Optimization of the extraction of azo colorants used in toy products

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Abstract

Azo dyes are widely used in formulations intended for children use. But their potential toxicity raised the need of an efficient and fast method of analysis. A study for the optimization of the extraction of some azo colorants used in toys was conducted. Several extraction methods for the selected analytes were evaluated and compared, i.e., supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and Soxhlet extraction. Poly(vinyl chloride) samples spiked with known quantities of the studied dyes were prepared. The influence of critical variables on analyte recoveries in SFE and MAE was investigated by using a full-level factorial design, where most significant parameters as well as order interactions were studied in each case. The analytes were subsequently detected by high-performance liquid chromatography with UV detection. The three extraction techniques were compared in terms of reproducibility, selectivity and analyte recoveries. MAE showed higher recoveries (above 98%), except for the diazo dye (nearly 60%). Reproducibilities were generally good for the three methods (relative standard deviation lower than 2.0%).   © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Azo dyestuffs are widely used as colorants in consumer and industrial products, such as food, cosmetics, medicines, toys, plastics, printing links, paints, textiles, etc. [1]. They are commonly prepared by coupling a diazotized aromatic amine with a phenol or another aromatic amine. But, these compounds are of human health concern due to the possibility of reduction and formation of potentially carcinogenic aromatic amines, such as naphthylamines, substituted phenylamines or benzidine analogues [2]. But, even considering their extended use, an efficient and reproducible method for the determination of azo dyes in toy products is lacking at the present time.

Several methods were proposed for the analysis of dyes in specific matrices, such as gasoline or water [3–7]. The general process consists of concentrating samples onto solid-phase extraction (SPE) cartridges in order to extract the azo dyes from the solutions, and their subsequent analysis by HPLC with mass spectrometric (MS) or ultraviolet spectrophotometric (UV) detection. However, none of these methods can be directly applied to solid plastic materials.

Several new approaches for the extraction of organic analytes from different matrices have been proposed, including supercritical fluid extraction (SFE) [8–16] and microwave-assisted extraction (MAE) [17–19]. The use of these techniques has improved recoveries in the determination of most organic additives, as well as permitted considerable reductions in solvent volume and extraction time [20,21]. However, the comparison of extraction methods was usually reduced to relative recoveries

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of target analytes, ignoring important analytical parameters of the extraction method. Selectivity is one of them, as the co-extraction of other organics from the matrix usually requires a post-extraction clean-up step before chromatographic analysis [22].

Factorial design techniques were widely used for the simultaneous study of the influence of several analytical SFE parameters in different samples [23–29]. It is possible to obtain information in a few runs by varying several factors at once, as well as estimating of interactions, which is not possible with classical experiments [30]. In this way, factorial designs are very useful for SFE studies where interactions between several SFE variables usually make the interpretation of results difficult.

This work presents the optimization of the extraction of some azo colorants normally used in toys. From those used most commonly (the majority from the solvent type and a few from the disperse type), three were selected for this study: Solvent Yellow 14 (monoazo), Solvent Red 24 (diazo) and Disperse Red 1 (monoazo) [31,32]. Extraction methods, including SFE and MAE, were optimized by means of two-level factorial designs. Pressure, temperature, static and dynamic time and volume of modifier were studied for SFE; and extraction temperature and heating time for MAE. Soxhlet extraction was also evaluated and compared to SFE and MAE.

2. Experimental

2.1. Chemicals and samples

Analytical-grade standards of dyes, Solvent Yellow 14, Solvent Red 24 and Disperse Red 1, were obtained from Sigma (St. Louis, MO, USA). The main characteristics of each colorant are shown in Table 1. Stock solutions of each compound (20

$\mu g/g$) were prepared in methanol (LC grade) obtained from Merck (Darmstadt, Germany).

Vestolit B 7021 (Hüls, Germany) was the poly-(vinyl chloride) (PVC) resin selected for this study. The plasticizer, tributylacetyl citrate (reagent grade), as well as calcium carbonate (analytical grade), were obtained from Merck (Darmstadt, Germany). PVC samples spiked with known quantities of each dye were prepared. The procedure included the mixture of powdered PVC resin with $CaCO_3$ (2 parts per 100 parts of resin, phr) to avoid the compaction of dye particles, and an adequate amount of a single solid dye. After an adequate homogenization, the plasticizer was added (48 phr). The mixture was then cured in an oven at 140 °C for 10 min. Dye content in all samples was approximately 0.01% (w/w). Samples were cut into sheets (4.0×1.2 cm large and 0.3 cm width) for extraction.

2.2. Extractions

SFE was performed using an ISCO Model SFX-220 extraction system (Lincoln, NE, USA) consisting of a SFX-220 extractor, a SFX-200 controller and a 100DX syringe pump. Supercritical grade CO_2 was supplied by Abelló Linde (Valencia, Spain). Samples (0.4 g) were introduced into stainless steel cartridges (internal volume, 2.5 ml), and then modifier (methanol) was directly added to the cartridge before extraction. Collection was carried out by inserting the restrictor into a graduate dark vial containing 8 ml of methanol. The capillary restrictor was coaxially heated and the temperature was 90 °C for the experiments at 13.8 MPa, and 105 °C for those at 55.2 MPa. A full 2^5 factorial design for each dye-spiked sample was carried out in order to optimise the extraction parameters.

MAE was performed using an MSP-1000, 950-W, laboratory microwave extraction system (CEM, NC,

Table 1
General properties of the dyes

Dye	CAS number	CI number	Molecular mass (g/mol)	Melting point (°C)
Solvent Yellow 14	842-07-9	12055	248.3	132
Solvent Red 24	85-83-6	26105	380.4	199
Disperse Red 1	2872-52-8	11110	314.3	161

USA) equipped with a 12-vessel position carousel. Duplicate 0.5-g samples were put into the PTFE microwave extraction vessels and mixed with 15 ml of methanol. Extraction was performed with the 80% maximum microwave power, with 3 min needed to reach the selected extraction temperature. After extraction, vessels were allowed to cool to room temperature before opening, and the final extracts were filled with methanol to the mark of a 20-ml graduated flask. A full 2^2 factorial design for each sample was carried out in order to study the influence of MAE parameters on recovery.

Soxhlet extraction was performed by extracting 1-g samples with 70 ml of methanol in a reflux for 7 h. The final extract was filled with methanol to the mark of a 100-ml graduated flask.

2.3. Analysis

Analysis of extracts was carried out by HPLC, using a Waters liquid chromatograph (Milford, USA) equipped with a 600E multisolvent delivery system, an in-line degasser, and a 996 UV–Visible photodiode array detector. Chromatographic conditions include a Tracer Excel 120 ODSA, 25×0.46 cm I.D. with 5 μm film thickness column (Teknokroma, Barcelona, Spain), 20 μl injection volume and 1

ml/min methanol flow-rate. Quantification of dyes was performed by comparing chromatographic peak areas for sample extracts with those of standards in the same concentration range. Calibration curves were run at seven concentration levels (0.2–20 μg/g) using appropriately diluted standards. Each concentration level was injected in triplicate and chromatographic peak areas were fitted by linear regression. The linearity was observed for the whole concentration range with a correlation coefficient higher than 0.999. An example of the HPLC–UV separation of dyes is shown in Fig. 1. Maximum absorbance for Solvent Yellow 14 and Solvent Red 24 was 230 nm, while it was 280 nm for Disperse Red 1.

3. Results and discussion

3.1. SFE optimization

The effect of different variables affecting SFE of dyes was studied by a five-factor design with two levels for each factor (low and high). This design requires 32 experiments plus one in the middle, which are run in a randomized manner. Six replicates of the central experiment were run in order to
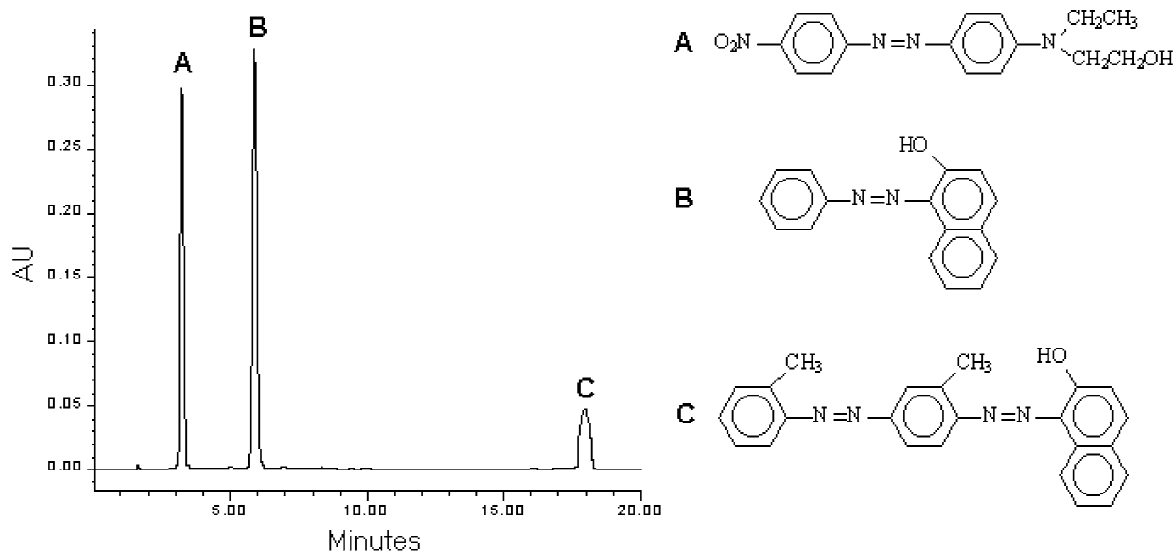

Fig. 1. HPLC–UV chromatogram at 230 nm for the analysis of the azo dyes. (A) Disperse Red 1; (B) Solvent Yellow 14; (C) Solvent Red 24.

Table 2
Factor levels and design matrix in the 2^5 factorial design for SFE

Factor			Units						Levels									
Variable		Key							Low (−)			High (+)			Middle (0)			
Pressure		P	MPa						13.8			55.2			34.5			
Temperature		T	°C						50			120			85			
Static time		S	min						2			15			8			
Methanol volume		V	µl						0			200			100			
Dynamic time		D	min						2			15			8			

Run	P	T	S	V	D	Run	P	T	S	V	D	Run	P	T	S	V	D
1	−	−	−	−	−	12	+	+	−	+	−	23	−	+	+	−	+
2	+	−	−	−	−	13	−	−	+	+	−	24	+	+	+	−	+
3	−	+	−	−	−	14	+	−	+	+	−	25	−	−	−	+	+
4	+	+	−	−	−	15	−	+	+	+	−	26	+	−	−	+	+
5	−	−	+	−	−	16	+	+	+	+	−	27	−	+	−	+	+
6	+	−	+	−	−	17	−	−	−	−	+	28	+	+	−	+	+
7	−	+	+	−	−	18	+	−	−	−	+	29	−	−	+	+	+
8	+	+	+	−	−	19	−	+	−	−	+	30	+	−	+	+	+
9	−	−	−	+	−	20	+	+	−	−	+	31	−	+	+	+	+
10	+	−	−	+	−	21	−	−	+	−	+	32	+	+	+	+	+
11	−	+	−	+	−	22	+	−	+	−	+	33	0	0	0	0	0

estimate the standard error of the design. Table 2 lists the design matrix as well as the values given to each factor, which were selected according to the instrumental limitations. The investigated parameters were supercritical CO_2 pressure (P), extraction temperature (T), static time (S), volume of methanol (V) and dynamic time (D).

SFE maximum recovery for dyes was found for experiment 32 (55.2 MPa, 120 °C, 15 min static time, 200 µl of methanol and 15 min dynamic time), as indicated in Table 3. As can be observed, a high recovery is obtained for Solvent Yellow 14 (95.2%), while 41.1 and 66.6% were obtained for Solvent Red 24 and Disperse Red 1, respectively. A statistical analysis of results was performed considering all possible interactions, with the results presented in Table 4, where only statistically significant effects are presented (95% probability). The most significant parameters as well as some second-, third- and fourth-order interactions ($PTSD$) were identified. From this table, we can see that the main effects are all significant for each dye, having an important influence on extraction. High temperature is necessary for high recoveries, meaning that diffusion of dyes is an important step in the extraction process [33]. In terms of solubility, high pressure is necessary for all dyes, and the presence of modifier seems to be convenient for the extraction of Disperse Red 1. $P–T$ interactions suggest that a compromise between an increase in CO_2 polarity and diffusion

Table 3
Maximum mean recoveries (%) of dyes extracted from dye-spiked samples by different methods

Dye	SFE[a]		MAE[b]		Soxhlet[c]	
	Mean[d]	RSD (%)	Mean[d]	RSD (%)	Mean[d]	RSD (%)
Solvent Yellow 14	95.2	1.6	99.4	0.8	81.0	1.8
Solvent Red 24	41.1	1.8	59.5	1.7	22.6	3.4
Disperse Red 1	66.6	1.0	99.8	0.4	95.3	0.1

[a] 55.2 MPa, 120 °C, 15 min static time, 200 µl of methanol and 15 min dynamic time.
[b] 120 °C, 20 min heating time.
[c] 70 ml of methanol, 7 h.
[d] Based on the extraction of four replicates.

Table 4
Standardised significant effects for SFE and MAE of dyes

Factors	Effects		
	Solvent Yellow 14	Solvent Red 24	Disperse Red 1
SFE: P	39.28 ± 2.82	15.19 ± 0.36	26.22 ± 1.86
SFE: T	14.39 ± 2.82	9.67 ± 0.36	10.61 ± 1.86
SFE: S	3.04 ± 2.82	1.69 ± 0.36	3.97 ± 1.86
SFE: V	5.89 ± 2.82	3.49 ± 0.36	19.15 ± 1.86
SFE: D	14.82 ± 2.82	5.74 ± 0.36	6.09 ± 1.86
SFE: PT	19.14 ± 2.82	9.03 ± 0.36	14.58 ± 1.86
SFE: PV	-4.88 ± 2.82	0.65 ± 0.36	–
SFE: PD	11.64 ± 2.82	5.87 ± 0.36	6.73 ± 1.86
SFE: TS	–	–	-1.93 ± 1.86
SFE: TV	-3.48 ± 2.82	1.61 ± 0.36	-2.33 ± 1.86
SFE: TD	4.15 ± 2.82	3.88 ± 0.36	4.16 ± 1.86
SFE: SV	–	0.76 ± 0.36	3.12 ± 1.86
SFE: VD	–	-0.67 ± 0.36	-2.63 ± 1.86
SFE: PTS	–	0.72 ± 0.36	–
SFE: PTV	–	0.55 ± 0.36	–
SFE: PTD	7.87 ± 2.82	4.12 ± 0.36	4.31 ± 1.86
SFE: PSV	–	–	–
SFE: PVD	–	–	-1.94 ± 1.86
SFE: TSD	4.05 ± 2.82	–	–
SFE: $PTSD$	3.15 ± 2.82	0.76 ± 0.36	–
SFE: $PSVD$	3.18 ± 2.82	–	–
MAE: T	60.90 ± 8.54	46.35 ± 2.30	56.66 ± 4.66
MAE: t	–	4.20 ± 2.30	10.44 ± 4.66

coefficients of the analytes is advisable in order to increase recovery [33]. Other important interactions are *PD*, *TD* and *PTD*, where dynamic time reflects its positive significance in solubility and diffusion processes.

Some additional experiments near the point of maximum recovery were carried out in order to improve Solvent Red 24 and Disperse Red 1 recoveries. Volume of methanol was increased to 400 μl, dynamic time was increased to 30 min, temperature was raised to 150 °C, and pressure to 68.9 MPa. No significant improvement was observed in any case. Therefore, results shown in Table 3 indicate that SFE conditions are efficient for the extraction of Solvent Yellow 14, but less efficient in the case of

Disperse Red 1 and Solvent Red 24, where stronger conditions are necessary (higher P and T). Unfortunately this was not possible due to instrumental limitations.

3.2. MAE optimization

Microwave-assisted extraction was evaluated by a two-factor design with two levels for each factor. This design requires four experiments plus one in the middle, which were performed in duplicate and were run randomized. The parameters selected were extraction temperature (T) and heating time (t). Table 5 lists the design matrix for experiments.

MAE maximum recovery was found for experi-

Table 5
Factor levels and design matrix in the 2^2 factorial design for MAE

Factor		Units	Levels			Run				
Variable	Key		Low (−)	High (+)	Middle (0)	1	2	3	4	5
Temperature	T	°C	40	120	80	−	+	−	+	0
Heating time	t	min	5	20	12.5	−	−	+	+	0

ment 4 (120 °C, 20 min heating time), as indicated in Table 3. Recoveries are higher than 98% for the mono-azo dyes, while is around 60% for the diazo dye. A statistical analysis of results was performed considering all possible interactions appearing in Table 4, where only the statistical significant effects are presented (95% probability). Extraction temperature is significant for all dyes. It is well known that temperature is an important parameter in all liquid–solid extraction processes. The polymer particles undergo a swelling effect caused by heating, making them more permeable to the solvents and helping the extraction of additives [21]. Heating time appears to be significant for Solvent Red 24 and Disperse Red 1.

Results shown in Table 3 indicate that MAE conditions are efficient for the extraction of Solvent Yellow 14 and Disperse Red 1, but less efficient for Solvent Red 24. These results, compared to those obtained for SFE, show that MAE seems to be a more adequate extraction method for dyes in PVC samples in terms of recovery.

3.3. Soxhlet extraction

Soxhlet extractions were carried out in order to compare SFE and MAE with a traditional extraction method. Recoveries are also presented in Table 3. As can be observed, recoveries in the extraction of Disperse Red 1 are similar to those obtained with MAE, but less effective for the other two dyes compared to both SFE and MAE techniques.

3.4. Comparison of techniques

MAE can be effectively applied to the extraction of azo dyes, giving higher recoveries than SFE and Soxhlet methods. All extraction methods show relative standard deviations lower than 2.0% for all dyes (Table 3). Table 6 summarizes optimum experimental conditions for SFE, Soxhlet and MAE. Additional advantages of the use of MAE are the considerable reduction of time and solvent waste when compared to Soxhlet extraction.

In terms of selectivity, as PVC samples were prepared with known amounts of each component, only citrate and dye were always extracted. However, when comparing the ratio between peak areas

Table 6
Optimum experimental conditions for SFE, MAE and Soxhlet extraction

	SFE	MAE	Soxhlet
Sample size (g)	0.4	0.5	1
Extraction solvent	CO_2	MeOH	MeOH
Temperature (°C)	120	120	b.p. of solvent
Pressure (MPa)	55.2	0.5	ambient
Time	30 min (static + dynamic)	20 min	7 h
Solvent volume (ml)	8	15	70

for dyes and citrate, we noticed that this ratio was similar for SFE and Soxhlet, but lower for MAE, which seems to be less selective for the target analytes.

The most notable differences in dye recoveries were observed for Solvent Red 24, a diazo dye with molecular mass 380.4 g/mol (Table 1), especially for Soxhlet and to a lower degree, for SFE. The larger the molecule the slower the diffusion through the polymer, and hence the slower the extraction. In addition, high-molecular mass compounds tend to be less soluble in supercritical CO_2 and hence extraction is limited by solubility for larger molecules [34]. In addition, the high melting point of Solvent Red 24 causes some difficulties for diffusion in pressurised extraction processes, like SFE.

4. Conclusions

A thorough optimization of the extraction of azo colorants in PVC samples is presented. Factorial design techniques permitted the simultaneous study of the different parameters affecting SFE and MAE recoveries for the studied azo dyes. Soxhlet extraction was also evaluated and compared to the other extraction techniques. Extraction temperature and CO_2 pressure were the more critical parameters in SFE, indicating that diffusion and solubility processes are important for the extraction of dyes. MAE yielded higher recoveries for dyes when compared to SFE and Soxhlet extraction. Maximum MAE recoveries were 99.4% for Solvent Yellow 14, 59.5% for Solvent Red 24 and 99.8% for Disperse Red 1 at 120 °C and 20 min. Extraction temperature appeared to be significant for the extraction of all

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dyes. A lower recovery was found for Solvent Red 24, a diazo dye with a high molecular mass, especially for Soxhlet, and to a lower degree, for SFE. Reproducibility was comparable for all extraction methods, but MAE seems to be the less selective method.

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