Membrane Water Treatment, *Vol. 8, No. 3 (2017) 245-257*
DOI: https://doi.org/10.12989/mwt.2017.8.3.245

A high-effective method to separate nicotine from the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) mixtures using electrodialysis

Shaolin Ge[1,2], Wei Li[3], Zhao Zhang[2], Chuanrun Li[1,3] and Yaoming Wang*[1,3]

[1]CAS Key Laboratory of Soft Matter Chemistry, Laboratory of Functional Membranes,
School of Chemistry and Materials Science, University of Science and Technology of China,
Hefei, Anhui 230026, P.R. China
[2]China Tobacco Anhui Industrial Co., LTD, Hefei, Anhui, 230088, P.R. China
[3]Hefei ChemJoy Polymer Materials, Co., LTD, Hefei, Anhui, 230601, P.R. China

(*Received March 9, 2016, Revised December 13, 2016, Accepted December 22, 2016*)

Abstract. The separation of nicotine and tobacco-specific N-nitrosamines is a tough problem in tobacco industry. In this study, separation of nicotine from 4-(methylnitrosamino) -1-(3-pyridyl)-1-butanone (NNK) mixtures was investigated using electrodialysis by taking the principle of the protonation status difference between these two components. The results indicated that the solution pH has a dominant impact on the separation process. In a pH range of 5-7, nicotine molecules are existed as mono- and di-protonated ions and can be separated from the uncharged NNK molecules. The acidic electrolyte is conducive to the separation process from the point of flux and energy consumption; while the alkaline electrolyte has negative impact on the separation process. A current density of 10 mA/cm^2 is an appropriate value for the separation process. The lowest energy consumption of the separation process is 0.58 kWh/kg nicotine with the process cost to be estimated at only \$0.208 /kg nicotine. Naturally, electrodialysis is a high-efficiency, cost-effective, and environmentally friendly process to separate and purify nicotine from tobacco juice.

Keywords: 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); nicotine; electrodialysis; ion exchange membranes

1. Introduction

Tobacco use is a global public crisis, which is the leading preventable cause of disease, disability, and death in many countries. There are more than 1 billion smokers worldwide (WHO 2012, Hecht 2003). The World Health Organization estimates that tobacco kills nearly 6 million people each year. Approximately one person dies every six seconds due to tobacco which is responsible for one in 10 adult deaths. More than half of the current smokers will eventually die of a tobacco-related disease. The global consumption of tobacco is increasing even through the number of smokers in the developed countries is decreasing, since about 80% of smokers worldwide live in low- and middle-income countries. There are thousands of extremely complex

---

*Corresponding author, Ph.D., E-mail: ymwong@ustc.edu.cn

http://www.techno-press.org/?journal=mwt&subpage=7 ISSN: 2005-8624 (Print), 2092-7037 (Online)

compounds including nicotine and carcinogens in the tobacco products. Numerous studies have indicated that the combination of nicotine and carcinogens is devastating and casually associated with various cancers. Nicotine is one of special and natural compound of all types of tobacco products. It is released after inhalation and has a stimulant effect on the human. Nicotine can increase the flow of epinephrine which is a stimulating hormone and neurotransmitter. This is also the reason for the commercial value of tobacco use. Even though nicotine is non-carcinogenic matter, it is able to activating many signaling pathways to generate the tobacco-specific N-nitrosamines (TSNAs). TSNAs are a major group of carcinogens in tobacco, which are formed by the reaction between nicotiana alkaloids and nitrate or nitrite salts during the processing, curing and smoking process. There are mainly four kinds of TNSAs identified in tobacco products, i.e. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosonornicotine (NNN), N'-nitrosoanabasine (NAB) and N'-nitrosoanatabine (NAT). NNK is the strongest carcinogen which induces tumors in lung, liver, nasal, mucosa, and pancreas in rodents (Yang *et al.* 2004), and plays a significant role in causing cancer in the humans (Magee 1996). NNK can induce the methylation, pyridyloxobutylation and pyridylhydroxybutylation of nucleobases in DNA and form DNA adducts, leading to the carcinogenic functions (Zheng *et al.* 2011, Hoffmann *et al.* 1996, Bedard *et al.* 2002). There is a directly correlation between TSNAs and nicotine in the tobacco. Nicotine can be metabolized or degraded to generate the secondary metabolites, including the cotinine, demethylation nicotine, and pseudooxynicotine. The latter two matters are the intermediates of NNK and NNN. Even though much attention has been dedicated to identify the correlation between nicotine and TNSAs accumulation during the curing process, the detailed mechanism of the formation of TNSAs is still a matter of controversy. To elucidate the detailed correlation between nicotine and TNSAs, the first and foremost thing is to purify nicotine from TNSAs mixture compound. Since the similar properties between nicotine and TNSAs, which have a similar molecular weight and a similar solubility to solvent, it is difficult to separate nicotine from TNSAs mixtures.

In addition, the commercial demand of high-purity nicotine is sharply increased due to medical and pesticide use of nicotine, and especially due to rise in popularity of electronic cigarettes (Ayers *et al.* 2011, Grana *et al.* 2014). Currently, nicotine is prepared from the tobacco by "dilution-organic solvent extraction", "distillation with alkaline", "carbonization-decarboxylation", "ion-exchange resin", "supercritical fluid extraction", and etc (Houston 1952, Leslie *et al.* 1993, Fischer *et al.* 1996, Ng *et al.* 2003). All these methods can be referred to separate nicotine and TNSAs, but the separation performance is poor due to the low selectivity in the liquid-liquid extraction process. Therefore, it is of significance to explore some novel technologies to separate nicotine from TNSAs, which is not only conducive to elucidate the formation mechanism of TNSAs but also to increase the purity of nicotine during the preparation process.

Electrodialysis (ED) is a powerful separation technology, which has found more and more significant applications in chemical cleaner production, environmental protection and energy conversion (Zhang *et al.* 2014, Aghajanyan *et al.* 2013, Ali *et al.* 2016, Majewska-Nowak 2013). As opposed to other separation techniques, electrodialysis (ED) does not suffer from major drawbacks, such as generation of large amounts of waste, use of hazardous solvents, short lifetimes of adsorbents, etc. ED is thus considered an environmentally friendly and sustainable technology and may be a very competitive one when it uses in the tobacco industry. Since the ionization status of nicotine is sensitive to surrounding pH, it can be existed as the free molecule, mono-protonated and di-protonated ions (Dixon *et al.* 2000, Morie 1972). According to the protonation status difference between nicotine and TNSAs, it is possible to separate nicotine from

Table 1 The main properties of the prepared tobacco extract solution

Tobacco extract solution	Nicotine concentration (g/L)	NNK Concentration (g/L)	pH	Conductivity (mS/cm)	TDS (g/L)
Properties	46.7	0.9	7.5	12	~0

Table 2 The main characteristics of membranes used in the experiments

Properties	Homogenous cation exchange membrane CJ-MC-2	Homogenous anion exchange membrane CJ-MA-2
Thickness/mm	0.200	0.145
Ion exchange capacity/mmol/g	1.50	1.25
Water Uptake/%	35	32
Resistance/$\Omega\cdot cm^2$	1.5	1.2
Transfer number/%	98	99
Break stress/MPa	>3.5	>3.5

the TNSAs mixtures. Therefore, the main objective of this study is to test the feasibility of separation of nicotine from NNK, the most representative component of TNSAs, by using electrodialysis process.

2. Experimental

2.1 Materials

Nicotine and NNK contained mixture solution, prepared from burley tobacco, was supplied by China Tobacco Anhui Industrial Co., Ltd, Hefei, Anhui Province, China. The main properties of prepared tobacco extract solution were presented in Table 1. The membranes used for the electrodialysis (ED) experiments were CJ-MA-2 (Hefei ChemJoy Polymers Co., Ltd, China) and CJ-MC-2 (Hefei ChemJoy Polymers Co., Ltd, China), and their properties are listed in Table 2. All the chemicals were of analytical grade.

2.2 ED Set-up

A schematic diagram of ED set-up is illustrated in Fig. 1. The ED set-up was composed of (1) a cathode and an anode were respective fixed on two organic glasses plate each with a rectangular notch; the electrodes were made of Iridium-Tantalum with a thickness of 1.5 mm. The electrodes were fixed into the notch with epoxy glue to make an even surface on the organic glasses. A direct current power supply (WYL1703, Hangzhou Siling Electrical Instrument Ltd.) was connected on the electrodes. The voltage drop across the stack was directly read from indicators on the power supply. The electrodialysis was initially operated at a constant current mode but then swifted into the constant voltage mode when the stack voltage reach the maximum voltage drop of the direct current power. (2) Eleven pieces of cation exchange membranes and ten pieces anion exchange membranes, which are alternatively arranged. The effective area of each membrane was 200 cm^2.

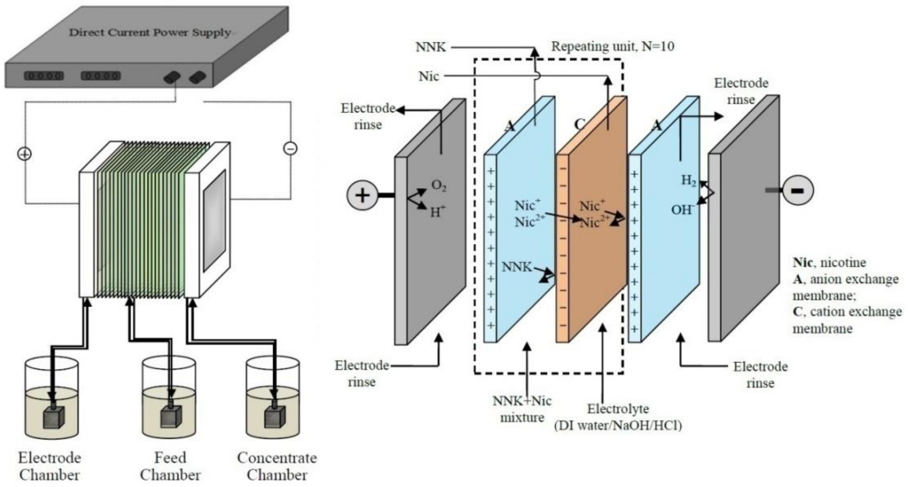

Fig. 1 Schematic diagram for the electrodialysis experiment and the principle of the separation process

(3) Sealing spacers made from PE with a thickness of 0.75 mm was used to separate the membranes; (4) Beakers to store the feed. Each beaker was connected with a submersible pump (AP1000, Zhongshan Zhenghua Electronics Co. Ltd., China, flow rate of 22 L/h) to form a circulating loop. In our experiments, three circulating loops, namely, electrode chamber, concentrate chamber, and diluted chamber were established in this ED stack. A 500 ml Na_2SO_4 solution (0.3 mol/l) was fed into the electrode chambers as the rinse electrolyte. A 400 mL nicotine and TNSAs mixed solution was fed into the dilute chambers. A 500 mL electrolyte solution (0.05 mol/L HCl, 0.05 mol/L NaOH and deionized water) was fed in the concentrate chamber. Before the experiment, each chamber was circulated for 30 min to eliminate the visible bubbles. Because the bubbles were trapped in the compartment, it will increase the resistance, voltage drop, and energy consumption of the stack. Current and bubbles collaboratively divert to localized “hot-spots” at high current density on the interface between solution and membrane which may lead to membrane damage and thus its lifetime is reduced. All the experiment runs were kept at room temperature.

2.3 Energy consumption

The energy consumption (E, kWh/kg) of the ED process was calculated as Eq. (1)

$$E = \int_0^t \frac{UIdt}{C_t VM} \tag{1}$$

U(V) is the voltage drop across ED stack; I (A) is the current applied; V is the volume of feed compartment; C_t is the concentration of nicotine; t is the time. The selectivity (S) of nicotine compared with NNK during the electrodialysis process was calculated as Eq. (2)

$$S = \frac{J_{Nic}}{J_{NNK}} \tag{2}$$

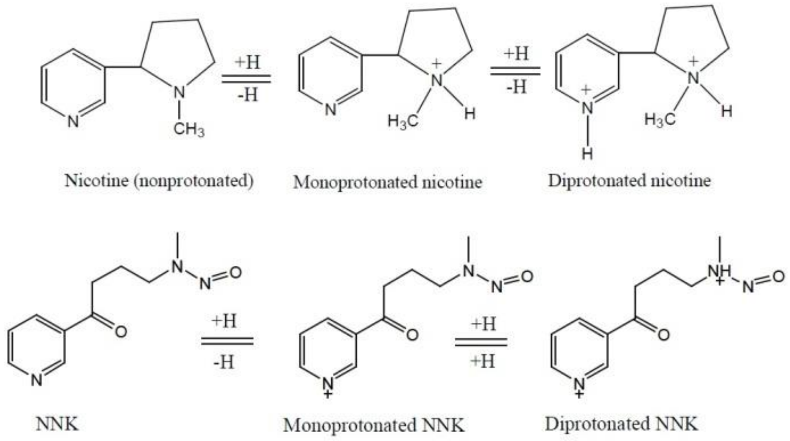

Fig. 2 The ionisation status of nicotine and NNK upon different pH range

Where J_{NIC} ($mol \cdot m^{-2} \cdot s^{-1}$) is the mean flux of nicotine transported from feed into the concentrate chamber during the experiment; J_{NNK} ($mol \cdot m^{-2} \cdot s^{-1}$) is the mean flux of NNK transported from feed into the concentrate chamber.

2.4 Analytical methods

The conductivity of the feed solution (dilute chamber) was monitored by a conductivity meter (DDS 307, Shanghai INESA Scientific Instrument Co. Ltd., China). The nicotine concentration was determined using the continuous flow method according to China Tobacco Industry standard YC/T 160-2002 in the literature (Liu *et al*. 2002). The TNSAs concentrations were determined by LS-MS (Acquity H-Class UPLC, Waters Corp. USA; 4000Q Trap, AB Sciex, USA) according to the literature (Ding *et al*. 2005).

3. Results and discussion

3.1 Effect of initial pH in the feed solution

Since nicotine molecule contains two heterocyclic nitrogen rings and exists as a weak base. Nicotine can exist as free base (nonprotonated) or as monoprotonate or diprotonatedsalts under different pH range, as depicted in Fig. 2. The pH value of the nicotine and NNK mixtures will have a significant impact on the separation process. Fig. 3 shows the effect of initial pH on the conductivities of dilute chamber. It should be noted that the conductivity is used to just give a directly information of the desalination process since the solution conductivity is significantly affected by the proton and hydroxide ions concentration. It can be seen that the conductivities in the dilute chambers are decreased with the elapse of time. At the same time, the lower initial pH value in the dilute chamber, the higher the conductivities will be. Since conductivities is the most extensive hint of the ionic strength in the aqueous solution. The decrement in the conductivity suggests the depletion of ionic electrolyte in the dilute chamber of ED stack. It is expected that

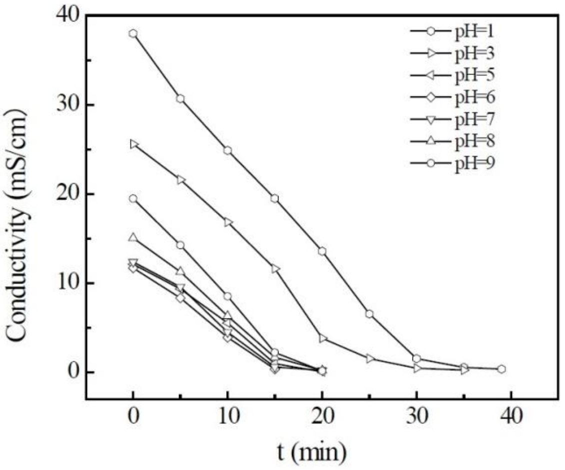

Fig. 3 The conductivity-time curves in the dilute chamber under different initial pH value

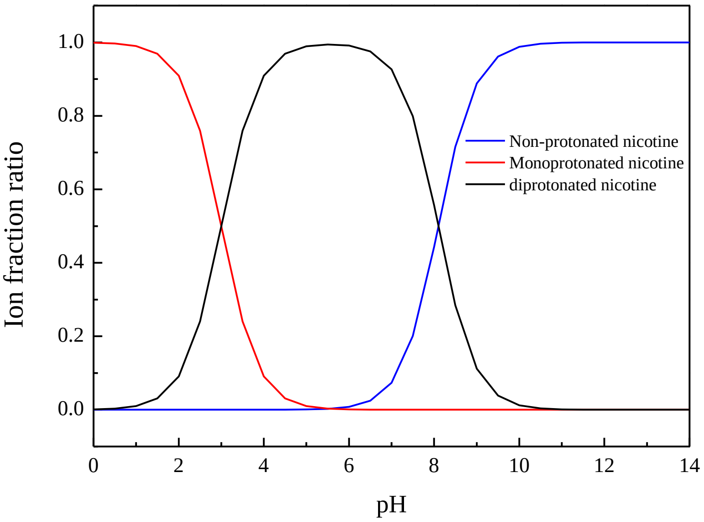

Fig. 4 The distribution percentage of nonprotonated, monoprotonated, and diprotonated nicotine throughout the pH range (Clayton *et al*. 2014)

nicotine is removed from the feed solution during the ED process. Meantime, the conductivities in the dilute chamber follow the order: low pH levels> high pH levels >middle pH levels. This is mainly two reasons for this. One is that the high equivalent conductivity of protons in acidic conditions compared with that of hydroxyl ions in alkali conditions. The other one is that more ions will be ionisated under the acidic conditions considering the ionization status of nicotine. This can also be seen from the percentage curve of the nicotine at different pH ranges in Fig. 4. More than 95% of nicotine molecules are existed as the diprotonated form at the initial pH of 1, while nearly 100% of nicotine molecules are existed as the nonprotonated form at the initial pH of 9. The conductivities at the high pH levels are contributed by the inorganic base during the pH adjustment process.

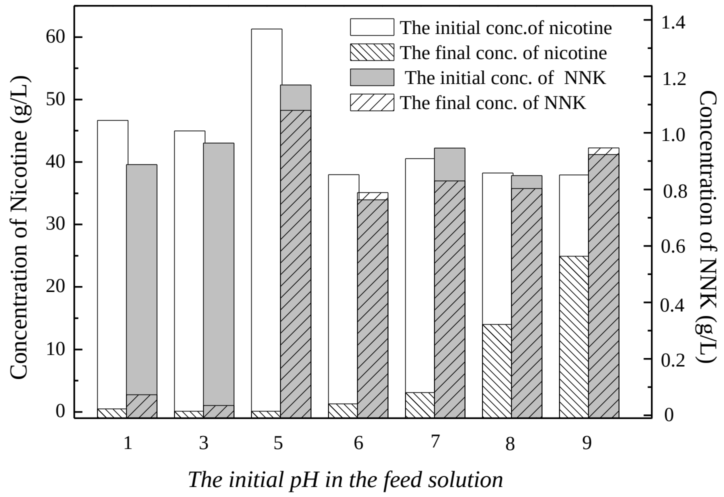

Fig. 5 Concentrations of nicotine and NNK before and after the experiments under different initial pH value

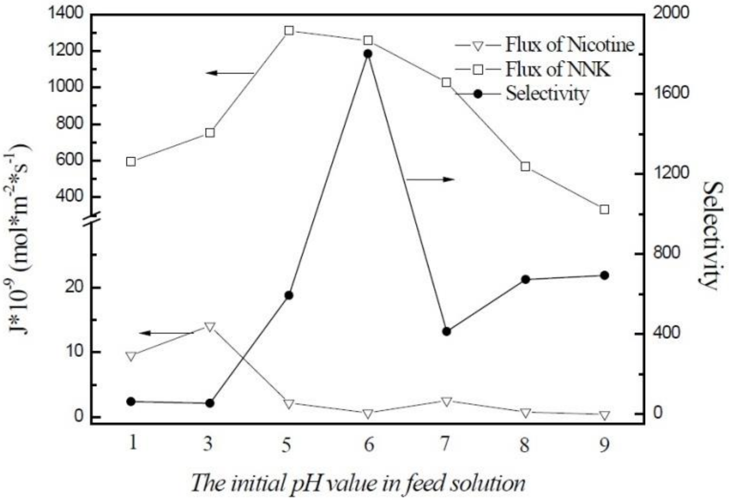

Fig. 6 The flux of nicotine and NNK, and the selectivity of separation process

Fig. 5 shows the changes in concentrations of nicotine and NNK before and after the ED experiments. It is noted that these concentrations are referred to the diluates. It is indicated that nicotine are almost removed from the feed solution at the acidic pH levels (1, 3, and 5), while the residual number of nicotine is increased with an increase in pH value. In comparison, NNK are nearly completely removed at acidic pH levels (1 and 3), but was almost retarded in the feed chamber in the pH range of 5-9 after the ED process. This indicated that it is feasible to separation nicotine and NNK in the pH range of 5-7 using electrodialysis process. The majority of nicotine molecules are existed as the monoprotonated ions in this pH range, and it is easy to separate nicotine from no-charged NNK mixtures by taking the principle of permselectivity in ion exchange membranes. It is interesting to note that the separation performance by electrodialysis can give some information about the ionization status of NNK molecule throughout pH change. There is not

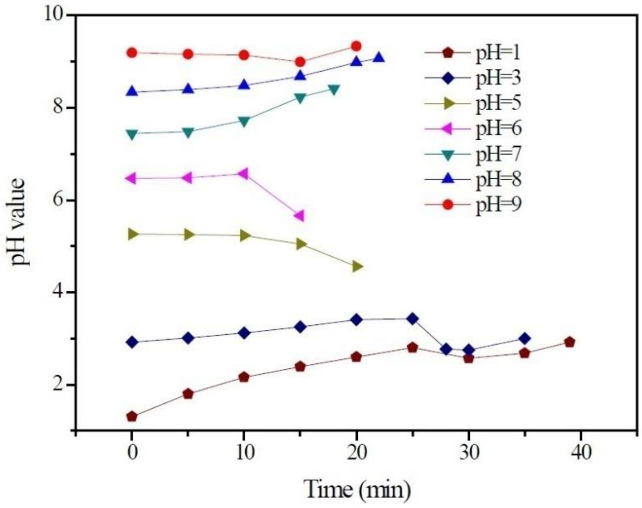

Fig. 7 The pH changes in the feed solution during the experiments under different initial pH value

much information about this NNK ionization status in the literature. The current experiment result indicates that the logarithmic acidity constant (pKa) of NNK is lower than that of nicotine. The fluxes of nicotine and NNK during the ED process as well as the selectivity of separation process are depicted in Fig. 6. It is clearly indicated that the fluxes of nicotine increase at first and then decrease with the increase of initial pH in the feed solution. The fluxes of NNK in the acidic pH levels (1, and 3) are much higher than that of the remaining investigated pH ranges. The selectivity of separation process also increases at first and then decreases with the increase of initial pH in the feed solution with the highest selectivity near the neutral pH range.

Fig. 7 indicates the pH changes in the feed solution during the experiments under different initial pH value. Three different trends are observed. In a high pH range (pH=7, 8, and 9), the pH values in feed solution are increased with the elapse of time. Under this alkali condition, nicotine exists as the free molecule. Nicotine is separated from the mixture solution by the transformation of free molecule into the protonated nicotine, leading to the introduction of hydroxyl ions in the feed solution. In near neutral pH (pH=5 and 6), the pH values in feed solution keep stable at first and then decrease at the late stage of experiment with the elapse of time. In this case, nicotine is existed as the protonated ions and a small fraction of diprotonated ions. Because the protonated nicotine are continuous removed from the feed solution using electrodialysis process. This will facilitate the transformation of diprotonated nicotine into the protonated nicotine, leading to decrease of the pH in the feed solution. In a low pH range (pH=1 and 3), the pH values increase at first and then slightly decrease at the last stage of experiments. In this case, the nicotine molecules are existed as the diprotonated ions. These protons carriers are removed during the ED process, leading to pH increment in feed solution. But when most diprotonated nicotine ions are depleted in the feed solution, the free NNK molecules begins the protonation reaction, leading to introduction of hydroxyl ions in the feed solution.

3.2 Effect of electrolyte type and current

Due to the poor ionic conductivities in the nicotine contained mixture solution, the ED

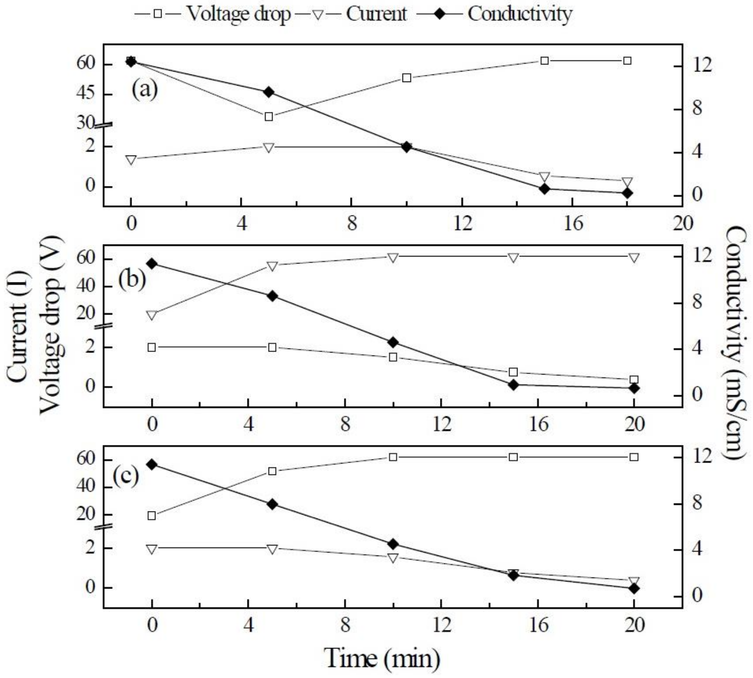

Fig.8 The effect of electrolyte type on the current and voltage drop in the ED stack as well as on the conductivity in the feed solution. (a)electrolyte, deionized water; (b) electrolyte, 0.05mol/L HCl solution; (c) electrolyte, 0.05 NaOH solution

experiment was operated under high voltage. To increase the voltage drop in the ED stack, three kinds of electrolytes, i.e., the 0.05 mol/L NaOH solution, 0.05 mol/L HCl solution, and deionized water are investigated. Fig. 8 shows the effect of electrolyte type on the current and voltage drop in the ED stack as well as on the conductivity in the feed solution. The initial pH in dilute chamber is 5. It is indicated that introduction of external electrolyte can decrease the starting voltages. But the voltage drops in the acidic and alkaline electrolytes exhibit the similar trend. It means that the voltage drops increase sharply after initiation of experiment and remain at a plateau on the maximum direct current supplier. In comparison, the voltage drop of ED stack with deionized water as the electrolyte decreases at first and then increases to the maximum voltage thereafter. This is because the migration of nicotine and NNK ions from the feed solution into the concentrate chamber can decrease the resistance of stack at the early stage of experiment. The voltage drop thereafter increases with the depletion of the charged ions.

Fig. 9 shows the effect of electrolyte type on the separation performance of nicotine from the mixture solution. The initial pH in the dilute chamber is 5. It should be point out that the feed solution is obtained in different batch of samples. The initial concentrations of nicotine are tested at the time of applying a direct current field after the feed solution circulated for 30 min. So there is a little difference for the initial concentration of nicotine concentrations in three kinds of electrolytes. Nevertheless, the separation performance is clearly indicated by the flux of nicotine and the energy consumption of process. It is clearly indicates that the HCl electrolyte is the most suitable choice from the point of flux and energy consumption. An alkaline electrolyte has negative impact on the separation performance. A possible reason is that nicotine is oxidized into

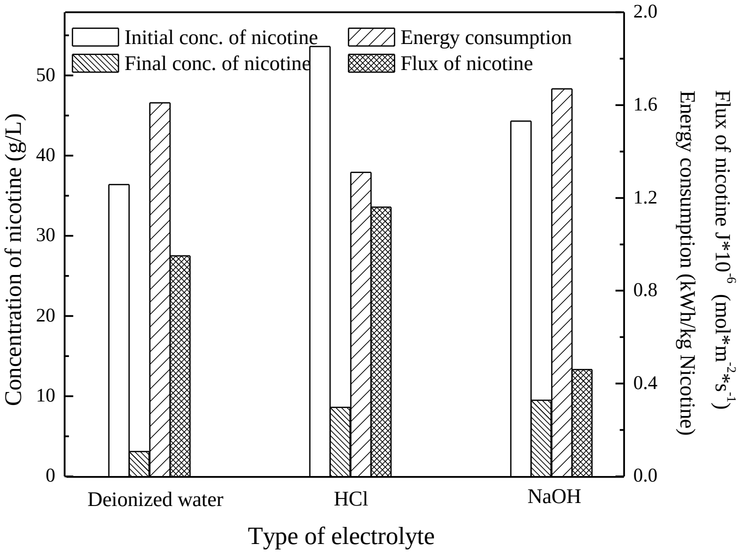

Fig. 9 Effect of electrolyte type on the separation performance of nicotine from the mixture solution

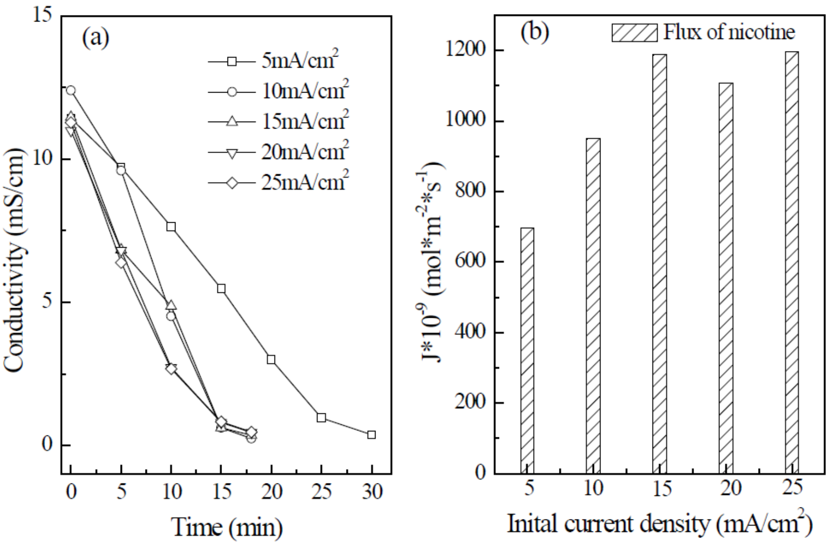

Fig. 10 Effect of applied initial current density on the separation performance of nicotine from the mixture solution

NNK under alkaline condition .Because it is observed that this is a slight increase of NNK in the concentrate chamber after the experiment compared with the initial value. It is also should be noted that the separated nicotine is mixed with the electrolyte when choosing an acidic or alkaline electrolytes. A further separation process, though not difficult, is still needed to obtain a high purity nicotine sample.

Fig. 10 shows the effect of applied initial current density on the separation performance of nicotine from the mixture solution with the deionized water as the electrolyte solution. It can be

Table 3 Estimation of process cost for the separation of nicotine from NNK mixtures using electrodialysis

Parameters	Mixture solution
Batch separation nicotine, g	24.45
Batch experiment time, min	20
Initial current density, mA/cm^2	10
Effective area of membrane, cm^2	200
Number of anion exchange membrane	10
Number of cation exchange membrane	11
Price of membrane,\$/m^{-2}	100
Energy consumption, kW·h/kg nicotine	0.59
Energy cost for the peripheral equipment	0.03
Treatment capacity, kg nicotine/year	563.3
Electricity charge, \$/kWh	0.1
Energy cost， \$/kg nicotine	0.062
Membrane cost, \$/year	42
Stack cost, \$/year	63
Peripheral equipment cost, \$/year	94.5
Total investment cost, \$/year	157.5
Amortization, \$/year	52.5
Maintenance, \$/year	15.75
Total fixed cost, \$/year	80.85
Total fixed cost, \$/kg nicotine	0.144
Total process cost, \$/kg nicotine	0.208

seen in Fig. 10(a) that the conductivities in the feed solution are sharply decreased with the elapse of time. The 5 mA/cm^2 current density requires more experimental time compared with the other current densities. It is logically truth that the higher the current is, the higher the drive force will be in an electrodialysis process. There are no significant differences in conductivity for current density in the range of 10-25 mA/cm^2. There is due to the limitation of direct current supply with the maximum voltage of 60 V. The ED operation mode shifts from current-constant mode to the voltage-constant mode when the voltages reach the maximum voltages. Due to the poor conductivity in mixture solution, there are no significant differences in the real current supplied to the ED stack at the initial current density higher than 10 mA/cm^2. The fluxes of nicotine in Fig. 10 (b) demonstrate the similar trend. There is a similar value in fluxes at initial current density higher than 10 mA/cm^2, that is high than the flux at current density of 10 mA/cm^2. There is consistent with the conductivity curve. The initial current density of 10 mA/cm^2 is an appropriate value for the separation process.

3.3 Process economy

Estimation of the operating cost of ED for separation of nicotine from NNK mixtures is paramount important to determine the economical feasibility of this process. The process cost is calculated by referring the literature (Wang *et al*. 2011, Strathmann and Koops 2000), and the result is listed in Table 3. The process cost contains the investment cost and energy consumption. The total investment cost is the sum of the stack cost and peripheral equipment cost. The stack cost is proportional to the membrane cost and is estimates to be 1.5 times the total membrane cost. The peripheral equipment cost (pumps, monitoring, control panels, etc.) is estimated at 1.5 times the stack cost. The energy consumption is mainly comprised of energy for ion transport and peripheral equipments such as pumps, control devices, and electrode reactions. The energy required for peripheral equipments is estimated at 5% of energy for ion transport. This process cost is estimated under the initial feed pH of 5 and current density of 10 mA/cm^2 with deionized water as electrolyte. The total process cost is estimated to be 0.208 $/kg nicotine. The energy consumption accounts 30% of the total process cost. The price of high-purity nicotine is higher than 100 $/kg nicotine. Therefore, it is a high-efficiency, cost-effective and environmentally friendly method to separation nicotine from NNK and nicotine contained mixtures using electrodialysis.

4. Conclusions

Laboratory-scale experimental set-up was used to verify the feasibility of electrodialysis for the separation of nicotine from the tobacco-specific carcinogen 4-(Methylnitrosoamino)-1-(3-pyridyl)-1-butanone(NNK) mixtures. Results indicated that the initial pH in the feed solution has a significant impact on the separation. By maintaining the feed solution pH in the range of 5-7, nicotine is dissociated into the mono- and diprotonated and can be separated from the NNK free molecule. It can be speculated that the logarithmic acidity constant (pKa) of NNK is lower than that of nicotine from the experiment. The feed solution pH fluctuates during the electrodialysis process. The acidic electrolyte is conducive to the separation process from the point of flux and energy consumption, while the alkaline electrolyte has negative impact on the separation process. A current density of 10 mAcm^{-2} is the appropriate value for the separation process. The lowest energy consumption of the separation process is 0.58 kWh/kg nicotine with the process cost to be estimated at only $0.208 /kg nicotine. Therefore, the electrodialysis is a powerful technique and has the potential to challenge the conventional preparation route of high-purity nicotine.

Acknowledgements

This research is supported in part by the National Natural Science Foundation of China (Nos. 21676259, 21476220), and Anhui Key Laboratory of Tobacco Chemistry (0920150623002).

References

Aghajanyan, A.E., Tsaturyan, A.O., Hambardzumyan, A.A. and Saghyan, A.S. (2013), “Obtaining the zwitterionic form of L-lysine from L-lysine monohydrochloride by electrodialysis”, *Membr. Water Treat.*, **4**(1), 1-9.

Ali, M.B.S. and Hamrouni, B. (2016), “Development of a predictive model of the limiting current density of an electrodialysis process using response surface methodology”, *Membr. Water Treat.*, **7**(2), 127-141.

Ayers, J.W., Ribisl, K.M. and Brownstein, J.S. (2011), “Tracking the rise in popularity of electronic nicotine delivery systems (electronic cigarettes) using search query surveillance”, *Am. J. Prev. Med.*, **40**(4), 448-453.

Bedard, L.L., Smith, G.B.J., Reid, K.R., Petsikas, D. and Massey, T.E. (2002), "Investigation of the role of lipoxygenase in bioactivation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in human lung", *Chem. Res. Toxicol*., **15(**10**)**, 1267-1273.

Clayton, P., Vas, C. and McAdam, K. (2014), "Use of chiroptical spectroscopy to determine the ionisation status of (S)-nicotine in electronic cigarette formulations", *ST 49, Coresta Congress*, Québec City, Canada.

Ding, S.C., Du, W.J., Ren, X., Qian, X.C. and Liu J.F., "Quantitative determination of tobacco specific TSNAs in leaf and cigarette smoke using LC-MS/MS", *Acta Tabacaria Sinica*., **11**, 17-22.

Dixon, M., Lambing, K. and Seeman, J. (2000), "On the transfer of nicotine from tobacco to the smoker. A brief review of ammonia and "pH" factors", *Beitr. Tabakforsch. Int*., **19(**2**)**, 103-113.

Fischer, M. and Jefferies, T.M. (1996), "Optimization of nicotine extraction from tobacco using Supercritical fluid technology with dynamic extraction modeling", *J. Agric. Food Chem*., **44(**5**)**, 1258-1264.

Grana, R.A. and Ling, P.M. (2014), "Smoking revolution: A content analysis of electronic cigarette retail websites", *Am. J. Prev. Med.*, **46(**4**)**, 395-403.

Hecht, S.S. (2003), "Tobacco carcinogens, their biomarkers and tobacco-induced cancer", *Nat. Rev. Cancer*ete, **3**, 733-744.

Hoffmann, D., Rivenson, A. and Hecht, S.S. (1996), "The biological significance of tobacco- specific N-nitrosamines: smoking and adenocarcinoma of the lung", *Crit. Rev. Toxicol*., **26(**2**)**, 199-211.

Houston, F.G. (1952), "Separation and determination of nicotine and nornicotine in tobacco", *Anal. Chem*. **24(**11**)**, 1831-1832.

Leslie, D., Millen, C. and Raymond, M.W. (1993), "Countercurrent extraction of nicotine from tobacco juice", *Ind. Eng. Chem. Res*., **32(**12**)**, 3056-3060.

Liu, H.M., Li, R., Li, M. and *et al*. (2002), "Tobacco and tobacco products-Determination of total alkaloids continuous flow method", China YC/T 160-2002, 402-407.

Magee, P.N. (1996), "Nitrosamines and human cancer: introduction and overview", *J. Cancer Prev*., **5**, 7-10.

Majewska-Nowak, K.M. (2013), "Treatment of organic dye solutions by electrodialysis", *Membr. Water Treat.*, **4(**3**)**, 203-214.

Morie, P. (1972), "Fraction of protonated and unprotonated nicotine in tobacco smoke at various pH values", *Tob. Sci.*, **16**, 167.

Ng, L.K. and Hupé, M. (2003), "Effects of moisture content in cigar tobacco on nicotine extraction: similarity between soxhlet and focused open vessel microwave assisted techniques", *J. Chromatog. A*, **1001(**1-2**)**, 213-219.

Strathmann, H. and Koops, G.H. (2000), "Process economics of electrodialytic water dissociation for the production of acid and base", Ed. A.J.B. Kemperman, Handbook on bipolar membrane technology, Enschede: Twente University Press.

Wang, Y.M., Wang, A.L., Zhang, X. and Xu, T.W. (2011), "Simulation of electrodialysis with bipolar membranes: estimation of process performance and energy consumption", *Ind. Eng. Chem. Res*., **50(**24**)**, 13911-13921.

World Health Organization (2002), "Reducing risks, promoting healthy life", The World Health Report; WHO: Geneva, Switzerland.

Yang, Y., Zhang, Z., Mukherjee, A.B. and Linnoila, R.I. (2004), "Increased susceptibility of mice lacking clara cell 10-kDa protein to lung tumorigenesis by 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone, a potent carcinogen in cigarette smoke", *J. Biol. Chem*., **279**, 29336-29340.

Zhang, Z.H., Ge, S.L., Jiang, C.X., Zhao, Y. and Wang, Y.M. (2014), "Improving the smoking quality of papermaking tobacco sheet extract by using electrodialysis", *Membr. Water Treat.*, **5(**1**)**, 31-40.

Zheng, H.C. and Takano, Y. (2011), "NNK-induced lung tumors: a review of animal model", *J. Oncol.* **2011**, 1-8.

CC