Migration study of optical brighteners from polymer packing materials to jam squeeze and fruit drink by spectrofluorimetry and RP-HPLC methods

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Abstract Optical brighteners are commonly used to modify the appearance and to improve polymer properties of packaging. They are not chemically bound to polymers and able to migrate from packaging into the foods. These migrants are potentially harmful to human health. In concern with human safety an approach was made to analyze three optical brighteners such as diphenylbutadiene, Uvitex-OB, benzophenone in commercial fruit juice and jam. The migration level of these optical brighteners from low density poly ethylene packaging into fruit juice and jam was studied. Two optimized and validated analytical techniques such as spectrofluorimetry and high performance liquid chromatography with photo diode array detector used for migration study. Both methods have shown high correlation coefficients (>0.999), over a concentration range of 0.1–3.2 $\mu\text{g/mL}$, 0.1–1 $\mu\text{g/mL}$, 0.05–3.2 $\mu\text{g/mL}$ for diphenylbutadiene, Uvitex-OB and benzophenone respectively. The preliminary studies confirm that the low density poly ethylene layer taken for study contained of diphenylbutadiene and the other two were absent. The migration level of diphenylbutadiene was studied at room temperature and different elevated temperature from 30 °C to 60 °C for up to 3 weeks. At room temperature no migration of diphenylbutadiene was observed where as at higher temperature migration could be observed. The maximum quantity of diphenylbutadiene

migrated was found to be 0.0462 mg/kg from tetrapak, and 0.0382 mg/kg from jam squeeze after 3 weeks treatment at 60 °C. The migration of diphenylbutadiene was found to be less than allowable concentration during the study period.

Keywords LDPE · Diphenylbutadiene · Uvitex-OB · Benzophenone · Spectrofluorimetric · HPLC-PDA

Introduction

Packaging is an important procedure in food manufacturing. This practice has the important task of protecting foodstuff from spoilage and degradation through storage and distribution, increasing its shelf life. In this area, plastic is one of the packaging materials most widely used by the food industry. This is not an inert material and is able to interact with the surrounding environment, allowing food-packaging interactions, such as sorption, permeation and migration (Crompton 2007).

Plastic additives, commonly used to modify their appearance and to improve polymer properties, and residual monomers and oligomers are not chemically bound to the polymer molecules and can, therefore, move freely within the polymer matrix. Plastic generally ages rapidly under the effects of light, oxygen and heat, leading to loss of strength, discoloration, scratching. Typical polymer additives include anti-oxidants, blowing agents, catalysts, colorants, filters and re-enforcements, flame retardants, impact modifiers, lubricants and slip agents, plasticizers and stabilizers, fluorescent white agents and a large variety of chemicals with different technical functions. Substances from food packaging are able to migrate into the foodstuff. It claims special attention in the aspect of food safety because the chemicals that migrate into food may be potentially harmful to human

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Table 1 Fixed spectrofluorimetric parameters

Parameters	Optical Brighteners		
	DPBD	UVX	BPN
Emission wavelength	375 nm	425 nm	418 nm
Excitation wave length	330 nm	375 nm	252 nm
Emission bandwidth	10 nm	10 nm	10 nm
Excitation bandwidth	5 nm	5 nm	5 nm
Response time	0.05 sec	0.05 sec	0.05 sec
Sensitivity	Medium	Medium	Medium

health. Safety thresholds for chemical impurities and leachables in consumer products such as foods and drugs have helped to ensure public health while establishing scientifically sound limits for identification and risk assessment of these compounds (Douglas et al. 2007).

The optical brighteners namely 1,4-diphenylbutadiene (DPBD), Uvitex-OB (UVX) and benzophenone (BPN) are the potential migrants reported frequently. Fluorescence spectra of DPBD was obtained by Takao Itoh et al. (2007). Determination of DPBD from food stuff using HPLC was reported by Raquel et al. (2004). Diffusion coefficient of DPBD was studied by Sanches et al. (2010) and migration study of the same into powder milk was carried out by Sanches et al. (2008). Al Malaika et al. (1991) studied migration of benzophenone into low density polyethylene. Sanches et al. (2006) reviewed a compilation of analytical methods and guidelines for the determination of selected model migrants such as Irganox 1076, DPBD, Chimassorb-81, Uvitex-OB, Caprolactam, Benzophenone, Diphenylphthalate and DEPA in ethanol, olive oil, sunflower oil.

This paper describes migration study of three optical brighteners namely 1,4-diphenylbutadiene, Uvitex-OB, and benzophenone from low density polyethylene film to food products. The migration level of optical brighteners was determined in two selected foods such as fruit juice and jams using two newly developed, optimized and validated

analytical techniques such as spectrofluorimetry and high performance liquid chromatography with photo diode array detector (HPLC-PDA).

Materials and methods

Chemicals and reagents Acetonitrile, ethanol, acetone, carbon tetrachloride, and n-Hexane were HPLC grade; supplied by Merck India Ltd., and ultrapure water was prepared using a milli-Q filter system (Millipore). The stock standard solutions were prepared by dissolving 100 mg of DPBD, UVX, BPN in 100 mL of acetonitrile, acetone, carbon tetrachloride respectively.

Spectrofluorimetric conditions A Jasco Spectrofluorometer model FP-750 with pair of quartz cuvette was used for the estimation of optical brighteners in foods and LDPE layer. The spectrofluorimetric conditions were optimized and applied for the study (Table 1).

Chromatographic conditions A Shimadzu HPLC system of VP series equipped with LC10AT-*vp* gradient solvent delivery modules, Rheodyne injector (7725i) of 20 µl capacity fixed volume loop and SPD M10A-*vp* photo diode array detector with Class 10A software was employed for migration study. The optimized and validated chromatographic conditions used for the estimation of optical brighteners in foods and LDPE layer is as given in Table 2.

Migration study A fluorescent whitening agent is a substance that is added to a material to absorb ultraviolet rays in sunlight and release them as blue rays will interact with the yellowish color and give the plastic the appearance of being whiter. The migration study was conducted for optical brighteners (DPBD, UVX, BPN) on two food products such as fruit juice and jam squeeze.

Table 2 Fixed chromatographic conditions of optical brighteners

Parameters	Optical Brighteners		
	DPBD	UVX –OB	BPN
Stationary phase (column)	Thermoquest, C18column (150 mm×4 mm, 5 µm)		
wavelength	330 nm	375 nm	252 nm
Mobile phase	ACN:Water	ACN: 0.5% triethylamine Water	ACN:Water
Solvent ratio	70:30%v/v	50:50%v/v	70:30%v/v
pH	3	6.8	3.2
Retention time (min)	5.6	3.45	8.4
Flow rate	1 mL/minute		
Temperature	Room temperature		

Sampling for migration study Among two food items chosen mango fruit juice is a liquid, acidic medium, contain carbohydrate packed in tetra pak. The strawberry jam is a semi-solid and acidic in nature and packed in squeeze tube. Both containers have inner LDPE polyethylene lining and they were purchased from local market.

Fig. 1 Spectrofluorimetric emission spectrum of (a) diphenyl butadiene (b) uvitex-OB (c) benzophenone standard solutions

Three different batches (B318, B292, B362) of tetra pak and jam squeeze (U100504c06:59, U100504c09:80, U100504c13:24) were collected between 6 month time intervals. Each lot containing 36 samples among which 12 samples were used to detect the initial concentration of the optical brighteners in LDPE layer and 24 samples were subjected to 7 weeks of migration study.

Extraction of optical brightening agents from LDPE layers Twelve samples of tetra pak and jam squeeze were emptied and opened carefully and washed well in the warm water, they can ensure to be free from sticky materials. The LDPE layers of peeled out from the containers and thoroughly wash with water and allowed to dry for 30 minutes. The layers were weighed individually and cut into small pieces. A quantity of 1.5 g of LDPE layers were taken separately. The migrants DPBD, UVX were extracted by using 10 ml of hexane and BPN was extracted by using 10 ml of chloroform from LDPE layers. From each extract

Fig. 2 HPLC chromatogram of (a) diphenyl butadiene, (b) uvitex - OB, (c) benzophenone standard solutions

Table 3 Method validation parameters of spectrofluorimetry for optical brighteners (OB), diphenyl butadiene (DPBD), benzophenone (BNP), uvitex-OB (UVX)

OB	Linearity (µg/mL)	Precision%RSD	Correlation co-efficient, Slope, Intercept	LOQ (µg/mL)	Stability hrs-RT
DPBD	0.1–3.2	<2	0.999, 966.1, 72.1	0.05	7
UVX-OB	0.1–1	<2	0.998, 565.4, 7.78	0.05	5
BPN	0.05–3.2	<2	0.999, 775.6, 64.47	0.02	5

5 ml of solvent was dried under nitrogen gas stream. The solid residue was dissolved in 5 mL of acetonitrile, acetone and acetonitrile respectively.

The preliminary studies were conducted on the extracts of LDPE layer and analyzed by spectrofluorimetry, for the presence selected optical brighteners. It has proved that only DPBD is present in the selected LDPE packing of tetrapak and jam squeeze containers and other two were absent. Hence further the migration study of DPBD from LDPE layer to food was conducted at room temperature and elevated temperature.

Determination of DPBD present in LDPE Layer at Room & Elevated Temperature LDPE layers were peeled out from the containers and an accurately weighed 1.5 g was thoroughly washed with water and allowed to dry for 30 min. The layers were weighed individually and cut into small pieces. 1.5 g of LDPE layers were taken separately. 10 ml of n-hexane was added into the beaker & they were subjected to room temperature and (30 °C to 60 °C) for 15 min and 5 ml of the supernatant liquid was collected and dried with N₂ gas and reconstituted with 5 ml of acetonitrile, analysed simultaneously by spectrofluorimetric and HPLC methods.

Migration study of DPBD from LDPE packing materials to fruit juice and jam at room & elevated temperature Twelve containers of tetrapak and jam squeeze from each batch were used for the study of migration of DPBD to food, were subjected to temperature from 30 °C–60 °C for 30 min in a hot air oven up to 3 weeks. The samples were withdrawn at the end and extracted by the procedure mentioned earlier and analysed by spectrofluorimetric and HPLC for the leaching of DPBD into jam & juice the study was carried out for 12 months, where samples collected

every 2 months once from different batches and performing the study mentioned earlier.

Double extraction procedure of DPBD from fruit juice/ jam The tetra pak contain mango juice and squeeze pack contain strawberry jam. Extraction of DPBD from these food materials was carried out by single extraction initially but resulted in poor recovery (<50%), where as double extraction procedure produced very good recovery (>85%) with a%RSD less than 0.95. About 5 g of each food sample were accurately weighed in a 10 ml centrifuge tube. Then, 5 ml of n-Hexane were added and the tubes were shaken manually for 10 min. To separate the organic phase the tubes were centrifuged at 3000 rpm for 15 min and organic phase was separated. The extraction was repeated with 5 mL of hexane twice. All hexane phases were collected in test tubes and dried with nitrogen gas. The solid residue obtained was dissolved in 5 mL of acetonitrile and analyzed.

Results and discussion

- Preliminary studies** The important properties of DPBD, UVX, BPN such as ultraviolet and fluorescence were evaluated using the solutions of optical brighteners to help the development of the spectrofluorimetric and HPLC-PDA methods in order to detect them in selected food products. Thus the maximum UV absorbance obtained at 330 nm, 375 nm, 252 nm respectively, for DPBD, UVX, BPN and used for detecting them in HPLC-PDA method. In HPLC method optical brighteners were separated using water and acetonitrile as mobile phase except UVX. For UVX, a 0.5% v/v solution of triethylamine in water was used in mobile phase as peak modifier, to achieve symmetrical peak.

Table 4 Method validation parameters of HPLC method for optical brightener (OB), diphenyl butadiene (DPBD), benzophenone (BNP), uvitex-OB (UVX)

OB	Linearity (µg/mL)	Precision% RSD	Correlation co-efficient, Slope, Intercept	LOQ (µg/mL)	Stability hrs-RT	Resolution	Efficiency	Tailing factor
DPBD	0.1–3.2	<2	0.999, 125872, 7072.1	0.05	7	8.2	4298	1.3
UVX-OB	0.1–1	<2	0.998, 2013.4, 70.78	0.05	5	6.4	6754	1.6
BPN	0.05–3.2	<2	0.999, 3375.6, 85.47	0.02	5	8.2	5643	1.1

LOQ is limit of quantification, RT is room temperature

Fig. 3 Overlay chromatogram of diphenyl butadiene (DPBD), benzophenone (BNP), uvitex-OB (UVX) and low density poly ethylene layer (LDPE) after extraction (aqueous solution)

In the spectrofluorimetric method the pair of wavelengths of excitation at 330 nm, 375 nm, 252 nm and emission at 375 nm, 425 nm, and 418 nm was used for DPBD, UVX, BPN respectively. The optimized conditions

of spectrofluorimetry and HPLC-PDA were used to carry out the migration study of optical brighteners. The emission spectrum and chromatogram of three optical brighteners are shown in Figs. 1 and 2.

Fig. 4 (i). Overlay chromatogram of diphenyl butadiene (DPBD), benzophenone (BNP), uvitex-OB (UVX) in low density poly ethylene (LDPE) layer of (a). Tetrapak, (b). Jam squeeze. (ii). Overlay emission spectrum of diphenyl butadiene (DPBD), benzophenone (BNP), uvitex-OB (UVX) in low density poly ethylene (LDPE) layer of (a). Tetrapak, (b). Jam squeeze

- ii. *Validation of analytical procedures* The two new analytical procedures used in the study were validated using series of standards in respective solvents and the linearity obtained over the concentration range of 0.1–3.2 µg/mL, 0.1–1 µg/mL, 0.05–3.2 µg/mL for DPBD, UVX, BPN respectively. Limit of quantification (LOQ), precision and stability were calculated for both methods, in accordance with the ICH guidelines and are shown in Tables 3 and 4; indicate that the methods are suitable for quantification of selected optical brighteners.
- iii. *Extraction of optical brighteners from LDPE layer* The aqueous solution containing optical brighteners were extracted and analysed by HPLC. The overlay of standard chromatogram of DPBD, Uvitex-OB, BPN and low density polyethylene from aqueous extract is shown in Fig. 3. LDPE doesn't interfere with retention time of any of optical brighteners showing the selectivity of this method to detect them without interference.

The overlay chromatogram and overlay emission spectrum of LDPE layer extracts of tetra pak and jam squeeze for various optical brighteners are shown in Fig. 4. From the chromatogram and emission spectrum it was observed that among three optical brighteners, only diphenylbutadiene (DPBD) was present in the LDPE layer of selected packaging. Further the peak purity of DPBD peak was found 0.9999, which further confirms the presence of DPBD only. Hence further migration study was carried out only for DPBD at room temperature and elevated temperature. The initial concentration of DPBD in layer was found to be 0.19–0.24 mg/kg. Keeping this as initial concentration in LDPE layer the amount of DPBD migrated was calculated in food products.

- iv. *Migration study* The Migration of DPBD from time to time was calculated by using given formula as below:

$$M = \frac{m \cdot a_1}{a \cdot q} \cdot 1000$$

Where,

M = the migration of DPBD in mg/kg, m = the mass in mg of DPBD released by the tetrapak and jam squeeze as determined by the migration test, a_1 = the surface area in dm^2 of the tetra pak and jam squeeze in contact with the fruit and jam respectively, during the migration test, a_2 = the surface area in dm^2 of the tetrapak or jam squeeze in real conditions of use, q = the quantity in grams of fruit juice or jam in contact with the tetra pak or jam squeeze in real conditions of use.

Migration of DPBD to food at room temperature and elevated temperature A mixture of known additives commonly used in fruit juice and jams squeeze such as preservatives, antioxidants, colouring agents and sweetening agents was prepared and spiked with DPBD. After extraction it was found that the additives were not interfering the recovery of DPBD. At room temperature DPBD was not identified from the samples of jam or juice, which shown that there is no leaching of DPBD from LDPE layer to foods occurred at room temperature.

Migration levels (mg/kg) of DPBD at the end of seventh week migration study to food for three different batches of tetrapak and jamsqueeze at elevated temperatures is shown in Table 5. A blank containing known additives of jam/fruit drink was prepared and they were spiked with DPBD and extracted to find interference. It was observed that no additional peak was found and no interference at the retention time of DPBD.

Table 5 Migration level of DPBD to food at elevated temperature for selected Batches

7thweek	Migration level (mg/kg)							%RSD
	Method	I	II	III	IV	V	VI	
Batch I								
TETRA PAK	SF	0.045	0.045	0.045	0.045	0.049	0.046	<0.76
	HPLC	0.046	0.046	0.048	0.046	0.046	0.048	
Jam SQUEEZE	SF	0.037	0.036	0.039	0.037	0.038	0.039	<1.14
	HPLC	0.038	0.036	0.036	0.036	0.036	0.035	
Batch-II								
TETRA PAK	SF	0.045	0.046	0.046	0.045	0.046	0.045	<0.53
	HPLC	0.046	0.045	0.046	0.046	0.046	0.046	
Jam SQUEEZE	SF	0.034	0.035	0.035	0.036	0.036	0.035	<1.41
	HPLC	0.037	0.037	0.038	0.038	0.038	0.035	
Batch-III								
TETRA PAK	SF	0.045	0.045	0.046	0.043	0.043	0.049	<1.22
	HPLC	0.045	0.045	0.045	0.046	0.046	0.045	
Jam SQUEEZE	SF	0.033	0.032	0.034	0.033	0.032	0.034	<0.81
	HPLC	0.034	0.034	0.034	0.031	0.034	0.034	

Conclusion

The migration study results confirm that among three optical brighteners taken for study, diphenylbutadiene was found to be present in packaging layer and it leached into food via low density polyethylene layer into fruit juice. In accordance with study results the spectrofluorimetric and HPLC methods allow the identification and quantification of diphenylbutadiene leaching to food from low density polyethylene layers. The migration level of DPBD into tetrapak and jam squeeze was successfully studied and it was found to be *negligible* under study period time. If these products are exposed to high temperature leaching occur more progressively. Hence, maintaining of storage conditions important to avoid any unpleasant reaction due to leachable from packaging.

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