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Short communication

Hydrotrope-driven disruption of micellar encapsulants for voltammetric detection of triclosan

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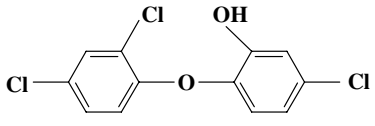
Abstract

The effect of surfactant matrices on the voltammetry of an antimicrobial agent, triclosan (TCN) and its release into the aqueous continuous phase for voltammetric detection have been studied in this paper. As the surfactant concentration (wt%) was increased, the peak current was found to decrease in the order: Tween-80 > Tween-60 >>> sodium laurylsulphate. The results of these experiments are found to correlate with the published data obtained using microcalorimetry. Attempts have been made to overcome the trapping effect of surfactant matrices by designing a solution composition based on *hydrotrope-driven disruption of micellar encapsulants* for complete release of TCN into the analyte solution for voltammetric detection. The hydrotropes employed in this study are sodium *p*-toluenesulphonate and sodium benzenesulphonate. The experiments intended to show the effect of hydrotropes on the 'pre-adsorbed' triclosan-surfactant ''pseudo-complex'' proved that hydrotrope-driven disruption of micelles takes place even on the electrode surface. Thus, immersion of the surfactant-covered electrode into a hydrotrope solution can ''strip'' the antimicrobial agent off the surfactant. The results of these *proof-of-concept* experiments were applied to the analysis of low concentrations (0.1–0.8 mM) in product formulations containing TCN. A linear relationship was found between TCN concentration and current magnitude with a correlation coefficient of 0.97, the sensitivity of the measurement towards the TCN oxidation being 12.8 μA mM^{-1}.

Keywords: Triclosan; Phenolic; Antimicrobial; Micelle; Hydrotrope; Voltammetry

1. Introduction

Antimicrobial agents are widely used as ''actives'' in healthcare product formulations and surfactant matrices play a significant role in controlling the availability of these ''actives''. Since these active agents are designed to be lipophilic/hydrophobic and are sparingly water-soluble, the addition of surfactants, in the concentration range of 0.5–2.0 wt% [1–3] ensures their aqueous solubility. The lipophilic molecules like triclosan (TCN) must be hydrophilic to be able to penetrate the aqueous mucus layer into the lipophilic mucosa [4], but at the same time, it must also be in a lipophilic form to be able to partition into mucosa and its sites of action. High initial concentration of the aqueous available TCN will enhance its delivery to the various sites of action (substantivity).



Structure of TCN (2,4,4_-trichloro-2_-hydroxy-diphenylether)

Regulatory requirements dictate the assay of TCN to be made for every production batch and determination of its contamination in rivers and lakes [5–7]. The analysis is beset with problems due to poor analyte

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peak shapes, matrix interference and even column degradation in chromatographic [5–8] techniques, which require time-consuming pre-treatment steps. Recently, Pemberton and Hart [9] have successfully exploited the electroactive nature of TCN to demonstrate its voltammetric determination in oral care products (PVM/MA copolymers in the formulations do not seem to "deactivate" as seen in our studies and as observed by Prencipe et al. [10]). Though sensitive, we found this methodology to underestimate TCN in formulations containing surfactants, e.g., polyoxyethylene sorbitan monoalkanoates, Tween and sodium lauryl sulphate (SLS) that interact strongly with TCN. Ironically, the surfactants, which favour solubility of the active agents, are also known to deactivate them [10]. It is thus challenging to track down the reason for the diminished voltammetric current signals and overcome the matrix interference especially due to surfactant matrices. Surfactants in aqueous solutions manifest as micelles and liquid crystal structures, solubilise the lipophilic active agent by micellar encapsulation and incidentally render it partially electroinactive. Obviously, disruption of the micelles will free the active agent to the aqueous phase. The issue is *akin* to the situation in chemical systems that release drugs from micellar compartments triggered by various types of stimuli [11–15] and different from that of Saji et al. [16] wherein the electroactive surfactant was oxidised to form organic thin films on the electrode surface. Solvents such as acetone and cyclohexane and hydrotropes can *loosen* surfactant micelles [17] and release TCN into the aqueous phase. The release profile was found to be linear with respect to the concentration of acetone. However, these solvents, needed in large excess, resulted in phase separation while dispersing the products containing the active. Ex situ extraction of TCN using hexane has also been reported [18]. In contrast to the aqueous systems, organic solvents pose environmental concerns and hence the use of hydrotropes was resorted to for electroanalytical determination of TCN. Hydrotropes are often used to optimise the cloud point of non-ionic and anionic surfactants in alkaline cleaners and increase the aqueous solubility of other organic compounds [18–20]. Thinning of the viscous products by addition of hydrotropes happens through loosening of the micellar aggregates. Hydrotropes themselves normally do not form aggregates and hence do not contribute to viscosity of the products [19]. In many instances, the micellar phase is not attained with the well-known surfactants [19,20].

Hence, a novel approach based on hydrotrope-driven disruption of the micellar encapsulants for the release of electroactive antimicrobials into the aqueous continuous phase is attempted in this work. Potential of this approach is examined for the direct voltammetric analysis of triclosan.

2. Experimental

Acetone, Tween-60 [polyoxyethylene (20) sorbitan monostearate] and Tween-80 [polyoxyethylene (20) sorbitan monooleate], sodium laurylsulphate, sodium *p*-toluenesulphonate were of analytical grade (Sigma–Aldrich) and were used as received. Triclsoan (TCN) was a sample from Ciba-Geigy. Phosphate buffer solution (PBS) of pH 11 was prepared using the following composition in 18 MΩ (Millipore) water: 0.05 disodium orthophosphate (50 mL) + 0.1 M sodium hydroxide (26.9 mL) made up to 100 mL to arrive at a pH ~ 11. Sodium *p*-toluenesulphonate was desiccated using $CaCl_2$ before use.

The electrolyte solution consisted of a pH 11 (0.1 M) PBS (typically, 30 mL solution volume). Calibration plots for various concentrations of TCN were obtained in a range of 0.1–1.0 mM dissolved in 0.1 M PBS. Surfactants were added in concentrations starting from sub-*cmc* values to examine their trapping effect on the electrooxidation of TCN, studied through cyclic voltammetry.

For release experiments, the hydrotrope was added incrementally to a solution containing TCN with surfactant. Alkalinity at pH 11 was maintained for all these experiments. For cyclic voltammetric (CV) experiments, a three-electrode configuration was used with a glassy carbon disk (3 mm diameter; BAS, Inc., USA) as working electrode; platinum foil (1 cm^2) as counter and normal calomel electrode (1.0 M KCl, NCE) as reference electrodes. The working electrode was mechanically polished with fine abrasives to obtain minimum and reproducible background response before each CV measurement. The CV set-up consisted of a EG&G PARC Potentiostat/Galvanostat (Model 273) coupled with a Rikadenki X-Y/t Recorder (Model RW 201T).

3. Results and discussion

In the course of our investigations on developing voltammetric protocols for the estimation of TCN in two different personal care products differing in their ingredients (specifically the surfactant matrices), voltammetric peak currents were found to be different for the same content of TCN. Interestingly, the ratio of peak current values of one formulation to the other is found to be a constant over a wide range of TCN content. Using this ratio as a correction factor (i.e.,"matrix interference factor"), it is possible to estimate TCN in a variety of products. To understand the origin of the above matrix interference, we have studied the oxidation of TCN in a pH 11 phosphate buffer solution (PBS) since alkalinity (pH 9–11) favours solubility (though limited) and electrooxidation of TCN containing the widely used surfactants, Tween (non-ionic) and sodium laurylsulphate

(SLS, anionic). As the surfactant concentration (wt%) was increased, the peak current was found to decrease in the order: Tween-80 > Tween-60 >>> SLS. The peak current decrease (Fig. 1) and the peak potential shifts (Fig. 2A) towards more anodic values point to the presence of strong-to-moderate interactions between TCN and the added surfactant. Surfactant addition usually increases the resistance of the buffer solutions thus contributing to iR drop. Due to this iR drop, the peak potential of the TCN oxidation shifts to more anodic values and decreases the peak current. Hydrotropes as thinners of the surfactant solutions reverses this trend by offsetting the iR drop. These observations are in consonance with those reported by Prencipe et al. [10] using microcalorimetric data. Understanding these interactions would lead to *direct* (overcoming matrix interference) electroanalytical protocols for phenolic antimicrobials, *a paradigm similar to a mobile phase in chromatography*.

In pH 11 PBS, TCN has a very limited solubility (<1.0 mM). It undergoes oxidation at $E_{p,a} = 0.55$ V vs. SCE (Fig. 1, inset) and the peak current scales linearly with its concentration up to 0.8 mM. It may be noted that the electrooxidation of TCN results in the fouling of the electrode surface. However, electrode activity can be regained by mechanical polishing or electrochemical cleaning [21]. The oxidation process, similar to that of simple phenols, appears to involve the hydroxyl group [21]. It is likely that the hydroxyl group interacts strongly with the surfactant present in the formulation, e.g., Tween, as shown by its ready solubility in neat liquid. In these, surfactants affect the percent saturation of TCN in the continuous aqueous phase of the composition. This implies that the active agent TCN is distributed between the continuous phase and the micellar

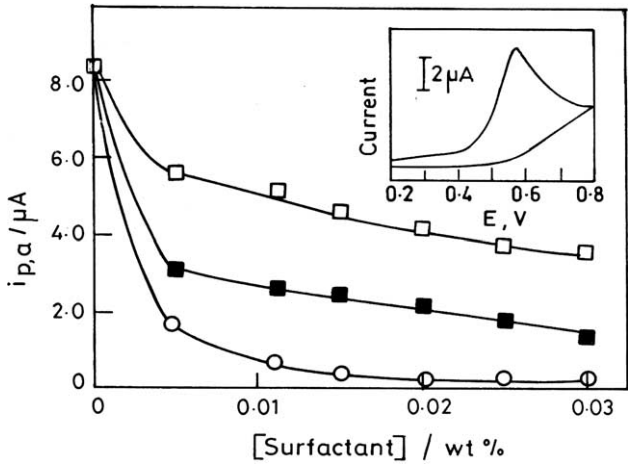

Fig. 1. Trapping of TCN by surfactants. Effect of addition of surfactants on the voltammetric peak current of TCN: (□) sodium laurylsulphate; (■) Tween-60; (○) Tween-80. (Inset) Typical voltammetric response of TCN in PBS (pH 11) on a glassy carbon electrode; $v = 0.04$ V s^{-1}.

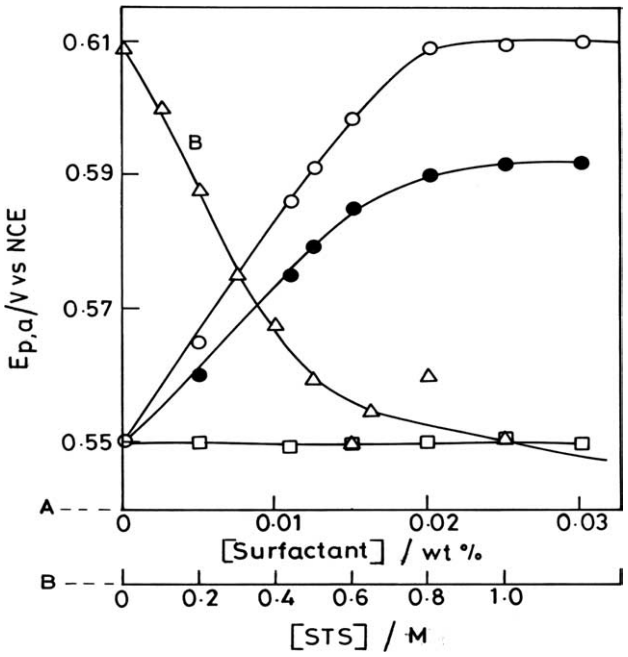

Fig. 2. Hydrotrope-driven release of TCN from surfactant-phase to aqueous phase. Effect of STS addition on the peak potential of TCN bound in surfactant solution: (□) sodium laurylsulphate; (●) Tween-60; (○) Tween-80; (△) STS.

pseudophase. Because of its low aqueous solubility, the distribution is shifted strongly towards the micelles. TCN solubilised in the micellar pseudophase is not *free* and hence not available for antimicrobial action and in the present case, for electroanalysis. However, TCN present in the micellar pseudophase, can serve as a reservoir to replenish the continuous aqueous phase with TCN (depleted due to antimicrobial action in physiology or electrooxidation in the voltammetry). While the surfactant micelles release TCN slowly under physiological conditions, for analytical estimation they need to be disrupted for a rapid and complete release.

For the purpose of electroanalysis of TCN, we attempted the in situ release of TCN completely into the aqueous continuous phase by the addition of hydrotropes to the solution of the formulation containing TCN. This effect is more pronounced in the case of non-ionic surfactants. Typical hydrotropes are the alkyl sulphates or alkyl sulphonates such as sodium-*p*-toluenesulphonate (STS), -xylenesulphonate, and -cumenesulphonate. They are electroinactive in the potential range employed here. In this work, we have chosen STS based on its efficacy and its well-understood behaviour [19,20,22–24]. For this, a particular concentration of Tween-80 or SLS is added to suppress the voltammetric response of TCN (ca. 0.5 mM) to a small but noticeable peak current value.

Fig. 3 shows the effect of the addition of STS on the voltammetric peak current of 0.5 mM of TCN bound by Tween-80 in a PBS solution (0.1 M). On increasing

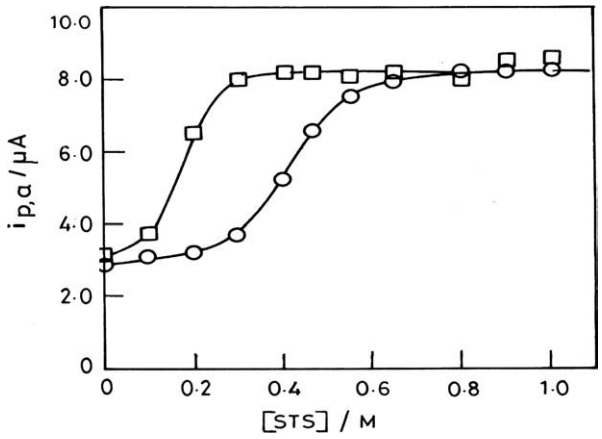

Fig. 3. Release profiles of TCN on addition of hydrotrope (STS); effect of hydrotrope concentration on the release of TCN into aqueous phase from surfactant micelles, measured as peak current in cyclic voltammetry. Surfactant: (□) sodium laurylsulphate; (○) Tween-80.

the concentration of STS, the peak current initially increases slowly, and then reaches almost the maximum value for TCN without Tween-80 (Fig. 3). The curves observed in the concentration range of 0.2–0.6 M are *sigmoidal* in shape and show a rise beyond a minimum (0.2 M) hydrotrope concentration. This behaviour can also be interpreted using adsorption arguments assuming that while the adsorbed surfactant micelles block the electrode surface, hydrotrope adsorption unblocks it. It is also conjectured that the permeability of the adsorbed layers to TCN may be different. Electrode surfaces with adsorbed surfactants as *admicelles* can limit the mass transport of TCN to the electrode surface thus contributing to the decrease in peak current [25]. In order to probe the effect of the adsorbed surfactants on the peak current of TCN oxidation, the electrode surface was exposed to a solution containing Tween-60 (0.2%) for about 60 min before carrying out cyclic

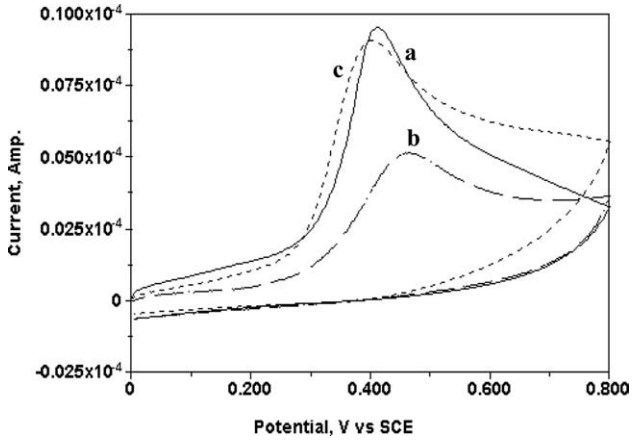

Fig. 4. (a–c) Voltammetric response of TCN at (a) bare GC electrode; (b) GC exposed to surfactant (Tween-60) solution; (c) surfactant-adsorbed GC in contact with sodium *p*-toluenesulphonate.

voltammetric examination of TCN oxidation behaviour. Fig. 4(a) shows the electrooxidation behaviour of TCN (0.5 mM in a PBS 11.4 buffer solution) on a bare glassy carbon electrode and Fig. 4(b) shows the response for the electrode subjected to prolonged exposure to the surfactant.

It is clear that the adsorbed surfactant decreases the peak current of TCN. Even on repeated use of this exposed electrode, the response continues to present a CV with currents lower than that at a bare GC electrode. It may be recalled that adsorbed layers of surfactants influence the rates of electrochemical reactions as in the case of *hemi-micelles* on the electrode surface [26,27]. In these systems, an aqueous solution of surfactants above a critical concentration constitutes a complex *mobile-phase modifier*. Experiments were carried out in order to examine the effect of hydrotrope addition on the adsorption of surfactants and in turn on the peak current of TCN. Surfactant-adsorbed electrode, when immersed in a solution containing the hydrotrope, sodium *p*-toluenesulphonate or sodium benzenesulphonate (0.3 M) even for shorter periods of time, presents a CV with full peak current corresponding to that of 0.5 mM TCN, whereas rinsing in water does not affect its behaviour (Fig. 4(c)). (Hydrotrope adsorption does not affect the TCN current as found from independent experiments). It is thus evident that the hydrotrope disrupts the surfactant micelles adsorbed on the electrode, releasing TCN. These experiments prove that hydrotrope-driven disruption of micelles takes place even on the electrode surface. Thus, immersion of the surfactant-covered electrode into a hydrotrope solution can "strip" the surfactant off the antimicrobial agent.

It may be noted that beyond the minimum hydrotrope concentration, micelles may not even exist [19,20,22,23]. The hydrotrope structures contain alternate, planar configurations of hydrophilic regions and open relatively loose hydrophobic regions. Assuming this structure is maintained, at least in part, in concentrated hydrotrope solutions, these portions would represent hydrophobic *niches* to host non-polar molecules [25]. In other words, the trapping/deactivating effect of surfactants is reversed by the hydrotropic action of STS. Similar investigations were carried out using SLS as the surfactant and the minimum hydrotrope concentration in this case was lower than that for Tween-80.

In the absence of surfactant, TCN exhibits oxidation peak at 0.55 V vs. NCE and the presence of the surfactant shifts it anodically. It is seen that the addition of STS results in shifting of the oxidation peak potential back to 0.55 V (Fig. 2B). This shows that the effect of surfactant is completely nullified and free TCN is available in the solution. The oxidation is also found to be amenable to voltammetric measurement as the peak current vs. (scan rate)$^{1/2}$ relationship is satisfied by both (i) free TCN in a PBS (without surfactant) and (ii) TCN

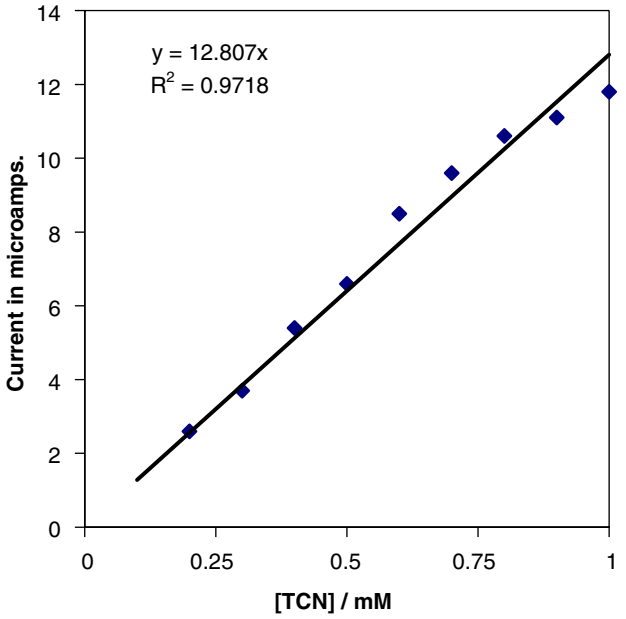

Fig. 5. Calibration plot of TCN in phosphate buffer at pH 11 at scan rate of 0.04 V s^{-1}.

released by hydrotrope-driven micellar disruption, indicating diffusion controlled oxidation of TCN [24].

The results of these *proof-of-concept* experiments were applied to the analysis of low concentrations (0.1–0.8 mM) in real products containing TCN. Further, a good linear relationship (Fig. 5) was found between TCN concentration and current magnitude with a correlation coefficient of 0.97, the sensitivity of the measurement towards TCN oxidation being 12.8 μA mM^{-1}. The detection limits were further enhanced with the use of pulse techniques, a detailed study of which will be reported separately.

4. Conclusion

In summary, an approach for in situ extraction of TCN into the aqueous electrolyte medium for direct voltammetric detection has been developed. The approach involves the incorporation of hydrotropes into the surfactant-containing formulation, which disrupt the micellar pseudophase, releasing the active agent into the electrolyte medium. We believe that this concept will have immense potential in (a) devising analytical protocols for the estimation of electroactive components in product formulations containing non-ionic- and anionic-surfactants and (b) minimising mass transport-related problems with aqueous solutions, using hydrotropes.

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