function of the irradiation time, with aldehyde and carboxylic species being the predominant groups under their conditions. The results on total oxygen functionality correlated with the contact angle, IR and AFM measurements [13].

It should be noted that, although the calibration curves in Figs. 3 and 4 appear linear, this may not always be the case. As the fluorescence dye density increases on the surface, fluorescence quenching may become more significant, decreasing the fluorescence yield per molecule. From this point of view, it is important

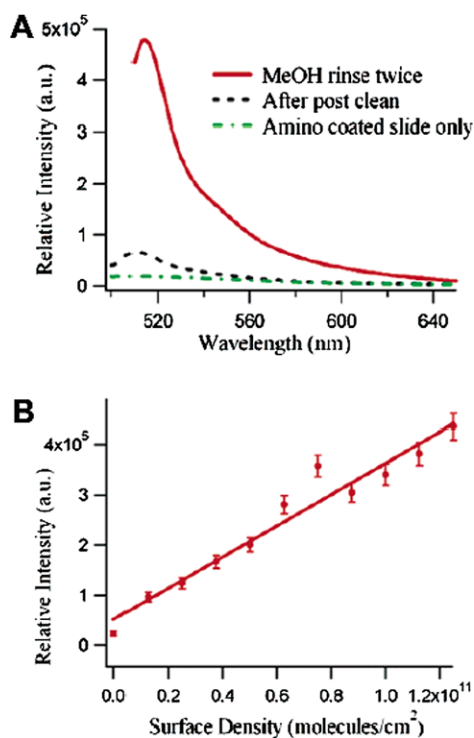


Fig. 4. (A) Fluorescence detection of two D2183-reacted amine coated slides (D2183 is a carboxyl terminated fluorescence probe provided by Invitrogen, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid). One sample is only rinsed in MeOH twice after the reaction (solid line), the other sample is subjected to the full post-reaction cleaning procedure (dashed line). The dashed-dotted line is the intrinsic fluorescence of the clean amine-coated slide. (B) Calibration curve. All samples were excited at 470 nm. Reprinted with permission from [18]. Copyright (2007) American Chemical Society.

to use calibration curves that explore a similar signal range as the FLOSS signal. As discussed earlier, quenching effects may limit the highest surface functional density that FLOSS can detect.

The specificity of FLOSS was studied by checking the interference of carboxyl group on the detection of aldehyde groups by amine modified dyes [18]. It was found that, after a proper post-cleaning procedure (rinsed twice with neat methanol and then sonicated successively in less polar solutions (CH_2Cl_2 , acetone, and hexane) for 10 min, respectively) to remove dye molecules weakly adsorbed on the surface, the number of carboxyl-modified dyes still bound to the surface was 0.36% of the number of aldehyde-modified dyes. This result indicates that the presence of carboxyl groups does not affect significantly the detection of aldehyde groups (Fig. 4) [18]. By choosing appropriate dyes, FLOSS can be applied to variety of surfaces, including polymer, silicon, glass, carbonaceous materials and particles.

3.1. FLOSS of carbon materials

FLOSS has been applied to carbonaceous materials, for example, carbon fibers [61], and single-walled carbon nanotubes (SWCNTs) [62]. The fluorescent active moieties act as “beacons” upon covalent attachment to the specific functional groups on the surface (Fig. 5) [62].

FLOSS on carbon fibers was performed using 1-naphthaleneethanol, 1-pyrenemethylamine, and triphenylmethylchloride to label carboxylic, carbonyl, and hydroxyl groups, respectively, revealing low concentrations ($\sim 10^{12}$ groups/ cm^2) of CHO and CO_2H groups, while the hydroxyl groups were below the detection limit ($\sim 10^{10}/\text{cm}^2$) [61]. It is worth noting, that contrary to the Boehm titration analysis, FLOSS detects primarily oxygen containing functionalities on external carbon fiber surfaces, and molecularly accessible pores rather than in pores smaller than FLOSS probes (i.e. < 1.5 nm). FLOSS provides knowledge of the surface functionality which is a key to applications such as the dispersion of carbon fibers for the formation of composites. Differences between FLOSS and Boehm titration will occur when materials contain pores that might be too small to be accessible for FLOSS label molecules [61]. Boehm titration does not differentiate between oxygen groups on the external surface, in molecularly large or small pores [61].

FLOSS experiments on SWCNTs used dansyl hydrazine (DH), panacyl bromide (PB), and 5-DTAF to label carbonyls, carboxyls, and hydroxyls, respectively [62]. The results of FLOSS on SWCNTs were consistent with the Boehm titration results, implying that all functional groups on SWCNTs are accessible to the FLOSS fluorophores [62]. According to FLOSS, even as-received arc-produced SWCNTs (which are supposed to be almost defect free) have $> 0.6\%$ content of total oxygen containing functional groups. The more aggressive the oxidative purification technique used, the

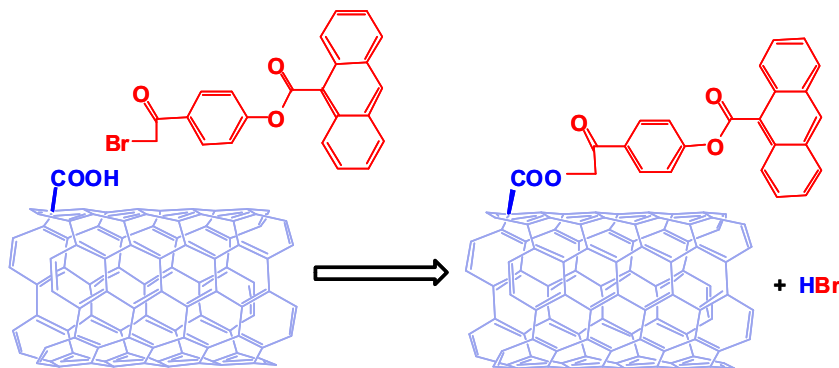


Fig. 5. Fluorescence labeling of carboxylic groups on single-walled carbon nanotubes by panacyl bromide.

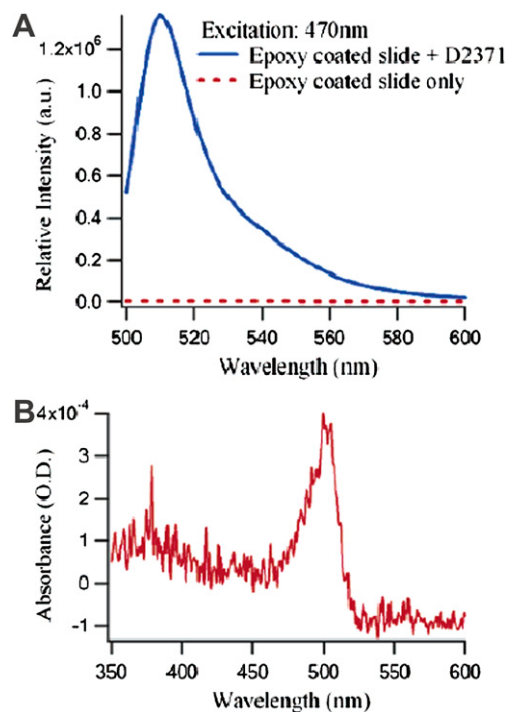


Fig. 6. Fluorescence detection (A) and UV-Vis detection (B) of D2371-reacted glass slide E (epoxy-coated). Reprinted with permission from [18]. Copyright (2007) American Chemical Society. (D2371 is an aldehyde reactive fluorescence probe provided by Invitrogen, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid, hydrazide.)

greater the concentrations of oxygen-containing functionalities on SWCNTs. Somewhat surprisingly, carbonyls are the most abundant oxygen containing functionalities on HNO₃ treated SWCNTs, suggesting that the chemical modifications of SWCNTs should include the chemistry of carbonyls as well as that of carboxyls.

4. Other chemical labeling based surface characterization techniques

Absorption spectroscopy techniques like UV-Vis can be used to determine the concentration of surface bound chemical labels [18,32]. One advantage is that UV-Vis spectrometers are routine instruments and can be accessed easily. The operation procedure for UV-Vis is relatively simple compared to other techniques. Furthermore, the signal measured can be translated to absolute concentration value based on the Beer-Lambert law, so no complicated calibration procedure is needed. However, sensitivity of UV-Vis spectroscopy is limited as illustrated in Fig. 6 [18].

Similar to XPS, SIMS is also a sensitive surface characterization technique. There are a few reports using chemical labeling based ToF-SIMS to quantify the concentration of carboxyl group and amine group polymer surfaces [33,34]. The detection limit is estimated to be 10¹³/cm² [33,34]. However, we are not aware of any systematic study of chemical labeling based ToF-SIMS techniques, probably due to the lack of access to this instrument and complicated operating and calibration procedures. As a result, the detection limit may not be fully optimized.

Electron spin resonance (ESR) of chemically bound spin-labels has been applied to the quantitative study of functional group density [35–37]. ESR active spin labels are chemically attached to functional groups and the ESR signal is measured. Similar to SIMS, chemical labeling based ESR characterization has not been fully explored.

5. Summary

We have reviewed most of the commonly used chemical labeling based surface characterization techniques with a focus on XPS and fluorescence characterizations. We discussed the advantages and limitations of each technique. Among all of the characterization techniques, FLOSS is probably the most promising one because of its high sensitivity, ease of operation, and in situ applicability. However, more systematic work needs to be done to investigate the specificity and accuracy of chemical labeling reactions. As fluorescence spectroscopy is in principle capable of single molecule detection, more effort is needed to reach this limit of sensitivity and even the development of FLOSS based chemical microscopy.

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