

Primary and secondary amine material based on crosslinked polystyrene: synthesis and initial application for multiresidue pesticides analysis

Huynh Minh Chau, Vo Dinh Thien Vu, Nguyen Thao Nguyen, Nguyen Anh Mai

Abstract—Weak anion exchange sorbent based on cross-linked polystyrene with primary secondary amine group was prepared by substitution nucleophilic reaction (S_N2) between methylene chloride group and 1,2-ethylene diamine. The effect of factors, namely the weight ratio of amine over methylene chloride, reaction time and temperature on nitrogen percentage were studied using experimental design approach. The amination yield rose as all of factors increased but was reduced while both temperature and time increased simultaneously. Nitrogen percentage of the products were varied from 4.0% to 6.3%. Sorbents with predicted capacity of 4.5%, 5.0%, 6.3%, and 6.5% were synthesized. The results showed that the actual capacities of the products were close to the predictions, especially for those in the experimental domain, indicating a good model that can be used to prepare sorbents of any desired capacity. The sorbent application ability of multiresidue pesticides analysis in food were initially investigated through both aspects: interference elimination and analyte content conservation.

Keywords—Anion exchange, crosslinked polystyrene, experimental design, multiresidue pesticides analysis, primary and secondary amine, QuEChERS

1 INTRODUCTION

Crosslinked polystyrene and its modified materials are popular materials which were

applied as sorbent of various analytes [1-4] due to their advantageous properties, namely, high surface area, chemical resistance, rigid structure. Crosslinked polystyrene with full of phenylene rings in its structure offers π - π interaction to aromatic analytes [5]. Nevertheless, surface modification by polar or ionic functional groups, e.g. sulfonated -SO₃⁻, would support polar-polar and electrostatic interaction [6].

QuEChERS (stand for Quick, Easy, Cheap, Effective, Rugged, and Safe) – introduced by Anastassiades *et al.* [7] – was developed as a sample preparation method for multiresidue-pesticide determination in fruits and vegetables. The method includes three main steps (i) the extraction of pesticides with acetonitrile (ii) partition the analytes into acetonitrile phase by adding salts and (iii) a dispersive solid phase extraction for clean-up. This method and several modified versions have been applied for different types of matrices and pesticides [8-11]. In the third step of QuEChERS, adsorbents, such as C18, primary secondary amine (PSA), graphitized carbon black (GCB), play an important role in interference elimination process. Silica is generally used as support for C18 and PSA sorbent thanks to its availability and hydrophilic surface. The aim of this work was to synthesize and test whether the PSA sorbent based on cross-linked polystyrene can be used in QuEChERS method.

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2 MATERIALS AND METHODS

Chemicals, apparatus and software

1-Dodecanol, toluene, 1,2-ethylene diamine, sodium hydroxide, sodium carbonate, formic acid, hydrochloric acid, nitric acid, sulfuric acid, boric acid, ammonium acetate, styrene, divinylbenzene, and vinylbenzyl chloride were of synthesized grade and purchased from Merck (Germany). Cross-linked polystyrene materials which contain various levels of vinylbenzyl chloride (VBC) were synthesized based on the procedure of our previous work [1].

Methanol, acetonitrile and ethyl acetate (HPLC grade) were purchased from Merck (Germany) and degassed prior to use. Standards of 13 pesticides (acetamiprid, carbendazim, fenpyroximate, flusilazole, hexaconazole, methamidophos, thiabendazole, myclobutanil, tebuconazole, lufenuron, tricyclazole, methomyl, trifloxystrobin) were provided by Sigma-Aldrich (Germany).

HPLC UV LC-20AD (Shimadzu), HPLC MS micrOTOF-Q II (Bruker), LC column Spherisorb S50DS2 (Waters) and ACE 3 (ACE) were used for investigation of interference elimination and simultaneously multiresidue pesticides analysis in food.

MODDE 8 (Umetrics, Sweden) was employed for experimental design work.

Preparation of polymer-based primary secondary amine sorbent (2MA)

2MA is the name assigned for the cross-linked polymer prepared from monomers (divinylbenzene and vinylbenzyl chloride) and then modified in order to have primary secondary amine groups on the surface. 2MA was synthesized via two steps, (1) preparation of ethylene chloride cross-linked polystyrene, and (2) amination of the polymer. The synthesis procedure of crosslinked polystyrene with methylene chloride group was conducted as in a previous work. Briefly, the monomers (14g VBC, 26g DVB), porogen (19g toluene, 41g dodecanol) and benzoyl peroxide (3g) were mixed. The polymerization was performed at 80°C for 24h. The un-polymerized components were removed by

Shoxlet extraction with methanol for 20h and dried at 60°C for 6h.

The polymer was then wetted with toluene, to which 1,2-ethylene diamine was added for the amination. The products were washed three times with 30mL of 2% hydrochloric acid in acetone and drying at 60°C overnight. To study effect of reaction conditions capacity of the sorbents, the mole ratio of amine to methylene chloride was varied from 10 to 70 times, temperature from 30 to 80°C, reaction time from 8 to 24h while mass of polymer (1g) and toluene volume ((20-V_{amine}) mL) were fixed.

Chloride and amine content analysis

The chloride contents of pre- and post-amination materials were determined by the procedure described in our previous publication [12]. Briefly, samples were treated by alkaline fusion method with mixture of Na₂CO₃ and NaOH. Then, their aqueous solutions were analyzed by indirect spectrophotometry of the chloride based on the adsorption at 460 nm of Fe(SCN)²⁺, a product of the reaction between chloride ion and a mixture of mercury (II) thiocyanate and ferric ion.

Additionally, %N was determined by Kjeldahl method. Sample (0.200 g) was digested with a mixture of 0.5 g CuSO₄, 5.0 g Na₂SO₄ and 10 mL H₂SO₄ (conc.). The solution was then alkalized with 60mL 7M NaOH. The ammonia gas was absorbed into a solution containing an excess of H₃BO₃. The nitrogen content is then determined by titration of the NH₄HBO₃ formed with standardized HCl solution using Tashiro as indicator.

Design of experiment (DOE) for amination reaction

Two-level full factorial design (denoted as 2³) was chosen for the design of experiment (DOE) in this study. Reaction time (Time), temperature (Temp), and the mole ratio of amine to methylene chloride (Ratio) were factors; and %N was the response. The reaction conditions of 11 experiments were tabulated in Table 1. Experiments (N1–N8) were at high and low levels of each factor. Three replicated

experiments at the center values (N9–N11) were used to evaluate the reproducibility of synthesis

and model. The run order of the experiments was randomized by software.

Table 1. Details of the factor and response values of 11 experiments in DOE model

Experiment name	Factors			Response	Experiment name	Factors			Response
	Ratio	Temp	Time	%N		Ratio	Temp	Time	%N
N1	30	8	10	3.76	N7	30	24	70	6.26
N2	80	8	10	5.44	N8	80	24	70	6.64
N3	30	24	10	5.18	N9	55	16	40	5.97
N4	80	24	10	5.67	N10	55	16	40	5.83
N5	30	8	70	5.51	N11	55	16	40	5.99
N6	80	8	70	6.48					

Investigation of interference elimination

Food, namely cucumber, lemon, cabbage, green bean, garlic, onion, strawberry, green tea, tomato, and apple, consisting of chlorophyll, organic acid, sugar, dye, and essential oil as interference was extracted by QuEChERS [13]. 10g of grinded sample was extracted with 10mL of ACN, 4g MgSO₄, and 1g NaCl for 1 min. 1mL of the decant was mixed with 25 mg 2MA and 150mg MgSO₄. Resulted solution was analyzed by HPLC UV at 210nm, gradient eluent (mixture

of acetonitrile: ammonium formate) from 50:50 to 95:5 (v/v) for 5 min, then keep in 5 min before returned to the initial condition.

Investigation of pesticide content conservation

10 µg of each pesticide was added to 100 g of grinded samples and kept at room temperature overnight. QuEChERS sample preparation was carried out same as procedure of interference elimination investigation. However, resulted solution was analyzed by HPLC MS with instrumental parameters shown in Table 2.

Table 2. HPLC-MS/MS conditions for multiresidue analysis of 13 pesticides

Time (min)	Eluent		Mass spectrometer	
	%NH ₄ COOH	%ACN	Parameter	ESI (+)
0.0	80	20	Capillary voltage	4.5kV
5.0	65	35	End Plate Offset	-500V
15.0	55	45	Collision Cell RF	300V _{pp}
40.0	0	100	Nebulizer	1.2bar
50.0	End		Dry heater	200°C
			m/z Range	50–3000

3 RESULTS AND DISCUSSION

Regression model for the amination of cross-linked polystyrene

Based on the experimental data the regression model was built for the amination procedure (Eq. 1). It should be noted that the regression coefficients are scaled and centered. This means that they are not expressed in original measurement scales of the factors, but in the coded -1/+1 unit corresponding to the lowest and the highest values.

$$Y = 5.932 + 0.394x_1 + 0.287x_2 + 0.542x_3 - 0.178x_1x_2 \text{ (Eq. 1)}$$

Where Y, x₁, x₂ and x₃ denoted Capacity, Ratio, Temp and Time, respectively.

After refining the model i.e. removing coefficients, which had uncertainty covering zero value, the resulting model having large goodness of fit factor (R² = 0.974) and prediction power (Q² = 0.775). Regression coefficients and factors evaluating the quality of the model are presented in Fig. 1. It was found that all of factors, including temperature, time, and amine to methylene chloride ratio give an increase in capacity. The results also revealed that the three main factors were not independent. In fact, there were significant interaction coefficients, which only can be investigated using the DOE approach.

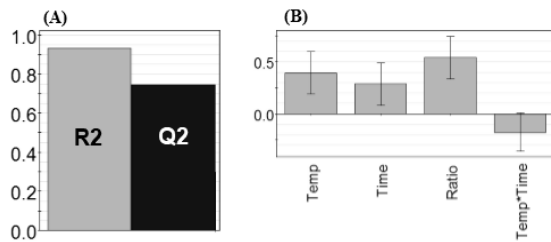


Fig. 1. (A) Model statistics, (B) coefficients charts for amination

It is obvious that increases in amine level (Ratio) led to high yields of the reaction because of the higher chance of amine reagent and methylene chloride site get into contact. The same effect of temperature could be explained by the reduced viscosity of the reaction medium which promoted the contact between the reagent and

surface of the porous material. Moreover, the longer reaction time, the more effective reaction sites between methylene chloride and amine reagent. However, the coefficient “Temp*Time” had the most profound negative effects which showed in response surface plot illustrated a quadratic regression between Temp and Time factors (Fig. 2A). The reason of their negative effect can be the destruction of peripheral reacted layers of 2MA sorbent to submicron scale particle which eliminated in post-synthesis treatment process while reaction was carried out at high temperature for a long time.

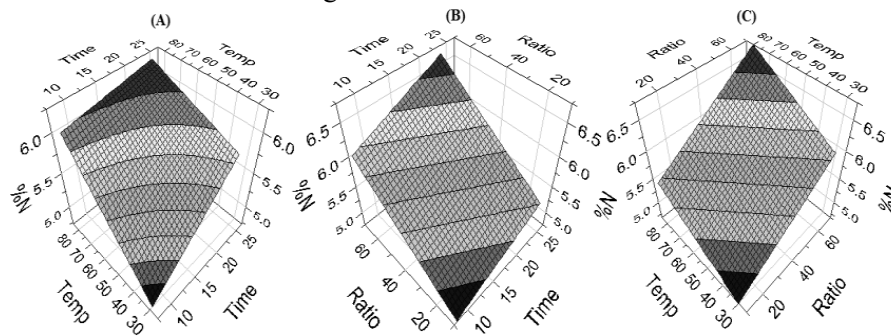


Fig. 2. Response surface plots showing the effects of (A) Temp-Time, (B) Ratio-Time and (C) Ratio-Temp on the percentage of Nitrogen of 2MA

The model can be visualized by means of response surface plots. The curvature in plots involving the factor “Temp*Time” confirmed its negative effect on the capacity when the reaction time and temperature were further increased simultaneously (Fig. 2B, C). At the bottom, higher ratio of amine to methylene chloride and longer reaction time gave high nitrogen content resulted sorbent.

Preparation of primary secondary amine sorbents with desired nitrogen percentage

To examine a model applicability, the model

of amination process was used to design suitable conditions to prepare 2MA with nitrogen percentage from 4.5% to 6.5%. It should be noted that there were several reaction conditions for a desired nitrogen percentage. Considering the fact that nitrogen content would be fallen down while both temperature and time increased simultaneously, the conditions were selected with low temperature to facilitate the procedure (Table 3). It was found that, the predicted and actual values were well agreed, indicating a very good model.

Table 3. Predicted and actual %N of the sorbents synthesized based on DOE prediction

Factors			%N	
Ratio (time)	Time (h)	Temp (°C)	Predicted	Actual
120	8	30	6.33 ± 0.77	5.65
50	8	30	4.92 ± 0.41	5.31
100	16	30	6.47 ± 0.62	6.43
30	8	30	4.52 ± 0.53	4.06

Initial application in simultaneous multiresidue pesticide analysis

The extracts of ten kinds of food which were treated by 2MA were analyzed by HPLC-UV and HPLC-MS/MS to examine the interference elimination of the home-made sorbents.

The results illustrated that extracts without sorbent treated would content many UV-responsive compounds which are interferences in pesticide analysis in food. After sample preparation procedure with adsorbents (2MA or commercial PSA, there are the losses of peaks of chromatograms (Fig. 3A). However, the interference elimination ability depended on the sample nature. In case of simple matrices, like apple, tomato, green bean, onion, and cabbage, both of 2MA and commercial PSA offered

effective elimination. With complex sample matrices, such as lemon, garlic, strawberry, and green tea, both of adsorbents could not remove their interferences. Moreover, the total ion chromatograms (Fig. 3B) showed that most of polar compounds which eluted before 40 mins had been removed by 2MA in simple matrix samples, the later peaks were washed out of the reversed phase column by neat acetonitrile, while in case of other complex matrix ones, early 40 mins peaks still appeared. The reason could be the high content of essential oils, polyphenols, organic acids and other polar compounds in garlic, green tea or lemon which cannot be eliminated completely by 2MA. It could be overcome by the combination of 2MA and other sorbents (C18, GCB) in QuEChERS.

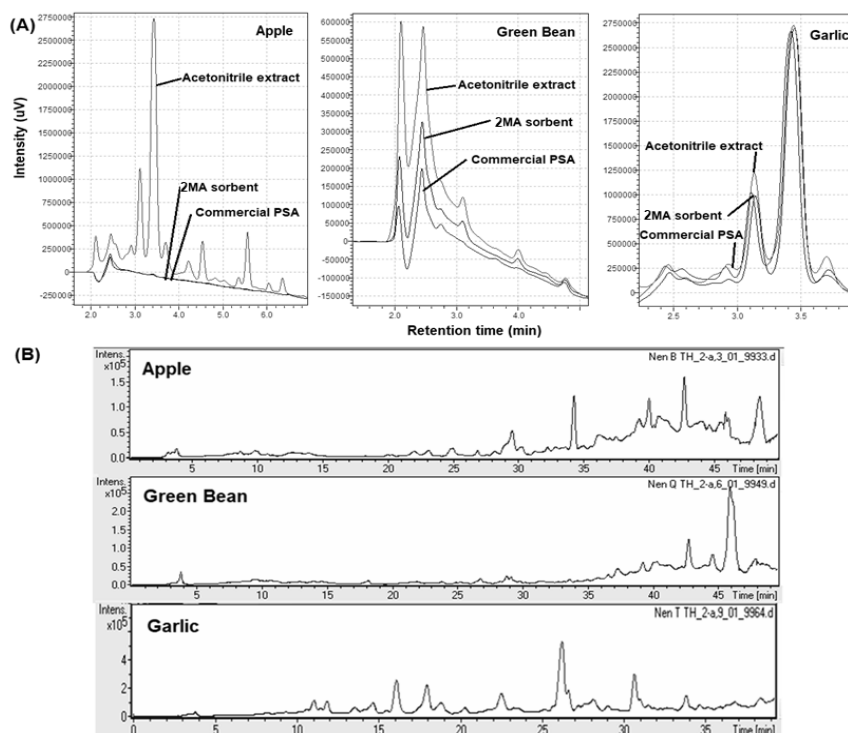


Fig. 3. (A) HPLC-UV chromatograms of apple, green bean and garlic acetonitrile extracts before and after treated by 2MA or commercial PSA sorbents. (B) HPLC-MS total ion chromatograms of these sample extracts treated by 2MA sorbent

Besides interference elimination, analyte conservation is one of the most important requirement of adsorbent. Recoveries of 13 pesticides (retention time from 10 mins to 37 mins) in 10 matrices which were prepared by 2MA or commercial PSA as sorbent in QuEChERS were shown in Table 4. Data

compatibility was checked by Student's t-test which provided p value equals 0.216, higher than 0.05 (with 95% significance level). It means the null hypothesis is accepted, there is no significant difference between recoveries of 13 pesticides in 10 kinds of food samples which were prepared by both home-made 2MA and commercial PSA.

Table 4. Recoveries of 13 pesticides (100 ng/g) in 10 matrices

Analyte	2MA	PSA	2MA	PSA	2MA	PSA	2MA	PSA	2MA	PSA
	Cucumber		Strawberry		Apple		Tomato		Green bean	
Propamocarb	102%	98%	98%	112%	93%	109%	105%	111%	118%	121%
Acetamiprid	103%	85%	95%	113%	95%	94%	104%	109%	104%	109%
Tricyclazole	88%	93%	94%	97%	94%	95%	97%	106%	104%	109%
Methomyl	84%	113%	93%	104%	99%	108%	108%	118%	116%	121%
Carbendazim	95%	100%	94%	96%	99%	103%	88%	93%	98%	103%
Cyproconazole	84%	94%	113%	107%	95%	59%	99%	102%	105%	113%
Myclobutanil	75%	71%	86%	85%	72%	77%	87%	91%	87%	85%
Tebuconazole	80%	74%	88%	94%	76%	82%	103%	96%	91%	92%
Flusilazole	86%	81%	92%	95%	81%	87%	90%	92%	96%	92%
Hexaconazole	71%	66%	76%	82%	57%	73%	77%	78%	94%	89%
Trifloxystrobin	98%	92%	100%	101%	84%	86%	99%	102%	97%	97%
Lufenuron	53%	59%	103%	106%	62%	56%	106%	105%	77%	78%
Fenpyroximate	95%	97%	90%	97%	93%	100%	96%	101%	93%	96%
	Onion		Lemon		Cabbage		Garlic		Green tea	
Propamocarb	102%	111%	85%	132%	100%	94%	99%	111%	81%	83%
Acetamiprid	92%	98%	107%	125%	97%	92%	104%	110%	70%	76%
Tricyclazole	104%	99%	72%	80%	113%	113%	103%	113%	60%	61%
Methomyl	110%	115%	74%	80%	82%	86%	89%	114%	53%	51%
Carbendazim	89%	88%	90%	96%	99%	98%	80%	81%	97%	100%
Cyproconazole	120%	114%	68%	71%	71%	115%	136%	124%	103%	102%
Myclobutanil	37%	55%	90%	84%	89%	95%	16%	22%	82%	84%
Tebuconazole	98%	93%	91%	94%	108%	105%	69%	63%	104%	102%
Flusilazole	82%	91%	29%	30%	99%	100%	45%	36%	83%	88%
Hexaconazole	88%	96%	75%	74%	90%	87%	66%	64%	79%	81%
Trifloxystrobin	92%	93%	99%	99%	99%	106%	50%	45%	81%	81%
Lufenuron	54%	65%	106%	94%	91%	103%	43%	33%	74%	68%
Fenpiproximate	105%	111%	135%	135%	105%	99%	102%	115%	89%	92%

4 CONCLUSION

In this work, primary secondary amine adsorbent based on crosslinked polystyrene had been synthesized via solution polymerization and substitution nucleophilic (S_N2) reaction. The content of nitrogen was modelled and controlled by Design of Experiment method which showed the effect of each factors as well as their combination. Resulted materials were applied as QuEChERS adsorbed material to prepare samples for simultaneously multiresidue pesticide analysis by HPLC-MS/MS. The results illustrated their initial ability of not only interference elimination but also analyte conservation.

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Vật liệu hấp phụ amine bậc một và bậc hai trên nền polystyrene khâu mạng: tổng hợp và bước đầu ứng dụng phân tích đa dư lượng thuốc bảo vệ thực vật

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Tóm tắt—Vật liệu hấp phụ anion yếu trên nền polystyrene khâu mạng với nhóm amine bậc một và bậc hai được tổng hợp từ phản ứng thế thân hạch (S_N2) giữa nhóm methylene chloride và 1,2-ethylene diamine. Ảnh hưởng của các yếu tố như tỉ lệ amine trên nhóm methylene chloride, thời gian và nhiệt độ phản ứng đến phần trăm nitrogen được khảo sát bằng phương pháp quy hoạch thực nghiệm. Hiệu suất phản ứng tỉ lệ thuận với điều kiện phản ứng nhưng có xu hướng giảm khi tăng đồng thời nhiệt độ và thời gian phản ứng. Phần

trăm nitrogen trong sản phẩm thay đổi từ 4% đến 6,3%. Chất hấp phụ với dung lượng dự đoán lần lượt 4,5%, 5%, 6,3% và 6,5% được tổng hợp. Kết quả cho thấy dung lượng thực tế phù hợp với dự đoán, cho thấy khả năng ứng dụng của mô hình quy hoạch thực nghiệm trong việc tổng hợp vật liệu như mong muốn. Khả năng ứng dụng của vật liệu trong quá trình phân tích đồng thời các chất bảo vệ thực vật trong thực phẩm được bước đầu khảo sát: khả năng loại bỏ nền mẫu và bảo toàn chất phân tích trong suốt quá trình xử lý mẫu.

Từ khóa—Trao đổi anion, polystyrene khâu mạng, quy hoạch thực nghiệm, phân tích đồng thời các chất bảo vệ thực vật, amine bậc một và bậc hai, QuEChERS