Research Article

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Study on controlling nicotine release from snus by the SIPN membranes

<https://doi.org/10.1515/epoly-2021-0048>

received March 31, 2021; accepted May 28, 2021

Abstract: Snus is one of the types of smokeless tobaccos, which is popular in many countries and regions. The nicotine level in the plasma samples obtained from snus users is similar to the nicotine level obtained from cigarette smokers; hence, the snus users find it difficult to quit. The aim of this study is to develop new semi-interpenetrating polymer (SIPN) membranes that control the stable release of nicotine from snus, achieving the purpose of quitting smoking. Here, the SIPN comprising a polyacrylate network and PEG chains was synthesized through *in situ* radical polymerization. The SIPN was characterized by Fourier transform infrared spectroscopy, contact angle, differential scanning calorimetry, scanning electron microscopy, cytotoxicity, and *in vitro* release experiments. Furthermore, this SIPN was used to evaluate the release properties of nicotine in different snus samples varying in moisture, pH, and the tobacco's particle size. The results indicated that the new SIPN could control release of nicotine at a specific rate, and the release rate could be adjusted according to the customer requirements. Thus, the new SIPN was contributed to build a new nicotine replacement therapy that served the snus users.

Keywords: semi-interpenetrating polymer, controlling release membrane, controlled drug release, snus, nicotine replacement therapy

1 Introduction

Nowadays, due to the adverse health effects of tobacco use, many countries and regions have issued a strict anti-smoking regulation in public places (1,2). More and more smokers choose smokeless tobaccos including dipping tobacco, chewing tobacco, and electronic cigarette. Wherein dipping tobacco is colloquially called snuff tobacco, which is a good choice for smokers because of no burning, no second-smoke hazard, and good privacy. In Sweden, the moist form of snuff that is placed under the upper lip is called snus. Now, snus is an alternative to smoking, chewing, and dipping tobacco. Swedish snus typically has a pH in the range of 7.8–8.5; thus, in snus, the nicotine is in free-base form that is rapidly absorbed through the oral mucosal membrane (3,4). The nicotine level in the plasma samples obtained from snus users is similar to the nicotine level obtained from cigarette smokers, and the nicotine level in the plasma samples obtained from snus users is about twice as high as the nicotine concentration obtained from nicotine patch users (5,6). Thus, the snus users have nicotine dependence and find it difficult to quit.

With increasing publicity of tobacco-related diseases, many smokers are aware of the necessity of smoking cessation (7). Nicotine replacement therapy (NRT) is the most widely applied approach for smoking cessation. The different types of NRT include the transdermal patch (8,9), chewing gum (10), nasal spray (11), and inhaler (12). These NRT approaches have similar success rates in terms of smoking cessation. However, the chewing gum commonly has side effects including nausea, hiccups, and mouth irritation. The inhaler commonly results in a cough and spray results in a runny nose. Although the patch commonly results in skin irritation, a nicotine patch is an excellent option among the NRT products because it offers a convenient once-a-day treatment in comparison with chewing gum taken every hour and nasal spray 8 to 20 times a day (13).

Usually, the release of nicotine from the patch is controlled by polymer membrane permeation (14) or polymer

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matrix diffusion (15,16). Herein, we have a new NRT strategy of combining a snus and a rate-controlling membrane, so that the user can take in less nicotine but does not change the intake habit. This newly developed NRT is applied under the upper lip like typical snus, and nicotine delivery rate is controlled by the rate-controlling membrane and oral mucosal membrane. The speed of nicotine passing through the oral mucosa is very fast; thus, the absorption rate of nicotine is limited by nicotine passing through the rate-controlling membrane. This new rate-controlling membrane used in the mouth should have some characteristics including (a) good hydrophilicity, saliva can infiltrate the film and nicotine quickly dissolves from the snus; (b) good permeability, free-base form nicotine can pass through this rate-controlling membrane; and (c) good biocompatibility, rate-controlling membrane is safe to the users.

In this study, a new semi-interpenetrating polymer network (SIPN) composed of a polyacrylate network and polyethylene glycol (PEG) chains was used as the rate-controlling membranes. It is well known that the SIPNs usually have innovative properties due to a combination of favorable properties of each constituent polymer (17) and have advantages of easily formed film from polyacrylate (18) and good biocompatibility from PEG (19). These SIPNs were characterized by FTIR, contact angle, DSC, SEM, and cytotoxicity. And *in vitro*, nicotine release properties were tested on several snus products. Moreover, the effects of particle size, moisture, and pH of snus on *in vitro* nicotine release were studied.

2 Materials and methods

2.1 Chemicals and cells

Several commercial snus products were kindly provided by Shanghai New Tobacco Product Research Institute (China) including General One Portion, General White Mint, General White Portion, and Catch Licorice Dry Mini made in Sweden and Golden Deer made in China. 2-Hydroxy-3-phenoxypropyl acrylate, 4-hydroxybutyl acrylate, and 1,6-hexanediol diacrylate were purchased from TCI Chemical Industry Co., Ltd. (Shanghai, China). Benzoyl peroxide (BPO) and PEG were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Nicotine standard product with purity 98.54% was purchased from Shanghai Yaji Biotechnology Co., Ltd. (Shanghai, China).

Fetal bovine serum (FBS) was purchased from Gibco Company (USA). High-glucose DMEM and phosphate-buffered saline (PBS) were purchased from Haoyang Biological Products Technology Co., Ltd. (Tianjin, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) was purchased from Aladdin company (Shanghai, China). All other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

The L929 murine fibroblast cell was kindly provided by Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China).

2.2 Preparation of SIPN membranes

2-Hydroxy-3-phenoxypropyl acrylate, 4-hydroxybutyl acrylate, and 1,6-hexanediol diacrylate were purified by vacuum distillation under reduced pressure and stored at 4°C for further use. The SIPN membrane was synthesized by the following procedure:

- (1) 2-Hydroxy-3-phenoxypropyl acrylate, 4-hydroxybutyl acrylate, and 1,6-hexanediol diacrylate were mixed with a weight ratio of 9:9:2, then 3% (weight ratio) BPO was added, and the acrylate solution was obtained.
- (2) PEG10000 and PEG400 were mixed with a weight ratio of 1:2, and the PEG solution was obtained.
- (3) The acrylate solution and the PEG solution were mixed with different weight ratios of 8:1, 6:1, and 4:1, and the reaction solution was obtained.
- (4) The reaction solution was poured into the round stainless-steel molds at different depths and treated under the UV light (wavelength: 200–400 nm; power: 1.5 kW) for about 4 min; thus, the SIPN membrane was formed.
- (5) The SIPN membrane was carefully stripped from the stainless-steel mold and stored in deionized water.

2.3 Characterization of the SIPN membranes

FTIR was performed on the NICOLET iS10 (Thermo, USA) equipped with an attenuated total reflectance (ATR) accessory. The freeze-dry SIPN membrane was scanned ranging from 700 to 4,000 cm^{-1} with a resolution of 1 cm^{-1} and an average of 32 scans.

The glass transition temperature (T_g) of the freeze-dry SIPN membrane was tested on the DSC8500 (PerkinElmer, USA).

The samples were heated from -60°C to 120°C at a rate of $10^{\circ}\text{C min}^{-1}$ in the N_2 atmosphere.

The morphology observation of the SIPN membrane was tested on the Sirion 200 (FEI/Philips, USA). Before SEM observation, freeze-dried SIPN samples were coated with a layer of gold under vacuum using a Cressington 108 auto sputter coater.

The static contact angle of the freeze-dried SIPN membrane was determined using the ThetaLite 101 (Biolin Scientific, Sweden). One drop of deionized water was placed on the surface of the sample, and the left and right contact angles were recorded till the water drop reached equilibrium within 10 s. The average contact angle was obtained from five independent experiments.

The film thickness of the freeze-dried SIPN membranes was measured using a digital micrometer (Shanghai Measuring and Cutting Tools Factory, Shanghai, China) with 0.001 mm accuracy. Five measurements were taken for each sample.

2.4 Cytotoxicity studies of the SIPN membranes

The cytotoxicity tests of samples were done according to GB/T 16886.5-2017/ISO 10993-5:2009. L929 cells were cultured in DMEM supplied with 10% FBS and incubated at a standard culture condition (37°C , 5% CO_2 in air) (Thermo Fisher Scientific, Waltham, MA USA). The culture medium was refreshed every 2–3 days.

Before cytotoxicity tests, the SIPN membranes were sterilized by soaking in a 75% ethanol solution for 24 h and exposure to UV light overnight. The samples tested for cytotoxicity included two types. The first type of sample was an extracting solution of the SIPN membrane, that is, the membrane was cut to a square with 1 cm^2 area, three pieces of square membranes were immersed in 1 mL of DMEM supplied with 5% FBS for 24 h at 37°C , and then the extracting solution was incubated with L929 cells. The second type of sample was SIPN material itself, that is, the membrane was cut to a round shape with a 4 mm diameter. This round disc was directly incubated with L929 cells.

The cytotoxicity of the samples was tested by the MTT assay method. First, L929 cells in the logarithmic growth phase were seeded into 96-well plates with a seeding density of 10,000 cells per well and incubated overnight. Then, 100 μL of fresh complete medium was changed and 100 μL of the extracting solution was added or the round disc into each well was placed. The blank controlling

group was prepared by the same procedure without sample treatment. The negative controlling group was the complete medium, and the positive controlling group was phenol (0.64% v/v in PBS). Each sample was set to five replicates on each plate. After 24 or 48 h of incubation, original medium and samples were removed, and 100 μL of fresh complete medium and 50 μL of MTT solution (5 mg/mL in PBS) were added to each well. After 4 h incubation, the medium was carefully discarded, 100 μL of DMSO was added to each well, and then the plate was shaken for 10 min to completely dissolve formazan crystals. The optical density (OD) was measured on the Multiskan MK3 microplate reader (Thermo Fisher Scientific, Waltham, MA USA). The detection wavelength was 570 nm, and the reference wavelength was 630 nm. The OD value of the sample in each well was the OD value at 570 nm subtracting the OD value at 630 nm.

The relative growth ratio (RGR) was calculated using the following equation:

$$\text{RGR}(\%) = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{blank}}} \times 100 \quad (1)$$

The cytotoxicity level was evaluated according to ISO 10993-5:2009: if $\text{RGR} \geq 100$, the material has no cytotoxicity; if $75 \leq \text{RGR} < 99$, the material has light cytotoxicity; if $50 \leq \text{RGR} < 74$, the material has medium cytotoxicity; and if $25 \leq \text{RGR} < 49$, the material has serious cytotoxicity.

2.5 In vitro nicotine release

The SIPN membrane was fixed between horizontal Valia-Chien diffusion cells with the parameters of 37°C water bath temperature and 200 rpm stirring speed. One portion of snus was soaked in 10 mL of artificial saliva in the donor chamber. The receptor chamber was full of 10 mL of phosphate buffer solution (PBS, pH 7.4). About 1 mL of PBS in the receptor chamber was taken out at a predetermined time, then an equal volume of PBS was immediately replenished. The nicotine content in the sample was tested on the HPLC (Waters, USA). Independent three batches of membranes were used, and the diffusion experiment was replicated 3 times.

The cumulative amount of nicotine was calculated as follows:

$$Q = \frac{C_n V + \sum_{i=1}^{n-1} C_i V_i}{A}, \quad (2)$$

where Q is the cumulative amount of the drug ($\mu\text{g}/\text{cm}^2$), V is the volume of receptor solution (mL), V_i is the volume

of sample withdrawn (mL), C_n and C_i are the drug concentrations of the receptor solution and the sample withdrawn ($\mu\text{g/mL}$), respectively, and A is the diffusion area (cm^2) (20).

2.6 HPLC analysis of nicotine

The column was C18 column, $5\mu\text{m}$, $4.6\text{ mm} \times 250\text{ mm}$ (Supersil ODS2; Elite, China). The mobile phase was methanol–phosphate buffer solution ($0.02\text{ mol/L Na}_2\text{HPO}_4$, 0.2% triethylamine, pH 6.5 adjusted by phosphoric acid) with a volume ratio of 6:4. The flow rate was 1 mL/min , the column temperature was 35°C , the UV detection wavelength was 259 nm , and the injection volume was $20\mu\text{L}$ (21).

Meanwhile, the method of HPLC was validated, including linearity and range, recovery, precision, detection, and quantitation limits:

- Linearity and range: A series of diluted standard solutions of nicotine ($0.2\text{--}20\mu\text{g/mL}$) were tested on HPLC, peak areas of nicotine ($n = 5$) versus concentrations were plotted and fitted to be linear in the entire concentration range.
- Recovery: Three samples at low, middle, and high concentrations were analyzed on HPLC. The experiment was conducted in triplicate.
- Precision: The intraday variability was checked at three time points on the same day, and the interday variability was checked on three consecutive days. The results were expressed as percent relative standard deviation (%RSD) of concentration.
- Detection and quantitation limits: The LOD (limit of detection) and LOQ (limit of quantitation) were defined as the concentrations which yielded a measured peak with S/N (signal-to-noise ratio) of 3 and 10, respectively.

2.7 Mathematical modeling of *in vitro* nicotine release

The kinetic evaluation of *in vitro* nicotine release was performed by plotting the results as cumulative release of nicotine amount against time. The data were fitted to zero-order, first-order, and Higuchi models using Origin Pro 2016 software. The correlation coefficient (r) for each kinetic model was calculated to determine the model that was better fitted.

2.8 Statistical analysis

Significant differences were evaluated using a one-way analysis of variance. Differences among different samples were considered significant at $p < 0.05$.

3 Results and discussion

3.1 Method validation of HPLC

3.1.1 Linearity and range

The calibration curve for nicotine on the HPLC method is shown in Figure 1. As shown, the peak areas of nicotine were obtained to be strictly linear in the concentration range of 0.2 to $20\mu\text{g/mL}$, and the correlation coefficient value was 0.9997 .

3.1.2 Recovery

The percentage recoveries of the three concentrations from low to high were found to 99.18 ± 0.60 , 100.45 ± 0.31 , and 101.40 ± 0.20 , respectively, which confirmed that the method was accurate.

3.1.3 Precision

The %RSD of intraday of the developed HPLC method was 1.26 , and the %RSD of interday was 1.44 , which

Figure 1: Calibration curve for nicotine by the HPLC method.

suggested an excellent precision of the developed HPLC method.

3.1.4 Detection and quantitation limits

The LOD and LOQ were found to be 1.5 and 3.8 ng/mL, respectively.

3.2 Synthesis of SIPN membranes

In previous studies, we knew that monofunctional acrylate monomers were easy to form linear polyacrylates at the UV lights (22,23). When using bifunctional acrylate monomers, the network polyacrylate would be formed (24). Herein 1,6-hexanediol diacrylate was used as a cross-linker. The SIPNs of polyacrylates and PEG were prepared by the *in situ* procedure. The acrylates, PEGs and BPO, were mixed together and poured into a stainless mold; afterwards, the mixture was exposed under UV lights, and the SIPN was formed in minutes. Although the SIPN material was easy to obtain by free radical polymerizations, to obtain stable SIPN in different batches, the photopolymerization need to be conducted using the same synthesis parameters including light time, light intensity, light distance, component ratio, and so on (25).

3.3 Characterization of the SIPN membranes

The SIPN membranes made of the acrylate monomers and the PEGs at different weight ratios 4:1, 6:1, and 8:1 were named SIPN-1, SIPN-2, and SIPN-3, respectively.

The FTIR spectra of the three SIPN membranes are recorded in Figure 2. Due to the same membrane constituents but a difference in the ratio, the results showed that there were similar characteristic peaks in three pieces of FTIR spectra. Taking SIPN-1 as an example, the absorption characteristic features are assigned as follows: 3,600–3,100 cm^{-1} (ν_{OH}), 2,938 cm^{-1} (ν_{CH}), 1,599, 1,495, and 1,454 cm^{-1} ($\nu_{\text{C}=\text{C}}$, aromatic ring), 755 cm^{-1} (δ_{CH} , aromatic ring), 1,727 cm^{-1} ($\nu_{\text{C}=\text{O}}$), 1,160 and 1,241 cm^{-1} ($\nu_{\text{C}-\text{O}-\text{C}}$), and 1,041 cm^{-1} ($\nu_{\text{C}-\text{OH}}$). The characteristic absorption features of acrylate monomers at 1,630 cm^{-1} nearby (corresponding to stretching vibration of $\text{C}=\text{C}$) disappeared, illustrating difunctional acrylate monomers cure to a network (26).

The DSC thermograms of three SIPN membranes and PEG 10000 are recorded in Figure 3. A sharp molten peak

Figure 2: FTIR spectra: SIPN-1 (blue), SIPN-2 (purple), and SIPN-3 (red).

Figure 3: DSC curves: (a) SIPN-1, (b) SIPN-2, (c) SIPN-3, and (d) PEG 10000.

Figure 4: SEM image of SIPN-1 membrane: 400,000 $\times$ amplification (left) and 10,000 $\times$ amplification (right).

was observed in pure PEG 10000, and this peak value (T_g) was 67.2°C. However, this sharp molten peak disappeared in the DSC thermograms of three SIPN membranes. The molten peak of PEG 10000 became wide and weak in the SIPNs, which meant PEG 10000 forms a homogeneous system with other polymers, and there is a weak interaction force between PEG 10000 and other polymers. And this weak interaction force was the intermolecular hydrogen bonding due to hydroxyl groups. Moreover, the glass transition temperature (T_g) values of SIPN-1, SIPN-2, and SIPN-3 were -6.8°C , -5.6°C , and -4.3°C , respectively. It was known that PEG was linear polymers with flexible molecular chains; however, herein polyacrylate was crosslinked polymers with rigid networks. Thus, the T_g values of final polymers with more PEG were lower.

The SEM images of SIPN-1 are shown in Figure 4. There were no micro- or nano-level pores in the film,

indicating that PEGs didn't take on the role of the porogen like in the literature (27), but interpenetrated into the polyacrylate network and resulted to generate a homogeneous structure.

The static contact angles (θ) of the SIPN membranes are listed in Table 1. The θ values of all membranes were less than 90° , which indicated the membranes had good hydrophilicity.

3.4 Cytotoxicity of the SIPN membranes

The RGRs of the SIPN membranes are listed in Table 2. Herein, the RGR values of 24 and 48 h negative controlling group were 120.0% and 124.6%, respectively. The RGR values of 24 and 48 h positive controlling group were 34.5% and 13.3%, respectively. The results showed that the SIPN membranes had good biocompatibility.

Table 1: The contact angle values of SIPN membranes

Materials	θ ($^\circ$)
SIPN-1	46.93
SIPN-2	47.02
SIPN-3	57.10

3.5 *In vitro* nicotine release

It is known that the nicotine in the snus is so quickly absorbed through the oral mucosal membrane that the

Table 2: The RGR values of SIPN membranes

Materials	RGR (%)		
	24 h – Extracting solution	48 h – Extracting solution	24 – Contacting membrane
SIPN-1	118.7	129.2	97.3
SIPN-2	109.1	132.0	111.6
SIPN-3	110.3	137.0	119.3

Table 3: Information of snus products

Snus	Product name	Portion weight (g)	Nicotine content (mg)
C2	General one portion	0.85	8.29
C3	General white mint	1	8.27
C4	General white portion	0.9	8.31
C5	Catch licorice dry mini	0.3	4.37
S1	Golden deer	0.37	4.5

Table 4: The fitting parameters by a zero-order kinetic model of *in vitro* release from SIPNs with 10 μm thickness

The parameters	snus C4			snus C5		
	SIPN-3	SIPN-2	SIPN-1	SIPN-3	SIPN-2	SIPN-1
J ($\mu\text{g}/(\text{cm}^2 \text{h})$)	2.1437	3.0249	5.5046	1.4208	1.8309	3.7357
r	0.9962	0.9962	0.9994	0.9930	0.9957	0.9996

snus users have nicotine dependence and find it difficult to quit. Thus, if SIPN was used as the controlling membrane in snus, then the user is expected to control the absorption of nicotine and at last quit smoking.

Here, five commercial snus products (C2, C3, C4, C5, and S1) are used to evaluate *in vitro* nicotine release; the portion weight and nicotine content of snus products are listed in Table 3.

Three SIPN membranes with 10 μm thickness, namely SIPN-1, SIPN-2, and SIPN-3, were fixed between two horizontal Valia-Chien diffusion chambers to choose a suitable rate-controlling membrane possessing both good physicochemical properties and controlling the drug-release properties. The results showed that the curve of cumulative nicotine release amount against time was well fitted to a zero-order kinetic model with a high correlation coefficient (r). The flux namely the release rate (J) is presented in Table 4. To the same snus product, the flux increased with an increase in the PEGs content in SIPNs. It may be explained by the physicochemical characteristics of SIPNs. When compared with SIPN-2 and SIPN-3, SIPN-1 had a lower T_g value, which meant SIPN-1 had better flexibility of the polymer and consequently nicotine passed through the SIPN membrane faster.

It is well known that the membrane thickness was an important factor to release flux in the membrane-controlling-rate drug delivery system. When the SIPN membrane's thickness was changed to 13 μm , the SIPN membranes still controlled nicotine release from the snus products followed zero-order kinetic models (Table 5). For example, when nicotine was released from snus C4 through SIPN-1 with a thickness of 10 and 13 μm , the flux values were

5.5046 and 3.9899 $\mu\text{g}/(\text{cm}^2 \text{h})$, respectively. When nicotine released from snus C5 through SIPN-1 with a thickness of 10 and 13 μm , the flux values were 3.7357 and 2.7489 $\mu\text{g}/(\text{cm}^2 \text{h})$, respectively. Hence, the differences in the effects of membrane thickness on the flux were significant ($p < 0.05$). These results indicated that the thickness was a facile and an available way to adjust the flux in comparison with the adjustment of the membrane components. This was also the most outstanding advantage of the membrane-controlling-rate drug delivery system when compared with the polymer matrix-type drug delivery system. The kinetic model of the former was fitted by the zero-order release equation, and it had an outstanding advantage of a controlled releasing rate. The kinetic model of the latter was fitted by the Higuchi equation (28) or first-order equation (29,30), and it had the property of sustained release rate.

The SIPN-1 membranes with 13 μm thickness were also used to evaluate other snus products C2, C3, and S1. The curves of cumulative nicotine release amount

Table 5: The flux of snus *in vitro* release through SIPN-1 with 13 μm thickness

Snus	J ($\mu\text{g}/(\text{cm}^2 \text{h})$) mean (S.D.)	Correlation coefficient (r) mean (S.D.)
C2	40.2353(3.6161)	0.9990(0.0007)
C3	16.2743(1.8797)	0.9988(0.0003)
C4	3.9899(0.4084)	0.9939(0.0052)
C5	2.7489(0.4163)	0.9927(0.0033)
S1	4.6571(0.2853)	0.9961(0.0008)

Figure 5: Nicotine release curve from SIPN-1 with 13 μm thickness.

against time (Figure 5) were well fitted to zero-order kinetic models with high correlation coefficient (r). The fitting parameters are presented in Table 5.

In five commercial snus products, C2, C3, and C4 had similar nicotine amounts, C5 and S1 had nearly half the amount of nicotine when compared with C2, C3, and C4. However, they showed different release properties by the SIPN-1 controlling. For example, the values of J decreased in the order of C2, C3, and C4 and increased in the order of C5 and S1. No clear correlation between nicotine release and nicotine amount indicated that there were other factors affecting the nicotine release. In the production process of snus, it is necessary to consider tobacco's particle size, moisture, and pH besides the nicotine amount. However, it was difficult to get the information of the commercial snus products in detail.

To further study the effects of the moisture, pH, and tobacco particle size on nicotine release, here we prepared some snus samples referring to different production parameters (Table 6). The SIPN-1 with 13 μm thickness was used as the rate-controlling membrane. The results of *in vitro* nicotine release amount against time

Table 7: The flux of snus samples with different production parameters

Sample	J ($\mu\text{g}/(\text{cm}^2 \text{ h})$)	Correlation coefficient (r)
S2	8.8642(1.472)	0.9966(0.0012)
S3	19.1465(1.0854)	0.9566(0.0057)
S4	13.6785(4.2857)	0.9901(0.0063)
S5	5.8926(0.3748)	0.9973(0.0015)
S6	6.5567(1.0920)	0.9889(0.0117)
S7	6.5949(1.2689)	0.9983(0.0005)

well fitted to zero-order kinetic models (Table 7). Samples S5 and S6 had the same particle size, similar moisture, and nicotine content, but they had different pH, the former was weak acid and the latter was weak base. The flux of S6 was higher than that of S5 ($p < 0.05$). The reason was that the nicotine was free-base form in an alkaline pH, which promoted nicotine rapid releasing from snus samples (31).

Samples S6 and S7 had similar moisture and nicotine content, but they had different pH, theoretically, the flux of S7 was higher than S6 because S7 showed a weak base and S6 showed weak acid. But the particle size of S7 was much higher than the S6; as a result, the fluxes between S6 and S7 were not significantly different ($p > 0.05$). Hence, the particle size was inversely related to the flux.

The release situations among S2, S3, and S4 were complicated, they had the same particle size and their pH was all weak base. The moisture obviously decreased and the nicotine content slightly increased following the sequence of S2, S3, and S4, but the order of the flux was $S3 > S4 > S2$. Thus, the effects of moisture on the flux were difficult to determine.

4 Conclusion

Commercial nicotine transdermal patches need to contain the pressure-sensitive adhesive (PSA) to apply the patch on the skin; however, PSA is a key factor inducing skin irritation and sensitization (32). Snus is placed under the upper lip, no PSA, no irritation which shows better patient compliance. Herein, new SIPN membranes composed of polyacrylates and PEG were synthesized. It showed the excellent properties of controlling nicotine release. It was expected to use as the rate-controlling membrane in snus, which would help snus user quit smoking. We can deduce the absorption of nicotine in this new administration is readily to tailor by the SIPN membrane's thickness and constituent. In summary, this

Table 6: The production parameter of snus samples

Sample	Moisture (%)	pH	Particle size (mesh)	Nicotine content (mg)
S2	40.00	7.90	18	3.78
S3	30.00	7.80	18	4.06
S4	20.00	7.67	18	4.32
S5	35.20	7.70	18	4.17
S6	36.15	6.70	18	4.31
S7	34.57	8.00	60	4.41

study proposes a new NRT hypothesis that the new nicotine administration not only change the way of snus usage but also decrease the nicotine intake. Moreover, the rate-controlling membrane developed in this study could be used to evaluate the relation of nicotine *in vitro* release flux of the snus with production parameters.

Funding information: This work was supported by Shanghai Tobacco Group Co., Ltd. (Project No. 2018310036700038).

Author contributions: Xiaoping Zhan: writing – original draft, methodology, data collection, and analysis; Xiaopeng Jian: project administration, resources offering, and funding acquisition; Zhenmin Mao: writing – review and editing.

Conflict of interest: Authors state no conflict of interest.

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