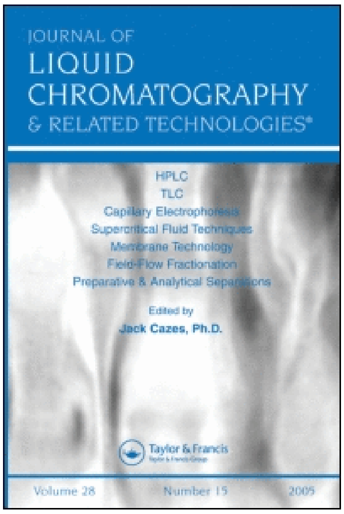

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Attenuated Total Reflectance as an Alternative of Diffuse Reflectance Infrared Detection in the Identification of Compounds Separated by Thin Layer Chromatography

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Abstract: Diffuse Reflectance (DRIFT) and Attenuated Total Reflectance (ATR) are infrared sampling methods, based on the detection of infrared radiation reflected by the sample. They are widely used in surface analysis and are both capable of detecting small amounts of organic compounds. DRIFT is a well known on-layer method for the identification of analytes, separated on thin layers. In the present work, the possibility of using a diamond ATR unit to collect IR spectra of sample spots was examined.

Keywords: Diffuse Reflectance, Attenuated Total Reflectance, Thin-layer chromatography, Detection

INTRODUCTION

The importance of chromatographic separation techniques coupled with spectroscopic identification methods is well known in the analysis of complex mixtures. Along with mass spectrometry, infrared spectroscopy is the most informative method available for identifying small amounts of unknown organic substances. Several techniques were developed to identify substances by FTIR, previously separated on thin layers.[1] These can be divided into two

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main groups: transfer- and in situ methods. Transfer methods consist of removal of the separated compound from the thin layer and preparation of a conventional IR sample.[2] The most obvious way is scraping off the TLC spot and extracting the analyte via a solvent to KBr powder, which is then pressed to form a pellet. Transfer methods eliminate the problems caused by the strong infrared absorbance of adsorbents, however, they are time consuming and have a potential to decompose and contaminate the sample. Using in-situ methods, the separated compounds are analysed directly on the plate. Fuller and Griffith[3] were the first to collect infrared spectra from TLC plates, using a diffuse reflectance (DRIFT) unit. The IR spectra is generated by focusing an IR beam onto the surface of the sample and collecting the reflected radiation by the aid of an elliptical mirror, which directs it to the detector. In order to get spectra on a transmittance scale from the reflectance intensities, a mathematical transformation has to be done, which is named after Kubelka and Munk.

Several studies have been performed to explore the potential of TLC–DRIFT methods. These studies were reviewed by Brown.[4] On conventional TLC phases, the lowest identification limits were found to be about 0.1 ug. When the TLC plate is moved stepwise under a DRIFT unit by the aid of a computer controlled XY stage, a series of IR spectra can be collected as the function of distance, and can be plotted in 3D.[5] This simplifies the localisation of analytes and enables differentiation of spots on the chromatogramm.

The greatest problem due to the storng absorption bands of the adsorbent is that some parts of the spectra have poor signal to noise ratios, and are only minimally informative. Silica absorbs infrared between 3700–3100 cm^{-1} and 1600–800 cm^{-1}, and aluminiumoxide under 900 cm^{-1}. Cellulose has multiple absorption bands in the region of 1500–600 cm^{-1}, therefore the fingerprint region of IR spectra, collected from cellulose layer is hard to use for identification. Reversed phase TLC plates can be used as well as silica plates, but water as eluent should be avoided if possible, or careful drying is required before collecting the spectra. To overcome the adverse effects of adsorbents, zirconium oxide, an IR transparent stationary phase, was tested but never came into general use.[6] Using diffuse reflectance for spectrum collection has an other disadvantage. Reflectance spectra differs from transmittance spectra considerably, which makes identification difficult without building a spectral library under the same conditions.[7]

Attenuated Total Reflectance (ATR) is a sensitive infrared sampling method and it can be an alternative for diffuse reflectance measurements in thin layer chromatography. ATR-FTIR methods are used for the detection of small amounts of compounds bonded or adsorbed on solid surfaces, for example, solid phase organic synthesis can be monitored without removing the product from the resin.[8] It's based on the phenomenon that an IR beam, arriving to the interface of a solid sample and the diamond prism is totally reflected, but meanwhile it penetrates into the sample by a few microns depth. A portion of the IR beam is absorbed by the sample, and by detecting the reflected infrared light, the surface spectra of the sample can be obtained.

Diamond ATR, likewise diffuses reflectance and is widely used for collecting IR spectra from the surface of solids. The two methods complement each other, ATR is used for smooth surfaces and diffuse reflectance for rough surfaces. ATR requires uniform contact with the sample, and diffuse reflectance requires no contact.

EXPERIMENTAL

Infrared spectra were collected on a Perkin Elmer Spectrum One FTIR spectrophotometer equipped with a diamond ATR unit (3 × reflection, 6 μm penetration of the IR beam into the sample). The spectra were evaluated with Spectrum ES 5.0 software. All the ATR spectra were recorded against air background, and are uncorrected. For comparison, diffuse reflectance measurements were also done using silica background, and only automatic Kubelka-Munk transformation was applied in order to get IR spectra on a transmittance scale from the reflectance data.

Reagent grade solvents and commercial grade organic compounds were used from Merck and Sigma Aldrich. The following TLC plates were examined: Kieselgel 60 F^{254}; aluminiumoxide 60 F^{254} neutral (Typ E, Merck 5550); cellulose (Merck 5552); silanised silicagel 60 (C1 on glass plate, Merck 5746).

The concentrated solutions of the samples were spotted with a 0.5 μL capillary pipette onto the TLC plates, in order to get the smallest spot possible. Only short path elution was applied to prevent spot growth and achieve uniform distribution of the compound in the spot area. The circle shaped ATR diamond had a diameter of 3 mm. For optimal detection the sample spot should have the same shape and size as the prism, because the overreaching part remains undetected. To achieve this, the TLC plate has to be precisely positioned on it. The spots should not be cut around, because the adsorbent easily peels down from the aluminium plate. It is better to cut off the upper part and one side of the plate just near the edge of the spot, in order get the spot on the corner of the remaining plate. Now, the prism can be easily covered with the corner of the plate containing the spot to be measured. In the case of glass plates, the spot can be drawn around with a soft pencil. This can be seen from the back side of the plate, and enables positioning of the spot onto the prism. After positioning, a thin silicon septum was placed on the back of the TLC plate and pressed down by the built in tension wrench of the ATR unit with a uniform force. Eight scans were collected from every spot. Measuring the spots against air background usually gives adequate results, but in the case of non volatile eluents a background spectra has to be collected from the area near the spot.

RESULTS AND DISCUSSION

Silica and aluminium oxide layers, which are good alternatives to each other were examined in detail; compounds that have characteristic bands in the region of the silica adsorption can be measured on aluminium oxide and the other way around.

From 1900 to 2300 cm^{-1} the ATR diamond has strong IR absorption, which causes strong noise in this region (Figure 1).

During our investigations, 37 compounds were tested for identifiablity from their ATR spectra recorded on silica-, and/or aluminiumoxide thin layers, after a short (few centimeters) elution with different eluents. The quality of the collected spectra strongly depends on the IR activity of the compound examined. In the case of weakly absorbing compounds, a relatively great amount of substance had to be spotted on the layer (more than hundred microgramms in some cases), but 5–6 characteristic bands generally appeared, enabling differentiation between each compound. In the case of strongly absorbing compounds, the collected spectra contained 8–12 characteristic bands with high intensity (Figure 2). Diffusion of the analyte on the layer makes the identification harder, because with increasing size of the sample spot, the undetected proportion of analyte increases. It's hard to keep the diameter of the spots under 3 millimeters along with acceptable separation, that's why limits of detection were not determined for each compound.

In the case of the marked compounds, the IR activity is too low to get valuable information from the spectra, even by using concentrated solutions. The absorbance of the most intensive absorption bands of these

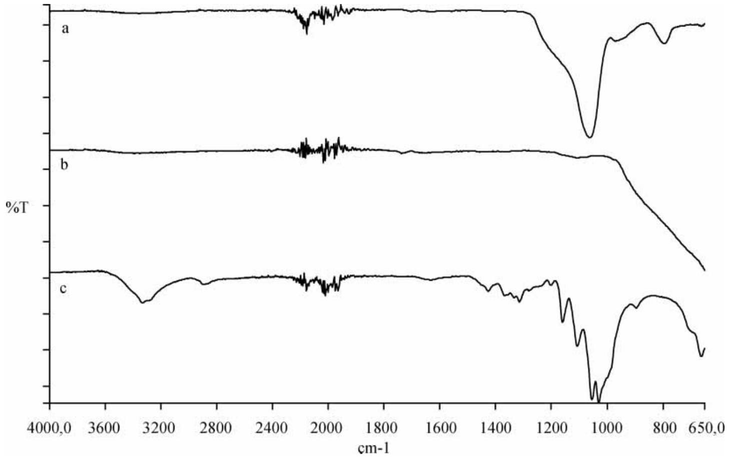
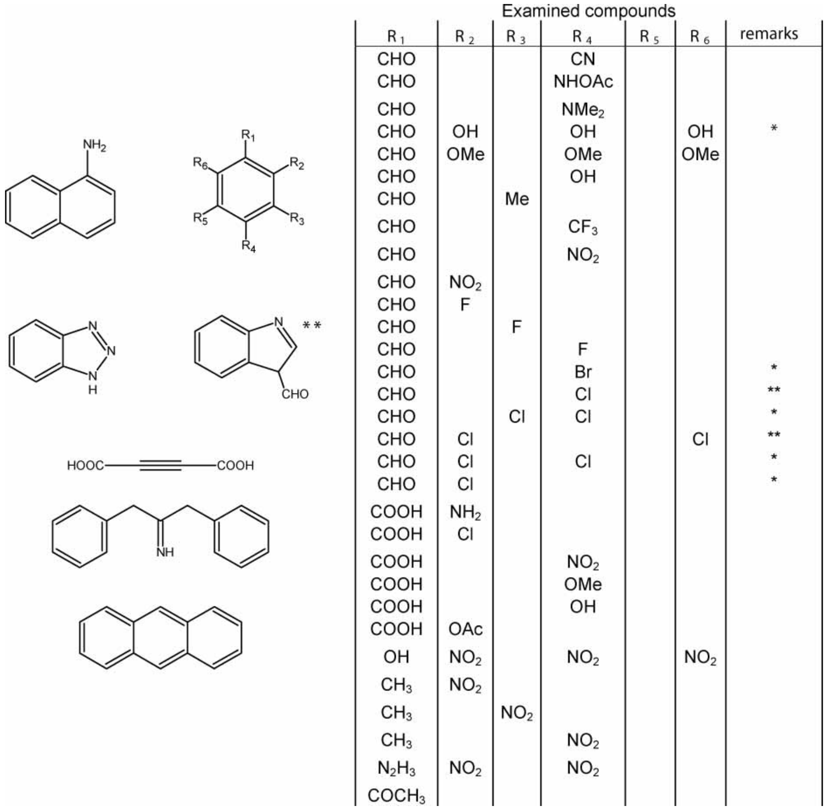


Figure 1. ATR spectra of common thin layers: Kieselgel 60 F$_{254}$, aluminiumoxide, cellulose.



Examined compounds

R_1	R_2	R_3	R_4	R_5	R_6	remarks
CHO			CN			
CHO			NHOAc			
CHO			NMe_2			
CHO	OH		OH		OH	*
CHO	OMe		OMe		OMe	
CHO			OH			
CHO		Me				
CHO			CF_3			
CHO			NO_2			
CHO	NO_2					
CHO	F					
CHO		F				
CHO			F			
CHO			Br			*
CHO			Cl			**
CHO		Cl	Cl			*
CHO	Cl				Cl	**
CHO	Cl		Cl			*
CHO	Cl					*
COOH	NH_2					
COOH	Cl					
COOH			NO_2			
COOH			OMe			
COOH			OH			
COOH	OAc					
OH	NO_2		NO_2		NO_2	
CH_3	NO_2					
CH_3		NO_2				
CH_3			NO_2			
N_2H_3	NO_2		NO_2			
$COCH_3$						

Figure 2. Examined compounds. No mark: good quality spectra with five or more characteristic absorption bands. *: low intensity spectra, with less than five characteristic bands. **: no bands analogous with the KBr spectra.

compounds, recorded from the pure substance on ATR is in the range 0.1–0.5, while for the other examined compounds 0.8–1.6.

Spectra for calibration were recorded from the spotted samples without elution, to avoid sample loss derived from spot growing. The limit of detection for 3-nitrotoluene is about 10 μg (Figures 3 and 4), but it is more informative that the limit of identification for 2- and 3-nitrotoluene is 25 μg (Figure 5). There are small differences in the spectra of the two compounds, but these are well reproducible, and can be used to differentiate between them. The position of the two bands around 1530 and 1350 cm^{-1} is shifted to greater wavenumbers compared to the pure substance, but the wavenumbers of 3-nitrotoluene are always greater than those of 2-nitrotoluene.

DRIFT measurements has lower detection limits, and the spectra are rich in detail, but ATR results in spectra which are less different than transmission spectra. This means that an unknown compound can be identified by

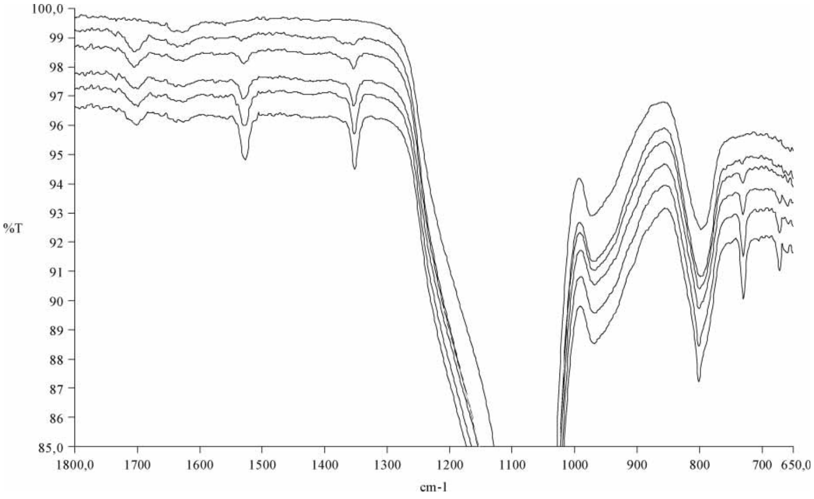


Figure 3. 3-nitrotoluene on silica, increasing quantities from top to bottom: blank, 5, 12, 17, 25, 37, and 49 μg compound.

comparing the position of its strongest bands with spectra collected from pure substances.

If we compare the spectra of 3-nitrotoluene collected with DRIFT and ATR (Figure 6), we can observe that the DRIFT spectra significantly differs from that collected from the pure compound on a KBr pellet. ATR spectra contains all the five strongest bands with minor differences in position.

In our survey, liquid samples gave better quality spectra with more intensive peaks than solid materials. Its probable reason is that a liquid uniformly covers the surface of adsorbent particles after the evaporation of the eluent, and this surface layer becomes in direct contact with the diamond prism and the IR beam penetrates it during the measurement. In the case of solid compounds, small crystals are formed among the silica particles, which have a poor contact with the surface of the diamond. In

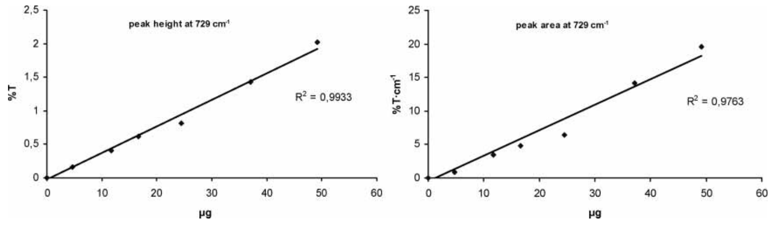


Figure 4. Calibration curves for 3-nitrotoluene on silica. Peak height and peak area are also proportional to the sample amount.

Figure 5. a: 2-nitrotoluene, b: 3-nitrotoluene, 25 μg of each on silica. The wavenumbers of the pure compounds are represented in brackets, after the wavenumbers collected on silica.

fact the spectra are recorded from a powder mixture, which consists of a small amount of organic compound diluted with an IR absorbing adsorbent. This results in one order of magnitude increase of detection- and identification limits.

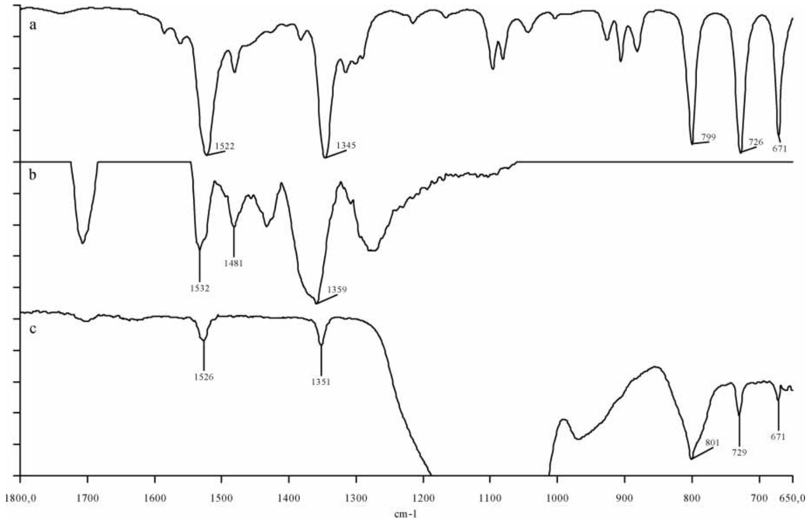


Figure 6. 3-nitrotoluene a: pure liquid, b: DRIFT spectra on silica, c: ATR spectra on silica.

CONCLUSIONS

The ATR method results in lower intensity absorption bands compared to DRIFT, but there are only minor differences to KBr spectra, and requires no mathematical transformation, which has a potential for information loss. The sensitivity could probably be enhanced by using an ATR diamond with 9 × reflection, which has a penetration depth of 18 microns. Due to the relatively great surface of the diamond prism, only 2–3 well separated spots can be measured on a chromatogramm by ATR, but it can give valuable information, for example, in the identification of a byproduct. This method can't be a substituent of DRIFT, due to its lower sensitivity, but it can be useful when the positions of the 4–5 strongest absorption bands are enough to identify the compound. In these cases, it can be superior to DRIFT because the differences to KBr-spectra are smaller, and identification can be done without assembling a TLC-ATR spectrum library.

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