A Novel Model Mouth System for Evaluation of In Vitro Release of Nicotine and Tobacco-Specific Nitrosamines from Moist Snuff

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Contents

1. Introduction

➢ **Moist snuff is a sub-category of oral smokeless tobacco products, which is usually placed between the upper jaw and gum and sucked.**

➢ **Nicotine is a toxic addictive alkaloid.**

➢ **Tobacco specific nitrosamines (TSNA) are widely considered to be among the most important carcinogens in smokeless tobacco products.**

1. Introduction

➢ **The release of nicotine and TSNA from moist snuff is an essential first step in nicotine and TSNA absorption.**

➢ **The estimation of the extraction by moist snuff users is of particular importance to product safety evaluation and quality control of moist snuff.**

1. Introduction

◆ **Our study:**

➢ **A novel model mouth system for evaluating the in vitro nicotine and TSNA release behavior of smokeless tobacco product s (moist snuff) has been developed.**

➢ **The model mouth system was applied to evaluate nicotine and TSNA release in 23 brands of commercially available moist snuff.**

2. Experiment

◆ **The Design Principle of the Model Mouth System**

➢ Moist snuff consumption does not involve chewing, as the pouch is held in a largely stationary fashion between the upper jaw and gum.

➢ The release of nicotine and TSNA is likely to involve diffusion from moist snuff into the thin layer of saliva coating the mouth. The extractive components from moist snuff are then transferred across the oral mucosa to be absorbed into the bloodstream.

➢ The system can be used to simulate buccal condition from three aspects: buccal temperature, saliva composition and the rate of saliva production.
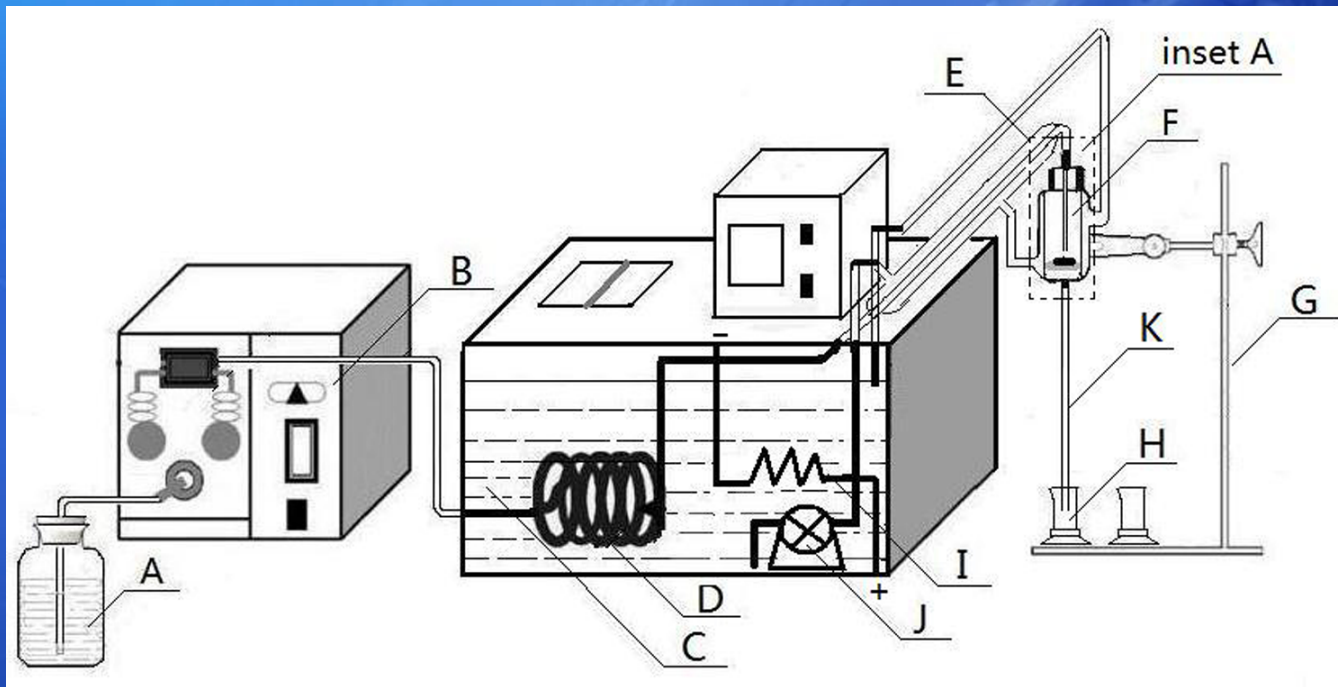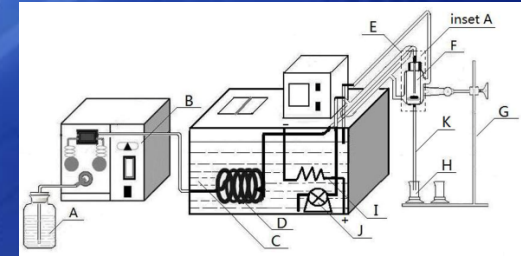
Figure 1. Schematic diagram of the model mouth system. Release medium reservoir (A), constant flow pump (B), water bath (C), pre-warming coils (D), water jacket tubing (E), release cell (F), release cell support bracket(G), and fraction collector (H), heating wire(I), circulating pump(J) , collection tube (K).

2. Experiment

◆ The release medium was poured into the release medium reservoir.

◆ The release medium was pumped through the release cell at a desirable constant flow rate.

◆ The temperature control module consists of a water bath, pre-warming coils and water jacket tubing, which bring the release medium to and maintain at desired temperature before supplying the release medium to release cell.

◆ The in vitro release test run lasted for 60 min, and the released solution of samples was collected at 5-min intervals.
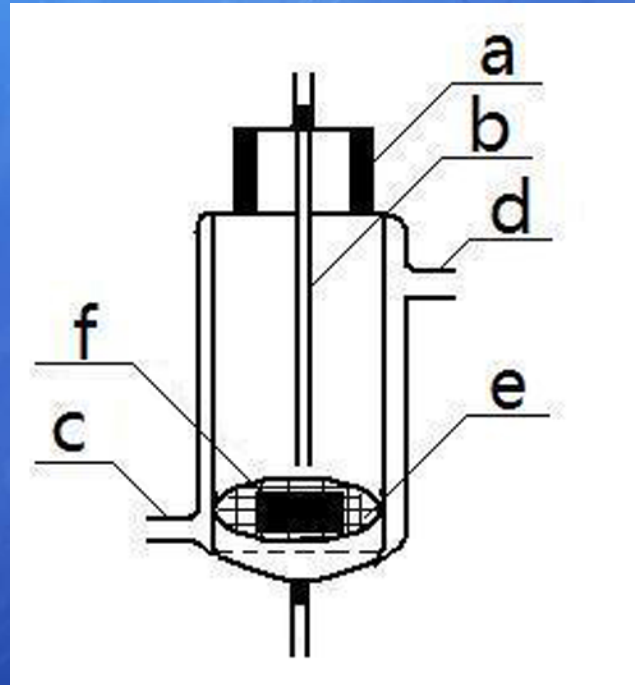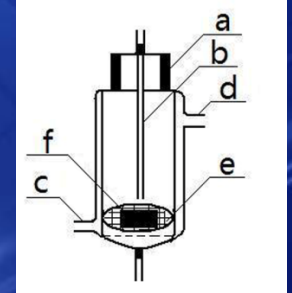
Figure 2. Schematic diagram of the release cell (inset A.). Glass cap (a), release medium supply tube (b), water jacket inlet (c), and water jacket outlet (d), sample holding mesh (e), moist snuff sample (f).

2. Experiment

◆The main part of the release cell is a columniform glass cannula.

◆The interlayer of release cell, which is filled with water of desired temperature, can ensure the release process is conducted at desired temperature.

◆The lower part of the release cell had a sample holding mesh.

◆ The glass cap plug had a glass catheter, which provided release medium to the center of moist snuff samples.

◆The release cell was transparent which facilitated visual inspection during the operation.

2. Experiment

◆**The condition of in vitro release test**

Release temperature	Release medium	Flow rate
37±0.5℃	**Artificial saliva**	**0.1-10.0 mL/min**

2. Experiment

Experiment process

In vitro release test was performed by the model mouth system.

The concentration of nicotine and TSNA of the released solution and the residual samples were determined by HPLC and LC–MS/MS.

3. Results and discussion

◆ *3.1 In vitro in vivo correlations*

➤ **To validate the model mouth system, nicotine release was evaluated and compared in healthy volunteers versus in vitro using the model mouth system.**

➤ ***In vivo*** **release of nicotine from two brands of moist snuff was assessed in 15 healthy volunteers, male, aged 27–48, according to study protocol.**

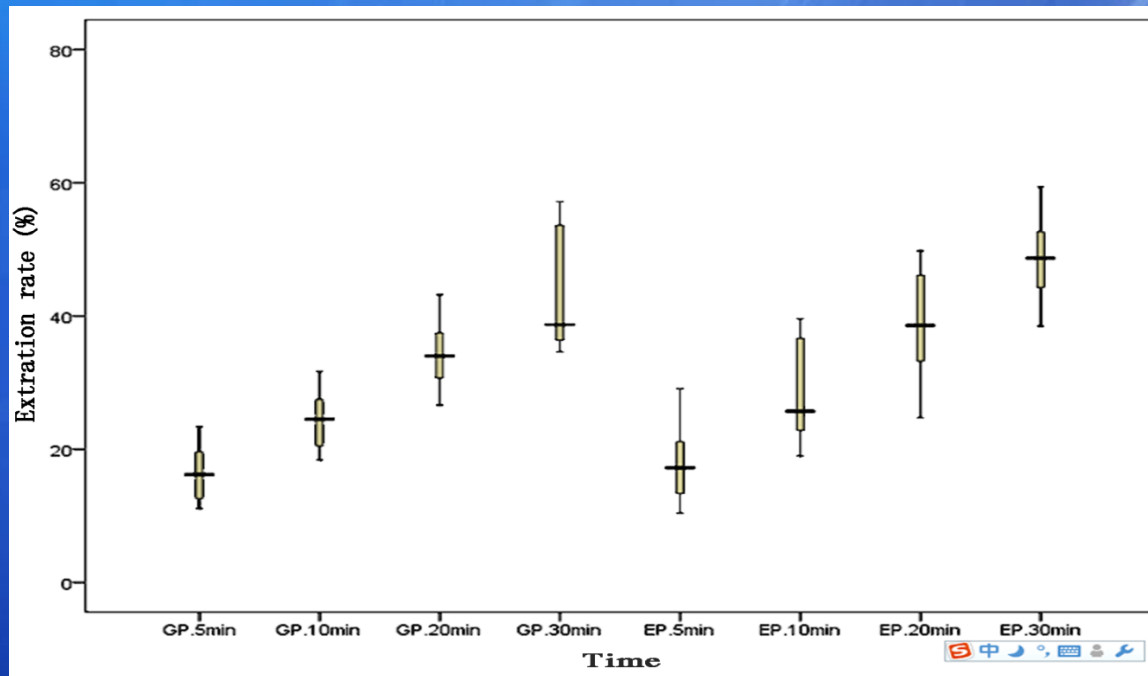# 3. Results and discussion

Fig. 3 Boxplot of the results for the extraction rates of nicotine from two moist snuff by volunteers.

➢There were significant inter-individual variations in nicotine release rate under the same experimental conditions.

➢ Nicotine release rate from two moist snuff were ranged from 34.6% to 57.2% and ranged from 38.5% to 59.4% at 30 min, respectively.
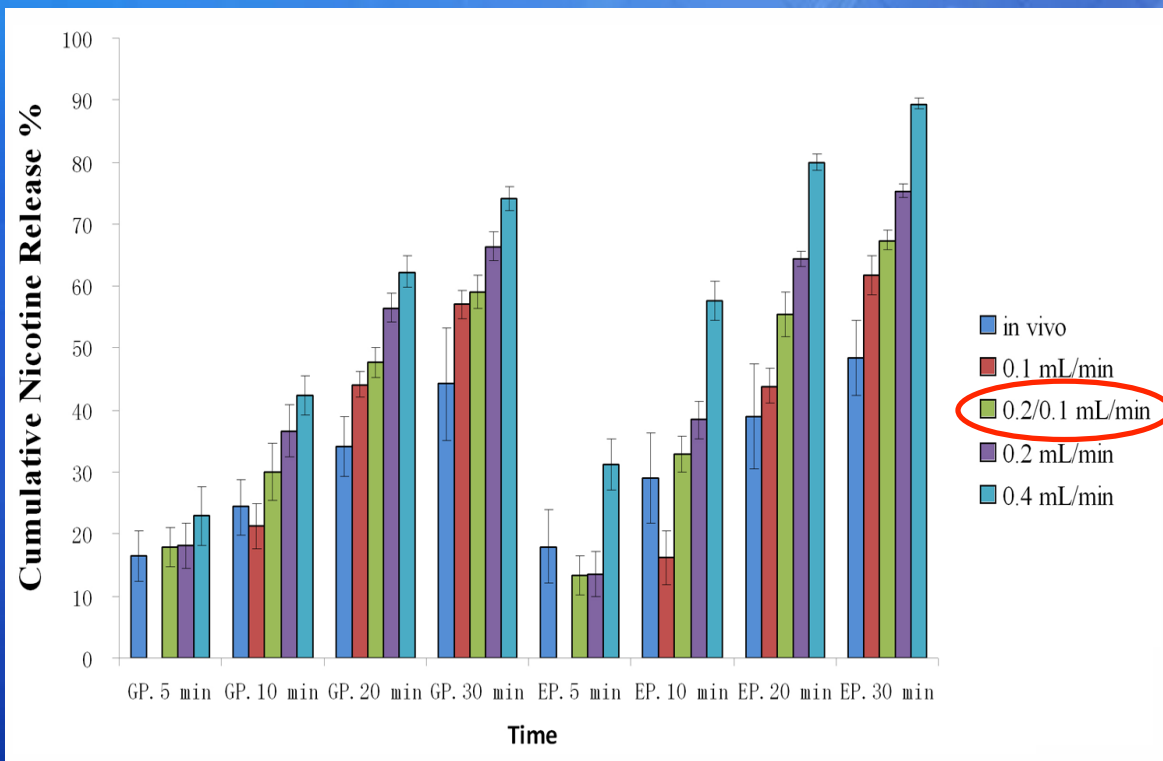
Fig. 4 Comparison of in vivo and in vitro nicotine release.

➤The cumulative release rate at lower flow rate was a better match to human data.

➤The flow rate was set to 0.2 mL min^{-1} in the first 5 min, and then it was changed to 0.1 mL min^{-1} after 5 min to better match in vivo nicotine release process.

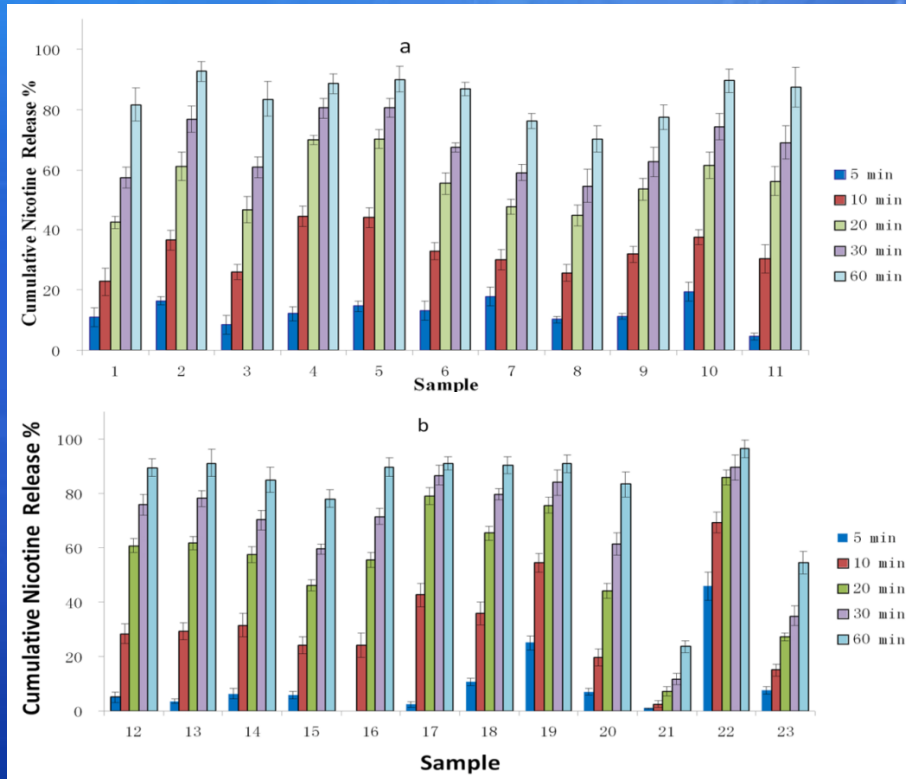# 3. Results and discussion

Fig. 5 The cumulative release rates of nicotine from 23 brands of moist snuff.

➢Nicotine release rates increased with extraction time and about 60-90% of nicotine was released after 30 min of extraction in most of the samples.

➢There were differences in nicotine release rates among the tested brands of moist snuff, and the differences were significant for a couple of brands.
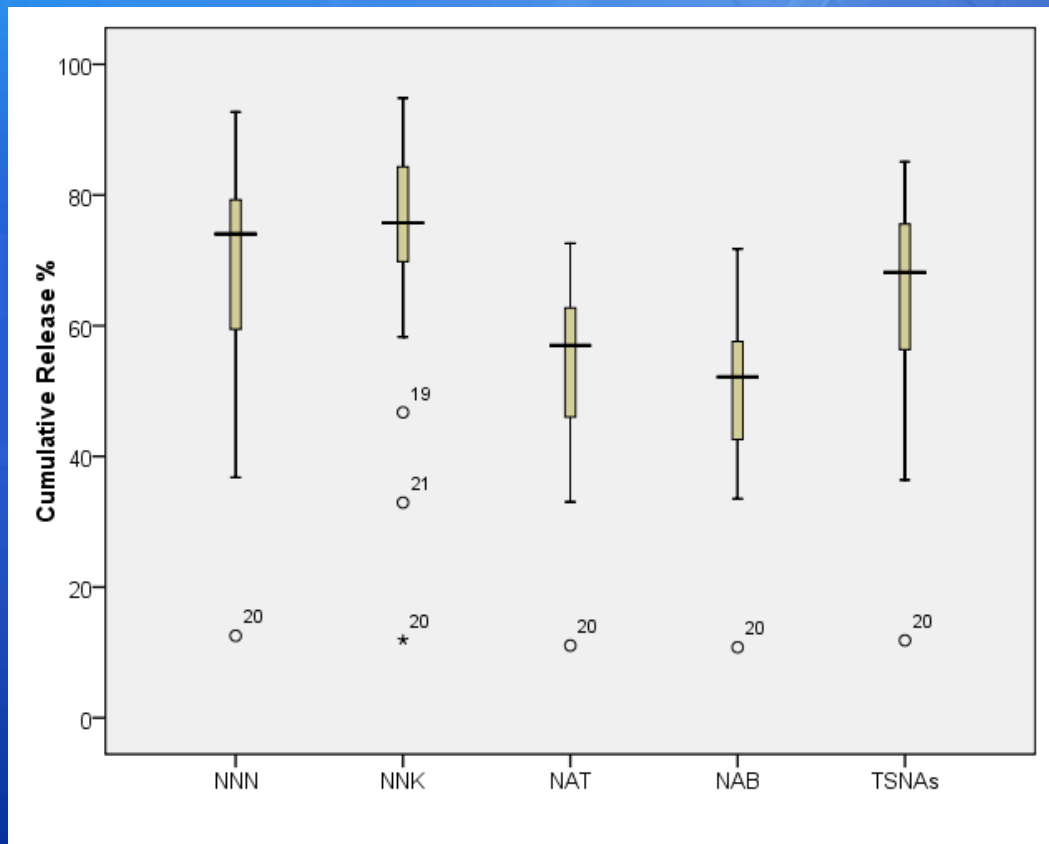
3. Results and discussion

> About 35-92% of TSNA was released after 30 min of extraction in most of the samples.

> The release rate is faster of NNK and NNN than that of NAT and NAB，and NNK is the fastest.

Fig. 6 Boxplot of the results for TSNA release rates at 30min from 21 brands of moist snuff.

4. Conclusion

◆A novel model mouth system for evaluating the *in vitro* release behavior of oral use smokeless tobacco products was designed.

◆The system performance is reliable, replicable, and consistent with human data under proper parameter settings.

◆The model mouth system can be used for comparative product evaluation studies for product development, quality control monitoring, and post-market surveillance, etc.

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Thank you for your attention and

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