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Addition of Porphyrins to Cigarette Filters To Reduce the Levels of Benzo[*a*]pyrene (B[a]P) and Tobacco-Specific *N*-Nitrosamines (TSNAs) in Mainstream Cigarette Smoke

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ABSTRACT: Tobacco-specific *N*-nitrosamines (TSNAs) and benzo[*a*]pyrene (B[a]P) in mainstream cigarette smoke (MSS) cause smoking-related diseases and environmental pollution. Porphyrins were added to cigarette filters to reduce B[a]P (porphyrins A−E) and TSNAs (porphyrin F) in MSS. The porphyrin−B[a]P and porphyrin F−TSNAs (*N*′-nitrosoanabasine (NAB), *N*′-nitrosoanatabine (NAT), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and *N*-nitrosonornicotine (NNN)) interactions were investigated by fluorescence quenching and UV−visible spectroscopy. The correlation coefficients were 0.987−0.997 (B[a]P) and 0.994−0.999 (TSNAs), and the binding constants were $(1.67-5.02) \times 10^5$ (B[a]P) and $3.42 \times 10^3-1.40 \times 10^4$ (TSNAs). Up to 36.72% of B[a]P and 46.67% of the TSNAs were eliminated from MSS, with greater reductions when more porphyrin was included in the filter. With the same mass of porphyrin in the filter, the reduction trend for B[a]P by porphyrins A−E was A > B > C > D > E. The reduction trend for TSNAs by porphyrin F was NNN > NAB > NNK > NAT. The porphyrin mode of action is possibly through strong $\pi-\pi$ interactions.

KEYWORDS: benzo[*a*]pyrene, binding constant, cigarette smoke, porphyrin, tobacco-specific *N*-nitrosamines

INTRODUCTION

Cigarette smoke has a public health impact on smokers as well as on other individuals through passive smoking of environmental tobacco smoke. Multiple epidemiological studies have shown that cigarette smoke is related to the development of cardiovascular disease, stroke, lung carcinoma, chronic bronchitis, chronic obstructive pulmonary disease, and emphysema.[1] Cigarette smoke contributes largely to indoor air pollution and has been implicated in the reduction of lung function[2] and the development of cancer[3] in nonsmokers. Although the best solution to reduce the incidence of these diseases is to reduce the number of smokers, there are still large numbers of individuals who smoke. Consequently, it is important to reduce the harmful components in cigarette smoke for smokers, nonsmokers, and the environment.

Cigarette smoke is a complex mixture of more than 4700 chemicals and contains hundreds of toxicants.[4] Two important groups of carcinogens in cigarette smoke are the tobacco-specific *N*-nitrosamines (TSNAs) and polycyclic aromatic hydrocarbons (PAHs).[5] Benzo[*a*]pyrene (B[a]P), an IARC Class 1 carcinogen, is the most potent among the PAHs in cigarette smoke.[6−9] Consequently, it is important to reduce the content of B[a]P in cigarette smoke. The carcinogenic TSNAs specific to tobacco and cigarette smoke include *N*′-nitrosoanabasine (NAB), *N*′-nitrosoanatabine (NAT), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and *N*-nitrosonornicotine (NNN). Among these TSNAs, NNN and NNK are potent IARC Class 1 carcinogens and have been linked to the development of lung, oral cavity, esophagus, pancreas, and liver diseases.[10−13] Therefore, it is also very important to reduce the content of PAHs and TSNAs in cigarette smoke.

Previous studies have investigated the removal of toxicants from cigarettes by adding pine bark extract,[5] zeolite,[14] *Ginkgo biloba* leaf extract,[15] charcoal, or palladium to the filter and/or cut fillers. Although zeolite effectively reduced TSNAs in mainstream cigarette smoke (MSS) by 30−60%,[14] it is expensive for large-scale application. *G. biloba* leaf[15] and pine bark extracts[5] were used to scavenge free radicals from MSS, but would not be useful for the removal of TSNAs and B[a]P. To our knowledge, no studies have investigated the removal of B[a]P from MSS.

Hemoglobin and other porphyrins have been shown to be promising cigarette filter additives to remove compounds, such as carcinogenic *N*-nitroso compounds, from MSS.[16] Porphyrins are a group of complex chemical structures that have important roles in photosynthesis, gas transport (hemoglobin, myoglobin), vitamin structure (cobalamin), and the metabolism of living organisms. Examples include heme, which is the iron porphyrin in hemoglobin, and chlorophyll, which is a magnesium porphyrin.[17] Because of their unique structures, porphyrins have a wide range of applications in bionics, materials chemistry, pharmaceutical chemistry, electrochemistry, optical physics and chemistry, analytical chemistry, and organic chemistry.[18−24] Consequently, many studies have focused on the applications of porphyrins. Furthermore, porphyrins are readily synthesized from pig blood, which makes them economical for large-scale applications. However, their applications to the reduction of harmful components and tar produced by cigarettes, and especially their mechanism of

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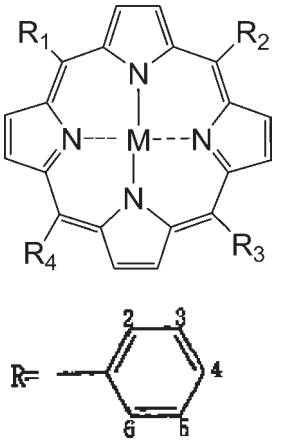

Figure 1. Structures of the porphyrins used as cigarette filter additives for the reduction of B[a]P (porphyrins A−E) and TSNAs (porphyrin F). The substituents on the porphyrins were as follows: porphyrin A, R_1 = R_2 = R_3 = R_4 = 4-methylphenyl; porphyrin B, R_1 = R_2 = R_3 = R_4 = 4-chlorophenyl; porphyrin C, R_1 = 4-hydroxyphenyl, R_2 = R_3 = R_4 = 4-methylphenyl; porphyrin D, R_1 = R_2 = R_3 = R_4 = 4-dodecoxylphenyl; porphyrin E, R_1 = 4-hydroxyphenyl, R_2 = R_3 = R_4 = 4-methylphenyl; porphyrin F, R_1 = R_2 = R_3 = R_4 = 4-octoxylphenyl. Porphyrins A−D had no metal ion at the heme core, porphyrin F had a manganese ion at the heme core, and porphyrin E had a cobalt ion at the heme core.

reaction with harmful components in cigarette smoke, have not been thoroughly investigated.

In this study, porphyrins were added to cigarette filters to investigate their effects on reducing TSNAs and B[a]P in MSS. The interactions of porphyrins with TSNAs and B[a]P were studied using UV−visible absorption spectroscopy (UV−vis) and fluorescence quenching (FQ), respectively. A possible mechanism for porphyrin reduction of TSNAs and B[a]P is discussed.

MATERIALS AND METHODS

Reagents. Standardized TSNAs and B[a]P were obtained from Sigma-Aldrich (St. Louis, MO). Meso-tetra(4-methylphenyl) porphyrin, meso-tetra(4-chlorophyenyl) porphyrin, 5-(4-hydroxyphenyl)-10,15, 20-tri(4-methylphenyl) porphyrin, meso-tetra(4-dodecoxylphenyl) porphyrin, 5-(4-hydroxyphenyl)-10,15,20-tri(4-methylphenyl) manganese porphyrin, and meso-tetra(4-octylphenyl) cobalt porphyrin (Figure 1) were synthesized in our laboratory[25−27] and are referred to here as porphyrins A, B, C, D, E, and F, respectively. All other reagents were of analytical grade.

Addition of Porphyrins to Cigarettes. Cigarette filters consisting of acetate cellulose and cut fillers of brand A were obtained from Chengdu Cigarette Factory (Chengdu, China). During machine molding of the filters, porphyrin was added to the filter through the carrier. Porphyrin-modified filters were then attached to cigarettes. Thirty different porphyrin-modified cigarettes were produced containing 0.0, 2.0, 4.0, 6.0, or 8.0 μg of one of the porphyrins (A−F) per filter. Control cigarettes were prepared using unmodified acetate cellulose filters of brand A. Cigarettes containing porphyrins A−E were used to investigate the reduction of B[a]P in MSS, whereas cigarettes containing porphyrin F were used to investigate the reduction of NAB, NAT, NNK, and NNN in MSS. Porphyrin F was used only in the investigation of TSNAs because it is a cobalt porphyrin and should be effective at reducing TSNAs, whereas porphyrins A−E are not cobalt porphyrins and are less effective at reducing TSNAs.

Analytical Method. A fully automatic smoking machine (RM200, Borgwaldt Technik GmbH, Hamburg, Germany) was used to generate and trap MSS from the 20 cigarettes.

Established methods were followed for the detection of B[a]P[6] and TSNAs[28] in the MSS. B[a]P was detected by gas chromatography−mass spectrometry (7890A/5975, Agilent Technologies Inc., Santa Clara, CA). NAB, NAT, NNK, and NNN were detected by gas chromatography−thermal energy analysis spectrometry with an Agilent 6890 GC and a Thermo 610 TEA (Thermo Fisher Scientific, Waltham, MA). Gas chromatography−mass spectrometry and gas chromatography−thermal energy analysis spectrometry results were used to calculate the percentage reductions in B[a]P and TSNAs in the MSS from porphyrin cigarettes in comparison with the control cigarettes.

The interaction between porphyrin and B[a]P was studied by FQ[29] using an F-7000 fluorometer with autoagitators (Hitachi Ltd., Tokyo, Japan). The effect of B[a]P on the level of porphyrins A−E was determined by adding 5 μL of B[a]P (200 μg/mL) to 3 mL of methylene dichloride and measuring the fluorescence intensity. The fluorescence intensity was measured again following the addition of 5 μL of porphyrin. The fluorescence intensityies for different concentrations of porphyrins were plotted to obtain the B[a]P fluorescence spectrum. PAHs such as B[a]P usually have characteristic fluorescence spectra. The fluorescence intensity of B[a]P can be reduced or quenched by other substances, which are called fluorescence quenchers. The fluorescence of B[a]P is quenched to a certain degree by porphyrin, and this process conforms to the Stern−Volmer equation (eq 1).[28,31]

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

In eq 1, F_0 represents the fluorescence intensity of B[a]P without a fluorescence quencher, F represents the fluorescence intensity of B[a]P with a fluorescence quencher, K_{SV} is the binding constant (quenching constant), and [Q] is the quencher concentration. K_{SV} and the interaction between the quencher and fluorophore are positively correlated, which means that larger binding constants indicate that the interaction of the quencher and fluorophore is stronger.

The interaction between porphyrin and the TSNAs was studied by UV−vis (HP8453, Agilent Technologies Inc.).[29,30] Generally, porphyrins can be detected by UV−vis, and they have a characteristic absorption peak at 414 nm. The effect of porphyrin F on the level of TSNAs was investigated by adding 25 μL of porphyrin (3.0×10^{-4} mol/L) to 3 mL of methylene dichloride and measuring the spectral intensity (A_0). The spectral intensity (A) was measured again following the addition of 5 μL of TSNAs (200 μg/mL). The spectral intensities for the different concentrations of TSNAs were plotted to obtain the porphyrin UV−vis spectrum. The adsorption of TSNAs by porphyrins conforms to the Benesi−Hildbrand (B−H) equation:

$$\frac{C_A}{d} = \frac{1}{C_B K \varepsilon_c} + \frac{1}{\varepsilon_c} \quad (2)$$

C_A represents the analytical concentration of fixed component A (porphyrin), C_B represents the analytical concentration of component B (TSNAs), ε_c is the molar absorptivity, and K is the binding constant (equilibrium constant). The parameter d can be calculated from $d = D/b$, where D is the absorbance and b is the optical path length.

RESULTS AND DISCUSSION

Interaction between Porphyrin and B[a]P or TSNAs. Fluorescence spectra (Figure 2) of the B[a]P fluorophore were obtained with different doses of porphyrins A−E. According to eq 1, the linear equation, coefficient, and slope (K_{SV}) were obtained from the F_0/F porphyrin concentration curve (Table 1). With all five porphyrins (A−E), the fluorescence intensity of B[a]P decreased as the dose of porphyrin in the cigarette

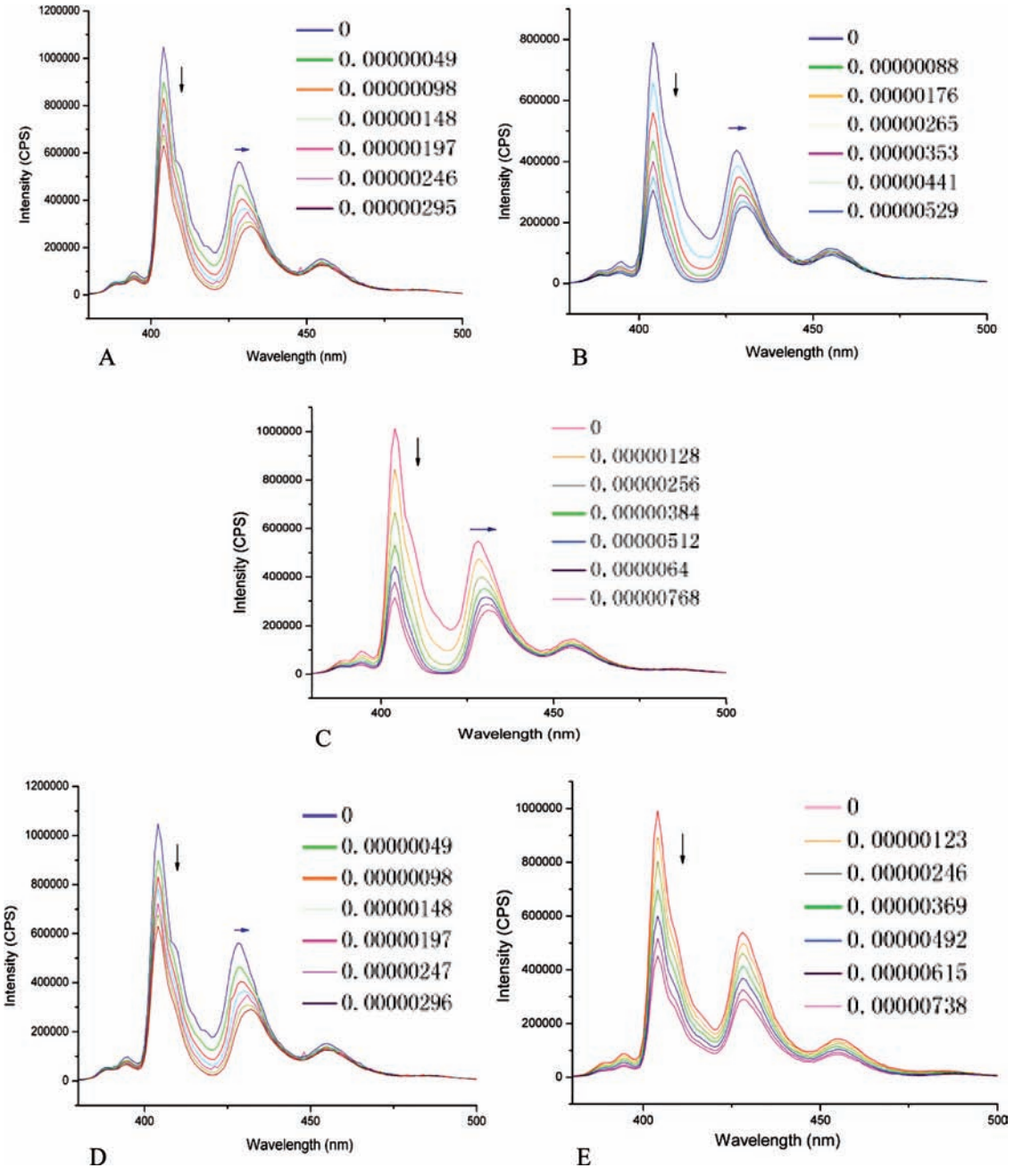

Figure 2. Fluorescence quenching spectra of B[a]P after addition of porphyrins A−E (panels A−E, respectively) at different concentrations (mol/L).

Table 1. Linear Equations, Correlation Coefficients, and Binding Constants for the Interactions of Porphyrins A−E with B[a]P

porphyrin	linear equation	correlation coefficient (r)	binding constant (K_{SV})
A	$F_0/F = 0.879 + 5.02 \times 10^5[Q]$	0.987	5.02×10^5
B	$F_0/F = 0.936 + 2.97 \times 10^5[Q]$	0.997	2.97×10^5
C	$F_0/F = 0.862 + 2.87 \times 10^5[Q]$	0.993	2.87×10^5
D	$F_0/F = 1.036 + 2.12 \times 10^5[Q]$	0.995	2.12×10^5
E	$F_0/F = 0.897 + 1.67 \times 10^5[Q]$	0.989	1.67×10^5

increased, and there was a red shift in the fluorescence spectra. The binding constant (K_{SV}) ranged over $(1.67-5.02) \times 10^5$, and the correlation coefficient (r) ranged from 0.987 to 0.997. The binding constants indicate that the interaction between the porphyrin and B[a]P was strong, and the interaction order of the different porphyrins and B[a]P was porphyrin A > porphyrin B > porphyrin C > porphyrin D > porphyrin E (Table 1). With reference to the structures of the porphyrins (Figure 1), the

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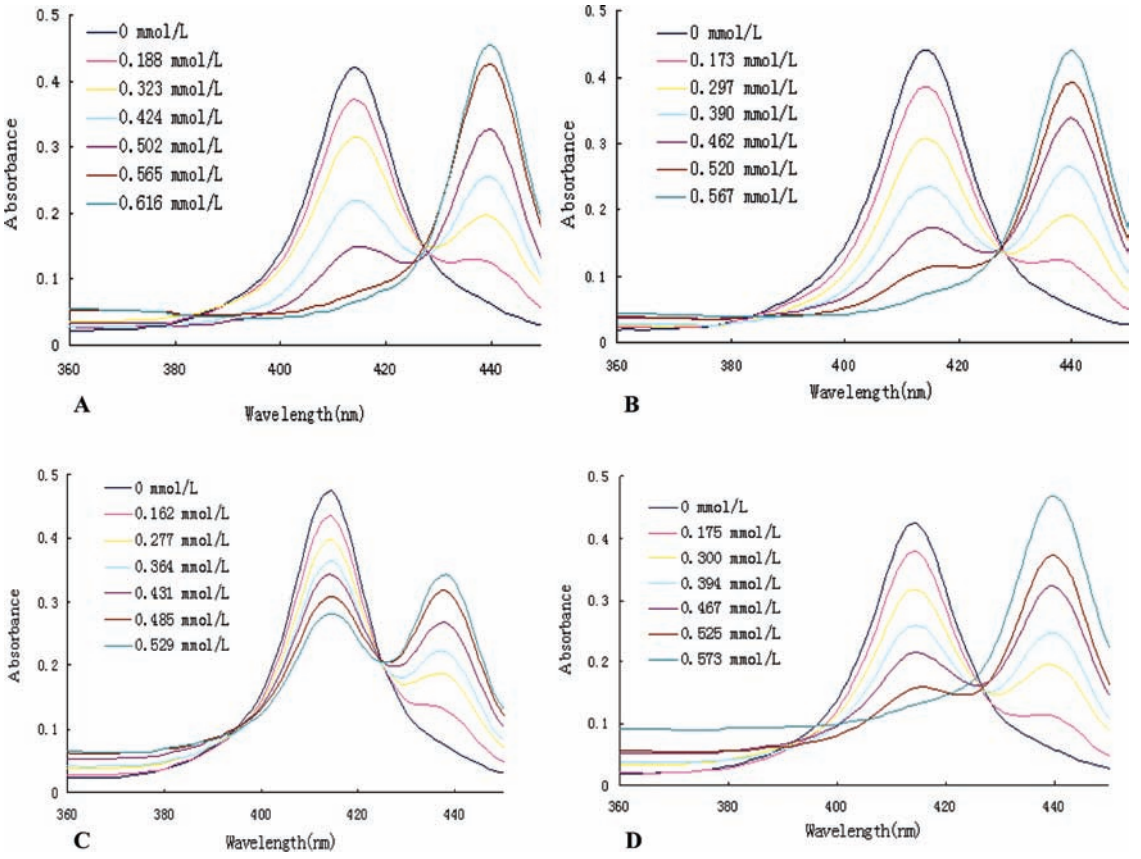

Figure 3. UV−vis spectra of porphyrin F after addition of different concentrations of the following TSNAs: (A) NNN; (B) NAB; (C) NNK; (D) NAT.

interaction between the porphyrin and B[a]P was affected by the addition of particular substituents to the porphyrin. Relatively large binding constants were observed for porphyrins A−C, which had four relatively small substituents, as compared with porphyrin D, which had four relatively large substituents and a smaller binding constant. This indicates that the interaction with B[a]P is stronger for porphyrins with smaller substituents than for those with larger substituents. The addition of a manganese ion in the central position (porphyrin E) resulted in a smaller binding constant than for the porphyrin with the same substituents without a metal ion (porphyrin C). These results indicate that adding a metal ion to the heme core reduces the interaction (i.e., lower binding constant) between the porphyrin and B[a]P.

The UV−vis spectra (Figure 3) of porphyrin F with different concentrations of TSNAs were obtained. The porphyrin−TSNA interaction could be described by the B−H equation (eq 2), and the linear equation, coefficient, and slope (K) were obtained from the $C_A/d - 1/C_B$ curve (Table 2). The intensity of the characteristic absorption peak of porphyrin at 414 nm decreased as the concentration of TSNA increased. A red shift was observed in the UV−vis spectra, and a new peak appeared at 438 nm, which gradually increased in intensity as the TSNA concentration increased. This suggests that a new compound is formed when porphyrin interacts with the TSNAs. The interaction binding constants (K) ranged from 3.42×10^3− to 1.40×10^4 and the correlation coefficients (r) from 0.994 to 0.999. These results suggest that porphyrin F interacts strongly with the four TSNAs. However, compared with the interactions of porphyrins A−E with B[a]P, the porphyrin F interactions were not as strong.

Table 2. Linear Equations, Binding Constants, and Correlation Coefficients for the Interaction of Four TSNAs with Porphyrin F

TSNA	linear equation	binding constant (K)	correlation coefficient (r)
NNN	$y = 7.13 \times 10^{-5}x - 1.529$	1.40×10^4	0.997
NAB	$y = 9.51 \times 10^{-5}x - 0.666$	1.05×10^4	0.994
NNK	$y = 1.70 \times 10^{-4}x + 0.748$	5.86×10^3	0.999
NAT	$y = 2.92 \times 10^{-4}x - 0.367$	3.42×10^3	0.995

The order of magnitude of the binding constants between porphyrin and B[a]P was 10^5, and that between porphyrin and TSNAs was 10^4. The strength of the porphyrin F interaction with the TSNAs was in the following order: NNN > NAB > NNK > NAT (Table 2).

Reduction of B[a]P and TSNAs in MSS by Porphyrins. Both B[a]P (Table 3) and TSNAs (Table 4) in MSS were greatly reduced by adding porphyrin compounds to the cigarette filters. Porphyrin reduced the levels of the B[a]P and TSNAs in MSS by up to 36.72 and 46.67%, respectively. Generally, the reductions in B[a]P and TSNA levels were greater for cigarettes that contained more porphyrin in the filter than for those that contained less porphyrin in the filter. However, with porphyrin F, the reduction in TSNAs did not increase much between the filters containing 6.0 and 8.0 μg. For B[a]P, in the case of cigarettes with the same mass of porphyrin added, the level of B[a]P reduction differed with the different porphyrins. The trend was consistent with the

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Table 3. Percentage Reduction in the Level of B[a]P in MSS (n = 5) after the Addition of Different Masses of Porphyrins A−E to the Cigarette Filter[a]

	percentage reduction (%) in B[a]P				
porphyrin (μg)	porphyrin A	porphyrin B	porphyrin C	porphyrin D	porphyrin E
0.0	0	0	0	0	0
2.0	17.02	14.8	14.27	12.37	11.42
4.0	24.89	22.93	22.68	20.45	19.05
6.0	31.42	28.92	28.8	26.05	25.18
8.0	36.72	33.57	32.93	30.07	29.03

[a] The percentage reduction was calculated in comparison to the levels in control cigarettes with unmodified filters.

Table 4. Percentage Reduction in the Levels of Four TSNAs in MSS (n = 5) after the Addition of Different Masses of Porphyrin F to the Cigarette Filter[a]

	percentage reduction (%) in TSNA			
porphyrin (μg)	NNN	NAB	NNK	NAT
0.0	0	0	0	0
2.0	20.21	19.56	14.27	10.97
4.0	32.57	29.53	21.76	15.68
6.0	45.71	40.40	28.47	20.63
8.0	46.67	41.51	29.62	21.49

[a] The percentage reduction was calculated in comparison to the levels in control cigarettes with unmodified filters.

binding constants for the porphyrins and followed the order porphyrin A > porphyrin B > porphyrin C > porphyrin D > porphyrin E. For cigarettes containing the same mass of porphyrin F, the reduction in TSNAs followed the order NNN > NAB > NNK > NAT, which was again consistent with the trend for the binding constants.

Mechanism of B[a]P and TSNAs Reduction Using Porphyrin. Porphyrin molecules have rigid structures and large faces, and the position and orientation of edge functional groups can be precisely controlled. This can be used to produce porphyrins that specifically recognize particular molecules, which can be used to identify compounds with certain molecular sizes, properties, functional groups, and chirality.[20] Strong $\pi-\pi$ interactions can occur between porphyrins and aromatic molecules,[32,33] and this is exploited in their use as a liquid chromatography stationary phase. In this application, the strong $\pi-\pi$ interactions of porphyrin with PAHs rich in π-electrons allow for identification of the PAHs. The interaction consists of face−face and face−edge $\pi-\pi$ interactions.[21,22,32−35] Chlorophyll has been shown to reduce the harmful aromatic components in cigarette smoke by strong $\pi-\pi$ interactions between the porphyrin ring of the chlorophyll and the aromatic ring of the harmful components.[32] Therefore, it is likely that porphyrins A−F interact with B[a]P and TSNAs in MSS by these strong $\pi-\pi$ interactions. This interaction will be investigated in detail in future studies. The strength of the $\pi-\pi$ interactions was affected by the structure of the porphyrin, as shown by the different binding constants obtained for the different porphyrins with B[a]P (Table 1). Adding the metal ion to the heme core (porphyrin E) probably increased the rigidity of the porphyrin and reduced its ability to interact with B[a]P. These interactions could be exploited to design specific porphyrin filter additives to reduce the levels of particular MSS components in the environment.

Porphyrin is a potent additive in cigarettes to reduce TSNAs and B[a]P levels in MSS and the environment. The addition of porphyrin to cigarette filters is feasible for cigarette production, economical, and environmentally friendly. The results of this study could facilitate the development of low-harm cigarettes and reduce the levels of TSNAs and B[a]P in the environment to decrease indoor air pollution. However, the potential transfer of porphyrins to cigarette smoke or their dissolution in the mouth and the toxicological consequences of this need to be evaluated in future studies.

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ABBREVIATIONS USED

B[a]P, benzo[*a*]pyrene; B−H, Benesi−Hildbrand; FQ, fluorescence quenching; MSS, mainstream cigarette smoke; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAB, *N*′-nitrosoanabasine; NAT, *N*′-nitrosoanatabine; NNN, *N*-nitrosonornicotine; PAHs, polycyclic aromatic hydrocarbons; TSNAs, tobacco-specific *N*-nitrosamines; UV−vis, UV−visible absorption spectroscopy.

REFERENCES

(1) Tønnesen, P.; Vermeire, P. A. Promoting a future without tobacco: also a continuing task for respiratory medicine in Europe. *Eur. Respir. J.* **2000**, *16*, 1031–1034.

(2) Hersoug, L. G.; Husemoen, L. L.; Sigsgaard, T.; Madsen, F.; Linneberg, A. Indoor exposure to environmental cigarette smoke, but not other inhaled particulates associates with respiratory symptoms and diminished lung function in adults. *Respirology* **2010**, *15*, 993–1000.

(3) Vineis, P.; Hoek, G.; Krzyzanowski, M.; Vigna-Taglianti, F.; Veglia, F.; Airoldi, L.; Overvad, K.; Raaschou-Nielsen, O.; Clavel-Chapelon, F.;

Linseisen, J.; Boeing, H.; Trichopoulou, A.; Palli, D.; Krogh, V.; Tumino, R.; Panico, S.; Bueno-De-Mesquita, H. B.; Peeters, P. H.; Lund, E. E.; Agudo, A.; Martinez, C.; Dorronsoro, M.; Barricarte, A.; Cirera, L.; Quiros, J. R.; Berglund, G.; Manjer, J.; Forsberg, B.; Day, N. E.; Key, T. J.; Kaaks, R.; Saracci, R.; Riboli, E. Lung cancers attributable to environmental tobacco smoke and air pollution in non-smokers in different European countries: a prospective study. *Environ. Health* **2007**, *6* (7).

(4) Rodgman, A.; Perfetti, T. A. *The Chemical Components of Tobacco and Tobacco Smoke*; CRC Press: Boca Raton, FL, 2009.

(5) Zhang, D. L.; Tao, Y.; Gao, J. T.; Zhang, C. N.; Wan, S. J.; Chen, Y. X.; Huang, X. Z.; Sun, X. Y.; Duan, S. J.; Schönlau, F.; Rohdewald, P.; Zhao, B. L. Pycnogenol® in cigarette filters scavenges free radicals and reduces mutagenicity and toxicity of tobacco smoke in vivo. *Toxicol. Ind. Health* **2002**, *18*, 215–224.

(6) Xia, Q. L.; Zhao, M. Y.; Feng, X.; Xie, F. W.; Wu, M.; Wang, S.; Nie, C. Cigarette—determination of benzo[α]pyrene in total particulate matters of smoke. In *National Standards of the People's Republic of China GB/T 21130−2007*; General Administration of Quality Supervision Inspection and Quarantine of the P.R.C. and Standardization Administration of the P.R.C.: Beijing, China, 2008; pp 1−7.

(7) Rodgman, A. *The N-Nitrosamines in Tobacco and Tobacco Smoke*; R. J. Reynolds Tobacco Company Press: Winston-Salem, NC, 1993.

(8) Zhao, Z. H. *Environment Health Chemistry of Polycyclic Aromatic Hydrocarbons*; Chinese Science and Technology Publishing House Press: Beijing, China, 1993.

(9) U.S. Department of Health and Human Services, Toxicological Profile for Polycyclic Aromatic Hydrocarbon, 1995.

(10) Zhang, T. M.; Lai, B. T. Relationship between the peculiar nitrosamine NNK of tobacco and lung cancer. *Chin. J. Epidemiol.* **2005**, *2*, 140–142.

(11) Shi, Y. X. *Content Analysis of TSNAs in Tobacco Leaves and Smoke and the Relation between Them and Precursors*. Master's thesis, Yunnan Normal University, Kunming, China, 2006.

(12) Hayes, J. R.; Meckley, D. R.; Stavanja, M. S. Effect of a flue-curing process that reduces tobacco specific nitrosamines on the tumor promotion in mice by cigarette smoke condensate. *Food Chem. Toxicol.* **2007**, *45*, 419–430.

(13) Hecht, S. S. Biochemistry, biology, and carcinogenicity of tobacco-specific *N*-nitrosamines. *Chem. Res. Toxicol.* **1998**, *11*, 559–603.

(14) Gao, L.; Cao, Y.; Zhou, S. L.; Zhuang, T. T.; Wang, Y.; Zhu, J. H. Eliminating carcinogenic pollutants in environment: reducing the tobacco specific nitrosamines level of smoke by zeolite-like calcosilicate. *J. Hazard Mater.* **2009**, *169*, 1034–1039.

(15) Wang, C. G.; Dai, Y.; Li, D. L.; Mao, K. Y. *Ginkgo biloba* leaf extract action in scavenging free radicals and reducing mutagenicity and toxicity of cigarette smoke in vivo. *J. Environ. Sci. Health: Part A, Toxic/Hazard. Subst. Environ. Eng.* **2010**, *45*, 498–505.

(16) Deliconstantinos, G.; Villiotou, V.; Stavrides, J. C. Scavenging effects of hemoglobin and related heme containing compounds on nitric oxide, reactive oxidants and carcinogenic volatile nitrosocompounds of cigarette smoke. A new method for protection against the dangerous cigarette constituents. *Anticancer Res.* **1994**, *14*, 2717–2726.

(17) Biesaga, M.; Pyrzynska, K.; Trojanowicz, M. Porphyrins in analytical chemistry. A review. *Talanta* **2000**, *51*, 209–224.

(18) Qiu, H.; Liu, Y. Q.; Han, S. T. Application and research progress of porphyrin in medicine. *J. Hebei Norm. Univ. (Nat. Sci. Educ.)* **2000**, *24*, 497–500.

(19) Zhang, H. F.; Pan, J. H. Application of porphyrins and metalloporphyrins. *Chin. J. Chem. Educ.* **2005**, *4*, 3–6.

(20) He, Y.; Zhang, M. R. Applications of porphyrins and their analogs in HPLC. *Chin. J. Pharm.* **2004**, *35*, 431–435.

(21) Mifune, M.; Shimomura, Y.; Saito, Y. High-performance liquid chromatography stationary phases based on $\pi-\pi$ electron interaction. Aminopropyl silica gels modified with metal phthalocyanines. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1825–1829.

(22) Chen, S.; Fetzer, J. C.; Meyerhoff, M. E. Retention behavior of large polycyclic aromatic hydrocarbons on metalloprotoporphyrinsilica stationary phases. *Fresenius' J. Anal. Chem.* **2001**, *3−4*, 385–392.

(23) Zhang, A. H. Orthogonal analysis for hemoglobin reducing tar content in cigarettes. *Tob. Sci. Technol.* **1992**, *2*, 27–28.

(24) Liu, S. M.; Xu, P.; Yan, X. Y. Study on NOx retention in cigarette mainstream smoke. *J. Environ. Health* **2002**, *19*, 186–187.

(25) Pan, J. M.; Li, Z. J.; Fang, G. Z. *New Chromogenic Reagents and Their Application in Spectrophotometry*; Chemical Industry Press: Beijing, China, 2003; pp 120−150.

(26) Li, Z. J.; Pan, J. M. Advances in synthesis and application of the derivatives of porphyrin as reagents in analytical chemistry. *Rev. Anal. Chem.* **2002**, *3*, 167–231.

(27) Shanmugathasan, S.; Boyle, R. W.; Edwards, C. Advances in modern synthetic porphyrin chemistry. *Tetrahedron* **2000**, *8*, 1025–1046.

(28) Xie, F. W.; Jin, Y. M.; Zhao, M. Y.; Wang, S.; Liu, K. J.; Wu, M. Cigarette—determination of tobacco specific *N*-nitrosamines in total particulate matter of mainstream cigarette smoke−GC-TEA method. In *National Standards of the People's Republic of China GB/T 23228−2008*; General Administration of Quality Supervision, Inspection and Quarantine of the P.R.C. and Standardization Administration of the P.R.C.: Beijing, China, 2009; pp 1−9.

(29) Qi, C.; Li, W. G.; Zhang, Y. J.; Liu, L. Y.; Zhao, D. Q. Researching the antibody−porphyrin interaction with fluorescence spectrum and ultraviolet−visible differential spectroscopy. *Chem. Res. Chin. Univ.* **2002**, *23*, 1453–1456.

(30) Chen, G.; Huang, X.; Zheng, Z. *Fluorescence Analysis Method*, 2nd ed.; Science Press: Beijing, China, 1990.

(31) Novaira, A. I.; Avila, V.; Montich, G. G. Fluorescence quenching of anthracene by indole derivatives in phospholipid bilayers. *J. Photochem. Photobiol. B.* **2001**, *60*, 25–31.

(32) Zhang, Y. H.; Liu, C. J.; Shi, W. Q. Researching the $\pi-\pi$ interaction between pyrene and graphite with single-molecular force spectroscopy. In *Proceedings of National Polymer Academic Thesis Public Lecture*; Sichuan University Press: Chengdu, China, 2007; Vol. 1, pp 6−8.

(33) Christopher, A. H.; Jeremy Sanders, K. M. The nature of $\pi-\pi$ interactions. *J. Am. Chem. Soc.* **1990**, *112*, 5525–5534.

(34) Yutaka, S.; Masaki, M.; Junichi, O. Copper(II)-phthalocyanine sulfonyl-aminopropyl silica gel for separation of π-electron rich compounds by high-performance liquid chromatography. *Anal. Sci.* **1991**, *7*, 805–806.

(35) Dashwood, R. H.; Yamane, S.; Larsen, R. Study of the forces stabilizing complexes between chlorophylls and heterocyclic amine mutagens. *Environ. Mol. Mutagen.* **1996**, *27*, 211–218.