

SS 16 - Mueller - Application of ^{13}C tracer technique to study fate and behaviour of ingredients applied on tobacco

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Application of ^{13}C tracer technique to study fate and behaviour of ingredients applied on tobacco.

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Summary

The fate of ingredients on tobacco is of major interest and has been subject of many publications and CORESTA papers. For this study ^{13}C labelled vanillin, benzaldehyde and D-glucose were applied on tobacco and test cigarettes produced. The cigarettes were smoked and smoke collected using the Sidestream Fishtail Chimney device.

For the investigation of fate and behaviour of these ingredients, the ^{13}C abundance in CO , CO_2 and volatile organic carbons were determined for mainstream and sidestream smoke, the smoked cigarette butt and ash. The $^{12}\text{C}/^{13}\text{C}$ ratio for the different smoking traps was determined using isotope mass spectrometry and the contribution of tracer labelled ingredient to the natural abundance was estimated. The newly developed analytical setup for the simultaneous determination of ^{13}C in CO and CO_2 and for volatile organic carbons (by oxidation) is described in detail.

It was found that noticeable amounts of the most volatile compound (benzaldehyde) are transferred into smoke without decomposition whereas the semi-volatile and non-volatile constituents (vanillin and glucose) contribute significantly to the ^{13}C concentration in CO and CO_2 . Based on the results it can be concluded that up to 94 % of the labelled compounds can be accounted for in the different smoking traps, butt and ash.

Introduction

Pyrolysis experiments on tobacco additives have been used to predict possible breakdown products formed and to estimate their possible contribution to Hoffmann Analytes yield [1-3]. However, reliable predictions are limited since combustion of ingredients on tobacco cannot be simulated by pyrolysis in a straightforward manner. As an alternative, tracer investigations with radioactive (^{14}C) or ^{13}C (stable isotope) labelled compounds have been described to track volatile and non-volatile ingredients and combustion products in mainstream and sidestream tobacco smoke [4-6]. For a successful outcome from such ^{13}C tracer

investigations a highly sensitive and specific method for the determination of the ^{13}C abundance of the ^{13}C containing components in cigarette smoke – especially CO and CO_2 – is needed. Sophisticated methodologies for the determination of the natural ^{13}C abundance of CO and CO_2 in atmospheric air are well known [7-12] and usually comprise catalytic oxidation of CO to CO_2 after GC separation. However, it turned out that the catalysts reported in the literature are deactivated shortly when exposed to cigarette smoke and thus, the methods described were modified for a fast ^{13}C one step determination of slightly ^{13}C enriched CO and CO_2 in cigarette smoke with sufficient precision. The methodology described below comprises the oxidation of CO to CO_2 and consecutive ^{13}C determination of CO_2 from CO and originally formed CO_2 . A second analytical run allows determining the total ^{13}C amount of all carbon containing compounds in mainstream and sidestream smoke after total oxidation to CO_2 .

Experimental

Smoking and sampling

The spiked test cigarettes and a control cigarette were smoked on a 4-channel Borgwaldt smoking machine following the protocol of the CORESTA Recommended Methods 54 and 55 [13, 14]. Sampling of mainstream and sidestream vapour phase was performed using aluminium coated Teflon bags. The yields of CO_2 and CO were determined by infrared spectroscopy.

Vanillin and benzaldehyde were quantified by HPLC and GC in mainstream and sidestream cigarette smoke (vapour phase, particulate matter, filter and butt) as well as in the unburned cigarette. The $^{12}\text{C}/^{13}\text{C}$ isotope ratio for CO_2 , CO and volatile organic carbons as well as for the filter pads (mainstream, sidestream), smoked cigarette filters, butts and ashes were determined by isotope mass spectrometry using the ^{13}C tracer technique described below.

^{13}C determination of CO and CO_2

Before entering the six-port sample valve, the gas sample from filtered smoke is passed through an iced trap filled with glass beads to remove traces of tar still present. The head of the GC column is kept at 0°C ; this cryo-focussing helps to improve peak sharpness. CO and CO_2 are then separated by GC followed by low temperature oxidation of CO to CO_2 using a catalyst and subsequent online quadrupole MS (QMS) ^{13}C detection of the resulting two CO_2 peaks (see Figure 1).

The use of a quadrupole MS provides sufficient precision for ^{13}C measurements even for only slightly ^{13}C enriched samples.

The catalyst consists of copper oxide (fine CuO wire) that was cut into 0.6 x 1.5 mm pieces and filled into a glass tube (150 mm length, 5 mm diameter).

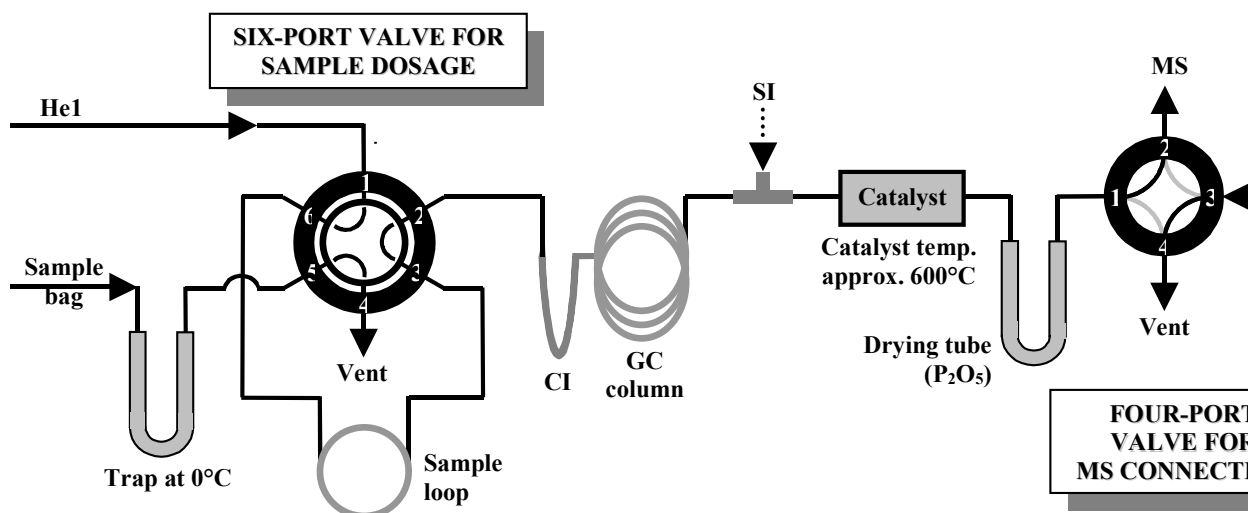


Figure 1: Analytical set-up for simultaneous ^{13}C determination of CO and CO_2 : CI – Part of GC column for cryo-focussing, He1 – Helium flow for QMS measurement; He2 – Helium flow for QMS purging, SI – Sample injection by syringe (optional).

The GC and QMS parameter listed in the following Table 1 allow a GC separation performance that results in a nearly baseline separation of CO originally present in smoke from those deriving from (partial) hydrocarbon oxidation.

Table 1: GC and QMS parameter

GC parameter	QMS parameter
GC column: Porapak Q 80/100 mesh, 180 cm x 2.1 mm (Supelco)	Type GAM 400 with electronmultiplier detection (InProcess Instruments Bremen, Germany)
Hel: Carrier gas Helium at 20 mL/min	High voltage, 1750 V
Column temp: Room temperature	Measuring mode: selected ion monitoring
Column head: 0°C (ice) cryo-focussing	Measuring time:
Sample volume: 2 mL	20 ms for $m/z = 44$; 35 ms for $m/z = 45$

^{13}C determination of total carbon (C_t)

Besides CO and CO_2 , vapour and particulate phase of cigarette smoke are composed of numerous other carbon containing compounds in minor concentrations. The total carbon contents including these constituents – expressed as total carbon (C_t) – has been analysed together with the originally formed CO and CO_2 . Regarding the C_t deriving from the ^{13}C labelled ingredient, the total of all ^{13}C containing components other than CO and CO_2 is described as breakdown products (C_{VOC}) and can be calculated using Equation 1:

$$C_{\text{VOC}} = C_t - (C_{\text{CO}} + C_{\text{CO}_2})$$

(Eq. 1)

The ^{13}C determination of C_t is based on a total oxidation of all carbon containing compounds to CO_2 on copper oxide at high temperature and subsequent online MS analysis of CO_2 . The analytical set-up for the C_t analysis can be derived from Figure 1 with the following modifications. The iced cooling must be removed and the temperature of the catalyst needs to be increased to 850°C . GC separation is not required because all carbon containing compounds are oxidized to CO_2 . The He1 carrier gas flow is set to 10 mL/min . and the injection volume amounts to 1 mL . The QMS parameters are the same as described in Table 1 but only CO_2 must be recorded.

Calculation of results

The ^{13}C abundance of CO and CO_2 (in at %) is calculated from the recorded current of $m/z = 44$ and 45 for both CO_2 peaks, the ^{13}C abundance of CO and CO_2 is calculated using Equation 2. Assuming a constant natural ^{17}O abundance of 0.0378 at \% , the influence of R_{45} from the isotope ^{17}O can be simply corrected by subtracting R_{45} deriving from $^{12}\text{C}^{16}\text{O}^{17}\text{O}$ [15].

$$a = (R_{45} - 0.000758)/(1 + R_{45} - 0.000758) \times 100$$

(Eq. 2)

All measured abundances were corrected by a calibration factor obtained from measurements of a precise ^{13}C Carbon dioxide standard gas with a ^{13}C abundance of 1.11129 at \% . This standard gas was prepared from calcium carbonate whose abundance was exactly determined versus VPDB (“Vienna Pee Dee Belemnite”, a carbonate rock).

Studied Material

For this study additive-free American blended cigarettes without filter ventilation were spiked with defined amounts of ^{13}C labelled vanillin, benzaldehyde and D-glucose (Figure 2).

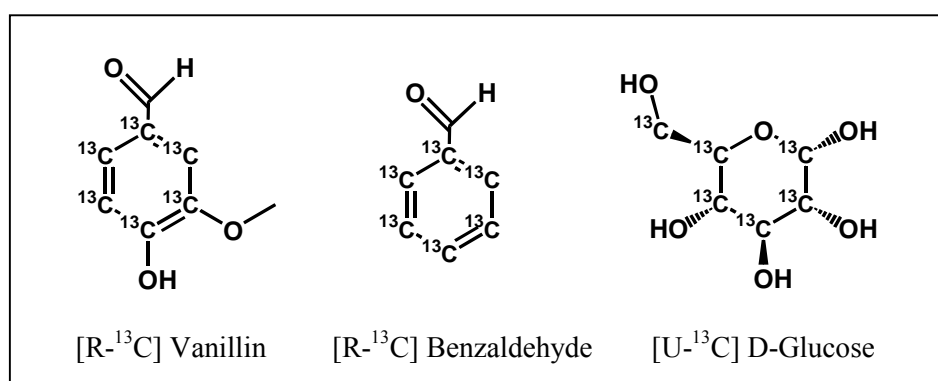


Figure 2: Chemical structures and labelling pattern of ^{13}C labelled ingredients used for spiking the test cigarettes.

For details on the spiked amounts of labelled ingredients see the following Tables 2-4.

Results and Discussions

The following tables summarise the amounts of ^{13}C labelled vanillin, glucose and benzaldehyde spiked on the test cigarettes and their distribution on the different smoking traps when smoked for sidestream smoke collection [13, 14].

Table 2: Spiked amount of labelled vanillin (analysed by HPLC), its distribution on different smoking traps and the respective CO/CO₂ yields (figures based on 5 replicates).

		[R-¹³C] Vanillin		CO₂		CO	
		mean	SD	mean	SD	mean	SD
		µg/Cig	µg/Cig	mg/Cig	mg/Cig	mg/Cig	mg/Cig
Unsmoked cigarette	<i>Filter</i>	<i>n.d.</i>	-	-	-	-	-
	<i>Tobacco</i>	565.0	13.0	-	-	-	-
	Total unsmoked	565.0	-	-	-	-	-
Mainstream smoke (MS)	<i>MS CF pad</i>	50.1	2.3	-	-	-	-
	<i>MS VP liquid trap 1</i>	<i>n.d.</i>	-	-	-	-	-
	<i>MS VP liquid trap 2</i>	<i>n.d.</i>	-	-	-	-	-
	Total MS	50.1	-	33.7	1.1	9.2	1.7
Sidestream smoke (SS)	<i>SS Fishtail</i>	6.5	0.5	-	-	-	-
	<i>SS CF pad</i>	49.3	2.2	-	-	-	-
	<i>SS VP liquid trap</i>	16.2	1.0	-	-	-	-
	Total SS	71.9	-	284.4	17.2	40.0	4.1
Smoked ash & butt	<i>Ash & Butt</i>	8.7	0.5	-	-	-	-
Smoked filter	<i>Cig. filter</i>	31.3	1.5	-	-	-	-
Total detected in butt, filter, MS and SS smoke		162.0		318.1		49.2	

Using HPLC analysis, about 30 % of the spiked vanillin was detected intact in the different smoking traps, smoked filter, butt and ash.

Table 3: Spiked amount of labelled benzaldehyde (analysed by GC-FID), its distribution on different smoking traps and the respective CO/CO₂ yields (5 replicates).

		[R-¹³C] Benzaldehyde		CO₂		CO	
		mean	SD	mean	SD	mean	SD
		µg/Cig	µg/Cig	mg/Cig	mg/Cig	mg/Cig	mg/Cig
Unsmoked cigarette	<i>Filter</i>	312.9	3.8	-	-	-	-
	<i>Tobacco</i>	174.0	3.1	-	-	-	-
	Total unsmoked	486.9	-	-	-	-	-
Mainstream smoke	<i>MS CF pad</i>	32.0	3.3	-	-	-	-

(MS)	MS VP liquid trap 1	2.3	0.04	-	-	-	-
	MS VP liquid trap 2	-	-	-	-	-	-
	Total MS	35.3	-	32.9	2.8	9.6	0.5
Sidestream smoke (SS)	SS Fishtail	8.4	0.2	-	-	-	-
	SS CF pad	11.6	0.44	-	-	-	-
	SS VP liquid trap	0	-	-	-	-	-
	Total SS	20.1	-	310.7	23.4	41.7	5.3
Smoked ash & butt	Ash & Butt	11.5	0.8	-	-	-	-
Smoked filter	Cig. filter	243.1	61.0	-	-	-	-
Total detected in butt, filter, MS and SS smoke		308.8		343.6		51.3	

For benzaldehyde about 65 % of the spiked compound can be accounted for in the different smoking traps, smoked filter, butt and ash using GC-FID. Even after a short time of storage (within a couple of days), about 60 % of the spiked compound has been migrated onto the filter. Due to the high volatility of benzaldehyde, losses during sample storage and handling are likely to occur.

Table 4: Distribution of ^{13}C deriving from labelled additives spiked on tobacco on different smoking traps, smoked filter, butt and ash (in $\mu\text{mol}/\text{cig.}$). Yields for CO , CO_2 , VOCs and all glucose data based on isotope QMS measurements (5 replicates).

		[R-^{13}C] Vanillin		[U-^{13}C] Glucose		[R-^{13}C] Benzaldehy	
		^{13}C amount	^{13}C recovery	^{13}C amount	^{13}C recovery	^{13}C amount	^{13}C re
		[$\mu\text{mol}/\text{cig}$]	[%]	[$\mu\text{mol}/\text{cig}$]	[%]	[$\mu\text{mol}/\text{cig}$]	[%]
Unsmoked cigarette		20.4	100	162.9	100	25.3	100
Mainstream vapour phase (MS)	CO	0.5	2.5	4.1	2.5	0.1	0.3
	CO_2	0.6	3.0	6.6	4.1	0.1	0.6
	VOC	not detected	-	1.3	0.8	not detected	-
	Total MS	1.1	5.5	12.0	7.4	0.2	0.9
Sidestream vapour phase (SS)	CO	2.4	11.6	16.3	10.0	0.3	1.2
	CO_2	8.6	42.3	70.1	43.0	1.7	6.7
	VOC	1.1	5.4	10.3	6.3	not detected	-
	Total SS	12.1	59.3	96.7	59.4	2.0	7.9
Total recovery vapour phase			64.8		66.8		8.8
Particulate matter (PM)	Smoked cig. filter	1.1	5.5	4.6	2.8	12.6	49.9
	MS CF pad	2.0	9.8	6.1	3.7	0.5	7.0
	SS CF pad	2.5	12.2	6.1	3.7	1.1	4.1
Total recovery PM		5.6	27.5	16.8	10.3	14.2	61.1
Smoked butt and ash		0.4	1.9	16.0	9.8	0.3	2.4
Overall ^{13}C Recovery			94.2		86.9		72.2

Recovery rates for ^{13}C from labelled glucose and vanillin were determined to account for 87 and 94 % respectively. About 60 % of vanillin and glucose were estimated from isotope

measurements to be oxidized to CO and CO₂. The combustion of glucose resulted in measurable contributions to volatile organic carbons accounting for 6.3 % of the labelled glucose in sidestream smoke and 0.8 % in mainstream smoke.

Due to migration onto the filter, only 36 % of added benzaldehyde was available for combustion in this experiment. However, about 25 % of benzaldehyde found on the tobacco rod was transformed into CO and CO₂. Considering all smoking traps and the remaining butt after smoking, 72 % of the labelled benzaldehyde could be accounted for.

It has been shown for vanillin, glucose and benzaldehyde that labelled and unlabelled compounds behave in the same way [16].

Conclusion

A simple and fast on-line method – based on GC-reaction-continuous flow-mass spectrometry – has been developed which allows determining the ¹³C abundance of CO and CO₂ in the vapour phase of cigarette smoke from ¹³C tracer experiments with a relative standard deviation of ≤ 0.5 % (typical ≤ 0.3 %, about 3 ‰) in one analytical run. In a second run, the ¹³C abundance of total volatile carbon can be determined by oxidation of all ¹³C containing species to CO₂ prior to MS measurement.

For the non volatile or less volatile compounds glucose and vanillin, ¹³C recovery rates of 87 % and 94 % were calculated and their distribution on the different smoking traps was found to be very similar. About 60 % of vanillin and glucose were estimated to be oxidized to CO and CO₂ during combustion.

For the most volatile additive benzaldehyde, recovery rates of 72 % were determined. Due to migration onto the filter, only 36 % of labelled benzaldehyde was available for combustion in this experiment. As a consequence, distribution on the different smoking traps was significantly different.

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References

- [1] S. Stotesbury, H. Digard, L. Willoughby, A. Couch: “The Pyrolysis of Tobacco Additives As A Means of Predicting Their Behaviour in A Burning Cigarette”, Beitr. Tabakforsch. Int. 18, 147-163 (1999).
- [2] R.R. Baker, L.J. Bishop, S. Coburn, C. Liu: “Pyrolysis studies to assess chemical aspects of tobacco ingredients”, presentation at the 58th Tobacco Science Research Conference, Winston-Salem, North Carolina, September 2004.
- [3] S. Coburn, R.R. Baker, C. Liu: “The effect of nitrogenous compounds on the generation of formaldehyde from pyrolysis of sugars”, presentation at the CORESTA Smoke Science &

Product Technology Meeting, Stratford-upon-Avon, UK, September 2005.

- [4] S.J. Stotesbury, L.J. Willoughby, A. Couch: "Pyrolysis of Cigarette Ingredients Labelled with Stable Isotopes", *Beitr. Tabakforsch. Int.* 19, 55-64 (2000).
- [5] J.D. Green, J. Chalmers, P.J. Kinnard: "The transfer of tobacco additives to cigarette smoke: Examination of the possible contribution of pyrolysis products to mainstream smoke composition", *Beitr. Tabakforsch. Int.* 14, 283-288 (1989).
- [6] R.W. Jenkins Jr., R.H. Newman, M.K. Chavis: "Cigarette smoke formation studies", *Beitr. Tabakforsch.* 5, 299-301 (1970).
- [7] A. R. Swanson, M. W. Anders: "Method for the analysis of [¹³C]carbon monoxide by gas chromatography-mass spectrometry", *J. Chromatography*, 207, 365-372 (1981).
- [8] A. R. Swanson, M. W. Anders: "Gas chromatographic-mass spectrometric analysis of [¹³C]carbon monoxide", *J. Chromatography*, 234, 268 (1982).
- [9] J. E. Mak, W. Yang: "Technique for Analysis of Air Samples for ¹³C and ¹⁸O in Carbon Monoxide via Continuous-Flow Isotope Ratio Mass Spectrometry", *Anal. Chem.*, 70, 5159-5161 (1998).
- [10] U. Tsunogai, F. Nakagawa, F. Hachisu, Y. Yoshida: "Stable carbon and oxygen isotopic analysis of carbon monoxide in natural water", *Rapid Commun. Mass Sp.*, 14, 1507-1512 (2000).
- [11] U. Tsunogai, F. Nakagawa, D. D. Komatsu, T. Gamo: "Stable carbon and oxygen isotopic analysis of atmospheric carbon monoxide using continuous-flow isotope ratio MS by isotope ratio monitoring of CO", *Anal. Chem.*, 74, 6595-5700 (2002).
- [12] M.S. Torn, S. Davis, J.A. Bird, M.R. Shaw, M.E. Conrad: "Automated analysis of ¹³C/¹²C ratio in CO₂ and dissolved inorganic carbon for ecological and environmental applications", *Rapid Commun. Mass Sp.* 17, 2675-2682 (2003).
- [13] CORESTA Recommended Method 54: "Determination of nicotine and nicotine-free dry particulate matter in sidestream smoke using a fishtail chimney and a routine analytical/linear smoking machine", June 2002.
- [14] CORESTA Recommended Method 55: "Determination of carbon monoxide in the vapour phase of cigarettes sidestream smoke using a fishtail chimney and a routine analytical/linear smoking machine", June 2002.
- [15] I.T. Platzner: "Modern Isotope Ratio Mass Spectrometry", John Wiley & Sons, Chichester, UK, 449-456 (1997).
- [16] Unpublished results (2006).