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Development of a Method To Study the Migration of Six Photoinitiators into Powdered Milk

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The aim of the present work was to develop a rapid multimethod for the analysis of six photoinitiators (PIs) in powdered milk and to study the migration of these PIs from LDPE packaging into powdered milk. The optimized HPLC-DAD method showed high correlation coefficients (>0.9999) over a concentration range of 0.1–10.9 mg/L. The kinetics of migration of the photoinitiators from LDPE packaging into powdered milk were determined at different temperatures. The key parameters of migration phenomena (diffusion and partition coefficients) were determined. The diffusion coefficients at 5 °C ranged between 8.4×10^{-12} (for ITX) and 5.1×10^{-10} (for benzophenone) and those at 40 °C between 5.9×10^{-10} (for ITX) and 6.1×10^{-9} (for Irgacure 184). The diffusion coefficients of the six model migrants under study increased with temperature and showed a good Arrhenius relationship between 5 and 40 °C.

KEYWORDS: Photoinitiators; inks; food packaging; powdered milk

INTRODUCTION

In the past few years health authorities and consumers have been affected by several food safety alarms. One of the most recent alarms was related to the photoinitiator isopropyl thioxanthone (ITX), which is present in printing ink. When printed packaging is rolled in bobbins, ITX can come into contact with the inside of the packing material and, thus, with food, into which it may then migrate. It can also migrate from the outside to the inside face of the packaging if no barrier layer is applied (*1*). The European Food Safety Authority (EFSA) has found ITX in milk products, fruit juices, fruit nectars, and drinks (*2*).

Photoinitiators (PIs) are catalysts for photopolymerization. Traditionally inks were cured thermally, and their formulations included organic solvents or water, which then had to be eliminated by a drying process. Nowadays, most curing processes are based on exposure to UV radiation. This process is environmentally friendly because it does not involve use of organic solvents. The curing process is initiated by a photoinitiator (catalyst), which is highly prone to adsorbing a photon of light and creating active species (radicals, cations or anions) that initiate and complete the curing process (*3–5*).

According to framework Regulation (EC) 1935/2004 (*6*), all materials and articles intended to come in contact with foods should not transfer their constituents into food in quantities that could endanger human health or bring about unacceptable changes in composition or characteristics of foodstuffs. In addition to all specific directives related to Food Contact Materials (FCM), the European Union (EU) has also recently approved Commission Regulation 2023/2006 (*7*), which lays down the rules on good manufacturing practice (GMP) for groups of materials and articles intended to come into contact with food and details processes involving printing inks.

In line with this, the optimization and validation of methods to determine potential low-weight substances included in packaging formulations in foodstuffs are essential to ensure the safety of packaged foods. Nevertheless, this is not easy to achieve because food samples are complex matrices and there exists a vast range of possible migrant substances, packaging materials, and applications. In the specific case of PIs, there is little information available regarding the determination of these substances in food items. Most relevant studies have involved the determination of ITX (*1, 8, 9*). One group has published two studies concerning multimethods for the determination of PIs (*10, 11*).

Studies of migration of model migrants into real foodstuffs were carried out by the pioneering FOODMIGROSURE project (*12*). In recent studies key parameters of the migration (diffusion and partition coefficients) were estimated in different food matrices (chocolates, margarines, and meat products) (*13–15*).

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Table 1. Solvent Ratios Used for HPLC

time (min)	ACN (%)	water (%)
0	20	80
2	20	80
23	100	0
30	100	0

The aim of the present study was to optimize and validate an HPLC-DAD method for the determination of six PIs in powdered milk and to study the migration of these PIs from LDPE packages into powdered milk.

EXPERIMENTAL PROCEDURES

Chemicals, Standards, and Samples. Powdered milk was purchased in a local supermarket. Acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared with a Milli-Q filter system (Millipore, Bedford, MA).

Irgacure 184 [CAS Registry No. 947-19-3; methanone, (1-hydroxy-cyclohexyl)phenyl- (9Cl)], benzophenone [CAS Registry No. 119-61-9; methanone diphenyl - (9CI)], Irgacure 651 [CAS Registry No. 24650-42-8; ethanone, 2,2-dimethoxy-1,2-diphenyl- (9CI)[, Irgacure 907 [CAS Registry No. 71868-10-5; 1- propanone, 2-methyl-1-[4-(methylthio)phenyl]-2-(4-morpholinyl- (9CI)], Quantacure ITX, mixture of two isomers [CAS Registry No. 5495-84-1 and 84846-86-0; 9*H*- thioxanthen-9-one, 2-(1-methylethyl)-(9CI)], and Quantacure EHA [CAS Registry No. 21245-02-3; benzoic acid, 4-(dimethylamino)-, 2-ethylhexyl ester (9CI)] (Irgacure and Quantacure are trademarks) were supplied by Aldrich (purity = 99%).

A primary stock solution of each PI was prepared in ACN (1.0 mg/mL). Standard solutions were prepared in ACN (0.1, 0.5, 1, 2, 5, 7, and 10 μg/mL) for construction of the HPLC-UV calibration curve. Solutions were stored in a refrigerator (5 °C).

Additivation of Photoinitiators in Plastic Films. The polyolefin was a low-density polyethylene film (LDPE) film of 450 μm thickness and 0.92 g/cm^3 density.

The polyethylene wax used was Licowax PE 520 (nonpolar and low molecular polyolefin waxes; drop point = 120 °C; density = 0.93 g/cm^3). PE granules were ground with a commercial grinder, and the resulting powder was homogenized. PE waxes were supplied by Clariant Ibérica, S.A. (Barcelona, Spain).

The method used to additivate the LDPE film was optimized in a previous study and involves the preparation of two reservoirs (sources) with a polyethylene (PE) wax to incorporate model migrants in LDPE films (*13*). To prepare reservoirs of these compounds, 40 g of PE wax was weighed and carefully mixed with approximately 0.5 g of each PI. The concentrations of the different PIs achieved in the LDPE film were 1876 mg/kg for Irgacure 184, 2717 mg/kg for benzophenone, 1976 mg/kg for Irgacure 651, 1787 mg/kg for Irgacure 907, 5223 mg/kg for ITX. and 3254 mg/kg for EHA.

Migration Tests. Samples of powdered milk were accurately weighed (approximately 4.5 g) to fill a glass washer of 0.1 dm^2 diameter and 0.8 cm high and were then placed in contact with the plastic containing the PIs (one side only). Samples were then wrapped in aluminum foil and placed inside a transparent plastic bag. Samples were vacuum packed to achieve intimate contact between the powdered milk and the film additivated with the PIs and were then stored under the different conditions, arranged so that the plastic film was on the underside of the packets.

Powdered milk was stored for up to 30 days at three different temperatures: 5 °C (refrigeration temperature), 25 °C (room temperature), and 40 °C (worst case scenario for migration test of product storage at room temperature). A total of 12 samples were prepared for each kinetic curve for a given temperature. For each time point of the migration kinetics two samples were removed and analyzed as described below.

Powdered Milk Extraction. Each powdered milk sample (approximately 4.5 g) was placed in 10 mL of ACN, shaken for 5 min at room temperature, and centrifuged at 3000 rpm for 5 min. The solid residue was then discarded, and the solution was filtered prior to prior to HPLC-DAD injection.

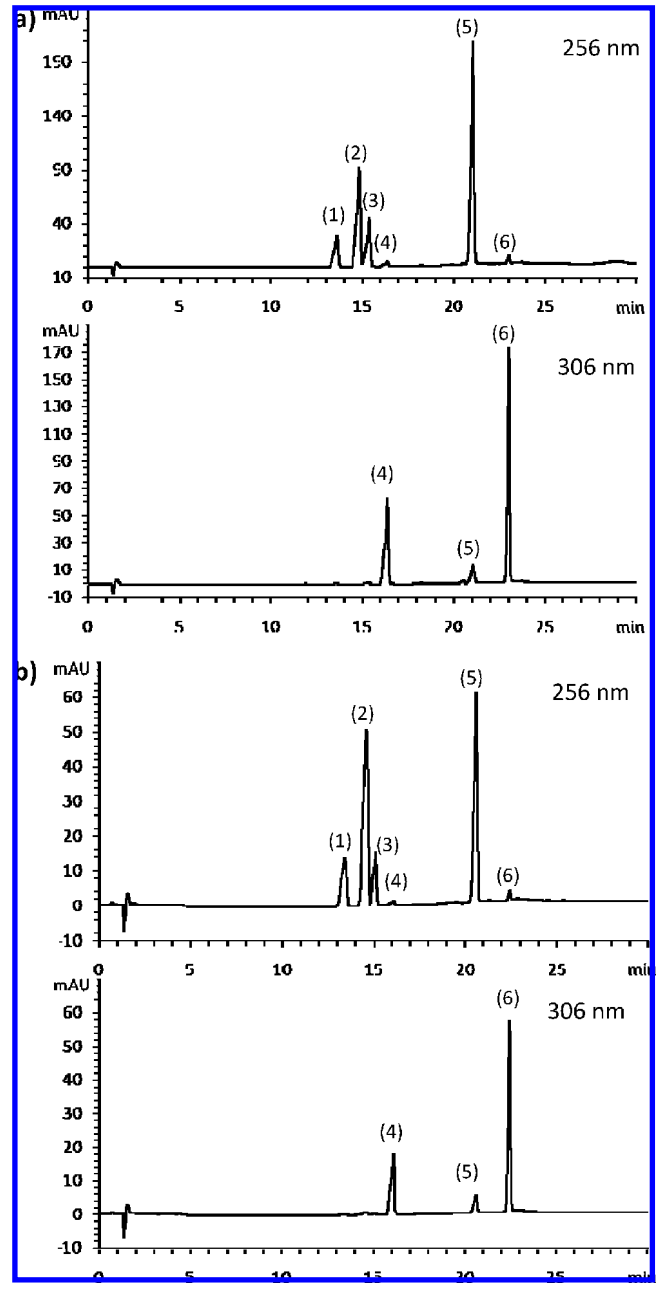

Figure 1. HPLC chromatograms of (**a**) standard solution and (**b**) powdered milk sample. Peaks: (1) Irgacure 186; (2) benzophenone; (3) Irgacure 651; (4) Irgacure 907; (5) ITX; (6) EHA.

HPLC-DAD Conditions. The HPLC system (Hewlett-Packard, Waldbronn, Germany) was fitted with a HP1100 quaternary pump, a degassing device, an autosampler, a column thermostatting system, and a diode array UV detector. The detector was continuously performing a scan in the range of 190–400 nm. The wavelengths used for each PI were selected on the basis of their highest absorbance peaks in UV scanning. Irgacure 184 was quantified at 246 nm, benzophenone and Irgacure 651 at 256 nm, Irgacure 907 at 306 nm, EHA at 310 nm, and ITX at 256 and 386 nm. The bandwidth used was 4 nm for both wavelengths.

The analysis was performed with ACN and water as mobile phase. The mobile phase gradient is shown in **Table 1**. HP ChemStation chromatographic software was used for data acquisition. Chromatographic separation was performed with a Kromasil 100 C18 column (15 cm × 0.4 cm i.d., 5 μm particle size) (Teknokroma, Barcelona, Spain) at 30 °C. The flow rate was 1.0 mL/min, and the injection volume was 50 μL. Photoinitiators were identified by comparison of their retention time and UV spectra with those of a pure standard injected under the same HPLC conditions.

Table 2. Linearity and Range of the HPLC Method for the Six Photoinitiators in Standard Solutions

	$y = ax + b$			
PI	*a*	*b*	r^2	range (mg/mL)
Irgacure 184	56.87	1.18	1.000	0.1–10.4
benzophenone	112.85	3.50	1.000	0.1–10.7
Irgacure 651	52.76	2.00	1.000	0.1–10.5
Irgacure 907	72.56	1.92	1.000	0.1–10.4
ITX, 256 nm	198.29	9.86	1.000	0.1–10.9
ITX, 386 nm	28.02	0.27	1.000	0.1–10.9
EHA	11.75	4.60	0.9999	0.1–10.3

RESULTS AND DISCUSSION

Method Validation. An HPLC multimethod was optimized for the identification and quantification of six photoinitiators. Several conditions were tested to obtain good chromatographic separation. The solvent gradient that gave best results started with 20% ACN and 80% of water (**Table 1**). HPLC chromatograms of a standard solution with 10 μg/mL of the six PIs and a powdered milk sample are shown in **Figure 1a**, and in **Figure 1b** are shown chromatograms of a powdered milk sample after 10 days of contact at 25 °C with the plastic film.

Calibration curves were linear over the concentration range of 0.1–11 μg/mL (**Table 2**) and presented excellent correlation coefficients ($\geq$0.9999). This indicates suitability for quantification of the PIs.

Detection limits were determined as the signal at 3 times the height of the noise level according to the American Chemical Society (ACS) (*16*). Detection limits were 33 ng/mL for Irgacure 184, Irgacure 651, and ITX (at 386 nm) and 17 ng/mL for benzophenone, Irgacure 907, ITX (at 256 nm), and EHA.

Recovery was tested by standard addition procedure. Powdered milk ($n = 6$) was spiked before extraction at four different concentration levels (2, 1, 0.4, and 0.2 mg/kg). Results (see **Table 3**) were satisfactory for all spiking levels and ranged from 91.5 to 97.8% at 2 mg/kg, from 89.2 to 95.7 at 1 mg/kg, from 81.7 to 86.6% at 0.4 mg/kg, and from 63.52 to 84.6% at 0.2 mg/kg.

Mathematical Models and Determination of Key Parameters. Fick's second law (eq 1) is a differential equation that can be used to describe migration of an additive or contaminant from an amorphous polymeric packaging film (*17*)

$$\frac{\partial C_p}{\partial t} = D\frac{\partial^2 C_p}{\partial x^2} \quad (1)$$

where C_p (mg/kg) is the concentration of the migrant in the packaging film at time t (s) and position x (cm) and D is the diffusion coefficient (cm^2/s). This equation was used to carry out mathematical modeling of the migration of PIs from packaging (additivated LDPE films) to powdered milk.

Equation 1 can be resolved to produce eq 2, which expresses the amount of migrant released from the polymer (P) into food (F) at time t (*18–20*):

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

with

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

where $m_{F,t}$ is the mass of migrant from P into F after time t (μg), A is the area of P in contact with F (cm^2), $C_{P,0}$ is the initial concentration of migrant in P (mg/kg), ρ_P is the density of P (g/cm^3), t is the migration time (s), d_p is the thickness of P (cm), V_P is the volume of P (cm^3), V_F is the volume of F (cm^3), q_n are the positive roots of the equation tan $q_n = -\alpha q_n$, D_P is the diffusion coefficient of migrant in P (cm^2/s), and $K_{P/F}$ is the partition coefficient of the migrant between P and F.

In the present study an effective D will be calculated rather than D_P. This corresponds to the diffusion coefficient of the whole polymer-powdered milk system.

The first step to predict migration, on the basis of this mathematical model, is to calculate the positive roots of the equation tan $q_n = -\alpha q_n$. Twelve roots ($1 \leq n \leq 12$) were calculated for $0.01 \leq \alpha \leq 1000$. The greater the number of roots, the more reliable the results are.

Experimental data were fitted to the proposed model with Solver of Microsoft Excel 2003 software, by nonlinear regression. From the series of experimental data on migration level (μg/dm^2) versus time, the model parameters α and D were calculated for each sample and storage temperature. The α and D values for powdered milk at different temperatures are shown in **Table 4**. Irgacure 184 and benzophenone presented the highest diffusion coefficients. This is probably because of the lower molecular weight of the latter two substances in comparison with the other PIs studied. The MW had the same effect on the migration process as found in another study carried out with diphenylbutadiene (DPBD) and triclosan (*13*).

Table 3. Recoveries of Photoinitiators at Four Different Levels of Spiking

	spike							
	2 mg/kg		1 mg/kg		0.4 mg/kg		0.2 mg/kg	
PI	mean recovery (%) $n = 6$	RSD (%)	mean recovery (%) $n = 6$	RSD (%)	mean recovery (%) $n = 6$	RSD (%)	mean recovery (%) $n = 6$	RSD (%)
Irgacure 184	94.23	1.36	91.66	0.83	84.81	2.01	71.72	1.74
benzophenone	97.81	1.01	95.74	1.01	83.87	2.01	68.62	3.48
Irgacure 651	95.24	2.12	94.79	2.94	86.60	2.21	66.88	5.42
Irgacure 907	96.71	1.08	93.70	0.87	86.08	1.30	70.93	1.68
ITX, 386 nm	91.49	1.52	89.22	1.22	84.75	4.19	84.59	10.45
EHA	93.84	1.39	91.44	1.90	81.69	3.18	63.52	5.10

Table 4. Diffusion Coefficients and α Values for All Photoinitiators Studied

PI	temp (°C)	MW	log K_{ow}[a]	D (cm^2/s)	α	RMSE (%)	$K_{P/F}$
Irgacure 184	5	204.3	2.34[b]	1.9×10^{-10}	1.50	8.1	11.8
	25			2.5×10^{-9}	2.33	4.6	7.6
	40			6.1×10^{-9}	1.90	7.0	9.4
benzophenone	5	182.2	3.18[c]	5.1×10^{-10}	3.00	12.5	5.9
	25			3.0×10^{-9}	2.13	4.5	8.4
	40			5.8×10^{-9}	2.64	10.2	6.7
Irgacure 651	5	256.3	4.75[b]	1.8×10^{-10}	1.10	1.5	16.2
	25			2.0×10^{-9}	1.60	3.7	11.1
	40			5.0×10^{-9}	1.67	5.0	10.7
Irgacure 907	5	279.4	2.99[b]	1.3×10^{-10}	1.48	3.2	12.0
	25			5.3×10^{-10}	0.67	2.8	26.5
	40			1.1×10^{-9}	1.20	3.5	14.8
ITX	5	254.3	5.33[b]	8.4×10^{-12}	0.38	0.3	46.8
	25			1.7×10^{-10}	0.35	0.9	50.8
	40			5.9×10^{-10}	0.40	0.4	44.4
EHA	5	277.4	6.15[b]	1.8×10^{-11}	1.20	0.5	14.8
	25			3.9×10^{-10}	0.48	2.1	37.0
	40			1.1×10^{-9}	0.51	2.9	34.9

[a] Values obtained from the Scifinder 2006 database. [b] Estimated. [c] experimental.

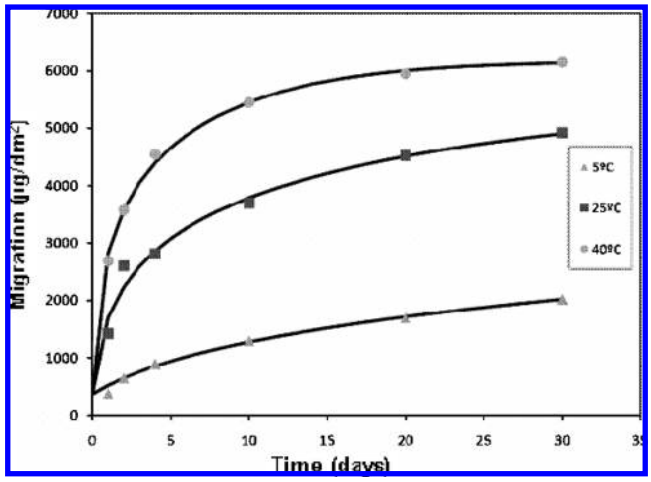

Figure 2. Experimental and predicted migration values of ITX into powdered milk at 5, 25, and 40 °C (lines represent the predicted values, and dots represent the experimental values).

The fit between experimental and estimated values was calculated by means of the mean-square error (% RMSE), with eq 3 (*21*)

$$\text{RMSE}\,(\%) = \frac{1}{C_{P,0}}\sqrt{\frac{1}{N}\sum_{i=1}^{N}((M_{F,t})_{\text{exptl},i} - (M_{F,t})_{\text{pred},i})^2} \times 100 \quad (3)$$

where N is the number of experimental points per migration curve and i is the number of observations.

There was a good correlation between experimental and estimated migration values as observed by the low RMSE values (see **Table 4**). This indicates that the proposed model can be used to predict migration of PIs into powdered milk. The migration kinetics with the experimental and predicted migration values of ITX into powdered milk at 5, 25, and 40 °C are shown in **Figure 2**. In **Figure 2** lines represent the predicted values and dots the experimental values.

The partition coefficient ($K_{P/F}$), that is, the relative solubility of the migrant at equilibrium between the plastic and the foodstuff, was calculated from the α value and polymer and food volumes (see eq 2). The V_P for all assays was 0.44 cm^3, and the V_F was 7.9 cm^3. The highest $K_{P/F}$ values (**Table 4**) corresponded to ITX and EHA, which are the PIs with the highest log K_{ow}.

Table 5. Arrehenius Equation Parameters for All Photoinitiators Studied

	D_0 (cm^2/s)	E_A (kJ/mol)	r^2
Irgacure184	11.4×10^3	73.1×10^3	0.973
benzophenone	2.1	51.0×10^3	0.982
Irgacure 651	3.1×10^3	70.2×10^3	0.978
Irgacure 907	1.8×10^{-2}	43.2×10^3	0.995
ITX	533×10^3	89.1×10^3	0.985
EHA	379×10^3	86.4×10^3	0.974

Diffusion coefficients increased with temperature (**Table 4**). The Arrhenius relationship (eq 4) was used to check for linearity in the range between 5 and 40 °C

$$D = D_0 e^{\frac{-E_A}{RT}} \quad (4)$$

where D is the diffusion coefficient (cm^2/s), D_0 is the pre-exponential factor (cm^2/s), E_A is the activation energy (kJ/mol), R is the gas constant (kJ/mol·K), and T is the temperature (K).

The correlation coefficients of Arrhenius' equation (**Table 5**) show that this equation can be used to estimate D values at any temperature in the range between 5 and 40 °C. These values were always >0.973. The values of D_0 and E_A are also shown in **Table 5**.

Concluding Remarks. An effective multimethod was developed to determine PIs by HPLC-DAD. The results will contribute to validate a model for migration estimation because there are few data regarding the migration behavior of photoinitiators in food matrices.

The good correlation between experimental and predicted results indicates that this model can be used to predict migration of PIs into powdered milk. The good correlation coefficients of the Arrhenius relationship allow prediction of diffusion coefficients at any temperature in the range from 5 to 40 °C. This is important because mathematical prediction of migration can minimize the number of time-consuming and expensive experiments required.

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