Factors affecting the amount of free-base nicotine in mainstream cigarette smoke and determination of same by SPME

Mingliang Bao, PhD, Peter J. Joza, William S. Rickert, PhD
Labstat International, ULC, Kitchener, ON N2C 1L3, Canada
John H. Lauterbach, PhD, DABT
Lauterbach & Associates, LLC, Macon, GA, USA 31210-4708

Outline for presentation

- Background for presentation
- Objectives for the presentation
- Factors affecting determination of free-base nicotine (FBN) in mainstream cigarette smoke (MSS) and mainstream particulate matter (MPM)
- Factors affecting concentration of FBN in MSS and MPM
- Considerations for the use of FBN methodology for the regulation of cigarette products
- Conclusions

Background for presentation

- Free base nicotine (FBN) remains a controversial topic
  - FBN and tobacco products
    - FBN is a misnomer, it is really just plain neutral nicotine
    - FBN in mainstream cigarette smoke (MSS)
    - FBN in aqueous extracts of smokeless tobacco products (STE)
    - Relationships between FBN and pH in MSS or STE
  - Numerous journal articles, company memos, and news reports about relationships between FBN (or pH) levels and product acceptability
  - FBN is difficult to measure in complex matrices such as STE or MSS (whole smoke or just particulate matter)
  - Amount of FBN in MSS depends on how cigarette is smoked

Objectives for presentation

- Brief review of FBN in MSS
  - Physical chemistry of FBN in MSS
  - Methods and techniques for determination of FBN in MSS
    - Whole smoke
    - Total particulate matter (TPM) only
  - Solid phase microextraction (SPME) methods for FBN in MPM
    - Watson *et al*., *J. Agric. Food Chem.* 2004, 52, 7240-7245
    - Lee et al., CORESTA Congress Paris 2006, SSPOST 25
    - Bao et al., CORESTA Congress Shanghai 2008, SSPT 42
- Summarize factors affecting amount of FBN in MSS
  - Methodological factors
  - Smoke chemistry factors
  - Smoking dynamics (e.g., puff volume, flow rate)

Brief review of FBN in MSS – 1

- Physical chemistry of FBN in MSS
  - MSS gas-vapor phase (GVP)
    - Permanent gases, very volatile smoke components
    - Semivolatile compounds that may be in GVP or PP depending on concentration and gas-particle partitioning coefficient
  - MSS particulate phase (PP)
    - Semivolatile compounds that may be in GVP or PP depending on concentration and gas-particle partitioning coefficient
    - Nonvolatile smoke components
  - Semivolatile compounds
    - Bases: nicotine, ammonia, amines, pyridines
    - Acids: carbonic, formic, acetic, lactic, glycolic
    - Neutrals: water, propylene glycol, glycerin, triacetin, menthol
    - Must assume semivolatiles in dynamic equilibria between GVP & PP

Brief review of FBN in MSS – 2

- Gas-particle partitioning (GPP) theory (J.F. Pankow)
  - Equations for GPP of nicotine in MSS
    1. $P_{g,nicotine}(\%) = 100\%\{1/(1+ K_{p,nicotine}\ TSP)\}$
    2. $K_{p,nicotine} = F_{nicotine}/TSP/A_{nicotine} = c_{p,nicotine}/c_{g,nicotine}$
    3. $K_{p,nicotine} = (f_{om}760RT)/(MW_{om}\gamma_{nicotine}p_L^{\circ}{}_{nicotine}\ 10^6)$
    4. $K_{p,nicotine} = K_{p,fb}/\alpha_{fb} = 10^{-5.03}/\alpha_{fb}$

$P_{g,nicotine}(\%)$ = Percent of nicotine in GVP

TSP = total suspended particulates (µg/m^3)

$K_{p,nicotine}$ = GPP constant for nicotine (m^3/µg)

TSP =10^9*TPM (mg/cig)/[puffs*(mL/puff)] (µg/m^3)

f_{om} = fraction TSP that is organic matter

$\gamma_{nicotine}$ = activity coefficient nicotine in TPM

$K_{p,fb}$ = GPP constant for FBN (m^3/µg)

$c_{p,nicotine}$ = conc. nicotine in PP (ng/µg)

$c_{g,nicotine}$ = conc. nicotine in GVP (ng/m^3)

$F_{nicotine}$ = conc. nicotine in PP (ng/m^3)

$A_{nicotine}$ = conc. nicotine in GVP (ng/m^3)

MW_{om} = Number avg. mol. wt. of TSP (g/mol)

$p_L^{\circ}{}_{nicotine}$ = vapor pressure nicotine (torr)

α_{fb} = FBN/Total nicotine (µg / µg both on CFP)

Brief review of FBN in MSS – 3

- Whole smoke techniques
  - pH of whole smoke aerosol or aqueous suspension of whole smoke (pH most always acidic with such techniques)
  - Traps after Cambridge filter pads (CFP)
  - Denuders
- Particulate-phase techniques
  - pH of aqueous suspension of total particulate matter (TPM)
  - Hexane extraction of TPM
  - Desorption of FBN in TPM before and after NH_3 treatment
  - SPME (solid-phase microextraction)
- $\alpha_{fb} = \boldsymbol{FBN/TN} = 10^{-pKa}/(10^{-pKa} + 10^{-pH})$ only if true solution

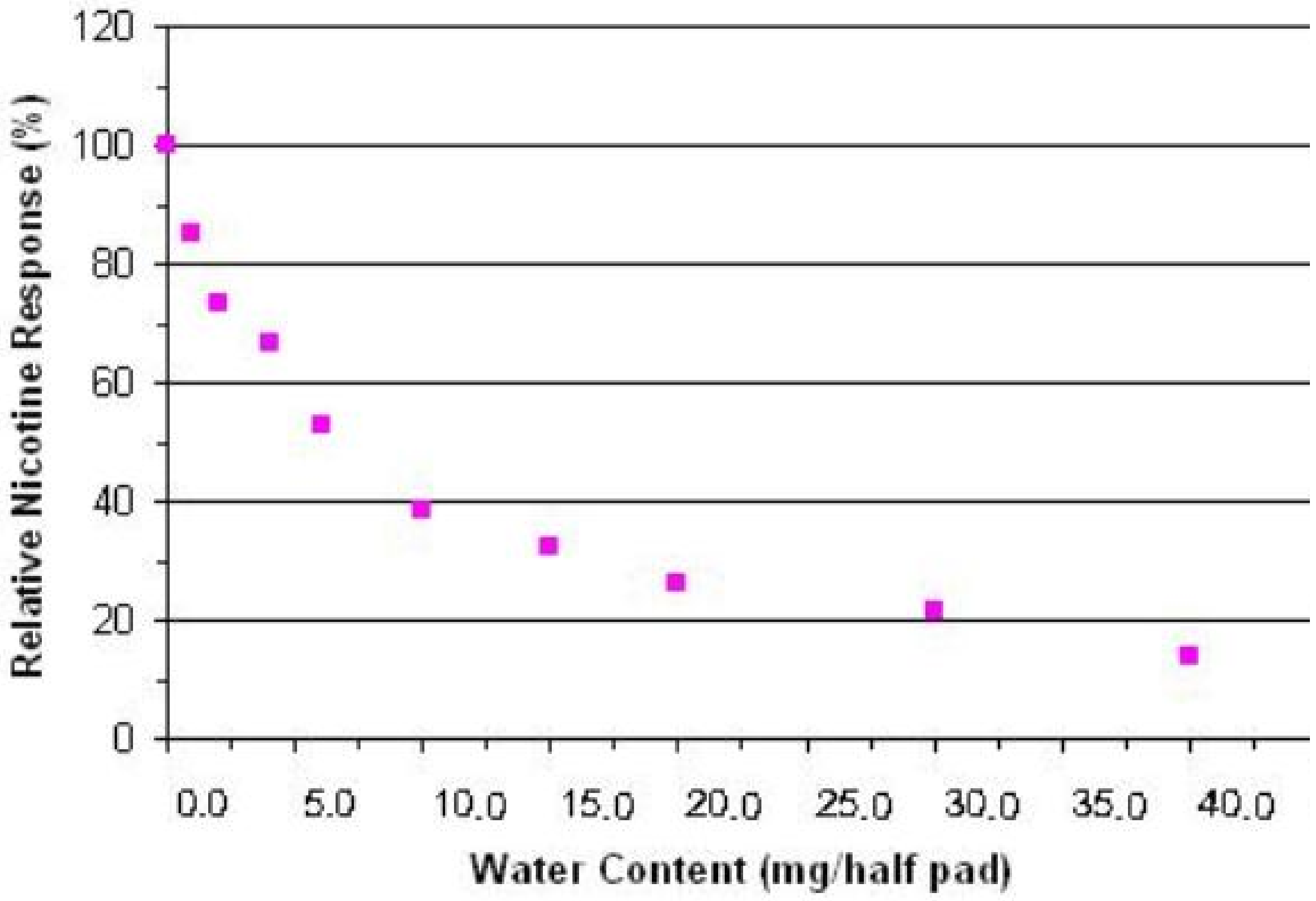
Why SPME for determination of FBN in MPM?

- Methods relying on aqueous suspensions inaccurate?
  - pH is defined only for dilute aqueous solutions
  - Acidity from volatile acids (H_2CO_3, CH_2O_2) may be artifactual
  - Trapping may alter smoking dynamics
- Advantages of SPME for FBN in MSS PP?
  - Works with standard smoking protocols: FTC/ISO (35/60/2, 0% VB), MDPH (45/30/2, 50% VB), HCI (55/30/2, 100% VB)
  - Does not require special smoking equipment and reagents
  - Uses conventional Cambridge filter pads
- But, two assumptions required
  - Equilibrium between MSS aerosol and MSS on CFP
  - Can correct for changes in SPME response from PP water

The two assumptions

- Equilibrium between MSS aerosol and MSS on CFP
  - As MSS is drawn through pad, particulate matter remains but volatiles come onto pad but can also be stripped off
  - Once the smoke run is finished and the pad is removed from holder, it can gain/lose water and other volatiles
  - Determination of water on half-pad allows measure of changes versus results from determinations with whole pad
- Correcting SPME response for PP water content
  - Nicotine and FBN are in TPM on CFP
  - SPME response depends on vapor pressure of FBN above CFP
    - Vapor pressure of FBN depends on mole fraction of FBN in TPM
    - As water content of TPM increases, mole fraction of FBN decreases
    - Must correct for response due to water content of TPM as that water content also affects gas-particle partitioning coefficient

Effect of water in TPM on SPME response to FBN



Factors known to affect relative amount of water in TPM

- Cigarette design is a major factor
  - Filter ventilation, higher levels give drier TPM
  - Humectants, such as propylene glycol, glycerin give wet TPM
- Intensive smoking gives wetter TPM (Counts *et al*., 2005)

Initial filter Ventilation (%)	ISO	ISO	ISO	MDPH	MDPH	MDPH	HCI	HCI	HCI
	0-30	31-60	>60	0-30	31-60	>60	0-30	31-60	>60
TPM (mg/cig)	15	8.1	2.5	37	23	11	53	44	36
H_2O (mg/cig)	1.5	0.45	0.09	9.6	4.0	0.81	18	16	13
H_2O/TPM (%)	10	5	4	26	17	7	34	36	36

- Conditioning of cigarettes prior to smoking
  - ISO 3402:1999 does not specify how well cigarettes should be conditioned prior to smoking; only minimum time specified

Table 1 – Contemporary reference & commercial cigarettes

Brand style	Regimen	Puff count	TPM (mg/cig)	Water (mg/cig)	Nicotine (mg/cig)	Tar (mg/cig)	Tar/ Nicotine	CO (mg/cig)	FBN (µg/cig)	α_{fb}	Smoke pH
KY2R4F	ISO	8.4	10.3	0.36	0.76	9.19	12.1	10.1	36.1	0.048	6.10
CIM 8	ISO	8.3	15.1	1.09	1.09	12.9	11.9	10.6	34.6	0.032	5.98
Camel Filters KS	ISO	8.4	16.2	0.86	1.08	14.3	13.2	11.7	45.3	0.043	6.07
Player's KS	ISO	8.1	18.2	1.7	1.12	15.3	13.7	13.3	15.7	0.014	5.83
Gauloises Blondes KS	ISO	7.1	10.4	0.36	0.79	9.27	11.7	9.38	43.3	0.055	6.10
KY2R4F	HCI	11.0	45.3	13.1	1.91	30.3	15.9	27.8	44.5	0.023	5.90
CIM 8	HCI	11.6	46.0	11.2	2.50	32.3	12.9	24.6	32.6	0.013	5.80
Camel Filters KS	HCI	9.3	55.1	14.1	2.41	38.6	16.0	30.5	34.1	0.014	5.78
Player's KS	HCI	11.3	53.1	13.6	2.67	36.9	13.8	29.0	18.4	0.007	5.68
Gauloises Blondes KS	HCI	8.7	45.9	12.0	2.05	31.8	15.5	30.1	24.7	0.012	5.88

Comparison of our results with those of other researchers

- Watson *et al.*, *JAFC* 2004, 52, 7240-7245 (ISO)
  - α_{fb} ~ 0.012 for KY1R4F, we found α_{fb} ~ 0.048 for KY2R4F
  - α_{fb} ~ 0.072 for Brand T (32% FV, 9 mg tar, 0.7 mg nic), we found α_{fb} ~ 0.055 for GBKS (31% FV, 9 mg tar, 0.8 mg nic)
- Lee *et al.*, CORESTA Congress 2006, SSPOST 25 (ISO)
  - α_{fb} ~ 0.13 for KY2R4F, we found α_{fb} ~ 0.048 for KY2R4F
  - α_{fb} ~ 0.051 for Test Cig 3 (30% FV, 9 mg tar, 0.7 mg nic), we found α_{fb} ~ 0.055 for GBKS (31% FV, 9 mg tar, 0.8 mg nic)
- Lee *et al.*, CORESTA Congress 2006, SSPOST 25 (HCI)
  - α_{fb} ~ 0.03 for KY2R4F, we found α_{fb} ~ 0.02 for KY2R4F
  - α_{fb} ~ 0.01 for Test Cig 3 (100% VB, 32 mg tar, 1.9 mg nic), we found α_{fb} ~ 0.01 for GBKS (100% VB, 32 mg tar, 2.1 mg nic)

Table 2 – Contemporary & historical Canadian cigarettes (ISO)

Brand style	Year	Puff count	TPM (mg/cig)	Water (mg/cig)	Nicotine (mg/cig)	Tar (mg/cig)	Tar/ Nicotine	CO (mg/cig)	FBN (µg/cig)	α_{fb}	Smoke pH
du Maurier KSFT	1970	10.3	26.2	1.70	1.41	23.1	16.4	23.9	13.2	0.009	5.66
du Maurier KSFT	1980	9.3	26.8	1.63	1.29	23.2	18.0	22.6	10.1	0.008	5.60
du Maurier KSFT	1993	8.1	15.6	1.00	0.94	13.7	14.6	14.4	11.0	0.012	5.63
du Maurier KSFT	1999	8.8	17.7	0.86	1.25	15.6	12.5	15.2	26.7	0.021	5.88
du Maurier KSFT	2007	8.5	21.6	1.90	1.24	18.4	14.8	16.4	12.8	0.010	5.65
Export A FF RSFT	1970	8.7	24.7	1.86	1.26	21.5	17.1	17.7	9.29	0.007	5.54
Export A FF RSFT	1980	8.6	32.0	4.63	1.32	26.1	19.7	21.9	6.20	0.005	5.43
Export A FF RSFT	1990	7.6	20.4	1.98	1.06	17.6	16.6	14.8	9.36	0.009	5.63
Export A FF RSFT	1999	7.4	18.6	1.79	1.23	15.6	12.7	13.9	20.0	0.016	5.77
Export A FF RSFT	2007	6.8	18.6	2.03	1.02	15.6	15.3	13.5	11.1	0.011	5.76
Rothmans KSFT	1971	10.8	25.5	1.67	1.40	22.5	16.1	21.8	14.0	0.010	5.46
Rothmans KSFT	1979	9.8	20.5	1.33	1.14	18.1	15.9	18.6	12.2	0.011	5.61
Rothmans KSFT	1996	8.4	18.2	1.02	1.25	16.0	12.8	14.3	26.0	0.021	5.64
Rothmans KSFT	1999	8.2	16.4	0.90	1.07	14.4	13.5	14.3	20.0	0.019	5.77
Rothmans KSFT	2007	8.4	18.1	0.65	1.27	16.2	12.8	16.2	32	0.025	5.81

Explanation of data in Table 2 and Figures 1 – 3

- Data taken from larger study of Canadian brands
  - Study began in 1970 and is continuing
  - Includes data on tobacco composition and smoke deliveries
  - Only includes cigarettes made with traditional ***additive-free***, all flue-cured Canadian blends
- Data shown in Figures 1 – 3
  - α_{fb} ***decreases*** when tar-to-nicotine ratio ***increases***
  - α_{fb} ***decreases*** when water in TPM (%) ***increases***
  - α_{fb} ***increases*** when smoke pH ***increases***
- Note that these effects occurred in the ***absence*** of ammonia-containing additives

Figure 1. α_{fb} versus Tar-to-Nicotine Ratio

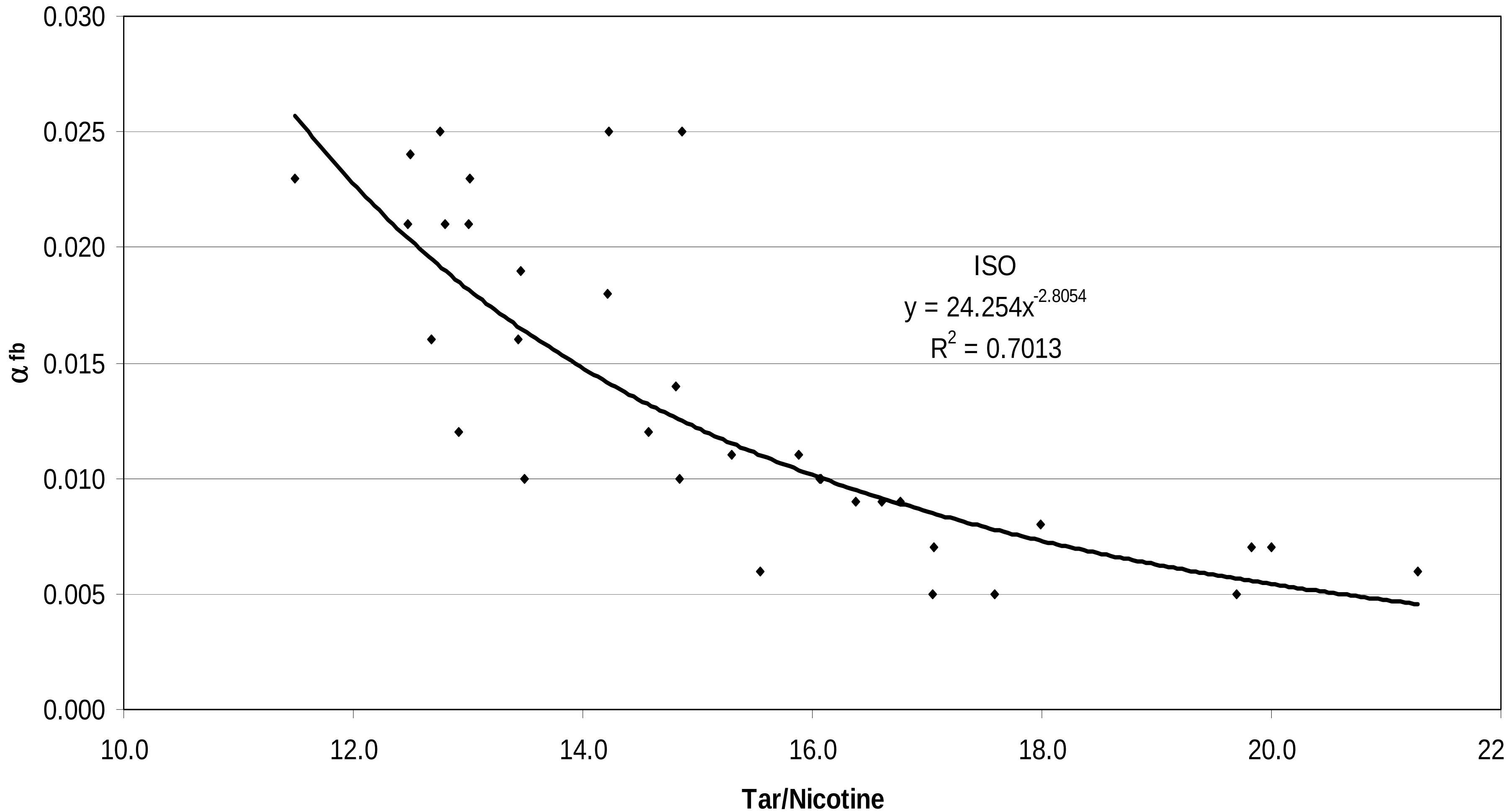


Figure 2. α_{fb} versus water in TPM (%)

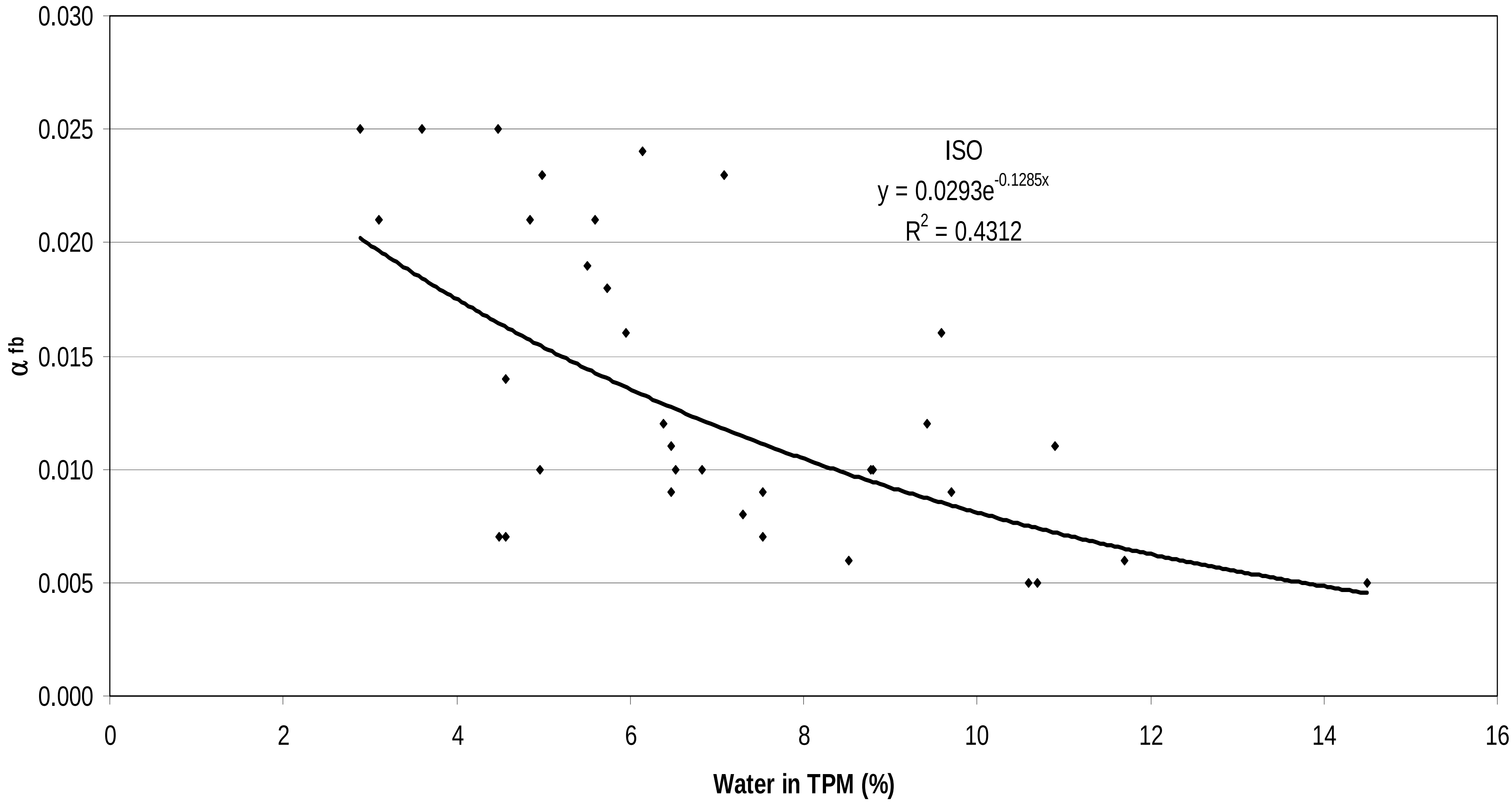
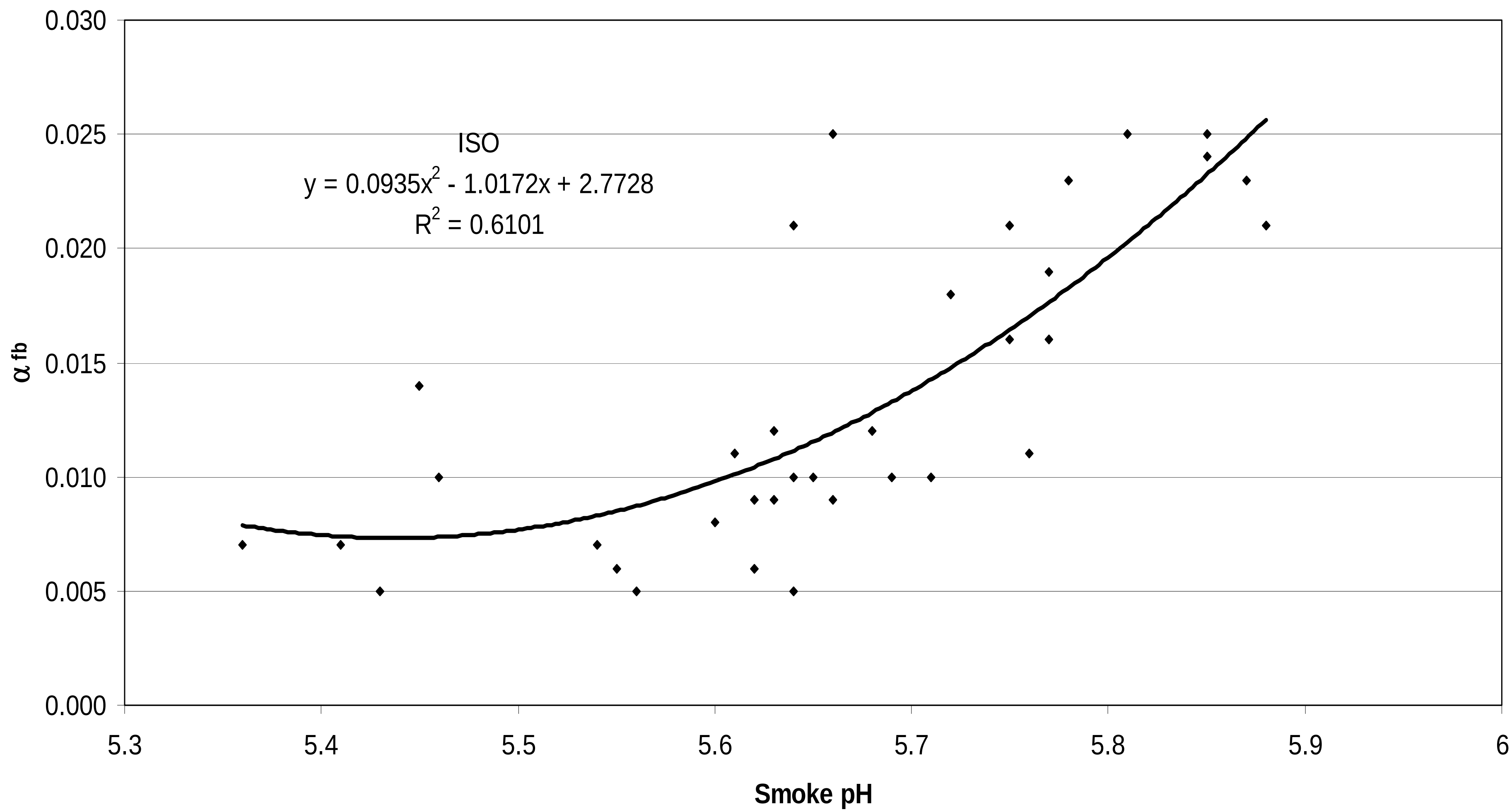


Figure 3. α_{fb} versus Smoke pH



Does α_{fb} equal $P_{g,nicotine}(\%)$? Definitely not!

- α_{fb} and $P_{g,nicotine}(\%)$ are two ***different*** parameters
  - α_{fb} is the fraction of MPP nicotine that ***not protonated***
  - $P_{g,nicotine}(\%)$ is the percentage of total smoke nicotine that is in the GVP of MSS and believed to be ***not protonated***
- Examples of differences between α_{fb} and $P_{g,nicotine}(\%)$
  - Data from Chen and Pankow, JAFC, 2009, 57, 2678-2690
    - MDPH puffing regimen and 50% vent blocking
    - For Camel KS, α_{fb} ~ 0.028, $P_{g,nicotine}(\%)$ = 0.0062 ± 0.0009%
    - For Marlboro KS, α_{fb} ~ 0.015, $P_{g,nicotine}(\%)$ = 0.0037 ± 0.0014
  - Watson *et al.*, *JAFC* 2004, 52, 7240-7245
    - ISO puffing regimen and 0% vent blocking
    - For Cigarette U (82% FV, 0.1 mg nicotine, 1 mg tar), α_{fb} = 0.3, $P_{g,nicotine}(\%)$ ~ 0.74% (exp. value of 0.76% for similar cigarette)

Regulatory aspects of FBN determinations

- Based on current information, SPME appears to be the best technique for development of a standard method for the determination of the amount of FBN in MPM
- Several aspects of the technique may need further study and optimization
  - Conditioning of the cigarettes to an equilibrium moisture
  - Minimization of changes in MPM between termination of smoking and sealing the CFP in the SPME sampling bottle
  - Minimization of changes in MPM during the SPME experiment
  - Optimization of the procedure for determining the correction factor for water content of the MPM

Conclusions

- SPME is currently the preferred technique for the determination of FBN in MPM
- Insufficient conditioning of the cigarettes before smoking may lead to FBN values that are too high
- There is the potential for changes in the composition of the MPM from the time smoke collection ends to the time the SPME experiment is finished
- Correction factors must be used to account for changes in FBN response due to changes in the water content of the MPM