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Analysis of human bodily fluids on superabsorbent pads by ATR-FTIR

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Abstract

Superabsorbent pads are composed of different layers with different grades of absorbent capacity, being the lower one the most absorbent layer. Due to their complexity, the analysis of bodily fluids on superabsorbent pads is certainly difficult.

In this study, semen, vaginal fluid and urine stains placed on superabsorbent pads including sanitary napkins, panty-liners and diapers were non-destructively detected by Attenuated Total Reflectance (ATR) Fourier Transform Infrared spectroscopy (FTIR). In spite of the higher absorbent capacity of the lower layers, this technique was able to detect the three fluids on the upper layer of all pads, showing that bodily fluids are distributed within all layers. Additionally, mixtures of these bodily fluids prepared on superabsorbent pads and cotton were studied, since real forensic investigations involving sexual abuse cases usually deal with mixtures of these fluids. Due to their IR marked protein region (1800-1480 cm^{-1}), semen and vaginal fluid were easily distinguished from urine. However, since semen and vaginal fluid have both a high protein composition, that region of their IR signatures were quite similar, except for slight visual differences, that should be further analysed. Therefore, we propose ATR-FTIR as a suitable, presumptive, non-destructive and rapid approach to detect stains of human bodily fluids on the upper layer of superabsorbent pads from sexual crimes.

Keywords: ATR-FTIR; Semen; Urine; Vaginal fluid; Mixtures of bodily fluids; Superabsorbent pads.

1. Introduction

Analytical approaches for bodily fluid detection currently used in forensic laboratories usually involve biochemical and immunological tests, which are destructive and applicable for only one bodily fluid [1, 2]. Specifically, to detect the presence of semen, forensic laboratories usually perform the presumptive assay of seminal Acid Phosphatase, followed by the confirmatory observation of spermatozoa by optical microscopy with the Christmas Tree test, and also the confirmatory immunological assays of Prostate Specific Antigen (PSA) detection and Semenogelin antigen detection, among other presumptive and confirmatory assays [3-5]. Regarding the detection of vaginal fluid and urine, there are only presumptive assays, including immunological, electrophoretic and chemical tests [4-6]. With regard to spectroscopic techniques, only UV-vis spectroscopy is already being used in forensic laboratories, as a presumptive technique for bodily fluids detection; even though this technique has low selectivity and, thus, produces a high number of false positive results [4, 6, 7].

In recent years, vibrational spectroscopic techniques such as IR and Raman spectroscopy have started to be explored for the fast, solvents-free and non-destructive identification of bodily fluids [3, 4, 7-23]. In fact, Raman spectroscopy has provided quite satisfactory results to identify and discriminate bodily fluids including semen, vaginal fluid, blood, saliva and sweat when they have been analysed on microscope slides, [8, 15-19]. However, Raman does not seem to be proper for the analysis of stains of bodily fluids on fabrics, because of the interference of the intense Raman signals from fabrics and its dyes, and the difficulty to focus the Raman spot just on the surface of the stain removing the fabric background [7, 20]. On the contrary, IR spectroscopy in the attenuated total reflectance (ATR) or any other reflection mode, is particularly suitable for analysing the surface of samples, which seems to be ideal for detecting stains of bodily fluids on fabrics and any other supporting materials. In fact, several applications in medicine were described for FTIR, such as cancer and microbiological infection diagnosis [11-14]. In the forensic field, this technique is already used for drugs, paints and fibres chemical analysis [10, 21, 24]. However, only a few years ago, Elkins [22] and Orphanou [10] proposed ATR-FTIR as a suitable method for the presumptive non-destructive detection and differentiation of bodily fluids, demonstrating that blood, vaginal fluid, saliva, tears, nasal mucus, semen, urine and different cosmetic materials and foodstuff had a unique spectrum due to their distinct composition, even when they were as stains on cotton. In

fact, the analysis was as simple as placing the stains onto the ATR window. Similarly but using external reflection FTIR rather than ATR, Zapata *et al* [23] analysed stains of semen, vaginal fluid, urine and mixtures of vaginal fluid and semen on cotton fabrics. However, until now, it has not been verified whether ATR-FTIR or any other vibrational spectroscopic technique is able to detect and differentiate semen, vaginal fluid, urine, and mixtures of these fluids on hygienic superabsorbent pads. These supporting materials are highly relevant in sexual assault and rape cases because, although immediately after sexual intercourse most of the semen flows back out, the remaining is slowly expelled during the following several days to the victim's underwear and superabsorbent pads [25]. Thus, the underwear and superabsorbent pads, including diapers, used by the victim during and after a sexual assault [26] are often collected and examined. Superabsorbent pads are composed by several layers with different absorbent capacity: the upper layer, that is an hydrophobic and porous layer composed mostly by polypropylene and polyethylene; the intermediate layer (not always present), which is a thin layer generally composed of cellulose and cotton; and the lower layer, usually composed of cellulose and superabsorbent salts such as cross-linked sodium polyacrylate salts [27-30]. Their complexity often hinders the semen detection using the current techniques, leading to a wrong dismissal of forensic samples that may actually contain semen's DNA. Therefore, the behaviour of stains of bodily fluids on superabsorbent pads needs to be known to perform an appropriate and non-destructive detection of the low quantities of semen along the pad, essential for a posterior DNA profiling.

To this aim, this study was focused on the differentiation of human bodily fluids stains on superabsorbent pads using ATR-FTIR. For this, due to the complexity of these supporting materials, the distribution of bodily fluids stains throughout the different layers of the studied materials as well as the spectral behaviour of bodily fluids on the different layers was previously aimed.

2. Materials and Methods

The superabsorbent pads analysed were: Pad 1 (feminine sanitary napkins from Evax (Procter & Gamble, Ohio, USA)), Pad 2 (panty-liners from Carefree (Johnson & Johnson, New Jersey, USA)) and Pad 3 (diapers from Dodot (Procter & Gamble, Ohio, USA)). In addition, cotton fabric was also considered in this study as a preliminary proof of concept. All pads were acquired from a local supermarket. The pores sizes of all layers of the three

pads were observed with a 10x magnification objective of a Trinocular Microscope with a 9MP camera T490B-9M (AmScope, Irvine, USA), and measured with the software AmScope ToupView, version 3.7.929 (AmScope, Irvine, USA).

Samples were collected from healthy anonymous donors (3 semen donors, 3 vaginal fluid donors and 3 urine donors), who previously signed an informed consent with the description of the project aims. All bodily fluids and their mixture were analysed on Pad 1, Pad 2 and cotton. However, as females start to produce vaginal fluid at puberty [31], only semen, urine and their mixture were analysed on Pad 3. To use a more representative sample, the samples of semen from the three donors were mixed previously to their use, as well as the urine samples. Stains of semen and urine were prepared by adding 0.5 mL of each bodily fluid, around 488±30 mg of semen and/or 560±40 mg of urine, on each one of the three pads and the cotton fabric, according to the procedure described in [23]. Briefly, this procedure was based on the collection of each fluid with a 0.5 mL Pasteur pipette and placing it on the surface of each supporting material. Regarding vaginal fluid stains, donors directly prepared these stains by swabbing the exterior of the vagina with Pad 1, Pad 2 or the cotton fabric.

The mixture stains were prepared by applying, consecutively, the different bodily fluids on the same supporting material. On Pad 1, Pad 2 and cotton, the mixture contained the three bodily fluids. First, vaginal fluid was added by the donors, then 0.5 mL of semen were added followed by 0.5 mL of urine, in a 1:1 ratio. On the contrary, on Pad 3, the mixture only contained semen and urine (1:1). Following this procedure, two stains of each bodily fluid or mixture were prepared per type of pad, 30 stains in total (8 stains on cotton fabric, 8 stains on Pad 1, 8 stains on Pad 2 and 6 stains on Pad 3).

All stains were left to dry overnight in a biological safety cabinet (BSC) prior to analysis. The weight of fluids added was measured with an Analytical Balance DV215CD (OHAUS, Nanikae, Swiss) prior to impregnation and 24 hours after impregnation. According to Zapata *et al* [23], these bodily fluids stains on cotton fabric lose a high percentage of weight after this step (between 80-96%, depending on the bodily fluid) due to water evaporation. Therefore, it was necessary to study the behaviour of these stains on superabsorbent pads.

FTIR spectra were obtained with a Thermo Nicolet IS10 (Thermo Fisher Scientific Inc., Massachusetts, USA), using an ATR-FTIR accessory (smart iTR) and the OMNIC software version 9.1.26 (Thermo Fisher Scientifc Inc., Massachussets, USA).

Spectra were collected in absorbance mode (log 1/R). For each measure, 16 scans were accumulated. The resolution was 4, the window aperture was at medium resolution, the gain was 2 and the optical velocity was 0.4747. At these parameters, good quality spectra with less spectral noise were obtained. As described in previous literature [23], the stains were measured between the range 2000-800 cm^{-1}, since the main bands of stains of bodily fluids occur between 1800-1500 cm^{-1} [23]. Between samples, the ATR-crystal was cleaned with isopropanol and the background was updated.

For each stain, 8 random spots were analysed on superabsorbent materials, and 6 random spots were analysed on cotton. A higher number of spots were studied in superabsorbent materials due to their higher grade of complexity of these supporting materials regarding cotton. Data were saved as .spa and .csv files.

The analysis of results and their graphical plots were performed with the software OriginPro 8 (OriginLab Corporation, Massachusetts, USA). The spectrum of each spot was analysed separately and then their average spectrum was calculated.

3. Results and Discussion

Since superabsorbent pads are complex samples in which the distribution of bodily fluids throughout their different layers has not been properly studied, this aspect was examined prior to the spectral discrimination of bodily fluids on the superabsorbent pads.

3.1. Distribution of bodily fluids stains throughout the different layers of superabsorbent pads

Superabsorbent pads have, at least, one hydrophobic porous layer (upper layer), and one absorbent layer with superabsorbent polymers (lower layer) [27, 28]. Pad 1 has three layers (upper, intermediate and lower), all detachable. Pad 2 only has the upper (hydrophobic) and the lower (absorbent) layers, also both detachable. With another format, Pad 3 has three layers (upper, intermediate and lower), but the intermediate layer is not detachable from the lower one. Consequently, the three layers from Pad 1, the two layers from Pad 2 and the upper and the intermediate layer's surface of Pad 3 were analysed.

To understand how bodily fluids are distributed in each pad, a measurement of the pores size of each layer of the superabsorbent pads was made by optical microscopy (Fig. 1).

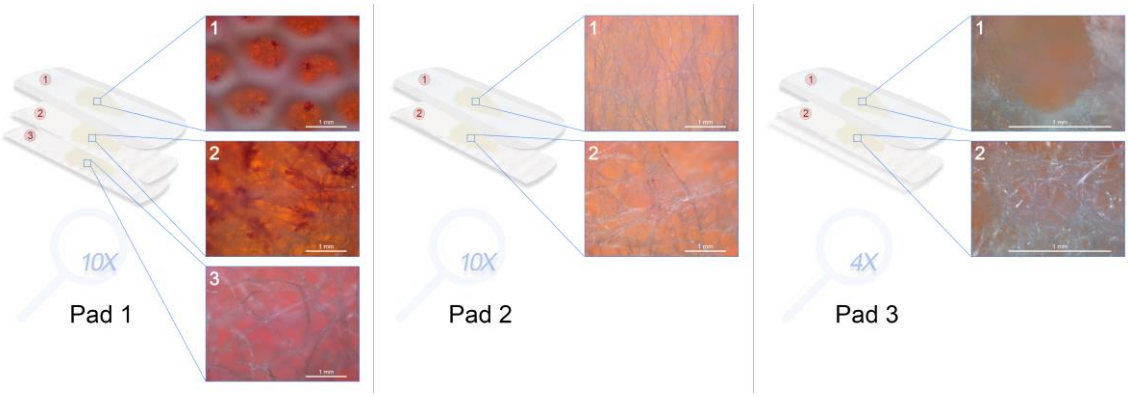

Fig. 1: Diameter measure of pores from all superabsorbent pads.

The size of the pores, of both upper and intermediate layers, of all pads (Table 1) is larger than the size of cells (epithelial cells: 30 μm; sperm cell: 4 μm with a tail of 50 μm; oocyte: 130 μm [32, 33]), and of the Stokes radius of proteins (<1 μm, for example albumin, common on semen and vaginal fluid, has around 3.5 nm [34]).Therefore all cells, proteins and any other compound from bodily fluids might easily pass through these initial layers until the lower layer. If that was completely true, the total amount of bodily fluids would be recovered from the lower layer and no remains of the bodily fluid would be found on the upper layer.

Table 1. Pore sizes of all layers of the three studied pads.

	Pad 1 (μm)	**Pad 2 (μm)**	**Pad 3 (μm)**
Upper layer	790 – 980	240 – 110	2500 – 2200
Intermediate layer	470 – 160	Not existing	600 – 20
Lower layer	430 – 150	160 – 50	Not measured

Therefore, in order to verify or dismiss this fact, the accurate distribution of bodily fluids along the different layers of pads was studied by weighing each layer before being stained, and 24 hours after depositing bodily fluids. It is important to refer that on this assay only semen and urine were used since the collection of vaginal fluid precluded an accurate measurement of the mass of this bodily fluid. Unexpectedly, results confirmed that all layers of stained pads contained some amount of bodily fluids' remains, since there was an increase in the mass of all layers from all pads (see Table 2).

Table 2. Average mass percentage of semen and urine remains on each layer (n = 2). Mass percentages were calculated as result of the bodily fluid mass weighed in each layer divided by the total mass of bodily fluid weighed on all layers.

Semen	**Pad 1**	**Pad 2**	**Pad 3**
Upper layer	1.04%	6.61%	0.85%
Intermediate layer	8.53%	93.39%	99.15%
Lower layer	90.43%		
Urine	**Pad 1**	**Pad 2**	**Pad 3**
Upper layer	0.22%	6.06%	0.03%
Intermediate layer	4.53%	93.94%	99.97%
Lower layer	95.25%		

According to Table 2, it was observed a clear influence of the fluidity of the bodily fluids on the passage through layers, shown by the fact that semen is more retained on the upper layer than urine. Regarding the vaginal fluid (for which an accurate mass measurement was not possible), it could be estimated that vaginal fluid would be more similarly retained on the upper layer as semen instead of urine, due to the vaginal fluid composition which is more similar to semen than urine.

Although the upper layer is hydrophobic, these results may be understood for the ability of the main components of the upper layer, polypropylene and polyethylene, to adsorb cells, DNA and proteins, as previously described in literature [35-37].

3.2. Spectral behaviour of bodily fluids on the different superabsorbent pad's layers

Once verified that bodily fluids were distributed in the different layers of the superabsorbent pads, the spectral analysis of stains was performed taking into account all layers. First, it was examined in which wavelength range bodily fluids displayed their characteristic bands without the supporting material overlapping them. In brief, the major bands from pads were located at 1470 and 1375 cm^{-1} for upper layer from Pad 1 (respectively due to either antisym. CH_3 bending or CH_2 scissoring and sym. CH_3 bending); 1740, 1460, 1375 and 1240 cm^{-1} for intermediate layer from Pad 1 (respectively assigned to C=O stretching, antisym. CH_3 bending or CH_2 scissoring, sym. CH_3 bending and C-OH stretching); 1240, 1150, 1100 and 1010 cm^{-1} for the lower layer of Pad 1 (due to C-O stretching); 1705, 1470, 1240, 1090 and 880 cm^{-1} for the upper layer from Pad 2 (due to C=O stretching, CH_3 and CH_2 bending, C-O stretching and CH_3 rocking, respectively); 1730, 1375, 1240, 1100 and 1020 cm^{-1} for the lower layer from Pad 2 (again due to C=O stretching, sym. CH_3 bending and C-O stretching); 1705, 1470, 1375, 1240 and 1060 cm^{-1} for the upper layer from Pad 3 (respectively due to C=O stretching,

antysym. CH_3 bending or CH_2 scissoring, sym. CH_3 bending and C-O stretching); and 1705, 1440, 1400, 1240, 1100, 1010, 975 and 880 cm^{-1} for the intermediate-lower layer from Pad 3 (again due to C=O stretching, antysym. CH_3 bending or CH_2 scissoring, sym. CH_3 bending and C-O stretching) [38], as shown by the IR spectra from pads' layers represented as dotted spectra in figures. For cotton, the major bands were located at 1425, 1365, 1330, 1315, 1150, 1100 and 1050 cm^{-1}, which are in accordance with previous literature [23, 39].

Consequently, the range less hindered by the pads was over 1480 cm^{-1}, limit below which numerous intense bands from the pads become significant and overlapping. The spectra from pads were not subtracted to the spectra of stains, because their intensity varied from spot to spot according to the amount of bodily fluid. However, despite differences in pads composition, the 1800-1480 cm^{-1} range, presented almost none interference from superabsorbent pads' spectra. Therefore, the IR spectral signatures of bodily fluids stains were mainly studied within the 1800-1480 cm^{-1} range.

According to previous studies [10], this range is suitable to detect these bodily fluids mainly through the characteristic spectral bands of amide I and amide II's vibrations from the different proteins and urea contained in these fluids. However, because of the wide spectral bands, which resulted from the combination of the vibrations from the different proteins contained in each bodily fluid, the specific assignment of bands to a unique compound was unfeasible, except for the case of urine stains in which the larger amount of urea determined its IR bands.

Therefore, Table 3, which summarizes the bands assignment for the bands observed for bodily fluids within the 1800-1480 cm^{-1} range, does not correlate each band with a specific compound but only with the respective vibrational modes involved.

Table 3. ATR-FTIR bands assignment for stains of bodily fluids on superabsorbent pads within the range 1800-1480 cm^{-1} according to the literature [10, 22, 23, 38, 40, 41].

Bodily fluids	Band frequency (cm^{-1})	Vibrational mode	Components (referenced band frequency)
Semen	1635	C=O st	Amide I β-sheets (1624-1642 cm^{-1})
	1550	C-N st & N-H ip bend	Amide II (1480-1575 cm^{-1})
Vaginal fluid	1650	C=O st	Amide I random coil; Amide I alpha helix (1640-1658 cm^{-1})
	1540	C-N st & N-H ip bend	Amide II (1480-1575 cm^{-1})
Urine	1670	C=O st	Urea - Amide β turn (1665-1685 cm^{-1})
	1635	N-C=O st	Urea - Amide I β-sheets (1624-1642 cm^{-1})
	1550	C-N st & N-H ip bend	Urea - Amide II (1480-1575 cm^{-1})

For instance, semen is composed of acid phosphatase, PSA, citric acid, calcium, choline, spermine, semenogelin, urea, immunoglobulins, albumin and spermatozoa [42, 43]. Acid Phosphatase, albumin and the rest of the proteins contribute to the wide bands at 1635 and 1550 cm^{-1} observed in the spectra of semen stains, as shown in Fig. 2. The shape and intensity of these bands varied for each layer and each pad, according to the semen concentration and the pad influence. The highest intensity for semen bands was measured on the upper layer of all pads, although it had been previously checked that the upper layer was the one with less remains of the bodily fluid. This fact could be explained by 2 factors: the less interference from the upper layer to the IR spectrum than the other layers, and/or the heterogeneous distribution of semen throughout the different layers in terms of composition remaining the most IR (1800-1480 cm^{-1}) active compounds from semen (*i.e.* proteins) in the upper layer. This fact was evident for the upper layer of Pad 1 (Fig. 2A) in which the bands of semen were highly intense and well defined in contrast with the intermediate and lower layers in which the intensity of semen bands vastly decreased to such an extent that it was not so clear the presence of the fluid. This also occurred for pads 2 and 3, though the differences in intensity of semen bands between upper and lower layer was not so pronounced as in Pad 1. In addition, on the upper layer of Pad 3 (diaper) (Fig. 2C), the bands of semen were not as well defined as on Pad 1 (Fig. 2A), proving the interference of the supporting material.

According to Fig. 3, vaginal fluid seems to have a similar spectral signature as semen within the 1800-1480 cm^{-1} range, as expected, due to the protein composition. Vaginal fluid is composed of transudate from the vaginal mucosa, acid phosphatase, glucose, prostaglandins, sialic acid, lactic acid, urea, peptidase, acetic acid, epithelial cells, bacteria and leukocytes [6, 22]. As occurred with semen stains, the highest intensity of the bands of vaginal fluid was also obtained on the upper layer of pads, and again, their intensity decreased for the intermediate and lower layers. Due to the differences in the proteins' composition that semen and vaginal fluid contain [22], the two major and specific bands of vaginal fluid related to amide I and II, were located at 1650 and 1540 cm^{-1}, slightly shifted from the semen bands, although it is such small shift that it is difficult to visually notice it, especially in those spots with low concentration of fluid.

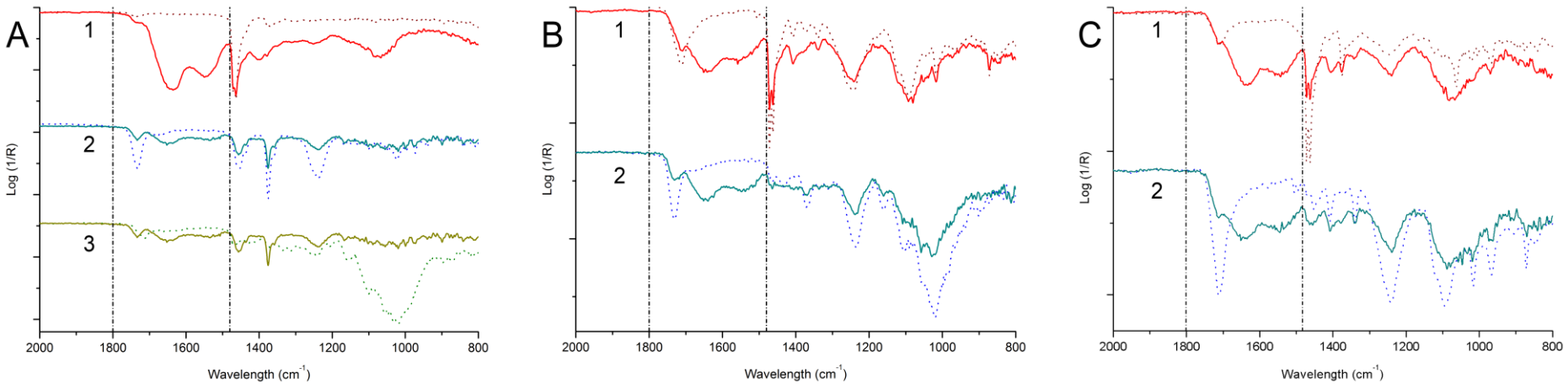

Fig. 2: ATR-FTIR spectra of Semen stains on the different layers of Pad 1 (A), Pad 2 (B) and Pad 3 (C). The different layers are presented: 1 – upper layer; 2 – intermediate layer; 3 – lower layer. The dotted spectra represent the layers without stain (blanks). ATR-FTIR measurements in the 2000-800 cm^{-1} range were taken: 16 scans, resolution 4, gain 2 and optical velocity of 0.4747.

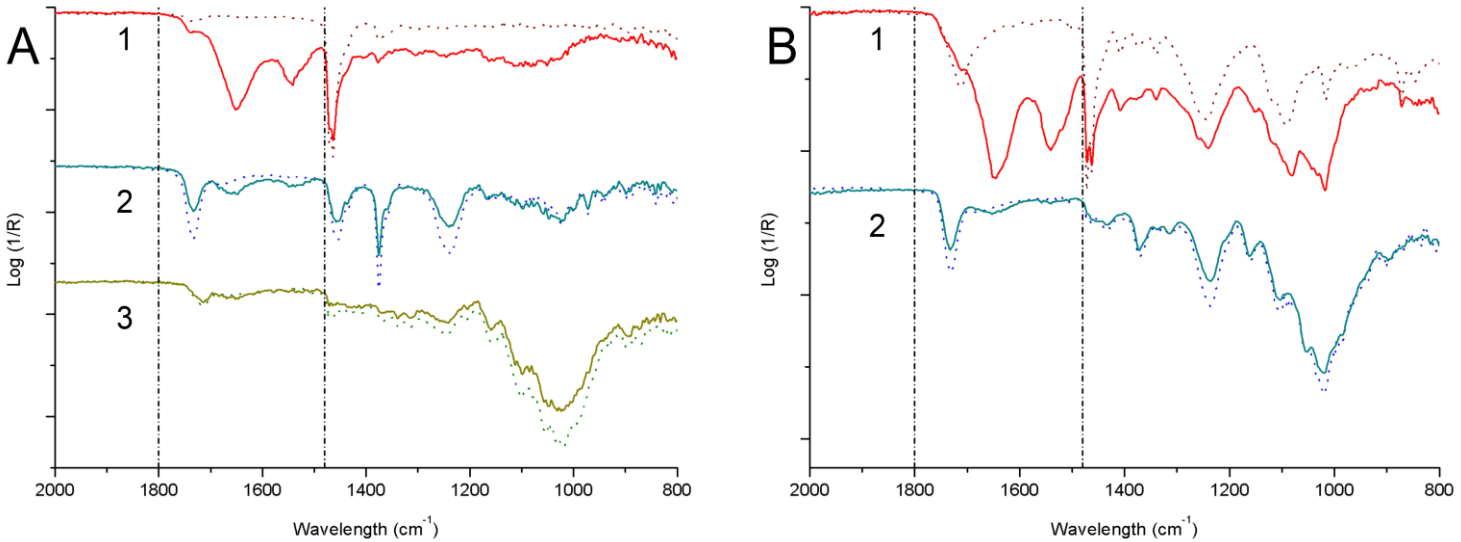

Fig. 3: ATR-FTIR spectra of Vaginal fluid stains on the different layers of Pad 1 (A) and Pad 2 (B). The different layers are presented: 1 – upper layer; 2 – intermediate layer; 3 – lower layer. The dotted spectra represent the layers without stain (blanks). Instrumental parameters as in Fig. 2.

Regarding urine stains (Fig. 4), their spectrum was markedly different to those from semen and vaginal fluid. This can be explained because urine has lower content of proteins and a considerably high content of urea [22]. In fact, the spectrum of urine displayed a wide band divided into two small bands at 1670 and 1635 cm^{-1}, which are due to the molecular vibrations of urea as summarized in Table 3. However, the same result with regards to the different intensity of the bands among the layers was observed for urine stains. The highest intensity was obtained on the upper layer, and it decreased for the intermediate and lower layers.

Finally, mixtures of bodily fluids impregnated on superabsorbent pads (Fig. 5) and cotton (Fig. 6) were analysed to test if semen was detectable in spots of the mixture stain.

In the mixture of semen, vaginal fluid and urine on cotton (Fig. 6), the presence of urine was not detected and it is not possible to visually specify if the spectrum obtained was from vaginal fluid, semen or both. The same occurred for mixtures on Pad 1 (Fig.5A) and Pad 2 (Fig. 5B).

Regarding Pad 3 (Fig. 5C), although urine was mostly detected, in some spots the presence of semen was verified through the detection of the bands at 1635 and 1550 cm^{-1} bands. However, only the bands of urine are noticeable in Fig. 5C, because it is displayed the average spectrum from all spots which were measured.

3.3. Semen differentiation within mixtures of bodily fluids on superabsorbent pads

Although Elkins [22] described a weak band at 1016 cm^{-1} in semen spectrum that would allow differentiating it from other fluids, it was not detected in any of the supporting materials tested. In cotton samples, the strong bands of cotton, located between 1200-800 cm^{-1} would have overlapped it, while in the superabsorbent pads, the noise would have mask such weak band. However, the IR band of semen at 1084 cm^{-1}, also described in [22, 23] and probably given by prostate specific antigen (PSA) and fructose according to Orphanou [10], although not visible on cotton, may help to differentiate semen and vaginal fluid on the superabsorbent pads studied. Also, according to [22], another band, around 1450 cm^{-1} which is due to the asymmetric methyl bends in amino acid side chains of proteins [10], appears stronger in semen spectrum and it would help to differentiate semen from vaginal fluid. However, in superabsorbent pads, the identification of these bands and the respective bodily fluid becomes subjective, due to their own complex spectra within this range.

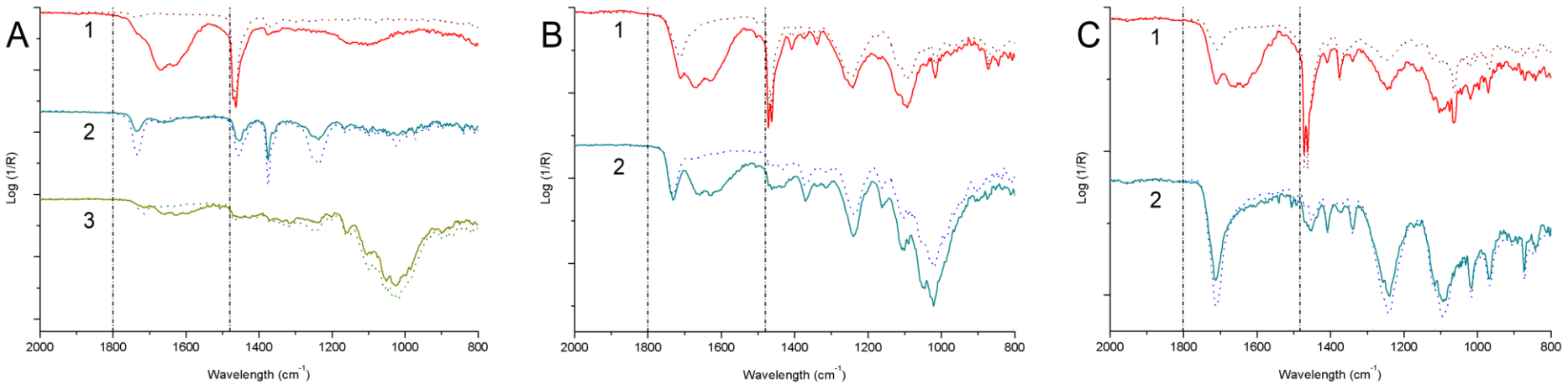

Fig. 4: ATR-FTIR spectra of Urine stains on the different layers of Pad 1 (A), Pad 2 (B) and Pad 3 (C). The different layers are presented: 1 – upper layer; 2 – intermediate layer; 3 – lower layer. The dotted spectra represent the layers without stain (blanks). Instrumental parameters as in Fig. 2.

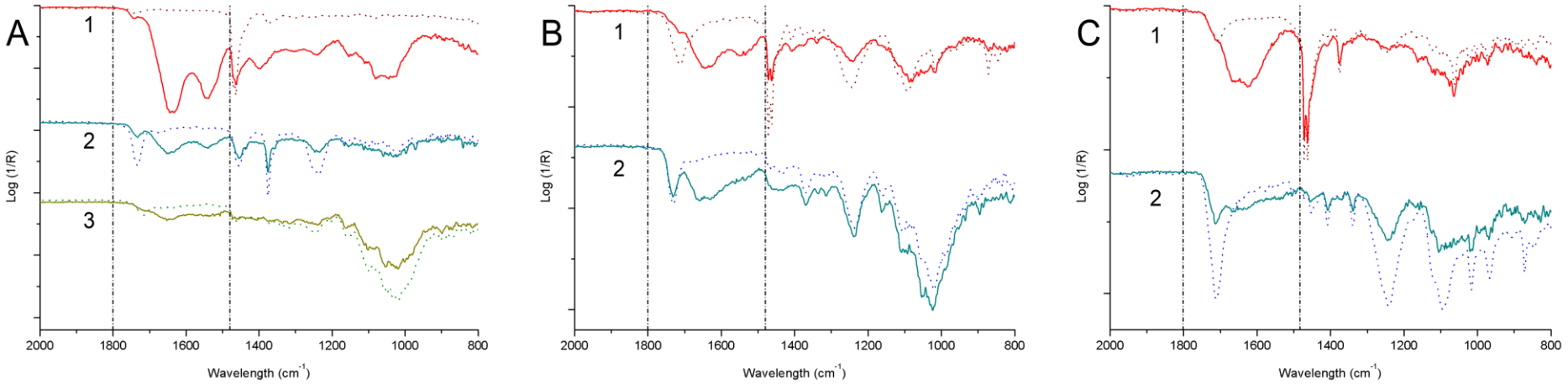

Fig. 5: ATR-FTIR spectra of Mixture stains on the different layers of Pad 1 (A), Pad 2 (B) and Pad 3 (C). The different layers are presented: 1 – upper layer; 2 – intermediate layer; 3 – lower layer. The dotted spectra represent the layers without stain (blanks). Instrumental parameters as in Fig. 2.

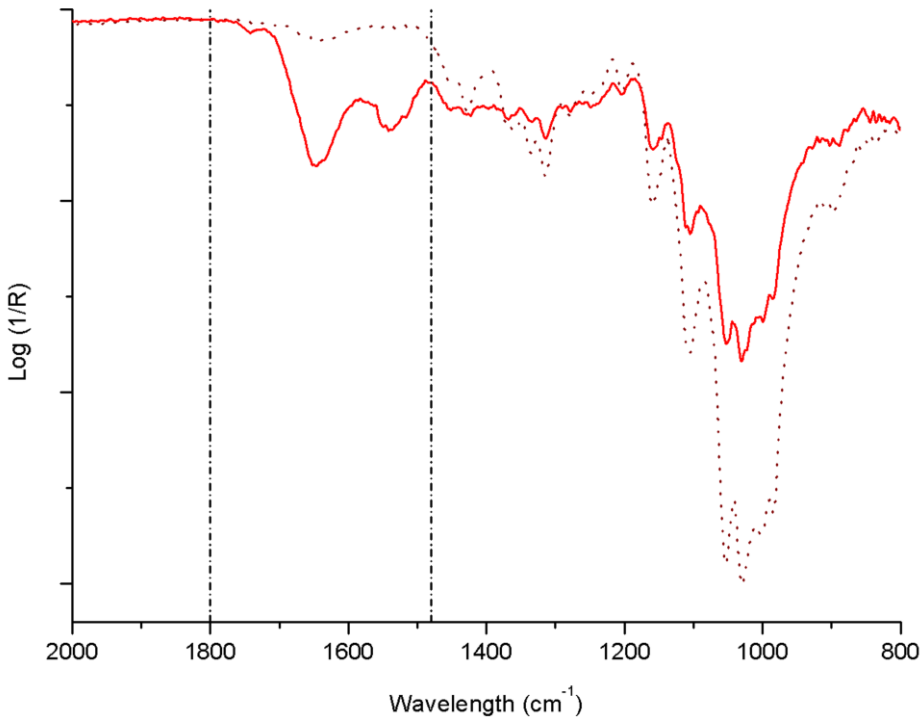

Fig. 6: ATR-FTIR spectra of Mixture stains on cotton. The dotted spectrum represents the cotton without stain (blanks). Instrumental parameters as in Fig. 2.

Thus, assuming that most real samples are mixtures of these fluids, and assuming the importance of detecting semen, more research using chemometrics and statistical analysis is necessary to increase the power of discrimination of this technique.

It was observed that semen and urine samples left a yellow stain on the intermediate and lower layers, which was not observed for stains of vaginal fluid. From this fact, the relative intensity of the bands of bodily fluids on the lower layers was compared among each other. For instance, by comparing simultaneously Fig. 2B, Fig. 3B and Fig. 4B (*i.e.* stains of each bodily fluid on Pad 2) it is quite noticeable that the intensity of bands of vaginal fluid on the lower layer (spectrum 2 in Fig. 3B) is almost negligible in comparison to the intensity of semen and urine bands in their corresponding spectra (spectrum 2 in Fig. 2B or Fig. 4B, respectively). This fact seems to indicate that vaginal fluid is more retained by the upper layer of pads than semen or urine, because there are lower remains of vaginal fluid than semen or urine in the lower layers, which are detected by ATR-FTIR. The higher viscosity of vaginal fluid could explain it. If this was the case, the different layers of superabsorbent pads would play as filters which would affect differently the pass of each bodily fluid. However, this is a preliminary observation, which needs to be further tested.

Another important discussion topic is if semen with sperm cells and semen without sperm cells would be detected in the same way through IR spectroscopy. In 2007, Barcot *et al* [40] demonstrated different spectra for sperm cells and semen by FTIR, on the absorbance mode. In this work, that result was not verified due to the complexity of superabsorbent pads, since the spectrum obtained by ATR-FTIR from semen stains on superabsorbent pads with low semen concentration was similar to the sperm cells spectrum given in [40]. Another future trend could be analysing semen from oligospermic (low sperm cells) and azoospermic (no sperm cells) men and compare them with samples from normal sperm.

Conclusions and future trends

Considering the structure of the superabsorbent pads studied (sanitary napkin, panty-liner and diaper), it could be presumed that fluid's components would be on the lower, more absorbent layer. However, this study shows that some components of the fluids remain on the upper layer, probably due to its adsorptive capacity. This fact was verified by ATR-FTIR, since semen, urine and particularly vaginal fluid were detected with higher intensity on the upper layer of all pads.

Positively, the IR region from 1480 to 1800 cm^{-1} was workable to detect remains of bodily fluids on the layers of the three different pads tested, even though they varied in composition, which is a preliminary proof of its reproducibility for superabsorbent materials in general.

Using this spectral range, urine was easily identified because of the molecular vibrations of urea. However, it showed limitations to visually discriminate between semen and vaginal fluid, because of having both similar bands due to proteins within the studied range. This is particularly crucial when both fluids are mixed in a stain. In addition, in those mixture stains containing urine and semen only, the mostly detected fluid was urine, although it was possible to detect semen on a few spots.

Regarding the technique's parameters, 40 sec (16 scans) were spent per spot and it was possible to detect the same stain 4 weeks later (data not shown), which means that this approach is rapid and non-destructive. In addition, this technique does not need treatment of samples, since the detection is possible on the upper layer, and it is fast, easy-to-use, non-destructive and non-invasive, and as it preserves the sample and the integrity of DNA, needed for further identification of the aggressor. Thus, considering all the benefits of using ATR-FTIR and also its problems with bodily fluids discrimination, this technique shows a potential that needs further research for its practical implementation.

As a mandatory future trend, it is necessary to overcome the difficulties related to the discrimination of bodily fluids in mixtures by statistically studying the spectral data with bioinformatics tools, as multivariate analysis.
Another future trend would be to define a plan of extraction of the bodily fluids from the superabsorbent pads for a subsequent identification of the semen's DNA profile.

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Declaration of interest: The authors declare they have no competing interests.

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