Optimization of Nicotine Extraction from Tobacco Using Supercritical Fluid Technology with Dynamic Extraction Modeling

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The influence of particle size, cell geometry, and packing of the extraction cell was investigated for the extraction of nicotine from tobacco using supercritical CO_2 modified with methanol using the hot-ball model of Bartle et al. (1990). When the tobacco was powdered to a particle size of 125−355 μm, the optimum extraction time was reduced by 15 min. Within this particle size range, particle size did not influence the recovery of nicotine so that a time-consuming sieving step is not required. The presence of water during the extraction step, which was introduced either by the packing material or by addition to the mobile phase, is important in order to desorp the nicotine from the cellulose matrix in the shortest possible time.

Keywords: *Supercritical fluid extraction (SFE); tobacco, nicotine*

INTRODUCTION

Since the Drug Abuse Advisory Committee has classified nicotine as an addictive substance (Kleiner, 1994), this enables the U.S. Food and Drug Administration to control and monitor the levels of nicotine in cigarettes and tobacco. There are several reports on the extraction of nicotine from tobacco using liquid extraction techniques (Saunders et al., 1981; Severson et al., 1981; Sudan et al., 1984); however, since some of these suggest the use of aqueous buffers (Saunders et al., 1981; Sudan et al., 1984), subsequent liquid extractions are necessary to allow screening for pesticides using gas chromatography−mass spectrometry (GC−MS).

The use of supercritical fluids for the extraction of organic compounds from a wide variety of matrices is increasing due to the favorable properties of these fluids. Supercritical fluids have lower viscosities and higher solute diffusivities, hence improving the mass transfer and reducing the time needed for the extraction. The applicability of CO_2 for the extraction of nicotine from moist snuff has been demonstrated by Sharma et al. (1991), and the extraction of nicotine from tobacco as an industrial process has been patented (Roselius et al., 1979).

There are two factors which may limit the extraction of a solute from a matrix. First, the solubility of the solute in the supercritical fluid is usually not the limiting factor if the solute is present in low concentrations and the flow rates of the supercritical fluids are high enough to ensure that the solute concentration in the fluid is well below its solubility limit. Second, the mass transfer of the analyte out of the matrix may limit the extraction. This may be a matter of diffusion within a homogeneous medium or, as more likely in real samples, involves processes such as diffusion out of pores, migration from one adsorption site to another, and replacement of solute molecules on adsorption sites by fluid molecules. These processes were taken into account by Pawliszyn (1994) when he developed a kinetic model of supercritical fluid extraction (SFE).

The present paper describes the extraction of nicotine from tobacco using the model of dynamic extraction to investigate the effects of particle size, extraction conditions, the method of packing the cell, and cell geometry on extraction recovery profiles. These effects have been evaluated mathematically using the hot-ball model of Bartle et al. (1990).

The recoveries using SFE have also been compared with "accelerated solvent extraction". "ASE" is a new technique developed by Dionex Ltd., in which solvents are used at elevated temperatures and pressures in order to increase analyte solubility and reduce solvent viscosity which improves solvent penetration into matrix pores.

EXPERIMENTAL PROCEDURES

Instruments. All experiments were performed using a Jasco SFE system (Jasco U.K. Ltd., Great Dunmow, U.K.) equipped with two pumps to allow the use of modifier. An ethylene glycol/water mixture was used with a cooler to maintain the head of the model 980-PU carbon dioxide pump at −5 °C. The extraction vessels (internal volume of 10 or 1.67 mL, Jasco, U.K.) were kept at a set temperature in a model 860-CO column oven. A model 880-81 back-pressure regulator kept the entire system under a selected, constant back-pressure which is regulated by an electronic feedback regulator that is flow-independent. A six-port Rheodyne valve was installed in place of an injection valve which enabled both dynamic and static extraction conditions to be employed.

All extracts were analyzed by high-performance liquid chromatography (HPLC) using a modular system consisting of an SP 8100 gradient pump (Spectra-Physics Analytical, Stone, U.K.) and a model 3100 UV-absorbance detector (LDC/Milton Roy, Stone, U.K.). Chromatographic data were collected using a HP 3395 integrator (Hewlett-Packard, Stockport, U.K.).

The tobacco was powdered using an electric mill M20 IKA-universal mill (Sartorious, Epsom, U.K.)

The powdered tobacco was sieved on a Fritsch "analysette" type 03.502 (Christison Scientific Equip. Ltd., Gateshead, U.K.) using test sieves (Endocotts Ltd., London, U.K.) with a diameter of 20 cm and the following mesh sizes: 355, 250, 180, and 125 μm.

Materials. Nicotine was purchased from Aldrich (Gillingham, U.K.). Methanol, 2-propanol, acetone, and acetonitrile (all HPLC grade) were obtained from Fisons (Loughborough, U.K.). Dark shag tobacco was purchased in Spain. α-Cellulose was obtained from Sigma (Poole, U.K.).

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Table 1. Levels of Water and Nicotine in Different Tobacco Fractions

tobacco fraction (μm)	fraction	water (%)	nicotine level[a] (mg/g)
125−180	1	10.0	18.02
180−250	2a	12.4	20.86
180−250	2b[b]	0	19.78
250−355	3	14.9	22.71

[a] Determined by SFE; level calculated on dry weight basis. [b] Fraction 2b was dried at 60 °C for 24 h.

Preparation of the Tobacco. Fresh tobacco was loosely distributed in the electric mill and powdered twice for 1 min. About 25 g of powdered tobacco was then transferred onto the top sieve (355 μm), attached onto the Fritsch analysette, and sieved for 15 min at an amplitude of 3. The sieves were then emptied and cleaned gently with a brush and the tobacco fractions returned to the appropriate sieve. The sieving process was continued for another 15 min.

The water content of the powdered tobacco fractions was determined by drying the fractions in an oven at 60 °C for 24 h to a constant dry weight. Table 1 lists the water content of the fractions as used for SFE and their nicotine content, which was determined by time-dependent extractions using the hot-ball model equation (eq 1).

Supercritical Fluid Extraction. *Packing 1.* First, 1.5 g of α-cellulose was packed into the 10 mL extraction cell, and then 0.5 g of tobacco was thoroughly mixed with 1.0 g of α-cellulose and packed into the cell. This was topped up with 1.0 g of α-cellulose to minimize the void volume. The extraction cell was then tightened and connected vertically into the SFE unit so that the 1.5 g of α-cellulose was on top and left for 10 min for temperature equilibration in the oven. The pump delivering the cooled carbon dioxide was then switched on, and the system was pressurized. After pressurization, the modifier pump was switched on and the extraction was started. Nicotine was collected in 5 mL of methanol in a vial which was wrapped with aluminum foil to avoid exposure of the extracts to light. The vial was cooled in an ice−water bath. Following the extraction, the contents in the receiver were transferred to a 20 mL volummetric flask and made up to volume with methanol. The extracts were diluted with the mobile phase and filtered using a 0.45 μm nylon filter before HPLC analysis.

Packing 2. α-Cellulose (3.5 g) was packed into the 10 mL extraction cell, and 0.5 g of tobacco was added. The extraction cell was connected to the SFE unit as before so that the 3.5 g of cellulose was at the top. The packing of the cell required approximately 10 min.

The packing of the 1.67 mL cell was easier as only 0.5 g of tobacco was filled into the cell.

The extracts were separated on a 4.6 mm inside diameter (i.d.) × 25 cm Hichrom ODS 5 μm column using the method of Zuccaro et al. (1993). The nicotine levels were calculated on a dry weight basis.

Gas Chromatography and Identification. The tobacco extracts were analyzed on an HP 59970 C GC−MS system (Hewlett-Packard, Stockport, U.K.). Hydrogen was used as carrier gas (flow rate of 1 mL/min), and the injector and transfer line were kept at 250 and 280 °C, respectively. The chromatographic separation was performed on a 50 m DB-5 bonded phase (0.25 μm thickness, 0.22 mm i.d.) capillary column (SGE, Milton Keynes, U.K.). The initial temperature was kept at 60 °C for 2 min and was raised at increments of 10 °C/min to 150 °C and held for 10 min. This was followed by a further increase of 5 °C/min to a temperature of 200 °C, which was held for 10 min. A final increase of 5 °C/min brought the temperature to 275 °C.

The peaks were identified using the library search facility.

Collection Efficiency. The extraction cell was completely filled with α-cellulose and extracted for 10 min using the following conditions: 8 mol % methanol, 50 °C, 200 kg/cm^2, and a 3 mL/min total flow rate. The cell was disconnected, opened, and spiked with 100 μL of approximately 0.2 g/mL nicotine standard, which correlates to the amount present in 1 g of tobacco. The nicotine was then extracted for 30 min using different amounts of methanol as collection solvent.

Time-Dependent Extractions. Tobacco was packed into the cell in the same way as described under Supercritical Fluid Extraction; however, samples were taken after the following time intervals: 5, 10, 20, 30, 60, 90, 120, and 150 min. This procedure has been described previously for studying the extraction of free fatty acids from soy and cottonseed meals (Fischer et al., 1995). The nicotine present in the original sample was calculated using the equation (Bartle et al., 1990a)

$$m_0 = m_1 + m_2^2/(m_2 - m_3) \quad (1)$$

in which m_0 is the total amount of nicotine in the original sample, m_1 is the extracted amount after a time t_1, and m_2 and m_3 are the amounts of nicotine extracted in subsequent, equal time intervals t_2 and t_3. The extracted mass, m_1, should be taken from the nonexponential part of the plot $\ln(m/m_0)$ versus time, whereas m_2 and m_3 should be taken from the exponential part. This is important for the correct calculation of m_0 in eq 1. After m_0 is calculated, the plot of $\ln(m/m_0)$ versus time is plotted to ensure that the masses m_1, m_2, and m_3 are taken at the correct times. The value of m represents the mass of nicotine that remains in the matrix after a certain time.

Equation 1 is derived from the hot-ball model, which was used by Bartle et al. (1990a) to evaluate the effects of matrix shape, size variation, and solublity limitation on dynamic extraction. The model makes three assumptions, which, when fulfilled, result in complete conformity of the extraction behavior with the model. First, the particles of the matrix should be spherical, the size of the particles should have a narrow size distribution, and the analyte is assumed to be evenly distributed within the particles. Second, the flow rate is fast enough to ensure that the analyte concentration is zero at the particle's surface. Third, the analyte moves through the matrix by diffusion. The resulting plot of $\ln(m/m_0)$ versus time of a dynamic extraction is characterized by a steep initial decline which is continued by an exponential decay whose slope is $1/t_c$ and is dependent on particle size and the diffusion coefficient, and an intercept (I) of a theoretical value of −0.49977. When real samples are extracted, the three assumptions are often not fulfilled and hence influence the theoretically derived plot. If the particle has an irregular shape and hence a greater surface:volume ratio, the initial fall will be larger; the slope however remains the same. A smaller particle size, which can be attained by grinding, will allow faster extraction which can be observed by a larger slope. The grinding process presses the solute to the particle surface and thus yields a steeper initial fall and a longer time to establish a smooth concentration profile. The time, $0.5t_c$, is the time when the curve $\ln(m/m_0)$ versus time becomes linear and a smooth concentration profile is established. Another deviation of the theoretical curve can occur when the extraction is solubility-limited. This reduces the initial rate of the extraction and therefore delays the establishment of the linear portion.

Liquid Extraction. The liquid extraction was performed according to Saunders et al. (1981). Tobacco (0.5 g) was extracted for 24 h under constant agitation with 12 mL of 25 mM of KH_2PO_4 buffer (pH 7.8). The extract was filtered using a Buchner flask and funnel with a Whatman no. 1 filter paper. The extract was diluted for analysis. This lengthy extraction procedure was chosen to ensure that all the nicotine is extracted in order to provide a comparison for the SF extractions.

Accelerated Solvent Extraction. The Jasco SFE unit was rebuilt to allow accelerated solvent extraction. Tobacco (0.5 g) was weighed into the 10 mL extraction cell and the cell connected vertically into the SFE unit. A mixture of 12 mL of methanol and 41.2 mM KH_2PO_4 (2:3) was pumped into the cell, and the temperature was set to 100 °C and the back-pressure regulator to 140 kg/cm^2. The system was left for 5 min to heat up and 10 min for temperature equilibration. Ten minutes were then allowed for static extraction, which was

Table 2. Recoveries of Nicotine in Milligrams per Gram of Tobacco Using Different Modifiers

modifier	tobacco fraction (μm)	recovery[a] (mg/g)
methanol	180 (air-dry)	11.08
2-propanol	180 (air-dry)	10.98
acetone	180 (air-dry)	9.12
acetonitrile	180 (air-dry)	10.14
methanol	180 (dry)	13.62
methanol/H_2O (97.5:2.5)	180 (air-dry)	11.22
methanol/H_2O (97.5:2.5)	180 (dry)	13.39

[a] Recovery calculated on dry weight basis. Conditions were as follows: 200 kg/cm^2, 50 °C, 3 mL/min total flow, 8 mol % modifier, 10 min extraction.

followed by releasing the pressure by changing the setting on the back-pressure regulator. The cell was flushed with 6 mL of new solvent mixture and purged for 10 min using CO_2, before being flushed with another 6 mL of solvent and finally purged with CO_2 for 10 min. The extracts were transferred into a 50 mL volumetric flask and made up to volume.

RESULTS AND DISCUSSION

Initially, several extractions as described under Collection Efficiency were conducted to investigate the efficiency of the solvent-trapping step. As pointed out by Hawthorne et al. (1993), it is crucial to test the trapping efficiency as low recoveries could be due to inefficient trapping. The experiments revealed that 3 mL of methanol trapped only 92.6% of the nicotine; therefore, the amount was increased to 5 mL of methanol which increased the trapping efficiency to 97.3%.

Tobacco (fraction 2b) and the following conditions were used to determine the most appropriate modifier: 8 mol % modifier, 200 kg/cm^2, 50 °C, and a 3 mL/min total flow rate. Table 2 lists the recoveries which show that methanol and 2-propanol seem to be the most efficient modifiers. Additionally, the influence of water content on the extraction recoveries was investigated to see whether it is necessary to adjust the moisture level of the tobacco or whether the tobacco can be used as received. As seen from Table 2, for air-dry tobacco, methanol and the methanol/water mixture were equally effective (11.08 and 11.22 mg/g), and similarly for dry tobacco (13.62 and 13.39 mg/g). However, the higher recoveries of the dried tobacco after a 10 min extraction are due to the drying process, which transports nicotine to the outer surface and makes it more readily extractable. This further proves that the extraction conditions allow even more nicotine than 11.2 mg/g to be extracted with a 10 min extraction. If the extraction had been solubility-limited, the same recoveries would have been obtained in both cases. Hence, for the subsequent time-dependent extractions, the same extraction conditions, as above and with methanol as a modifier, were used.

Influence of Particle Size. For all the fractions listed under Preparation of the Tobacco, time-dependent extractions were carried out to investigate the influence of particle size on extraction profile. The extracts were analyzed, and the results obtained were used to calculate $\ln(m/m_0)$. The initial amount was calculated by extrapolation using the model of Bartle et al. (1990). Figure 1 shows the calculated value of $\ln(m/m_0)$ plotted versus extraction time.

Considering the results of the dried tobacco fraction 2b (180−250 μm) first, the curve of $\ln(m/m_0)$ versus time has the form of the hot-ball model, characterized by its initial steep slope which becomes linear after a certain

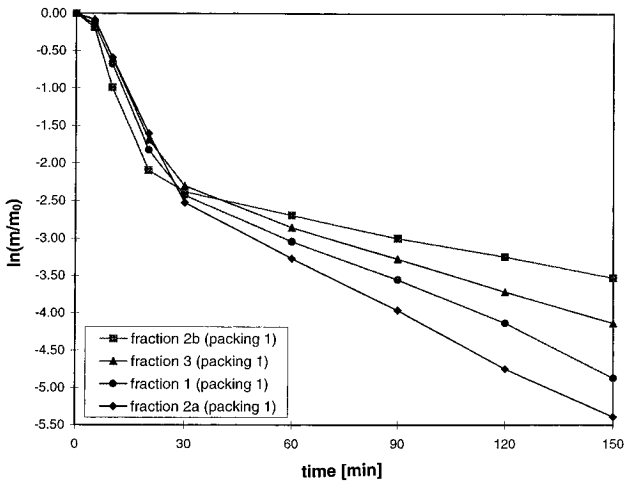

Figure 1. Dynamic extraction of nicotine by supercritical fluid CO_2 and methanol from tobacco prepared into four fractions as in Table 1. Conditions were as follows: packing 1, 200 kg/cm^2, 50 °C, 8 mol % methanol.

Table 3. Values Characterizing the Linear Part of Plots of $\ln(m/m_0)$, Obtained by Calculation or Graphical Evaluation, for the Different Tobacco Fractions

tobacco fraction (μm)	nicotine recovery[a] (%)	calcd slope (min^{-1})	calcd $0.5t_c$ (min)	graphical value $0.5t^c$(min)	intercept (I) of the linear part
125−180	91.2	0.0187	26.7	24.9	−1.886
180−250	92.0	0.0240	20.8	30.0	−1.815
180−250 (dry)	90.8	0.0095	52.6	22.3	−2.117
250−355	90.0	0.0142	35.2	32.6	−2.003

[a] After extraction for 30 min.

time. According to the model, the time at which the linear portion starts should be $0.5t_c$. The slope of the linear portion is $1/t_c$, and t_c can therefore be calculated. In the case of the dried tobacco, it was calculated to be 52.6 min. However, the graphical evaluation gives a value of ca. 22 min. This indicates that the slope of dried tobacco should be steeper, and hence, diffusion out of the matrix seems to be a more complex process than assumed by the hot-ball model. This limitation has already been mentioned by Bartle et al. (1992). The graph also shows by the initial steep fall that the dried tobacco yielded the highest recovery, which is caused by the drying process transporting more nicotine to the proximity of the surface. The high value of the intercept (I) confirms the nonuniform distribution of the tobacco; however, this could also be caused by the particle shape being nonspherical.

Looking at the remaining graphs in Figure 1, one can evaluate the influence of the particle size on the extraction profile. Theoretically, it is expected that fraction 1 (125−180 μm) should have the steepest slope and the linear part should be established in the least time. Using the mean radii of the different fractions, the theory predicts a 2-fold increase in the slope when fraction 3 (r = 151.3 μm) is compared to fraction 2 (r = 107.5 μm) as listed in Table 3. The slope in the graph increases only 1.7 times, which shows quite good correlation to the theory. If fraction 1 (r = 76.3 μm) is compared to fraction 2, one expects again a 2-fold increase for the smaller particle size; however, one observed a 1.3-fold decrease. As already seen from the dried tobacco, the slopes present a more complex process than diffusion through a homogeneous matrix; therefore, it seems the influence of the particle size is not the dominant factor in the extraction of nicotine.

Table 4. Nicotine Extracted from Tobacco Fraction 2a during the Time-Dependent Extraction and the Calculated Values of $\ln(m/m_0)$

time (min)	extracted nicotine (mg/g)	total amount of nicotine (mg/g)	remaining amount of nicotine (mg/g)	$\ln(m/m_0)$[a]
5	1.75	1.75	19.11	−0.088
10	7.55	9.30	11.56	−0.590
20	7.37	16.67	4.19	−1.605
30	2.52	19.19	1.67	−2.525
60	0.876	20.07	0.79	−3.269
90	0.397	20.46	0.40	−3.962
120	0.216	20.68	0.18	−4.747
150	0.086	20.77	0.09	−5.392

[a] $m_0 = 20.86$ mg/g.

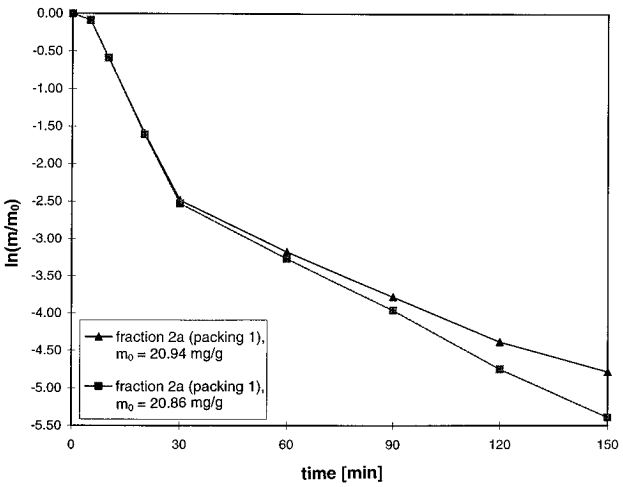

Figure 2. Influence of m_0 on the slope of $\ln(m/m_0)$ versus time.

Moreover, after 30 min, around 90% is extracted from all four fractions as seen from Table 3, and hence, the sieving step can be omitted.

Table 3 also lists the calculated times $0.5t_c$ at which the linear part should approximately start; however, these do not correlate to the graphical evaluation. The graphical results appear to be more logical as they demonstrate that the largest particle took the longest time to establish the linear portion. Additionally, as the intercept (I) is influenced by both the nonuniform distribution and the nonspherical particle shape, no qualitative conclusions should be drawn.

Table 4 lists the recovery levels from fraction 2a during a time-dependent extraction. Subsequently, m_0 was calculated using the values in Table 4 and the following time intervals: m_1, $t_1 = 0-30$ min; m_2, $t_2 = 30-90$ min; m_3, $t_3 = 90-150$ min; $m_0 = 20.86$ mg/g. The graph in Figure 1 confirms that m_2 and m_3 are taken from the exponential part. However, in order to show how the graph can be influenced by the value calculated for m_0, different time intervals were used to calculate m_0: m_1, $t_1 = 0-60$ min; m_2, $t_2 = 60-90$ min; m_3, $t_3 = 90-120$ min; $m_0 = 20.94$ mg/g. The graphs in Figure 2 demonstrate how a difference between the m_0 values (0.38%) influences the slopes. The slope for the m_0 value of 20.84 mg/g is 1.2 times greater than that for the m_0 value of 20.94 mg/g. Therefore, when interpreting the effects of different parameters on the slopes, one has to take this uncertainty into account.

Influence of Extraction Conditions. Fraction 3 (250−355 μm) was used to investigate the influence of pressure and modifier levels upon the extraction efficiency, and the calculated $\ln(m/m_0)$ values are listed in Table 5. Increasing the level of methanol from 8 to

Table 5. Values Characterizing the Linear Part of Plots of $\ln(m/m_0)$, Obtained by Calculation, for the Different Extraction Condition

extraction conditions[a]	nicotine recovery[b] (%)	calcd slope (min^{-1})	intercept (I) of the linear part
200 kg/cm^2 and 8 mol % methanol	90.0	0.0142	−2.003
200 kg/cm^2 and 12 mol % methanol	90.2	0.0187	−1.881
300 kg/cm^2 and 12 mol % methanol	89.8	0.0159	−2.022

[a] 50 °C, 3 mL/min total flow rate. [b] After extraction for 30 min.

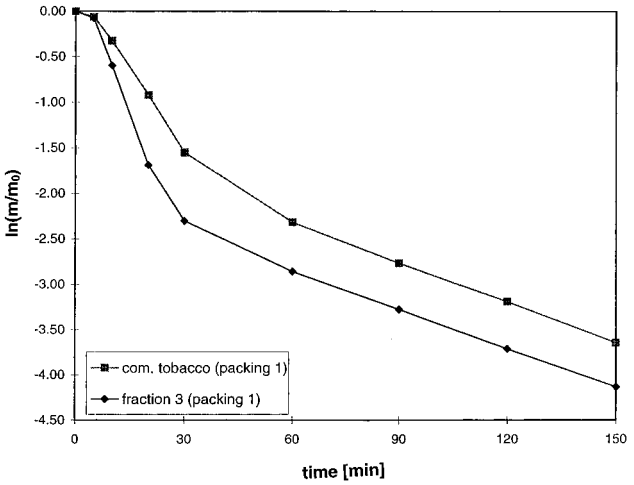

Figure 3. SFE of untreated and powdered tobacco (fraction 3).

12 mol % at 200 kg/cm^2 did not increase the initial steep fall, and it actually decreased slightly, which can be seen from the higher value of the intercept (1). This was caused however by the inhomogeneous nature of the sample rather than being influenced by the modifier level. Next, the modifier was kept at 12 mol % methanol and the pressure increased to 300 kg/cm^2. As seen in Table 5, the initial steep fall remained the same, indicating that maximum solubility was reached using 8 mol % methanol, 50 °C, and 200 kg/cm^2.

An untreated commercial tobacco (0.5 g) with a water content of 17.68% was extracted using the same conditions, and the cell was packed according to packing 1. The graph $\ln(m/m_0)$ versus time is plotted in Figure 3, showing also the graph obtained from the extraction of tobacco fraction 3 for comparison. Theoretically, one would expect a less negative intercept (I) for the untreated tobacco as the nicotine should be more evenly distributed compared to the powdered sample. At the same time, the untreated tobacco deviates more from the ideal sphere, and hence, this could cause a more negative intercept. However, this is only true if the surface-to-volume ratio increases (Bartle et al., 1990) which is not the case for the untreated tobacco as the strands used actually have a lower surface:volume ratio. Additionally, $0.5t_c$ should be larger as the linear part takes longer for larger particles to be established and the slope is expected to be flatter.

As seen from Figure 3, the intercept is less negative, hence confirming the more even distribution of nicotine in the tobacco matrix compared to that of the powdered tobacco. Therefore, the time needed to reach the linear part is greater compared to that of fraction 3 which is also in agreement with the theory. This means that, if tobacco is extracted without prior treatment, the opti-

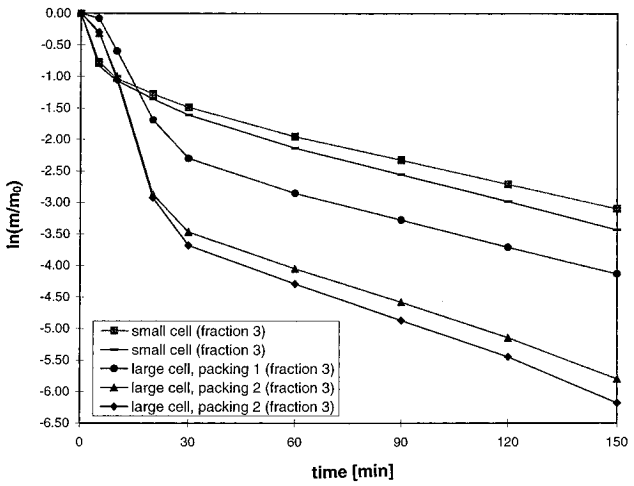

Figure 4. Influence of cell geometry and packing upon the extraction profile of nicotine from tobacco fraction 3.

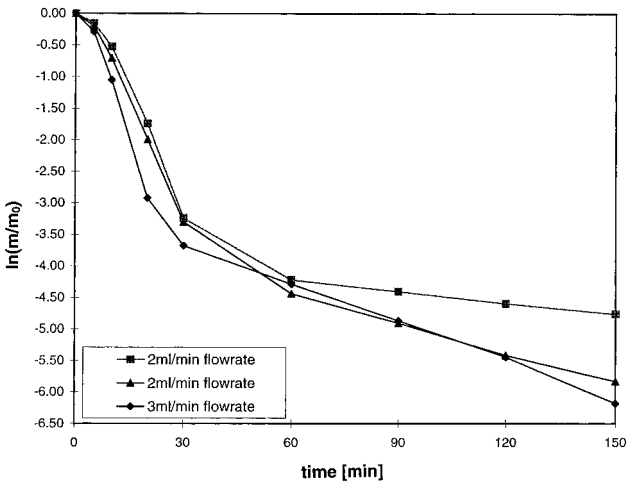

Figure 5. Influence of flow rate on the extraction profile (fraction 3, packing 2).

mum extraction time is longer compared to the time necessary to extract the powdered tobacco. The optimum extraction time is the time at which the linear portion of the graph is reached, as the majority of the tobacco is extracted at that time. The extraction rate slows considerably, which means that low standard deviations are obtained in repetitive experiments.

Influence of Packing the Cell and Cell Geometry. In order to investigate the influence of the method of packing the cell, time-dependent extractions using packing methods 1 and 2 were carried out. Tobacco fraction 3 and the same conditions as earlier were used to conduct duplicate time-dependent extractions. As seen in Figure 4, packing 2 moved the graph downward without changing the slope significantly. The reason for this was that more nicotine had been extracted by the time the linear portion was reached, suggesting that the extraction using packing 1 was solubility-limited. This however can be excluded as in both extractions the same extraction conditions were used. It seems as if the profile of the initial slope is influenced by the length of the distance the nicotine has to be transported before being flushed out of the cell. Hawthorne et al. (1993) reported insufficient flushing out of the extraction cell when the cell was horizontally connected or when vertically positioned having a large void volume and being extracted from bottom to top. All these factors can be dismissed as irrelevant, as the extraction cell was vertically mounted, the void volume was filled with α-cellulose, and the extraction occurred from top to bottom.

However, α-cellulose with its hydroxyl groups interacts strongly with nicotine via hydrogen bonding, causing the retention of the nicotine. Therefore, the additional α-cellulose in packing 1 after the tobacco is probably responsible for the slow desorption kinetics of the nicotine, which influences the amount being extracted after 30 min. However, it leaves the time at which the linear part of $\ln(m/m_0)$ starts unchanged.

A small cell with an internal volume of 1.67 mL was therefore used to perform replicate time-dependent extractions as before. The results are also plotted in Figure 4, and surprisingly, the graph moved upward. The shape of the initial steep slope changed, and the same shape was predicted by Pawliszyn (1994) in his theoretic kinetic model when the flow rate was infinite, suggesting that the maximum amount of nicotine was recovered. The cell dimension of the 1.67 mL cell is 10 × 0.46 cm; therefore, the distance required by the nicotine to reach the cell exit is longer than with the 10 mL cell (6.1 cm × 1.45 cm). The first interval recovered roughly twice as much nicotine compared to the 10 mL cell as the linear velocity using the small cell is ca. 3 times faster. Therefore, the cell is flushed more often, causing greater recovery in the beginning. However, as soon as the water present in the tobacco is extracted, the extraction rate slows considerably. This means that after 30 min only 76.7% nicotine is extracted using the 1.67 mL extraction cell compared to 90% with the 10 mL extraction cell (packing 2).

Influence of Flow Rate. As the flow rate of 3 mL/min using the 1.67 mL cell did not accelerate the extraction, a lower flow rate using the 10 mL cell was investigated to determine whether the flow rate can be reduced to 2 mL/min. Time-dependent extraction was performed using tobacco fraction 3 with a flow rate of 2 mL/min. Figure 5 shows the plot $\ln(m/m_0)$ versus time, including the extraction with a 3 mL/min flow rate for comparison. The slopes of the two extractions using a 2 mL/min flow rate deviate quite considerably from each other which is caused by the inhomogeneous nature of the tobacco. It is clearly demonstrated that the initial steep fall is less pronounced when the flow rate is reduced and additionally that $0.5t_c$ is greater. The graph shows the theoretical features of a solubility-limited extraction, which are a reduction at the beginning of the extraction, a decrease in the slope, and a move of the slope upward on the graph (Cotton et al., 1993).

The flow rate of 2 mL/min was not sufficient to reach the linear part in the least time. Therefore, higher initial recoveries could be achieved with the 10 mL cell using flow rates larger than 3 mL/min. However, the use of a 4 mL/min flow rate causes the trapping efficiency to decrease. An experiment using a 4 mL/min flow rate to test the trapping efficiency revealed that only 91.4% was trapped. The increase in flow rate would further only be beneficial for the first interval as in the subsequent intervals the flow rate does not influence the recovery. A flow rate of 3 mL/min seemed therefore to be the optimum flow rate for 0.5 g of tobacco.

Influence of Water Content. The initial experiments (Table 2) led to the conclusion that there was no difference between the extraction of air-dry and dried tobacco when using the large cell packed with α-cel-

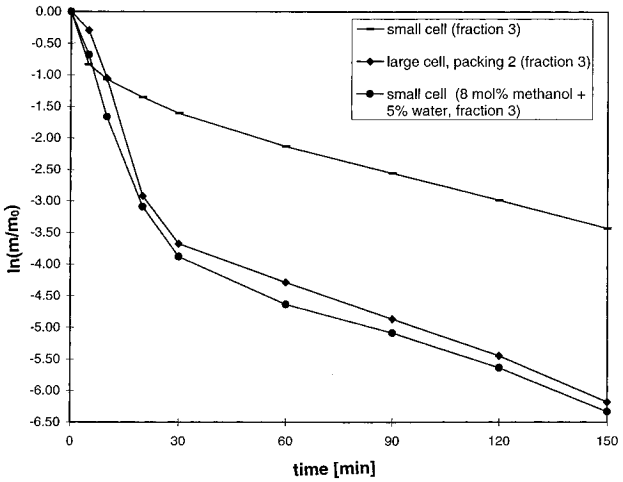

Figure 6. SFE of nicotine from tobacco fraction 3 using the large cell (packing 2) and the small cell.

lulose. The α-cellulose was therefore dried at 100 °C to constant weight and showed that it had a water content of 2.76%.

Therefore, 0.069 g of water from the α-cellulose was present in the extraction of dried tobacco in Figure 1, and this amount seemed to be sufficient to yield the same recovery as the extraction of air-dry tobacco, which has a total water content of 0.130 g, within 10 min. This leads to the assumption that the lower recovery in the beginning using packing 1 compared to packing 2 (Figure 4) must be caused by the presence of additional α-cellulose, which introduces more adsorption sites. Additionally, when tobacco fraction 3 was extracted using pre-extracted α-cellulose, the recovery of nicotine after 30 min was about the same as the recovery using the small cell. One can therefore conclude that water is necessary to allow the shortest possible extraction time.

In order to achieve the same recovery with pre-extracted α-cellulose, the water content had to be increased to 5 vol % in the methanol when 8 mol % modifier was used. Using this mixture for the extraction of tobacco held in the small cell, one would expect the same extraction profile for the small cell as for the large cell (packing 2). Figure 6 shows the extraction profile of tobacco fraction 3 when 5 vol % water is added to the modifier using the small cell and for comparison the extractions using the large cell and the small cell without addition of water to the modifier. The extraction profile using the small cell changed dramatically, and 97.5% is recovered after 30 min which is the same as with the large cell.

Constituents of Tobacco. As water and the addition of α-cellulose have a great influence on the recovery, this can be used to selectively extract constituents of tobacco such as flavorings, pesticides, and other contaminants. Additionally, around 50% less nicotine is recovered when dried tobacco is extracted with methanol as modifier without further water addition.

Screening for pesticides in tobacco is necessary and will be even more important when transgenic tobacco plants are introduced, which are resistant to the herbicide bromoxynil, and higher levels can be applied to the crop without damaging the plant (MacKenzie, 1994). In order to screen for a wide range of constituents, tobacco fraction 3 was extracted using the large cell (packing 2). The collected extract was evaporated and dissolved in 200 μL of dichloroethane and analyzed by GC−MS. The following compounds were identified using the reference library: 2-methylphenol, 3-methylphenol, 2,4-dimethylphenol, 3,4-dimethylphenol, 4-ethyl-2-methoxyphenol, dehydroacetic acid (*), 2,6-bis(1,1-dimethylethyl)-4-methylphenol (*), 2-ethyl-1,1′-biphenyl, [*R*-[*R**,*R**-(*E*)]]-4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one, and [*S*-(*E*,*Z*,*E*,*E*)]-3,7,11-trimethyl-14-(1-methylethyl)-1,3,6,10-cyclotetradecatetraene. Two of the above substances (*) are listed as pesticides in the reference library. Benzoic acid could be identified in the extract when ethyl acetate was used as solvent.

Table 6. Recoveries of Nicotine after Extraction for 30 min

tobacco	packing	recovery (%)
1	1	91.2
2a	1	92.0
3	1	90.0
3	2	97.5
3	small cell	77.4
3	small cell	80.0

Table 7. Recoveries of Nicotine Using Different Extraction Methods

method	no. of repetitions	recovery (mg/g)	rel SD (%)
SFE[a]	6	22.71	1.38
SFE[b]	6	23.06	2.08
liquid extraction	6	22.63	2.14
ASE	3	21.35	2.93

[a] 10 mL extraction cell (packing 2). [b] Small cell.

Comparison of Different Extraction Methods. As seen from the graphs, the linear part using both the small and large cell (packing 2) is established after 30 min, and hence, using 30 min dynamic extraction, one recovers the majority of the nicotine. Good standard deviation should be achieved with repetitive extractions. Table 6 lists the recoveries after 30 min obtained in the time-dependent extractions using the extrapolated values of m_0 as reference values.

Subsequently, six extractions each using the small and large cell were performed with the following conditions: 8 mol % methanol, 50 °C, 200 kg/cm^2, and a flow rate of 3.0 mL/min. Additionally, six extractions according to Saunders et al. (1981) and three extractions using the new accelerated solvent extraction method using fraction 3 were conducted for comparison.

As seen from Table 7, the SFE using both cells and liquid extraction showed excellent correlation. However, ASE showed higher relative standard deviation which may be due to the short period for temperature equilibration and to the fact that the purging with CO_2 was not as efficient as high-pressure purging with nitrogen as carried out by Dionex.

CONCLUSIONS

Use of the hot-ball mathematical model allowed the calculation of the original mass, m_0, of nicotine in the tobacco sample and hence allowed optimum extraction times to be determined. The comparison of these results with those of liquid solvent extraction showed excellent correlation.

The resulting graphs however have to be evaluated carefully in order not to draw wrong conclusions, as the model does not account for all parameters influencing the extraction. Bartle et al. (1992) pointed out that real SFE systems are complex in terms of geometry, solute distribution, and the effect of the matrix on the extractability of the solute. A comprehensive treatment would

therefore be daunting (Bartle et al., 1992). Nevertheless, the model is an excellent help in the calculation of the initial mass. If more than one compound (e.g. pesticides) needs to be determined, the hot-ball model allows after an initial time-dependent extraction the initial amounts of each compound to be calculated.

The experiments showed that the particle size had no major influence upon 30 min extractions as after 30 min 92.0% nicotine was extracted from fraction 2 and 90.2% from fraction 3 and 91.1% from fraction 1. One can conclude that no time is gained by sieving the tobacco, whereas grinding achieved a 10% higher recovery and hence is beneficial. Time-dependent extractions must be carried out initially in order to calculate the original mass of nicotine in a particular type of tobacco.

The experiments also showed that it is important to determine the water content of samples and any additives (e.g. α-cellulose) added to the extraction cell. The influence of these parameters can be used to introduce additional selectivity and hence makes SFE very attractive for selective determination of compounds.

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