Evaluation of Factors Impacting Nicotine Permeation from Oral Pouch Products

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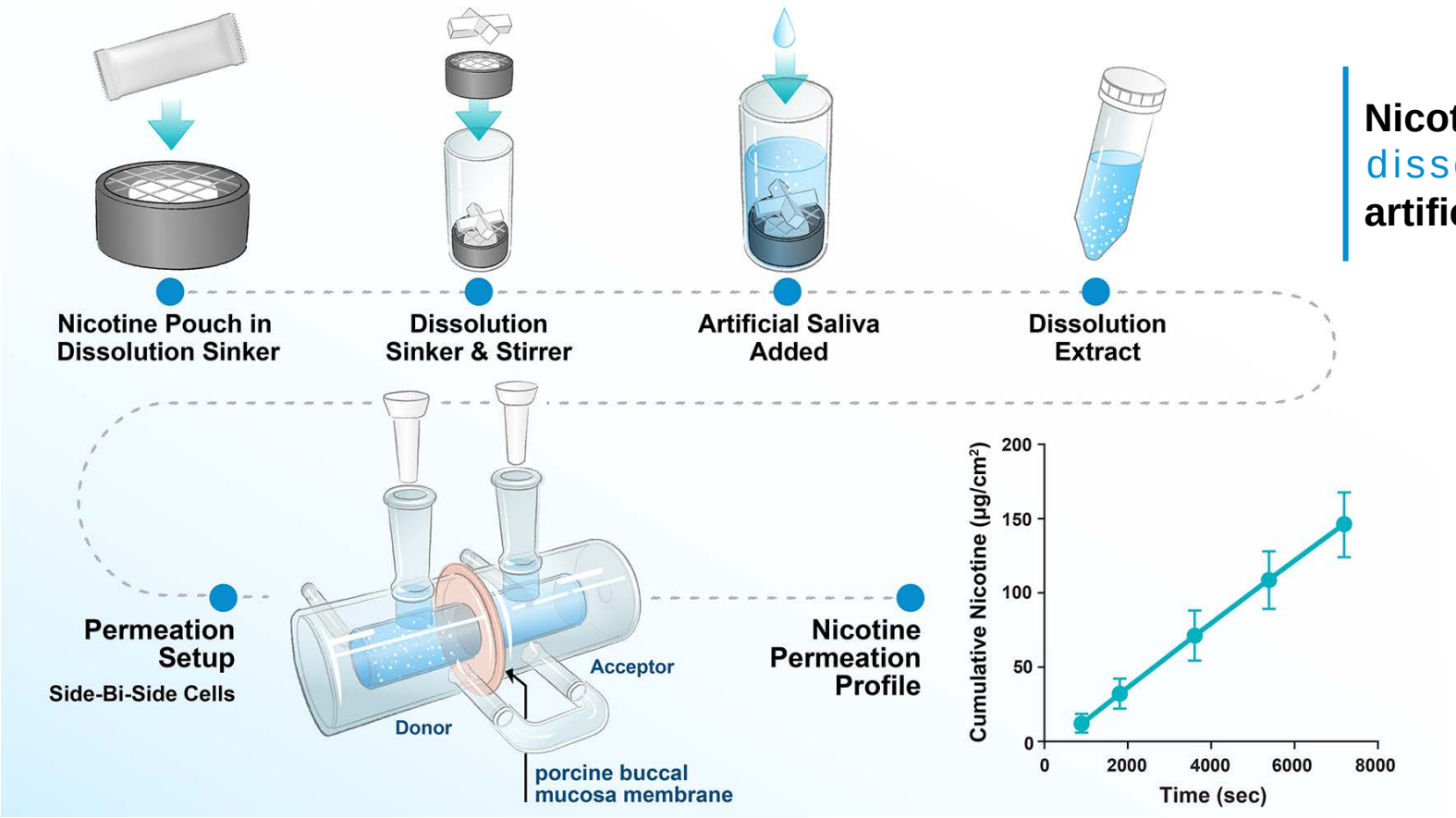
Objective

Establish a screening tool to evaluate product attributes **influencing nicotine release and buccal permeation from pouch products, supporting evaluation of product performance and formulation consistency.**

Nicotine pouch dissolution **into artificial saliva**



Nicotine permeation

Chemical Characterization of Nicotine Pouch Products

- Nicotine
- pH
- Dissolution
  - USP-4
  - Sinker

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Nicotine Content and pH Values

Product	Nicotine Content (mg/pouch) (SD)	pH (SD)
 ***Zyn*® 6 mg Cool Mint**	5.58 (0.31)	8.22 (0.02)
 ***Dryft*® 7 mg Spearmint**	5.94 (0.50)	8.32 (0.05)
 ***Velo*® 4 mg Mint**	4.16 (0.24)	7.40 (0.02)

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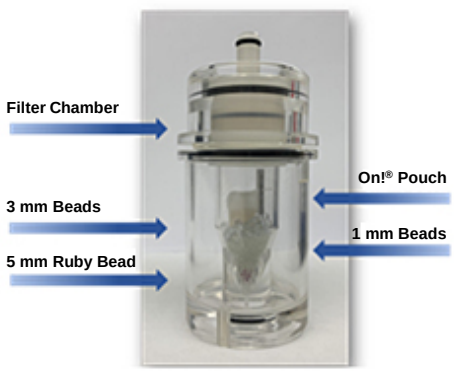
Dissolution Testing in Various Artificial Saliva

Product	Saliva: Buffer	Saliva: Salivea	Saliva: Orthana
***Zyn* 6 mg Cool Mint**	✓	✓	✓
***Dryft* 7 mg Spearmint**	—	—	✓
***Velo* 4 mg Mint**	—	—	✓
Saliva Composition	Sodium Chloride Potassium Chloride Calcium Chloride Potassium Phosphate dibasic (anhydrous) Magnesium Chloride Hexahydrate Potassium Carbonate (anhydrous)	Propylene Glycol Xylitol Hydrogenated Starch Hydrolysate Poloxamer 407 Hydroxyethyl Cellulose Sodium Benzoate Benzoic Acid Disodium Phosphate Zinc Gluconate Aloe Vera 7 Salivary Enzymes Potassium Chloride Sodium Chloride Sodium Bicarbonate	Porcine Gastric Mucin Methyl-4-hydroxybenzoate Benzalkonium Chloride EDTA Disodium Salt Potassium Hydroxide Xylitol Sodium Chloride Potassium Chloride Calcium Chloride Potassium Carbonate (anhydrous)

*53160-1., D.V.T.M., *Colorfastness to Saliva; Determination of the Colorfastness of Articles in Common Use Part 1: Resistance to Artificial Saliva*. DIN V Test Method, 2022.

USP-4 Dissolution Methodology

Flow-Through Cell



Cells Holder

Pump

Fractions Collector

Analysis

UPLC-PDA*

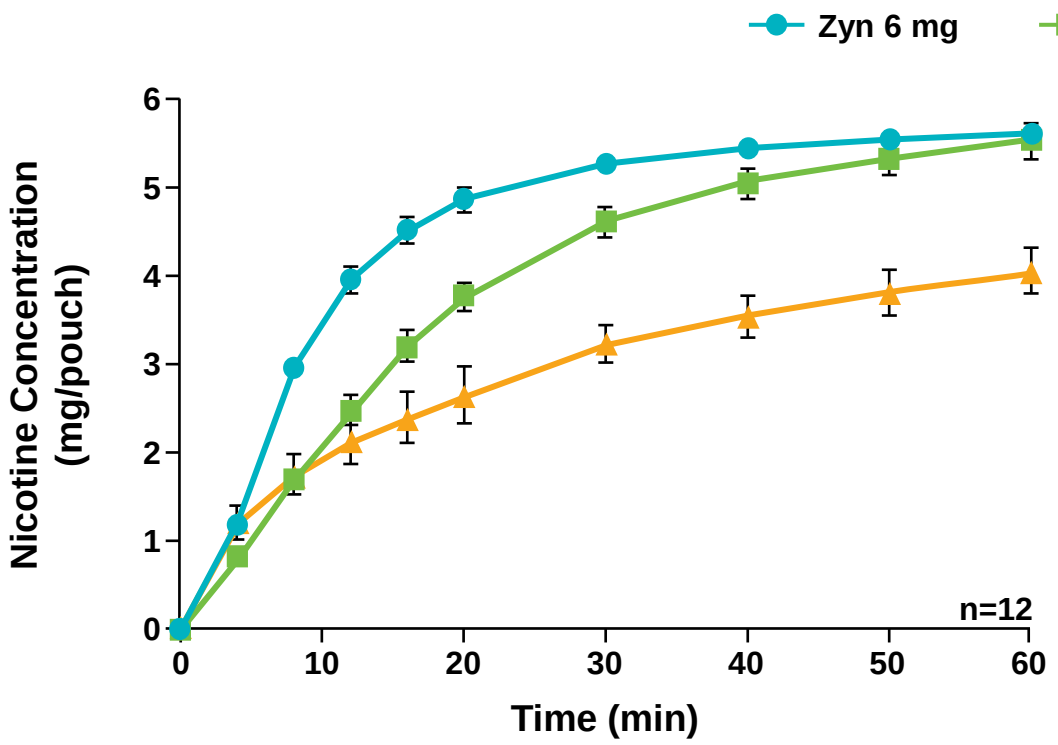
Flow rate 4.0 mL/min | Temperature 37° C | 12 replicates | 9 fractions per replicate

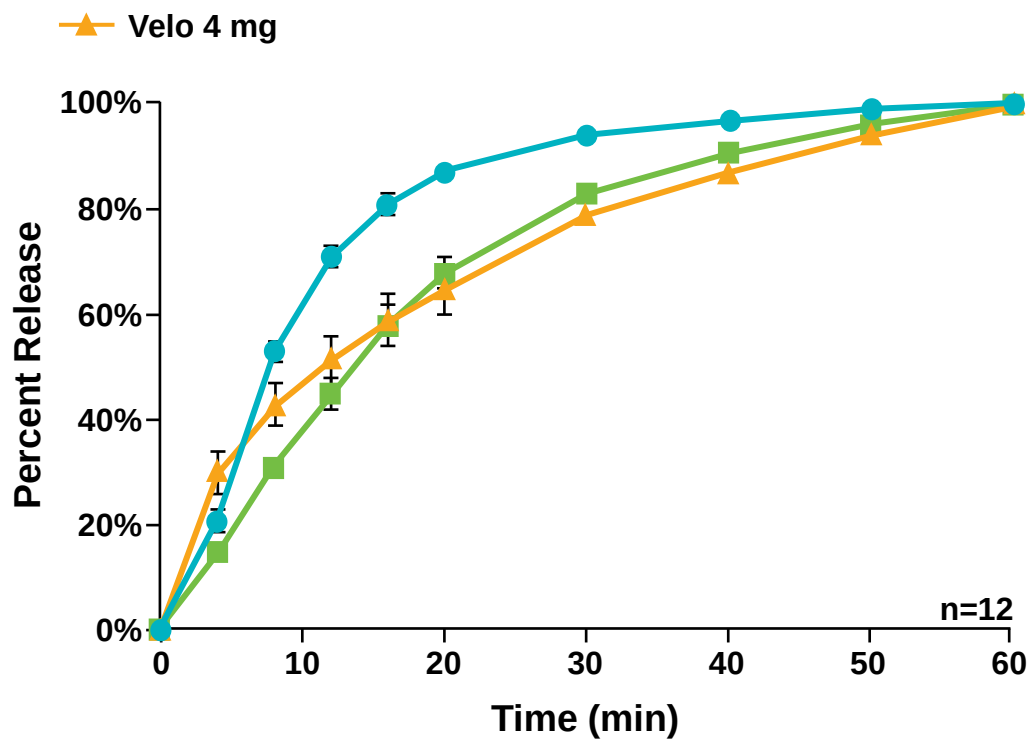
*UPLC-PDA: Ultra-high Performance Liquide Chromatography (UPLC)-Photodiode Array (PDA)

J. H. Miller, T. Danielson, Y. B. Pithawalla, A. P. Brown, C. Wilkinson, K. Wagner, and F. Aldeek. Development and validation of dissolution testing for nicotine release from smokeless tobacco products using flow-through cell apparatus and UPLC-PDA. J. Chromatogr B 2020, 1141, 122012.

F. Aldeek, N. McCutcheon, C. Smith, J. H. Miller, and T. Danielson. Dissolution testing of nicotine release from OTDN pouches: product assessment and product-to-product comparison. *Separations*. 2021, 8 (1), 7.

USP-4 Nicotine Dissolution Profiles in Buffer Saliva



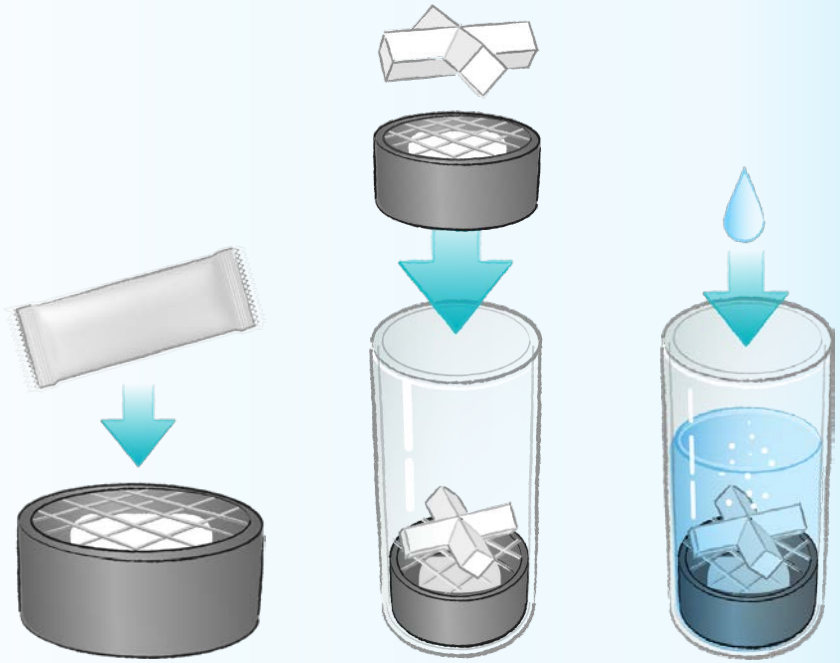


The total amount of nicotine released after 60 minutes of dissolution in buffer saliva was found to be 100%, 93% and 98% for *Zyn, Dryft* and *Velo*, respectively

Velo* and *Dryft* exhibited equivalent release rates, both of which were slower than *Zyn. This could be attributed to differences in the inherent product characteristics.

Sinker Dissolution Methodology

Low Volume Dissolution Setup



Rotational Speed =
- **100 rpm**

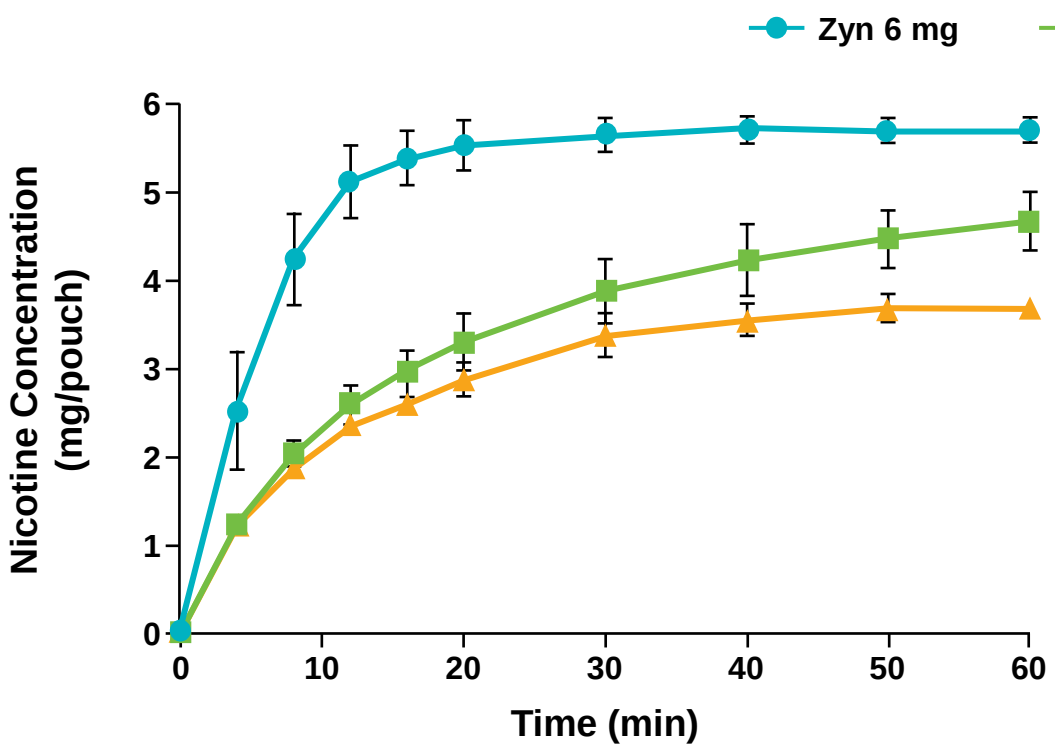
Volume of Artificial Saliva =
- **10 mL**

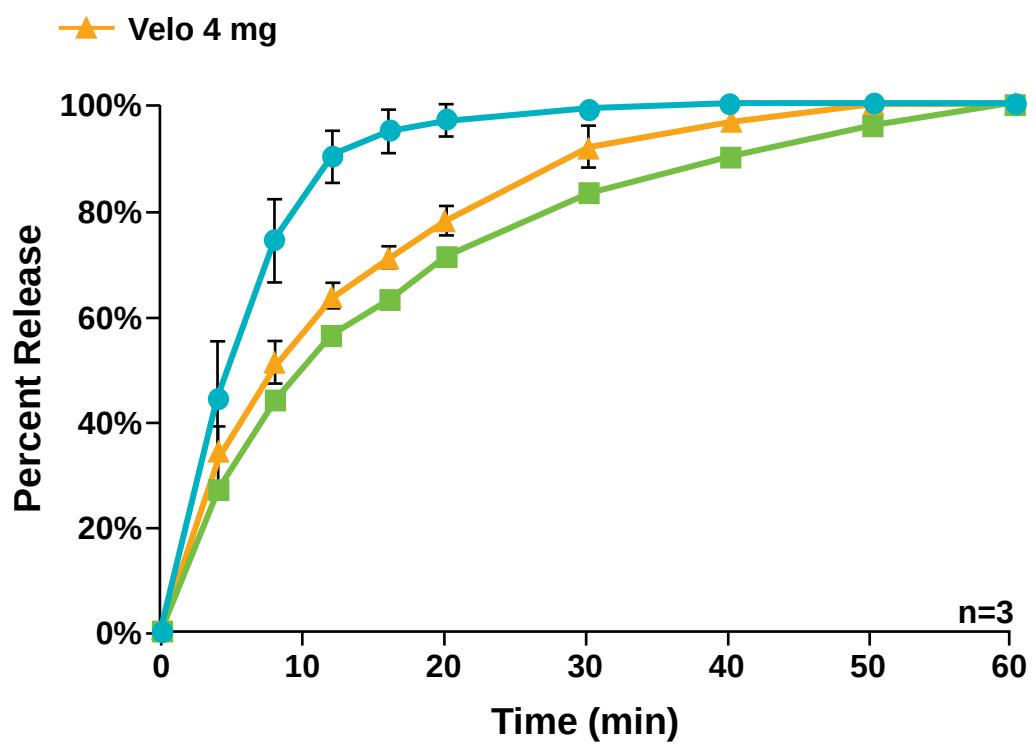
Sampling Volume =
- **250 µL**

Sampling Time Points =
- **4, 8, 12, 16, 20, 30, 40, 50 and 60 minutes**

Knopp, M. M.; Kiil-Nielsen, N. K.; Masser, A. E.; Staaf, M., Introducing a Novel Biorelevant In Vitro Dissolution Method for the Assessment of Nicotine Release from Oral Tobacco-Derived Nicotine (OTDN) and Snus Products. Separations 2022, 9 (2), 52.

Sinker Method Dissolution Profiles in Orthana Saliva

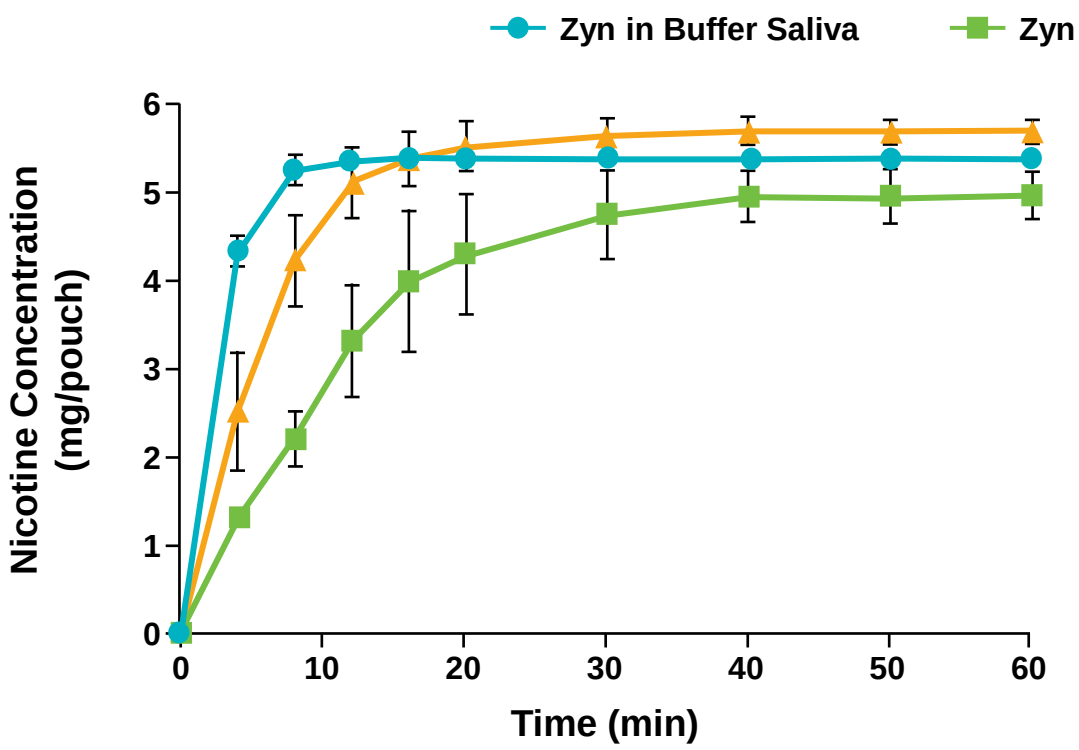


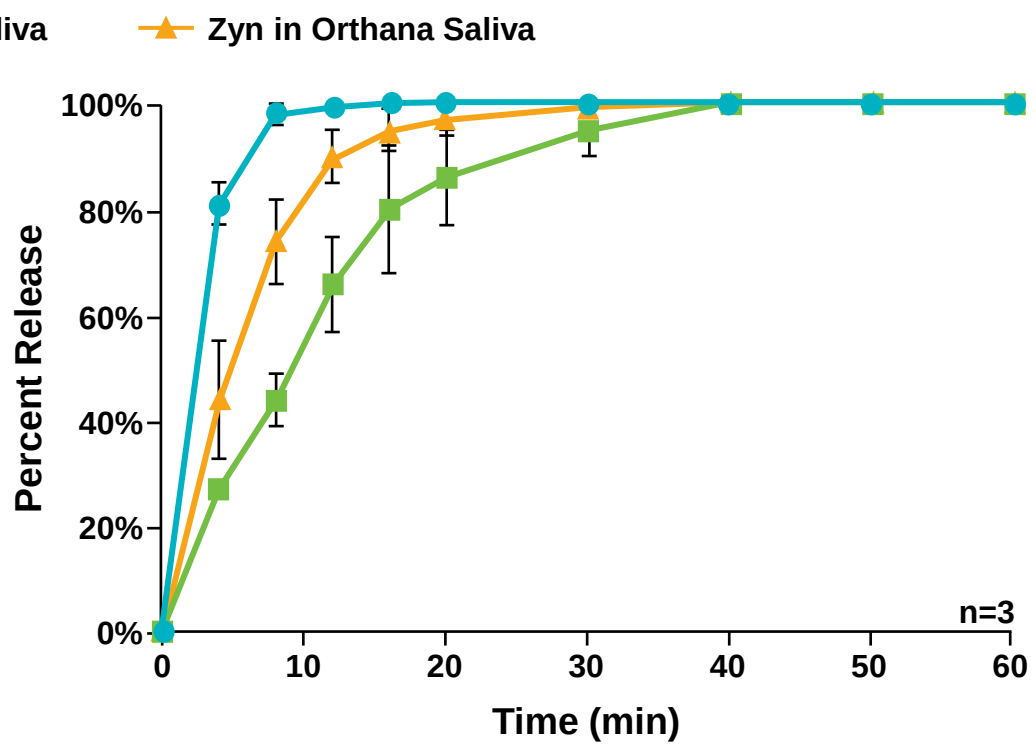


The total amount of nicotine released after 60 minutes of dissolution in orthana saliva, was found to be 102%, 79% and 89% for *Zyn, Dryft* and *Velo*, respectively

Release rates of the three nicotine pouches showed similar trend to USP-4, despite differences artificial saliva composition

Influence of Saliva on Nicotine Release Rate





The rate of nicotine release was found to increase in the following order:
Salivea saliva < Orthana saliva < Buffer saliva

Conclusion

Differences in release rates

may be due to inherent product characteristics

Nicotine dissolution trends were similar

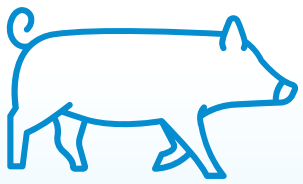
across USP-4 and sinker methods

Saliva type

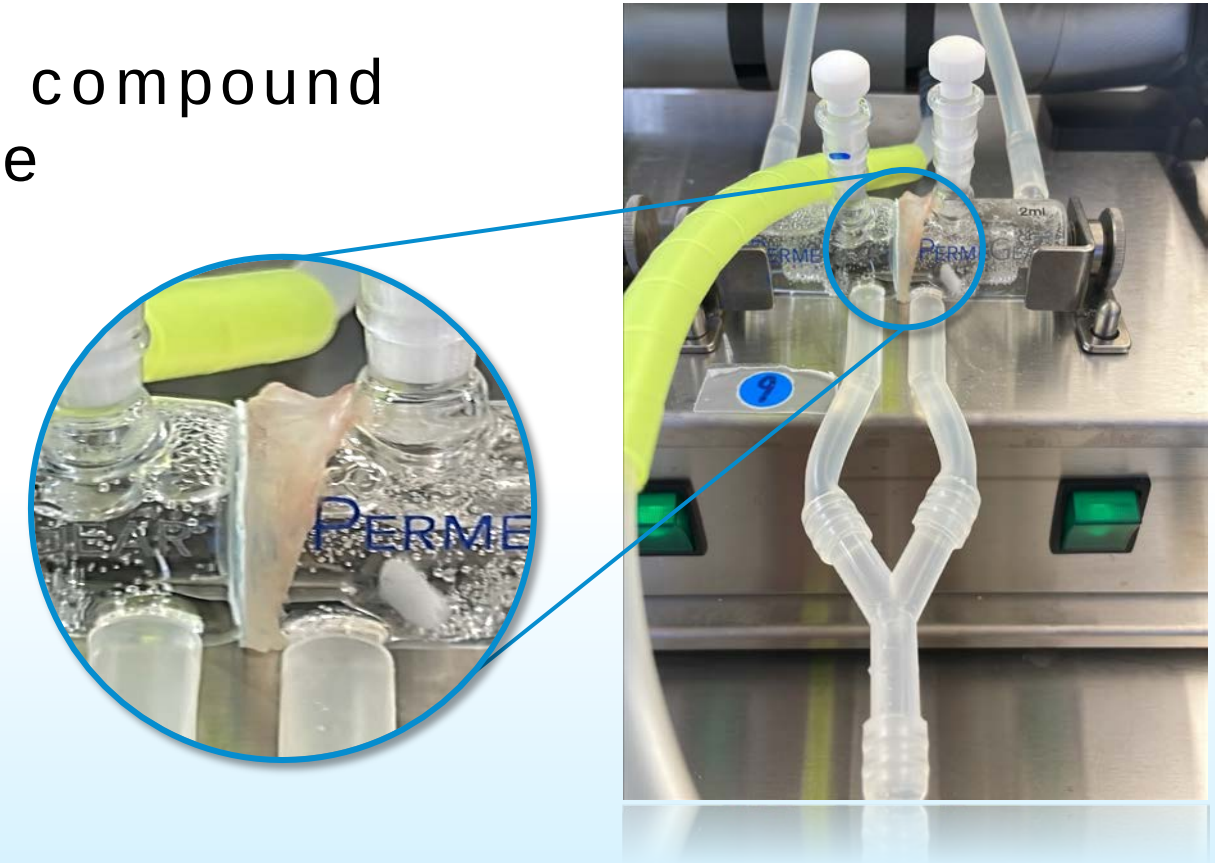
affects nicotine release rate

Buccal Permeation Test

Test the delivery of active compound through the oral membrane



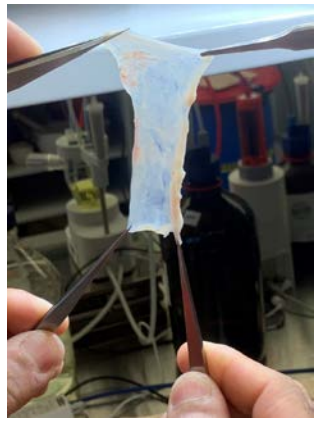
Use porcine oral membrane to emulate human oral membrane



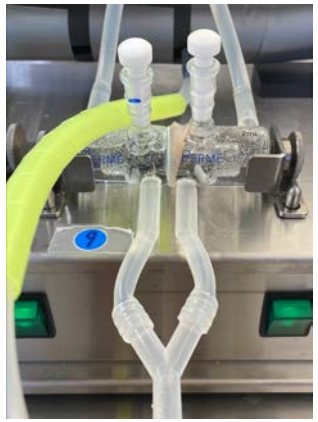
Useful tool for formulation development of certain oral products, such as nicotine pouches

Diagram of *Ex Vivo* Permeation Experiment

Tissue Preparation



Permeation Setup



Permeation Experiment Duration: 2 hrs

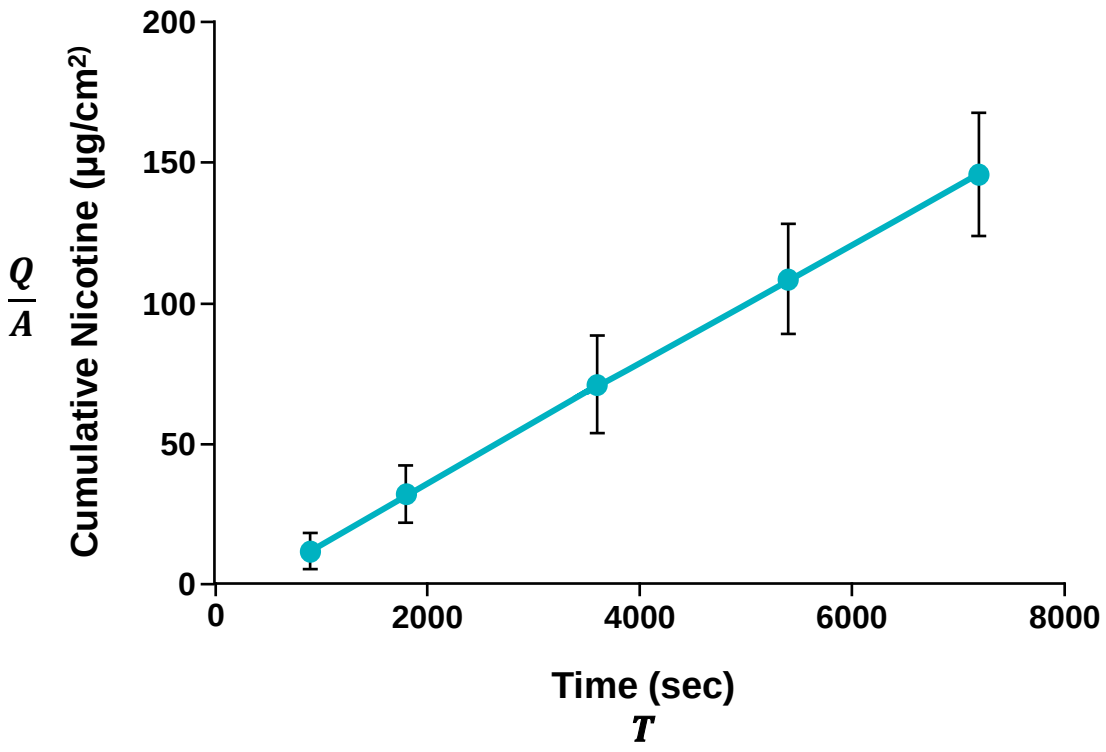
Sample Collection

Sample collections at: 15, 30, 60, 90 and 120 min

Sample Analysis

Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS)

Introduction to Apparent Permeability Coefficient (P_{app})



Q: Cumulative amount of permeated drug

T: Time interval (t)

Flux: Slope of the linear regression of the cumulative plot of the drug through the barrier normalized by the surface area (A)

Apparent permeability coefficient (**P_{app}**)**:** Flux divided by concentration of the drug in the donor compartment at T_0 (C_0)

$$Flux = \frac{dQ}{dt * A} \qquad P_{app} = \frac{Flux}{C_0}$$

Dissolution Samples with Different Nicotine Concentrations, pH, and Artificial Saliva

Dissolution Samples of *Zyn* 6 mg Cool Mint in Various Artificial Saliva

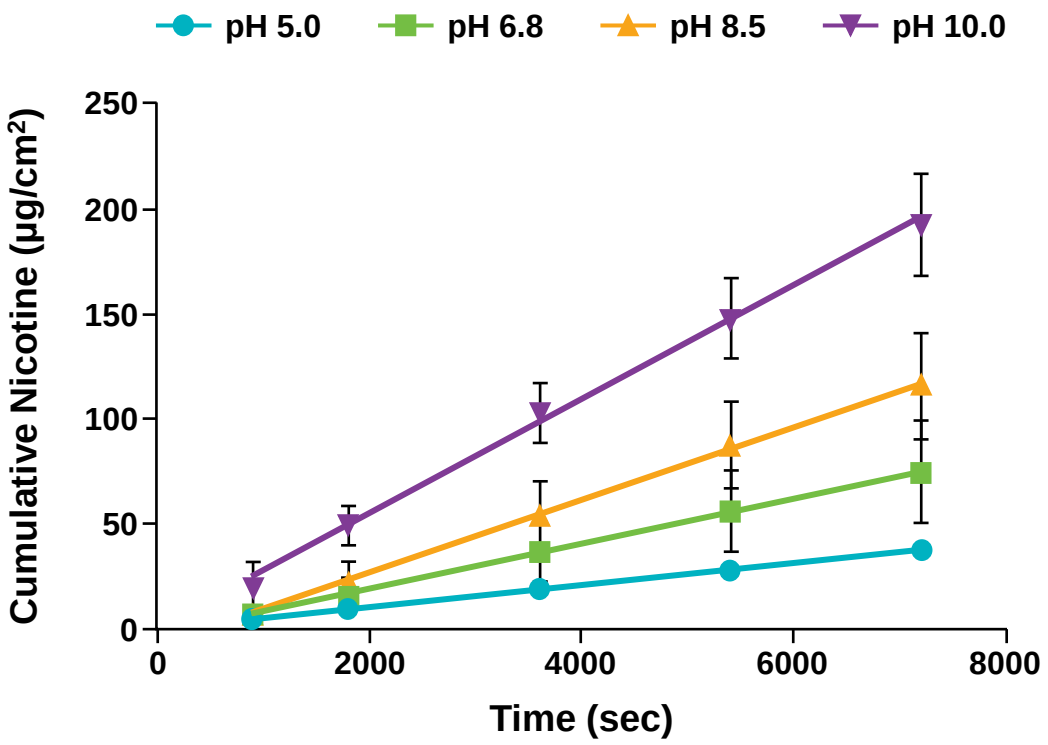
Sample Name	Extract pH	Conc. of Nicotine (mg/mL)
Buffer saliva	8.06	0.60
Salivea saliva	8.04	0.48
Orthana saliva	7.79	0.60

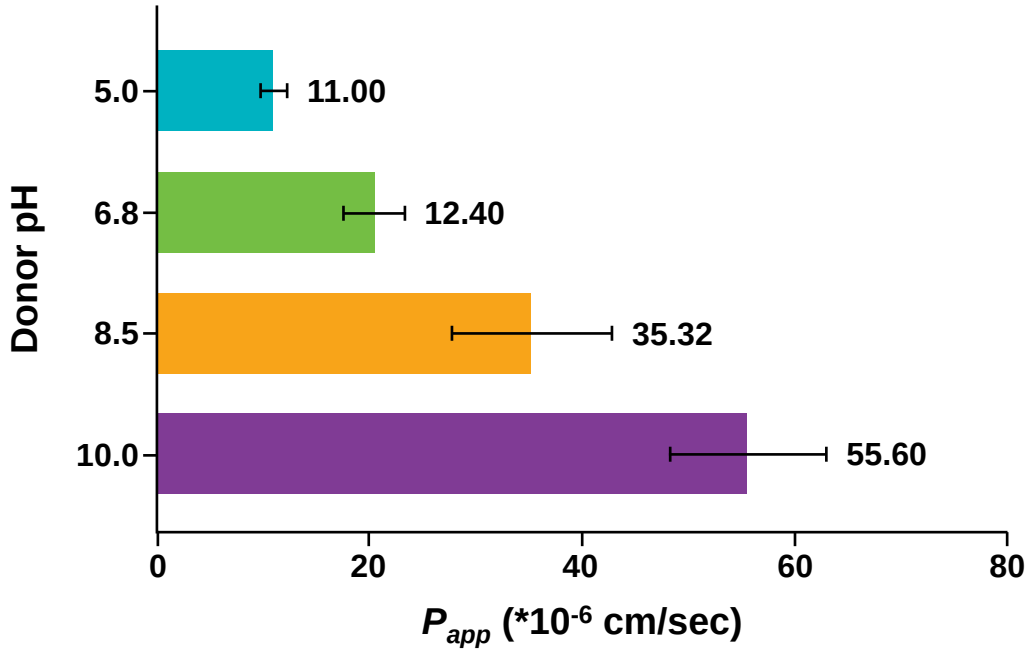
Dissolution Samples of Commercial Products in Orthana Saliva

Sample Name	Extract pH	Conc. of Nicotine (mg/mL)
***Zyn* 6 mg Cool Mint**	7.79	0.60
***Dryft* 7 mg Spearmint**	7.74	0.45
***Velo* 4 mg Mint**	6.94	0.38

Nicotine Permeability Increases as Donor pH Increases

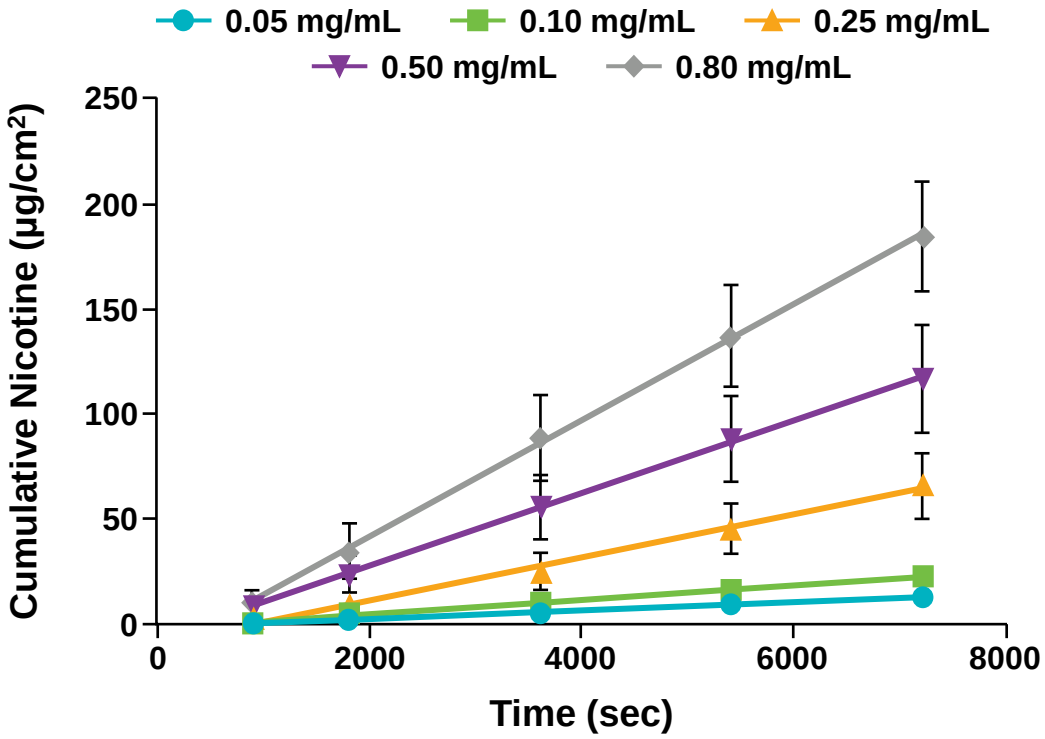
Donor: 0.5mg/mL Neat Nicotine Solution in PBS, n=12

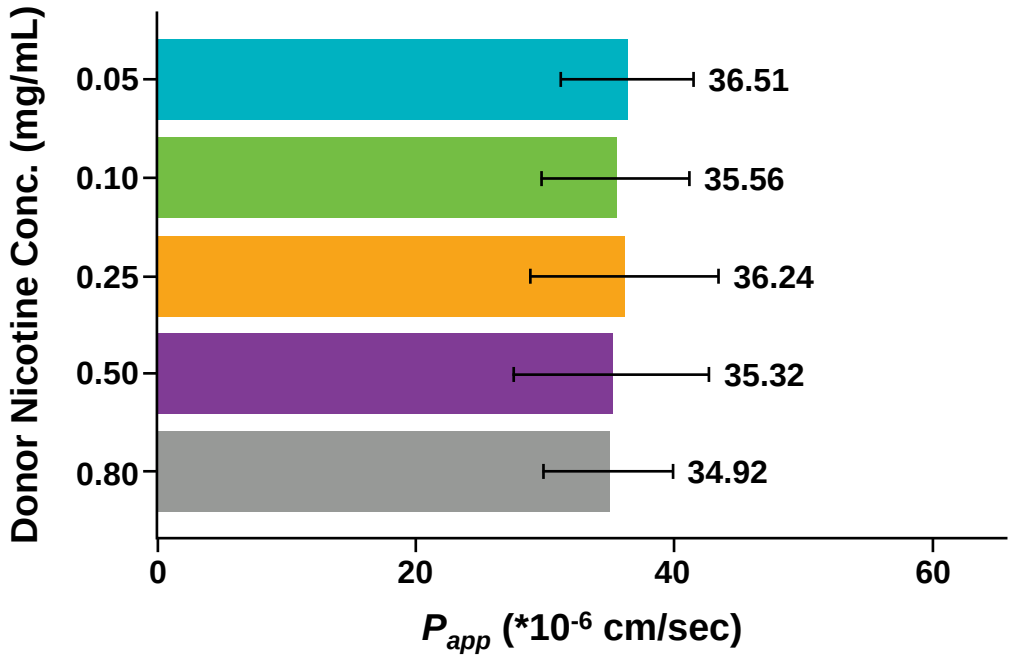




Concentration Does Not Impact Nicotine Permeability within the Tested Range

Donor: Neat Nicotine Solution in PBS, pH 8.5, n=12

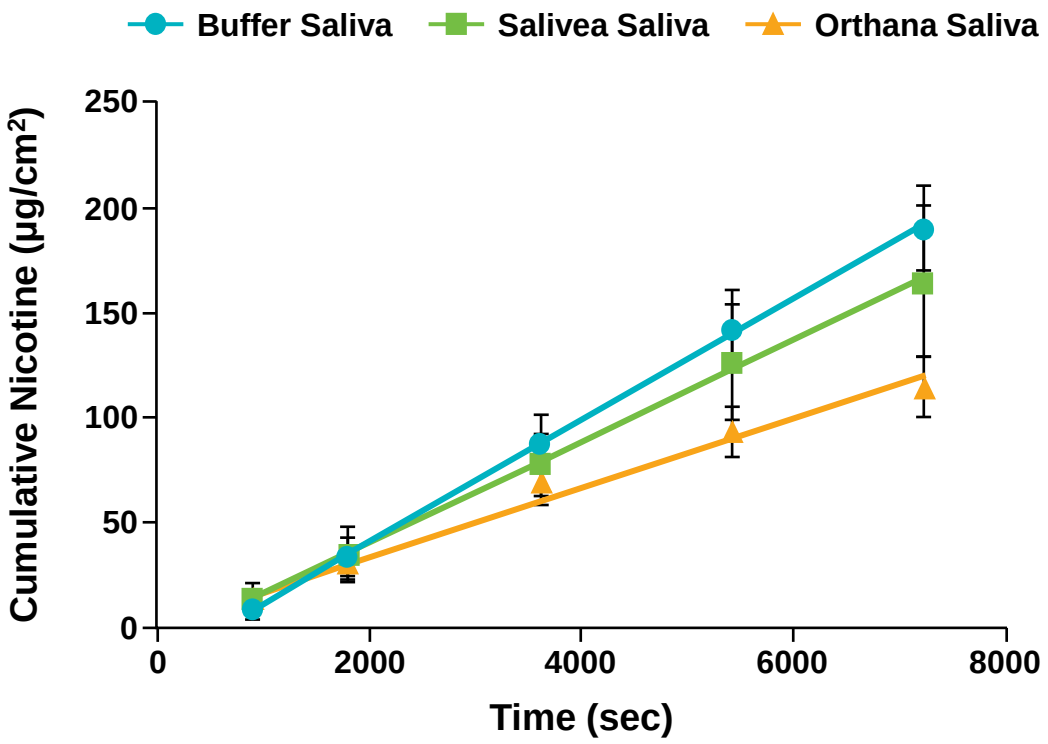


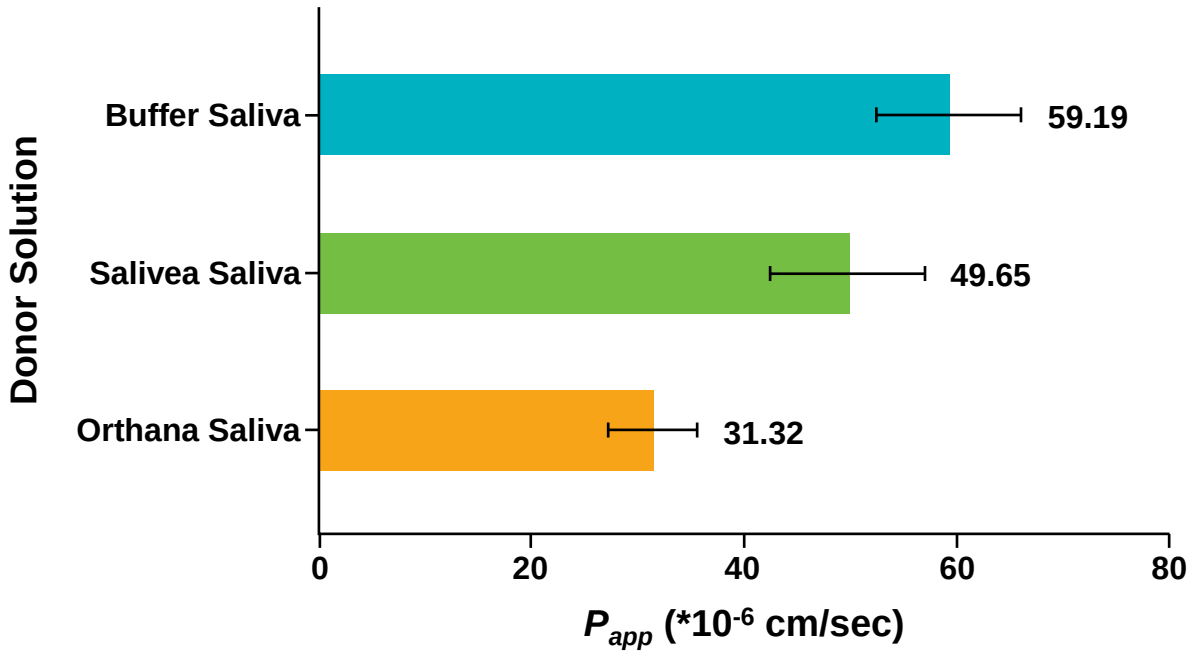


Nicotine permeability is normalized against C_0 and is not dependent on concentration in the tested range

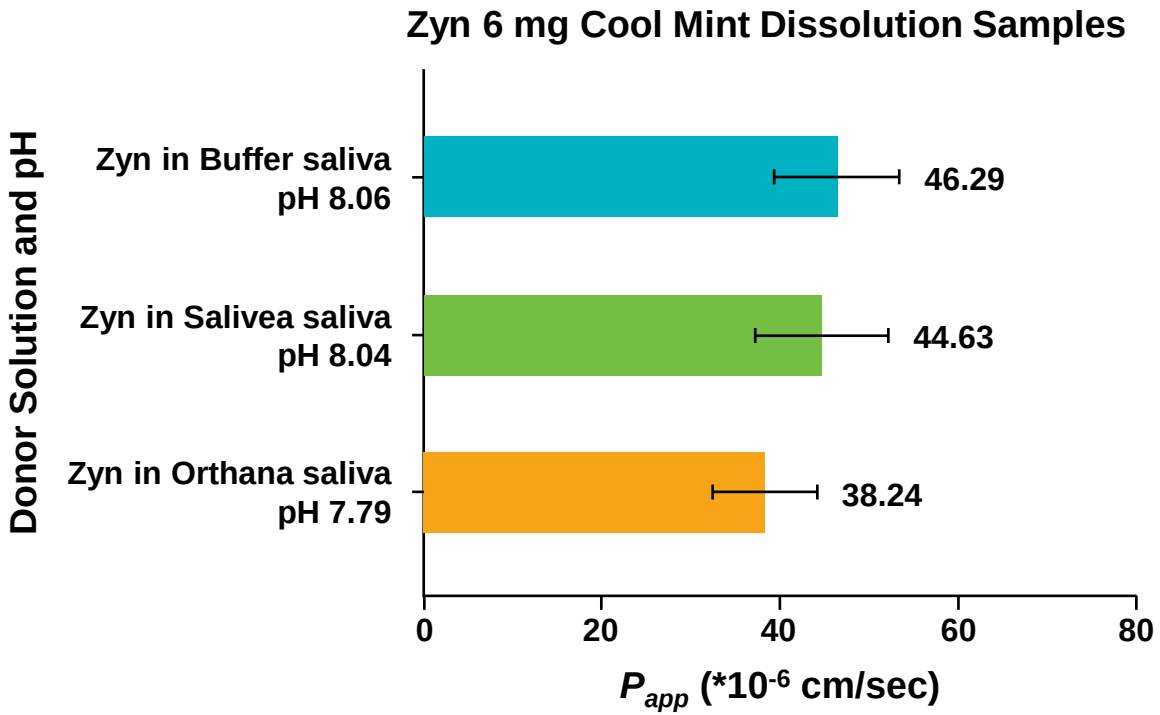
Artificial Saliva Composition Affects Nicotine Permeability

Donor: 0.5 mg/mL Neat Nicotine Solution in Artificial Saliva, pH 8.5, n=12





Permeability of Nicotine from *Zyn* 6 mg Cool Mint Dissolution Samples – Comparison of Artificial Saliva



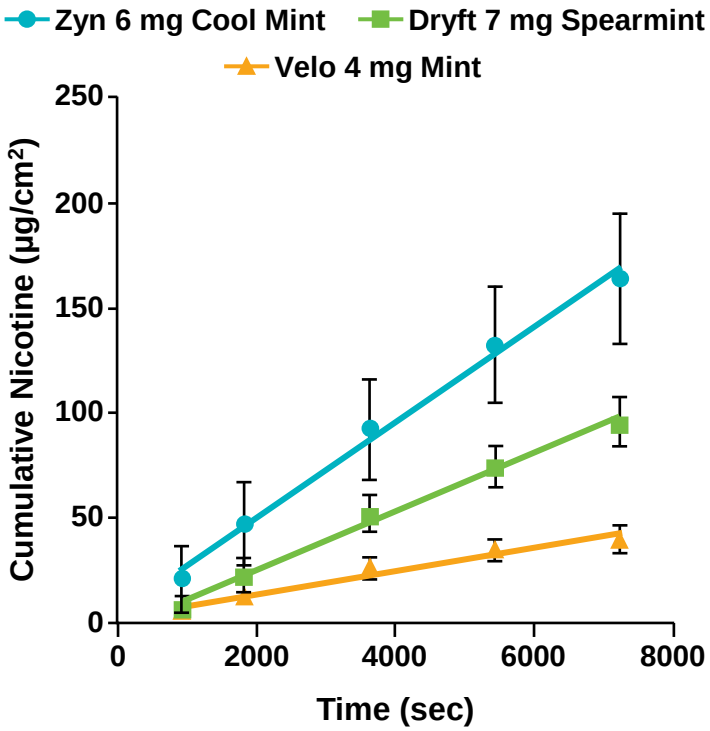
Donor	Extract pH	Conc. of Nicotine (mg/mL)
Buffer saliva	8.06	0.60
Salivea saliva	8.04	0.48
Orthana saliva	7.79	0.60

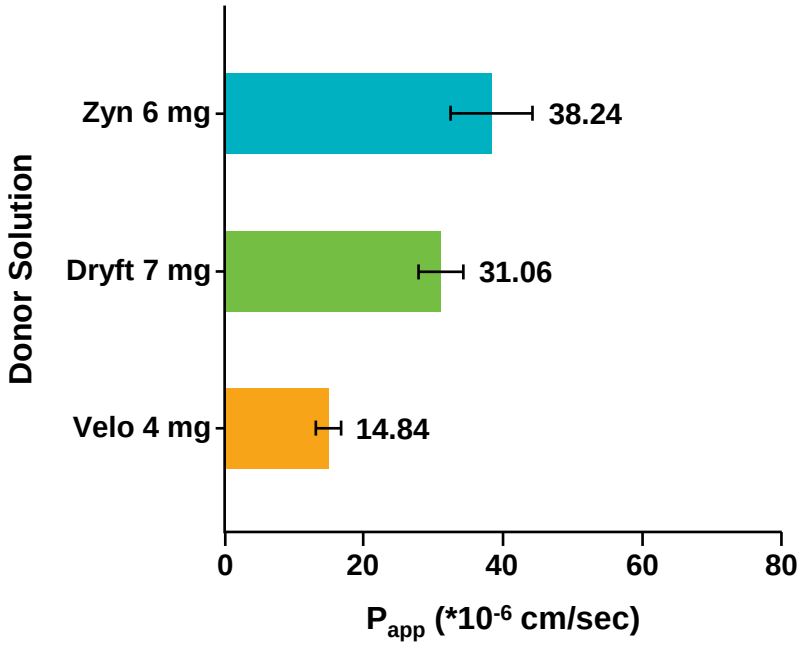
The differences in three extracts:

- Nicotine concentration
- pH of the donor solution
- Type of artificial saliva
- Other ingredients released from the product

Permeability of Nicotine in Orthana Saliva – Comparison of Products

Donor: Dissolution Samples in Orthana Saliva, n=12





Donor	Extract pH	Conc. of Nicotine (mg/mL)
***Zyn* 6 mg Cool Mint**	7.79	0.60
***Dryft* 7 mg Spearmint**	7.74	0.45
***Velo* 4 mg Mint**	6.94	0.38

Zyn 6 mg Cool Mint has higher permeability than _Dryft_ 7 mg Spearmint at the same pH and in the same solvent

Conclusion

Zyn has HIGHER permeability than *Dryft*, may be due to formulation

Velo has the LOWEST permeability, may be due to pH and / or formulation

Permeability is INDEPENDENT of nicotine concentration in the tested range

Permeability can be INFLUENCED by pH and artificial saliva composition

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THANK YOU!