EFFECT OF SINGLE AND MIXED SURFACTANTS ON ANTIOXIDANT ACTIVITY OF BIO-ACTIVE MOLECULES

DISSERTATION

Submitted in partial fulfillment of the requirements provided for the award of Degree of

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In

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By

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Dedicated to my beloved and caring

Parents

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CERTIFICATE FROM SUPERVISORS

This is to certify that the work presented in this dissertation entitled "*EFFECT OF SINGLE AND MIXED SURFACTANTS ON ANTIOXIDANT ACTIVITY OF BIO-ACTIVE MOLECULES* " is original and has been carried out by **Ms. Suraya Jabeen** under our supervision. This piece of work is suitable for submission for the award of M.Phil Degree in Chemistry. It is further certified that the work has not been submitted in part or full for award of any degree in this or any other University.

(Dr. G. M. Rather)
Supervisor

(Dr. Aijaz Ahmad Dar)
Co-supervisor

DECLARATION

I hereby declare that the work incorporated in the present dissertation was carried out by me in the Department of Chemistry, University of Kashmir, Srinagar 190006. The entire work or any part of it has never been submitted before for any prize or degree anywhere.

(Suraya Jabeen)

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Chapter 1
Introduction

Surfactants have unique physico-chemical properties as a result of their amphiphillic molecular structure and are fundamental to life and living bodies.[1] Most of amphiphiles display very important phenomena such as surface activity, wetting adsorption and micelle formation with the resultant functions like solubilization, emulsification, dispersion, drug delivery, ion transport etc.[2,3] Micelles are colloidal particles with the size in the nanometre range, into which many amphiphillic molecules self assemble spontaneously.[4] They are versatile products and have found application in emulsion polymerization,[5,6] enhanced oil recovery,[7] biomedical materials,[8] and biomemitism.[9] Surfactant mixtures have become more interesting than single surfactant solutions due to their wide technological applications and their molecular interactions on complex supramolecular systems.[10] The interactions between water soluble polymers and surfactants are of considerable interest from an industrial point of view as well as because they mimic protein membrane interactions.[11] The use of aqueous miceller media in kinetic studies is rapidly increasing with the aim to replace the conventional organic solvent based syntheses by micelle based syntheses, which not only provides a greater control over stereoselectivity but is environment friendly as well.[12,13]

The preferential solubilization of antioxidants of different nature in interior hydrophobic cores of micelles formed by the long hydrocarbon chains or in outer hydrophilic corona formed by the head groups enables the micelles to play an important part in the mechanism and hydrogen abstraction kinetics of antioxidants. Thus, the location of an antioxidant in the emulsifier/surfactant environment can be of crucial importance for its activity. Micelles are ideal model systems for comparing organic residues in respect of their interaction at biological membrane surface, since they provide similar hydrophobic/hydrophilic interface as the membrane surface.[14]

There is a considerable interest in the interaction between organic solutes and miceller structures as models for understanding of even more complex phenomena such as those occurring in biological systems.

1.1: Surfactants and micellization

Surfactants are amphiphillic molecules composed of a hydrophilic or polar moiety known as head and a hydrophobic or nonpolar moiety known as tail. The surfactant head can be charged (anionic or cationic), dipolar (zwitterionic), or non-charged (non-ionic). The surfactant tail is usually a long chain hydrocarbon residue and less often a halogenated or oxygenated hydrocarbon or siloxane chain.[15, 16]

In aqueous solution dilute concentrations of surfactant act much as normal electrolytes, but at higher concentrations very different behaviour results. This behaviour is explained in terms of the formation of organized aggregates of large numbers of molecules called micelles, in which the lipophilic parts of the surfactants associate in the interior of the aggregate leaving hydrophilic parts to face the aqueous medium. An illustration presented by Hiemenz and Rajagopalan[17] is given in figure 1.1. The formation of micelles in aqueous solution is generally viewed as a compromise between the tendency for alkyl chains to avoid energetically unfavourable contacts with water, and the desire for the polar head groups to maintain contact with the aqueous environment.

A thermodynamic description of the process of micelle formation includes a description of both electrostatic and hydrophobic contribution to the overall Gibbs energy of the system. Hydrocarbons and water are not miscible; the limited solubility of hydrophobic species in water can be attributed to the hydrophobic effect. This effect spontaneously minimizes the unfavourable hydrocarbon-water contact and increases the entropy of the system. But while the hydrocarbon chains pack closer to

minimize water contact, the polar head groups of identical charge tend to stay away from each other as a result of electrostatic repulsion and extensive group hydration. Thus in a miceller aggregate, the equilibrium distance between the polar heads is maintained as a result of compromise between the two opposing tendencies.

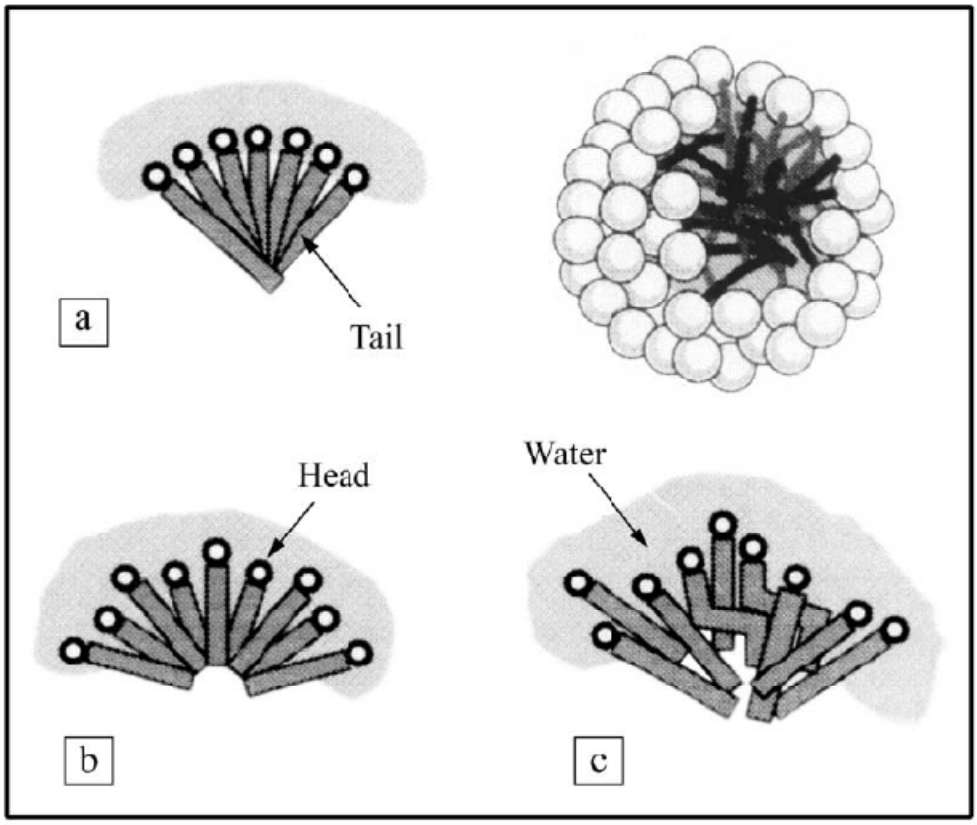


Figure 1.1: *Organization of surfactant molecules in a micelle. From Hiemenz and Rajagopalan.*[17]

Micelle formation is a cooperative process that occurs over a narrow range of concentration, where the transition from the monomeric solution to a solution containing both monomers and micelles takes place. It is customary to define a single concentration within this narrow range as the Critical Micellar Concentration (*cmc*). The *cmc* is considered as the saturation concentration for monomers, and further increase of surfactant concentration leads to an increase in the number of micellar aggregates, prior to any growth in their size.[18] The determination of surfactant *cmc* is

accomplished by use of several physical properties, such as surface tension (γ), conductivity (*k*) – in case of ionic surfactants, osmotic pressure( ), detergency, etc. When these properties are plotted as a function of surfactant concentration (or its logarithm, in case of surface tension), a sharp break can be observed in the curve obtained evidencing the onset of micellization at that point.

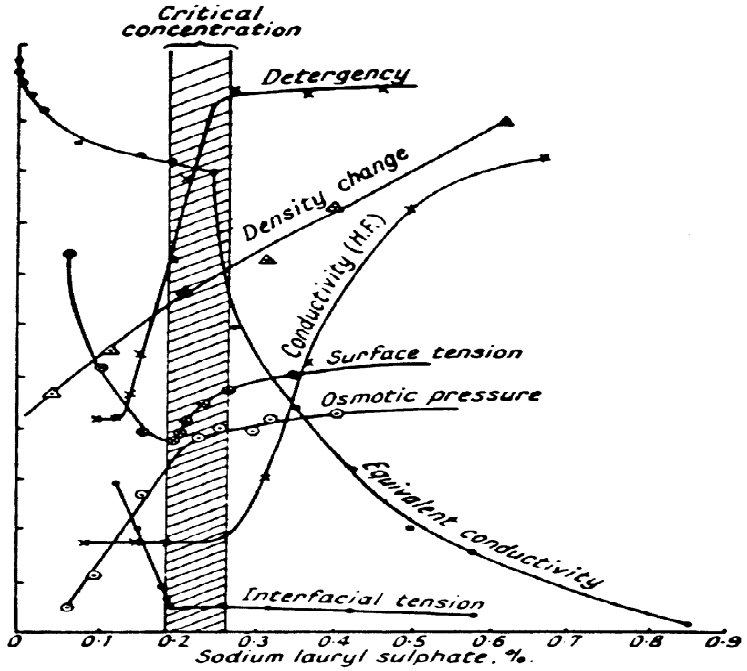


Figure 1.2: *Changes in the physical properties detergency, conductivity (k), osmotic pressure ( ), surface tension (γ) of an aqueous solution of surfactant as a function of surfactant concentration. The break in the curve of each property corresponds to the Critical micelle concentration (cmc).*[19]

1.2: Aggregation Number and Miceller Morphology

Micelles can be characterized by their aggregation number, N_{ag}, that corresponds to the average number of surfactant monomers in each micelle of a miceller solution. Micelles are formed by the noncovalent aggregation of individual surfactant monomers. Therefore, they can be spherical, cylindrical, or planar. Micelle shape and size can be controlled by changing the surfactant chemical structure as well as by

varying solution conditions such as temperature, overall surfactant concentration, surfactant composition, ionic strength and pH. In particular, depending on the surfactant type and on the solution conditions, spherical micelles can grow one-dimensionally into cylindrical micelles or two-dimensionally into bilayers or discoidal micelles. Micelle growth is controlled primarily by the surfactant heads, since both one-dimensional and two-dimensional growth require bringing the surfactant heads closer to each other in order to reduce the available area per surfactant molecule at the micelle surface, and hence the curvature of the micelle surface. [20, 21]

For all these micellar structures in aqueous media, the surfactant molecules are oriented with their polar heads towards the water phase and their tail away from it. In ionic micelles, the interfacial region between the micelle and the aqueous phase contains the ionic head groups - the Stern Layer of the electrical double layer related to these groups - approximately half of the counter ions associated with the micelle, and water. The remaining counter ions are contained in the Gouy-Chapman portion of the double layer that extends further into the aqueous phase. The thickness of the double layer is a function of the ionic strength of the solution and it can be highly compressed in the presence of electrolytes.[22] For the non-ionic surfactants having a polyethylene oxide (PEO) head group, the structure is essentially the same, except that the counter ions are not present in the outer region, but rather coils of hydrated polyethylene oxide chains. The interior of the micelle containing the hydrophobic groups presents a radius of approximately the length of the fully extended hydrophobic chain.[16] Another important characteristic of micelles is that the aqueous phase penetrates into the micelle beyond the hydrophilic head groups, and the first few methylene groups adjacent to the head are considered in the hydration sphere.

Therefore, we can divide the interior region of the micelle into an outer core penetrated by water and an inner core completely water-excluded.[21]

Based on the geometry of various micellar shapes and the space occupied by the hydrophilic and hydrophobic groups of the surfactants, it is possible to estimate the structure of a micelle.[23] Accordingly, the parameter $V_H/l_c a_o$ can determine the shape of the micelle, with V_H corresponding to the volume of the hydrophobic group in the micellar core, l_c is the length of the hydrophobic group in the core and a_o the cross-sectional area occupied by the hydrophilic group at the micelle-solution interface. According to Tanford, [24] V_H = 27.4 +26.9 *n* Å, where *n* is the number of carbon atoms in the chain less by one, and l_c depends upon the extension of the chain. For a fully extended chain, l_c = 1.5 + 1.265 *n* Å.

Table 1.1: Correlation between the parameter $V_H/l_c a_0$ and the micelle structure.

Value of ($V_H/l_c a_0$)	***Structure of micelle***
0-1/3	Spherical in aqueous media
1/3-1/2	Cylindrical in aqueous media
1/2-1	Lamellar in aqueous media
>1	Reversed micelles in nonpolar media

1.3: Mixed Micellization

The study of mixed surfactant systems is important, because surfactant systems are often superior in performance to individual components. There is a substantial

difference in the micellization tendency of mixtures of two or more surfactants as compared to a single pure species. This results in a dramatic change in properties and behavior of mixed surfactants as compared to any single surfactant. In some cases, the two surfactants interact in such a fashion that the *cmc* of the mixture is always intermediate in value between those of the pure components. In other cases, they interact in such a way that the *cmc* of the mixture at some ratio of the two surfactants is less than either of the *cmc*. When this situation arises, the system is said to exhibit synergism in mixed micelle formation. In still other cases, when *cmc* of the mixture is larger than *cmc* of either surfactant, the system is said to exhibit antagonism (negative synergism) in mixed micelle formation. Interest in mixed micelles has largely been driven by industry, in search of properties that lie beyond those defined by each surfactant component. Synergistic effect greatly improves many technological applications in areas such as emulsion formulation, interfacial tension reduction, cosmetic products, pharmaceuticals, and petroleum recovery, etc. In this regard, the specific interaction between two components of a mixture and their physicochemical properties including adsorption behavior and micellization is of paramount importance. Various theoretical models have been proposed to interpret and explain the composition and interaction within mixed micelles and mixed monolayers. While the Clint model[25] is applicable for ideal mixing of surfactant systems, the Rubingh model, [26] based on regular solution theory, is applicable for non ideal mixing and gives the estimate of deviation of experimental *cmc* values from cmc_{ideal}.

1.4: Flavonoids as antioxidants

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea.[27] etc. These

natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruits, and leaves.[28] Biological activities of flavonoids exploitable in the biomedical field include antiinflammatory,[29,30] antiviral,[31] anticancer,[32] anticoagulant,[33] antiatherosclerosis, low-density lipoprotein (LDL) oxidation inhibitory,[34] antioxidant,[35] immunomodulatory,[36] and antitumor[37] activities. In addition, they are also known as potential cell growth inhibitors[38] and multidrug resistance modulators.[39] By virtue of their capacity to inhibit LDL oxidation; flavonoids have demonstrated unique cardio protective effect.[40] Flavonoids can be divided into various classes on the basis of their molecular structure.[41] The 4 main groups of flavonoids includes, the flavones are characterized by a planar structure because of a double bond in the central aromatic ring. One of the best-described flavonoids, quercetin, is a member of this group. Quercetin is found in abundance in onions, apples, broccoli and berries. The second group is the flavanones, which are mainly found in citrus fruit. An example of a flavonoid of this group is narigin. Flavonoids belonging to the catechins are mainly found in green and black tea and in red wine, [28] whereas anthocyanins are found in strawberries and other berries, grapes, wine, and tea.

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. The flavones and catechins seem to be the most powerful flavonoids for protecting the body against reactive oxygen species. Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced

by exogenous damage.[42, 43] The mechanisms and the sequence of events by which free radicals interfere with cellular functions are not fully understood, but one of the most important events seems to be lipid peroxidation, which results in cellular membrane damage. Free radicals can attract various inflammatory mediators, contributing to a general inflammatory response and tissue damage.

Humans have evolved with antioxidant systems to protect against free radicals. These systems include some antioxidants produced in the body (endogenous) and others obtained from the diet (exogenous). Owing to the deficiency of our endogenous defense systems and the existence of some physiopathological situations (cigarette smoke, air pollutants, UV radiations, high polyunsaturated fatty acid diet, inflammation, ischemia/reperfusion,etc.) in which reactive oxygen species (ROS) are produced in excess and at the wrong time and place, dietary antioxidants are needed for diminishing the cumulative effects of oxidative damage over the life span.[44,45] Well established antioxidants derived from the diet are vitamins C, E, A and carotenoids, which have been studied intensively.[46] Besides these antioxidant vitamins, other substances in plants might account for at least part of the health benefits associated with vegetable and fruit consumption. Over the past decade evidence has been accumulated that plant polyphenols are an important class of defense antioxidants. These compounds are wide spread virtually in all plant foods, often at high levels, and include phenols, phenolic acids, flavonoids, tannins, and lignans. Many of these natural antioxidants, especially flavonoids, seem to be very important in the prevention of diseases that have their etiology and pathophysiology in ROS.[47,48] Indeed the level of intake of flavonoids through diet is considerably high as compared to those of vitamin C (70mg/day), vitamin E (7-10 mg/day), and

carotenoids (β-carotene 2-3 mg/day).[49] Flavonoid intake can range between 50 and 800 mg/day, depending on the consumption of vegetables and fruits, and of specific beverages.[50]

Flavonoids can prevent injury caused by free radicals in various ways. One way is the direct scavenging of free radicals. Flavonoids reduce radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive component of the radical.

Selected flavonoids can directly scavenge superoxides, whereas other flavonoids can scavenge the highly reactive oxygen-derived radical called peroxynitrite. Epicatechin and rutin are also powerful radical scavengers.[51] the scavenging ability of rutin may be due to its inhibitory activity on the enzyme xanthine oxidase. By scavenging radicals, flavonoids can inhibit LDL oxidation in vitro.[52] This action protects the LDL particles and, theoretically, flavonoids may have preventive action against atherosclerosis.

1.5: Antioxidant properties and structure - activity relations

The basic flavonoid structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C_6-C_3-C_6), labeled A, B, C **(Figure 1.3).** The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the A and B Rings.

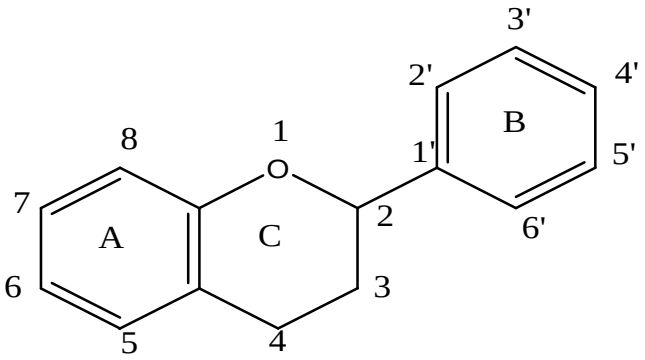


Figure 1.3: *Basic flavonoid structure*

Flavonoids are benzo-γ-pyrone derivatives consisting of phenolic and pyran rings, and most possess high antioxidant and free radical scavenging activities.[35, 53] The antioxidant activity of flavonoids and their metabolites in vitro depends on the arrangements of functional groups about the nuclear structure.

Many studies have been performed to established the relationship between flavonoid structure and their radical scavenging activity and provide clear evidence that the radical scavenging activity depends on the structure and the substituents of the heterocyclic and B rings, as suggested by Bors et al.[54] More specifically, the major determinants for radical-scavenging capability are: (1) the ortho-dihydroxy (catechol) structure in the B-ring, imparting a greater stability to the formed aryloxy radicals as a result of flavonoid oxidation, possibly through H- bonding and electron delocalization[55]; (2) the 2,3-double bond, in conjugation with the 4-oxo function, enhancing electron-transfer and radical scavenging actions through electron delocalizations.[56] The presence of both 3- and 5-OH groups, enables the formation of stable quinonic structures upon flavonoid oxidation.[57] Substitution of the 3-OH results in increase in torsion angle and loss of co planarity, and subsequently reduced antioxidant activity.[58] A typical flavonoid which meets the above three criteria is quercetin, showing the highest antioxidant capacity.

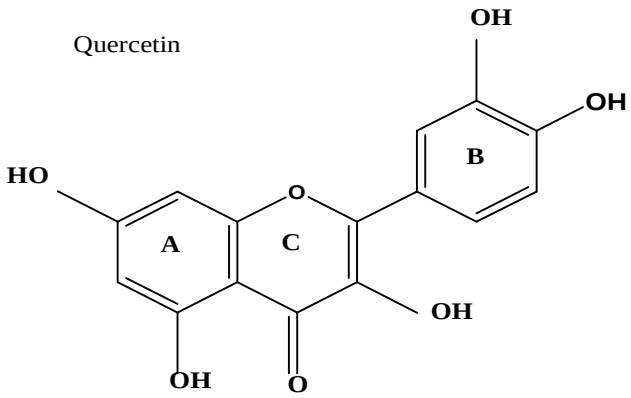


Figure 1.4: *Structure of the flavonol quercetin showing features important in defining the classical antioxidant potential of flavonoids. The most important of these is the catechol or dihydroxylated B-ring. Other important features include the presence of unsaturation and a 4-oxo function in the C ring.*

Aside from these structural requirements, the number of hydroxyl substituent's on the flavonoid molecule, the position of these hydroxyls, the presence of glycosides (-OR) or aglycons (-OH), and the overall degree of conjugation are important in determining antioxidant activity.[59] For phenolic compounds having the same number of –OH groups, the presence of electron-donating-OMe groups in ortho- and para- positions with respect to the –OH substituents (especially in hydroxycinnamic acids) stabilizes the formed aryloxy radicals resulting from one-electron oxidation, and thereby increases antioxidant-activity.[60] With the same number of hydroxyl and methoxy groups, hydroxycinnamic acids tend to be more effective in antioxidant-capacity than the corresponding hydroxybenzoic acids, possibly due to the aryloxy radical stabilizing effect of the –CH=CH-COOH linked to the phenyl ring by resonance.[41,61] Thus, flavonols and flavones containing a catechol group in ring B are highly active, with flavonols more potent than the corresponding flavones because of the presence of the 3-hydroxyl group. Glycosylation of this group, as in Rutin, reduces greatly the radical-scavenging capacity. An additional hydroxyl group in ring B (Pyrogallol

group) further enhances the antioxidant capacity, as exemplified by myricetin. On the contrary, the presence of only one hydroxyl in ring B diminishes the activity. Flavonols and flavanones, due to the lack of conjugation provided by the 2, 3-double bond with the 4-oxo group, are weak antioxidants.[56]

1.6: Antioxidants in micellar media

Lipid oxidation is one of the main factors limiting the shelf life of bulk oils, since it adversely affects flavor and quality, and potentially produces toxic reaction products.[62] Preventing or inhibiting the oxidation of bulk oils is, therefore, of great importance to consumers and the food industry. A variety of mechanisms have been proposed to be responsible for the oxidation of bulk oils during processing and storage: photosensitized oxidation, metal-promoted and autoxidation being the most well-known. Some factors that impact the oxidative stability of bulk oils include: oil extraction and processing conditions, light exposure, temperature, fatty-acid composition, antioxidant composition, oxygen levels, and the presence of minor components.[63] Manipulation of these factors can be used to retard lipid oxidation in edible oils.

One of the most effective ways of inhibiting lipid oxidation in bulk oils is to incorporate antioxidants.[64]Among numerous compounds reported to possess antioxidant properties, the phenolic compounds, synthetic or natural, have been extensively examined as lipid oxidation retardants in an array of lipid substrates. Activity of food phenolics (antioxidants), mostly in bulk oils, has been studied by number of researchers.[65-68] Antioxidants are substances that when present in low concentrations relative to the oxidizable substrate significantly delay or reduce the oxidation of the substrate. Antoxidants that combat oxidation,[69] protect the body from

adverse effects of free radicals and reactive oxygen species by converting the free radicals into more stable substances.[70] They have greater application in the food industry for increasing the stability and shelf life of food products. Moreover, they also find use as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention.[71]

The lipophilic character of an antioxidant is determined by its partitioning between phases differing in polarity. One important driving force for partitioning is the energy of removing a loosely held water sheath which appears to form around the antioxidant in the aqueous phase. The forces of interaction between molecules that result from attraction of different functional groups can lead to different partition behaviour. On the other hand, the overall composition of the discrete environments can cause differences in polarities which affect the partition behaviour of the antioxidant.[72] several studies indicate that the relative activity of antioxidants can vary when comparing systems which differ in the distribution of the lipid phase. As per porter et al.,[73, 74] a remarkable example of the effect of hydrophobicity on the relative reactivity of antioxidants is the so called "polar paradox", the observation that polar antioxidants are more effective in polar, oil-in-water emulsion.

Several studies have shown that antioxidants can partition into different physical locations in emulsions, and the activity of a given antioxidant depends not only on the environmental pH but also on a number of factors including its partitioning between different regions of the system, making such an evaluation of the antioxidant activity a difficult task.[75,76-78] Micelles and other colloidal systems have been extensively used as models for understanding the effects of heterogeneous environments on reaction dynamics and mechanisms, providing relatively simple models for understanding the complex behaviour encountered in food and biological assemblies.[79,1,80,81] Micellar

systems are usually characterized as "two phase" systems where separation or concentration of the reactants between the aqueous and interfacial regions may occur, allowing one to analyze some of the complexities that arise in real systems in a relatively simpler fashion; for example, partitioning of substrates, local concentration or dilution effects, and so forth.

The organized surfactants are well known to affect the structural and electronic properties of the antioxidants which not only can solubilize them to increase their aqueous solubility but also can influence their antioxidant ability greatly.[82] The solubilization of antioxidants in the different phases and environments of micelles results in different physicochemical interactions compared to homogeneous systems. Many studies have demonstrated that the activity of antioxidants can vary strongly depending on the systems in which they have been solubilized.[83-85] For careful study of the location of the antioxidants and therefore to be able to characterize the chemical microenvironment of the antioxidants in micelle solutions, the partitioning behaviour [86,87] of antioxidants between the micellar phase and the aqueous phase is crucial for understanding differences in antioxidant activity as a function of surfactants with different charges. Interest in understanding the parameters that influence the activity of antioxidants in complex or multiphase systems is increasing as actual food products are multicomponent matrices. [88-90, 86, 91] As per Frankel, [92] -interfacial phenomena are key to better understanding of antioxidant action in heterogeneous foods and biological systems.

Aims and Objectives of the study

Reactive oxygen species (ROS) behave both as a positive and negative agent in many living physiological processes. In order to balance the physiological generation of free radicals, organisms have evolved a wide array of enzymatic and nonenzymatic endogenous antioxidant defences [93-94]. Nevertheless, in situations of increased free radical generation the reinforcement of endogenous antioxidants with dietary antioxidants may be particularly important in diminishing the cumulative effects of oxidatively damaged molecules. Flavonoids, a group of naturally occurring benzo-γ-pyrone derivatives, have been reported to possess multitude of biological properties and proven to be strong antioxidants and free radical scavengers.[95, 41.]

Micellar systems have been employed as models in investigations concerned with understanding colloidal physicochemical phenomena. The similarities between self assembled surfactant aggregates, such as micelles and biological lipid membranes have not gone unnoticed.

Quercetin, (3, 5, 7, 3', 4'pentahydroxyflavone; scheme 1) is one of the most common flavonoids present in nature. Abundant in the human diet, quercetin has potent antioxidant and metal ion chelating capacity, possesses various biological and biochemical effects including anti-inflammatory, antineoplastic and cardio-protective activities. [96-100] In addition, quercetin is among the group of phytoestrogens (plant derived molecules with estrogenic or anti-estrogenic effects) suggested to reduce risks of certain cancers.[101] It has been reported that such activity of polyphenols is sensitive to the environmental changes like change in solvent polarity, use of miceller media etc [102-104]. Evaluation of antioxidant activity in presence of micelles that mimic physiological environment will not only lead to a better understanding of life

processes, but will also be helpful in the development of novel medicines and biological sensors.[105] Therefore, the investigation of antioxidant capacity of Quercetin in these organized assemblies is important for understanding its antioxidant mechanism in bio - membranes.

In view of this, the present study was carried out to investigate the interaction of various surfactants viz; cationic surfactants DTPB, DDEAB, anionic surfactants SDBS, SDS, non-ionic surfactant Brij30 and some of their mixed binary and ternary mixtures DTPB-Brij30, DDEAB-Brij30, SDBS-Brij30, SDS-Brij30, DTPB-DDEAB-Brij30, SDBS-SDS-Brij30 towards the standard antioxidant Quercetin and hydroxyl radical ($^{\cdot}OH$) generated by Fenton's reagent to focus on the influence of such microstructures on the hydroxyl radical scavenging activity of quercetin so as to optimize their activity.

This piece of work may throw some light on the importance of simple microheterogeneous environments within ionic, non-ionic and mixed micelles on the antioxidant activity of Quercetin having some correlation with complex biological systems. The radical scavenging activity of Quercetin against hydroxyl radical ($^{\cdot}OH$) in micellar media was studied employing spectrophotometric and tensiometric techniques.

Chapter 2
Review of literature

Review of literature

Free radicals, usually generated during normal cellular metabolism, [106-112] are reactive due to the presence of unpaired electrons. Normally, their production is maintained in balance by endogenous antioxidants like superoxide dismutase (SOD), catalase and glutathione peroxidase, glutathione urate, etc.[107, 110] this balance is disturbed by several pathological conditions leading to oxidative stress and to remedy the excessive production of free radicals, several extrogenous antioxidants are being developed.

There is increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. Therefore, in the last decade considerable progress has been made in understanding the nature and reactions of biologically important free radicals. Biochemical, free radical scavenging and fast reaction techniques have provided valuable information in the development of new antioxidants.

Flavonoids, a group of phenolic compounds widely occurring in the plant kingdom, are believed to be good antioxidants, and their inhibition of lipid oxidation has been widely investigated.[100, 113]

Organized assemblies formed by surfactant molecules have various structures, including micelles, microemulsions, lamellar liquid crystals, monolayer membranes and liposome. These organized assemblies are of great importance as a convenient model for studying bio-macromolecules such as protein, cell and phospholipids bilayer due to their similarity to the basic structure of the life system.[79, 114] Analysis and mimicry of physiological environment will not only lead to a better understanding of life phenomena, but will also be helpful in the development of novel medicines and

biological sensors. [105] Therefore, the investigation of the antioxidant capacity of antioxidants in these organized assemblies is important to understanding the antioxidant mechanism of different bioactive molecules in bio membranes.

Some of the recent research papers that address the issue of the interaction and antioxidant activity of different bioactive molecules in the micellar or microemulsion media are:

- Pekkarinen, et al. (1999) reported the scavenging of DPPH radicals reflecting the Antioxidant activity in bulk oil systems but not in an emulsion. Specific interaction of the antioxidants with other compounds, for example the emulsifier and intramolecular hydrogen bonds may play an important role in reducing the antioxidant activity. Moreover, interactions of antioxidants with emulsifier have a strong influence on their partitioning. The proportion of antioxidant solubilized in the lipid phase and particularly in the interface does not necessarily reflect the efficiency of the antioxidants.[86]
- Schwarz, et al. (2000) reported that antioxidants can partition into different physical locations in emulsions and this partitioning dramatically influences antioxidant effectiveness rates.[115]The activity of different antioxidants was studied in different oil in water (O/W) and water in oil (W/O) emulsions, and in bulk oil with and without added emulsifiers. Partitioning of antioxidants, hydrogen bonding, interphase transport, surface accessibility, and interaction of emulsifier with antioxidants are considered to be important parameters that determine antioxidant activity in lipid –containing systems.
- Richards, et al. (2002) reported that surfactants can influence the physical location of antioxidants in oil-in-water emulsions by causing solubilization of lipid soluble antioxidants into the aqueous phase.[116] The Physical location of

antioxidants can be an important determinant in their activity. Excess Brij micelles in oil-in-water emulsion were found to increase the partitioning of phenolics into the continuous phase with polar antioxidants partitioning more than nonpolar antioxidants. Solubilization of polar antioxidants was rapid coming to equilibrium in less than 5 min. increasing surfactant concentration from 0.3-2.8% increased the solubilization of polar antioxidants by 2-3 folds. Solubilization of phenolic antioxidants into the aqueous phase by Brij micelles did not alter the oxidative stability of salmon oil-in-water emulsions, suggesting that surfactant micelles influenced oxidation rates by mechanisms other than antioxidant solubilization.

- Weiya Liu and Rong Guo, (2005) reported that the organized surfactant (SDS) not only can solubilize morin (antioxidant) to increase its solubility and concentration in the aqueous solution but also can influence the antioxidant ability greatly with its diversification in structure and microenvironment.[82]The electronic absorption and fluorescence emission spectra studies showed that the embedment of the 2',4'- dihydroxyl group linked on the B- ring into a more hydrophobic environment makes the oxidant peak potential become higher accompanied with decreasing peak currents, but the solubilization did not change the redox electrode reaction process, which directly reflects the antioxidant capability of morin. Morin can be located in the palisade layer of the SDS micelles, and its binding to SDS micelles is a spontaneous and exothermic process. However small value of Δ indicated that the force driving the binding is the weak intermolecular force.

  In 2006 the same authors reported the interaction between the flavonoid Quercetin with SDS (anionic surfactant) and CTAB (cationic surfactant)

micelles, using cyclic voltammetry. The interaction has been compared from interaction force to binding mode and to the final influence on micellar morphology. The charge distribution either in Quercetin molecule itself or in micelles is both vital to the interaction between them.[117]

- Heins, et al. (2007) reported that the close proximity of radical and antioxidant is a crucial prerequisite for the radical reducing action of antioxidants and also reported antioxidant activity is more in brij then in SDS because the depth of intercalation for galvinoxyl in the interface depended on the surfactant used and increased in the order SDS < Brij < CTAB. CTAB increased the antioxidant efficiency due to solubilization of antioxidant and hydrophobic radical in close proximity in the micelle interior and thereby elevating their concentrations. In interfaces modelled by Brij a longer alkyl chain of the antioxidant (from methyl to butyl) resulted in increasing antioxidant efficiency. In contrast, interfaces modeled by SDS micelles caused a segregation of galvinoxyl (palisade layer) and antioxidant (stern layer), thus no antioxidant action took place. The hydrophilic Fremy's radical was exclusively solubilized in the aqueous environment of SDS systems but partitioned partly into the large head group region of Brij micelles. As gallates were solubilized to substantial amounts in micelles, the antioxidant efficiency was higher in Brij than in SDS micellar systems.[103]
- Rong Guo and Ping Wei, (2008) reported that spectral property and antioxidant capacity of rutin in CTAB rod-like micelles are different from that in spherical micelles. Rutin molecules are partly solubilized in CTAB spherical micelles through electrostatic attraction and partly through hydrophobic force. In a more hydrophobic environment solubilization leads to

the reinforcement of planarity and the extension of pi conjugation of the whole rutin molecule, but the most antioxidant parts on the molecule (3', 4'-hydroxyls) are shielded, which results in decreasing hydroxyl radical scavenging activity with the CTAB concentration. But the compact structure of the rod-like micelles, which cannot provide enough solubilization space on their surface, the probability of reaction between rutin and hydroxyl radical is heightened. [102]

- Weiya Liu and Rong Guo, (2008) reported that the anti-oxidant and the free radical scavenging capabilities of anti-oxidant lies in its ability to function as reducing reagent and terminator of radicals by rapid donation of one or two hydrogen atoms to the radicals. Thus in the micelles, anti-oxidants can protect HSA (Human Serum Albumin) from the damage induced by hydroxyl radicals effectively and can form an anti-oxidant –HAS complex which is more thermally stable than the original protein with the denaturing temperature 20^{o}C higher. [118]
- Aliaga, et al. (2008) reported the use of substituted nitro oxide radicals as probes to determine the anti-oxidant activity in micelles. This approach takes into account both the hydrophobicity of the anti-oxidant and also the high selectivity of the nitroxide radical towards very reactive phenols such as flavonoids. [119]
- Medina, et al. (2009) reported that the antioxidant efficiency of hydroxytyrosol is greatly affected by the lipophilic chain. Maximum anti-oxidant efficiency seemed to appear when the chain length of the hydroxytyrosol derivative was that of 8 carbons, which is probably associated

with a preferential location of the diortho phenolic moiety in the right geometry.[120]

- Bushra, et al. (2010) reported the availability of flavonoid in micelles of sodium dodecyl sulfate is reflected in term of partition co-efficient. The partition coefficients of structurally related flavonoid are correlated with their antioxidant activities. The presence of ionized hydroxyl grouping in the interferential area and the attainment of particular geometry by a flavonoid could allow for differentiation between antioxidant potential of these flavonoids obtained in organized solution.[121]
- Chat, et al. (2011) reported that the radical scavenging activity of Rutin in the solubilized form was higher within ionic micelles than in non-ionic micelles. However, the antioxidant exhibited enhanced activity for the radical in mixed cationic- nonionic micelles compared with any of the single component micelles. In contrast, anionic-nonionic mixed micelles modulated the activity of Rutin in between the pure anionic and non-ionic micelles .[122]The activity was found to be in direct correlation with the solubilizing efficiency of cationic surfactants of varying chain length towards both Rutin as well as DPPH. The higher activity of Rutin in SDS than in DTAB with same chain length was attributed to more favorable orientation of Rutin within SDS micelles. Stronger H-bonding effect of Rutin with non-ionic Brij was observed to be a key factor for their low RSA within such systems. The activity in binary cationic–nonionic surfactant systems correlated well with their solubilizing efficiency for both Rutin and DPPH.
- Noipa, et al. (2011) developed a simple and sensitive method to evaluate the antioxidant capacity using 2, 2 –diphenyl- 1-picrylhydrazyl ($DPPH^{\cdot}$) radical

incorporated in surfactants. Various parameters affected the performance of the assay such as the CTAB concentration; buffer pH and concentration were optimized. The IC_{50} values of various antioxidants were calculated and compared to those prepared in methanol. The role of reaction between $DPPH^{\cdot}$ and antioxidants were also investigated and the rate constants in the micelle system were found significantly faster than those in methanol, allowing shoter analysis time.[123]

- Chen, et al. (2011) investigated the influence of phospholipid reverse micelles on the activity of non-polar (α-tocopherol) and polar (Trolox) antioxidants in stripped soybean oil (SSO). Phospholipid reverse micelles were found to improve the activity of low α-tocopherol or Trolox concentrations but decreased the activity at high concentrations. Hydrophillic Trolox had better antioxidant activity than hydrophobic α-tocopherol. The differences in the antioxidant activity of Trolox and α-tocopherol could be due to differences in their physical location in phospholipid reverse micelles.[124]

Though there are a good number of studies devoted towards the effect of single surfactants on the antioxidant activity of bioactive molecules, but as per our literature survey, reports regarding the effect of binary surfactant system are scanty and no report regarding the influence of ternary mixed micelles on the antioxidant activity of bioactive molecules, inspite of the fact that surfactant mixtures perform better in most of the applications than single surfactant systems.

Chapter 3
Experimental

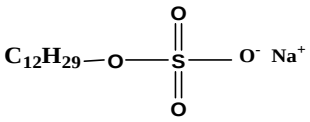
3.1: MATERIALS

The non-ionic amphiphile Polyoxyethylene (4) mono-n-dodecyl ether (Brij - 30), cationic amphiphiles dodecyldimethylethylammonium bromide (DDEAB) and dodecyltriphenylammonium bromide (DTPB), anionic amphiphiles sodium dodecylsulfate (SDS) and sodium dodecylbenzenesulphonate (SDBS), the antioxidant quercetin dihydrate (quercetin, > 98%) were all Aldrich products, and were used as received. Methanol (Merk) was used after distillation. The purity of the surfactants was further ensured by the absence of minimum in surface tension vs. the logarithm of surfactant concentration plots. $FeSO_4.7H_2O$, H_2O_2 were of analytical grade. The structures of the surfactants, and antioxidant used are presented in **Scheme 3.1**. Surfactant solutions were prepared in triple distilled water.

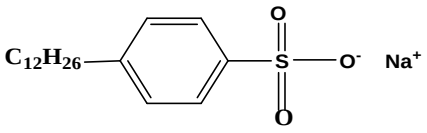
3.2: METHODS

3.2.1: Determination of cmc

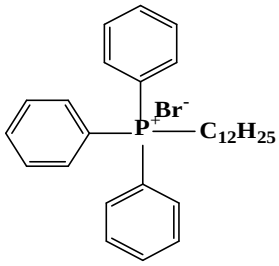
The *cmc* values of all surfactant solutions were determined from the plot of surface tension (γ) vs. logarithm of surfactant concentration (log Ct) as shown in **Figure 3.1**. Surface tension measurements were made by the platinum ring detachment method with a Krüss-9 (Germany) tensiometer equipped with a thermostable vessel holder. Surfactant concentration was varied by adding solution of known surfactant concentration in small installments using a Hamilton micro syringe to water in the sample vessel placed in the vessel holder. Measurements were made after thorough mixing and temperature equilibration at 25 °C (±0.1 °C) by circulating water from a HAAKE GH thermostat through the vessel holder. The accuracy of measurements was within ±0.1 dyne cm^{-1} and the readings were taken in triplicate to ensure reproducibility.



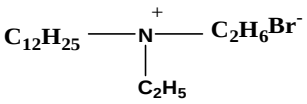
SDS



SDBS



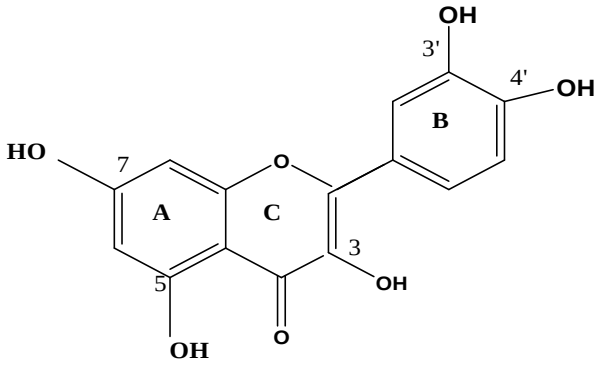
DTPB



DDEAB

$C_{12}H_{25}.(OCH_2CH_2)_4OH$

Brij 30



Quercetin

Scheme 3.1: Structure of Surfactants and Quercetin used in this study.

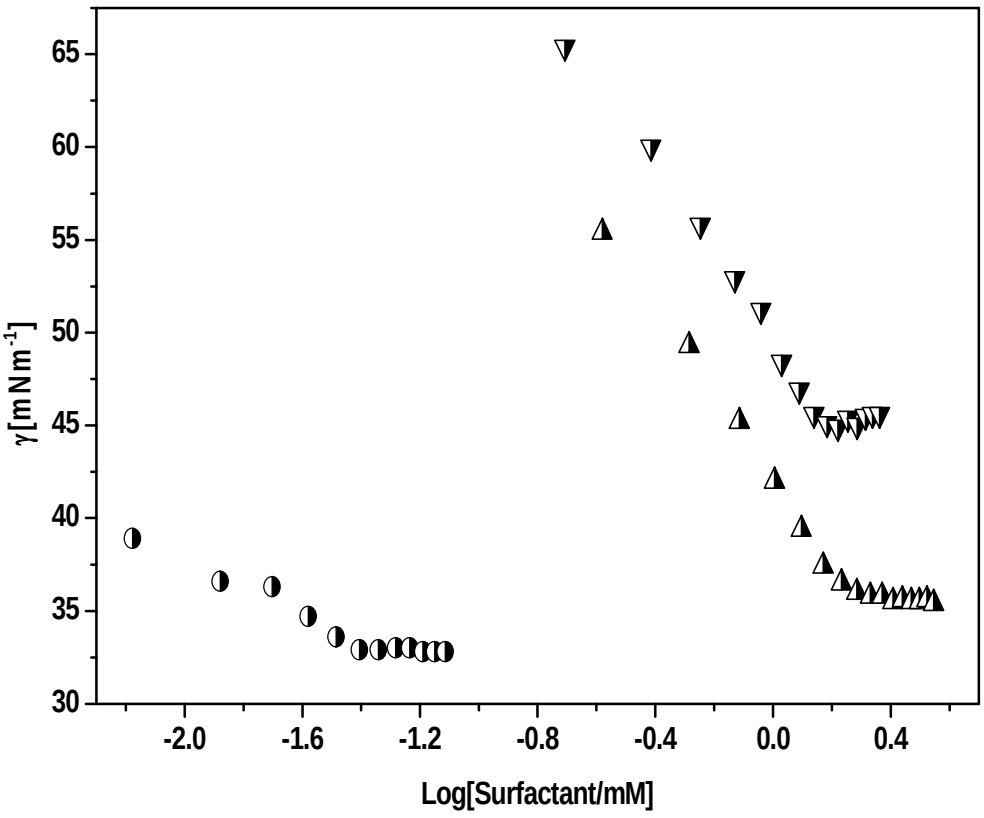


Figure 3.1: *plots of surface tension versus logarithm of surfactant concentration for various surfactants.*

3.2.2: Evaluation of hydroxyl-radical ($^{\cdot}OH$) scavenging activity of Quercetin

Hydroxyl radical scavenging potential of the antioxidant quercetin in each surfactant solution was determined by first dissolving the antioxidant in surfactant solution followed by addition of Fenton's reagent to the mixture after thorough shaking by hand at 25 °C. The decrease in absorbance at the absorption wavelength of quercetin after 60s intervals was monitored with a Schimadzu 1650 PC spectrophotometer (**Figure 3.2** and **3.3)** for the determination of the hydroxyl radical scavenging activity. In the total 3ml volume of solution in cuvette the concentrations of quercetin 0.05mM, $FeSO_4$ 0.0125mM, H_2O_2 0.125mM were fixed. Three different surfactant concentrations in the pre-micelle, micelle and post-micelle range were used for each of the single, binary and ternary surfactant system. All the experiments were performed in triplicate. i.e. the radical scavenging activity (antioxidant activity) was calculated using the following equation:

$$RSA = 100 \times (1- A_t/A_0)$$

Where A_t is the absorbance of sample at time t while A_0 is the absorbance at time t_0.

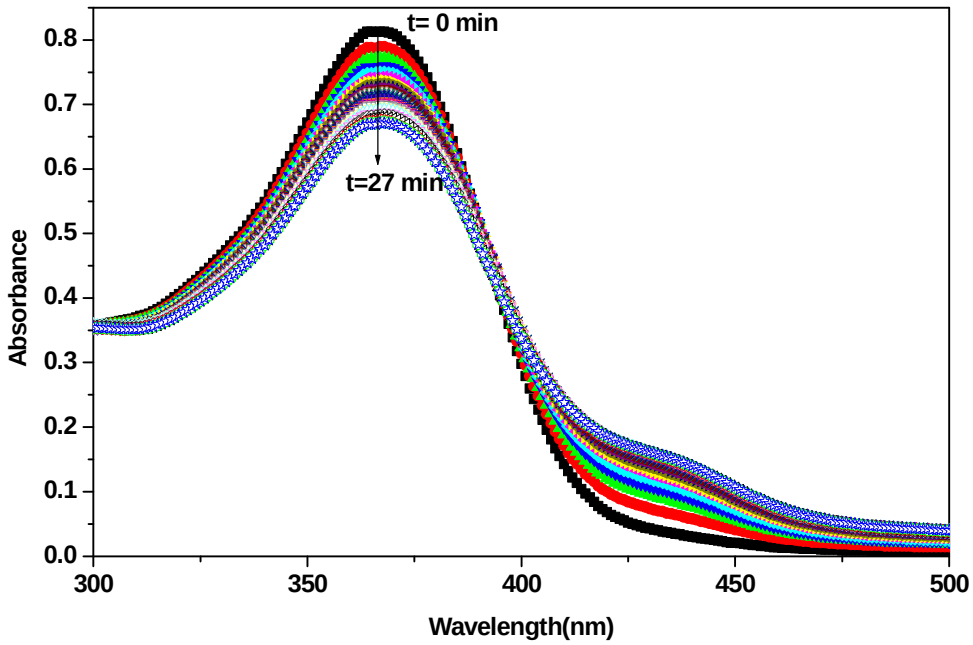


Figure 3.2: *Absorption spectra of quercetin at different times obtained during its reaction with hydroxyl radical in surfactant system.*

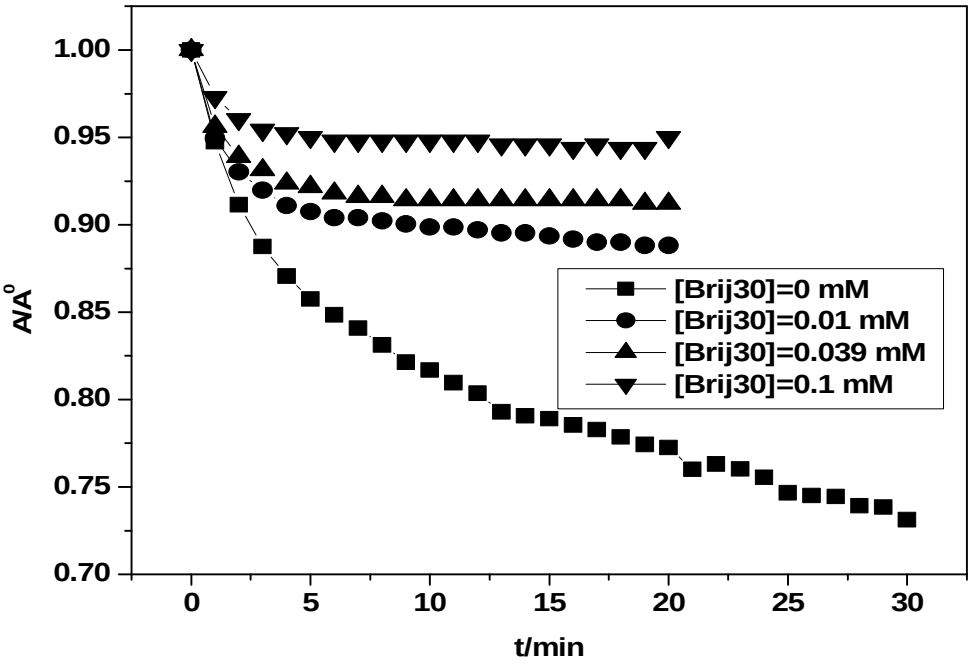


Figure 3.3: *Degradation of quercetin as a function of time during its reaction with hydroxyl radicals at different surfactant concentrations.*

Chapter 4
Theoretical

4.1: MODELS FOR MIXED MICELLE FORMATION

Several theoretical formulations are available for describing the behaviour of multicomponent ideal (e.g., homologous series of surfactants with similar head groups) and binary nonideal (e.g., mixtures of ionic and non-ionic surfactants) systems. [125, 25, 26,126] The models provide simple tools for analysis and prediction of the main properties of mixed micelles, including mixed *cmc* values, micellar mole fractions, and monomer concentrations. Various theoretical models of mixed micellization used in the present study are discussed briefly as follows:

4.1.1: Clint Model

The Clint model is applicable for ideal mixing of surfactant systems. For a mixture of surfactants, cmc_{ideal}, according to Clint model [25] is given as

$$\frac{}{} = \frac{}{} + \frac{}{} + \frac{}{} + \cdots \qquad (4.1)$$

Where cmc_i and α_i are the experimental critical micellization concentration and mole fraction of the ith component in the bulk surfactant mixture.

4.1.2: Rubingh Model

This model, based on the regular solution theory and applicable for nonideal mixing, gives the estimate of deviation of experimental *cmc* values from cmc_{ideal}. Analysis of the *cmc* as a function of net mole fraction α_1 of component 1 in the mixed surfactant systems in terms of micellar composition ( ) at the *cmc* has been made in the light of Rubingh's[26] equation:

$$\frac{(\qquad)}{(\quad) \{ \quad (\quad) \quad \}} = 1 \qquad (4.2)$$

Where cmc_1, cmc_2, cmc_{12} denote the *cmc* values of the surfactants 1, 2 and mixed system respectively. The interaction parameter, β, of mixed micelle formation given by

$$= \frac{(\qquad\qquad)}{(\qquad)} = \frac{(\qquad\qquad)}{(\qquad)} \qquad (4.3)$$

β is an indicator of the degree of interaction between two surfactants in mixed micelle and accounts for deviation from ideality. A negative value of β implies attractive interactions the more negative its value, the greater the interaction. The activity coefficients, f_i, of individual surfactants within the mixed micelles are related to the interaction parameter through the eqs.

$f_1 = \exp\{\beta(1 - \qquad)\}$ (4.4a)

$f_2 = \exp\{\beta \qquad\}$ (4.4b)

4.1.3: Holland and Rubingh Model

This is a generalized multicomponent nonideal mixed micelle model, based on the pseudo-phase separation approach, and has been successfully applied in the case of many ternary surfactant systems[127-129] for evaluation of micellar composition, activity coefficients, and *cmc* values. According to this model, the activity coefficients f_i, f_j, ... of micelle forming surfactant species *i*, *j*, ...in an *n*-component mixture are represented, on a general basis, by the equation.

$$\ln f_i = \sum_{(\quad)} \qquad + \sum_{(\quad)} \sum \quad (\qquad\qquad) \qquad (4.5)$$

where β_{ij} *represents* the net (pair wise) interaction between components *i* and *j* and Xj^M is the mole fraction of the *j*-th component in the micelles. At *cmc*, the relation

$$X_i^M = \frac{\qquad\qquad}{\qquad\qquad} \qquad (4.6)$$

holds, where terms cmc_i and cmc_j are *cmc* values of the *i*- and *j*-th components in their pure state, respectively. The interaction parameter, β_{ij} can be obtained independently from binary mixtures using the Rubingh method. The activity coefficients for a three component system, i.e., f_1, f_2, and f_3 at mixed cmc can be calculated from the above

equations by using the method of successive substitutions subject to the constraint that the sum of Xi^M values equals unity. The values of f_i so obtained can then be used to find the mixed micellar *cmc*, cmc_{RH}, of ternary systems by the equation:

$$\frac{}{} = \sum \frac{}{} \qquad (4.7)$$

Chapter 5
Results and Discussions

5.1: CMC and Surfactant-Surfactant Interactions

The *cmc* values of selected single, mixed binary and ternary surfactant systems, obtained from plots of surface tension (γ) vs logarithm of surfactant concentration (c_t) shown respectively in **Figure 5.1**, **5.2** and **5.3**, are presented in **Table 5.1** along with the ideal *cmc* values, cmc_{ideal}, of binary as well as ternary surfactant systems based on the Clint equation (4.1).

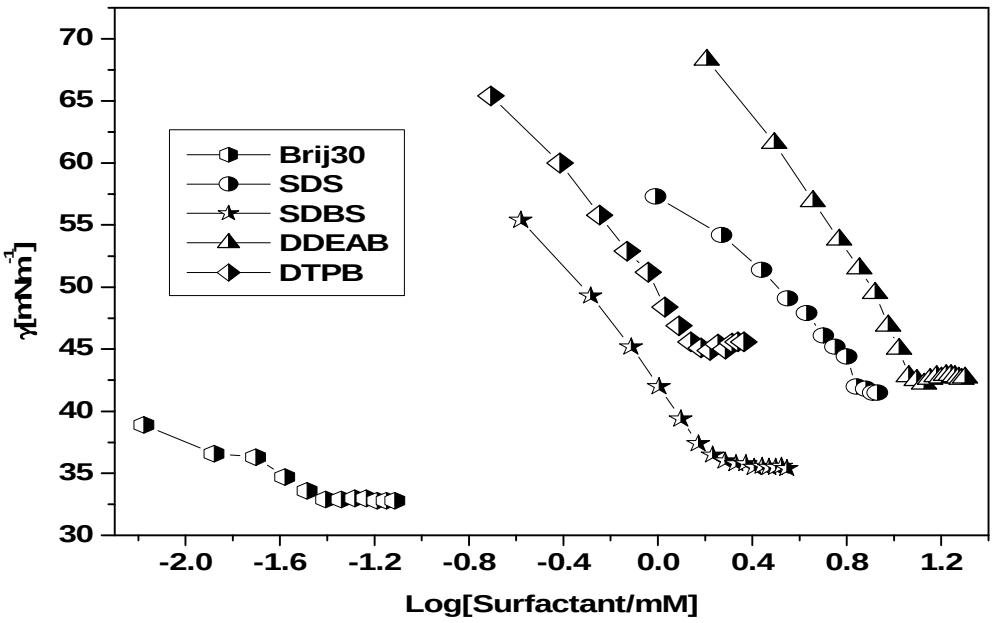


Figure 5.1: *Plots of surface tension versus logarithm of surfactant concentration for single surfactant systems.*

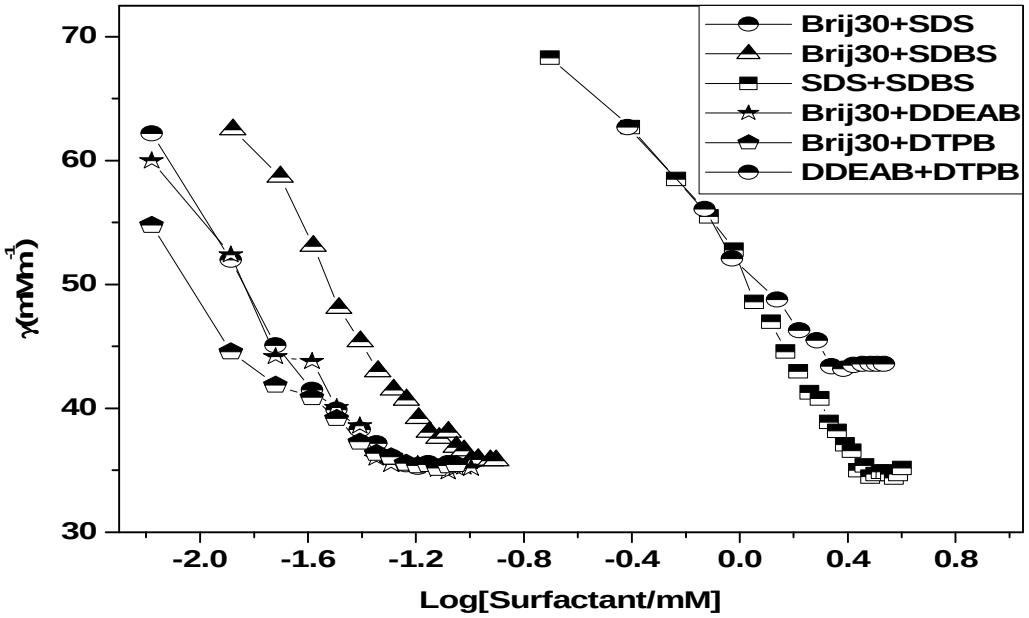


Figure 5.2: *Plots of surface tension versus logarithm of surfactant concentration for binary surfactant systems*

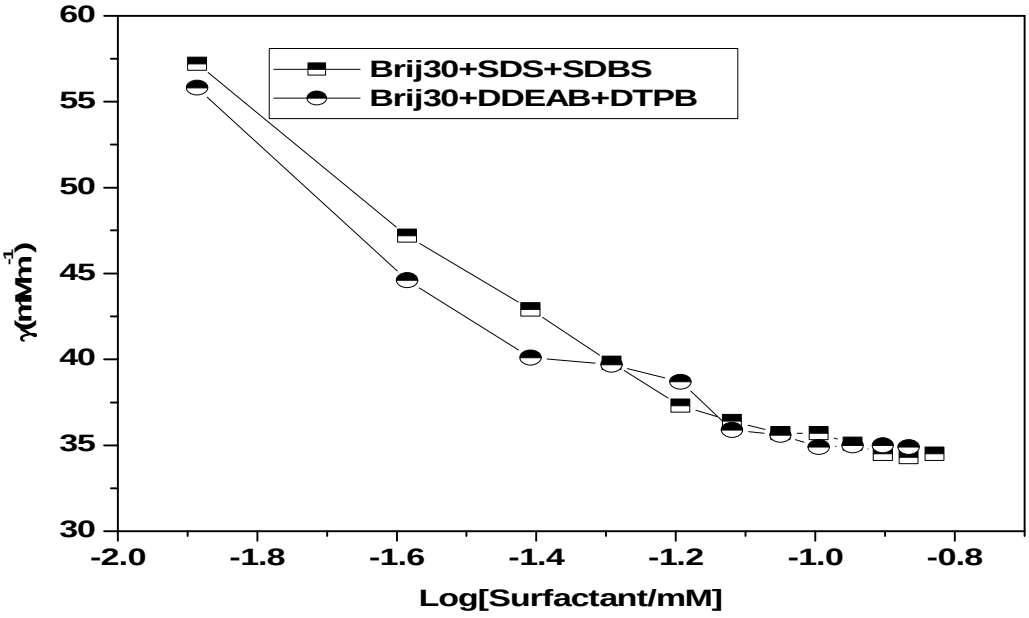


Figure 5.3: *Plots of surface tension versus logarithm of surfactant concentration for ternary surfactant systems.*

Table 5.1: *Experimental and literature critical micelle concentration values (cmc_{exp} and cmc_{lit}) of single, binary and ternary surfactant systems, along with the miceller mole fraction (x_i^M), interaction parameter (β) and activity coefficients (f_i) for binary and ternary surfactant system calculated by Rubingh and Rubingh Holland methods respectively at 25^oC.*

System	cmc_{exp} (cmc_{lit}) (mmol dm^{-3})	system	cmc_{exp} (cmc_{ideal}) (mmol dm^{-3})	β	X_1^M/X_2^M	f_1/f_2
Single surfactant systems		***Binary surfactant systems***				
Brij30	0.0392 (0.0351)[a]	Brij30-DDEAB	0.051 (0.078)	-6.88	0.82/0.18	0.80/0.10
DDEAB	14.02 (14)[b]	Brij30-DTPB	0.057 (0.076)	-3.3	0.81/0.19	0.89/0.11
DTPB	1.37 (2)[c]	DDEAB-DTPB	2.17 (2.5)	-1.22	0.82/0.18	0.96/0.44
SDS	7.4 (8.1)[d]	Brij30-SDS	0.057 (0.077)	-5.34	0.84/0.16	0.87/0.02
SDBS	2.02 (2.2)[e]	Brij30-SDBS	0.07 (0.076)	-2.02	0.91/0.09	0.98/0.19
		SDS-SDBS	3.04 (3.184)	-0.25	0.24/0.76	0.87/0.99

system	$cmc_{exp}(cmc_{ideal})$ (mmol dm^{-3})	cmc_{RH}	$X_1^M/X_2^M/X_3^M$	$f_1/f_2/f_3$
Ternary surfactant systems				
Brij30-DDEAB-DTPB	0.101(0.115)	0.069	0.77/0.14/0.09	0.76/0.01/0.18
Brij30-SDS-SDBS	0.088(0.115)	0.082	0.82/0.15/0.03	0.85/0.02/0.37

[a]Ref.[130], [b]Ref.[131], [c]Ref.[132], [d]Ref.[133], [e]Ref.[134]

All the observed *cmc* values were found to be lower than ideal values, indicating negative deviation from ideal behaviour for mixed micelle formation.

The estimate of the negative deviation and hence nonideality of mixed binary surfactant systems has been obtained from Rubingh's model.[26] The interaction parameter, β , that accounts for deviation from ideality is an indicator of the degree of interaction between two surfactants in the mixed micelles. β values along with the micellar mole fraction , X_i^M , and activity coefficient, f_i, of the ith surfactant within mixed micelles calculated through Rubingh equations[26] are also presented in **Table 5.1**. The negative values of β indicate synergistic interactions. It is well known[135-136] that in ionic–nonionic mixed surfactant systems the significant electrostatic self-repulsion of ionics and weak steric self-repulsion of non-ionics (depending on the headgroup size) before mixing are weakened by dilution effects after mixing and that the electrostatic self-repulsion of the ionic surfactant is replaced by ion–dipole interactions.

However, in our study less negative value of β in the case of SDBS-Brij30 mixed surfactant system over SDS-Brij30 system may be due to the presence of the benzene substituent in SDBS, contributing to steric repulsion and hence less stability for mixed micelles. Similarly, less negative value of β in DTPB-Brij30 mixed surfactant system over DDEAB-Brij30 could be due to the larger head group size of the DTPB which contains three phenyl groups, thus making a larger steric self repulsion contribution towards inter-headgroup interactions. A small negative value of β and small deviation of f_i values from unity in the case of anionic-anionic (SDBS-SDS) mixed surfactant systems indicate their almost ideal behaviour for mixed micelle formation, since there is only a slight difference between head groups of SDBS and SDS surfactants. However greater negative value of β indicates considerable deviation from ideal

behavior and existence of synergistic interaction in the cationic-cationic DTPB-DDEAB mixed surfactant system. It could be related to the presence of three phenyl rings in the head group of DTPB posing appreciable steric self repulsion in its pure micelles which gets diluted when mixed micelles are formed, thereby leading to synergism. Although both the surfactants in this system are positively charged, such a negative value of β is in tune with the results reported in the literature[131,137-138] for other cationic-cationic surfactant mixtures.

Holland and Rubingh[128] have proposed a generalized muticomponent nonideal mixed micelle model on the basis of pseudo-phase separation approach. It has been successfully applied in the case of many ternary surfactant systems[126-128] for evaluation of micellar composition, activity coefficients, and *cmc* values. It makes an effective use of net interaction parameters determined experimentally from *cmc* measurements on binary systems. In the present study, values of binary interaction parameters β_{12} , β_{13} , and β_{23} following Rubingh's method and *cmc* values of pure surfactants were used in the Rubing-Holland (RH) equations (4.7) to evaluate f_1, f_2, f_3, X_1^M , X_2^M, X_3^M . The calculations were done using solver in MS Excel. These values were then used to predict cmc of the ternary system, cmc_{RH}, according to the Rubing-Holland (RH) formulation. The results are presented in **Table 5.1**.

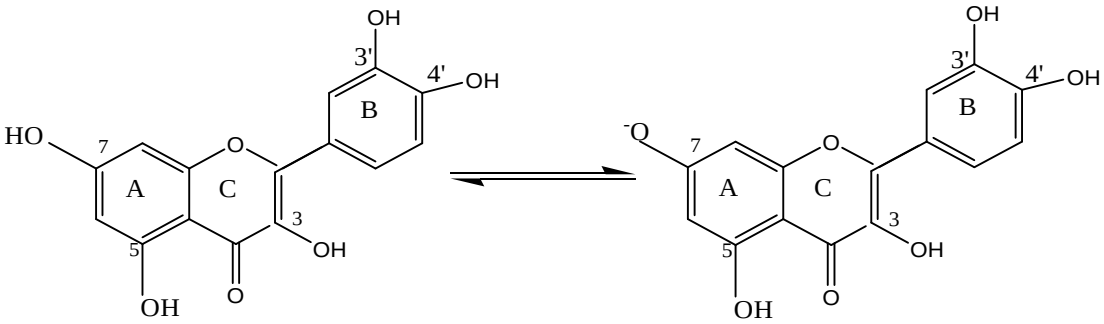
The mole fractions of individual amphiphiles in the mixed micelles X_i are different from stoichiometric composition α_i: X_{ionic} values are much lower than α_{ionic} values, but $X_{nonionic}$ values are fairly higher than $\alpha_{nonionic}$ values. The activity coefficients of ionics are very low but are close to unity for nonionics. The Brij30-SDS-SDBS is found to be in fair agreement with experimental *cmc* value while a deviation of cmc_{RH} value from experimental *cmc* value was observed for Brij30-DDEAB-DTPB system. It could be the manifestation of high steric repulsion related to the presence of three

phenyl groups in the DTPB. However, both experimental *cmc* and cmc_{RH} are lower than the ideal *cmc*, indicating synergistic nonideal nature of mixed ternary micellar systems. Fair agreement between cmc_{RH} and cmc_{exp} in case of Brij30-SDBS-SDS indicates fair applicability of the RH method for such system.

5.2: Hydroxyl radical scavenging activity (RSA) of Quercetin

Quercetin (3, 3', 4', 5,7-pentahydroxyflavone, **scheme 3.1**) has been selected because it is abundant in plants and food and displays the structural requirements (C-2-C-3 double bond, and ortho-dihydroxy substitution on ring B and the presence of a 4 -oxo in the C ring) favourable to strong antioxidant activity.[139]

As we know, quercetin exists in the anionic state with one or two charges in aqueous solutions as shown below. The most acidic phenolic OH groups of quercetin are in the 3,7 positions of the molecule, which can dissociate and result in the mixture of neutral and anionic species.[140-141]



3', 4' two hydroxyl groups on the B ring are the most active antioxidant parts in the quercetin molecule having ability to scavenge hydroxyl radicals. With the hydroxyl radicals cleared, the quercetin molecule itself will degrade and its absorption peak intensity will decrease accordingly. **Figure 5.4, 5.5, 5.6** and **5.7** shows the absorption spectra of quercetin at different times during its reaction with hydroxyl radicals in different single (non-ionic, ionic) and mixed (binary and ternary) surfactant systems. Therefore, we analysed ability of quercetin to scavenge hydroxyl radicals generated

by fenton's reagent in the different micellar media by monitoring changes in its characteristic UV-VIS spectrum.

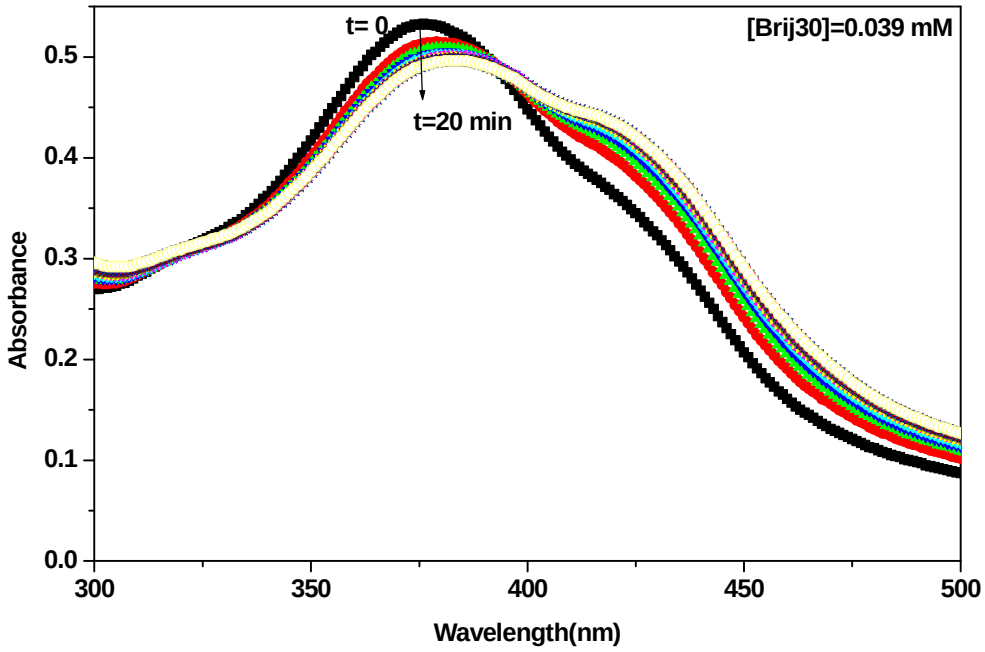


Figure 5.4: *Absorption spectra of quercetin at different times during its reaction with hydroxyl radical in* 0.039 *mM Brij30.*

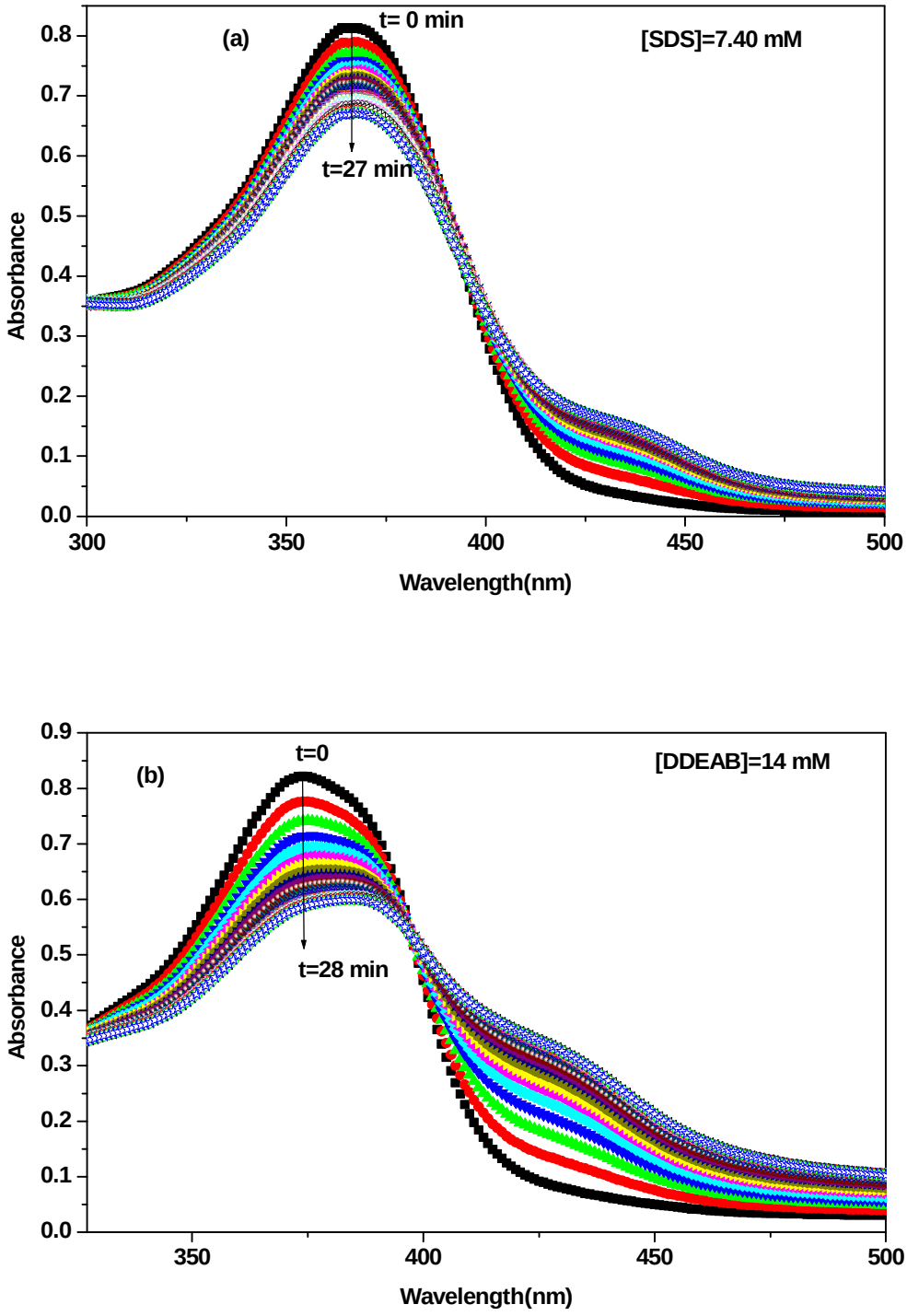


Figure 5.5: *Absorption spectra of quercetin at different times during its reaction with hydroxyl radical in presence of: (a) 7.40 mM SDS, (b) 14.0 mM DDEAB.*

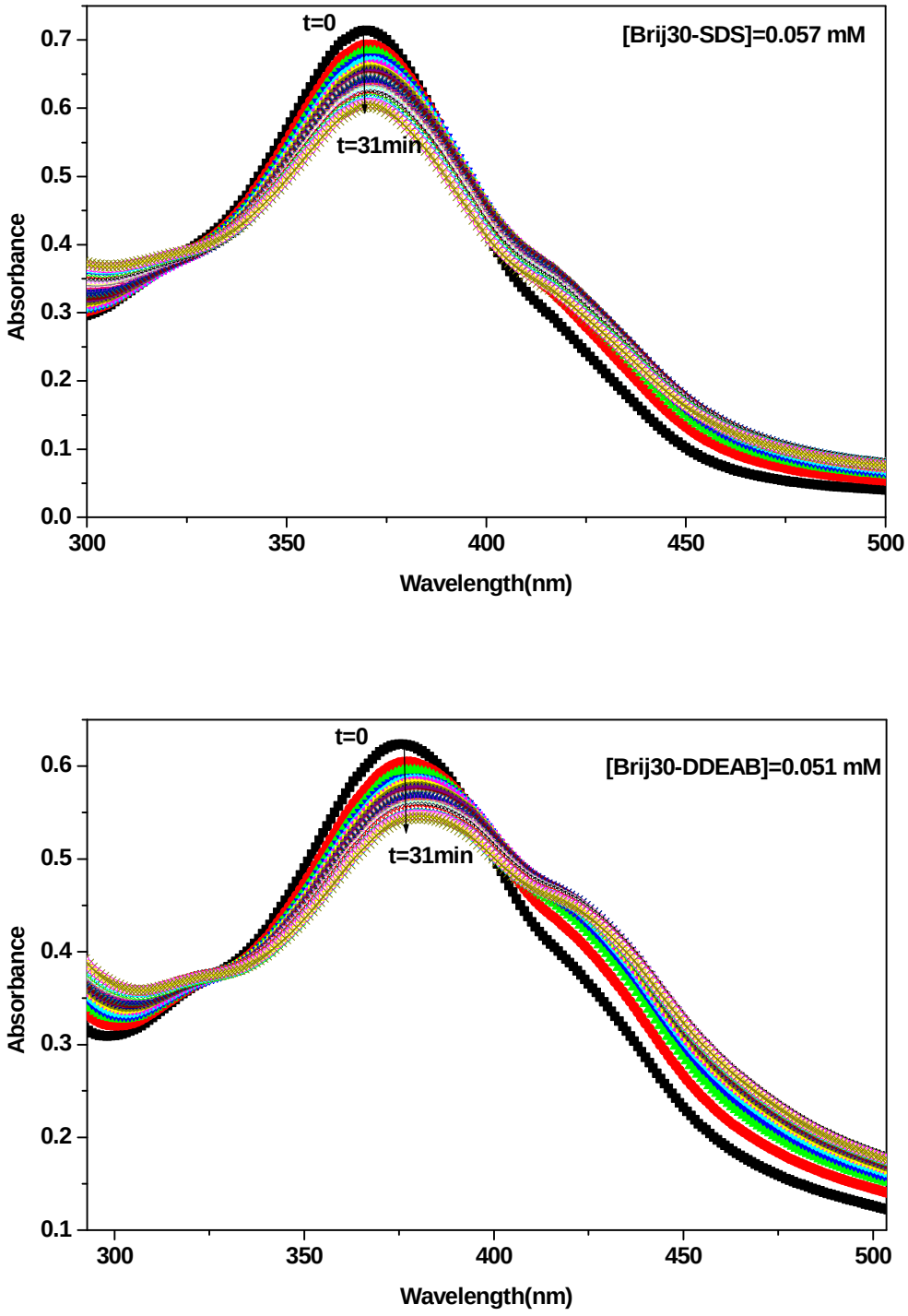


Figure 5.6: *Absorption spectra of quercetin at different times during its reaction with hydroxyl radical in presence of different binary surfactant systems.*

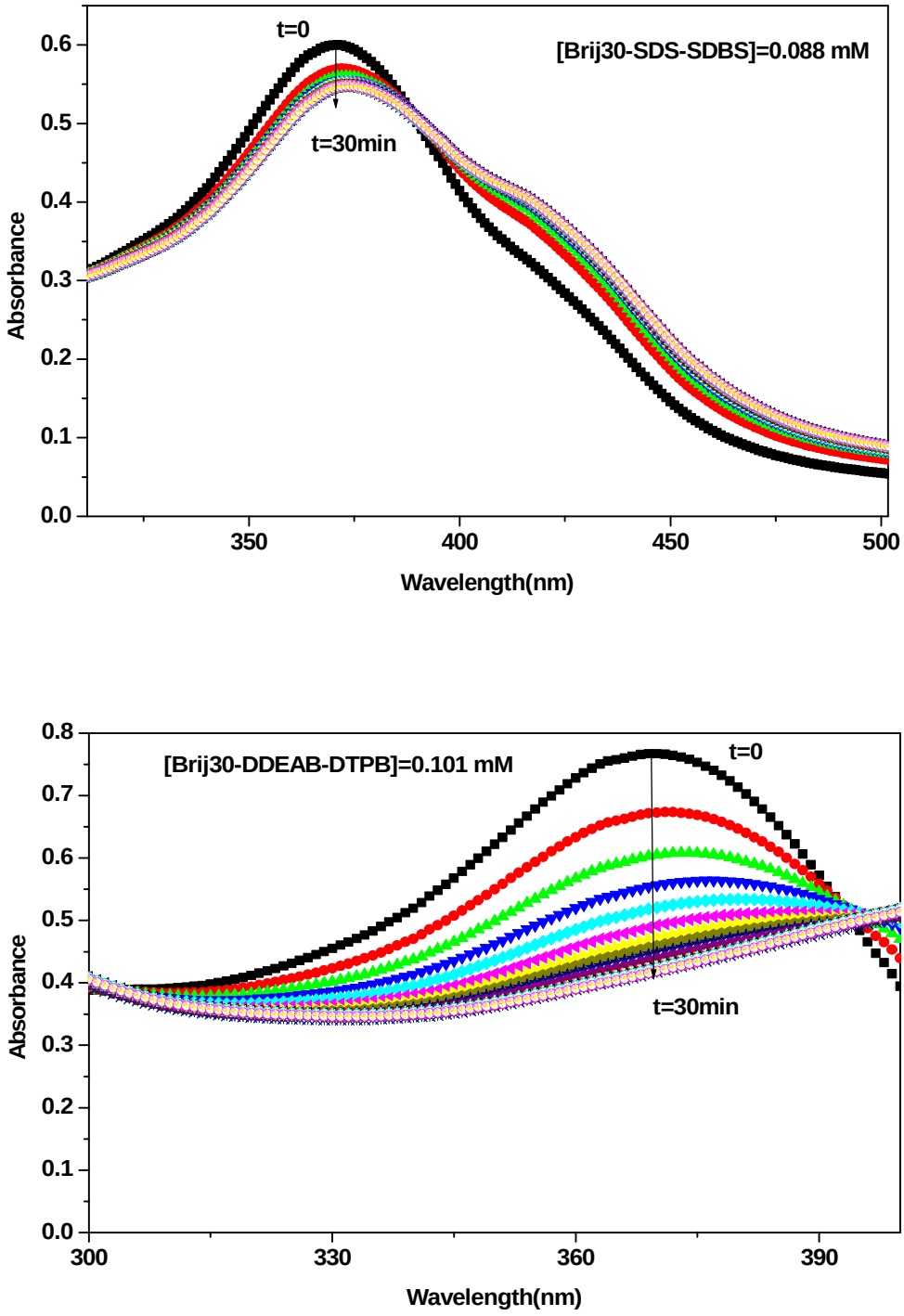


Figure 5.7: *Absorption spectra of quercetin at different times during its reaction with hydroxyl radical in presence of different ternary surfactant systems.*

The influence of surfactant concentrations on the degradation of quercetin upon attack of hydroxyl radicals is shown in **Figure 5.8, 5.9 and 5.10**. After mixing with Fenton's reagent, the absorption peak of quercetin drops rapidly within the first ten minutes then slows down and finally levels off.

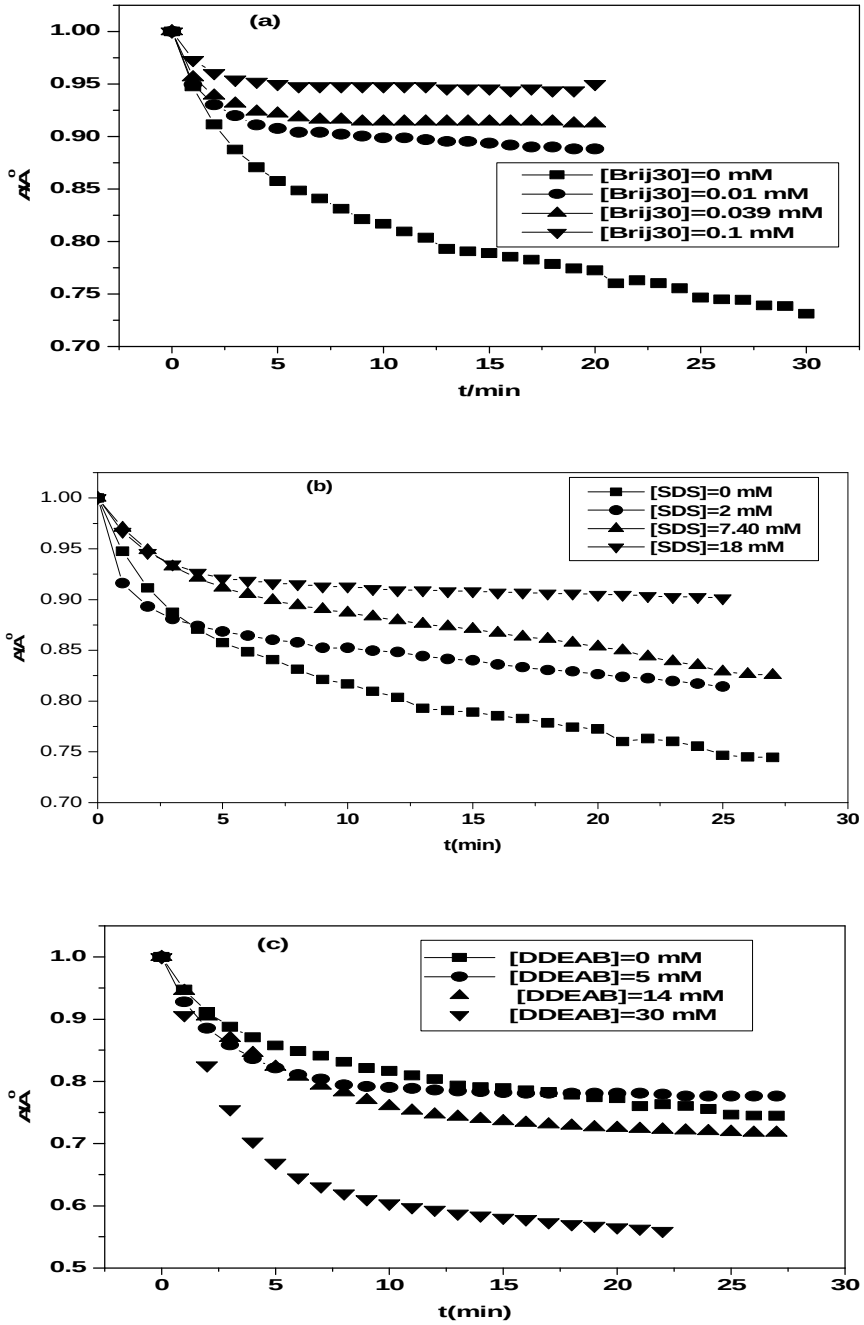


Figure 5.8: *Degradation of quercetin as a function of time during its reaction with hydroxyl radicals at different surfactant concentrations in (a) non-ionic Brij30,(b) anionic SDS and (c) cationic DDEAB surfactant media.*

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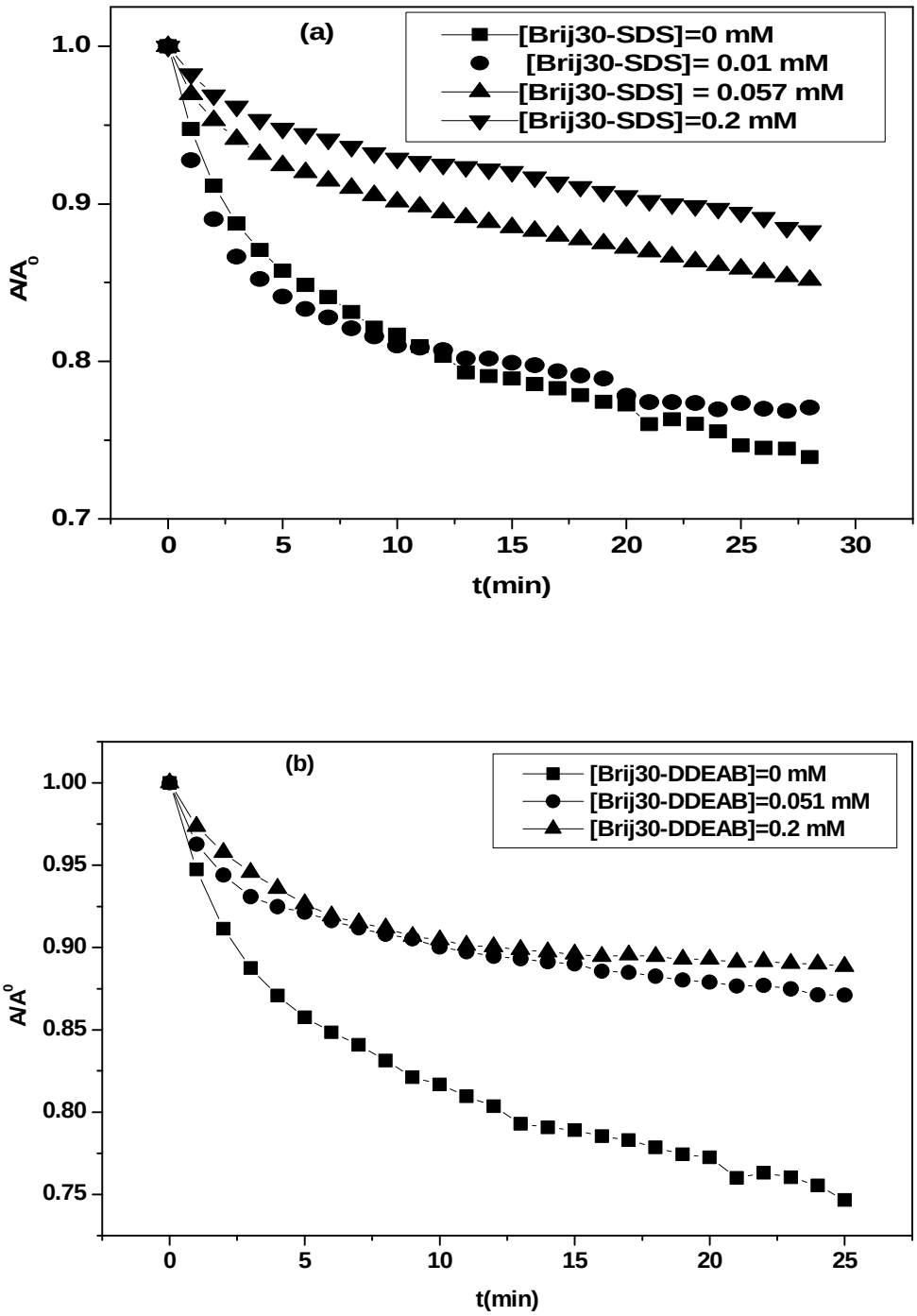

Figure 5.9: *Degradation of quercetin as a function of time during its reaction with hydroxyl radicals at different surfactant concentrations in (a) anionic- nonionic and (b) cationic-nonionic surfactant media.*

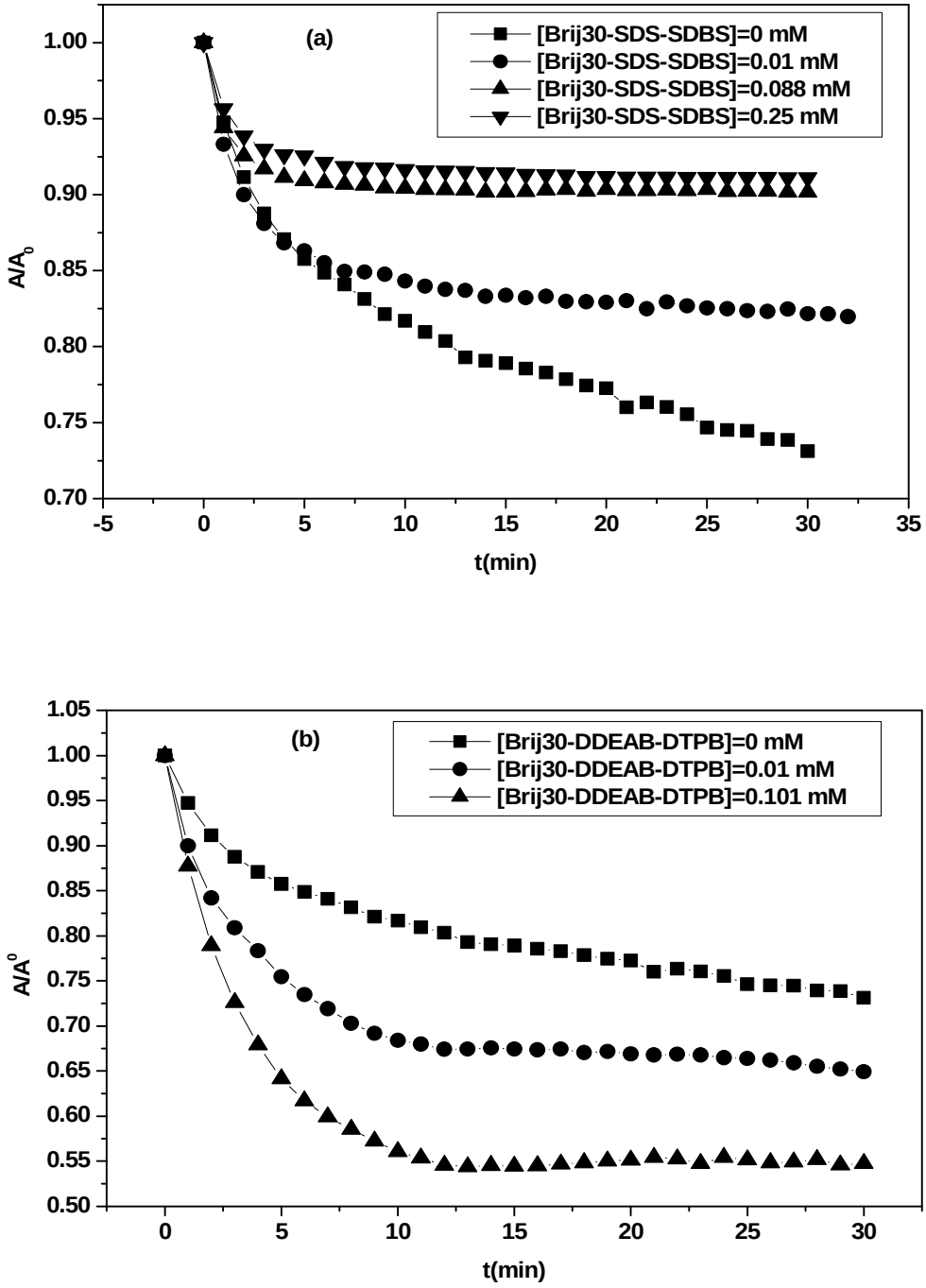

Figure 5.10: *Degradation of quercetin as a function of time during its reaction with hydroxyl radicals at different surfactant concentrations in (a) nonionic anionic-anionic and (b)nonionic- cationic-cationic surfactant media.*

The hydroxyl radical scavenging activity (RSA) of quercetin was determined as described in experimental section and was measured after ten minutes. The results in different surfactant systems are listed in **Table 5.2** and plotted in **Figure 5.11** for different single surfactant systems as a function of surfactant concentration.

Table 5.2: *Radical scavenging activity (RSA) of quercetin in single, binary and ternary surfactant systems.*

Single system			Binary system		
system	**[surfactant]/mM**	**RSA**	**System**	**[surfactant] /mM**	**RSA**
Brij30	0	18.32	**Brij30-SDS**	0	18.32
	0.01	10.12		0.01	19.21
	0.039	8.59		0.057	9.87
	0.10	5.22		0.2	7.11
SDS	0	18.32	**Brij30-SDBS**	0	18.32
	2.0	14.77		0.01	19.72
	7.4	11.32		0.07	8.90
	18	8.72		0.2	6.21
SDBS	0	18.32	**Brij30-DDEAB**	0	18.32
	0.1	16.09		0.051	9.98
	2.0	58.38		0.2	9.53
	6.0	72.39			
DDEAB	0	18.32	**Brij30-DTPB**	0	18.32
	5.0	18.98		0.01	18.21
	14.0	24.20		0.057	25.21
	30.0	39.61		0.200	33.75
DTPB	0	18.32			
	0.50	17.34			
	1.37	23.82			
	5.0	40.64			

Ternary system		
System	**System**	**RSA**
SDS-SDBS-Brij30	0	18.32
	0.01	15.69
	0.088	9.58
	0.25	8.42
DTPB-DDEAB-Brij30	0	18.32
	0.01	31.6
	0.101	43.94

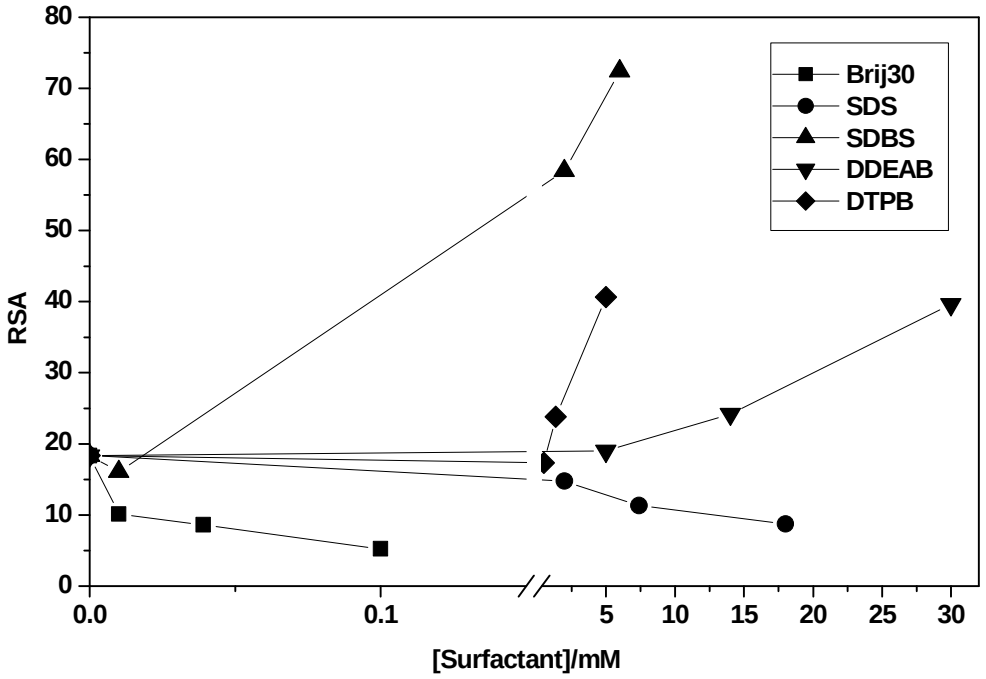

Figure 5.11: *Hydroxyl radical scavenging activity of quercetin in different single surfactant systems.*

As seen in **Figure 5.11**, the hydroxyl radical scavenging activity of quercetin decreases with the increase in the concentration of Brij30 and SDS, both below and above their *cmc* values, indicating that the reaction between quercetin and hydroxyl radical is partly blocked due to the solubilization of quercetin within these micelles at or above *cmc* values. It has been reported[116] that the possible orientation of quercetin molecule within SDS micelles is such that the B-ring, containing the electroactive hydroxyl groups, is embedded within the palisade layer of micelle while ring A and C, having negative charge, lie outside the micelle away from negatively charged head groups to avoid unfavourable electrostatic repulsions. In other studies[79,85,103] it has been established through spectroscopic and electrochemical studies that Rutin (glycoside derivative of quercetin) molecules are located in the palisade layer of polyoxyethyene surfactant micelles involving hydrophobic and hydrogen bonding

interactions thereby solubilizing it preferably with its B-ring pointing towards micellar core. In addition, these studies also showed that hydrogen abstraction kinetics of Rutin by the radical is inhibited due to strong hydrogen bonding of electroactive hydroxyls with the OE units of non-ionic surfactants. These orientation effects of quercetin are schematically shown in **Scheme 5.1** As such, the OH radical scavenging activity of quercetin in non-ionic surfactants is lowest compared to that in cationic micelles since OH radicals are mainly present in the aqueous phase. Decrease in RSA of Quercetin in Brij30 surfactant system below its *cmc* indicates the role of hydrogen bonding in premicellar concentration to slow the hydrogen abstraction kinetics in contrast to that in SDS wherein the change is small in premicellar region. The strong hydrogen bonding tendency along with the orientation effect of quercetin with polyoxyethylene groups of Brij30 reduces the RSA of quercetin more than in SDS micelles as shown in **Figure 5.11.**

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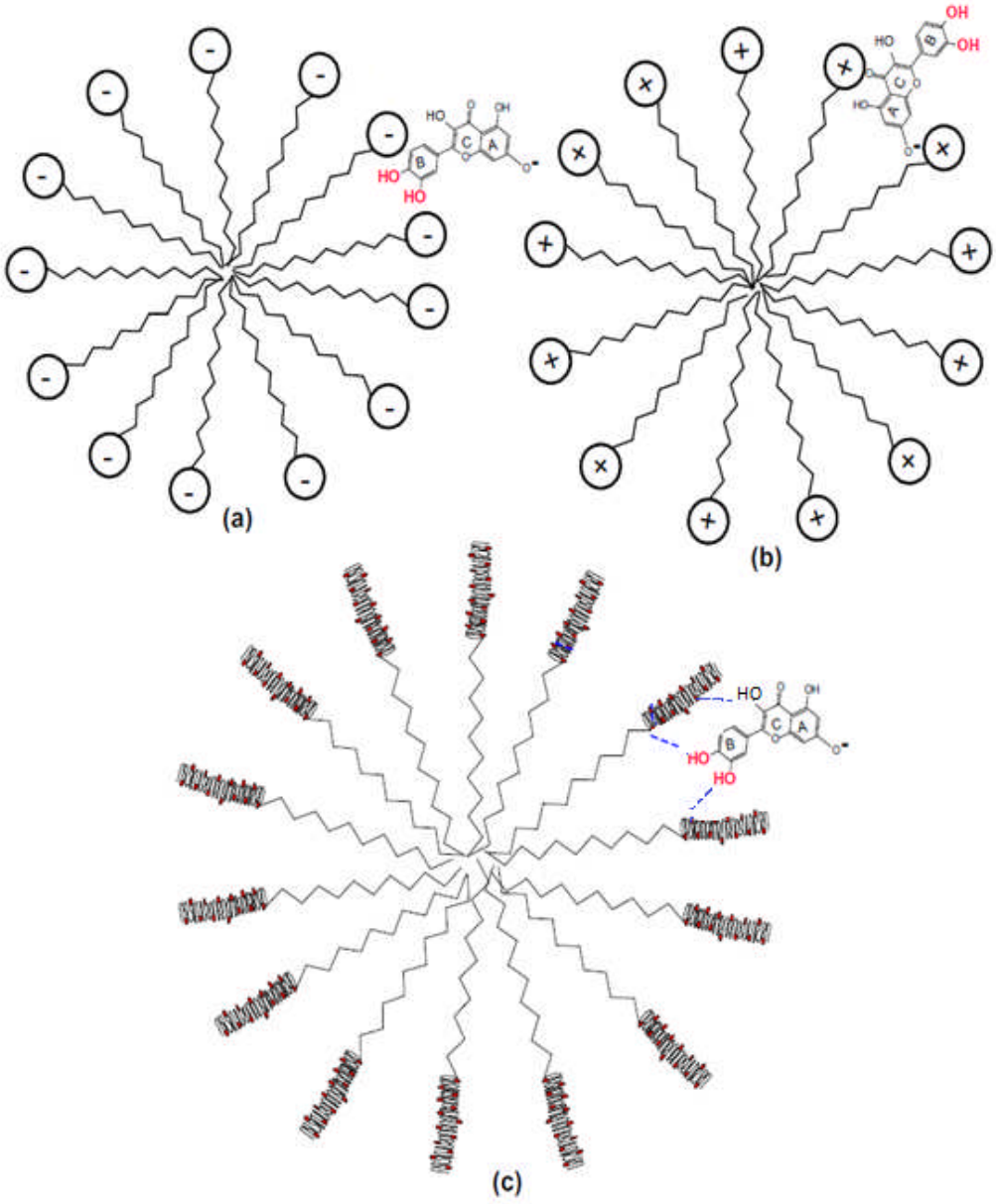

Scheme 5.1: *Probable location of quercetin in: (a) anionic, (b) cationic and (c) non-ionic micelles.*

The RSA of quercetin in SDBS micellar system initially decreases slightly upon the addition of surfactant in the premicellar region as observed with SDS, followed by a large increase at the surfactant concentration at or above its *cmc* i.e. when micelles of SDBS are formed in the solution. This observation is quite opposite to that in SDS micelles although both the surfactants have same hydrocarbon chain length and the charge on head group. This unusual behaviour could not be explained.

In cationic surfactant systems, DDEAB and DTPB the antioxidant activity of quercetin was almost constant in premicellar region but increased with increases in the surfactant concentration above their *cmc.* Moreover, the antioxidant activity of quercetin was more in these cationic surfactant systems than in nonionic Brij30 and anionic SDS surfactant systems. Liu and Guo[116] have demonstrated that quercetin interacts with cationic surfactants via rings A and C due to favourable interaction between negatively charged center of quercetin and positively charged head groups of surfactants (**Scheme 5.1**). Therefore, such orientation effect within the cationic micelles increases the accessibility of hydroxyl radicals in the aqueous phase to electroactive hydroxyl groups present in the quercetin molecules. This increases the chance of electroactive 3',4' hydroxyl groups to transfer their hydrogens to OH radical present in the aqueous phase, leading to higher RSA in cationics micelles than in anionic/non ionic micelles in which quercetin interacts via ring B.

Figures 5.12 and 5.13 give a comparison of the activity of quercetin in scavenging OH radical in equimolar nonionic-anionic and nonionic-cationic mixed binary surfactant systems respectively with that in their single component systems. As observed from the figures the activity of quercetin in the binary nonionic-cationic, nonionic-anionic surfactant systems lies in between the values observed in anionic, cationic and nonionic single surfactant systems except in equimolar binary DTPB and

Brij30 surfactant system in which exhibits a higher RSA than the corresponding single surfactant systems.

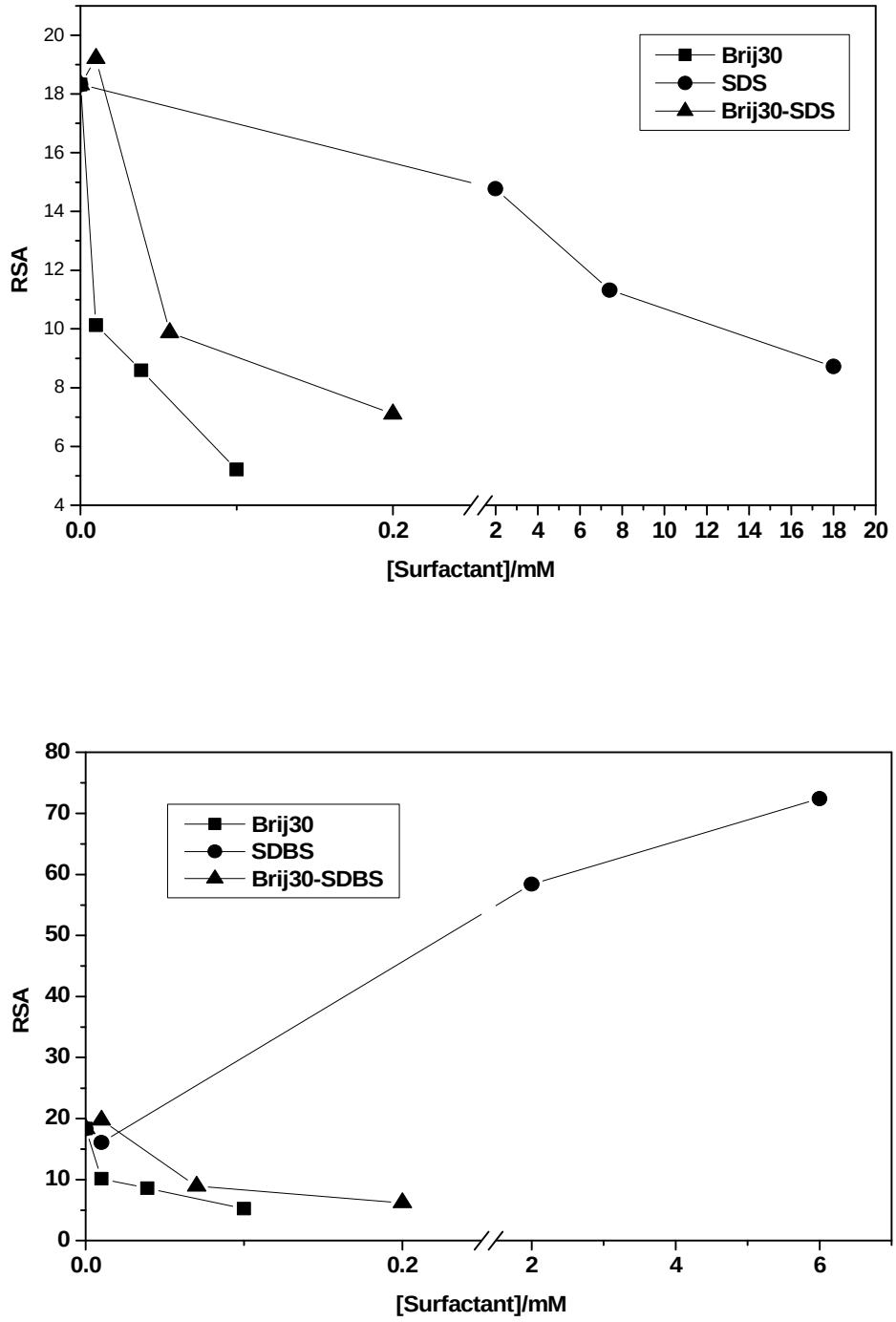

Figure 5.12: *Hydroxyl radical scavenging activity of quercetin in mixed nonionic-anionic surfactant systems and their comparison with single surfactant systems.*

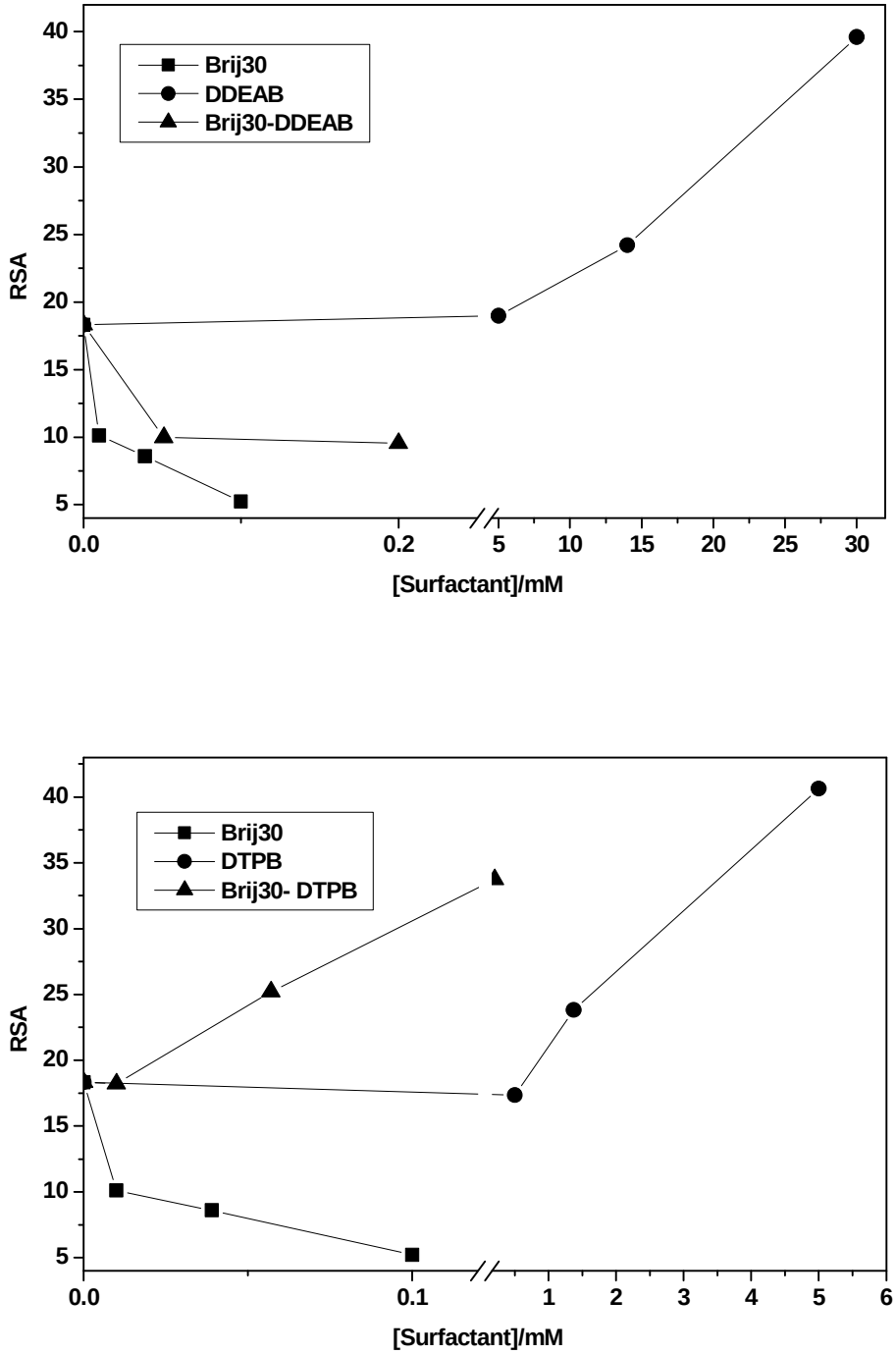

Figure 5.13: *Hydroxyl radical scavenging activity of quercetin in mixed nonionic-cationic surfactant systems and their comparison with single surfactant systems.*

In case of Brij30-SDS and Brij30-SDBS binary systems, activity of quercetin was observed to be intermediate between that in single surfactant systems. It is known from the literature[136, 142] that in an aqueous anionic – non-ionic binary surfactant solution the weakly basic POE head group gets protonated to acquire positive charge, even at neutral pH. Therefore, owing to higher micellar mole fraction of Brij30 within the mixed Brij30-SDS and Brij30-SDBS surfactant systems (**Table 5.1**) the presence of slight positive charge would increase its interaction with quercetin via rings A and C, leading to solubilization of quercetin molecules such that their 3',4' electroactive hydroxyls point outwards facilitating their antioxidant activity. However, there is also hydrogen bonding effect characteristics of pure nonionic micelles which reduces the antioxidant activity of quercetin, by directing a few quercetin molecules to interact via ring B making them to point inwards leading to reduction of antioxidant activity. Thus, due to these two opposing effects the antioxidant activity of quercetin in these anionic–nonionic surfactant systems lies in between the single surfactant systems.

In the case of Brij30-DDEAB binary surfactant system, the antioxidant activity of quercetin was observed to be intermediate between that in single surfactant systems. Since nonionic-cationic mixed micelles are predominantly made up of the nonionic component (**Table 5. 1**), therefore most of the quercetin molecules would interact in such mixed micelles via ring B having 3', 4' electroactive hydroxyls pointed inwards, thereby reducing the antioxidant activity. In addition, strong hydrogen bonding effect of nonionics would also reduce the antioxidant activity of quercetin. However, due to positive charge on Brij30-DDEAB mixed micelles the quercetin would also interact via ring A and C with micelles making 3' 4 'electroactive hydroxyls pointing towards aqueous phase. Both these opposite effects taken together are responsible for the

intermediate antioxidant activity of quercetin than that in pure Brij30 and DDEAB micelles.

In Brij30-DTPB binary system the antioxidant activity is more than in either of the single surfactant systems. Since the head group of DTPB contains three phenyl groups attached to the phosphorous, the positive charge would be delocalised over all the phenyl rings due to conjugation leading to spread of charge over the larger surface area of micelles, though the micellar mole fraction of DTPB in Brij30-DTPB mixed micelles is comparable to that of DDEAB in Brij30-DDEAB mixed micelles where such effect is absent. This would lead to interaction of quercetin via ring A and C with micelles forcing 3', 4 'electroactive hydroxyls to point toward the aqueous phase thereby enhancing its RSA. It has been reported that synergism in mixed micelle formation may lead to the enhancement of extent of solubilization towards water insoluble compounds.[143, 144] In this context, mixed micelles of Brij30-DTPB might be involved in enhanced solubilization of quercetin in the palisade layer compared to pure DTPB micelles leading to further enhancement of RSA. Such an effect could have been observed in Brij30-DDEAB system as well, but their mixed micelles would be more compact as a result of lower steric hindrance of DDEAB head group, leading to lesser solubilization of quercetin and hence lesser RSA, in addition to other effects already discussed. Comparing the RSA of quercetin in nonionic -cationic and nonionic-anionic binary surfactant mixtures, the antioxidant activity is found to be greater in cationic nonionic binary surfactant systems because of favourable orientation effect in the former than in latter.

Figure 5.14 gives a comparison between the activities of quercetin in scavenging OH radicals in equimolar nonionic-cationic-cationic and nonionic-anionic-anionic ternary surfactant systems. As observed from the figure, the antioxidant activity of quercetin

in the ternary anionic-anionic-non ionic surfactant system decreases with increase in the total concentration of the surfactant. Since the micellar mole fraction of Brij-30 is higher than the sum of mole fraction of the SDS and SDBS in this surfactant system (**Table 5.1),** therefore, the activity profile would be the same as observed in anionic-nonionic binary surfactant systems due to the reasons explained earlier.

On the other hand in Brij-30-DTPB-DDEAB ternary system the antioxidant activity of quercetin increases with increase in concentration of the surfactant. In the pre micellar range, the reasons of increase in RSA could not be figured out. However, the increase in the activity in the post-micellar region even more than the Brij30-DTPB system might be due to the higher combined micellar mole fraction of the two cationic surfactants in the ternary surfactant system resulting in more positive charge on their mixed micelles. Since the calculated cmc_{RH} of this system is slightly less than the experimental value, it indicates that the combined micellar mole fraction of cationics would be even more than shown in the **Table 5.1.** Hence most of the quercetin molecules are expected to interacts with ternary nonionic-cationic-cationic surfactant system via ring A and C pointing towards the micelle and ring B having 3' 4' hydroxyls pointing towards the aqueous phase thereby increasing the proximity of the hydroxyls towards the quercetin and consequent increase in the antioxidant activity. In addition, the steric factor of DTPB surfactant would also increase the solubilisation of quercetin in the micellar palisade layer contributing to enhanced antioxidant activity as explained earlier.

The antioxidant activity is more in nonionic-cationic-cationic ternary surfactant system than in the nonionic-anionic-anionic system. This is attributed to the favourable orientation effect in the former for reaction between quercetin and OH.

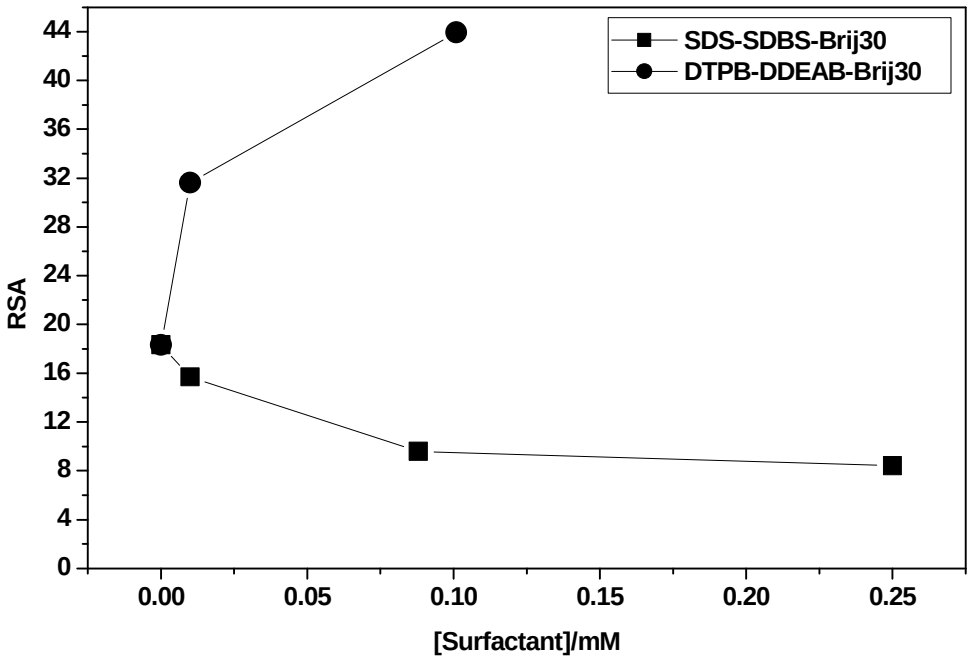

Figure 5.14 *Hydroxyl radical scavenging activity of quercetin in mixed ternary surfactant systems.*

Chapter 6
Main Highlights of the work

1. The present study represents effects of single and mixed (binary and ternary) surfactants on antioxidant activity of Quercetin, followed by evaluation of hydroxyl radical scavenging activity (RSA) of quercetin in studied micellar systems.
2. Hydroxyl RSA of Quercetin in cationic surfactant systems (DDEAB, DTPB) increased with increase in surfactant concentration above their *cmc*. In addition the antioxidant activity of Quercetin was more in these cationic surfactant systems than nonionic (Brij30) and anionic (SDS) surfactant systems due to favorable orientation effect of Quercetin within these micelles.
3. The activity of Quercetin in nonionic Brij micellar system was observed to be lower than that of in SDS micellar system attributed to stronger H-bonding effect in such micelles that hampers H-abstraction kinetics. Hydroxyl radical scavenging activity of Quercetin in Brij30 and SDS decreased with the increase in surfactant concentration, indicating that the reaction between Quercetin and hydroxyl radical was partly blocked due to the solubilization of Quercetin within these micelles at or above *cmc* values.
4. The activity of Quercetin in the binary nonionic-cationic, nonionic-anionic surfactant systems lies in between the values observed in anionic, cationic and nonionic single surfactant system except in equimolar binary DTPB and Brij30 surfactant system in which the RSA was higher than the corresponding single surfactant systems.
5. The activity of Quercetin in ternary surfactant system was more in nonionic-cationic-cationic than in nonionic-anionic-anionic surfactant system. This is attributed to the favorable orientation effect in the former for reaction between Quercetin and OH.

6. The study is supposed to be essential to understand and control the antioxidant activity at interfaces present in wide range of foods, cosmetics, pharmaceuticals and of biological membranes and gives the importance of simple micro heterogeneous environments on the antioxidant activity of Quercetin having some correlation with the complex biological systems.

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Appendices

Appendix I

Table (i): Surface Tension Data of Various single Surfactant Systems at 25^0C.
Units used are γ (mNm^{-1}), C_t (mM)

Brij30		SDS		SDBS		DDEAB		DTPB	
log c_t	**γ**	**log c_t**	**γ**	**log c_t**	**γ**	**log c_t**	**γ**	**log c_t**	**γ**
-2.177	38.9	-0.01	57.3	-0.58	55.4	0.207	68.3	-0.707	65.4
-1.879	36.6	0.27	54.2	-0.2848	49.3	0.494	61.6	-0.415	60
-1.703	36.3	0.44	51.4	-0.1141	45.2	0.657	56.9	-0.247	55.8
-1.58	34.7	0.55	49.1	0.00518	42	0.769	53.8	-0.13	52.9
-1.485	33.6	0.63	47.9	0.09691	39.4	0.853	51.5	-0.041	51.2
-1.406	32.9	0.7	46.1	0.17056	37.4	0.92	49.5	0.029	48.4
-1.341	32.9	0.75	45.2	0.23223	36.5	0.975	46.9	0.089	46.9
-1.283	33	0.8	44.4	0.28488	36	1.022	45	0.139	45.6
-1.235	33	0.84	42	0.33082	35.8	1.062	42.8	0.183	45.1
-1.19	32.8	0.88	41.8	0.37144	35.8	1.096	42.5	0.221	44.9
-1.15	32.8	0.91	41.5	0.4079	35.5	1.127	42.2	0.255	45.4
-1.114	32.8	0.93	41.5	0.44059	35.6	1.154	42.6	0.286	45
				0.47041	35.5	1.179	42.8	0.314	45.5
				0.49776	35.5	1.201	42.7	0.339	45.6
				0.52284	35.6	1.221	42.9	0.363	45.6
				0.54605	35.4	1.24	42.9		
						1.257	42.8		
						1.273	42.7		
						1.287	42.6		
						1.301	42.7		

Table (ii): Surface Tension Data of Various Binary Surfactant Systems at 25^0C.

Units used are γ (mNm^{-1}) , C_t (mM)

Brij30 SDS		Brij30 SDBS		SDS SDBS		Brij30 DDEAB		Brij30 DTPB		DDEAB DTPB	
log C_t	**γ**	**log C_t**	**γ**	**log C_t**	**γ**	**log C_t**	**γ**	**log C_t**	**γ**	**log C_t**	**γ**
-2.18	62.2	-1.879	62.5	-0.703	68.3	-2.18	60	-2.18	54.8	-0.415	62.7
-1.886	52	-1.703	58.7	-0.406	62.7	-1.886	52.4	-1.886	44.6	-0.13	56.1
-1.721	45.1	-1.58	53.1	-0.235	58.5	-1.721	44.2	-1.721	41.9	-0.029	52.1
-1.585	41.5	-1.485	48.1	-0.114	55.5	-1.585	43.8	-1.585	40.9	0.139	48.8
-1.494	39.9	-1.406	45.4	-0.021	52.8	-1.494	40.1	-1.494	39.2	0.221	46.3
-1.408	38.3	-1.341	43	0.053	48.6	-1.408	38.6	-1.408	37.3	0.286	45.5
-1.346	37.2	-1.283	41.5	0.116	47	-1.346	36	-1.346	36.4	0.339	43.4
-1.292	36.1	-1.235	40.7	0.17	44.6	-1.292	35.5	-1.292	36.1	0.384	43.2
-1.236	35.5	-1.19	39.2	0.217	43	-1.236	35.5	-1.236	35.6	0.422	43.5
-1.193	35.3	-1.15	38.1	0.259	41.3	-1.193	35.5	-1.193	35.4	0.455	43.6
-1.154	35.6	-1.114	37.6	0.296	40.8	-1.154	35.4	-1.154	35.5	0.485	43.6
-1.119	35.3	-1.08	38.1	0.33	38.9	-1.119	35.1	-1.119	35.2	0.51	43.6
-1.08	35.6	-1.05	36.9	0.361	38.2	-1.08	34.9	-1.08	35.4	0.534	43.6
		-1.022	36.6	0.39	37.1	-1.05	35.2	-1.05	35.5		
		-0.995	35.8	0.416	36.6	-1.022	35.3				
		-0.97	35.9	0.44	35	-0.995	35.2				
		0	35.7	0.463	35.4						
		-0.924	35.8	0.484	34.5						
		-0.903	35.8	0.504	34.7						
				0.522	34.9						
				0.54	34.9						
				0.557	34.7						
				0.572	34.4						
				0.587	34.7						
				0.602	35.2						

Table (iii): Surface Tension Data of Various Ternary Surfactant Systems at25^{0}C.
Units used are γ (mNm^{-1}) , C_t (mM)

Brij30-SDS-SDBS		**Brij30-DDEAB-DTPB**	
logC_t	**γ**	**LogC_t**	**γ**
-1.886	57.2	-1.886	55.8
-1.585	47.2	-1.585	44.6
-1.408	42.9	-1.408	40.1
-1.292	39.8	-1.292	39.7
-1.193	37.3	-1.193	38.7
-1.119	36.4	-1.119	35.9
-1.05	35.7	-1.05	35.6
-0.995	35.7	-0.995	34.9
-0.946	35.1	-0.946	35
-0.903	34.5	-0.903	35
-0.866	34.3	-0.866	34.9
-0.829	34.5		