Preparation of Dual-Templated Filter Material Using Grafted Molecularly Imprinted Chitosan for Sequestration of Toxic Tobacco Smoke Products

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Abstract **---- Smoke from Tobacco contains known toxicants and carcinogens. Outcomes from efforts at containing their impacts by the use of filters have had the challenges of biodegradability and non-recyclability. An imprinting strategy is used in preparing filter materials for sequestration of the degradation products by using a bio friendly, degradable polymer matrix of 'Chitosan', grafted with methacrylic acid thus, molecularly imprinted and non-imprinted polymers were synthesized. Evaluated interaction with the filter materials and sequestered target compounds, confirmed their utility value as studied. Fourier transformed infra-red spectroscopy showed the polymerization and products' characteristic functional groups. The morphology and cavity integrities of the samples were determined by scanning electron (SEM) and transmission electron (TEM) microscopies. 1g each of filter samples, P5, P10, MP5, and MP10, were introduced into improvised cigarette holders and using an adapted machine-aided smoking regime, rolled Marlboro brand of cigarettes each weighing 0.75g (without butts), measuring 5.5cm length (without filter butt) and diameter of 0.8cm, were fixed into the improvised cigarette holders and toxicants were sequestered from them. Liquid chromatographic analysis of extracts from the filters was carried out. The fabricated filters sequestered over 60% (max) and above 25% (min) of the Nicotine and other toxicants from the smoked cigarette while comparatively the commercial filter sequestered a little above 10% of these from the stream. The filter materials with dual (blend) template cavities (P10 and MP10) sequestered much higher amount of the smoke stream toxicants than the commercial filters specifically in a 9:1 concentration ratio. This shows the advantaged utility potential of the fabricated MIPs over the conventional filters currently being used.**

Keywords**----- grafted-chitosan, dual-templated, molecularly imprinted, tobacco smoke stream, toxin-sequestration.**

I. INTRODUCTION

The incidence of damage to health from cigarette smoking, both to the smoker and the non-smoker exposed to second and third hand smokes, is on the increase. This is consequent to the fact that efforts geared towards mitigating the impact from smoking are still on-going. Outcomes from machine aided smoking experiments, presents a misconception that filters filter tar, Nicotine and carcinogens from mainstream smoke there by giving the impression that it makes smoking safer. Armed with this manufacturer allowed an increase in the amount of TSNAs in cigarette with the aim of compensating for the filtered fraction of Nicotine particularly. The increased amount of Nicotine and the design of filters with ventilation encourage deep and prolonged smoke drags by smokers. The smoker, non-smoker as well as the environment are exposed to the exhaled higher doses of the dangerous carcinogenic TSNAs. It is nearly impossible to achieve good success in preventing negative impacts from cigarette smoking. Cigarette manufacturers with reasons are not ready to modify their products to meet world health standards while individuals find it difficult to break the addiction. The smoker and non-smoker ought to be protected from the impact of this age-old practice.

Synthetic non-biodegradable materials are currently being used in the manufacturing of filter materials. However, in recent time, biodegradable materials are being employed in the manufacturing process to cater for the environmental issues like biodegradability and pollution. Greenbuttts filter is an example of the biodegradable class of filter products. This product is made with organic cotton, degummed hemp and wheat flour is used as the binder. The green butt filter brand is marketed under the illusion that it is an improvement over the common cellulose acetate filter being currently used worldwide. Filter vents or ventilating holes are included in the design of modernized filters; the holes allow more air to be drawn in to dilute the inhaled smoke. This diluted smoke stream encourages the active smoker to inhale deeply and consequently dangerously from the cigarette. This deep inhalation has been shown to be a primary route to adenocarcinoma, (Song et al., 2017). The attempt to curtail

the inhalation of the toxic tobacco smoke by the use of filters, created a dangerous exposure level. A study (Hoffmann, Rivenson, and Hecht, 1996.), showed that the nitrate content of the filter-cigarette is higher than that of non-filter cigarette. This situation is because the filter reduces the accessible amount of nicotine to a smoker thereby prompting an intense dragging and inhalation of smoke than from a non-filter cigarette. This exposes the peripheral lung to higher amounts of nitrogen oxides, nitrosated compounds and lung-specific smoke carcinogens.

II. MATERIALS AND METHODS

Materials

Chitosan (from SIGMA-ALDRICH Ltd. Dorset, England), Methacrylic acid, 1,4 Bis (Acryloyl)piperazine (BAP), Nicotine, 2-Pyridinemethanol; ammonium persulphate and 2% acetic acid. Chitosan was. Methacrylic acid (MAA), Ammonium persulphate, Phenylalanine amide (3-PhenylPiridine), sodium phosphate monobasic monohydrate and Ammonium chloride were all of analar grade and procured from SIGMA-ALDRICH Ltd. Sodium phosphate dibasic anhydrous, Sodium Hydroxide pellets and Methanol were all bought from Merck, Darmstadt, Germany. Sodium phosphate dibasic heptahydrate and Sodium chloride were sourced from Thermo Fisher Scientific Ltd. All reagents were of analar grade and the MMA was redistilled under reduced pressure (removal of the preservative contained), before being used.

Method

Nicotine and Phenylalanine amide blend template materials (238.2µl); Chitosan 20ml (0.12mmol/l); MAA (1.2ml); cross linker (1,4 Bis (Acryloyl)piperazine 5mls); Ammonium persulphate (2.4ml), were dissolved in 2% Acetic acid solution(porogen/solvent). The mixture was sonicated for 2mins then purged with nitrogen, closed and placed in a temperature regulated water bath set at 70^{O}C for 12hrs. The resultant MIPs were washed with different solvent regimes aided by sonication until no template material was detected in the rinse solution. Non-imprinted polymers (NIPs) for control experiments were obtained following the same procedure but without template material. Adapting and modifying the method by Ying Liu, Xueliang Liu and Junde Wang (2003), 1g each of MIP samples, P5, P10; MP5, and MP10, were introduced into 1cm diameter tubes that served as improvised cigarette holder. The number '5' indicates BAP-Nicotine-Phenylalanine amide blend and number '10' indicates Geranic Acid-Nicotine-Phenylalanine amide blend that were heated using a temperature regulated water bath. The MP5 and MP10 indicate samples that were produced using microwave induced heating.

Rolled Marlboro brand of cigarettes each weighing 0.75g (without butt), measuring 5.5cm length (without filter butt) and diameter of 0.8cm, were fixed into the improvised MIP containing cigarette holders. The cigarettes were machine smoked by aid of application of suction from the vacuum pump connected to the column. TSNAs were evaluated by HPLC analysis of eluents from washed MIP filter samples after the adapted machine-aided cigarette smoking experiment as presented in Plates 1, 2 and 3. Stripping of Sequestered Nicotine and TSNA analogues after the machine-aided smoking regime was carried by using Methanol to strip the MIPs of the sequestered degraded cigarette smoke constituents using the method by (SaharTaghavi et al. 2012), but with slight modifications. Here, the MIP samples were put in an ependorf tube and 15mls of Methanol was introduced into the tube. The set-up was placed in a vortex stirrer overnight and sequestered components were extracted. A 50% dilution of the extracts was made with a 7.4 pH Phosphate buffer saline solution before filtering through a 0.5 µm PTFE Millipore filter. This served as the stock solution from which 20µl portion was taken for HPLC studies. 1 ml of the extract was made up to 10mls as sample solution by the addition of a 0.01 M solution of Phosphate Buffer Saline (pH 7.0).

Using Shimadzu Lc-2030 3d HPLC equipment, serial no. L214552, Shimadzu Corp 00405 with Diode Array Detector(DAD) and column chamber temperature control system; at a pump pressure set at 2030psi, temp., 30^{O}C, flow rate of 1ml/min, injection volume of 20µl; the presence and concentrations of the adsorbed and eluted degraded products from the cigarette were determined. A 50:50 methanol: PBS pH 7.4 buffer solution was employed as mobile phase using a Kromasil C18 column (250mm, 4.6 mm, 5 mm) as stationary phase. 20µl volume injections were introduced into the HPLC machine and analyzed at wavelengths of 254, 259 and 260nm for Phenylalanine amide analogues, Nicotine and its analogues as well as analogues of their blends respectively. Isocratic elution method was adopted with elution time of 10mins. Theprocedure was carried out in triplicate and average of the readings taken.

Figure 1: Set-up of the machine aided cigarette smoking experiment.

Figure 2: improvised cigarette holders with polymer samples.

Figure 3: The MIPs packed in the adapted column before and after the cigarettes is attached.

Figure 4: The smoking regime and entrapment of TSNAs by the prepared MIP samples. Smoked Cigarettes from the machine smoked experiment showing the discoloration of the MIP samples in the column due to entrapped cigarette smoke constituents.

III. RESULTS

FTIR results

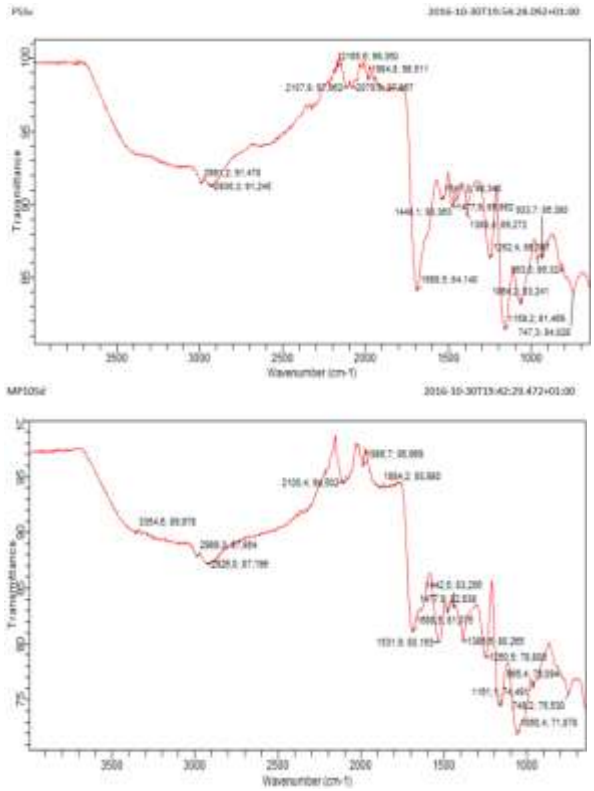

Figure 5: Representative FTIR spectra of both P5and P10 as well as MP5 and MP10 samples of MIP.

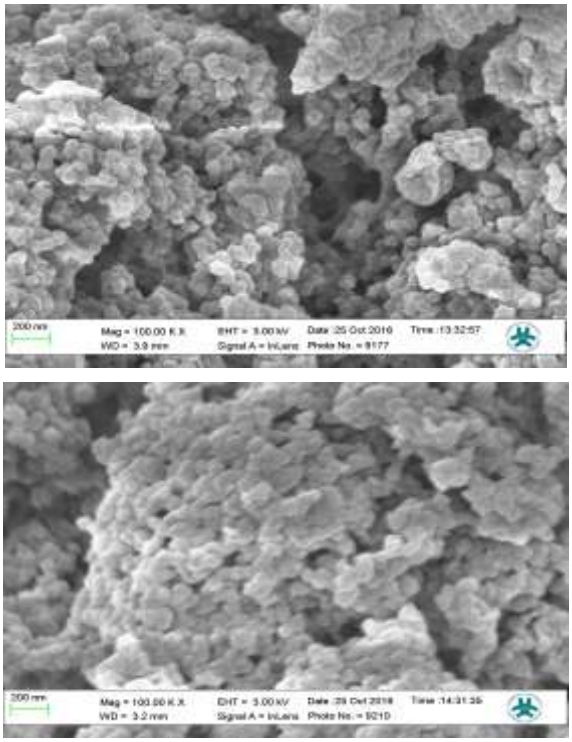

Figure 6:Microgram of P5(BAP-cross linked) MIP and microgram of P10(Geranic acid cross linked) MIP.

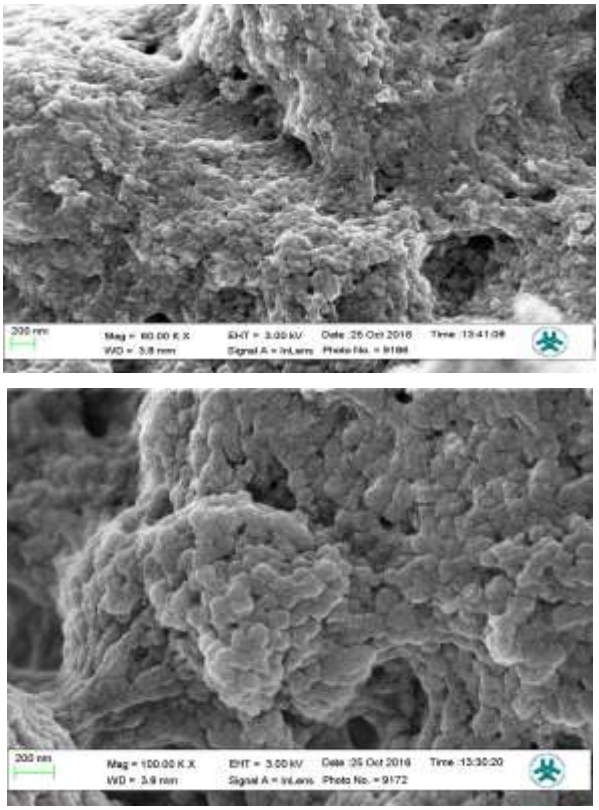

Figure 7: Microgram of MP5 (BAP-cross linked) MIP and microgram of MP10(Geranic acid cross linked) MIP.

TEM Results

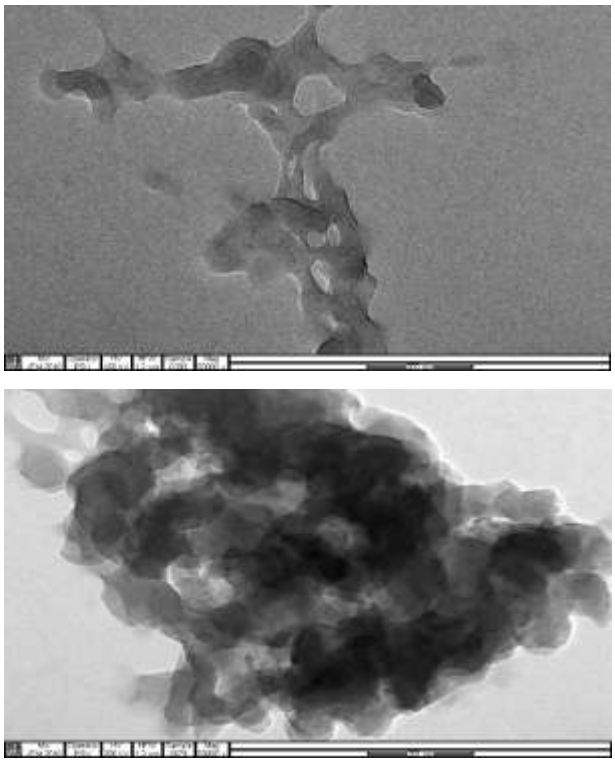

Figure 8: Temogram of P5(BAP-cross linked) MIP and microgram of P10(Geranic acid cross linked) MIP.

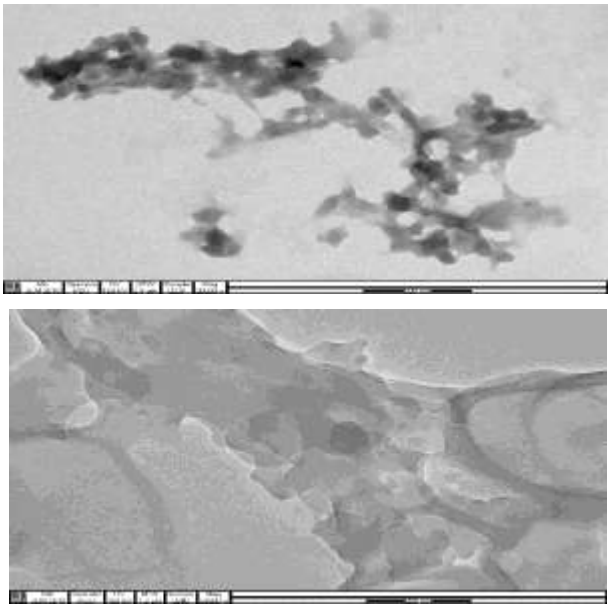

Figure 9: Temogram of MP5 (BAP-cross linked)MIP and microgram of MP10(Geranic acid cross linked) MIP.

Surface area, pore size and pore volume results.

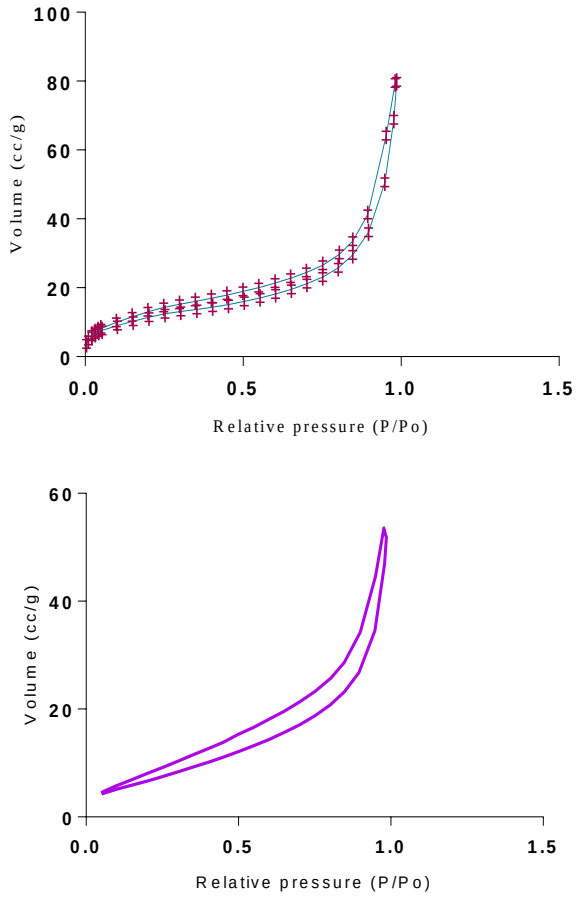

Figure 10: Representative BET Isotherm for BAP-cross linked MIP and Geranic acid cross linked MIP

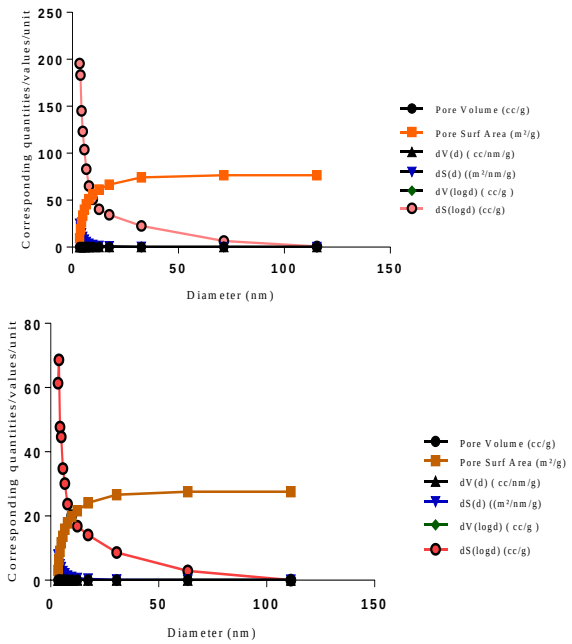

Figure 11: BJH desorption plot for BAP-cross linked MIP and Geranic acid cross linked MIP.

Table 1: BJH desorption result summary

Sample	Surface Area (m^2g^{-1})	Pore Volume (ccg^{-1})	Pore Diameter (nm)
P5 (BAP cross linked blend templated)	**27.072**	**0.112**	**3.823**
P10(Geranic acid cross linked blend templated)	**27.577**	**0.078**	**3.826**
MP10(Geranic acid cross linked)	5.339	0.010	4.302
Non-imprinted sample	76.635	0.219	3.411

HPLC rebinding studies.

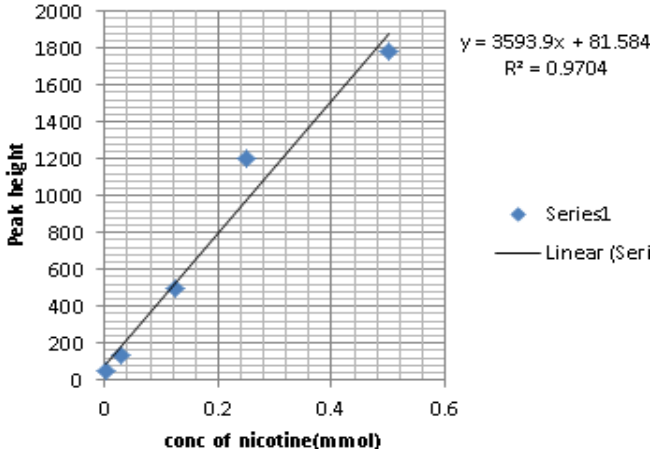

Figure 12a: Calibration curve for Nicotine

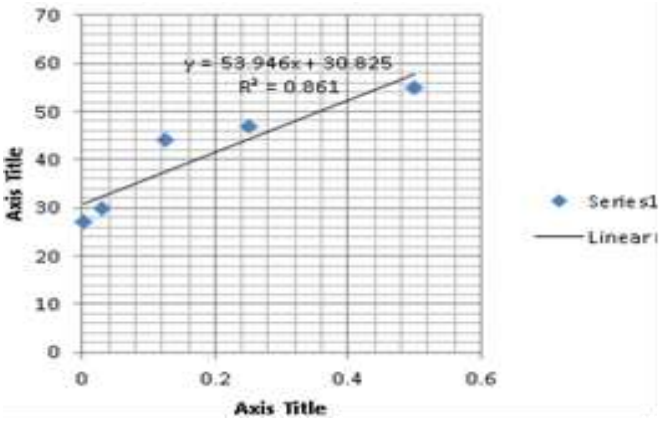

Figure 12b: Calibration curve for Phenylalanine amide

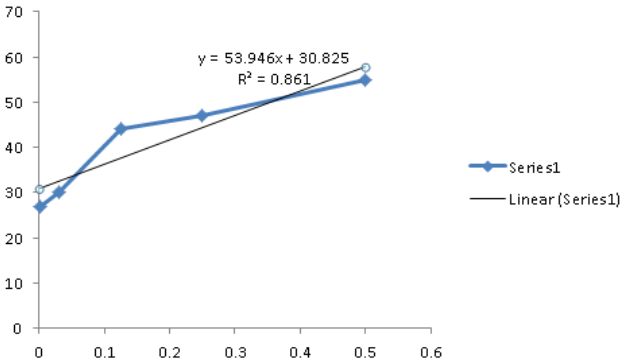

Figure 12c: Calibration curve for Nicotine-Phenylalanine 50:50 blend.

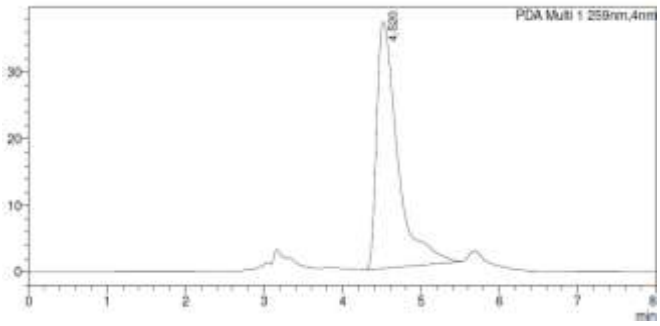

Figure 13: Chromatoram of Nicotine from the smoked cigarette stream at 259nm.

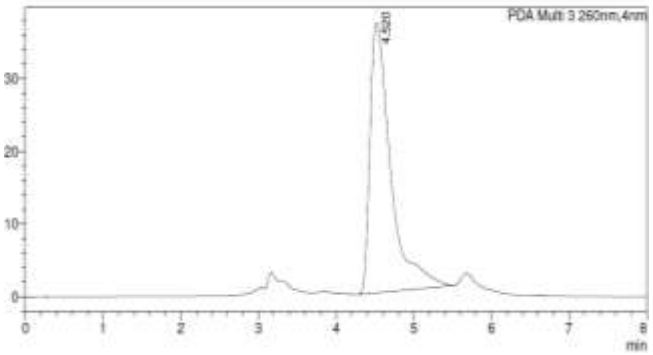

Figure 14: Chromatoram of Nicotine from the smoked cigarette stream at 260nm.

Figures 12, 13 and 14 presents the HPLC chromatogram of the Nicotine from the machine smoked cigarette. The retention time of 4.5min was specific for the nicotine even at the two different wavelengths of 259 and 260nm. This shows the specificity of the separation and identification of the process method and equipment.

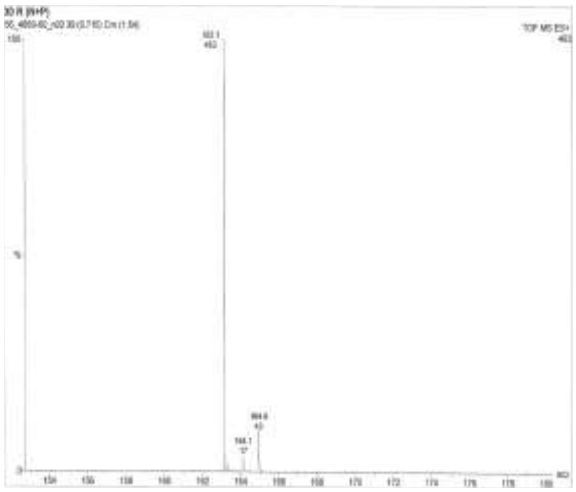

Figure 15a: chromatogram of P5 MIP

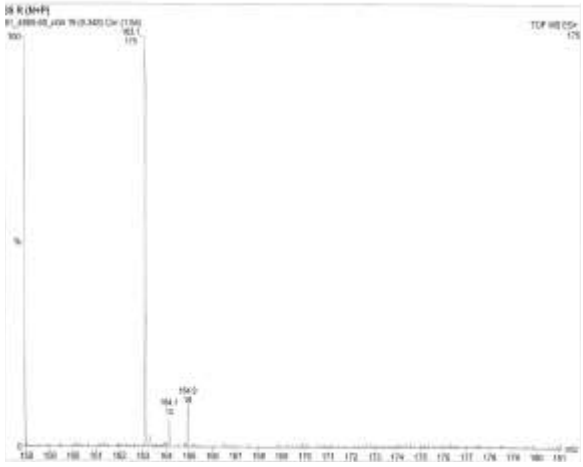

Figure 15b: P10 MIP samples

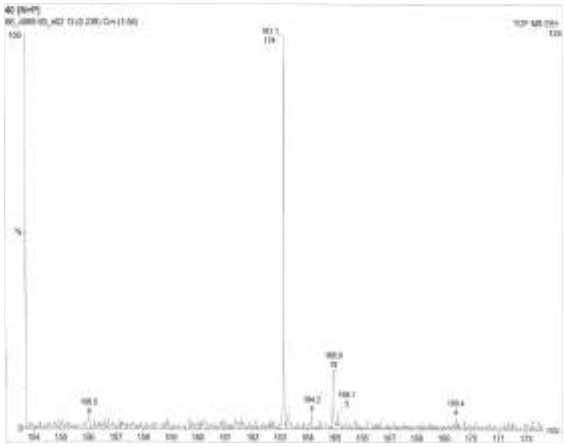

Figure 15c: chromatogram of MP5 MIP

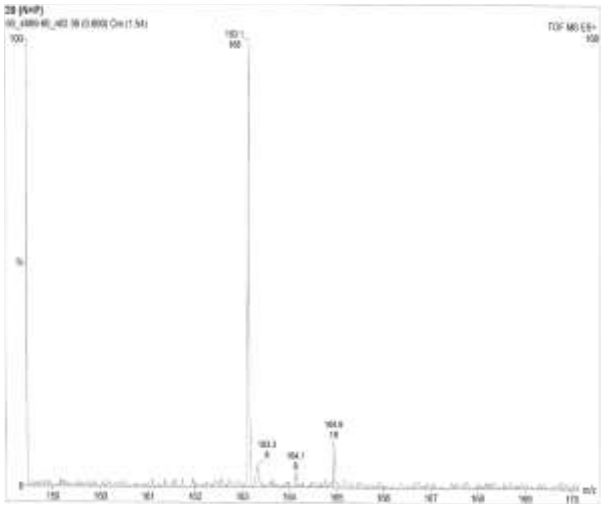

Figure 15d: chromatogram of MP10 MIP samples

IV. DISCUSSION

FTIR

Observed transmittances from Figure 5, indicates that at wavenumbers, 3073.13, 2174.9, 1695.94, 1435.03 and 954.19 $cm.^{-1}$, exists overlaps from the interactions between the chitosan, the methacrylic acid and cross linker. Chitosan's characteristic peak at 3328.51 and MAA's peaks at 2963.23, 2817.87, 2629.64 and 946.74cm-1 are distinctive prior to cross linking while on cross-linking, distinct peaks at 1110.75 of C-O secondary alcohol stretching and 1298.98 of OH in-plane bending of a cross-linked matrix became distinct. The carboxylic acid of MAA after cross-linking with Chitosan appeared at 1470.43cm^{-1}. Other noticeable peaks are the1649.35 (primary amine N-H bend), 2126.45 and 3324.79cm-1(normal polymeric OH stretch and N-H stretch of primary amine).

Surface area, pore size and pore volume.

From the isotherms (Figure 10), the samples present a typical composite type IVa and type II isotherm, that is an isotherm having the features of a type II isotherm with potential unrestricted monolayer-multilayer adsorption with characteristic "sharp knee" point, (IUPAC, 2015). The "sharp knee" point indicates the completion of monolayer coverage. The curve then graduates as a less distinctive curve relative to the 'sharp knee", and this is a proof of substantial degree of overlap of the monolayer coverage and the inception of a multilayer adsorption. This phenomenon combines with the type IV isotherm property of conical and cylindrical mesopores that are closed at the tapered end as deduced from results of the TEM analysis (Figures 8 and 9). The tapered ends of the pores are presented as converging cavities. Meanwhile the pores' widths are averagely wider than 4nm, therefore the adsorption may have occurred via capillary condensation and hysteresis supposedly experienced as part of the adsorption mechanism (Eddaoudi, 2005; Thommes and Cychosz, 2014 and Landers, Gor and Neimark, 2014), the

samples exhibited the H3 type of hysteresis,(IUPAC, 2015), which is hysteresis type with characteristic adsorption branching phenomenon of the Type II isotherm's "sharp knee" and this substantiates the earlier deduction that the samples are type IV isotherm members. This phenomenon is also characterized by the branching at the lower limit of desorption being normally cited at the cavitation induced pressure point (p/p0).

The hysteresis loop occurring at relatively high pressure is distinctive of mesoporous samples and is in agreement with reports by other researchers, (Merino et al., 2013). From the BJH desorption result summary, Table 1, the Geranic acid cross-linked blend templated sample (P10), gave a slightly larger surface area at 27.577 m^2g^{-1} but with a smaller pore volume of 0.078 ccg^{-1} for the same value of pore diameter 3.826nm with the BAP cross-linked contemporary (P5). This may be attributed to Geranic acid's larger molecular size/structure imparting the observed larger surface area to the MIP without necessarily influencing the pore volume and dimensions. This is in agreement with (Yoshimatsu et al. 2007) and is suggestive of the possible influence of template materials on the MIP's cavity architecture and identity irrespective of cross linker material used. This is at variance with the report from (Sun et al. 2017), where it is stated that the cross linker impacts on the polymer cavity's physiochemical integrity. None the less no other reason comes readily to mind as to why the observed event between the samples played out. The values of the surface areas, pore volumes and pore diameters were all below 100 $m2g^{-1}$ for surface, $2ccg^{-1}$ for pore volume and 4nm for pore diameter. This confirms that the MIPs and NIPs are nanoparticle sized materials as is substantiated by the report of (Yan et al. 2016).

The microwave heated sample (MP10) from Table 1 which has 5.339 m^2g^{-1} (surface area), $0.0102ccg^{-1}$ (pore volume) and 4.302nm (pore diameter), when compared to its contemporary P3 with 26.455 m^2g^{-1}(surface area), $0.067ccg^{-1}$(pore volume) and 3.411nm (pore diameter); it becomes evident that the use of microwave heating gives materials with more compact physio-structural attributes. Meanwhile the values obtained from the NIP sample having a higher amount of cross linker than the MIPs show a larger surface area, pore volume but smaller pore diameter.

Deductions made from the results are in agreement with report by Fu, et al. (2015), that the formation of cavities is influenced by template-monomer polymerization interactions and not by the porogen employed in the polymerization reaction. The result from the TEM analysis, presents non-uniform cavity dimensions for both the MIPs and NIP (due to the weak template-monomer interactions), with peak centers above 4 nm. This supports the finding that the pore dimensions were generated during the polymerization from template-monomer interactions rather than porogen/solvent influences.

Toxicant extraction potentials of MIPs

Table 2: Concentrations of adsorbed templates by representative sample materials.

Sample ID	Conc. Of Nicotine adsorbed (mM)	Conc. Of Phenylalanine adsorbed (mM)	Conc. Of Nicotine-Phenylalanine adsorbed (mM)	
MIP samples cross-linked with BAP				
P5 (single washing)	Not Detected as an individual moiety	Not Detected as an individual moiety	0.171(Nic.)	0.067(Phe.)
P5 (double washing)	Not Detected as an individual moiety	Not Detected as an individual moiety	0.140(Nic)	0.158(Phe.)
P5 Non imprinted (single wash)	Not Detected	Not Detected	0.005(Nic)	0.003(Nic)
MIP samples cross-linked with Geranic acid				
P10	Not Detected as an individual moiety	Not Detected as an individual moiety.	0.041(Nic.)	0.108(Phe.)
MP10(single washing)	Not Detected as an individual moiety.	Not Detected as an individual moiety.	0.112(Nic.)	0.113(Phe.)
P10 non-imprinted (single wash)	Not Detected	Not Detected	0.002(Nic.)	0.003(Phe.)

The P5 (BAP cross linked) samples with cavities from dual-templating that were washed twice with methanol: acetic acid (9:1) gave better sequestration results than the ones that were washed/rinsed ones (Ofoegbu Obinna, 2018). The geranic acid cross-linked samples performed as well as their BAP cross-linked counterparts. The microwave heated sample recorded closely related concentration as the water bath samples. A reversal of trend was observed between the samples from the different cross linkers P5 and P10 particularly with respect to the template material (Phenylalanine). The single wash gave 0.171mmol (Nicotine), 0.067 (Phenylalanine) for P5 and 0.140mmol (Nicotine), 0.158mmol (Phenylalanine) for the double wash sample. The trend was reversed with the P10 (geranic acid cross linked) sample where the adsorbed Nicotine is lower (0.041mmol) than the adsorbed Phenylalanine (0.108mmol). The geranic acid cross linked, microwave heated sample followed the same trend but with a higher amount of adsorbed toxicant 0.112(Nic.) and 0.113(Phe.). From Table 2, the integrity of the MIPs with respect to selectivity and specificity can be argued in the positive direction. The inability of the samples not to adsorb non-targeted templates based on fabrication design and the ability of the dual-templated samples to selectively adsorb specific amounts of individual templates without the influence of their respective interacting potentials,

confirms the selectivity of the MIP samples. The most striking feature is the templated MIPs not adsorbing single template materials where they are brought into contacts. This is shown by the non-detection of the template materials from the eluate obtained from the adsorbed materials that contacted MIP samples. This shows the ability of the dual templated MIPs having the potential of sequestering toxicants in real life streams where they occur as mixtures and blends in synergy with each other. The fabricated MIPs adsorbed over 60% (max) and above 25% (min) of the content of the smoked cigarette while the commercial filter was only able to adsorb a little above 10% of the substances from the stream. The MIPs with blend template cavities (P10 and MP10) were also able to adsorb much higher amount of the smoke stream toxicants than the commercial filters specifically in a 9:1 concentration ratio. This shows the advantaged utility potential of the fabricated MIPs over the conventional filters currently being used.

Table 3: Concentrations of adsorbed degraded products from the machine smoking experiment using representative MIP samples and commercial cigarette Butt filter.

Cigarette smoke constituent	Adsorbent and concentration (mmol) of adsorbed degraded product(s).	
cigarette TSNA and other products	P5	0.612
	MP 5	0.501
	P10	0.927
	MP10	0.708
	Commercial filter	0.149

The Marlboro menthol brand of cigarette contains approximately 0.8mg of nicotine, an average of 65% of which is pyrolyzed or lost from side stream smoke (Ying Liu, Xueliang Liu and Junde Wang, 2003). The rest of the stream delivers approximately 20–80µg of nicotine and TSNAs through the filter butt. This amount exposes the smoker to 0.8mmol of Nicotine/cigarette by conversion alongside the TSNAs. From Table 3 the fabricated MIPs adsorbed over 60% (max) and above 25% (min) of the Nicotine content of the smoked cigarette while the commercial filter was only able to adsorb a little above 10% of the Nicotine from the stream. The MIPs with blend template cavities (P10 and MP10) were also able to adsorb much higher amount of the smoke stream toxicants than the commercial filters specifically in a 9:1 concentration ratio. This shows the advantaged utility potential of the fabricated MIPs over the conventional filters currently being used. Figures 13, 14 and 15 show the specific identification of Nicotine by the MIP sample as displayed on the chromatograms.

The challenge of having bio friendly and degradable filters for manufactured cigarette had being a mainstream driver in recent research activities of the tobacco industry. The litter caused by the non-degradable cellulose acetate filter butts has posed great concern for the environment and the environmentalist. Chitosan presented a good biomaterial resource for various industrial applications such as was required in thin film sensors, fluid environments and pH influenced set-ups. This was primarily due to the inherent property of Chitosan to swell when influenced by certain conditions of existence. These stated challenges have been addressed by the result of this work.

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CONFLICT OF INTEREST

There exists no conflict of interest with respect to this work.

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