MODELLING OF BISPHENOL A MIGRATION FROM LDPE INTO FOOD SIMULANTS

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Packaging

2012

ABSTRACT

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Migration testing of bisphenol A (BPA) from low-density polyethylene (LDPE) into food simulants was performed with three factors taken into account: temperature, initial BPA concentration and food simulant type. BPA analysis was carried out by a HPLC-UV method. Fick's diffusion equations were applied to the migration modeling. Diffusion coefficients (D_P) and partition coefficients ($K_{P,F}$) were determined by fitting the migration curve with the diffusion equation. D_P values obtained under different conditions ranged from 10^{-10} to 10^{-8} cm^2 s^{-1}. Statistical analysis showed significant effects of all factors on the diffusion coefficient. No interaction effect was shown significant, except for the interaction between temperature and food simulant type. The dependence of diffusion coefficients on temperature followed an Arrhenius type of relationship with the activation energy (E_a) ranging from 118 to 134 kJ mol^{-1} for different food simulants. An exponential relationship was found between the diffusion coefficient and initial BPA concentration for each food simulant. Based on the statistical analysis, an empirical model was developed to express the diffusion coefficient as a function of temperature and initial BPA concentration.

Acknowledgements

This thesis is based on nearly two years' research. During the time, I have received precious support from my friends and colleagues. With their contribution either directly or indirectly to my work, I was able to overcome many difficulties and push the research forward. Here, it is my pleasure to express my heartfelt thanks.

First, I would like to say thank you very much to Dr. Maria Rubino. As my advisor, she gave me many valuable suggestions both on my experiment and thesis writing. I appreciate the freedom and trust she gave me that I had an opportunity to design and arrange my research. I appreciate her praise and encouragement, making me brave and confident to finish my research successfully. I appreciate her tolerant heart on mistakes in my research. I also appreciate her warm heard for many books and articles she offered me, to greatly broaden the knowledge on my research. Overall, I'm so happy to work with Dr. Rubino and this experience will become a good memory in all my life.

I would like to thank Suheewan from School of Packaging for her great help on HPLC training. Another person I wish to say thank you is Michelle Sanderland, a technician from Waters Company, for her hard work on the maintenance of HPLC, which is very essential to my research. I also learned a lot about the instrument through our conversation.

I would like to convert my gratitude to my Friend Jun Lai from Mathematical Department who taught me MATLAB. I would also like to thank Katya and Wenzhao Yang from CANR statistical consulting center with their great help on the statistical analysis.

During my thesis writing, I receive many help from my writing course (NSC 840). Dr Snider, professor from writing center, has made a lot effort on the correction of grammar and modification of sentences. My classmates also gave me many valuable suggestions.

Finally, I wish to send my great appreciation to my dear parents. My academic performance at MSU is their most concern. My success toward graduation is their greatest wish. They are always my spirit pillar.

Thank you all

Yining Xia

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ABBREVIATIONS AND SYMBOLS

D	Diffusion coefficient
μ	Chemical potential
x	Diffusion distance in the polymer
C	Permeant concentration
t	Diffusion time
T_g	Glass transition temperature
E_a	Activation energy
R	Gas constant
T	Absolute temperature
D_0	Pre-exponential factor
D_p	Diffusion coefficient in the polymer
C_F	Migrant concentration in the food or food simulant
$M_{F,t}$	Migration level at time t
$C_{p,0}$	Initial migrant concentration
ρ_p	Density of the polymer
ρ_F	Density of the food or food simulant
L_p	Polymer film thickness
V_p	Volume of the polymer
V_F	Volume of the food or food simulant

$K_{P,F}$	Partition coefficient of the migrant
$M_{F,\infty}$	Migration level at equilibrium
A	Area of the polymer in contact with the food simulant
λ_{ex}	Excitation wave length
λ_{em}	Emission wave length
$RMSE$	Root mean-square error
R_a	Solubility parameter distance
δ_D	Dispersion parameter
δ_P	Polar parameter
δ_H	Hydrogen bonding parameter

CHAPTER 1

Introduction

1.1 Background

In the food industry, packaging plays a very important role to the food product. The main functions of food packaging [1] are: (a) to provide containment of the food product; (b) to afford protection of the food product from the outer environment; and (c) to give the consumer detailed information of the food product it contains. Various kinds of materials are used for food packaging such as metal, glass, paper, wood and plastic. Compare to other materials, plastic is a relatively new material and is used extensively in food packaging due to its ability to adapt to specific requirements.

The synthetic plastic industry first started in 1909, with the development of a phenol formaldehyde plastic by *Baekeland* [2]. After that, different types of plastic materials were developed and used for packaging purposes. The demand for plastics as packaging materials has grown year by year and they have been a good alternative to other types of materials such as glass and metal. Plastics have some advantages that have made them very useful as packaging materials, especially for food product applications [3], such as easy to shape, low in cost, almost chemically inert, lightweight, superior sealing ability, and relatively good barrier properties.

One important feature of plastic packaging materials is their semi-crystalline or even non-crystalline morphology. The crystalline region helps to improve the mechanical and barrier properties of the packaging materials. The amorphous region makes the packaging

materials more flexible and easier for processing. However, the existence of amorphous region is one of the factors that enable the transfer of small molecules (such as gases, liquids and solids) through the boundary layers of plastic materials [4]. One phenomenon of the transfer of small molecules in the packaging system is migration, which corresponds to the release of compounds from the packaging materials [5]. The released components can be residual monomers, oligomers, processing aids and additives. Additives, such as plasticizers, stabilizers, UV absorbers and anti-oxidants, make the packaging materials more processable and durable. When those components go into the food product, they may affect the quality and safety of the food product.

Bisphenol A (BPA) (Figure 1), or 2, 2-bis (4-hydroxyphenyl) propane, is a chemical primarily used as a precursor in the synthesis of polycarbonate (PC) and epoxy resins, to be used as rigid containers and metal can linings. It can also be used as an additive in various plastic materials such as PVC and rubber to improve the durability (UV resistance, heat stability, etc.) of the materials [6-8]. Migration of BPA happens when those packaging materials are in direct contact with the food system [9-14]. Another source of BPA migration could take place when the packaging materials are either recycled or discarded in the landfill. In this situation, BPA migrates into the surrounding environment such as river water [15, 16] and soil [17]. However, the migration of BPA due to the direct contact of the packaging materials with the food system is the primary concern since the food constitutes the main route of human exposure to BPA [18].

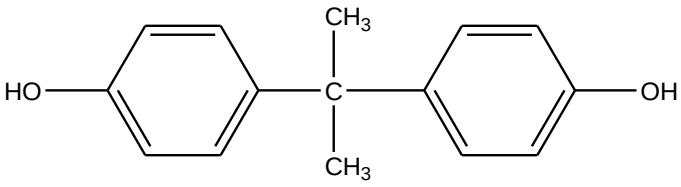

Figure 1.1 Molecular Structure of BPA.

BPA is also known as one of the endocrine disrupting compounds (EDCs), a group of chemicals that interact with steroid hormone receptors of human and animals and disrupt normal endocrine functions [19]. Since BPA is widely used in food packaging, in recent years, there is an increasing concern regarding the level of BPA in the food system which could impact the human health [20, 21]. Thus, there is a need to determine the level of BPA that migrate into the food system and how BPA is released into the food system from the packaging materials in order to ensure food safety.

1.2 Motivation

It is important to assess the level of BPA in the food system promoted by the packaging materials being in direct contact with the food product. It is also important to understand how the migration takes place and how fast or slow that BPA is released from the packaging materials into the specific food or food simulant under specific conditions. The conventional migration testing proposed by the US Food and Drug Administration (FDA) usually measures the level of additives that migrate into a specific food simulant [22]. But this method does not provide the profile of migration process. Mathematic models have been developed in recent years aiming to predict the migration of additives

and other low molecular weight components from plastic packaging materials into the food or food simulants [23]. For example, diffusion equations derived from Fick's second law are applied to describe the migration process as a function of time, by solving parameters such as diffusion coefficient (D_P) and partition coefficient ($K_{P,F}$). The prediction of migration using mathematical models may overcome some disadvantages associated with conventional migration testing [22], such as (a) time consuming, (b) difficult for the analysis of migrants at ultra-low concentrations, (c) expensive in analyses used in migration testing, and (d) generating hazardous laboratory waste. Therefore, model prediction is considered a promising alternative to the conventional migration testing.

1.3 Goal and objectives

The overall goal is to describe the migration profile of BPA from plastic packaging materials into the food system at different conditions. To reach the goal, the following objectives are addressed, with special focuses on the development of methodologies to describe BPA migration.

(1) Set up an analytical method for the quantification of BPA;

(2) Implement mathematical models in order to describe the migration process;

(3) Evaluate the effect of temperature, initial BPA concentration and food simulant type on the migration process.

CHAPTER 2

Literature Review

This chapter starts with an introduction on bisphenol A (BPA), including the characteristics and properties, potential health risks, public concerns and some regulatory issues. Then, a brief description of mass transfer in packaging system will be given. Fick's diffusion theory is addressed to express the migration process involved in mass transfer. Some diffusion models derived from Fick's laws of diffusion are outlined. These models can be used to describe the migration process within the packaging system. In order to ensure food safety, migration testing of BPA is quite necessary. Methods of migration testing recommended by the US Food and Drug Administration (FDA) are introduced. The inspection of migration level is an important aspect of migration testing. Instrumental techniques regarding the determination of BPA are listed, including the conventional methods such as liquid chromatography and gas chromatography, as well as a new method called immunochemical technique.

2.1 A brief description of BPA

2.1.1 Characteristics and properties of BPA

The synthesis of BPA was first reported by *Zincke* [24] using acid catalyzed condensation of acetone and phenol (Figure 2.1). Chemical and physical properties [25] of BPA are listed in Table 2.1. Commercial production of BPA began in 1950's when it was widely used in the manufacture of polycarbonate (PC) plastics and epoxy resins. The demand of BPA has been grown worldwide with the continuous growth of the uses for these plastic materials. Today, BPA is one of the world's most widely produced chemicals, with an annual production of over 2.2 million tones [26]. In the US, BPA ranks in the top two percent of high production volume chemicals, with annual production exceeding a billion pounds (0.5 million tons) [27]. Over 70% BPA are made into PC plastics and about 21% BPA go into epoxy resins [28]. For food contact applications, less than 5% BPA are used [29].

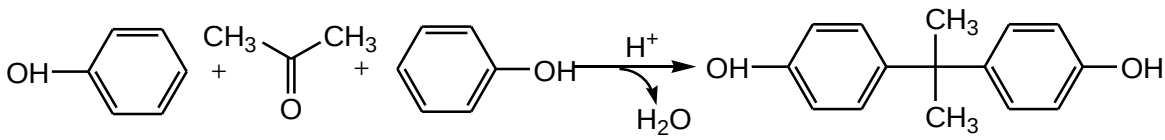

Figure 2.1 Synthesis of BPA.

Table 2.1 Chemical and physical properties of BPA.

Formula	Mw	Mp (℃)	Bp/Fp[a] (℃)	Td[b] (℃)	Density (g/cm^3)	Solubility
$C_{15}H_{16}O_2$	228	153	250/79	180	1.195	not soluble in water; soluble in acetic acid; very soluble in ethanol, diethyl ether, benzene

Note: a. Fp = flash point; b. Td = thermal decomposition point.

2.1.2 Potential risks of BPA

BPA was identified as a weak estrogenic chemical; approximately 1000-2000 fold less potent than the natural estrogenic chemical 17-β estradiol [30]. A potential risk of BPA is its estrogenic activity [30, 31], firstly proved by experiments on rats in the 1930s [32]. Due to the accumulation of BPA in the body, adverse health effects are caused by BPA at doses much lower than that would normally be expected, which is also known as low dose effects [33]. Some examples of low dose effects in laboratory animals such as rats and mice are: (a) early onset of sexual maturation in females [34], (b) increased postnatal growth in both males and females [35, 36]; (c) altered immune function [37]; and (d) behavioral effects such as hyperactivity [38] and increase in aggressiveness [39]. The most serious problem of BPA is its carcinogenic activity [40] which can be correlated to cancer such as breast cancer [41]. The potential risks of BPA on breast cancer are attributed to two aspects: (a) BPA can alter the growth of mammary tissue that increase the risk of breast cancer as well as increase the sensitivity of breast tissue to cancer

causing agents [42]; and (b) BPA can significantly promote the growth of cancer cells. An example is the proliferation in MCF-7 human breast cancer cell line induced by BPA at low doses [43].

2.1.3 Public concerns and regulatory issues of BPA

The public doubt whether present regulations on BPA are adequate to protect human health according to the study on low dose effects of BPA. Adverse effects of low-dose exposure to BPA on laboratory animals were first reported in 1997 [44]. By December 2004, there were 115 published *in vivo* studies that dealt with low dose effects of BPA [45]. Among those studies, 94 out of 104 government-funded studies have reported significant adverse health effects, and 31 of them have reported effects caused by doses at or below the current reference dose (RfD) which was set to be 50 μg kg^{-1} day^{-1} by the US Environmental Protection Agency (EPA). However, none of the remaining 11 industry-funded studies reported any significant biological impact of BPA [30]. Thus, there comes a debate regarding the safety of BPA [46]. One group suggests a higher restriction on BPA and eventually a ban on its use in any food contact application. The other group claims that the current use of BPA is safe.

The FDA has shown its concern regarding the safety of BPA for many years. A draft assessment of BPA for its use in food contact applications was published in 2008 with particular focuses on its developmental toxicity [47]. By far, the FDA considers that the current level of exposure of BPA to adults and infants is safe based on the current RfD.

However, the FDA will keep on reviewing the safety of BPA as new data of BPA become available, and the current regulations on BPA might be changed in the future.

Some actions have already been taken out to protect the human health by minimizing the exposure of BPA to the human body. In 2008, Canada became the first country to designate BPA as a toxic substance. As a consequence, Canada banned the import, sale and advertisement of polycarbonate baby bottles containing BPA and carried out efforts to reduce BPA contamination of infant formula in metal cans [48, 49]. In 2009, Connecticut became the first state in the US to ban the use of BPA in any infant formula and baby food containers, as well as in any reusable food or beverage container [50]. The European Union will ban the use of BPA in plastic baby bottles from 2011 with the support from the majority of its members [51].

2.2 Mass transfer

2.2.1 Mass transfer in packaging system

Interactions between plastic packaging materials and the food product are always connected with mass transfer occurring within the packaging system including sorption, permeation and migration. The driving force for the transport of a substance in the packaging system is the gradient of chemical potential of that substance. Here, the chemical potential can be interpreted as concentration or partial pressure of the substance. The transport of the substance from higher chemical potential side to lower side is a spontaneous process, in order to equilibrate the chemical potential between the two sides.

Sorption

Sorption refers to the uptake of food components such as flavor, lipids and moisture by the plastic packaging materials. The extent of sorption depends on the initial concentration of the sorbent in the food as well as the polymer properties [52]. The sorption process causes the loss/change of flavor or quality of the food product which will be unacceptable to the consumer [53].

Permeation

Permeation is the exchange of small molecules (gases, vapors and liquids) across the packaging materials and can be expressed in three steps: (a) absorption of the substance by the polymer surface at the higher concentration side; (b) diffusion of the substance through the polymer toward the lower concentration side; and (c) desorption of the substance at the lower concentration side.

Migration

Migration can be considered the opposite process of sorption which is the release of components from the plastic packaging materials into the product. The components released are also called migrants. Monomers and additives are two common types of migrants existed in most of the plastic materials. Those components are usually under intense legal control by the regulatory agencies to minimize their potential risk to human health due to their migration into the food.

The migration process in the packaging system is controlled by both thermodynamics and kinetics, or partition and diffusion, respectively [54-56]. The partition (thermodynamics process) of the migrant between the polymer phase and the liquid (food simulant) phase at equilibrium of migration is affected by the solubility and affinity of the migrant in the two phases. The diffusion (kinetics process) provides information on the migration velocity and is influenced by [57]: (a) molecular structure and molecular weight of the migrant, (b) affinity of the migrant to the food simulant, and (c) affinity between the polymer and the food simulant.

The affinity can be described by solubility parameter δ [58]. The principle for the use of solubility parameter is "like dissolve like", which means two liquids with similar δ values are miscible with each other. This principle may also extend to the miscibility between solid and liquid and solid and solid. The solubility parameter can be divided into three components in order to precisely define the degree of likeness in a given system. The three components are also known as Hansen solubility parameters [59] which are given as δ_D, δ_P and δ_H, for dispersion, polar and hydrogen bonding contribution, respectively. Therefore, the affinity can be calculated and compared based on the Hansen solubility parameters.

2.2.2 Fick's laws of diffusion

Mass transfer of the substance within the packaging system is usually associated with the diffusion process in the polymer (Figure 2.2). Fick's laws of diffusion [60] are useful

to quantitatively describe this process. For steady state, one dimension diffusion of a substance in the polymer, Fick's first law is used [57]:

$$F = -D\frac{\partial C}{\partial x} \tag{2.1}$$

where **F** is the transfer rate of the substance per unit area; **C** is the substance concentration in the polymer; x is the diffusion distance; and **D** is the diffusion coefficient of the substance in the polymer. The negative sign indicates that the substance travels from the higher concentration region to the lower one. For unsteady state, one dimensional diffusion of the substance in the polymer, Fick's second law is used [57]:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{2.2}$$

where t is the diffusion time.

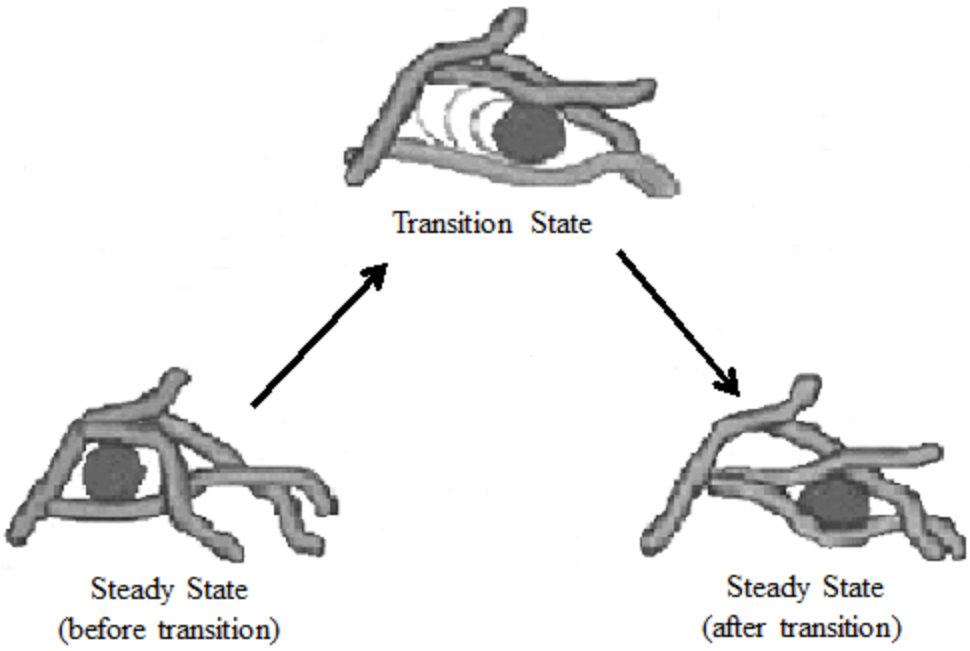

Figure 2.2 Diffusion process of a small molecule in the polymer matrix.

Note: The graph was modified from the original graph on the website of Dr. Mauritz's research group: http://www.psrc.usm.edu/mauritz/diffuse.html. The existence of free volume and mobility of polymer chains enable the diffusion of small molecules [4, 61].

Regarding equations 2.1 and 2.2, some assumptions [57] are made here:

(1) The value of D is assumed to be independent of both, the substance concentration and the polymer chain relaxation;

(2) Diffusion processes through packaging materials are generally unidirectional and perpendicular to the surface of the package;

(3) Solutions of diffusion equations are obtained for particular cases derived from the corresponding boundary and initial conditions.

2.2.3 Effect of temperature on diffusion

When dealing with the problem of diffusion of the substance in the polymer, one important feature that should be addressed is temperature as it significantly affects the mobility of polymer chains. Diffusion mechanisms are different at temperatures above and below the glass transition temperature, Tg, of the polymer. At temperature T>Tg, polymers are at a "rubbery" state and respond rapidly to changes in their physical condition. The time required for the substance-polymer system to reach a new equilibrium state is much shorter than that required for the diffusion of the substance through the polymer matrix, due to the fast relaxation of polymer chains [62-64].

When T<Tg, polymers are at a “glassy” state, polymer chains are “frozen” and their mobility is restricted. There is not enough time for the relaxation of polymer chains to completely reach a new equilibrium state when the substance transports through the polymer matrix. Therefore, the polymer is not in a true equilibrium state below the glass transition temperature [62, 65].

From these considerations, the diffusion behavior of a substance in polymers can be summarized as followed:

(1) In rubbery polymers, the diffusion behavior is generally Fickian, excepting the case when the sorption does not reach equilibrium at the polymer interfaces [66];

(2) In glassy polymers, the diffusion behavior can be categorized into three aspects [67]:

(a) Case I or Fickian diffusion, when the relaxation of the substance-polymer system is faster than the diffusion of the substance; (b) Case II diffusion [68], when the relaxation of the substance-polymer system is slower than the diffusion of the substance; and (c) non-Fickian diffusion, when the relaxation rates of substance-polymer system are comparable with the diffusion rates of the substance. In this case, the diffusion process is mainly affected by the existence of holes and micro-cavities in the polymer matrix.

2.2.4 Diffusion models for migration process

Fick’s second law of diffusion is useful to describe the migration process in

packaging system with proper initial and boundary conditions [60]. This second order differential equation can be resolved to express the amount of migrant released per unit area A from the polymer into the food simulant at time t [69]:

$$\frac{M_{F,t}}{A} = C_{P,0}\rho_P d_P \left(\frac{\alpha}{1+\alpha}\right) \times \left[1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} exp\left(-D_P t \frac{q_n^2}{d_P^2}\right)\right] \quad (2.4)$$

with

$$\alpha = \frac{1}{K_{P,F}} \frac{V_F}{V_P}$$

where $M_{F,t}$ is the amount of migrant in food simulant at time t, mg; A is the contact area between the polymer and food simulant, cm^2; $C_{P,0}$ is the initial migrant concentration in the polymer, mg g^{-1}; ρ_P is the polymer density, g cm^{-3}; d_P is the film thickness, cm; V_P and V_F is the volume of the polymer and food simulant, cm^3, respectively; q_n is the positive roots of equation $tan\ q_n = -\alpha q_n$; and $K_{P,F}$ is the partition coefficient of migrant in the polymer/simulant system and can be calculated from the ratio of migrant concentration in the polymer ($C_{P,\infty}$) and food simulant ($C_{S,\infty}$):

$$K_{P,F} = \frac{C_{P,\infty}}{C_{S,\infty}} \quad (2.5)$$

To get a more reliable result on the theoretical migration with equation 2.4, a very large number of positive roots of equation $tan\ q_n = -\alpha q_n$ are required. To avoid the heavy work of calculation, a simplified model can be used [70]:

$$\frac{M_{F,t}}{M_{F,\infty}} = (1+\alpha)[1 - \exp(\omega)\, erfc(\omega^{0.5})] \quad (2.6)$$

with

$$\omega = \frac{D_P t}{\alpha^2 d_P^2}$$

where $M_{F,\infty}$ is the amount of migrant in food simulant at equilibrium, mg. Equations 2.4 and 2.6 are applicable to both one-sided and two-sided contact migration. A two-sided contact migration can be considered as the combination of two one-sided contact migration due to the axis of symmetry at x=0 (Figure 2.3). For two-sided contact migration, half layer thickness, $d_P/2$, is used without any change of the equation [4].

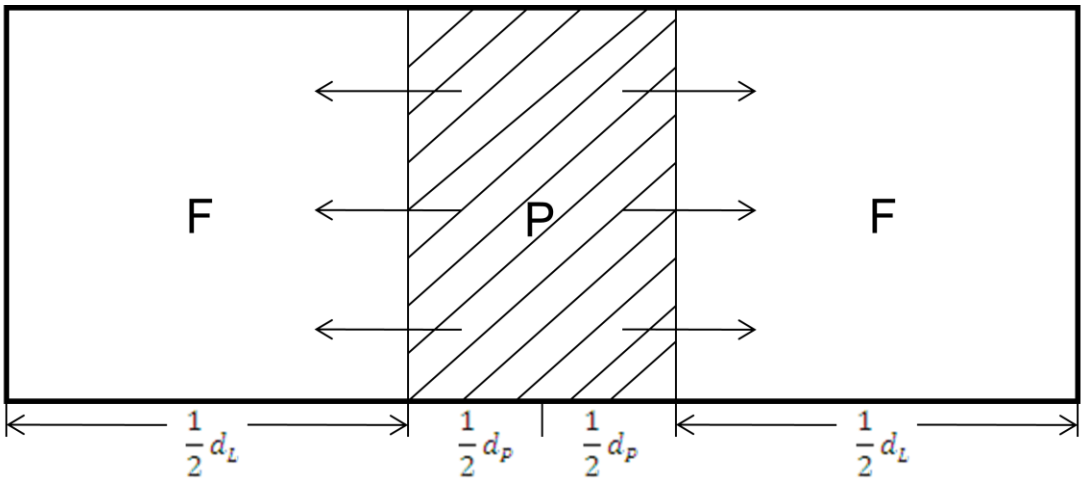

Figure 2.3 Two-sided contact migration between the polymer (P) and food simulant (F).

In the case that migration is only diffusion controlled, a further simplified equation can be used [57]:

$$\frac{M_{F,t}}{A} = 2C_{P,0}\rho_P\left(\frac{D_P t}{\pi}\right)^{1/2} \tag{2.7}$$

Equation 2.7 can be reorganized to express the migration process for a finite polymer-finite food system or an infinite polymer-infinite food system [4]:

$$\frac{M_{F,t}}{M_{F,\infty}} = \frac{2}{d_P}\sqrt{\frac{D_P t}{\pi}} \tag{2.8}$$

In addition to the above-mentioned equations, several other semi-empirical models for diffusion have been applied to match some specific requirements of the migration testing [71-74].

2.3 Methodology of migration testing

In the US, the FDA is the institute to set regulations for migration testing under 21 CFR 170.39 (Threshold of regulation for substances used in food-contact articles). Migration testing is usually carried out under finely controlled laboratory conditions and designed to: (a) simplify the experimental operations, and (b) simulate the migration in real case. Some recommendations for the design of migration experiment [75] are listed below in three parts.

Description of the migration cell

The design of a migration cell should fully enable the characterization of migration testing. Usually, the food container such as water bottle can be directly used as the migration cell. Otherwise, a specifically designed migration cell should be considered when: (a) the surface area of the food container cannot make sufficient extractives for characterization; and (b) a soft film was used as the packaging material. A specimen of known surface area and a food simulant of known volume are required for the use of a migration cell. The specimen can be either one-sided contact or two-sided contact with the food simulant (immersed into the food simulant). For the latter case, a two-sided

migration cell is usually adopted [76] with two essential features: (a) separation of polymer films or sheets by inserting spacers (such as glass beads) to allow the free flow of food simulant around each film or sheet; and (b) minimization of headspace with gas-tight or liquid-tight seals. Two-sided contact migration testing is not suitable in some cases, e.g., when a multilayer film is used. Therefore, the two-sided migration cell should be replaced by other cell designs such as a one-sided migration cell [77]. For volatile migrant or solvent, the migration cell must be sealed and kept away from the light.

Selection of Food simulants

Due to the complexity of food matrices, the extraction of migrant from the food is difficult and time consuming [78]. Thus, migration testing is usually performed by using food-simulating liquids to avoid the complicated extraction process. Food simulants recommended by the FDA are: water for aqueous foods, 3% acetic acid for acid foods, 10 to 50% ethanol for low and high alcoholic foods, food oil (e.g. olive oil) or HB307 or Miglyol 812 for fatty foods. When oil is used as food simulant, an extra extraction step is required before injection. To avoid this step, some aqueous-based solvents are recommended as alternatives for fatty-food simulants. For instance, absolute or 95% ethanol is an effective fatty-food simulant for polyolefins, and 50% ethanol is used as fatty-food simulant for rigid PVC, PS and rubber-modified PS [79]. The simulant volume-to-specimen surface area ratio should match the value in actual food packaging. In general, a ratio of 10 ml in^{-2} is recommended. Other ratios may also be acceptable

depending on the migrant solubility in the polymer and the food simulant.

Temperature and exposure time

The FDA has recommended the short-term accelerated testing to reflect the migration for real applications. For room temperature applications, a temperature of 40 ℃ for 10 days is recommended, which is approximate equivalent to the migration for 6 months under room temperature. For refrigerated or frozen food applications, the test temperature of 20 ℃ is used. Other temperatures and exposure times could also be used to match the conditions of different applications. Portions of the testing solution should be analyzed during the migration testing. At least four samplings should be taken with variant time intervals. Analysis of a control is also recommended.

2.4 Instrumental analysis for the quantification of BPA

A variety of analytical methods have been reported for the determination of BPA. Chromatographic techniques including liquid chromatography (LC) and gas chromatography (GC) are commonly used. Immunochemical techniques have also been developed for the determination of BPA in recent years. Instrumental analysis for BPA usually consists of three steps: sample preparation, separation and detection. Different techniques have different requirements for each step.

2.4.1 Chromatographic techniques: liquid chromatography

Liquid chromatography (LC), especially high performance liquid chromatography (HPLC), is commonly applied in the separation, identification and quantification of BPA, for it is relatively simple in use [80]. Sample preparation for instrumental analysis involves complicated extraction procedures if the food was directly used in the migration testing. Solvent extraction and solid extraction [25] are two commonly applied techniques for BPA extraction from the food. When food simulating solutions are used, procedures involved in the extraction from the food can be omitted and a portion of the extracting solution can be directly injected into the LC system.

Isolation of BPA from other compounds in the solvent is mainly carried out in reverse-phase C_{18} columns, while other types of columns can also be used such as a shield RP-18 column [81, 82]. The column is usually kept at room temperature during the analysis to extend the life-time of the column. A higher temperature [83, 84] can also be adopted to accelerate the analytical process while maintain a good resolution. The elution conditions depend on the composition of the sample and the detector coupled to the LC [85]. The elution can be either isocratic or gradient. The mobile phase is usually a binary solution of water and an organic solvent such as methanol [86, 87] or acetonitrile [88, 89]. The mobile phase is sometimes adjusted to a lower PH value with the addition of acetic acid [84] or trifluoroacetic acid [90] or formic acid [91] to achieve a better resolution. Other than the mobile phase mentioned above, *Szymanski et al.* [82] adopted a special mobile phase called micellar mobile phase (an aqueous solution with the anionic

surfactant sodium dodecyl sulphate) to improve the detection limit.

Various detection techniques are applied for the determination of BPA. Among these techniques, ultra violet (UV) detection, fluorescence (FL) detection and mass spectrometry (MS) detection are frequently applied, while electrochemical detection (ED) is utilized in a lesser extent.

UV and FL detection

BPA has a positive absorption of UV light at the wavelength of 275 nm and 225 nm. The wavelength of 225 nm is commonly used since the absorption is more intensive so a better sensitivity can be reached [92]. Other wavelengths used [82, 88, 89] are usually closed to the two wavelength designated above. BPA also shows negative fluorescence with the excitation wavelength of around 275 nm and emission wavelength of around 305 nm. The characteristic wavelengths of BPA for both UV and FL detection are stable in solvents typically used as the LC mobile phase: water, acetonitrile and methanol. The sensitivity depends on the sample preparation and the mobile phase composition. A better sensitivity is usually obtained in a mobile phase with higher organic composition [85]. The detection limits of BPA are usually in the range of 0.2 - 20 μg L^{-1} for UV detection [93] and can go as low as 0.01μg L^{-1} [94]. The typical detection limits of BPA using FL detection range from 0.1 to 5 μg L^{-1} [85] and can reach ppt (ng L^{-1}) level [95].

MS detection

MS Detection is carried out by using electrospray ionization (ESI) in negative mode [86, 96] and atmospheric pressure chemical ionization (APCI) in negative mode [97, 98]. ESI is more frequently applied since it provides better sensitivity [85]. Chromatographic conditions for MS detection are identical to those used in UV and FL detection. The response of BPA is highly dependent on the mobile phase composition [99]. Mobile phases made up of water-methanol are preferred than those consist of water-acetonitrile to achieve a better response. The response can be modified by adding the modifier such as 0.5% ammonia [100] and 0.01% acetic acid [86]. The most abundant ion in BPA mass spectrum used for quantitative analysis is m/z 227, which corresponds to the loss of H^+ during ionization. Other fragments used are m/z 211 and m/z and m/z 212, corresponding to the loss of a hydroxyl group and a methyl radical, respectively. The detection limits are ranged from ppt (ng L^{-1}) level to ppb level (µg L^{-1}) [93]. Compared to UV and FL detectors, MS detector applies more reliable identification of BPA, and thus can be conjunct with those two detectors for the qualitative analysis [97].

Electrochemical detection

Electrochemical detection (ECD) is mainly used for the determination of BPA in biological fluid [101-103] and environmental water [16, 104]. It also has similar chromatographic conditions to those applied in UV and FL detection. Isocratic elution is recommended than gradient elution to avoid the large equilibrium time during the

analysis [105]. The mobile phase is usually modified with some electrolyte content and adjusted to a proper PH to achieve a better sensitivity [85]. For example, *Rezzano et al.* [106] used a mobile phase of methanol/water containing 10mM KNO_3 and 0.25mM H_2SO_4 as supporting electrolyte and the method detection limit was lowered to 0.2 ppb (μg L^{-1}). A better sensitivity can also be obtained by using a chemically modified electrode prepared by coating a glassy carbon electrode with a Ni-Protoporphyrin IX dimethyl ester film [107]. Compare to UV and FL detection, ECD is highlighted for its good sensitivity [108]. The detection limit for BPA can be below ppt level (ng L^{-1}) [16].

2.4.2 Chromatographic techniques: gas chromatography

Gas chromatography (GC) is more sensitive and better in resolution than liquid chromatography (LC), because of the absence of chromatographic effect that happens in LC [80]. Other than the sample preparation procedures for LC, a derivatization step on BPA is usually adopted in GC such as silylation [109, 110] and acetylation [111, 112]. The derivatization step leads to an easy volatilization of BPA and consequently, better separation from other analytes and a higher sensitivity [113]. However, a drawback is the time consuming work and the probable contamination of the sample involved in these steps.

The compounds are separated at their gas state in either a packed column or a capillary column. A capillary column is preferred since it provides much higher separation efficiency, while the amount of sample injected at one time is limited [114].

The programmed temperature is applied to the column to achieve better separation of the compounds. The mobile phase is made of chemically inert gas, so there will be no interaction between the compounds and the mobile phase during the analysis. Helium is usually used as the mobile phase or carrier gas. Other types of carrier gases are nitrogen, argon and hydrogen.

A mass spectrometry (MS) detector is usually coupled with GC for the determination of BPA. MS detection is operated with electron impact (EI) ionization in selected ion monitoring (SIM) mode [115-118]. The fragments selected for quantitative analysis are similar to those selected in LC-MS. The commonly used fragments for GC-MS method are m/z 213, m/z 228 and m/z 119. The detection limits of BPA are usually in the ranges of 0.5 to 6 ng L^{-1} and 0.04 to 0.6 μg L^{-1} [93].

2.4.3 Immunochemical techniques

The determination of BPA in foods (mainly in liquid foods) by immunochemical techniques is very recent [119, 120]. Immunoassays provide good sensitivity and specificity, though they are easy to perform and low in cost [85]. The analysis is carried out by using polyclonal mammalian [121] and chicken [122] antibodies in enzyme linked immunosorbent assays (ELISA). BPA is conjugated with a protein to form a complete antigen, and then to initiate an immune response [119, 123]. The detection limits are in a range of 0.05 to 500 μg L^{-1} and mainly affected by the immunogen and the type of antibody produced [85].

CHAPTER 3

Materials and Methods

This chapter mainly sets up the methodology for the migration of BPA from plastic packaging materials into food simulants. A HPLC-UV method was adopted for the quantitative analysis of BPA. The film samples used for migration testing were prepared through melt-mixing followed by compression molding. Migration testing was performed according to ASTM D 4754-98 under finely controlled laboratory conditions. Migration modelling was carried out using MATLAB to fit the migration curves to the experimental data. Statistical analysis was conducted by SAS. Methods used in this study can be described by a flow chart (Figure 3.1) below.

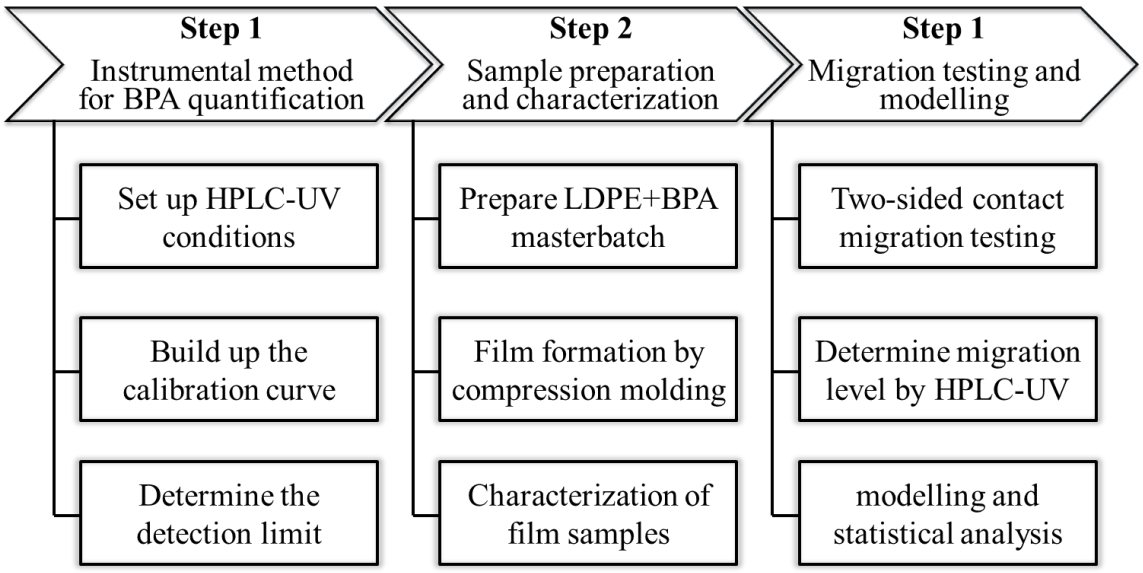

Figure 3.1 Schematic diagram of the study on BPA migration from LDPE into food simulants.

3.1 Materials

The main chemicals used were: BPA (99%+ pure, Sigma-Aldrich, Milwaukee, USA); water (HPLC grade, Mallinckrodt Baker Inc., NJ, USA), acetonitrile (HPLC grade, EMD Chemicals Inc., NJ, USA), ethanol (200 proof, Decon Labs Inc., PA, USA) and acetic acid (ACS grade, EMD Chemicals Inc., NJ, USA). LDPE resin (Petrothene NA960000) was obtained from Lyondell Chemical Company, TX, USA. Apparatus for migration testing included: 40-ml pre-cleaned amber vials with slide valve caps with PTFE-silicon septa (Cole-Parmer, USA), stainless steel wire, and glass beads (McMaster-Carr, USA).

3.2 Instrumental method for the quantification of BPA

High performance liquid chromatography (HPLC) analysis was performed using an Alliance 2695 HPLC (Waters Co., MA, USA) equipped with an automatic sampler/injector. An XBridge C18 3.5μm, 3.0 x 150 mm column with an XBridge guard column (Waters Co., MA, USA) was kept at the temperature of 25℃. The isocratic elution was carried out using acetonitrile/water (40:60, v/v) as the mobile phase at a flow rate of 0.5 ml/min. BPA was detected by an Alliance 2487 UV detector (Waters Co., MA, USA) set at 225nm. The injection volume was 10 µl. All samples were tested in triplicate.

A stock solution of 1 mg/ml was prepared by weighting 100 mg BPA and dissolving in acetonitrile in a 100 ml volumetric flask. The stock solution was stored in the refrigerator at -4℃. Standard solutions were prepared by diluting the stock solution with

the mobile phase. The calibration curve was established by measuring standard solutions at six concentration levels ranging from 5 to 120 μg L^{-1} with triplicate per concentration. Linear regression was applied using the analyte peak area vs. analyte concentration.

The limit of detection (LOD) was estimated by running successive dilutions of the stock solutions until the height of the BPA peak was about three times of the background noise level at the retention time. The limit of quantification (LOQ) was defined as 10 times of the background noise level.

Within-run precision was expressed as the relative standard deviation of ten replicate measurements of a standard solution at three different concentrations (20, 40 and 80 μg L^{-1}). Between-run precision was expressed as the relative standard deviation of eight independent replicate analyses (preparation and measurement) of a standard solution of 20 μg L^{-1}.

3.3 Sample preparation for migration testing

3.3.1 Preparation of LDPE + BPA masterbatch

Masterbatches consisting of LDPE and BPA were obtained by melt mixing with three different BPA concentration levels: 0.10, 0.25 and 0.50 wt%. A control (without BPA) was also prepared. LDPE resin was ground in a Laboratory Mill with 1 mm mesh (Arthur H. Thomas Company, PA., USA), and then pre-mixed with BPA at a specific concentration in a blender. The mixture (40 g) with each BPA concentration was placed in an electrically heated three-piece mixer with two roller style mixing blades (C.W.

Brabender Instruments Inc., NJ, USA) (Figure 3.2). The temperature was set at 150 ℃ in order to achieve proper viscosity for mixing. The mixer was set at a rotation speed of 50 rpm and ran for 5 min per batch. The mixture was then kept in a refrigerator at -4 ℃ for 1 hr and ground again.

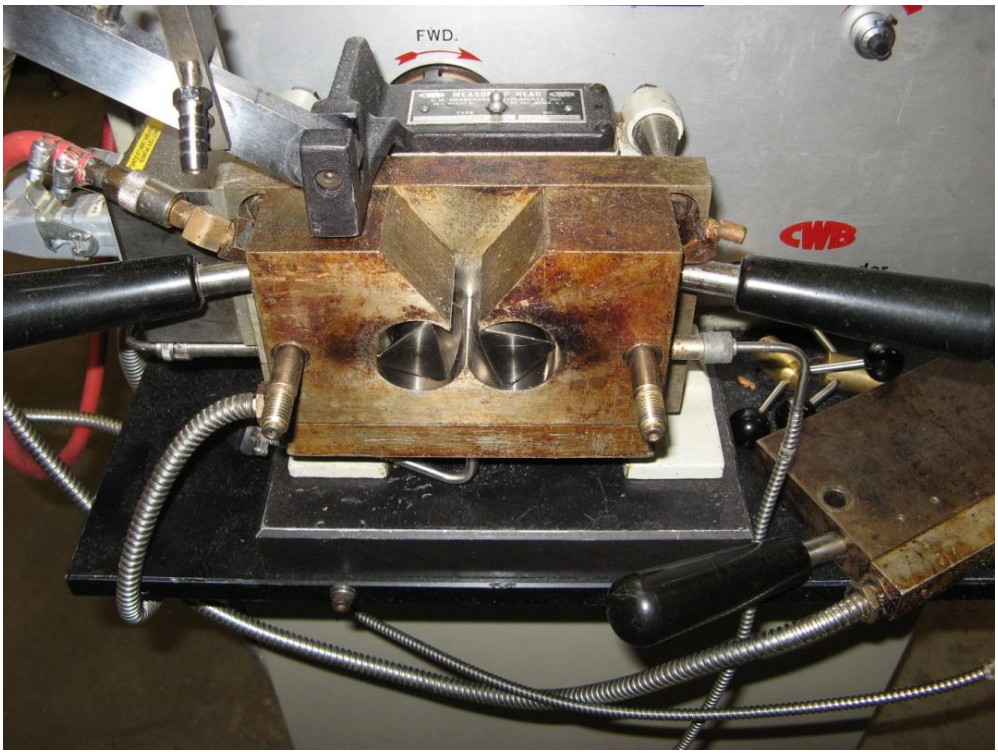

Figure 3.2 Electrically heated three-piece mixer with two roller style mixing blades.

Note: For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation

3.3.2 Film sample formation for migration testing

Masterbatch at each BPA concentration level was used for film formation. Unoriented film samples for migration testing with a thickness of 117 ±4 μm (N=24) were

made by compression molding using a Carver Laboratory Press (Carver Inc., IN, USA) (Figure 3.3). The temperature of both upper and lower plates of the compression molder was set at 120 ℃. Masterbatch sample of about 1 g was placed on the lower plate and melted under atmospheric pressure. Then, the pressure was increased to 10,000 pounds gradually and kept for 5 min. The film was cooled with a flow of cooling water to room temperature. The pressure was then released, and disks (1 cm in diameter) were cut from the film and used for the migration testing. A control film (without BPA) was also made by the same procedures.

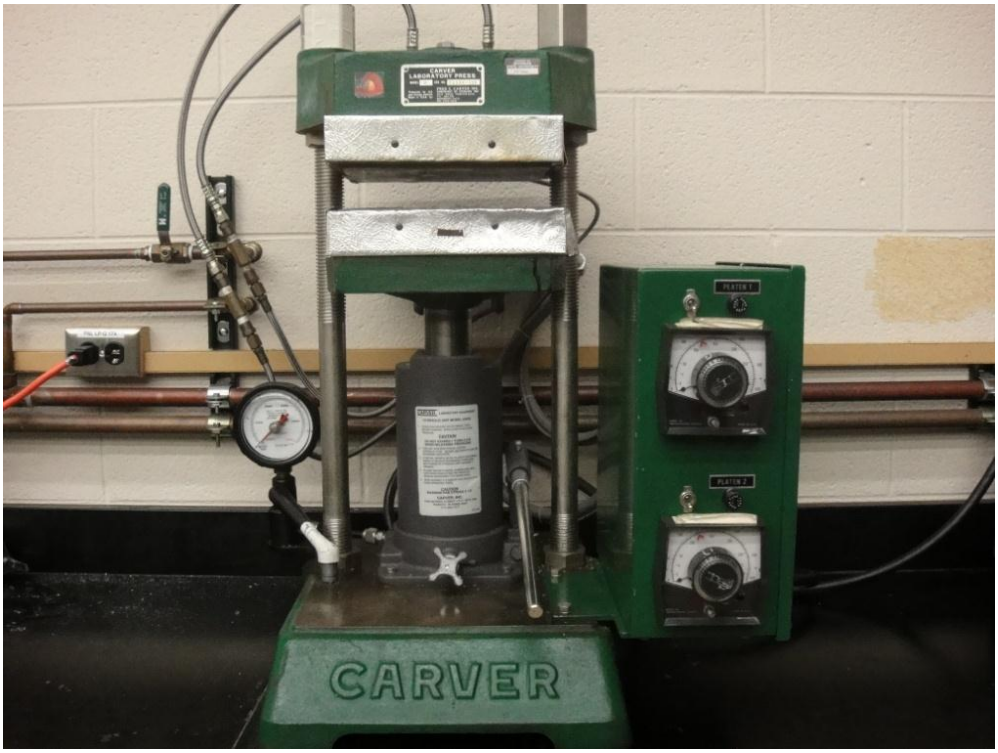

Figure 3.3 Carver Laboratory Press used for compression molding.

3.4 Characterization of LDPE film

3.4.1 Determination of initial BPA content in LDPE film

Initial BPA concentrations in LDPE film were determined by reflux extraction [124] before the samples were used for the migration testing. Film disks at each BPA concentration level as well as the control film weighting approximately 0.3 g were placed in a 250 ml round-bottom flask, and reflux-extracted by 100 ml ethanol for 60 min. A small portion of the extracting solvent was diluted, transferred to an auto-sampler vial, and analyzed by HPLC-UV. To ensure that all BPA was extracted, the reflux extraction was repeated. The first extraction was considered complete if <2 wt% BPA was found in the second one. The extraction was conducted in triplicate.

3.4.2 Determination of BPA distribution in LDPE film

Infrared spectrometry was performed on a Shimadzu IRPrestige-21 FTIR apparatus (Shimadzu Co., Japan) equipped with a Pike Technologies horizontal attenuated total reflectance (HATR) accessory. The system was operated by Shimadzu IRsolutioin software. In order to obtain a higher absorbance for the analysis, LDPE film samples with the initial BPA concentration of 0.5 wt% (nominal) were selected and tested in triplicate. Each film sample was scanned by transmission spectroscopy at five different places and HATR spectroscopy of both sides with fifteen different places on each side within the range 4000-400 cm^{-1}. Absorbance (peak intensity) at 827 cm^{-1} was recorded for each scan and correlated to the BPA concentration in LDPE film. Those absorbance values were

considered an indication of BPA distribution in LDPE film.

3.4.3 Thermal analysis

The melting temperature (T_m) and percent crystallinity (X_c) of LDPE films without and with BPA (0.5 wt% in nominal) were determined using a differential scanning calorimeter (DSC Q-100, TA Instruments Inc., DE, USA). Transition temperatures were obtained in accordance with ASTM D3418-03. The percent crystallinity was calculated with the equation below:

$$X_c = \frac{\Delta H_m}{\Delta H_m^0} \tag{3.1}$$

where ΔH_m is the enthalpy of fusion of the sample, and ΔH_m^0 is the heat of fusion of 100% cystalline LDPE (290 J g^{-1}) [125]. T_m and X_c values were determined from the first heating cycle from 20 ℃ to 180 ℃ with a ramp rate of 10 ℃ min^{-1}. All tests were done in triplicate and the data were analyzed by TA Universal Analysis software (TA Instruments Inc., DE, USA).

3.5 Migration experiment

Migration testing was carried out according to ASTM D 4754-98, at three different temperatures (40, 60 and 80 ℃), three different initial BPA concentrations (determined after the film was produced), and three different food simulants (water, 3% (w/v) acetic acid and 95% ethanol). Two-sided liquid extraction (Figure 3.4) was performed by placing 10 film disks (around 0.35 g) of each BPA concentration in a 40-ml amber glass

vial, and 30 ml food simulant of each type was added. The disks were placed on the stainless steel wire and separated by glass beads. The vials were kept in a water bath (±1 ℃) at each temperature. An extra experiment was conducted at room temperature (22 ℃) to simulate food storage conditions. At this situation, two-sided extraction of film disks with the initial BPA concentration of 0.25 wt% (nominal) was conducted in each food simulant. A small portion of solvent was taken with an injection syringe at variable time intervals until the equilibrium of migration was achieved. The solvent sample was properly diluted, transferred to an auto-sampler vial, and analyzed by HPLC-UV.

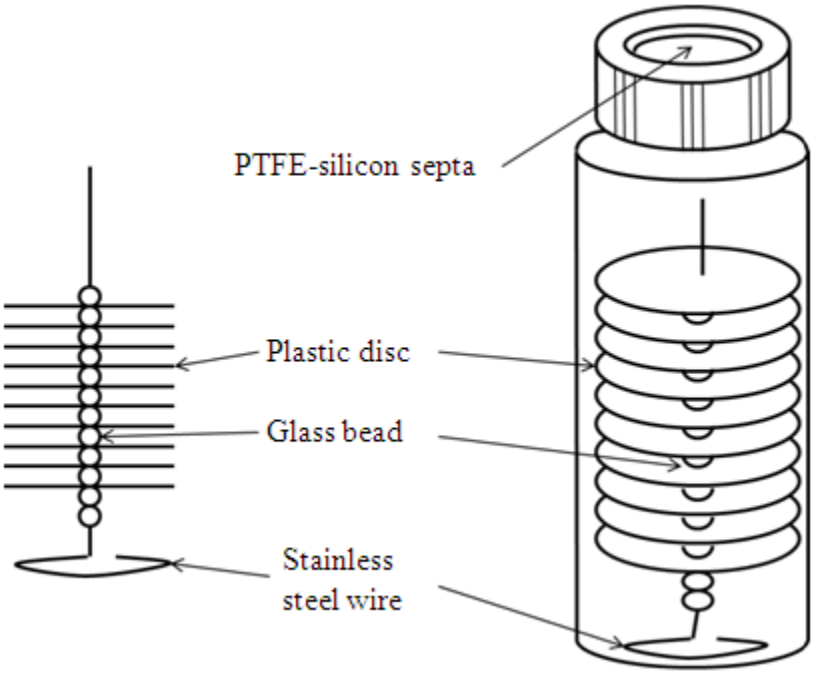

Figure 3.4 Apparatus for two-sided contact migration testing.

3.6 Estimation of D_p and $K_{P,F}$

Diffusion coefficients (D_P) and partition coefficients ($K_{P,F}$) were derived from equations 2.6 and 2.8. Non-linear regression was performed by the curve fitting tool of MATLAB (version 7.0, The MathWorks, Inc., MA, USA). The migration curve was manually fitted until the best fit was achieved. The fit of the applied equation to the experimental data can be expressed by the root mean square error (RMSE) [126]:

$$RMSE = \frac{1}{M_{P,0}} \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left[(M_{F,t})_{experimental,i} - (M_{F,t})_{predicted,i} \right]^2} \qquad (3.2)$$

where N is the number of experimental points per migration curve; i is the number of observations; $M_{F,t}$ is the amount of migrant in the food simulant at time t; and $M_{P,0}$ is the initial amount of migrant in the polymer.

Some assumptions were made to enable the application of the two models [127]:

(1) The film disks should be even in thickness;

(2) Migrant initially is homogeneously distributed through the plastic film;

(3) There is no migrant in food simulants at the beginning of the migration;

(4) D_P is a constant at dilute migrant concentrations (<1%);

(5) There is no swelling effect caused by food simulants.

3.7 Statistical analysis

A 3^3 full factorial design was adopted for the migration testing, with three factors: temperature, initial BPA concentration and food simulant type, each at three levels. Food

simulant type was an incontinuous variable and taken as the category. Temperature and initial BPA concentration were taken as continuous variables under each category. The diffusion coefficient, D_P, as an indicator of the migration rate, was the response variable. To investigate the effect of the three factors and their interactions on the diffusion coefficient, a general linear model [128] was introduced:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 * X_2 + \beta_{23} X_2 * X_3 + \beta_{13} X_1 * X_3 + \beta_{123} X_1 * X_2 * X_3 \quad (3.3)$$

There were seven effects involved, including three main effects (effect of temperature, initial BPA concentration and food simulant type), three two-way interactions and one three-way interaction. A normal distribution of the response variable was assumed for the use of the model. To meet this assumption, natural log transformation on D_P values was carried out without changing the nature of the interaction term. Three-way analysis of variance (ANOVA) was conducted by SAS (version 9.2, SAS Institute Inc., NC, USA) to determine whether each of the effects was significant ($Pr < 0.05$) on the diffusion coefficient. Based on the analysis, an empirical model was developed to express the diffusion coefficient as a function of temperature and initial BPA concentration for each food simulant. Parameter estimation was carried out by SAS as well.

3.8 Modelling of BPA concentration profiles in LDPE film

The concentration profile in the polymer film can be expressed by Fick's second law in one dimension:

$$\frac{\partial C(x,t)}{\partial t} = D_P \frac{\partial^2 C(x,t)}{\partial x^2} \tag{3.4}$$

D_P values were generated from the experimental data. $C(x,t)$ was solved by using the following initial and boundary conditions.

Initial conditions:

$$C_P(x,0) = C_{P,0}$$

$$C_F(0) = 0$$

Boundary conditions:

$$C_P(0,t) = C_P(L,t) = C_F(t)$$

where $C_{P,0}$ is the initial migrant concentration in the polymer; L is the polymer thickness; x is the position in the polymer ranges from 0 to L. $C_F(t)$ is the migrant concentration in the food simulant.

To solve $C(x,t)$ based on the initial and boundary conditions, the following assumptions are made.

(1) Initially, the migrant is evenly distributed throughout the polymer and there is no migrant in the solvent.

(2) There is no swelling effect caused by the solvent. Migration behavior in the polymer is Fickian, and the diffusion coefficient is constant during the migration.

(3) The migrant concentrations at both sides of the interface between the polymer and the solvent should be equal.

(4) The migrant has a good solubility in the solvent. Finally, most of the migrant migrate into the solvent.

(5) The migrant concentration at the interface of the polymer is assumed to be equal to that in the solvent.

The BPA concentration profiles in LDPE film was plotted by MATLAB using a “pde” (partial differential equation) function. Diffusion coefficients, initial and boundary conditions were manually input to generate the concentration profiles.

CHAPTER 4

Results and Discussion

4.1 Performance of HPLC-UV method

BPA was eluted out around 6.5 min and fully separated from other chemicals (Figure 4.1). The calibration curve is generated from standard solutions (5-120 μg L^{-1}), and plotted based on the average response of three replicates. A linear relationship was obtained with the correlation coefficient of 0.9990. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 1 μg L^{-1} and 3 μg L^{-1}, respectively. Within-run precision, expressed as relative standard deviation (RSD) (N=10), at 20, 40 and 80 μg L^{-1} of the mobile phase, was 1.62%, 0.98% and 0.56%, respectively. Between-run precision, expressed as RSD (N=8) at 20 μg L^{-1} of the mobile phase, was 3.31%. These values indicate a good method performance.

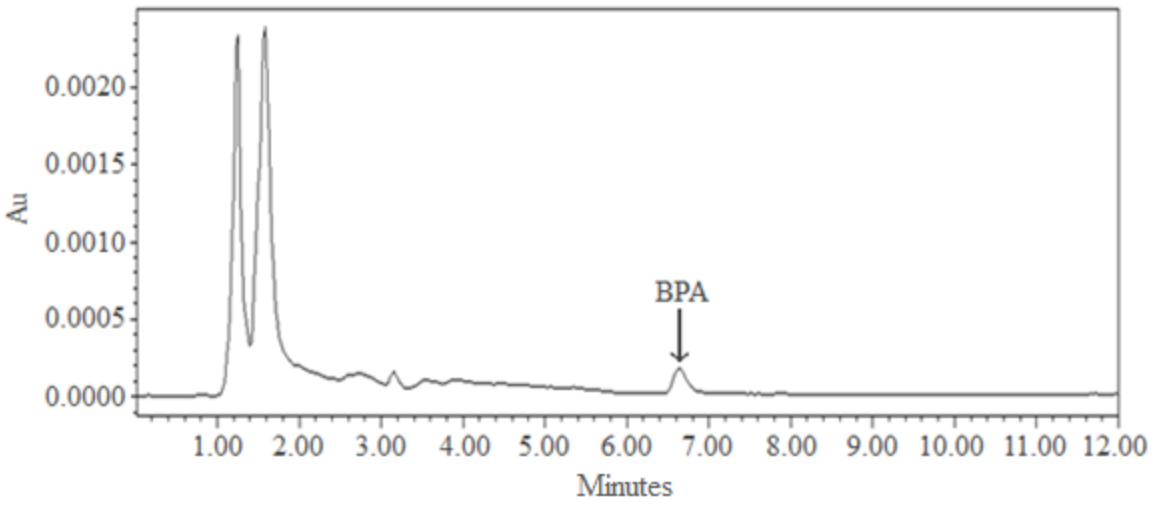

Figure 4.1 HPLC-UV chromatogram of a standard solution containing 10 μg L^{-1} BPA.

4.2 Properties of LDPE film

Initial BPA concentrations in LDPE film were determined to be 0.41±0.01, 1.42 ±0.08 and 2.66±0.14 mg g^{-1}. No BPA was detected in the control sample. A 50% decrease was found between the amount of BPA when it was initially added into the polymer and after the film was produced and ready to be tested for migration. This was mainly due to the heat and high pressure during the manufacture.

Homogeneous distribution of BPA throughout the film samples was essential for the application of diffusion models. FTIR analysis on the film samples demonstrate that BPA was approximately even distributed throughout the film as shown in Table 4.1. For each film sample, similar absorbance values were obtained for both sides and the whole sample. The variability (relative standard deviation) for each absorbance value was below 4% in most cases and no more than 5%. The absorbance values were also close to each other between different film samples.

Thermal properties of LDPE films without and with BPA (0.5 wt% in nominal) were determined by DSC. The melting temperatures were 110.2±0.2 ℃ and 110.8±0.8 ℃, respectively. The percent crystallinity was 37.3±0.6% and 36.7±0.2%, respectively. It can be seen that the addition of BPA did not significantly change the morphology of the film.

Table 4.1 Absorbance of BPA (0.5 wt% in nominal) in LDPE film obtained by HATR spectroscopy and transmission spectroscopy.

Sample name	Absorbance x 10^{-2}					
	Top side	SD	Bottom side	SD	Whole sample	SD
Sample 1	6.16	0.24	6.01	0.29	6.12	0.29
Sample 2	6.03	0.27	5.89	0.23	5.92	0.19
Sample 3	5.95	0.18	6.08	0.24	6.04	0.21

Note: Absorbance values (15 replicates) for top and bottom sides were obtained by HATR spectroscopy; absorbance values (5 replicates) for the whole sample were obtained by transmission spectroscopy.

4.3 D_p and $K_{P,F}$ determination

LDPE is selected because it is very commonly used as food packaging materials. Also, as mentioned previously (section 3.6), the use of diffusion models requires some assumptions. LDPE was selected as a simple system to better meet these assumptions and examine the applicability of these models. A weak interaction was expected between polar BPA and non-polar LDPE. A weak interaction was also expected between the polar food simulants and non-polar LDPE, so the food simulant would not have a significant effect on the morphology of the polymer. Therefore, diffusion models can be more easily applied to describe the migration process.

Experimental data were applied for the determination of diffusion parameters by equations 2.6 and 2.8. Equation 2.6 was used for the parameter estimation of both D_P and $K_{P,F}$, while equation 2.8 was only used for the calculation of D_P. Diffusion coefficients

derived from both equations are listed in Table 4.2-4.3. D_P values were in a scale of 10^{-10} to 10^{-8} cm^2 s^{-1}. A small variance of D_P values was observed under the each condition with a relative standard deviation (RSD) below 5% in most cases. This might be caused by the variable film thickness under each condition.

Table 4.2 Mean (±SD, N=3) diffusion coefficients (D_P) (generated from equation 2.6) of BPA migration from LDPE under different conditions.

Food simulant	Initial concentration ($mg\ g^{-1}$)	D_P x 10^{-10} ($cm^2\ s^{-1}$)		
		40 ℃	60 ℃	80 ℃
Water	0.41	2.03 ±0.074	33.4 ±0.18	371 ±31
	1.42	0.969 ±0.016	23.3 ±0.42	145 ±11
	2.66	0.467 ±0.018	7.41 ±0.082	84.5 ±1.8
3% Acetic acid	0.41	2.45 ±0.15	36.7 ±0.93	397 ±8.8
	1.42	1.01 ±0.034	21.5 ±0.80	174 ±8.5
	2.66	0.479 ±0.033	7.96 ±0.093	88.7 ±1.5
95% Ethanol	0.41	1.17 ±0.048	23.3 ±1.6	373 ±6.7
	1.42	0.454 ±0.012	15.2 ±0.73	158 ±8.0
	2.66	0.237 ±0.012	5.42 ±0.19	81.4 ±1.2

Table 4.3 Mean (±SD, N=3) diffusion coefficients (D_P) (generated from equation 2.8) of BPA migration from LDPE under different conditions.

Food simulant	Initial concentration (mg g^{-1})	D_P x 10^{-10} (cm^2 s^{-1})		
		40 ℃	60 ℃	80 ℃
Water	0.41	2.06 ±0.061	33.4 ±0.36	372 ±31
	1.42	1.00 ±0.028	23.5 ±0.43	147 ±11
	2.66	0.498 ±0.018	7.62 ±0.079	85.2 ±1.8
3% Acetic acid	0.41	2.48 ±0.14	36.8 ±1.0	398 ±9.6
	1.42	1.04 ±0.038	21.7 ±0.81	175 ±7.9
	2.66	0.507 ±0.034	8.14 ±0.092	89.4 ±1.5
95% Ethanol	0.41	1.17 ±0.048	23.3 ±1.6	373 ±6.7
	1.42	0.471 ±0.013	15.3 ±0.86	159 ±8.0
	2.66	0.254 ±0.065	5.51 ±0.19	82.0 ±1.3

D_P values obtained from equations 2.6 and 2.8 were compared. Student's T-test (α=0.05) was applied for statistical analysis and no statistical difference was found between the D_P values from each equation under the same condition. However, equation 2.6 is preferred since it provides both parameters D_P and $K_{P,F}$, while equation 2.8 is a further simplified model based on many assumptions, which limits its application.

Partition coefficients derived from equation 2.6 are listed in Table 4.4. $K_{P,F}$ represents the partition of the migrant between the two phases: polymer and food simulant. The small $K_{P,F}$ values indicate that BPA is more likely to migrate into the food simulant rather than stay in the polymer during the migration testing. BPA is insoluble in LDPE due to its polar nature and the non-polar nature of the polymer. But it was very soluble in ethanol and a little soluble in water with a solubility of 300 mg L^{-1} at room temperature [93]. It was found that the maximum BPA concentration in each food

simulant was about 30 mg L^{-1} (calculated according to an initial BPA concentration of 2.66mg g^{-1}) which was far below the solubility limit in each food simulant. Due to the large solvent (food simulant) to polymer volume ratio (about 80 in this study), there would be a huge concentration gradient causing most of BPA migrate from the polymer into the food simulant to try to equilibrate this gradient. A BPA migration level of >90% was mostly found in the experiment, which has an agreement with the small $K_{P,F}$ values presented in Table 4.4.

Table 4.4 Mean (N=3) partition coefficients ($K_{P,F}$) (generated from equation 2.6) of BPA between LDPE and food simulants under different conditions.

Food simulant	Initial concentration (mg g^{-1})	$K_{P,F}$		
		40 ℃	60 ℃	80 ℃
Water	0.41	1	1	0.3
	1.42	3	1	1
	2.66	8	3	1
3% Acetic acid	0.41	1	0.3	0.3
	1.42	2	1	0.8
	2.66	8	3	1
95% Ethanol	0.41	1	1	0.2
	1.42	2	1	1
	2.66	3	2	0.5

The fit of equation 2.6 to the experimental data was evaluated by RMSE in equation 3.2 (Table 4.5). The small RMSE values indicate a good fit of the applied equation to the experimental data. An example of the experimental data with the fitted

graph (generated from equation 2.6) is shown in Figure 4.2. The migration curve fits the experimental data well within a wide range. A larger deviation is usually observed at a higher migration level (>50%). As mentioned in section 4.2, there was a slightly uneven distribution of BPA in LDPE film, which should be responsible to the deviation of experimental data from the migration curve. Another source of the deviation could be the experimental error.

Table 4.5 Mean (N=3) RMSE values as a measure of fit between the experimental data and the applied diffusion equation.

Food simulant	Initial concentration ($mg\ g^{-1}$)	RMSE		
		40 ℃	60 ℃	80 ℃
Water	0.41	0.046	0.052	0.055
	1.42	0.068	0.053	0.054
	2.66	0.055	0.043	0.056
3% Acetic acid	0.41	0.037	0.068	0.050
	1.42	0.038	0.077	0.025
	2.66	0.034	0.049	0.039
95% Ethanol	0.41	0.047	0.024	0.042
	1.42	0.055	0.018	0.050
	2.66	0.033	0.045	0.024

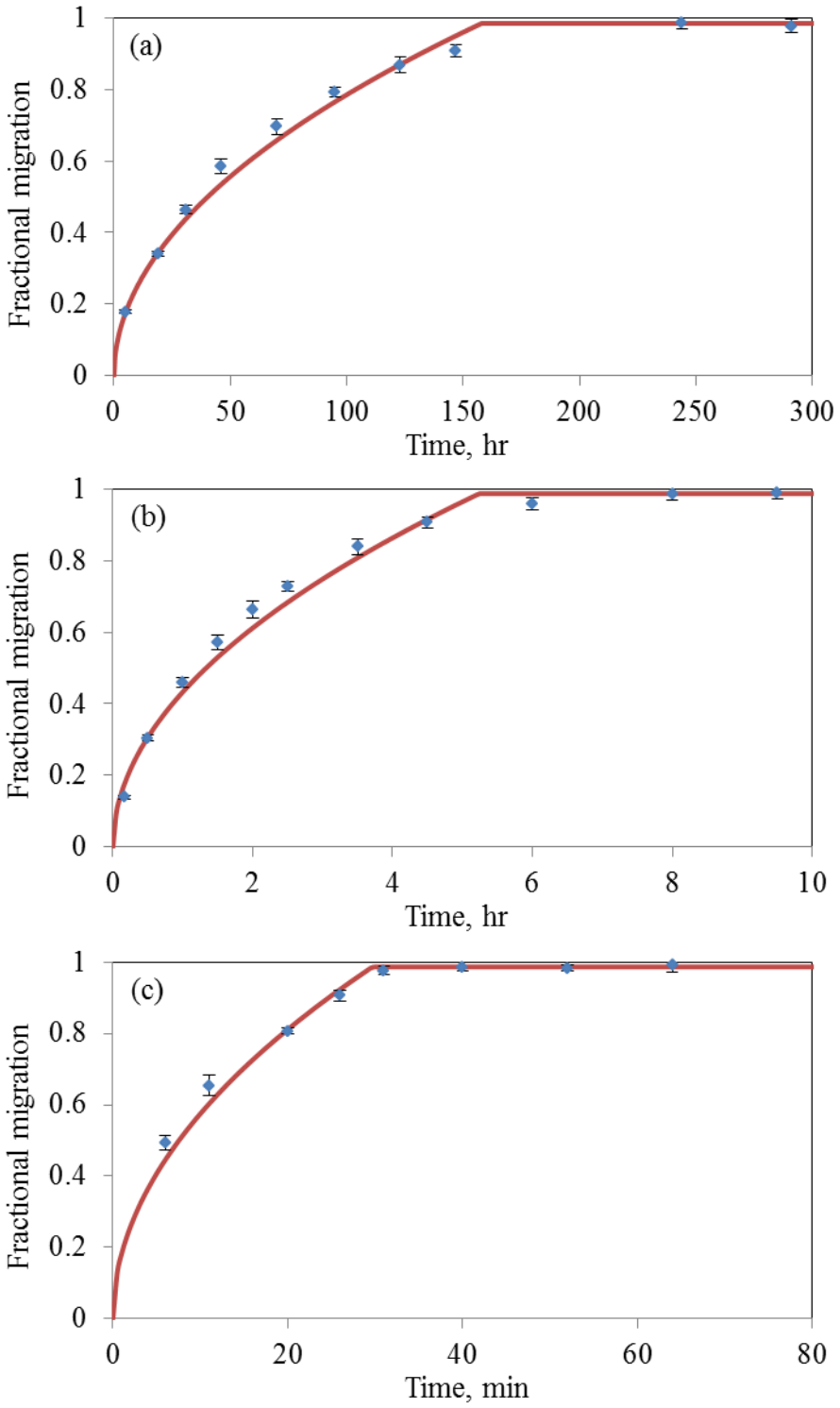

Figure 4.2 Amount of BPA migrated from LDPE into 95% ethanol at (a) 40 ℃, (b) 60 ℃ and (c) 80 ℃ relative to the initial amount in the polymer (1.42 mg g^{-1}).

4.4 Effects of various factors on the diffusion coefficient

Diffusion coefficient can be taken as an indicator for the migration rate, or how fast the migrant moves through the polymer. It is also important to evaluate the effects of various factors and their interactions on the diffusion coefficient in order to predict how the diffusion coefficient behaves under different conditions. The evaluation was conducted by SAS software using equation 3.3 and the results are listed in Table 4.6. The effects of all the factors (temperature, initial BPA concentration and food simulant type) are significant on the diffusion coefficient. The interaction between temperature and food simulant type was also significant on the diffusion coefficient, but not for the other two-way and three-way interaction of the factors.

Table 4.6 Effect of temperature, initial BPA concentration, food simulant type and their interactions on the migration rate at $\alpha = 0.05$.

Effect	Df	F value	$Pr^a > F$	Power
Temperature	1	2767.81	<.0001	0.999
Concentration	1	63.85	<.0001	0.999
Simulant	2	8.54	0.0005	0.961
Temp*Conc	1	0.24	0.6285	0.077
Temp*Simulant	2	4.85	0.0107	0.784
Conc*Simulant	2	0.19	0.8300	0.078
Temp*Conc*Simulant	2	0.11	0.8934	0.067

Note: a. Pr value ≤0.05 indicates a significant effect on the diffusion coefficient.

Effect of temperature

In rubbery polymers, the relationship between the diffusion coefficient and temperature (above the polymer glass transition temperature, Tg) can be described using an Arrhenius type of equation [129]:

$$D = D_0 \exp\left(-\frac{E_a}{RT}\right) \quad (4.1)$$

where E_a represents the activation energy of diffusion; R is the gas constant; and T is the absolute temperature. A linear relationship was obtained by plotting $\ln(D_p)$ as a function of inverse temperature ($R^2 > 0.99$). Activation energies of BPA migration were calculated to be 118±2.6 kJ mol^{-1} in water, 118±1.9 kJ mol^{-1} in 3% acetic acid and 134±1.4 kJ mol^{-1} in 95% ethanol. Similar E_a values were obtained for each food simulant regardless of the initial BPA concentrations. The reason could be the very small amount of BPA (< 0.5 wt% in nominal) added to LDPE, which has nearly no effect on the polymer morphology according to the DSC results. This behavior coincides with the non-significant interaction effect between temperature and initial BPA concentration from the statistical analysis.

D_P values obtained at 22 ℃ were compared with the ones predicted by the Arrhenius equation (Figure 4.3). The difference between the experimental and predicted values were within the range of $\pm 6\%$, indicating a reliable application of the Arrhenius equation.

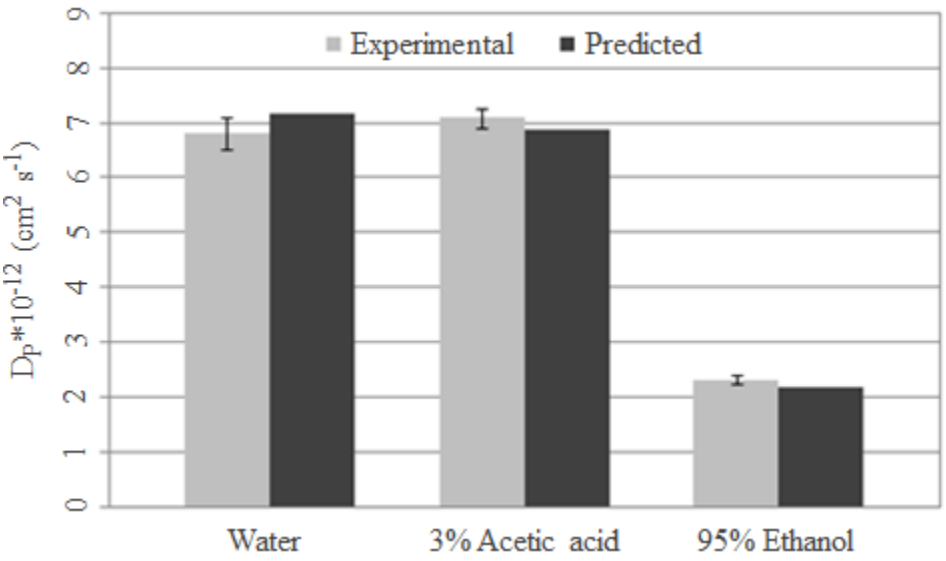

Figure 4.3 Mean (±SD, N=3) experimental and predicted diffusion coefficients of BPA in LDPE contacting with three different food simulants.

Note: The experiment was conducted at room temperature with an initial BPA concentration of 1.42 mg g^{-1}.

Effect of initial BPA concentration

As initial BPA concentration increased, the diffusion coefficient decreased. The transport of BPA in the polymer was restricted by the mobility of polymer chains and the free volume in the polymer [4, 61]. Since the addition of BPA was too little in amount to cause an effect the polymer matrix, there should be nearly no modification on the mobility of polymer chains by BPA. A longer time for the equilibrium of migration was required at a higher initial BPA concentration. The initial concentration dependence of diffusion coefficients can be expressed in an exponential form, as an approximately linear relationship ($R^2 > 0.95$) was obtained after natural log transformation on D_p values. An

example is shown in Figure 4.4.

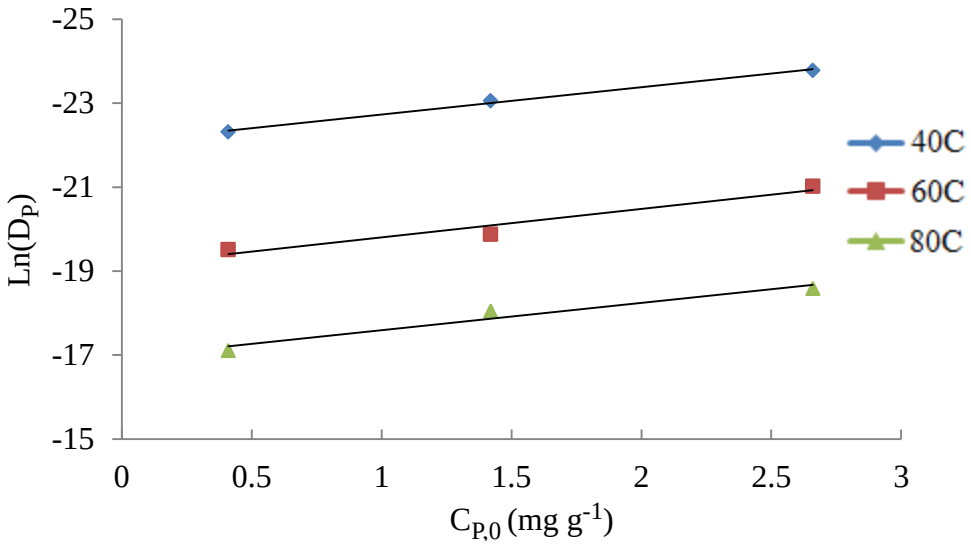

Figure 4.4 Diffusion coefficient of BPA in LDPE in contact with water as a function of initial BPA concentration at different temperatures.

Note: Each data point represents the mean values of triplicate analysis. The parallel lines indicate that the interaction between temperature and initial BPA concentration is not significant.

Effect of food simulant type and its interaction with temperature

The transport property of the polymer phase varies with different types of food simulants [130]. Table 4.2 shows that the diffusion coefficients were higher in water and 3% acetic acid than in 95% ethanol at 40 and 60 ℃. This phenomenon may be attributed to the affinity between LDPE and the solvent (food simulant). According to regular solution theory (RST), the affinity between the polymer and the solvent can be quantified

by solubility parameter distance, Ra, expressed as [59]:

$$R_a = \sqrt{4(\delta_{Dp} - \delta_{Ds})^2 + (\delta_{Pp} - \delta_{Ps})^2 + (\delta_{Hp} - \delta_{Hs})^2} \quad (4.2)$$

where δ_D, δ_P and δ_H are dispersion, polar and hydrogen bonding parameters, respectively. The second subscript p and s represent the polymer and the solvent, respectively. The smaller R_a, the higher the affinity between the polymer and the solvent. The solubility parameters for LDPE and each food simulant at room temperature, and the calculated R_a values are listed in Table 4.7. Water was used to represent 3% acetic acid since similar diffusion coefficients were obtained for the two simulants.

Table 4.7 Dispersion (δ_D), polar (δ_P) and hydrogen bonding (δ_H) solubility parameters for LDPE and different food simulants.

Material	δ_D $MPa^{1/2}$	δ_P $MPa^{1/2}$	δ_H $MPa^{1/2}$	R_a from LDPE $MPa^{1/2}$	Ref.
LDPE	17.9	0	0	0	[131]
Water	15.5	16	42.3	45.5	[132]
Ethanol	7.7	4.3	9.5	22.9	[132]

The affinity between LDPE and ethanol was higher than that between LDPE and water. During the migration, the solvent penetrated the polymer matrix and a higher affinity between polymer and the solvent would slow the movement of BPA. Therefore, there was a delay in the diffusion of BPA in LDPE when exposed to ethanol and BPA migrated faster into water and 3% acetic acid. At 80 ℃, the diffusion coefficients of BPA for all food simulants were higher than the other temperatures, but there was no much

difference among different food simulants. It was expected that the whole system got very high mobility at high temperatures; therefore affinity did not play an important role on the migration. The effect of food simulant type on the diffusion coefficient at lower temperature tended to disappear at higher temperatures. The influence of food simulant type on the diffusion coefficient with the change of temperature showed an interaction effect indicated by the statistical analysis.

4.5 Empirical model for BPA migration from LDPE film

A relationship can be established to express the diffusion coefficient as a function of temperature and initial BPA concentration for each food simulant. Again, a general linear model was introduced:

$$\ln(D_P) = a_0 + a_1 * T + a_2 * C + a_3 * T * C \quad (4.3)$$

Parameters estimation was carried out by running "glm" function of SAS software, and the significance of each parameter was evaluated by comparing with 0 (non-significant). The results are shown in Table 4.8. R^2 values showed a good fit of the model to the experimental data. The positive a_1 values indicated that diffusion coefficient increased with the increase of temperature and the effect of temperature on the diffusion coefficient is stronger in 95% ethanol than in the other simulants. The negative a_2 values showed an inverse relationship between the diffusion coefficient and the initial BPA concentration, and the effect of initial BPA concentration on the diffusion coefficient was stronger in 3% acetic acid. It also can be seen that the interaction between temperature and initial BPA

concentration did not have a significant effect on the diffusion coefficient since a_3 values were very close to 0 with $Pr > 0.05$.

Table 4.8 Parameter estimation of the empirical equation 4.3 for BPA migration from LDPE into different food simulants.

Food simulant	a_0	a_1	a_2	a_3 [a]	R^2
Water	-27.1006	0.1285	-0.6446	0.0002	0.9914
3% Acetic acid	-26.8664	0.1266	-0.7779	0.0015	0.9950
95% Ethanol	-28.3486	0.1442	-0.7246	0.0008	0.9947

Note: a. Parameter for a_3 was not significant ($Pr > 0.05$).

4.6 BPA concentration profiles in LDPE film

With the diffusion coefficients obtained from the kinetic study, one can model the concentration profiles of BPA in LDPE film during the migration. Concentration profiles were generated by solving Fick's second law with initial and boundary conditions. An example of BPA concentration profiles in LDPE film are shown in Figure 4.5. Both 2-D and 3-D concentration profiles are generated by MATLAB. The migrant concentration in the solvent can be considered to be 0 due to the large volume of the solvent compared to that of the polymer, as well as the extremely low initial migrant concentration in the polymer. Therefore, a concentration of 0 can be applied to the boundary condition that made the modeling of concentration profile easier.

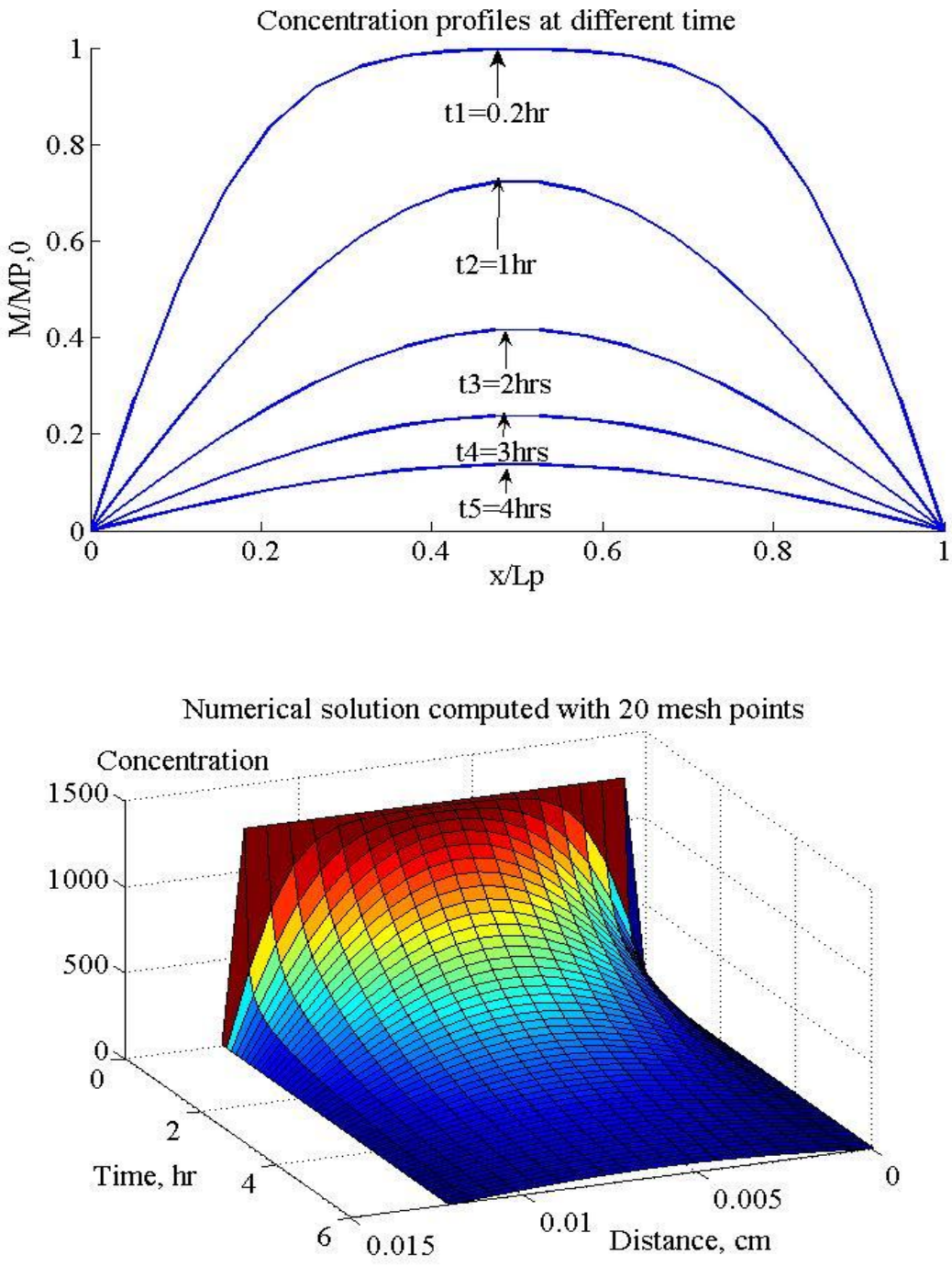

Figure 4.5 Concentration profiles (2D and 3D) of BPA in LDPE contact with water at 60 ℃ with an initial BPA concentration of 1.42 mg g^{-1}.

CHAPTER 5

Conclusion

5.1 Outcomes from the study

Due to the potential adverse heath effect of additives such as BPA in food packaging materials, migration testing on these low molecular weight components is required. Migration level is determined from the experiment and used for estimating the daily intake, in order to protect human health. In this study, A HPLC-UV method was successfully built for BPA analysis. The detection limit was found to be 1μg L^{-1} which is quite capable to determine the trace amount of BPA in the food simulant. The small variance and excellent repeatability of the instrumental method enables the requisition of accurate data, which is essential when dealing with the migration modelling.

Migration of BPA from LDPE was diffusion controlled and followed the Fickian diffusion behavior. The migration process was affected by chemical properties of the migrant, the food simulant and the polymer. Parameters related to migration process such as diffusion coefficients and partition coefficients can be determined by Fick's diffusion equations through a kinetic study under finely controlled laboratory conditions. D_P values obtained under different conditions ranged from 10^{-10} to 10^{-8} cm^2 s^{-1}. These equations could also be applied to other migrant-polymer systems with weak interaction between the migrant and the polymer.

The statistical analysis showed that temperature, initial BPA concentration, and food

simulant type, all significantly affected the diffusion coefficient. However, the interaction effects of the factors on the diffusion coefficient were not significant, except for the interaction of temperature and food simulant type. Among these factors, temperature dependence of diffusion coefficients can be described using an Arrhenius type of equation. Activation energies obtained were independent from the initial BPA concentration, indicating that there was no obvious effect on polymer morphology caused by the addition of BPA at very low concentration levels. The relationship between diffusion coefficients and initial BPA concentration followed an exponential form. Based on the statistical analysis, a general linear model can be applied to correlate the diffusion coefficient to temperature and initial BPA concentration. This model may also be applied to other polymer-migrant systems.

5.2 Prospects for the future work

The future work may concentrate on the following areas:

(1) Apply other instrumental methods for BPA analysis such as UV-Fl and GC-MS, and make comparison between different methods;

(2) Perform one-sided migration testing using either film samples or containers, and compare with the results of two-sided migration testing;

(3) Perform migration testing of BPA and its derivatives from other polymers such as PP and PC, apply mathematical models to the migration process and validate these models, and investigate the effect of various factors on the migration rate.

APPENDICES

APPENDIX A

Graphs for IR and thermal analysis of LDPE+BPA

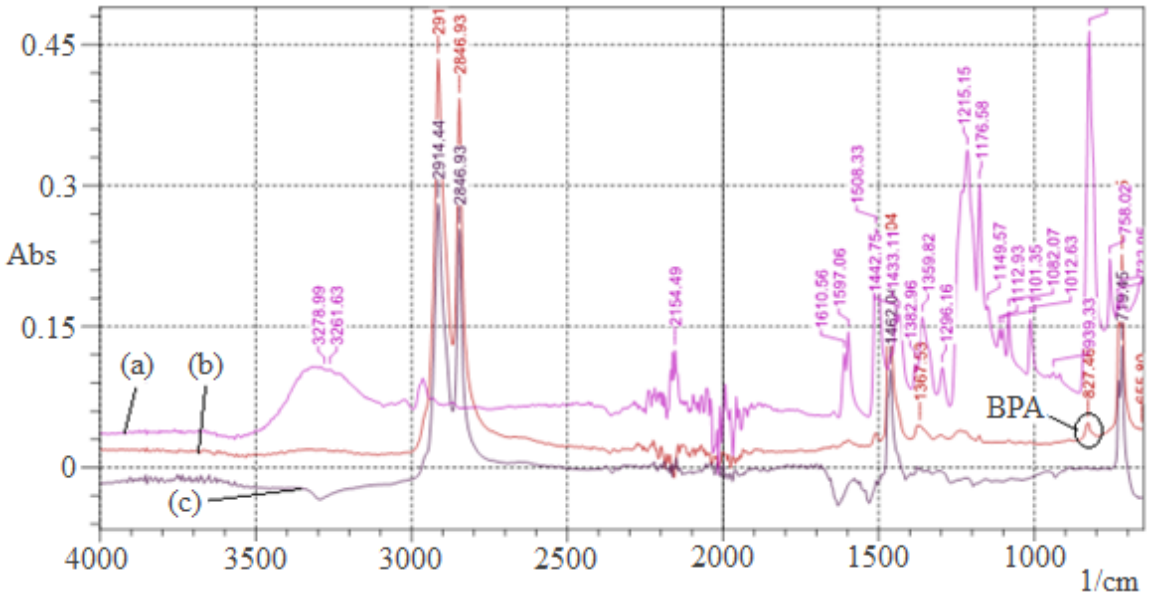

Figure A1 FTIR graph of (a) BPA, (b) LDPE + BPA (0.5 wt%) and (c) LDPE.

Note: The absorbance at 827 cm^{-1} was used as an indicator for BPA distribution in LDPE.

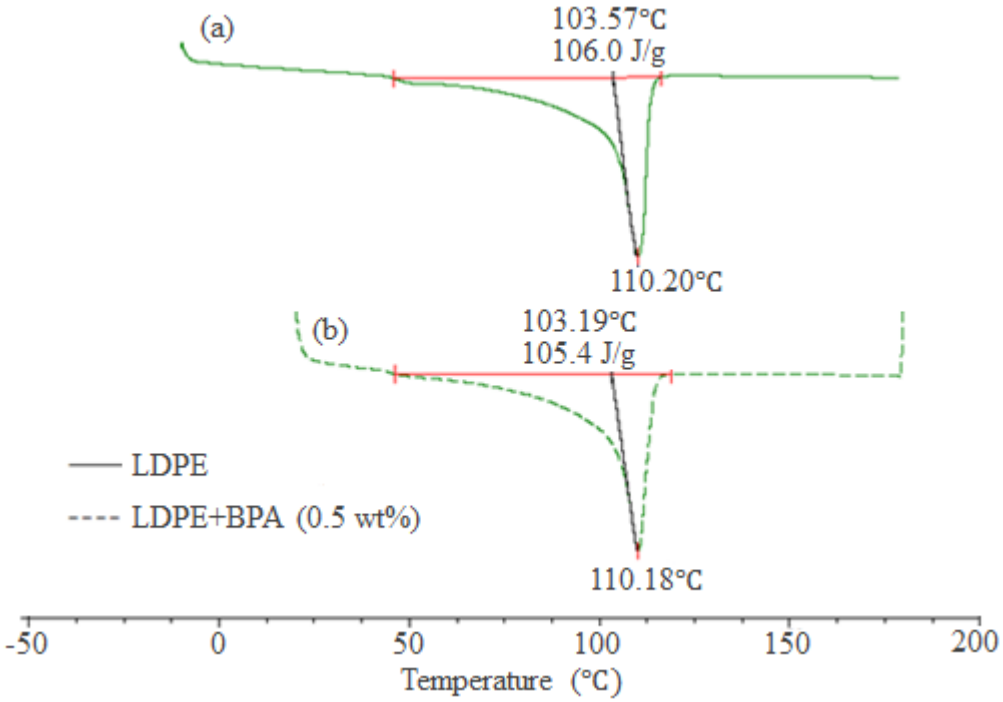

Figure A2 DSC graph for (a) LDPE and (b) LDPE + BPA (0.5 wt%).

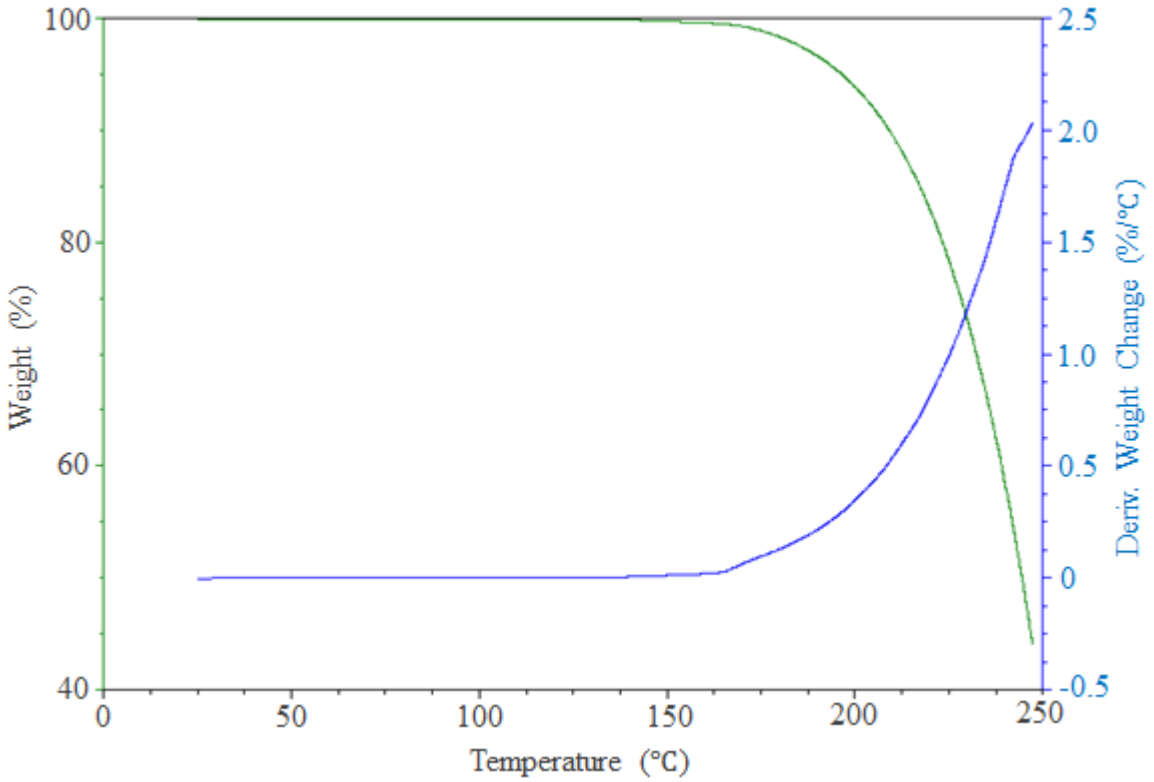

Figure A3 TGA graph of BPA.

APPENDIX B

Migration graphs obtained under different conditions

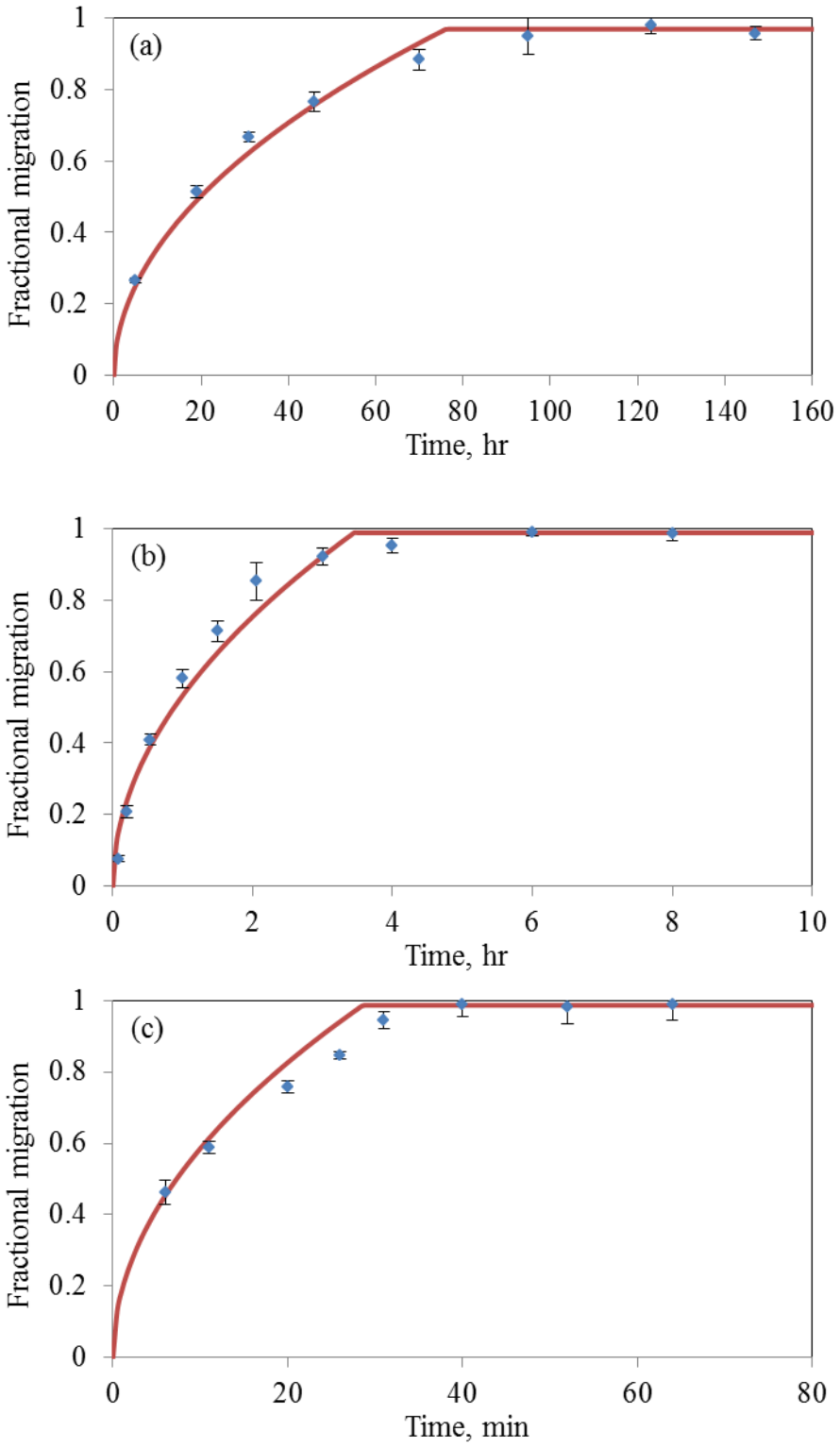

Figure B1 Amount of BPA migrated from LDPE into water at (a) 40 ℃, (b) 60 ℃ and (c) 80 ℃ relative to the initial amount in the polymer (1.42 mg g^{-1}).

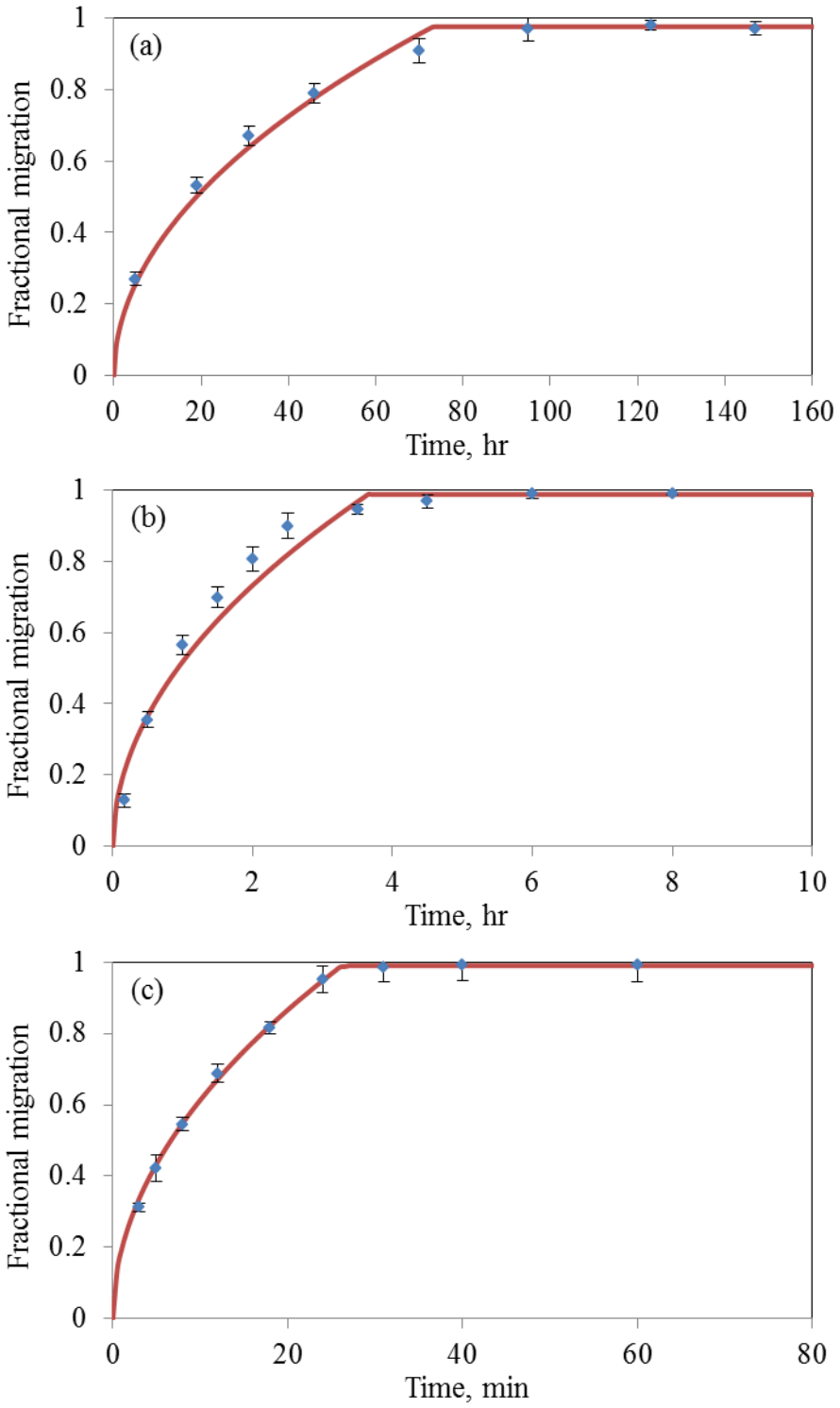

Figure B2 Amount of BPA migrated from LDPE into 3% acetic acid at (a) 40 ℃, (b) 60 ℃ and (c) 80 ℃ relative to the initial amount in the polymer (1.42 mg g^{-1}).

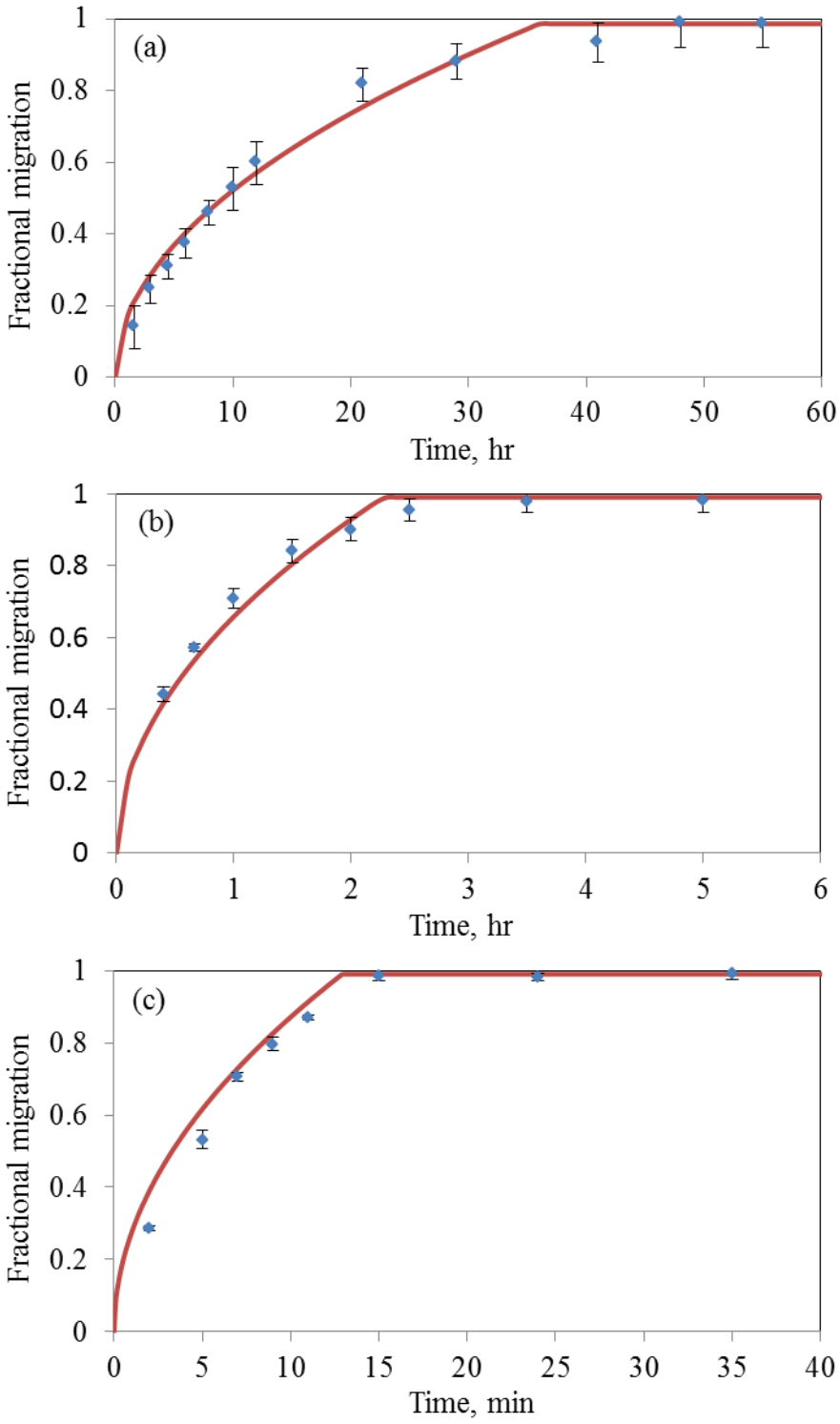

Figure B3 Amount of BPA migrated from LDPE into water at (a) 40 ℃, (b) 60 ℃ and (c) 80 ℃ relative to the initial amount in the polymer (0.41 mg g^{-1}).

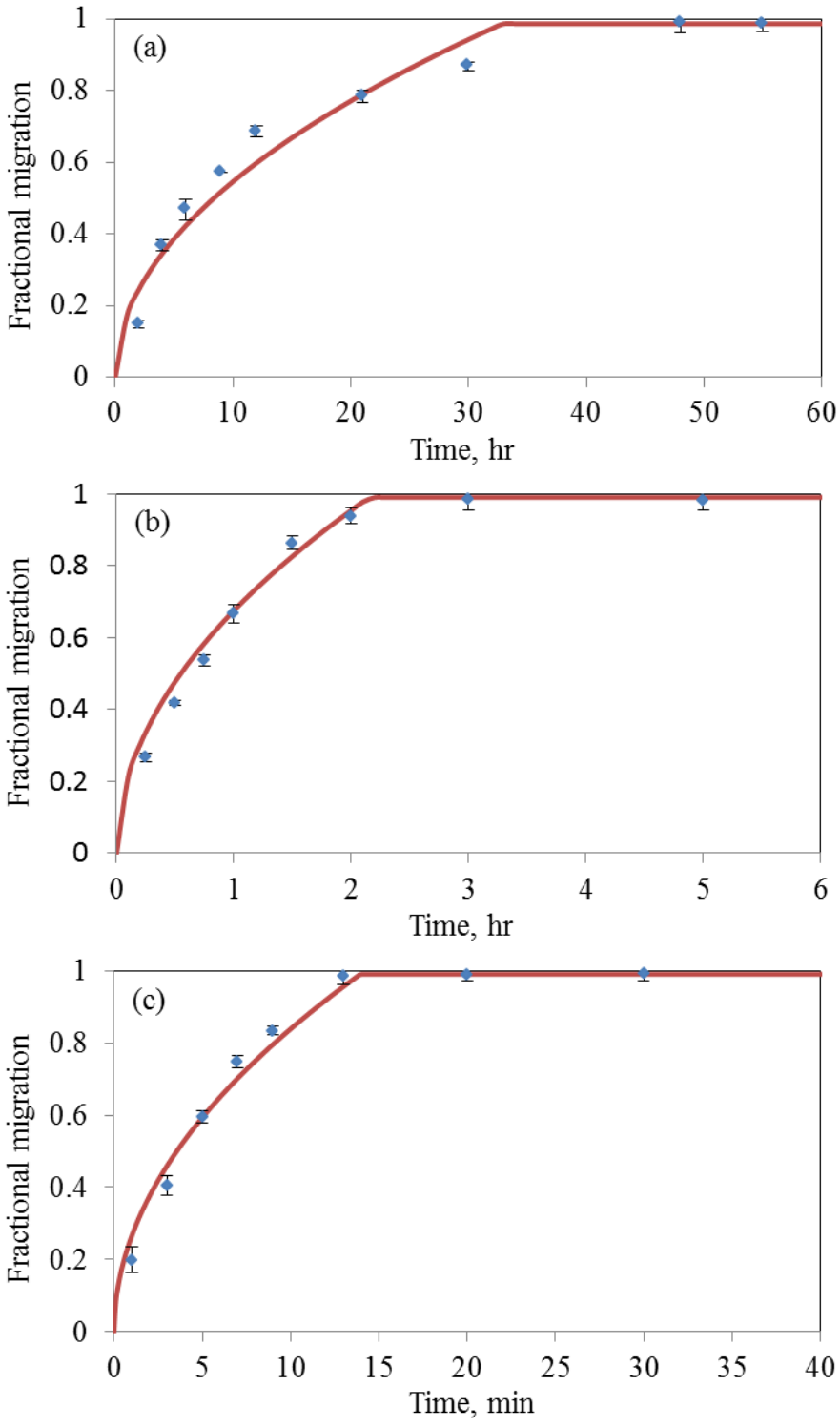

Figure B4 Amount of BPA migrated from LDPE into 3% acetic acid at (a) 40 ℃, (b) 60 ℃ and (c) 80 ℃ relative to the initial amount in the polymer (0.41 mg g^{-1}).

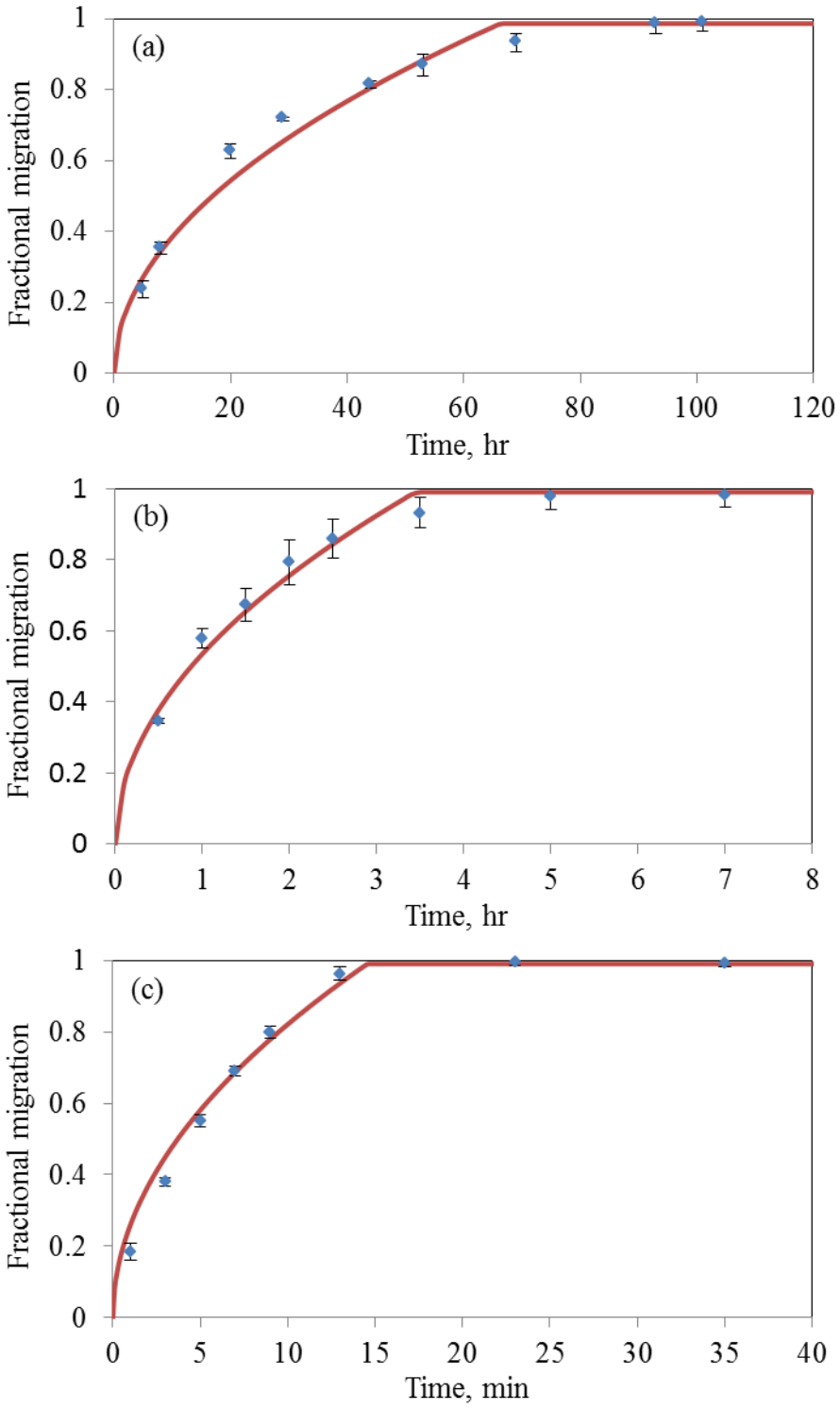

Figure B5 Amount of BPA migrated from LDPE into 95% ethanol at (a) 40 ℃, (b) 60 ℃ and (c) 80 ℃ relative to the initial amount in the polymer (0.41 mg g^{-1}).

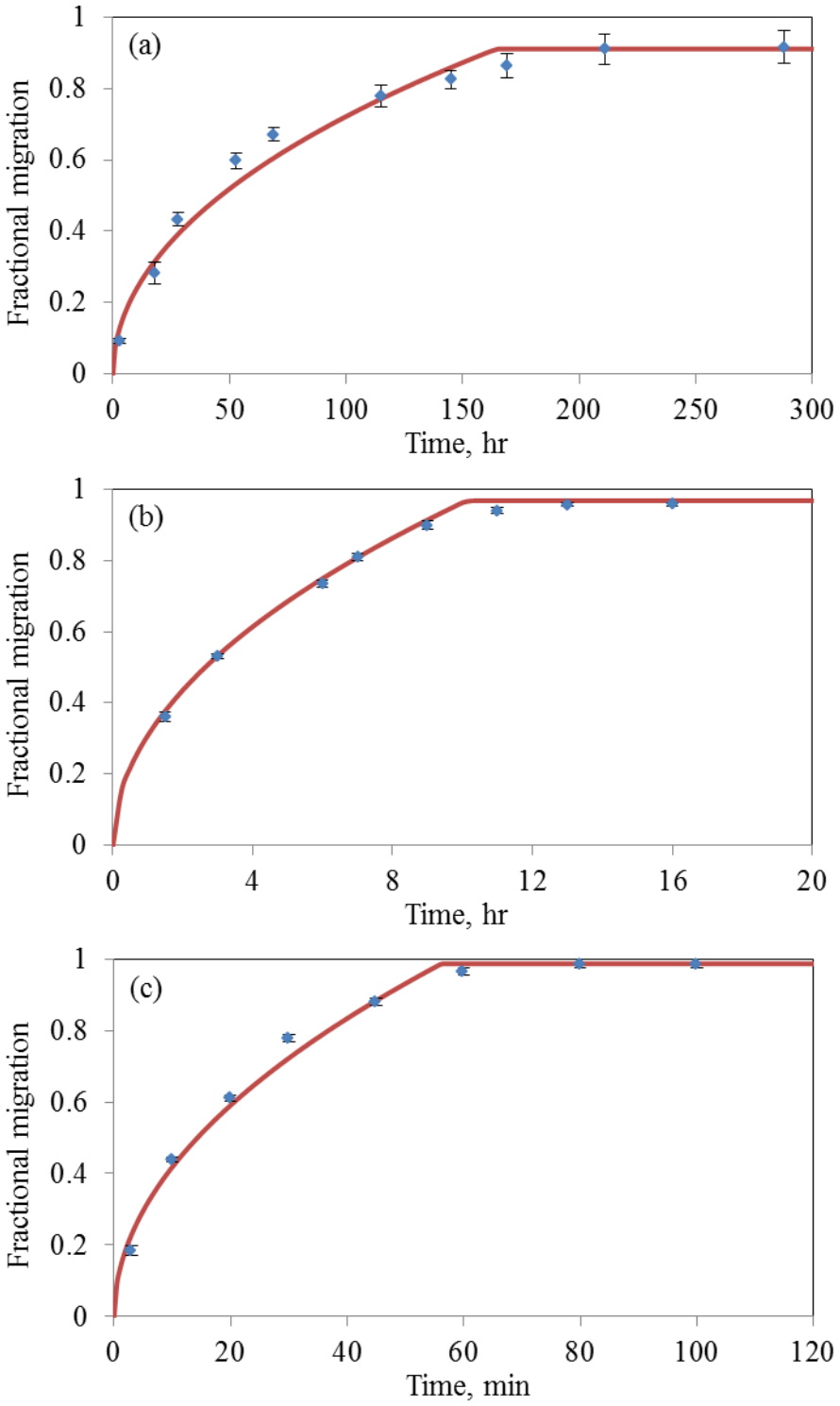

Figure B6 Amount of BPA migrated from LDPE into water at (a) 40 ℃, (b) 60 ℃ and (c) 80 ℃ relative to the initial amount in the polymer (2.66 mg g^{-1}).

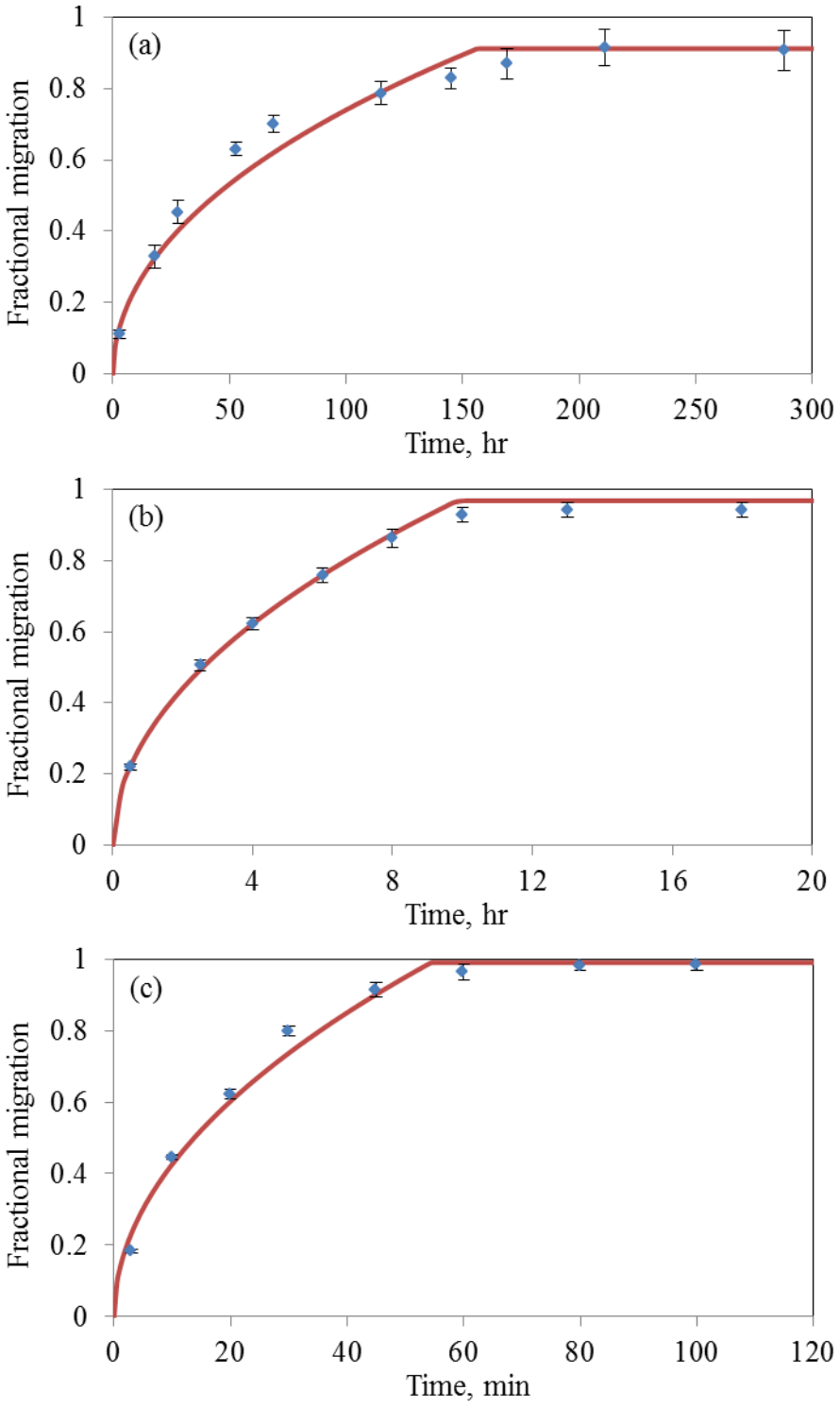

Figure B7 Amount of BPA migrated from LDPE into 3% acetic acid at (a) 40 ℃, (b) 60 ℃ and (c) 80 ℃ relative to the initial amount in the polymer (2.66 mg g^{-1}).

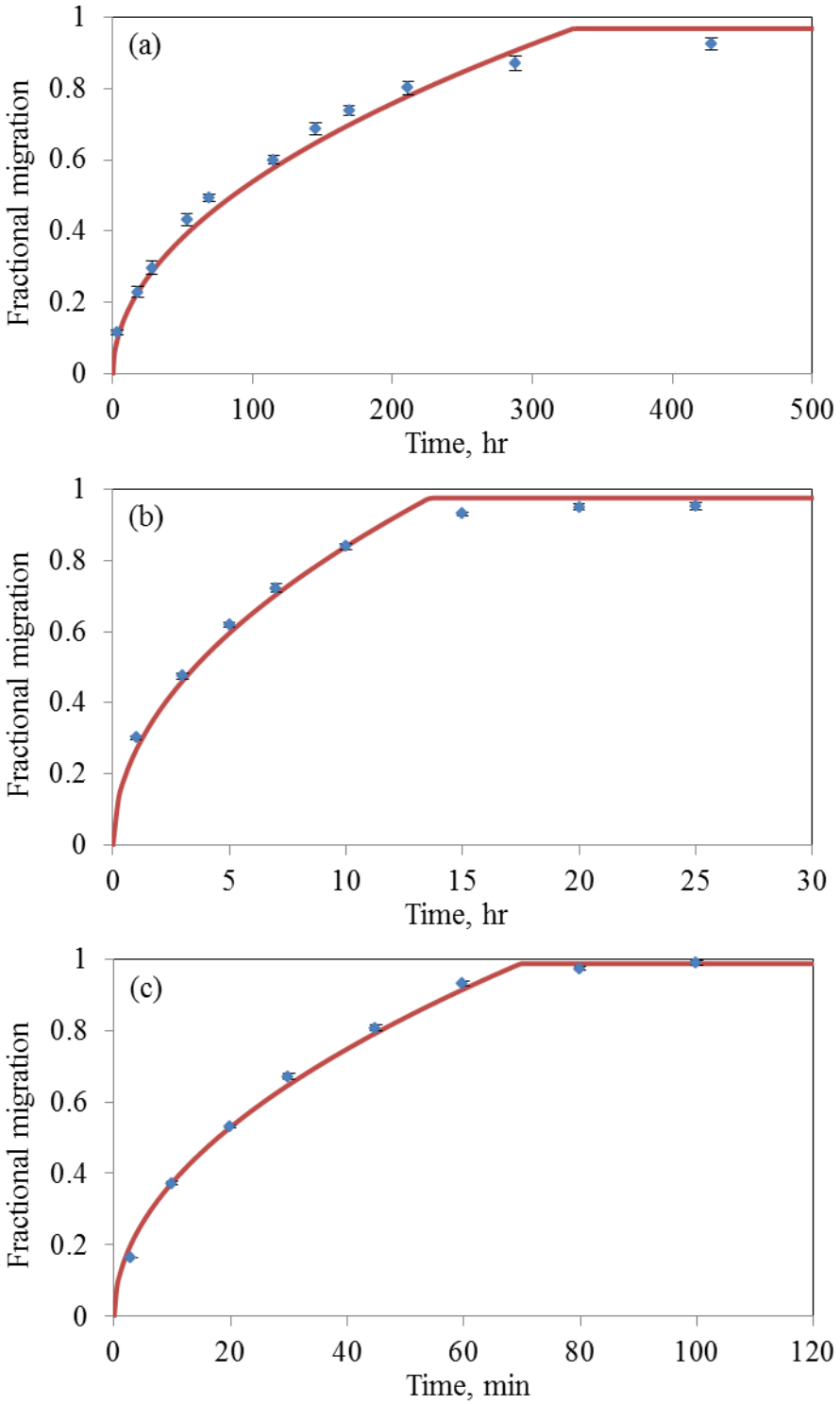

Figure B8 Amount of BPA migrated from LDPE into 95% ethanol at (a) 40 ℃, (b) 60 ℃ and (c) 80 ℃ relative to the initial amount in the polymer (2.66 mg g^{-1}).

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