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Strategies for continuous on-line high performance liquid chromatography coupled with diode array detection and electrospray tandem mass spectrometry for process monitoring of sulphonated azo dyes and their intermediates in anaerobic–aerobic bioreactors

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Abstract

On-line HPLC with diode array detection (DAD) coupled to electrospray tandem mass spectrometry (ESI-MS/MS) is presented as an integrated quality control and process integrated optimisation tool for the continuous monitoring of sulphonated azo dyes (SADs) and their intermediates in anaerobic and aerobic bioprocesses. Ion-pair RP-HPLC is found out to be more suitable for simultaneous monitoring of aromatic amines (AAs), sulphonated aromatic amines (SAAs) and sulphonated azo dyes in comparison to RP-HPLC with polar embedded phases. Monitoring of the anaerobic degradation of the diazo Reactive Black 5 displays the dependency of a two stage azo group reduction mechanism on the redox potential of the bioreactor. An autoxidation sensitive intermediate released from the anaerobic reduction is characterised by ESI-MS/MS for the first time. The functionality of the method is demonstrated by the control and evaluation of selective adaptation of bacteria to certain pollutants and the identification of unknown intermediates causing re-gaining colour released from azo dye treatment.

Keywords: Sulphonic acids; Azo dyes; Aromatic amines; On-line LC–MS/MS; Reactive Black 5

1. Introduction

Complementary information from various analytical techniques is often required for the unambiguous identification and quantification of organic dyes [1]. Dyes have always been the objective of analytical interest of scientists concerning the identification of natural dyes as well as synthetic dyes produced by oneself or by competitors. Brunner in 1929 recommended a technique called capillary analysis which was first described by Goppelsröder for the purity analysis of natural or synthetic dye mixtures [2–4]. In the 1920s, the analytical task of the separation and analysis of more than 1000 azo dyes encouraged the use of chromatographic techniques as described by Tswett [5] and Fierz-David [6]. About 30 years after Tswett's invention of the chromatographic adsorption method, dye chemists assessed the new analytical method as useful. However, this method can only be applied to simple systems, which only consist of few dyes [7–9]. Since this time, an approach has been made to separate azo dyes by liquid chromatography. Today, the number of known azo dyes and other commercial dyes exceeds 10,000 [10]. In 1979 Sträule and von Wattenwyl [11] developed a RP-HPLC method to separate anionic azo dyes and intermediates. However, dye manufacturers and end users are often more focused on performance and intensity of dyes rather than on a comprehensive characterisation of the content of their products. Therefore, the required chromatographic conditions to effectively resolve the organic impurities in dyes are not readily available from manufactures [12]. Following the studies of the carcinogenic potential of benzidine derived dyes initiated by National Institute for Occupational Safety and Health (NIOSH) in 1978 and the health hazard alert for

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benzidine, *o*-toluidine and *o*-dianisidine based dyes in 1980 by NIOSH analytical methods for the identification and quantification of azo dyes became more and more important in the field of environmental and toxicological analytics [13,14]. The combination of HPLC with UV–vis detection has been found to be a useful method for the determination of benzidine impurities in azo dyes and for the analysis of structurally similar dyes [12,15,16]. In 1986 the proposed US Environmental Protection Agency (EPA) regulatory program for the dye industry required the development of mass spectrometry (MS) methods for the qualitative analysis of sulphonated azo dyes (SADs) that are easily adaptable to inexpensive routine analysis procedures [17]. LC–MS appears to offer good performance for the analysis of dyes in industrial wastes and treated effluents since the complex nature of these wastes and effluents will require HPLC separation for unambiguous mass spectrometric identification [18]. The detection of azo dyes (azo benzene) by mass spectrometry was performed first by Bowie et al. in 1967 [19]. Coupling of liquid flow injection with thermospray ionisation tandem mass spectrometry without previous chromatographic separation was introduced for the analysis of azo dyes, driven by the concern over the discharge of wastes into the aquatic environment from the industries manufacturing and using dyes [18,20,21]. Improvements in the LC–MS interfacing technology lowered detection limits for dyes and increased the potential for LC–MS based structural elucidation analysis by fragmentation experiments [22–25]. An overview about the recent development of mass spectrometry for azo dye analysis and its different applications is given by Richardson [26]. Reemtsma [27] recently reviewed analytical LC–MS methods suitable for the analysis of sulphonated compounds. Effects of ion-pairing agents on ESI signal suppression were investigated by Holčapek et al. [28]. Due to an increasing use of biological wastewater treatment concepts for synthetic compounds such as sulphonated azo dyes in industrial wastewater, analytical interest is focused on the degradation of these compounds. Textile dye producers and textile colouration plants contribute to the release of sulphonates in the aquatic environment which represents a major problem in drinking water plants in south-eastern Spain [29]. Analysis of industrial wastewater from dye producers and colouring plants showed the demand for selective analytical methods for the monitoring of sulphonated aromatics (SAs), sulphonated aromatic amines (SAAs) and aromatic amines (AAs) in water [30–38].

Textile dyes of various categories, i.e. acid, direct, mordant and reactive dyes, are sulphonated in order to induce high water solubility, which is important for the dyeing process. Water solubility results from full dissociation of the dyes in aqueous solutions as anionic solutes. The analytical strategies used for the determination of SAs, SAAs and SADs include a wide range of classical and modern techniques. Many of these techniques make use of the negative charge of the dye. Small SAs and SAAs such as sulphanilic acid are not retained on RP phases because they are rejected from the pores by the Donnan exclusion effect originated by negative-charged free residual silanol on the particle surface.

Jandera et al. [32] investigated the separation of SAs by reverse-phase chromatography in the presence of various inorganic electrolytes including sodium nitrate, potassium phosphate and sodium sulphate in order to suppress the Donnan exclusion effect on the solutes. Jandera et al. [33] found that isomeric naphthalene mono- to tetrasulphonic acids are best separated with aqueous-methanolic mobile phases containing sodium sulphate and various aromatic aminosulphonic acids by ion-pair reversed-phased chromatography with mobile phase containing tetrabutylammonium ions. Several researchers [30,34,35] investigated the use of ammonium acetate (5–30 mM) as an electrolyte in the HPLC separation of dyes because of its weak ion-paring (IP) properties and volatility which allows coupling to LC–MS. Ammonium formate was used for RP-HPLC separation of benzene and stilbene sulphonic acids coupled to diode array detection (DAD) with electrospray mass spectrometry (ESI-MS) [36]. The use of amine bases was found to enhance the sensitivity of ESI-MS towards complex polysulphonated azo dyes [37]. It was observed that the use of triethylamine (TEA) and diethylamine (DEA) as organic modifiers in LC–MS follows the concept of total cation removal to combine individual ions from a series into a single intensive peak. The base gas-phase proton and sodium affinity is considered to be responsible for the cation removal mechanism and also to effect the observed sensitisation.

The most commonly used IP agents for the separation of anionic solutes are tetraalkylammonium salts, such as tetrabutylammonium salts (TBA) which allow separation of very strong acidic SAAs (for example benzene and naphthalene sulphonic acids). However, non-volatile TBA salts obstruct the interface if IP-RP-HPLC is coupled with ESI or APCI to MS. Socher et al. [38] developed a method to determine sulphonated compounds by RP-IP-HPLC–MS with on-line removal of non-volatile TBA ion-paring agent. Based on the same coupling method Bauer et al. [39] combined ion chromatography (IC) with ESI-MS for the analysis of polar organic micropollutants, such as EDTA, belonging to the group of polar persistent pollutants (P^3).

Azo dyes, SAs and SAAs are known to be poorly degradable in communal biological wastewater treatment plants. An approach has been made to adapt micro-organisms to these compounds whereby a biodegradation could be achieved in industrial wastewater treatment plants [40]. However, many SAs and SAAs still belong to the group of recalcitrant synthetic compounds, which are known to pass aerobic biological wastewater treatment without degradation. Knowledge about the degradation mechanism and pathway of SAs and SAAs such as azo dyes is necessary for a process optimisation of biological treatment processes of such compounds and analytical control of the process, respectively. SAAs can be found in the untreated production and colouring wastewater of the dye and dyeing industry [27,29,41,42]. In addition