The Malaysian Journal of
ANALYTICAL SCIENCES
ISSN 1394-2506

A STUDY ON THE MIGRATION OF STYRENE FROM POLYSTYRENE CUPS TO DRINKS USING ONLINE SOLID-PHASE EXTRACTION LIQUID CHROMATOGRAPHY (SPE-LC)

(Kajian Mengenai Migrasi Stirena daripada Cawan Polistirena kepada Minuman Menggunakan Teknik Ekstraksi Fasa Pepejal Kromatografi Cecair (SPE-LC))

Norashikin Saim, Rozita Osman[*], Hurin Ain Wan Abi Sabian, Mohamad Rafaie Mohamed Zubir, Nazarudin Ibrahim

Faculty of Applied Sciences,
Universiti Teknologi MARA, 40450, Shah Alam, Selangor

*Corresponding author: rozit471@salam.uitm.edu.my

Abstract

The migration of residual styrene from polystyrene cups to drinks has been a major concern in recent years worldwide. In this study, a three-factor response surface experimental design was used to evaluate the interactive effects of temperature (30-80 ℃), pH (5-7) and contact time (35-120 min) on the migration of styrene from polystyrene cups into water. Online solid-phase extraction liquid chromatography (SPE-LC) with diode array detector (DAD) was used for the separation and detection of styrene. Initial temperature of water was found significant in increasing the rate of migration of styrene whereas the effect of contact time was less significant. In terms of interactions between the effects, the relation between temperature and pH was the most significant. The amount of styrene in water varied from 0.3 to 4.2 µg/L with maximum migration obtained at initial water temperature of 80 °C and pH 5.

Keywords: migration of styrene, experimental design, polystyrene cups

Abstrak

Migrasi residu stirena daripada cawan polistirena ke minuman menjadi kebimbangan utama di seluruh dunia sejak kebelakangan ini. Dalam kajian ini, rekabentuk eksperimen menggunakan kaedah respon permukaan 3-faktor digunakan untuk menilai interaksi di antara suhu (30-80 ℃), pH (5-7) dan tempoh sentuhan (35-120 min) ke atas migrasi stirena daripada cawan polistirena kepada air. Pengekstrakan fasa pepejal dan analisis dijalankan selari menggunakan teknik kromatografi cecair (SPE-LC) dengan alat pengesan foto diod (DAD). Suhu awal air merupakan faktor yang paling signifikan bagi peningkatan kadar migrasi stirena berbanding kesan masa sentuhan air dengan cawan polistirena. Daripada segi interaksi di antara faktor, hubungkait di antara suhu dan pH adalah paling signifikan. Amaun stirena yang dikesan di dalam air berubah daripada 0.3 ke 4.2 µg L^{-1} dengan migrasi tertinggi didapati pada suhu 80 °C and pH 5.

Kata kunci: migrasi stirena, rekabentuk eksperimen, cawan polistirena

Introduction

Styrene monomer is one of the most widely used foods packaged contact polymers. Several adverse health effects are attributed to styrene [1,2,3,4]. Styrene is considered a possible human carcinogen by the World Health Organization International Agency for Research on Cancer. Evidence already shows that styrene causes cancer in animals. The migration of styrene monomers from polystyrene containers into foods has been extensively studied [5,6]. Previous study showed that the migration of styrene from a polystyrene cup containing cold or hot beverages has been observed to be as high as 0.025% of the total styrene for a single use [7]. Fat content, temperature, and contact time were the common parameters studied in predicting the potential exposure of consumers to styrene. Study conducted by Till et al. [8] found that increases in temperatures used in polymer processing may cause degradation and migration of polymers in food products. In this study, the effect of temperature, pH and contact

times on migration of styrene from polystyrene cups to drinks were evaluated using an experimental design approach (Design Expert 6.0.4). This approach allows all three parameters to be investigated individually, as squared terms, and to consider two-component interaction effects. The separation of styrene achieved using an online solid-phase extraction liquid chromatography (SPE-LC) approach enabled direct injection of samples resulted in faster analysis and good reproducibility.

Experimental

Chemicals and Reagents

HPLC Grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Methanesulfonic acid (MSA) and styrene standard were bought from Fluka and Sigma-Aldrich (Steinheim, Germany), respectively. Ethyl acetate and ammonia solution 25% were purchased from System (ChemAR). Acetic acid was bought from R & M chemical (Essex, United Kingdom).

Preparation of styrene standard solutions

Styrene standard stock solution of 1000 mg L^{-1} was prepared by dissolving 0.2778 ± 0.01 mL of styrene standard in methanol. The standard solution was mixed thoroughly in 250 mL volumetric flask. The quantitative analysis of styrene was achieved using a 5-point calibration curve.

Sample preparation

Polystyrene cup samples were purchased from local market. Deionized water was used and the pH was adjusted using acetic acid for acidic conditions and ammonia solution 25% for basic conditions. The initial temperature of each sample (100 mL) was adjusted and directly transferred into the polystyrene cup.

Online SPE-LC analysis

Dionex Ultimate 3000 Online SPE-LC instrument was used for styrene separation and detection. The major components of this instrument involved online SPE column, C_{16} Acclaim Polar Advantage, 5μm 120 Å (4.6 m x 50 mm), C_{18} analytical column C_{18} Acclaim Polar Advantage II, 5μm 120 Å (4.6m x 150 mm), two pumping systems, auto-sampler and photo diode array detector. The prepared styrene standards and polystyrene cup samples of large volume (3.5 mL) were loaded directly onto the system, and delivered to the SPE column for enrichment, followed by interferences washed out. Then, the SPE column is switched into the analytical column for analyte separation. The analytical column for separation is equilibrated with the second pump at the same time. The solvents system used were a mixture of ultra-purified water and 10 mM methanesulfonic acid (MSA), acetonitrile (ACN), and ethyl acetate. Table 1 shows the gradient for online SPE using the loading pump while Table 2 the gradient for separation using an analysis pump. The wavelength range used for detection of styrene by photo diode array detector in the range 214 - 282 nm, then styrene was detected at wavelength of 248 nm.

Table 1: Gradient Program for Online SPE

Time (min)	Flow rate (mL/min)	Solvent A H₂O +10mM MSA, (% vol.)	Solvent B, ACN, (% vol.)	Solvent C Ethyl Acetate, (% vol.)	Curve
0.0	1.0	95.0	5.0	0.0	
3.5	1.0	95.0	5.0	0.0	5
6.5	1.0	70.0	30.0	0.0	5
6.5	1.0	0.0	100.0	0.0	5
7.5	1.0	0.0	100.0	0.0	5
7.5	1.0	0.0	0.0	100.0	5
9.0	1.0	0.0	0.0	100.0	5

Table 2: Gradient Program for Separation (Analytical Column)

Time (min)	Flow rate (mL/min)	Solvent A H$_2$O (% vol.)	Solvent B, ACN, (% vol.)	Solvent C Ethyl Acetate, (% vol.)	Curve
0.0	1.0	70.0	30.0	0.0	
0.0	1.0	70.0	30.0	0.0	5
14.0	1.0	5.0	95.0	0.0	7
21.0	1.0	0.0	100.0	0.0	5
22.0	1.0	0.0	0.0	100.0	5
24.0	1.0	0.0	0.0	100.0	5
24.0	1.0	0.0	100.0	0.0	7
25.0	1.0	0.0	100.0	0.0	7
27.0	1.0	70.0	30.0	0.0	7
28.0	1.0	70.0	30.0	0.0	7

Experimental Design Approach
The effect of three independent variables at five levels, t; contact time (t:35–120 minutes) temperature (T: 30 – 80 °C), and pH (p: 5 – 7). Each of the variables had levels set at five coded levels: -α, -1, 0, +1, and +α (Table 2), requiring 20 experiments in total (Table 3). Experiments were randomised in order to minimize the effect of unexplained variability in the actual responses due to extraneous factors. The centre point was repeated 6 times to calculate the repeatability of the method [9].

Table 3: Central composite design used for the analysis of styrene in drinks

	t: contact time (min)	T: Temperature (°C)	p:pH
-α	6	13	4
- 1	35	30	5
0	78	55	6
1	120	80	7
+α	149	97	8

Results and Discussion
Online Solid Phase Extraction-Liquid Chromatography (SPE-LC) analysis
Optimization of the online SPE-LC conditions indicated that a mixture of acetonitrile, ultra-purified water, 10 mM methanesulfonic acid (MSA) and ethyl acetate were the mobile phases for conditioning the extraction column, washing the impurities, and elution of styrene. Using large volume injection of 3.5 mL, styrene in water was simultaneously concentrated and separated. Styrene was detected at wavelength of 248 nm using UV-DAD detector based on the comparison of its retention times (10.1 min) and UV-Vis absorption (Figure 1a and 1b). Calibration linearity for the determination of styrene was investigated by making replicate injections of styrene standards at four different concentrations. The calibration curve was found linear in the range of 0.01 to 1.00 µg mL^{-1} with correlation coefficients, r = 0.9992.
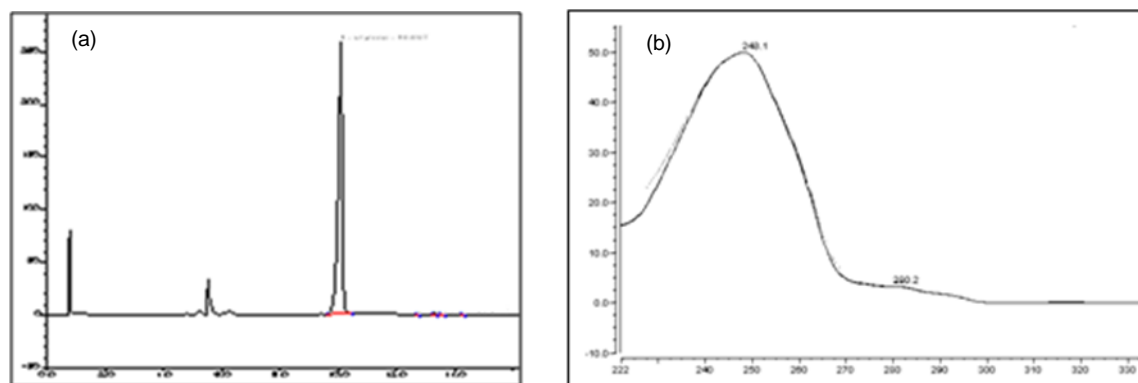
Figure 1: Chromatogram of styrene (a) and UV-Vis spectrum of styrene (b)

Experimental Design Approach

The results based on the amount of styrene recovered are tabulated in Table 3. High amount of styrene migration was observed at high initial temperature and in acidic condition. Multilinear regression was applied to the results of the central composite design (CCD). The effect of independent variables; time (t), temperature (T) and pH (p) and interactive effect (interaction between pairs of variables) on the migration of styrene were evaluated by second order (quadratic) (Table 4). The adequacy of the models were determined using model analysis, lack of fit test and coefficient of determination (R^2). The significance of the equation parameters for each response variable was also assessed by F-ratio at a probability (p) of 0.05. The closer the value of R^2 to unity, the better the empirical model fits the actual data. Criteria for a good fit of a model, the R^2 should be at least 0.80 [10]. In this study, the R^2 was 0.8923. The high value of R^2 (> 0.80) indicates the adequacy of the applied quadratic model.

Table 4: Central Composite Design (CCD) and the amount of styrene detected

Experiment no.	Contact time (min)	Temperature (°C)	pH	Amount of Styrene (µg L^{-1})
1	35	30	5	2.2
2	35	80	7	3.4
3	120	80	5	3.9
4	78	55	6	2.6
5	120	30	7	2.7
6	78	55	6	2.7
7	120	30	5	2.8
8	78	55	6	2.8
9	35	80	5	4.2
10	78	55	6	3.1
11	120	80	7	3.1
12	35	30	7	1.2
13	78	97	6	3.7
14	78	55	6	0.3
15	78	55	4	2.5
16	78	55	6	2.4
17	78	13	6	2.6
18	6	55	6	2.4
19	78	55	8	2.4
20	149	55	6	2.1

Table 5: Multilinear Regression Results for analysis of styrene in drinks

Variable	p value
t	0.2177
T	**0.0006**
p	0.4970
t^2	0.4458
$\mathbf{T^2}$	**0.0046**
p^2	0.7230
tT	0.7182
tp	0.0831
Tp	**0.0058**

Evaluation on each factor individually, showed that the migration of styrene was strongly dependent upon initial temperature of water as shown in Table 5 ($p<0.05$). The results concurred with the previous studies [5,7]. The influence of other individual factors such as contact time and pH was not significant. However, based on the evaluation between two factors revealed that the interaction between initial temperature and pH gave a significant impact on the migration of styrene with the p value less than 0.05 (p=0.0058) (Table 4). Figure 1a shows that increase in initial water temperature resulted in an increased amount of styrene. The effect of the interaction between initial water temperature and pH is shown in Figure 1b. In acidic condition (pH 5), the migration of styrene increased significantly with temperature.
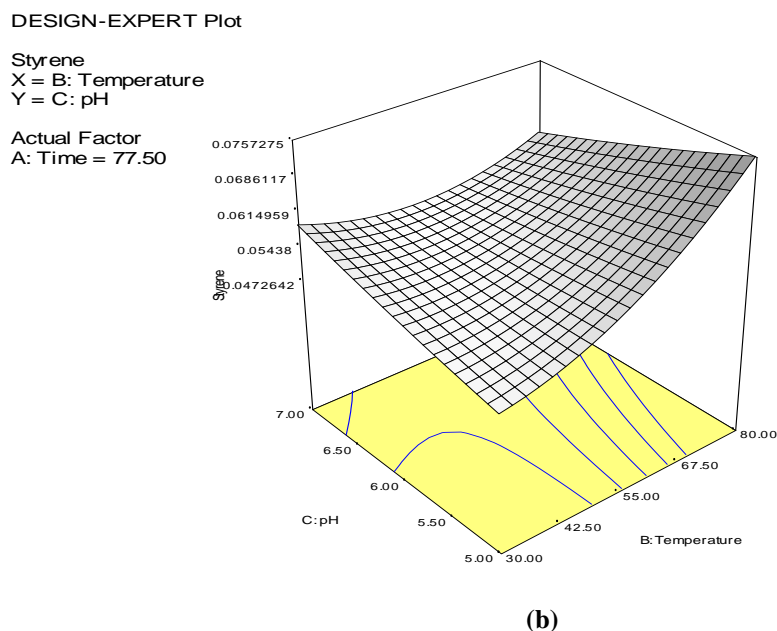

(a)

(b)
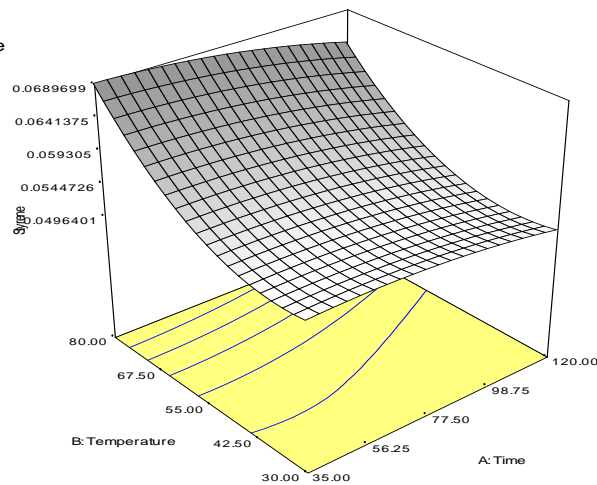
Figure 1: Contour and response surface plot (a) time against temperature, (b) temperature against pH
for the migration of styrene using Central Composite Design

Conclusion

The online SPE-LC was able to determine the migration of styrene from polystyrene cups to water with minimum sample preparation as the sample concentration and separation steps were achieved on-line using two columns. Based on individual independant variable, only initial temperature was significant whereby higher migration was observed at high initial water temperature. However, using experimental design approach, the interactions betweeen the variables were also considered. The results showed that the interaction of initial water temperature and pH was also significant and highest migration of styrene was observed at high temperature (80 °C) and in acidic condition (pH 5.0). This study showed the advantage of experimental design approach compared to conventional approach (changing one variable at a time) in studying the effect of several variables with minimal number of experiments.

Acknowledgement

References

1.   Varner, S. L., and Breder, A. (1981). C.V. Headspace sampling and gas chromatographic determination of styrene migration from food-contact polystyrene cups into beverage and food simulants. *J. Ass. Offic. Anal.Chem. Int.* 64:1122–1130.
2.   Varner, S. L., Breder, C. V., and Fazio, T. (1983). Determination of styrene migration from food-contact polymers into margarine, using Azeotropic distillation and head space gas chromatography. *J. Ass. Offic. Anal.Chem. Int.* 66:1067–1073.
3.   Muratak, A., Avakis, S., and Yokoyama, K. (1991). Assessment of the peripheral, central and autonomic nervous system function in styrene workers. *Am. J. Index Medicus* 20:775–784.

4. Cohen, T., Carlson, G., Charnley, G., Coggon, D., Delzell, E., and Graham, J. D. (2002). A comprehensive evaluation of the potential health risks associated with occupational and environmental exposure to styrene. *J. Toxicol. Env. Health B* 5:1–263.

5. Khaksar, M.R and Ghazi-Khansari M (2009). Determination of migration monomer styrene from GPPS (general purpose polystyrene) and HIPS (high impact polystyrene) cups to hot drinks. *Toxicology Mechanisms and Methods* ; 19(3): 257–261.

6. Arvanitoyannis, I. S., and Bosena, L. (2004). Migration of substances from packaging materials to foods. Crit. Rev. Food Nutr. 44:63–76.

7. Tawfik, M.S. and Huyghebaert, A (1998). Polystyrene cups and containers: styrene migration. *Food Addit. Contam.* 15(5), 592-599.

8. Till, D. E., Ehntholt, D. J., Reid, R. C., Schwartz, P. S., Schwopc, A. D., Sidman, R. R., and Whelan, R. H. (1982). Migration of styrene monomer from crystal polystyrene to food and food simulating liquid. *Ind. Eng. Chem. Prod. Res. Dev.* 21:161–185.

9. Mirhosseini, H., Tan, C. P., Hamid, N. S. A., & Yusof, S. (2008). Optimization of the contents of Arabic gum, xanthan gum and orange oil affecting turbidity, average particle size, polydispersity index and density in orange beverage emulsion. *Food Hydrocolloids 22*, 1212-1223.

10. Joglekar, A. M., & May, A. T. (1987). Product excellent through design of experiments. *Cereal Foods World, 32*, 857-868.