การวิเคราะห์ลิปสติกด้วยเอทีอาร์เอฟทีไออาร์ไมโครสเปกโทรสโกปี

นางสาวประพิมพรรณ ทรัพย์เมือง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2549

ANALYSIS OF LIPSTICKS BY ATR FT-IR MICROSPECTROSCOPY

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2006

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ประพิมพรรณ ทรัพย์เมือง: การวิเคราะห์ลิปสติกด้วยเอทีอาร์เอฟทีไออาร์ไมโครสเปกโทรสโกปี (ANALYSIS OF LIPSTICKS BY ATR FT-IR MICROSPECTROSCOPY) อ. ที่ปรึกษา: รศ.ดร. สนอง เอกสิทธิ์, อ. ที่ปรึกษาร่วม: พันตำรวจเอกธาดา ตั้งศรีสุข, 115 หน้า.

อุปกรณ์ไมโครไออาร์อีชนิดใหม่แบบสไลด์ที่ใช้เจอร์มาเนียมและเพชรที่เจียระไนแล้วเป็นหัวตรวจวัด อุปกรณ์นี้ถูกนำมาใช้งานร่วมกับกล้องจุลทรรศน์อินฟราเรดเพื่อศึกษาสมบัติทางเคมีของคราบลิปสติกบนพื้นผิว เนื่องจากไมโครไออาร์อีชนิดใหม่มีพื้นที่ในการตรวจวิเคราะห์ขนาดเล็กจึงสามารถนำมาใช้ศึกษาสารตัวอย่างที่มีปริมาณน้อยมากๆ ได้ แถบการดูดกลืนที่ได้จากการตรวจวิเคราะห์ด้วยอุปกรณ์ไมโครไออาร์อีชนิดใหม่มีประสิทธิภาพเหมือนกับอุปกรณ์เพิ่มเติมเอทีอาร์ที่มีขายเชิงพาณิชย์ งานวิจัยนี้ศึกษาลิปสติกที่มีจำหน่ายโดยทั่วไปจากบริษัทเครื่องสำอางต่างๆ แถบการดูดกลืนที่ได้จากเทคนิคนี้มีความสัมพันธ์โดยตรงกับโครงสร้างทางโมเลกุล และองค์ประกอบทางเคมีของสารตัวอย่าง จึงสามารถใช้แถบการดูดกลืนที่ได้ไปใช้ในการระบุลักษณะเฉพาะของลิปสติก เทคนิคคอนแทคแอนด์คอลเลกถูกนำมาประยุกต์ในการวิเคราะห์ลิปสติกที่อยู่บนพื้นผิวรองรับ เทคนิคนี้สามารถวิเคราะห์ลิปสติกที่มีปริมาณน้อยมากๆ ได้ และยังให้ข้อมูลทางเคมี และองค์ประกอบทางเคมีของลิปสติกโดยไม่มีอิทธิพลของพื้นผิวรองรับชนิดต่างๆ ต่อการวิเคราะห์ลิปสติก เทคนิคนี้สามารถตรวจวิเคราะห์โดยไม่ต้องเตรียมตัวอย่างและไม่ทำลายตัวอย่าง จึงสามารถนำเทคนิคนี้ไปใช้ในการพิสูจน์เอกลักษณ์ของลิปสติกที่มีปริมาณน้อยมากๆ ได้ และสามารถนำข้อมูลที่ได้ไปประยุกต์ใช้ในเชิงนิติวิทยาศาสตร์

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต ประพิมพรรณ ทรัพย์เมือง
ปีการศึกษา 2549 ลายมือชื่ออาจารย์ที่ปรึกษา
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

##4872353223: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEYWORD: TRACE LIPSTICK SMEARS / ATR FT-IR MICROSPECTROSCOPY / FORENSIC ANALYSIS.

PRAPIMPAN SUPMUANG: ANALYSIS OF LIPSTICK BY ATR FT-IR MICROSPECTROSCOPY. THESIS ADVISOR: ASSOC. PROF. SANONG EKGASIT, Ph.D., CO-ADVISOR: POL. COL.THADA TANGSRISUK, 115 pp.

The slide-on Ge μIRE and the slide-on diamond μIRE for ATR FT-IR spectral acquisition with an infrared microscope were employed for molecular characterization of trace lipstick smear on various surfaces. Due to the small sampling area of the IREs, a small quantity of lipsticks can be analyzed. The spectra acquired by diamond μIRE and Ge μIRE were in good agreement to those acquired by conventional ATR technique. In this work, commercial lipsticks from various manufactures were investigated. Direct correlation between the molecular structures and the infrared absorptions enable the identification of lipstick based on the observed ATR FT-IR spectra of the trace lipstick smear. The *"contact-and-collect"* operation was employed for transferring the trace lipstick onto the tip of the IREs. A minute smear of lipstick on the μIRE can be analyzed while the chemical information of lipsticks on various substrates was obtained without any interference from the substrate. The operation was non-destructive and could be performed without an additional sample preparation. This method is suitable for the unique identification of minute lipstick amount as well as forensic analysis.

Field of student...Petrochemistry and polymer science...Student's signature...ประพิมพ์พรรณ ทรัพย์เมือง
Academic Year...2006...Advisor's signature...
Co-advisor's signature...

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Associate Professor Dr. Sirirat Kokpol, Pol. Col. Thada Tangsrisuk, Associate Professor Dr. Supason Wanichweacharungruang, Assistant Professor Dr. Varawut Tangpasuthadol for the insightful suggestions and contribution as thesis committees.

I also would like to acknowledge Associate Professor Chuchaat Thammacharoen and Dr. Pimthong Thongnopkun for guidance, suggestion, encouragement during my entire study.

I am thankful to Mrs. Nuchsara Daokrajang and Mr. Att Poojang for the lipstick samples and substrate samples and my friends and colleagues at the Sensor Research Unit the everlasting friendship throughout the time of study.

Above all, I am profoundly grateful to my wonderful parents and the endearing family for their patient love, perpetual encouragement, and overwhelming support.

Finally, this thesis would never be successfully completed without the excellent advice from my thesis advisor, Associate Professor Dr. Sanong Ekgasit, who always provides me the useful guidance, suggestion, encouragement, and understanding. He also patiently trained my technical skill during the whole research.

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LIST OF ABBREVIATIONS

ATR	: attenuated total reflection
FT-IR	: Fourier transform infrared
Ge	: germanium
IRE	: internal reflection element
MCT	: mercury cadmium telluride
MSEF	: mean square electric field
μm	: micrometer
PET	: polyethylene terephthalate
PP	: polypropylene
S/N	: signal-to-noise
Si	: silicon
TIR	: total internal reflection
ZnSe	: zinc selenide

LIST OF SYMBOLS

μ : micrometer (10^{-6})

A : absorbance

I : intensity of incident radiation

I_A : intensity of absorbed radiation

I_R : intensity of reflected radiation

I_S : intensity of scattered radiation

I_T : intensity of transmitted radiation

θ : angle of incidence

θ_C : critical angle

v : wavenumber

R : reflectance

d_p : penetration depth

CHAPTER I

INTRODUCTION

1.1 Forensic Analysis

Forensic analysis may generally be defined as the application of scientific technique, or other specialized knowledge to assist courts in resolving question of fact in civil and criminal trials. Important evidences for identification are sometimes found at the crime scene such as fingerprint, footprint, blood, hair, paint chips, fibers, stain, and etc. The investigation of the evidences can be fundamental in resolving a criminal act and can frequently provide useful information about linkage between a victim and a suspect. Forensic evidence is also used to link crimes that are thought to be related to one another. For example, DNA evidence can link one offender to several different crimes (or exonerate the accused). Linking crimes helps law enforcement authorities to narrow the range of possible suspects and to establish patterns for crimes, which are useful in identifying and prosecuting suspect. The lipstick smear may deposit on the forensic evidence as a result of the attack or an unintentional contact. A minute amount of lipstick smears are sometimes found on the different surfaces such as smooth, rough, porous, and non-porous surface.

1.2 Lipstick

Lipstick is a cosmetic product that uses for the lip and is variety in color. The primary ingredients found in lipstick are waxes, oils, alcohols, and pigments. The wax uses to prepare a lipstick base usually involves some combination of beeswax, candelilla wax, and carnauba wax. Wax enables the mixture to be formed into the easily recognized shape of the cosmetic [1]. Oils such as mineral oil, castor oil, lanolin oil, or vegetable oil are added to the wax. Fragrance and pigment are also added as preservatives and antioxidants which prevent lipstick from becoming rancid. While every lipstick contains these components, a wide variety of other ingredients can also be included to make the substance smoother or glossy or to moisten the lips. Other composition for improving qualities of lipstick such as sunscreen [2-4] was also normally added.

Waxes are largely used in cosmetic and pharmaceutical industries. The world production amounts to be about 7000 tons per year and 60% are used in cosmetic and pharmacy. In general, wax and oil make up about 60 percent of the lipstick (by weight), with alcohol and pigment accounting for another 25 percent.

Table 1.1 Types of waxes

Animal waxes	
• Beeswax	produced by honey bees
• Chinese wax	produced by scale insects *Coccus ceriferus*
• Shellac wax	from the lac insect *Coccus lacca*
• Lanolin (wool wax)	from the sebaceous glands of sheep
Vegetable waxes	
• Bayberry wax	from the surface of the berries of the bayberry shrub
• Candelilla wax	from the Mexican shrubs *Euphorbia cerifera* and *E. antisyphilitica*
• Carnauba wax	from the leaves of the Carnauba palm
• Castor wax	catalytically hydrogenated castor oil
• Jojoba oil	pressed from the seeds of the jojoba bush
Mineral waxes	
• Ozocerite (Ozokerite)	found in lignite beds
Petroleum waxes	
• Paraffin wax	made of long-chain alkane hydrocarbons
• Microcrystalline wax	with very fine crystalline structure
Synthetic waxes	
• Polyethylene waxes	based on polyethylene
• Fischer-Tropsch waxes	from Fischer-Tropsch reaction
• Chemically modified waxes	usually esterified or saponified

All waxes are water-resistant materials made up of various substances including hydrocarbons (normal or branched alkanes and alkenes), ketones, diketones, primary and secondary alcohols, aldehydes, sterol esters, alkanoic acids, terpenes (squalene), and monoesters (wax esters), all with long or very long carbon chains (from 12 up to about 38 carbon atoms) and solid in a large range of temperature (fusion point between 60 and 100°C). More commonly, waxes are esters of an alcohol other than glycerol (long chain alcohol, sterol, hydroxycarotenoids, and vitamin A) and a long chain acid (wax esters).

Table 1.2 Composition in waxes

Compound	General structure
n-alkane	$CH_3(CH_2)_nCH_3$
ketone	R_1COR_2
Secondary alcohol	$R_1CH(OH)R_2$
Monoester	R_1COOR_2
Alkanoic acids	$RCOOH$
Aldehyde	$RCHO$
Primary alcohol	RCH_2OH
Dicarboxylic acid	$HOOC(CH_2)_nCOOH$
β-Diketone	$R_1COCH_2COR_2$
ω-Hydroxy acids	$HOCH_2(CH_2)_nCOOH$

R_1 and R_2 commonly have 10 to 20 carbon atoms or more.

A common problem of forensic analysis is to identify the provenance trace of lipstick smears and minute lipstick smears are sometimes found on clothing, cigarette butts, glass, tissue paper, and miscellaneous crime scene surfaces.

There are various of sampling techniques for specifically application of forensic of lipsticks such as developing techniques [5-8], color comparison [9-10], extraction [17-18], chromatographic techniques such as thin layer chromatography (TLC) [10-11], gas chromatography (GC) [9, 11, 20-23], and high performance liquid chromatography (HPLC) [10, 15, 17, 18], and scanning electron microscope (SEM) [10, 14].

For the developing techniques, lip print is unique and can be used to identify individual person. The possibility of using the lip cosmetic as a DNA source has to be considered [24]. When dealing with latent traces, the first step is visualizing them by a developing process. The developing of latent lip prints on porous surfaces is more recent than that for fingerprints. The first research of the developing technique showed that traditional reagents used for fingerprints (i.e., aluminium powder, cobalt oxide powder, and magnetic powder) were not successful [5]. It has recently been determined that lysochromes (i.e., Sudan black, Oil red O, Sudan III) are quite effective in developing recent latent lip prints, as well as older ones, on porous surfaces [6]. In addition, lip marks made on paper and developed with Sudan Black can be used as DNA source for forensic identification [25]. Moreover, the effectiveness of lysochromes on the developing of invisible lipstick on human skin can be obtained [7]. Although lysochrome is the useful reagents to obtain lip print, it may occur on colored or multicolored surface. The developing technique is not perceived due to contrast problem between the reagent and the surface where lip print is searched. To solve this problem, Nile Red is studied as a potential developer for latent lip prints. Considering the results obtained, it is already possible to affirm that Nile Red is very efficient for searching and developing [8].

The first step in forensic examination is visual comparison of color under white light or fluorescence at three excitation wavelengths [9-10]. Several techniques have been used for further characterizing and discriminating of the chemical composition of lipsticks such as thin-layer chromatography (TLC) of dyestuffs extracted from lipstick smear [10-11], high performance liquid chromatography (HPLC) [10,15, 17-18], gas chromatography (GC) of waxes [9, 11], and GC/mass spectroscopy of transesterified derivatives of oil in lipstick [20-23]. For the analysis of trace element in lipsticks, neutron activation analysis has been used [12]. Additional research, elemental analysis of inorganic component was analyzed by an electron probe microanalyzer with an energy-dispersive X-ray spectrometer [10, 14]. Moreover, surface enhanced resonance Raman scattering is used for the *in-situ* characterization of chromophores in lipstick smears on glass and cotton surfaces [13].

Although several publications employed various techniques for the forensic examination of lipsticks, most methods used large samples and are destructive techniques. These methods are not suitable for routine analysis of forensic investigation and comparison the trace amounts of lipstick smear in a crimes scene because a lipstick smear is minute in quantities. Therefore, non-destructive analysis is required. The destructive techniques such as solvent extraction which required complicated extraction procedure must be lastly used in order to keep their original sample for further analysis. Fourier Transform Infrared (FT-IR) Spectroscopy is particularly important because of the rich information content of the chemical information and chemical structure. This technique can be applied for forensic analysis of lipsticks.

1.3 Fourier Transform Infrared (FT-IR) Spectroscopy

For many years, FT-IR spectroscopy has been used to identify a compound and to investigate the composition of a sample. Infrared spectroscopy is a molecular spectroscopy which has information of chemical composition and molecular structure. In compounds, a complete identification of all compounds is often possible from the functional group. This technique is widely used in both research and industry as the biotechnology, food industry, clinical analysis, qualitative analysis, and etc.

Infrared radiation covers the range of the electromagnetic spectrum between 0.78 and 1000 μm. In the context of infrared spectroscopy, wavelength is measured in wavenumbers unit. IR radiation does not have enough energy to induce electronic transitions as seen with UV radiation. Absorption of IR radiation is restricted to compounds with small energy differences in the vibrational and rotational states. A molecule to absorb IR radiation, the vibrations or rotations within a molecule must cause a change in the dipole moment of the molecule. The electrical field of the radiation interacts with fluctuations in the dipole moment of the molecule. If the frequency of the radiation matches the vibrational frequency of the molecule then radiation will be absorbed, causing a change in the amplitude of molecular vibration.

In order to measure a sample, a beam of infrared radiation is passed through the sample and the amount of energy absorbed at each wavelength is recorded. FT-IR spectra provide information directly related to the chemical structure, chemical composition, and other composition in material. There are many sampling technique in FT-IR spectroscopy for each type of sample such as transmission, specular reflection, and diffuse reflection. Each technique has its advantages and disadvantages. In FT-IR spectroscopy, very few sampling techniques are available for non-destructive technique and applied to forensic analysis. ATR FT-IR spectroscopy is one of such sampling technique.

1.4 ATR FT-IR Spectroscopy

Attenuated total reflection Fourier transform infrared (ATR FT-IR) spectroscopy is a surface characterization technique. The technique is one of the sampling techniques of infrared spectroscopy. This technique has been used in the application including qualitative analysis, quantitative analysis, surface analysis, and depth profiling. In ATR experiment, the sample is placed against an internal reflection element (IRE) or ATR crystal or waveguide. The IRE is made of a material that has high refractive index such as silicon (Si), zinc selenide (ZnSe) or germanium (Ge). When infrared radiation traveling into the IRE, total internal reflection phenomena occurs at the interface as the angle of incidence greater than the critical angel is utilized. The chemical information of material against with IRE was achieved. This technique can be applied to forensic analysis of lipstick.

In 2001, Bartick et al. [26] studied the application of microscopical infrared internal reflection spectroscopy for the analysis of forensic evidences such as single fiber, hair, paint chips, writing ink, and lipstick on tissue and on black electrical tapes.

1.5 The Advantages of ATR FT-IR Spectroscopy

ATR FT-IR spectroscopy is a non-destructive technique which is suitable for forensic analysis of lipsticks. The technique does not require additional sample preparation and has short analysis time for spectral acquisition. The obtained spectra provide information directly correlated with chemical structure, chemical composition, and other composition in analyzing sample. Therefore, this technique was employed for acquisition of chemical information of lipsticks.

1.6 The Limitations of ATR FT-IR Spectroscopy

Although, ATR FT-IR spectroscopy has several advantages but it has limitation. A contact between the sample and IRE is required for the good ATR spectra which have the high of spectral intensity. From physical properties of the sample, a perfect contact between a liquid sample must be achieved. On the other hand, the solid sample rarely has a good contact with the IRE. When optical contact is not achieved, there is an air gap between the sample and IRE while an ATR spectrum cannot be observed. In the general practice, applying pressure to a solid sample against the IRE is the main technique for improving the contact between the sample and IRE. A great care must be taken when making a contact since the smooth surface of IRE can be easily scratched and damaged. It should be preventing a possible damage of the brittle surface of the hemispherical IRE due to an excessive force as a solid sample was pressed against the IRE.

A conventional ATR accessory (the Seagull^{TM}, Harrick Scientific, USA) with a hemispherical Ge IRE (diameter of 25 mm) was employed for ATR FT-IR spectral acquisitions of lipsticks. A lipstick smear was made at the center of the flat surface of the hemispherical IRE with a diameter of at least 5 mm [27]. Due to the large sampling area of the hemispherical Ge IRE, the small sample cannot be analyzed. Thus, this conventional technique is not suitable for case of forensic examination.

1.7 ATR FT-IR Microspectroscopy

ATR FT-IR microspectroscopy is defined as the coupling of a microscope to an infrared spectrometer. Infrared microscope has been developed in order to reveal the sample in both transmission and reflection mode. By coupling the focused radiation into specially designed IREs made of high refractive index materials and by making the angle of incidence at the sampling surface of the IRE greater than their corresponding angle of incidence, the ATR FT-IR application of the radiation can be realized.

ATR FT-IR microspectroscopy is an analytical technique for chemical identification. This technique has been widely used in various applications including surface characterization, quantitative analysis, and qualitative analysis. ATR FT-IR microspectroscopy has been applied to the forensic examination. The application of this rapid, non-destructive, analytical technique to the forensic study of lipsticks has the potential to add a new layer of information available to investigators.

To overcome the limitation of the conventional ATR FT-IR technique, the homemade μIRE accessories, the slide-on Ge μIRE and the slide-on diamond μIRE were developed. The novel μIRE accessories for spectral acquisition using a hemispherical dome of cone Ge IRE and gem quality round brilliant cut diamond [27-28] as internal reflection element (IRE) were introduced and were employed for forensic analysis of lipsticks. An infrared microscope can be utilized for a spectral acquisition of sample. The advantage of the homemade μIRE accessories is the small contact area of diamond tip and cone-shaped Ge μIRE, a small sample size can be analyzed. The chemical information of lipsticks on various substrates can be acquired. Spectra without interference were observed when substrates such as glass, tissue paper were analyzed together with lipsticks. In addition, the homemade μIRE accessories required short time for spectral acquisition. There was no separation of the trace lipstick prior the analysis. The operation was non-destructive and could be performed without an addition sample preparation.

In this research, different aspect of lipsticks analysis is explored. Commercial lipsticks from various manufactures were investigated. The demonstration experiment shows that ATR FT-IR microspectroscopy is a rapid, powerful, and non-destructive tool for accurately identifying lipstick smears. The results obtained from these studies are presented and discussed. These potential applications could have a significant impact on forensic science and could dramatically enhance the amount of information that can be obtained from the study of lipstick smears.

1.8 The Objective of This Research

The objective of this research is to develop ATR microspectroscopic technique for analysis of trace lipstick smears and apply for forensic analysis.

1.9 The Scope of This Research

1. To study the chemical information of lipsticks from various cosmetic manufactures using the homemade μIRE accessories.
2. To investigate a trace lipstick smear on the several surfaces such as glass, tissue paper, straw, and etc. using ATR FT-IR microspectroscopy.
3. To study time dependent changes of lipstick.

CHAPTER II

THEORETICAL BACKGROUND

2.1 Fundamental of Light Propagation

Light is an electromagnetic wave. Electromagnetic radiation is described as a self-propagating wave in space with electric and magnetic components. These components oscillate at right angles to each other and to the direction of propagation, and are in phase with each other. Electromagnetic radiation is classified into types according to the frequency of the wave. In order to increase frequency, radio waves, microwaves, infrared radiation, visible light, ultraviolet radiation, X-rays and gamma rays. In some technical contexts the entire range is referred as just light. According to classical wave theory, the electric field vector (E) and the magnetic field vector (H) of the radiation are oscillating periodically in space, as depicted in Figure 2.1. The electric and magnetic vector components and the direction of propagation are perpendicular to each other.

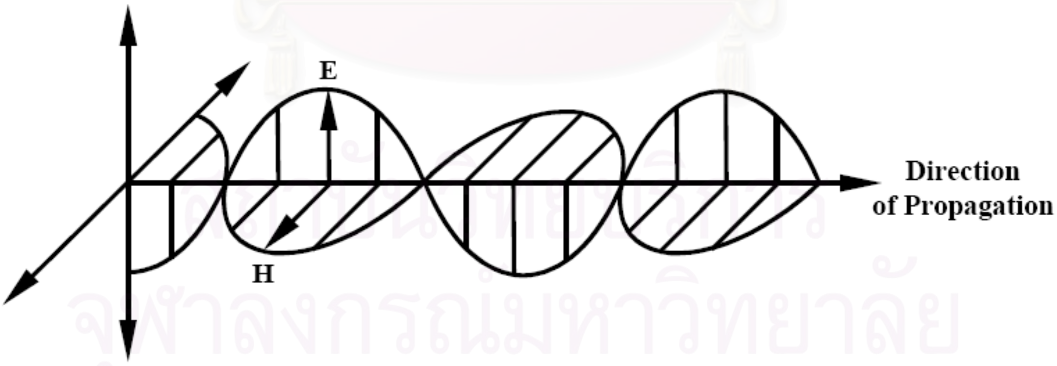


Figure 2.1 Propagation of a linearly polarized electromagnetic wave in the direction of propagation. Electric (E) and magnetic (H) vector are always perpendicular to each other and to direction of propagation.

2.2 Concepts of Spectroscopy

Spectroscopy is the study of matter and its properties by investigating light that is emitted, absorbed or scattered by the matter under investigation. When electromagnetic radiation impinges on the matter, interactions between incident beam and the molecule will alter the incident radiation. The incident beam can be transmitted, scattered, or absorbed by the matter. As seen all spectroscopic techniques, it is used to identify a compound and investigate the composition of sample. Since a schematic illustration for an interaction between light and the matter is illustrated in Figure 2.2.

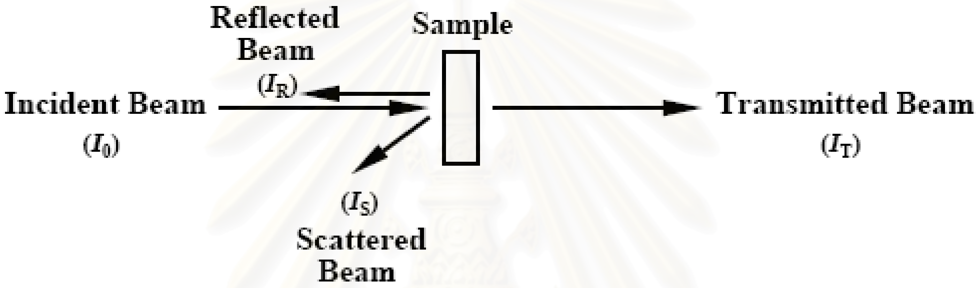


Figure 2.2 Interaction of light with matter.

This process can be expressed by the following relationship [27]. The total amount of the incident energy is the sum of the reflected, transmitted, scatted, and absorbed light.

$$I_0 = I_R + I_S + I_T + I_A \tag{2.1}$$

where I_O is the intensity of the incident radiation and I_R, I_S, and I_T are the reflected, scattered and transmitted radiation, respectively. I_A is the radiation absorbed by the matter. The intensity of the each radiation depends on the intensity and wavelength of the incident of radiation, the optical properties of the specimen, the concentration specimen, and the geometry of the experimental setup. Consider the electromagnetic radiation when a sample is inserted between a source of light and detector. The sample absorbs a fraction of the incident radiation. In order to measure the ratio and amount of light being absorbed by the sample, we need to measure the ratio of the sample attenuated (I) and non-attenuated (I_O) intensities of the radiation. The ratio is proportional to the transmittance of the sample. The relationship can be quantitatively related to the chemical composition of the sample by the Beer-Lambert law as follows [27]:

$$I / I_0 = e^{-A(\nu)} = e^{-c_2 \varepsilon(\nu) l} \tag{2.2}$$

where $A(v)$ is the sample absorbance at the a given wavenumber v, c_2 is the concentration of the absorbing functional group, $\varepsilon(v)$ is the wavenumber-dependent absorption coefficient, and l is the film thickness for the IR beam at a normal incidence to the sample surface.

2.3 Infrared Spectroscopy

Infrared spectroscopy is the subset of spectroscopy that deals with the infrared region of the electromagnetic spectrum. Infrared spectroscopy is a molecular spectroscopy which has information of chemical composition and molecular structure. In compounds, a complete identification of all compounds is often possible from the identification of the functional group. This technique is widely used in both research and industry such as biotechnology, food industry, clinical analysis, quantitative analysis, qualitative analysis, and etc.

Infrared is divided into three regions; the near, mid, and far infrared named for their spectrum. The far-infrared (~400-10 cm^{-1}) has low energy. The mid-infrared or MIR (~4000-400 cm^{-1}) used to study the vibration associated the rotation-vibration, while the high energy near-infrared (~1400-4000 cm^{-1}) can excite overtone and harmonic vibrations. Infrared spectroscopy works because chemical bonds have specific frequencies at which they vibrate corresponding to energy levels. For a vibrational mode in molecule, it must be associated with changes in the permanent dipole. Simple diatomic molecules have only one bond, which may stretch. More complex molecules may have many bonds, and vibrations can be conjugated, leading to infrared absorptions at characteristic frequencies that maybe related to chemical groups. The atoms in a CH_2 group, commonly found in organic compounds can vibrate in six different ways, symmetrical and asymmetrical stretching, scissoring, rocking, wagging and twisting.

There are many sampling techniques in FT-IR spectroscopy depend on the nature of the sample and the required information such as transmission, specular reflection, and diffuse reflection. Each has ones advantages and disadvantages. This technique can accept a wide range of type of sample such as gases, liquids or solids.

The simple transmission technique implicates passing the infrared radiation throughout the whole sample and detecting a portion of the radiation that is transmitted. This technique may provide a significant amount of information concerning molecular structures and their properties. However, transmission mode is not applicable to surface analysis but the information obtained is an average information coming from both the surface and the bulk. Moreover, transmission is a destructive technique. ATR FT-TR technique is a surface characterization technique. This technique provides the information which directly associated to chemical composition of the sample.

2.4 Principles of Light Reflection and Refraction

When the electromagnetic radiation strikes a boundary between two media with different refractive indices, refraction and reflection occur. The law that governs the reflection process requires that the angle of incidence is equal to the angle of reflection. In this case, reflection is specular. If electromagnetic radiation passes from one medium to another that has a different refractive index, a suddenly change of beam direction takes place because of the differences in propagation velocity through two media. If light propagates through an incident media with refractive index n_1 and enters a medium with refractive index n_2 (Figure 2.3), the light path will be changed and the extent of refraction is given by the following expression, known as Snell's law [27]:

$$n_1 \sin \alpha_1 = n_2 \sin \alpha_2 \tag{2.3}$$

where α_1 and α_2 are the angles of incidence and refraction, respectively.

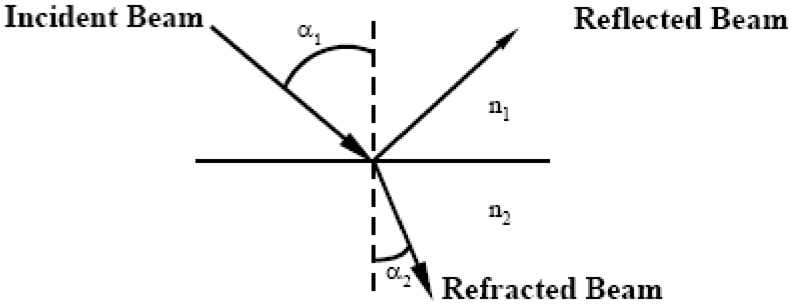


Figure 2.3 Reflection and refraction of a plane wave at a dielectric based on Snell's Law.

Total internal reflection occurs when light traveling through an optically denser medium of high refractive index is incident on the interface with a lower index (i.e., $n_1 > n_2$) with an incident angle greater than the critical angle. The critical angle can be derived from the Snell's Law and given by the Equation 2.4. As illustrated in Figure 2.4 [27-28].

$$\theta_C = \sin^{-1}(n_2 / n_1) \tag{2.4}$$

where n_1 and n_2 are the refractive indices of the denser medium and that of rarer medium, respectively.

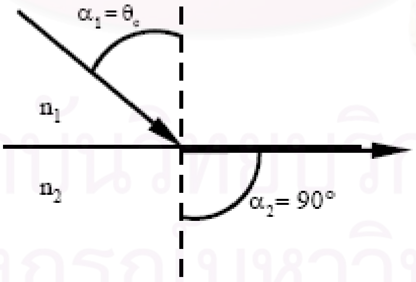


Figure 2.4 Condition under which total internal reflection (TIR) occurs. Light travels from an optically denser medium and impinges at the surface of the optically rarer medium (n_1>n_2) with angle of incidence is equal to the critical angle.

According to Figure 2.4, when an incident angle equals the critical angle, θ_C, the refracted angle equals 90°. This implies that under a total internal reflection, TIR, phenomenon there is no light from the optically denser medium travels across the interface into the optically rarer medium. If the rarer medium is non-absorbing, then all incident radiation (i.e., at angle greater than the critical angle) are internally reflected. If the rarer medium absorbs radiation at a specific wavelength, the intensity of the reflected radiation is reduced at that particular wavelength. This attenuation of the total reflection produced ATR spectra.

Total internal reflection spectroscopy is the technique of recording the spectrum of the material that attaches to the optically denser medium. The wavelength depends on the reflectivity of this interface being measured by introducing light into the denser medium. In this technique, the reflectivity is a measurement of the interaction between the electric field and the material. The resulting spectrum is the characteristic of the material.

2.5 Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) Spectroscopy

ATR FT-IR spectroscopy is a characterization technique based on an internal reflection principle. The major advantages of this technique are reduced sample preparation, non-destructiveness, the ease-of-use of internal reflection spectroscopy attachments, and its ability to give molecular information, chemical structure, and chemical information.

ATR FT-IR technique is used to obtain the spectra of the solids, liquids, semisolids, and thin films. ATR is performed by using an accessory that mounts in the sample compartment of an FT-IR. At the heart of the accessory is the crystal of the infrared transparent material of high refractive index. Mirrors on the accessory bring infrared radiation to a focus on the crystal. After radiation passing through the crystal and reaching its top on surface, and the expected radiation should leave the crystal. However, if the crystal has the proper refractive index and the light has the proper angle of incidence, the radiation undergoes what is called *total internal reflection,* TIR.

In an ATR configuration, a denser medium (*i.e.*, an internal reflection element, IRE) is optically contacted with rarer medium (*i.e.*, a sample) as shown in Figure 2.5. The IRE is infrared transparent and has a refractive index n_0. The sample is infrared absorbing and has a complex refractive index at frequency ν (cm^{-1}) of [21]:

$$\hat{n}_1 = n_1 + ik_1 \tag{2.5}$$

where n_1 and k_1 are refractive index and absorption index, respectively.

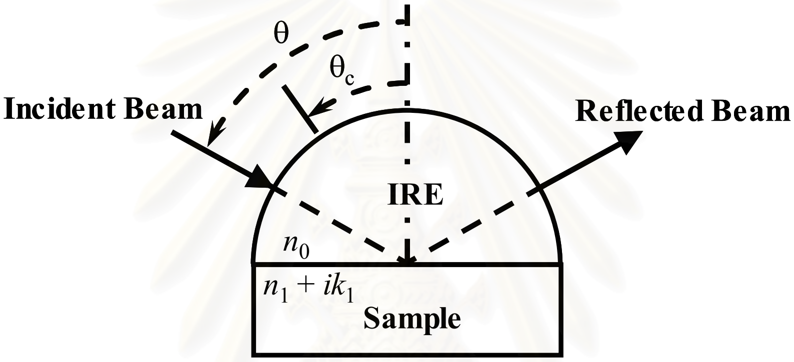


Figure 2.5 Ray tracing under total internal reflection.

Once the infrared radiation is inside the crystal, a standing wave of radiation is set up, called an *evanescent wave*. A unique property of the evanescent wave is that it is slightly for deeper than the crystal, and it penetrates a small distance beyond the crystal surface into space. A sample brought into contact with the crystal can interact with the evanescent wave, absorbs infrared radiation, and has its infrared spectrum detected.

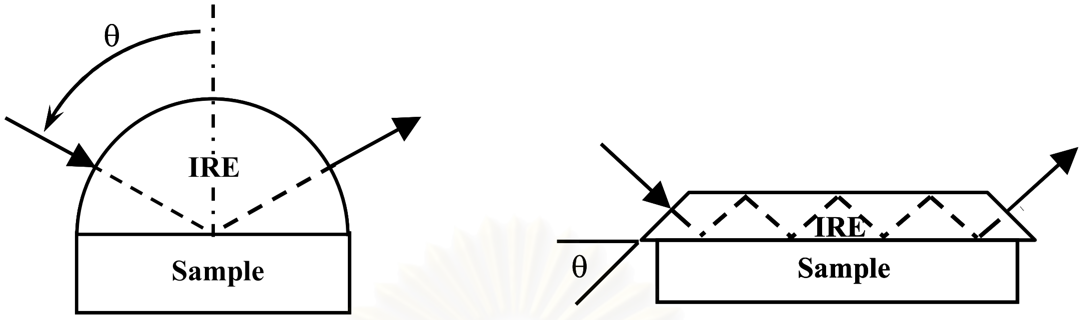
2.5.1 Internal Reflection Element (IRE)

The internal reflection element (IRE) or ATR prism is made of a material that has high refractive index such as silicon (Si), zinc selenide (ZnSe) or germanium (Ge). IRE must be transparent throughout the mid-infrared spectral region. Listed in Table 2.1 are the properties of the material that used for internal reflection element.

Table 2.1 Properties of materials used for internal reflection elements.

Material	Reflective index At 1000 cm^{-1}	ATR spectral range (cm^{-1})	Hardness (kg mm^{2})	Uses
Germanium	4.02	5,500-600	780	Good for most samples and strong absorbing samples
Silicon	3.42	8,300-660	1150	Resistant to basic solutions
Zinc selenide	2.43	20,000-460	120	General use
Diamond	2.417	45,000-~2,500, ~1650-200	8820	Good for most samples and extremely caustic of hard samples

In ATR FT-IR technique, IRE is a necessary material to establish the condition which to obtain the internal reflection spectra of the sample. Radiation propagates through the IRE by mean of reflection. ATR spectra are easily obtained by placing the sample against the IRE while the infrared radiation is coupled into the IRE. The information obtained from the spectrum depends on several factors such as properties of IRE, angle or range of angle of incidence, number of reflection, aperture, number of passes, surface preparation, and material which it was made from. In general, the IRE configuration included variable-angle hemispherical crystal with single reflection and multiple reflection planar crystal (Figure 2.6).



A. Single Reflection **B. Multiple Reflection**

Figure 2.6 Selected IRE configurations commonly used in ATR experimental setups: (A) Single reflection variable-angle hemispherical crystal, and (B) Multiple reflection single-pass crystal.

2.5.2 ATR Spectral Intensity

In an ATR configuration, a medium with a high refractive index is optically contacted with a medium with lower refractive index (i.e. sample). The IRE is an infrared transparent. On the other hand, the sample is infrared absorbing and has a complex refractive index at frequency ν of $n_1(\nu) \overset{\wedge}{=} n_1(\nu) + ik_1(\nu)$, where $n_1(\nu)$ and $k_1(\nu)$ are refractive index and absorption index, respectively. Incident light travels from the IRE and impinges the IRE-absorbing medium interface with an angle of incidence is greater than the critical angle, total internal reflection can be occurred.

Under non-absorbing condition [i.e., $k_1(\nu) = 0$], the incident light is totally reflected at the interface. Since no light travels across the interface and there is no reflection loss due to absorption [i.e., refractance $R(\theta,\nu) = 1$], this phenomenon is then called the *total reflection phenomenon*.

Where a rarer medium is absorbing [i.e., $k_1(\nu) > 0$], there is reflection loss due to absorbing by the material [i.e. $R(\theta,\nu) < 1$]. This phenomenon is called the *ATR phenomenon*. Although no light travels across the boundary, there is a strong electric field at the interface region of the absorbing medium. Interaction between the electric field and the absorbing medium is the cause of reflection loss in ATR experiment. The magnitudes of the interaction between light and the sample can be expressed in term of absorbance. The absorbance depends on both the material properties (e.g. refractive index of the IRE and refractive index of the sample) and the experimental parameters (e.g. the angle of incidence, frequency, and polarization of the incident beam). The relationship between absorbed and reflected intensity in an ATR spectrum is given by [29-30]:

$$A(\theta,v) = 1\text{-}\ R(\theta,v) \tag{2.6}$$

where $A(\theta,v)$ and $R(\theta,v)$ is absorptance and reflectance, respectively. In general, absorptance in ATR can be expressed in term of experimental parameters and material characteristic by the following expression [29-30]:

$$A_{\bar{p}}(\theta,\nu) = \frac{4\pi\nu}{n_0 \cos\theta} \int_0^{\infty} n_1(\nu) k_1(\nu) \left\langle E_z^2(\theta,\nu) \right\rangle dz \tag{2.7}$$

where $A(\theta,v)$ is the absorptance and l indicates the polarization of the incident beam. is *the mean square electric field* (MSEF) at depth z, $n_1(v)$, and $k_1(v)$, respectively, are the refractive index and the absorption index of the sample, and n_0 is the refractive index of the IRE.

The MSEF is a function of both experiment parameters and material characteristics. The relationship between the absorption strength and the material properties shown in equation 2.6 is nonlinear. In general, the MSEF is strongest at the IRE/sample interface. Its strength decreases exponentially as a function of depth. The strength and decay characteristic of the MSEF vary with the absorption strength are shown in Figure 2.7.

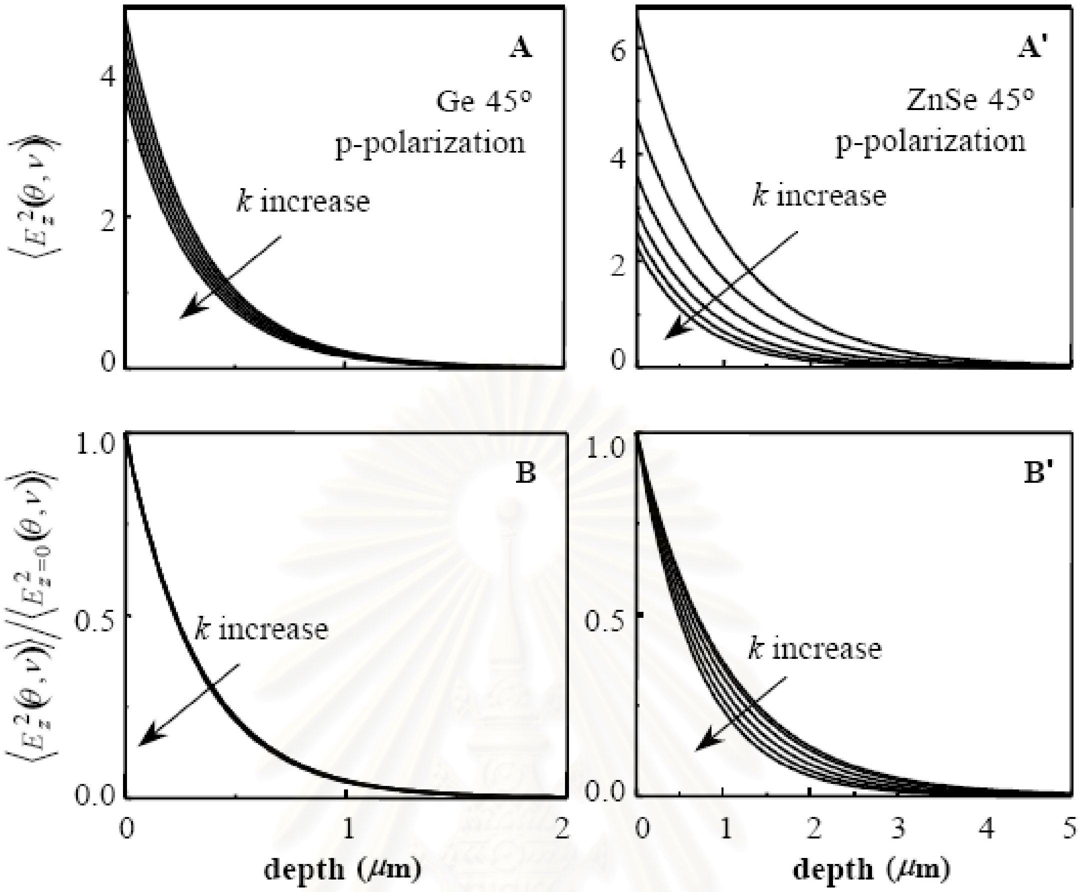


Figure 2.7 The MSEF at various experimental condition (A, A’) and its decay characteristic (B, B’). The simulation parameters are n0 = 4.00 for Ge, n_0 = 2.40 for ZnSe, ν = 1000 cm^{-1}, $n_1(\nu)$ = 1.50, k1(ν) = 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5, respectively.

The MSEF strength is greatest at the interface between the IRE and the sample. It decays exponentially as a function of distance from the interface. The distance from the interface where the field strength decays to 1/e of that the interface is called *penetration depth*. The penetration depth is given in terms of material characterization and experimental parameters by [28]:

$$d_p = \frac{1}{2\pi\nu n_0\left(\sin^2\theta - (n_1/n_0)^2\right)^{1/2}} \tag{2.8}$$

where ν is the frequency of the infrared radiation and θ is the angle of incidence. By the altering the angle of incidence while ensuring that it remains larger than the critical angle, the depth of penetration is consequently varied. Example of the penetration depth at various experimental conditions and material characteristics are shown in Figures 2.8 and 2.9.

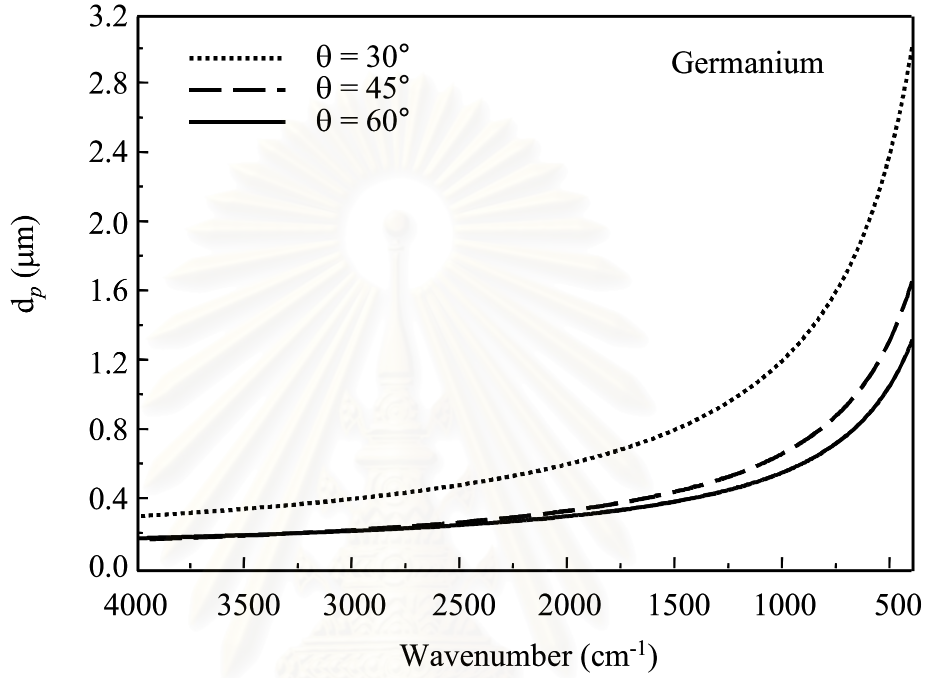


Figure 2.8 Relationship between the penetration depth and wavenumber for Ge crystal (n_0 = 4.0) at different angle of incidence.

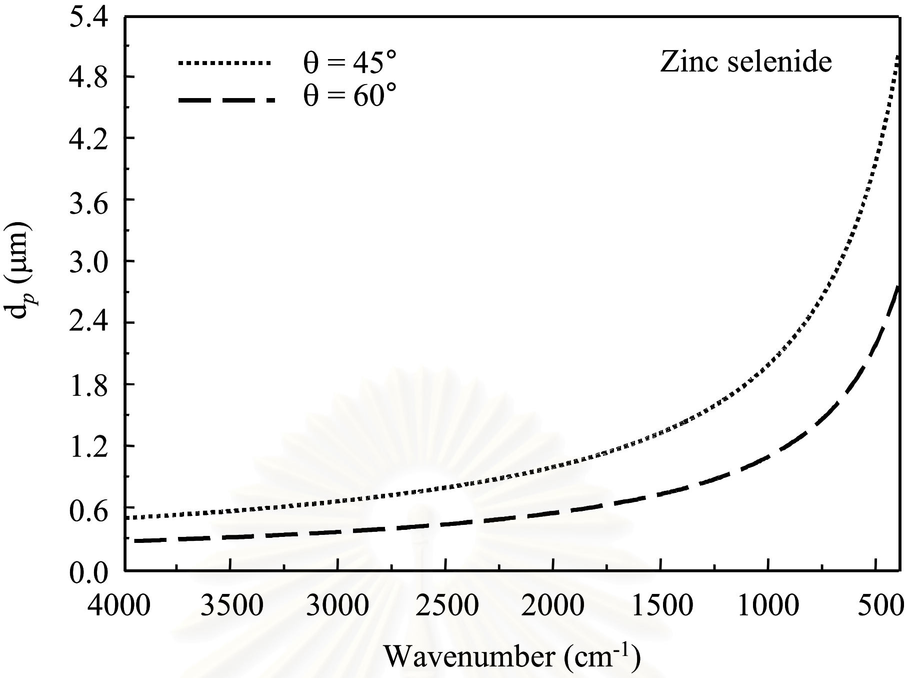


Figure 2.9 Relationship between penetration depth and wavenumber for ZnSe crystal (n_0 = 2.4) at different angle of incidence.

In order to obtain good ATR spectra, a very good contact between the sample and IRE is required [30]. Liquid samples always have a perfect contact with IRE. On the other hand, the solid sample rarely has a good contact with IRE especially the hard and rigid solid. When the system does not achieve an optical contact, there is always an air gap between the sample and the IRE. The larger is the air gap, the smaller is the observed spectral intensity. If an air gap is large enough the spectrum cannot be obtained. To solve this problem, the high pressure is applied at the sample against the IRE. However, the IRE may be damaged by an excessive force. Since the ATR spectra are sensitive to physical phenomena in a few micrometers near the interface, spectral information at the greater depth cannot be obtained.

2.6 ATR FT-IR Microspectroscopy

2.6.1 Infrared Microscope

FT-IR microscope defines as the coupling of a microscope to an infrared spectrometer. This coupling allows one to focus infrared radiation onto the specimen, to collect transmitted or reflected infrared radiation from the sample by a detector. Infrared microscope has been developed in order to reveal the small samples that are beyond the capability of normal infrared spectrometer. Infrared microscope is designed with two paths from the sample to the detector; transmission and reflection. For transmission mode, the infrared light passes through the sample. The transmitted radiation is collected by the second infrared objective and sends the radiation to the detector.

According to the optical design of the objective, the focused radiation contains rays with angles of incidence ranging from 15° to 35°. By coupling this focused radiation into specially designed IREs making of the high refractive index material and by making the angle of incidence.

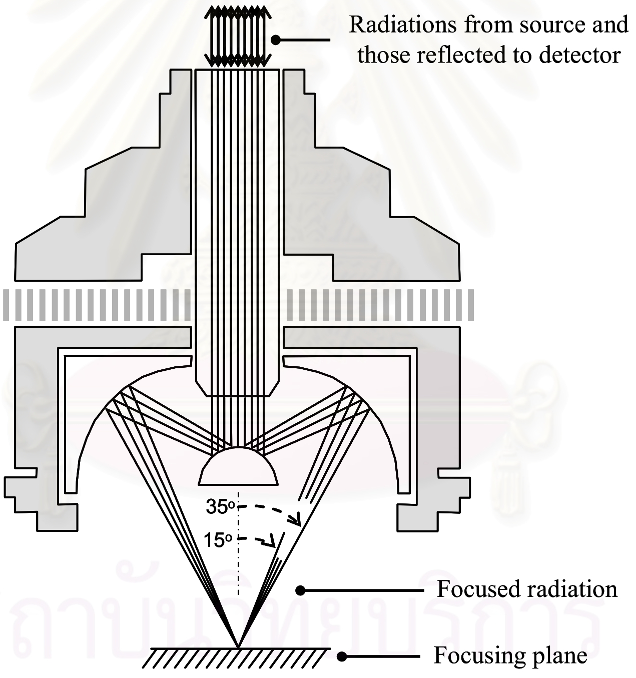


Figure 2.10 Path of infrared radiation through an infrared objective of the infrared microscope.

2.6.2 The Ge μATR Technique

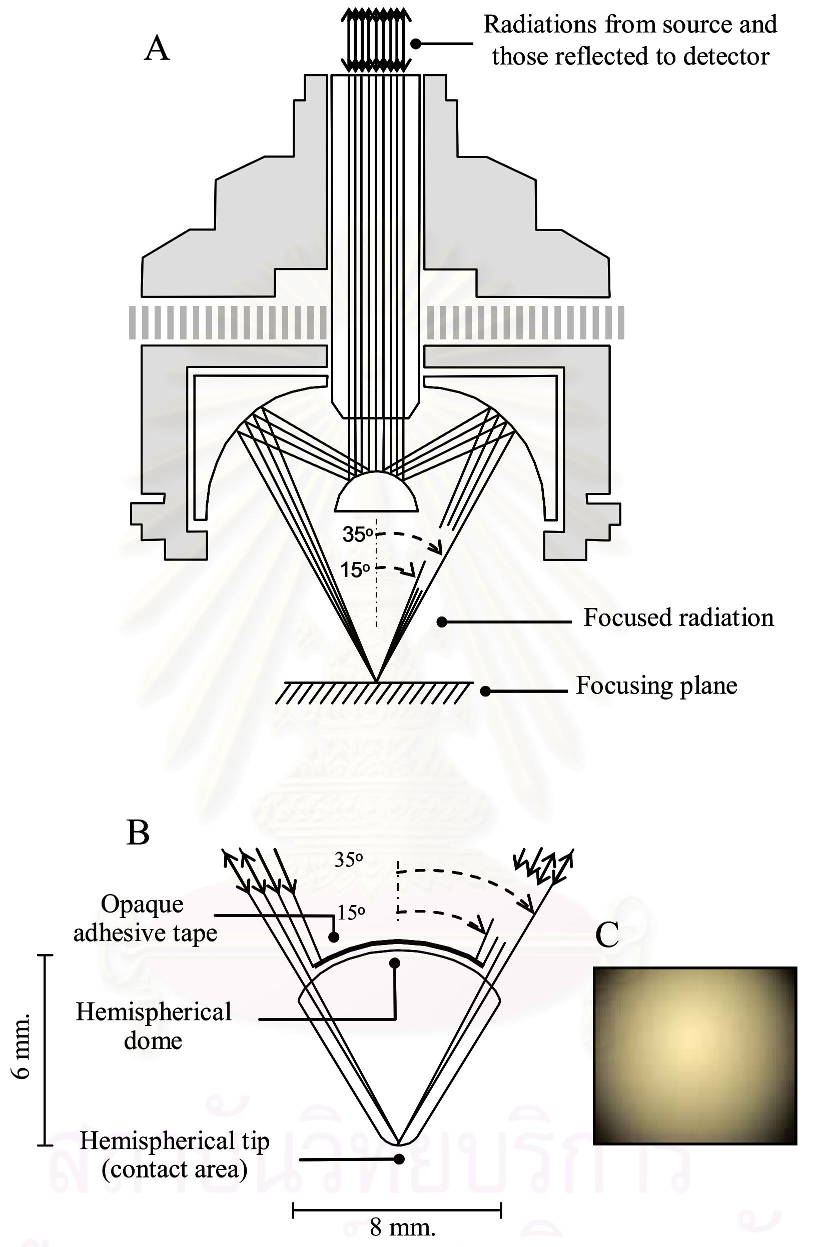


Figure 2.11 Ray tracing within the 15X Schwarzschild-Cassegrain infrared objective and the coupling of the focused beam into the homemade slide-on Ge μIRE.

A schematic illustration of ray tracing within the 15X Schwarzschild-Cassegrain infrared objective (Figure 2.11A) and the coupling of the focused beam into the homemade Ge μIRE are shown in Figure 2.11B. According to the optical design of the objective, the focused radiation contains rays with angles of incidence ranging from 15° to 35°. By the coupling this focused radiation into specially designed IREs making of high refractive index materials and by making the angle of incidence at the sampling surface of the IRE greater than their corresponding angle of incidence, the ATR FT-IR application of the radiation can be realized.

According to Figure 2.11B, the hemispherical dome of the miniature cone-shaped Ge IRE facilitates the coupling of the focused radiation traveling into the IRE by minimizing the reflection loss at the air/Ge interface. If a nearly perfect coupling was assumed, the radiation transmitted through the air/Ge interface of the dome and impinged the Ge/air interface of the tip without a significant change in the angle of incidence (i.e., the refraction at the air/Ge interface was minimized). To ensure a good contact, the circular tip of the IRE was made a hemispherical surface. Since the contact area is small (~ 100 μm in diameter, Figure 2.11C), a good contact is achieved with a minimal force exerting on the tip. For the Ge IRE ($n_{\mathrm{Ge}} = 4.0$), the critical angle for the total internal reflection (TIR) at the interface with air ($n_{\mathrm{air}} = 1.0$) and an organic medium ($n_{\mathrm{organic}} = 1.5$), respectively, are 14.48° and 22.02°. As the results, parts of the coupled radiation can be employed for ATR FT-IR investigation of a material having an optical contact with the tip of the miniature IRE. To eliminate the interference from the internal reflection associated with the radiation having an angle of incidence smaller than the critical angle, an opaque circular adhesive tape was placed on the center of the hemispherical dome. Once the unwanted radiation was blocked, the observed spectrum was free of refractive-index type peak shape originated by internal reflection at the tip. Due to an effective condensation of the coupled radiation and an efficient light-matter interaction under the ATR condition at the tip of the IRE, ATR FT-IR spectra of a small specimen or a small area can be acquired with superb spectral quality.

A "*contact-and-collect*" operation was developed in order to measure ATR FT-IR spectra of a trace lipstick smear without interference from the substrate by making a gentle contact between the tip of the μIRE and the samples. When a lipstick trace on a substrate was brought into an optical contact with the IREs, some of the lipstick stuck at the tip of the IREs after removing the substrate. Several contacts can be made in order to collect a sufficient amount of the lipstick. The trace lipstick on the IRE can then be characterized under the ATR mode. The observed spectrum can be employed for the unique identification. Since the contact areas of the IREs were very small, there was virtually no noticeable change on the sample. The specimen can be employed for further analysis or kept as a reference.

To collect an ATR FT-IR spectrum of a trace lipstick stain on a substrate with the miniature IREs, the analyzing specimen was placed on the microscope stage. The sampling spot of the lipstick stain was selected through a built-in objective (optical or infrared). A miniature IRE was slid into the position and the microscope stage was elevated until the selected position touched the tip of the IRE. To achieve the same degree of contact for all measurements, a contact alert sensor plate was employed. The degree of contact was reproducible as the LED of the sensor plate was lid as a pre-defined pressure was attained. The elevation of the stage was stop when the sensor plate indicated an onset of a critical force exerting on the sensor plate. Once a good contact was achieved, ATR FT-IR spectrum of the sample at the position of contact was collected. After the spectral acquisition was performed, the stage was lowered. The IRE was then cleaned and was readied for the next acquisition. It should be noted that the contact alert sensor plate also prevents a possible damage of the brittle surface of the miniature-Ge IRE due to an excessive force as a solid sample was raised against the IRE. A great care must be taken when making a contact since the smooth surface of the miniature Ge IRE can be easily scratched.

2.6.3 The diamond μATR Technique

2.6.3.1 Transflectance Phenomena

A modern brilliant cut diamond consists of 57 + 1 faces depending on the presence of the culet (Figure 2.12). Since the reflective index of the diamond is greater than that of air ($n_{\text{air}} = 1.0$), total internal reflection at the diamond/air interface is observed when radiation traveling inside the diamond impinges on the interface with an angle greater than the critical angle. The critical angle, θc is given in the terms of the refractive indeces by $\theta c = \sin^{-1}(n_{\text{air}} / n_{\text{diamond}})$ and is equal to 24.44 ° for the diamond/air interface. In principle, a faceted diamond is cut such a proportion that the total internal reflection within the diamond is enhanced. To increase the number of total internal reflections, the cutting proportion of the diamond is carefully designed with respect to refractive index, size, shape, and carat weight. The number of total internal reflection depends on the angle and positions at which light enters the diamond. The greater the number of reflections within the diamond, the better the *fire* and *brilliance* of the facet diamond [31-32].

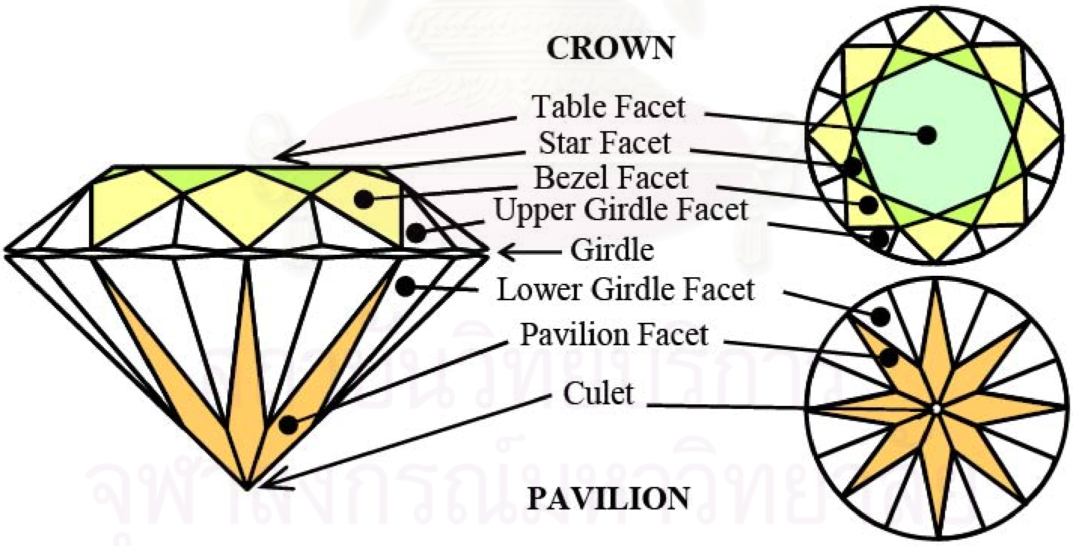
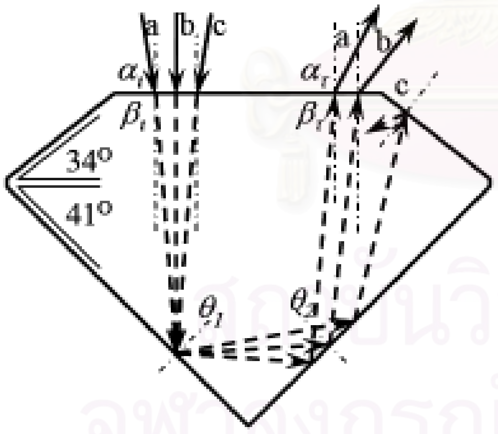


Figure 2.12 A schematic drawing of a round brilliant cut diamond.

In order to collect transflectance spectra of a faceted diamond using an infrared microscope, the infrared radiation is coupled into while the transflectance radiation is collected from the table faceted by the 15X Schwarzschild-Cassegrain infrared objective. A schematic illustration of ray tracing a round brilliant cut diamond is shown in Figure 2.13. For the coupled radiation with normal incidence to the table facet, the radiation totally reflects at the pavilion facet. Under the employed cut proportion (*i.e.*, a set of Tolkowsky's recommended proportion with pavilion angle of 41° and crown angle of 34°), the angles of reflection at the pavilion facet are 41° and 57 ° for the first and second reflection, respectively. The radiation reaches the diamond/air interface at the table facet with an angle of 41.77°. Due to the complex cut surfaces of the facet diamond, the coupled radiations that impinge the table facet with different angles and/or position undergo different reflections inside the facet diamond before emerging into air at any facet [31-32].

According to the traveling path of the coupled radiations, the outgoing radiation from the table facet of the diamond is defined as the *transflectance radiation* [31-32]. The evanescent field generated under the total internal reflection at the pavilion facets can interact with material attached to the diamond. The coupled radiation can be employed for ATR FT-IR absorption of samples having a contact with the pavilion facet of diamond.



Ray	Angle (degree)					
	α_i	β_i	θ_1	θ_2	β_r	α_r
a	4.84	2	43	55	14	35.78
b	0	0	41	57	16	41.77
c	4.84	2	39	59	38	N/A

Figure 2.13 Ray tracing of the coupled radiations within the faceted diamond. Angle of reflections and refractions defined with respect to the direct normal are summarized.

2.6.3.2 Using a Faceted Diamond as an IRE

Diamond can be employed as an IRE due to its high refractive index (n_{diamond} = 2.417) that is greater than that of sample (n_{organic} = 1.5). The critical angle, θc is given in the terms of the refractive indeces by $\theta c = \sin^{-1} (n_{\text{organic}} / n_{\text{diamond}})$ and is equal to 38.36° for the diamond/organic material interface and partial optical transparency in the mid-infrared region.

Diamond has three major absorption bands in the mid infrared region, namely *one-phonon* (1400-900 cm^{-1}), *two-phonon* (2650-1500 cm^{-1}), and *three-phonon* (3900-2650 cm^{-1}) absorptions. The absorptions are associated with impurities and defects in diamond crystal structure. The absorption magnitude in the one-phonon region depends strongly on the concentration of nitrogen impurity within the diamond structure. Diamond with high nitrogen content always shows over absorption while that with low nitrogen content does not show significant absorption in this region. Transflectance spectra of round brilliant cut diamonds (*i.e.*, ATR spectra of air) with different magnitude of nitrogen impurities are shown in Figure 2.14. A diamond with low nitrogen content can be employed as an IRE. The two-phonon region is the intrinsic absorption of diamond crystal structure. Although the two-phonon region is always over absorbing, it has little effect on analytical application since most organic materials do not absorb light in this region. The three-phonon region, on the other hand, is very weak. It imposes insignificant interference with the absorption of the materials. Although the diamond has an absorption in mid-infrared region, the single beam spectrum obtained from spectral acquisition of the diamond IRE with no sample contacting was used as a background spectrum. Therefore, the absorption band of diamond has no interference with the absorption of the sample [31-32].

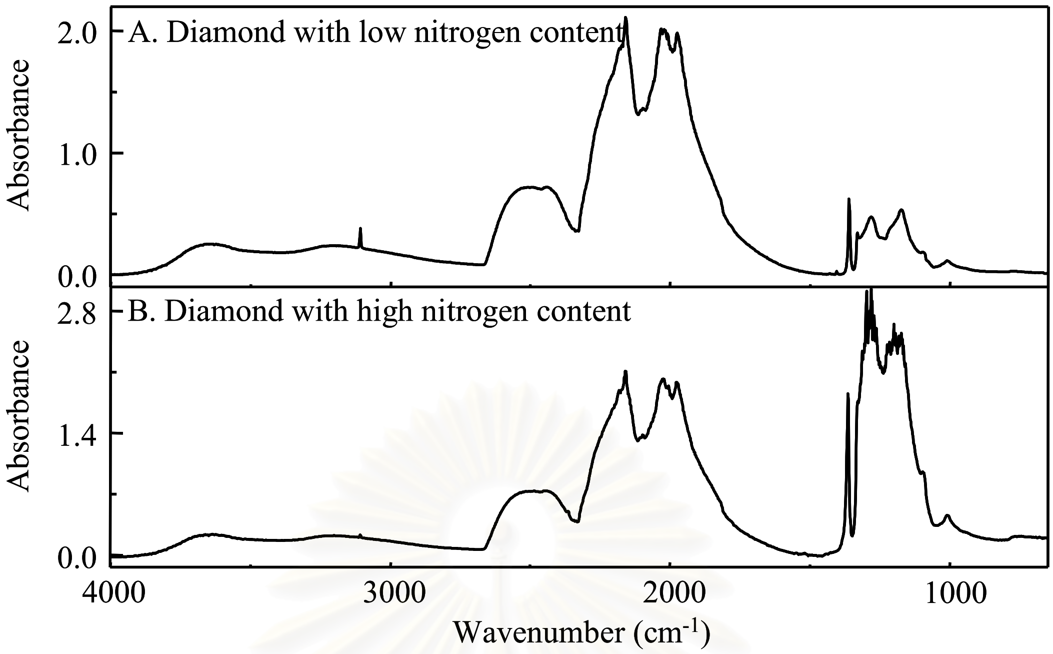


Figure 2.14 Transflectance spectra of (A) a brilliant cut diamond with low nitrogen content, and (B) diamond with high nitrogen content.

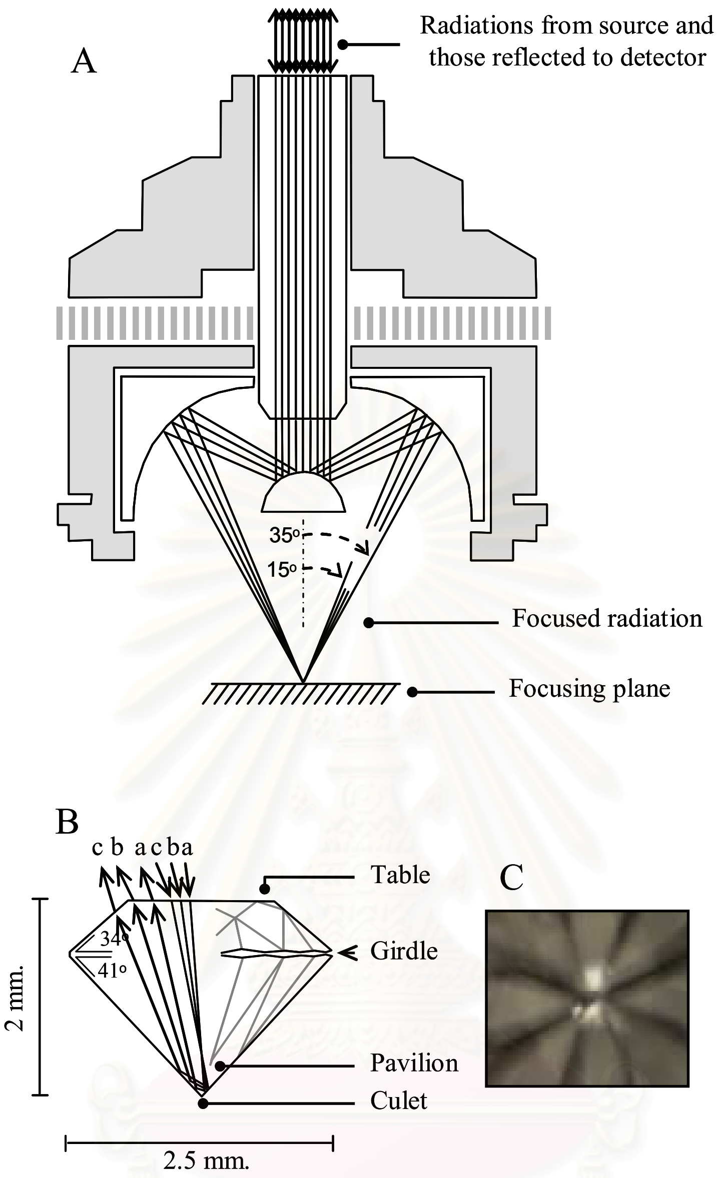


Figure 2.15 Ray tracing within the 15X Schwarzschild-Cassegrain infrared objective and the coupling of the focused beam into the homemade slide-on diamond μIRE.

Figure 2.15 illustrated ray tracing within the diamond IRE (~0.1 ct round brilliant cut natural diamond type IaB). The diamond tip (i.e. the part of the pavilion) is utilized as sampling area where the evanescent field interacts with the sample. Pressure can be applied onto a sample against the diamond μIRE in order to ensure the optimal contact during spectral acquisition. For the coupled infrared radiation with normal incident to the table facet, unlike the Ge IRE, the total internal reflection (TIR) phenomenon occurs at the pavilion facet instead of the culet, which is the tip of the diamond IRE as shown in Figure 2.15B. This is due to the geometry and size of the diamond IRE. For a diamond with a nearly ideal cut proportion, the angle of incidence at the pavilion facet of diamond/air or diamond/organic medium is greater than the critical angle [29-30]. As the results, ATR FT-IR absorption of a material having an optical contact near the diamond tip can be realized. The small contact area (i.e. as small as 50 μm in diameter, Figure 2.15C) and the inherent hardness of the diamond IRE enable spectral acquisition of a surface with high spatial resolution.

CHAPTER III

EXPERIMENTAL SECTION

From the objective of this study, this chapter is divided into the six sections, each section describing the result of the examination of the lipsticks form cosmetic manufactures that acquired with different accessories, the slide-on Ge μIRE and the slide-on diamond μIRE. The advantage and disadvantage of different accessories will be discussed. The next section shows application of homemade μIRE accessories for forensic examination of lipsticks on various substrates. An influence of effect on lipstick analysis such as time and environment were studied.

3.1 Materials and Equipments

3.1.1 Instruments

1. Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI, USA) equipped with a mercury cadmium-telluride (MCT) detector.
2. Continuμm^{TM} infrared microscope with 15X Schwarzschild-Cassegrain infrared objective and 10X glass objective.
3. Variable-angle single-reflection attenuated total reflection accessory (The Seagull^{TM}, Harrick Sceintific, USA) with a hemispherical Ge IRE at the incident angle of 30°.
4. The homemade slide-on germanium (Ge) accessory.
5. The homemade slide-on diamond accessory (gem-quality round brilliant cut diamond IRE (0.1005 ct type IaB natural diamond)).

3.1.2 Lipstick Samples

Commercial lipsticks from various cosmetic manufactures were collected. All employed samples are shown in Table 3.1

Table 3.1 Lipstick samples

Sample	Manufacture	Model number	Model	Color shade
1	Clinique	16	Pink chocolate matte	brown-red
2	Clinique	133	Plum sicle	violet
3	Clinique	11	Tawny spice	orange-pink
4	Clinique	04	A different grape	violet
5	Christian Dior	984	Bibelot rubyglass	dark violet
6	Christian Dior	814	Brun photo brown	brown
7	Christian Dior	005	Corail	orange
8	Elizabeth Arden	074	Spiced pecan	brown-orange
9	Oriental Princess	05	Tropical Orange	brown-orange
10	CHANEL	06	Pourpre	red
11	Kanebo	OR-508	Mandarin	orange
12	No brand	04	Tommy lipstick	brown-orange
13	Pond	-	Lip care-smooth	-
14	Body shop	-	Raspberry lip balm	pink
15	Lancôme	50	Rouge essential	red
16	Yves Saint Laurent	80	Rouge flammable poinsettia	red
17	Esteē Lauder	13	Beautiful pink	pink

3.1.3 Default Spectral Acquisition Parameters

Nicolet 6700 FT-IR spectrometer

Instrumental Setup	
Source	Standard Globar^{TM} Infrared Light Source
Detector	MCT/A
Beam splitter	Ge-coated KBr
Acquisition Parameters	
Spectral resolution	4 cm^{-1}
Number of scans	256 scans
Spectral format	Absorbance
Advanced Parameters	
Zero filing	none
Apodization	Happ-Genzel
Phase correction	Mertz

Continuμm^{TM} infrared microscope

Instrumental Setup	
Objective	15X Schwarzchild-Cassegrain
Aperture size	150 x 150 μm

3.2 The Homemade μIRE Accessories

3.2.1 The Homemade Slide-on Ge Accessory

The homemade slide-on Ge accessory was developed from the hemispherical Ge IRE to hemispherical dome with a cone-shaped as shown in Figure 3.1. Due to the small sampling area of the slide-on Ge IRE (~100 μm in diameter), the small sample can be employed for forensic analysis.

The homemade slide-on Ge accessory consists of two parts including the slide-on μIRE and slide-on housing (Figure 3.1). The first component is the slide-on Ge μIRE which was designed for Ge positioning and Ge alignment in order to achieve high energy throughput. The second component is the slide-on housing which fixed on continuμm infrared microscope. The slide-on Ge μIRE is slid into the position of slide-on housing as illustrated in Figure 3.2. The homemade Ge slide-on IRE was locked by a knob. Ray tracing within infrared microscope and the infrared radiation traveling into the IRE are shown in Figure 3.3. The infrared radiation from microscope was coupled at the hemispherical dome-shaped and impinges at the tip of cone-shaped Ge μIRE while total internal reflection occurs at the tip of cone-shaped Ge μIRE. The coupled radiation can be employed for ATR FT-IR spectral acquisition of samples having a contact with the tip of the Ge μIRE.

In this study, due to the small sampling area of the slide-on Ge μIRE, a minute amount of lipstick smear on the several surfaces was analyzed.

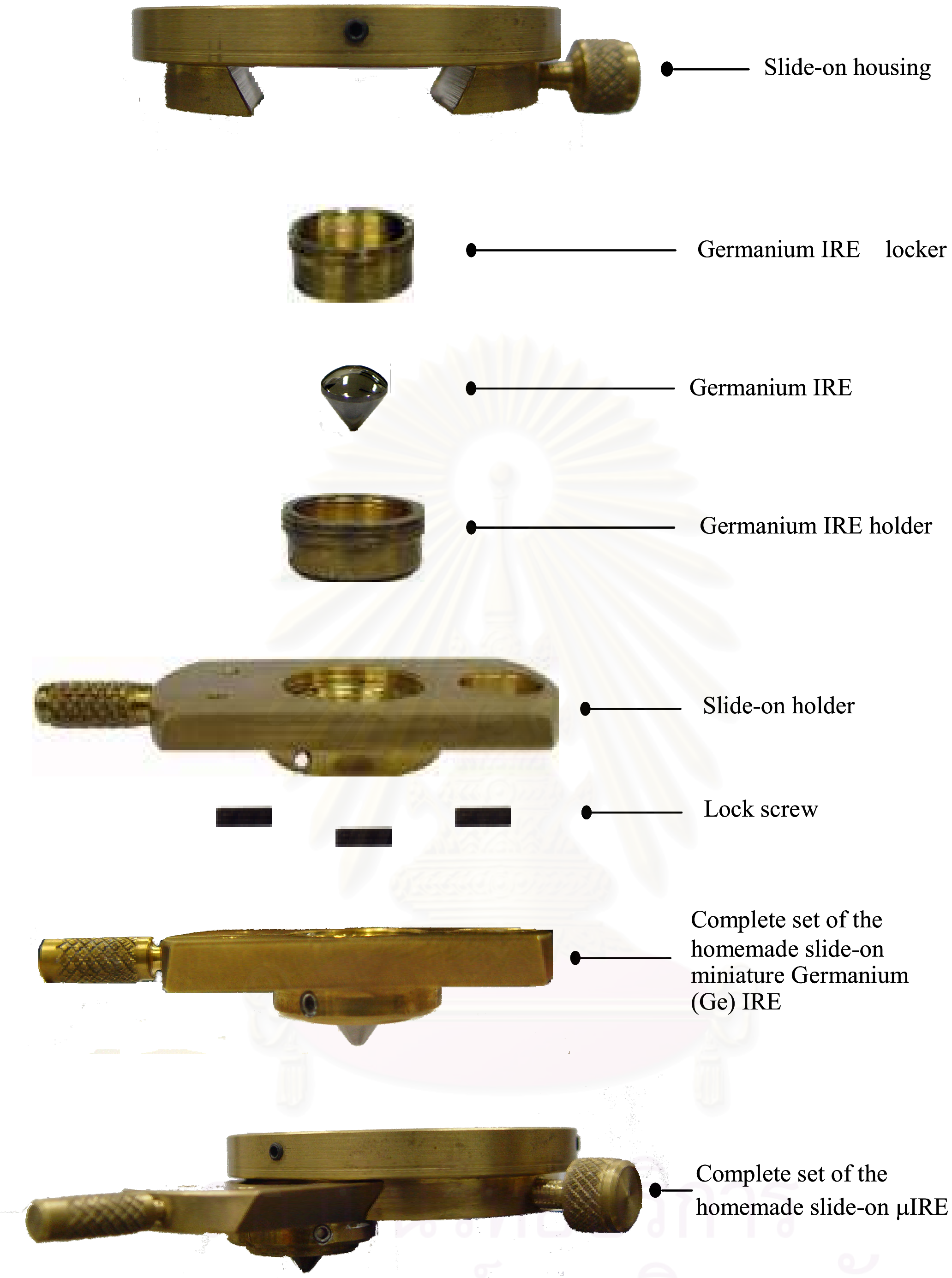


Figure 3.1 The homemade slide-on germanium (Ge) µIRE.

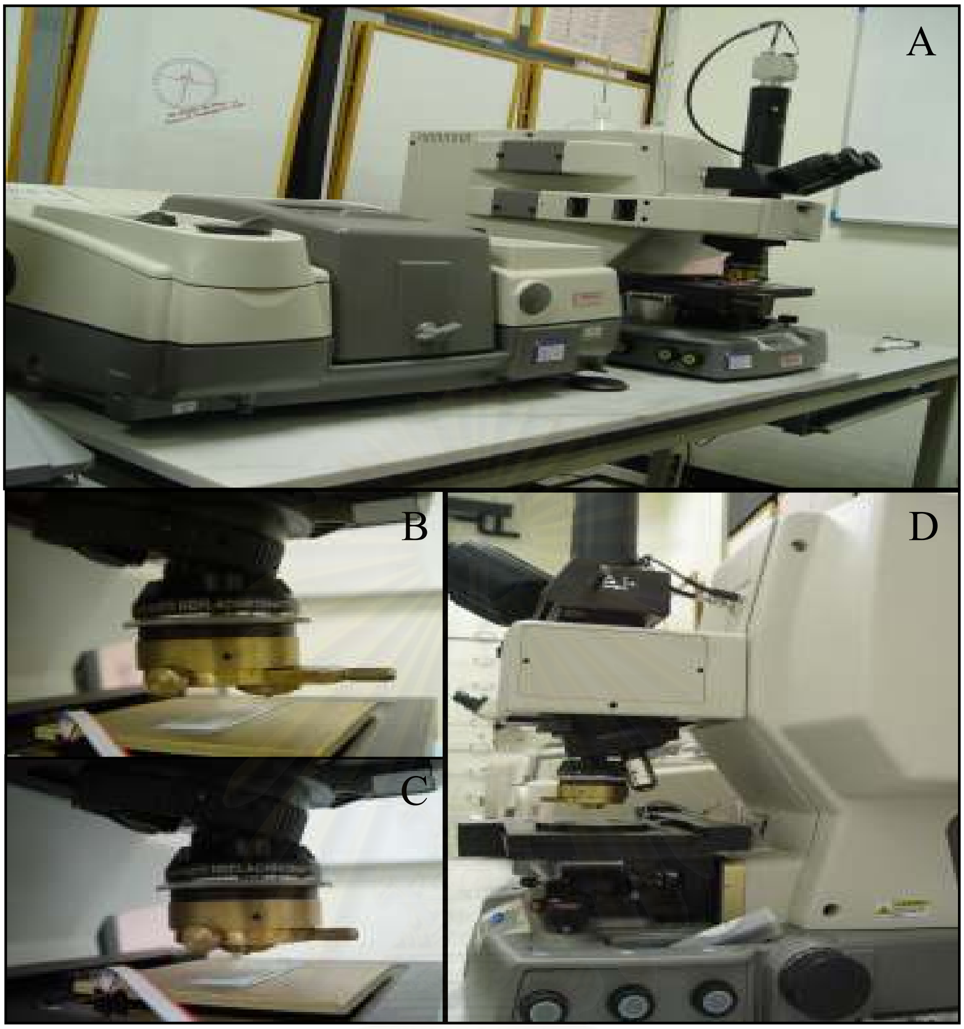


Figure 3.2 (A) continuμm infrared microscope attached to the Nicolet 6700 FT-IR spectrometer (B) slide-on Ge μIRE was slid into the position of the slide-on housing on the 15X Schwarzschild-Cassegrain infrared objective (C) The slide-on IRE was fixed on the slide-on housing (D) complete set of the homemade μIRE accessory.

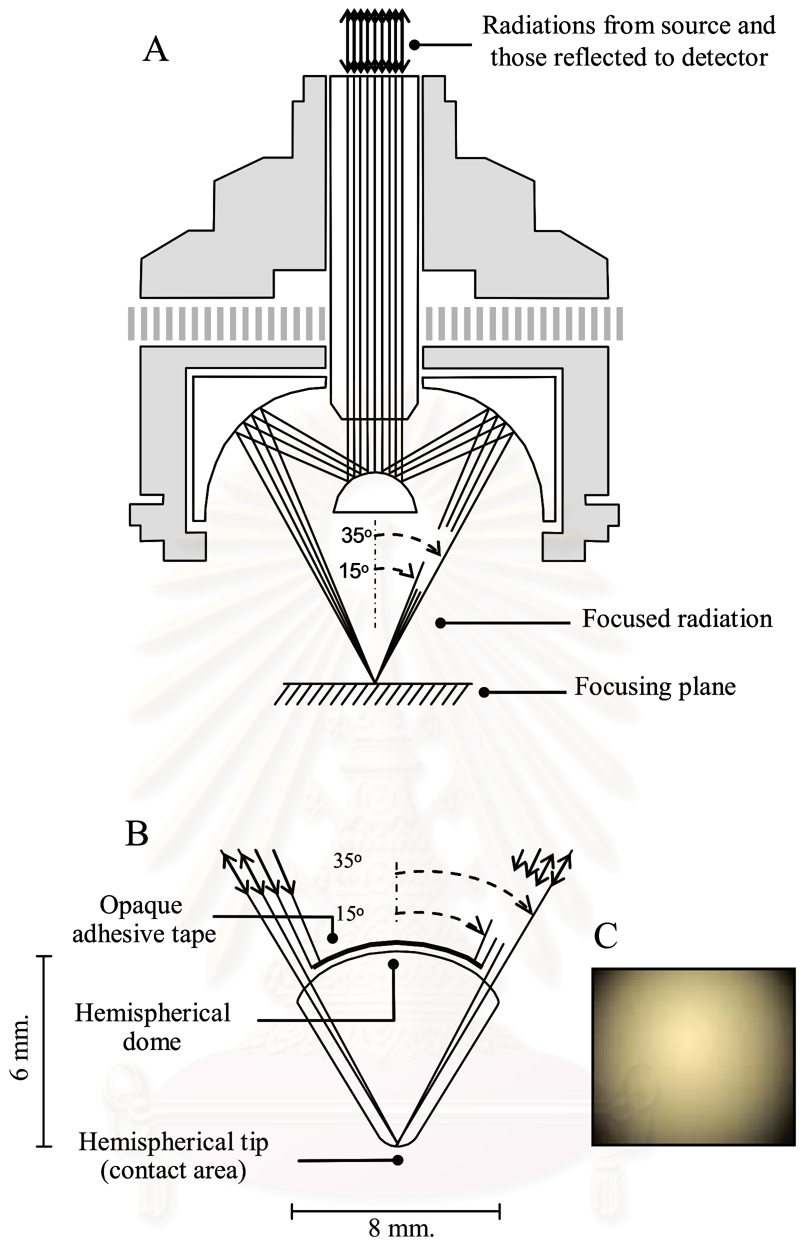


Figure 3.3 A schematic illustration of ray tracing within (A) the 15X Schwarzchild-Cassegrain infrared objective microscope and (B) the infrared radiation traveling into the Ge μIRE (C) Image of the tip of Ge μIRE.

3.2.2 The Homemade Slide-on Diamond Accessory

The homemade slide-on diamond accessory consists of two components including the diamond slide-on IRE and the slide-on housing (Figure 3.4). The first component, the slide-on diamond µIRE, was designed for place diamond and diamond alignment to achieve the maximum energy throughput. The second component is the slide-on housing which was fixed on the continuµm infrared microscope. The homemade slide-on diamond µIRE is slid into the position of slide-on housing as illustrated in Figure 3.5. The homemade slide-on diamond µIRE was locked with a knob when a high energy throughput was obtained. Ray tracing within infrared microscope and the infrared radiation traveling into the IRE are shown in Figure 3.6. Unlike the Ge µIRE, the infrared radiation from the microscope was coupled at the table facet of the diamond while total internal reflection occurs at the pavilion facet of diamond. The coupled radiation can be employed for ATR FT-IR absorption of samples having a contact with the pavilion facet of the diamond IRE.

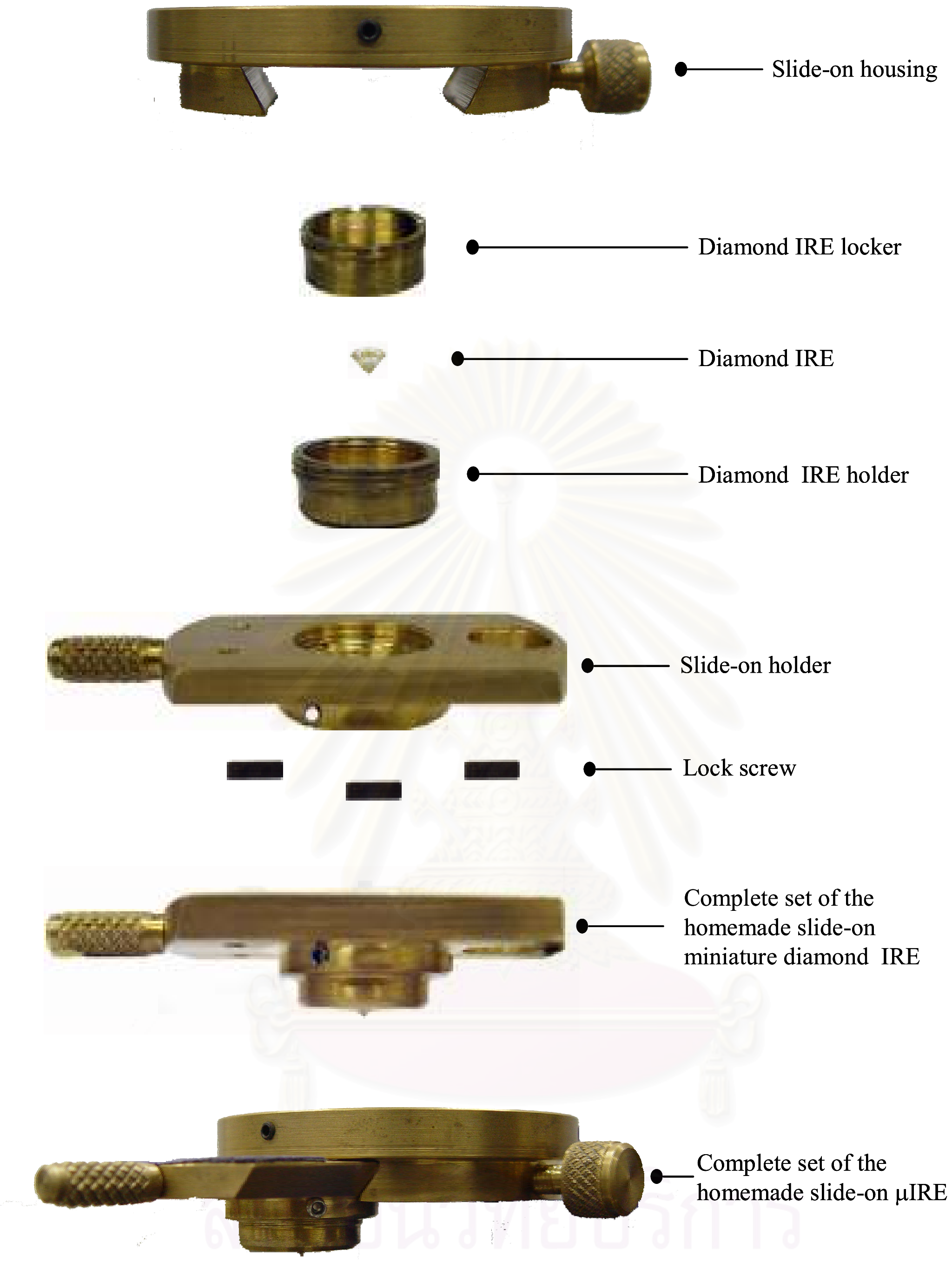


Figure 3.4 The homemade slide-on diamond μIRE.

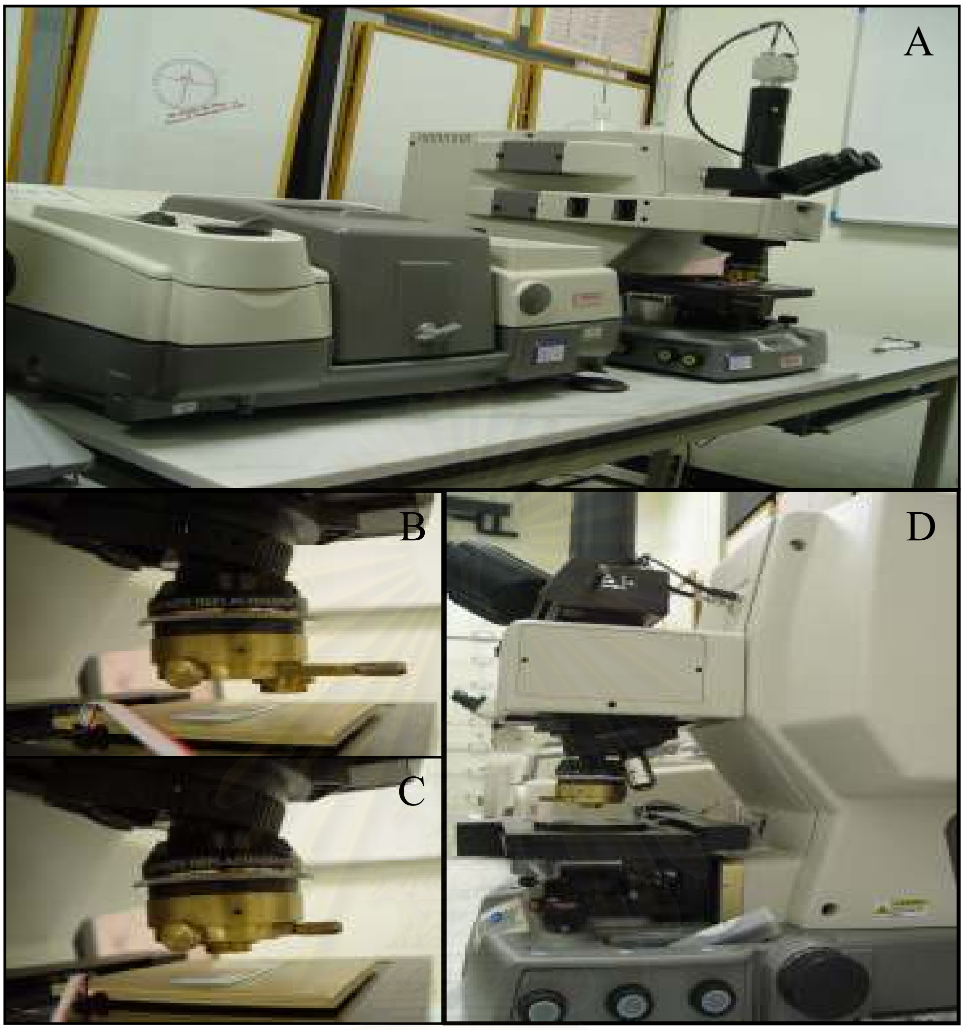


Figure 3.5 (A) continuμm infrared microscope attached to the Nicolet 6700 FT-IR spectrometer (B) slide-on diamond μIRE was slid into the position of the slide-on housing on the 15X Schwarzschild-Cassegrain infrared objective (C) slide-on IRE was fixed on the slide-on housing (D) complete set of the homemade μIRE accessory.

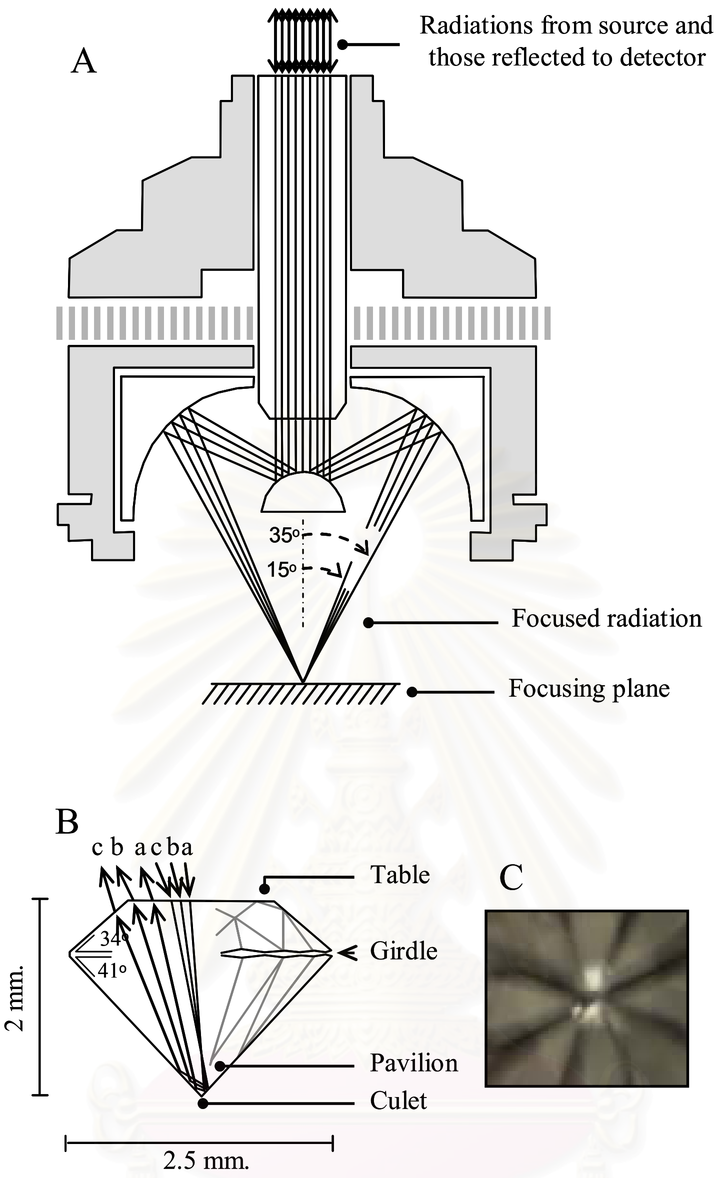


Figure 3.6 A schematic illustration of ray tracing within (A) the 15X Schwarzchild-Cassegrain infrared objective microscope and (B) the infrared radiation traveling into the diamond μIRE (C) Image of the tip of diamond μIRE.

3.3 Spectral Analysis of Lipsticks

3.3.1 Sample Preparation

The samples are some variety of lipsticks from cosmetic manufacturers for measurement with the homemade μIRE accessories, the slide-on Ge μIRE and the slide-on diamond μIRE. Lipstick smears were made on a glass slide**.** After that the spectrum of a lipstick sample on the glass slide was collected.

For conventional ATR experiment**,** lipstick smears was made at the center of surface of the hemispherical Ge IRE. After that the spectrum of lipstick samples on IRE was collected.

3.3.2 Experimental Procedure for the Homemade μIRE Accessories

All ATR spectral acquisition was collected with a Continuμm^{TM} infrared microscope equipped with a mercury-cadmium-telluride (MCT) detector. The microscope was connected with a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI USA). Spectra in mid-infrared region (4000-650 cm^{-1}) at spectral resolution of 4 cm^{-1} were collected with 256 co-addition scans. Two homemade μIRE accessories with a slide-on germanium (Ge) μIRE and a slide-on diamond μIRE were employed for ATR spectral acquisitions of lipsticks. The IREs were aligned and fixed on the slide-on housing at the 15X Schwarzschild-Cassegrain infrared objective. The ATR spectral acquisitions were performed in the reflection mode. The single beam spectrum obtained from the Ge μIRE without a sample was used as a background spectrum.

To collect ATR spectrum of a trace lipstick smear on the glass slide with the slide-on Ge μIRE, a lipstick sample on the glass slide was placed on the microscope stage. The microscope stage was elevated until the selected position touched the tip of the IRE. To achieve the same degree of contact for all measurement, a contact alert sensor plate was employed. The elevation was stop when the sensor plate indicated an onset of the critical force exerted on the sensor plate. The degree of contact was reproducible as the LED of the sensor plated was lid when a pre-defined pressure was attained. After the stage was lower, ATR spectrum of the residual lipstick on the tip of the slide-on Ge μIRE was collected. The contact alert sensing plate was employed for prevention of the possible damage of the surface of Ge μIRE, since the surface of Ge μIRE can be easily scratched.

For the slide-on diamond μIRE, the trace lipstick was deposited at the pavilion facet of the diamond μIRE. ATR spectrum of a trace lipstick smear was collected with the same procedure of Ge μIRE.

3.3.3 Experimental Procedure for Conventional ATR Accessory

A commercial single reflection ATR accessory (the Seagull^{TM}, Harrick Scientific, USA) was employed for ATR spectral acquisition of lipsticks. A hemispherical Ge IRE (diameter of 25 mm) was used as IRE. The Seagull^{TM} was placed in the compartment of the spectrometer. A lipstick smears was made at the center of the flat surface of the hemispherical Ge IRE with a diameter of at least 5 mm. The spectrum was acquired by the Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI USA) with a 30° angle of incidence and spectra in mid-infrared region (4000-650 cm^{-1}) at spectral resolution of 4 cm^{-1} were collected with 256 co-addition scans. The single beam spectrum obtained from spectral acquisition of the hemispherical Ge IRE with no sample contacting was used as a background.

Figure 3.7 Single-reflection attenuated total reflection accessory (The Seagull^{TM}, Harrick Sceintific, USA) with a hemispherical Ge IRE at the incident angle of 30°.

3.4 Influence of Substrates on Lipsticks Analysis

3.4.1 Sample Preparation

This section, a lipstick sample was choosed as volunteers for the studying of substrate influence. The lipstick was applied on various types of substrates such as glass, tissue paper, straw, cigarette butt, cotton cloth, single cotton bundle, poly(ethylene terephthalate) (PET) fiber, and chopstick. After that the lip print was analyzed by both of μATR accessories.

3.4.2 Experimental Procedure for the Homemade μIRE Accessories

All ATR spectra were collected by using a Continuμm^{TM} infrared microscope equipped with a mercury-cadmium-telluride (MCT) detector. The microscope was connected with a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI USA). Spectra in mid-infrared region (4000-650 cm^{-1}) at spectral resolution of 4 cm^{-1} were collected with 256 co-addition scans. Two homemade μIRE accessories with a slide-on germanium (Ge) IRE and a slide-on diamond IRE were employed for ATR spectral acquisitions of lipsticks. The IREs were aligned and fixed on the slide-on housing at the 15X Schwarzschild-Cassegrain infrared objective. The ATR spectral acquisitions were performed in the reflection mode. The single beam spectrum obtained from spectral acquisition of the hemispherical Ge IRE with no sample contacting was used as a background spectrum.

To collect ATR spectrum of a trace lipstick smear on a substrate with the IREs, A "*contact-and-collect*" operation was performed for ATR FT-IR spectra measurement of a minute amount of lipstick on the substrate. A trace lipstick smear on the substrate was placed on the microscope stage. The microscope stage was elevated until the selected position touched the tip of the IRE. ATR spectrum of a trace lipstick smear on substrate was collected. The observed spectrum is a spectrum of lipstick included substrate. After the stage was lowed, some of the residual lipstick stuck at the IREs tip after removing of substrates that the trace of lipstick on the IREs was identified. The contact alert sensing plate was acquired for prevention of the possible damage of the surface of Ge μIRE since the surface of Ge μIRE can be easily scratched.

The advantage of the slide-on diamond μIRE is that it can be used to analyze a trace lipstick smear on a hard or rough surface due to the inherent hardness of the diamond μIRE. The ATR spectrum of a trace lipstick smear was collected with the same procedure of the slide-on Ge μIRE.

3.4.3 Experimental Procedure for Conventional ATR Measurement

A commercial single reflection ATR accessory (the Seagull^{TM}, Harrick Scientific, USA) with a hemispherical Ge IRE (diameter of 25 mm) was employed for ATR spectral acquisitions of lipsticks. The Seagull^{TM} was placed in the compartment of the spectrometer. A lipstick smears on substrate were made at the center of the flat surface of the hemispherical Ge IRE with a diameter of at least 5 mm. A spectrum was acquired with the Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI USA) with a 30° angle of incidence and spectra in mid-infrared region (4000-650 cm^{-1}) at spectral resolution of 4 cm^{-1} were collected with 256 co-addition scans. The single beam spectrum obtained from spectral acquisition of the hemispherical Ge IRE with no sample contacting was used as a background spectrum.

3.5 Time Dependence Analysis of Lipstick Smears

Sample Preparation

This section, lipstick samples from cosmetic manufacturers was collected. Then, the lipsticks were applied on volunteers. After that lip prints were made on substrates**.** Lipstick samples on substrate were kept at the ambient condition for 1, 2, 15, and 30 days. After that the lip print was analyzed by the slide-on Ge μIRE.

Experimental Procedure for the Homemade Slide-on Ge Accessory

ATR spectra of a trace lipstick smear on various substrates were collected with the same procedures that of the homemade slide-on Ge μIRE in the section 3.4.2.

3.6 Influence of Environmental Effects on the Change of Chemical Composition on Lipstick Analysis

Sample Preparation

Lipstick samples from cosmetic manufacturers were collected. Then, the lipsticks were applied on volunteers. After that lip prints were made on substrates. Lipstick samples on substrate were kept in the car, under sunlight, and in the water for 1, 7, and 30 days. After that the spectrum of lipstick samples on substrate from each condition was collected.

Experimental Procedure for the Homemade Ge Slide-on Accessory

ATR spectra of a trace lipstick smear on various substrates were collected with the same procedures that of the homemade slide-on Ge μIRE in the section 3.4.2.

CHAPTER IV

RESULTS AND DISCUSSION

Forensic analysis is one of the applications of ATR technique. The homemade μIRE accessories, the slide-on Ge μIRE and the slide-on diamond μIRE, were applied for forensic examination of lipsticks. They were employed for ATR FT-IR spectral acquisition using infrared microscope. The spectral acquisition was performed in reflection mode.

4.1 Comparison of the ATR Spectra acquired by Three Accessories

Several researches employed various techniques for the forensic analysis of lipsticks such as thin layer chromatography (TLC) [10-11], high performance liquid chromatography (HPLC) [10, 15, 17, 18], and gas chromatography (GC) [9, 11, 20-23]. However, most method used fairly large samples. These methods are destructive sampling technique and need sample preparation. As a result, they were not suitable for analysis of trace lipstick smears in the crime scene. In case of forensic analysis, the technique must be capable of analyzing small amount of sample and non-destructive analysis is required for kept the further analysis. In order to perform forensic examination of small sample, the homemade μIRE accessories were developed for the ATR measurements. Due to the small sampling area of diamond and cone-shaped Ge μIRE, small quantities of lipsticks can be analyzed. The operation was non-destructive and could be performed without a sample preparation.

A lipstick generally consists of many ingredients, such as waxes (which used to prepare a lipstick base), oils, fats, color additives, perfume, flavoring, and other additives. In this work, lipstick samples from various manufactures were investigated. The ATR FT-IR spectra of lipstick samples from the cosmetic manufacture were collected by all three accessories: hemispherical Ge IRE (30°), the slide-on diamond μIRE, and slide-on Ge μIRE, were shown in Figure 4.1A, 4.1B, and 4.1C, respectively.

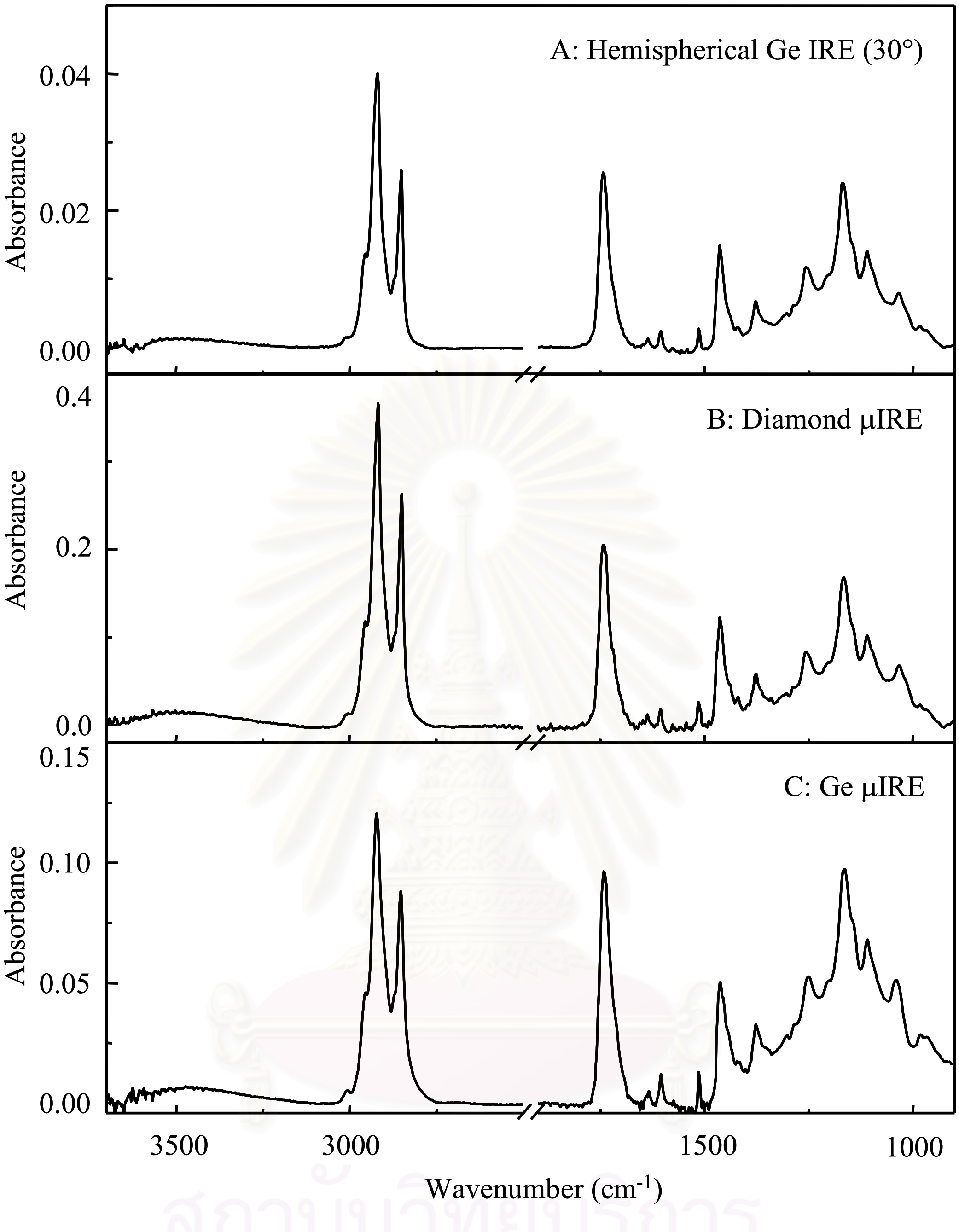


Figure 4.1 ATR FT-IR spectra of a commercial lipstick (Oriental Princess, 05 Tropical Orange) were acquired by: (A) Hemispherical Ge IRE (30°), (B) The homemade slide-on diamond μIRE, and (C) The homemade slide-on Ge μIRE.

The observed spectra of a lipstick were obtained by the slide-on Ge μIRE (Figure 4.1B) and slide-on diamond μIRE (Figure 4.1C) were the same as those acquired by the hemispherical Ge IRE (30°) (Figure 4.1A). Although the same spectral features were observed, the slide-on Ge μIRE and slide-on diamond μIRE gave better spectral quality than that of hemispherical Ge IRE (30°). The ATR spectra acquired by both μIRE accessories were noticeable of the high noise level due to the nature of incident radiation penetrating into the sample. In addition, due to the small sampling area of the homemade μIRE accessories, the smaller quantities of lipsticks can be analyzed. This technique was used as a direct analytical technique for lipsticks analysis without any pre-treatment while the operation was non-destructive.

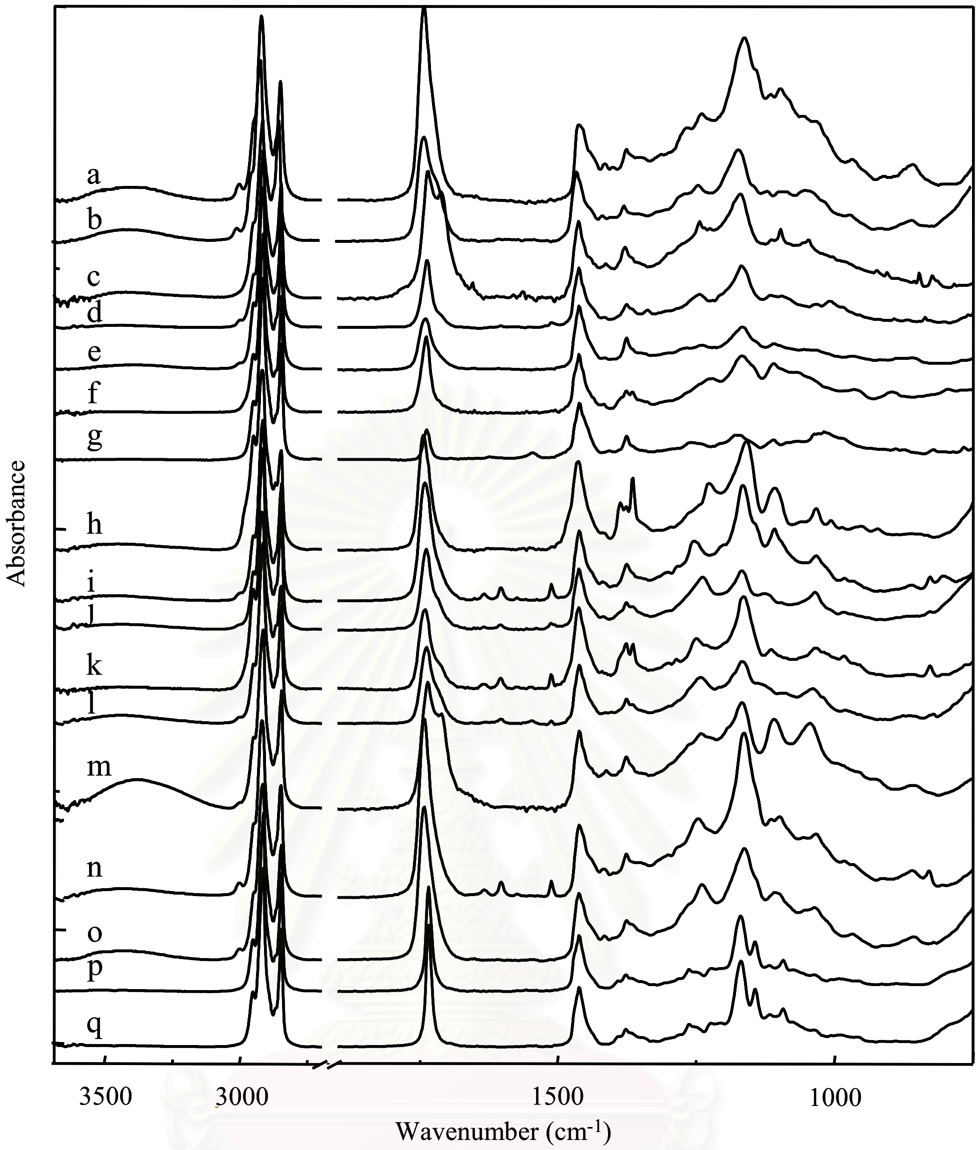


Figure 4.2 ATR FT-IR spectra of some lipstick samples from various cosmetic manufactures acquired by Hemispherical Ge IRE (30°). The lipstick samples included: (a) Body shop (Rasberry lip balm), (b) Estēe Lauder (13 Beautiful pink), (c) Yves Saint Laurent (80 Rouge flammable poinsettia), (d) Lancôme (50 Rouge essential), (e) Ponds (Lip care-smooth), (f) No brand (Local made), (g) CHANEL (06 Pourpre), (h) Kanebo (OR-508 mandarin), (i) Oriental Princess (05 Tropical Orange), (j) Christian Dior (005 Corail), (k) Christian Dior (Brun photo brown), (l) Christian Dior (984 Bibelot rubyglass), (m) Elizabeth Arden (074 Spiced pecan), (n) Clinique (135 Plum sicle), (o) Clinique (A different grape), (p) Clinique (11 Tawny spice), (q) Clinique (16 Pink chocolate matte).

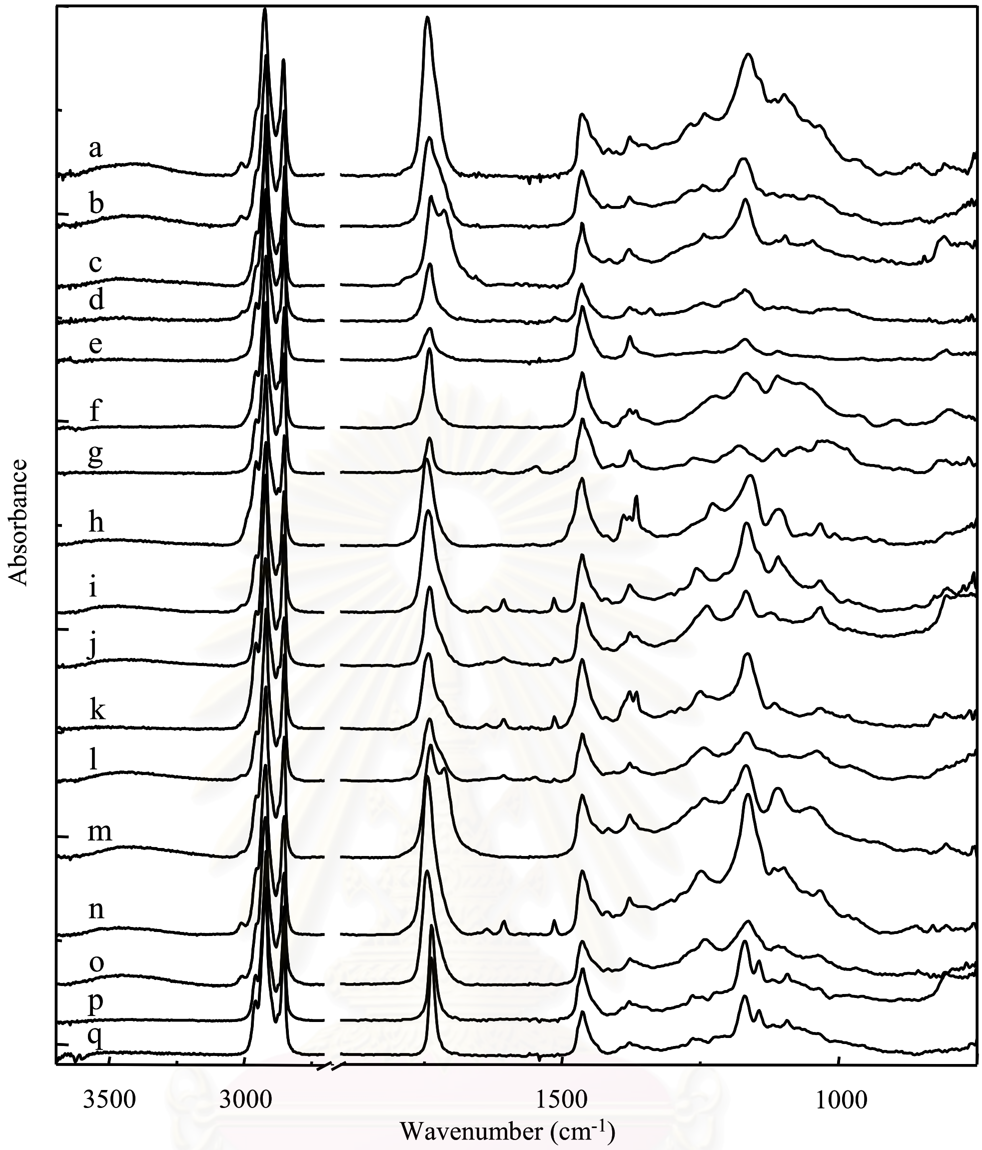


Figure 4.3 ATR FT-IR spectra of some lipstick samples from various cosmetic manufactures acquired by slide-on Ge μIRE. The lipstick samples included: (a) Body shop (Rasberry lip balm), (b) Estēe Lauder (13 Beautiful pink), (c) Yves Saint Laurent (80 Rouge flammable poinsettia), (d) Lancôme (50 Rouge essential), (e) Ponds (Lip care-smooth), (f) No brand (Local made), (g) CHANEL (06 Pourpre), (h) Kanebo (OR-508 mandarin), (i) Oriental Princess (05 Tropical Orange), (j) Christian Dior (005 Corail), (k) Christian Dior (Brun photo brown), (l) Christian Dior (984 Bibelot rubyglass), (m) Elizabeth Arden (074 Spiced pecan), (n) Clinique (135 Plum sicle), (o) Clinique (A different grape), (p) Clinique (11 Tawny spice), (q) Clinique (16 Pink chocolate matte).

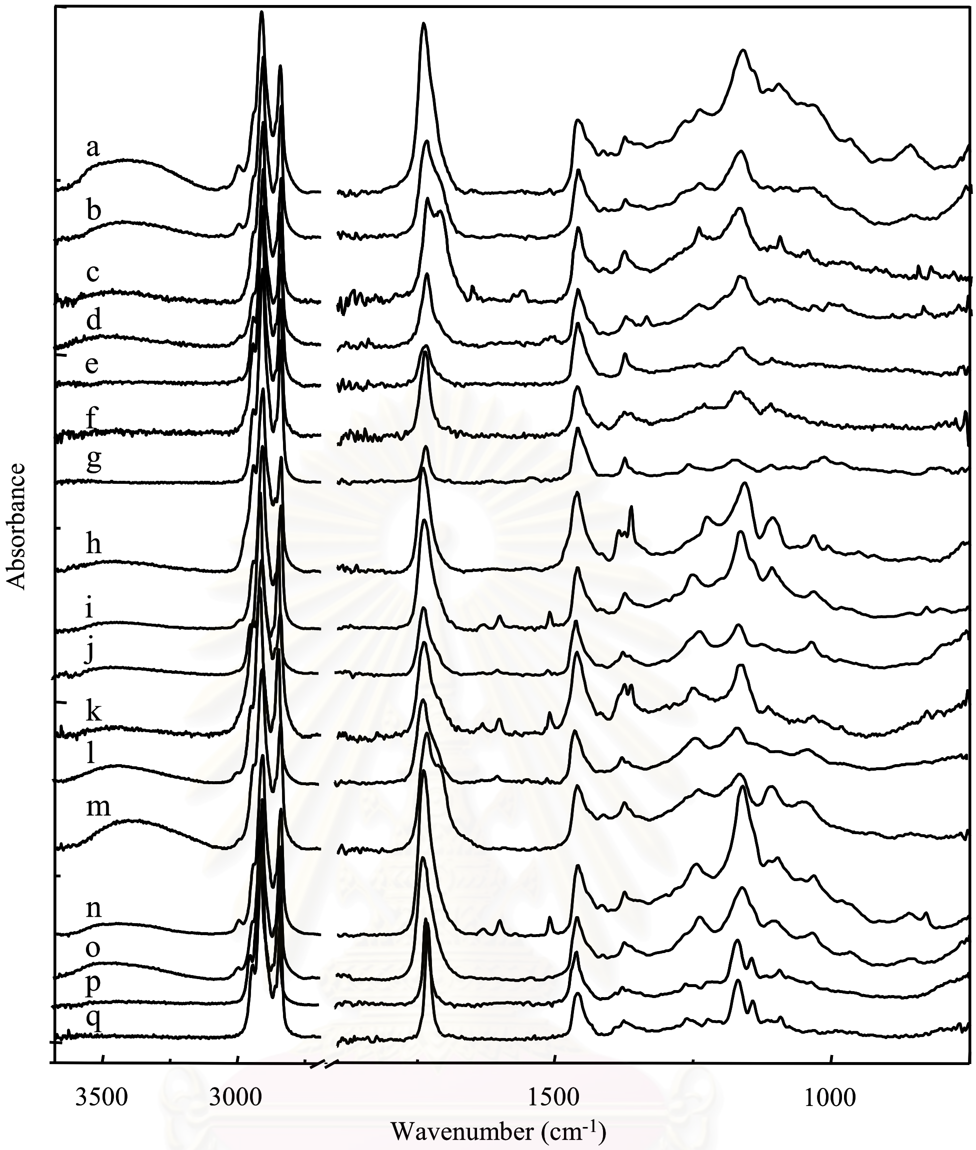


Figure 4.4 ATR FT-IR spectra of some lipstick samples from various cosmetic manufactures acquired by slide-on diamond μIRE. The lipstick samples included: (a) Body shop (Rasberry lip balm), (b) Estēe Lauder (13 Beautiful pink), (c) Yves Saint Laurent (80 Rouge flammable poinsettia), (d) Lancôme (50 Rouge essential), (e) Ponds (Lip care-smooth), (f) No brand (Local made), (g) CHANEL (06 Pourpre), (h) Kanebo (OR-508 mandarin), (i) Oriental Princess (05 Tropical Orange), (j) Christian Dior (005 Corail), (k) Christian Dior (Brun photo brown), (l) Christian Dior (984 Bibelot rubyglass), (m) Elizabeth Arden (074 Spiced pecan), (n) Clinique (135 Plum sicle), (o) Clinique (A different grape), (p) Clinique (11 Tawny spice), (q) Clinique (16 Pink chocolate matte).

The ATR spectra acquired by the diamond μIRE (Figure 4.4) were noticed the high noise level in two-phonon region (2650-1500 cm^{-1}). This is due to the saturated absorption of the diamond. Although the two-phonon region is always over absorbing, it has little effect on the analysis of lipsticks since most of the materials do not have absorption in this region.

The slide-on Ge μIRE (Figure 4.3) and the slide-on diamond μIRE (Figure 4.4) present the same spectral features for all lipstick samples. The spectra acquired by both μIRE accessories were the same as those acquired by the conventional ATR technique (Figure 4.2). The distinct advantage of the μIRE was that they worked with small sample size. As a result, it is possible to use the μIRE for forensic analysis of lipstick smears.

4.2 Chemical Information of Lipsticks

ATR FT-IR microspectroscopy is an analytical technique for chemical identification. The acquired spectra provided information directly correlated with chemical structure, and chemical composition of the analyzed samples. Therefore, this technique was performed for chemical information of lipsticks.

4.2.1 Spectral Assignment of Lipsticks

In general, lipsticks compost of ester of fatty acid as the major component. Some of the primary ingredients in lipstick included waxes, oils, and fats. Although there were many components in a lipstick, the composition (i.e., chemical structure and its content) is unique for an individual lipstick. The spectral feature associated with the chemical structure and composition can then be employed for positive identification or discrimination of lipstick samples. Two lipsticks of the same color may contain the different coloring material such as organic pigment, inorganic pigment or chemical ingredients and compositions. ATR FT-IR spectra of some commercial lipsticks from various manufactures are shown in Figure 4.5. The spectral signature of lipstick in mid-infrared region can be divided into three regions. First, the strong absorption band at 3000-2900 cm^{-1} is the C-H stretching mode of the methyl and methylene group in long chain hydrocarbons. Second, the characteristic absorption in the 1700-1600 cm^{-1} corresponds to C=O stretching vibration of the carbonyl group. And finally, the absorption band in the 1500-900 cm^{-1} is assigned to the fingerprint region. The peak assignments of absorption band, a summary of unique absorption band associated with the functional group of various components in lipsticks are shown in Table 4.1

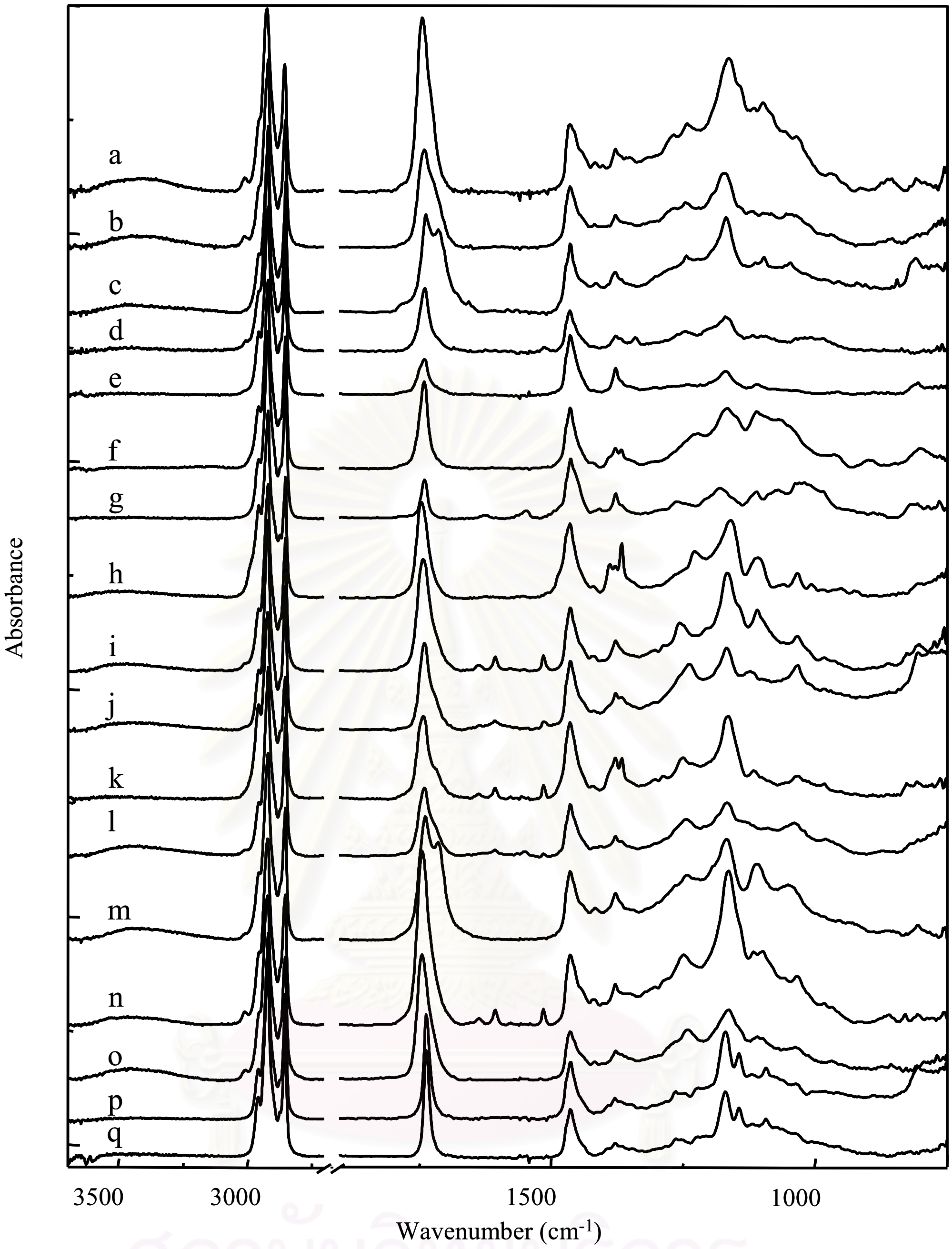


Figure 4.5 ATR FT-IR spectra of the 17 commercial lipsticks from various manufactures acquired by the slide-on Ge μIRE a: Body shop (Rasberry lip balm) b: Estēe Lauder (13 Beautiful pink) c: Yves Saint Laurent (80 Rouge flammable poinsettia) d: Lancôme (50 Rouge essential) e: Ponds (Lip care-smooth) f: No brand (Local made) g: CHANEL (06 Pourpre) h: Kanebo (OR-508 mandarin) i: Oriental Princess (05 Tropical Orange) j: Christian Dior (005 Corail) k: Christian Dior (Brun photo brown) l: Christian Dior (984 Bibelot rubyglass) m: Elizabeth Arden (074 Spiced pecan) n: Clinique (135 Plum sicle) o: Clinique (A different grape) p: Clinique (11 Tawny spice) q: Clinique (16 Pink chocolate matte).

Table 4.1 Peak assignments of aliphatic hydrocarbon in lipsticks [29-30]

Wavenumber (cm^{-1})	Vibrational mode	Functional group
3300-2500	OH stretching	carboxylic acid
3040-3010	CH stretching	RCH=CHR
2975-2950	asymmetric CH stretching	CH_3
2940-2915	asymmetric CH stretching	CH_2
2885-2865	symmetric CH stretching	CH_3
2870-2840	symmetric CH stretching	CH_2
1750-1725	C=O stretching	ester
1725-1700	C=O stretching	carboxylic acid
1640-1635	C=C stretching	Acrylates (CH_2=CHCOOR)
1490-1460	O-CH_2 deformation	O-CH_2
1463	asymmetric out-of-phase CH deformation	CH_2 (alkane)
1475-1450	asymmetric out-of-phase CH deformation	CH_3 (alkane)
1455	asymmetric out-of-phase CH deformation	CH_3 in -OCH_2CH_3
1420	CH deformation	CH_2 (next carbonyl)
1390-1360	O-CH_2 wagging	O-CH_2
1383-1377	symmetric in-phase CH_3 deformation	CH_3 (alkane)
1385, 1368	symmetric in-phase CH deformation	Gem dimethyl group -$C(CH_3)_2$
1375	symmetric in-phase CH deformation	CH_3 in -OCH_2CH_3
1300-1200	CH_2 wagging	CH_2 (alkane)
1305-1295	C-C twisting	-$(CH_2)_n$- (alkane)
1290-1280	C-O-C stretching	CH_2=CHCOOR
1275-1185	asymmetric C-O-C stretching	C-O-C
1250	C-C-O stretching	C-C-O
1300-1150	C-O stretching	-COOR (ester)
1180-1130	C-O stretching	-COOR (carboxylic acid)
1160-1050	symmetric C-O-C stretching	C-O-C
1120-1090	symmetric CCCC stretching	CCCC
1110-1080	asymmetric CCCC stretching	CCCC
1060-1035	O-CH_2 stretching	(O=C)-O-CH_2
990-980	CH deformation	CH_2=CHCOOR
970-960	CH_2 deformation	CH_2=CHCOOR

4.2.2 Spectral Comparison of the Different Types of Lipstick from the Same Manufacture

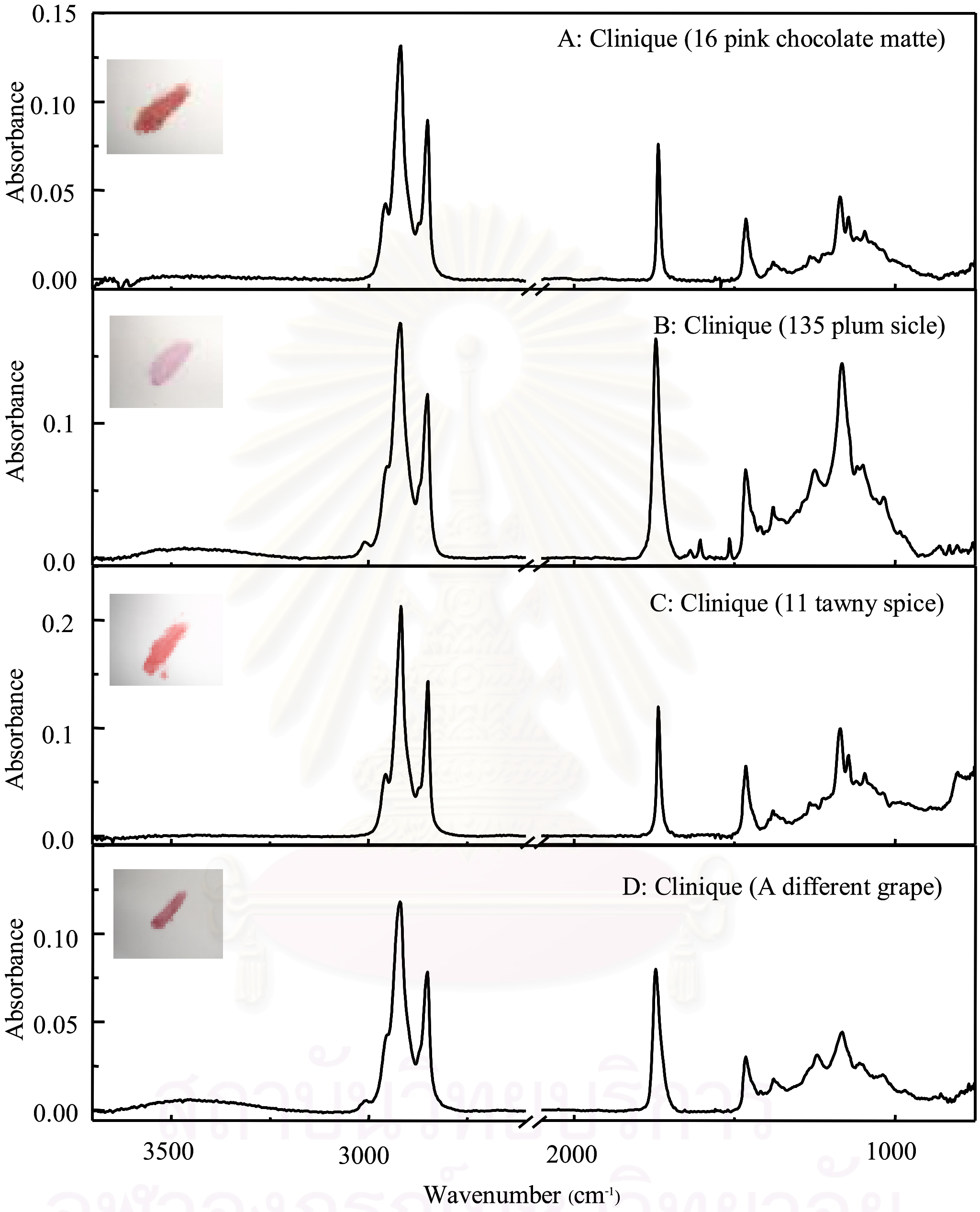


Figure 4.6 ATR FT-IR spectra of different type of Clinique lipstick acquired by the homemade slide-on Ge μIRE.

Although all of the lipstick samples from various manufactures have absorption band in 1700-1600 cm^{-1} region which corresponding to C=O stretching vibration of carbonyl group, the spectral feature of C=O group of each lipstick sample is different. The carbonyl group is contained in a large number of the different classes of compounds from the different type of carbonyl group, e.g. aldehydes, ketones, carboxylic acids, etc. for which a strong absorption band due to C=O stretching vibration is observed in the 1850-1550 cm^{-1} [32]. The frequency of this carbonyl stretching vibration is dependent on various factors such as the structural environment of the C=O group or the physical state of the sample. Thus, the different types of carbonyl have different frequencies. According to the ATR FT-IR spectra in Figure 4.6 and 4.7, each observed spectrum shows the different spectral feature of carbonyl band due to the type of the carbonyl group (i.e. carbonyl of carboxylic acid, ester or ketone) in ingredient of each commercial lipstick. Due to the different composition i.e. raw material, type of carbonyl used in lipstick formulation, different spectral feature and different peak position in carbonyl band of each lipstick were observed. For example, Figure 4.6A shows the sharp peak of carbonyl group (at ~1700 cm^{-1} region) from Clinique brand. On the other hand, a broad carbonyl band was shown in Figure 4.6B. This peak can be possible identified as the carbonyl group which occurred from many of the type carbonyl group such as carbonyl of carboxylic acid and ester group. The broad band is also associated with the appearance of OH stretching (at 3500 cm^{-1} region) which is possible as a result from the OH group of carboxylic acid.

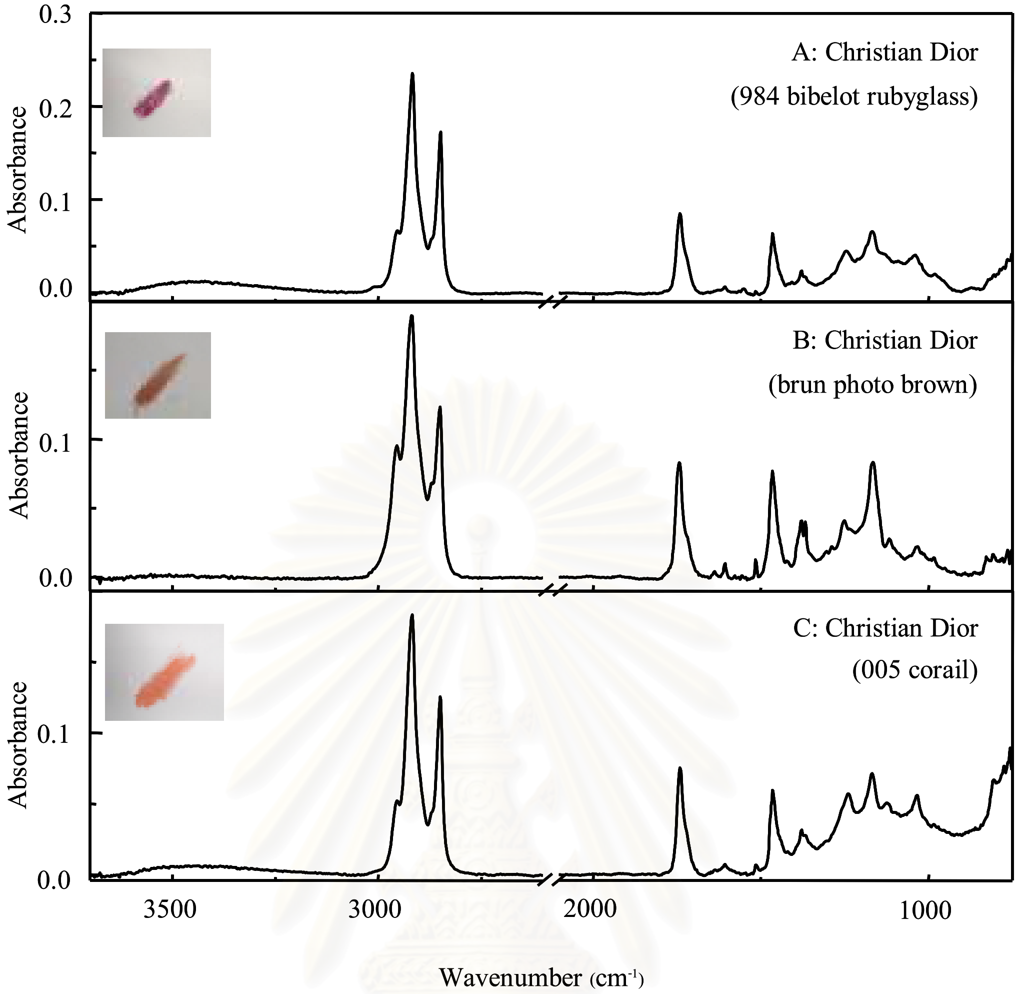


Figure 4.7 ATR FT-IR spectra of different type of Christian Dior lipstick acquired by the homemade slide-on Ge μIRE.

ATR FT-IR spectra of various models of lipstick from the same manufacture acquired by the slide-on Ge μIRE were shown in Figure 4.6 and 4.7. The lipsticks compose of different raw material of each manufacture such as type of waxes, oils, proportion of raw material, coloring materials (i.e., organic pigment or inorganic pigment), type of fragrance, preservation, and antioxidant. Since manufactures of cosmetics frequently alter ingredient in their formulations for various reasons, it is possible that unique absorption bands of an identical lipsticks may be specifically identified by ATR FT-IR spectra. The unique spectral feature associated with chemical information can be employed for forensic analysis. Therefore, it was possible to identify different brands of lipstick and the model of lipstick. They are also included to illustrate potential information to solve a criminal case.

4.2.3 Spectral Comparison of Different Shade Color of Lipstick

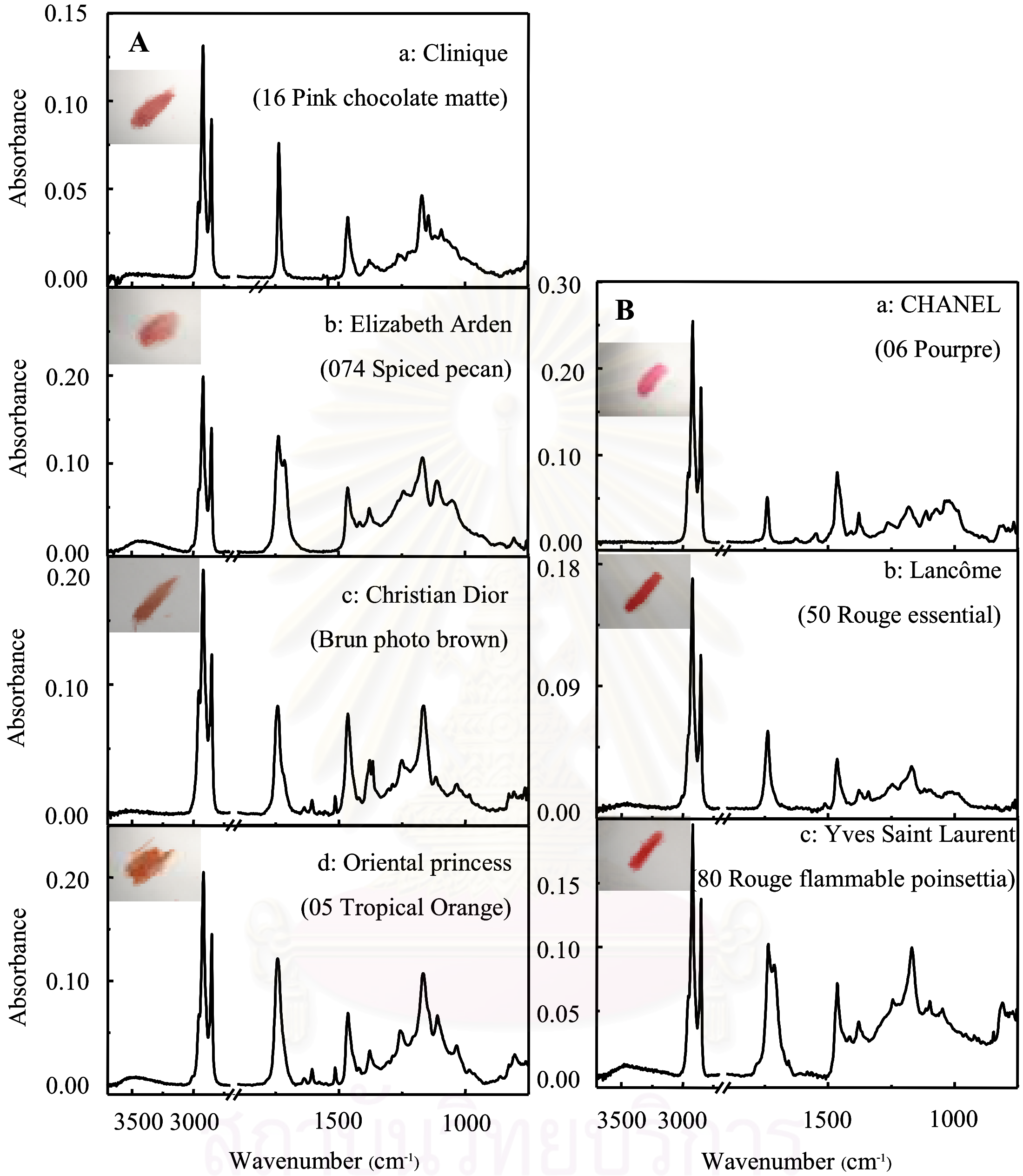


Figure 4.8 ATR FT-IR spectra of the commercial lipsticks from different shade color lipstick: (A) brown shade color, (B) red shade color acquired by the homemade slide-on Ge μIRE.

In general, lipstick with the same color may not come from the same piece. Lipsticks from different manufacture or model may have the same color or similar color. Those lipsticks may not be differentiated based on color or physical appearance alone.

The same color lipsticks from different manufactures contain different pigment and chemical composition. Lipsticks with different compositions showed different spectral feature as shown in Figure 4.8A and 4.8B. The obtained spectra can be compared with the provenance of trace lipsticks for the identification brand and type. ATR FT-IR spectroscopy allowed nearly all samples to be discriminated. Moreover, the requirement of small sample size enables the slide-on Ge μIRE to acquire spectrum of a trace lipstick. The observed spectra had same quality as that from the bulk lipstick.

4.3 Influence of Substrate on Lipsticks Analysis

For some forensic evidences, minute lipstick smears were sometimes found as evidence on various types of surfaces such as clothing, cigarette butts, glass, tissue paper, and etc. The lipstick smear may deposit on the forensic evidences as a result of attack or an unintentional contact. The smear on the surface may be large or small. The ATR FT-IR spectra of selected substrates acquired by the slide-on Ge μIRE were shown in Figure 4.9.

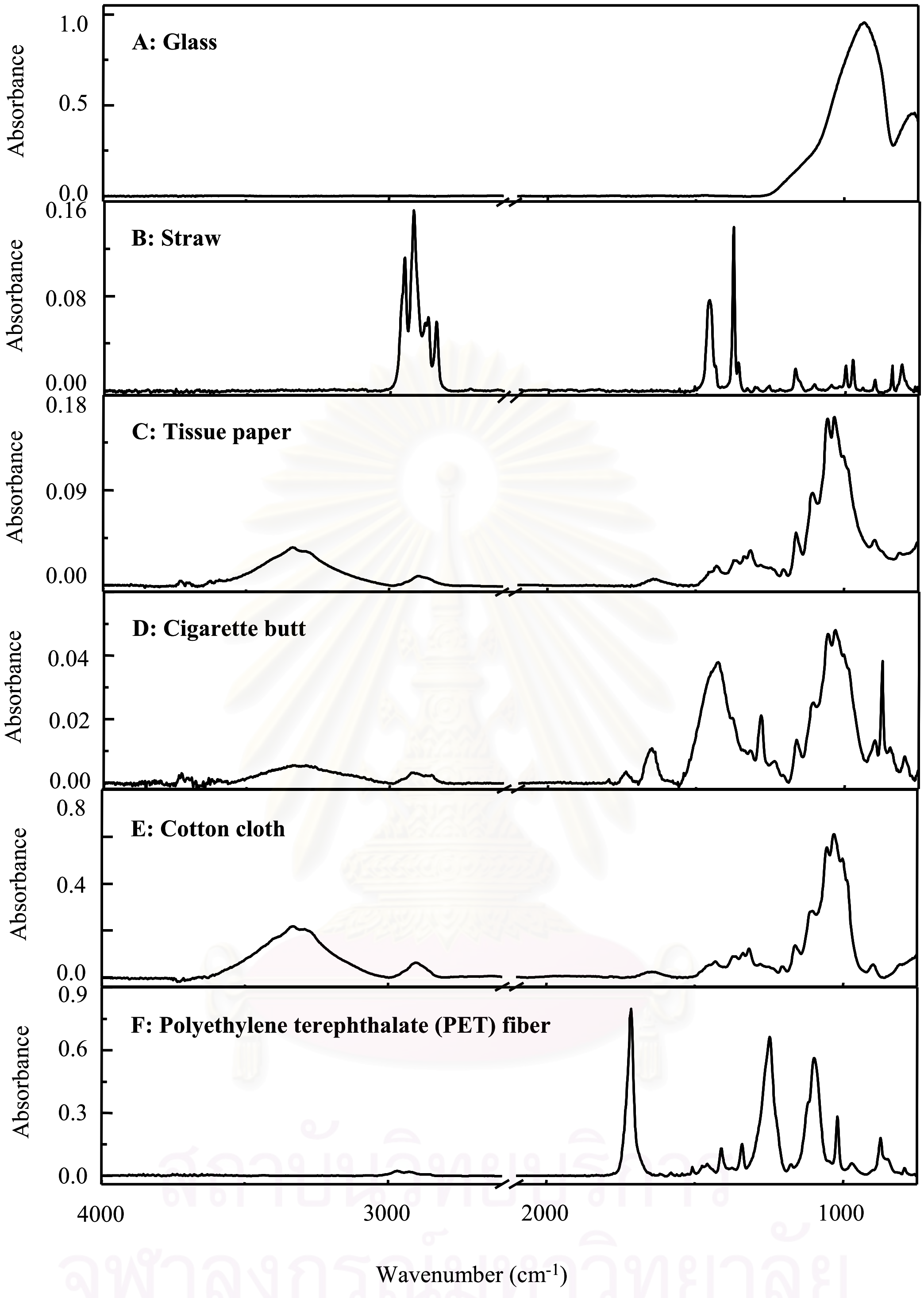


Figure 4.9 ATR FT-IR spectra of potential substrates for lipstick smear: (A) glass, (B) straw, (C) tissue paper, (D) cigarette butt, (E) cotton cloth, and (F) poly(ethylene terephthalate) fiber. The spectra were acquired by the slide-on Ge μIRE.

4.3.1 The Ge μATR Technique

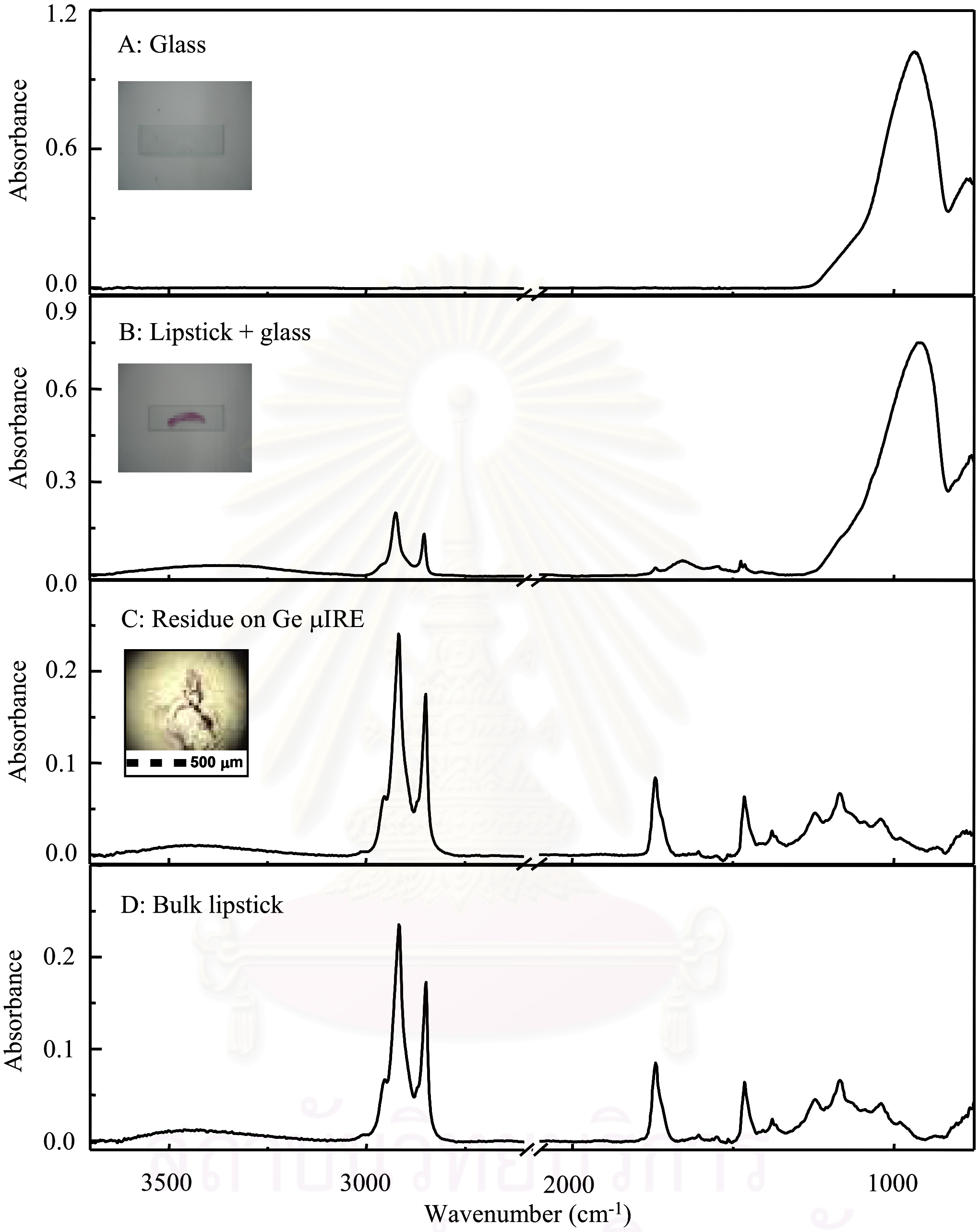


Figure 4.10 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 Bibelot rubyglass) on a glass slide acquired by the slide-on Ge μIRE: (A) glass slide, (B) a lipstick stain on glass slide, (C) spectrum of making a contact between the tip of the Ge μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

In order to acquire an ATR FT-IR spectrum of a trace lipstick on a substrate, a novel *"contact-and-collect"* technique was developed in order to perform the operation nondestructively. Due to the unique advantage of the Ge μIRE where the contact area or the sampling area of the IRE is very small (i.e., smaller than 100x100 μm^2), a small lipstick smear on any substrate can be selectively picked up at the tip of the IRE. ATR FT-IR spectrum of the collected lipstick smear can then be acquired without interference from the substrate. In this section, an influence of the first substrate as a glass was studied. The most familiar form of glass is the silica-based material used for various applications. Absorption band of glass was acquired by the slide-on Ge μIRE and was shown in Figure 4.10A. The characteristic absorption band in the 1100-1000 cm^{-1} is assigned to Si-O-Si stretching vibration of glass [30]. When glass substrate that has a lipstick stain (Figure 4.10B) was analyzed, spectral feature is change by appearance of absorption of a lipstick stain at 3000-2900 cm^{-1} is the C-H stretching mode of the methyl and methylene group of aliphatic hydrocarbons in lipstick and C=O stretching vibration (at ~1700 cm^{-1} region) of carbonyl group in lipstick. When the lipstick stain on the substrate was brought into an optical contact with the Ge μIRE, some of the lipstick was stucked at the tip of the IRE after the removal of the substrate. Several contacts were performed in order to collect a sufficient amount of the lipstick. The trace lipstick on the IRE can be characterized under the ATR mode without any interference of the substrate. As shown in Figure 4.10C, the observed spectrum of the lipstick on the tip of the IRE can be employed for the unique identification. It is identical to that of the bulk lipstick (Figure 4.10D).

An example of common substrate is straw. Straw is a material made of polypropylene (PP) which has characteristic absorption band as shown in Figure 4.11A. ATR FT-IR spectrum of trace amount of lipstick smear on a straw acquired by the Ge μIRE was shown in Figure 4.11B. When straw that has a trace lipstick smear was analyzed, spectral feature was changed by the appearance of absorption of a lipstick stain at 1700 cm^{-1} corresponds to the C=O stretching mode of carbonyl. Peak shape and peak position at C-H stretching region change in 3000-2900 cm^{-1} region due to absorption band of C-H stretching of straw. As shown in Figure 4.11C, the *"contact-and-collect"* operation was performed for ATR FT-IR spectra measurement of a minute amount of lipstick on the substrate by making a gentle contact between the tip of the μIRE and the lipstick smear on a surface. A minute amount of the lipstick was transferred onto the tip. The transferred lipstick smear can be employed for the unique identification, and matching with the bulk lipstick (Figure 4.11D).

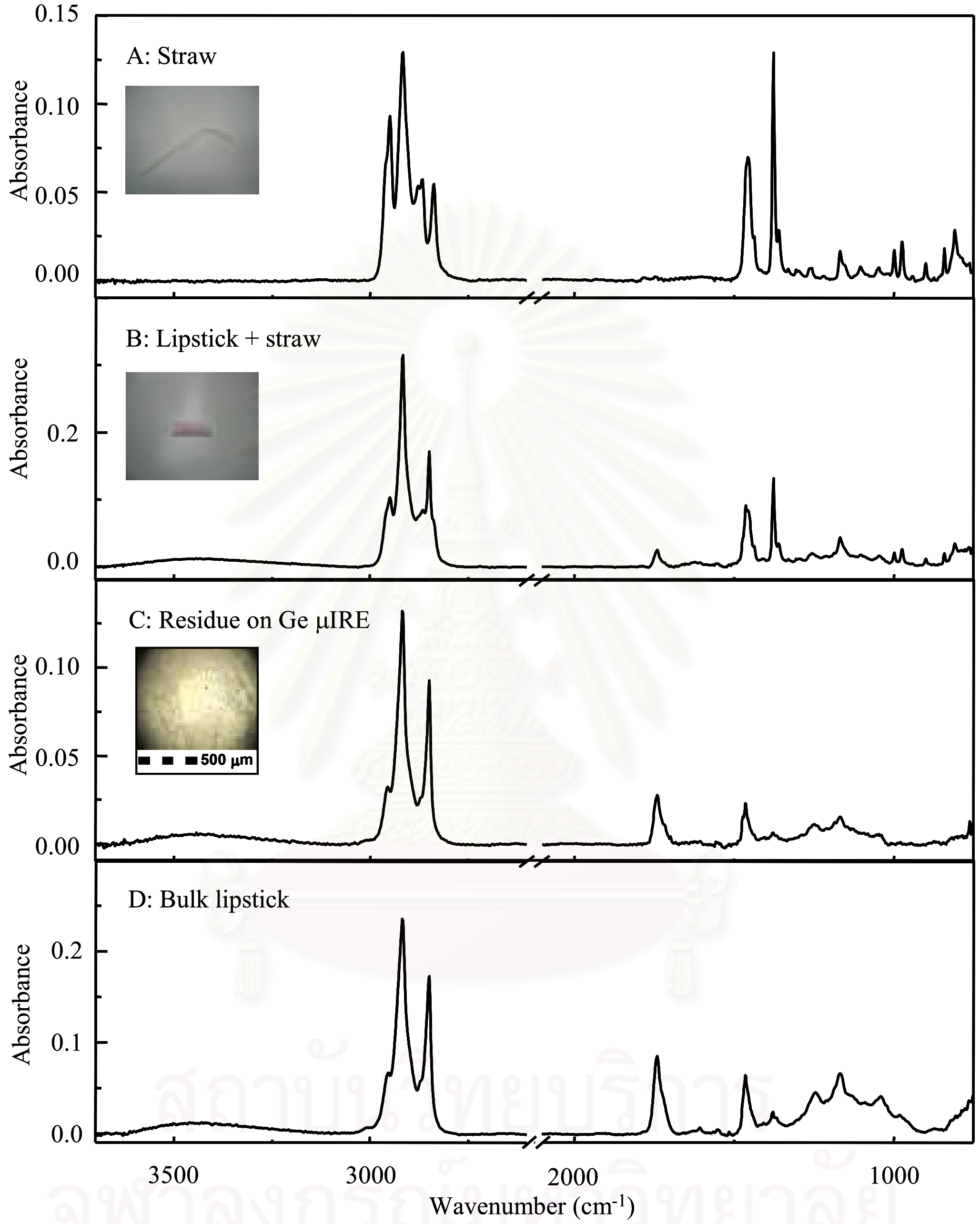


Figure 4.11 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 Bibelot rubyglass) on straw acquired by the slide-on Ge μIRE: (A) straw, (B) a lipstick stain on straw, (C) spectrum of making a contact between the tip of the Ge μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

The characteristic absorption band of tissue paper, which is a commodity of the material produced by the amalgamation of fibers, typically composed of cellulose were shown in Figure 4.12A. By composition spectrum of tissue paper and tissue paper which have a lipstick stain, difference is clearly that in their spectra. In spectrum of tissue paper that has a lipstick stain (Figure 4.12B), there are peaks in C-H stretching mode of aliphatic hydrocarbons in a stain lipstick and the absorption band at 1700 cm^{-1} region corresponding to absorption of carbonyl group. The spectrum of lipstick smears was acquired by the *"contact-and-collect"* operation by making a gentle contact between the tip of the μIRE and trace lipstick smear on the substrate. Some of the lipstick smear at the tip of the μIRE after the removal of the substrate can be characterized under the ATR mode. The observed spectrum was shown in Figure 4.12C. It can be utilized for the unique identification purpose and matching with the bulk lipstick in Figure 4.12D. According to the observed spectrum, its spectral feature is the same to those of the spectrum of the bulk lipstick. Therefore, it was possible to identify different brands of lipstick and the model of lipstick.

In case of the lipstick stain on the substrate with porous surface such as tissue paper or cotton cloth, the investigation was usually difficult. A numbers of contacts can be made in order to collect a sufficient amount of the lipstick on the tip of the Ge IRE before spectral acquisition.

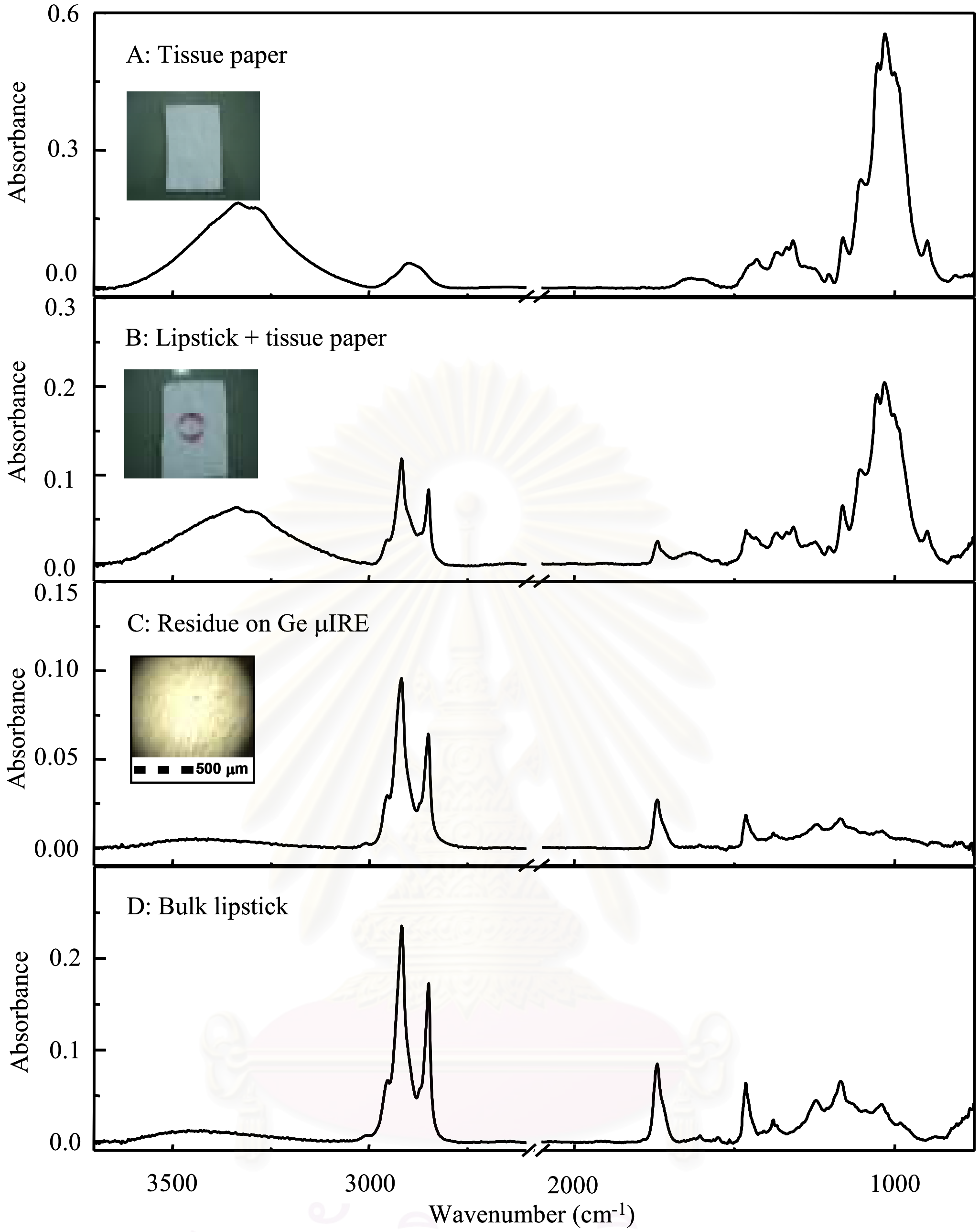


Figure 4.12 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 Bibelot rubyglass) on tissue paper acquired by the slide-on Ge μIRE: (A) tissue paper, (B) a lipstick stain on tissue paper, (C) spectrum of making a contact between the tip of the Ge μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

Cotton is a soft fiber that grows around the seeds of the cotton plant. The fiber is most often spun into thread and used to make a soft, breathable textile. It is the most widely used natural-fiber cloth in clothing today. Cotton fiber consists of nearly pure cellulose, a natural polymer. The characteristic absorption of cotton cloth like the absorption band of tissue paper since they were made from material of the same chemical composition, as shown in Figure 4.13A.

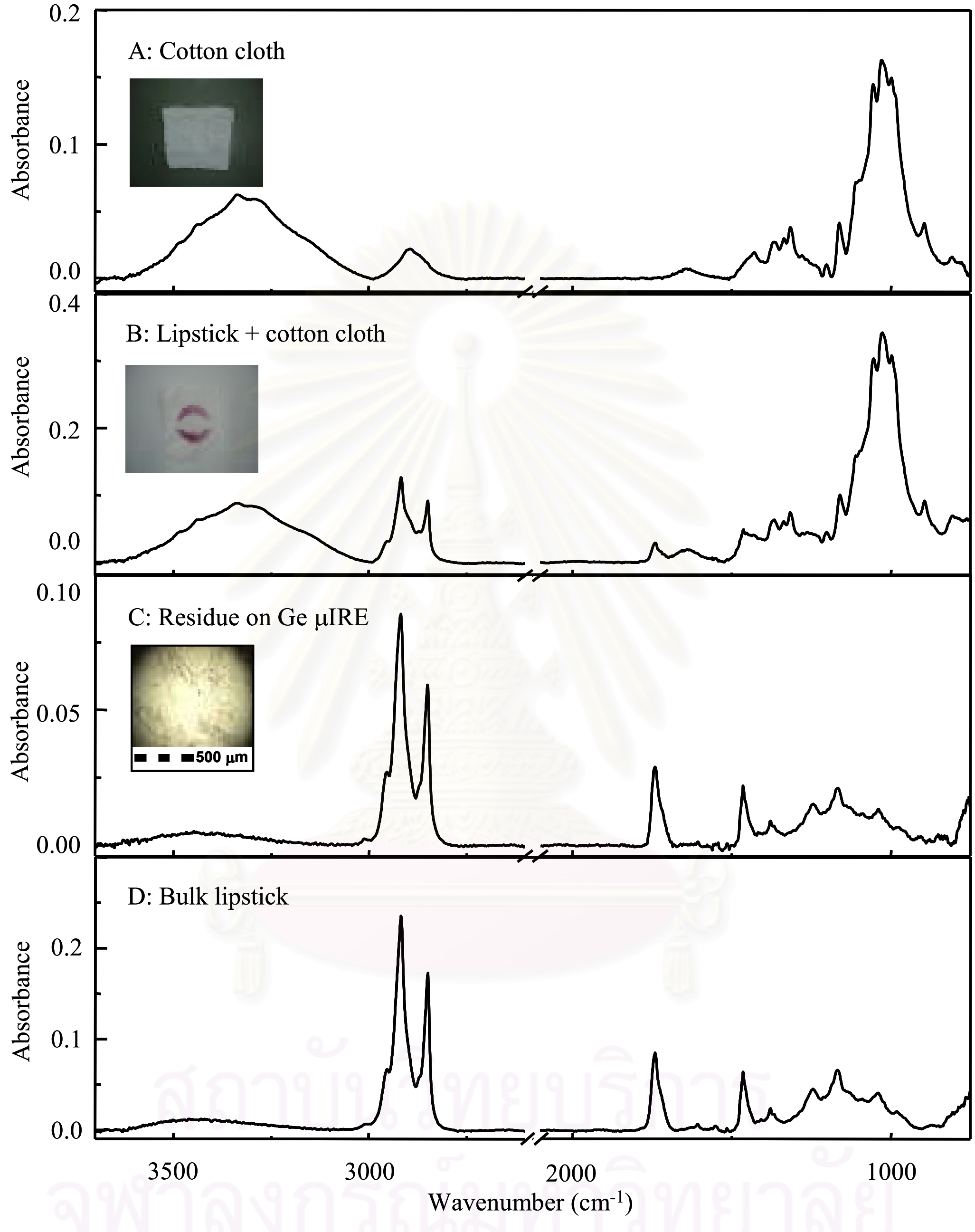


Figure 4.13 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 Bibelot rubyglass) on cotton cloth acquired by the slide-on Ge µIRE: (A) cotton cloth, (B) a lipstick stain on cotton cloth, (C) spectrum of making a contact between the tip of the Ge µIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

From the result in Figure 4.13C, the spectral feature is the same as those of the spectrum of the bulk lipstick in Figure 4.13D. Therefore, this technique can be utilized for the unique identification propose and matching with the bulk lipstick.

As the results, like the other substrates, a trace lipstick on cigarette butt can be analyzed by the *“contact-and-collect”* operation. ATR FT-IR spectrum of cigarette butt was acquired by the Ge μIRE as shown in Figure 4.14A. Appearance of absorption band in C-H stretching region (3000-2900 cm^{-1}) of a trace lipstick stain was observed in Figure 4.14B. Moreover, absorption band of C=O stretching vibrational region (at the ~1700 cm^{-1}) of lipstick stain has higher spectral intensity than absorption band in the same region of cigarette butt. ATR FT-IR spectrum of the sample at the position of contact was collected (Figure 4.14C). By comparison, both ATR FT-IR spectra of residual lipstick at the tip of Ge μIRE (Figure 4.14C) and the bulk lipstick (Figure 4.14D) were the same.

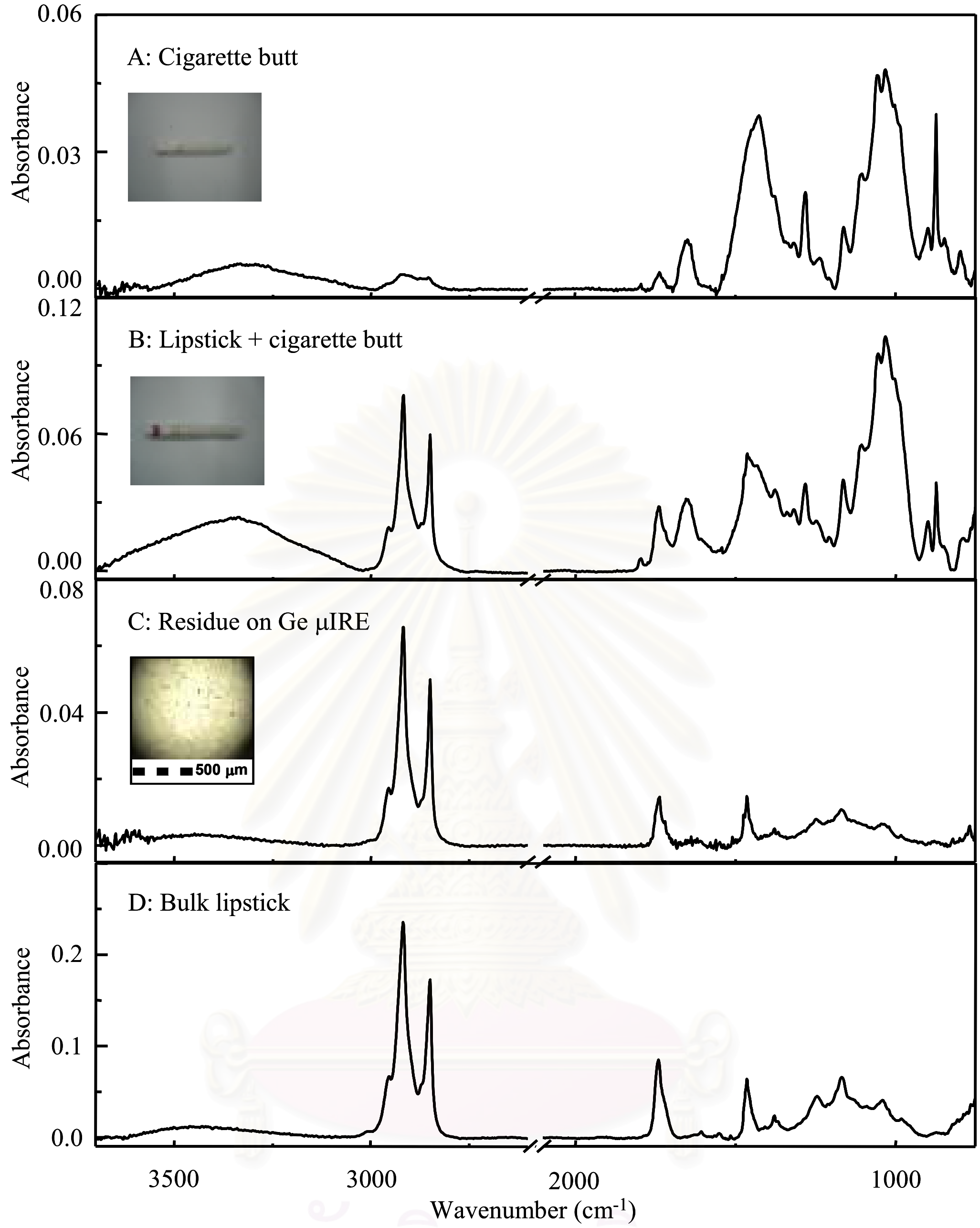


Figure 4.14 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 Bibelot rubyglass) on cigarette butt acquired by the slide-on Ge μIRE: (A) cigarette butt, (B) a lipstick stain on cigarette butt, (C) spectrum of making a contact between the tip of the Ge μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

4.3.2 The Diamond μATR Technique

The slide-on diamond μIRE has a gem quality round brilliant cut natural diamonds as internal reflection element (IRE). It was employed for ATR FT-IR spectral acquisition using an infrared microscope. The total internal reflection phenomenon occurs at the pavilion facet of diamond. The evanescent field generated at the interface can be employed for spectral acquisition for a material attached to the pavilion facet.

ATR FT-IR spectra of trace lipstick smears on several surfaces such as glass, straw, tissue paper, cotton cloth, cigarette butt acquired by the slide-on diamond μIRE are shown in Figure 4.15, 4.16, 4.17, 4.18, and 4.19 respectively. The ATR spectra acquired by the slide-on diamond μIRE have the higher signal to noise (S/N) ratio than that acquired by the Ge μIRE. It is noted that the relative intensities are different due to the nature of the incident radiation.

$$d_p = \frac{1}{2\pi\nu n_0 (\sin^2\theta - (n_1/n_0)^2)^{1/2}} \tag{4.1}$$

According to equation 4.1, there are several parameters that affect the spectral qualities. One of them is refractive index. When an organic material (refractive index of 1.5) is attached to with a diamond IRE (refractive index of 2.4), the ratio of the refractive indices is $(n_1/n_0) = 0.625$. On the other hand, when an organic material is attached to with a Ge IRE (refractive index of 4.0), the ratio of the refractive indices becomes 0.375. As the results, the penetration depth with diamond as the IRE is greater than that with Ge IRE.

Like the Ge μIRE, the observed spectra of lipstick smears on several substrates acquired by the slide-on diamond μIRE are the same as those of the bulk lipstick. In case of the glass substrate (Figure 4.15), ATR spectrum acquired by the diamond μIRE has a poor spectral quality because the optical contact between the diamond μIRE and glass slide is not achieved. The problem is due to the sensor plate indicated an onset of the critical force exerting on the sensor plate before a material attach to the pavilion of the diamond.

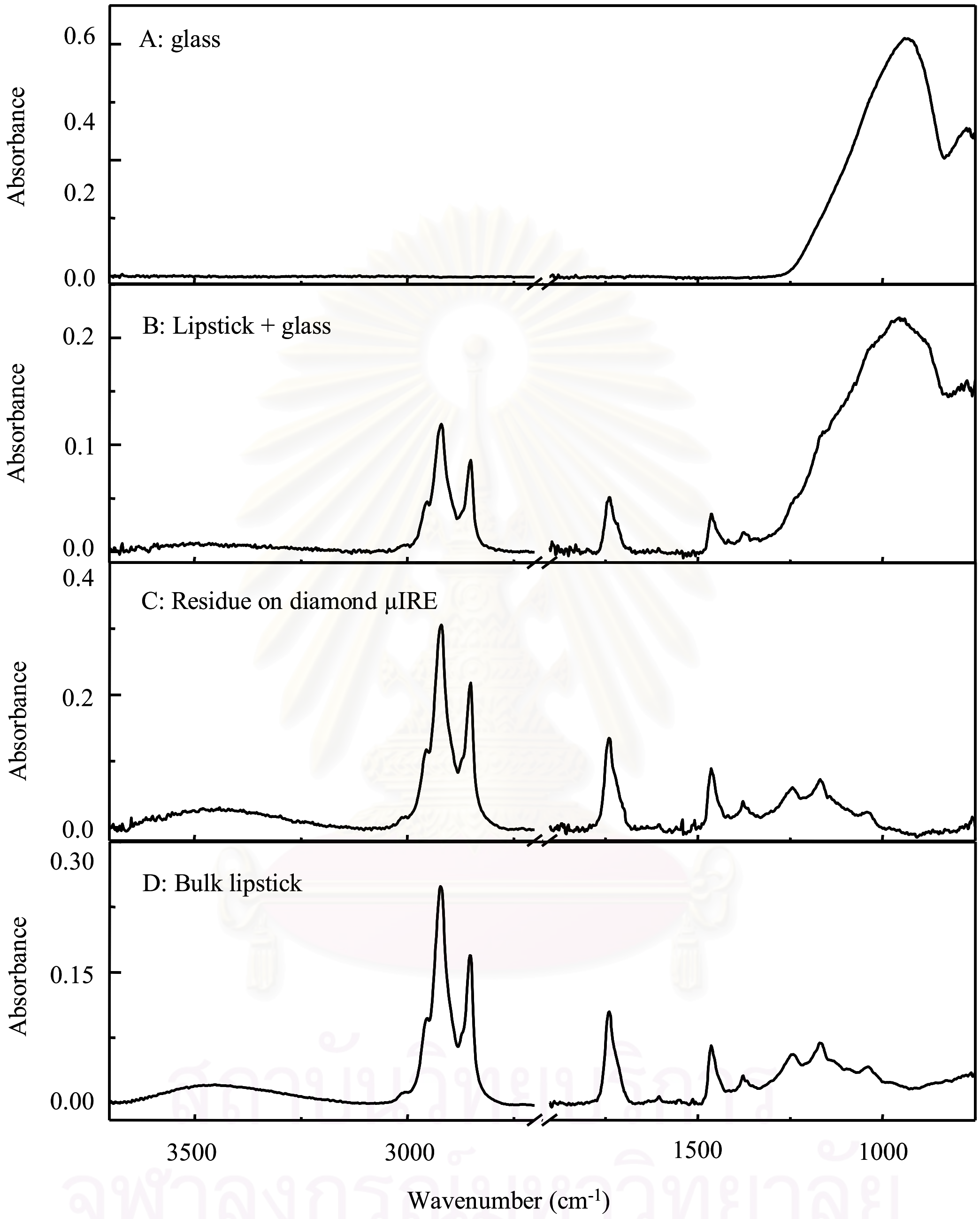


Figure 4.15 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 Bibelot rubyglass) on glass acquired by the slide-on diamond μIRE: (A) glass, (B) a lipstick stain on glass, (C) spectrum of making a contact between the tip of the diamond μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

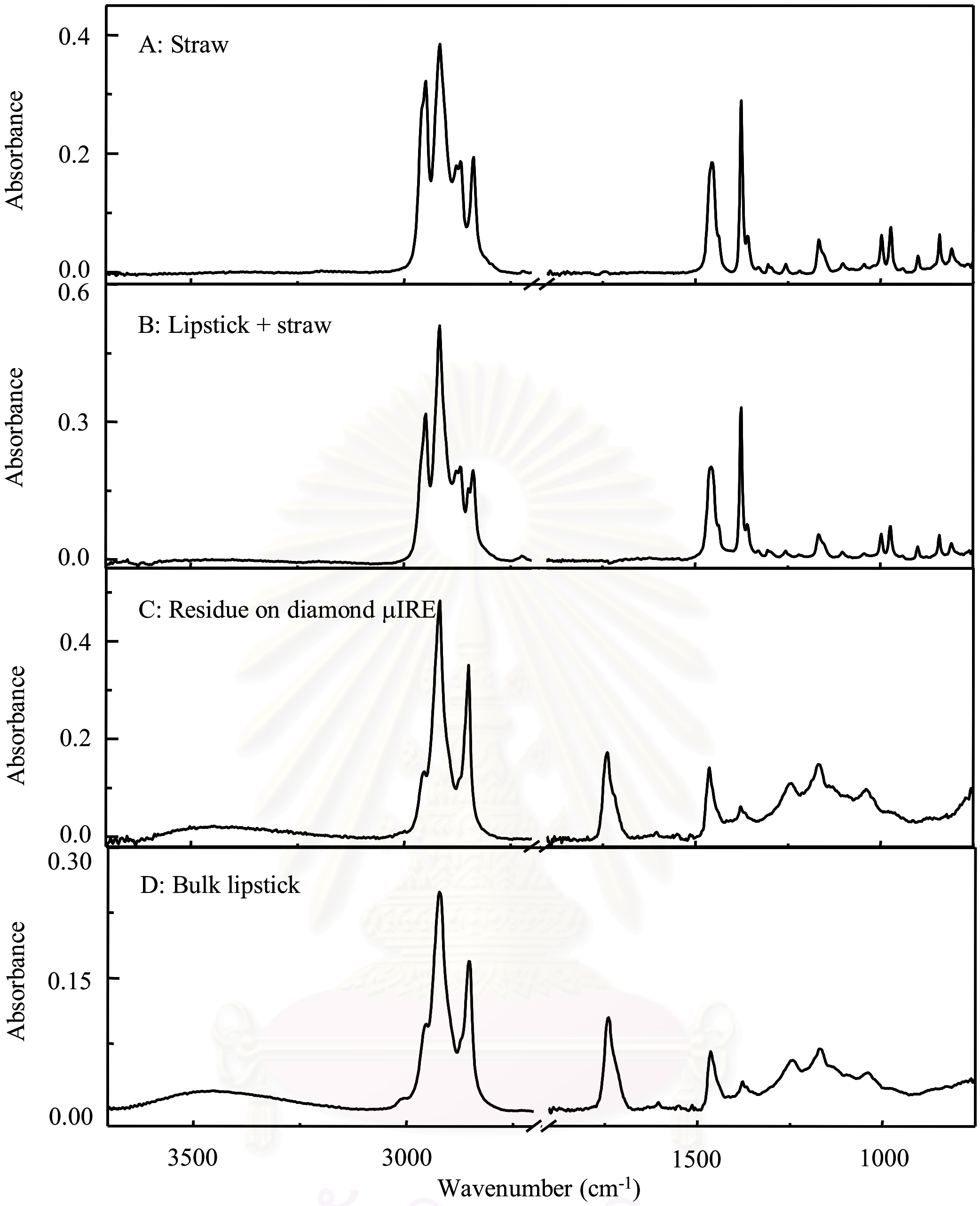


Figure 4.16 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 Bibelot rubyglass) on straw acquired by the slide-on diamond μIRE: (A) straw, (B) a lipstick stain on straw, (C) spectrum of making a contact between the tip of the diamond μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

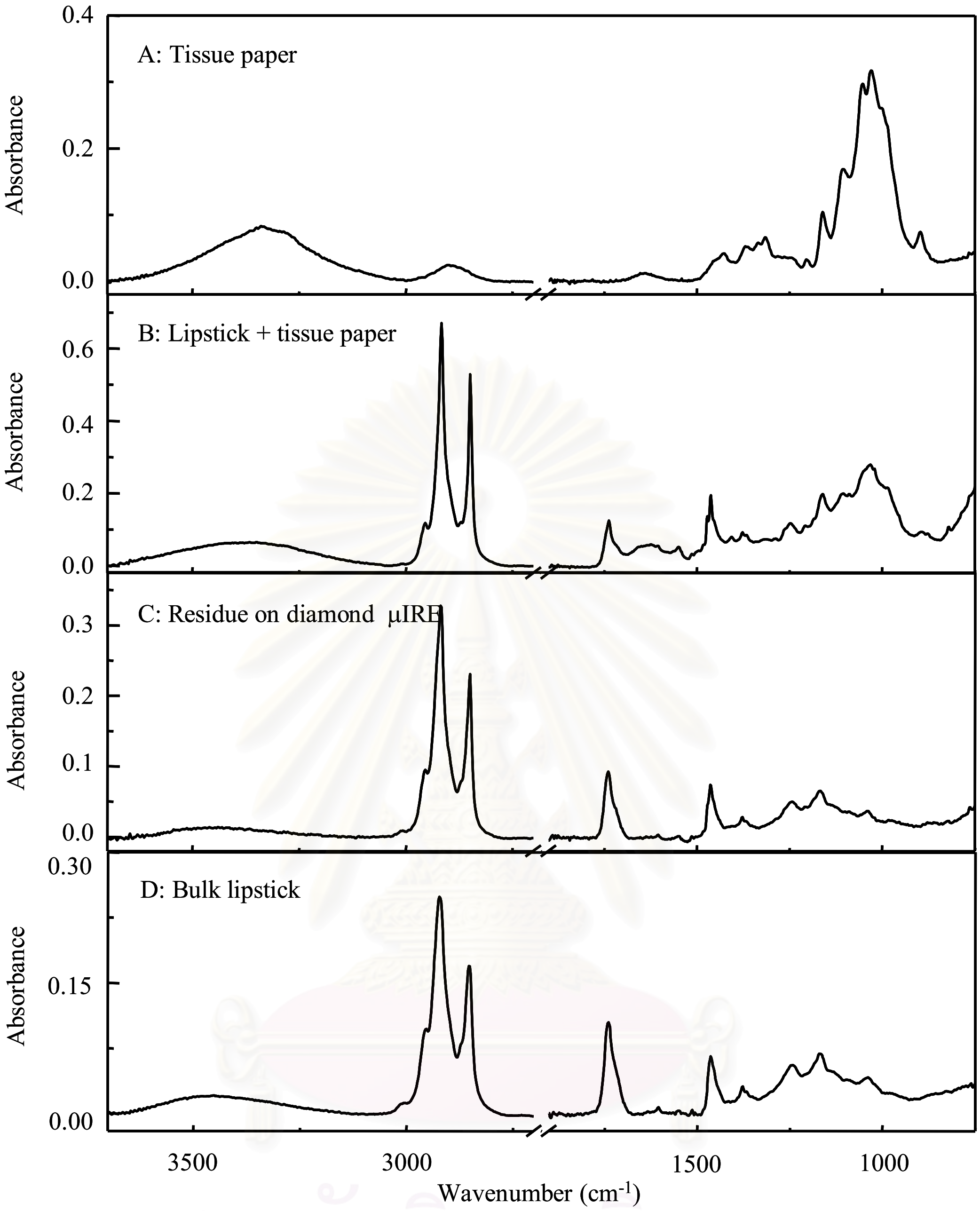


Figure 4.17 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 bibelot rubyglass) on tissue paper acquired by the slide-on diamond μIRE: (A) tissue paper, (B) a lipstick stain on tissue paper, (C) spectrum of making a contact between the tip of the diamond μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

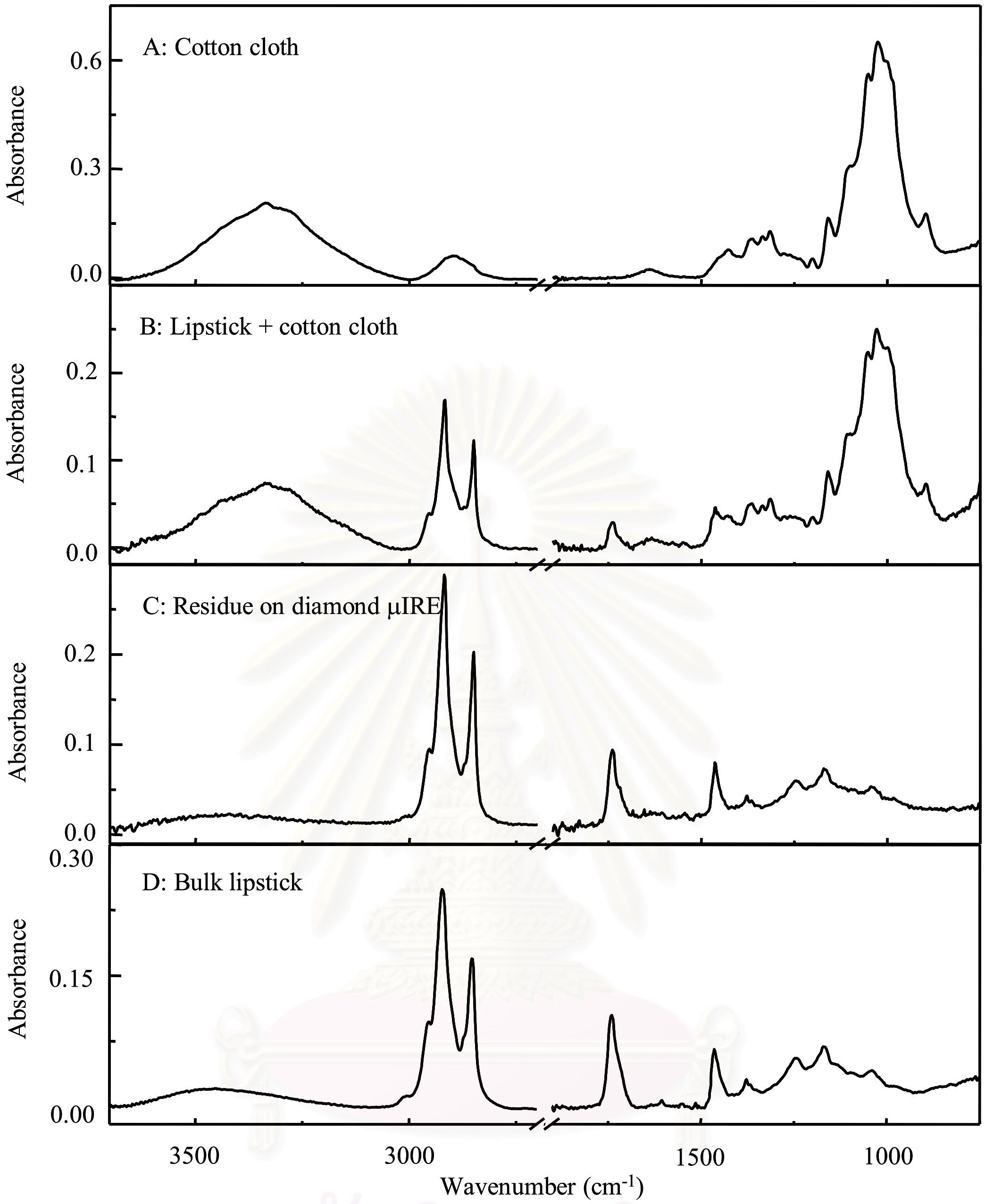


Figure 4.18 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 bibelot rubyglass) on cotton cloth acquired by the slide-on diamond μIRE: (A) cotton cloth, (B) a lipstick stain on cotton cloth, (C) spectrum of making a contact between the tip of the diamond μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

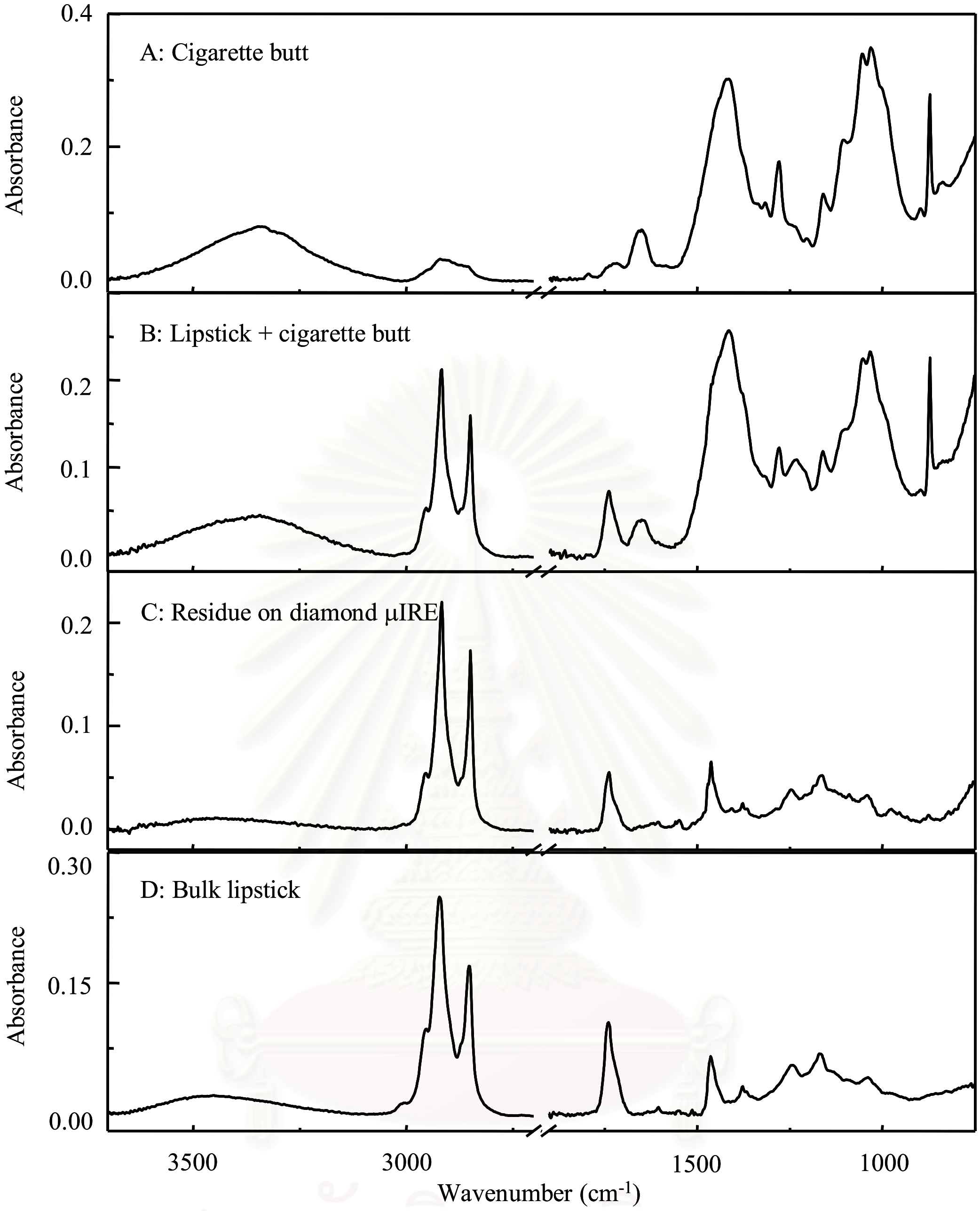


Figure 4.19 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 bibelot rubyglass) on cigarette butt acquired by the slide-on diamond μIRE: (A) cigarette butt, (B) a lipstick stain on cigarette butt, (C) spectrum of making a contact between the tip of the diamond μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

In an ATR experiment, optical contact between the IRE and the sample plays an important role on obtaining a good spectral quality. In order to get a good-quality ATR spectrum, an optical contact between IRE and sample must be achieved. From the physical properties of the sample, liquid sample always wet the surface of the IRE. A perfect contact between the sample and IRE is always achieved. On the other hand, the solid sample rarely has a good contact with the IRE. In the general practice, applying pressure to a solid sample against the IRE is the technique for improving the contact between the sample and IRE. A great care must be taken when making a contact since the smooth surface of IRE can be easily scratched and damaged. A damage of the brittle surface of the Ge μIRE due to an excessive force as a solid sample was raised against the Ge μIRE must be avoided. Therefore, it should be noted that the contact alert sensor plate also prevents a possible damage.

The diamond IRE for spectral acquisition using a gem quality round brilliant cut diamond as IRE was introduced. Due to the geometry and small size of the diamond IRE, the TIR phenomenon occurs at the pavilion facet instead of culet. ATR absorption of sample having an optical contact with the pavilion facet can be analyzed. Because of the inherent hardness, geometry, and small contact area of the diamond IRE, the IREs can be employed for collecting small quantities of lipstick smears on a hard, irregular shape sample and/or rough surface such as wood chopstick (Figure 4.20).

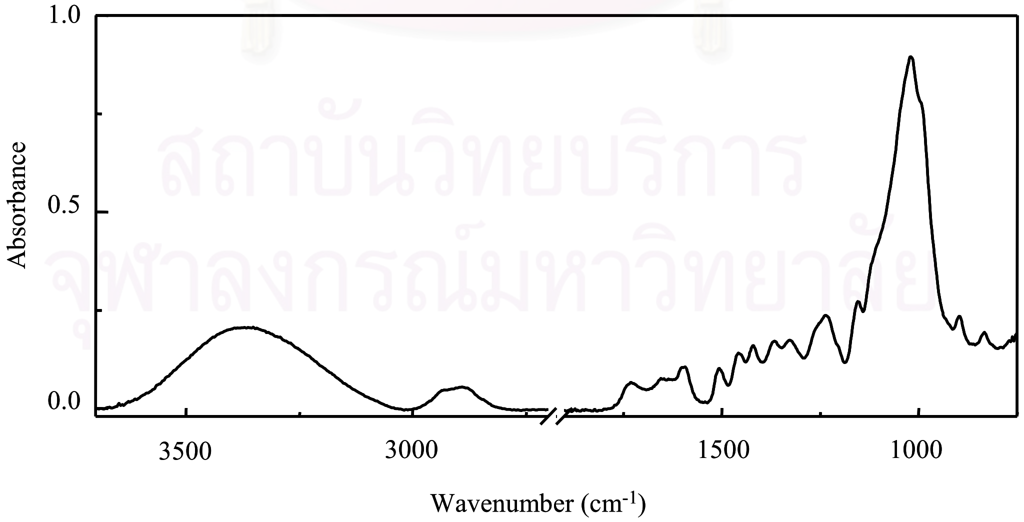


Figure 4.20 ATR FT-IR spectrum of the wood chopstick acquired by the slide-on diamond μIRE.

Once a trace amount of lipstick was deposited at the pavilion facet near the culet, ATR FT-IR spectrum of lipstick can be collected, as shown in Figure 4.21.

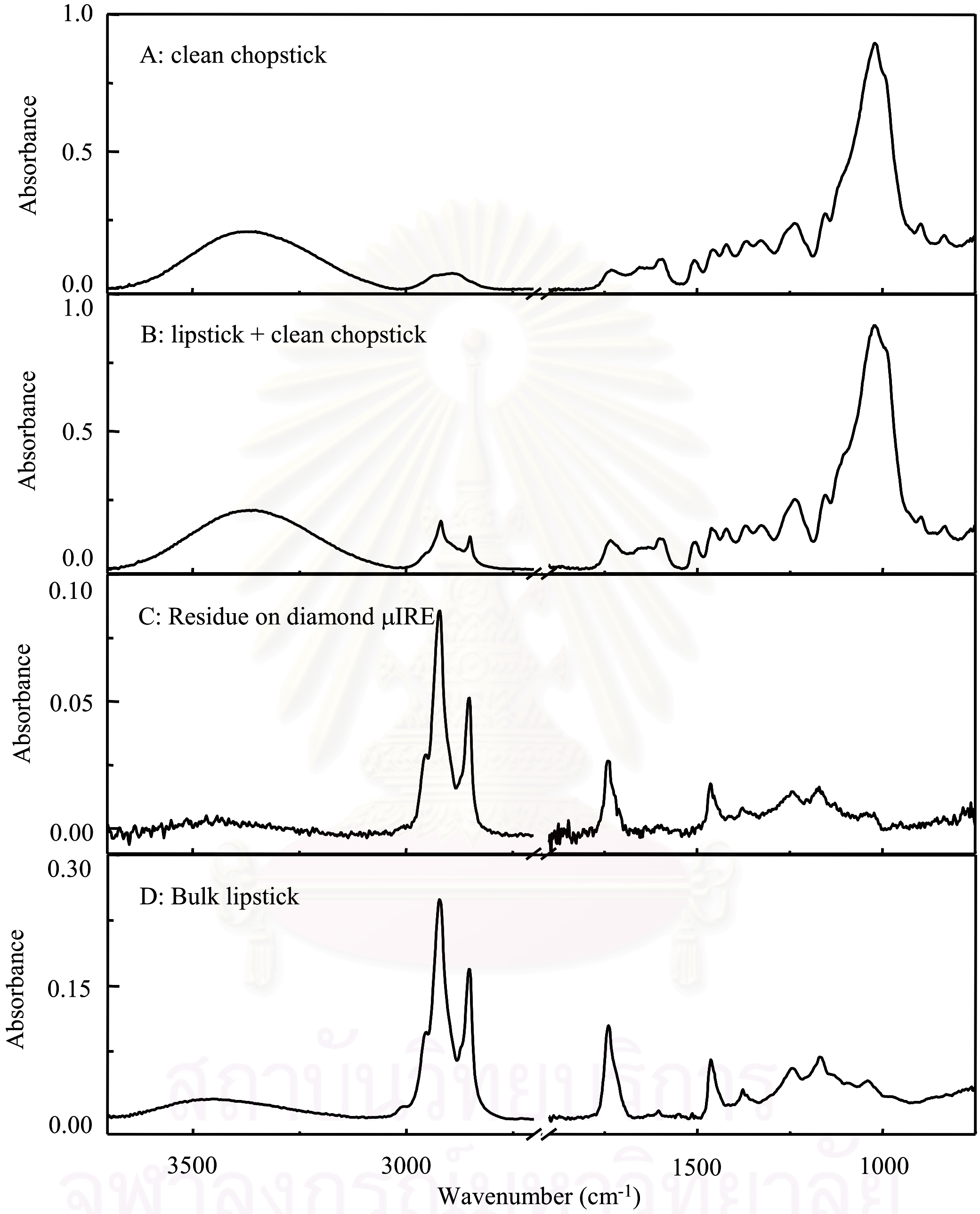


Figure 4.21 ATR FT-IR spectra of a lipstick stain (Christian Dior, 984 bibelot rubyglass) on chopstick acquired by the slide-on diamond μIRE (A) spectrum of chopstick, (B) spectrum of a lipstick stain including chopstick, (C) spectrum of making a contact between the tip of the diamond μIRE and the lipstick stain, and (D) spectrum of bulk lipstick.

In order to demonstrate the small sampling area of the homemade μIRE accessories, the Ge μIRE (100x100 μm^2) and the diamond μIRE (30x30 μm^2) for forensic analysis of lipsticks, minute lipstick smears which sometimes found at the substrates were analyzed by the IREs. The easily operation was achieved by placing the sample on the microscope stage and the advance feature an infrared microscope; any part of trace lipstick smears can be selectively analyzed. This technique does not require an additional sample preparation. There was no separation of the trace lipstick prior the analysis. The entire operation was non-destructive. The chemical information of lipsticks on various substrates can be achieved. In case of the porous surface such as tissue paper and cotton cloth, the investigation was usually difficult. Several contacts can be made in order to collect a sufficient amount of lipstick. Since the contact area of the IREs was small, the characterization process was non-destructive, and there was virtually no noticeable change in the original sample, the specimen can be employed for further analysis. This method is suitable for identification of trace amount lipsticks on various substrates in forensic science investigation.

4.4 Analysis of Trace Lipstick Smears

The lipstick smear may deposit in the forensic evidence as a result of attack or an unintentional contact. The smear on the surface may be large or small. In this section, efficiency of the homemade μIRE accessories for minute of lipstick analysis was introduced. Lipstick smear that is found at the small substrates such as single fiber (cotton cloth) and poly(ethylene terephthalate) (PET) fiber was investigated.

Like the cotton cloth, absorption band of single fiber of cotton cloth which typically fiber composed of cellulose was shown in Figure 4.22A. From the picture, single fiber has a diameter of ~90 μm. When single fiber that has a trace lipstick smear was analyzed, appearance of C-H stretching region was observed in their spectrum (Figure 4.22B). According to the ATR FT-IR spectrum in Figure 4.22C, it shows that the "*contact-and-collect*" operation can acquire the spectrum of small quantities of lipstick stains on the single cotton bundle. In order to demonstrate the efficiency of the slide-on Ge μIRE for minute of lipstick analysis, due to the small sampling area of Ge μIRE (less than 100x100 μm^2 in diameter), minute amount of trace lipstick smears can be analyzed. ATR FT-IR spectra of small quantities of sample can be acquired with superb spectral quality.

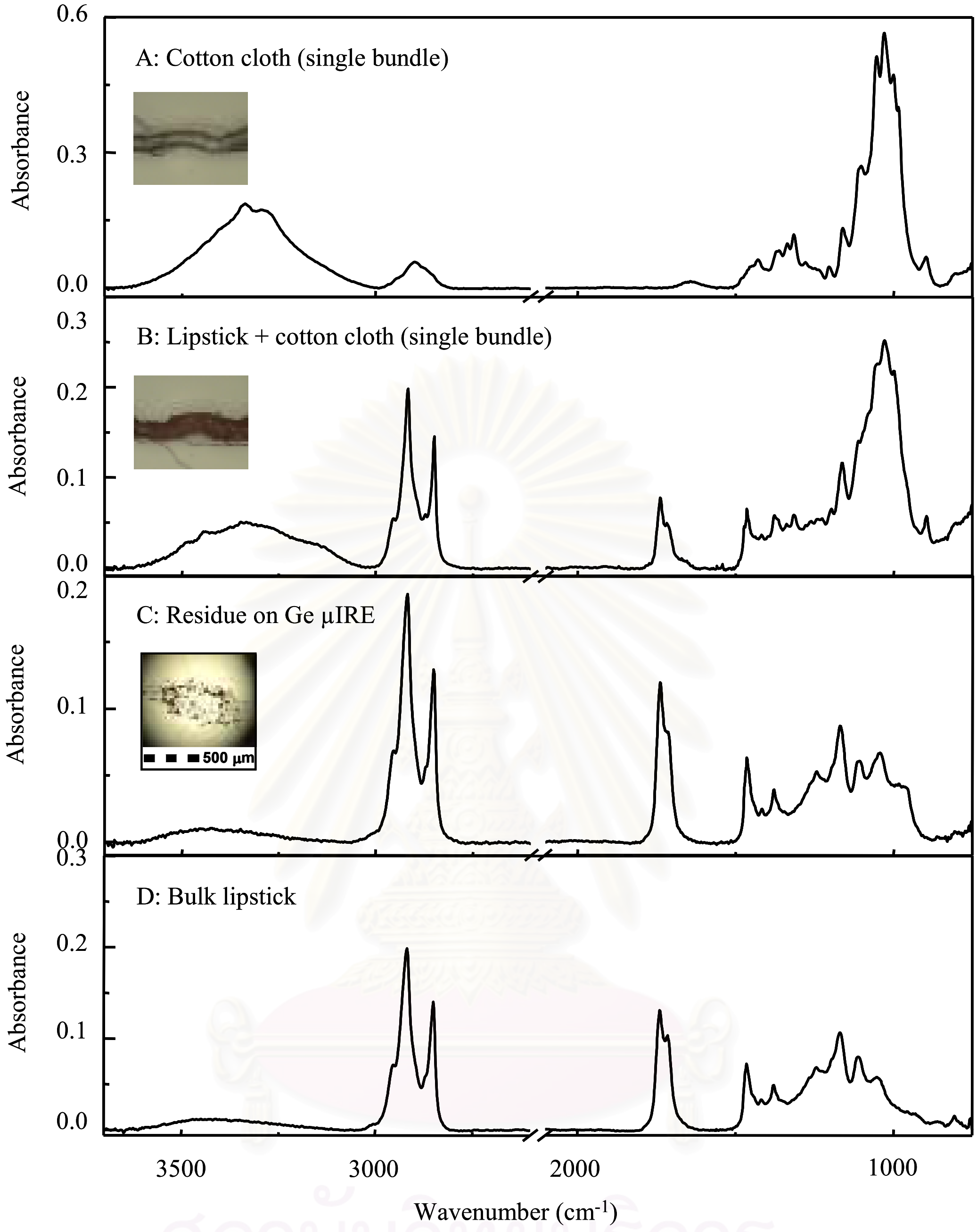


Figure 4.22 ATR FT-IR spectra of lipsticks acquired by slide-on Ge μIRE: (A) spectrum of cotton cloth (single bundle), (B) spectrum of lipstick stains on cotton cloth (single bundle), (C) spectrum of residual lipstick on Ge μIRE, and (D) spectrum of bulk lipstick (Elizabeth Arden, 074 spiced pecan).

Poly(ethylene terephthalate) (PET) is a thermoplastic polymer resin of the polyester family that is used in synthetic fibers; beverage, food and other liquid containers. It can be synthesized by the esterification reaction between terephthalic acid and ethylene glycol. The majority of the world's PET production is for synthetic fibers (60%). In this study, PET fiber which has a diameter of ~50 μm was analyzed. The characteristic absorption band of PET fiber was shown in Figure 4.23A. In Figure 4.23C, the *"contact-and-collect"* operation was performed for ATR FT-IR spectrum measurement of the minute amount of lipstick smear on the PET fiber. By comparison, the observed spectrum from the making a contact between the tip of the Ge μIRE and the lipstick stain (Figure 4.23C) were the same as that of the bulk lipstick (Figure 4.23D).

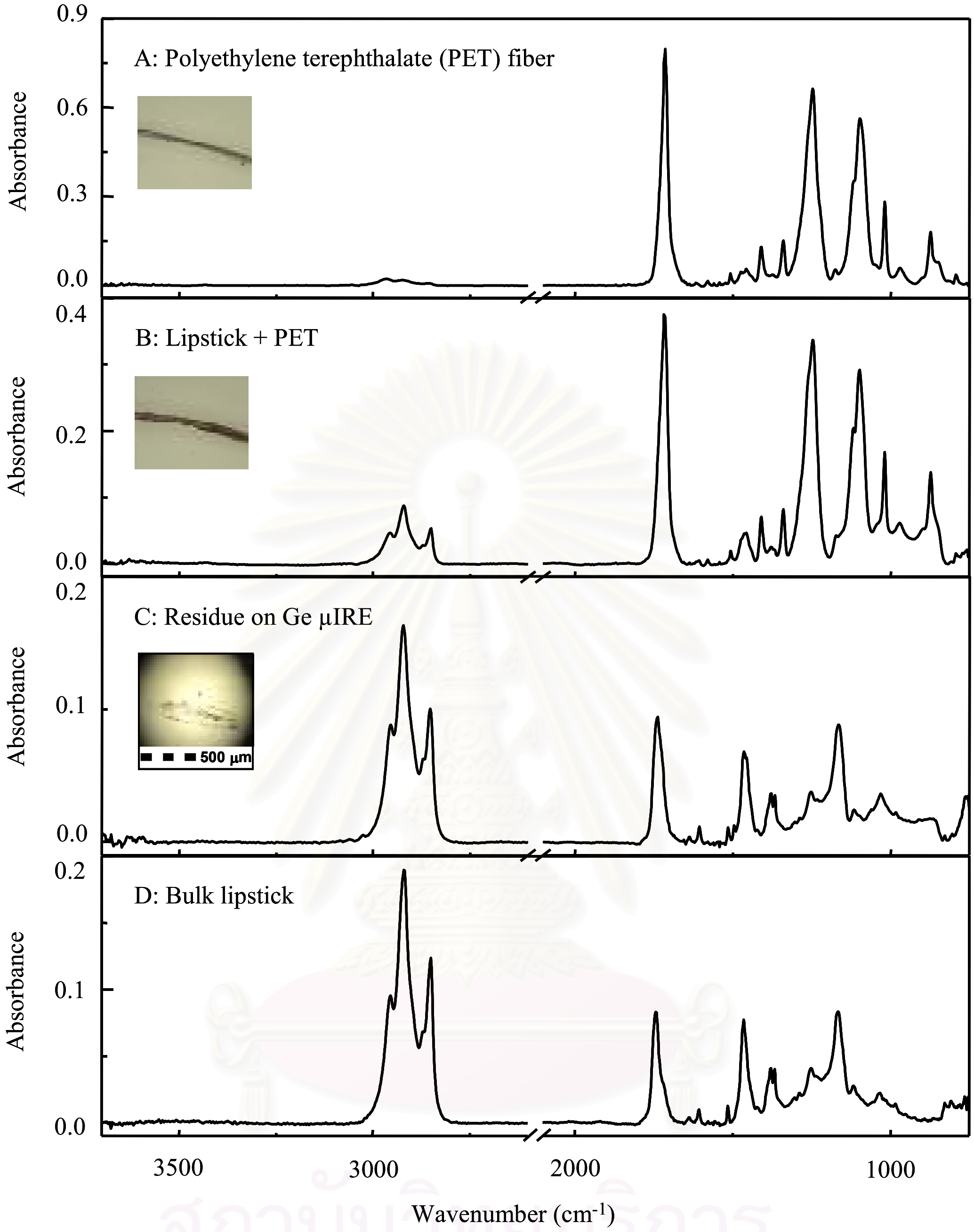


Figure 4.23 ATR FT-IR spectra of lipsticks acquired by the slide-on Ge μIRE: (A) spectrum of PET fiber, (B) spectrum of lipstick stains including PET fiber, (C) spectrum of residual lipsticks on GeμIRE, and (D) spectrum of bulk lipstick (Christian Dior, brun photo brown).

To summarize, because of the small of contact area of the Ge μIRE (less than 100x100 μm^2), a minute amount of lipstick smear which are found at the small substrate such as single fiber of cotton cloth and PET fiber can be analyzed.

4.5 Time Dependence Analysis of Lipstick Smears

After a crime action, it is not immediately found a place where a crime happened. Important evidences such as blood, hair, fingerprint, and etc., may be changed in chemical properties and/or physical properties. In this section, the influence of time on the change of the chemical composition on lipstick was studied. Lipstick smears are found at several surfaces that maybe changed when time changed.

From the prior experimental conditions, the lipsticks were applied on volunteers. After that, lip prints were made on substrates**.** Lipstick samples on substrates were kept at the ambient condition at different time intervals after impression (1 day, 2 days, 15 days, and 30 days). Subsequently, the lipstick smears from each condition with intervals ranging from 1 day to 30 days from impression were analyzed by the slide-on Ge μIRE. The *"contact-and-collect"* operation was performed for ATR FT-IR spectra measurement of a minute amount of lipstick on the substrate by making a gentle contact between the tip of the μIRE and the lipstick smear on the surface.

The problem of the study of time dependence (an influence of time) on the lipstick analysis, the lipsticks became tightly attached to the substrates when lipstick was exposed to air for a long time. The trace of lipstick stain is difficult to obtain due to the lipsticks adhere with the substrate. Figure 4.24 shows the ATR FT-IR spectra of lipstick smears on glass slide from each condition. As the result, the different spectral intensity was observed in each condition. In principle, spectral intensity provided information directly correlated with the quantities of the analyzed sample. Therefore, spectral intensity depends on quantities of lipstick on the μIRE. When the small sample was analyzed, the smaller is the observed spectral intensity. When the small quantities of lipstick at the tip of μIRE were analyzed, the good spectral quality could not be acquired, an ATR spectrum could not be observed. Thus, several contacts between the tip of the Ge μIRE and the lipstick stain were necessary required for obtaining the large amount of lipstick for comparison.

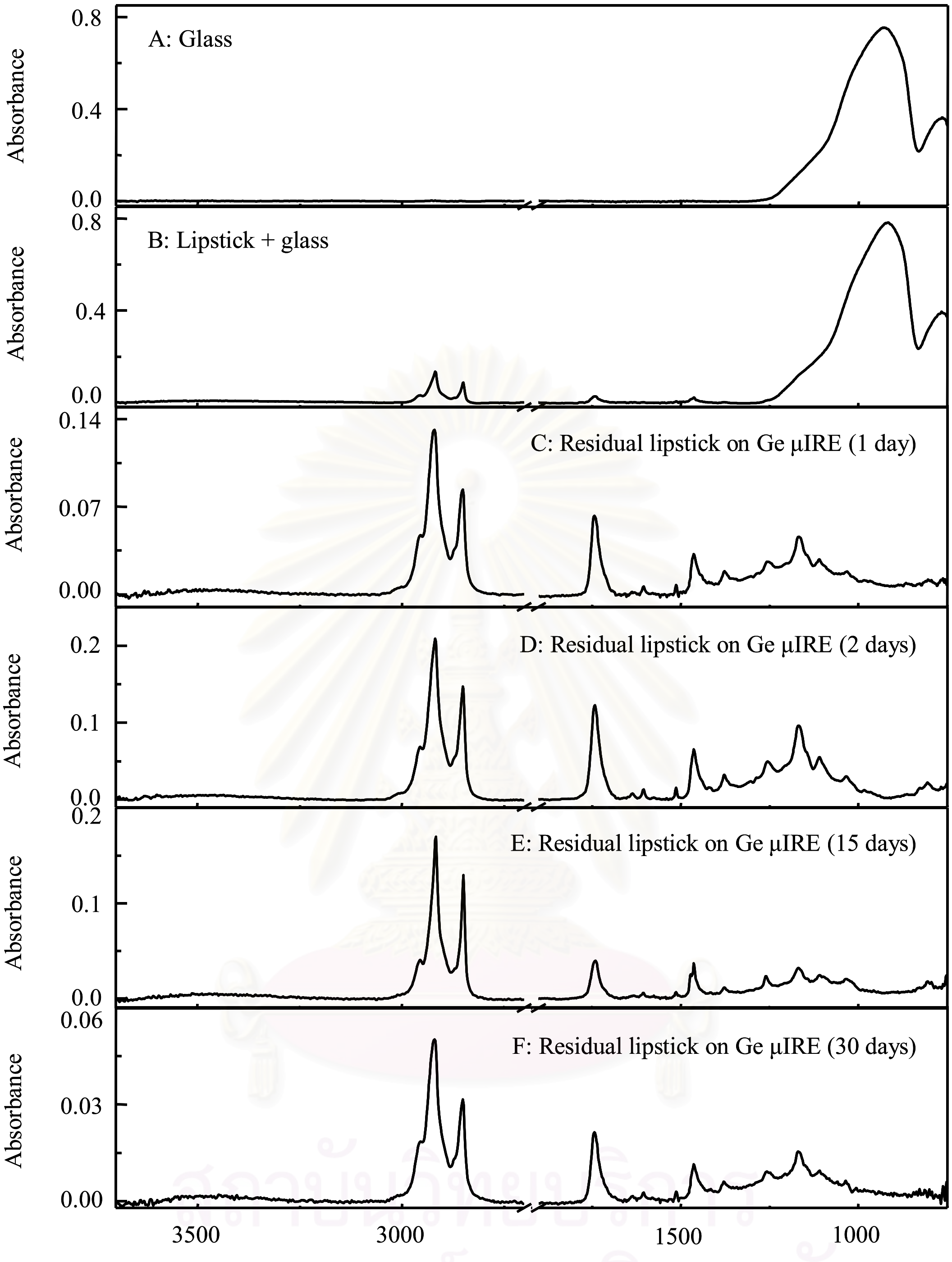


Figure 4.24 ATR FT-IR spectra of trace lipsticks (Oriental Princess, 05 Tropical Orange) on glass acquired by the side-on Ge μIRE. The specimens were kept at the ambient condition.

According to Figure 4.24, for comparisons purposes, each observed ATR FT-IR spectrum of lipstick stains on glass at different time intervals after impression showed the same spectral feature. Some variations of the intensities were observed but the frequencies of the main peaks remained the same. This variation in intensities can possibly be attributed to the number of contact. The spectral feature directly correlated with chemical structure, chemical composition, and other composition in analyzed lipstick. Thus, the lipsticks kept at the ambient condition did not show any change in the composition during the investigated period (30 days).

Figure 4.25 shows the ATR FT-IR spectra of trace lipstick on straw at different time after the impression acquired by the Ge μIRE. In order to collect ATR FT-IR spectra, the "*contact-and-collect*" operation was employed. The observed spectrum in Figure 4.25E showed greater noise level than those of other spectra. This is due to the quantities of the analyzed lipstick is small. Several contacts were performed in order to acquire a good spectral quality as illustrated in Figure 4.25F. Like the glass substrate, each observed ATR FT-IR spectrum of lipstick stains on the straw at each condition showed the same spectral feature. Some variations of the intensities were observed but the spectral frequencies of the main peaks remained the same. Thus, in 30 days intervals after impression, lipsticks on straw were kept at the ambient condition that did not show any change of the composition.

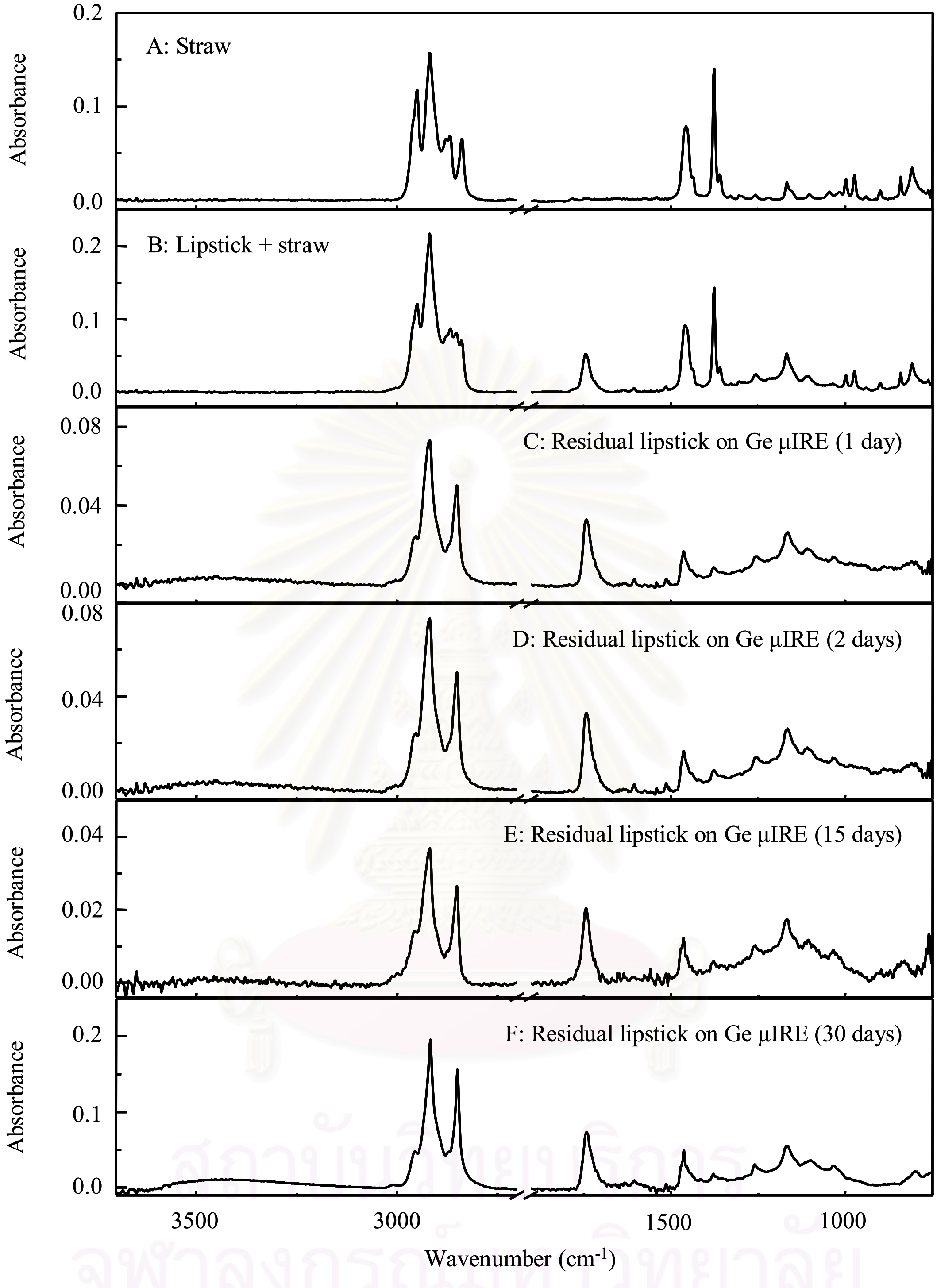


Figure 4.25 ATR FT-IR spectra of trace lipsticks (Oriental Princess, 05 Tropical Orange) on straw acquired by the slide-on Ge μIRE. The specimens were kept at the ambient condition.

The quality of the investigation for μATR accessories studied depends on two factors: the type of surface and the age of lipstick [6]. In case of an old lipstick, the lipstick investigation was usually difficult. Especially, the lipsticks are found at the porous surface such as tissue paper (Figure 4.26) and cotton cloth (Figure 4.27).

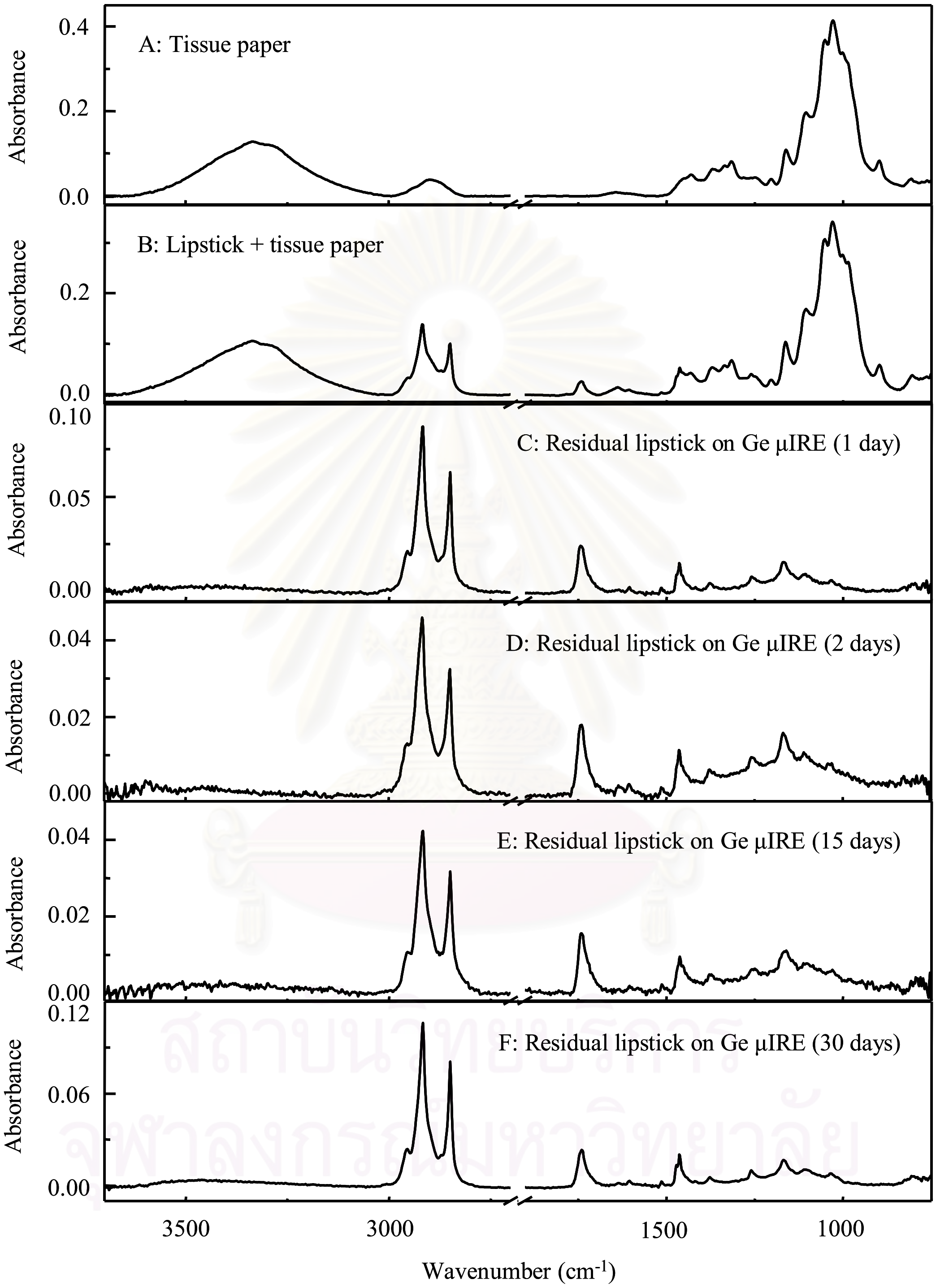


Figure 4.26 ATR FT-IR spectra of trace lipsticks (Oriental Princess, 05 Tropical Orange) on tissue paper acquired by the slide-on Ge μIRE. The specimens were kept at the ambient condition.

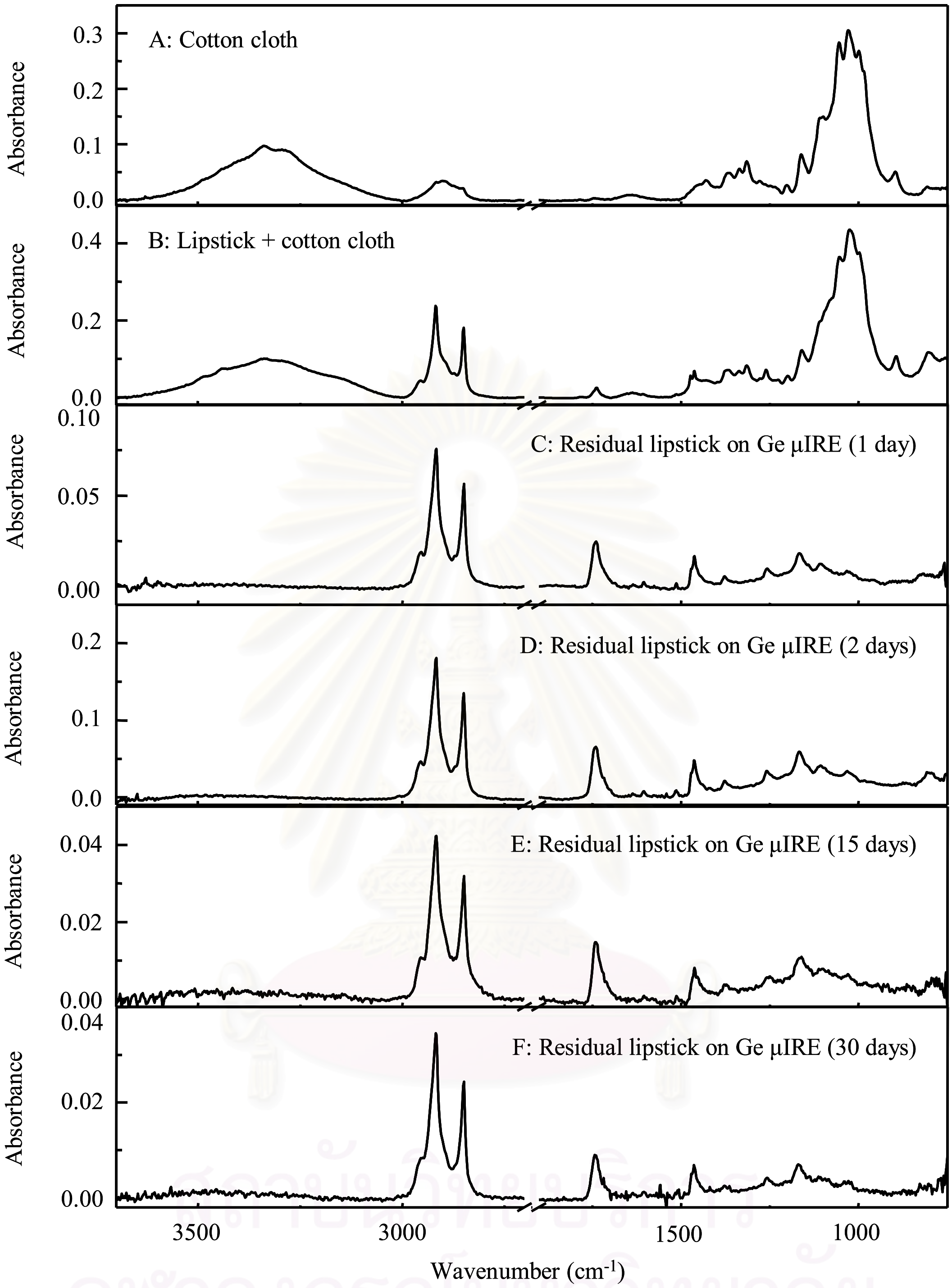


Figure 4.27 ATR FT-IR spectra of trace lipsticks (Oriental Princess, 05 Tropical Orange) on cotton cloth acquired by the slide-on Ge μIRE. The specimens were kept at the ambient condition.

As show in Figure 4.26 and 4.27, the effectiveness of the investigation diminished with the age of lipstick smears and the poor quality on the older lipstick. Analyzing the results obtained from the two porous surfaces, the analysis quality of the lipstick was poorer on porous surface than the other surface. The softer surface of tissue paper allows the investigation process to affect the structure, producing poor results. Since it is more absorbent than the other surface, the lipstick adheres to the porous surface better, therefore reducing the effectiveness of the analysis. In the "*contact-and-collect*" operation, several contacts were performed in order to collect a sufficient amount of the lipsticks for investigation and comparison. In case of both porous surfaces, no changes of chemical ingredient of trace lipstick smear occur due to no significant change in their composition.

ATR FT-IR spectra of a trace lipstick on the small surface i.e., single fiber and PET fiber were acquired by the Ge μIRE as shown in Figure 4.28 and 4.29, respectively.

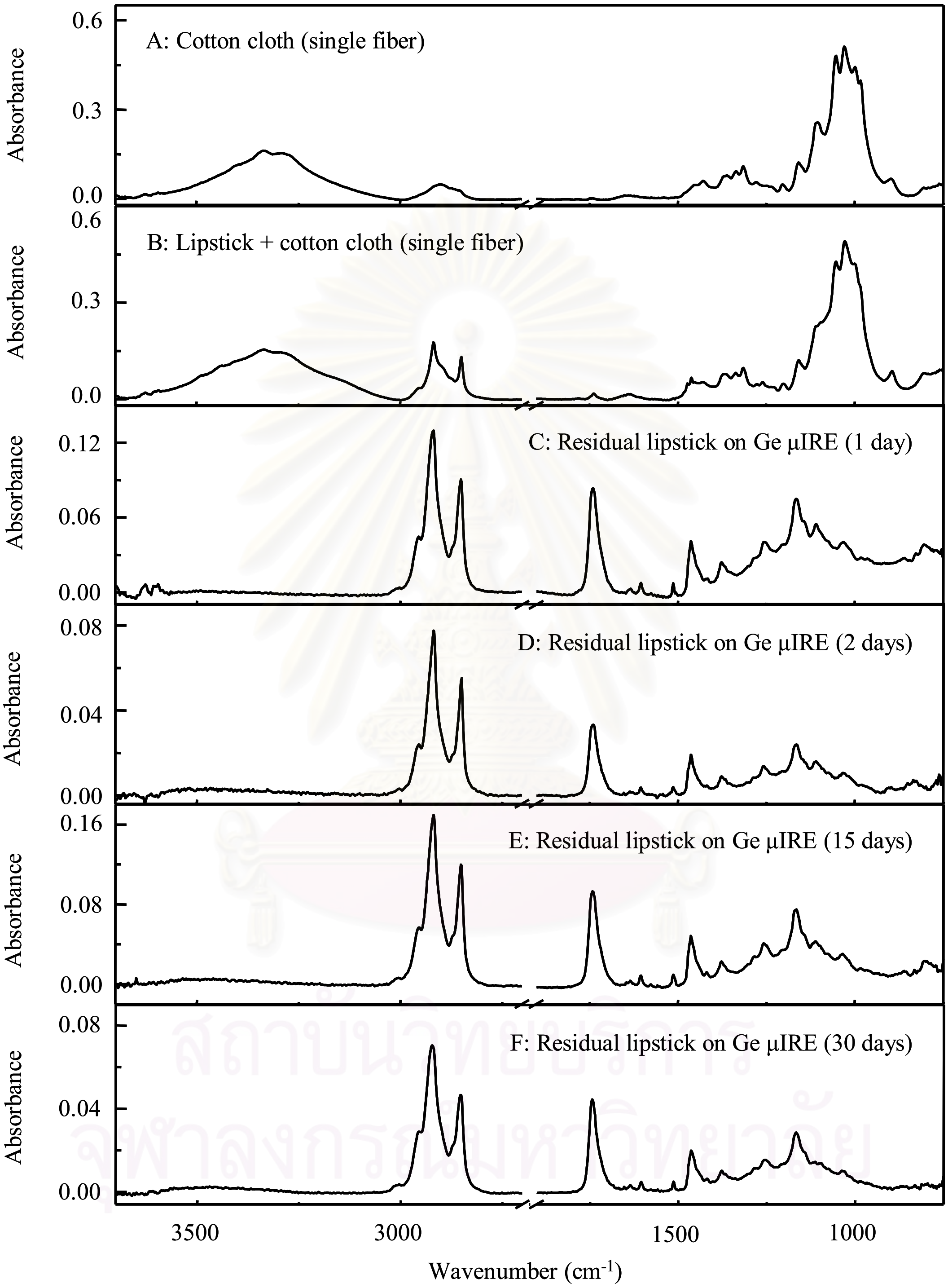


Figure 4.28 ATR FT-IR spectra of trace lipsticks (Oriental Princess, 05 Tropical Orange) on cotton cloth (single fiber) acquired by the slide-on Ge μIRE. The specimens were kept at the ambient condition,.

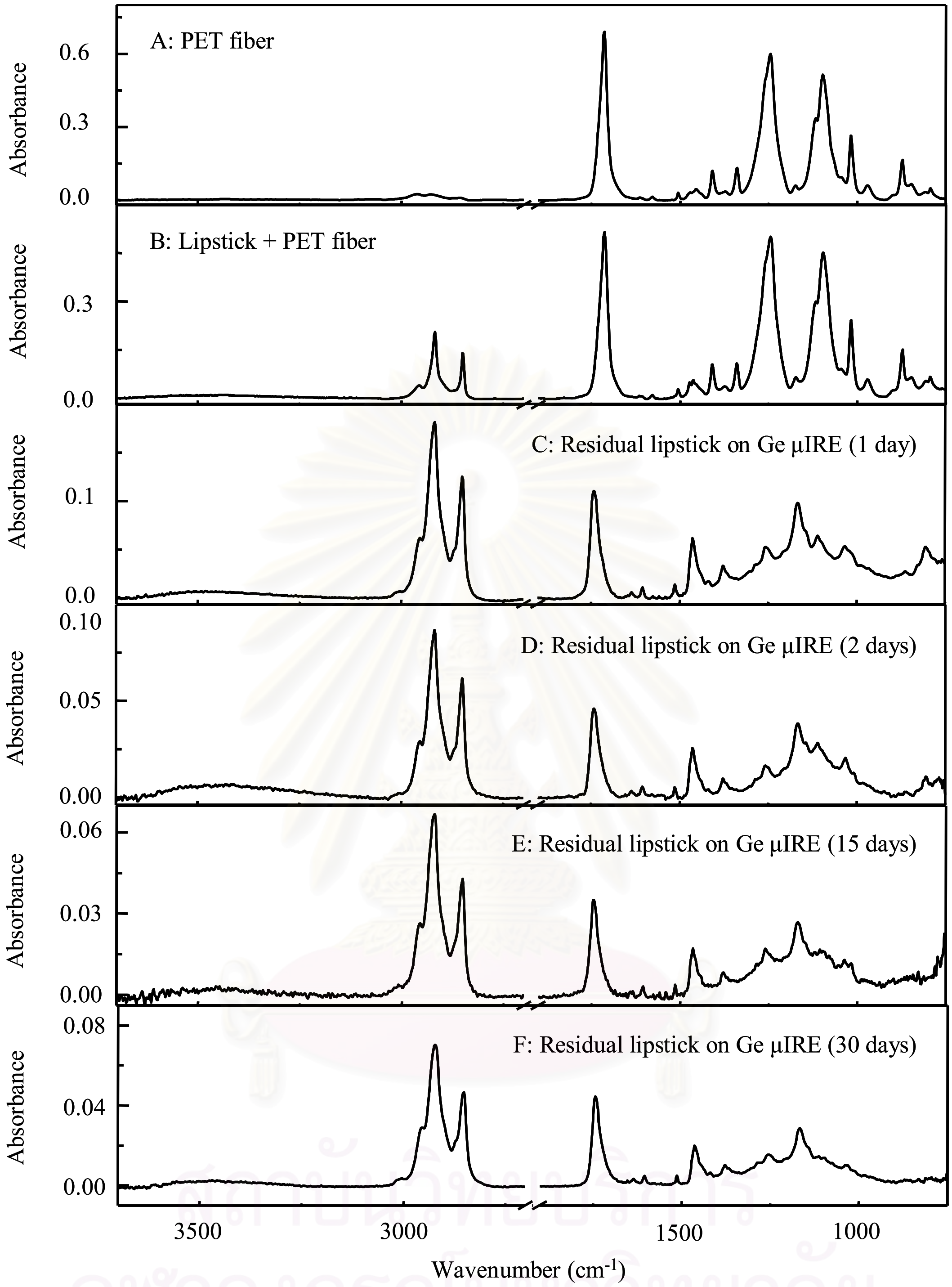


Figure 4.29 ATR FT-IR spectra of trace lipsticks (Oriental Princess, 05 Tropical Orange) on PET fiber acquired by the slide-on Ge μIRE. The specimens were kept at the ambient condition.

Although the trace lipsticks on the small substrates such as the single fiber (cotton cloth) or PET fiber are difficult to analyze, it can be analyzed by ATR FT-IR microspectroscopy. The acquired spectra of trace lipsticks on the small substrates at each condition were the same as that on other substrates. Lipsticks were kept at the ambient condition that did not show any change in the composition during the investigated period.

To summarize, the effectiveness of the investigation diminished with the age of lipstick smears and the poor quality on the older lipstick. A number of contacts are required for the sufficient amount of lipstick for a good spectral quality. The "*contact-and-collect*" operation is effective when analyzed lipstick smears on porous surfaces, such as tissue paper and cotton cloth, where detection is usually difficult. No changes in spectral feature of all lipstick stain on various substrates were observed in their ATR FT-IR spectra. Thus, chemical composition of lipsticks at different time intervals after impression (1 day, 2 days, 15 days, and 30 days) that was kept at the ambient did not change. The results show that identifiable lipstick by ATR FT-IR microspectroscopic technique can be obtained up to 30 days after being produced.

4.6 Influence of Environmental Effects on the Change of Chemical Composition on Lipsticks Analysis

In the crime scene, a minute amount of trace lipsticks on various substrates may found at the place under the influence of several factors such as moisture or water, heat, dust, and etc. Chemical ingredient of lipsticks may be changed because of various environmental effects. Therefore, forensic analysis of lipsticks is necessary to consider several factors on the change of chemical composition or factors that have interference on lipstick analysis. In this section, several influences were studied (i.e., moisture, heat, and dust).

4.6.1 An Influence of Moisture

Because of the main ingredient of lipstick is waxes, which are water-resistance materials make up of various substances including long chain hydrocarbon, ketones, diketones, primary and secondary alcohols, aldehydes, esters, alkanoic acids, and monoesters (wax esters), all with long or very long carbon chains (from 12 up to about 38 carbon atoms). Waxes are relatively insoluble in water. Wax molecules have these properties because they consist largely of long hydrocarbon tails which are hydrophobic in nature. The ATR spectra of trace lipstick stain on wet tissue paper are shown in Figure 4.30. By comparing the ATR FT-IR spectra of tissue paper (Figure 4.30A) and wet tissue paper (Figure 4.30B), difference in spectral features were observed. Spectrum of wet tissue paper, shown in Figure 4.30B, have high relative intensity in OH stretching region (3600-3000 cm^{-1}) corresponding to absorption of water molecule and an absorption band at 1633 cm^{-1} attributable to OH bending absorption of water molecule.

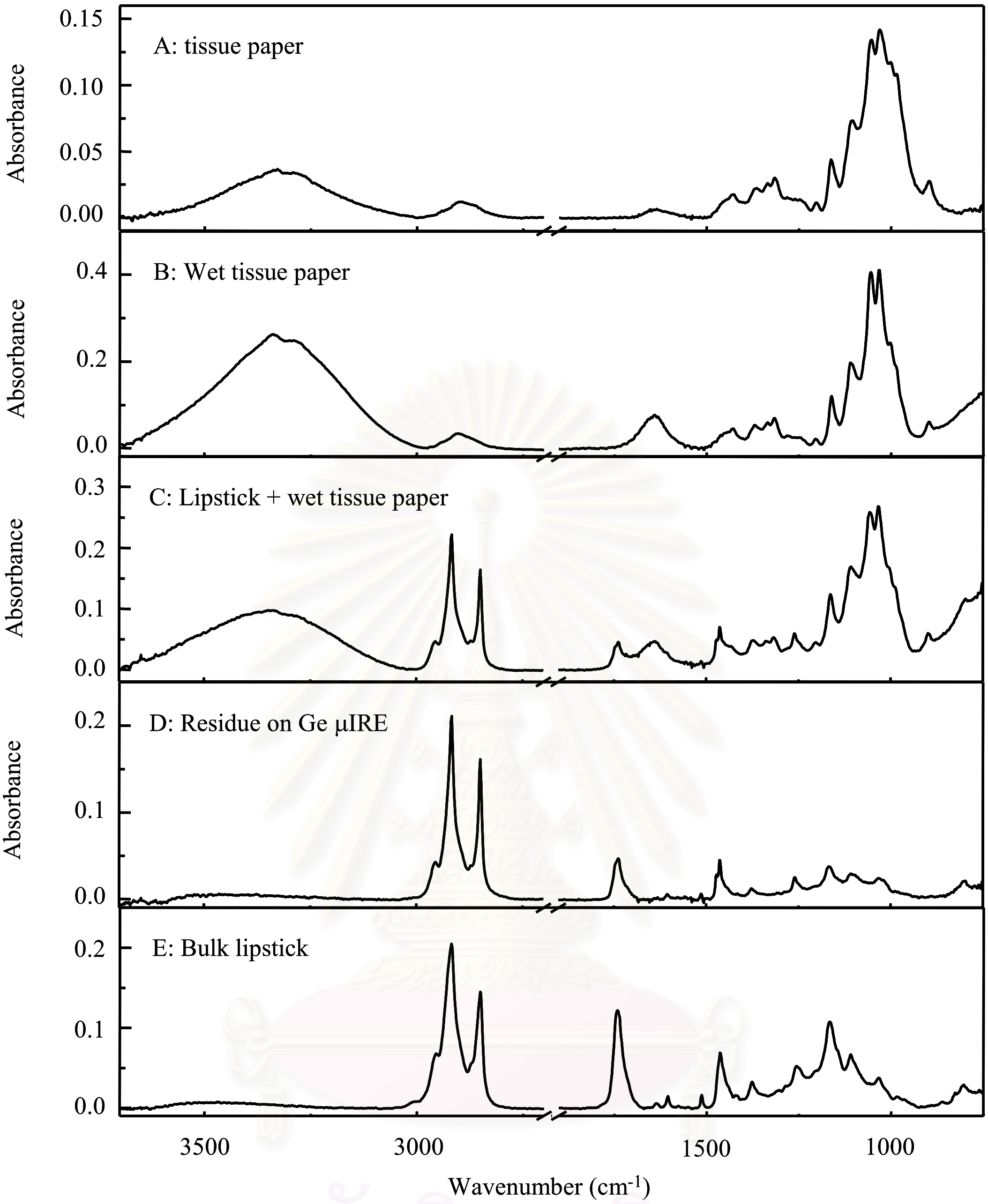


Figure 4.30 ATR FT-IR spectra of lipstick smears (Oriental Princess, 05 Tropical orange) on wet tissue paper acquired by the slide-on Ge μIRE.

From the results in Figure 4.30C, no change in spectral feature of lipstick from the provenance of lipsticks (Figure 4.30D) which consists of waxes as a lipstick base was observed. This is due to the fact that lipsticks are insoluble in water. Therefore, no influence of the moisture or water on the change of chemical composition on lipsticks analysis was observed in ATR FT-IR spectra. To ensure the result, lipsticks which were soaked in water were analyzed as shown in Figure 4.31. Lipsticks are found on the wet substrate or the place where wet can be analyzed without an influence of moisture.

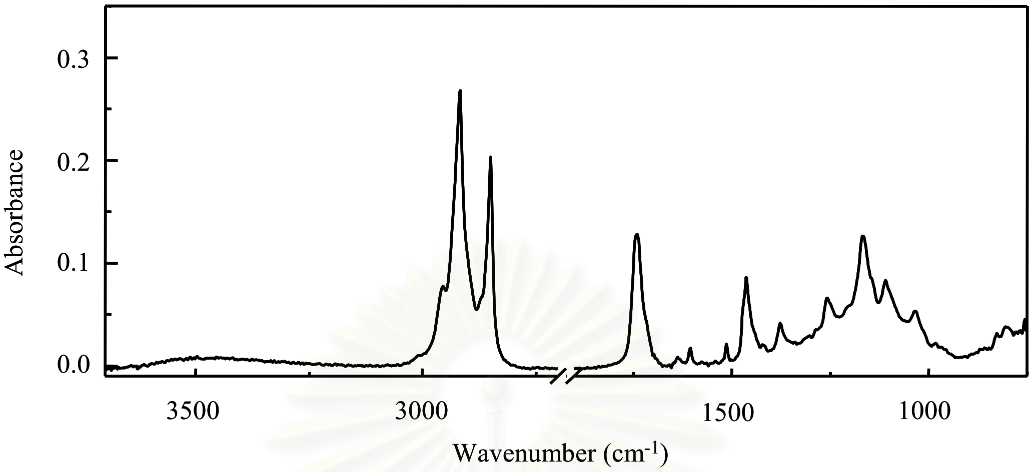


Figure 4.31 ATR FT-IR spectrum of lipsticks (Oriental Princess, 05 Tropical orange) which soaked in water was acquired by the slide-on Ge μIRE.

4.6.2 An Influence of Heat and Dust

4.6.2.1 In Car

At the crime scene, various factors made changes in chemical composition of lipstick. In this section, influence of heat and dust were studies. Figure 4.32, 4.33, and 4.34 show ATR FT-IR spectra of analyzing trace of lipsticks on different substrates such as straw, tissue paper, and cotton cloth which were kept in car at different time intervals after impression (1 day, 7 days, and 30 days).

From previous experimental condition, the lipsticks kept in the car were exposed to heat from the sunlight, and from within the car. It was found that the heat did not induce any significant change on the chemical composition of lipsticks. However, dust has interference on lipstick analysis. The lipsticks were analyzed with intervals ranging from 1 day from impression up to 30 days from impression. ATR FT-IR spectra of lipstick smears on straw as illustrate in Figure 4.32.

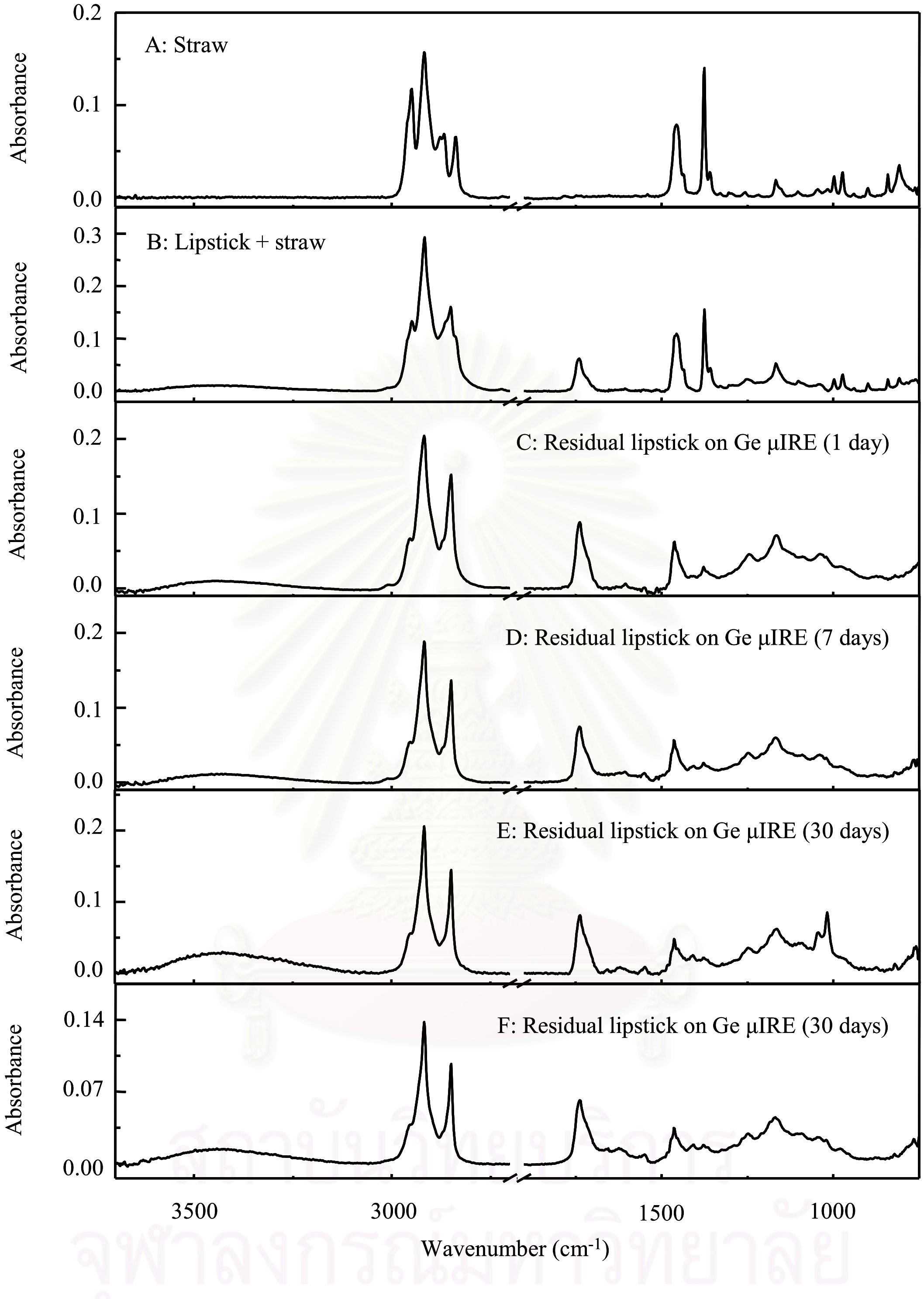


Figure 4.32 ATR FT-IR spectra of lipstick stains on straw which was kept at the car were acquired by the slide-on Ge μIRE.

As the results in Figure 4.32C, 4.32D and 4.32F, due to no changes in the chemical information of lipsticks occur within 30 days, lipsticks in each condition do not have any change in spectral features. Spectral frequencies or peak position remained the same. To summarize, heat from several factors do not make any change in ingredient of lipsticks within ranging 30 days. Although it has any influence of dust which has absorption band at 1017 cm^{-1} [30] on the observed ATR spectrum in Figure 4.32E, the advance features an infrared microscope, any part of trace lipstick smears can be selectively analyzed while it does not have any influence on the observed ATR spectrum as shown in Figure 4.32F.

In case of lipstick that is produced for the long time and the lipsticks that are found at the particularly difficult surface such as tissue paper (Figure 4.33) and cotton cloth (Figure 4.34), the investigation of lipstick was usually difficult. As described previously, the effectiveness of the analysis diminished with the age of lipstick. Several contacts were performed in order to collect a sufficient amount of the lipstick. Considering the result, according to the observed spectrum of both porous surfaces, its spectral feature is same to those of the spectrum of the bulk lipstick. Thus, within 30 days intervals after impression, lipsticks were kept in the car that did not change of the composition in lipsticks.

To summarize, lipsticks that were kept in the car within 30 days did not change in their chemical composition. No influence of the heat from sun and from within the car was observed in the ATR spectrum. By using the advance features of the infrared microscope, any part of trace lipstick smears can be selectively analyzed while it does have any inference of the dust. Therefore, this method is useful for forensic identification of lipsticks that were kept under heat and dusty condition.

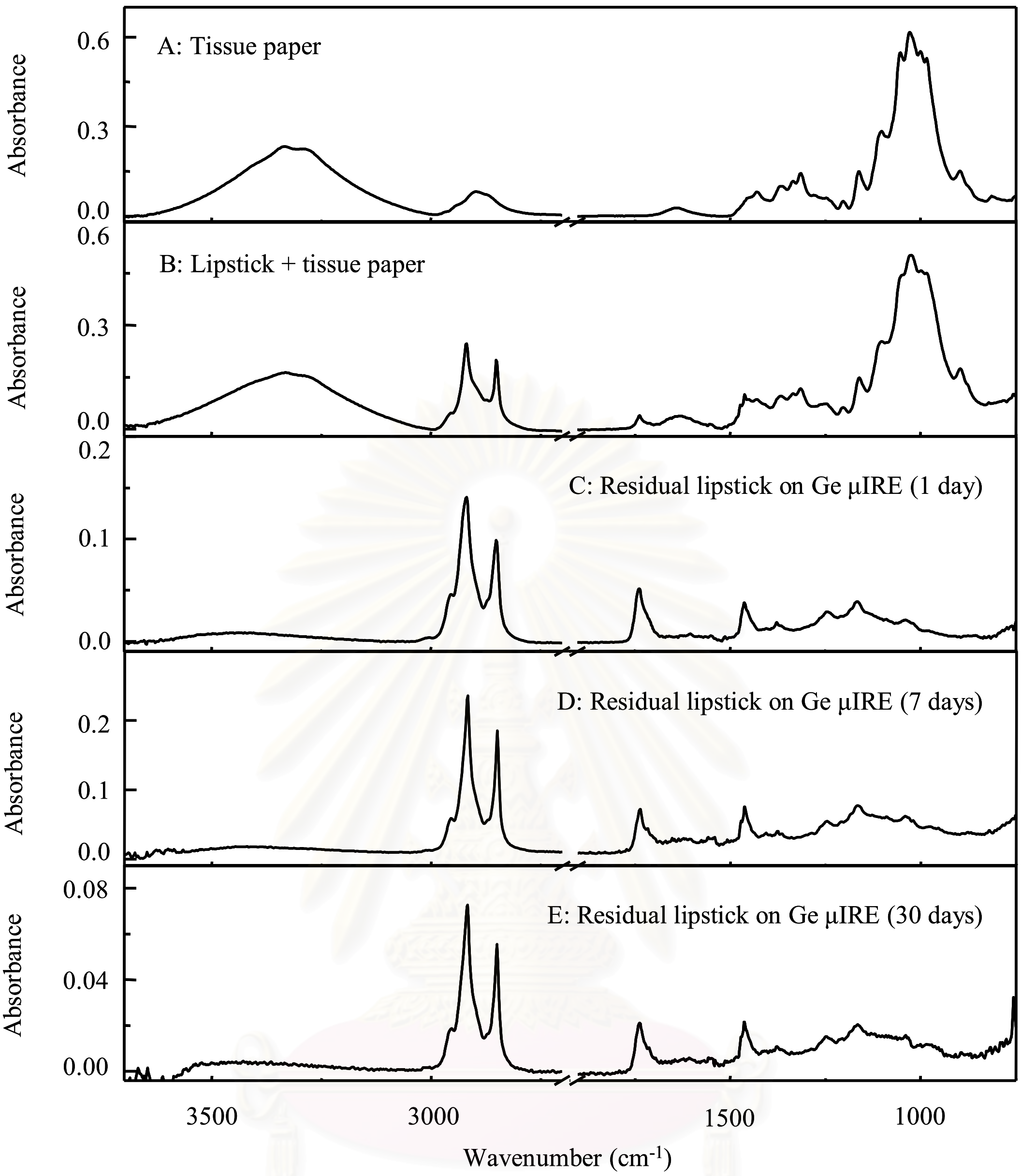


Figure 4.33 ATR FT-IR spectra of lipstick stains on tissue paper which was kept at the car were acquired by the slide-on Ge μIRE.

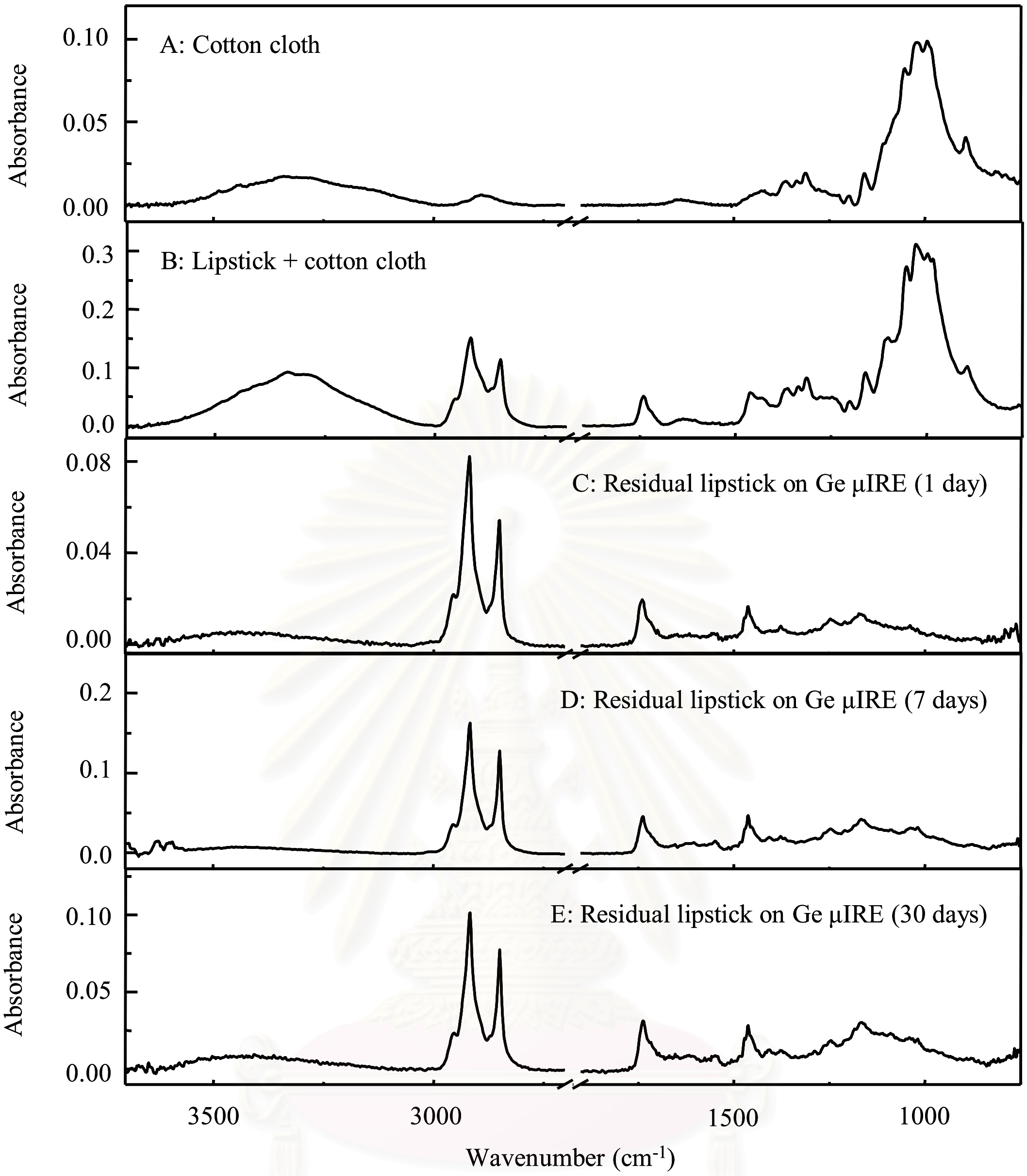


Figure 4.34 ATR FT-IR spectra of lipstick stains on cotton cloth which was kept at the car were acquired by the slide-on Ge μIRE.

4.6.2.2 Outdoors

In this section, lipstick smears on the different surfaces which were kept outdoors were analyzed. The outdoor environment has more influence of heat and dust than the interior of the car. Examples of non-porous surfaces i.e., glass and straw were analyzed. ATR FT-IR spectra of lipstick stains on glass that were kept outdoors are shown in Figure 4.35.

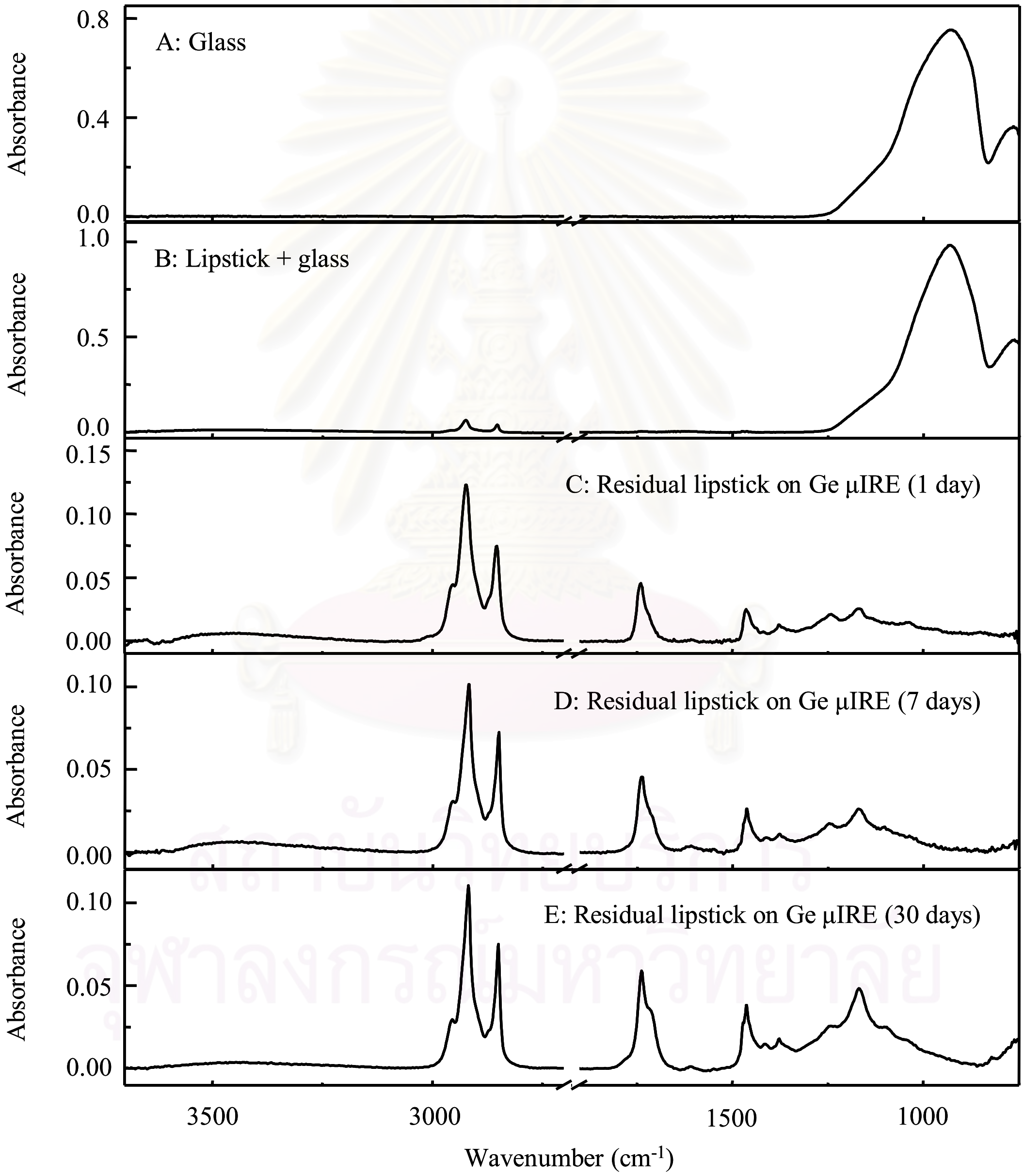


Figure 4.35 ATR FT-IR spectra of lipstick stains on glass which was kept at the outdoors were acquired by the slide-on Ge μIRE.

According to Figure 4.35, each observed ATR FT-IR spectra of lipstick stains on glass at different time intervals after impression show the similar spectral feature but the different in spectral features at ~1700 and ~1525 cm^{-1} region were observed. The spectral feature directly correlated with chemical structure, chemical composition in analyzed lipstick. Thus, lipsticks were kept at the outdoor condition that maybe changed of the minor ingredients in lipsticks when time changed but major component did not show any change. Although the influence of factors such as weather condition (i.e., heat from the sun on the lipsticks analysis of the lipstick on the different surfaces which were kept at the outdoor condition for 30 days after impression) were observed in their ATR FT-IR spectra, the major components which did not change showed unique characteristic of the type of lipstick in the ATR spectrum. Therefore, the observed spectra at the each condition can be employed unique identification.

Figure 4.36 shows ATR FT-IR spectra of the trace amount of lipstick on straw at different times after impression acquired by the Ge μIRE. A "*contact-and-collect*" operation was performed for ATR FT-IR spectrum measurement of trace lipstick by making a gentle contact between the tip of the μIRE and the lipstick smear on a surface. Several contacts were performed in order to acquire a good spectral quality. Like the glass substrate, the ATR FT-IR technique gave ATR FT-IR spectrum of lipstick stains on the straw at each condition with the similar spectral feature. Some variations of the intensities were observed but the frequencies of the main peaks remained the same. The different spectral features show at ~1700 and ~1525 cm^{-1} region Thus, within 30 days intervals after impression, lipsticks that kept at the outdoor condition maybe changed in the minor composition due to influence of heat. The major ingredients which did not change show unique characteristic of the type of lipstick in the ATR spectrum.

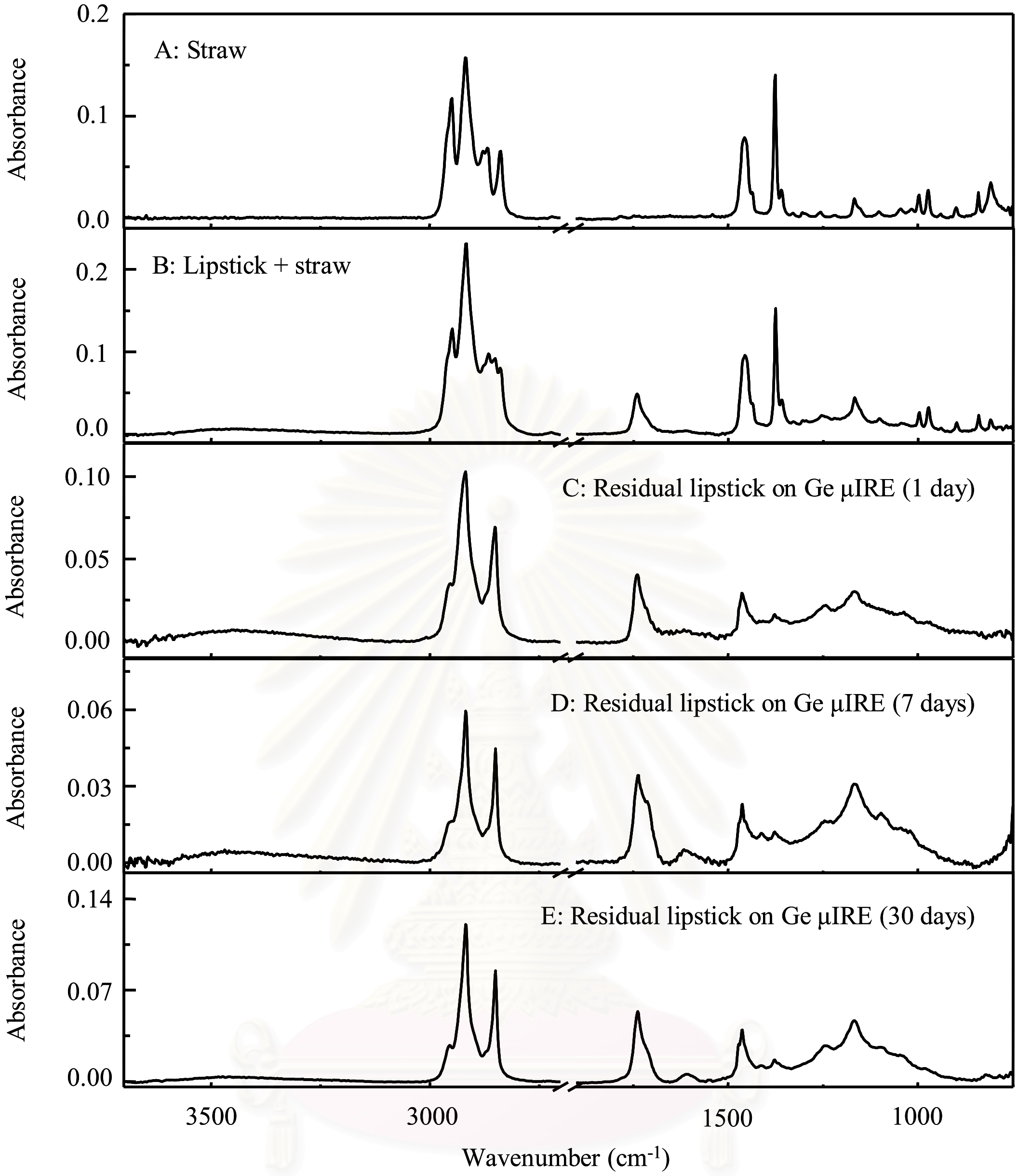


Figure 4.36 ATR FT-IR spectra of lipstick stains on straw which was kept at the outdoors were acquired by the slide-on Ge μIRE.

Example of porous substrates i.e., tissue paper and cotton cloth were studied. The porous surface is the difficult surface for investigation. The quality of the investigation for µATR accessories studied depends on two factors: the type of surface and the age of lipstick.

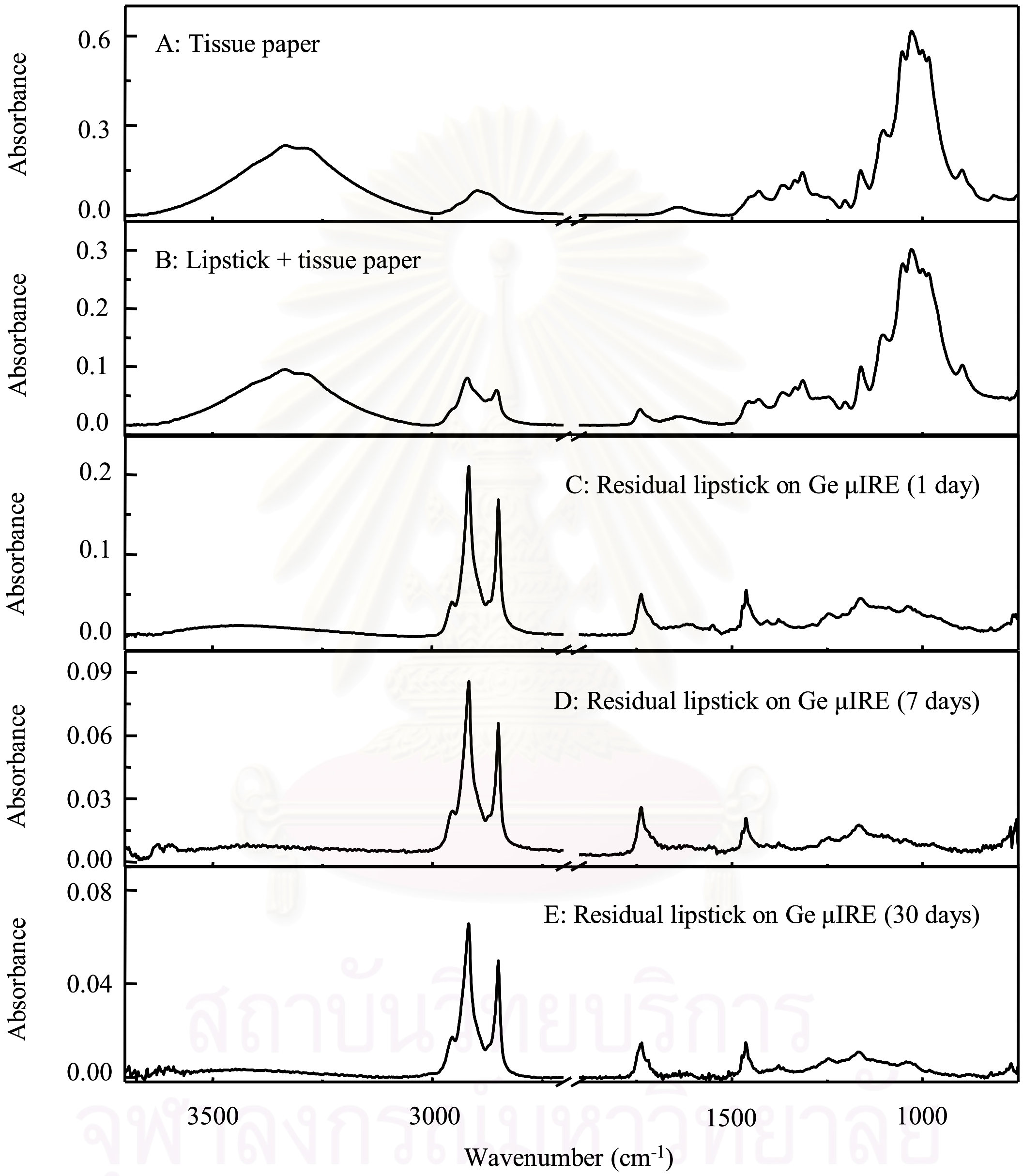


Figure 4.37 ATR FT-IR spectra of lipstick stains on tissue paper which was kept at the outdoors were acquired by the slide-on Ge µIRE.

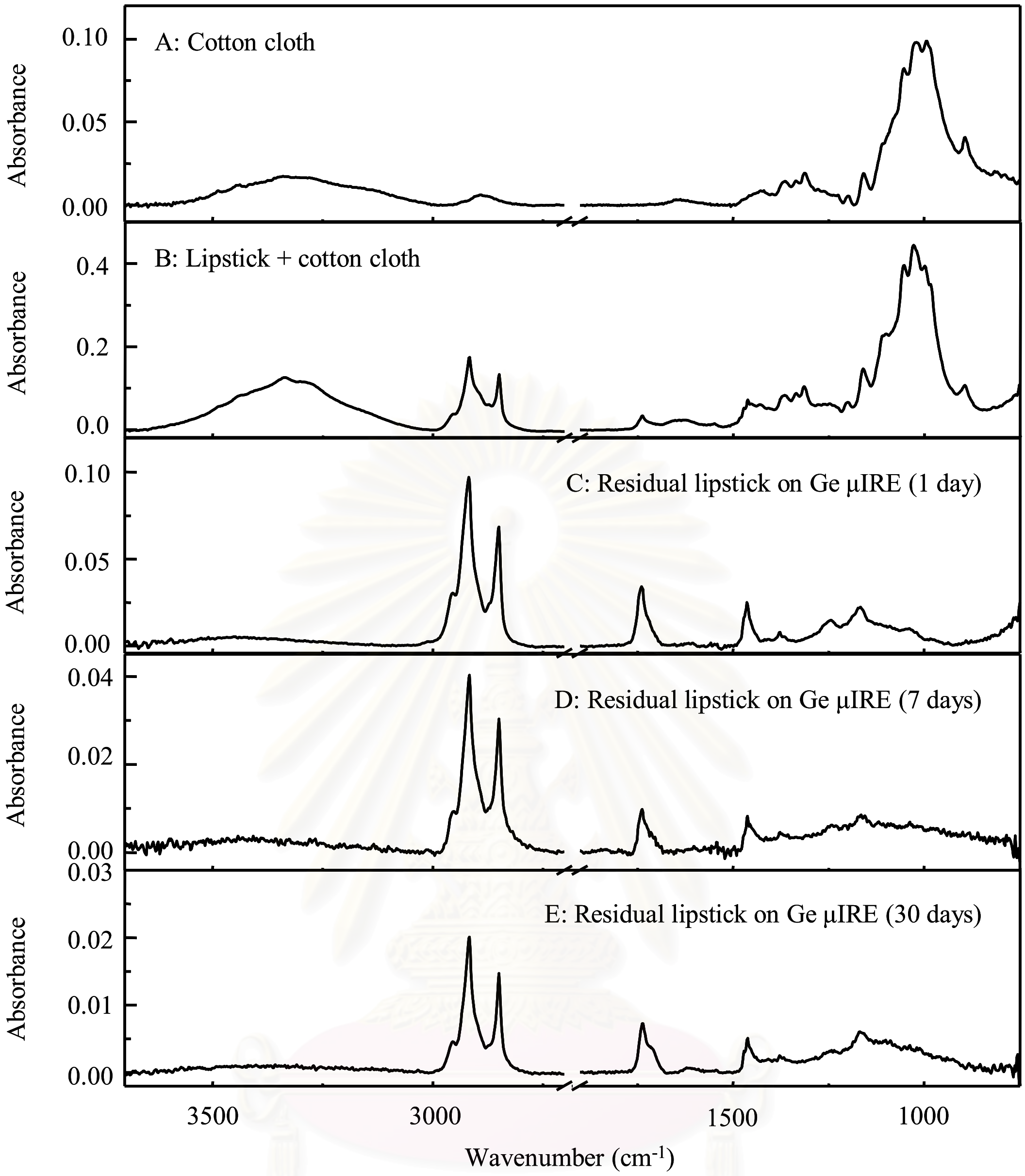


Figure 4.38 ATR FT-IR spectra of lipstick stains on cotton cloth which was kept at the outdoors were acquired by the slide-on Ge μIRE.

The effectiveness of the investigation diminished with the age of lipstick, the poor quality on the older lipstick. Analyzing the results obtained for the two surfaces, the quality of the analysis of lipstick was poor on porous surface than the other surface. The softer surface of porous material allows the investigation process to affect the structure of the surface, producing poor results. Since it is more absorbent than the other surface, the lipstick adheres to the porous surface better, therefore reducing the effectiveness of the analysis. In order to collect a sufficient amount of lipstick, several contacts were required for a good spectral quality for investigation and comparison.

The observed spectrum in Figure 4.38D has more noise level than that of other time due to the amount of analyzed lipstick is small. When the small sample was analyzed, the weak absorption was observed. Considering the results obtained (Figure 4.37 and 4.38), change in the spectral feature was observed in ATR FT-IR spectra at the ~1700 cm^{-1} region. Thus, lipsticks were kept at the outdoor condition maybe changed of the minor ingredients in lipsticks when time changed but major component did not show any change. Therefore, influences of heat from the sun but no interference from dust were observed when lipsticks keeping at the outdoors were analyzed. Heat from the sun and dust may be making any change in minor ingredient of lipsticks within ranging 30 days.

Analyzing and identifying lipsticks at the crime scene may provide important evidences. ATR FT-IR microspectroscopy was used successfully for the analysis of lipstick smears on the different surfaces. The technique is simple, fast and sensitive, and required a minute amount of sample, the results are reliable and there is no pre-separation step. It concludes that this method is suitable for identification of trace amounts of lipsticks for forensic science investigation. The results show that identifiable lipstick can be obtained up to 30 days after being produced. No any influences were observed on the lipstick analysis.

CHAPTER V

CONCLUSIONS

The homemade μIRE accessories, the slide-on Ge μIRE and the slide-on diamond μIRE were employed for ATR FT-IR spectral acquisition using an infrared microscope. The evanescent field generated at the interface can be employed for spectral acquisition of the sample that attached to the tip of Ge μIRE and the pavilion facet of diamond. Regarding to the small sampling area of diamond tip and Ge tip, the small quantities of lipsticks can be analyzed. The spectra acquired by diamond μIRE and Ge μIRE were in good agreement to those acquired by the conventional ATR technique. However, the relative intensity of ATR FT-IR spectra acquired by two accessories is greater than that of conventional Ge IRE (30°) due to the nature of incident radiation penetrating into the sample. A novel *"contact-and-collect"* operation was developed in order to measure ATR FT-IR spectra of a trace lipstick smear without interference from the substrate. The trace lipstick on the IRE can then be characterized under the ATR mode. The spectrum acquired by the μIRE shows unique characteristic of lipstick. The observed spectrum can be employed for the unique identification, matching, or library searching. This technique does not require an additional sample preparation. There was no pre-separation step of the trace lipstick prior the analysis. The entire operation was non-destructive. The chemical information of lipsticks on various substrates can be acquired. Since the contact area of the IREs were small, the characterization process was non-destructive, and there was virtually no noticeable change in the original sample, the specimen can be employed for further analysis. Due to advance features an infrared microscope, any part of trace lipstick smears can be selectively analyzed. The distinct advantages of the μIREs were suitable for very small sample size. Overall, analyzing and identifying lipsticks at the crime scene may provide important evidence. This ATR FT-IR microspectroscopic method has considerable advantages over other qualitative analytical techniques used for the forensic investigation. The results are accurate and reliable. The application of this technique applying to forensic studies has a potential significantly enhance the amount of information that can be obtained from the study of lipstick.

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APPENDIX

APPENDIX

1. Peak assignments of polypropylene

Wavenumber (cm^{-1})	Peak assignments
2952	Asymmetric C-H stretching, CH_3
2915	Symmetric C-H stretching, CH_3
2816	Asymmetric C-H stretching, CH_2
2833	Symmetric C-H stretching, CH_2
1455	CH_3 bending
1375	CH_3 bending
1165	C-C stretching
970	CH_3 rocking

2. Peak assignments of cellulose

Wavenumber (cm^{-1})	Peak assignments
3575-3125	O-H stretching
1750-1725	C=O stretching
1635-1600	O-H deformation
1480-1435	CH_2 deformation
~1375	C-H deformation
~1340	O-H deformation

3. Peak assignments of poly(ethylene terephthalate)

Wavenumber (cm^{-1})	Peak assignments
3076	Aromatic C-H stretching
2973, 2908	Aliphatic C-H stretching
1715	C=O stretching of aromatic ester
1371, 1338	$-CH_2-$ wagging
1239, 1097	asymmetric & symmetric C-O-C stretching of aromatic ester
1613	C=C stretching vibration of aromatic ring
1578, 1505, 1408	Skeletal vibration of conjugation
1173, 1117, 1017	Skeletal ring indicated 1, 4-substitution
871	C-H deformation of two adjacent coupled hydrogen on aromatic ring

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