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Separation of pigment formulations by high-performance thin-layer chromatography with automated multiple development☆

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ABSTRACT

Food packaging is designed to provide sufficient protection for the respective filling, legally binding information for the consumers like nutritional facts or filling information, and an attractive appearance to promote the sale. For quality and safety of the package, a regular quality control of the used printing materials is necessary to get consistently good print results, to avoid migration of undesired ink components into the food and to identify potentially faulty ink batches. Analytical approaches, however, have hardly been considered for quality assurance so far due to the lack of robust, suitable methods for the analysis of rarely soluble pigment formulations. Thus, a simple and generic high-performance thin-layer chromatography (HPTLC) method for the separation of different colored pigment formulations was developed on HPTLC plates silica gel 60 by automated multiple development. The gradient system provided a sharp resolution for differently soluble pigment constituents like additives and coating materials. The results of multi-detection allowed a first assignment of the differently detectable bands to particular chemical substance classes (e.g., lipophilic components), enabled the comparison of different commercially available pigment batches and revealed substantial variations in the composition of the batches. Hyphenation of HPTLC with high resolution mass spectrometry and infrared spectroscopy allowed the characterization of single unknown pigment constituents, which may partly be responsible for known quality problems during printing. The newly developed, precise and selective HPTLC method can be used as part of routine quality control for both, incoming pigment batches and monitoring of internal pigment production processes, to secure a consistent pigment composition resulting in consistent ink quality, a faultless print image and safe products. Hyphenation of HPTLC with the *A. fischeri* bioassay gave first information on the bioactivity or rather on the toxicological potential of different compounds of the pigment formulations. The results of the bioassay might be helpful to choose pigment compositions that provide both, a high printing quality but at the same time guarantee a high consumer safety, especially in regard to smaller pigment components, which tend to migrate through the packaging.

1. Introduction

The original function of food packaging is the protection of the food product contained to guarantee its faultless quality and safety for the labeled shelf-life [1]. A printing of food packaging is mainly used to provide the consumer with sufficient information, allowing him to make an informed buying decision. This includes partly legally binding information such as weight and composition of a product, the presence of allergens, nutrition labeling or information about the manufacturer [2,3]. Furthermore, the printed packaging plays an important role for the presentation and advertisement of a foodstuff [4]. A faultless print image, bright and appealing colors, light fastness and a good adhesion to the packaging material are important quality parameters that need to be fulfilled. Migration of the printing materials through the packaging needs to be avoided or at least reduced to a technical feasible and acceptable minimum, to guarantee the highest possible consumer protection [5].

In only very few cases, printing inks are applied on the inner side of a packing, leading to a direct contact between the ink and the food. In most cases, printing inks are applied on the non-food contact surface [6]. Once the colors printed on the package are dried or cured, they are an integral part of the packaging [7]. Although there is currently no specific EU-wide legislation regarding printing

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inks, printed food packaging falls under the general requirements of the EU regulation (EC) No. 1935/2004. Accordingly, food packaging shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could endanger human health, bring about an unacceptable change in the composition of the food or deteriorate the organoleptic characteristics [8].

Printing inks are complex multi-component mixtures, but mostly consist of four main components, namely the colorants (pigments, less often dyes), solvents that enable the transfer of the pigment mixture to the substrate, resins that acts as a film former and binder of the colorant, and further additives like plasticizers or UV absorbers that improve exemplarily the stability, flexibility or flow of the inks [9]. For coloring, mostly organic pigments are used, which are produced by classical synthesis (e.g., azo pigments by coupling of a diazonium compound and a coupler like 2-hydroxynaphthalene-3-carboxylic acid). To further reduce their water solubility, some pigments are precipitated as insoluble calcium, strontium or barium salts (lake pigments). Additionally, many pigments are coated with a further layer of inorganic (e.g., silica) and/or organic (e.g., siloxanes and silanes) material to improve their distribution and application properties, without having a substantial influence on the color itself [10]. After synthesis and modification, the pigments are dried to form powders and are used in most cases without further purification.

The classification of the different colorants is based on the listing according to the Color Index Generic Names/Numbers (C.I. numbers). The C.I. numbers are grouped according to the chemical structure of the pigments, e.g., thiazole dyes can be found under the numbers C.I. 49000–49399 [10].

To guarantee the printing ink quality and thus good printing results, to ensure compliance with legal requirements and to minimize possible interfering contaminations, an efficient in-house control during the whole production process and an effective and standardized quality assurance during the incoming inspection control of globally purchased materials are crucial [6]. In this context, different aspects may be of interest for the manufacturer: to compare different batches of one supplier to set comparable and verifiable quality parameters, to examine differences of pigments with the same C.I. number provided by different suppliers and to assign different coating materials, which may influence the printing performance.

Currently, quality control is mainly based on the examination of the finished ink and includes testing of the pH, the density, the viscosity (rheological properties), the opacity and the color strength of the mixture as well as the adhesiveness and drying ability of the ink during a test print [11]. Analytical approaches, however, have hardly been considered for quality assurance so far due to the lack of robust, suitable methods for the analysis of rarely soluble pigment formulations.

For the examination and identification of writing inks, however, analytical methods have been used since the 1950s, particularly due to forensic purposes. These methods include paper chromatography, thin-layer chromatography (TLC), paper electrophoresis, luminescence photography, spectrometric measurements, infrared spectroscopy, high-performance liquid chromatography (HPLC), and capillary electrophoresis [12]. Also different high-performance TLC (HPTLC) methods are described, while they mainly concentrate on the separation of well-soluble dyes [13–15]. In 1993, Aginsky studied 120 synthetic pigments, dyes and printing inks with only slight solubility in different solvents [16]. The samples were dissolved in dimethylformamide or concentrated sulfuric acid. A three step separation was applied, starting with chloroform for the separation of basic and acidic dyes. With ethyl acetate/isopropanol/water/acetic acid (30:15:10:1, *v*/*v*/*v*/*v*) also oil, ethanol or water soluble dyes were separated, while the third development using concentrated sulfuric acid was applicable for phthalocyanines and other slightly soluble organic pigments. HPTLC also proved to be suited for analysis of water-soluble food dyes in bakery ink formulations, which were used for printing on food, e.g., for printing images on fondant covers of cakes [17–19] and might get increasing attraction in 3D food print. Apart from writing and printing inks, also textile colorings were analyzed by HPTLC [20,21].

HPTLC may fill the lack in chromatographic methods for the analysis of pigment formulations. Due to their complex matrix, the multiplicity of components and their partly slight solubility in any kind of organic solvents, like in the case of some pigments, HPTLC was seen as promising method to compare the composition of different pigment batches as part of regular quality control. Via an automated gradient separation by multiple developments, it is possible to further improve the chromatographic resolution, resulting in sharper substance bands and a better separation over a wide polarity and sample range. Post-chromatographic derivatization of the HPTLC plate can further enable an additional gain of information, what facilitates the comparison of different, supposedly identical pigment batches and may explain different printing results in praxis. Due to the single use of the HPTLC plates, sample preparation can be minimized and a possible contamination of the system, especially due to slightly soluble pigment components or matrix, is no issue, quite contrary to other analytical techniques like column chromatography. In contrast to latter techniques, substances retained at the starting zone are detectable and accessible in HPTLC.

Hence, the aim of the present work was the development of a suitable, automated HPTLC gradient method that allowed the comparison of different coated pigments from the azo, diazo, dioxazine and phthalocyanine family with different C.I. numbers in only one run to reduce both, the analysis time and the consumption of chemicals to a minimum. Additionally, hyphenation of HPTLC with high resolution mass spectrometry (HRMS) and Fourier transform infrared spectroscopy (FTIR) was investigated for the examination and assignment of different unknown, characteristic substance bands separated on the HPTLC tracks. This would enable the manufacturer to identify possible impurities, undesired by-products of the synthesis and unknown coating materials. Pigment formulations, which led to problems during the printing process, could be compared to faultless samples to identify characteristic differences in their fingerprint. Based on these differences further pigment batches can be analyzed during regular quality control to recognize discrepancies at an early stage. Such a profiling would avoid potential problems during the printing process and variations in quality in advance. Hyphenation with the *Aliivibrio fischeri* bioassay will give additional information of bioactive, potentially toxicologically active pigment components. This may be of particular interest with respect to pigment constituents, for which a tendency for migration into the food is conceivable due to their chemical and physical properties.

2. Materials and methods

2.1. Materials

Methanol, *n*-butanol, toluene, ethyl acetate, tetrahydrofuran, *N*,*N*-dimethylformamide, dimethyl sulfoxide, sulfuric acid (96%), ammonia (25%) and natural product reagent (99.9%) were purchased from Roth, Karlsruhe, Germany. Fast Blue Salt B, primulin, β-naphthol, polyethylenglycol (PEG)-400 (all of analytical grade), acetonitrile and acetone were obtained from Fluka Sigma Aldrich, Steinheim, Germany. Ninhydrin (analytical grade) and HPTLC