Reactive Oxygen Species in Laundry Applications

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Abstract

Reactive Oxygen Species in Laundry Applications

PhD Thesis Anna M. Stańczak February 2018

Chapter 1 is divided into two parts, the first gives a brief review of oxygen-based bleaching agents commonly used in powder laundry detergent with emphasis on the properties of hydrogen peroxide and peroxy acids. In the second part of the literature review, different methods of detection of Reactive Oxygen Species (ROS) are discussed.

Chapter 2 discusses analytical techniques used throughout this thesis, mainly focusing on the fluorescence and UV-Vis spectroscopy, as well as imaging techniques: Image Analysis and Diffuse Reflectance Spectroscopy. This chapter provides also detailed experimental procedures.

Chapter 3 includes the results and discussion part of the thesis. The process of the development of fluorescent probes for the most relevant ROS in laundry applications: hydrogen peroxide, peroxy acid, hydroxyl radicals and singlet oxygen is discussed. The established toolbox of molecular probes consists of: terephthalic acid, 9,10-anthracenediyl-bis(methylene)dimalonic acid and 2-naphthylboronic acid for detection of hydroxyl radicals, singlet oxygen and peroxides, respectively.

Chapter 4 reports the kinetic parameters of the ROS generated from genuine bleach source in simplified model system (alkaline solution) established with the developed toolbox of molecular probes.

Chapter 5 introduces four model dyes which represent genuine stains: curcumin, crocin, betanin and chlorophyllin. The impact of pH on the bleaching of the model dyes was investigated as well as bleaching kinetic parameters. The behaviour of peroxy acids generated from bleach sources in the presence of model dyes was determined with the toolbox of molecular probes developed in Chapter 3.

Chapter 6 examines the bleaching performance of different ROS on genuine stains deposed on fabric: grass, tomato, curry, red wine and tea with Image Analysis technique and Diffuse Reflectance Spectroscopy. In the second part of the chapter the behaviour of the bleaching agents in the presence of stains was investigated.

REACTIVE OXYGEN SPECIES IN LAUNDRY APPLICATIONS

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List of Abbreviations

ABA	Aromatic boronic acids
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ADMA	9,10-Anthracenediyl-bis(methylene)dimalonic acid
C12E8	Octaethylene glycol monododecyl ether
CAPB	Cocamidopropyl betaine
CCA	Coumarin-3-carboxylic acid
CCD	Charge-coupled device
CIE	Comission Internationale de l'Eclairage
CTAB	Cetrimonium bromide
DAED	Diacetylethylenediamine
DAP	Diacyl peroxide
DOBA	Decanoyloxybenzoic acid
DPBF	1,3-Diphenylobenzofuran
DRS	Diffuse reflectance spectroscopy
EDTA	Ethylenediaminetetraacetic acid
EPR	Electron paramagnetic resonance
FFA	Furfuryl alcohol
FL	Fluorescence
HPAA	Hydroxyphenylacetic acid
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
HTA	2-Hydroxyterephthalic acid
IA	Image Analysis
IFE	Inner filter effect
IR	Infrared
LED	Light emitting diodes
LMG	Lecuomalachite green
LOBS	Sodium lauroyloxybenzene sulfonate
MS	Mass spectrometry

NBA	Naphthyl-2-boronic acid
NMR	Nuclear magnetic resonance
NOBS	Sodium nonanoyloxybenzene sulfonate
NOH	Naphth-2-ol
OH-CAA	7-Hydroxy-coumarin-3-carboxylic acid
PAA	Peracetic acid
PAP	6-(Phthalimido)peroxyhexanoic acid
PNA	Pernonanoic acid
RB	Rose Bengal
ROS	Reactive oxygen species
SC	Stain change
SDS	Sodium dodecyl sulfate
SRI	Stain removal index
TA	Terephthalic acid
TAED	Tetraacetylethylenediamine
TAGU	Tetraacetylglycoluril
TriAED	Triacetylenediamine
UV	Ultraviolet
Vis	Visible
WN	Washed noticeability

Declaration

The work described in this thesis was carried out at the Department of Chemistry, University of Durham and Procter & Gamble Brussels Innovation Centre, between October 2014 and September 2017. This thesis is the sole work of the author, except where acknowledged by reference, and has not been submitted for any other degree.

Statement of Copyright

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1 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Laundry detergents are products that contain a mixture of surfactant and other ingredients to clean fabrics in the wash process. Laundry detergent has traditionally been a powdered or granular solid. In 1960 liquid laundry detergents were introduced and their use has gradually increased over the years.[1] Since 2012 liquid laundry detergents have been sold also in form of single dose pods, which were introduced by Procter & Gamble as *Tide Pods*.[2]

Cleanliness and hygiene are among mankind's basic needs. Detergents and cleaners play a major role in ensuring basic standards of living. The additional use of bleach brings further improvements regarding stain and germ removal from fabrics and hard surfaces. Bleach in a powder laundry detergent is a product that provides oxidizing capacity to remove bleachable stains on fabrics.[3] Oxygen-based bleaching agents typically refer to hydrogen peroxide and related compounds, and are differentiated from those bleaches based on chlorine. Chlorine-bleaches (containing ClO^-) are used in hard surface cleaners as well as in some laundry additives for washing white fabrics. The main function of a bleaching agent is to provide whiter garments by removing stains, either alone or in combination with a surfactant system.[4]

This introduction starts with a brief description of laundry detergents and is followed by a short history of bleach development. In the next sections the physical and chemical properties of hydrogen peroxide and peroxy acids are described. The subsequent parts focus upon currently used peroxide-based bleaching systems in domestic laundry products. Finally at the end of the 'bleach' part of the review, other Reactive Oxygen Species (ROS) present in laundry are presented and mechanisms of the bleaching process are discussed. The second part of the review focuses on ROS detection methods and lists the most applicable probes and techniques. The ROS discussed are: hydroxyl radicals, singlet oxygen, hydrogen peroxide and peroxy acids.

1.2 Domestic laundry detergents

A modern laundry product is a complex mixture of components, each filling a role, but working together to improve the cleaning process. A first look at the ingredients section of a domestic laundry powder might be overwhelming. However, the main classes of component are builders (water softeners), surfactants, bleach and enzymes (Figure 1.1).

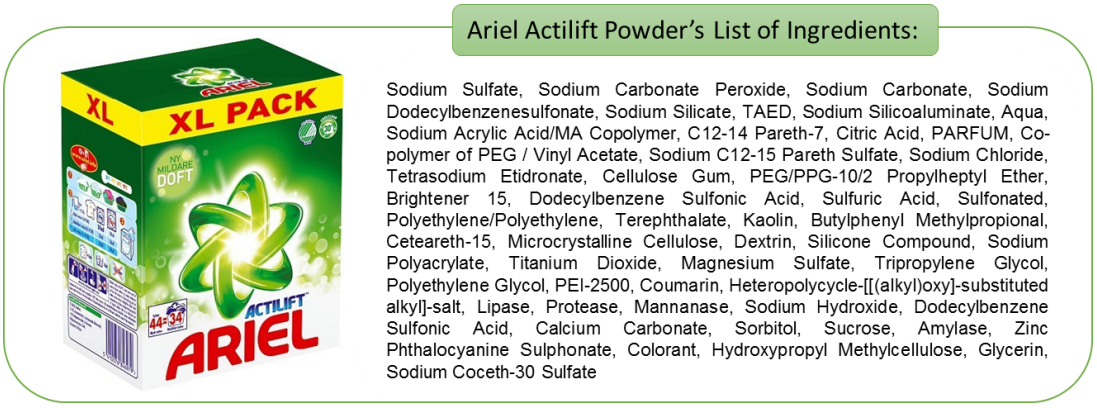
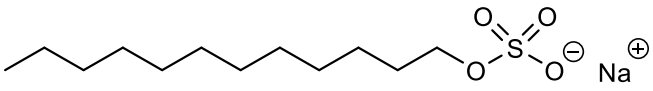


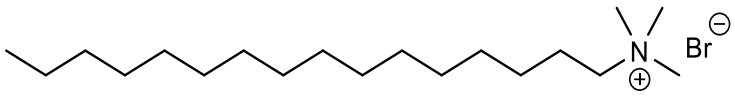
Figure 1.1 List of ingredients of laundry powder. [5]

The formulation is strongly affected by the temperature of the cleaning process and varies from country to country. Currently there are global trends which encourage people to use lower temperature washes for environmental and financial reasons and it can be assumed that the common laundry temperature in Europe lies around 30 – 40 °C.

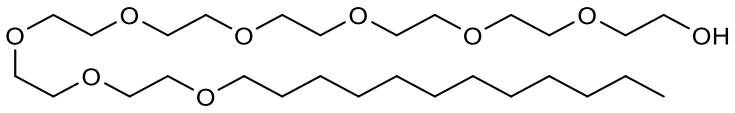
The most important components of the powder laundry detergent are surfactants. The term surface-active agent or surfactant represents a heterogeneous and long-chain molecule containing both hydrophilic and hydrophobic moieties, which are able to lower the surface tension between two immiscible liquids or between a liquid and solid. Due to this property surfactants are able to improve the wetting ability of water, helping loosen and remove soil with the aid of wash action as well as emulsify, solubilise and suspend soils in the wash solution. [6] Laundry detergent may contain more than one kind of surfactant. These can differ in their ability to remove certain types of soil, in their effectiveness on different fabrics and in their response to water hardness. Surfactants are classified by the nature of their polar, hydrophilic portion. Four main group can be distinguish: cationic, anionic, nonionic and amphoteric surfactants. The most common examples of surfactants from each group that are used for laundry can be found in Figure 1.2. [1]



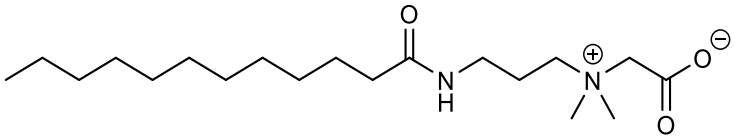
Anionic - Sodium dodecyl sulfate (SDS)



Cationic - Cetrimonium bromide (CTAB)



Nonionic - Octaethylene glycol monododecyl ether (C12E8)



Amphoteric - Cocamidopropyl betaine (CAPB)

Figure 1.2 Examples of surfactant structures.

Builders are the second most important ingredient of detergent because they enhance or "build" the cleaning efficiency of the surfactant. Builders are designed to:

- Soften water by binding the hard water minerals (Ca^{2+}, Mg^{2+})
- Help surfactant concentrate on removing soil from fabrics
- Increase the efficiency of the surfactant
- Provide a desirable level of alkalinity to aid the cleaning process
- Disperse and suspend soils so they cannot redeposit themselves on the clothing

Modern examples of builders include: citric acid, gluconic acid, ethylenediaminetetraacetic acid (EDTA) or ion exchange agents such as zeolites. [7]

Laundry is usually performed in alkaline pH (usually pH 10.5), which has several advantages and can be created by alkaline agents such as sodium carbonate or silicate. The basic condition in a cleansing

solution is useful to give negative charges to the soils and the substrates. Oily soils containing fatty acids can be spontaneously removed in alkaline solutions owing to the formation of soaps (sodium or potassium salts of higher fatty acids) in the dirt.[8]

Bleaching agents and activators are used to make white clothes clean. In addition, oxygen-based bleach provides detergents with an all-fabric bleaching agent for stain and soil removal. The bleach system acts via aggressive oxidation chemistry, generating ROS that trigger degradation reactions. Unfortunately, the bleach system is not able to differentiate between unwanted food or dirt residues and certain synthetic textile dyes. Very often the bleach system attacks both in parallel, leading to colour fading which becomes visible after a few washing cycles. In the worst case, the bleach system may also react with the cellulose of cotton fibers leading to significant damage and destruction of the garment.[9]

Enzymes are now very commonly blended into detergent formulations. Enzymes aid in breaking down complex soils, especially proteins such as found in blood or grass, so they can be more easily removed from fabrics. Protease, lipase and amylase are used to digest the soils of protein, lipids and starch respectively.

Fragrances do not playing a direct role in soil removal, but are able to provide some benefits. They can cover the chemical odour of the detergent and odour of soils in the washing solution. In addition, they impart a pleasant scent to fabrics, thus reinforcing the perception of cleaning performance.

Laundry detergents may also contain many other components than listed above, i.e. corrosion inhibitors, processing aids, colourant or optical brighteners.[3] Optical brighteners (fluorescent whitening agents) are chemicals that absorb light in the ultraviolet and violet region (usually 340-370 nm), and re-emit light in the blue region (typically 420-470 nm) by fluorescence. These additives are often used to enhance the appearance of colour of fabric, causing a whitening effect, they make intrinsically yellow or orange materials look less so, by compensating the deficit in blue and purple light reflected by the material, with the blue and purple optical emission of the fluorophore.[3] Is it important to emphasize that the main focus of this thesis are the bleaching agents and they are discussed in more detail in subsequent part of this work.

1.3 Short history of bleaching systems

Up to the 18th century, a hard soap was a basic product used for personal hygiene, laundry and medicinal purposes. The essential recipe included boiling together vegetable and/or animal fats with alkaline salts; potash (potassium carbonate) or soda ash (sodium carbonate). The alkaline salts were usually sourced from burning wood and trees.[10] In the 19th century, soap powders and flakes became available; however these products were mostly pulverized soap, sometimes mixed with soda ash, and therefore not detergents. Additives began to appear which improved performance. These were used alongside, or sometimes incorporated into, the soap products. [11]

In 1878 the German manufacturer Henkel released a product known as *Bleich-Soda* (washing soda), which contained sodium carbonate (soda) and sodium silicate (water glass), to the German market.[12] When used with soap this system prevented the build-up of water-insoluble soap residues; a problem which arose in areas of hard water.[8] Despite the name, *Bleich-Soda* did not actually contain bleach. The first detergent to contain bleach was *Persil*, which was released by Henkel in 1907 (also in Germany, Figure 1.3).[13] *Persil's* main ingredients were soap, sodium carbonate, sodium silicate and hydrogen peroxide – in the form of sodium perborate tetrahydrate. The name *Persil* stemmed from the two active ingredients 'per' from perborate, and 'sil' from silicate. Use of *Persil* eliminated the need for clothes to be scrubbed by hand or hung outside for prolonged periods to be bleached by the sun.

Figure 1.3 *Bleich-Soda* from 1878 and *Persil* from 1907 packages. [12,14]

When *Persil* was introduced in 1907, laundry washes in Europe were typically carried out at 95 °C, and under these conditions sodium perborate was an efficient stain remover. However, in the USA, washing conditions were milder with lower wash temperatures (40-60 °C) and shorter wash times

(10-20 min). Under these conditions, sodium perborate was ineffective.[15] Instead, sodium hypochlorite solution became the traditional bleaching agent. In the 1910s and 1920s, dilute sodium hypochlorite solutions were developed and launched in the USA for use in household laundry applications. Due to the compatibility issues with other detergent components (such as fluorescent dyes and enzymes), sodium hypochlorite solutions were sold as a separate product and added separately to the wash process. In the 1950s, products containing dry sources of hypochlorite were introduced, but these disappeared by the late 1960s, most likely due to lower efficiency than their liquid counterparts and claims of their causing fabric damage.[16]

Although a cost-effective, safe and environmentally sound source of bleaching, hydrogen peroxide has one significant disadvantage for today's laundry applications: it has a reduced effectiveness at lower temperatures. During the 1970s, the combination of the need for energy efficiency and the increased usage of synthetic fibers in Europe brought about a downward trend in wash temperatures. Instead of the traditional boil wash, washing was now commonly done at 40-60 °C. Moreover in North America, domestic laundry conditions are quite unlike those in Europe. Dilutions are greater and wash temperatures are on average *ca.* 40 °C. If bleach-systems based on hydrogen peroxide were going to remain effective under European conditions and also challenge the market dominance of hypochlorite bleaches in North America, some way would have to be found to boost their performance. [17]

The earliest reference to bleach activation dates back to the 1920s, when two patents emerged regarding the use of mixtures of compounds for bleaching foodstuffs. [18,19] These patents claimed that fatty acid peroxides derived from oils (such as coconut) could act as bleaching agents, and could also 'act as activators' for other organic peroxides (such as benzoyl peroxide), increasing the overall bleaching efficiency of those peroxides. In particular, higher fatty acids such as the 'soap-forming fatty acids' were found to work best. Three decades later, organic nitriles (1953) and aliphatic carboxylic acid amides, *e.g.* RC(O)NR'R", (1955) were discovered to give improved bleaching at lower temperatures (60-70 °C) when combined with sodium perborate.[20,21] The bleaching performance attained at this temperature was as good as could be achieved at 95-100 °C using perborate alone. It was proposed that the amide 'activators' were able to increase the amount of oxygen liberated by the perborate. In 1959, Unilever revealed that acyl organoamides, *e.g.* R'C(O)NHR", also had an 'activating effect' on the bleach activity of inorganic persalts. [22] The proposed mechanisms suggested that the persalt releases hydrogen peroxide, which then reacts with the amide forming a new bleaching species which is more active than hydrogen peroxide, although no suggestions were made regarding

the nature of this species. This new bleach was found to be efficient at temperatures 50-60 °C. Further work continued into compounds which could 'activate' hydrogen peroxide at lower temperatures.

Activated bleach systems were far superior to simple persalt systems and, from the 1970s, 'activating compounds' began to be produced on a commercial scale and included in detergent formulations. The first bleach activator to be incorporated in a *Cid* detergent in 1972 by Henkel was tetraacetylglycoluril (TAGU, Figure 1.4). Biodegradation problems have prevented its wider commercial exploitation. [7]

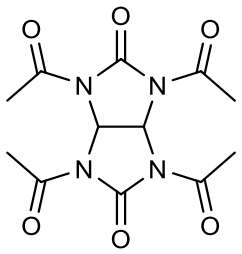
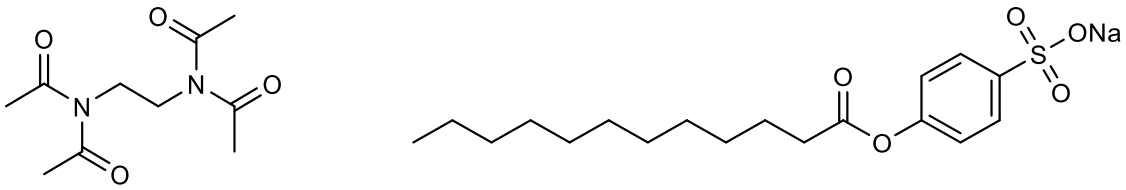


Figure 1.4 Structure of TAGU.

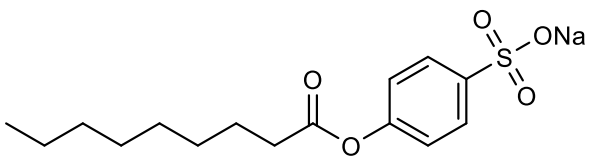
Up to 1990, when the presence of a 'new' oxidation species was experimentally proven, the chemistry of bleach activators was not completely understood. Experiments showed the presence of peracetic acid formation in a solution of sodium perborate and the bleach activator - tetraacetylethylenediamine (TAED, Figure 1.5) and the occurrence of perhydrolysis reaction was proven for the first time.[23] The chemistry of bleach activators and perhydrolysis reactions are discussed in more detail in section 1.4.3.

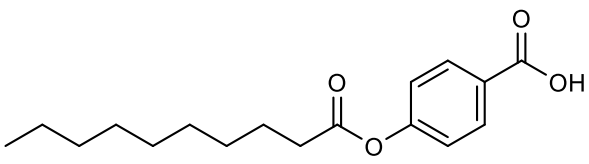
Despite the large number of bleach activators that have been investigated and patented, only a few are used commercially. Currently, there are four main activators which are used in commercial products across the globe: tetraacetylethylenediamine (TAED, Europe), sodium nonanoyloxybenzene sulfonate (NOBS, America and Asia), sodium lauroyloxybenzene sulfonate (LOBS, Asia) and decanoyloxybenzoic acid (DOBA, Asia) (Figure 1.5), although 98% of the market value is accounted for by the first two. [9]



Tetraacetylethylenediamine (TAED)

Sodium lauroyloxybenzene sulfonate (LOBS)





Sodium nonanoyloxybenzene sulfonate (NOBS)

Decanoyloxybenzoic acid (DOBA)

Figure 1.5 Currently used bleach activators.

Nowadays, on a global basis about 30% of all detergents contain a built-in bleach system. That corresponds to around 750,000 tons of bleaching agents based on active oxygen consumed worldwide per year. For several reasons the remaining market cannot be reached with current technology. In Europe, there is a general trend for energy savings, e.g., shorter washing cycles. Furthermore, in many countries the washing is done at ambient temperature. Under both conditions TAED and NOBS are not very effective bleach activators. In parallel, liquid detergents are gaining in importance. In such formulations, current bleach systems are not stable to storage.[9]

1.4 Bleaching systems

1.4.1 Hydrogen peroxide

Hydrogen peroxide – H_2O_2 is the simplest peroxide (Figure 1.6) and it is used widely as an oxidizer, bleaching agent and disinfectant. The chemistry of hydrogen peroxide is dominated by the nature of its unstable oxygen-oxygen bond.

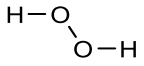


Figure 1.6 Structure of hydrogen peroxide.

As mentioned before, the bleach-active ingredient in *Persil* was sodium perborate, which is a class of compound known as a persalt or solid hydrogen peroxide carrier. Persalts release hydrogen peroxide when they come into contact with water. Pure hydrogen peroxide is a pale blue, viscous liquid and is extremely unstable and reactive, but is typically sold either as a persalt or an aqueous solution (up to 70% w/v as more concentrated solutions are hazardous).[16] In all forms, hydrogen peroxide is thermodynamically unstable and undergoes a disproportionation reaction, evolving oxygen and generating heat (Eq. 1.1 and 1.2). [24]

1.1 $$2\,H_2O_2\,(g) \rightarrow 2H_2O(g) + O_2(g);\ \Delta H = -105.8\ \text{kJ mol}^{-1}$$

1.2 $$2\,H_2O_2\,(l) \rightarrow 2H_2O(l) + O_2(g);\ \Delta H = -98.3\ \text{kJ mol}^{-1}$$

Hydrogen peroxide is kinetically stable in the absence of catalyst or other accelerating factors (e.g. light, heat), and strict storage conditions (typically involving radical scavengers, the minimization of transition metal ion sources, and an acidic pH) can minimize decomposition. However, even low levels of decomposition generates oxygen, which can pressurise sealed equipment and create 'oxygen enrichment' in the headspace of the container. [25]

Hydrogen peroxide is weakly acidic in aqueous solution, with a dissociation constant of $1.78 \cdot 10^{-12}$ (pK_a 11.75) at 20 °C. As a weak acid, hydrogen peroxide forms salts with various metals.

Hydrogen peroxide can behave as an oxidising and as a reducing agent (Table 1.1, standard potential of redox reaction measured against a hydrogen electrode (25 °C, 100 kPa)). Systems with a redox potential $E_0 < -1.80$ V at pH 0 cannot be oxidised by hydrogen peroxide and systems with a redox potential $E_0 > -0.66$ V at this pH cannot be reduced by hydrogen peroxide.

		Redox reaction	Standard potential E_0
Oxidising agent	pH 0	$HOOH + 2H^+ + 2e^- \rightarrow 2\,HOH$	+1.80
		$HSO_3^- + HOH \rightarrow SO_4^{2-} + 3H^+ + 2e^-$	-0.17
		$NO_2^- + HOH \rightarrow NO_3^- + 2H^+ + 2e^-$	-0.94
		$2Cl^- \rightarrow Cl_2 + 2e^-$	-1.36
		$2Br^- \rightarrow Br_2 + 2e^-$	-1.07
		$2I^- \rightarrow I_2 + 2e^-$	-0.54
	pH 14	$HOOH + 2e^- \rightarrow 2OH^-$	+0.87
		$Mn(OH)_2 + 2OH^- \rightarrow MnO(OH)_2 + HOH + 2e^-$	+0.05
Reducing agent	pH 0	$HOOH \rightarrow 2H^+ + O_2 + 2e^-$	-0.66
		$5e^- + MnO_4^- + 8H^+ \rightarrow Mn^{2+} + 4HOH$	+1.51
		$e^- + Ce^{4+} \rightarrow Ce^{3+}$	+1.61
	pH 14	$HOOH + 2OH^- \rightarrow 2HOH + O_2 + 2e^-$	+0.08
		$e^- + ClO_2 \rightarrow ClO_2^-$	+1.16
		$2e^- + ClO^- + HOH \rightarrow Cl^- + 2OH^-$	+0.89

Table 1.1 Hydrogen peroxide as an oxidizing and reducing agent.[26]

Hydrogen peroxide is used in the textile industry for bleaching cotton, linen, wool, silk, polyester fiber, and polyurethane fiber. In the pulp and paper industry, it is used to bleach sulfate and sulfite cellulose, wood pulp, and wastepaper, and to brighten wood veneers and wooden structures. The chemical industry employs hydrogen peroxide for the production of peroxy compounds such as sodium perborate, sodium percarbonate, metallic peroxides, or percarboxylic acids.[27] Hydrogen peroxide is very important in organic chemistry for epoxidation and hydroxylation (manufacture of plasticizers and stabilizers for the plastics industry), oxidation (manufacture of amine oxides as surfactants for detergents), oxohalogenation, and initiation of polymerization.[25]

When sodium perborate was first included in detergents, it was used in the form of sodium perborate tetrahydrate. A second grade, known as sodium perborate monohydrate, became available later. The two grades differ by the number of waters of crystallization present and dissolution rates, with a faster dissolution profile in the case of monohydrate. However, both names are misleading as they are not directly related to the composition, due to an inaccurate initial understating of the structures. When the tetrahydrate was first prepared, its structure was reported as $NaBO_3 \cdot 4H_2O$. However in 1961, the

crystal structure was determinated [28], and shown to be disodium salt of the 1,4-diboratetrooxane dianion $[Na_2B_2(O_2)_2(OH)_4 \cdot 6H_2O]$ (Figure 1.7). The IUPAC name is sodium tetrahydroxo-di-μ-peroxodiborate (III) hexahydrate, however the commercial product is still referred to as sodium perborate tetrahydrate as its molecular structure is almost always reported as $NaBO_3 \cdot 4H_2O$, with an associated molecular mass of 154.[15]

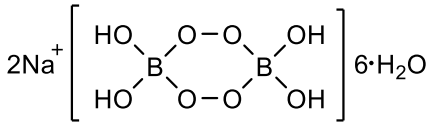


Figure 1.7 Structure of sodium perborate tetrahydrate.

Sodium perborate monohydrate was later prepared by dehydration of the tetrahydrate form. The nomenclature of the monohydrate is also inaccurate, and is based on the structure incorrectly being described historically as $NaBO_3 \cdot H_2O$. The crystal structure of the monohydrate has not been determined, but it is believed to contain the same cyclic structure as the tetrahydrate (Figure 1.7), and does not have any water of crystallisation. The monohydrate has a faster dissolution profile compared to the tetrahydrate and this advantage increased the use of the monohydrate over the tetrahydrate. Both forms of sodium perborate are stable and can be introduced to the wash with the detergent. On coming into contact with water, sodium perborate hydrolyses to form hydrogen peroxide and sodium borate (Eq.1.3).

1.3 $$[(HO)_2B(\mu O_2)_2B(OH)_2]^{2-} + 4H_2O \rightarrow 2[B(OH)_4]^- + 2H_2O_2$$

In the 1990s, a second persalt, sodium percarbonate (SPC), began to replace sodium perborate in laundry detergents, due to the environmental concerns over ecotoxicity of boron products.[29] Sodium percarbonate ($2\,Na_2CO_3 \cdot 3\,H_2O_2$) is an adduct of sodium carbonate and hydrogen peroxide. [24] When SPC is dissolved in water, it yields a mixture of hydrogen peroxide and sodium carbonate (Eq.1.4).

1.4 $$2\,Na_2CO_3 \cdot 3\,H_2O_2 \rightarrow 2\,Na_2CO_3 + 3\,H_2O_2$$

Unlike sodium perborate, sodium percarbonate contains no peroxocarbonate bonds (C-O-O-C).[30] In sodium percarbonate the hydrogen peroxide is hydrogen bonded to the carbonate ions and held loosely bound in a crystal lattice.[31] This makes it inherently less stable than the perborate. SPC releases hydrogen peroxide in water at least as quickly as sodium perborate monohydrate, which is advantageous, but requires more protection in granular form to improve its stability than sodium perborate. [11] SPC is generally considered to be an eco-friendly and relatively gently bleaching agent.

At typical wash temperatures sodium percarbonate has a minimal effect on fabric and dyes, making it the preferred choice of primary bleach in the global laundry market.

1.4.2 Peroxy acids

As mentioned before the active species generated by reaction of hydrogen peroxide and bleach activators are peroxy acids, which have superior cold water bleaching capability compared to hydrogen peroxide because of the greater electrophilicity of the peracid peroxygen moiety. [32]

Peracids are compounds containing the functional group –C(O)-OOH derived from an organic or inorganic acid functionality. An example of a simple peracid is peracetic acid (PAA, pK_a 8.2, Figure 1.8). Formally, organic peroxides can be regarded as derivatives of hydrogen peroxide in which the hydrogen atoms are substituted by various organic groups.

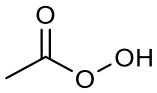


Figure 1.8 Structure of peracetic acid.

Short-chain aliphatic peroxycarboxylic acids are miscible with water and possess an unpleasant, pungent odour. They are explosive when pure or highly concentrated. The tendency to explode decreases with increasing chain length. Long-chain peroxycarboxylic acids (> C_6) are increasingly water insoluble solids. Aromatic peroxycarboxylic acids are only slightly soluble in water; both types, are however, soluble in various organic solvents. Peroxycarboxylic acids exhibit pKa values that are 3-4 units greater than those of the corresponding carboxylic acids due to the absence of resonance stabilisation in the peroxycarboxylate anions. As a result of the weak substituent effect, the pKa values all lie in a small range (pK_a ~ 7-10). [32]

Peroxy acids have the highest oxidation potential of all organic peroxides, whereby the corresponding carboxylic acid is formed with release of active oxygen. The stability of aqueous solutions of peroxycarboxylic acids depends on the pH. Bases cause decomposition, whereas in acid media an equilibrium is established (Eq. 1.5). [33]

1.5 $$RCO_3H + H_2O \rightleftharpoons RCO_2H + H_2O_2$$

The peracids may decompose by a number of routes (Figure 1.9).

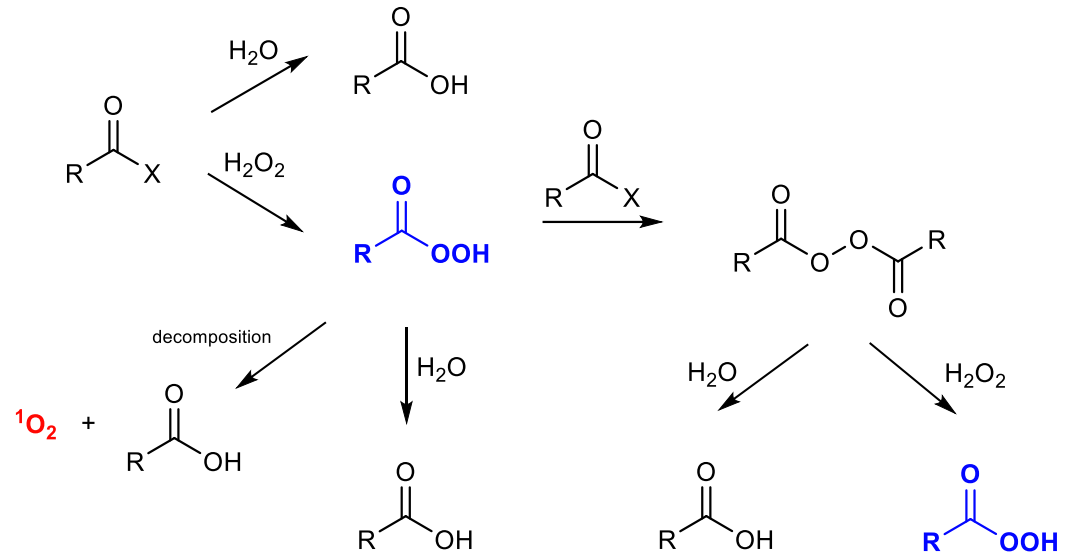


Figure 1.9 Routes of peroxyacid decompositions.

The peracid may react with another molecule of activator, generating a diacyl peroxide. Diacyl peroxides have not generally been thought of as contributors to bleaching action since they break down *via* short-lived radical species. Diacyl peroxides may be perhydrolysed back to peracids but this reaction is slower than their rate of formation and results in the decomposition of peracids. Additionally, the peracid may hydrolyse to its carboxylic analog and may also decompose spontaneously to singlet oxygen and carboxylic acid. Hydrolysis is obviously undesirable, and free radical decomposition is not recognized as being an effective bleach mechanism. The activator may also undergo hydrolysis rather than perhydrolysis to give a carboxylic acid, again an undesirable reaction.[18]

Homolytic cleavage of the O-O bond, readily induced by light or heat, is a typical reaction of organic peroxides. The reactivity depends strongly on the structure of the organic compound. The largest use of peroxycarboxylic acids is in the production of epoxides. Compounds with an isolated double bond that cannot be oxidized with hydroperoxides or hydrogen peroxide in the absence of a catalyst are epoxidized smoothly and stereospecifically by peroxycarboxylic acids. Cyclic ketones, such as cyclohexanone, are converted to lactones; the production of ε-caprolactone, an important intermediate for the production of polyesters and polyurethanes, is carried out on an industrial scale using this reaction. Phenols can be hydroxylated with peroxycarboxylic acids to give dihydroxybenzenes. Amine N-oxides and -sulfoxides, obtained by reaction of peroxycarboxylic acids with amines and sulfides are used as detergents and in the pharmaceutical industry. [32]

Peroxycarboxylic acids have a wide range of applications in preparative and industrial chemistry on account of their high oxidation potential. Due to their instability some peroxyacids are generated in situ.

1.4.3 Bleach activators

Peracids can be introduced into the bleaching system by two methods. They can be manufactured separately and delivered to the bleaching bath with the other component or as a separate product. Peracids can be also be formed *in situ* utilizing the perhydrolysis reaction between activator and hydrogen peroxide (Figure 1.10).

Figure 1.10 Perhydrolysis reaction.

R can be a variety of groups. The leaving group X^- is typically the conjugate base of a weak acid. The hydrogen peroxide is typically incorporated into the wash liquor bath by adding a solid source of peroxide such as sodium percarbonate or the mono- or tetrahydrate of sodium perborate. It was realized in 1990 that only the perhydroxyl anion, and not the hydrogen peroxide molecule, reacts with the activator, since the rate of peroxyacid formation was observed to increase with increasing alkalinity [23], as a consequence of the equilibrium presented in Eq.1.6. [34]

1.6 $$H_2O_2 \rightleftharpoons H^+ + [HO_2]^-, \text{ pK}_a\ 11.64$$

The rate of formation of peracids (PA) by perhydrolysis may be expressed by the Eq.1.7, where k_{ph} = perhydrolysis rate constant, k_h = hydrolysis rate constant, and k_d = decomposition rate constant (this has multiple components) and A is the activator.

1.7 $$\frac{d[PA]}{dt} = k_{ph}[A][H_2O_2] - k_h[A] - k_d[PA]$$

The perhydrolysis reaction is second-order although under more concentrated 'European conditions', it is pseudo-first-order.[35] As with the rate of bleaching, the perhydrolysis rate will decrease with decreasing concentration and decreasing temperature. However, one effect of lower wash temperatures is that decomposition processes are slowed, leading to longer lifetime of the peracid. [17]

Bleach activators are essentially acylating agents and consist of an acyl moiety to which is attached a leaving group of some sort. They usually take the form of imides or esters, examples of which are TAED, and NOBS respectively. Properties of the activators are modified by alteration of acyl moiety (peroxy acid precursor) and the leaving group. The peroxy acid precursor affects the bleaching properites of the peroxy acids: determining activity, selectivity, hydrophilic/lipophilic balance, and oxidation potential. The leaving group influences the solubility, perhydrolysis rate, and storage stability of the activator. [36]

A number of advantages of an activated bleaching systems were identified by Croud.[37] Firstly, an activated system allows bleaching to occur at lower temperatures, which is more energy efficient, better for the environment, and maintains clothes longer. The activated system also provides low temperature sanitisation: peroxy acids reduce the level of microorganisms present, minimizing the spread of disease and infections. Activated systems also attack microbes and inhibit bacterial growth. This has a number of follow up benefits: inhibition of odours caused by a high microbial loading on fabric (the musty smell of damp laundry); inhibition of fabric damage caused by microorganisms; inhibition of microbially-induced washing machine corrosion (some bacteria liberate ammonia which erodes aluminium). Activated oxygen systems are also advantageous when compared with chlorine-based bleaches as these are incompatible with many additives in the detergent formulation such as optical brighteners and enzymes, both of which are oxidized by chlorine bleach but are affected to a much lesser extent by oxygen bleaching systems.

1.4.3.1 Tetraacetylethylenediamine (TAED)

TAED, a crystalline amide, is a hydrophilic bleach activator that is the main activator used in European detergents.[38] In aqueous alkaline solution, the perhydroxyl anion reacts with TAED to form triacetylethylenediamine (TriAED) and diacetylethylenediamine (DAED) consecutively, and releases (approximately) two equivalents of peroxyethanoic acid (Figure 1.11). TAED, TriAED and DAED are all completely biodegradable and substantially removed during wastewater treatment.[39]

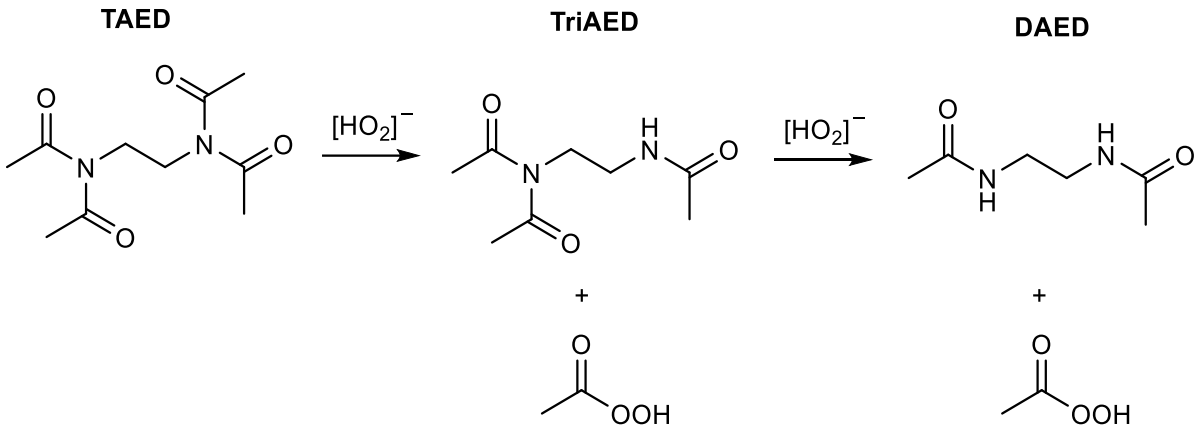


Figure 1.11 Perhydrolysis reaction of TAED.

Theoretically, the perhydrolysis of TAED could yield four equivalents of peroxyethanoic acid per mole of TAED, as there are four acyl groups present. However, a molecule of TAED can only provide a maximum of two equivalents of peroxyethanoic acid due to the substantial increase in the conjugate acid pK_a of the leaving group going from an amide (pK_a 17) to an amine (pK_a 35).[16] However, due to a competing reaction of TAED with hydroxide anions (saponification), the full two equivalents are not quite released, and various values have been measured *e.g.* 1.5 eq. [23] or 1.7 eq. [40] and reported.

The benefits of TAED are lost at temperatures of > 60 °C due to the activity of the persalt at higher temperatures, combined with the thermal instability of peroxyethanoic acid. [37]

TAED gives a highly favourable perhydrolysis-to-hydrolysis ratio, which has been attributed to the formation of hydrogen bonds between TAED and hydrogen peroxide during perhydrolysis, stabilising the transition state and favouring perhydrolysis.[32] Moreover, TAED does not form diacyl peroxides.

1.4.3.2 Nonanoyloxybenzene sulfonate (NOBS)

NOBS is a hydrophobic bleach activator that was developed by Procter & Gamble in 1983.[41] Its structure is designed to give it a degree of hydrophobicity for easier approach of the bleach to oily soils at the fabric surface. This is achieved by an optimal chain length in the C8 - C10 range.[38]

Once delivered in the alkaline wash, the hydroperoxide anion attacks NOBS to produce pernonanoic acid (PNA), which exists mostly deprotonated at a wash pH of 10.5, and hydroxybenzene sulfonate leaving group (Figure 1.12).

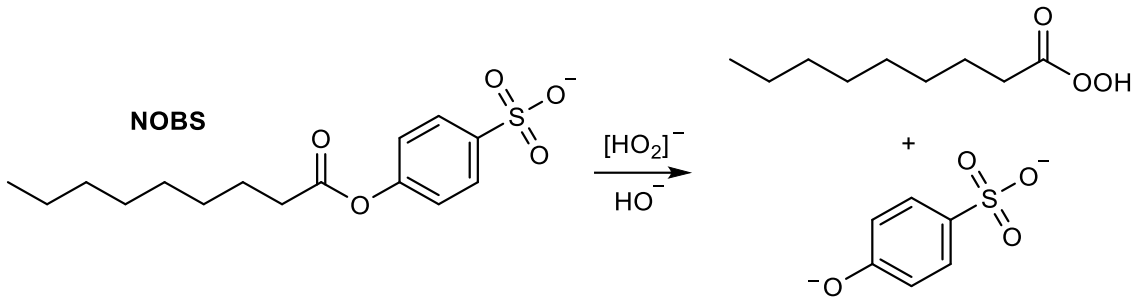


Figure 1.12 Perhydrolysis reaction of NOBS in pH 10.5.

An important side reaction is the nucleophilic attack of the pernonanoic acid anion with a second NOBS molecule, producing di-*n*-nonanoyl peroxide (generically 'DAP' for diacyl peroxide).This is a second active bleaching agent and can provide bleaching benefits on different stain types than pernonanoic acid (Figure 1.13).[4]

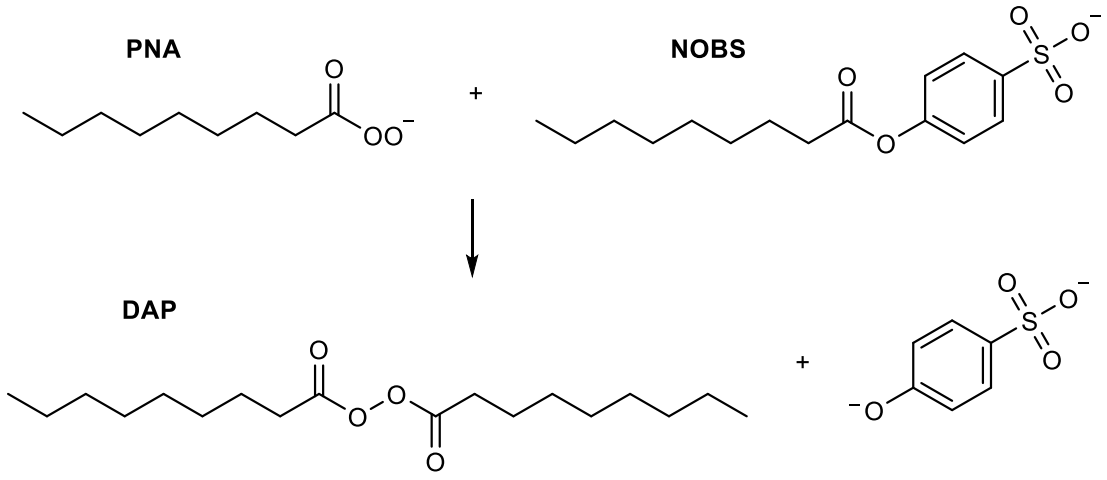


Figure 1.13 The formation of DAP.

The formation of a small amount of DAP is beneficial, but too much is considered a waste of NOBS. The degree of DAP formation to NOBS can be controlled by manipulating the persalt to NOBS ratio and the wash pH; high persalt ratios and high pH disfavour DAP formation, as perhydroxyl anion formation is favoured and the excess of NOBS is minimised. If excess persalt is not used, the yield of peroxynonanoic acid falls dramatically and ratios of persalt/NOBS of at least 3:1 are recommended. NOBS can also hydrolyse to sodium nonanoate and sodium hydroxybenzene sulfonate in alkaline solution.[11]

Pernonanoic acid is effective at bleaching the chromophores in certain stains. Some stains where this effect is noticeable are grass, tea, wine, and grape juice. Equally important, DAP can effectively decolourise tomato-based stains such as spaghetti and barbecue sauce, possibly via the radical mechanism (Figure 1.14). [4]

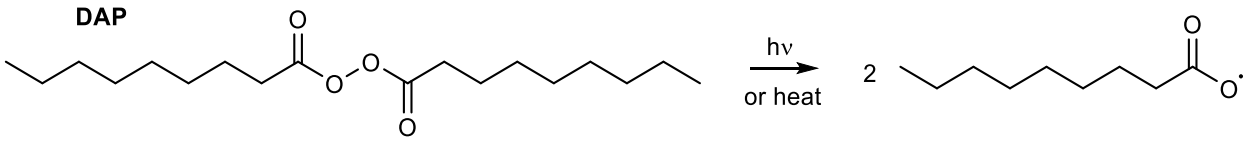


Figure 1.14 Decomposition of the DAP.

1.4.4 Preformed peroxy acids

Some peracids can be generated at a manufacturing site and be directly incorporated into formulations without the need for *in situ* generation. This approach removes the need to add a source of hydrogen peroxide, saving material space and reducing waste. Preformed peroxy acids would also avoid the extra mass associated with the leaving group of a bleach activator. However, in practice, there are many technical challenges encountered in the use of preformed peroxy acids, including thermal instability, product compatibility and cost which have limited their use.

The incorporation of a polar functional group such as an amido or imido linkage into an alkyl peroxycarboxylic acid was found to increase the thermal stability of peroxy acids. One successful example of this is 6-(phthalimido)peroxyhexanoic acid (PAP, Figure 1.15) which is a white, odourless powder.[32]

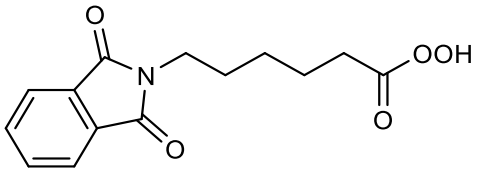


Figure 1.15 Structure of PAP.

Hydrogen bonding results in infinite chains of molecule in the solid state, and provides stability.[42] Even in the pure state PAP is not explosive. Optimum bleach activity is achieved at 20-50 °C and pH 7-10, which correlates well with current washing conditions. PAP is only sparingly water-soluble (0.2 g L^{-1} at 20 °C, pH 4.5), allowing stable aqueous dispersions to be prepared in an acidic pH range.

The solubility of PAP improves with increasing pH and temperature: at pH 10 and 20 °C, 80% of PAP dissolves within 5 min, so it functions well under typical alkaline washing conditions. [23] Under alkaline conditions, the phthalimido ring may open irreversibly. At pH 9.2 and 40 °C, the half-life of PAP is 20 min. The ring-opened peroxy acid is considerably less active as a bleach due to the negative charge on the ring.[19] Overall, the issues highlighted above, including the thermodynamic instability of peroxy acids, have focused research into developing bleach activators which form the peroxy acid *in situ* in the wash. When bleach activators are used a source of hydrogen peroxide must also be included in the formulation.

1.4.5 Other ROS relevant in laundry applications

Hydrogen peroxide and peroxy acids are primary oxidants in the laundry products, however one might also expect the presence of other ROS in the laundry process. Hydroxyl radicals can be present in the wash solution due to Fenton chemistry with traces of transition metal ions present in tap water, or be an important intermediate species during the photodegradation of the fabrics when they are exposed to the sunlight. Singlet oxygen is a peroxy acid decomposition product, and can also be generated during drying the fabric outside by special photobleaching agents, which have become a more and more popular additive to the laundry detergents, especially in those parts of the world where the wash is carried in cold water or is done by hand and drying is done outside in sunlight. [43]

In theory, superoxide $O_2^{\bullet-}$ and its protonated form hydroperoxyl radicals $HOO^{\bullet}$ might be present as well; however they will not be further discussed in that work.

1.4.5.1 Hydroxyl Radicals

The hydroxyl radical is a non-selective oxidant than can be generated from a variety of sources, including photolysis of nitrate and nitrite ions (Eq. 1.8),[44] transition metal ions (e.g. Fe^{2+}, Co^{2+},Cu^{+}), and Fenton-type reactions between peroxides and transition metal ions (Eq. 1.9).[45]

1.8 $$NO_3^- + h\nu_{254\,nm} \xrightarrow{H_2O} NO_2 + OH^- + HO^{\bullet}$$

1.9 $$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH^- + HO^{\bullet}$$

All natural and synthetic textile fibers undergo some degree of photodegradation when exposed to sunlight in presence of oxygen. The most affected parameters of the fabrics are: abrasion resistance,

tensile strength, elasticity and colour. For undyed white fabrics, direct sunlight containing UV wavelengths down to 285 nm causes photoyellowing, but exposure to sunlight filtered through window glass, which removes much of the high-energy UV (<350 nm), can result in photobleaching.[46]

A generally accepted mechanism of polymer photodegradation involves the formation of free radicals as a consequence of the excitation of chromophores, particularly in the UV region.[47] These chromophores can be either part of the chemical structure, for example the benzoyl group in polyester (polyethylene terephthalate), or small concentrations of impurities capable of sensitizing photooxidation, such as the lignin present in cotton that is not part of the cellulose chains. The aromatic amino acids residues tyrosine (λ_{max} = 275 nm) and tryptophan (λ_{max} = 280 nm) are the most significant chromophores in wool and silk fibers. In nylon the NHCO group can absorb below 340 nm, and absorption at longer wavelengths has been attributed to excitation of chromophores present as structural defects in the polymer chain or to impurities. The presence of trace amounts of certain metal ions has been shown to significantly increase the production of free radicals in irradiated polymers. [48]

Once free radical species are formed via photochemical initiation, they react rapidly with molecular oxygen and initiate the polymer photodegradation chain. During the propagation stages significant levels of polymer hydroperoxides are produced. In the presence of transition metal ions or light, hydroperoxides can decompose to produce aggressive hydroxyl radicals. Another suggested route to hydroxyl radicals is via the superoxide radical anion, which undergoes dismutation to produce hydrogen peroxide. This can also form hydroxyl radicals through the Fenton reaction in the presence of trace metal ions and through direct photolysis of hydrogen peroxide.[47]

1.4.5.2 Singlet oxygen

Ground state molecular oxygen exists as a triplet state ($^3\Sigma_g^-$) with the lowest lying excited state of oxygen being a singlet state [O_2 ($^1\Delta_g$) or $^1O_2$] that lies 94 kJ/mol above the ground state.[49] The singlet state can be generated in solution by energy transfer from excited photosensitizers (S, e.g., Rose Bengal, Eq. 1.10); or chemically, for example via the reaction between hypohalites and hydrogen peroxide (Eq. 1.11)[50,51]

1.10 $$S \xrightarrow{h\nu} S^* \xrightarrow{O_2} S + {}^1O_2$$

1.11 $$OCl^- + H_2O_2 \rightarrow Cl^- + H_2O + {}^1O_2$$

Singlet oxygen is an electrophilic agent, and is able to oxidize unsaturated double bonds, sulfide,phenolic, and amino groups as well as other electron-donor groups in organic compounds. The lifetime of singlet oxygen in aqueous solution is constrained through quenching by water with its lifetime in pure water being ~4 μs.[52]

Peroxycarboxylic acids decompose spontaneously in aqueous solution to give the corresponding carboxylic acids and oxygen; the reaction can be regarded as a nucleophilic attack of the anion upon the peroxy acids (Figure 1.16).[53,54] The decomposition is sensitive to the presence of transition metal ions, such as Co^{2+}, but their effect can be largely eliminated by adding a chelating agent.[55,56]

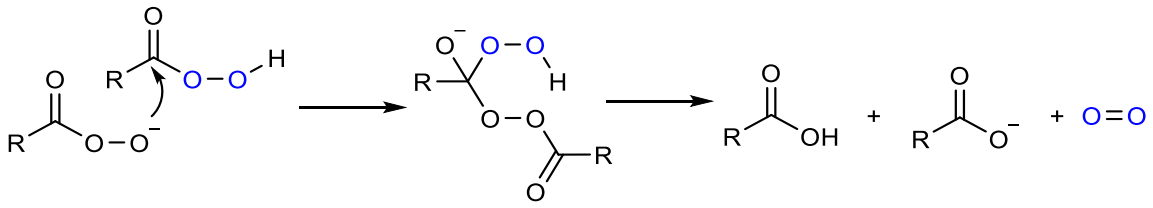


Figure 1.16 Decomposition of peroxycarboxylic acid.

On the basis of conservation of spin, the mechanism of decomposition of peroxocarboxylic acid might be expected to give singlet oxygen, rather than ground state oxygen; this was proven in 1985 by Evans and Upton.[57] During the experiment they obtained essentially quantitative yields of singlet oxygen from decomposition of peracetic acid in pH = pK_a = 8.2.

1.4.6 Mechanism of bleaching

Bleaching is a term to describe a decolourisation or whitening process that can occur in solution or on a surface. The colour-producing materials in solution or on fibers are typically organic compounds that possess extended conjugated chains of altering single and double bonds and often include heteroatoms, carbonyl groups, and phenyl rings. The portion of molecule that absorbs a photon of light is referred to as the chromophore. For a molecule to produce colour, the conjugated systems must result in sufficiently delocalized electrons such that the energy gap between the ground and excited states is small enough so that photons in the visible portion of the light spectrum are absorbed.

Bleaches remove a coloured stain by altering the stain chromophore so that is no longer coloured. This action may also break up the stain molecule into smaller, more soluble pieces, which can then be removed by surfactants/wash liquor, or simply make the stain less apparent on the fabric.

Bleaching and decolourisation can occur by destroying one or more of the double bonds in the conjugated chain, by cleaving the conjugated chain, or by oxidation of one of the other moieties in the conjugated chain.[16] Stain chromophores are large structures with long stretches of conjugation, they have multiple points of attack. Peroxy species can break up conjugation by a number of different routes:

- Addition of oxygen across a double bond to give an epoxide
- Oxidation of aldehydes to acids
- Oxidation of sulfur compounds to sulfoxides and sulfones
- Oxidation of nitrogen compounds to amine oxides, hydroxylamine, or nitro compounds
- Oxidation of α-diketone compounds to anhydrides
- Oxidation of ketones to esters

The results of any one from the listed reactions is an increase in the energy gap between the ground and excited states, so that the molecule then absorbs light in the ultraviolet region, and no colour is produced. Bleaching by perhydroxyl anions or peroxy acid anions proceeds via nucleophilic attack on electrophilic centers (e.g. alkene groups, Figure 1.17). [58,59]

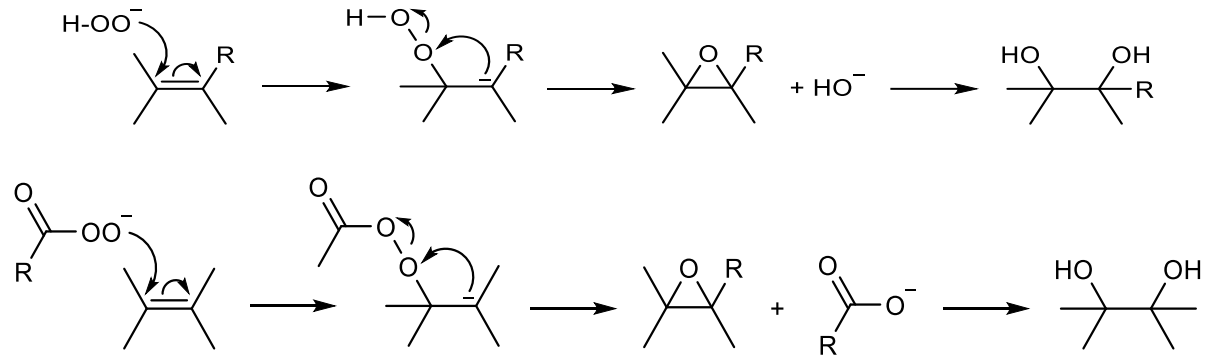


Figure 1.17 Bleaching by perhydroxyl ion and peroxy acid anion through nucleophilic attack on an alkene group. R is an electron-withdrawing group.

1.5 Detection of Reactive Oxygen Species

1.5.1 Reactive Oxygen Species (ROS) and their detection

Although oxygen it is remarkably unreactive, it is the substrate for the generation of a variety of reactive oxygen species (ROS). ROS are, generally speaking, oxygen molecules or some compounds of oxygen, which as their name implies, present higher reactivity than molecular oxygen.

The most important molecules with oxidizing capacity in laundry applications are: hydroxyl radicals, singlet oxygen, hydrogen peroxide and peroxyacids (Figure 1.18). For the purposes of this review, peroxyacids will be included as a ROS.

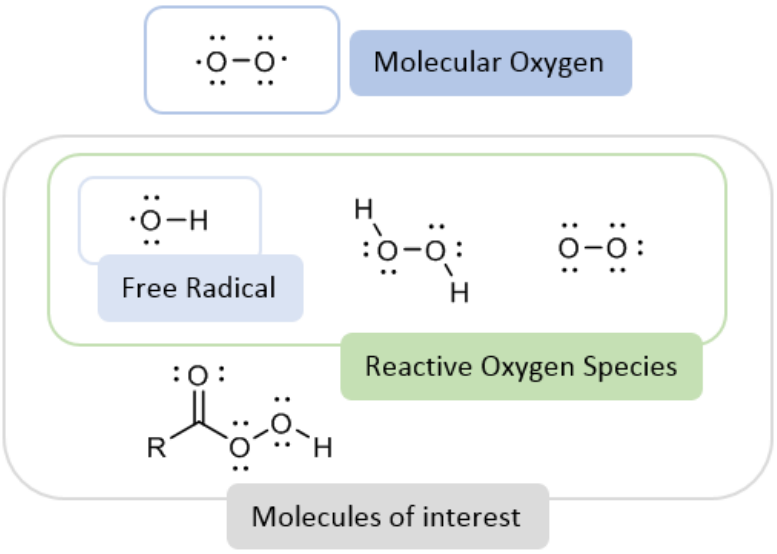


Figure 1.18 Lewis structures of ROS.

Hydrogen peroxide and singlet oxygen have paired electrons and they are not considered as a free radicals, but they are usually included under ROS. A radical (also called a 'free radical') refers to molecules with at least one unpaired electron in its outermost shell of electrons. Due to the presence of an unpaired electron, free radicals are typically highly reactive and as a consequence of this are also short-lived. Among the ROS, the hydroxyl radical is the most reactive species.[60]

ROS are usually generated by photolysis, electron transfer or energy transfer reactions.[61] In the absence of other sinks, most free radical ROS undergo self-reaction (e.g. dimerization or disproportionation) or atom abstraction, while the electronically excited singlet oxygen rapidly decays through vibronic

coupling with water to return to the ground state. Hydrogen peroxide does not react with itself but catalytically degrades through rapid reactions with trace metal ions and enzymes to form a variety of species.[62] Consequently, analyses of ROS have proven challenging because the short lifetime of these species, with the exception of peroxides, making ex-situ analysis difficult.[64]

Methods of ROS detection can be broadly classified as either direct or indirect. Due to the short lifetimes and typically low concentrations of ROS, their direct observation is only possible on the sub-milliseconds timescale, with the relatively stable peroxides being an exception. Indirect methods involve the reaction of a particular ROS with a probe molecule to yield a more stable, long-lived analyte.[63] Such methods typically involve specific chemical derivatisation (e.g. trapping a radical with a nitroxide or other spin trap) or are based on competitive kinetics.

Specificity varies widely between methods, and should be carefully considered when choosing a method for ROS qualification and/or quantification. Additional analytical considerations are availability, robustness, portability (for field or off-site studies), the cost of the necessary instrumentation, and in some cases, the cost of the probe molecules. The interferences caused by other species present in ithe nvestigated solution e.g. optical brighteners should also be considered.

Largely due to these latter factors, much of the method development for aqueous ROS analysis has focused on UV-Vis light absorption spectroscopy and the use of relatively common probes.[65] The lower limit of detection of such a chromophore by absorption spectroscopy is typically ~ 10^{-7} – 10^{-8} mol dm^{-3}, limiting the sensitivity of this technique. Additional problems often arise from undesired reactions of the probes from their uneven distribution in the studied system, production of ROS by the probes themselves and perturbation of the system under investigation by the probes. [64]

Fluorescent probes permit detection of ROS with much higher sensitivities than chromophoric probes with a limit of detection of <pM using suitable probes. Especially useful are the 'positive' fluorogenic probes, which are non-fluorescent (or weakly fluorescent), prior to reaction and which yield fluorescent products upon reaction with ROS. Advantages of assays based on the increase of fluorescence are: higher sensitivity, linear response to a wide range of ROS concentrations and low background fluorescence. [64]

Other analytical techniques for ROS detection; such as electron paramagnetic resonance (EPR), high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and derivatisation

with attendant mass spectrometric (MS) analysis can also be quite useful but are less portable and often require considerable technical expertise (and expense) to operate. [60]

1.5.2 Hydroxyl Radical

HO• reacts at near-diffusion-controlled rates with many substrates, resulting in a low steady-state concentration of hydroxyl radicals. The corresponding low concentration and brief lifetimes (~μs) for hydroxyl radicals pose significant challenges in quantifying this ROS. While HO• absorbs light in the UV region (230 nm), direct observation is typically not possible because of its low concentration, limited lifetime and the presence of other chromophores absorbing in a similar wavelength region. Therefore, hydroxyl radicals are quantified either through the loss of a reagent or accumulation of a product.

The ability of hydroxyl radical to undergo H-abstraction reactions has been employed for detection, typically using aliphatic alcohols/acids or halogenated alkenes as probe compounds. Typical probe compounds employed in early studies involved monitoring chromatographically the loss of 1-chlorobutane or the reaction of 2-propanol with hydroxyl radical, yielding acetone. [66,67]

Aromatic hydroxylation is often employed for hydroxyl radical quantification.[68] A wide variety of compounds have been reported for use in this type of hydroxyl radical assay, including terephthalate, benzoic acid, *p*-chlorobenzoic acid or benzene. [69–71] These methods utilize the ability of HO• to add to an aromatic ring to initially form a hydroxycyclohexadienyl radical which can be further oxidized to a phenolic moiety by a range of oxidants (e.g. dissolved oxygen, Figure 1.19). [72]

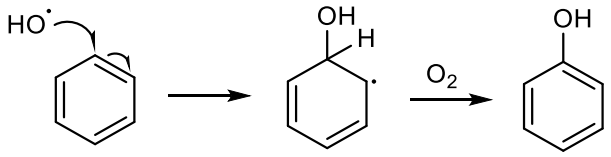


Figure 1.19 Aromatic hydroxylation reaction with hydroxyl radicals.

With aromatic hydroxylation probes, quantification can be undertaken by monitoring spectroscopically the loss via UV/Vis or fluorescence of the probe itself or the formation of the hydroxylated product(s), with the latter typically considered to be more selective for hydroxyl radicals than other strong oxidants and to offer sensitivity advantages. [73]

Terephthalic acid (TA) has proven to be an especially convenient hydroxyl radical trap, which can be used to estimate relative amounts of hydroxyl radicals formed under varying conditions. TA reacts with hydroxyl radicals to form hydroxyterephthalate via aromatic hydroxylation (HTA, Figure 1.20). HTA is highly fluorescent with excitation and emission at 315 and 425 nm, respectively.[74] Reaction of TA to form HTA is highly specific towards hydroxyl radicals; other common oxygen species, such as the superoxide anion and organic hydroperoxides, do not produce HTA directly.[69] As the terephthalate molecule is symmetric with respect to ring-hydroxylation, there is only one form of the monohydroxylated adduct (soluble in water), and dihydroxylated derivatives do not form. [71]

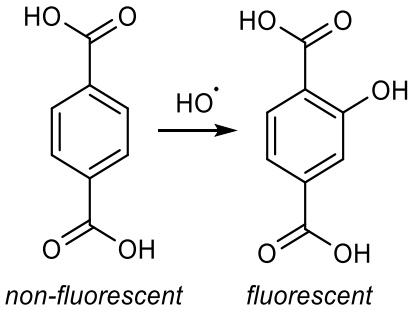


Figure 1.20 Reaction of TA and hydroxyl radical.

Another example of an aromatic hydroxylation probe is coumarin-3-carboxylic acid (CCA).[75] CCA is virtually non-fluorescent, but upon aromatic hydroxylation it produces the highly fluorescent and water soluble 7-hydroxycoumarin-3-carboxylic acid (OH-CCA, Figure 1.21). Dihydroxylated forms do not form during the reaction. This reaction can be monitored at λ_{ex} = 400 nm, and λ_{em} = 450 nm. The main advantage of CCA is that the excitation wavelength is shifted to the visible part of the spectrum in comparison to TA. This parameter reduces the absorption of light in experiments by other species present in laundry detergents, which can interfere with the measurement.

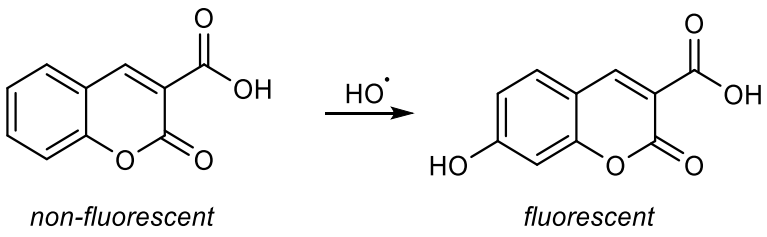


Figure 1.21 Reaction of CCA with hydroxyl radical.

1.5.3 Hydrogen Peroxide

Hydrogen peroxide is the simplest peroxide and it is a strong oxidizing agent, which has been widely employed as a bleach and as a cleaning reagent in industrial water treatment.

Hydrogen peroxide can be detected directly using spectrophotometric techniques, although its molar absorption coefficient ($\varepsilon_{200\ nm}$ = 189 M^{-1} cm^{-1}) is low, limiting this analytical method to relatively pure solutions where the H_2O_2 concentration is high or the analytical pathlengths are long. [76]

A wide variety of hydrogen peroxide assays utilise a combination of a probe and a peroxidase enzyme (e.g. horseradish peroxidase – HRP) to achieve the required specificity. Use of HRP can result in measurement of not only H_2O_2, but also other peroxy species such as a peroxyacetic acid, methyl hydroperoxide, hydroxymethylperoxide, and several propylperoxides formed through HRP-catalyzed reactions. The most common probes, which involve HRP-catalyzed oxidation yield products that either exhibit fluorescence (e.g. *p*-hydroxyphenylacetic acid) or whose fluorescence is diminished (e.g. scopoletin) after oxidation. These fluorometric methods make use of readily available fluorophores, do not require specialised equipment other than a reliable fluorometer, and generally afford greater specificity, sensitivity and lower limits of detection compared to absorbance-based methods. [77]

The first H_2O_2 probe in this review, which use HRP as a catalyst is *p*-hydroxyphenylacetic acid (PHPAA). This molecule is oxidised by hydrogen peroxide in the presence of peroxidase to a dimer that is optimally fluorescent above pH 8.5 with excitation and emission maxima at 315 and 414 nm respectively (Figure 1.22). [78]

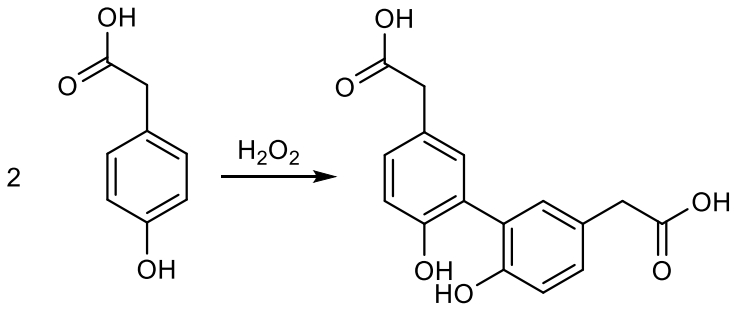


Figure 1.22 Reaction of PHPAA with hydrogen peroxide.

Scopoletin is an example of negative fluorescence probe used in the inverse fluorescence detection of hydrogen peroxide (Figure 1.23). The probe is fluorescent while the products of HRP-catalysed oxidation of hydrogen peroxide are not; decreased fluorescence is a measure of hydrogen peroxide

formed in the system. The main problem associated with inverse fluorescence is the poor linearity of decreased fluorescence versus oxidant concentration and false readings due to other factors.[64,79]

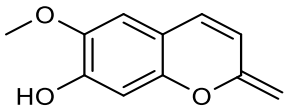


Figure 1.23 Structure of scopoletin.

Another hydrogen peroxide probe is N-acetyl-3,7-dihydroxyphenoxazine (Amplex Red), which is a non-fluorescent molecule that yields resourfin when oxidised by H_2O_2 in the presence of HRP, a stable, highly fluorescent product (Figure 1.24, λ_{ex} = 563 nm; λ_{em} = 587 nm). The sensitivity of Amplex Red when detecting H_2O_2 is at least 10 times higher than that of scopoletin under the same conditions. The pH range of the assay should be kept between 7.5 and 8.5, to maintain the stability of HRP. [77,80]

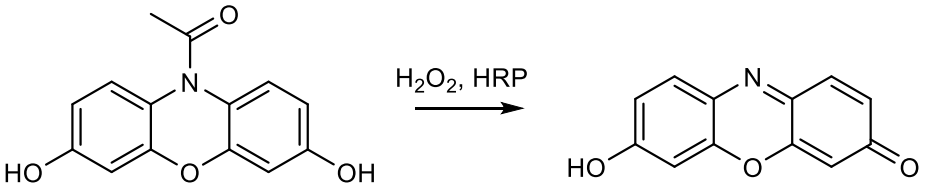


Figure 1.24 Reaction of Amplex Red with hydrogen peroxide to yield resourfin.

A novel fluorescent hydrogen peroxide sensor has been developed based on the peroxidase-like activity of copper(II) oxide nanoparticles which effectively catalyse the decomposition of hydrogen peroxide into hydroxyl radicals. Copper(II) oxide nanoparticles are considerably more stable than peroxidase and possess an almost unchanged catalytic activity over a wide range of pH and temperature. Resultant hydroxyl radicals can be easily detected using by e.g. terephthalic acid (Figure 1.25).[81,82]

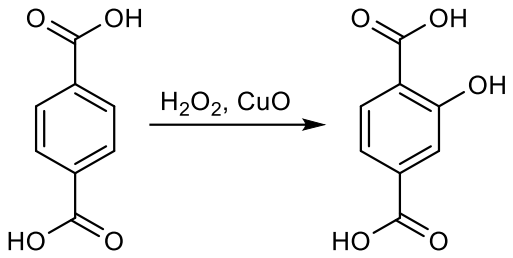


Figure 1.25 Generation of HTA after catalytic decomposition of hydrogen peroxide by CuO nanoparticles.

Finally the reaction of boronic acids with H_2O_2 is well known in synthetic chemistry, as a way to produce different phenols (Figure 1.26). The formation of phenols could be then determined either by absorbance or fluorescence. A wide range of boronic acids are commercially available, allowing fluorescent molecules to be chosen which can be excited across the UV and visible region of the spectrum. [83–86]

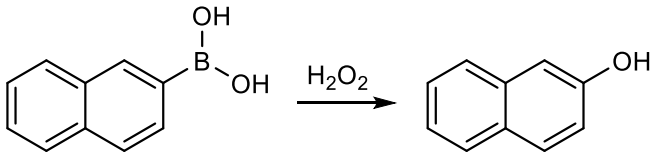


Figure 1.26 Reaction of 2-naphthylboronic acid with hydrogen peroxide.

The mechanism of reaction probably involves one molecule of boronic acid and one molecule of perhydroxyl anion (Figure 1.27).[87] Reaction is faster at the alkaline pH due to the faster formation of the reactant - perhydroxyl anion.

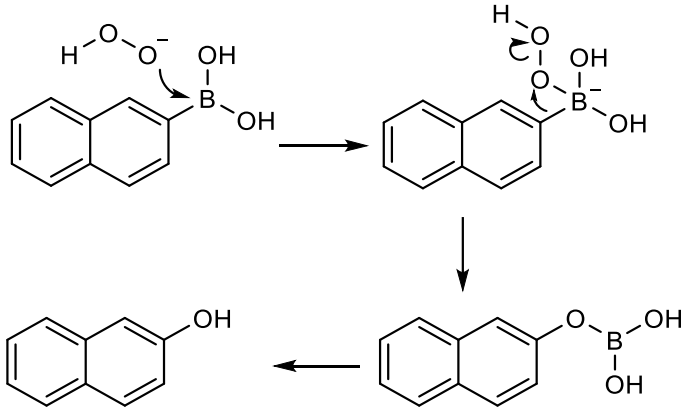


Figure 1.27 Mechanism of naphthol generation from arylboronic acid.

1.5.4 Peroxy acids

Organic compounds containing RC(O)-OOH, where R is an organic group, are important substances from the industrial and biochemical point of view. In industry and in other practical contexts, they act as free radical initiators, bleaching agents, oxidants and disinfectants. [88,89]

Despite the lack of a characteristic chromophore, ultraviolet-visible methods of peroxy acid analysis are significantly represented in the literature, mainly because of the various possible redox reactions in which coloured products may be formed.[90] An important problem in peroxy acid analysis is that they typically co-exist with hydrogen peroxide. As both reagents are strong oxidants, they are difficult to distinguish. Chemiluminescence and fluorescence-based methods are less popular, but the most significant progress can be seen in the field of mass spectrometry, probably due to developments in soft ionization techniques, superior sensitivity, and the growing number of available instruments.[88]

The classical method of peroxy acid analysis is the iodometric method. Products of the reaction between peracid and iodide are carboxylic acid, iodine and oxygen (Eq.1.12). Iodine can be easily determined by simple titration with thiosulfate (Eq.1.13). [91] The sensitivity of this method could be improved by the spectroscopic determination of I_3^- (Eq.1.14), at λ_{max} = 350 nm. [92]

1.12 $$CH_3COOOH + 2I^- \rightarrow 2\ CH_3COOH + I_2 + O_2$$

1.13 $$I_2 + 2\ S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2\ I^-$$

1.14 $$I_2 + I^- \rightarrow I_3^-$$

Iodide is also used as a catalyst in other methods of peracid determination. The method is characterised in that sample solution is mixed with a chromogen, an iodide and, where appropriate, a buffer-containing reagent, and the colour reaction is evaluated by spectrophotometry or visually. Under these conditions, hydrogen peroxide does not react or reacts only slowly, whereas the peracid reacts rapidly and evidently. A range of chromogens are able to be oxidised in the presence of peroxides.[93] Suitable examples include but are not limited to benzidine derivatives such as guaiacol, or leuco dyestuffs such as leucomalachite green (Figure 1.28).

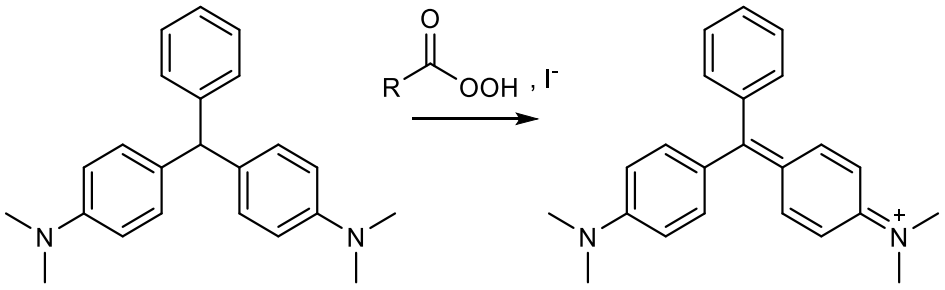


Figure 1.28 Reaction of leucomalachite green with peroxy acid.

2,2'-Azino-bis(3-ethylbenzothiazoline)-6-sulfonate (ABTS) is one of the most popular substrates for the enzyme peroxidase, and it is oxidised selectively over hydrogen peroxide by peracids to an intensely green radical cation ($ABTS^{\bullet+}$, Figure 1.29). Photometric detection of the product can be performed in four characteristic regions of the visible and near infrared spectrum in the range 405-815 nm. [94]

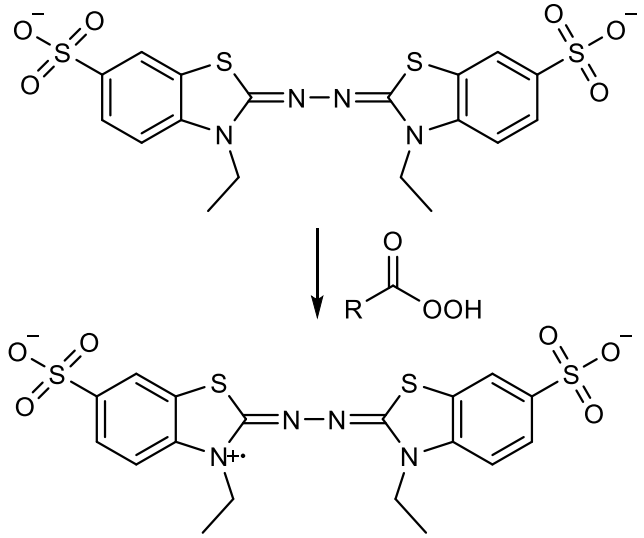


Figure 1.29 Reaction of ABTS with peroxy acid.

1.5.5 Singlet oxygen

Direct measurement of the concentration of singlet oxygen is possible through observation of its phosphorescence at 1268 nm.[95] However, the intensity of this emission is very weak and detection sensitivity is low at this wavelength which combine to make it a poor method at low singlet oxygen concentrations. Consequently, use of this technique has been largely restricted to transient luminescence studies initiated by pulsed laser irradiation.

A successful singlet oxygen probe should meet several criteria that are well summarized by Nardello: 'The probe must be highly reactive towards singlet oxygen, specific, compatible with aqueous media and must not perturb the system under study. Moreover, it must be transparent in the spectral range of the incident light in order to avoid photosensitization by the trap itself.'[96]

Typical singlet oxygen probes include anthracene- and pyrene-based compounds which are poorly soluble in water and absorb strongly in the UV-A and UV-B ranges. Widely used are also furan derivatives, such as furfuryl alcohol (FFA), which react with singlet oxygen to yield the corresponding molozonide and other products (Figure 1.30). The loss of the furfuryl alcohol can be monitored by absorbance at 219 nm. [97]

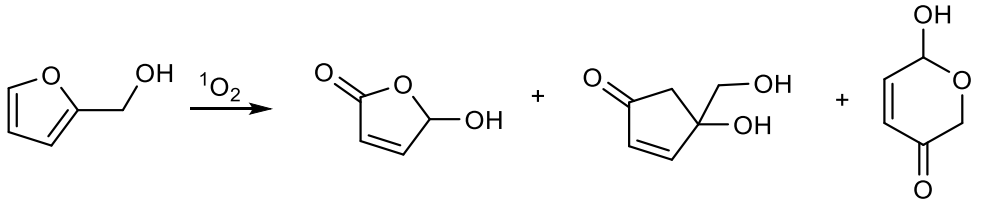


Figure 1.30 Reaction of FFA with singlet oxygen.

The presence of singlet oxygen can also be tested through the addition of quenchers that promote non-radiative decay of this ROS back to the ground state. The addition of these materials result in an effective reduction in the rate of an observed process, to a degree that is predictable (and therefore testable) based on the known rates of reaction between the added quencher and singlet oxygen. Examples of frequently cited quenchers are azide ions, iodide ions and diazabicyclooctane (DABCO).[98–100] As with the furan derivatives, the interpretation of the results of these experiments is complicated by the additional reactivity of the quencher with hydroxyl radicals.[101]

The use of D_2O as the reaction solvent avoids interference from the hydroxyl radical in singlet oxygen assays. Singlet oxygen has a longer lifetime than hydroxyl radicals in D_2O solutions than H_2O as a result of the relatively poor vibronic coupling between $^1O_2$ and D_2O relative to H_2O.[49] The reduced decay rate of singlet oxygen in D_2O and/or D_2O-water mixtures results in higher steady-state $^1O_2$ concentrations leading to higher rate constants for oxidation of singlet oxygen acceptors. [52]

1,3-Diphenylbenzofuran (DPBF) has been widely used as a singlet oxygen probe. Loss of absorbance at 420 nm can be used to determine the presence of $^1O_2$ (Figure 1.31). The product of the reaction can decompose in solution. This probe possibly has limited response to superoxide. [102]

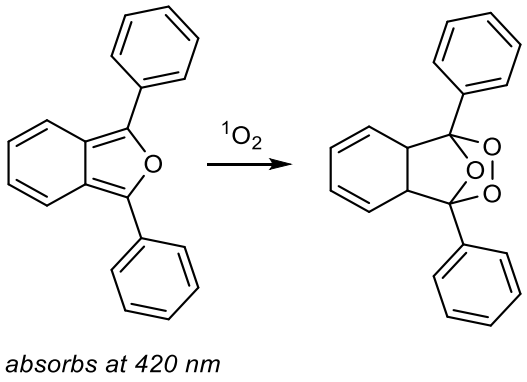


Figure 1.31 Reaction of DPBF with singlet oxygen.

1.6 Conclusion and Project Aims

Bleaching agent(s) are key active ingredients in a laundry detergent, delivering a wide range of consumer benefits such as coloured stain removal, real item cleaning, solution bleaching and malodour control. Current laundry detergents rely on a longstanding combination of a hydrogen peroxide source (typically sodium percarbonate), and an amide or ester activator that together generate an organic peracid and hydrogen peroxide *in situ*. A range of novel bleach catalysts and boosters have been developed over recent years.[11] However, the difficulties in identifying the active bleaching species of these innovations has hindered the understanding of the bleaching mechanism in a washing environment. This project will undertake fundamental scientific research to properly understand the identity and the role of reactive oxygen species in bleach formulations to allow new product development. These new products will have reduced energy and materials requirements, potentially making a transformative change to the laundry business.

This project seeks to develop an understanding of the process by which chemical bleaching agents added to laundry products operate, in particular understanding the identity and changes to concentration of ROS over time during a wash cycle. In addition, it will go on to understand how their overall performance is modulated by their interaction with other materials present in laundry cleaning products, e.g. surfactants. This work will allow optimisation of formulations to ensure that all components work in unison to achieve the desired end result with the smallest environmental chemical footprint.

In the first step of the project, a comprehensive review of laundry bleaching systems and related areas, particularly the detection of ROS, will be carried out. A toolbox of molecular probes and analytical techniques will be developed to selectively detect specific ROS that are thought to be generated from detergent bleach sources. The potential probes should be able to detect ROS with appropriate sensitivity and selectivity in aqueous environment and alkaline pH range. Target ROS are the hydroxyl radical, hydrogen peroxide, peroxy acids and singlet oxygen.

In the second step of the project, this toolbox of molecular probes and analytical techniques will be employed to determine which ROS are generated from a bleach source. Detergent bleach sources include: SPC, TAED, NOBS and PAP systems. Kinetic studies of identified ROS will be performed.

In the third part of the project, the established toolbox will be used to explore the oxidation of model substrates which will be selected to mimic challenging stains. Monitoring the oxidizing species present through the course of the process and the degradation of the substrates will provide a far greater level of understanding of the cleaning process. This will allow the rational use of existing bleaching systems as well as offering an insight into new bleaching systems.

In the last step of the project the presence, concentration and rate of ROS will be determined when detergent bleach sources are exposed to a bleachable surface in a typical washing solution environment (complex system). The complex washing solution environment will include other detergent ingredients, water hardness and typical laundry soils. Realization of all of these steps will allow optimization of formulations to ensure that all components work in unison to achieve the desired end result with the smallest environmental chemical footprint.

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2 EXPERIMENTAL

2.1 Experimental techniques

2.1.1 Basic molecular photophysics

The absorption of radiation by an atom or molecule can lead to the production of a range of electronically excited states that may exhibit chemical and physical properties that are different from the ground state. The energy absorbed can be dissipated in many ways, including heat, emission of radiation of longer wavelength, or initiation of chemical reactions.

The processes which occur between the absorption and emission of light are usually illustrated by a Jabłoński diagram (Figure 2.1). The singlet ground, first and second electronic states are depicted by S_0, S_1 and S_2, respectively. At each of these electronic energy levels the fluorophores can exist in a number of vibrational energy levels. Relative transitions between states are depicted as vertical lines to illustrate the instantaneous nature of light absorption. These transitions occur in about 10^{-15} s, a time too short for significant displacement of nuclei.

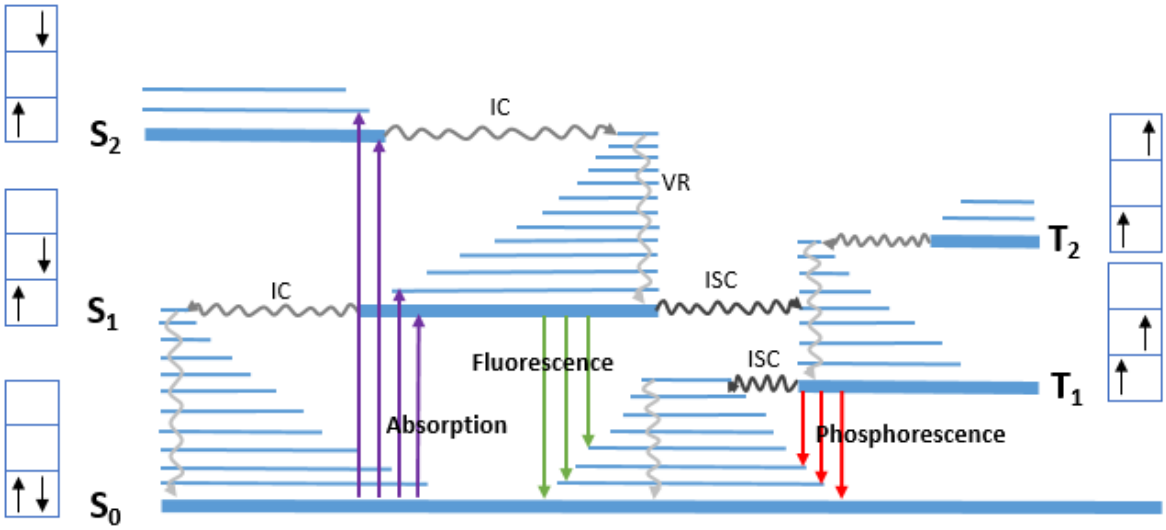

Figure 2.1 Jabłoński diagram

Absorption typically occurs from molecules with the lowest vibrational energy, due to the typical Boltzmann distribution at 298 K. The transition between the S_0 and S_1 excited state can be induced by optical frequencies, and due to this energy gap it is unlikely to excite the molecule to this state by thermal population, and this is the reason why light and not heat is used to induce fluorescence.

Following light absorption, several processes can occur. A fluorophore may be excited to some higher vibrational level of either S_1 or S_2. With a few rare exceptions, molecules in a condensed phase rapidly relax to the lowest vibrational level of S_1 by a process e.g. called internal conversion and vibrational relaxation and generally occurs in 10^{-12} s or less. Since the fluorescence lifetimes are typically near 10^{-8} s, internal conversion to the lowest level of the singlet state is generally complete prior to emission. Hence, fluorescence emission generally results from a thermally equilibrated excited state, that is, the lowest-energy vibrational state of S_1 (Kasha's rule).

Return to the ground state is unrestricted regarding which vibrational level it can enter, except by the overlap integral of the initial and final states of vibrational wavefunctions; this then quickly (10^{-12}) reaches thermal equilibrium. An interesting consequence of emission to higher vibrational ground states is that the emission spectrum is typically a mirror image of the absorption spectrum of the $S_0 \rightarrow S_1$ transition. This similarity occurs because electronic excitation typically does not greatly alter the nuclear geometry. Hence, the spacing of the vibrational energy levels of the excited state is similar to that of the ground state. Excitation to higher vibrational levels requires higher frequencies but the relaxation into higher vibrational level results in lower frequencies. As a result, the vibrational structures seen in the absorption and the emission spectra are similar.

Molecules in the S_1 state can also undergo a spin conversion to the first triplet state, T_1. At lowest energy state that is triplet in nature, i.e. T_0 is effectively unheard of in simple organic molecules (the ground state is usually a singlet state (S_0), and only excited states have a subscript number greater than 0). Emission from T_1 to the ground state (S_0) is termed phosphorescence and is shifted to longer wavelength (lower energy) relative to the fluorescence. Conversion of the singlet excited state to triplet excited state is called intersystem crossing. Transitions from T_1 to the singlet ground state are forbidden and, as a result, the rate constants for triplet emission or back intersystem crossing are several orders of magnitude smaller than those for fluorescence. Molecules containing heavy atoms such as bromine and iodine are frequently phosphorescent. The heavy atoms facilitate intersystem crossing and thus enhance phosphorescence quantum yields by enhancing k_{isc} and k_p. [1]

2.1.2 UV-Vis spectroscopy

UV-Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions, but solids and gases may also be studied.

The instrument used in UV-Vis spectroscopy is called a UV-Vis spectrophotometer. It measures the intensity of light passing through a sample ($I_t(\lambda)$), and compares it to the intensity of light before it passes through the sample ($I_0(\lambda)$). The ratio $I_t(\lambda)/I_0(\lambda)$ is called the transmittance (T), and is usually expressed as a percentage (%T). The absorbance, A, is based on the transmittance (Eq. 2.1).

$$A(\lambda) = -log_{10}\left(\frac{I_t(\lambda)}{I_0(\lambda)}\right) \quad (2.1)$$

The basic parts of a spectrophotometer are a light source, a holder for a sample, a diffraction grating or prism in a monochromator to separate the different wavelengths of light, and a detector. The radiation source is often a tungsten filament (300 – 2500 nm); a deuterium lamp, which is continuous over the ultraviolet region (190 – 400 nm), a xenon arc lamp, which is continuous from 160 – 2000 nm, or more recently, light emitting diodes (LED) for visible wavelengths. The detector is typically a photomultiplier tube, a photodiode, a photodiode array or a charge-coupled device (CCD). A spectrophotometer can be either single beam or double beam. In a single beam instrument, all of the light passes through the sample cell. In a double beam instrument, the light is split into two beams before it reaches the sample (Figure 2.2)[2], where one passes through the sample and the other is used as a reference, allowing $I_0(\lambda)$ and $I_t(\lambda)$ to be determined.

In Durham University the UV-Vis absorption spectra were obtained using a Unicam UV-Vis Spectrometer UV2-100. A tungsten lamp is used as the source of wavelengths greater than 325 nm, and a deuterium lamp for wavelengths below 325 nm. The spectrometer was controlled using Unicam Vision Software Version 3.0 running on PC. In Procter & Gamble Brussels Innovation Centre spectra were recorded on Genesys 10S UV-Vis spectrophometer, controlled by VISIONlite 5 software.

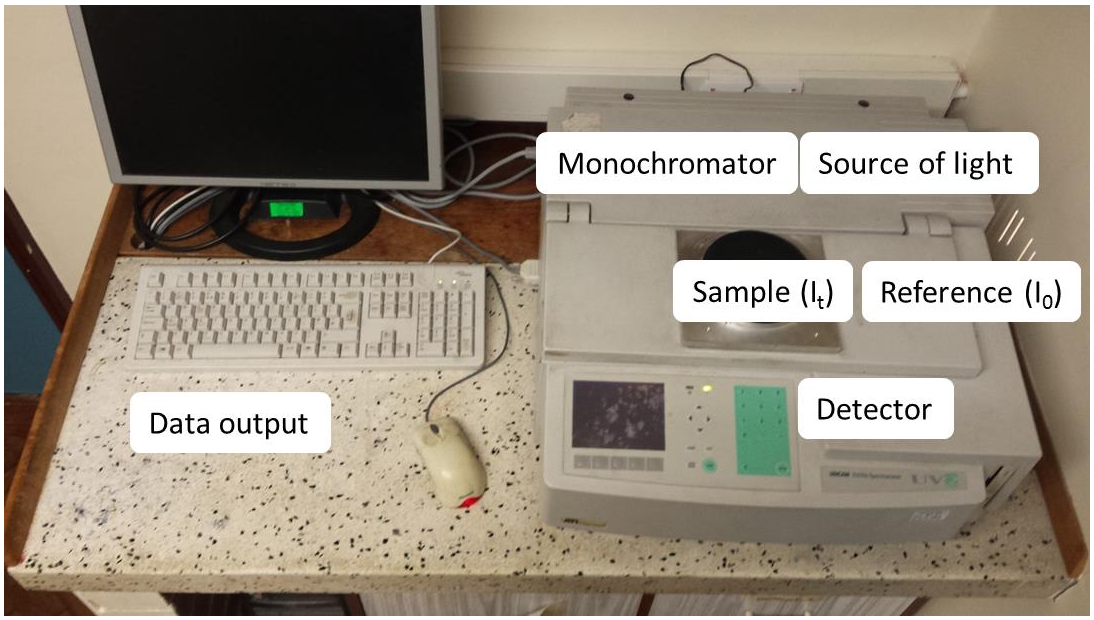

Figure 2.2 Unicam UV-Vis Spectrometer UV2-100.

Samples were contained in quartz absorption cuvettes with a path length of 1 cm, unless otherwise stated. A baseline was determined with the appropriate solvent or buffer prior to running each spectrum.

Extinction coefficients were calculated using the Beer-Lambert Law (Eq. 2.2), where A = absorbance at wavelength in question, ε = extinction coefficient at wavelength in question, c = concentration in mol·dm^{-3}, and l = path length in cm.

$$A = \varepsilon \cdot c \cdot l \qquad (2.2)$$

2.1.3 Fluorescence spectroscopy

Fluorescence spectroscopy is an extremely versatile, sensitive experimental technique used in identification and quantification of many industrial related compounds. Through judicious selection of excitation and emission wavelengths, one can often analyse a single desired fluorophore in complex mixtures containing several absorbing and fluorescing species.

Spectrofluorometers use diffraction grating monochromators to generate the incident light and analyse the fluorescence. Light from an excitation source passes through a monochromator, and strikes the sample. A proportion of the incident light is absorbed by the sample, and some of the

molecules in the sample fluoresce. The fluorescent light is emitted in all directions. Some of this fluorescent light passes through a second monochromator and reaches the detector, which is usually placed at 90° to the incident light beam to minimise the risk of transmitted or scattered incident light reaching the detector (Figure 2.3).[3]

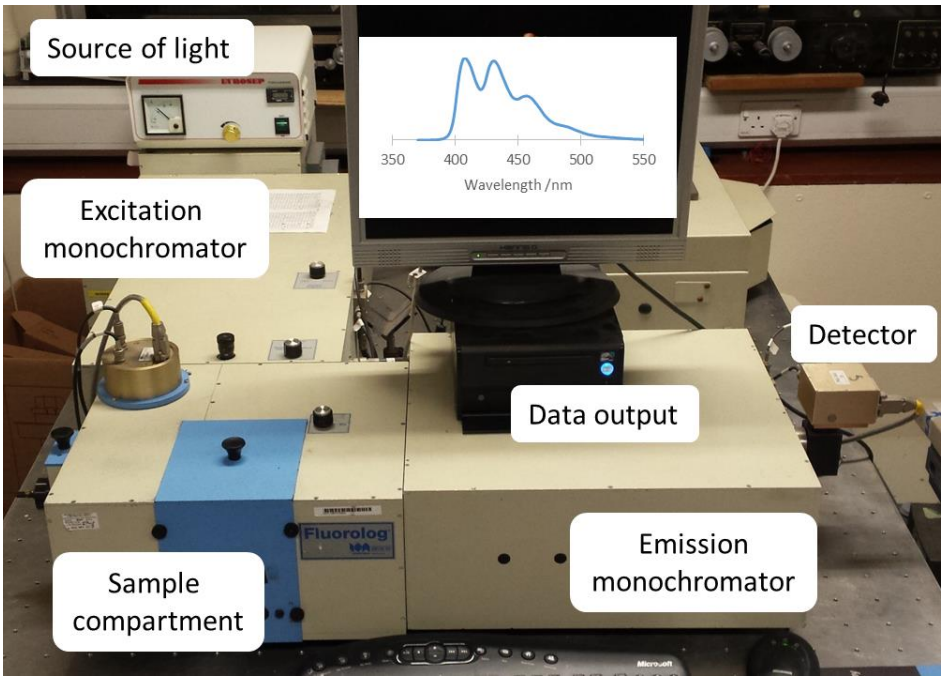

Figure 2.3 Spectrofluorimeter Fluorolog 3-22.

Various light sources may be used as excitation sources, including lasers, LED, and lamps; xenon or mercury arc lamps in particular. A monochromator transmits light of a selected wavelength with an adjustable bandwidth. The most common type of monochromator utilizes a diffraction grating; collimated light illuminates a grating and exits with a different angle depending on the wavelength. The monochromator can then be adjusted to select which wavelengths to transmit. The detector can either be single-channelled or multichanneled. The single-channelled detector can only detect the intensity of one wavelength at a time, while the multichanneled detects the intensity of all wavelengths simultaneously, making the emission monochromator or filter unnecessary. [4]

The presence of strongly absorbing molecules, like dyes and other ingredients of laundry detergent can affect the observed fluorescence spectra by decreasing the intensity of the peak or changing its shape. This phenomenon is called inner filter effect (IFE). The primary IFE refers to the absorption

of the excitation beam prior to reaching the interrogation zone – the area from which the emission is detected, and secondary IFE refers to the absorption of the emitted fluorescence photons.[5]

An exact correction is required for both primary and secondary IFE, requiring measurement of the absorbance of the solution at all wavelengths used. The correction for both primary and secondary IFE can be calculated as (Eq.2.3) [6]:

$$I_f = I_0\left(10^{-b(A_{ex}+A_{em})}\right) \quad 2.3$$

Where I_f = detected fluorescence intensity, I_0 = fluorescence in the absence of IFE, b = path length for both excitation and emission beam, A_{ex} = absorbance at the excitation wavelength, A_{em} = absorbance at the emission wavelength.

Fluorescence measurements were performed in diluted solutions with an absorbance of < 0.1, using a Horiba Jobin-Yvon Fluorolog 3-22 Tau 3 spectrofluorimeter or a bespoke system based upon a Maya 2000 Pro spectrometer supplied from Ocean Optics Inc. The fluorescence spectra were corrected for the spectral response of the machines. The correction takes into account that the output of the xenon lamp is not uniform throughout the entire wavelength range and the efficiency of the gratings and detectors.

Two types of fluorescence spectra can be recorded. For an emission spectrum, the sample is excited at a chosen wavelength, and the emission spectrum is recorded over a range of wavelengths. An excitation spectrum is similar to an absorption spectrum in form; a fixed emission wavelength is chosen, and the excitation wavelength varied. This builds a profile of which wavelength absorption results in emission at a specific wavelength.

Emission and excitation spectra were obtained in quartz cuvettes with 1 cm path length, unless otherwise stated.

2.1.4 Diffuse reflectance spectroscopy

The colour of the fabric or the stain can be measured by a technique called diffuse reflectance spectroscopy (DRS). The diffuse reflection is a phenomenon in which the incident light is reflected at many angles from the surface, rather than at just one angle as in the case of specular reflection (Figure 2.4).[7]

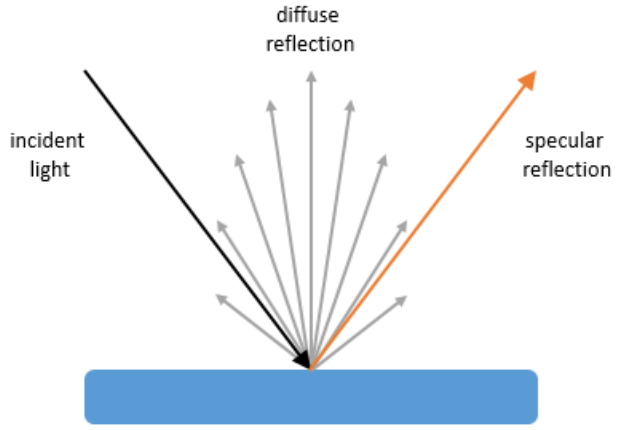

Figure 2.4 Diffuse and specular reflection from a glossy surface.

Visible light forms only a small part of the electromagnetic spectrum, with a spectral range from approximately 400 nm (violet) to 750 nm (red). If the spectral distribution throughout the visible region is unequal, then the sensation of colour is evoked by radiant energy reaching the retina of the eye. An even spectral distribution makes the light appear as white. The unequal distribution responsible for colour sensation may be characteristic of the source itself; such as atomic emission spectra composed of one or more monochromatic wavelengths, or may result from selective absorption by the system, making it appear coloured. The latter includes several systems that show selective absorption for light and exhibit colour as a result of reflection or transmission of unabsorbed incident radiant energy. [8]

The diffuse reflectance spectrum was obtained using the system shown in Figure 2.5. The setup consists of a tungsten halogen broadband light source, a reflectance head, a Maya 2000 spectrometer and computer. Diffuse reflectance spectra were measured in darkness, using a Spectralon® tile as a white standard. The standard is made of a fluoropolymer, and has a high diffuse reflectance over the UV, Vis, and near-IR regions of the spectrum. Spectra of both dry and wet fabric can be recorded on a vertical or horizontal configuration of the equipment, respectively.

In the horizontal configuration the fabric is dipped in the solution under investigation and applied to the surface of the container and held by the tongs. The reflectance head is at the right angle to the substance allowing measurement of the rapid changes in colour i.e. bleaching of the red wine stain.

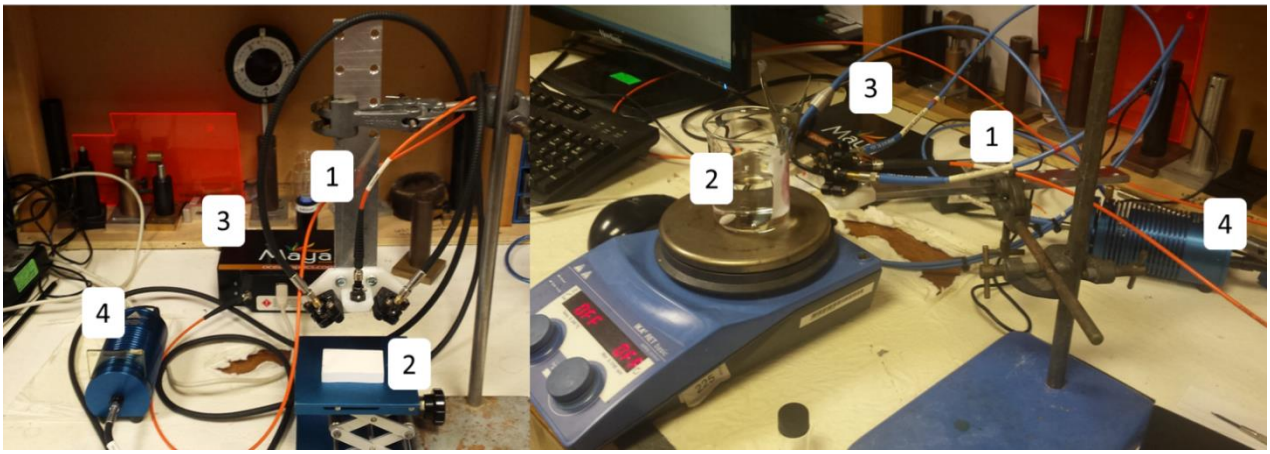

Figure 2.5 Vertical and horizontal configuration of the diffuse reflectance setup, 1 – reflectance head, 2 – sample, 3 – detector (spectrometer) and 4 – source of light + filter.

2.1.5 Image analysis

The Laundry Stain Removal Image Analysis System (IA) is an image analysis grading system used by Procter & Gamble to determine the stain removal performance of detergents. The typical setup contains a light booth, a lamp, camera lens and is computer controlled. The light booth is an enclosed, white painted cylinder with a sliding front door, where the swatches should be placed. The procedure starts from the calibration of the instrument, which is followed by taking pictures of the initial, unwashed fabrics. Before taking the pictures of washed fabrics, a repeat of the calibration is required. The final stage of the procedure is results analysis by software (Matlab).

Stain removal is monitored using CIE colourimetry using the CIE 1976 LAB *L*a*b** system.[10] The CIE Lab is a colour specification system for quantitative interpretation of the colour developed by the Comission Internationale de l'Eclairage (CIE). It provides a standard method for describing the stimulus of a colour, under controlled light and viewing conditions, based on the average known response of the human visual system.[11] Parameter L* represents the lightness of colour, the scale of L* is from 0 (black) to 100 (white). The chromaticity of a colour is represented in a two-dimensional diagram where axis *a** determines the ratio of green (negative) to red (positive), and axis *b** specifies the ratio of blue (negative) to yellow (positive, Figure 2.6).

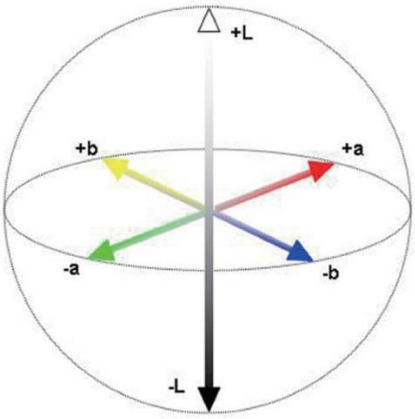

Figure 2.6 CIE Lab system.

The CIE 1976 *L*a*b** colour difference is widely used measure for overall colour change, and is simply calculated as the Euclidean distance in CIE Lab space. For colours specified by [L_1*, a_1*, b_1*] and [L_2*, a_2*, b_2*] parameters the difference, ΔE*, is calculated as follows (Eq. 2.4)[12]:

$$\Delta E^*_{a,b} = \sqrt{(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2} \quad (2.4)$$

Procter & Gamble have developed several parameters for stain removal determination by Image Analysis. Formulas are based on the initial and washed samples of stained fabrics (Figure 2.7), where **A** and **C** refers to initial and washed colour of the clean fabric and **B** and **D** to initial and washed colour of stain.

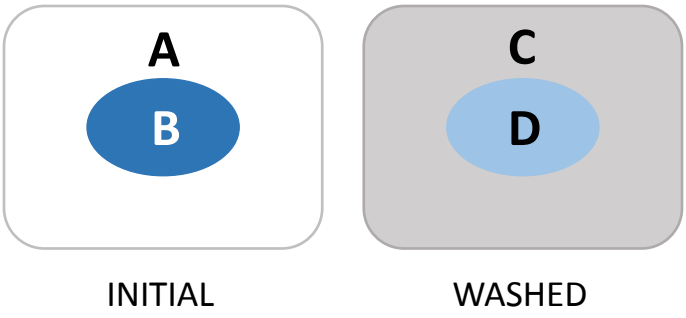

Figure 2.7 Initial and washed samples of stained fabrics.

The washing performance is often assessed by three parameters: stain removal index, washed noticeability and stain change. The Stain Removal Index (SRI) is the first parameter used in this work (Eq. 2.5). It can be thought of as the representation of the percentage of stain removal, and a higher value usually indicates a better detergent or washing product. It also demonstrates how close the

washed stain is to the initial fabric (how close is **D** to **A**). It is possible to obtain a negative value when the stain becomes darker, during wash cycle, for instance in the tea stains case.

2.5 $$SRI = 100 \cdot \frac{(\Delta E^*_{A,B} - \Delta E^*_{A,D})}{\Delta E^*_{A,B}}$$

Washed noticeability (WN) is analogous to what actually can be observed on the final fabric (Eq. 2.6). In this case a lower value usually indicates a better detergent or wash product.

2.6 $$WN = \Delta E^*_{C,D}$$

Stain Change (SC) indicates how much the stain alone changed without regard to what happened to the unsoiled fabric (Eq.2.7). A higher value of SC usually means a better wash product.

2.7 $$SC = \Delta E^*_{B,D}$$

2.1.6 Basic chemical kinetics

The rate law or rate equation for a chemical reaction is an equation that links the reaction rate with the concentrations or pressures of the reactants and constant parameters. For many reactions the rate is given by a power law such as Eq.2.8.

2.8 $$r = k[A]^x[B]^y$$

Where [A] and [B] express the concentration of the species *A* and *B*, respectively (usually in moles per litre). The exponents *x* and *y* are the partial reaction orders and must be determined experimentally; they are often not equal to the stoichiometric coefficients. The constant *k* is the reaction rate constant of the reaction. The value of *k* may depend on conditions such as temperature, ionic strength, or light irradiation. [13]

A zero order reaction has a rate that is independent of the concentration of the reactant(s). Increasing the concentration of the reacting species will not speed up the rate of the reaction i.e. the amount of substance reacted proportional to the time. The rate law for a zero order reaction is presented in Eq. 2.9.

2.9 $$r = k$$

Where *r* is the reaction rate and *k* is the reaction rate coefficient with unit of concentration or time. A reaction is zero order if concentration data are plotted versus time and the result is a straight line. A plot of [*A(t)*] vs. time t gives a straight line with a slope of –*k*.

A first order reaction depends on the concentration of only one reactant. Others reactants can be present, but each will be zero order. The rate law for a reaction that is first order with respect to a reactant A is presented on Eq.2.10, where k is the first order rate constant, which has units of s^{-1}.

2.10 $$\frac{-d[A]}{dt} = r = k[A]$$

The integrated first order rate law can be expressed as in Eq.2.11, or can be also be written in the form of the exponential decay equation (Eq.2.12). A plot of ln[A] vs. time *t* gives a straight line with a slope of –*k*.

2.11 $$ln[A] = -kt + ln[A]_0$$

2.12 $$A = [A]_0 e^{-kt}$$

The half-life of a reaction describes the time needed for half of the reaction to be depleted. The half-life of a first order reaction is independent of the starting concentration and is given by Eq.2.13.

2.13 $$t_{1/2} = ln(2) \cdot k^{-1}$$

A second order reaction depends on the concentrations of one second order reactant, or two first order reactants. The reaction rate is given by Eq.2.14, or by Eq.2.15.

2.14 $$r = k[A]^2$$

2.15 $$r = k[A][B]$$

Measuring a second order reaction with reactant A and B can be problematic; the concentrations of the two reactants must be followed simultaneously, which is more difficult; measurement of one with calculation of the other as a difference, which is less precise. A common solution for that problem is the pseudo-first order approximation. If the concentration of one reactant remains effectively constant because it is supplied in great excess, its concentration can be absorbed at the expressed rate, obtaining the pseudo first order constant: the rate then depends on the same concentration of only one of the two reactants. If, for example, [B] remains in excess, then rate equation can be express by Eq.2.16, and a new rate constants, as an Eq.2.17. [14]

2.16 $r = k[A][B] = k'[A]$

2.17 $k' = k[B]_0$

2.1.7 Stopped flow spectrophotometry

One of the most frequently used techniques to examine rapid reactions is the stopped-flow technique. During the procedure, the small volumes of solutions are rapidly driven from syringes into a high efficiency mixer to initiate a fast reaction. The resultant reaction volume then displaces the contents of an observation cell thus filling it with freshly mixed reagents. The volume injected is limited by the stop syringe which provides the 'stopped-flow'.[15] Just prior to stopping, a steady state flow is achieved. The solution entering the flow cell is only milliseconds old. The age of this reaction volume is also known as the dead time of the stopped-flow system. As the solution fills the stopping syringe, the plunger hits a block, causing the flow to be stopped instantaneously (Figure 2.8). The most common detection methods are absorption and fluorescence spectroscopy.

The work here was carried by using a SFA-20 Rapid Mixing Accessory (TgK Scientific) coupled with a spectrofluorimeter (Fluorolog FL3-22).

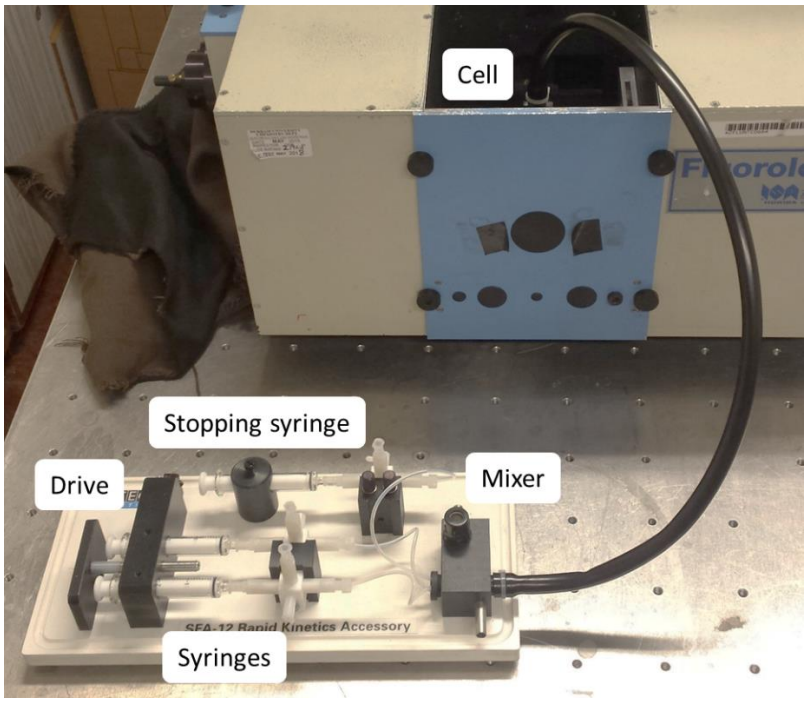

Figure 2.8 SFA-12 Rapid Kinetics Accessory.

2.2 Materials

The full list of used chemicals with their suppliers and purity can be found in the Table 2.1. In experiments deionised water was used, unless otherwise stated. Rubbed grass, tomato, curry, red wine and tea 4 cm diameter stains were purchased from Warwick Equest (Consett) on patches of white, knitted cotton.

	Compound	Supplier	Purity
Probes	Terephthalic acid	Sigma Aldrich	98%
	2-Hydroxyterephthalic acid	Sigma Aldrich	98%
	Coumarin-3-carboxylic acid	Sigma Aldrich	99%
	Iron(II) sulfate heptahydrate	Sigma Aldrich	99%
	Potassium nitrate	Sigma Aldrich	99%
	1,3-Diphenylisobenzofuran	Sigma Aldrich	97%
	9,10-Anthracenediyl-bis(methylene)dimalonic acid	Sigma Aldrich	90%
	2-Naphthylboronic acid	Sigma Aldrich	95%
	2-Naphthol	Sigma Aldrich	98%
	Naphthalene-2-boronic acid pinacol ester	Sigma Aldrich	-
	Leucomalachite Green	Sigma Aldrich	97%
	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt	Sigma Aldrich	98%
	Rose bengal	Sigma Aldrich	95%
	(4-Hydroxyphenyl)-acetic acid	Sigma Aldrich	98%
Dyes	Chlorophyllin sodium copper salt	Sigma Aldrich	90%
	Curcumin	Sigma Aldrich	65%
	Crocin	Sigma Aldrich	95%
	Cyanin chloride	Sigma Aldrich	90%
	Betanin	Sigma Aldrich	80%
Bleach	Hydrogen peroxide	Sigma Aldrich	30%
	Peracetic acid	Sigma Aldrich	38-40%
	Sodium percarbonate	Procter & Gamble	27%
	PAP	Procter & Gamble	71%

	PAP suspension	Procter & Gamble	16%
	TAED	Procter & Gamble	91%
	NOBS	Procter & Gamble	81%
	Neptune	Procter & Gamble	-
Others	Sodium bicarbonate	Sigma Aldrich	99%
	Sodium hydroxide	Sigma Aldrich	98%
	Sodium tetraborate decahydrate	Sigma Aldrich	99%
	Hydrochloric acid	Sigma Aldrich	36-38%
	Potassium iodide	Sigma Aldrich	99%
	Sodium thiosulfate	Sigma Aldrich	99%

Table 2.1 List of compounds used in the project.

2.3 Measurements

In this section a detailed experimental procedure can be found for experiments carried out in this work. The following reactions were performed in buffered solutions (0.1 M) unless otherwise stated:

- pH 10.5 : $NaHCO_3$ + NaOH
- pH 9.5: $Na_2B_4O_7$ + NaOH
- pH 8.5: $Na_2B_4O_7$+ HCl
- pH 7.5: KH_2PO_4 + NaOH

2.3.1 Development of a hydroxyl radical probe

Two approaches were examined for hydroxyl radical generation; the Fenton reaction and irradiation of potassium nitrate solution:

- Fenton reaction: A mixture of iron(II) sulfate heptahydrate (100 μM), H_2O_2 (100 μM) and TA (250 μM) was prepared in 100 mL of buffer. The resultant precipitant was removed by filtration and the fluorescence spectra of the remaining solution was recorded.
- Irradiation of KNO_3: A mixture of KNO_3 (1 mM) and TA (250 μM) was prepared in 100 mL of buffer and irradiated with 254 nm UV-TLC lamp for period of time (5-60 min), and monitored by recording of the fluorescence spectra of the solution.

2.3.2 Development of a singlet oxygen probe

Singlet oxygen was generated by the irradiation of Rose Bengal, which act as a photosensitiser. Reactions were carried out directly in quartz cuvettes (3.5 mL volume). A typical mixture contained: 2.4 mL of buffer, 0.3 mL of Rose Bengal (final concentration: 50 μM) and 0.3 mL of probe solution (concentration varies from 1 to 50 μM). The solution was then irradiated at 525 nm wavelength using a LED lamp for a period of time (5-60 min). In the last step the absorbance and fluorescence of the solution were recorded.

2.3.3 Development of a peroxide probe

During the development of a peroxides probe, commercially available stock solutions of PAA and H_2O_2 were used. Reactions were carried out directly in quartz cuvettes (3.5 mL volume). The most common approach was to add 2.4 mL of buffer to the cuvette, and solutions of peroxide (0.3 mL) and probe (0.3 mL). The concentrations were kept in the micromolar range (5-50 μM). NBA and NOH were dissolved in a solution of 20% ethanol and 80% deionised water.

2.3.4 Stopped flow

Solutions of PAA, PAP, SPC and NBA (250 μM) were prepared in pH 10.5 buffer. The peroxides were loaded in a syringe, and the NBA in a separate syringe. A stopped-flow cell was placed in the cell holder of the Fluorolog, and the fluorescence in time-based mode acquisition was collected (λ_{ex} = 340 nm, λ_{em} = 420 nm, time interval: 0.1 s, total $time_{SPC}$ = 15 min, total $time_{PAA/PAP}$ = 2 min). Experiments were started by initialising the measurement and depressing the syringes. Measurements for each peroxide were repeated 8 times. Data was fitted as an exponential growth by JMP Pro 12 software (statistical data analysis software) and the quality of the fit was judged by R^2 parameter.

2.3.5 Iodometric titration

Iodometric titration was used to confirm the concentration of PAA and H_2O_2 stock solutions, and was carried out by Procter & Gamble protocol: the reagents were mixed as follows in a beaker equipped with a magnetic stirrer:

1. H_2O_2/PAA/PAP – m <0.1 g
2. 40 mL of deionised water
3. 10 mL of glacial acetic acid
4. 10 mL of 10% potassium iodide solution
5. Few drops of 1% starch solution
6. Few drops of 1% potassium molybdate, as a catalyst for hydrogen peroxide analysis

A mixture prepared as above was then titrated with 0.01 mol/L sodium thiosulphate until decolourisation of the sample as the endpoint. In addition PAA samples were titrated in the ice bath,

to avoid interference from H_2O_2, which is present in samples of peroxy acids due to the equilibrium between them.

2.3.6 Detection of singlet oxygen and oxygen formation kinetics

100 mL of buffered 10 μM ADMA solution was placed in a beaker. The peristaltic pump was then connected to the beaker and Maya 2000 spectrometer. The setup of the experiment was followed by the initiation of the closed circuit flow of the ADMA solution by the pump and monitoring the fluorescence at 430 nm (λ_{ex} = 365 nm). Depending on the experiment, the solid SPC, PAP, TAED + SPC, NOBS + SPC and Neptune were added directly to the beaker to obtain 500 μM concentration of the bleach and the ADMA fluorescence was monitored for 45 min. The solution in the beaker was stirred for the throughout the experiment.

2.3.7 High temperature hydroxyl radicals experiment

100 mL of 500 μM buffered solution of TA was prepared in a beaker. The solution was stirred and heated to 60 °C. When the temperature was reached, the solid sources of SPC, PAP, SPC + TAED, SPC + PAP and SPC + NOBS were added to the beaker to obtain 5,000 μM concentration of SPC and 1,670 μM of the additive (PAP, TAED, NOBS). The solutions were kept at 60 °C for 45 min. A 3 mL sample of each solution was then placed in a quartz cuvette and the fluorescence emission spectra were recorded (λ_{ex} = 315 nm).

2.3.8 Bleaching performance as a function of pH

100 mL of 25 μM buffered solutions of curcumin (CUR), chlorophyllin (CHL), cyanin (CYN) and crocin (CRO) were placed in separated beakers, the closed circuit flow was provided by a peristaltic pump and the pump's cell was placed in the UV-Vis spectrometer. The absorbance of the dye was measured at 0 min. The sources of PAA, SPC, PAP, SPC + TAED and SPC + NOBS were added to obtain 250 μM concentration, then placed in the beaker and the absorbance was monitored at the wavelength of maximum absorbance in 15 min intervals.

For determination of the bleaching performance of hydroxyl radicals as a function of pH, 100 mL of the mixture of 25 μM buffered dyes solutions and 1000 μM of KNO_3 were poured in a beaker. The

absorbance at 0 min was recorded. Then, the beaker was placed under a UV lamp and irradiated for 45 min with 254 nm wavelength light. The absorbance was recorded in 15 min intervals.

For determination of the bleaching performance of singlet oxygen as a function of pH, 100 mL of the mixture of 25 μM buffered dyes solutions and 10 μM of RB were placed in a beaker, and the absorbance at 0 min was recorded. Then, the beaker was placed under a 525 nm LED and irradiated for 45 minutes. The absorbance was recorded in 15 min intervals. Prior to the 0 min absorbance measurement, the 10 μM RB solution absorbance was recorded and then subtracted from the final 0, each spectra.

Bleaching performance was calculated from the formula presented on Eq. 2.18.

$$BP = \frac{Abs_{0\ min} - Abs_{45\ min}}{Abs_{0\ min}} \cdot 100\% \qquad 2.18$$

2.3.9 Image analysis

The influence of pH on the bleaching of red wine, tea and coffee stains by PAA, SPC and TAED was investigated. For each set of variables (stain, bleach and pH value), 4 fabrics were used; in two beakers with two swatches inside each. Images of all of the unwashed fabrics were captured before the experiment by designated Image Analysis equipment (Section 2.1.5). In the next step 100 mL of buffered solutions of PAA (0.01 mol/L), SPC (0.01 mol/L) and TAED + SPC (0.01 mol/L + 0.03 mol/L) were prepared and poured into the beakers containing the swatches. The mixture was then stirred for 45 min, which is an average wash cycle in domestic laundry. Swatches were then placed on a tray and dried for 24 h in the dark. Images of the dry fabrics were collected again by the Image Analysis equipment.

2.3.10 Dye bleaching kinetics

The experiments were performed with peristaltic pump in pH 10.5 buffered solutions. 100 mL of 25 μM solutions of CHL or CRO or BET were placed in a beaker and the closed circuit flow was initiated with the pump. The cell was placed in the UV-Vis spectrophotometer and the absorbance was monitored at the wavelength of maximum absorbance. The absorbance was measured at time 0 min and then immediately a solid source of SPC and PAP or a small volume of PAA solution was added to the beaker to obtain final concentration of bleach at level of 250 μM. The absorbance was

then measured every 2.5 min for a total time of 45 min, which is the average time of a domestic wash. Recorded absorbance data was then fitted as an exponential decay with JMP Pro 12 software.

2.3.11 Kinetics with dyes and stains

The kinetics experiment were run on two spectrometers: Maya 2000 and Fluorolog, due to the different sensitivity of those instruments, two experimental procedures were employed.

2.3.11.1 Maya 200

Experimental work started with the preparation of:

- 24 cuvettes with 1.7 mL of 10.5 buffer
- setup of the spectrometer (λ_{ex} = 365 nm, λ_{em} = 420 nm, t = 2700 s, time interval = 1 s)
- 1500 μM solution of NBA (pH 10.5)

TAED and SPC samples were carefully weighed to obtain final concentration of TAED at 50 μM and SPC at 150 uM. A beaker was placed on a stirring plate and filled with solid bleaching species. A 100 mL of buffer or 10 μM buffered solution of CUR, CHL, CRO or BET solutions were prepared in a measuring cylinder. The fluorescence measurement was started immediately after pouring the solution from the measuring cylinder into the beaker. Then as fast as possible, 0.3 mL of the investigated solution was placed in the quartz cuvette, the cuvette then placed into the spectrometer and the 1 mL of the NBA added. The entire procedure was repeated 24 times over 45 min. In first 10 min the samples were measured approximately in 1 min intervals and the rest 16 measurements were measured approximately in 3 min intervals.

The calibration curve made from PAA (5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 μM) was performed in the same procedure as with the TAED.

In the case of stains, 1 stain of grass, curry, tomato, red wine or tea, together with the solid source of peroxides was placed into the beaker and the measurement was started when 100 mL of buffer solution was poured into the beaker from a measuring cylinder.

Due to the higher concentrations of reagent used in this experiment and due to the lower sensitivity of the Maya 2000 spectrometer in comparison to the Fluorolog, the IFE must be corrected. For this

purpose a beaker containing dyes or stains together with the source of peroxides (the same concentration as above) was equipped with the closed circuit flow (peristaltic pump) and the absorbance was recorded at two wavelength, corresponding to the excitation and emission wavelength of the fluorophore (λ_{ex} = 365 nm, λ_{em} = 420 nm). From the data an equation of absorbance as a function of time was obtained and the fluorescence data was corrected using these values.

The path length, *b* used in correction equation (Eq. 2.3) was determined experimentally to the 0.45 cm, by running a set of absorbance and fluorescence spectra of mixtures of NOH and, CHL and CUR at different concentrations (10, 20, 30, 40, 50 μM of each compound) and checking which path length (0.4, 0.425, 0.45, 0.475 and 0.5 cm) gave the closest results to unfiltered fluorescence of NOH.

2.3.11.2 Fluorolog

The experimental procedure was the same as in the case of the Maya 2000 spectrometer. Except the following concentrations were used:

- 10 μM NOBS + 30 μM SPC (+ 2 μM CUR/CHL/CRO/BET or 1 stain from the set)
- 5000 ppm Neptune; which contains 22 μM of TAED and 540 μM (+ 4 μM CUR/CHL/CRO/BET or 1 stain from the set)
- Calibration curve made from PAA (1, 2, 4, 6, 8, 10, 12, 15, 18, 20, 25, 30, 35, 40, 45 μM)

Due to the low concentration of the dyes, IFE was not observed in the measured samples and therefore the correction was not needed.

2.3.12 Diffuse reflectance of the fabrics

The diffuse reflectance and CIE Lab parameters (using D65 illuminant – sunlight) of the stained fabric were measured before and after the wash. Fabrics were washed for 45 min in 100 mL bleach or detergent solutions, buffered to the 10.5 pH value. The following concentrations were used:

- 1000 μM TAED + 3000 μM SPC
- 1000 μM Neptune + 3000 μM SPC
- 5000 ppm Neptune

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3 DEVELOPMENT OF THE MOLECULAR PROBES TOOLBOX

3.1 Introduction

This third chapter opens the results and discussion section of this work and presents the development of a toolbox of molecular probes and spectroscopic techniques for detection of important ROS in laundry applications. The experimental part starts from the generation and detection of hydroxyl radicals, due to the previous work of a Masters student in the research group.[1] The hydroxyl radical experiments were followed by determination of a suitable method for singlet oxygen detection and also its generation *in situ*. The last part of the work focused on the most important species in bleaching systems – hydrogen peroxide and peroxy acids. After an extensive study on peroxide probes, it has been realised that every probe for hydrogen peroxide will also give a response to peroxy acids and vice versa, due to the presence of peroxy moiety in both species. In laundry products in the majority of cases, peroxy acids are formed in the presence of big excess of hydrogen peroxide, which makes selective analysis challenging. A new analytical approach was needed and developed here.

It is important to characterise the behaviour of the investigated molecules as a function of pH in terms of their future applications. Currently laundry is usually performed at pH 10.5, but there are efforts to reduce the wash solution pH to less alkaline values (e.g. pH 8.5 or 9.5), following the global trend of milder and more environmentally-friendly washes.

3.2 Hydroxyl radicals

Hydroxyl radicals are highly reactive, and consequently short-lived, species. This ROS is thought to be present when textile fibres undergo photodegradation when exposed to sunlight in the presence of oxygen.[2] They can also be generated in laundry solution due to Fenton type chemistry between hydrogen peroxide and transition metal ions.

In this step of the project, an effective source of hydroxyl radicals had to be found to assess the performance of the HO• probes. For this purpose the Fenton reaction and irradiation of aqueous potassium nitrate were chosen for tests in alkaline conditions. The Fenton reaction is a well-known reaction between iron(II) ions and hydrogen peroxide, which produces hydroxyl radical and hydroxide anions (Eq. 3.1). Irradiation of potassium nitrate solution at 254 nm was also selected (Eq. 3.2), because of the reported high efficiency and simplicity of the method.[3]

3.1 $$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^\bullet$$

3.2 $$NO_3^- \xrightarrow{hv} ONOO^- + H^+ \rightarrow NO_2^\bullet + HO^\bullet$$

The most promising probes for laundry applications were terephthalic acid (TA) and coumarin-3-carboxylic acid (CCA), and therefore these were tested under simplified, model conditions – buffered, basic solutions. Both methods rely upon the aromatic hydroxylation of the aromatic molecules to yield a fluorescent product. TA was chosen, because of the reported high efficiency, selectivity and simple hydroxylation product. CCA has an advantage over TA in that the product of hydroxylation 7-OH-CCA can be excited with a longer wavelength than HTA. It is a desirable to excite the fluorescent probe at the lower energy to avoid the exciting of multiple species which are present in the laundry detergent. Laundry solution is a complex mixture and UV light can excite other molecules present in laundry detergents (e.g. surfactants or optical brightners), which can interfere with the probe signal.

3.2.1 Sources of hydroxyl radicals

The Fenton reaction is a well-known method for the generation of hydroxyl radicals in solutions (Eq.3.1). Unfortunately, preliminary experiments showed that the Fenton reaction is a poor source of hydroxyl radicals at alkaline pH and the yield of HO• is very low due to the precipitation of $Fe(OH)_n$. Terephthalic acid was used as a detection method (detailed information about TA in section 3.2.2).

Very weak fluorescence was observed, which is an indicator of a poor yield of HO• formation. In addition, as the pH increased, the fluorescence intensity decreased (Figure 3.1). The observation of the water Raman peak (350 nm) is an indication of a very weak fluorescence.

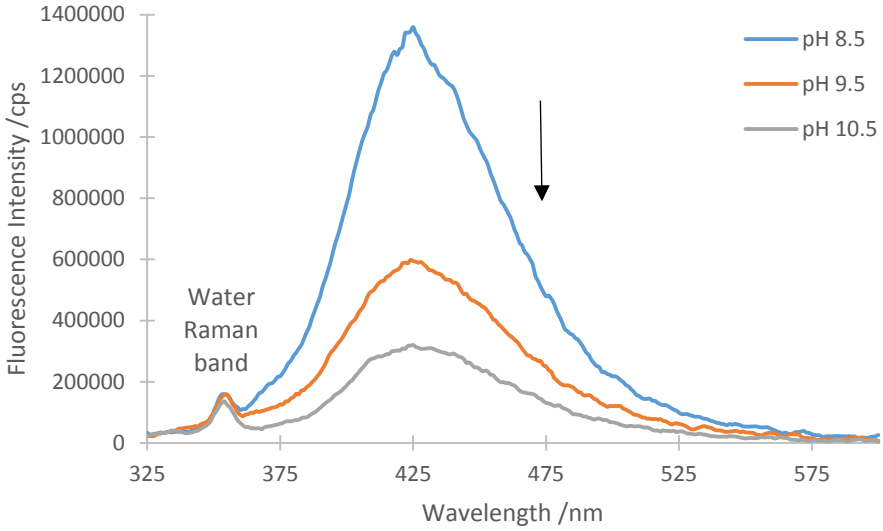

Figure 3.1 Generation of hydroxyl radicals by the Fenton reaction with TA detection in alkaline pH. $[Fe^{2+}]$ = 100 μM, $[H_2O_2]$ = 100 μM, [TA] = 250 μM, λ_{ex} = 315 nm.

Another issue with the Fenton reaction is the problematic, green precipitate of iron (II) hydroxide which was formed at pHs above 8. As found in the literature, an optimum pH for the Fenton reaction is pH 3.[4] Also in alkaline solution, the dominant reaction is the catalytic decomposition of hydrogen peroxide to oxygen by iron ions. Another disadvantage of this source of hydroxyl radicals is the instability of iron (II) solutions, which are quickly oxidised by atmospheric oxygen to iron (III) solution. The latter disadvantage arises from the precipitation of brown iron (III) hydroxide after reaction. [5]

After unsuccessful attempts to generate hydroxyl radicals by the Fenton reaction, tests focused on irradiation of KNO_3 (Eq.3.2). The potassium nitrate method can be utilised in two ways. First the solid KNO_3 can be subjected to shortwave UV radiation (254 nm), which upon dissolution in water yields hydroxyl radicals. The second is generating HO• *in situ* by the irradiation of potassium nitrate solution.

In this work the UV irradiation (254 nm) of a 1 mM KNO_3 solution was used. The yield of HO• formation was observed to be greater than in the case of Fenton reaction. The yield was determined

by the increase of the fluorescence of the hydroxyl-terephthalic acid. It seems that this method increases with solution alkalinity, but the same spectra - pH profile were observed for pure HTA solutions (section 3.2.2), which might indicate that the generation of the radicals is not pH dependent, but rather it is a feature of the fluorescence of the probe.

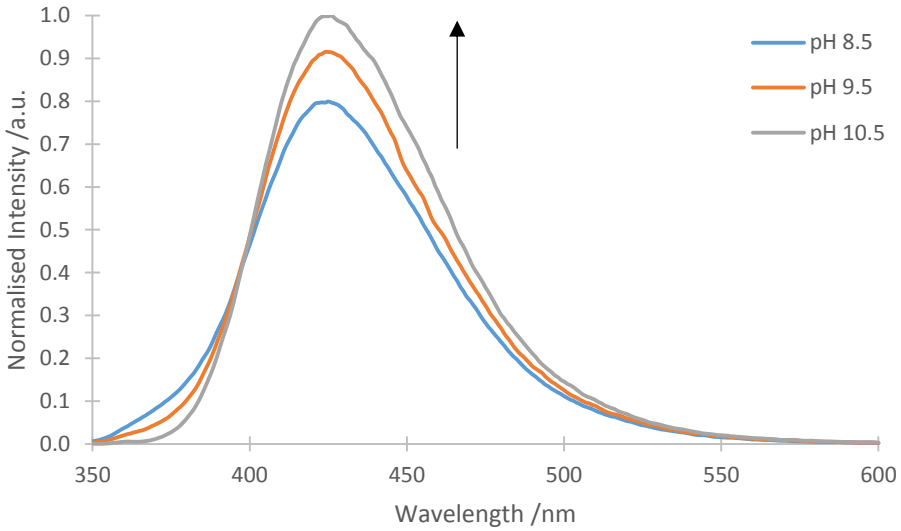

Figure 3.2 Generation of hydroxyl radicals with irradiation of KNO_3 as a function of pH. [KNO_3] = 1 mM, [TA] = 250 μM, λ_{ex} = 340 nm.

3.2.2 Terephthalic Acid

The first probe tested was terephthalic acid, TA. TA is not fluorescent, but upon reaction with hydroxyl radicals produces fluorescent 2-hydroxy-terephthalic acid, HTA.

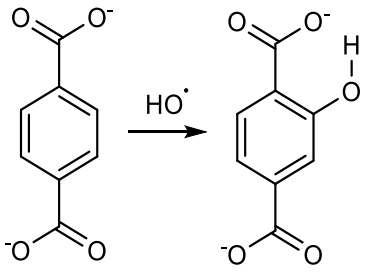

Figure 3.3 Reaction of terephthalic acid (TA) and hydroxyl radical which yields to 2-hydroxy-terephthalic acid (HTA).

The main advantage of this probe is a high specificity for the hydroxyl radicals. Other advantages of TA are: stability of the reagent and product, high sensitivity, and both the reaction and the product fluorescence are almost pH independent. A reference sample of HTA was characterised by emission (λ_{ex} = 310 nm), excitation (λ_{em} = 420 nm) and absorbance spectra (Figure 3.4). The calculated molar extinction coefficient at 310 nm was determined to be 3757 M^{-1} cm^{-1} (pH = 10.5.).

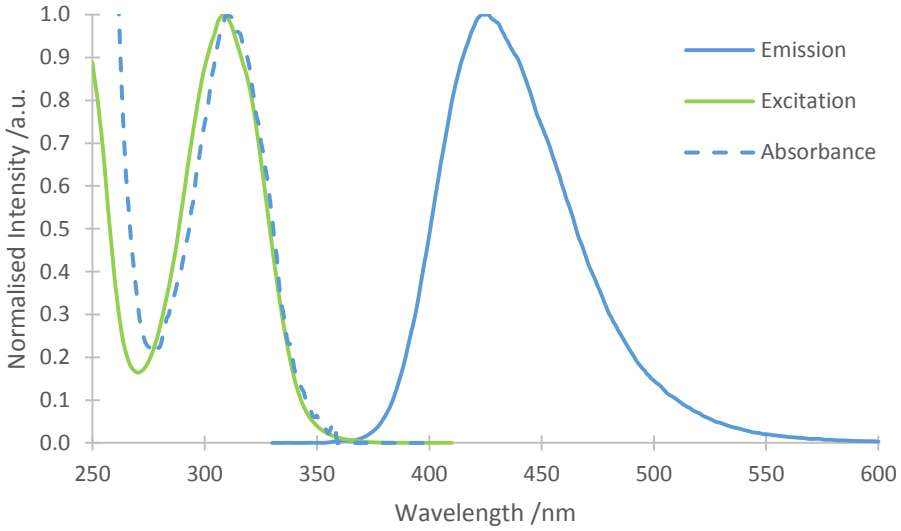

Figure 3.4 Spectral characteristic of HTA at pH = 10.5, λ_{ex} = 320 nm, λ_{em} = 420 nm.

Emission, excitation and absorbance of HTA were examined as a function of pH. The fluorescence intensity increased slightly with an increase in alkalinity (Figure 3.5). The first and second pK_a values of TA are: 3.51 and 4.82, respectively. It can be expected from the 2-hydroxy-benzoic acid example (pK_{a1} = 2.98 and pK_{a2} = 13.04) that HTA can have two lower pK_a values, similar to the TA and one higher, above pH 10.5, similar to 2-hydroxy-benzoic acid. The hydroxy moiety in the ortho position to the ionised carboxylic group is proposed to stabilise the structure of the molecule via an internal hydrogen bond. It can be assumed that the carboxylic groups of the HTA were both ionised and the amount of ionised hydroxyl groups increased with solution alkalinity, resulting in the small differences in the recorded fluorescence spectra.[6]

Experiments were also performed with TA in presence of hydrogen peroxide, peracetic acid and singlet oxygen and no response of TA was observed. The only disadvantage of the TA probe is excitation in UV region, which can be problematic in further studies of more complex systems.

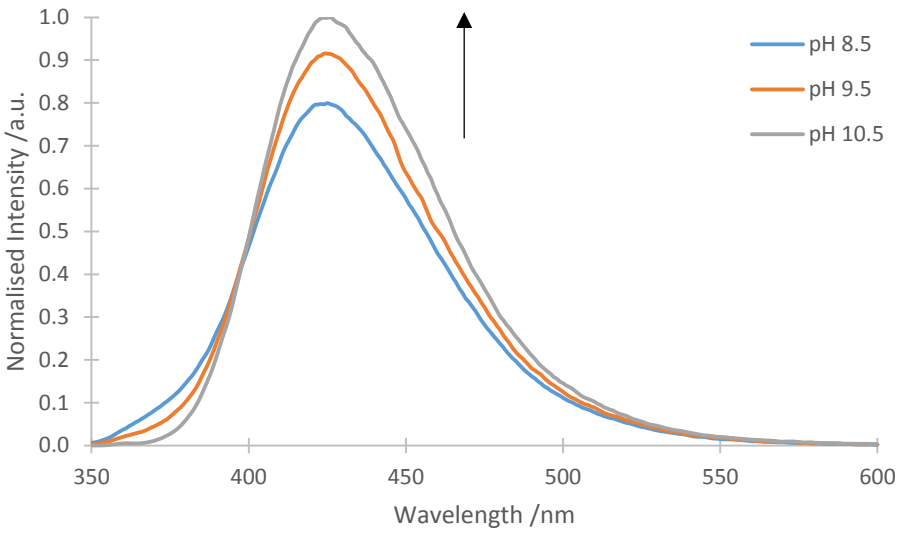

Figure 3.5 HTA fluorescence as a function of pH, λ_{ex} = 340 nm

3.2.3 Coumarin-3-Carboxylic Acid

Coumarin-3-carboxylic acid, CCA, reacts with hydroxyl radicals via aromatic hydroxylation. CCA is almost non fluorescent, but the hydroxylated product: 7-hydroxy-coumarin-3-carboxylic acid is highly fluorescent (Figure 3.6). The main advantage of the CCA probe is excitation at 405 nm. Unfortunately, after the first trial tests it was realised that CCA is not a specific probe.

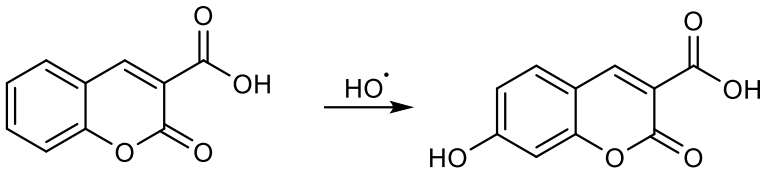

Figure 3.6 Reaction of coumarin-3-carboxylic acid (CCA) and hydroxyl radical which yields to 7-hydroxy-coumarin-3-carboxylic acid (7-OH-CCA).

A test with peroxyethanoic acid showed that CCA can be oxidised by peracids. When [CCA] = 50 μM was exposed to [PAA] = 250 μM, it exhibited fluorescence that was red-shifted in comparison to the 7-OH-CCA. The product of the reaction between CCA and PAA is probably not a 7-OH-CCA and was not identified. Reaction between CCA and peroxides would affect the final results of hydroxyl radical determination when they are analysed in presence of peroxides.

3.3 Singlet oxygen

Singlet oxygen is a key species in the photobleaching process and can be generated by sensitisers (added to the wash formulation) during the drying of fabrics in the sun. The determination of the presence and amount of singlet oxygen generated in the photobleaching process can be a useful tool in rating different photobleaching agents. Singlet oxygen is also a product of spontaneous and metal-catalysed decomposition of peroxy acids in solutions and may be formed during the wash cycle.

For the initial test, singlet oxygen was generated by irradiation of a photosensitiser Rose Bengal (RB) at 532 nm (more detailed information in section 3.3.1). While most of the singlet oxygen probes absorb light in the UV region, 2,5-diphenylisobenzofuran (DPBF) is an exception, and therefore was selected as a first probe for singlet oxygen sensing. However, during the initial test DPBF was found to be unstable in an aqueous environment as well as being susceptible to sunlight. After issues were encountered with DPBF, water soluble 9,10-anthracenediyl-bis(methylene)dimalonic acid was chosen as an alternative candidate for singlet oxygen probe. A number of anthracene derivatives have been established for the detection of $^1O_2$ in the literature. [7–11]

3.3.1 Photosensing

Rose Bengal (Figure 3.7) when exposed to the light from the visible region of the electromagnetic spectrum (520 – 550 nm), reacts as a photosensitiser with molecular oxygen dissolved in the solution and generates singlet oxygen.

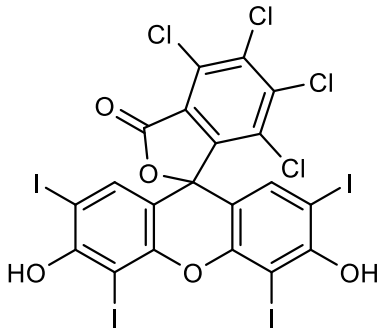

Figure 3.7 Structure of Rose Bengal.

Absorption of light (520 – 550 nm) results in the excitation of photosensitizer which undergoes rapid, efficient intersystem crossing (Φ_T = 1.0) and interacts with diatomic oxygen by energy transfer from the photosensitiser to triplet oxygen to form singlet oxygen (Figure 3.8). [12–14]

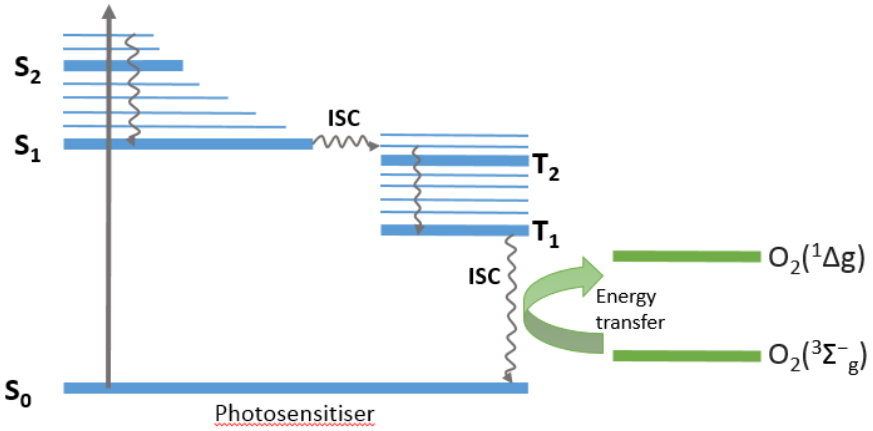

Figure 3.8 Generation of singlet oxygen by photosensitiser.

RB was chosen, because of its strong absorption at 550 nm (Figure 3.9), which allows excitation of the dye by a LED or laser in the presence of the UV-absorbing probe. Rose Bengal absorbance is not pH sensitive in the alkaline pH range (pH 7.5 – 10.5).

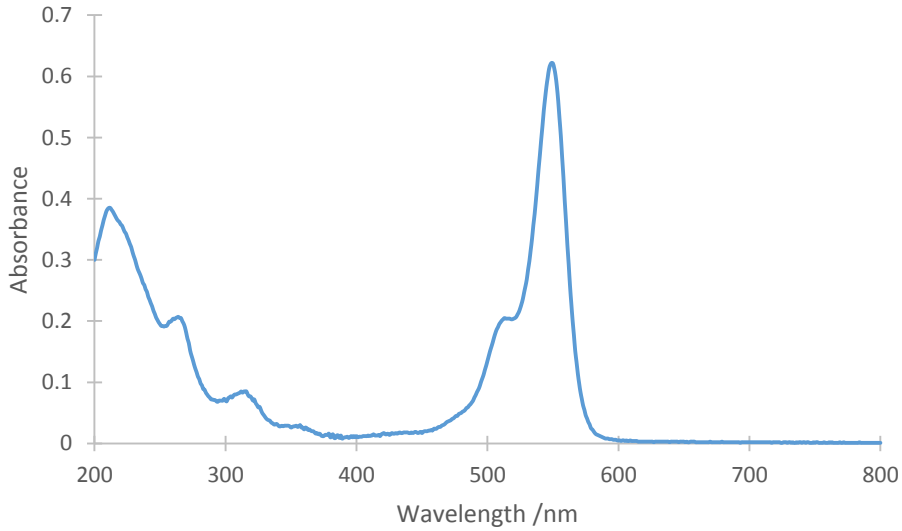

Figure 3.9 Absorbance of the RB in pH = 10.5, [RB] = 50 μM.

3.3.2 Diphenylisobenzofuran

The first singlet oxygen probe examined was diphenylisobenzofuran (DPBF). The reaction of DPBF with singlet oxygen results in a significant change in the absorption spectrum. The absorption spectrum of the probe is slightly pH dependent, with an increase of intensity with alkalinity of the solution and with the maximum around 415 nm (Figure 3.10).

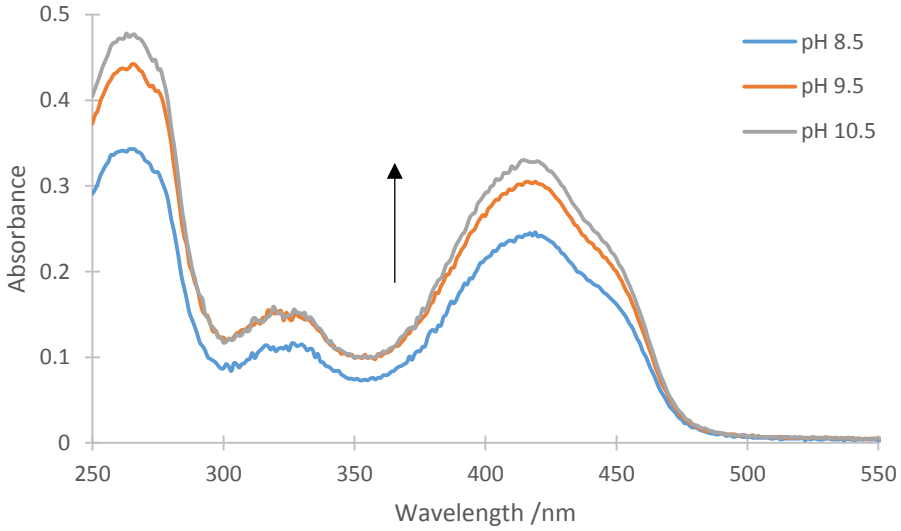

Figure 3.10 DPBF absorbance as a function of pH, [DPBF] = 50 μM.

During the experimental trials with DPBF some difficulties and uncertainties were encountered, such as a long dissolution time and inconsistent results. It is known that the compound is poorly soluble in water and tends to dimerise and become unreactive toward singlet oxygen in water-rich mixtures.[7] Another experiment also showed that DPBF exhibits strong fluorescence in ethanol solution while no sign of emission was observed in an aqueous environment. Furthermore, exposure of the DPBF to sunlight results in decreased probe absorbance indicating its decomposition (Figure 3.11).

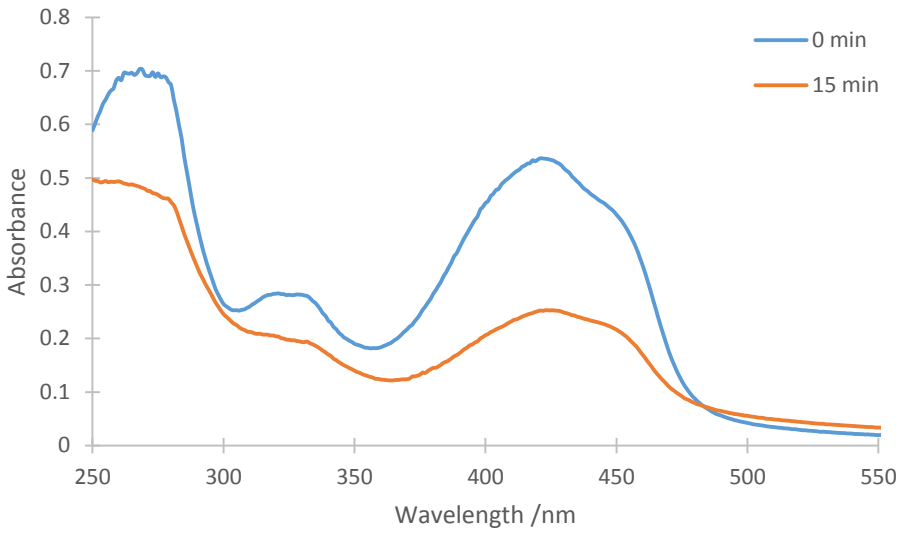

Figure 3.11 Decomposition of the DPBF in pH 10.5 buffered solution when exposed to sunlight, [DPBF] = 60 μM.

DPBF also showed a response to the presence of peracetic acid (decrease of the absorbance, [DPBF] = 50 μM and [PAA] = 250 μM), which makes this probe non-selective and unsuitable for this project purposes.

3.3.3 9,10-Anthracenediyl-bis(methylene)dimalonic acid

The studies of DPBF were followed by the investigation of anthracene derivatives as potential singlet oxygen probes. The first derivative tested was 9,10-dimethylanthracene, which was found to be insoluble in alkaline aqueous solutions. The second investigated derivative was 9,10-anthracenediyl-bis(methylene)dimalonic acid, ADMA, which is much more water soluble due to the presence of the two dimalonic acid groups. ADMA is highly fluorescent, and its reaction with singlet oxygen results in the formation of non-fluorescent endoperoxide (Figure 3.12) which lacks the anthracene chromophore.

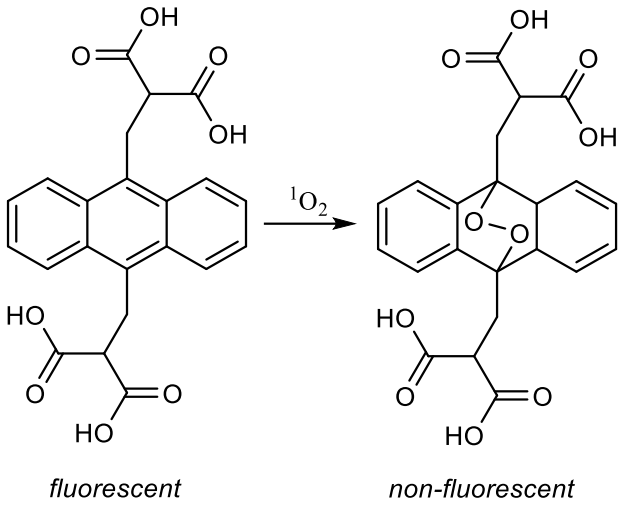

Figure 3.12 Reaction of ADMA and singlet oxygen results in a non-fluorescent endoperoxide.

The spectral characteristic of ADMA can be found in Figure 3.13. The absorption, emission and excitation spectra are not pH dependent. ADMA is a sensitive and selective probe towards the singlet oxygen and no response of the probe was found in the presence of peroxides.

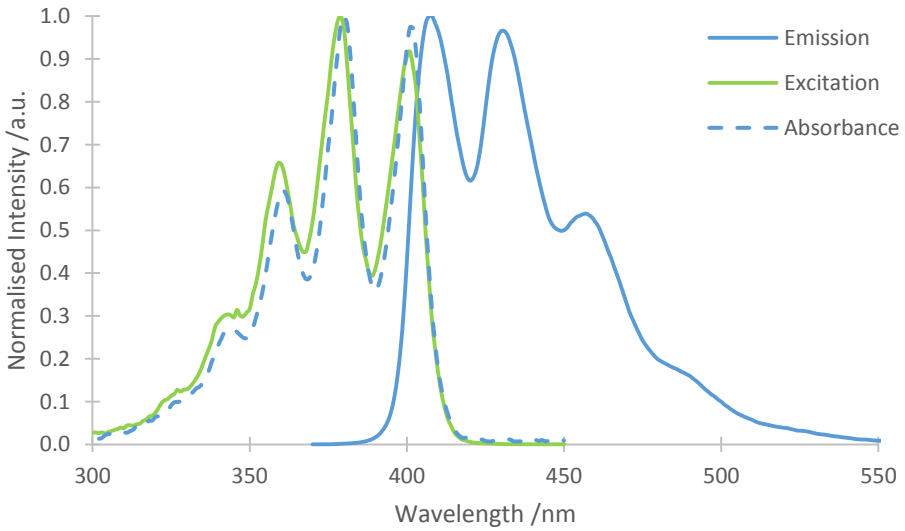

Figure 3.13 Spectral characteristics of the ADMA probe at pH = 10.5, [ADMA] = 25 μM, λ_{ex} = 360 nm, λ_{em} = 420 nm.

The kinetic plot of the fluorescence intensity as a function of time showing the bleaching of ADMA with singlet oxygen generated by irradiation of RB can be found in Figure 3.14. The pseudo-first order rate constant was found to be equal to $9 \cdot 10^{-4}$ s^{-1}.

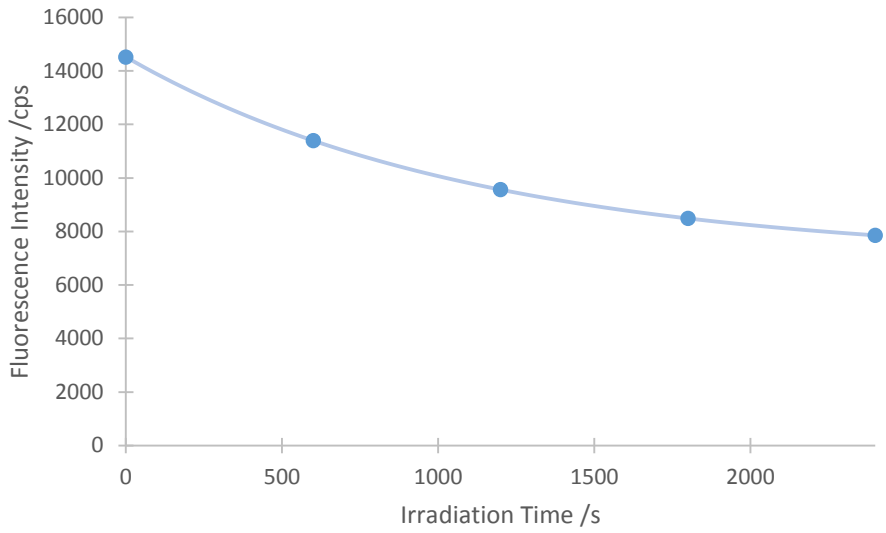

Figure 3.14 ADMA bleaching by singlet oxygen kinetic plot in pH 10.5, [ADMA] = 25 μM, [RB] = 50 μM, λ_{ex} = 340 nm, λ_{em} = 430 nm.

3.4 Hydrogen Peroxide

Hydrogen peroxide is a primary oxidant species in laundry detergent. Reaction of hydrogen peroxide and bleach activators leads to the production of secondary oxidant species e.g. peracids. A full understanding of the reactions of hydrogen peroxide will benefit the improvement of current laundry detergents and design of new products.

Hydrogen peroxide is widely available in the form of a stock solution or as a persalt, e.g. sodium percarbonate or sodium perborate. In this step of the project a 30% (v/v) stock solution of hydrogen peroxide was used, to ensure a lack of additional interactions between hydrogen peroxide source and the probes.

After successful trials with terephthalic acid as hydroxyl radicals probe, CuO nanoparticles were evaluated as a potential reagent for the determination of hydrogen peroxide. These are reported to decompose H_2O_2 to $HO^{\bullet}$, which can be subsequently detected with TA. This procedure would allow also a sequential analysis of hydroxyl radicals and hydrogen peroxide with one probe. The second probe evaluated was 2-naphthylboronic acid (NBA), which has been utilised with success in the literature. [15,16]

3.4.1 Copper Oxide Nanoparticles

The advantages of the copper oxide nanoparticles are their low cost and availability. According to the paper published by *Hu et al.*, CuO nanoparticles should effectively catalyse the decomposition of hydrogen peroxide to hydroxyl radicals, which can be easily detected (with TA) over a wide pH range.[17]

Unfortunately, the initial tests indicated that the copper oxide nanoparticles method works poorly in alkaline pH, making the determination and quantification of hydrogen peroxide impractical. Measurement at 30 min after mixing the reagents showed a very weak fluorescence of HTA, which demonstrates a low yield of hydrogen peroxide decomposition and therefore poor efficiency of the method in the alkaline pH range (Figure 3.15).

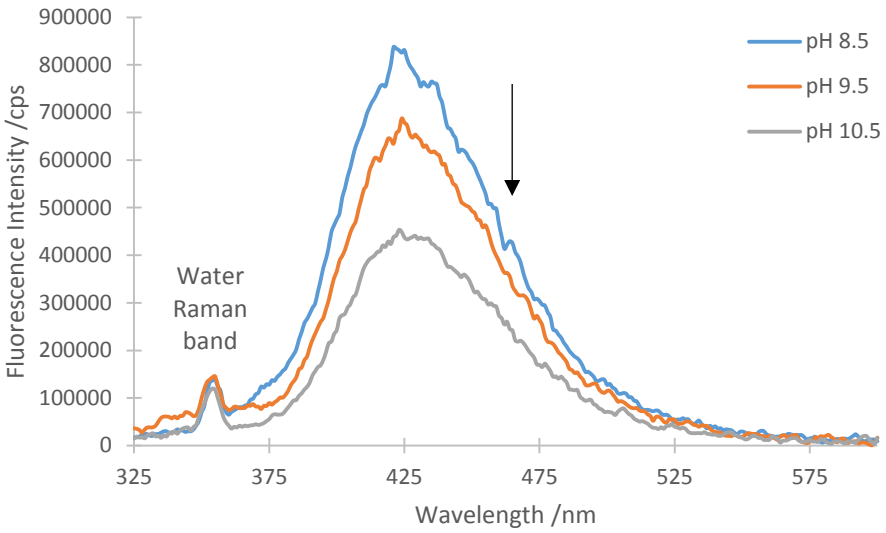

Figure 3.15 HTA fluorescence after 30 min in CuO assay of H_2O_2 detection. [CuO]= 50 μM, [H_2O_2] = 100 μM, [TA] = 250 μM, λ_{ex} = 315 nm.

3.4.2 Aromatic Boronic Acids

The next investigated category of hydrogen peroxide probes were aromatic boronic acids (ABA). ABA react with H_2O_2 to produce fluorescent phenols (Figure 3.16). A wide range of ABA derivatives are commercially available and the parameters of the molecule can be selected to match the conditions of the experiments.

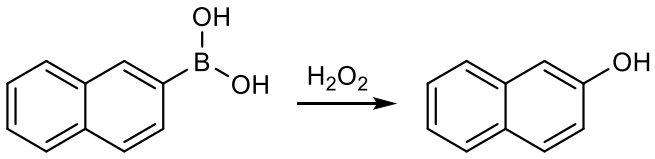

Figure 3.16 Reaction of 2-naphthylboronic acid with hydrogen peroxide which results in the generation of fluorescent 2-naphthol.

The first boronic acid evaluated was 2-naphthylboronic acid (NBA, Figure 3.16). This particular boronic acid was selected because of the reported earlier good performance.[15] The product of the reaction was fluorescent 2-naphthol (NOH). It is worth noticing, that although both NBA and NOH are fluorescent, their behaviour in the alkaline solutions can be considered as 'opposite' (Figure 3.17). With an increase of the alkalinity, NBA fluorescence drops almost to zero, while the fluorescence of

NOH is enhanced at higher pH values. Also, the absorption spectrum of NOH is shifted to longer wavelength than NBA and therefore it can be selectively excited. To avoid interference from NBA, the product of the reaction – NOH can be excited at 340 nm.

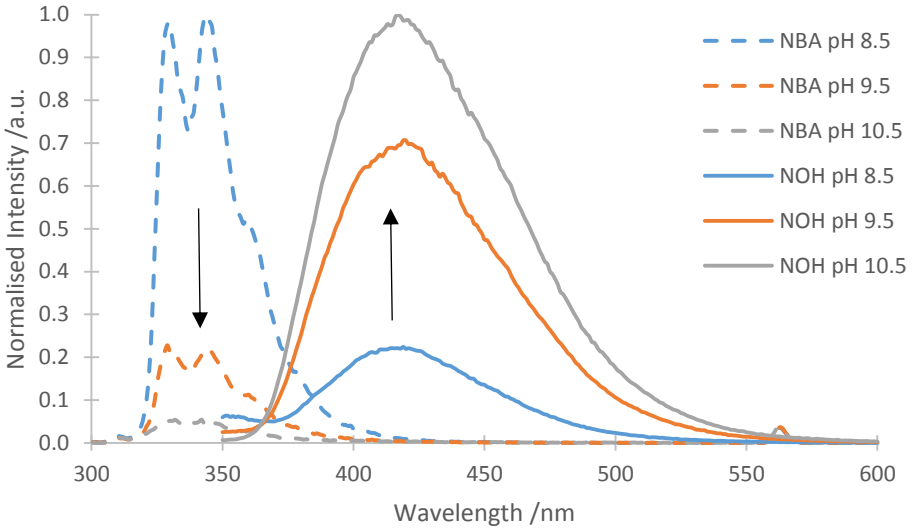

Figure 3.17 Fluorescence of the NBA and NOH as a function of pH, λ_{ex} = 280 nm, [NBA] = 25 μM, [NOH] = 25 μM.

The dependence between pH and fluorescence intensity was examined further. Tests were carried out in strongly acidic, neutral and strongly alkaline solutions and the results revealed that the undissociated form of the aromatic boronic acid is highly fluorescent and the deprotonated form is less fluorescent. For phenols the opposite situation is the case – the dissociated form is more fluorescent than protonated form. The spectral characteristic of NOH can be found in Figure 3.18 below.

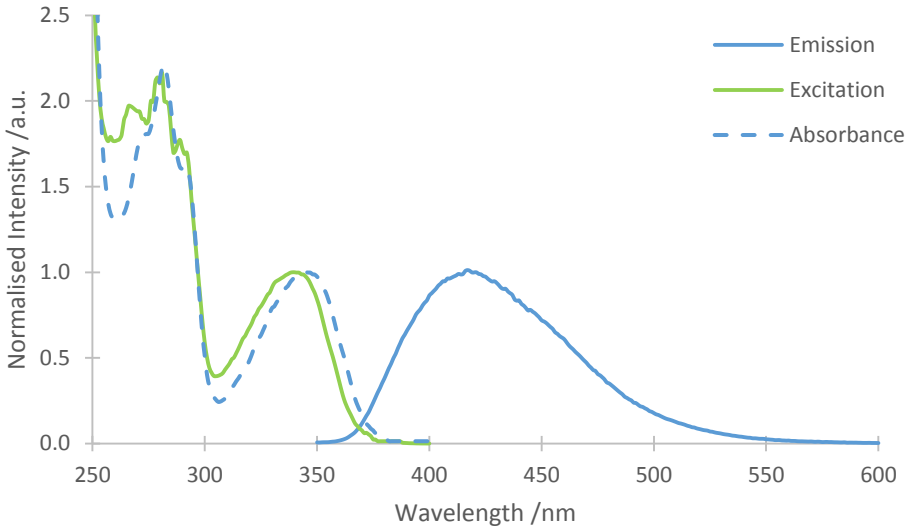

Figure 3.18 Spectral characteristic of the NOH in pH = 10.5, λ_{ex} = 340 nm, λ_{em} = 420 nm, [NOH] = 25 μM.

Unfortunately, although boronic acids show most promising results when compared to the other probes, some disadvantages of the NBA were observed. Firstly, the reaction required around 15 min to reach completion, but most importantly NOH fluorescence was observed after mixing the NBA with peracetic acid, which makes NBA a non-selective probe.

In the course of the study, naphthyl-1-boronic acid, pyrene-1-boronic acid and naphthalene-2-boronic acid pinacol ester were also tested, however the behaviour of the naphthyl-2-boronic acid was found to exhibit the bestoptical properties and was selected as the probe for further studies.

3.5 Peroxy acids

Organic compounds containing the R-C(O)-OOH moiety, where R is an organic group are important substances from an industrial and biochemical point of view. As they are derived from hydrogen peroxide, they are usually called organic peroxy compounds, organic peroxides or simply peroxides.

All of the probes mentioned in first chapter were tested experimentally, starting from the oldest method, but still widely used in industry – iodometry, followed by oxidation of leucomalachite green to produce a coloured product. And ending with a more complex molecule – ABTS.

3.5.1 Iodide

The most common method for the quantification of peracids utilises their reaction with iodide and is followed by the titration of the liberated iodine with thiosulfate (Eq.3.3 and 3.4). The sensitivity of the method can be improved by spectrophotometric determination of I_3^- ion, which is formed by the reaction of I_2 and I^- ion (Eq.3.5).

3.3 $$CH_3COOOH + 2I^- \rightarrow 2\ CH_3COOH + I_2 + O_2$$

3.4 $$I_2 + 2\ S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2\ I^-$$

3.5 $$I_2 + I^- \rightarrow I_3^-$$

The triiodide ion absorbs in the UV region, which might be difficult to analyse in a more complex mixture, for instance laundry detergent, where the majority of the molecules present absorb ultraviolet light. Another problem associated with iodide analysis is decreasing absorbance with an increase of alkalinity (Figure 3.19), as well as interference caused by the presence of hydrogen peroxide, which can be mitigated by dropping the temperature of analysis. Triiodide ion is stable in an aqueous environment, therefore the decrease of absorbance with pH can be associated directly with the reaction between peracetic acid and iodide anions.

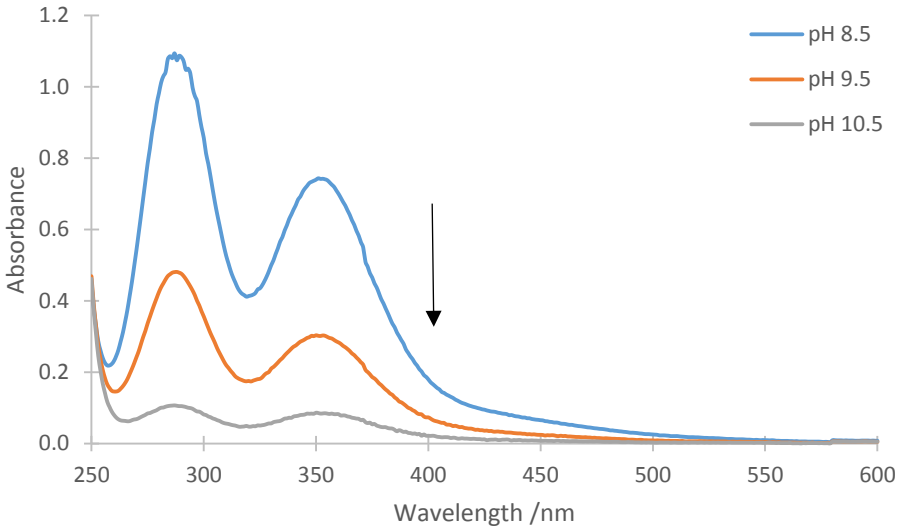

Figure 3.19 I_3^- absorbance as a function of pH, [PAA] = 25 μM, [KI] = 100 μM.

3.5.2 Leucomalachite Green

Leucomalachite green (LMG) can be oxidised by peracids to yield the bright green dye, malachite green (Figure 3.20), which has a maximum absorbance at 650 nm.

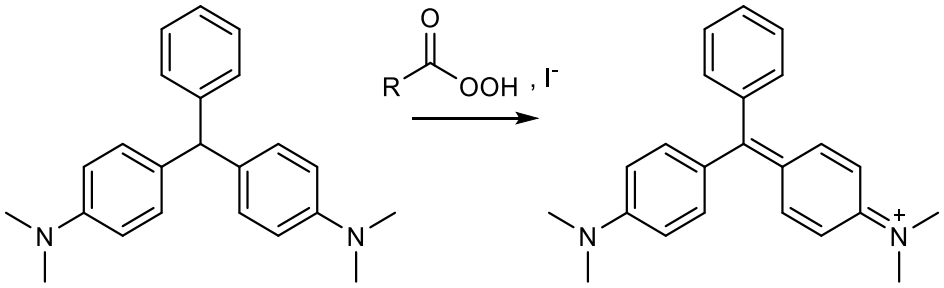

Figure 3.20 Reaction of LMG and PAA in presence of I^- results in generation of malachite green.

The dye precursor is light- and air-sensitive, thus experiments should be performed in darkness. The first problem encountered with LMG is its insolubility in water. Whilst LMG may be readily dissolved in methanol or ethanol, mixtures of methanol and water in different proportions are suspensions, thus immeasurable by absorption spectroscopy. In addition the reaction between LMG and PAA in methanol is very slow and requires *ca.* 30 min to complete.

3.5.3 Diamino 2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonate

Colourless and easily water soluble diamino-2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonate (ABTS) reacts with peracids to form the intense green radical cation (Figure 3.21), which can be quantified by spectrophotometry.

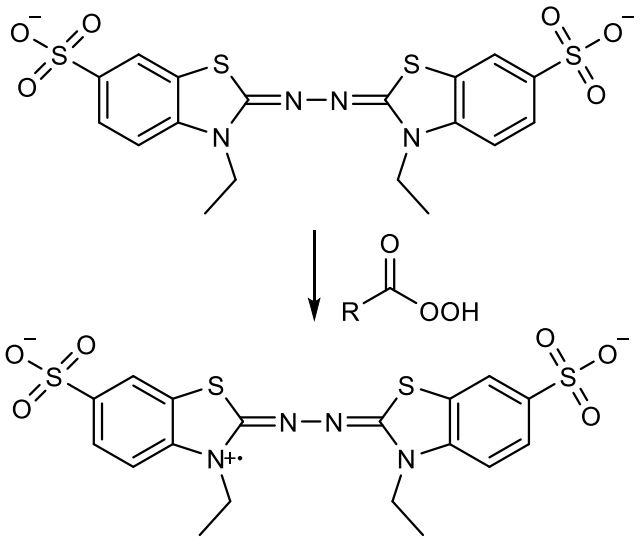

Figure 3.21 Reaction of ABTS with PAA results in ABTS radical cation.

This species exhibits a strong absorption around 420 nm and also in the region between 650 to 820 nm. Reactions can be accelerated by the addition of catalytic amount of the iodide. The main disadvantage of the ABTS assay is a strong pH dependence and low absorbance in more alkaline environments (Figure 3.22).

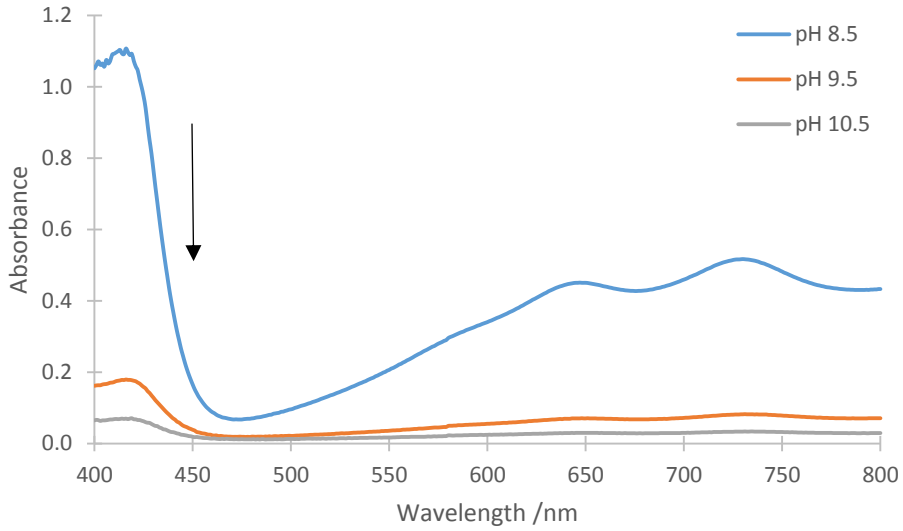

Figure 3.22 Absorbance of the ABTS radical cation as a function of pH, [ABTS] = 50 μM, [PAA] = 10 μM.

Further tests did not show any response of the probe to the hydrogen peroxide. However, measurements of the mixture of PAA and H_2O_2 revealed unexpected behaviour of the probe, and a significant decrease of absorbance in the case of a large excess of hydrogen peroxide over PAA, which possibly can be explained by some secondary oxidation processes (Figure 3.23).

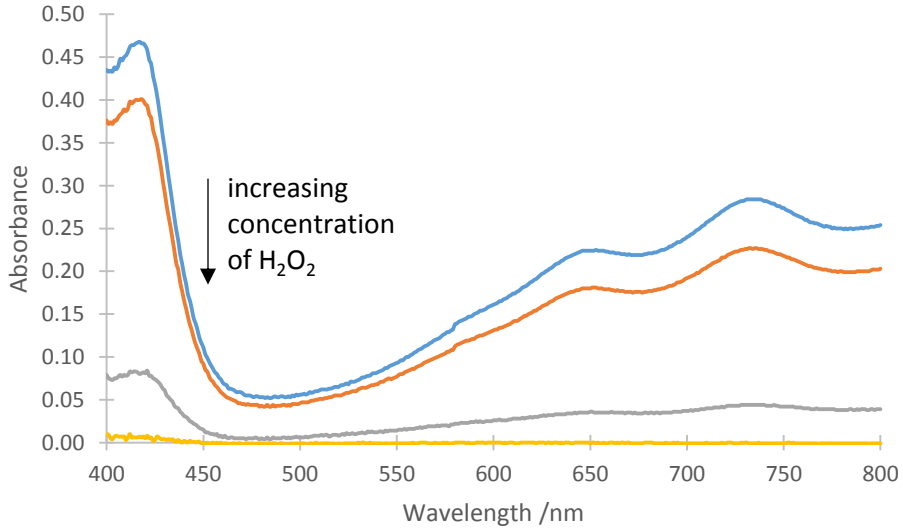

Figure 3.23 Absorbance of the mixtures of ADMA and H_2O_2 with constant PAA concentration at pH = 8.5, [ABTS] = 100 μM, [PAA] = 20 μM, [H_2O_2] = 5, 10, 20, 50 μM.

3.6 Peroxides

After tests with different kinds of hydrogen peroxide and peroxy acids probes, it was realised that none of the probes were single species selective, when the mixture of peroxides was analysed. A new assay was needed.

From the previous work, naphthyl-2-boronic acid proved to be the most promising probe for hydrogen peroxide and potentially peroxy acid detection. During the initial experiments with the probe, it was observed that reaction between NBA and H_2O_2 required a moderate reaction time for completion. In addition, according to the internal P&G experimental procedure, a mixture of the peracids and H_2O_2 can be analysed by iodometric titration in an ice bath, which slows down the reaction between iodine and hydrogen peroxide. Those two pieces of information led to the hypothesis that maybe a new assay could be based on competitive kinetics. (Figure 3.24).

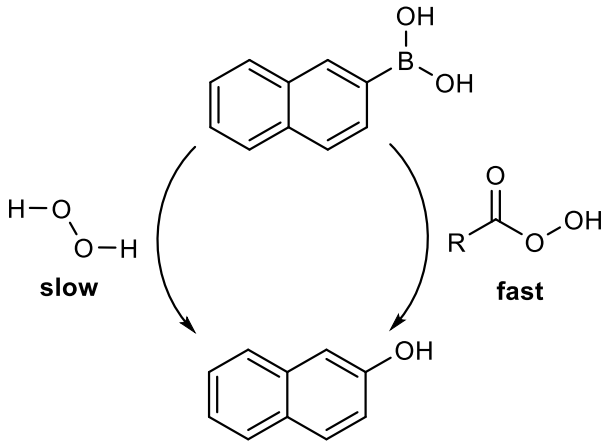

Figure 3.24 Concept behind assay for peroxides detection.

These properties were used as the foundation of a novel method of peroxide detection. Measurement of the fluorescence of the product of the reaction, naphthol, as a function of time delivers specific and accurate information about the presence and concentration of different peroxides (Figure 3.25, Figure 3.26 and Figure 3.27, the spectra were normalised in order to emphasize the shape of curve). Peroxy acids can be identified with this method even in the presence of a large excess of hydrogen peroxide, which is a common situation in laundry solutions.

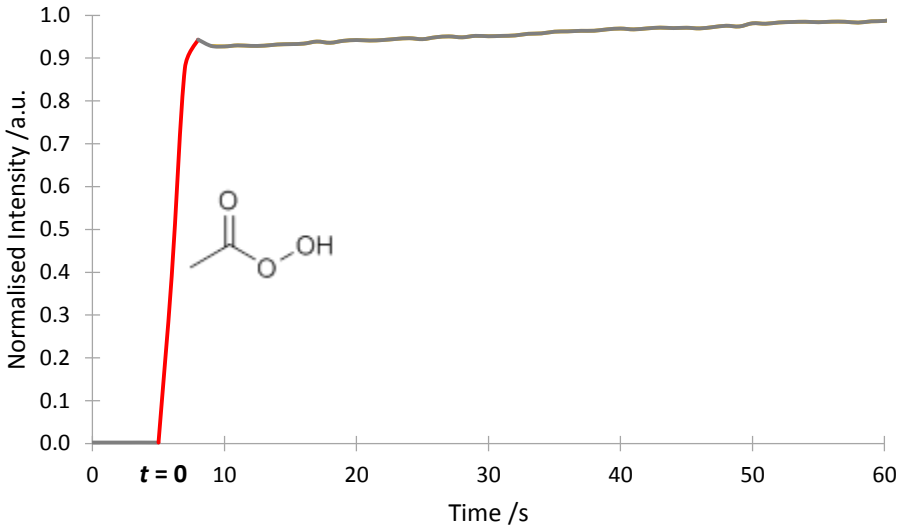

Figure 3.25 NOH fluorescence as a function of time, after the reaction between NBA and PAA, pH 10.5. PAA was added to the mixture in the 5th second of measurement, pH 10.5, [NBA] = 250 μM, [PAA] = 50 μM, λ_{ex} = 340 nm, λ_{em} = 420 nm. Red colour indicates the peroxy acid.

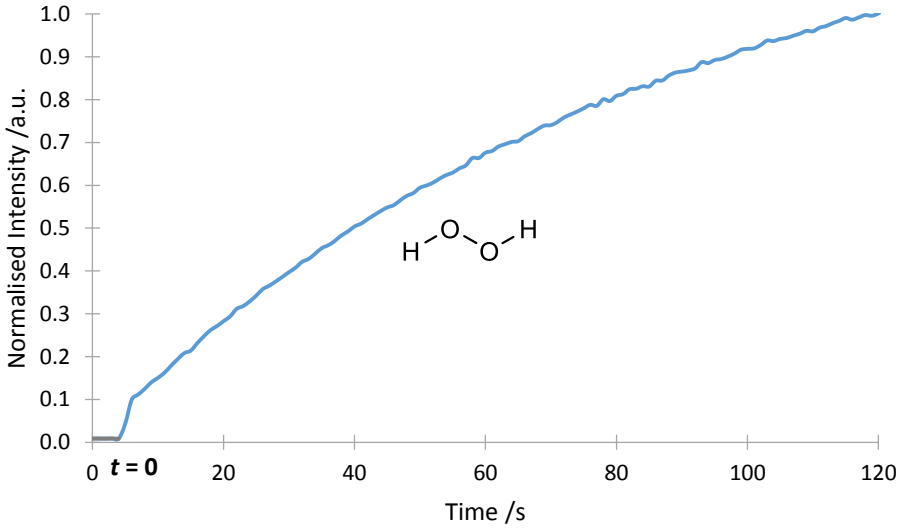

Figure 3.26 NOH fluorescence as a function of time in the presence of hydrogen peroxide, pH 10.5. H_2O_2 was added to the mixture in the 4th second of measurement, pH 10.5, [NBA] = 250 μM, [H_2O_2] = 50 μM, λ_{ex} = 340 nm, λ_{em} = 420 nm. Blue colour indicates the hydrogen peroxide.

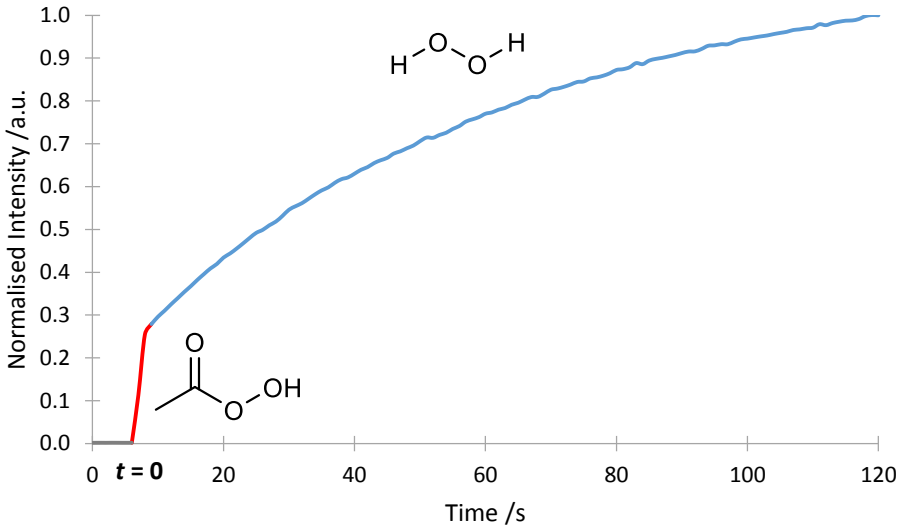

Figure 3.27 NBA fluorescence as a function of time in the presence of peroxyacid and hydrogen peroxide. PAA and H_2O_2 were added to the mixture in the 6th second of measurement, pH 10.5, [NBA] = 250 μM, [PAA] = 25 μM, [H_2O_2] = 25 μM, λ_{ex} = 340 nm, λ_{em} = 420 nm. Red colour indicates the peroxy acid and blue the hydrogen peroxide.

This fluorescence assay has more advantages than the classical method that is widely used in industry – iodometric titration. Samples do not have to be specially prepared before the analysis, the measurement is fast and can be done online. Fluorescence is a very sensitive technique and the detection limit is low (picomolar concentrations).

The foundation behind the novel analytical method was confirmed with a stopped flow technique, which allows the kinetic parameters of rapid reactions to be measured. Data obtained from the reaction of H_2O_2, PAA and PAP with NBA showed pseudo-first order reactions, and the rate coefficient determined with 95% confidence can be found in Table 3.1.

	k /s^{-1}	**$\tau_{1/2}$ /s**
H_2O_2	0.014 ± 0.002	51 ± 7.3
PAA	0.91 ± 0.09	0.76 ± 0.10
PAP	0.92 ± 0.10	0.75 ± 0.09

Table 3.1 Stopped flow results.

The experiments confirmed that reaction between NBA and peroxy acids is indeed very rapid (Figure 3.28) with the rate constant roughly 65 times greater than the reaction of NBA with H_2O_2.

Reaction of the probe and hydrogen peroxide consequently requires a much longer time to reach completion (Figure 3.29).

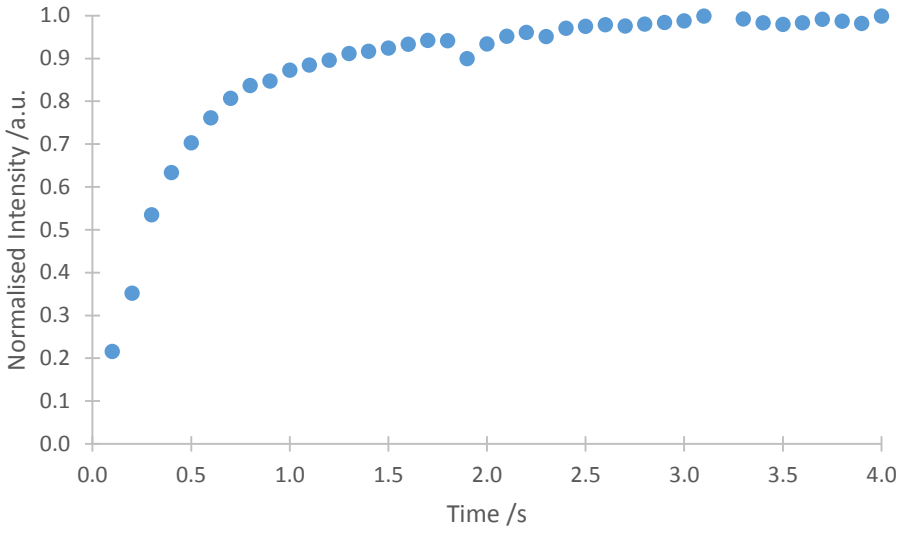

Figure 3.28 Fluorescence at 420 nm recorded using stopped flow during the reaction of NBA and PAA, pH 10.5, [NBA] = 50 μM, [PAA] = 20 μM, λ_{ex} = 340 nm, λ_{em} = 420 nm.

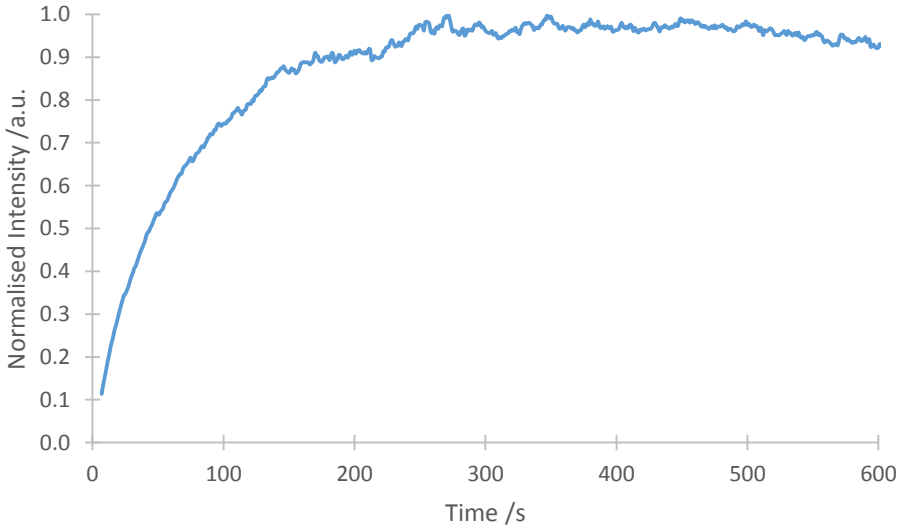

Figure 3.29 Fluorescence at 420 nm recorded using stopped flow during the reaction of NBA and H_2O_2, pH 10.5, [NBA] = 50 μM, [H_2O_2] = 20 μM, λ_{ex} = 340 nm, λ_{em} = 420 nm.

In addition, the order of the reaction between NBA and PAA was determined. The reaction was found to be first order, and depends on the concentration of only one reactant – NBA. Therefore the rate equation has a form presented on Eq. 3.6.

3.6 $$\boldsymbol{r = k[NBA]'}$$

The conclusion was based on the stopped flow results, when the rate constant was measured with different concentrations of one reactant and fixed concentration of the second reactant (Table 3.2).

C_{PAA} /μM	C_{NBA} / μM	k / s^{-1}
20	50	0.90 ± 0.09
30		0.88 ± 0.12
40		0.85 ± 0.10
50		0.90 ± 0.09
50	25	0.68 ± 0.07
	50	0.91 ± 0.09
	100	1.55 ± 0.12
	200	3.97 ± 0.83

Table 3.2 The rate constant of reaction between NBA and PAA, when different concentration of reactant were used.

The plot of the rate constants as a function of NBA concentration shows a straight line, which indicates that the reaction is only dependent on 2-naphthylboronic acid (Figure 3.30).

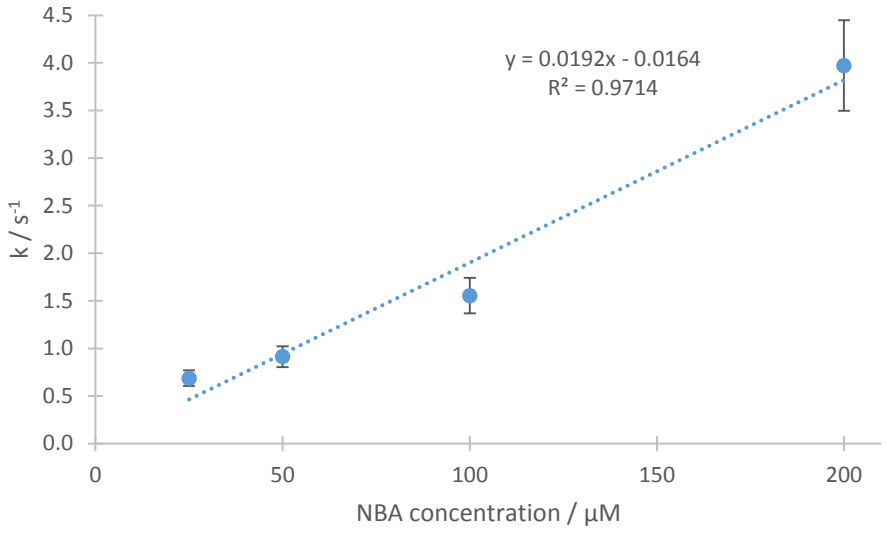

Figure 3.30 Linear plot of rate constant as a function of NBA concentration , while [PAA] = 50 µM, pH 10.5.

3.7 Summary of the toolbox

At this stage of the project a complete toolbox suitable for the study of ROS in wash liquor has been developed. The biggest challenge was to find probes which would work selectively in an alkaline pH range. All of the probes: TA, ADMA and NBA utilise fluorescence spectroscopy, which is much more sensitive than absorbance and allows detection of picomolar concentrations of analyte and can potentially be used in an on-line assay (Figure 3.31). All of the probes can be used for qualitative as well as quantitative analysis of ROS presented in laundry applications: hydroxyl radicals, singlet oxygen and peroxides.

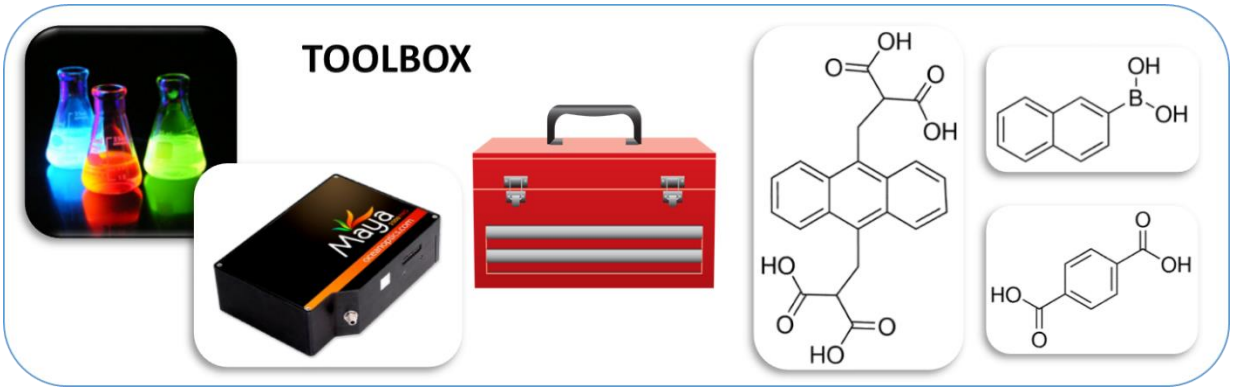

Figure 3.31 Toolbox of molecular probes for ROS detection in laundry applications.

The toolbox contains of terephthalic acid, which after reaction with hydroxyl radicals generates fluorescent product – hydroxyterephthalic acid. Detection of singlet oxygen is based on bleaching of fluorescent ADMA to endoperoxide. Finally, detection of hydrogen peroxide and peracids is based on measuring the fluorescence of naphthol as a function of time, which allows selective analysis of peroxy acids in big excess of hydrogen peroxide, as found in typical laundry conditions and discussed in the future chapter.

In addition the irradiation of potassium nitrate solution and photosensitising of Rose Bengal were proven to be suitable methods for generation in an alkaline environment of hydroxyl radicals and singlet oxygen, respectively.

3.8 References

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4 DETERMINATION OF ROS GENERATED FROM BLEACH SOURCES

4.1 Introduction

The toolbox of molecular probes which consists of TA, ADMA and NBA enables determination of the presence and the concentration of the most common reactive oxygen species in laundry applications (alkaline solutions): hydroxyl radicals, singlet oxygen, peroxy acids and hydrogen peroxide.

The work presented in the third chapter was conducted on commercially sourced, stock solutions of peracetic acid and hydrogen peroxide of high purity. In this chapter, the performances of the molecular probes was evaluated using genuine bleaching species such as sodium percarbonate (SPC) and tetraacetylethylenediamine (TAED). The presence and concentration of peroxides, hydroxyl radicals and singlet oxygen were examined in simplified model systems – alkaline solutions of bleaching species.

4.2 Detection of peroxides in bleach sources

The primary ROS in laundry applications are peroxides (hydrogen peroxide and peroxy acids) and therefore a new, analytical assay based on 2-naphthylboronic acid was tested. The solid sources of hydrogen peroxide and peracids (SPC and PAP), bleach activators (TAED and NOBS), as well as a model laundry detergent (Neptune) were investigated.

Solid SPC sample was dissolved (<1 min) in alkaline solution, mixed with 2-naphthylboronic acid and the fluorescence at 420 nm as a function of the time was recorded simultaneously. The data obtained (Figure 4.1) shows a slow rising profile, attributed to the presence of 'free' hydrogen peroxide. It is important to emphasize that the trace reflects the slow reaction between SPC and NBA, which is the basis of the analytical method, not dissolution of the solid SPC in alkaline solution. This type of experiment provides a qualitative analysis, regarding the type of peroxides present in the investigated solution.

The much smaller 'jump', at $t = 0$ in comparison to a peroxy acids sample, can also be observed, which demonstrates the existence of impurities in the sample.

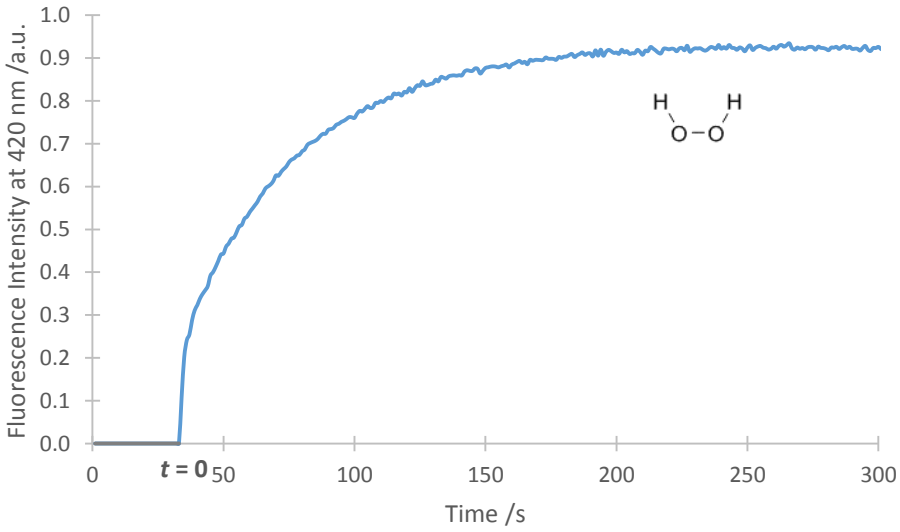

Figure 4.1 Fluorescence of NOH obtained after mixing SPC and NBA at 420 nm as a function of time (λ_{ex} = 340 nm) at pH 10.5, [SPC] = 50 μM, [NBA] = 250 μM, λ_{ex} = 340 nm, λ_{em} = 420 nm.

Iodometric titration of the SPC solution in pH 10.5 performed over 2 hours shows that after fast dissolution of sodium percarbonate, hydrogen peroxide is stable in the solution for at least 2 hours, which covers the average time of the domestic wash (45 min).

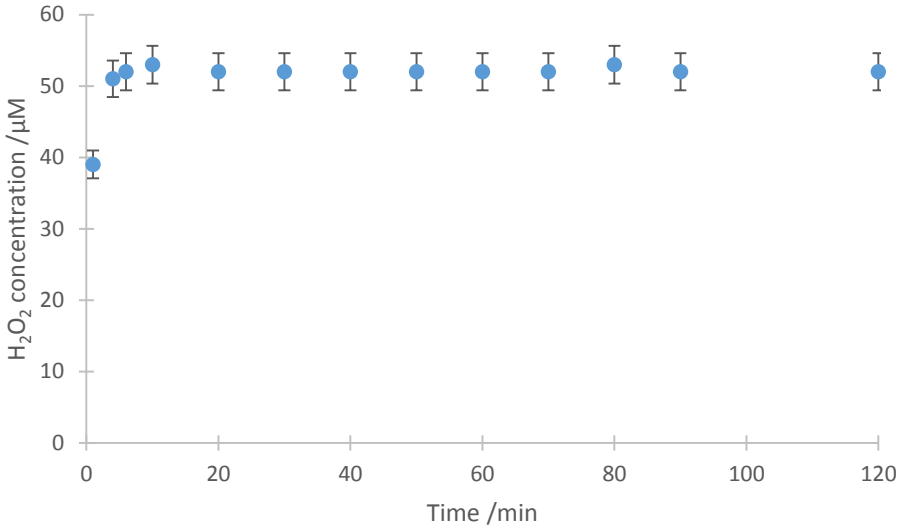

Figure 4.2 Iodometric titration of a 50 µM SPC sample over 2 hours. H_2O_2 is rapidly released from SPC and then stable in alkaline environment (pH 10.5).

After demonstrating the slow reaction of the NBA with SPC the test was repeated using a preformed solid peracid, PAP. As expected for the rapid reaction of the PAP with the 2-naphthylboronic acid, only a rapid increase in fluorescence was observed, confirming the presence of peroxy acids, PAP, in the sample and absence of hydrogen peroxide impurities (Figure 4.3).

PAP is widely used in professional laundry products, and because of special design of its structure, PAP is stable and can be stored for a long periods of time. Stability of the peroxy acid in alkaline solution (pH 10.5) was assessed experimentally over 45 min (average wash cycle) by fluorometric titration. Aliquots of the investigated solution were taken every 2-3 min and mixed with the NBA. The results show that PAP dissolves quickly in alkaline solution and is stable for the duration of experiment (Figure 4.4).

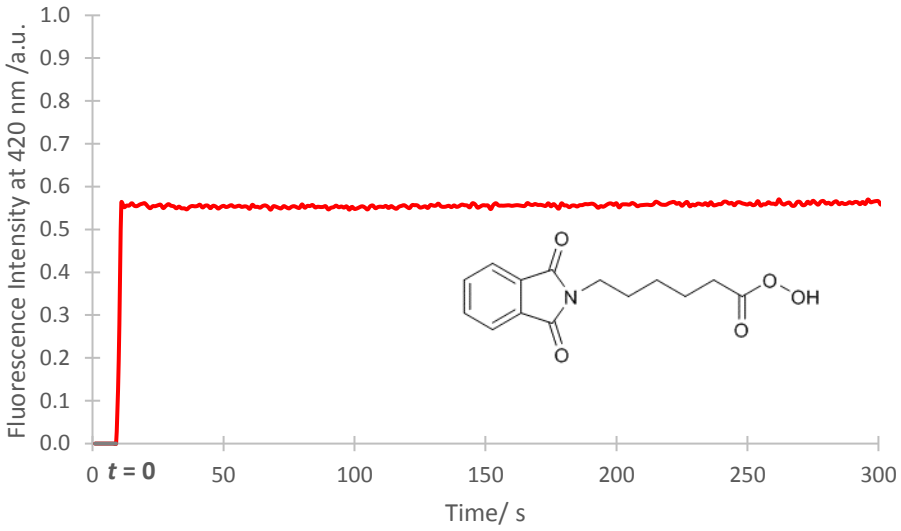

Figure 4.3 Fluorescence of NOH obtained after mixing PAP and NBA, recorder at 420 nm as a function of time (λ_{ex} = 340 nm) in pH 10.5, [PAP] = 50 µM, [NBA] = 250 µM, λ_{ex} = 340 nm, λ_{em} = 420 nm.

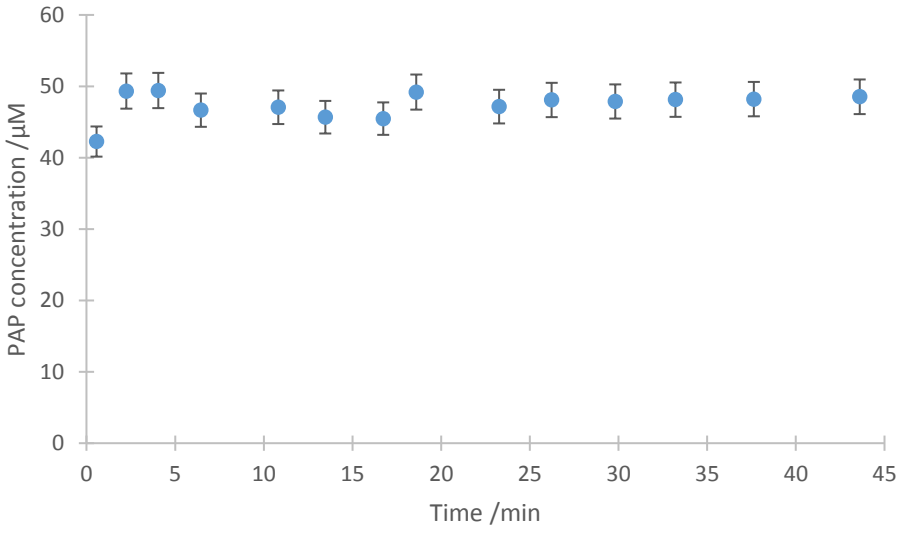

Figure 4.4 Stability of the PAP (50 µM) in pH 10.5 confirmed with NBA (250 µM) fluorescent assay, λ_{ex} = 340 nm, λ_{em} = 420 nm.

The most common bleach activator in Europe is TAED, which forms peracetic acid *in situ* upon reaction with hydrogen peroxide. To determine the nature of the peroxides present, TAED was mixed

with SPC in ratio 1:3 in buffered solution (pH 10.5). After 10 min (to allow components to dissolve and react), NBA was added to the mixture and the fluorescence at 420 nm recorded as a function of time. The fluorescence intensity shows a rapid rise, attributed to the reaction of the PAA with NBA, followed by a slower rise of intensity due to the reaction between NBA with residual hydrogen peroxide (Figure 4.5).

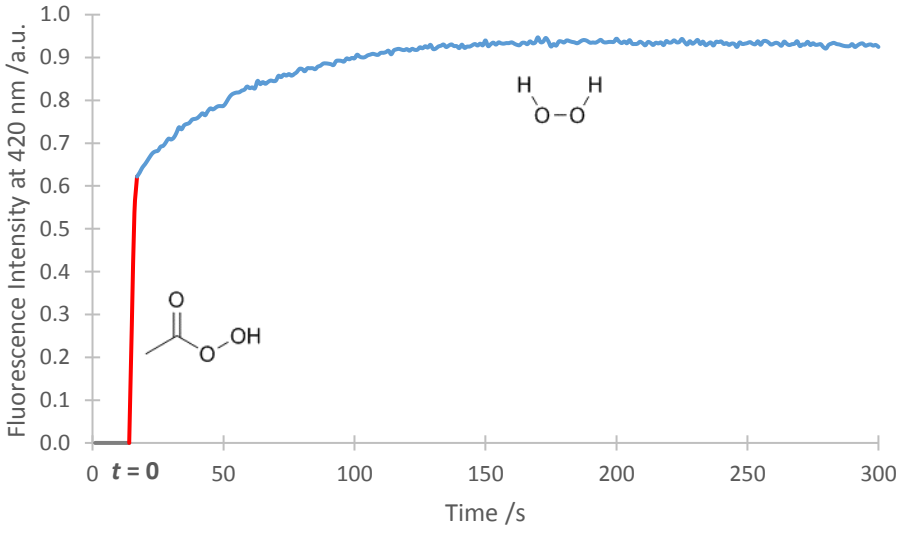

Figure 4.5 Fluorescence of NOH obtained after mixing TAED, SPC and NBA, recorded at 420 nm as a function of time (λ_{ex} = 340 nm, λ_{em} = 420 nm) in pH 10.5. The rapid rise (red) is attributed to the presence of formed PAA, and slow rise (blue) to the presence of hydrogen peroxide. [TAED] = 25 μM, [SPC] = 75 μM, [NBA] = 250 μM.

The fluorometric titration was employed to obtain the kinetic parameters of perhydrolysis reaction between TAED and SPC in pH 10.5 (1:3 ratio). The aliquots of pre-mixed solution of SPC and TAED were mixed with NBA in order to determine the concentration of formed PAA. Resultant concentrations were then plotted as a function of time (Figure 4.6). The figure shows that the perhydrolysis reaction between TAED and SPC is complete during first 10 min, and that the PAA formed is stable for the remaining time, 35 minutes.

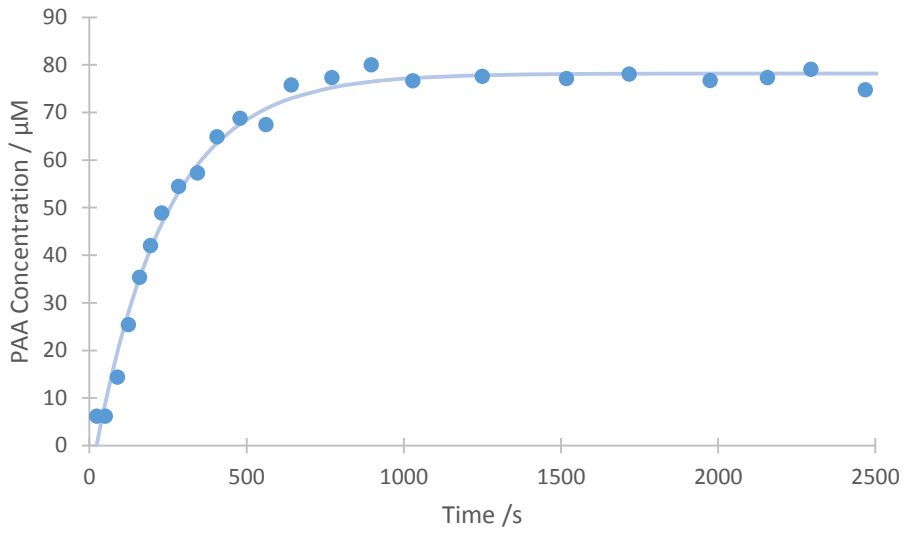

Figure 4.6 The formation of PAA during perhydrolysis reaction of TAED and SPC (1:3 ratio, pH 10.5), determined by the fluorometric titration with NBA, [TAED] = 25 µM, [SPC] = 75 µM, [NBA] = 250 µM, λ_{ex} = 340 nm, λ_{em} = 420 nm.

The perhydrolysis reaction between TAED and SPC is a second order reaction, although, when excess of hydrogen peroxide is used, the reaction becomes pseudo-first order. The pseudo-first order rate constants are directly proportional to the concentrations of TAED.[1] The rate constant for the TAED/SPC system (ratio 1:3) was of 0.0045 ± 0.0008 s^{-1} with 95 % confidence. Consequently, the half-life of the reaction was calculated, and $\tau_{1/2}$ = 154 ± 26 s.

Theoretically, the perhydrolysis of TAED could yield four equivalents of peroxyethanoic acid per mole of TAED, as there are four acyl groups present. However, a molecule of TAED can only provide a maximum of two equivalents of peroxyethanoic acid due to the substantial increase in the conjugate acid pK_a of the leaving group going from an amide (pK_a 17) to an amine (pK_a 35).[2] However, due to a competing reaction of TAED with hydroxide anions, the full two equivalents are not quite released, and various values have been measured *e.g.* 1.5 eq.[3] or 1.7 eq.[4] and reported. In this work during the experiments carried out in sodium bicarbonate buffer at pH 10.5 and with 3-fold excess of hydrogen peroxide to TAED, 1.6 ± 0.1 mole of peracetic acid from one mole of TAED was observed.

The next investigated bleach activator was NOBS – the most common bleaching additive used in North American domestic products. As expected, the fluorometric analysis with the naphthylboronic acid confirmed the presence of peroxynonanoic acid after mixing NOBS and SPC for 10 min (Figure

4.7). Mixing NBA with pre-mixed SPC and NOBS solution shows the characteristic rapid increase of fluorescence, attributed to the reaction between formed *in situ* peroxynonanoic acid with NBA. Rapid rise is followed by the slower, secondary phase attributed to the reaction of residual hydrogen peroxide with NBA.

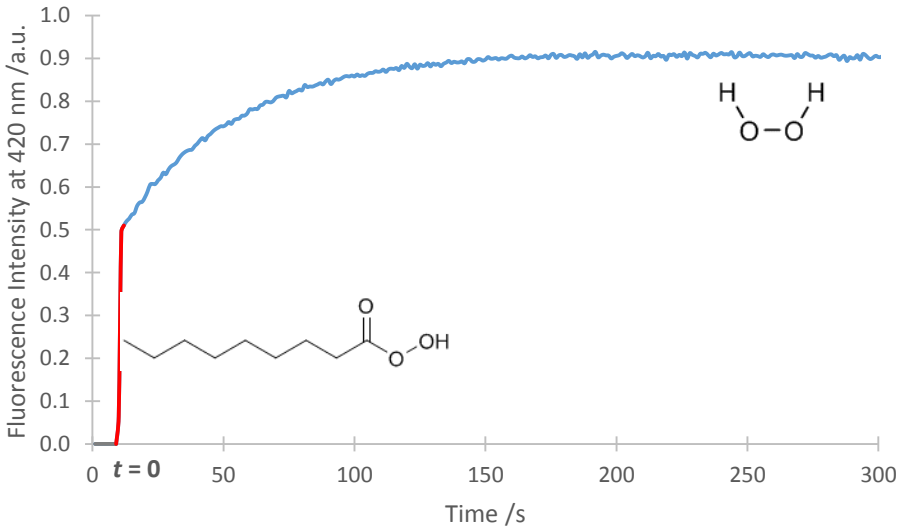

Figure 4.7 Fluorescence of NOH obtained after mixing NOBS, SPC and NBA, recorder at 420 nm as a function of time (λ_{ex} = 340 nm, λ_{em} = 420 nm) in pH 10.5. The rapid rise (red) is attributed to the presence of formed NPA, and slow rise (blue) to the presence of hydrogen peroxide. [NOBS] = 25 μM, [SPC] = 75 μM, [NBA] = 250 μM.

The fluorometric titration of pre-mixed solutions of TAED and SPC, was repeated for NOBS and SPC mixture with NOBS:SPC ratio 1:3. The obtained NPA concentrations were plotted as a function of time, and Figure 4.8 shows that the perhydrolysis reaction is complete within 5 min, and generated NPA very slowly decomposes (k_d = 0.00006 ± 0.00001 s^{-1}), which can be neglected.

The reaction follows a pseudo-first order and the rate constant was determined to be 0.0088 ± 0.0012 s^{-1} and the half-life of the reaction was 79 ± 10 s. One mole of NOBS should provide one mole of peroxynonoaic acid, however due to the competing reaction, which generates diacyl peroxide, 100% yield is not usually achieved. [5] In this work the yield was determined to be ~70%.

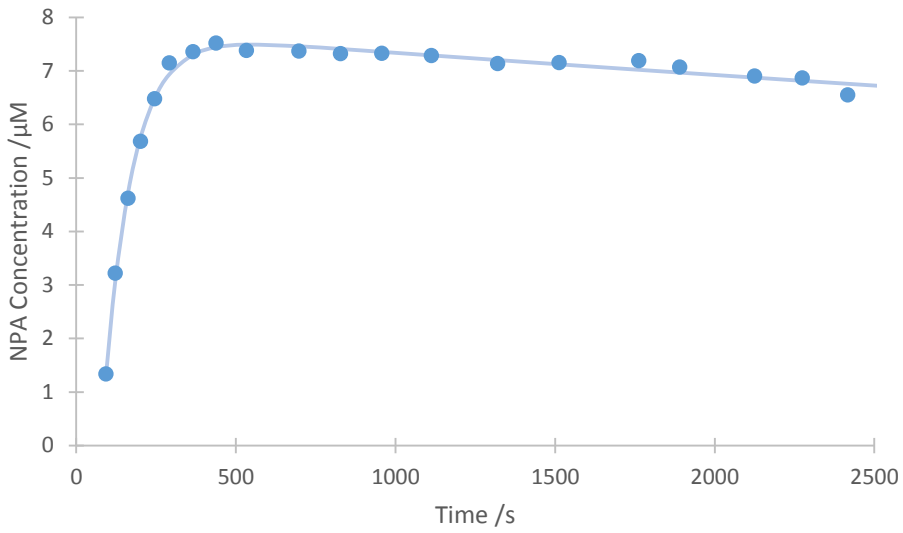

Figure 4.8 The formation of NPA during the perhydrolysis reaction of NOBS and SPC (1:3 ratio, pH 10.5), determined by the fluorometric titration with NBA, [NOBS] = 25 µM, [SPC] = 75 µM, [NBA] = 250 µM, λ_{ex} = 340 nm, λ_{em} = 420 nm.

The next system investigated was a much more complex mixture - a fully formulated, powder laundry detergent, Neptune, provided for tests by Procter & Gamble. The formulation utilised in these experiments lacks the optical brightener, which due to its own intense fluorescence, would prevent an adequate assessment of the probe's signal. Neptune was designed to be used at a dose 5 g/L and provides a wash liquor pH = 10.5. Neptune contains 11.01 % of sodium percarbonate (SPC) and 3.26 % of TAED by mass. Neptune contains also builders, surfactants and other typical ingredients, however their concentrations were unknown.

The time-concentration profile of the peracetic acid shows very similar behaviour in the Neptune sample for the reaction of SPC with TAED (Figure 4.9), which leads to the conclusion that the perhydrolysis reaction itself and stability of the generated peroxy acid are not affected by other ingredients of the Neptune. As observed with model systems one mole of TAED delivers a 1.5 ± 0.1 mole of PAA using this mixture.

The pseudo-first order rate constant of the PAA formation in the Neptune sample was determined to be 0.0098 ± 0.0015 s^{-1} with a reaction half-life of 71 ± 11 s.

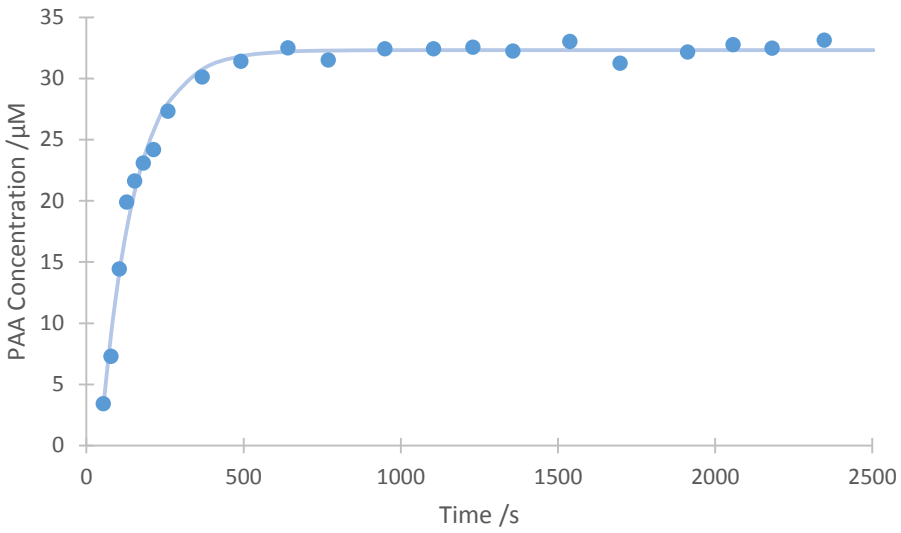

Figure 4.9 Time-concentration profile of PAA in Neptune sample, pH 10.5, [Neptune] = 5000 mg/L, [NBA] = 250 µM, λ_{ex} = 340 nm, λ_{em} = 420 nm.

This part of the project has demonstrated a novel analytical assay utilising the reaction of peroxides with 2-naphthylboronic acid. It allows the simultaneous analysis of both a peracid, and H_2O_2 and has allowed the kinetics of peroxide/bleach activator to be established. The main conclusions are that bleach activators (TAED, NOBS) reacts within a few minutes with SPC. The common feature of the investigated peroxides is their stability in alkaline pH range for at least 45 min (in the absence of the other substrates), which is an average, domestic wash cycle.

The rate constant of formation of PAA from pure TAED is roughly two times smaller than in the Neptune sample, which might be explained by faster dissolution of TAED and SPC due to the presence of a surfactant system in the detergent sample (Table 4.1). The perhydrolysis reaction of NOBS with SPC is almost as fast as the reaction of TAED and SPC in presence of a surfactant system, which might be attributed to the hydroxybenzene sulfonate being a better leaving group.

	k /s^{-1}	**$\tau_{1/2}$ /s**
TAED	$4.5 \cdot 10^{-3} \pm 8.0 \cdot 10^{-4}$	154 ± 26
NOBS	$8.8 \cdot 10^{-3} \pm 1.2 \cdot 10^{-3}$	79 ± 10
Neptune	$9.8 \cdot 10^{-3} \pm 1.5 \cdot 10^{-3}$	71 ± 11

Table 4.1 Kinetic data for bleach activator systems.

4.3 Detection of singlet oxygen in bleach source

In a laundry solution the most likely source of singlet oxygen is the spontaneous and metal-catalysed decomposition of peroxy acids (e.g. PAA, PAP or NPA). For all sources of peroxy acids, except PAP, it can be assumed that a metal-catalysed decomposition mechanism prevails over the spontaneous decomposition (Figure 4.10). The highest concentration of $^1O_2$ was observed in a solution of TAED + SPC dissolved in tap water, but that can be easily explained by the possibility of TAED to deliver ~ 1.5 equivalents of PAA from one equivalent of TAED.

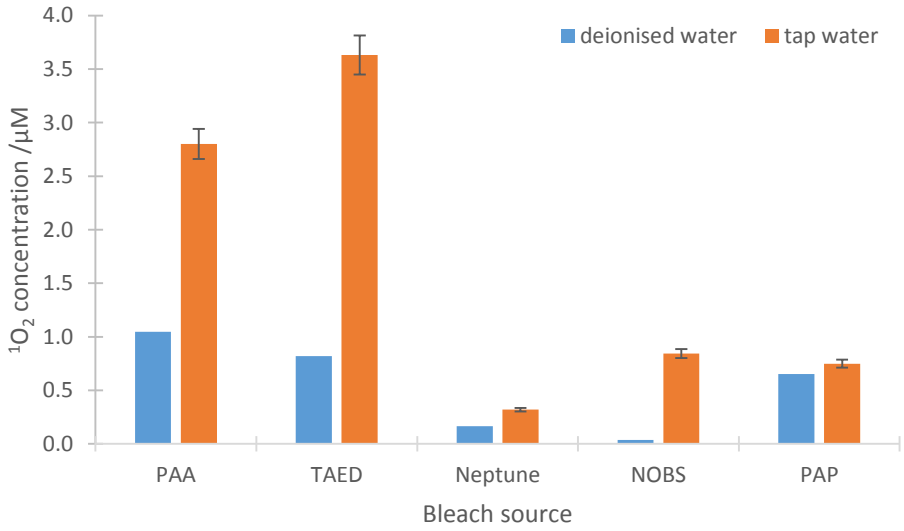

Figure 4.10 Singlet oxygen generated from bleach sources in 45 min in pH 10.5, [ADMA] = 100 µM, [PAA, PAP, TAED, NOBS] = 500 µM, [SPC] = 1500 µM, [Neptune] = 5000 mg/L, λ_{ex}= 360 nm, λ_{em} = 410 nm.

The yield of singlet oxygen generation from decomposition of peracids is lowest for Neptune for both deionised and tap water, which can be explained by the presence of various chelators in the Neptune formulation, which can prevent the decomposition by chelating transition metal ions, e.g. $Fe^{2+/3+}$, that can produce Fenton chemistry (Table 4.2). Similar low yields can be observed for peroxynonanoic acid, PNA, generated from NOBS in deionised water which may indicate that PNA is much more stable than PAA.

	Yield of $^1O_2$ formation /%				
	PAA	**TAED**	**Neptune**	**NOBS**	**PAP**
deionised water	$2.1 \cdot 10^{-1}$	$1.6 \cdot 10^{-1}$	$6.8 \cdot 10^{-3}$	$7.0 \cdot 10^{-3}$	$1.3 \cdot 10^{-1}$
tap water	$5.6 \cdot 10^{-1}$	$7.3 \cdot 10^{-1}$	$1.3 \cdot 10^{-2}$	$1.7 \cdot 10^{-1}$	$1.5 \cdot 10^{-1}$

Table 4.2 Yield of singlet oxygen formation from bleach sources. Error ± 8%. Yield is based on initial concentrations of PAA, TAED, Neptune, NOBS and PAP.

The decomposition of peroxyacids is very slow, and therefore kinetic parameters of the reaction were not obtained. The formation of singlet oxygen was found to follow first order kinetics (Figure 4.11). The experiment showed that the slope of the ADMA bleaching grows linearly with the concentration of PAA.

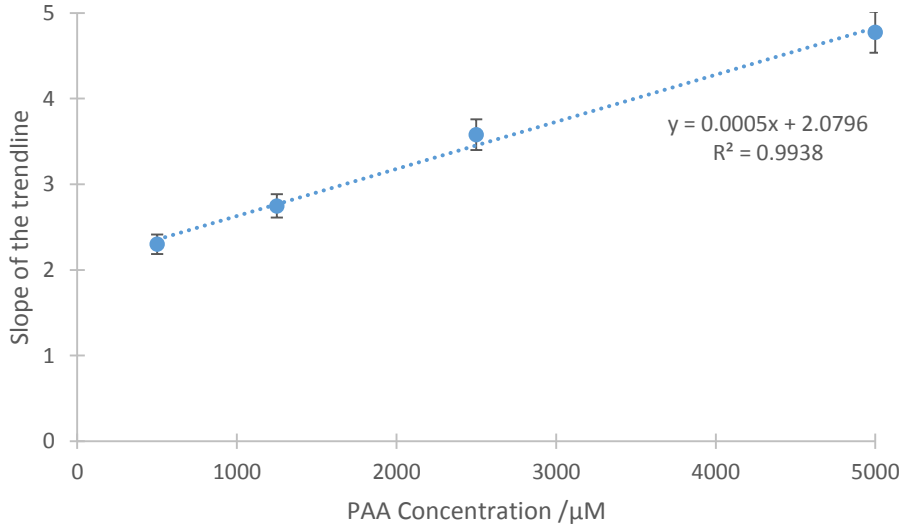

Figure 4.11 Determination of the order of the decomposition reaction. [ADMA] = 100 µM, λ_{ex}= 360 nm, λ_{em} = 410 nm.

Both the spontaneous and metal-catalysed decompositions are very slow and in general exhibit a very low yield. In addition the lifetime of singlet oxygen in an aqueous environment is very short, 4 µs,[6] thus it can be argued that singlet oxygen does not play an important role in bleaching during the wash. However, formation of singlet oxygen can be analysed with the fluorescence probe – ADMA, and might be used to assess for instance the performance of newly designed photobleaching agents.

4.4 Detection of hydroxyl radicals in a bleach source

The potential presence of hydroxyl radicals in bleach sources in the alkaline pH range was investigated with the turn-over fluorescent probe – terephthalic acid. However, for room temperature experiments no sign of HO˙ was found. As illustrated in Figure 4.12 no fluorescence signal could be detected after an extended period: the band at 350 nm is the weak Raman scattering of water.

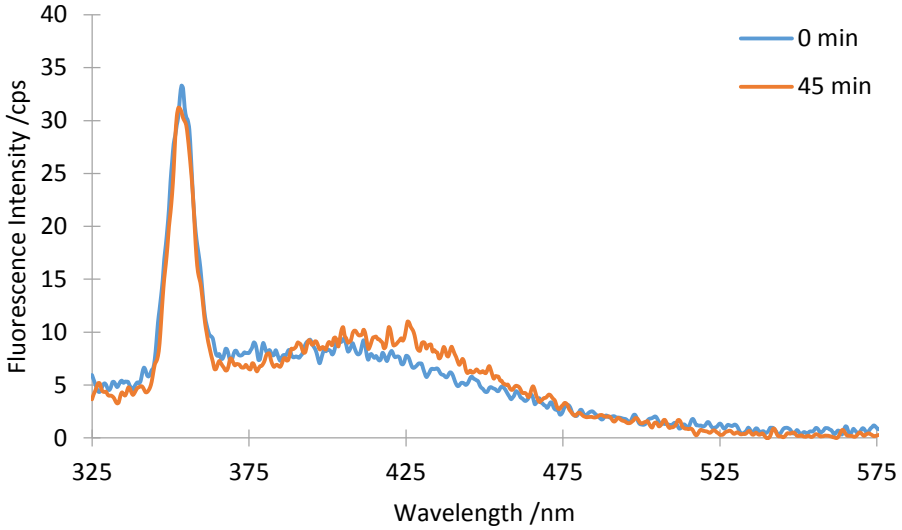

Figure 4.12 Raman scattering of water peak, obtained from mixture of PAA and TA in pH 10.5, [TA] = 100 μM, [PAA] = 500 μM, λ_{ex} = 315 nm.

Inspired by the paper of *Si et al.* [7] hydroxyl radical experiments were also performed at higher temperature, 60°C, which is nowadays the highest expected temperature of a laundry cycle. Experiments were conducted in deionised and tap water, to mimic the conditions of the domestic wash. *Si et al.* claimed that the presence of the bleach activators in the mixture with hydrogen peroxide enhanced the formation of hydroxyl radicals at higher temperatures. In addition they claimed that at higher temperatures hydroxyl radicals are the dominant agent responsible for bleaching, rather than the peroxy acids.

At this elevated temperature the highest concentrations of hydroxyl radical were observed in SPC dissolved in tap water, which can be explained by the occurrence of the Fenton reaction between hydrogen peroxide and transition metal ions presented in tap water (Figure 4.13). The next results stand in opposition to results from the paper – the presence of bleach activators significantly decrease

the amount of hydroxyl radicals detected. The quantity of hydroxyl radicals generated from PAP is negligible. Thus the question arises: what is the cause of the lower concentration of HO• when SPC was mixed with PAP (3:1 ratio)? From one hand this can be explained by possible scavenging properties of peroxy acids, and from other hand by the equilibrium between peroxy acids and hydrogen peroxide (Eq. 4.1). When hydrogen peroxide is in excess, then the equilibrium shifts to the left side of the equation.

4.1 $$RC(O)OOH + H_2O \rightleftharpoons RC(O)OH + H_2O_2$$

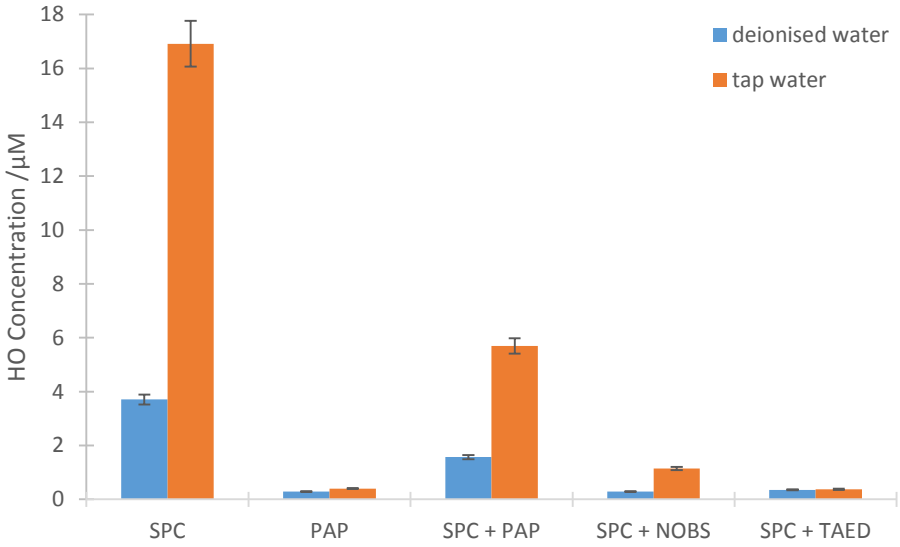

Figure 4.13 Hydroxyl radicals generated from bleach sources at 60 °C within 45 min. [SPC, PAP] = 5000 µM, [PAP, NOBS, TAED] = 1670 µM, [TA] = 500 µM, λ_{ex} = 315 nm.

Even lower concentrations of HO• can be observed for the mixtures of SPC and bleach activators: TAED and NOBS. This can be explained by considering that H_2O_2 released by the SPC was consumed for perhydrolysis reaction which generates peroxy acids, which are in equilibrium with hydrogen peroxide and/or exhibits scavenging effects.

The main conclusion from these experiments is that the hydroxyl radicals are present in higher temperature wash conditions, but at very at low concentration. They are most likely formed by Fenton chemistry and their presence is effectively suppressed by the presence of peroxy acids. Again, as in the case of singlet oxygen, the fluorescent probe proved that HO• are present, but again the contribution of this species can be considered of little relevance to the bleaching performance.

4.5 Sonochemistry

In order to investigate alternative ways of laundry which utilizes , the cleaning of the stains by ultrasounds was tested experimentally. Hydroxyl radicals can be generated by the sonification of water in an ultrasound bath and this process was also investigated. Ultrasound refers to sound waves beyond the frequency that can be detected by the human ear, frequencies greater than 20 kHz fall into this category. [8] Ultrasound creates rapidly forming and collapsing bubbles in liquids, known as an 'acoustic cavitation', generating extreme conditions of temperature and pressure that can be used for various processing applications and for promoting chemical reactions. Cavitation is defined as the cyclical formation (nucleation), rapid growth (expansion) and collapse (implosion) of micro bubbles in the liquid. [9] The consequences of these extreme conditions generated include radical generation, light emission (sonoluminescene), shock waves, microjets, microstreaming, shear forces and turbulence. [10]

In air-saturated water, a variety of radicals and molecular products such as H_2O_2, $HO_2^{\bullet}$, $O^{\bullet}$, O_3, HNO_2, HNO_3, H_2 and $OH^{\bullet}$ radicals are generated (Eq. 4.2 - 4.9).[11] Primary radicals of sonolysis $OH^{\bullet}$ and $HO_2^{\bullet}$ can react in three different zones: in the gas phase, at the cavitation bubble interface (recombination and formation of hydrogen peroxide (Eq. 4.3 and 4.7)) and in the solution bulk (formation of hydrogen peroxide and initiation of other oxidation reactions).[12] The generation of $H^{\bullet}$ and $OH^{\bullet}$ radicals during acoustic cavitation has been confirmed using a number of experimental techniques, e.g. Electron Spin Resonance spin traps,[13,14] hydroxylation of terephthalic acid [15,16] or oxidation of the luminol. [17]

4.2 $H_2O \rightarrow H^{\bullet} + OH^{\bullet}$

4.3 $2\ OH^{\bullet} \rightarrow H_2O_2$

4.4 $O_2 \rightarrow 2\ O^{\bullet}$

4.5 $O + O_2 \rightarrow O_3$

4.6 $H + O_2 \rightarrow HO_2^{\bullet}$

4.7 $2\ HO_2^{\bullet} \rightarrow H_2O_2 + O_2$

4.8 $N_2 + O_2 \rightarrow 2\ NO$

4.9 $2\ NO + O_2 \rightarrow 2\ NO_2$

Ultrasound can also accelerate conventional reactions, e.g. the Fenton reaction (Eq. 4.10). In the sono-Fenton process, the ultrasonic irradiation could promote Fenton reactions by the faster regeneration of ferrous ions and the generation of more hydroxyl radicals from water without hydrogen peroxide consumption (Eq. 4.11). [18]

4.10 $$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$$

4.11 $$Fe^{3+} + H_2O \rightarrow Fe^{2+} + OH^\bullet + H^+$$

A sample of 100 mL of water at pH 7 and 10 (sodium bicarbonate buffer) containing terephthalic acid (100 μM) was sonicated in a ultrasonic bath for 60 min. An aliquot of the solution was analysed for the formation of a hydroxyl-terephthalic acid by fluorescence spectroscopy and the concentration determined by reference to a calibration curve. The experiment confirmed the presence of HO$^\bullet$ in water exposed to the ultrasound (Figure 4.14). After 60 min of sonification the highest concentration of HO$^\bullet$ was found at level of 3.1 μM in tap water. The results show that the presence of transition metal ions significantly enhanced the HO$^\bullet$ generation, due to the sono-Fenton reaction.[19] In addition a neutral pH is more favourable than alkaline solution.

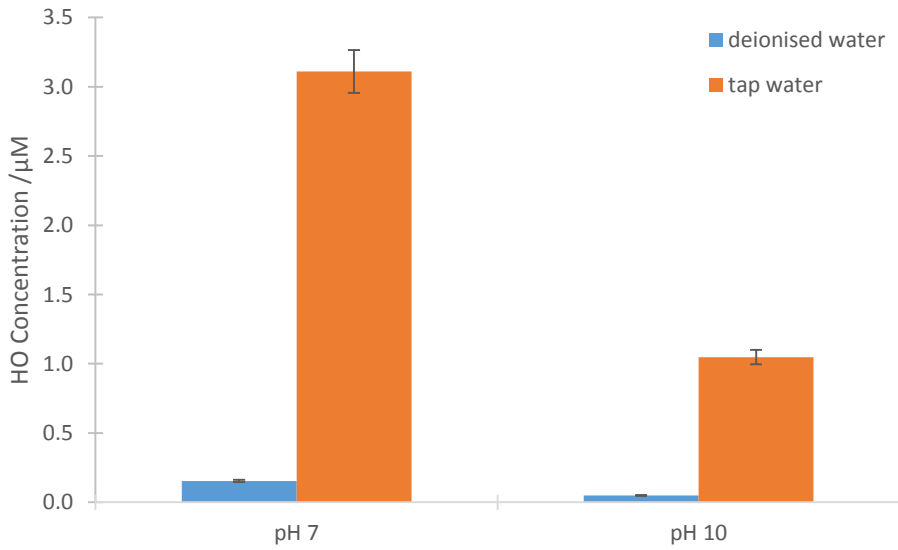

Figure 4.14 Hydroxyl radicals generated by acoustic cavitation, [TA] = 100 μM, t = 60 min, λ_{ex} = 315 nm.

4.6 Summary

The toolbox of molecular probes which had been shown to work under ideal conditions of pure ROS species was utilised with success on commercially important bleach sources. The presence of peroxides were confirmed in all of the investigated bleaching species (Table 4.3). In addition the kinetic parameters of the perhydrolysis reaction were obtained, and the reaction of TAED and SPC in a model laundry product 'Neptune' formulation was found to be the fastest.

Singlet oxygen was found to be a product of the decomposition of the peroxy acids in an aqueous, alkaline environment. The majority of the $^1O_2$ comes from the metal-catalysed decomposition, and peracetic acid was found to be most susceptible to the metal presence and also less stable than other peracids. Metal ions also play important role in decomposition of hydrogen peroxide in higher temperatures (60 °C) and in sono-Fenton reactions, when $HO^{\bullet}$ are generated by sonification of water via ultrasound bath. In room temperature experiments no signs of hydroxyl radicals were found in investigated bleach sources.

	H_2O_2	**RCO_3H**	**$^1O_2$**	**$HO^{\bullet}$**
SPC	✓			✓
PAP		✓	✓	
PAA		✓	✓	
TAED		✓	✓	
NOBS		✓	✓	
Neptune	✓	✓		

Table 4.3 Presence of ROS generated from bleach sources.

Research conducted in this chapter also confirmed that sodium percarbonate releases hydrogen peroxide upon dissolution in an aqueous environment. Bleach activators (TAED and NOBS) after reaction with hydrogen peroxide yield peracids (PAA, PNA) within a minute of reaction and that these peracids generated *in situ* are stable for a long time (i.e. wash period). The major bleaching species in a wash are hydrogen peroxide and peracid; singlet oxygen and hydroxyl radicals can be considered as minor bleaching species.

4.7 References

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5 DETERMINATION OF ROS IN THE PRESENCE OF MODEL DYES

5.1 Introduction

In the previous chapters a toolbox of molecular probes was developed to detect the most important ROS in laundry applications and applied to identify the presence of ROS on genuine bleach sources under simplified, model, conditions – alkaline, buffered solutions. In this chapter the behaviour of the bleach sources was investigated in more complex systems – in the presence of dyes used to model stains. It is important to first understand fully the behaviour of bleach when it is exposed to single dyes, before moving to the more complicated studies – with stains on fabrics. These results are able to give a first indication of the mechanistic understanding of how ROS react with the stains and will also provide direction for further studies.

Tomato and carotenoid based stains are a top stain in developed regions (various kinds of sauces, children food). Another example of common bleachable stains are these with beverage origins (tea, coffee, red wine). In a domestic laundry load also grass and curry based stains can be found. To mimic these most popular stains, four model dyes were chosen. The first one is crocin (CRO) a water soluble carotenoid, which represents stains of tomato origin. The second is chlorophyllin (CHL), a semi-synthetic and water soluble derivative of chlorophyll, which is used to mimic the behaviour of the grass stain. The next dye is curcumin, a natural yellow dye, which can be found in curry food products. A final pair of dyes, was selected to mimic the red stains other than tomato origin, and therefore cyanin and betanin were used, which can be found in e.g. red wine.

In the first part of this chapter, model dyes are introduced and the bleaching performance of hydroxyl radicals, singlet oxygen and peroxides (SPC, PAA, PAP, NOBS + SPC, TAED + SPC and Neptune) across the pH range 7.5 – 8.5 – 9.5 – 10.5 is presented. The next part focuses on the kinetics of bleaching these dyes by simple peroxides – SPC, PAA and PAP. The final part investigates the effects of the dye on behaviour of bleach activator systems – TAED, NOBS and Neptune.

The pH range in section 5.3 was expanded to include the value of pH 7.5 at Procter & Gamble's request. The results from that section were a part of a collaboration with Newcastle Innovation Centre and aimed to justify the bleaching performance of formulation closer to more neutral pH.

5.2 Model dyes

This section describes the absorption spectra of the model dyes, which were selected to mimic the most common bleachable stains, which customers need to remove almost on an everyday basis: grass, tomato, tea, curry and red wine from their laundry.

5.2.1 Grass stain - chlorophyllin

Chlorophyll is a natural dye, which occurs widely in green plants, and therefore can be found in grass stains, which are one of the most common stains in domestic laundry. Unfortunately, natural chlorophyll is not soluble in water, only in fats, and does not have good stability. For the purpose of this study, a water soluble, semi-synthetic copper derivative of chlorophyll was used – chlorophyllin (CHL, Figure 5.1).[1,2] Chlorophyllin is used as a food colouring agent and it is also known as Natural Green or as E141. [3]

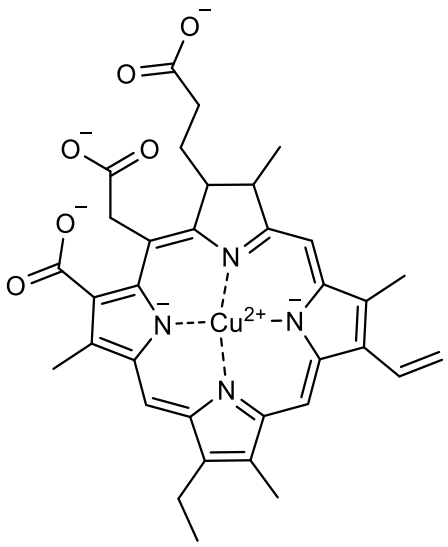

Figure 5.1 Structure of chlorophyllin.

The absorbance spectrum of the CHL in the visible region shows a strong band at 628 nm (Figure 5.2). It also can be concluded that absorbance of CHL is not affected by the pH from alkaline range.

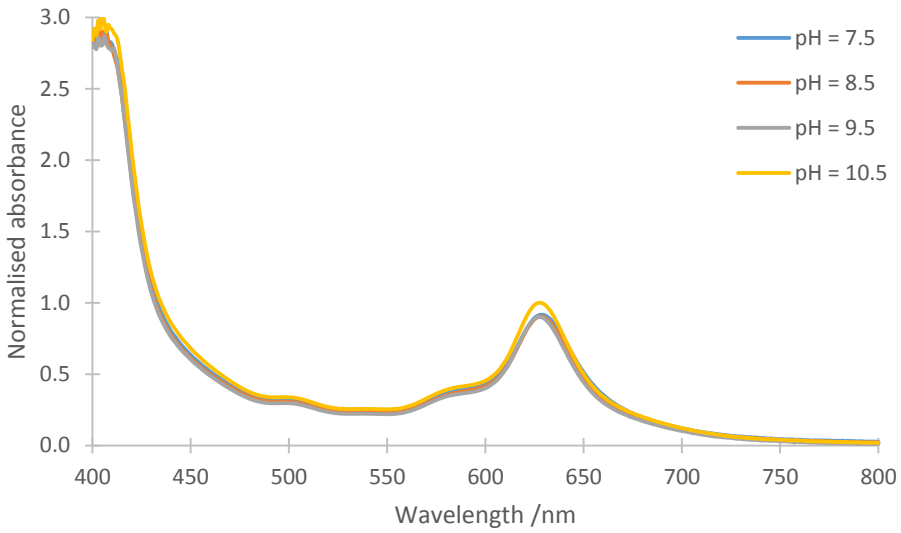

Figure 5.2 Absorbance of the CHL as a function of pH in visible region, [CHL] = 25 μM, in a buffer solutions (0.1 M).

5.2.2 Curry stain - curcumin

The second chosen dye was curcumin (CUR). In the solid state this is a bright yellow powder that is produced naturally by plants. It is the principal curcuminoid of turmeric, a member of the ginger family.[4] Curcumin is referred to E100 on the list of food additives or as a food colourant - Natural Yellow 3.[5] In the model dyes study this dye represents curry stains of which it is a major component. CUR is insoluble in water under acidic or neutral conditions but dissolves in alkaline conditions. [6]

Curcumin is a weak Bronsted acid, with three labile protons, and three pK_as have been estimated corresponding to three prototropic equilibria (Figure 5.3) with help of NMR and absorption spectroscopy. The first pK_a in the pH range of 7.5 to 8.5 changes curcumin from yellow to red. The chemical reactivity and solubility of the anionic curcumin increases with the alkalinity. [7]

In the work of *Manolowa et al.* it was shown that the tautomeric equilibrium between enol-keto and di-keto forms of curcumin (Figure 5.3) can be shifted by water and/or alcohol concentration in the mixture. The enol-keto form is only presented in ethanol, while in pure water the di-keto tautomer could be dominating. [8]

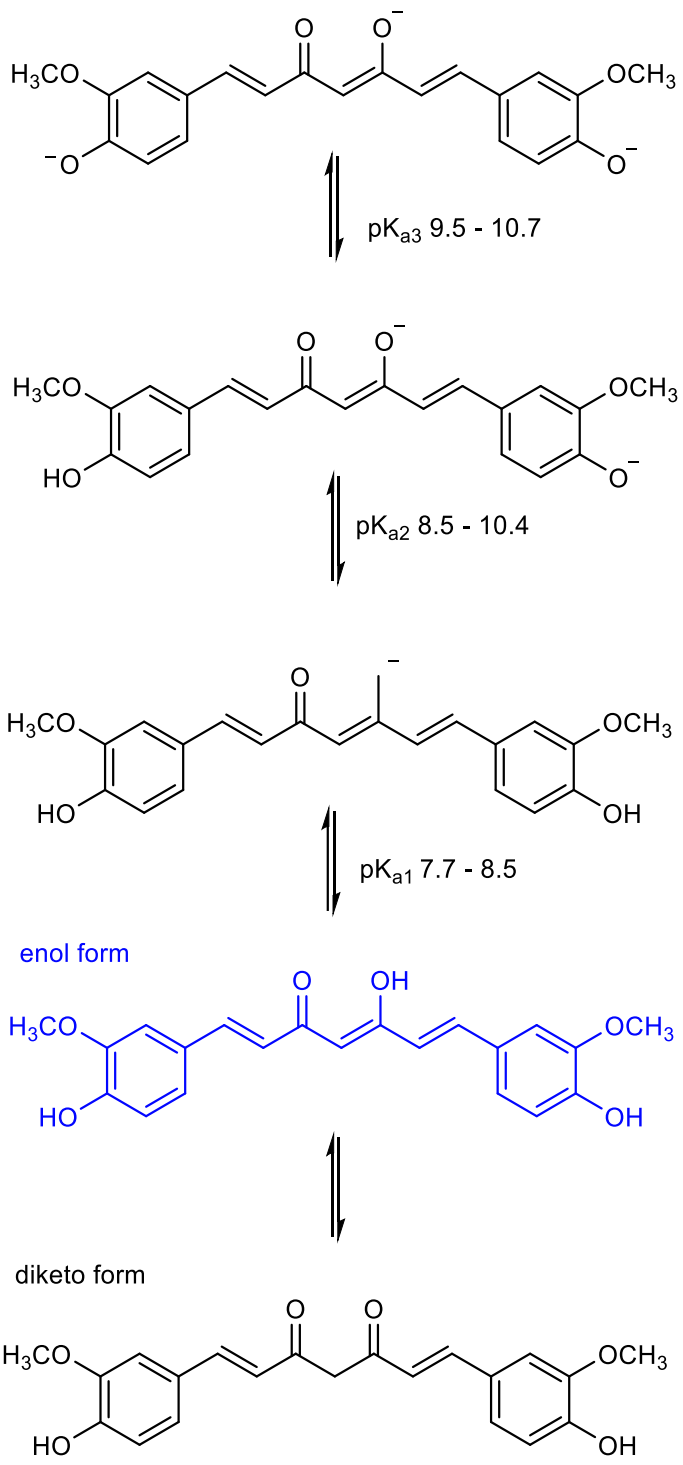

Figure 5.3 Keto-enol tautomerism and prototropic equilibria of curcumin; pK_{a1} 7.7 to 8.5, pK_{a2} 8.5 to 10.4, pK_{a3} 9.5 to 10.7. [7]

In Figure 5.4 the normalised absorbance of CUR as a function of pH in the alkaline range can be found. Probably due to the highest solubility, the strongest peak at 460 nm can be observed at pH

10.5. The position of the main peak changes it position through the pH range: 7.5 – 428 nm, 8.5 – 424 nm, 9.5 – 440 nm and 10.5 – 460 nm. In addition two smaller peaks around 550 nm can be observed at pH 8.5 and 9.5. The spectrum in pH 7.5 is attributed to nonionised curcumin, with the successive ionisations occurring at the higher pH values. However, the presence of coupled equilibria in the investigated system might prove difficult to correctly attribute the relevant curcumin form to the pH value. Investigated solutions contained 0.3 % of ethanol, therefore it can be assumed, that both tautomers are present with dominating diketo tautomer.

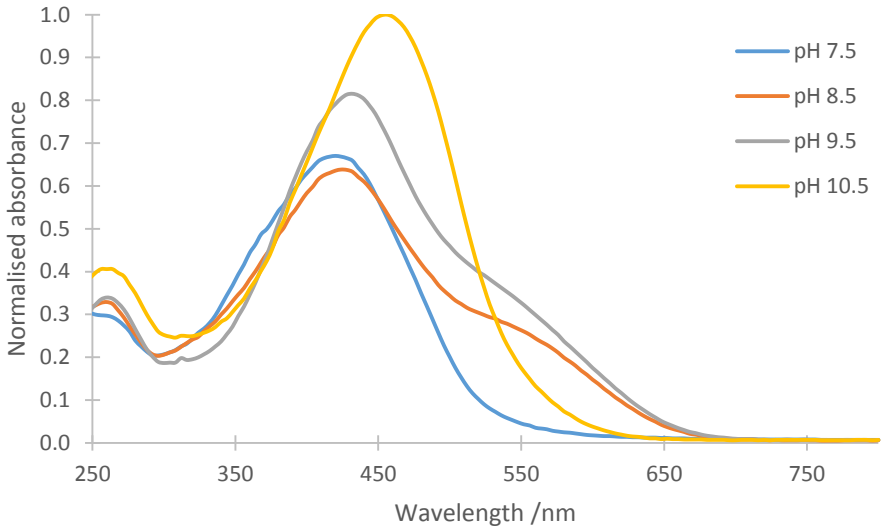

Figure 5.4 Absorbance of CUR as a function of pH, [CUR] = 25 μM in a buffer solutions (0.1 M) containing 0.3% of ethanol.

5.2.3 Tomato-based stain - crocin

Carotenoids, like beta-carotene and lycopene, are widely present in tomato-based stains. Crocin (CRO, Gardenia Yellow) is an example of a commercially available carotenoid (Figure 5.5). The primary source of the naturally occurring crocin is saffron.[9,10] It is one of the few carotenoids found in nature that is water soluble, and therefore chosen for this studies. The molecule is soluble due to the presence of sugar moieties. The pigment, in solid form, has a deep red colour, and alkaline solutions are brightly yellow. In addition, a crocin bleaching assay is a common method for evaluating the antioxidant activity of water soluble samples i.e. food. [11,12]

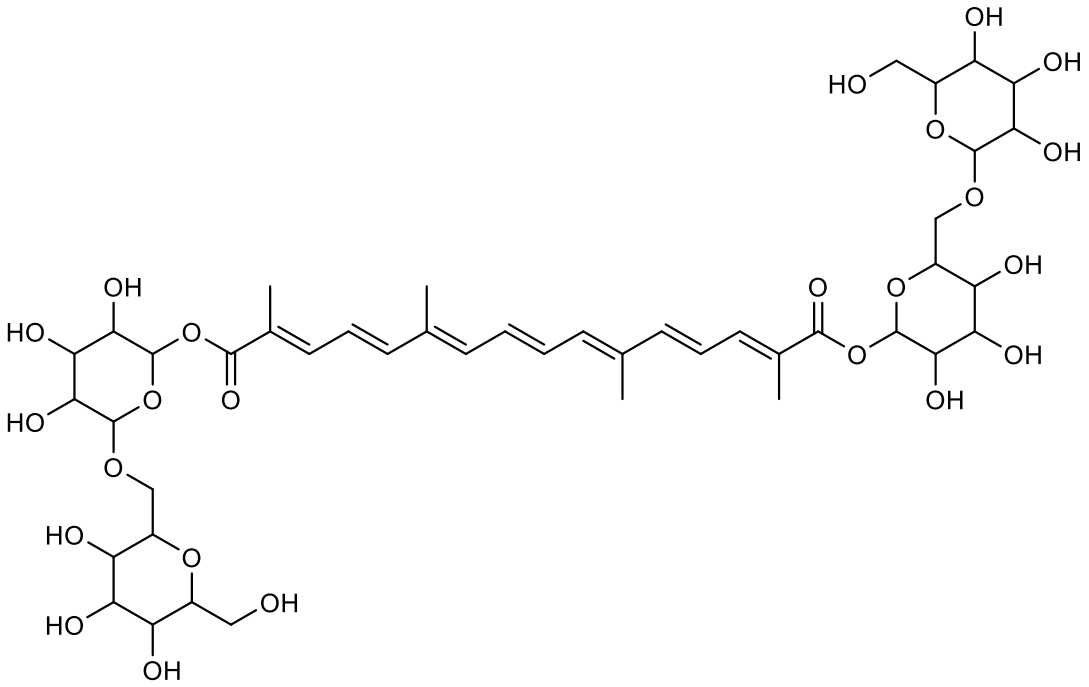

Figure 5.5 Structure of crocin.

The absorbance spectrum of crocin in the alkaline pH range shows a main peak in 444 nm (Figure 5.6). With the increase of the pH intensity of the absorbance drops, which is associated with alkaline degradation and is not reversible.

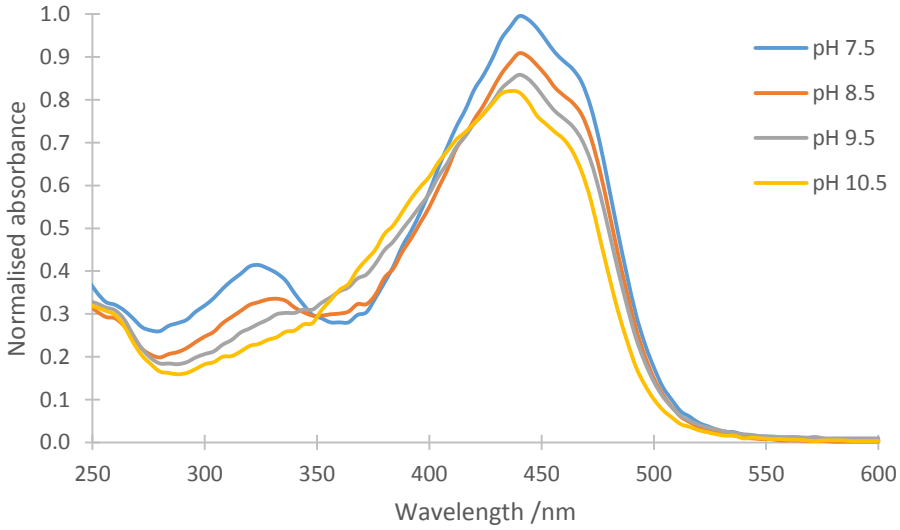

Figure 5.6 Absorbance of the CRO as a function of pH. [CRO] = 25μM in buffer solutions (0.1 M).

5.2.4 Red wine stain – cyanin chloride and betanin

Cyanin chloride (CYN) belongs to the anthocyanin family and this natural dye can be found in the red wine, and therefore is used to represent red wine stains. [13] Cyanin is the 3,5-O-diglucoside of cyanidin (Figure 5.7).

Anthocyanins together with carotenoids are amongst the most utilised vegetable colourants in the food industry. Carotenoids are liposoluble, stable and able to colour food products from yellow to red; they are obtained mostly from carrots, tomatoes and peppers. On the other hand, the anthocyanins are water-soluble, less stable than carotenoids and they are extracted from grapes, berries, red cabbage, apples, radishes, tulip, roses and orchids, amongst others.[14] Another significant property of anthocyanins is their antioxidant activity, which is thought to play a vital role in the prevention of various kinds of diseases. [15]

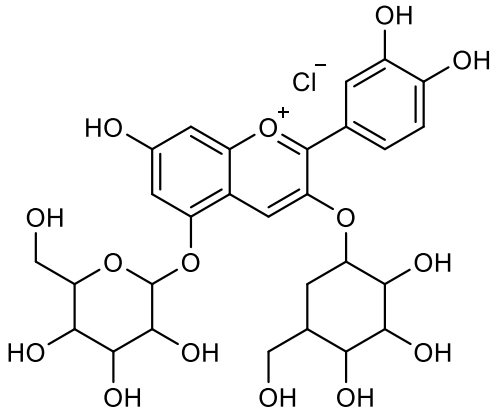

Figure 5.7 Structure of cyanin chloride (flavylium cation) at pH 1.

The isolated anthocyanins are highly unstable and very susceptible to degradation. Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, and the presence of enzymes, flavonoids, proteins and metallic ions.

Anthocyanins can be found in different chemical forms which depend on the pH of solution. At pH values higher than 7, the anthocyanins are degraded depending on their substituents groups. Investigations about anthocyanins stability and the colour variations with pH conclude that the changes in the colour of these compounds are more significant in the alkaline region due to their instability. [16]

Absorbance of cyanin shows one main peak at 596 nm in a pH 8.5 (Figure 5.8). With an increase of the pH, the position of the peak shifts to slightly longer wavelengths. The change of the spectra can be possibly correlated with the degradation of the pigment – the change of colour is nonreversible.

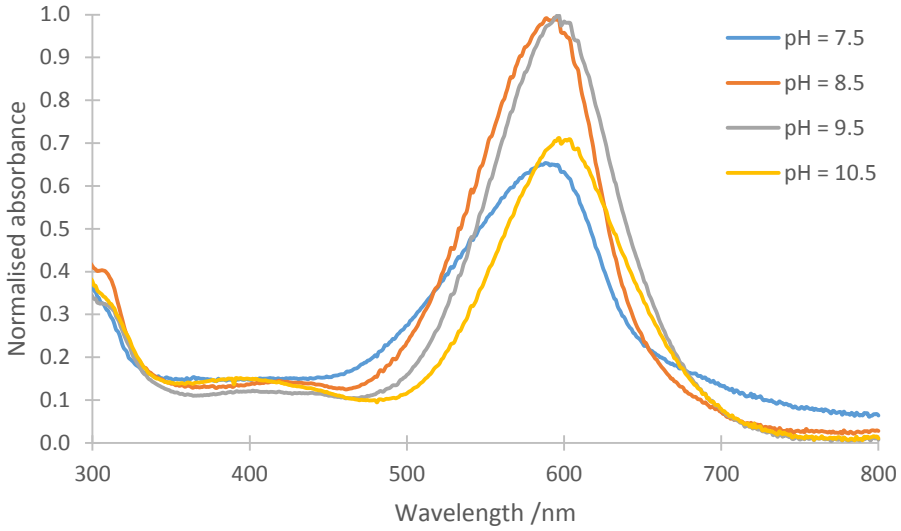

Figure 5.8 Absorbance of the CYN as a function of pH. [CYN] = 25 μM in buffer solutions (0.1 M).

In later work betanin (BET) was introduced as a replacement dye for cyanin due to practical and financial constraints. Betanin is also known under the name of Beetroot Red, and is a red glucosidic food dye obtained from beets (Figure 5.9). It is also used as a food additive, and is known as E162. Betanin degrades when subjected to light, heat, and oxygen. It's sensitivity to oxygen is highest in products with a high water content and/or containing metal cations (e.g. iron and copper). In the dry form betanin is stable in the presence of oxygen. The colour of betanin depends on pH; between 4 and 5 it is bright bluish-red, becoming blue-violet as the pH increases. At alkaline pH, betanin can degrade by hydrolysis, resulting in a yellow-brown colour.[17]

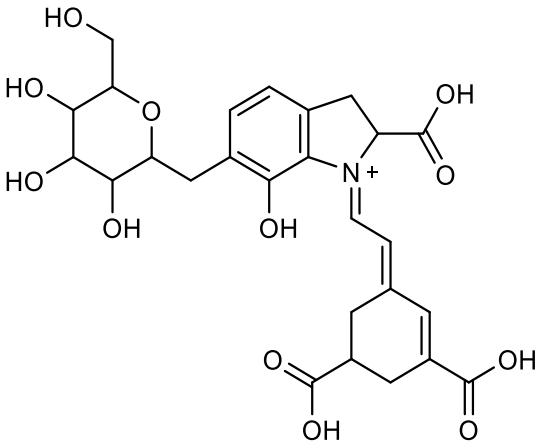

Figure 5.9 Structure of betanin.

The absorbance of previous pigments (CHL, CRO and CUR) were measured at a concentration of 25 μM. However, betanin solutions at this concentration were transparent and therefore a concentration of 200 μM was used (Figure 5.10). In addition, spectra obtained were smoothed with a Savitzky-Golay filter to increase the single-to-noise ratio. The absorbance of betanin as a function of pH shows a slight decrease as pH increases. The maximum of the peak shifts to longer wavelength with higher pH, which can be caused by the degradation of the dye progressing with the alkalinity. There are two isobestic points: first at around 450 nm and second one ~560 nm.

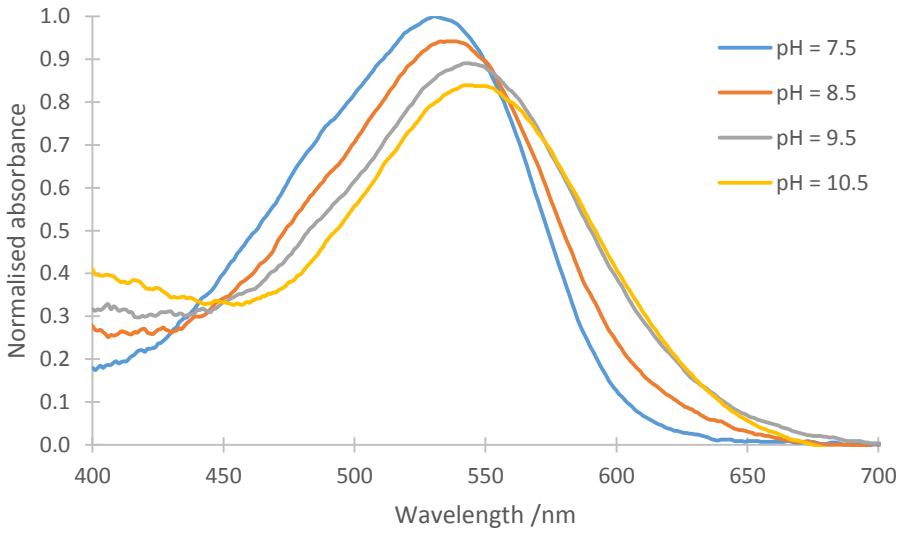

Figure 5.10 Absorbance of BET as a function of pH. [BET] = 200 μM in buffer solutions (0.1 M). Savitxky-Golay filter was used to smooth the spectra (n=5).

In summary, five dyes were chosen to mimic common stains for the purpose of experiments with bleaching systems, four of these can be found in a food product with plant origin and one is a semi-synthetic derivative of naturally occurring dye (CHL).

To mimic grass and plant-based stains chlorophyllin was chosen. Curcumin dye can be found in curry and orange food stains. Tomato-based food stains were represented by water soluble carotenoid – crocin. Cyanin chloride, and later betanin are examples of red food dyes originating in a food and beverages other than tomato.

Only the properties of chlorophyllin can be considered as not affected by pH. An alkaline environments cause deprotonation of curcumin and therefore increases its solubility and chemical reactivity. On the other hand, high basicity can cause the degradation of the crocin, cyanin chloride and betanin.

5.3 Bleaching performance of ROS – preliminary study

As mentioned earlier it is important to assess the performance of bleaching species in an alkaline pH range due to the current trend of the laundry conditions in terms of pH and temperature. However, the main objective of this part of the work, was to establish whether the bleaching agents currently used can be employed at lower formulations pHs.

In this section a general bleaching performance (BP) was assessed, based on bleaching of four model dyes (CHL, CUR, CRO, CYN) by peroxide sources (SPC, PAA, PAP, TAED, NOBS), hydroxyl radicals and singlet oxygen. The time-scale of the experiments was 45 min, to represent the average domestic wash cycle.

Bleaching performance was calculated from the formula 5.1 and it is expressed in percentage. The difference between absorbance at 0 min and absorbance at 45 min at the maximum of the peak, was divided by the absorbance at 0 min and multiplied by 100%, thus a BP of 100% represents a complete removal of the dye.

$$\text{BP} = \frac{\text{Abs}_{0\ \text{min}} - \text{Abs}_{45\ \text{min}}}{\text{Abs}_{0\ \text{min}}} \cdot 100\% \qquad (5.1)$$

The results below provide an overall image of bleaching capability as well as directions for further studies with the model dyes.

5.3.1 Chlorophyllin

Commercial food grade copper chlorophyllin is not a single, pure compound, but is a complex mixture of structurally distinct porphyrins, chlorin, and non-chlorin components with variable numbers of mono-, di- and tri- carboxylic acids that may be present as either the sodium or potassium salts,[18] therefore the composition of the mixture and products of CHL oxidation are not known. [19]

Thermally induced degradation of copper chlorophyllin (in deionised water) causing it to lose the central copper ion and affecting the porphyrin ring structure (breaking the bridge between five carbon rings). [20] It can be assumed that degradation of CHL caused by aggressive oxidation by bleach follows a similar mechanism.

Bleaching performance parameters obtained after oxidation of CHL for 45 minutes with various kinds of bleaching agents (peroxides, hydroxyl radicals and singlet oxygen) can be found in Table 5.1. The uncertainty of the BP values reported were based on the reproducibility of the measurement (5 repetitions) and was established at the 5 % level. Taking these uncertainties into account no direct pH trends can be observed when SPC, PAA, TAED + SPC, hydroxyl radicals and singlet oxygen were used, which indicates that the most common bleaching agents in Europe can be incorporated with success to lower the pH of laundry formulations. A drop in performance with increasing pH may be observed when CHL was bleached with PAP, which can be caused by non-reversible ring opening of the PAP molecule in higher pH which is associated with the loss of PAP activity. The mixture of NOBS and SPC however exhibits better performance in a more alkaline environment. This can be explained by the increase of concentration of $[HO_2]^-$ (pK_a H_2O_2 = 11.6) with pH, and therefore increase of concentration of peroxynonanoic acid generated from perhydrolysis reaction between NOBS and SPC.

	Bleaching performance /% ± 5%						
pH	SPC	PAA	PAP	TAED+SPC	NOBS+SPC	$HO^{\bullet}$ *	$^1O_2$ **
7.5	29	67	75	52	52	40	26
8.5	29	61	79	61	73	41	25
9.5	24	53	70	56	80	46	27
10.5	21	50	44	56	82	31	22

Table 5.1 Bleaching performance as a function of bleach and pH for chlorophyllin after 45 min. [CHL] = 10 μM, [SPC, PAA, PAP] = 100 μM, [TAED + SPC] = 100 μM + 300 μM, [NOBS + SPC] = 100 μM + 300 μM, * = generated by irradiation of KNO_3 (500 μM), ** = generated by irradiation of Rose Bengal photosensitiser (1 μM)

Figure 5.11 shows the absorbance spectra of CHL measured every 1 min, while bleaching with PAA in pH 10.5 and the slow oxidation of the groups responsible for CHL colour can be observed. The first big drop in the absorbance is caused by the small dissolution of the CHL sample which occurred when PAA was added to the cuvette. During oxidation no new absorption peaks appeared, which suggest that any intermediate products formed during the reaction were rapidly degraded. The intermediate products cannot be determined spectrophotometrically.

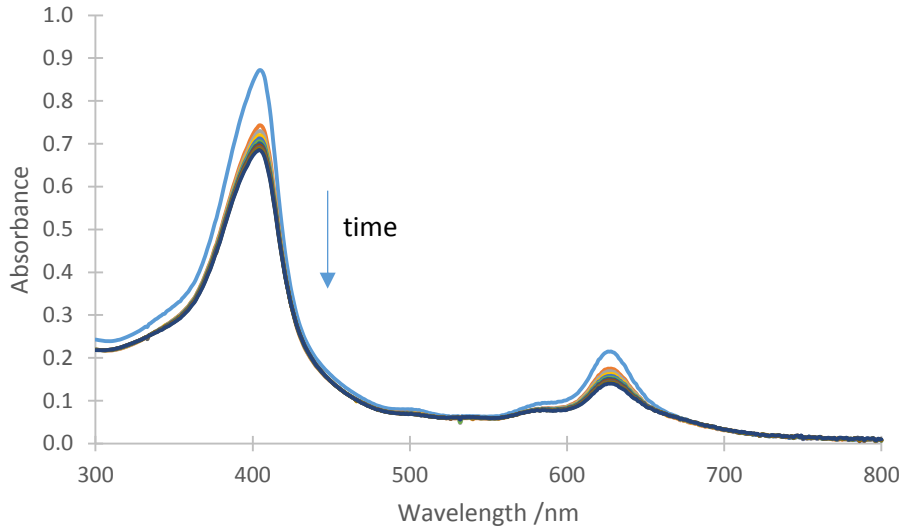

Figure 5.11 Bleaching of CHL with PAA at pH 10.5. [CHL] = 10 μM, [PAA] = 100 μM, in sodium bicarbonate buffer (0.1 M), total time = 10 min, measurement taken every 1 min.

5.3.2 Curcumin

Curcumin is poorly soluble in water and unstable in neutral and alkaline conditions. The main products of curcumin degradation in an alkaline environment are trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal (major degradation product), vanillin, ferulic acid and feruloyl methane. Curcumin is also light sensitive and photochemical degradation leads to the formation of vanillin, vanillic acid, ferulic aldehyde, ferulic acid, and 4-vinylguaiacol (Figure 5.12).[21] It can be expected that oxidation in alkaline pH of the curcumin molecule can result in a similarly complex mixture of products.

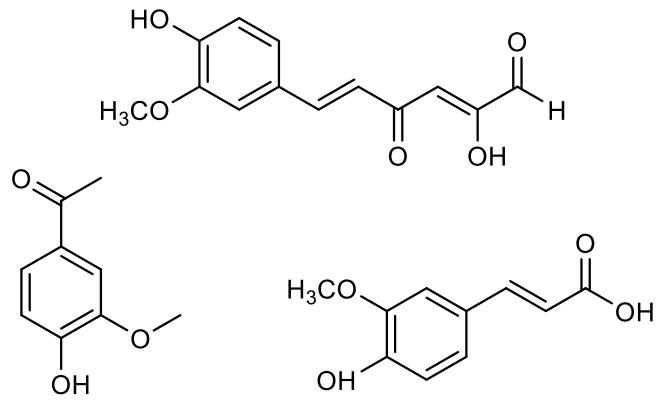

Figure 5.12 Structures of main curcumin degradation products: *top* - 6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal, *left bottom* - vanillin, *right bottom* - ferulic acid.

Bleaching performance values obtained from curcumin oxidation by various oxidants can be found in Table 5.2. It proved difficult to find a relationship between the bleaching behavior of SPC and PAP with CUR across a range of pH's. No direct pH trend was observed when SPC and PAP were used. By contrast, the data seems to be random and cannot be explained easily. Similarities can be found in bleaching performance of PAA, TAED + SPC and singlet oxygen – the lowest value was obtained for pH 7.5 and values obtained for pH 8.5, 9.5 and 10.5 are on very similar level. As mentioned before, at pH 7.5 curcumin is not dissociated and therefore less soluble and reactive than the dissociated form. In addition the TAED + SPC system is less effective than PAA alone, due to the low efficiency of perhydrolysis reaction between TAED and SPC at lower pH. NOBS is a more hydrophobic bleaching agent than TAED, and therefore exhibits much higher performance towards curcumin at pH 7.5. Hydroxyl radicals are the most aggressive oxidants and are efficient across the whole pH range.

Bleaching performance /% ± 5%

pH	SPC	PAA	PAP	TAED+SPC	NOBS+SPC	$HO^{\bullet}$ *	$^1O_2$ **
7.5	50	75	94	27	80	76	27
8.5	75	93	79	90	85	77	77
9.5	50	92	92	89	86	74	77
10.5	31	88	71	84	86	69	74

Table 5.2 Bleaching performance as a function of bleach and pH for curcumin after 45 min. [CUR] = 10 μM, [SPC, PAA, PAP] = 100 μM, [TAED + SPC] = 100 μM + 300 μM, [NOBS + SPC] = 100 μM + 300 μM, * = generated by irradiation of KNO_3 (500 μM), ** = generated by irradiation of Rose Bengal photosensitiser (1 μM)

The absorbance spectra of curcumin in the alkaline pH range were recorded for the first 10 minutes of bleaching with peracetic acid (Figure 5.13, Figure 5.14, Figure 5.15 and Figure 5.16). At pH 10.5 (Figure 5.13) both a big loss of intensity and spectrum change can be observed. The maximum of the intensity shifts to the shorter wavelengths and in addition new peak around 350 nm emerged. Curcumin is susceptible to pH and therefore at pH 10.5 the solution of the dye can be a complex mixture, containing the curcumin molecule with different level of ionisation and several degradation products, as well as several oxidation products.

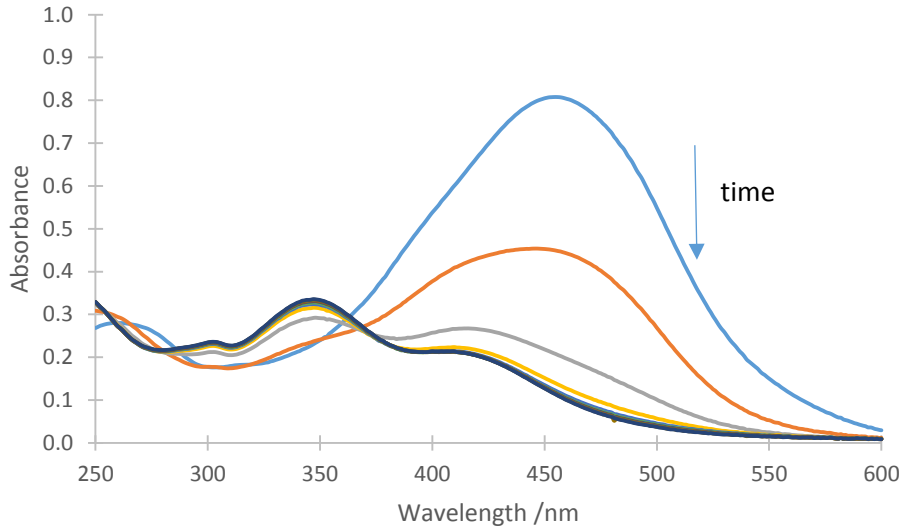

Figure 5.13 Bleaching of CUR with PAA at pH 10.5. [CUR] = 10 μM, [PAA] = 100 μM, in sodium bicarbonate buffer (0.1 M), total time = 10 min, measurement taken every 1 min.

The similar change of the spectra as in pH 10.5 can be observed in pH 9.5 but in slightly less intense manner (Figure 5.14). It can be explained by the lower solubility and reactivity caused by a lower deprotonation of CUR molecule.

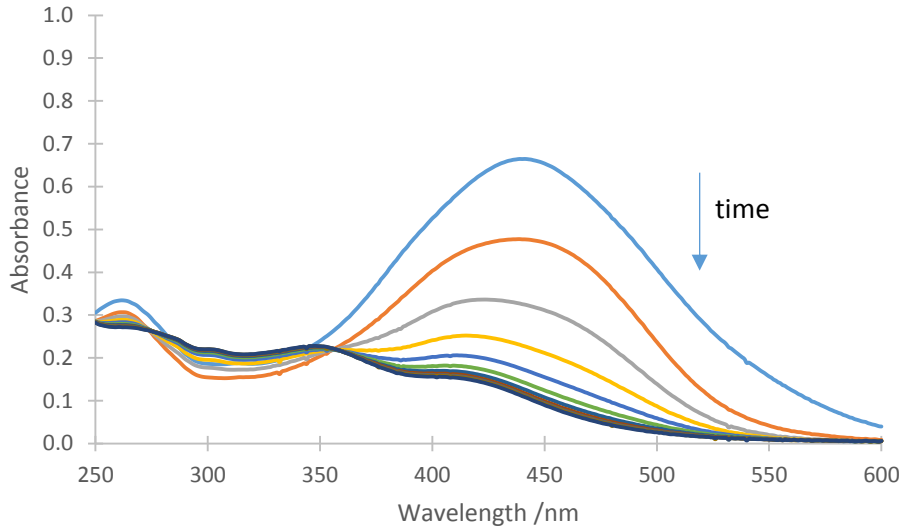

Figure 5.14 Bleaching of CUR with PAA at pH 9.5. [CUR] = 10 μM, [PAA] = 100 μM, in borax buffer (0.1 M), total time = 10 min, measurement taken every 1 min.

At pHs 8.5 and 7.5 (Figure 5.15 and Figure 5.16) rather slower bleaching can be observed, which shows that the chromophores are oxidised evenly and no intermediate products can be observed. In more alkaline solutions the rate of the reaction dramatically drops after ~ 5 min, however continues slowly at pH 7.5.

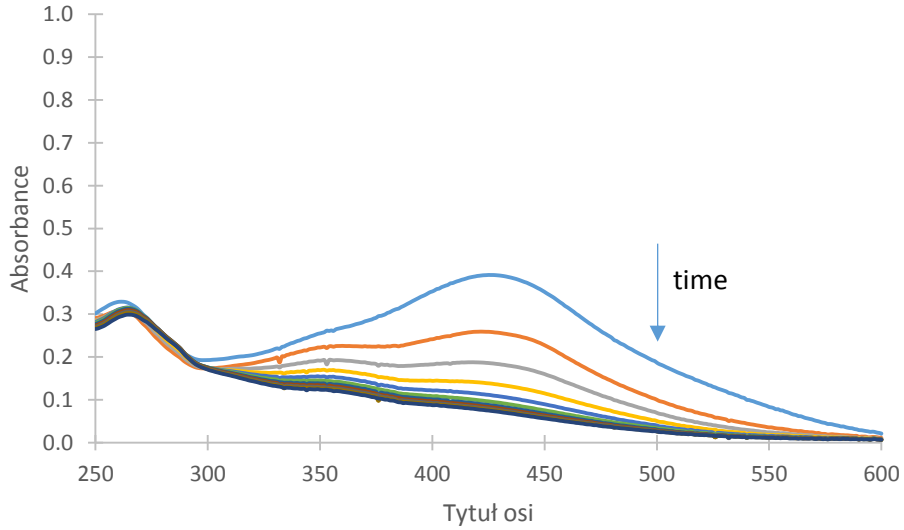

Figure 5.15 Bleaching of CUR with PAA at pH 8.5. [CUR] = 10 μM, [PAA] = 100 μM, in borax buffer (0.1 M), total time = 10 min, measurement taken every 1 min.

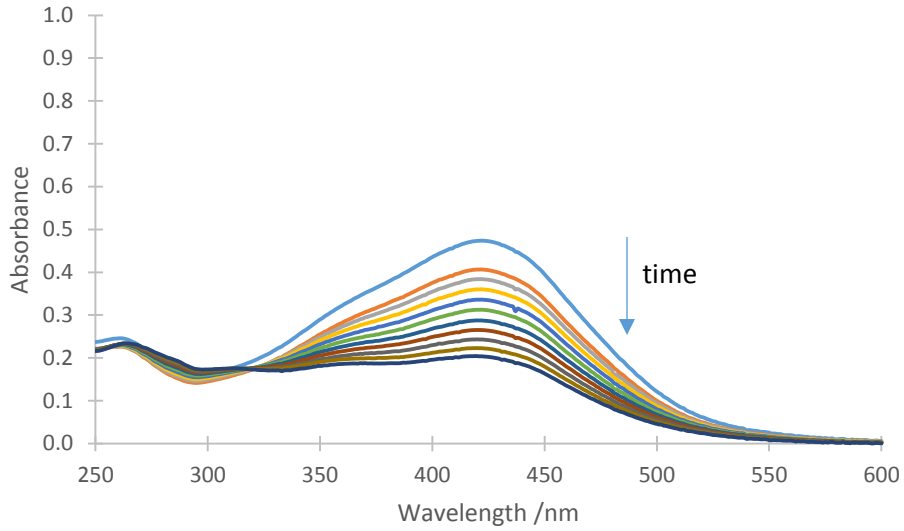

Figure 5.16 Bleaching of CUR with PAA at pH 7.5. [CUR] = 10 μM, [PAA] = 100 μM, in phosphate buffer (0.1 M), total time = 10 min, measurement taken every 1 min.

5.3.3 Crocin

Carotenoids are known for their antioxidant properties, which are closely related to their long-chain conjugated polyene structures. They are also known to be sensitive to oxygen, and are said to be unstable in presence of air. Because of their polyene structures, carotenoids are susceptible to reactions with the ROS that may be radicals ($O_2^{\bullet-}$, $HO^{\bullet}$) or non-radicals (H_2O_2, $^1O_2$).

The electron-rich conjugated double bond structure is primarily responsible for the excellent ability of carotenoids to physically quench singlet oxygen without degradation, the chemical reactivity of carotenoids with free radicals, and its instability toward oxidation. Oxidation, the major cause of carotenoid loss, depends on available oxygen and the carotenoid involved, and may be stimulated by light, heat, peroxides, metal such as iron, and enzymes.

One of the natural roles of carotenoids in plants is to physically quench the highly reactive singlet oxygen produced from triplet oxygen and the triplet state of chlorophyll produced in the presence of light. Through this action, carotenoids turn to a low energy excited triplet state that returns to the ground state by losing the extra energy in the form of heat. Carotenoid chemical structure is usually not affected by the physical quenching.[19] This was confirmed experimentally (Table 5.3) and indeed

low bleaching performance values were obtained for bleaching of crocin with singlet oxygen in alkaline environment and no pH trend can be directly observed.

Crocin can quench hydroxyl radicals chemically, however the exact mechanism has not been yet elucidated. Bleaching of crocin could be attributed to either H-atom abstraction and/or addition of the radical to the polyene structure.[22] Within the margin of error it can be stated that bleaching of crocin with hydroxyl radicals is not pH dependent (Table 5.3).

Bleaching performance /% ± 5%

pH	SPC	PAA	PAP	TAED+SPC	NOBS+SPC	$HO^{\bullet}$ *	$^1O_2$ **
7.5	3	3	22	22	0	76	4
8.5	2	1	3	19	1	77	6
9.5	3	1	5	3	3	74	9
10.5	6	2	9	6	7	70	10

Table 5.3 Bleaching performance as a function of bleach and pH for crocin after 45 min. [CRO] = 10 μM, [SPC, PAA, PAP] = 100 μM, [TAED + SPC] = 100 μM + 300 μM, [NOBS + SPC] = 100 μM + 300 μM, * = generated by irradiation of KNO_3 (500 μM), ** = generated by irradiation of Rose Bengal photosensitiser (1 μM)

Due to the presence of the conjugated chain in the structure of crocin, it can be effectively bleached by peroxides, however experimental results show that bleaching of CRO in model washing conditions is insignificant (Figure 5.17, first drop of absorbance is associated with the dilution of the sample with PAP).

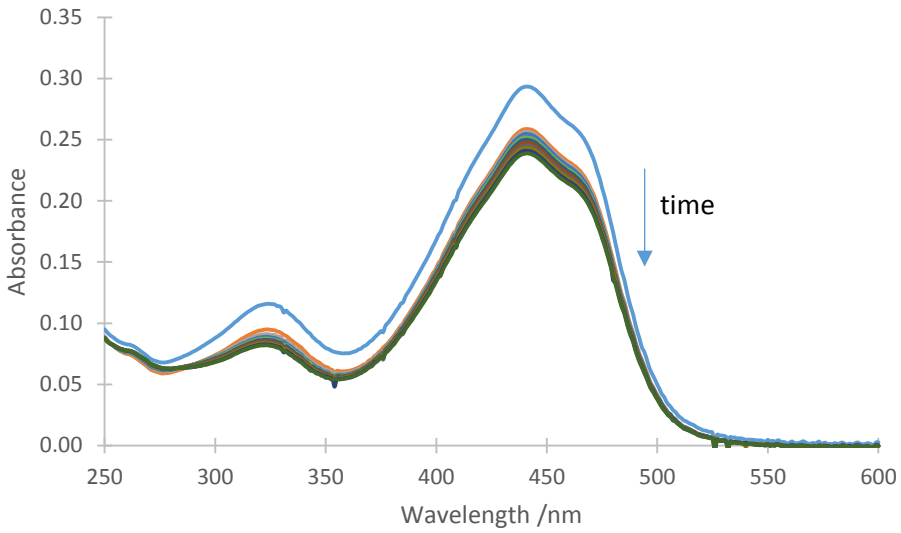

Figure 5.17 Bleaching of CRO with PAP at pH 7.5. [CRO] = 10 μM, [PAP] = 100 μM, in phosphate buffer (0.1 M), total time = 10 min, measurement taken every 1 min.

5.3.4 Cyanin chloride

Cyanin belongs to the anthocyanin family of the molecules and therefore is susceptible, inter alia, to light, oxygen and pH. Anthocyanins exhibit the highest stability as the red flavylium cation around pH 1-2 (Figure 5.7), whereas the other forms, especially the chalcone (pH > 7, Figure 5.18) are unstable and eventually lead to the degradation of the anthocyanins.[23] Peroxides under alkaline conditions reacts with the anthocyanin, breaking down the basic structure of the aglycon (C2 and C3 positions of the anthocyanin).

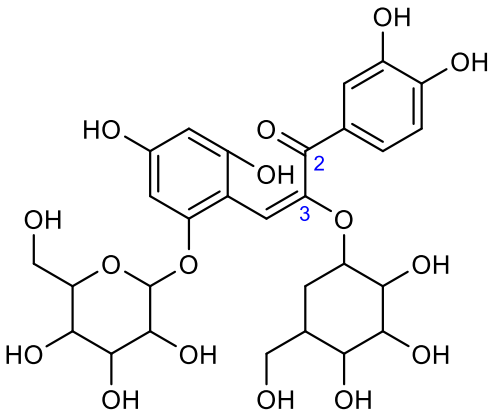

Figure 5.18 Structure of chalcone form of cyanin (pH > 7).

To obtain bleaching performance trends as a function of pH, the oxidation time was reduced from 45 min to 15 min, due to the almost complete bleaching of CYN by ROS after 45 min. It was found that the 254 nm irradiation used for $HO^{\bullet}$ generation is able to bleach cyanin in absence of KNO_3 and therefore hydroxyl radical bleaching performance data was not obtained (Table 5.4). With the exception of PAP, the most suitable pH for cyanin bleaching was 10.5. It is worth mentioning that the bleaching performances of both bleach activators, TAED and NOBS at lower pH values are much lower than in case of other, less oxidation resistant dyes (CHL, CUR). With almost stable performance of PAA and PAP over alkaline pH range, a strong trend can be observed for performance of SPC, TAED + SPC, NOBS + SPC and singlet oxygen. It can be associated with the decreasing stability of anthocyanins with increased pH, and in case of TAED and NOBS with perhydrolysis reaction, which prefers higher pH values.

	Bleaching performance /% ± 5%						
pH	SPC	PAA	PAP	TAED+SPC	NOBS+SPC	$HO^{\bullet}$ *	$^1O_2$ **
7.5	2	85	84	16	11	-	9
8.5	13	88	86	30	13	-	13
9.5	20	87	83	76	76	-	30
10.5	60	87	72	94	94	-	75

Table 5.4 Bleaching performance as a function of bleach and pH for cyanin after 15 min. [CYN] = 10 μM, [SPC, PAA, PAP] = 100 μM, [TAED + SPC] = 100 μM + 300 μM, [NOBS + SPC] = 100 μM + 300 μM, * = generated by irradiation of KNO_3 (500 μM), ** = generated by irradiation of Rose Bengal photosensitiser (1 μM)

5.3.5 Summary of model dyes bleaching performance

Four model dyes (chlorophyll, curcumin, crocin and cyanin chloride) were employed for these experiments, and the main purpose was to answer the question – can established bleaching agents such as PAP or TAED be used efficiently in lower pH laundry formulations? Nowadays laundry powders are optimised and designed to operate in pH 10.5, however lowering the pH would represent a more environmentally friendly option and hence there is a commercial reason to move to these conditions. No direct pH trends were observed when chlorophyllin was bleached by SPC, PAA, TAED + SPC, $HO^{\bullet}$ and $^1O_2$. Bleaching performance increases with rises in pH for bleach activator NOBS and decreases when PAP was used.

The behaviour of curcumin in alkaline environment is complex, due to the presence of enol and di-keto tautomers and level of the dissociation. CUR is not bleached effectively at pH 7.5 by PAA, TAED + SPC and singlet oxygen, possibly due to the low solubility of CUR itself at lower pH. On the other hand at pH 7.5 PAP and NOBS + SPC exhibit a high bleaching performance, due to the more hydrophobic nature of peracids, and therefore PAP and NPA can more easily approach the CUR molecule.

Crocin in model wash conditions can only be bleached by hydroxyl radical across the investigated pH range (7.5 – 10.5). CRO scavenges singlet oxygen via physical process, not chemical and therefore no destruction of the dye molecule was observed. In model washing conditions the bleaching of crocin by peroxides is very slow, and for the purpose of this work can be neglected.

Anthocyanins are very susceptible to degradation at alkaline pH and the highest bleaching efficiency was observed for all of the investigated bleaching species in pH 10.5. In addition exposure time to the oxidising agent was shorten from 45 min to 15 min, due to the dye sensitivity to the high pH.

Based upon the results obtained in this work PAP would be the best candidate for a laundry product designed to work close to neutral pH (7.5). It also has the advantage that it can be delivered in the solid form to the laundry formulation. PAP has the disadvantage of possible irreversible opening of the phthalimide ring over time with storage, and therefore a drop in product activity might be observed.

5.4 Kinetics of bleaching of model dyes by simple bleaching species

In this section bleaching kinetics were investigated over 45 min at pH 10.5by simple peroxides: sodium percarbonate (SPC), peracetic acid (PAA) and phthalimido-peroxy-hexanoic acid (PAP). Crocin was not included in these studies, because previous studies had demonstrated that peroxide based bleaches are not effective and the bleaching of crocin during a typical wash (45 min) can be neglected.

Figure 5.19 shows the results obtained for chlorophyllin. The kinetic profiles overlap with data from section 5.3.1. - SPC is not as effective as peracids in bleaching of CHL, and PAP exhibits similar performance to PAA.

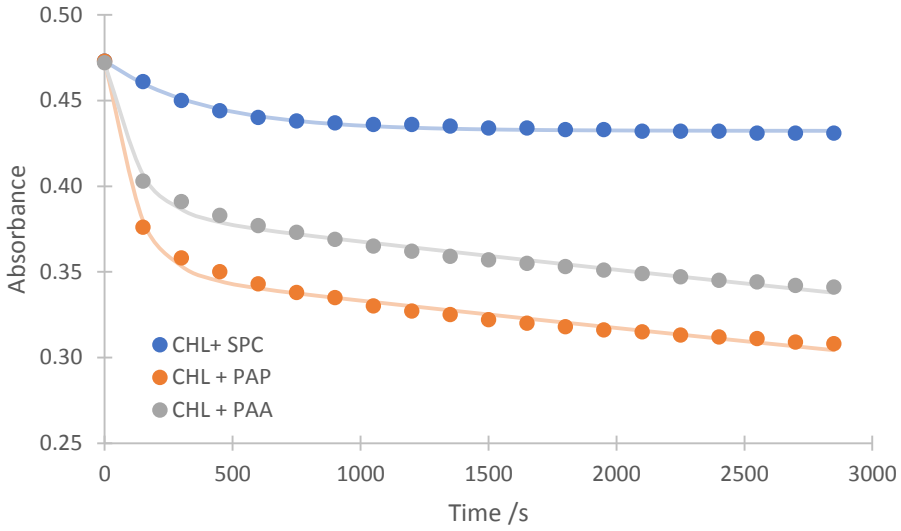

Figure 5.19 Absorbance of the CHL at 630 nm during bleaching by SPC, PAP and PAA over 45 min in pH 10.5, [CHL] = 25 μM, [SPC, PAP, PAA] = 250 μM.

A summary of the kinetic data can be found in Table 5.5. Bleaching of the chlorophyllin by sodium percarbonate can be fitted to single exponential function, and the rate constant was found at level of $2.6 \cdot 10^{-3} \pm 2.8 \cdot 10^{-4}$ s^{-1} with half-life of reaction of 270 ± 30 s. The best fit for peracid kinetics was obtained, when results were fitted to a double exponential, which would suggest the presence of two processes during the bleaching, first one fast ($\tau_{1/2}$ = 78 ± 9 s), and very slow second one ($\tau_{1/2}$ = 3 h 53 min). The slower process can be for instance a bleaching of the product of the reaction of faster oxidation. The rate constant of faster process is almost identical for two peracids

($k_{PAP} = 8.9 \cdot 10^{-3}$ s^{-1} $k_{PAA} = 8.4 \cdot 10^{-3}$ s^{-1}) and roughly 3.5 times higher than that determined for sodium percarbonate.

	k /s^{-1}	$\tau_{1/2}$ /s
SPC	$2.6 \cdot 10^{-3} \pm 2.8 \cdot 10^{-4}$	$2.7 \cdot 10^{2} \pm 3.0 \cdot 10^{1}$
PAP	$8.9 \cdot 10^{-3} \pm 1.0 \cdot 10^{-3}$	$7.8 \cdot 10^{1} \pm 9$
	$4.9 \cdot 10^{-5} \pm 5.4 \cdot 10^{-6}$	$1.4 \cdot 10^{5} \pm 1.6 \cdot 10^{4}$
PAA	$8.4 \cdot 10^{-3} \pm 9.8 \cdot 10^{-4}$	$8.2 \cdot 10^{1} \pm 9$
	$4.6 \cdot 10^{-5} \pm 5.4 \cdot 10^{-6}$	$1.5 \cdot 10^{5} \pm 1.6 \cdot 10^{4}$

Table 5.5 Rate constants and half-lives for bleaching of CHL by SPC, PAP and PAA in pH 10.5, [CHL] = 25 μM, [SPC, PAP, PAA] = 250 μM.

The second investigated dye was curcumin (Figure 5.20) and this did not overlap well with previous test (Section 5.3.2). In addition, the decay of the curcumin absorbance appeared to follow a zeroth order pathway showing a linear decrease in absorbance with time.

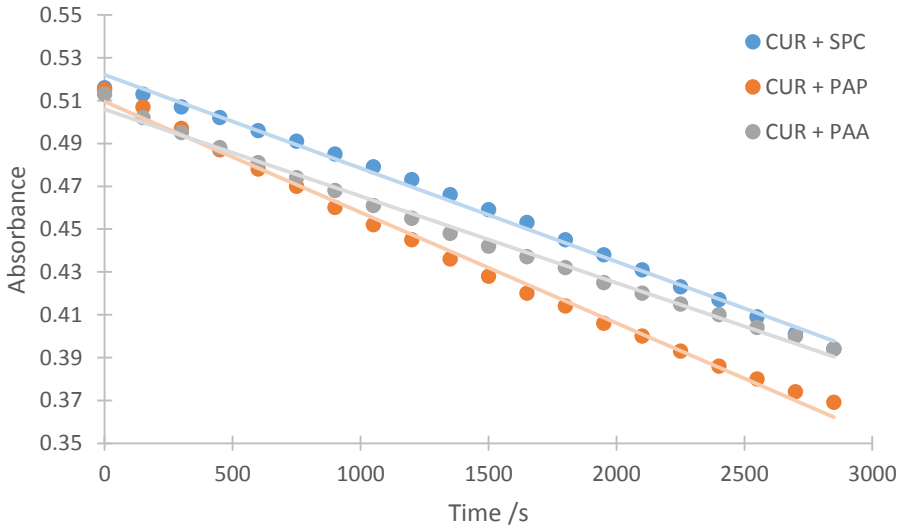

Figure 5.20 Absorbance of the CUR at 420 nm during bleaching by SPC, PAP and PAA over 45 min in pH 10.5, [CUR] = 25 μM, [SPC, PAP, PAA] = 250 μM.

Curcumin is almost insoluble in acidic aqueous solution but is soluble in alkali. According to the literature, at a pH above neutral, curcuminoids undergo hydrolytic degradation, giving raise to feruloyl methane, ferulic acid, vanillin, and coloured condensation products. The alkaline degradation of curcuminoids corresponds to pseudo-first order kinetics. The degradation rate increases from pH 7.45

to a maximum at pH 10.2 and decreases at higher pH levels.[24, 25] Based on these information, rate constants, which follow pseudo-first order kinetics were established (Table 5.6).

	$k\ /s^{-1}$	$\tau_{1/2}\ /s$
SPC	$1.6 \cdot 10^{-4} \pm 1.8 \cdot 10^{-5}$	$4.3 \cdot 10^{3} \pm 4.8 \cdot 10^{2}$
PAP	$1.9 \cdot 10^{-4} \pm 2.1 \cdot 10^{-5}$	$3.6 \cdot 10^{3} \pm 4.0 \cdot 10^{2}$
PAA	$1.8 \cdot 10^{-4} \pm 2.0 \cdot 10^{-5}$	$3.8 \cdot 10^{3} \pm 4.2 \cdot 10^{2}$

Table 5.6 Rate constants and half-lives for bleaching of CUR by SPC, PAP and PAA in pH 10.5, [CUR] = 25 µM, [SPC, PAP, PAA] = 250 µM.

The kinetic parameters of the bleaching of curcumin by peroxides (SPC, PAA and PAP) are similar ($k = 1.6 – 1.9 \cdot 10^{-4}\ s^{-1}$ and $\tau_{1/2} = 60 – 70$ min), with as expected the slowest process between SPC and CUR.

However, bleaching of curcumin by PAP over 5 hours revealed that oxidation process has a complex kinetics and only the assumption can be made from based on the 45 minutes kinetics (Figure 5.21). It is also possible, that the product of the bleaching is also orange coloured, and therefore this makes the measurement more complicated.

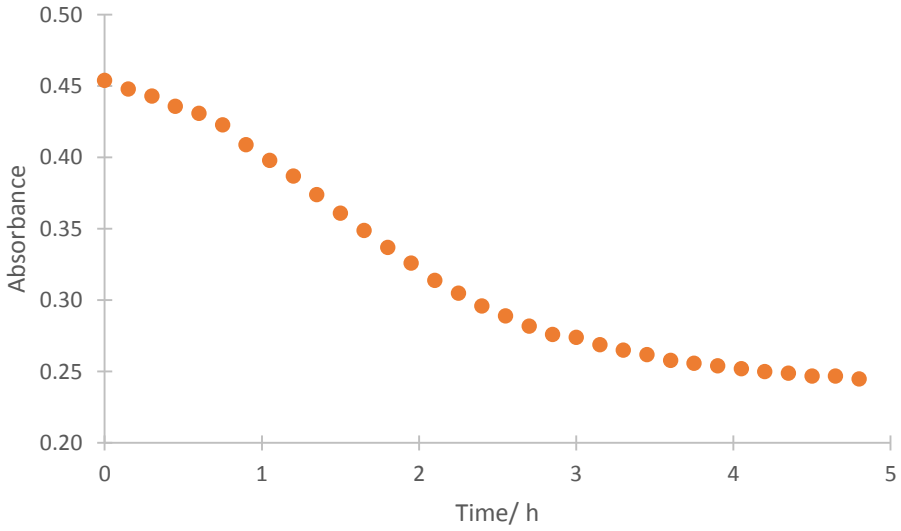

Figure 5.21 Absorbance at 420 nm of CUR while bleaching by PAP over 5 hours in pH 10.5, [CUR] = 25 µM, [PAP] = 250 µM.

The final dye investigated was betanin (Figure 5.22). Betanin is air and light sensitive, as well as unstable in an aqueous environment. The first experiments were made to investigate stability of betanin in buffer at pH 10.5. Decomposition of BET under the conditions of the oxidation experiments was found to be insignificant and did not affect the oxidation kinetics.

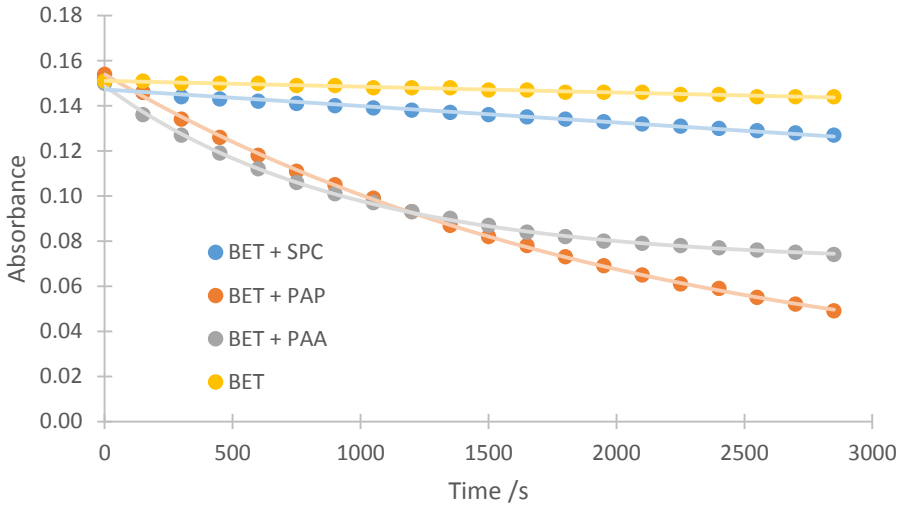

Figure 5.22 Absorbance of the BET at 550 nm and also in presence of agents, PAP and PAA over 45 min in pH 10.5, [BET] = 25 μM, [SPC, PAP, PAA] = 250 μM.

As in case of CHL, there was a big difference between the performance of hydrogen peroxide and peroxy acids. Accordingly to the data (Table 5.7), the reaction between betanin and peracetic acid was the fastest ($k = 1.0 \cdot 10^{-3} \pm 1.2 \cdot 10^{-4}\ s^{-1}$, $\tau_{1/2} = 66 \pm 7.2$ s). Contrary to the two previous cases (CHL and CUR), the reaction of BET with PAP is slower than with PAA.

	k /s^{-1}	**$\tau_{1/2}$ /s**
SPC	$3.0 \cdot 10^{-4} \pm 3.3 \cdot 10^{-5}$	$2.3 \cdot 10^{3} \pm 2.5 \cdot 10^{2}$
PAP	$4.8 \cdot 10^{-4} \pm 5.2 \cdot 10^{-5}$	$1.4 \cdot 10^{3} \pm 1.6 \cdot 10^{2}$
PAA	$1.0 \cdot 10^{-3} \pm 1.2 \cdot 10^{-4}$	$6.6 \cdot 10^{2} \pm 7.2 \cdot 10$

Table 5.7 Rate constants and half-lives for bleaching of BET by SPC, PAP and PAA in pH 10.5, [BET] = 25 μM, [SPC, PAP, PAA] = 250 μM.

5.5 Behaviour of bleach activators and Neptune in presence of model dyes

In this part of the project, the behaviour of model dyes (CHL, CRO, CUR and BET) with the species generated from bleach activator was investigated. Peracids formed from TAED + SPC, NOBS + SPC and Neptune samples were titrated fluorometrically with 2-naphthylboronic acid, while exposed to the model dyes and the data was then presented as a time – peracid concentration profiles. As in previous experiments, 45 min experiment time was used, which corresponds with the average, domestic wash cycle.

Peroxy acid consumption (PA Consumption) was calculated from the equation (expressed as a percentage) and is an indicator which reflects how much of the generated peroxy acid was consumed for dye bleaching in comparison to the solution of TAED and SPC without dye.

$$\text{PA Consumption} = \frac{[\text{PA}]_{\text{TAED}} - [\text{PA}]_{\text{dye}}}{[\text{PA}]_{\text{TAED}}} \cdot 100\% \qquad (5.2)$$

5.5.1 TAED

TAED is the most common bleach activator in the European powder laundry products[26] and generates peracetic acid *in situ*. The behaviour of the peracetic acid in the presence of model dyes can give the first indication of how PAA operates during the wash. The first conclusion from time-concentration profile is that peracetic acid is stable, when formed from TAED for the 45 min, which is in average wash cycle in investigated concentration range (Figure 5.23). Model dyes did not affect the rate constant of the reaction, but the final concentration of PAA does vary, attributed to reaction of the PAA with the dyes. When TAED was mixed with SPC in ratio 1:3, from 50 μM of TAED, 77 μM of PAA was generated, which corresponds well with previous results (Section 4.2.).

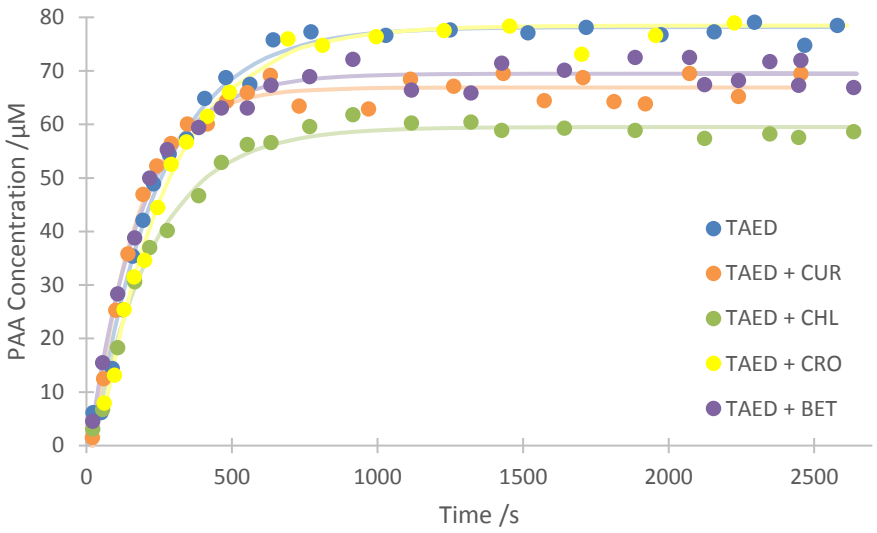

Figure 5.23 Time-concentration profiles of PAA generated from TAED in presence of dyes in pH 10.5, over 45 min, [TAED] = 50 µM, [SPC] = 150 µM, [CUR, CHL, CRO, BET] = 10 µM, [NBA] = 250 µM, λ_{ex} = 365 nm, λ_{em} = 420 nm.

As expected from the screening studies, there was no sign of reaction between crocin and PAA generated from TAED, therefore the PAA consumption in presence of crocin after 45 min is 0 % (Table 5.8). The biggest amount of the PAA was consumed when chlorophyllin present. PAA consumption was establish at level 23% (18 µM of PAA consumed for 10 µM of CHL), which can suggest that two moles of PAA react with one mole of CHL. Roughly 10 µM of PAA were consumed when curcumin and betanin were present, therefore a stoichiometry 1:1 can be assumed for both cases.

Concentration /µM ± 5%

	TAED	SPC	DYE	PAA final	PAA Consumption /%
TAED	49.0	150.5	-	77	-
TAED + CHL	49.4	148.9	10.0	59	23
TAED + CUR	49.4	152.1	10.0	67	13
TAED + CRO	49.4	150.5	9.9	77	0
TAED + BET	49.2	151.4	10.1	70	9

Table 5.8 PAA final concentration and consumption in presence of the dyes, generated from TAED.

5.5.2 NOBS

NOBS is the most popular bleach activator in Northern American laundry products and after reaction with hydrogen peroxide releases peroxynonanoic acid, PNA, which is more hydrophobic than peracetic acid. Under certain conditions NOBS molecule can react with PNA to form diacyl peroxide and trigger radical oxidation. Yield of perhydrolysis reaction between NOBS and SPC was determined to be roughly 70 %.

1.4 % of generated PNA was consumed, when crocin was present, however the error of the measurement was determined to be ± 5 %, thus it can be again assumed that bleaching of crocin in model wash conditions is very slow, and can be neglected (Figure 5.24).

In section 5.3.2 was demonstrated that curcumin can be effectively bleached by NOBS in alkaline pH range (bleaching performance = 86 % in pH 10.5), however fluorimetric titration shows that only a small amount of PNA was consumed during the reaction with CUR, it is possible that majority of the curcumin degradation is caused by the alkaline pH and not by the oxidation reaction with PNA.

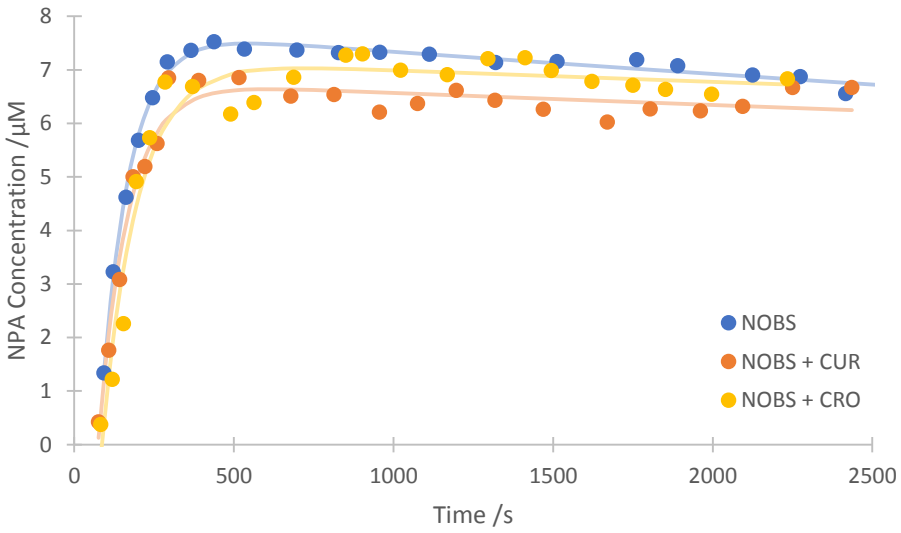

Figure 5.24 Time-concentration profiles of NPA generated from NOBS in presence of CUR and CRO in pH 10.5, over 45 min, [NOBS] = 10 µM, [SPC] = 30 µM, [CUR, CRO] = 2 µM, [NBA] = 250 µM, λ_{ex} = 365 nm, λ_{em} = 420 nm.

The behaviour of peroxynonanoic acid when exposed to chlorophyllin or even in bigger extent to betanin changed dramatically (Figure 5.25). Initially, as in previous case PNA was generated due to the perhydrolysis reaction between NOBS and SPC, subsequently a slow drop in the peracid's

concentration can be observed (k_{CHL} = 9.0 · 10^{-5} ± 9.9 · 10^{-6} s^{-1} and k_{BET} = 1.4 · 10^{-4} ± 1.5 · 10^{-5} s^{-1}). Those results might indicate that multiple molecules of peroxynonanoic acid react with one molecule of chlorophyllin or betanin. When taking to the account results from sections 5.3.1 and 5.3.4 it can be stated that in pH 10.5 PNA oxidises effectively anthocyanins and chlorophyllins.

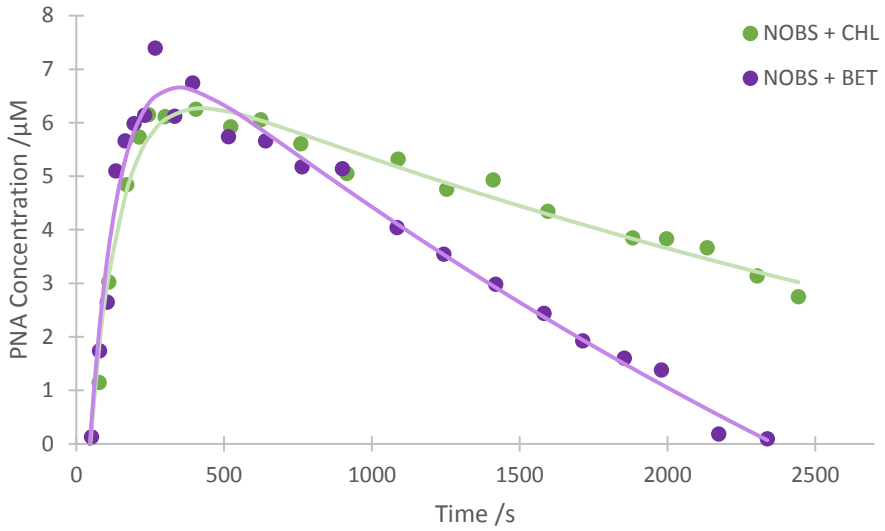

Figure 5.25 Time-concentration profiles of NPA generated from NOBS in presence of CHL and BET in pH 10.5, over 45 min, [NOBS] = 10 µM, [SPC] = 30 µM, [CHL, BET] = 2 µM, [NBA] = 250 µM, λ_{ex} = 365 nm, λ_{em} = 420 nm.

It also worth noticing that the consumption of NPA is higher than PAA, with the same ratio between bleach activator and dye (Table 5.9).

Concentration /µM ± 5%

	NOBS	SPC	DYE	NPA final	NPA Consumption /%
NOBS	10.3	31.1	-	6.9	-
NOBS + CHL	10.0	30.8	1.9	2.7	70
NOBS + CUR	10.2	30.3	2.4	6.4	7.3
NOBS + CRO	9.9	29.5	2.2	6.8	1.4
NOBS + BET	10.1	29.8	2.0	0.1	99

Table 5.9 NPA final concentration and consumption in presence of the dyes, generated from NOBS.

5.5.3 Neptune

The impact of the dyes on the perhydrolysis reaction was also investigated in Neptune samples (Figure 5.26). Neptune is a fully formulated powder laundry detergent which contains bleach activator TAED and persalt – SPC – which releases hydrogen peroxide when it comes in contact with water.

From previous results in this chapter, it is known that bleaching agents based on peroxides are not effective at bleaching crocin – a water soluble carotenoid, due to the very slow oxidation reaction (Table 5.10).

Surprisingly, the consumption of peracetic acid when curcumin is present is much lower (3.7 %) than in the TAED experiment (13 %). This might be explained by micellar interactions between dye and surfactant – the lipophilic dye may be trapped inside the micelle, leading to a less effective oxidation reaction. Micelles can act as a protective environment and reduce the availability of dyes e.g. curcumin towards peroxides. [27]

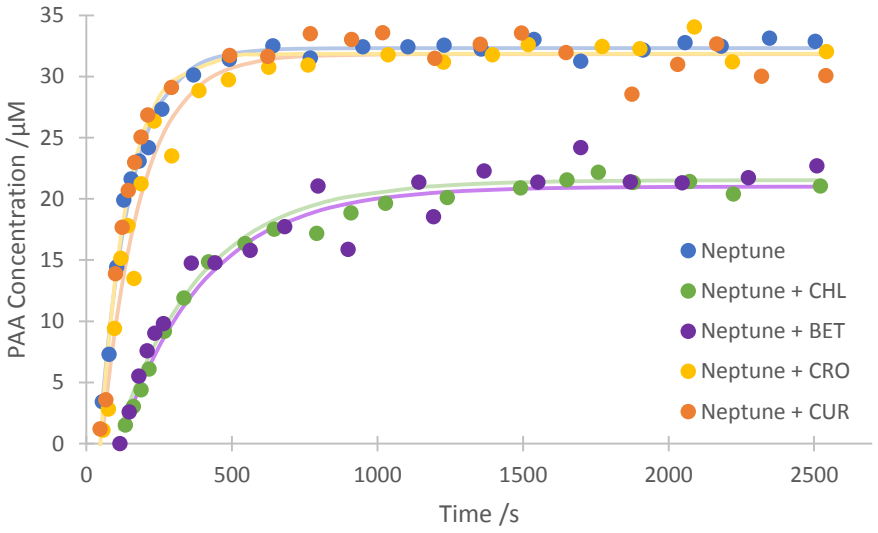

Figure 5.26 Time-concentration profiles of PAA generated from Neptune in presence of dyes in pH 10.5, over 45 min, [Neptune] = 5000 mg/L, [CHL, BET, CRO, CUR] = 4 µM, [NBA] = 250 µM, λ_{ex} = 365 nm, λ_{em} = 420 nm.

The measured peracetic acid consumptions can be found in Table 5.10. From the results obtained when Neptune was exposed to CHL and BET it can be assumed that roughly three moles of PAA, generated from Neptune react with one mole of the dye. The ratio is higher, than those found for TAED sample (PAA:BET = 1:1, PAA:CHL = 2:1). It is possible that other ingredients of the Neptune

boost the performance of the peracetic acid, e.g. surfactants increases the solubility of the dyes and therefore increase their availability.

Concentration /μM ± 5%

	TAED	SPC	DYE	PAA final	PAA Consumption /%
Neptune	22.0	540.5	-	32	-
Neptune + CHL	22.0	541.6	4.0	21	34
Neptune + CUR	22.0	541.3	4.2	31	3.7
Neptune + CRO	22.0	541.3	4.1	32	0.6
Neptune + BET	22.0	541.3	4.1	22	32

Table 5.10 PAA final concentration and consumption in presence of the dyes, generated from Neptune.

In addition a drop in rate constant of perhydrolysis was observed, when chlorophyllin and betanin were present (Table 5.11).The measured rate constants are roughly 3 times smaller than for just Neptune and this phenomenon was not observed in case of TAED. Both the consumption of peracetic acid and the rate of perhydrolysis reaction are affected with these two dyes. The higher PAA consumption can be explained by better solubility of the dyes in surfactant system and therefore easier access to them of the bleach system. However, this not explain decreased rate constant.

	k /s^{-1}	**$\tau_{1/2}$ /s**
Neptune	$9.8 \cdot 10^{-3} \pm 1.5 \cdot 10^{-3}$	71 ± 11
Neptune + CHL	$3.1 \cdot 10^{-3} \pm 4.0 \cdot 10^{-4}$	223 ± 29
Neptune + BET	$3.0 \cdot 10^{-3} \pm 4.0 \cdot 10^{-4}$	231 ± 28

Table 5.11 Rate constants and half-lives of perhydrolysis reaction in Neptune sample in presence of CHL and BET in pH 10.5, [Neptune] = 5000 mg/L, [CHL, BET] = 4 μM, [NBA] = 250 μM, λ_{ex} = 365 nm, λ_{em} = 420 nm.

5.6 Summary

In this chapter the interactions between bleach and dyes were investigated comprehensively: from the point of view of the dye, as well as from a ROS point of view. These studies are essential to understand fully the interaction in more complex systems, which contain a genuine stains deposed on fabrics.

In the first part of this chapter, dyes used as model stain compounds were introduced and also their behaviour in alkaline pH range was present. The pH is an important factor, and e.g. the presence on different tautomers and ionisation depends on it. In the following studies, it was presented that currently used bleaching species can be used in lower pH formulations and still exhibit a good bleaching performance. In addition it was examined, that the only effective oxidising agents of crocin, amongst investigated ROS are hydroxyl radicals. The kinetics of the bleaching process are not simple ones, and they were only assessed over 45 min period of time, which is an average domestic wash cycle.

The behaviours of the peroxyacids, generated from bleach activators were also examined, and in most cases, peracid were found to be stable, for the whole duration of the experiments (45 min – average wash cycle). The exceptions are results, when NOBS were exposed to chlorophyllin and betanin, where the formation of peracid was observed in the first stage of the experiment and then slow drop of the concentration. The rate constant of perhydrolysis reaction decreased, when Neptune was exposed to CHL and BET.

5.7 References

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6 DETERMINATION OF ROS FROM BLEACH SOURCE IN THE PRESENCE OF STAINS

6.1 Introduction

Stain is a discolouration caused by intensely coloured substances, which in small amounts can affect the colour and appearance of a textile. Commonly, the term stain denotes either a staining agent or the resulting discolouration, which needs a bleaching agent or an enzyme for its removal.

Most stains consist of coloured substances of natural origin belonging to the polyphenol, carotenoid, or chlorophyll class. Artificial food colourants, cosmetic ingredients, and decorative dyes complete the stain portfolio. Very often stains are complex mixtures of spilt food or beverages. Combined with oils, fats, or other organic material, such as proteins, starch, or waxes, the properties of stains are quite different from those of the isolated dyes.

The previous chapter provided a first indication and information regarding the interaction between bleaching species and dyes in an alkaline pH range. In this chapter more complex interactions are investigated – between reactive oxygen species and stains deposited on fabrics. Five stains were chosen as representative of those encountered or identified as problematic: curry, tomato passata, tea, red wine and grass. The results obtained from this work provide an insight into the interactions between stain and bleach.

Two experimental techniques were used to assess the bleaching performance by looking at stain removal: image analysis, as a basic technique, and diffuse reflectance spectroscopy, which is able to provide more detailed data. To determine the behaviour of peroxy acids (peracetic acid generated from TAED and Neptune, and peroxynonanoic acid generated from NOBS) fluorimetric titration with 2-naphthylboronic acid was used.

In the first part of this chapter, the chemistry of the laundry soils is presented and it is followed by the image analysis results discussion. The next part focuses on the use of reflectance spectroscopy to monitor stain degradation during a wash cycle. In the end of the chapter the time-concentration profiles of peroxy acids in the presence of stains are presented.

6.2 Chemistry of laundry soils

A wide variety of soils can be found on textiles and can be roughly divided by their origin. Coloured stains mostly come from food and drink. Within that category we can differentiate oily/greasy stains (unsaturated or saturated triglycerides), particulates (proteinaceous or starchy in nature) and stains containing strong chromophores such as wine and tea (polyphenol-polysaccharide-protein condensates), tomatoes (carotenoids), berries (anthocyanins) and spinach (ß-carotene and chlorophyll). Body soils are responsible for the biggest contamination of household laundry. This group includes blood (hemoglobin), sweat, sebum, urine, faeces (containing fats, protein, cellulose and the strongly coloured bilirubin) and applied products (e.g. cosmetics such as lipstick and mascara). The next category can be described as outdoor activities, where typical stains examples include grass (chlorophyll) and mud (clay or humus). The last group comes from commercial and leisure activities and the composition of these stains is highly variable, but grease (mineral oils), paint (pigments), ink (dyes) and particulates such as soot and rust are characteristic.

An alternative way to categorize soils is to group them according to the detergent functionality needed to remove the soil. Typical bleach responsive stains include tea, coffee, red wine and fruit and vegetable juices although often some mixed soils such as baby foods or curry show a response to a bleach system, in addition to detergency and/or enzymes.

A single stain can be a complex mixture of chromophores which show various degrees of ageing as a result of autoxidation, hydrolysis or pyrolysis. Only some of the structures of the chromophores are known. Most chromophores are large organic molecules ranging from macrocycles (e.g. chlorins, porphyrins) through polyunsaturated compounds (e.g. carotenoids) to oxygen-rich molecules (e.g. polyphenolic derivatives). These include anthocyanin and curcuma dyes derived from, for example, blueberries, cherries, red currants, mustard and curry.

The intensity of a stain depends on the nature of the fibres and the chemical composition of the staining substance. Most natural colours are non-ionic and do not dissociate in water although some natural colours are ionic and, in water, can form negatively or positively charged coloured ions. All synthetic food colours have sulfonic or carboxylic acid groups and are therefore anionic. Staining with natural colours or synthetic food colours is similar to dyeing with textile dyes. The staining propensity depends, in analogy to dyeing, on the affinity of the staining substances to fibres. Affinity depends on

the structure of the staining colourant and the nature of the fibres. Cotton is stained by cationic and non-ionic stains. Synthetic food dyes in a neutral medium only slightly stain cotton, but acidity enhances staining markedly.

Staining can occur by one of the two transport mechanisms – transfer of stain from a soiled substrate to the initially clean substrate or a direct deposition of the staining substance to the fabric. A liquid dropped onto a fabric spreads on the fabric by capillary sorption. Spreading depends on the wettability of fibres by the staining liquid. A colourant in the liquid can be sorbed by fibres, if the colourant has affinity to fibres and the diffusion rate is adequate. [1]

6.2.1 Coffee stain

Coffee is a popular beverage and coffee stains on textiles are common, especially on tablecloths and on carpets. The permanence of coffee stains on the textile depends on the nature of the fibres. The affinity of coffee stain to fibres, indicated by resistance of detergency, increases in the order polyester < cotton < nylon. Coffee stains have little affinity to polyester fibres but adhere to cotton and even more firmly to nylon. [2]

Although coffee has been consumed for centuries, the chemical composition of coffee is largely unknown. Coffee is a complex mixture, and over 700 components have been detected so far. The various classes of chemical compounds found in coffee are (approximately): caffeine (2-5%), acids (7-17%), trigronelline (1-2%), protein (1-6%), sugar (1-5%), polymeric carbohydrates (30-50%) and coloured ingredients (20-35%). [3]

The brown colour of coffee is formed when coffee beans are roasted. During roasting, several degradation, polymerization, dehydration, fragmentation, and recombination reactions occur. Monosaccharaides and sucrose are thermally degraded and polymerised to caramel – a brown substance. The caramel can react with chlorogenic acids (Figure 6.1) to form brown-black substances that resemble humic acids. [4]

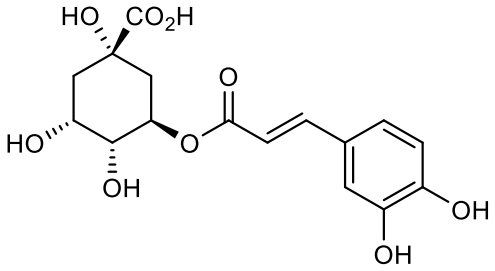

Figure 6.1 Structure of the chlorogenic acid.

6.2.2 Tea stain

Tea leaves contain tea polyphenols, which possess significant antioxidant activity due to their ability to scavenge reactive oxygen species and chelate metal ions. Polymerisation of tea polyphenols occurs in the presence of polyphenol oxidase contained in the leaves and constitutes a spontaneous reaction during the industrial processing of tea, which is known as 'fermentation'. During 'fermentation' some of the catechins combine to create complex theaflavins and other flavonoids that give distinctive flavour and colour to black tea.[5] In addition to flavonoids, black tea beverage contains approximately 15% undefined condensation products. Some of these products are likely to be related to melanins.[6] These dark pigments were found in different plants, such as chestnut, sunflower, beans and grapes, which contain abundant polyphenols.

Flavonoids and catechins can also polymerise into tannins (Figure 6.2), which can later bind and cross-link via metal ions to other tannins and fabric surfaces. [7]

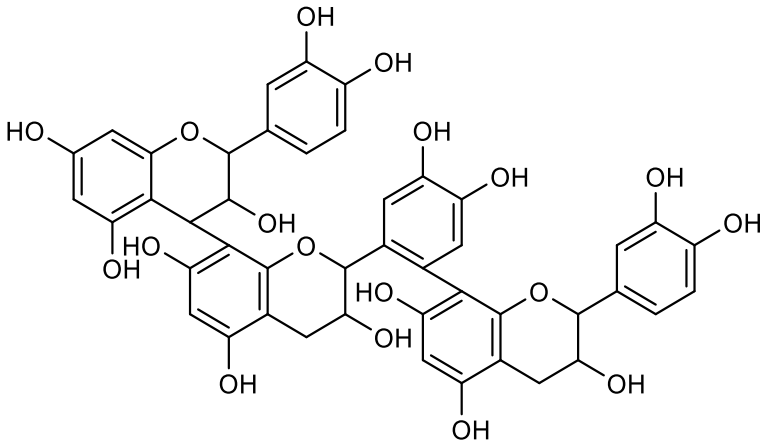

Figure 6.2 Example of polymerised catechin.

6.2.3 Red wine stain

The chemical composition of the red wine has proven to be exceptionally complex, with pigmented molecules continually changing from the moment grapes are brought to the winery, during the pressing, fermentation, pressing and then afterwards in the barrel and during bottle aging. [8]

The principal sources of red wine colour come from anthocyanins (Figure 6.3) or their further derivatives that are extracted or formed during the vinification process. [9]

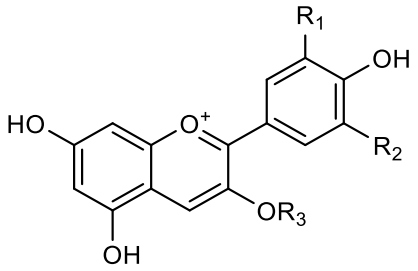

Figure 6.3 The basic chemical structure of anthocyanins - glycosides of anthocyanidins.

Anthocyanins are water soluble flavonoid pigments that, depending on pH, and in some cases complexing agents, can contribute diverse colours such as red, purple and blue. They are widely spread throughout the plant kingdom, and they can occur in almost all tissues of higher plants, including roots, stems, leaves, flowers, and fruits. Consequently they are considered to be a one of the major groups of natural pigments in the plant-derived food, including red wines. During fermentation and especially in the first one or two years of maturation, the monomeric anthocyanins in wine undergo a wide variety of reactions and various new anthocyanin-derived pigments are formed.[10]

6.2.4 Grass stain

The light-harvesting components in green plants are the third major group of natural chromophores. In food processing steps, especially during thermal treatment, chlorophylls are not very stable, resulting in colour change or colour loss. Exchange of the Mg(II) ion by Cu(II) ion stabilises the chromophore, which is used to stabilise semi-synthetic derivative of chlorophyll – chlorophyllin. Cu-chlorophyll is 1 of about 500 food additives that are allowed in Europe.

A grass stain is very complex, which may contain multiple layers of lipids, polysaccharides and protein cover the pigment itself, and makes an effective bleaching challenging.

6.2.5 Tomato-based and curry stains

Tomato and carotenoid based stains are the most common stains in Europe and North America. The most significant carotenoid soils are tomato-based foods (especially pasta sauces) and spices (paprika, saffron). Even a simple stain can contain multiple components, e.g. lycopene, β-carotene, γ-carotene, neurosporene, phytofluoenbe, phytoene and lutein.

Carotenoids, nature's most widespread pigments, are major components of red, orange and yellow fruits, root crops and vegetables. In principle, carotenes are easy to oxidize because of the large number of conjugated double bonds. However, their hydrophobic nature means that they are very often solubilized in oils and fats (e.g., spaghetti sauce) and therefore protected against hydrophilic bleach systems such as peracetic acid.[11]

6.3 Image Analysis test with the stains

Bleach contributes to stain removal by either oxidatively modifying the stain such that it becomes more water soluble and easier to remove, or by decolourising the stain such that it is no longer visible. The simplest and most common bleach is hydrogen peroxide. The ability of peroxide to decolourise and help remove hydrophilic stains like tea, coffee, and wine has been known for a very long time. The performance of the hydrogen peroxide is most powerful at high temperatures and long wash times. A bleach activator is a peracid precursor that reacts with peroxide in the wash solution to form peracid. They have the advantage being more reactive and therefore more effective than hydrogen peroxide. [12]

Optimising a bleach reaction is a very difficult task, as an incredible number of factors control the process. Besides the two reaction partners, the bleach active species and the stain, the reaction matrix, and especially the reaction conditions have a major impact on the results. Before oxidation can take place, both reaction partners have to come into close contact. In this respect, bleaching in solution is easier than fabric bleaching, as under alkaline washing conditions, cellulosic fibres are negatively charged, and electrostatic repulsion has to be overcome. Cationic charges such as ammonium groups in bleaching agents lead to favourable interactions, whereas negatively charged substituents often results in a poor performance. Increasing the ionic strength in the washing liquor or high temperatures can assist in overcoming the repulsion of negatively charged surfaces. [13]

The current trend is to move towards more environmentally friendly domestic detergents that operate at lower temperature and closer to neutral pH. Therefore, as an expansion of previous studies of dyes bleaching as a function of pH, the results from stained fabrics experiments are presented in this section.

An Image Analysis (IA) technique was used to obtain presented results. IA is primarily used in Procter & Gamble procedures for assessment of the performance of new detergent formulations. Usually a standard set of various stains is used, not just a single stain. The method is not as accurate as spectroscopic techniques (the equipment contains a regular camera). In addition difficulties in stains reproducibility should also be taken to the account when considering the experimental error. The error value was established on ± 5%, based on the results from repeated experiments.

Three common, beverage-based stain were chosen for this purpose: coffee, tea and red wine. PAA, SPC and TAED + SPC were used as a bleaching agents and the pH range: 7.5 – 8.5 – 9.5 – 10.5 were investigated. The bleaching performance was assessed by three parameters: Stain Removal Index, Washed Noticeability and Stain Change (Section 2.1.5). A very good detergent usually can be described with high SRI and SC parameters and low WN value.

The presented data was recorded using Procter & Gamble's standard protocol and disassembly of the analysis code was not possible,hence the estimation of the analysis errors.

6.3.1 Coffee stain

The comparison of the photographs of the coffee stains before and after bleaching (Figure 6.4) suggest that PAA and TAED + SPC are efficient in the investigated pH range and unwashed stain can be seen by naked eye only when sodium percarbonate was used.

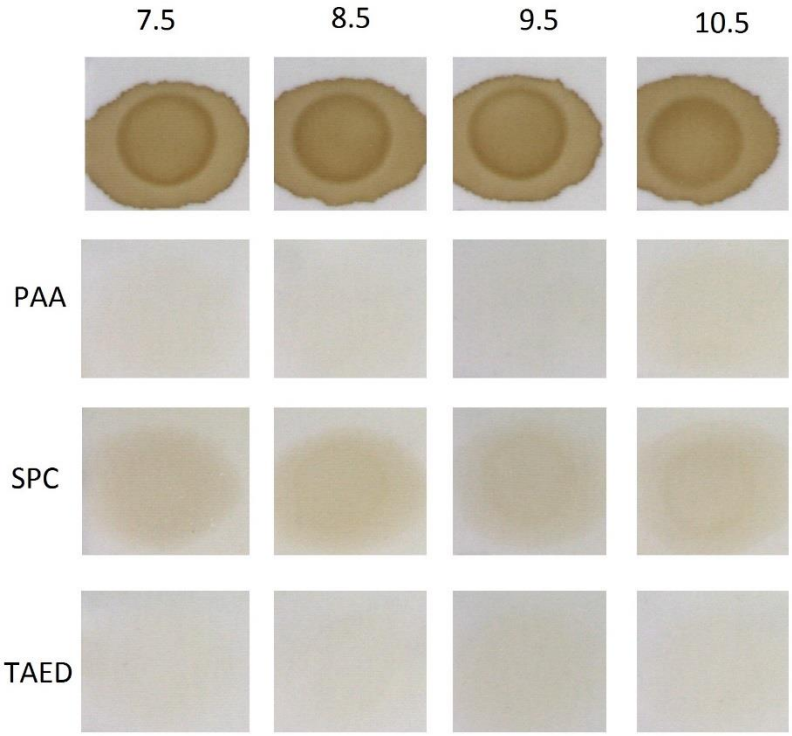

Figure 6.4 Pictures of coffee stains bleached by PAA, SPC and TAED over 45 min in alkaline pH range, [PAA] = 0.01 M, [SPC] = 0.01 M, [TAED + SPC] = 0.01 M + 0.03 M.

Analysis of the obtained parameters (SRI, WN and SC) reveals much detailed information about pH dependence of bleaching performance (Table 6.1). The SRI calculated for PAA drops from above 80 % to 54 – 60 %, when the pH rises, which indicate that peracetic acid itself performs better in pH values closer to the neutral. The behaviour of TAED correlates almost identically with behaviour of PAA, which is surprising, because TAED should release more PAA (1.5 – 1.7 equivalent) and also it would be expected that the perhydrolysis reaction would impact the final result. In case of SPC also drop in SRI parameter can be observed when pH increases from value 66% in pH 7.5 to 40 – 45 % in pH 9.5 and 10.5.

Bleach	pH	SRI /%	WN	SC
PAA	7.5	83.8	2.8	23.9
	8.5	84.3	2.6	24.4
	9.5	54.2	2.1	22.0
	10.5	60.5	2.9	20.6
SPC	7.5	66.0	5.7	18.0
	8.5	60.8	7.6	17.3
	9.5	39.8	6.6	16.7
	10.5	44.9	6.2	15.6
TAED + SPC	7.5	84.6	2.3	24.6
	8.5	85.7	2.1	24.2
	9.5	52.4	3.4	20.9
	10.5	61.7	2.1	20.4

Table 6.1 SRI, WN and ST parameters obtained for coffee stains after bleaching with PAA, SPC and TAED in alkaline pH range over 45 min, [PAA] = 0.01 M, [SPC] = 0.01 M, [TAED + SPC] = 0.01 M + 0.03 M.

6.3.2 Tea stain

The pictures of dry tea stains before and after 45 min treatment with PAA, SPC and TAED are shown in Figure 6.5. Tea stain is lighter than coffee stain, and therefore one might think that bleaching tea stains should be easier than coffee. However, visual comparison of the two figures (Figure 6.4 and Figure 6.5) shows that after 45 min bleaching the tea stain residues are more visible, especially when sodium carbonate was used.

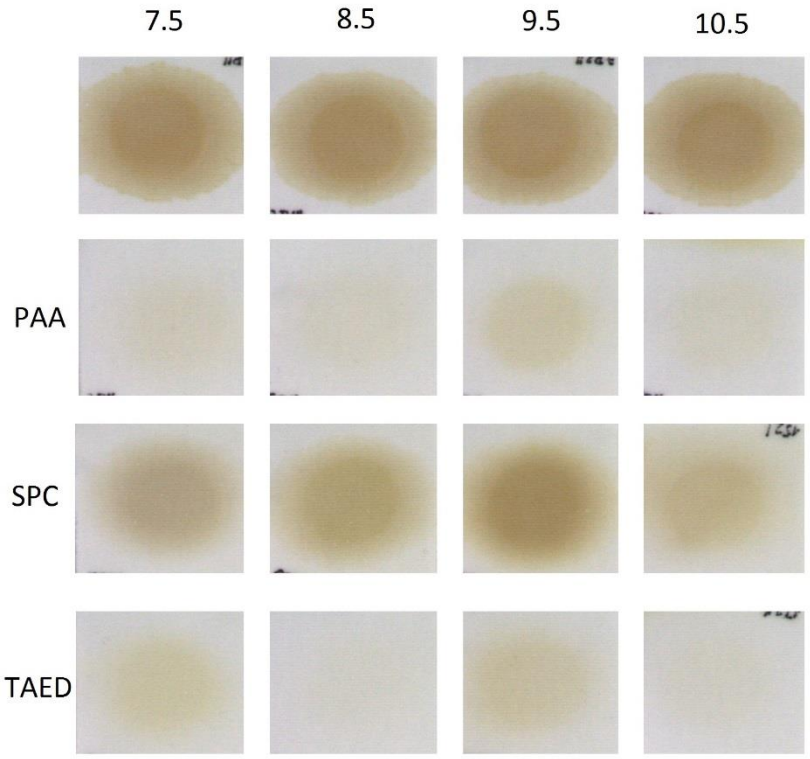

Figure 6.5 2 SRI, WN and ST parameters obtained for tea stains after bleaching PAA, SPC, TAED oxidants and in alkaline pH range over 45 min, [PAA] = 0.01 M, [SPC] = 0.01 M, [TAED + SPC] = 0.01 M + 0.03 M.

The analysis of the SRI, WN and SC parameters obtained for peracetic acid did not show any trends with pH (Table 6.3), within the margin of error (± 5%) the performance of PAA can be considered as stable in the alkaline pH range.

The results obtained from TAED showed great variation with pH and showed no trend or correlation between SRI and pH. When tea stains were bleached with sodium percarbonate, a decrease in performance was observed from pH 7.5 to pH 9.5, but then increased again to reach greatest performance in pH 10.5.

Tea stain is a complex system and it is difficult to find a reason behind the strongly pH dependent behaviour. Possibly it is a complementary effect of the pK_a of the components of the stain and bleaching species. For tea stain removal, as a general conclusion the most optimal pH would be 10.5.

Bleach	pH	SRI /%	WN	SC
PAA	7.5	75.5	4.0	18.0
	8.5	75.6	4.3	18.0
	9.5	65.3	5.9	15.7
	10.5	77.3	4.2	17.8
SPC	7.5	36.8	12.4	8.1
	8.5	17.1	15.7	5.8
	9.5	5.1	20.9	2.6
	10.5	42.9	9.5	11.1
TAED	7.5	56.3	8.3	15.0
+ SPC	8.5	78.1	3.5	18.4
	9.5	53.4	8.5	14.0
	10.5	77.8	3.4	18.3

Table 6.3 SRI, WN and ST parameters obtained for tea stains after bleaching with PAA, SPC and TAED in alkaline pH range over 45 min, [PAA] = 0.01 M, [SPC] = 0.01 M, [TAED + SPC] = 0.01 M + 0.03 M.

6.3.3 Wine stain

The last stain investigated by image analysis technique was red wine stain. The pictures of dry stains, before and after reaction with sodium percarbonate, peracetic acid and TAED +SPC are presented in Figure 6.6. A key feature of red-wine stain is the dramatic colour change that occurs due to the change in pH. The wine stains after bleaching not only faded but also changed colour – from purple to beige. This is associated with the chemical nature of anthocyanins, which change colour depending on the environment, i.e. they act as pH indicators. The colour change was not observed for coffee and tea stains, however this change is not clearly manifested by the SRI, WN and SC data.

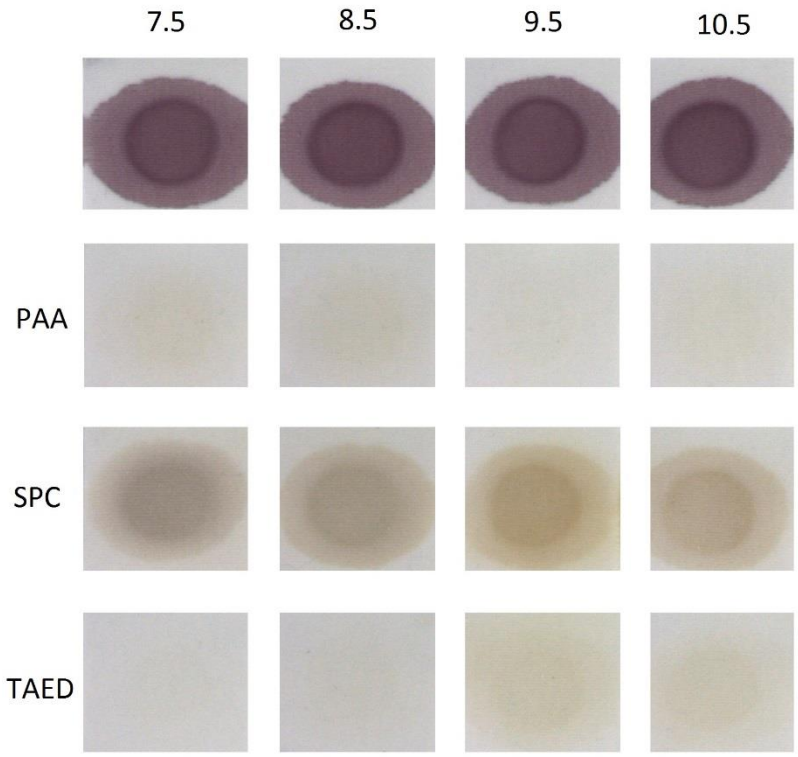

Figure 6.6 SRI, WN and ST parameters obtained for red wine stains after bleaching with PAA, SPC and TAED in alkaline pH range over 45 min, [PAA] = 0.01 M, [SPC] = 0.01 M, [TAED + SPC] = 0.01 M + 0.03 M.

Red wine stain is a hydrophilic stain and PAA, SPC and TAED are hydrophilic bleaching species, in addition red wine stain is susceptible to oxidation. Therefore the general removal of the stain is high (Table 6.4). PAA exhibits a high performance across the whole pH range investigated (SRI > 85 %).

Sodium percarbonate, brought about only partial stain removal. Within the margin of error, it can be assumed that performance of SPC in almost identical over investigated pH range.

SRI values obtained for TAED + SPC system are almost the same as those obtained for PAA, as expected.

Bleach	pH	SRI /%	WN	SC
PAA	7.5	85.5	4.1	41.8
	8.5	87.8	3.7	35.9
	9.5	89.4	2.6	38.0
	10.5	87.0	2.7	38.1
SPC	7.5	66.7	13.9	30.4
	8.5	64.2	10.7	28.2
	9.5	60.9	12.3	32.0
	10.5	67.6	10.9	31.5
TAED	7.5	87.4	1.9	43.0
+ SPC	8.5	92.1	2.1	35.8
	9.5	80.6	5.2	37.7
	10.5	81.9	5.0	37.5

Table 6.4 SRI, WN and ST parameters obtained for red wine stains after bleaching with PAA, SPC and TAED in alkaline pH range over 45 min, [PAA] = 0.01 M, [SPC] = 0.01 M, [TAED + SPC] = 0.01 M + 0.03 M.

Based upon the image analysis of the results obtained from beverage stains it can be stated that the investigated bleaching systems, PAA, SPC and TAED, will be similarly effective at lower pH (7.5 – 8.5) than in usually employed in laundry formulations (pH 9.5 – 10.5). However, it is important to remember that the performance at lower pHs can be further modified by the presence of other ingredients in the formulation and should be fully investigated.

Unfortunately, natural stains are complex mixtures of coloured compounds with unknown protonation constants. Even knowing the pK_a of the peroxy acid, the optimum reaction pH has to be determined experimentally. Using TAED + SPC system, maximal stain removal on tea and red wine is achieved at pH 8.5, whereas coffee stain has an optimum around pH 8. Each bleach system also has an optimum pH for removal of a given stain.

6.4 Diffuse Reflectance tests of the stains

Diffuse reflectance spectroscopy (DRS) is another useful measurement technique, which can be used to monitor stain removal. DRS spectroscopy is very closely related to UV-Vis spectroscopy. The difference in these techniques is that in UV-Vis spectroscopy one measures the relative change of transmittance of light passing through solution, whereas in diffuse reflectance, one measures the relative change in the amount of reflected light of a surface e.g. stained fabric.

In addition DRS measurement can also provide a CIE Lab coordinates of the stain. CIELAB is computed with reference illuminat (D65 – sunlight), which is normalised to a value of 100 at 560 nm. The reflection spectrum (0-100 % at each wavelength) is multiplied by illuminant before the individual colour values (X, Y, Z) are computed. Thus, the resulting X, Y and Z values are all relative to this normalised reference illuminant. Because of that, at no point can the reflection spectrum exceed the intensity (which shows up as the L* value in CIELAB) of the reference illuminant. [14]

The DRS technique can be used in two configurations (Section 2.1.4): horizontal and vertical. The horizontal allows the measurement of dry fabrics, before and after washing or bleaching, while the vertical configuration allows online monitoring of the wet stain colour and spectrum as a function of time during a wash cycle in a model reactor.

In the first part of this section results from DRS horizontal configuration are presented. Five stains (curry, grass, red wine, tomato passata and tea) were exposed to genuine bleach sources (Neptune, TAED + SPC, NOBS + SPC, PAP and SPC) and their diffuse reflectance spectra were collected before and after the exposure (dry fabric). In the second part, when the vertical configuration was utilised, reflectance spectra of the red wine and tomato stains were collected in the solution as a function of time, when exposed to the fully, formulated laundry detergent – Neptune.

6.4.1 DRS horizontal configuration

CIELAB parameters are used to present the colours of the stains after bleaching by various bleaching species (Table 6.5).

	Curry	Grass	Red Wine	Passata	Tea
Before					
Neptune					
TAED + SPC					
NOBS+ SPC					
PAP					
SPC					

Table 6.5 Colours of the stains after bleaching by Neptune, TAED, NOBS, PAP and SPC assessed by CIE Lab coordinates, relative to plain fabric, [Neptune] = 5000 mg/L, [TAED + SPC] = 1000 μM + 3000 μM, [NOBS + SPC] = 1000 μM + 3000 μM, [PAP] = 1000 μM, [SPC] = 1000 μM.

Neptune is the most effective bleach, which is not a surprise since it is a fully formulated laundry product with high concentration of TAED and SPC, as well as surfactants. However, no stain was bleached completely to give the clean, white fabric.

Table 6.6 shows the change of colour of the stain after treatment with bleaching agent and assessed by the Stain Change (SC) parameter, which is basically a ΔE value – a difference between the CIE Lab coordinates before and after bleaching (more detailed information in Section 2.1.5). A higher value of SC usually means a better bleach/wash product. In addition the results from ultrasound experiments are shown, when stained fabric were only exposed to ultrasound in neutral or alkaline buffered solution (Sono chemistry is explained is Section 4.5).

The comparison of the Stain Change parameter for the curry stain between oxygen-based oxygen bleach and sonication of the water reveals (Table 6.6) that hydroxyl radicals generated by sono-Fenton chemistry and agitation are, within experimental error almost as good as the fully formulated laundry detergent Neptune. However, as mentioned before, curry stain is difficult to remove, and performance of ultrasound should be considered 'as bad as' oxygen-based bleach. For tea and grass stain the performance of sonication can be compared to performance of sodium percarbonate alone. Ultrasonic washing clearly has potential and possibly, due to the fast technology development will be available in the near future. Ultrasonic washing would allow the reduction of the amount of chemicals used in the wash as well as the time and hence energy used.

	Stain Change parameter ΔE ± 5%				
	Curry	Grass	Red Wine	Passata	Tea
Neptune	14.9	20.8	26.6	24.0	12.6
TAED	16.8	8.5	26.5	8.5	9.7
NOBS	19.4	15.0	25.6	10.2	6.3
PAP	14.4	10.5	26.2	12.4	4.5
SPC	14.6	3.3	22.8	8.6	5.5
Sono, pH 7	18.0	4.8	14.4	4.1	4.2
Sono, pH 10.5	16.5	1.2	10.9	5.2	5.3

Table 6.6 Stain change parameter values for different stains in reflectance study, [Neptune] = 5000 mg/L, [TAED + SPC] = 1000 μM + 3000 μM, [NOBS + SPC] = 1000 μM + 3000 μM, [PAP] = 1000 μM, [SPC] = 1000 μM.

The reflectance spectra of the curry stain after 45 min treatment with a different bleaching agents can be found at Figure 6.7. From the preliminary experiment it was realised that curry stain is not easy to bleach and that the Neptune detergent is the most effective, which was also confirmed with the help of the reflectance spectra.

According to the spectra (Figure 6.7), after bleaching the curry stain with PAP or SPC, the stain is actually slightly darker than before treatment, which can be explained by only partial bleaching to more intensively coloured products. The long hydrophobic tail of peroxynonanoic acid allows the molecule to approach different components of the curry stain than hydrophilic bleach like peracetic acid, which can be seen in 425 – 525 nm region of the spectrum. In addition the peak around 675 nm can be observed, which indicates the presence of green-coloured dyes in the curry stain, such as chlorophyll.

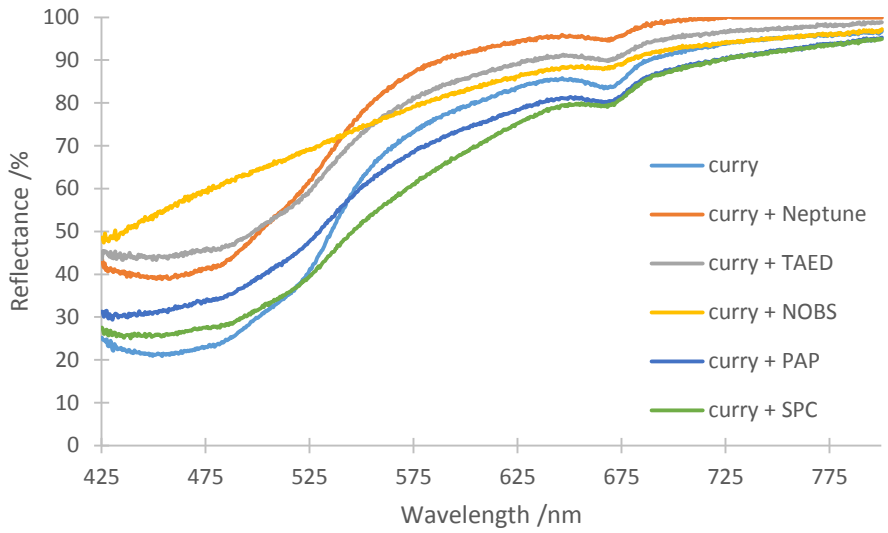

Figure 6.7 Reflectance spectra of curry stains before and after 45 min treatment with Neptune, TAED, NOBS, PAP and SPC, [Neptune] = 5000 mg/L, [TAED + SPC] = 1000 μM + 3000 μM, [NOBS + SPC] = 1000 μM + 3000 μM, [PAP] = 1000 μM, [SPC] = 1000 μM.

The reflectance spectra of the tea stain does not have any distinctive peaks or regions (Figure 6.8). The performance of Neptune, TAED and PAP are very similar and hydrophobic peroxynonanoic acid is the most effective oxidant.

The red wine reflectance spectrum shows the lowest reflectance around 550 nm (Figure 6.9), which is consistent with the absorbance spectrum of the betanin as presented in previous chapter (Section 5.2). All of the investigated oxidants, performed very well with the exception of sodium percarbonate. As in case of the tea stain, the reflectance above 650 nm is lower, than pre-treated stain itself, indicating a lower overall reflecting of the fabric after treatment by the SPC. It can suggest that SPC oxidised the wine stain to darker partially-oxidised products.

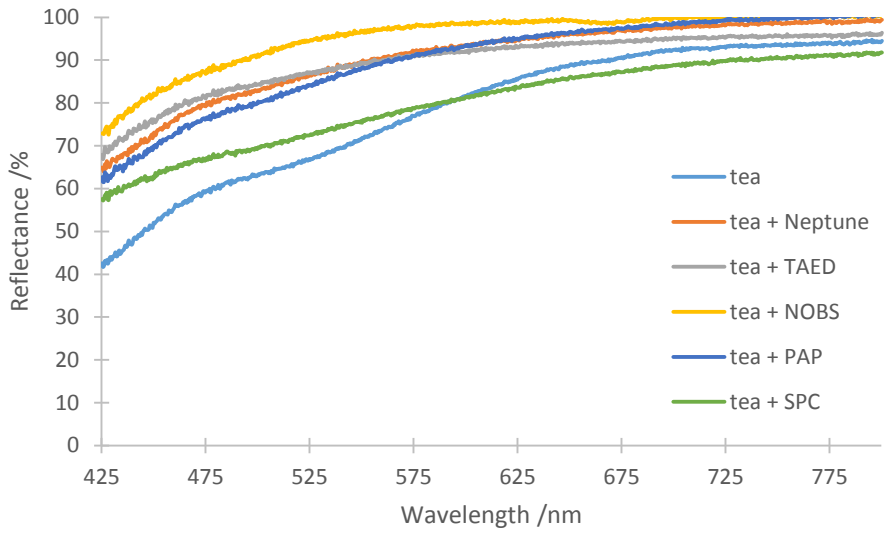

Figure 6.8 Reflectance spectra of the tea stains before and after 45 min treatment with Neptune, TAED, NOBS, PAP and SPC, [Neptune] = 5000 mg/L, [TAED + SPC] = 1000 μM + 3000 μM, [NOBS + SPC] = 1000 μM + 3000 μM, [PAP] = 1000 μM, [SPC] = 1000 μM.

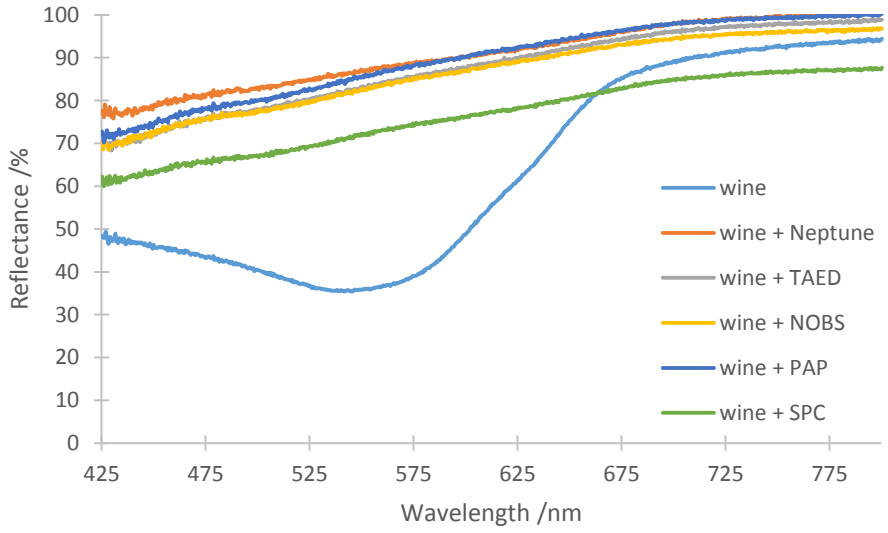

Figure 6.9 Reflectance spectra of the red wine stains before and after 45 min treatment with Neptune, TAED, NOBS, PAP and SPC, [Neptune] = 5000 mg/L, [TAED + SPC] = 1000 μM + 3000 μM, [NOBS + SPC] = 1000 μM + 3000 μM, [PAP] = 1000 μM, [SPC] = 1000 μM.

Chlorophylls are mostly responsible for very distinctive reflectance spectra of grass stain (Figure 6.10). Every one of the bleach investigated is able to oxidise some of the components of grass stain but to varying degrees. The bleaching performance can be ranked as: SPC < TAED < PAP < NOBS << Neptune. The porphyrin structure of the chlorophylls is responsible for the presence of the characteristic band at 675 nm and is not completely oxidised by any of the used bleaches. Rubbed grass stain contains porphyrin rings embedded in binding protein, therefore an enzyme strategy is the most suitable for removal of these kind of stains. In addition the presence of surfactant systems increase the solubility of the green dyes.

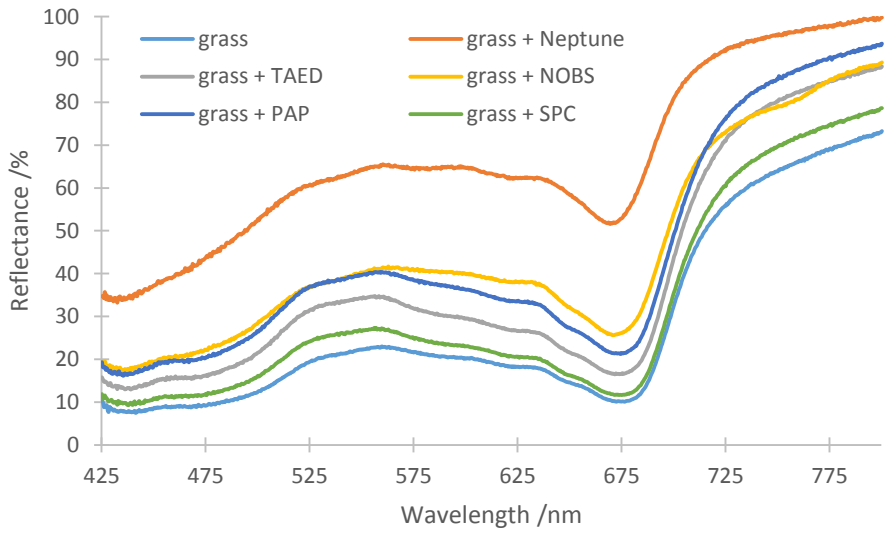

Figure 6.10 Reflectance spectra of the grass stains before and after 45 min treatment with Neptune, TAED, NOBS, PAP and SPC, [Neptune] = 5000 mg/L, [TAED + SPC] = 1000 μM + 3000 μM, [NOBS + SPC] = 1000 μM + 3000 μM, [PAP] = 1000 μM, [SPC] = 1000 μM.

Finally tomato passata stain was investigated by the dry reflectance measurement (Figure 6.11). Almost full bleaching was observed when Neptune powder was used. TAED + SPC and SPC alone are much less effective and only partial oxidation of the stain's components was observed, possibly due to the lack of surfactant system. As in the case of curry stain, which also contains various kinds of carotenoids, the reflectance spectrum measured for NOBS bleach activator differs significantly from the others and being more 'flat', without distinctive peaks, which might suggest some additional interactions between NPA, NOBS or diacyl peroxide and carotenoids.

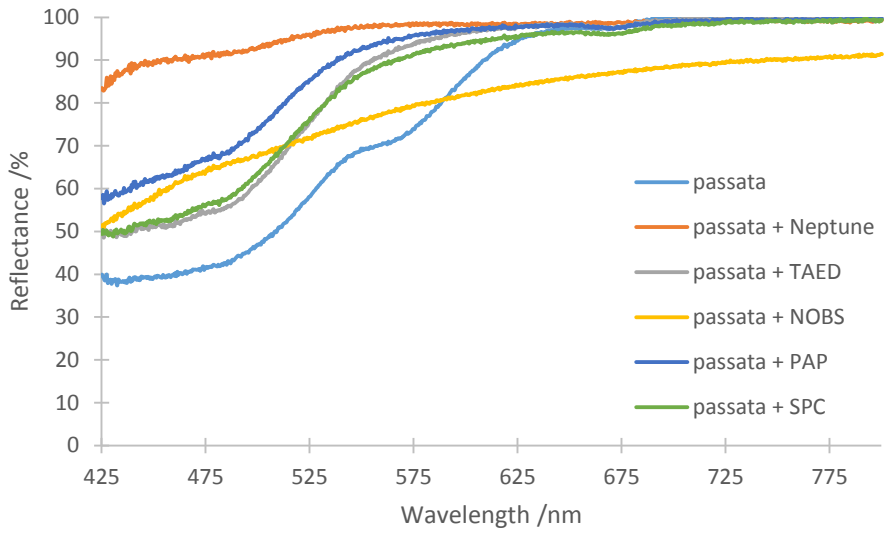

Figure 6.11 Reflectance spectra of the tomato stains before and after 45 min treatment with Neptune, TAED, NOBS, PAP and SPC, [Neptune] = 5000 mg/L, [TAED + SPC] = 1000 μM + 3000 μM, [NOBS + SPC] = 1000 μM + 3000 μM, [PAP] = 1000 μM, [SPC] = 1000 μM.

In summary, reflectance spectroscopy can deliver a new insight into the cleaning assessment of the stained fabrics. Additionally, it can be potentially applied to other fields of household detergency, for instance cleaning of surfaces or dishes.

The main conclusion from this part of the study is that bleaching is highly dependent of the type of the bleach, and the components of the stain and there are no straightforward trends. As a fully formulated detergent, Neptune is definitely the most effective at removing the stains tested here, however, in some cases the mixture of NOBS bleach activator and sodium percarbonate, which results in *in situ* generation of peroxynonanoic acid performed comparably. NPA is also able to oxidise different molecules, due to its hydrophobic character than for instance PAA, which might suggest that the formulation, which would contain the mixture of different bleach activators would be very efficient.

6.4.2 DRS vertical configuration

As shown in the previous section diffuse reflectance spectroscopy can provide a better insight into the bleaching of stained fabrics than the Image Analysis method. However, DRS is not only limited to the assessment of the dry fabric, when used in a horizontal configuration (more detailed information

in Section 2.1.4) but also allows online monitoring of the colour of the wet fabrics/stain as a function of time.

For the purpose of the experiment two stains were chosen: red wine and tomato passata, because of earlier observed more dramatic colour change during and after bleaching. Anthocyanins present in red wine stains are employed often as a pH indicators, due to the changes of their chemical structure when exposed to different pH values (Section 5.2.4). Anthocyanins can also be found in food product based on tomatoes.

Figure 6.12 shows a transition of the colour of the red wine stain during first 5 minutes after adding a solid detergent – Neptune. Neptune is a product designed to reach pH 10.5 when added to the water. A transition from purple colour (pH 7) to blue-greenish (pH 10.5) can be observed.

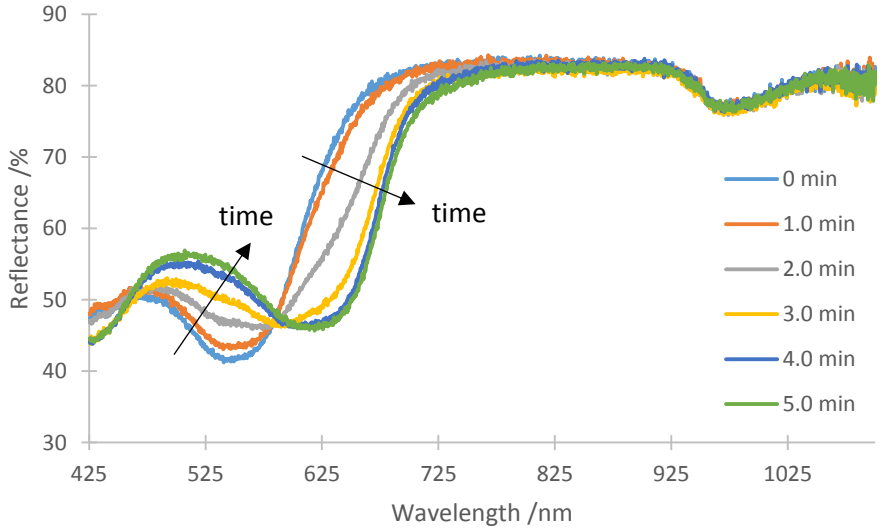

Figure 6.12 Reflectance spectra of red wine stain during treatment with Neptune over 5 min. Measurement taken every 0.5 min, [Neptune] = 5000 mg/L.

The next Figure 6.13 shows the reflectance spectra of the red wine stain during 45 minute of bleaching with Neptune detergent. The light blue line represent measurement taken at time = 0 min, the change of the colour of the stain can be observed firstly, associated with the reaching of the solution pH 10.5, and then slow fading of the colour.

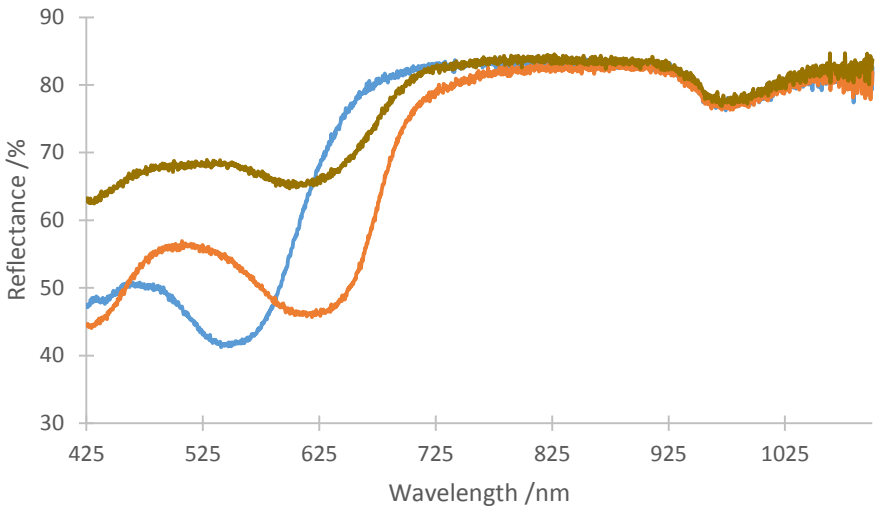

Figure 6.13 Reflectance spectra of red wine stain during treatment with Neptune over 45 min, [Neptune] = 5000 mg/L. Blue line: $t < 0$ min (without Neptune), orange line: $t = 0$ min (with Neptune), brown line: $t = 45$ min (bleaching).

During bleaching of the tomato stain with Neptune over 45 min, no change in bands positions nor appearance of new bands were observed (Figure 6.14). It can be concluded that the surfactant system present in Neptune detergent increases the solubility of the coloured components of the stain (e.g. carotenoids) which can be later oxidised by peracetic acid generated from reaction between TAED and SPC. It can be assumed that PAA is able to oxidise all of the dyes present in the stain – the colour is fading, but not changing.

Diffuse reflectance spectroscopy is a versatile method, which can be used for colour assessment of various surfaces (e.g. stains, fabrics, dishes) in various conditions (dry or wet). The technique was employed with success on both dry and wet stained fabrics and provided a new insight into bleaching stains with commercial used bleaching agents and allows both an estimate of the degree of stain removal and detailed information about any changes in spectral profile that occur during the wash.

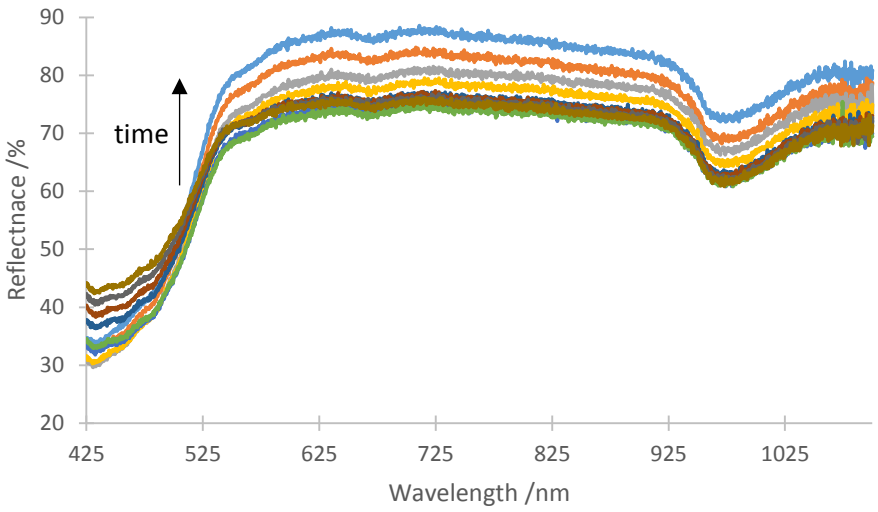

Figure 6.14 Reflectance spectra of tomato stain during treatment with Neptune over 45 min. Measurement taken every 5 min, [Neptune] = 5000 mg/L.

6.5 Behaviour of bleach activators and Neptune in the presence of stains on fabrics

The behaviour of the bleach activator (TAED and NOBS, when mixed with SPC) and Neptune detergent in presence of dyes was investigated in a previous chapter. In this section, more complex systems were investigated, where TAED, NOBS and Neptune were exposed to genuine stains deposited on white, knitted cotton.

Oxidation with peroxygen bleaches involves three reactions:

1. Perhydrolysis reaction between hydrogen peroxide and bleach activator (e.g. TAED or NOBS)
2. Bleaching in solution of stain desorbed from the fibres
3. Bleaching of the stain located within the fibres.

Bleaching stain residing within the fibres is much more difficult than bleaching in solution and the rate of bleaching of dyes in fibres is much slower than in solution. The bleaching rates observed in solution do not correlate with those for bleaching in fibres. Hence the dynamics of the bleach penetrating the fiber must be different. [15]

Bleaching of stain located within fibres has to occur by one of two plausible mechanisms:

1. The stain diffuses from fibres into the bath and is bleached there
2. The bleaching agent diffuses into the fibres and bleached the stain within the fibres.

It is possible that bleaching involves both mechanisms. [1]

The analytical method fluorometric titration with 2-naphthylboronic acid, was developed by the author of this work. The main errors in the results originate from the stains itself (complex system, difficult to obtain identical stains, e.g. rubbed grass) and to a smaller extent from calibration curve.

6.5.1 TAED

The behaviour of the TAED in presence of model dyes was examined in a previous chapter (5.5.1). In this part of the work, a more complex system was investigated – the perhydrolysis reaction between TAED and SPC in the presence of real stains deposited on a white, knitted cotton.

As in previous experiment with the dyes – the *in situ* generated peracetic acid that remains stable for 45 min (Figure 6.15), and the presence of the stains and fabrics does not affect the rate of PAA formation. PAA is consumed immediately as indicated by reduced final concentration of PAA.

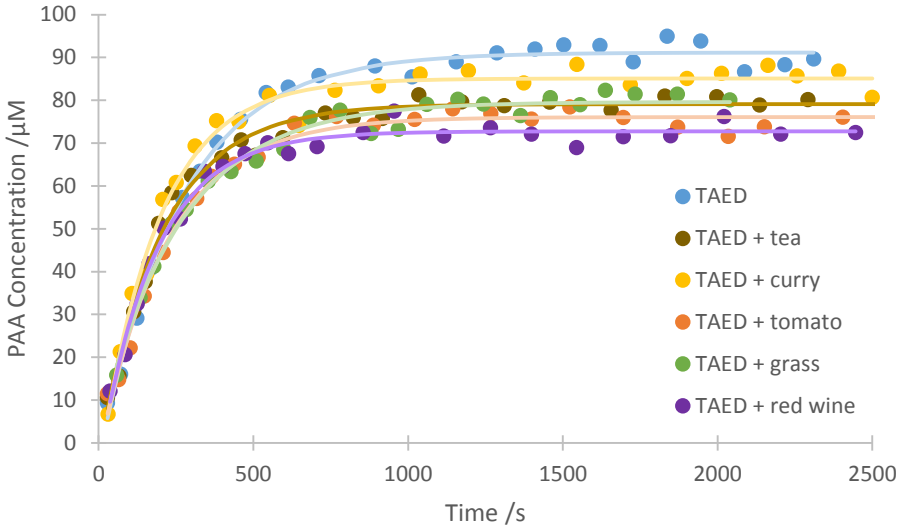

Figure 6.15 Time-concentration profile of PAA, generated from TAED in presence of stains, pH 10.5, [TAED] = 50 µM, [SPC] = 150 µM, two stains of curry, grass, tomato, wine and tea per experiment, [NBA] = 250 µM, λ_{ex} = 365 nm, λ_{em} = 420 nm.

The time-concentration profiles of PAA, generated from the reaction of TAED and SPC are very similar when the system was exposed to dyes and stains, however, the amount of PAA consumed differs. The largest amount of PAA was consumed when it was exposed to chlorophyllin (23 %), but the consumption of *in situ* formed PAA in the presence of a grass stain on knitted cotton set at 11 % level (Table 6.7). This can be explained by greater complexity of the grass stain and that the dye itself is mixed in with various other species (e.g. lipids, proteins) which cannot be easily bleached with peracetic acid.

Similar behaviour can be observed for curcumin or curry stain: less peracid was consumed for stain bleaching. More acid was consumed for bleaching the wine stain (20 %), than betanin itself (9 %), possibly due to the presence of various kinds of easily oxidised dyes from the anthocyanin family.

Similar amounts of peroxyacetic acid reacted with tomato stain (19 %) as with red wine stain (20 %), which might suggest that crocin is not the best model for tomato-based model dye. Crocin is a water soluble carotenoid, and due to its structure should be susceptible to oxidising agents, however

previous results showed that under laundry conditions (pH 10.5, 45 min experiment duration, presence of oxygen-based bleach) no signs of reaction between crocin and bleaching agents can be observed. Theoretically the bleaching of carotenoids present in tomato-based stain (e.g. β-carotene, lycopene) should be even more difficult, due to their lack of solubility in aqueous solution (only soluble in fats). Tomato stain contains not only carotenoid but other pigments (e.g. anthocyanins) and components as well, which can be more easily bleached, thus the observed higher consumption of PAA, than in presence of only crocin.

Concentration /μM ± 5%

	TAED	SPC	PAA final	PAA consumption /%
TAED	49.8	149.0	78	-
TAED + Tea	51.0	153.0	69	12
TAED + Curry	49.8	149.0	74	5.5
TAED + Tomato	50.2	151.5	63	19
TAED + Grass	50.6	151.5	69	11
TAED + Red Wine	49.8	148.9	62	20

Table 6.7 Consumption of PAA, when generated from TAED in presence of stains on fabrics, pH 10.5.

6.5.2 NOBS

In previous chapter, when the behaviour of peroxynonanoic acid was investigated in presence of the dyes: chlorophyllin and betanin (Section 5.5.2), it was found that the peracid was first generated via reaction between NOBS and SPC, and then over a period of time this was consumed completely when exposed to betanin (PNA_C = 99%) and chlorophyllin (PNA_C = 70%). However, this behaviour was not observed when NOBS was exposed to stains on fabrics. The time-concentration profiles resemble one obtained for TAED bleach activator (Figure 6.16). The presence of the stains does not affect the rate of perhydrolysis reaction between NOBS and SPC.

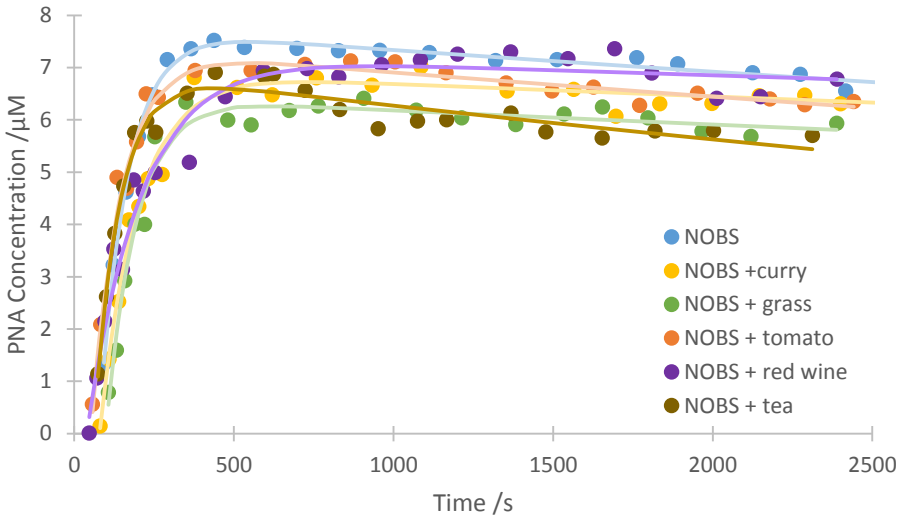

Figure 6.16 Time-concentration profiles of NPA, generated from NOBS in presence of stains, pH 10.5, [NOBS] = 10 µM, [SPC] = 30 µM, two stains of curry, grass, tomato, wine and tea per experiment, [NBA] = 250 µM, λ_{ex} = 365 nm, λ_{em} = 420 nm.

While the consumption of NPA, exposed to betanin was almost complete after 45 min, the opposite situation was observed, when NPA was exposed to the red wine stain (NPA consumption = 1.4 %, Table 6.8). This result is very surprising, because in previous experiments the red wine was found to bleach to the greatest extent. Also the consumption of the peroxynonanoic acid when used in presence of grass stain (14 %) is lower, than in case of chlorophyllin (70 %), possibly due to the difficulties accessing the coloured components of the stain, which is covered by multiple bio-layers. Curry and tomato passata experiments showed the same consumption of peroxy acid (7.2 %).

Lower consumption of the PNA in presence of stains on fabrics than when exposed to dyes is difficult to explain. Peroxynonanoic acid molecule has a long hydrophobic tail and is larger than peracetic acid molecule, it is possible that due to the size of PNA the reaction with the dyes located in the fibre's pores was more difficult.

Concentration /μM ± 5%

	NOBS	SPC	NPA final	NPA consumption /%
NOBS	10.9	29.8	6.9	-
NOBS + Tea	9.9	30.3	5.7	17
NOBS + Curry	10.1	29.8	6.4	7.2
NOBS + Tomato	10.1	29.8	6.4	7.2
NOBS + Grass	9.9	30.6	5.9	14
NOBS + Red Wine	10.0	30.3	6.8	1.4

Table 6.8 Consumption of NPA, when generated from NOBS in presence of stains on fabrics, pH 10.5.

6.5.3 Neptune

In the previous chapter it was observed that the presence of chlorophyllin and betanin is able to affect the rate constant of the perhydrolysis reaction between TAED and SPC in Neptune, effectively slowing it down. This phenomenon was not observed when Neptune detergent was exposed to the stained fabrics with genuine stains. The peracetic acid is formed rapidly within 100 seconds after mixing, for all of the samples tested, and only the consumption of the PAA is different, depends on the stain used (Figure 6.17).

The largest consumption of PAA occurred when the red wine stain was added to the investigated solution (PAA consumption = 33 %, Table 6.9), which is well comparable with the betanin dye experiment (PAA consumption = 32 %). The consumption of peracetic acid when curcumin or curry stain were introduced, are also at similar level – 3.7 % and 3.1 %, respectively. As in the TAED experiment, the consumption of the peracids is higher when actual tomato stain was used, and this is attributed to the stain on the fabric being removed.

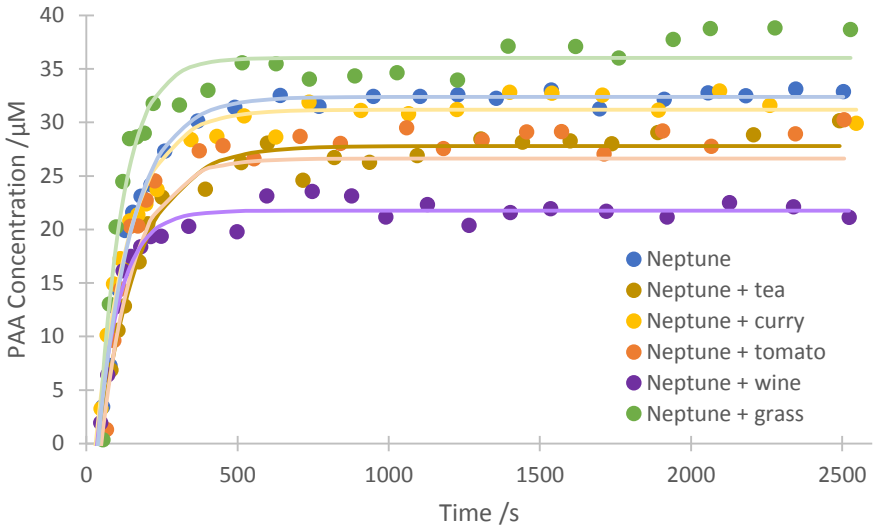

Figure 6.17 Time-concentration profile of PAA, generated from Neptune in presence of stains, pH 10.5, [Neptune] = 5000 mg/L, [NBA] = two stains of tea, curry, tomato, wine and grass per experiment, [NBA] = 250 µM, λ_{ex} = 365 nm, λ_{em} = 420 nm.

While the majority of the results compare well with the solution based dye experiments and can be easily explained, a very strange phenomenon was observed, when grass stain was employed. The PAA consumption value has a minus sign, which means that more peracetic acid was released than in reference Neptune experiment. Additional experiments were run in effort to explain that result.

Concentration /µM ± 5%

	TAED	SPC	PAA final	PAA consumption /%
Neptune	22.0	540.5	32	-
Neptune + Tea	22.0	542.0	29	12
Neptune + Curry	21.9	538.7	31	3.1
Neptune + Tomato	21.9	540.3	29	11
Neptune + Grass	22.0	542.1	38	-16
Neptune + Red Wine	22.0	540.7	22	33

Table 6.9 Consumption of PAA, when generated from Neptune in presence of stains on fabrics, pH 10.5.

During the development of the peroxides probe, the tests with 2-naphthylboronic acid and different variations of Neptune formulations were performed, in order to exclude possible interferences. Two variations of the Neptune formulation were used:

1. Neptune I – free from optical brightener
2. Neptune II – free from optical brightener and bleach (lacking of both SPC and TAED)

Neptune I and Neptune II were tested for the presence of background fluorescence, which could interfere with the response on the NBA probe (λ_{ex} = 340 nm, λ_{em} = 420 nm). In addition, a fluorescence spectrum was run for the mixture of Neptune II and NBA. In both cases, no sign of the any background fluorescence was found. This preliminary experiment can exclude components of the Neptune detergent as the cause of the unexpectedly high concentration of peracetic acid, generated from reaction between TAED and SPC in the presence of the grass stain.

The immersing of the grass stain in a solution of Neptune I and Neptune II results in bright green colour of the solutions. This effect was not observed, when grass stain was immersed into the alkaline solution of TAED and SPC, therefore it can be concluded that the presence of the surfactant system in Neptune formulation enhanced greatly the solubility of the grass stain components. To explain the higher than expected concentration of PAA in the presence of the grass stain deposed on white cotton, the following hypothesis was tested. Soluble in the presence of surfactant systems components of the grass stain can be or behave as a natural peroxy acids or bleach activator, and therefore generate *in situ* peroxy acids when a source of hydrogen peroxide is present (e.g. SPC). To examine that possibility, Neptune II formulation was mixed with SPC, grass stain and NBA. However, the peroxy acids were not detected, only slow rise of the fluorescence was observed, attributed to the presence of hydrogen peroxide in the sample. Also a lack of peracid was observed when solution of Neptune II, grass stain and NBA was analysed.

Currently, the unconfirmed hypothesis is that some component, or components, from the grass stain might react as a catalyst, and effectively increase the amount of released peracetic acid from TAED molecule. [16,17]

6.6 Summary

This chapter contains an attempt to understand the interaction between bleaching agents and various model stains. The bleach results strongly depend on the washing conditions, the bleach active species and the type of stain. Natural stains are complex mixtures of chromophores, food ingredients and additives, making the oxidation reaction very complex. As a result the bleaching process can be influenced in many ways, positively and negatively.

A significant factor influencing the stain removal performance is the pH of the washing solution, usually 9.5 – 10.5 for powder formulations. This does not necessarily match exactly with the pH optimum for the production of the individual bleaching agents. Bleach activators generally are not very effective below pH 8 due to the slow rate of perhydrolysis reaction, due to the low concentration of perhydroxyl anion, pK_a for H_2O_2

The hydrophilic/hydrophobic characters of the bleach system and the stain play an important role in the interaction of both components. Whereas hydrophilic peracids (e.g. peracetic acid) are optimum for bleaching polar polyphenolic stains, such as tea and red wine, they are normally less effective on water-insoluble stains like spaghetti sauce or grass. Long alkyl chain or aromatic ring-containing compounds are preferred, as they are attracted more easily and are able to penetrate hydrophobic stains.

Two successful imaging techniques were employed to assess the stain removal from stains point of view, image analysis and the more versatile diffuse reflectance spectroscopy, DRS. DRS results showed that the presence of surfactant systems and other ingredients of laundry powder are essential for competent stain removal. In addition the results confirmed the hypothesis that the NOBS system actually operates differently to those based of hydrophilic oxidising agents. In addition horizontal configuration of DRS allows *in situ* monitoring of the colour of the wet stain during a wash cycle.

The kinetics profiles of bleach activators in presence of stains revealed that stains themselves does not affect the perhydrolysis reaction kinetics and that in presence of fabrics the perhydrolysis reaction occurs rapidly and is complete within a few minutes after mixing of the SPC and activator. Furthermore, the PAA and NPA are stable for extended period under wash conditions (>40 min). Additionally, the direct comparison between dyes and stains results in some cases proved to be

difficult. Dyes results can give the first indication, but the full image of the system can be only obtained when real stains deposed on fabrics were used.

6.7 References

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7 SUMMARY AND FUTURE WORK

The key ingredient of the household laundry detergent is the bleaching agent, which delivers multiple benefits to the customers, i.e. coloured stain removal. Bleach systems usually consist of a combination of hydrogen peroxide source, and an amide or ester activator, which upon reaction with hydrogen peroxide can generate peroxy acid directly in a wash.

Investigated in this work bleaching agents are: hydrogen peroxide, peroxy acid, singlet oxygen and hydroxyl radical and can be referred also as a reactive oxygen species (ROS). The main goal of this work was to fully understand how the ROS are forming, operating and degrading during a wash cycle.

During the first phase of the project main efforts were directed into finding selective and sensitive toolbox of molecular probes, which allows the analysis towards selected ROS to be qualitative and quantitative. Prerequisite operating conditions for each molecular probe was alkaline environment (pH 7.5 – 10.5). Probes and ROS used in the experimental part were purchased from supplier on generated *in situ* (singlet oxygen, hydroxyl radical). Experimental part started from finding the most optimal method of hydroxyl radical generation in alkaline solution, and UV irradiation of the KNO_3 was found to work the best, due to the high efficiency and stability in basic solution. Experiments showed that terephthalic acid is the most suitable probe for hydroxyl radical detection, due to the formation of single, highly-fluorescent product. The most efficient way of singlet oxygen generation and detection in the alkaline solution was found to be photosensing of Rose Bengal and fluorescent probe – ADMA, respectively. The most difficult part was to found a selective way to detect peroxy acids in presence of large excess of hydrogen peroxide. Due to the presence of the peroxy moiety in both of the species, most of the probes discussed in the literature give the response to both peroxy acids and hydrogen peroxide without any selectivity, therefore new analytical assay was needed. Proposed procedure utilizes naphthylboronic acid, which after oxidation produces fluorescent naphthol, the 'trick' is based on measuring the naphthol fluorescence intensity as a function of time, which allows the distinction peroxy acid and hydrogen peroxide. All of the molecular probes uses the fluorescence phenomenon to allow detection of picomolar concentration of analyte.

In the second phase, developed toolbox of molecular probes was tested in more complex environment. This time ROS were sourced from genuinely laundry detergent ingredients. SPC was

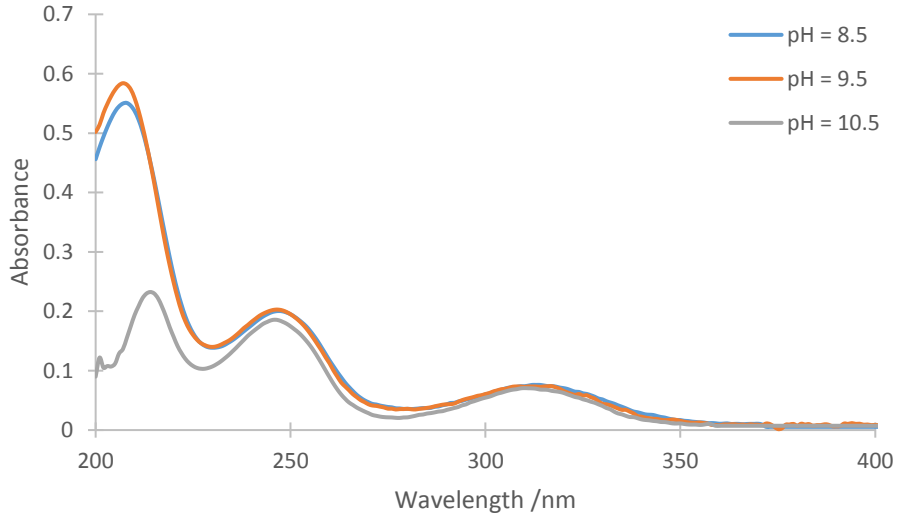
used as a solid precursor of hydrogen peroxide. PAA and PAP were used as a examples of bleaching agents based on peroxy acids. Peroxy acids were also generated via perhydrolysis reaction of bleach activators: TAED and NOBS. In addition fully formulated, powdered detergent – Neptune was used. Toolbox as expected confirmed the presence of peroxides in all investigated sources. Singlet oxygen was found to be a product of the peroxy acids decomposition in alkaline environment, catalyzed by the presence of metal ions. Hydroxyl raidcals were detected in SPC, due to the Fenton reaction. Kinetic parameters of the formation of ROS were also investigated with inter alia stopped-flow technique i.e. formation of PAA was found to be the fastest in Neptune sample, because of the faster dissolution of SPC and TAED, due to the presence of surfactant system. This experimental part prove that, the concepts behind the toolbox work very well in alkaline environment and with genuinely bleaching agents, and can be utilized with success for this type of analysis.

In phase three, model dyes were introduced to the investigated systems. Five model dyes were chosen in order to mimic the most common stains present on fabrics: tomato, red wine, grass and curry. Interaction between bleach and dyes were investigated comprehensively: from the point of view of the dye, as well as from ROS point of view. Firstly, the bleaching performance of the studied ROS were investigated across the alkaline pH range: 7.5 – 8.5 – 9.5 – 10.5 with absorption spectroscopy. However, obtained results are complex, due to the presence of various equilibria and pH-dependencies. In overall, investigated ROS were found to be effective in lower than designed form the to operate pH value (10.5), which could be a preliminary work for more milder pH laundry formulations, which are in addition more eco-friendly. In the second part, the kinetics of the ROS formation were investigated in the presence of the model dyes. In majority of the cases, generated peroxy acids were found to be stable for the duration of the experiment (45 min – average wash duration).

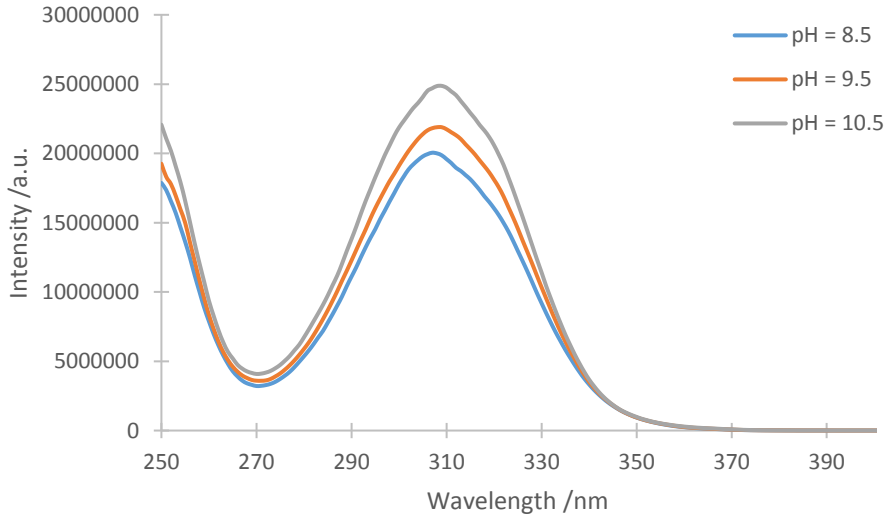
During the fourth phase complexity of the system increased again, by introducing the stain deposed on knitted cotton (tea, coffee, red wine, curry, tomato and grass). Two additional imaging techniques were employed in order to assess the stain removal from the stain point of view: image analysis, developed by Procter & Gamble and diffuse reflectance spectroscopy (DRS). DRS result showed that the presence of the completely, formulated detergent is essential for satisfying stain removal. Kinetic profiles obtained for bleaching agents in presence of the genuine stains showed that the perhydrolysis reaction is not affected by the soiled fabrics and is complete within a few minutes.

The main achievement of this work is the toolbox of molecular probes, which consist of: TA, ADMA and NBA. The probes can be used across the alkaline pH range and in very complex systems, yet still provide characteristic signal from the analyte. Future work suggested by the author of this work are measurements of the washing liquor probed directly from the washing machine. The combination of existing pumping adjustment to the washing machine with portable spectrometer can result in powerful tool for monitoring the presence and concentration of particular ROS. In addition interactions between bleach and other ingredients of the laundry detergent could be studied in more detail.

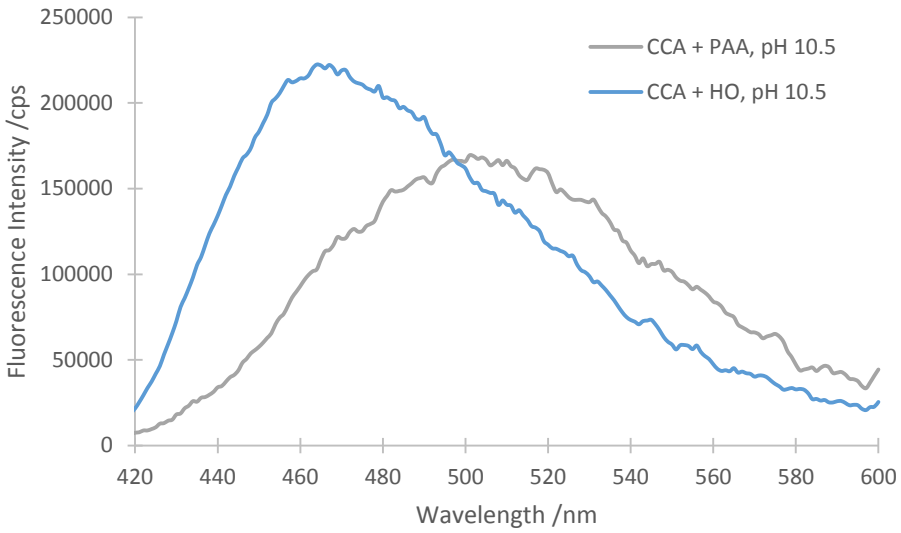
8 APPENDICES



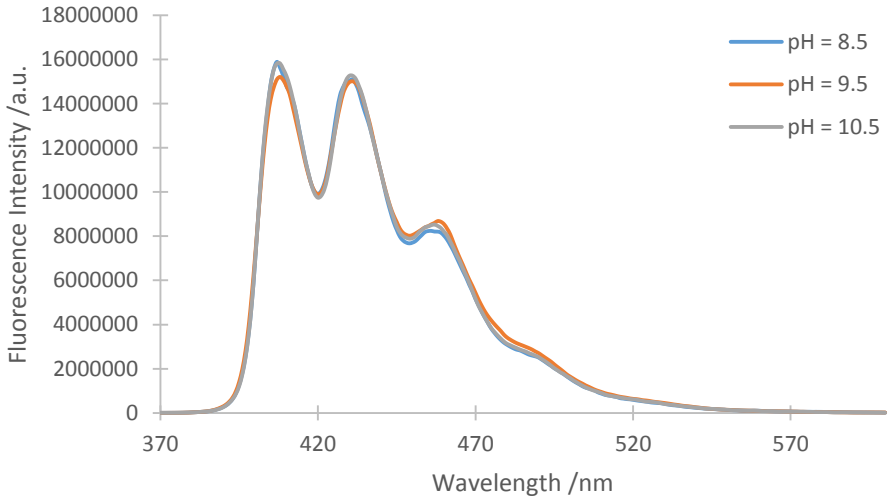
Appendix 1 HTA absorbance as a function of pH, [HTA] = 25 μM.



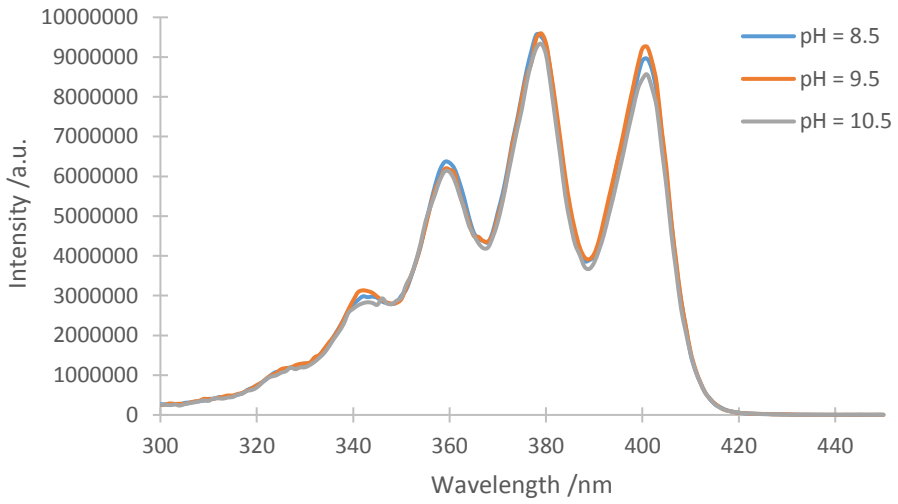
Appendix 2 HTA excitation spectra as a function of pH, [HTA] = 10 μM, λ_{em} = 320 nm.



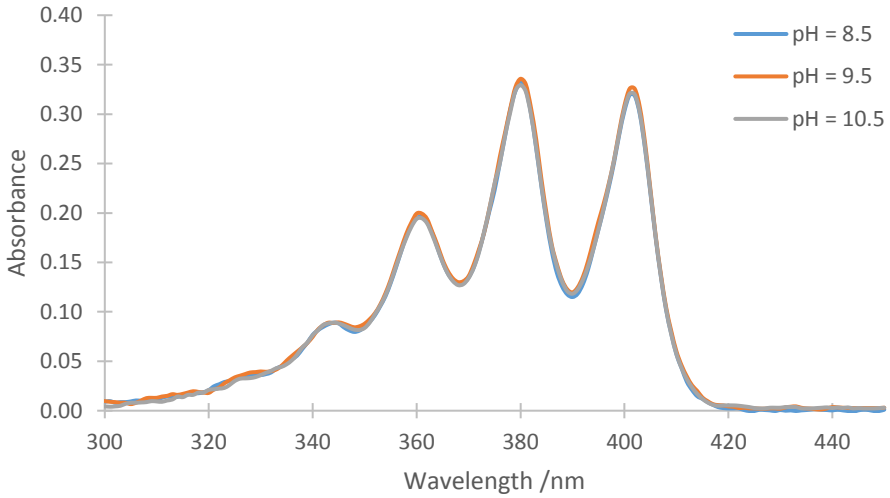
Appendix 3 Fluorescence of HO-CCA, after reaction of CCA with PAA and HO•, in pH 10.5, [CCA] = 10 μM, [PAA] = 50 μM, [KNO_3] = 250 μM, λ_{ex} = 410 nm.



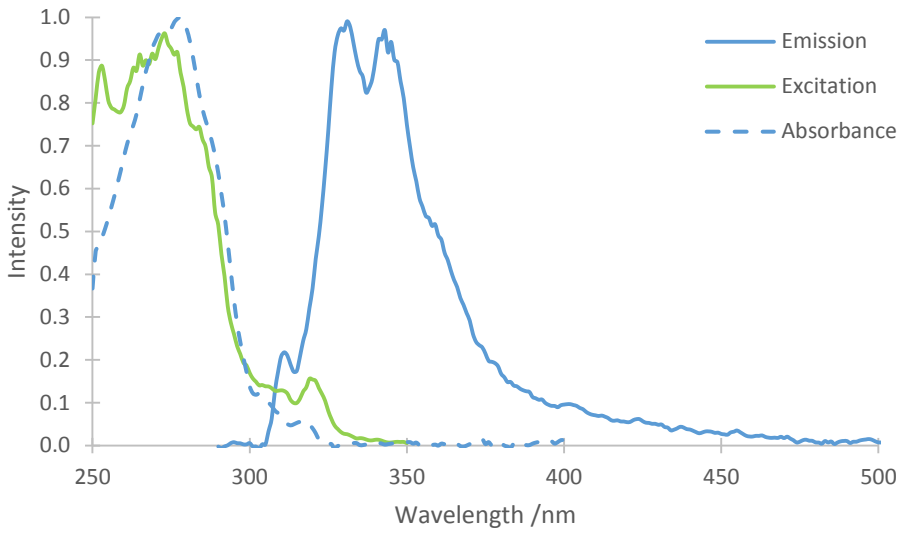
Appendix 4 Fluorescence of the ADMA as a function of pH, [ADMA] = 25 μM, λ_{ex} = 360 nm.



Appendix 5 Excitation spectra of the ADMA as a function of pH, [ADMA] = 25 μM, λ_{em} = 420 nm.



Appendix 6 Absorbance of the ADMA as a function of pH, [ADMA] = 25 μM.



Appendix 7 Spectral characteristic of the NBA in pH = 8.5, [NBA] = 10 μM, λ_{ex} = 280 nm, λ_{em} = 340 nm.