Rapid diagnostic method of tobacco products in saliva by fourier transform infrared spectroscopy (FTIR)

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Abstract: The present study was designed to explore the easy and fast method diagnosis of tobacco products in saliva of tobacco users (TU) by FTIR. Sixty four male tobacco users (TU) with mean age range 15.3 to 30.7 years were randomly selected for collection of saliva samples before and after tobacco use (smoking, chewing and dipping tobacco). Twenty were the smoking tobacco users (STU), 24 were chewing tobacco users (CTU) and 20 were dipping tobacco users (DTU). CTU were the users of Mainpuri (n=10) and users of PEN, FIT, 2100 (n=14). Forty eight saliva samples of age and gender matched healthy individuals with negative personal or family history of any addiction were also collected for comparison which served as controls. All were analyzed for their salivary flow rate, salivary pH and salivary diagnostic bands by FTIR. Significantly increased SFR ($p<0.05$) and salivary pH were found in after chewing tobacco as compared to before its chewing. The comparison between after tobacco use and controls we found decreased SFR and salivary pH for STU. Significant decreased SFR and increased salivary pH were found before or after use of dipping tobacco as compared to controls. Sharp bands at 735-745 cm^{-1} were found and may be used as salivary diagnostic bands for STU, 945-949 cm^{-1} for DTU and 900-915 cm^{-1} for CTU as well as DTU. In conclusion, the salivary diagnostic bands were found at 735-745 cm^{-1}, 900-915 cm^{-1} and 945-949 cm^{-1} for TU by easy and fast method using FTIR.

Keywords: Salivary flow rate, salivary pH, chewing tobacco, smoking tobacco, dipping tobacco, FTIR.

INTRODUCTION

Fourier Transform Infrared Spectroscopy (FTIR) nowadays becomes the most fast and easy method for the identification of organic or inorganic chemicals (Kumar *et al.*, 2014; Sahu and Mordechai, 2005; Conti *et al.*, 2005; Abstract book 2013; Kong & Shaoning, 2007; Dovbeshko *et al.*, 2000; Guo & Zhang, 2004; Gazi *et al.*, 2007; Li Q *et al.*, 2013; Griebe *et al.*, 2007; Channa *et al.*, 2007a; Channa *et al.*, 2007b; Erukhimovitch *et al.*, 2006; Mordechai *et al.*, 2004; Lin *et al.*, 2001). It can be used for the identification of unknown and unidentifiable mixture components. It is also used to analyze the solids, liquids, and gases. FTIR is useful in the field of biology for analyzing the chemical changes in a living cell. Interestingly it is valuable in studying the light induced reactions in photo biochemical systems such as rhodopsin, bacteriorhodopsin and photosynthetic reaction centers (Abstract book, 2013). Nowadays, FTIR is used extensively in studying the secondary structure of protein molecules (Kong & Shaoning, 2007); it also helps in studying nucleic acid damage (Dovbeshko *et al.*, 2000). FTIR has vast applications in identifying the structure of metal complexes with carbohydrates (Guo & Zhang, 2004). Direct evidence of lipid translocation between adipocytes and prostate cancer cells was also made possible due to imaging FTIR micro spectroscopy (Gazi *et al.*, 2007). It helps to detect cancer (Sahu and Mordechai, 2005), rapidly diagnose gastric endoscopic biopsies for gastric inflammation (Li Q *et al.*, 2013), diagnosis of Alzheimer's disease (Griebe *et al.*, 2007), composition of kidney stones and gallstones (Channa *et al.*, 2007a; Channa *et al.*, 2007b). Leukemia (Erukhimovitch *et al.*, 2006), cervical cancer, melanoma (Mordechai *et al.*, 2004), changes in the biochemistry of oral cavity tissues (Conti *et al.*, 2005) and congenital hypothyroidism (Lin *et al.*, 2001) are diagnosed rapidly by FTIR.

Previously the identification of smokers from serum by FTIR spectroscopy was investigated and remarkable results were found (Abhinav *et al*., 2010). Use of saliva in FTIR is an easy and fast method for identification of tobacco users, which is used in diagnosing the diabetic from non-diabetic patients (Scott *et al*., 2010). The present study was designed to explore the salivary diagnostic bands for TU by FTIR.

MATERIALS AND METHODS

Sixty four (n=64) male TU with mean age range 15.3 to 30.7 years randomly selected for collection of saliva samples pre and post use of tobacco (smoking tobacco and chewing tobacco). An informed consent form was signed in by all selected tobacco users as well as controls separately. Twenty were the STU, 24 were CTU and 20 were DTU. CTU were the users of Mainpuri (n=10) and users of PEN, FIT, 2100 (n=14). Forty eight (n=48) saliva samples of age and gender matched healthy individuals with negative personal or family history of any addiction

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(mean age range 15.4 to 30.9 years) were also collected for comparison which served as controls. The users of more than one type of tobacco addiction were excluded from the study. The study was approved by Institutional Ethical Committee.

Saliva collection

After an overnight fast all the TU as well as controls were advised to rinse their mouth with distilled water and relax for 5 minutes then they were instructed to swallow all saliva present in their mouths before starting saliva collection. They were also advised to keep their head down and not to speak and swallow during collection period of five minutes. After that they were asked to let saliva drool from their mouth for five minutes in the sterilized graduated plastic tube equipped with plastic funnel. The volume of saliva after each minute was recorded. All the samples were stored at -40□C until analyzed by FTIR. Salivary flow rate (SFR) was calculated as ml/min by computing mean of 5 minutes salivary volume at the time of collection.

FTIR analysis

The saliva samples were thawed at room temperature and 2µl of homogenous saliva sample was placed on diamond crystal plate of FTIR (Thermo Scientific Nicollet Avatar ISO 10) and analyzed by EZ Omnic software, version 7.3, of instrument. To obtain a high signal/noise ratio 64 scans were accumulated for each sample. Then the resultant spectrum for each sample was obtained by following method:

The saliva spectrum of controls was subtracted from the saliva spectrum of tobacco users. The subtracted spectra collected through EZ Omnic software were categorized in to Subtracted Spectra Before Tobacco Use (SSBTU) and the Subtracted Spectra After Tobacco Use (SSATU). Again the subtraction was performed between the SSBTU and SSATU to get the resultant spectra. The whole protocol was performed by EZ Omnic version 7.3, briefly given in fig. 1.

STATISTICAL ANALYSIS

Results of salivary pH and flow rate were expressed as mean ± SD. Student′s t test was used to compare the mean values between before and after addiction, between before addiction and controls and between after addiction and control subjects. The level of significance was kept at less than 0.05 and was considered statistically significant. The whole statistical analysis was carried out on MS Excel, 2007.

RESULTS

Table 1 revealed a significantly increased SFR after chewing tobacco as compared to before its chewing ($p=0.0048$). We did not find any significant variation of SFR in STU and DTU before and after use. The comparison between before tobacco use and controls we found significant decreased SFR for DTU. Whereas, significantly increased ($p<0.05$) SFR after tobacco use as compared to controls was found for CTU, and significantly decreased ($p<0.05$) in STU and DTU.

The comparison between salivary pH before tobacco use and after tobacco use is shown in table 2. It showed a significant increased salivary pH after chewing and dipping tobacco use when compared with salivary pH before tobacco use. When the salivary pH of before and after tobacco use were compared with controls, significant increase in salivary pH of DTU before tobacco use was found, whereas, significantly increased salivary pH in CTU and DTU after tobacco use.

Table 1: Comparison of salivary flow rate (ml/min) between TU and controls

Groups	Salivary flow rate before tobacco use Mean ± SD	Salivary flow rate after tobacco use Mean ± SD	P-value (<0.05)
Chewing Tobacco users (n=24)	0.494±0.03	0.765±0.09*	0.0048
Smoking tobacco users (n=20)	0.459±0.09	0.360±0.03*	0.4881
Dipping tobacco users (n=20)	0.448±0.06*	0.448±0.07*	1.0000
Total TU (n=64)	0.518±0.03	0.614±0.03	0.2366
Controls salivary flow rate (n=48)	0.6±0.08		

Table 2: Comparison of salivary pH between TU and controls

Groups	Salivary pH before tobacco use Mean ± SD	Salivary pH after tobacco use Mean ± SD	P-value (<0.05)
Chewing Tobacco users (n=24)	7.27±0.69	8.0±0.45*	0.00079
Smoking tobacco users (n=20)	7.11±0.58	6.89±0.50	0.07125
Dipping tobacco users (n=20)	7.43±0.18*	7.75±0.21*	0.03104
Total tobacco Users (n=64)	7.34±0.60*	7.70±0.63*	0.03297
Controls salivary flow rate (n=48)	7.09±0.29		

*p<0.05 compared with controls

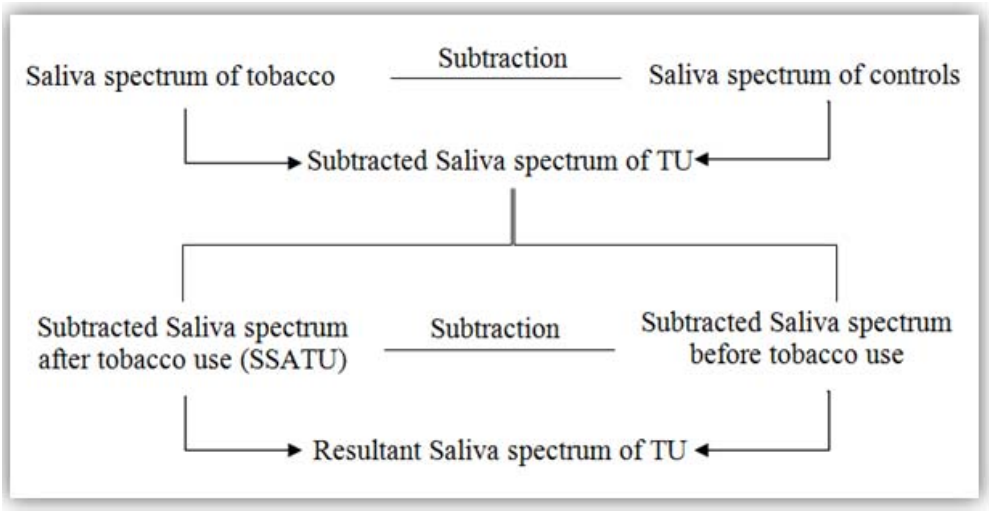

Fig. 1: Protocol to obtain the resultant spectrum for each sample

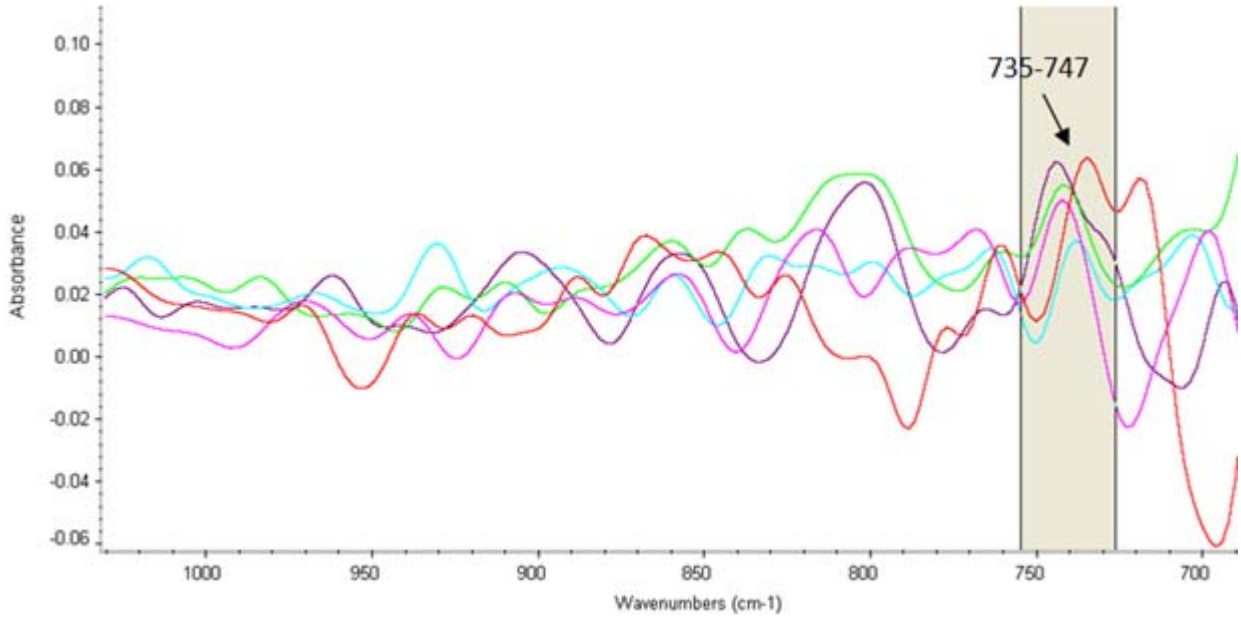

Fig. 2: Typical salivary FTIR spectrum of Cigarette smokers

Several important bands are identified as diagnostic bands for TU from saliva (figs. 2, 3 and 4). We found the bands at a range of 735-744 cm^{-1} in saliva of STU and 945-949 cm^{-1} in CTU (fig. 2 and 3).

Absorbance bands at 902-910cm-1, 945-949 cm-1 and 755cm-1 in saliva were found (fig. 3).In present study the bands at 902-910cm-1 in saliva of CTU and 905-915cm-1 in saliva of DTU were noted (fig. 4).

DISCUSSION

Some investigators found effect of nicotine on the taste nerve apparatus as initial stimulation of saliva followed by decreased SFR (Uematsu *et al*., 2001). Our results in contradiction, showed significantly increased SFR in CTU after its use ($p<0.05$), whereas, smoking and dipping tobacco caused decreased SFR as compared to controls (table 1).

In present study the alkaline pH in tobacco chewers (table 2) is due to the added slaked lime in chewing tobacco products which raise the pH (alkaline) in presence of saliva (Nair *et al.*, 1990; Nair *et al.* 1992). Furthermore, additives, such as ammonia, carbonate or bicarbonate in chewing products are also responsible to raise the pH (Nair *et al.*, 2004). However, in contradiction some studies have shown that long term consumption of tobacco, especially in the form of chewing tobacco, is one of the risk factors for decreasing salivary flow rate (Rad *et*

al., 2010; Kanwar *et al.,* 2013). Although our tobacco chewers in present study were the long term users but their increased SFR and salivary pH may be reflected towards the composition of chewing tobacco. The study opens the area to study the detailed composition of chewing tobacco used in Pakistan and detection of the components responsible to increase SFR and decrease salivary pH.

At pH 7.4 the nicotine as a weak base (pKa = 8.0) is rapidly absorbed across biological membranes. Smoke from tobaccos is more acidic (pH 6.5), and is well absorbed through the mouth (Armitage *et al*., 1978, Sensabaugh and Cundiff, 1967). We found non significantly ($p>0.05$) decreased (acidic) salivary pH and SFR in STU which may be due to long term effect of tobacco smoking (Kanwar *et al*., 2013). But, it is well documented that the SFR is significantly increased in case of new tobacco smokers, whereas it becomes normal after long term use (Heintz, 1984, Parvinen, 1984).

In fig. 2, the bands found were in consistent with the findings of other investigators who found similar band at 737cm^{-1}, which was identified in active smokers and past smokers when compared with the non-smokers (Ahmed *et al*., 2009). So, the band at 737cm^{-1} may be due to smoking induced alterations in saliva and can be used as diagnostic bands for detection of cigarette smokers.

We found absorbance bands at 902-910 cm^{-1}, 945-949 cm^{-1} and 755cm^{-1} in saliva (fig. 3). The spectral lines at 945-949 cm may be due to N-N stretching (Braibanti *et al*., 1968) present in tobacco specific N-nitrosoamines which are carcinogens (IARC, 2007). In present study the bands at 902-910 cm^{-1} in saliva of CTU and 905-915cm^{-1} in saliva of DTU were noted (fig. 4), which are in the range of 900-915cm^{-1}. The spectral bands at 900-915 indicates the epoxy compounds (Ahmad *et al*., 2001) which are present in the form of 7,8-epoxy-4-basmen-6-one in tobacco (Inger *et al*., 1984).

CONCLUSION

The salivary diagnostic bands may be used at the bands 735-745cm^{-1}, 900-915 cm^{-1} and 945-949cm^{-1} for TU by FTIR. Increased SFR may be associated with CTU, whereas, reverse is true for STU and DTU. Significantly increased salivary pH is associated with CTU and DTU. Further study into the potential utility of FTIR as diagnostic tool for tobacco use is warranted.\

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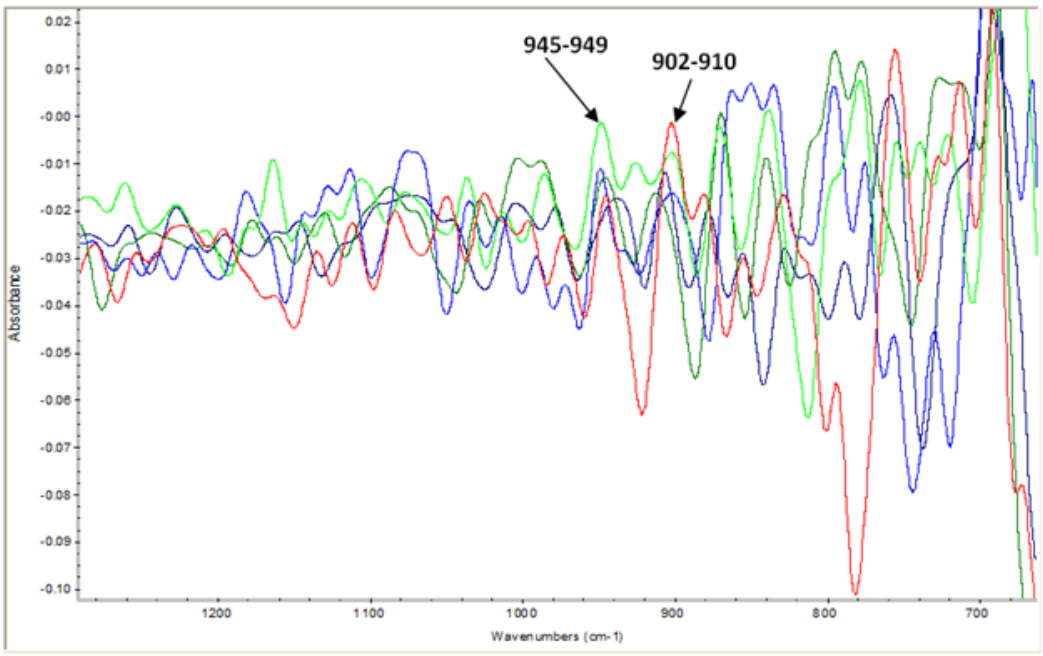

Fig. 3: Typical salivary FTIR spectra of Chewing tobacco users

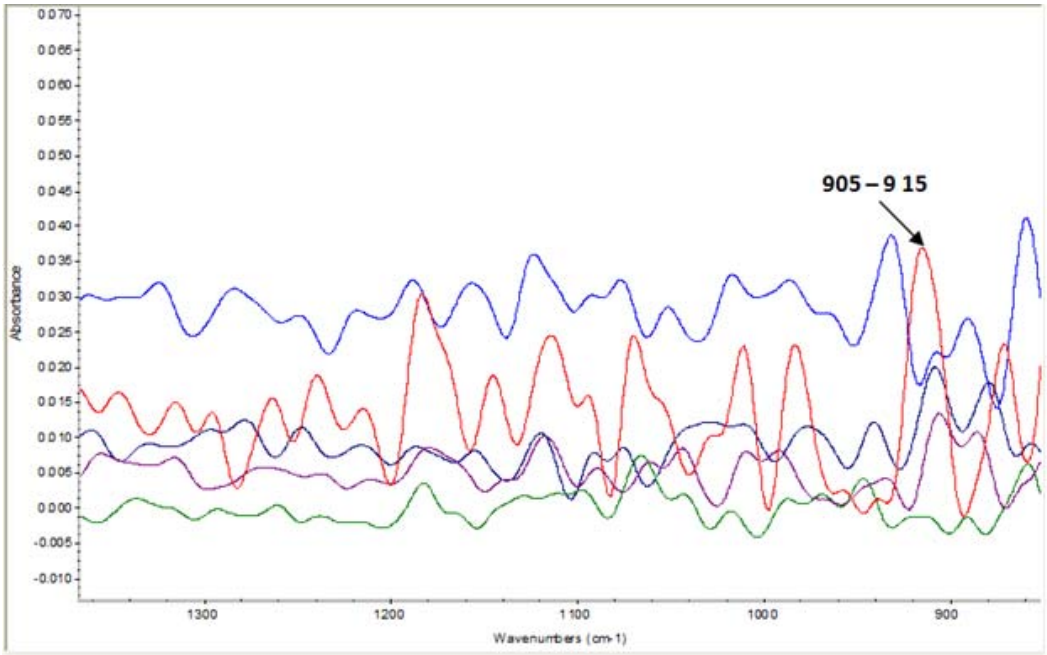

Fig. 4: Typical salivary FTIR spectrum of Dipping tobacco users

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