Oxidation Kinetics of Simvastatin Using Cetyltrimethylammonium Dichromate

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ABSTRACT: This article reports an attempt on the studies on resistance of oxidative stress by the prodrug, simvastatin (SV). Cetyltrimethylammonium dichromate has been used as a lipid compatible oxidant to study the oxidation kinetics of SV in organic media. The reaction undergoes via an ionic mechanism without any side product. The reaction is found to be acid catalyzed and sensitive to solvent polarity. The increase in the rate constant due to an increase in hydrophobicity (apolarity) of the solvent indicates the existence of a less polar transition state. Furthermore, the decrease in the rate constant due to an increase in [CTAB] suggests partitioning of the substrates and the oxidants into two different domains with different polar characteristics akin to a reversed micellar aggregates. Considering the above results and the thermodynamic parameters, a reaction mechanism has been proposed, wherein a complex formed at the interface of the two domains due to the reactant and the oxidant in a fast process decomposes to the products in a slow process in the nonpolar bulk. © 2012 Wiley Periodicals, Inc. Int J Chem Kinet 1–7, 2012

INTRODUCTION

Simvastatin (SV) is a lactone prodrug used for the treatment of hypercholesterolemia [1]. Following conversion of this lactone prodrug to its hydroxyl acid form, the compound is a potent competitive inhibitor of HMGCoA reductase, the rate-limiting enzyme in cholesterol biosynthesis [2]. The oxidative biotransformation of SV takes place at the heptanoic acid side chain. It acts as an antioxidant and inhibits the oxidation of low-density lipoproteins (LDL) [3] and also decreases aldehyde production derived from lipoprotein oxidation [4]. SV treatment induced an increase in autoantibodies against specific oxidized LDL antigens [5].

The electrochemical detection of SV in the form of drugs stems on its oxidation behavior in the presence of a multiwalled carbon nanotubes–dihexadecyl hydrogen phosphate composite modified glassy carbon electrode [6]. SV, on various stress degradation, such as acid and base hydrolysis, oxidation by hydrogen

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peroxide led to the formation of simvastatin acid and dehydrated products, respectively [7].

An onium ion, as the counterion for anionic oxidants, makes a lot of difference in oxidation potential of the oxidant as well as to the oxidizing system. It makes the oxidant lipid soluble, mild, and many a time chemoselective [8,9]. Tailor-made oniums have been used as the counterions, wherein heterocyclic bases such as pyridine, quinoline, caffeine, imidazole, and nicotine units become a part of the oxidant [8]. In different reaction conditions, these oxidants often show biomimetic characteristics due to the counterions, which help in providing a microheterogeneous environment with different solubilization pockets for the substrates as in the case of micelles, reversed micelles, microemulsions, vesicles for artificial systems, and proteins and lipid membranes in living systems [10]. Among these oxidants, Cr(VI) has been studied extensively.

In our efforts in exploring some biomimetic oxidants to oxidize organic substrates in organic solvents, we have reported the oxidation behavior of cetyltrimethylammonium permanganate (CTAP) [11–14], cerate (CTACN) [15], and dichromate (CTADC) [10,16] toward various organic substrates. These are inorganic oxidants with an organic amphipathic carrier, cetyltrimethylammonium (CTA+) ion, to carry the oxidants into the organic (lipid) phase. However, these oxidants are hydrophobic and thus support the existence of a tight ion pair of the cationic carrier and the anionic oxidant in nonpolar medium [17]. In organic solvents, CTAP oxidizes its carrier, CTA^+, in a manner similar to β-oxidation of fatty acids [11]. Other aforesaid oxidants are found to be inert toward their carrier. We have used CTAP and CTADC for oxidation of cholesterol to yield a diol at the double bond [14] and 7-dehydrocholesterol [10], respectively, while with addition of acetic acid to CTADC in dichloromethane (DCM) the product was found to be 5-cholesten-3-one. CTADC is devoid of an acidic proton and thus is relatively milder than other Cr(VI) oxidants [8]. In the absence of acid, CTADC exhibits some bizarre reactions with nonconventional products. Aromatic amines are found to yield corresponding diazo compounds [18], and arylaldoximes yielded corresponding nitriles [19].

In this paper, we have made an attempt to investigate the oxidation behavior of CTADC toward the prodrug, SV, in organic solvents. To follow up the objectives, the oxidation product was characterized and kinetics were run in different media with varied polarities and also in microheterogeneous systems, generated due to the presence of a cationic surfactant (CTAB: cetyltrimethylammonium bromide) and anionic surfactant (SDS: sodium dodecyl sulfate) at different concentrations. By analyzing the rate constants determined by varying [substrate], [acid], and [CTADC] in the reaction process, a suitable mechanism for the reaction has been proposed. Earlier, SV was subjected to oxidative degradation by using hydrogen peroxide to yield a variety of products through a free radical mechanism [20]. Cr(VI) oxidation of many biological substrates also encountered free radical intermediate, and the reactions become complicated. In most of the oxidations by CTADC, no free radical mechanism has been proposed. Thus the present study highlights the effect of Cr(VI) oxidant on SV to get a clear picture of the oxidative stress on SV.

EXPERIMENTAL

Materials

CTADC was prepared by the method reported earlier [21]. SV(**I**) was used without further purification. Glacial acetic acid was used as a source of hydrogen ion and was used without further purification. The organic solvents used were purified by standard methods [22]. The surfactants, CTAB and SDS, were obtained from Spectrochem (Mumbai, India) and were purified by recrystallization from ethanol solution.

Kinetic Measurements

The oxidation kinetics of SV by CTADC in the presence of acetic acid was monitored in different solvents and surfactant systems spectrophotometrically at the absorption maxima of CTADC (350 nm) by using a Hitachi U3010 spectrophotometer with a thermostatic cell holder attached to a water bath. The first-order rate constant, k_{obs}, was obtained from the linear ($r = 0.99$) plot of log[oxidant] against time upto 75% completion of the reaction in a pseudo-unimolecular condition by keeping a large excess of SV. The values reported are the average of triplicate runs and were reproducible within $\pm 4\%$ error.

Product Analysis

After keeping the reaction mixture of CTADC and SV in proper composition for 24 h in DCM, the volume of the reaction mixture was reduced to a pasty mass under low pressure. Acetic acid was added to the reaction mixture with CTADC as the oxidant. Then the organic compounds from the pasty mass were extracted by using diethylether in excess. On evaporation of the ether, the products were subjected to column chromatographic separation by using a mixture of ethyl acetate

and toluene (1:2 v/v). On evaporation of the eluents with a single spot in TLC, the isolated compound was subjected to fast atom bombardment mass spectrometry (FABMS), nuclear magnetic resonance (NMR), and infrared (IR) analyses. The compound is proposed to be the corresponding carbonyl compound (**II**).

Stoichiometry

The stoichiometry of the reaction was determined by performing the experiment at 303 K, under the conditions with fixed [Oxidant] and varying [SV]. The disappearance of Cr(VI) was followed until the absorbance values became constant, and then CTADC was estimated after 48 h. The stoichiometry ratios are found to be 1:2 for CTADC/SV.

RESULTS AND DISCUSSION

Under reflux conditions, the solution of CTADC and SV in DCM in the presence of acetic acid became green, indicating the reduction of Cr(VI) to Cr(III). The completion of the reaction was ascertained by monitoring the TLC of the reaction mixture. CTADC exists as a contact ion pair in aqueous medium as well as in organic solvents [17]. In the presence of acetic acid, the dichromate ion becomes free from the grasp of the quaternary onium ion due to the change in polarity of the medium and also the probable substitution of the onium ion by proton of acetic acid. Furthermore, acrylonitrile was added to the reaction mixture during the reaction process. As no turbidity of the medium in the reaction mixture was observed, the possibility of the free radical mechanism was ruled out. The reaction kinetics of the oxidation reaction was monitored in the presence of acid, and the kinetic data are presented in Table I.

The acid-catalyzed oxidation of SV with CTADC in DCM was found to increase linearly with an increase in the concentration of SV (Fig. 1). To obtain a relationship between the rate constants with the parameters of the reaction condition, i.e., [substrate], [oxidant], and [acid], log k_{obs} values obtained in different conditions were correlated with the above three parameters through multiple regression analysis. The regression model, thus obtained, is given by Eq. (1). The orders with respect to [CTADC], [SV], and [acetic acid] are found to be 0.634, 0.554, and 0.844, respectively:

$$\log k_{obs} = -5.114\,(\pm 0.321) - 0.634(\pm 0.074) \times \log[\text{CTADC}] + 0.554(\pm 0.074)\log[\text{SV}] + 0.844 \pm 0.107 \log[\text{acetic acid}]$$

Table I Effect of [SV], [CTADC], and [Acetic Acid] on the Oxidation of SV by CTADC at 303 K in DCM

[CTADC] $\times 10^4$ (M)	[SV] (M)	[Acetic Acid] (M)	$k_{obs} \times 10^4$ (s^{-1})	Rate $\times 10^7$ (mol L^{-1} s^{-1})[a]
0.5	0.02	4.86	17.27	0.86
1	0.02	4.86	11.13[b]	1.11
2	0.02	4.86	9.21	1.84
4	0.02	4.86	4.22	1.69
1	.005	4.86	5.76	0.58
1	0.01	4.86	6.91	0.69
1	0.04	4.86	17.27	1.73
1	0.02	6.48	14.97	1.50
1	0.02	3.24	8.06	0.81
1	0.02	1.62	4.61	0.46

[a] Rate $= k_{obs} \times$ [CTADC].

[b] $10^4\ k_{obs}$ at 293, 298, and 308 K were found to be 6.53, 9.98, and 14.97 s^{-1}, respectively.

$$R^2 = 0.964,\ F = 54,\ n = 10 \quad (1)$$

Using the regression model, the log k_{obs} values were calculated and plotted against the observed values (Fig. 2). A linear plot without any outlier supports the validity of the regression model.

Without acid, the reaction became too slow to measure. With increasing [acetic acid], the rate constant increases linearly (Fig. 3). The reaction is found to acid catalyzed with an uncatalyzed rate of 1.15×10^{-4} s^{-1}.

In an earlier report on oxidation of cholesterol, nonlinearity with the Michaelis–Menten relationship of the substrate with the k_{obs} was observed, indicating a complex mechanism for the oxidation reaction [10]. In the present study, the molecularity was found to be in decimal (Eq. (1)), indicating an occurrence of a complex reaction mechanism, which may be proposed vide infra (Scheme 1, where Q refers to CTA).

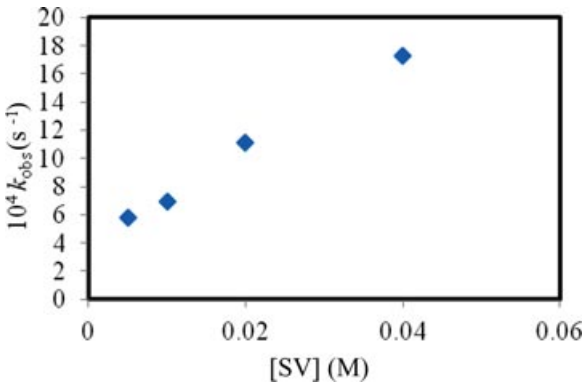


Figure 1 Plot of $10^4\ k_{obs}$ vs. [SV] in the oxidation reaction of CTADC with SV at 303 K.

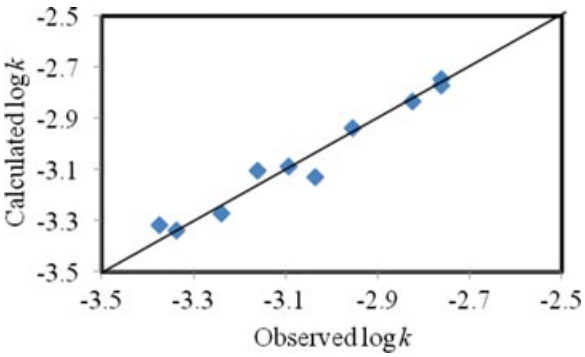


Figure 2 Plot of observed log k vs. calculated log k using the regression model equation (1).

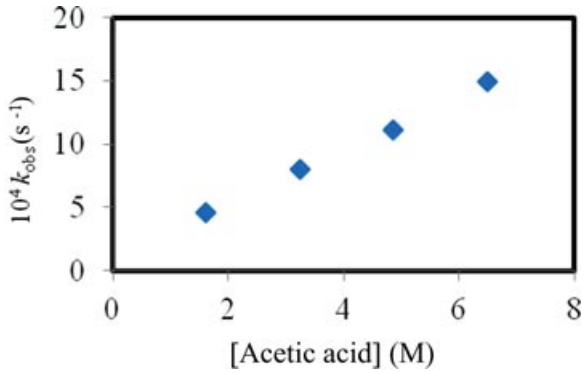


Figure 3 Plot of 10^4 k_{obs} vs. [acetic acid] in the oxidation reaction of SV with CTADC at 303 K.

Scheme 1 can lead to the derivation of a rate equation (Eq. (2)):

$$\text{Rate} = -\frac{\text{d[C]}}{\text{d}t} = k[\text{C}] = kK_1K_2\frac{[\text{Q}_2\text{Cr}_2\text{O}_7][\text{H}^+][\text{SV}]}{\text{Q}^+} \quad (2)$$

Cr(III) is found in the reaction products during the oxidation of various substrates by CTADC in organic medium [10]. The existence of Cr(III) in the product mixture is well established from the peak at 580 nm. However, reaction kinetics could not be studied at this wavelength due to nonreliability and low absorptivity of the spectrum. The formation of Cr(III) from Cr(VI)

$$\text{Q}_2\text{Cr}_2\text{O}_7 + \text{H}^+ \underset{}{\overset{K_1}{\rightleftharpoons}} \text{QCr}_2\text{O}_7\text{H} + \text{Q}^+$$

$$\text{SV} + \text{QCr}_2\text{O}_7\text{H} \overset{K_2}{\rightleftharpoons} \text{Complex (C)}$$

$$\text{Complex (C)} \xrightarrow{k} \text{Product}$$

Rate-determining step

Scheme 1

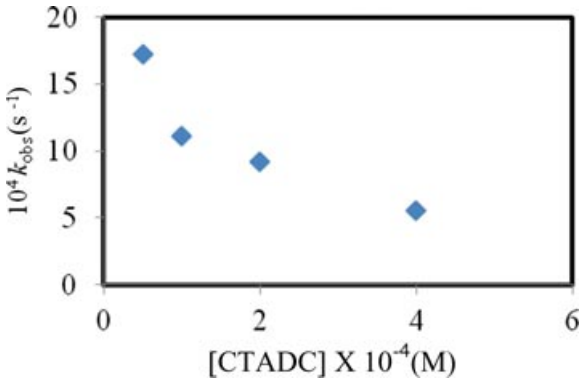


Figure 4 Plot of 10^4 k_{obs} vs. [CTADC] in the oxidation of SV at 303 K in DCM.

due to oxidation seems to be a complex phenomenon as shown below:

$$\text{Cr(VI)} + 2e \rightarrow \text{Cr (IV)}$$

$$\text{Cr(IV)} + \text{Cr(VI)} \rightarrow 2\text{Cr(V)}$$

$$\text{Cr(V)} + 2e \rightarrow \text{Cr(III)}$$

Cr(VI) is initially reduced to Cr(IV), which subsequently changes to Cr(V) with another Cr(VI). The formation of Cr(III) is a result of two-electron reduction of Cr(V). The existence of Cr(IV) as the reduced state in oxidation of benzyl alcohol by quinolinium fluorochromate has also been reported by Dave et al. [23]

The rate constant is found to decrease nonlinearly with increasing [CTADC] (Fig. 4). Similar observations have been made for the oxidation reaction of different substrates by CTADC in organic solvents. Earlier, it has been rationalized by proposing the occurrence of a reversed micellar phenomenon during the oxidation reaction [10]. This proposition was further supported by a drastic decrease in the rate constant with addition of CTAB (Table II), a reversed

Table II Rate Constant of Oxidation of SV at Different [CTAB] and [SDS] at 303 K in DCM ([CTADC] = 1×10^{-4} M, [SV] = 0.02 M, [Acetic Acid] = 4.86 M)

[CTAB] × 10^4 (M)	$k_{obs} \times 10^4$ (s^{-1})	[SDS] × 10^4 (M)	$k_{obs} \times 10^4$ (s^{-1})
1	9.6	1	17.66
5	5.76	5	36.08
10	3.45	10	46.06
20	2.30	15	58.73
–	–	50	74.46

micelle-forming surfactant. The spherical reversed micelle has various localization sites, including the polar inner core, where the ionic oxidant is partitioned more. Substrate, being nonpolar in characteristics, partitioned to the bulk and remains away from the reactive oxidant. With an increase in [CTADC], the inner polar core may assume a larger interfacial area so that the substrate can, relatively, be more in contact with the polar oxidant to facilitate the complexation of the SV and Cr(VI). Owing to the decrease in the polarity of the complex compared to the reactants, it is partitioned to nonpolar bulk and, therein, dissociates to the product. The larger the interfacial area, the less will be the partitioning leading to decrease in the rate. CTAB can form spherical micelle in aqueous medium and reversed micelle in nonaqueous medium. The decrease in the rate constant may be attributed to the enhanced reversed micellization in the presence of CTAB, which provides a common counterion with CTADC for the formation of reversed micelle.

Furthermore, as the reaction is acid catalyzed and the interface due to CTA^+ is positively charged which repels the proton, the rate is retarded. This proposition gets further support from the rate enhancement due to the addition of SDS, an anionic surfactant (Fig. 5). SDS is inert toward CTADC and provides an anionic environment to the reactant either through mixed micellization or through a reversed micellar aggregate, which can provide an anionic interface for the interaction between the proton, dichromate, and SV.

The rate law as derived in Eq. (2) gets support from the above observations. With increasing [CTADC], [acetic acid], and [substrate], the rates of the reaction (as mentioned in Table I) increase linearly. Similarly, the rate of reaction decreases with increasing [CTA]. The plot of rate vs. 1/[CTA] is also found to be linear ($R^2 = 0.99$).

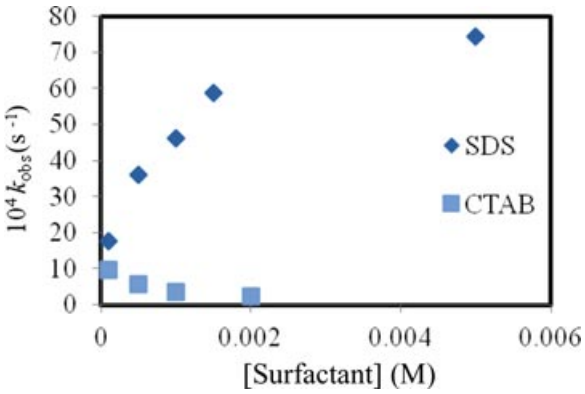


Figure 5 Plot of 10^4 k_{obs} vs. [surfactant] for the oxidation reaction of SV with CTADC at 303 K.

Table III Observed Rate Constants for the Oxidation Reaction of SV in Various Organic Solvents at 303 K, [CTADC] = 1×10^{-4}M, [SV] = 0.02 M, and [Acetic Acid] = 4.86 M

S. No.	Solvent	$k_{obs} \times 10^4$ (s^{-1})
1	Dioxan	6.91
2	Ethyl acetate	8.44
3	Acetone	8.06
4	Acetonitrile	5.76
5	Benzene	13.43
6	Toluene	15.74
7	Dichloromethane	11.13
8	Chloroform	17.66
9	Carbon tetra chloride	19.96

To investigate the effect of environment on the reaction mechanism, nine organic solvents with different polarities were used as reaction medium. CTADC was found to be stable in all these solvents in the presence of acetic acid for more than 24 h. The rate constant is found to be highly sensitive to change in polarity of the solvents (Table III). To elucidate the characteristics of the transition state of the reaction, the rate constants were plotted against various solvent parameters such as cation-binding (A) and anionic-binding (B) capacity, dielectric constant, π^*, dipole moment, and log P (where P being the partition coefficient of the substrate between octanol and water indicating the nonpolar characteristics of the solvents). The plots of the rate constants with all the polarity parameters delineate scattered relationship indicating the transition state to be sensitive to polarity without any specific trend. However, from the linear relationship of these parameters with the rate constants, the solvents can be classified into dipolar aprotic solvents (acetonitrile, dioxane, ethyl acetate, and acetone) and nonpolar solvents (benzene, toluene, carbon tetrachloride, chloroform, and DCM). In some corelationships, DCM is found to be in the boarder line of the classification. With the increasing dipole moment or dielectric constant of the solvent, in most of the cases, the rate constant decreases. In cognizance of this, the rate constant increases with increasing log P value of the solvent (Fig. 6). These observations support the formation of a relatively less polar transition state compared to the polarity of the reactants.

The thermodynamic parameters such as $\Delta H^{\#}$, $\Delta S^{\#}$, and $\Delta G^{\#}$ were calculated for the oxidation of SV with CTADC in the presence of 4.86 M acetic acid by using Arrhenius and Eyring equations and are found to be 36.5 ± 1.4 kJ mol^{-1}, –181.1 ± 6.9 J K^{-1}, and 91.4 ± 3.5 kJ mol^{-1}, respectively. A high negative value

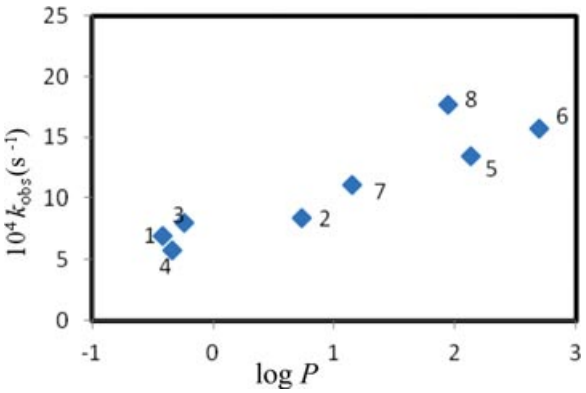


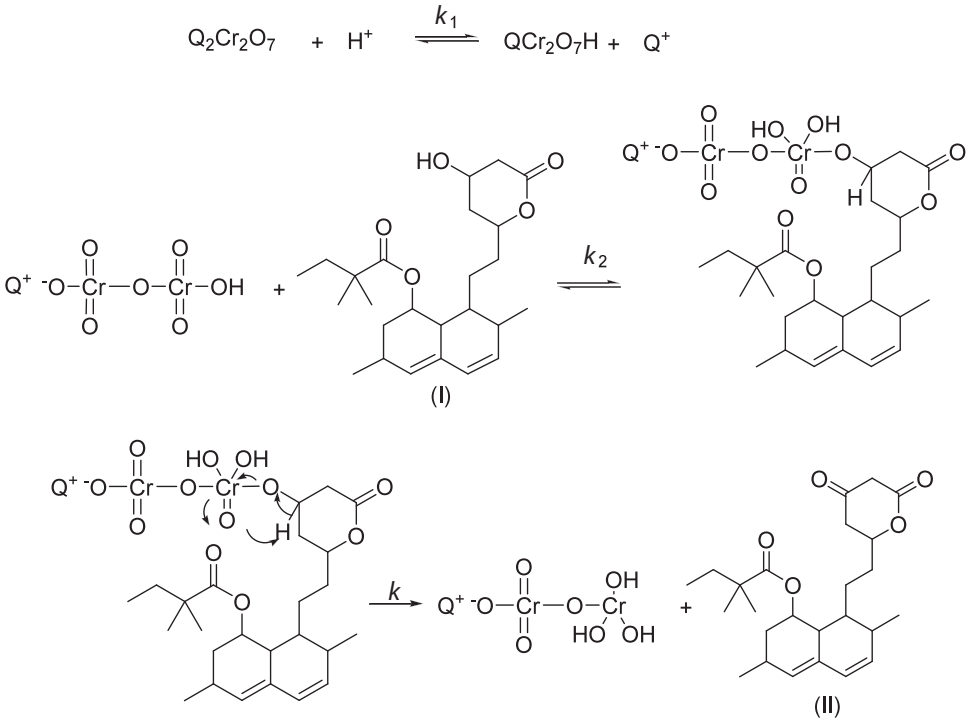
Figure 6 Plot of $10^4\, k_{obs}$ vs. log P for the oxidation reaction of SV with CTADC at 303 K .

of $\Delta S^{\#}$ supports the proposal of the involvement of a cyclic transition state [24].

From the above findings, a tentative mechanism has been proposed (Scheme 2), wherein the CTADC equilibrates with acetic acid to form the protonated dichromate, which subsequently reacts with SV, giving rise to a dichromate ester. The complex decomposes to the reduced Cr(IV), which on further disproportionation gives rise to stable Cr(III), and corresponding carbonyl compound by α-hydrogen abstraction from the substrate. The FABMS results of the carbonyl compound with a (M – H)/z peak at 415.3 corroborate the structure of the product. The appearance of characteristics IR band at 1577 cm^{-1} for β-diketone and disappearance of 3549 and 1265 cm^{-1} for –OH substantiate the oxidation of secondary –OH to corresponding carbonyl one. Furthermore, the disappearance of the NMR peak at 1.568 δ in the product is also an indicator of conversion of the hydroxyl group to a corresponding carbonyl group. (The spectra of the reactant and product are available as Supporting Information.)

CONCLUSION

SV is an established prodrug used in increasing the level of high-density lipoproteins and acts at cellular surface exposed to various oxidants. SV undergoes oxidative degradation by hydrogen peroxide through a free radical mechanism. In the lipid system, metallic oxidants like Cr(VI) can act to different substrates with the help of an amphiphilic carrier. SV when interacts with CTADC, Cr(VI) carried by cetyltrimethylammonium ion, leads to the formation of the corresponding carbonyl compounds catalyzed by an acid through an ionic intermediate. The non–free radical reaction leads to a mechanism with fewer or almost no side



Scheme 2

products. Furthermore, the reaction is proposed to occur in an organized media where the partition of substrates and oxidants into different domains retard the rate of the reaction. The use of amphiphilic compounds such as CTAB and SDS in the reaction media provides a biomimic environment to understand the reaction process. Thus CTADC is found to be an excellent model oxidant to study the oxidative degradation of different substrates in biological membranes.

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