ATTENUATED TOTAL REFLECTANCE SPECTROSCOPY: AN OVERVIEW

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ABSTRACT

Attenuated Total Reflectance (ATR) is today the most widely used FTIR sampling tool. ATR generally allows qualitative or quantitative analysis of samples with little or no sample preparation which greatly speeds sample analysis. The main benefit of ATR sampling comes from the very thin sampling path length or depth of penetration of the IR beam into the sample. This is in contrast to traditional FTIR sampling by transmission where the sample must be diluted with IR transparent salt, pressed into a pellet or pressed to a thin film, prior to analysis to prevent totally absorbing bands in the infrared spectrum. In transmission spectroscopy, the IR beam passes through the sample and the effective path length is determined by the thickness of the sample and its orientation to the directional plane of the IR beam.

Keywords: ATR,Critical angle,Total internal reflection,Evanescent waves.

INTRODUCTION

ATR is introduced in 1960s ATR is one of the most widely used Technique for Surface Analysis. It is used for analysis of solids, liquids, semi-solids and thin films. Total Internal Reflection is the main Principle involved in ATR [1].

Attenuated total reflectance (ATR) is a sampling technique used in conjunction with infrared spectroscopy which enables samples to be examined directly in the solid or liquid state without further preparation [2].

Attenuated Total Reflectance (ATR) is today the most widely used FTIR sampling tool. ATR generally allows qualitative or quantitative analysis of samples with little or no sample preparation which greatly speeds sample analysis. The main benefit of ATR sampling comes from the very thin sampling path length or depth of penetration of the IR beam into the sample. This is in contrast to traditional FTIR sampling by transmission where the sample must be diluted with IR transparent salt, pressed into a pellet or pressed to a thin film, prior to analysis to prevent totally absorbing bands in the infrared spectrum. A comparison of transmission Vs ATR sampling result for a thick polymer sample is shown in Figure 1 where the sample is too thick for high quality transmission analysis (shown in the lower blue spectrum). In transmission spectroscopy, the IR beam passes through the sample and the effective path length is determined by the thickness of the sample and its orientation to the directional plane of the IR beam. Clearlyin the example below the sample is too thick for transmission analysis because most of the IR bands are totally absorbing. However, simply placing the thick sample on the ATR crystal(Miracle with diamond crystal) and applying pressure generates a high quality spectral result (upper red spectrum) identified by library search as a polybutylene terephthalate.The total analysis time for the thick polymer by ATR was less than 1 minute [3].

Critical angle

When the angle of refraction is 90° from the normal, the angle of incidence in this case is called critical angle.

The critical angle θ_c is given by: n_1 and n_2 are refractive indices of less and more optically dense mediums respectively. The larger the angle to the normal, the smaller is the fraction of light transmitted, until the angle when total internal reflection occurs.

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$$\theta c = \arcsin\left(\frac{n_1}{n_2}\right)$$

When light crosses a boundary between materials with different refractive indices, the light beam will be partially refracted at the boundary surface, and partially reflected.However, if the angle of incidence is greater than the critical then the light will stop crossing the boundary altogether and instead be totally reflected back internally.This can only occur where light travels from a medium with a higher refractive index to one with a lower refractive index.

Evanescent Waves

The infrared radiation interacts with the sample through a series of standing waves, called evanescent waves. An evanescent wave is a penetrating electromagnetic field whose intensity quickly decays as it moves away from its source. Evanescent wave is a wave with an intensity that exhibits exponential decay with distance from the boundary at which the wave was formed. Evanescent waves are a general property of wave-equations. They are formed at the boundary between two media with different wave motion properties, and are most intense within one-third of a wavelength from the surface of formation [6].

Total Internal Reflection (TIR)

When the angle of incidence is greater than the critical angle, a phenomenon known as Total Internal Reflection (TIR) takes place. In TIR the incident ray is reflected inside the material instead of being refracte.It takes place at the interface of an optically denser material with one that is optically less dense.

Instrumentation

In this technique, the IR beam is guided in an IR transparent crystal by total reflection. Due to quantum mechanical properties of the IR light, the electromagnetic field may extend beyond the crystal surface for about one micron as a so-called evanescent field [7].

How an ATR Accessory Works

With ATR sampling we direct the IR beam into a crystal of relatively higher refractive index. The IR beam reflects from the internal surface of the crystal and creates an evanescent wave which projects orthogonally into the sample in intimate contact with the ATR crystal. Some of the energy of the evanescent wave is absorbed by the sample and the reflected radiation (some now absorbed by the sample) is returned to the detector. This ATR phenomenon is shown graphically for a single reflection ATR in Figure 2.

The radiation reflects in the crystal one or more times. ATR measures the total reflected beam which is in contact with a sample. In this way, an evanescent wave penetrates into the sample in contact with the crystal, producing a spectrum of the sample [8].

Crystal Selection

The selection of the ATR crystal characteristics should be matched to the type of samples we run. Selection can be made to control depth of penetration of the IR beam, for hardness to prevent crystal damage, for desired spectral range and for acceptable pH range for acid or caustic samples. No individual crystal type will solve all applications, so PIKE Technologies offer a very broad range of choices for ATR. Factors for selection of crystal type include water solubility, PH range, and hardness [9].

Crystals are typically made of
- Zinc Selenide (ZnSe)
- Germanium (Ge)
- Zinc Sulfide (ZnS)
- Silicon (Si)
- Diamond
- AMTIR: Germanium, Arsenic, Selenium (GeAsSe)

KRS-5

It is the trade name for thallium iodide/thallium bromide. It is a useful material because its transmission covers entire I.R range. Its low refractive index gives it the highest depth of penetration. The Disadvantage of KRS-5 is that it is a soft material that can be easily scratched and bent extremely. It is toxic.

Zinc selenide (ZnSe)

Most commonly used ATR crystal. Depth of penetration is similar to KRS-5. Ideal for analyzing liquids,non abrasive pastes and gels. Disadvantages with Zinc selenide crystals: It is not particularly robust at working PH range of 5-9. It cuts off at about 600cm (masking a small part of mid I.R)

Germanium

Highest refractive index of all the ATR crystals available. It is very hard and not attacked by strong acids. Usually employed when very small penetration depths are desired. Carbon black filled rubbers are typically analyzed.

Silicon

Hardest ATR crystal next to Diamond which makes it well suitable for abrasive materials. Chemically inert. It withstands thermal shocks therefore well suited for applications requiring temperature changes. Affected by strong Oxidising agents. Usefulness is limited to <1500cm

Critical parameter sinatr

Sample must be in direct contact with the ATR crystal, because the evanescent wave extends 0.5-5μ beyond the crystal.

Refractive Index of the ATR crystal must be significantly greater than that of the sample (or else light will be transmitted rather than internally reflected)

The refractive indices of the crystal and sample govern the basic phenomenon of the ATR sampling technique by virtue of the following equation,

$$\theta c = \sin^{-1}[n1/n2]$$

Where n2 is the refractive index of the sample, n1 is the refractive index of the crystal and c is the critical angle.

When we exceed the critical angle, we will observe a purely ATR spectral result. If the critical angle is not met, we will observe a combined ATR and external reflectance result. This occurs if the angle of incidence of the IR beam is too low, if the refractive index of the crystal is too low, if the refractive index of the sample is too high or a combination of these 3 factors
The sample is a carbon filled rubber gasket (high refractive index) run using a single reflection ATR accessory (MIRacle) with Ge and diamond crystals. The upper (blue) spectrum is excellent, using the high refractive index Ge crystal. However, using a diamond crystal with its lower refractive index, we see derivative shaped absorbance bands indicating that we have not satisfied the critical angle requirement.

Another way to correct the spectral artifacts observed (above) in the black rubber sample spectrum would be to increase the angle of incidence in the ATR accessory to a value above the critical angle. Adjustment or selection of the angle of incidence is available in several of the PIKE Technologies ATR accessories [10].

Factors Influencing ATR Analysis
- Wavelength of I.R. radiation λ
- Refractive Index of sample and ATR crystal n_{sp} n_{IRE}

- Angle of Incidence of I.R.radiation θ
- Depth of penetration   Dp
- ATR crystal characteristics
- Effective path length
- Number of reflections

for qualitative or quantitativeanalysis, then we need to increase the effective pathlength (EPL) by increasing the number of reflections (N) within the ATR crystal. The effective path length in ATR is derived by the following equation: *EPL = N de*. An example of the benefit of increased number of reflections for the spectral data for the analysis of carbohydrate content in a soft drink sample. The upper red spectrum is run using a 10 reflection HATR accessory. The lower blue spectrum is run using a single reflection ATR using an identical scaling factor. Clearlythe minor carbohydrate bands are more readily apparent in the multi-reflection ATR accessory [11].

Sample and IRE contact efficiency
The thin penetration of the evanescent wave into

the sample, it is obvious that intimate contact of the sample be made onto the surface of the ATR crystal. For liquid or pliable samples, quality of sample contact with the ATR crystal is generally not a problem. For rigid, irregular shaped or porous samples, high pressure sufficient to deform the sample will increase the extent of sample contact and thereby increase sample absorbance. This is shown in Figure.

In the spectral data collected for a porous foam polymer using a Miracle ATR with an AMTIR crystal. The lower blue spectrum was collected with low pressure applied to the foam sample, whereas the upper red spectrum is produced with high pressure. The ATR absorbance using high pressure is about 10 times greater than with low pressure – all other sampling factors are identical. For rigid, crystalline, or hard, irregular surface samples we recommend a single reflection Diamond Miracle ATR because it is relatively easy to apply high pressure onto the small crystal (1.8 mm diameter) with the high pressure clamp, producing over 10,000 PSI [12].

When an incident ray is totally internally reflected at the interface between two materials of different refractive index, the intensity of evanescent wave extending into the medium of lower refractive index decays exponentially with distance from the boundary
- The intensity of evanescent wave is calculated by following equation
- $I_{ev}=I_o \exp[-Z/Dp]$
- Where;
    I_{ev}=Intensity of the evanescent wave
    Z= distance normal to the optical interface
    I_o= Intensity at Z=0
    Dp= Depth of penetration

Depth of Penetration is defined as the depth at which the evanescent wave is attenuated to 36.8% of its total intensity

$$dp = \frac{\lambda}{2\pi n_{IRE} \sqrt{\sin^2\theta - (n_{smp}/n_{IRE})^2}}$$

- Longer is theλ, greater is the depth of penetration
- Depth of penetration is lower at higher wave number
- ATR intensity decreases
- At higher wave number when compared to transmission spectra
- Most FTIR software packages incorporate an ATR correction algorithm

Technically, this is defined as the distance required for the electric field amplitude to fall to e-1of its value at the surface and is further defined by: where λ is the wavelength of light and θ is the angle of incidence of the IR beam relative to a perpendicular from the surface of the crystal. Typical depth of penetration in ATR ranges from about 0.5 microns up to about 5 microns depending

upon these experimental values. As seen in the graphical representation of the ATR phenomenon, the strength of the evanescent wave decays rapidly as we progress from the surface of the ATR crystal. If we wish to compare the sample absorbance of the ATR measurement with that of a transmission measurement, we need to calculate the volume of the evanescent wave, known as the effective penetration of the IR beam. The effective penetration (de) is unique for parallel polarization and perpendicular polarization. The effective penetration for an unpolarized IR beam is the average of the parallel and perpendicular penetration.

ATR Data Collection
- Samples are run as % Reflectance or log (1/R)
- % Reflectance is equivalent to % Transmittance
- Log (1/R) is equivalent to Absorbance
- In summary, % Reflectance is similar to Absorbance.

Advantages ATR vs. Transmission
- Most samples can be run "neat", which means "in their natural state."
- ATR sampling is fast and easy because little or no sample preparation is required.
- Other techniques, such as infrared transmission, often require the sample to be heated, pressed or ground in order to collect the spectrum.

Solid analysis
- For measuring the composition of solids.
- Examples of solids are films, fabrics, paper, hard polymer sheets, glass and rubber.
- ATR is an ideal technique for measuring dark colored materials which often absorb too much energy to be measured by IR transmission.
- Some examples of strong infrared absorbers are inks, dyes, surfactants, detergents, soaps, paints and some solvents.
- Even highly absorbing liquids, such as aqueous solutions, can be measured accurately by ATR without dilution, because the IR beam penetrates the sample a small amount.
- The solid sample must be placed firmly against the crystal, because the IR beam from an ATR accessory migrates a short distance from the ATR crystal.
- Increasing the angle reduces the number of reflections, thus, the depth of penetration into the sample.
- Additional crystals that have different angle specifications or use a variable-angle ATR accessory can collect information about each layer in a multi-layered sample such as a laminate [13].

Liquid analysis
- ATR is an ideal technique for analyzing liquids Sample preparation is ATR can be used to analyze non-aqueous solutions such a lubricants, oils, paints, glues, solvents, inks and dyes.
- Gels and pastes can also be analyzed minimal.
- When analyzed by IR transmission, viscous liquids such as gels and pastes must be diluted with a solvent and placed in a liquid transmission cell. With the ATR accessory,
- Simply spread the paste or gel over the crystal surface a analyze.

Powder Analysis
- Powders are easier to run by ATR than by IR transmission, because little or no preparation is required.
- This category includes pure samples and mixtures that are available in powdered from.
- Some examples of pure samples and mixtures are pharmaceuticals and pigments.
- For transmission analysis, powders must be mixed with the proper amount of potassium bromide (KBr) and pressed into a pellet. The pellet is then placed in the path of the IR beam. When an ATR accessory is used, the powdered sample is placed directly on the ATR crystal. A powder is then pressed to achieve even distribution and good contact.

Merits
- Rapid and simple.
- Spectrum obtained is independent of the sample thickness.
- Minimum personal errors.
- Applicable to aqueous solutions and IR-opaque materials.
- Cleanup is easy and fast.

Demerits
- The data will be inaccurate if the sample is not in good contact with the crystal.
- ATR crystals have pH limitations.
- The region between 650^{cm-1}-400^{cm-1} cannot be analyzed by use of Ge.
- Temperature dependent transmittance of Ge, so difficult work at elevated temperature.
- Limited to specimen regions having an appropriate refractive indices [14].

Applications
- Characterize the structure and property of biological systems.
- Study the release from semisolid formulations non-invasively.
- Measure drug diffusion from relevant pharmaceutical system such as polymers, films, etc.
- Investigate drug penetration into appropriate acceptor system such as artificial and biological membranes.

- Identification and quantization of polymorphs.
- *In situ* reaction monitoring.
- Liposome adsorption at solid-liquid interfaces.
- Diffusion of transversal drugs through skin mimetic.
- Interactions of polymer-water systems.
- In-line process monitoring of polymers by real time FTIR/ATR.
- FTIR-ATR has emerged as one of the standard techniques utilized to study the structure of the stratum corneum at the molecular level and to evaluate the influence of various penetration enhancers on the SC barrier function.
- FTIR-ATR is now a well-established technique employed to monitor the penetration of drugs into membranes and the permeation of drugs across membranes as well as to determine diffusion coefficients of the diffusants.

An appropriate artificiator natural membrane acting as acceptor is sandwiched between an impermeable ATR crystal and reservoir of penetrant.

- The membrane is initially devoid of penetrant. As diffusion through the membrane occurs, there will be a buildup of penetrant concentration at interface between membrane and crystal, which can be monitored online by the appearance and increase of drug specific IR bands as function of time.
- In contrast to penetration studies, in a release experiment, the arrangement of donor and acceptor is inverted: The formulation is placed on the ATR crystal and the membrane used as acceptor compartment on the top of the ointment.
- In this manner, the evanescent IR beam can monitor the changes of the drug content in the ointment near the surface of the ATR crystal in the course of the release process [15].

STUDY OF DRUG RELEASE FROM SEMISOLID FORMULATIONS

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- In this manner, the evanescent IR beam can monitor the changes of the drug content in the ointment near the surface of the ATR crystal in the course of the release process [16-17].

FUTURE APPLICATIONS

- Forensic Investigation.
- Biomedical applications.
- For forensic investigation and biomedical applications a microscope is used to position a small sample on the ATR crystal. The sample size is 10 microns or smaller (the diameter of a human hair or smaller). The sample is then analyzed with the microscope and by IR [18].

CHALLENGES

- Alignment of the beam splitter.
- Technique will change with sample type.
- When the beam is not aligned less energy will pass through the sample.
- Optimum position of the beam will change over time, so the beam splitter will need to be aligned.
- If the sample is rigid it will need to be cut to the correct size in order to make good contact with the crystal.
- If the sample is a powder, a sufficient amount of sample will need to be placed on the crystal.
- If not enough pressure is applied, the sample will not make good contact with the crystal, giving an incorrect reading.
- If too much pressure is applied, there will be no sample left between the crystal and pressure point, giving an incorrect reading [19-25].

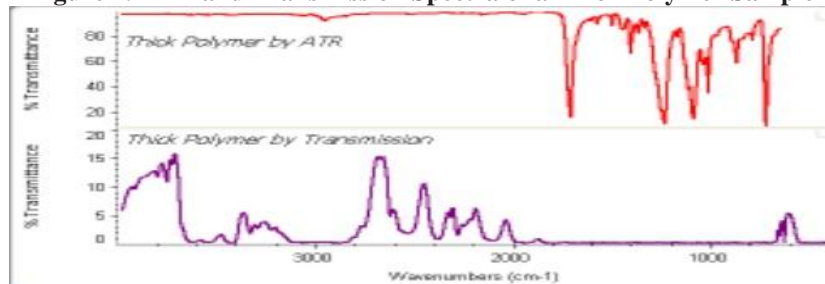**Figure 1. ATR and Transmission Spectra of a Thick Polymer Sample**
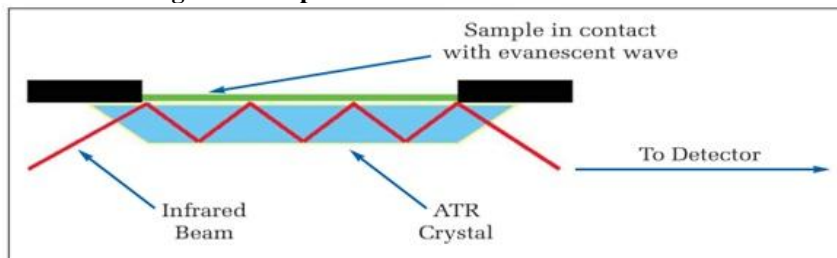
Figure 2.Sample in contact with evanescent wave
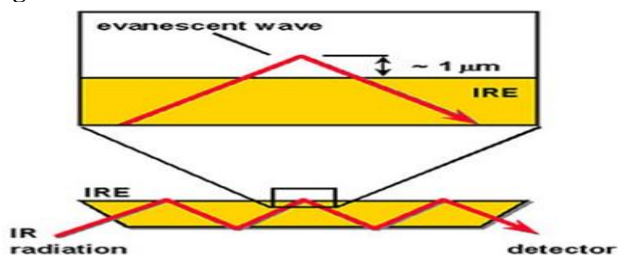

Figure 3. Evanescent wave

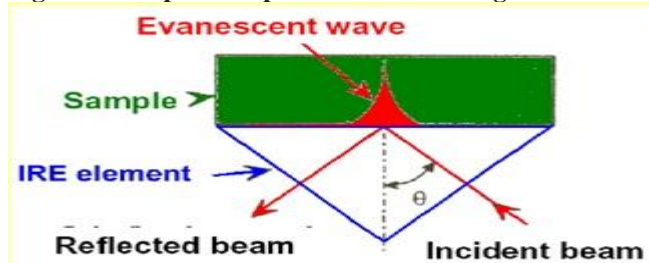**Figure 4. Graphical representation of a single reflection**
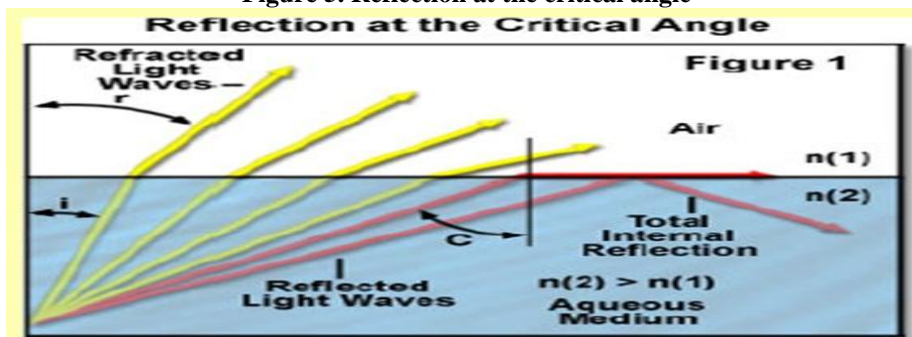

Figure 5. Reflection at the critical angle

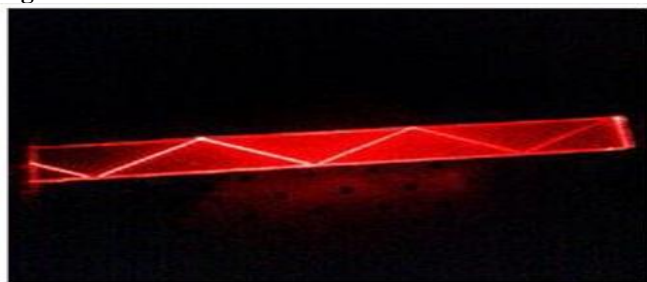

Figure 6. Total internal reflection

Figure 7. Instrumentation of ATR
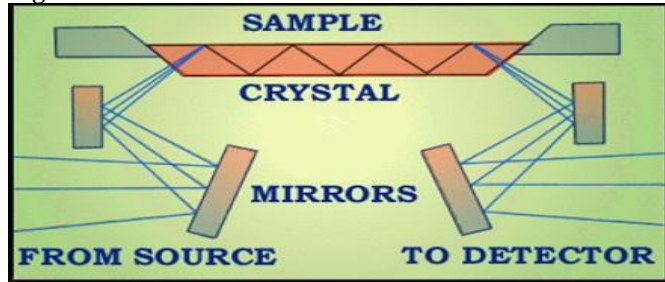
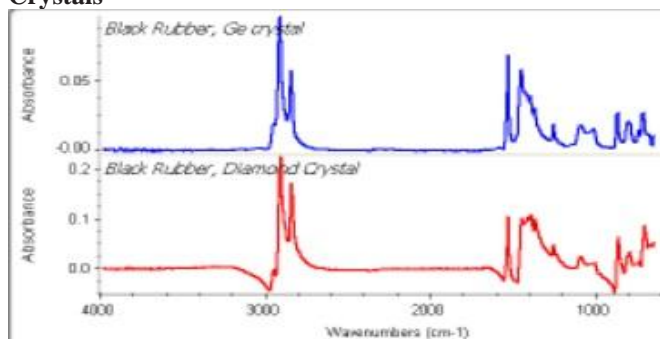**Figure 8. Perkin-Elmer Spectrum 100FT-IR Spectrometer**

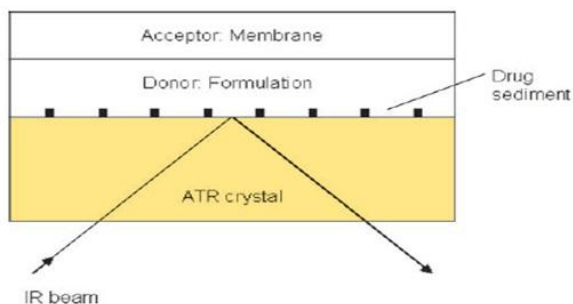| ATR Crystal	Useful Spectral Range	RI at 1000 cm⁻¹
AMTIR	11,000 -725	2.5
Diamond	4,500 - 2,500 1,667 - 33	2.4
Ge	5,500 - 830	4
Si	8,300 - 1,500 360 - 70	3.4
ZnSe	20,000 - 650	2.4

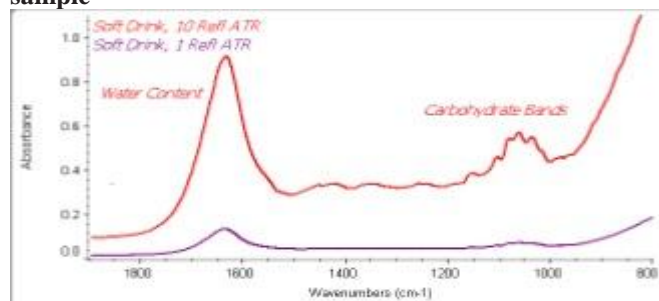**Figure 9. Spectra of Carbon Filled Rubber Gasket Sample Using MIRacle ATR with Ge and diamond Crystals**

Figure 10. Single reflection (lower blue spectrum) Vs multireflection(upper red) HATR spectra for a soft drink sample

Figure 11. Effect of pressure upon quality of ATR spectra shown for a foam sample

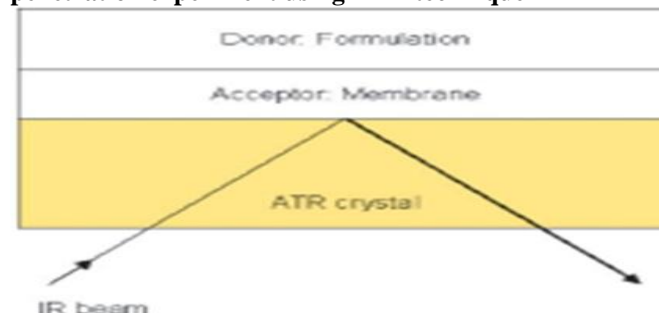**Figure 12. The sample arrangement for the drug diffusion experiment using ATR technique**

Figure 13. Sample arrangement for the drug release experiment in ATR Technique
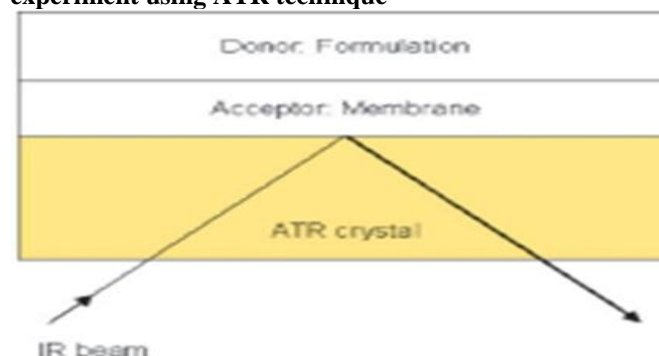
Figure 14. The sample arrangement for the drug penetration experiment using ATR technique

Table 1.

Chart of Common Crystal Materials

Material	ATR Spectral Range (cm⁻¹)	Refractive Index	Depth of Penetration (µ) (at 45° & 1000 cm⁻¹)	Uses
Germanium	5,500 - 675	4	0.66	Good for most samples. Strong absorbing samples, such as dark polymers.
Silicon	8,900 - 1,500 & 360-120	3.4	0.85	Resistant to basic solutions.
AMTIR	11,000 - 725	2.5	1.77	Very resistant to acidic solutions.
ZnSe	15,000 - 650	2.4	2.01	General use.
Diamond	25,000 - 100	2.4	2.01	Good for most samples. Extremely caustic or hard samples.

CONCLUSION

Attenuated Total Reflectance is an easy-to-use, fast, and versatile technique for infrared sampling. Solids, pastes, gels, liquids and powders can be analyzed with little or no preparation. When light crosses a boundary between materials with different kinds of refractive indices, the light beam will be partially refracted at the boundary surface, and partially reflected. However, if the angle of incidence is greater (i.e. the ray is closer to being parallel to the boundary) than the critical angle – the angle of incidence at which light is refracted such that it travels along the boundary – then the light will stop crossing the boundary altogether and instead be totally reflected back internally. This can only occur where light travels from a medium with a higher [n_1=higher refractive index] to one with a lower refractive index [n_2=lower refractive index]. For example, it will occur when passing from glass to air, but not when passing from air to glass.

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