Asian Journal of Chemistry; Vol. 25, No. 11 (2013), 6125-6129

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14283

Investigation on the Influence and Interaction Effect of Phytosterols on the Polycyclic Aromatic Hydrocarbons Delivery in Tobacco Based on Uniform Design

YOU DU[1], FANG XUE[2,*], HAILONG LIU[1], FENGCHENG SHI[2], YA DAI[2], DONGLIANG LI[2] and HUA CHEN[2,*]

[1]Key Lab of Green Chemistry and Technology, Ministry of Education; College of Chemistry, Sichuan University, Chengdu 610064, P.R. China
[2]Technology R & D Center, China Tobacco Chuanyu Industrial Corporation, Chengdu 610066, P.R. China

*Corresponding author: Fax: +86 28 86005067; Tel: +86 28 86005602; E-mail: xuefang@pride56.com

(*Received*: 9 July 2012; *Accepted*: 30 April 2013) AJC-13417

In this study, the impact of four free phytosterols (cholesterol, stigmasterol, β-sitosterol and campesterol) on the delivery of 15 polycyclic aromatic hydrocarbons in tobacco was analyzed by GC/MS, the experiments were designed and carried out basing on uniform design and the results were analyzed by a second-order polynomial stepwise regression model. With regression analysis, the results highlight that those four free phytosterols as well as their interaction effect significantly influenced the delivery of polycyclic aromatic hydrocarbons and there may existed an obvious linear relationship. The analysis results of benzo[a]pyrene indicated that cholesterol, stigmasterol and campesterol have a positive influence on the delivery of benzo[a]pyrene while the interaction effect between cholesterol and campesterol, between stigmasterol and campesterol, between β-sitosterol and campesterol have a negative influence on the delivery of benzo[a]pyrene.

Key Words: Phytosterols, Polycyclic aromatic hydrocarbons, Uniform design, Regression analysis.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of environmental pollutants which were primarily formed from incomplete combustion of various organic materials including tobacco[1]. Numerous research groups have reported that tobacco phytosterols, consisting of a tetracyclic cyclopenta[a]-phenanthrene ring and a long flexible side chain at the C-17 carbon atom (Fig. 1), are the important precursors of those tumorigenic polycyclic aromatic hydrocarbons[2-4].

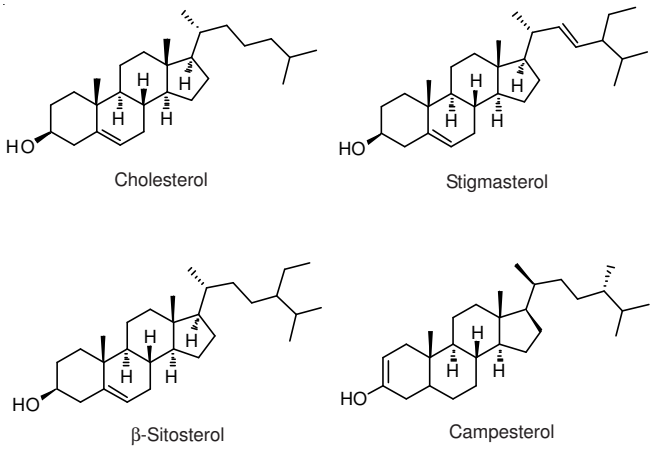

Fig. 1. Structure of phytosterols

Stigmasterol, cholesterol, β-sitosterol and campesterol are the major sterols present in tobacco. They existed in tobacco as free sterols (FSs) and as conjugates including steryl esters (SEs), steryl glycosides (SGs) and acylated steryl glycosides (ASGs)[5]. Up to date, there are many reports focused on the delivery of PAHs influenced by phytosterols. In 1980, Freudenthal *et al*. and Schepartz *et al*. reported that *ca.* 61 % BaP was produced in tobacco smoke through the pyrolysis of hexane-extractable fraction of phytosterols[6]. Meanwhile, Johnston *et al*.[7] and Stedman *et al*.[8] also reported that pyrolysis of stigmasterol under 750 ºC could produce benzo[a]pyrene. Britt *et al*.[4] reported that the steroid structure had an influence on the pyrolytic formation of PAHs and their yields were particular sensitive to the number of double bonds in the steroid B-ring. In cases of other groups shown that adding phytosterols into tobacco has a significant influence on the delivery of PAHs. It was proved by Rodgman *et al*.[9] when adding two times of phytosterols into cigarettes caused the delivery of PAHs in mainstream smoke increased by 13 % and by 18 % when adding three times. Liu *et al*.[10] reported that there were significantly positive correlation between the quantity of free phytosterols and the delivery of PAHs. In particularly, the delivery of four, five, six membered ring PAHs was significantly influenced by stigmasterol.

Although researchers have demonstrated that phytosterols are important precursors of PAHs, so far, few reports about

the interaction effect between phytosterols on the delivery of PAHs. Uniform design (UD) is a statistical experimental design method developed by Chinese mathematicians Fang Kaitai[11] and Wang Yuan[12]. Compared to other traditional experimental design methods, the larruping trait of uniform design is to achieve results with much fewer numbers of experiments, which uniformly scattered in the experimental region and highly representative in the experimental domain. Owing to its attractive futures, for example cost-efficiency, robustness and flexibility[13], uniform design has been proved to be a powerful experiment design method in many fields[14-16], particularly for multi-factor and multi-level experiments. Based on the traits of uniform design, it is a powerful approach to investigate the influence and interaction effect of experiment factors without strong assumption on the model. However, so far, no work has reported the application of uniform design to analyze the influence and interaction effect of phytosterols on the delivery of PAHs in tobacco.

In this paper, we utilized uniform design to analyze the influence of phytosterols on the delivery of PAHs in tobacco. In order to investigate the influence and interaction effect of the four main sterols on the delivery of PAHs in tobacco, the second-order polynomial stepwise regression model was chosen to analyze the experiment results.

EXPERIMENTAL

Tobacco samples were obtained from technology center of China Tobacco Chuanyu Industrial Corporation and were conditioned at 22 ºC and 60 % relative humidity for at least 48 h before experiment. Stigmasterol, cholesterol, β-sitosterol, campesterol were purchased from Sigma-Aldrich (95.0 % pure). The PAH standards, acenaphthylene (ACL), acenaphthene (AC), fluorene (FL), phenanthrene (PHE), anthracene (AN), fluoranthene (FA), pyrene (PY), benz[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]-fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenzo[a,h]anthracene (DahA), benzo-[g,h,i]perylene (Bghip) and their isotopically labeled analogues (ACL-d_8, FL-d_{10}, PHE-d_{10}, AN-d_{10}, FA-d_{10}, PY-d_{10}, BbF-d_{12}, BkF-d_{12}, BaP-d_{12}, IcdP-d_{12}, BghiP-d_{12}) were purchased from Sigma-Aldrich (97.0 % pure). Cyclohexane, methanol and dichloromethane were HPLC grade.

Tunnel-leave conditioning machine (Kunming Shipbuilding Co., Ltd.), SH315D tube plate cut tobacco drying machine (Qinhuangdao Tobacco Machinery Co., Ltd.); RM20/CS smoking machine (Germany BORGWALDT Company), HP6890GC/5973MSD (US Perkin Elmer Company), DELTA D200H ultrasonic generator (Taiwan Delta Company), AB204-S analysis balance (Switzerland Mettler-Toledo Company), V-805/R-205 vacuum rotary evaporator (Switzerland BUCHI Corporation); solid phase extraction cartridge (US Varian Company).

Experiment design: Based on the results of our previous report[17], we had investigated the contents of the four main sterols in each cigarette (Table-1), so the four main sterols, cholesterol (x_1), stigmasterol (x_2), β-sitosterol (x_3) and campesterol (x_4) were chosen as four independent variables in the uniform design approach. The investigation levels of each factor was shown in Table-2 and four levels for each factor were divided. In order to get more information and improve the accuracy of the experiment, the experiment were carried out using $U_{12}(12^4)$ table (Table-3). Meanwhile, the range and levels of each factor were based on the principle of uniform design as well as the actual production conditions and all the data analysis processes were conducted by the DPS statistical software (DPS ® v12.01 data processing system).

TABLE-1
CONTENT OF THE FOUR MAIN PHYTOSTEROLS AS FREE STEROL IN CIGARETTE

	Phytosterol			
Content (μg/cigarette)	Cholesterol	Stigmasterol	β-Sitosterol	Campesterol
	48	288	196	76

TABLE-2
FACTORS, THEIR SYMBOLS AND LEVELS FOR THE UNIFORM DESIGN $U_{12}(12^4)$

Factor	Symbol	Levels			
		1	2	3	4
Cholesterol (μg)	x_1	24	48	96	144
Stigmasterol (μg)	x_2	144	288	576	864
β-Sitosterol (μg)	x_3	98	196	392	588
Campesterol (μg)	x_4	38	76	152	228

TABLE-3
DESIGN MATRIX FOR THE UNIFORM DESIGN

Run	Factor x_1	Factor x_2	Factor x_3	Factor x_4
1	1	3	1	1
2	2	2	2	2
3	1	1	4	2
4	4	2	4	4
5	3	4	4	1
6	1	4	3	4
7	2	2	3	3
8	3	3	3	2
9	4	4	1	3
10	3	1	1	4
11	2	3	2	3
12	4	1	2	1

Analytical procedures

Sample pretreatment: According to the contents of phytosterols in each cigarette and the results of the uniform design (Tables 2 and 3), the suitable contents and combination of phytosterols was solved into dichloromethane and added into the cigarette evenly by using a microinjector. Three samples were made for each experiment trail in order to improve the accuracy of the experimental results. At last, all the samples were conditioned at 22 ºC and 60 % relative humidity for at least 48 h again.

Smoke collection: Mainstream smoke TPM generated under ISO smoking conditions (60 s puff interval, 2 s puff duration and 35 mL puff volume) was collected on individual CFPs using a RM20/CS smoking machine. One cigarette was smoked per pad for each individual sample and 20 pieces of cigarettes were smoked in each round, using the industry-standard Cambridge filter pad holder.

Sample preparation: The sample preparation method was based on a previous report[18]. After being smoked, each CFP was placed in 60 mL cyclohexane and added 1 mL internal standard solution. The mixture was then placed on the ultrasonic generator and shaken 40 min. Then 15 mL of the mainstream smoke particulate absorption liquid was pipetted into a solid phase extraction cartridge and washed with 50 mL of cyclohexane. The cyclohexane extracts were evaporated to approximately 0.5 mL and was used for GC/MS analysis, each sample was determined three times.

GC/MS analysis: GC separation was performed using a DB-5MS fused-silica capillary column (30 m × 0.25 mm I.D.; 0.25 μm film thickness), the splitless injector was set to 270 ºC, a constant flow of 1.2 mL min^{-1} of helium carrier gas was maintained through the column and injection volume was 1 μL. The following temperature program was used: the oven was heated with the initial temperature of 50 ºC and held for 1 min, after ramping up to 150 ºC at 25 ºC min^{-1}, then raised to 280 ºC at 4 ºC min^{-1} and held for 10 min, then raised to 300 ºC at 25 ºC min^{-1} and maintained for another 5 min, giving a total run time of 53.3 min. The mass spectrometer was operated in the electron impact mode (EI), the ion source temperature was 200 ºC and the GC-MS-interface set to 280 ºC. The analysis was performed by selected ion monitoring (SIM).

The standard solutions of 15 kinds of PAHs were prepared in cyclohexane, with the concentration of 12.5, 25, 50, 100, 200, 400, 800 and 1600 ng mL^{-1}, respectively. The concentration of internal standard was kept same in each standard solution. PAHs yields were calculated using a calibration curve obtained from the analysis of standard solutions containing the 15 individual PAHs and the reported yield was the average of three experimental runs. The response of PAHs to its concentration showed a good linear relationship with linear correlation coefficients higher than 0.999.

RESULTS AND DISCUSSION

Test results and regression models of uniform design: According to the uniform design table (Tables 2 and 3), 12 experiment trails were carried out to study the influence and the interaction effect of the four main phytosterols, including stigmasterol, cholesterol, β-sitosterol and campesterol, on the delivery of PAHs in tobacco. In this work, cholesterol (x_1), stigmasterol (x_2), β-sitosterol (x_3) and campesterol (x_4) were chosen as four independent variables and the 15 PAHs were selected as the dependent variables to represent the delivery of PAHs in tobacco. Since some interaction effect among the factors may occur, the results were analyzed by a second-order polynomial stepwise regression model.

$$Y = \beta_0 + \sum_{i=1}^{m}\beta_i X_i + \sum_{i=1}^{m}\beta_{ii} X_i^2 + \sum_{i=1}^{m}\beta_{ij} X_i X_j$$

where Y is the respose variable to be modeled; X_i and X_j are the independent variables; β_0, β_i, $ß_{ii}$ and $ß_{ij}$ are the regression coefficients; m is the number of factors. The significance of each coeffcient was determined by using *t*-test and *p*-value.

Based on uniform test results, the regression equations for the delivery of PAHs were shown in Table-4. In order to improve the accuracy of the regression model, all of the regression equations were made over 0.05 levels. It can be seen that most of the significant levels were smaller than 0.05, indicating that the models were significant and fitted the experiment well. And the determination coefficient after adjustment implied these equations were all showed significance with obvious linear relationship between independent variables and the dependent variables.

Model diagnosis and factor analysis for benzo[a]pyrene: Polycyclic aromatic hydrocarbons are known to be carcinogenic compounds and thus remain of public health concern. Benzo[a]pyrene is the prototype PAH carcinogen that has been most intensively investigated[19-21]. In our work, four free phytosterols were chosen as four independent variables and we tested the influence and interaction effect of the independent variables on the delivery of BaP based on uniform design. The following equation (eqn. 1) was the regression model over 0.05 levels to describe the delivery of BaP influenced by the four independent variables. The *p*-value ($p = 0.05$) was much smaller, implying that this model was significant and fitted the experiment well, the determination coefficient after adjustment was 0.9360, indicating that 93.60 % of the variability in the response could been explained by the model and all the used variables were necessary for building a correct model and this regression model had an obvious linear relationship between independent variables and BaP.

$$\begin{aligned} y = {} & 15.0133 + 1.5700\times10^{-3}x_2 + 2.6326\times10^{-2}x_4 \\ & + 9.2296\times10^{-5}x_1^2 - 3.8914\times10^{-5}x_4^2 \\ & - 1.0715\times10^{-4}x_1x_4 - 1.4052\times10^{-5}x_2x_4 \\ & - 4.5674\times10^{-6}x_3x_4 \end{aligned}$$

$$R^2 = 0.9360,\ P = 0.0041 \qquad (1)$$

Then this regression equation was made into standardized residual-predictive value chart so as to test the linear relationship, the standard deviation, the existence of distinguished value and to determine whether the model can be accepted. Standard residual-predictive value chart in Fig. 2 shown that the distribution of standard residuals of BaP is between ± 2.0, indicating that there is no distinguished value. The scatter diagram does not show any obvious trend, which demonstrated that the established linear regression equation model was eligible. Additional distribution of the standard residuals in scatter diagram was in ± 1.5, indicating that the equation meets the requirements of mean-square deviation. All diagnosis results showed that the regression equation of BaP and the four independent variables can be accepted for optimization analysis.

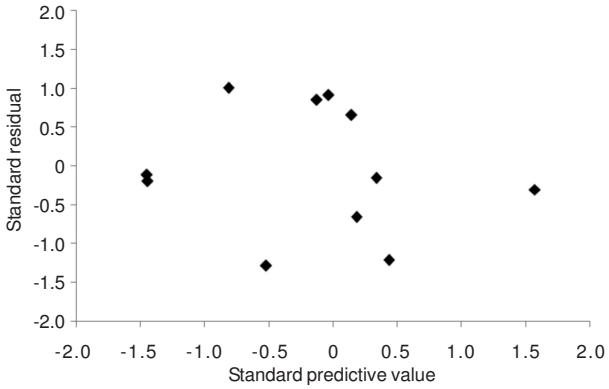

Fig. 2. Standard residual and standard predictive value of BaP

TABLE-4
REGRESSION EQUATIONS OF 15 PAHS BASED ON THE UNIFORM TEST RESULTS[a]

Index	Regression equation	R^{2} [b]	Significant level
ACL	$y = 372.6933 - 0.1268x_2 - 0.3999x_3 + 4.4228\times10^{-4}x_3^2 - 9.9156\times10^{-4}x_4^2 + 2.2733\times10^{-4}x_2x_3 + 4.0379\times10^{-4}x_2x_4$	0.0000	0.7782
AC	$y = 66.0145 + 5.9287\times10^{-2}x_2 - 0.1385x_3 - 1.7696\times10^{-2}x_4 - 4.4800\times10^{-5}x_2^2 + 1.9634\times10^{-4}x_3^2 - 9.0604\times10^{-5}x_1x_2$	0.8839	0.0047
FL	$y = 246.2168 + 0.2729x_1 + 0.5646x_4 + 3.9081\times10^{-5}x_3^2 - 1.0728\times10^{-3}x_4^2 - 2.4271\times10^{-4}x_1x_2 - 7.4929\times10^{-4}x_3x_4$	0.8436	0.0096
PHE	$y = 232.1701 + 0.2793x_1 - 3.4811\times10^{-2}x_3 + 0.8672x_4 + 3.5408\times10^{-5}x_2^2 - 1.9518\times10^{-3}x_4^2 - 2.1120\times10^{-3}x_1x_4 - 4.1222\times10^{-4}x_3x_4$	0.8860	0.0126
AN	$y = 88.4353 + 5.1415\times10^{-2}x_1 + 5.5296\times10^{-2}x_3 + 6.1961\times10^{-2}x_4 - 7.8211\times10^{-5}x_3^2 - 9.0632\times10^{-6}x_2x_3 - 1.3461\times10^{-4}x_3x_4$	0.9100	0.0025
FA	$y = 79.8062 + 9.1690\times10^{-2}x_4 + 2.7906\times10^{-6}x_3^2 - 3.2730\times10^{-4}x_4^2 + 7.4904\times10^{-6}x_2x_4$	0.9343	0.0001
PY	$y = 85.2487 - 4.1972\times10^{-2}x_2 - 5.4136\times10^{-4}x_1^2 + 3.6556\times10^{-5}x_2^2 + 1.4631\times10^{-4}x_1x_3 + 2.9549\times10^{-4}x_1x_4 - 1.0017\times10^{-4}x_3x_4$	0.2915	0.2770
BaA	$y = 17.5655 - 1.7333\times10^{-4}x_4^2 - 6.8799\times10^{-5}x_1x_2 - 1.2122\times10^{-4}x_1x_3 + 5.7387\times10^{-4}x_1x_4 + 2.2234\times10^{-5}x_2x_3$	0.8779	0.0018
CHR	$y = 19.4601 + 9.3389\times10^{-2}x_1 + 1.5710\times10^{-2}x_2 - 8.5617\times10^{-6}x_2^2 - 2.7983\times10^{-4}x_1x_3 + 1.3613\times10^{-5}x_2x_3 - 9.1722\times10^{-5}x_2x_4 + 1.2901\times10^{-4}x_3x_4$	0.9850	0.0002
BbF	$y = 22.6479 + 3.2774\times10^{-2}x_1 - 1.6454\times10^{-2}x_2 - 3.6699\times10^{-4}x_1^2 + 1.9269\times10^{-5}x_2^2 + 7.5643\times10^{-5}x_1x_3 - 1.2728\times10^{-5}x_2x_3$	0.3348	0.2450
BkF	$y = 5.4021 + 8.1720\times10^{-3}x_1 - 3.1226\times10^{-3}x_3 + 1.0902\times10^{-2}x_4 + 5.4945\times10^{-6}x_3^2 - 5.4059\times10^{-5}x_4^2 - 9.3964\times10^{-6}x_1x_2 + 7.4603\times10^{-6}x_2x_4$	0.8634	0.0179
BaP	$y = 15.0133 + 1.5700\times10^{-3}x_2 + 2.6326\times10^{-2}x_4 + 9.2296\times10^{-5}x_1^2 - 3.8914\times10^{-5}x_4^2 - 1.0715\times10^{-4}x_1x_4 - 1.4052\times10^{-5}x_2x_4 - 4.5674\times10^{-6}x_3x_4$	0.9360	0.0041
IcdP	$y = 8.3821 - 3.6989\times10^{-3}x_3 + 5.0008\times10^{-3}x_4 + 4.2529\times10^{-6}x_3^2 + 4.8441\times10^{-6}x_1x_3 - 9.3725\times10^{-6}x_3x_4$	0.1240	0.3707
DahA	$y = 9.2724 - 3.0104\times10^{-3}x_2 + 2.9674\times10^{-5}x_1x_3 - 5.6424\times10^{-5}x_1x_4 + 2.0148\times10^{-5}x_2x_4 - 1.6322\times10^{-5}x_3x_4$	0.3271	0.2009
BghiP	$y = 4.9559 + 1.1050\times10^{-2}x_4 + 3.6572\times10^{-5}x_1^2 + 1.2186\times10^{-6}x_2^2 + 1.8936\times10^{-6}x_3^2 - 3.3697\times10^{-5}x_4^2 - 1.2815\times10^{-5}x_1x_2 - 9.4844\times10^{-6}x_3x_4$	0.9522	0.0023

[a]x_1, x_2, x_3 and x_4 represents cholesterol, stigmasterol, β-sitosterol and campesterol respectively. [b]R^2 represents determination coefficient after adjustment.

According to the impact on BaP delivery from independent variables in the regression equation (Table-5), it can be concluded that cholesterol (x_1^2), stigmasterol (x_2) and campesterol (x_4) have a positive impact on the delivery of BaP. By contrast, the interaction effect between cholesterol and campesterol (x_1x_4), the interaction effect between stigmasterol and campesterol (x_2x_4), the interaction effect between β-sitosterol and campesterol (x_3x_4) have a negative influence on the delivery of BaP. In addition all the variables made a significant influence on the dependent variable, because the *p*-values were much smaller than 0.05.

TABLE-5
INFLUENCE AND INTERACTION EFFECT OF EACH INDEPENDENT VARIABLE ON BaP[a]

Index	Variables	Direct coefficient	*p*-Value
BaP	x_2	1.2813	0.0014
	x_4	5.7377	0.0004
	x_1^2	2.0955	0.0002
	x_4^2	-2.3751	0.0035
	x_1x_4	-2.9413	0.0003
	x_2x_4	-2.2884	0.0007
	x_3x4	-0.4953	0.0064

[a]x_1, x_2, x_3 and x_4 represents cholesterol, stigmasterol, β-sitosterol and campesterol respectively.

Conclusion

We utilized uniform design to investigate the influence of four main phytosterols, stigmasterol, cholesterol, β-sitosterol and campesterol, on the delivery of PAHs in tobacco. From the test results of uniform design and the second-order polynomial stepwise regression analysis models, it can be seen that the independent variables have a significant influence on

the dependent variables, except the delivery of ACL, PY, BbF, IcdP and DahA. Especially the regression model of BaP showed an obvious linear relationship between three main phytosterols (cholesterol, stigmasterol and campesterol) and the delivery of BaP, meanwhile the interaction effects between phytosterols also has a significant influence. Based on our experiment results, we confirmed that the uniform design was an effective and powerful approach to analysis the influence and interaction effect of phytosterols on the delivery of PAHs in tobacco.

ACKNOWLEDGEMENTS

This project was supported by a grant for the Research on the Relationship between the Phytosterols in Tobacco and the Content of Polycyclic Aromatic Hydrocarbons in Tobacco Mainstream Smoke. The author gratefully acknowledged the support of the Technology Center of China Tobacco Sichuan Industrial Corporation.

REFERENCES

1. Y.S. Ding, J.S. Trommel, X.J. Yan, D. Ashley and C.H. Watson, *Environ. Sci. Technol*., **39**, 471 (2005).
2. W.S. Schlotzhauer, R.F. Severson, O.T. Chortyk, R.F. Arrendale and H.C. Higman, *J. Agric. Food Chem*., **24**, 992 (1976).
3. P.F. Britt, A.C. Buchanan, M.M. Kidder, C.V. Owens, J.R. Ammann, J.T. Skeen and L. Luo, *Fuel*, **80**, 1727 (2001).
4. P.F. Britt, A.C. Buchanan, M.M. Kidder and C.V. Owens, *J. Anal. Appl. Pyrolysis*, **66**, 71 (2003).
5. Y.X. Liu, G.P. Yong, Y.B. Xu, D.L. Zhu, H.W. Tong and S.M. Liu, *Chromatographia*, **71**, 727 (2010).
6. A.I. Schepartz, J.J. Ellington and W.S. Schlotzhauer, *Tobacco Sci*., **24**, 55 (1980).
7. R.A.W. Johnstone and J.R. Plimmer, *Chem. Rev*., **59**, 885 (1959).
8. R.L. Stedman, *Chem. Rev*., **68**, 153 (1968).
9. A. Rodgman, http:// www.rjrtdocs.com, document ID 501008855 -8928 (1964).
10. S.M. Liu, B. Ding, H.W. Tong, X.Y. Yan, X.M. Ruan, H.T. Xu, Q.H. Wang and Y. Yue, *Tobacco Smoke Chem*., **13**, 10 (2007).
11. K.T. Fang, *Acta Math. Appl. Sin*., **3**, 363 (1980).
12. Y. Wang and K.T. Fang, *Kexue Tongbao*, **26**, 485 (1981).
13. Y. Huang, H. Lin, R. Song, Y. Tai and Z.J. Zhang, *Anal. Methods*, **3**, 857 (2011).
14. S.Y.L. Leung, W.H. Chan and C.H. Luk, *Chemom. Intel. Lab. Syst*., **53**, 21 (2000).
15. S.J. Lin, W. Zhao, Y.C. Li, S.Y. Tong and W.Q. Wang, *Res. Develop*., **8**, 30 (2010).
16. J.F. Li, H. Liao, B. Normand, C. Cordier, G. Maurin, J. Foct and C. Coddet, *Surf. Coat. Technol*., **176**, 1 (2003).
17. F. Xue, Y. Dai, D.L. Li, G.F. Song, L.J. Zhu, X.J. Tang and H. Chen, *Chem. Res. Appl*., **24**, 617 (2012).
18. Y.S. Ding, D.L. Ashley and C.H. Watson, *J. Agric. Food Chem*., **55**, 5966 (2007).
19. S.O. Baek and R.A. Jenkins, *Atmosp. Environ*., **38**, 6583 (2004).
20. K. Straif, R. Baan, Y. Grosse, B. Secretan, F.E. Ghissassi and V. Cogliano, *Lancet Oncol.*, **6**, 931 (2005).
21. H.V. Gelboin, *Physiol. Rev*., **50**, 1107 (1980).