



Surface Enhanced Raman Spectroscopic Analysis of Nicotine Release from Oral Nicotine Pouches in Artificial Saliva

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Altria



Introduction

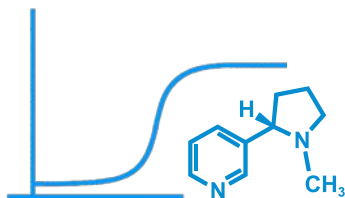


Nicotine Pouch Products

Growing category
in the smoke-free market

Tobacco-leaf-free pouches,
containing either synthetic or
tobacco-derived nicotine

They come in **various nicotine
levels and flavors**



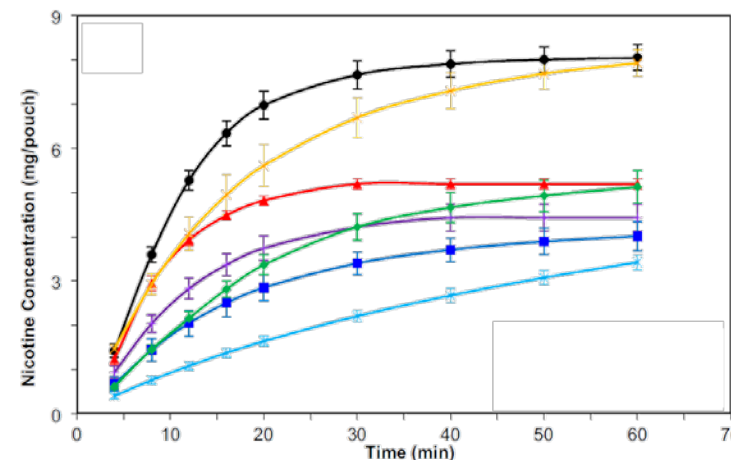
Accurate determination of nicotine
released from oral tobacco products

Essential for understanding

- ✓ Nicotine exposure
- ✓ Product performance
- ✓ Formulation behavior

Robust analytical methods

capable of precise and time-
resolved measurements



PLATT, Sean P., et al. Dissolution and physical characterization
of oral nicotine pouch products. *Scientific Reports*, 2026.

- Characterizing nicotine release kinetics
- Supporting formulation optimization
- Enabling meaningful product comparisons



Dissolution apparatuses and LC-based detection



USP-4 Apparatus (Flow Through Cell)

- MILLER, J. H., et al. Method development and validation of dissolution testing for nicotine release from smokeless tobacco products using flow-through cell apparatus and UPLC-PDA. *Journal of Chromatography B*, 2020.
- ALDEEK, F., et al. Dissolution testing of nicotine release from OTDN pouches: product assessment and product-to-product comparison. *Separations*. 2021



USP-1 and USP-2 Apparatus

- RAHMAN, Z., et al. Potential application of USP paddle and basket dissolution methods in discriminating for portioned moist snuff and snus smokeless tobacco products. *AAPS PharmaSciTech*, 2021



Small-volume Sinkers Setup

- LI, X., et al. Evaluation of factors impacting nicotine permeation in oral nicotine pouch products. *ACS Omega*, 2025
- KNOPP, M. M., et al. Development of a model to predict nicotine pharmacokinetics from oral nicotine pouches. *Scientific Reports*, 2026

Liquid Chromatography (LC) Detection

Regardless of the apparatus employed, the majority of dissolution studies rely on LC to quantify nicotine in the collected dissolution fractions.



*UPLC-PDA: Ultra-high Performance Liquid Chromatography (UPLC)-Photodiode Array (PDA)

Drawbacks of conventional LC analysis

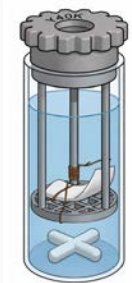
- Time-intensive workflow
- High reagent- and solvent-consumption



LC-based nicotine detection

LC-based detection of released nicotine

Nicotine dissolution
in a mucin containing
artificial saliva



Dissolution Fraction
Collection



Dilution



Precipitation



Centrifugation



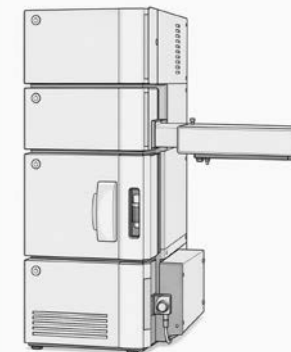
Filtration



Injection



UPLC-PDA measurement
Run time: ~10 min



Determination of released nicotine using LC-based detection for a standard dissolution study entails:

- 12 replicates per sample. 9 fractions per replicate yielding 108 injections
- With QC runs, ~140 injections → ~ 1400 minutes → ~ 23 hours
- Add hands-on work (preparing samples and mobile phase)



Raman spectroscopy as an alternative

Vibrational Spectroscopy

for analysis of dissolution fraction in an aqueous environment

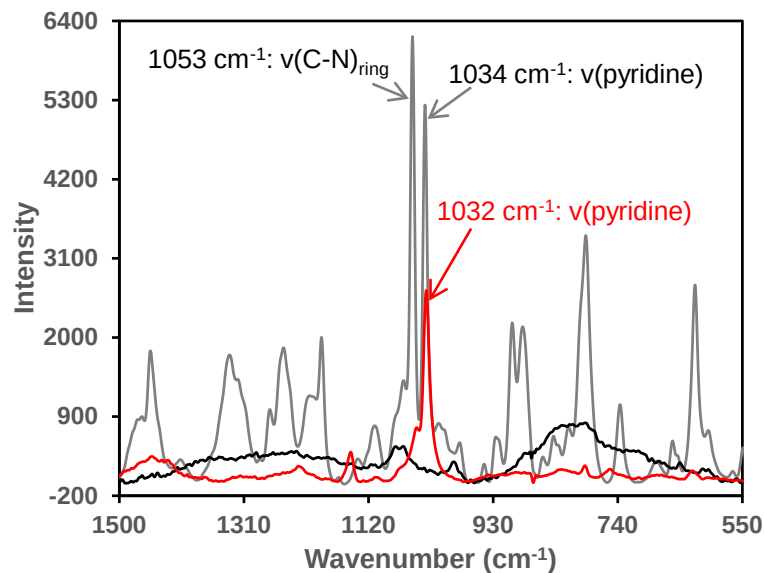
IR or NIR spectroscopy

Unsuitable due to the strong interference from water peaks

Raman spectroscopy

Free from water-related interference

Conventional Raman vs. SERS



Conventional Raman:
Nicotine bitartrate powder

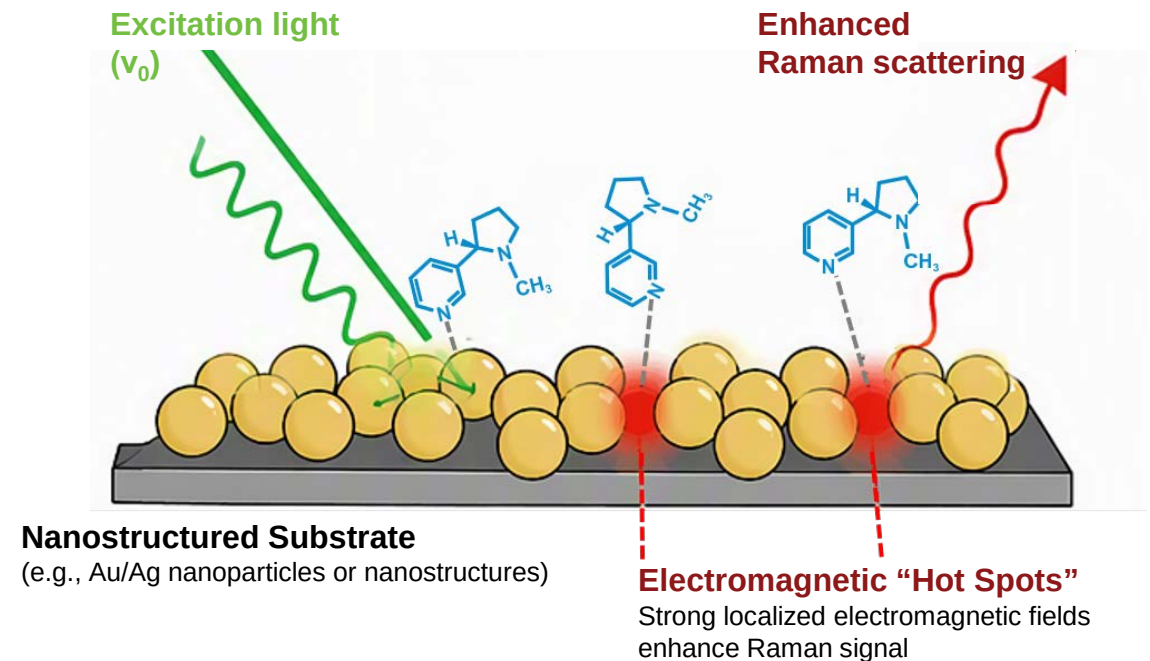
Conventional Raman:
61.6 μM Nicotine in
artificial saliva buffer

SERS:
61.6 μM Nicotine

61.6 μM = 10 μg/mL

Surface-Enhanced Raman Spectroscopy (SERS)

- Enhance Raman signal of molecules adsorbed on a metal surface
- Enhancement factor: up to 10^{12} ($10^4 - 10^{12}$) times



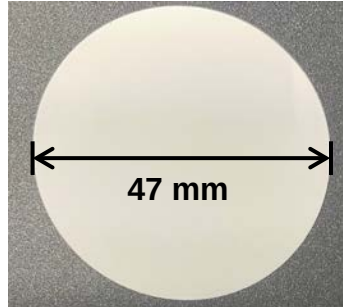
- Nicotine is SERS-active: Pyridine nitrogen atom
- Other ingredients in nicotine pouch exhibit minimal SERS response: lack nitrogen or sulfur functional groups

SERS provides both high sensitivity and selectivity

SERS Method Development

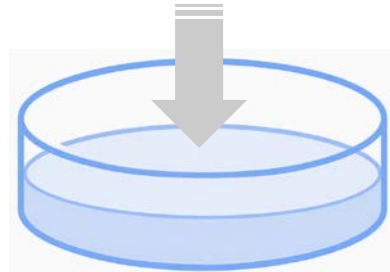
Cellulose acetate filter paper

- Pore size: 0.45 μm
- Diameter: 47 mm



Au (gold) reduction

- 12 mL gold precursor
 - HAuCl_4 (10 mM)
- 12 mL reducing agent:
 - *o*-methylhydroxylamine hydrochloride (50 mM)
- 24 h at room temperature



Filter paper loaded with Au nanostructures

- Easily fabricated over large areas
- Scaled to various sizes
- Ideal for the frequent measurements required in dissolution testing



Cut into square substrates (4.2 mm $\times$ 4.2 mm)

Micro-Raman

Laser illumination size: 10 μm

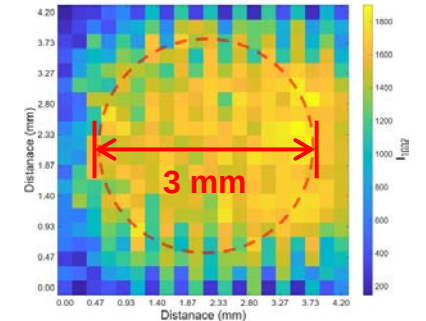
RSD

Inner red circle, 3 mm diameter, 148 pixels:
10.9 %

Whole area, 4.2 mm $\times$ 4.2 mm, 400 pixels:
34.9 %



Heat map from micro-Raman

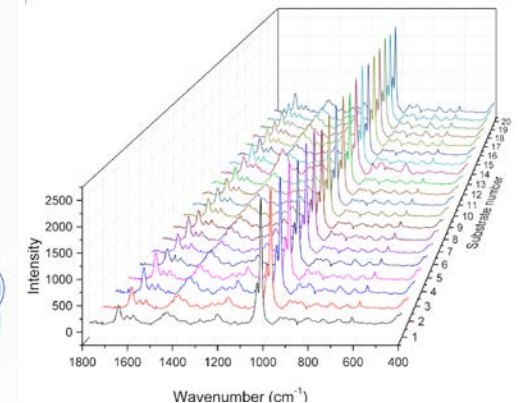
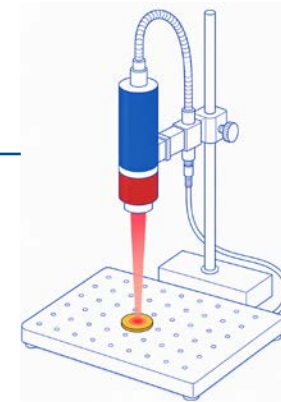


Wide Area Illumination Raman

Laser illumination size: 3 mm

RSD

20 separately prepared substrates:
3.01 %



Homogeneous formation of Au nanostructures
Effectiveness of wide-area intensity averaging

Reproducible quantitative analysis



Evaluation of Dynamic Response Range in SERS Measurement of Nicotine

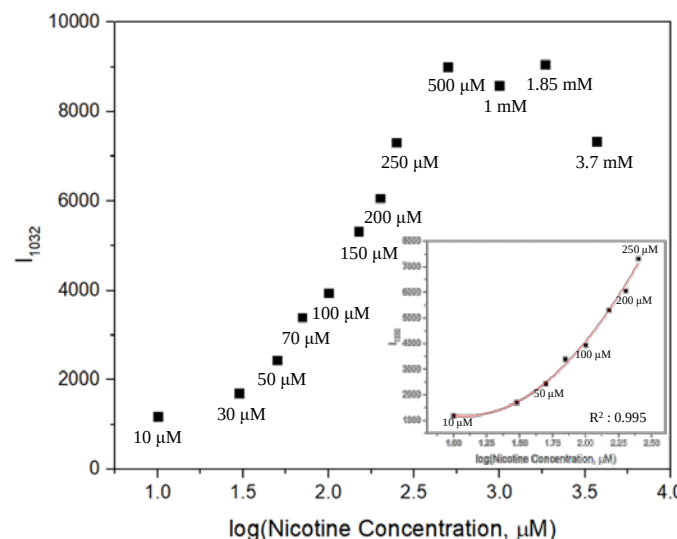
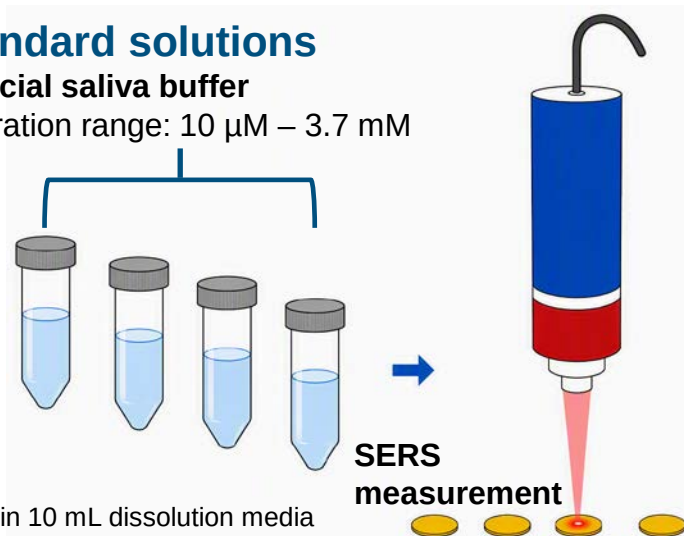
Nicotine standard solutions

Prepared in **artificial saliva buffer**

Nicotine concentration range: 10 μM – 3.7 mM

3.7 mM nicotine:

6 mg nicotine pouch in 10 mL dissolution media



- Peak intensity at 1032 cm^{-1} no longer increases above 500 μM
- Attributed to saturation of the available SERS hot-spots
- All dissolution fractions diluted 20-fold to remain within the dynamic range

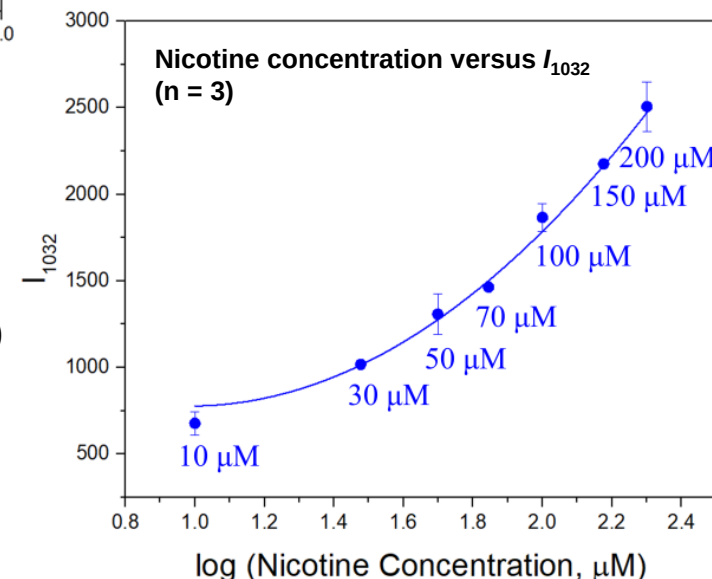
$$3.7 \text{ mM} \times 1/20 = 185 \mu\text{M}$$

Preparation of Working Standard Solutions

- Nicotine concentration range: 10 μM – 200 μM
- Prepared in **artificial saliva with mucin and artificial saliva buffer mixture (1:19 v/v)**

Construction of Calibration Curve

- Polynomial regression yielded $R^2 = 0.985$
- Good reproducibility and robustness of the SERS measurements



Three Different Artificial Saliva Formulations

Small-Volume Sinker Setup



Dissolution media
Volume: 10 mL

Nicotine pouch
6 mg nicotine/pouch

Magnetic bar
Stirring rate: 100 RPM

- Biorelevant sinker dissolution setup
- Closed system for dissolution testing for nicotine release

Artificial saliva buffer (AS-B)



- Lab-made artificial saliva
- Contains various salts (Na^+ , Cl^- , Ca^{2+} , K^+ , Mg^{2+} etc.)

Artificial saliva (AS-WM)



- Commercial artificial saliva
- Contains various salts and other components (Urea, KSCN, NH_4Cl)

Artificial saliva with mucin (AS-M)



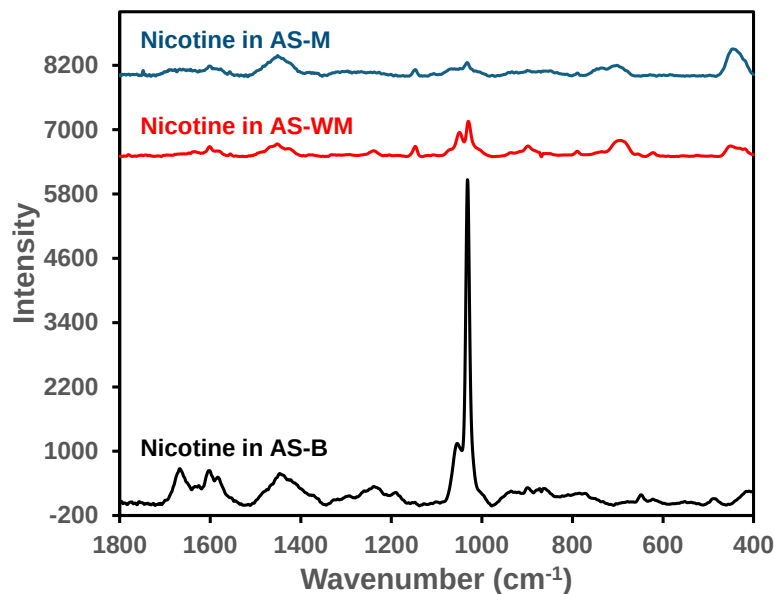
- Commercial artificial saliva
- AS-WM + mucin (0.3 wt%)
- Increased viscosity



Increasing similarity to natural human saliva

As biological relevance increases from AS-B to AS-WM and AS-M, the matrix composition becomes progressively more complex.

Assessment of Matrix Interference in Biorelevant Dissolution Media



Nicotine in AS-B

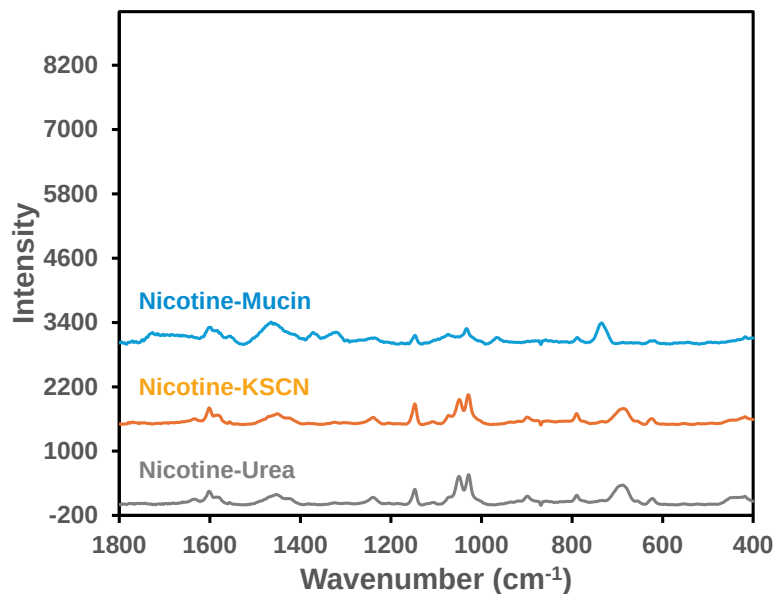
- 200 μ M Nicotine in Artificial Saliva Buffer

Nicotine in AS-WM

- 200 μ M Nicotine in Artificial Saliva without Mucin
- Contain urea (2.0 mM) and KSCN (1.96 mM)

Nicotine in AS-M

- 200 μ M Nicotine in Artificial Saliva with Mucin
- 0.3 wt% mucin in addition to the other components noted for the AS-WM formulation



Nicotine-Urea

- 200 μ M Nicotine, 2.0 mM Urea in DI water

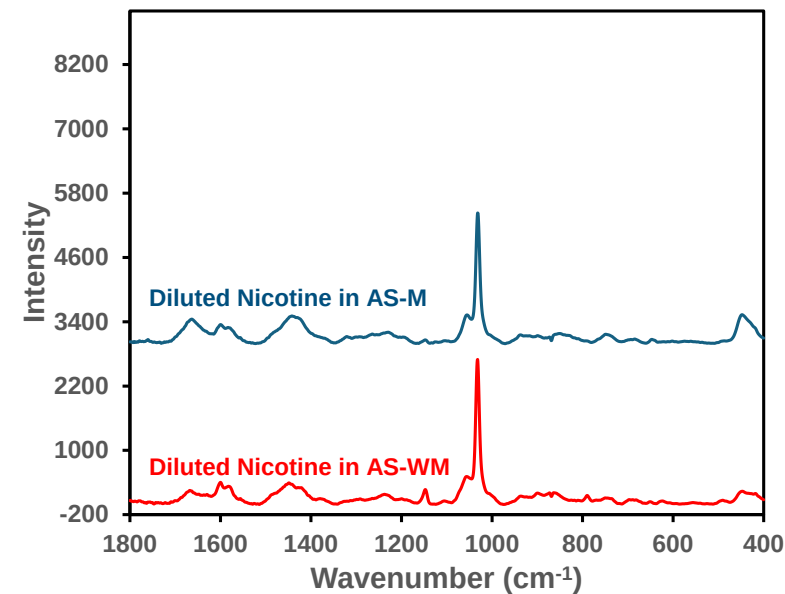
Nicotine-KSCN

- 200 μ M Nicotine, 1.96 mM KSCN in DI water

Nicotine-Mucin

- 200 μ M Nicotine, 0.3 wt% mucin in DI water

Urea, KSCN and mucin urea are the primary components responsible for suppressing nicotine SERS peaks



Diluted Nicotine in AS-WM

- 200 μ M nicotine solution in AS-WM was diluted 20-fold with AS-B

Diluted Nicotine in AS-M

- 200 μ M nicotine solution in AS-M was diluted 20-fold with AS-B

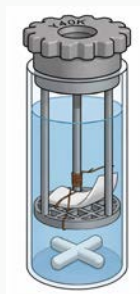
Straightforward dilution strategy reduces matrix interferences in complex artificial saliva media and recovers nicotine SERS sensitivity



SERS Detection for Monitoring of Released Nicotine in Dissolution Test

LC-based detection of release nicotine

Nicotine dissolution
in a mucin containing
artificial saliva



Dissolution Fraction
Collection



Dilution



Precipitation



Centrifugation



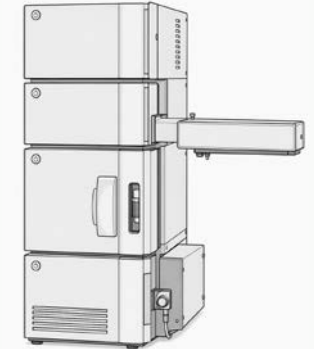
Filtration



Injection



UPLC-PDA measurement
Run time: ~10 min



SERS detection of released nicotine

Dilution



Loading



SERS measurement
Run time: ~10 s



Determination of released nicotine using SERS detection

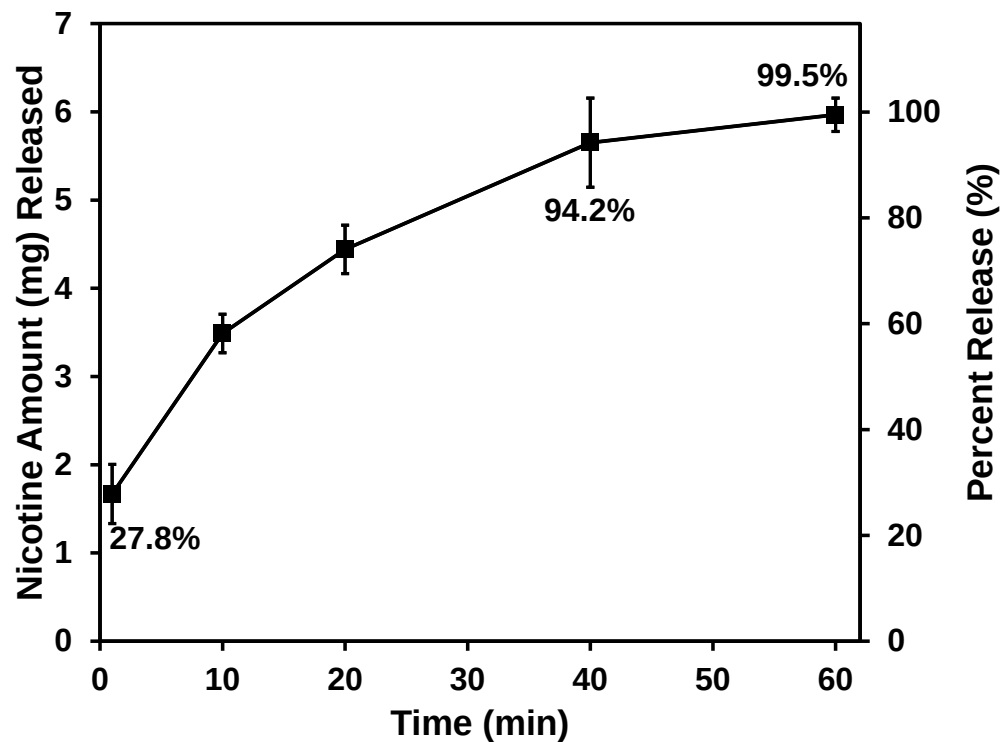
- 12 replicates across each of 9 fractions yields 108 measurements
- With calibration, ~130 measurements → ~1300 seconds → ~22 minutes



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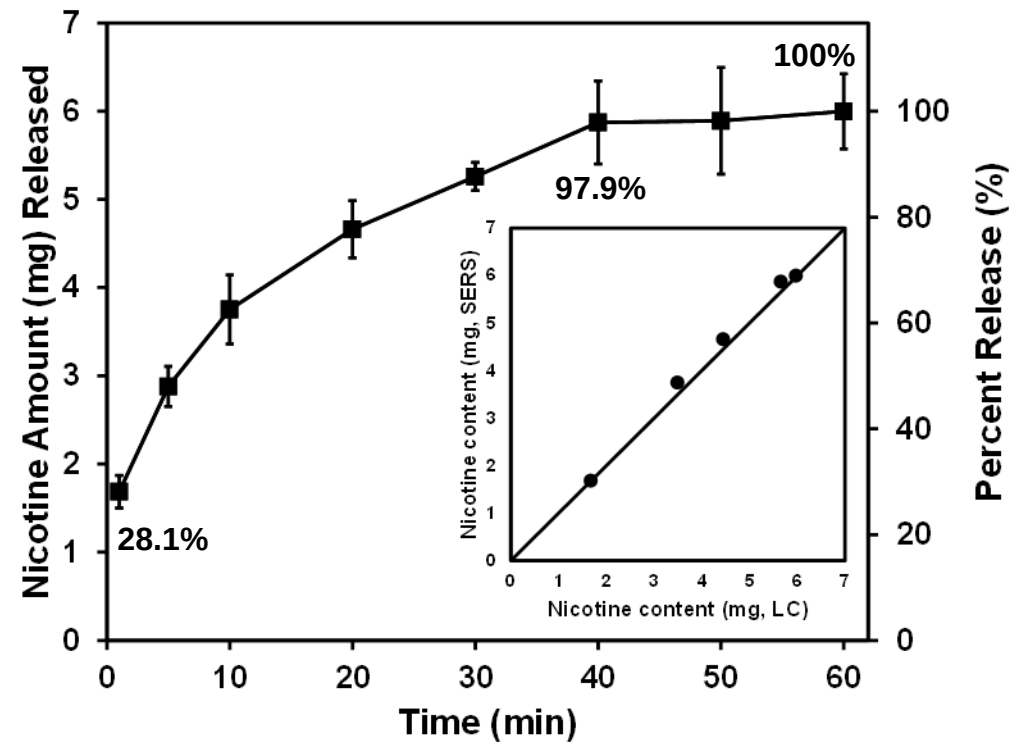
Monitoring of Released Nicotine in Dissolution Test Using AS-M

Dissolution profile based on LC detection



- **Detection:** LC-MS/MS
- **Replicates:** three independent dissolution runs
- **Error bars:** one standard deviation

Dissolution profile based on SERS measurements

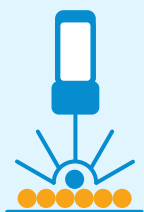


- **Detection:** SERS
- **Replicates:** three independent dissolution runs
- **Measurements:** two per fraction
- **Error bars:** one standard deviation of six I_{1032} values
- **Inset:** Correlation between the determined nicotine contents by the LC and SERS analysis





Conclusion



Surface-Enhanced Raman Spectroscopy

PROVIDED

a fast, reliable, and highly sensitive approach for nicotine dissolution testing in artificial saliva environments

- ✓ Selective nicotine detection achieved through the strong SERS activity of nicotine, with minimal interference from pouch filler ingredients
- ✓ Reliable nicotine quantification demonstrated across the full concentration range encountered during nicotine pouch dissolution
- ✓ Robust performance maintained in chemically complex artificial saliva matrices
- ✓ Homogeneous Au nanostructures and wide-area Raman sampling enabled high sensitivity and excellent substrate-to-substrate reproducibility
- ✓ Dilution effectively mitigated competitive adsorption effects, restoring nicotine signal intensity and reducing matrix-induced bias



Acknowledgements



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THANK YOU!

