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Determination of trace carvedilol by solid substrate–room temperature phosphorimetry, based on its activating effect on hypochlorite-oxidizing amaranth using sodium dodecyl benzene sulphonate as sensitizer

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ABSTRACT: This work proposes a simple and sensitive solid substrate–room temperature phosphorimetry (SS–RTP) for the selective determination of carvedilol (CV). The method is based on the sensitizing effect of sodium dodecyl benzene sulphonate (SDBS) on CV to activate the oxidation between NaClO and amaranth, resulting in the intense quenching of room temperature phosphorescence (RTP) of the system. Compared with non-SDBS system, the reduction of phosphorescence intensity (ΔI_p) with SDBS is 16.5 times higher and is directly proportional to the content of CV, covering a wide range 0.080–16.00 fg/spot. The regression equation of the working curve can be expressed as $\Delta I_p = 0.7780 + 7.057\ m_{CV}$ (fg/spot) (correlation coefficient (r) = 0.9976, n = 8), with a detection limit (LD) of 0.020 fg/spot (corresponding concentration is 5.1×10^{-14} g/mL, sample volume is 0.40 μL/spot). This sensitive method has also been applied to determine trace CV in human plasma and the results agreed with synchronous fluorimetry (SF). The activation energy (E) and rate constant (k) of this activating reaction were 69.04 kJ/mol and $3.580 \times 10^{-4}\ s^{-1}$, respectively. The reaction mechanism is also discussed. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: carvedilol; amaranth; sensitizing; activating; solid substrate–room temperature phosphorimetry

Introduction

Carvedilol (CV), a non-selective β-receptor blocker, is used as a hypertension drug for angina, and has also been accepted as an effective agent for the treatment of congestive heart failure (1,2). Meanwhile, CV is classified as a prohibited drug by the World Anti-doping Agency (3) and the International Olympic Committee. Therefore, the study of the methods for the determination of CV has great significance and application prospects for the early warning and prevention of human diseases and anti-doping in sports.

The methods available currently for the determination of CV in plasma, serum, urine or other biological samples mainly focus on HPLC–MS/MS (LD = 2.0×10^{-10} g/mL) (4), HPLC–fluorescence detection (LD = 1.6×10^{-9} g/mL) (5), HPLC–electrochemical detection (LD = 1.0×10^{-10} g/mL) (6), GC–MS (LD = 3.0×10^{-10} g/mL) (7) and LC/LC–FLD (LD = 7.0×10^{-10} g/mL) (8). However, these methods have many drawbacks, including laborious and toxic sample preparations, time-consuming analytical processes and the necessity for expensive equipment, which limit routine analysis. Some chemists have developed simple methods for determining CV in tablets, such as flow-injection spectrofluorimetry (LD = 1.5×10^{-9} g/mL) (9) and chemiluminescence (LD = 3.5×10^{-9} g/mL) (10), but they have significant background interference and poor selectivity and repeatability. Futhermore, the sensitivity of the methods described above is at the ng level, and can not meet the demand for the determination of CV at pg levels. Obviously, developing a new, highly sensitive, selective, simple and rapid method for the determination of CV would be important.

As a means of detection, room temperature phosphorimetry (RTP) has excellent features, such as a large Stokes' shift and long light-emission life, and it is easy to reduce or eliminate the interference of background fluorescence and scattering. Based on the RTP change, catalytic SS–RTP (LD = 5.2×10^{-20} g clenbuterol/mL) (11), inhibited SS–RTP (LD = 6.5×10^{-12} g terbutaline/mL) (12), SS–RTP sensors (13), phosphorescence switch SS–RTP (14), eosin Y molecular self-assembly SS–RTP (15), ion imprinting SS–RTP (16), ion association SS–RTP (17), SS–RTP immunoassay (18) and affinity adsorption SS–RTP (19) have been established. This work

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has shown the distinct advantages of SS–RTP, such as lower sample and reagent consumption, flexible operation, high sensitivity, and high selectivity. Catalytic SS–RTP, molecular self-assembly SS–RTP, SS–RTP immunoassay and ion association SS–RTP improved sensitivity by increasing the RTP signal from the biological target through a signal amplification effect of a catalytic reaction, eosin Y molecular self-assembly, use of dibromofluorescein nanospheres and the increasing number of luminescence ligands, respectively.

The aim of this study was therefore to develop a simple, rapid, selective and sensitive SS–RTP for the determination of CV. During the experiments we found that amaranth could emit stable RTP on a nitric acid cellulose membrane (NCM) and could be oxidized by NaClO to lead to the quenching of RTP. Amaranth could be further oxidized by chloramine generated from oxidation between CV and NaClO, indicating the activating effect of CV on this process. More importantly, SDBS could sensitize the activation reaction of CV, resulting in the intense quenching of the RTP signal. Compared with the system without SDBS, the ΔI_p of the system with SDBS was enhanced 16.5-fold, which provided a new method to increase the sensitivity of SS–RTP. We studied the feasibility, optimum measurement conditions, analysis parameters and analytical applications of this reaction.

Experimental

Apparatus and reagents

Phosphorescent measurements were carried out on a Perkin-Elmer (Norwalk, CT, USA) LS-55 luminescence spectrophotometer with a solid surface analysis apparatus. The instrument's main parameters were as follows: delay time 0.1 ms; gate time 2.0 ms; cycle time 2.0 ms; flash count 1.0; Ex. slit 10 nm; Em. slit 15 nm; scan speed 1500 nm/min. The acidity of all the systems was measured using a pHS-3B precision acidometer, and a 80-2 centrifuge (Shanghai Surgical Instruments Factory) was adopted for sample treatment. All the materials were weighed using an AE240 electron analytical balance (Mettler Toledo Instruments Co.). A 0.50 μL flat-head micro-injector (±0.010 μL; Shanghai Medical Laser Instrument Plant) was used to introduce the solutions.

CV working (stock) solution (0.10 mg/mL) stock solution was prepared by dissolving 0.0100 g of the drug (Sigma) in 5.00 mL 4.0% m/v ethanol and completing the volume up to 100.00 mL with water. Working standard solutions with CV concentrations in the range 5 – 1000 pg/mL were prepared daily by water dilution of the above stock solution. The solution was stored at 0°C and protected from light. Britton–Robinson (B-R) buffer solution, pH 11.20, was prepared as follows. Into 100 mL of a three-acid mixture (phosphoric, acetic and boric acids, concentration of each 0.040 mol/L), 85.00 mL 0.20 mol/L NaOH was added and mixed. 1.00×10^{-2} mol/L Amaranth (Sigma), 10% NaClO and 2.0% SDBS were also prepared with water for use. All the reagents were AR grade, except that CV was a primary standard regent. The water used was purified by triple quartz sub-boiling distillation.

Filter paper was purchased from Xinhua Paper Corporation (Hangzhou, China); polyamide membrane (PAM), acetylcellulose membrane (ACM) and NCM were purchased from Luqiaosijia Biochemical Plastic Plant. The paper sheets were pre-cut into wafers (diameter 15 mm) and a ring indentation was made at the centre of the strip using a standard pinhole plotter (diameter is 4 mm).

Preparation of human plasma samples

According to the method described previously (20), 10 healthy male volunteers were selected (aged 21.5 ± 1.42 years; height 170.0 ± 4.85 cm; weight 69.2 ± 8.13 kg; no heart, liver, or kidney disease or hypertension); they refrained from alcohol, tobacco and drug use before and during the tests. Plasma samples were collected from the volunteers before the oral dose and after pretest fasting for >10 h, and then each took a 1 mg CV tablet (cat. no. 20090103, Ningbo Tianheng Pharmaceutical Co. Ltd). Plasma samples (5 mL) were collected at 0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 24.0 h after the oral administration (blood sampling time ±2 min). The blood samples were added into centrifuge tubes containing heparin and centrifuged for 15 min at 3000 rpm, then transferred to plastic tubes and stored in the refrigerator at −20°C and measured within a week.

Experimental methods

To a 25 mL colorimetric tube, the CV working solution, 2.00 mL Amaranth, 1.50 mL SDBS, 2.00 mL B-R buffer and 2.00 mL NaClO were added, diluted to 25 mL with water, and then mixed. The colorimetric tube was heated at 50°C for 10 min, and then cooled by flowing water for 5 min. The NCM prepared was immersed in 1.0 mol/L Pb^{2+} solution for 10 s and then dried at 90 ± 1°C for 2.5 min. 0.40 μL test solution was suspended onto the centre of the NCM by a 0.50 μL flat-head micro-injector and then the NCM was dried at 90 ± 1°C for 2.5 min. At the same time, a blank test was also conducted. The phosphorescence intensities of the test solution (I_p) and the reagent blank (I_{p0}) were directly measured at 466/630 nm $\left(\lambda_{ex}^{max}/\lambda_{em}^{max}\right)$, then ΔI_p ($=I_{p0}-I_p$) was calculated.

Results and discussion

Phosphorescence spectra

The phosphorescence spectra of the Amaranth-NaClO-B-R buffer-SDBS-CV system are shown in Figure 1. As Table 1 shows, under the experimental conditions of 50°C and 10 min, Amaranth emitted RTP on the surface of the NCM with 1.0 mol/L Pb^{2+} as ion perturber, which increased the transition probability of Amaranth

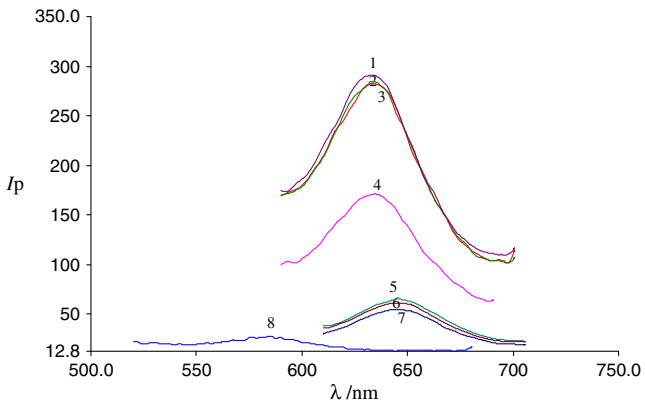

Figure 1. RTP spectra for the Amaranth-NaClO-B-R buffer–SDBS–CV system. Experimental conditions: sensitizer, SDBS; solid substrate, NCM; perturber, Pb^{2+}; pH range, 10.88–11.40; reaction temperature, 50°C; reaction time, 10 min; desiccation temperature, 90°C; desiccation time, 2.5 min; time for passing drying N_2, 10 min.

Table 1. RTP characteristics of Amaranth-NaClO-B-R buffer-SDBS-CV system

Curves of the system	λ_{em}^{max} (nm)	I_p	ΔI_p
1. 2.00 mL Amaranth + 2.00 mL B-R buffer + 1.50 mL SDBS	633.6	291.3 (I_{p0})	
2. 1 + 2.00 mL NaClO	633.9	284.8 (I_{p2})	6.5
3. 2 + 5.0 pg CV	633.6	282.2 (I_{p1})	2.6
4. 2 + 1000.0 pg CV	634.8	171.3 (I_{p1})	113.5
5. 2.00 mL Amaranth + 2.00 mL B-R buffer	644.7	65.2 (I_{p3})	
6. 5 + 2.00 mL NaClO	644.3	61.4 (I_{p4})	3.8
7. 6 + 1000.0 pg CV	644.6	54.9 (I_{p5})	6.5
8. NCM	584.6	28.3	

from the singlet to triplet state (Fig. 1, curve 5, $\lambda_{em}^{max} = 644.7$ nm, $I_p = 65.2$). When NaClO was added, Amaranth was oxidized and the RTP signal of the Amaranth-B-R buffer system was quenched (Fig. 1, curve 6, $\lambda_{em}^{max} = 644.3$ nm, $I_p = 61.4$, $\Delta I_p = 3.8$), and when 1000.0 pg CV was present, the RTP signal of the Amaranth-NaClO-B-R buffer system was further quenched (Fig. 1, curve 7, $\lambda_{em}^{max} = 644.6$ nm, $I_p = 54.9$, $\Delta I_p = 6.5$), indicating its activating effect on this oxidation. The ΔI_p was small, however, in the presence of SDBS, the RTP of the Amaranth-NaClO-B-R buffer-CV system was intensely quenched (Fig. 1, curve 4, $\lambda_{em}^{max} = 634.8$ nm, $I_p = 171.3$) and the ΔI_p of this system was 113.5 and was increased 16.5-fold (113.5/6.5). The λ_{em}^{max} had a blue shift of 11.1 nm, which could be due to the micelle formed by SDBS and Amaranth. This experimental finding provided the basis for the determination of CV by SS–RTP, using a sensitizer to activate NaClO to oxidize Amaranth.

Optimum measurement condition

Parameters of the apparatus. For the system containing 0.80 fg CV/spot, the effects of instrumental parameters on the ΔI_p of the system were studied and are listed in Figs 2 and 3; relative standard deviation (RSD; %) for each was ≤5; $n = 6$. The value of ΔI_p reached a maximum when the instrumental parameters were as follows: delay time 0.10 ms; gate time 2.0 ms; cycle time 2.0 ms; and flash count 1.0.

Optimization of working conditions. For the system containing 0.80 fg CV/spot, the effects of concentrations and volumes of the reagents, reaction acidity, reaction temperature and time, solid substrate, sensitizer, perturber and the time needed for passing dried N_2 on the ΔI_p in the system and the corresponding RSDs (%) of ΔI_p were investigated (Table 2). The results show that the ΔI_p of the system reached the maximum and the corresponding RSDs of ΔI_p were within ±5% when 2.00 mL 1.0×10^{-4} mol/L Amaranth, 2.00 mL 0.50% NaClO, 1.50 mL 1.00% SDBS, 2.00 mL B-R, pH 10.88, and 1.00 mol/L Pb^{2+} were used; the pH value of the system was 10.88–11.40, the reaction temperature and time were 50°C and 10 min, desiccation temperature and time were 90°C and 2.5 min, respectively, NCM was the solid substrate, SDBS was the sensitizer, Pb^{2+} was the perturber, and the N_2 flow time was 10 min. Under optimum measurement conditions, the ΔI_p of the system remained almost unchanged within 5–35 min after being cooled by flowing water for 5 min.

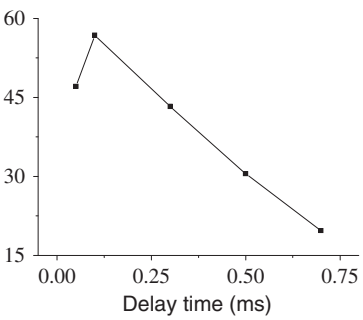

Figure 2. Correlation curve between delay time and ΔIp of the Amaranth-NaClO-B-R buffer–SDBS–CV system.

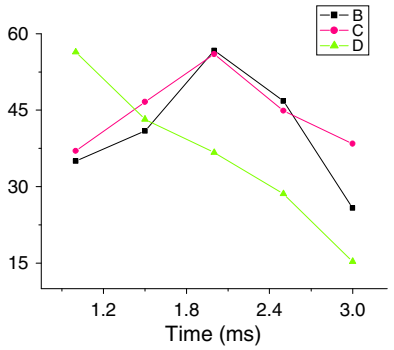

Figure 3. Correlation curve between gate time (B), cycle time (C) and flash count (D) and the ΔI_p of the Amaranth-NaClO-B-R buffer–SDBS–CV system.

Kinetic constants

For the system containing 0.80 fg CV/spot, $1/T$ was positively correlated with $-\log[\log I_{p0}/I_p]$ in the range 303–323 ^{0}K, and the regression equation was:

$$\log[\log I_{p0}/I_p] = -7.284 + 2.682 \times 1000/T$$

where r is 0.9963, T is 323 ^{0}K, E is 69.04 kJ/mol, calculated by $k \times (1/T) \times 1000 \times 8.314$. Meanwhile, t was linear with $\ln I_{p0}/I_p$ in the range 2–10 min, and the regression equation was:

$$\ln I_{p0}/I_p = -0.0259 + 0.0218\,t(\text{min})$$

where r is 0.9996, t is 10 min, k is 3.580×10^{-4} s^{-1}, calculated by $1/t \times \ln I_{p0}/I_p$.

Working curve, linear range and LD

Under the optimum conditions, the CV concentration was proportional to the ΔI_p of the system. The linear range, regression equation limit of detection (calculated based on 3Sb/k where Sb is the standard deviation and k the slope of the working curve), and limit of quantiation (LOQ, calculated by 10 Sb/k) and RSDs (%) and the comparison with previously described methods (7,10) are listed in Table 3.

Interference test

Under the optimum conditions, for the system containing 0.80 fg CV/spot (corresponding concentration was 20.0 pg CV/mL),

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Table 2. The effects of the concentrations and volumes of reagents, reaction acidities, time and temperature for reaction (RT, RTEM), solid substrate, sensitizer, perturber and the N_2 flow time on ΔI_p of the system when the desiccation temperature and time were 90°C and 2.5 min, respectively

Reagents	Conditions	The ΔI_p in Amaranth-NaClO-B-R buffer–SDBS–CV system	RSD (%)	Optimal
Amaranth (mol/L)	10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}	41.9, 48.8, 54.1, 35.7, 30.3	2.1, 1.8, 1.4, 2.5, 2.9	10^{-4} mol/L
Amaranth (mL)	0.50, 1.00, 1.50, 2.00, 2.50, 3.00	25.5, 36.1, 43.4, 54.8, 49.3, 38.6	3.4, 3.1, 2.0, 1.2, 1.7, 2.7	2.00 mL
NaClO (%)	0.010, 0.050, 0.10, 0.50, 1.00, 1.50	16.5, 26.4, 44.2, 54.0, 48.5, 39.7	3.7, 3.1, 1.9, 1.1, 1.7, 2.6	0.50%
NaClO (mL)	0.50,1.00, 1.50, 2.00, 2.50, 3.00	17.8, 33.9, 47.5, 54.4, 50.7, 41.2	3.5, 2.6, 1.8, 1.3, 1.5, 2.2	2.00 mL
SDBS (%)	0.050, 0.10, 0.05, 1.00, 1.50, 2.00	15.7, 27.3, 42.5, 54.3, 46.0, 33.8	3.8, 3.1, 2.0, 1.2, 1.8, 2.7	1.00%
SDBS (mL)	0.50,1.00, 1.50, 2.00, 2.50	22.4, 41.8, 54.6, 47.5, 35.6	3.2, 1.3, 1.1, 1.6, 2.5	1.50 mL
B-R (mL) RSD (%)	0.50,1.00, 1.50, 2.00, 2.50, 3.00	28.4, 36.3, 49.1, 54.7, 51.2, 48.7	3.0, 2.4, 1.7, 1.3, 1.6, 1.9	2.00 mL
Pb^{2+} (mol/L) RSD (%)	0.10, 0.50, 1.00, 1.20	39.5, 47.2, 54.0, 50.9	1.7, 1.5, 1.1, 1.3	1.00 mol/L
pH	6.09, 8.35, 10.38, 10.88, 11.20, 11.40, 11.58	16.7, 25.3, 44.1, 54.8, 54.4, 54.2, 49.0	3.7, 3.4, 1.5, 1.1, 1.3, 1.2, 2.0	10.88–11.40
RT (min)	3, 5, 7, 10, 12, 15, 18	17.8, 28.1, 40.6, 54.5, 47.3, 42.7, 36.4	3.6, 3.1, 2.0, 1.2, 1.6, 1.8, 2.1	10 min
RTEM (°C)	30, 35, 40, 45, 50, 55, 60	17.2, 24.0, 33.5, 41.8, 53.8, 46.4, 37.5	3.7, 3.5, 2.3, 2.0, 1.3, 2.1, 2.4	50°C
Solid substrate	Paper, NCM, PAM, ACM	23.9, 53.4, 27.8, 41.7	3.3, 1.3, 3.0, 2.0	NCM
Sensitizer	CPC, CPB, CTAB, SDBS, CMC, PAM, PEG, TritonX-100, Tween-80	25.6, 35.0, 23.5, 54.6, 30.6, 40.1, 26.0, 48.9, 43.9	3.1, 2.7, 3.4, 1.1, 2.9, 2.3, 3.0, 1.9, 2.1	SDBS
Perturber RSD (%)	Pb^{2+}, Cu^{2+}, Ag^{+}, Li^{+}, I^{-}	53.8, 38.7, 30.3, 22.9, 18.6	1.2, 1.8, 2.7, 3.5, 3.7	Pb^{2+}
Passing drying N_2 (min)	5, 10, 20, 30, 40, 50	54.3, 54.8, 54.6, 54.5, 54.4, 49.3	1.3, 1.1, 1.2, 1.0, 1.3, 1.7	10 min
Without drying N_2 (min)	5, 10, 20, 30, 40, 50	50.5, 44.6, 40.4, 38.5, 31.3, 28.7	1.5, 1.8, 2.1, 2.4, 2.7, 3.0	

CPC, cetylpyridinium chloride; CPB, cetylpyridinium bromide; CTAB, cetyltrimethylammonium bromide; CMC, sodium carboxymethyl cellulose; PAM, polyacrylamide; PEG, polyethylene glycol.

Table 3. Analysis parameters (Δh is the height of the recorded peak, reflecting the transient variation in the chemiluminescence intensity.)

Method	Linear range	Regression equation	r	RSD (%)	LD (g/mL)	LOQ (g/mL)
This method	0.200–40.0 (pg/mL)	$\Delta I_p = 0.7780 + 7.057\ m_{CV}$ (fg/spot)	0.9976	1.4 – 3.7	5.1×10^{-14}	1.6×10^{-13}
Ref. (7)	0.75–75.0 (ng/mL)	$Y = 0.5199x + 1.6235$	0.9958	1.4	3.00×10^{-10}	
Ref. (10)	0.410–1.20 (μg/mL)	$\Delta h = -10.274\,C - 0.6097$	0.9992	1.3	3.50×10^{-9}	

the allowed concentrations of coexistent ions (error was within ±5%) are listed in Table 4 and were larger than those found for previous methods (21) (0.03 μg CV/mL) and (22) (10.0 ng CV/mL), indicating the better selectivity of this method.

Sample analysis

The CV content of a series of plasma samples was determined according to the experimental method and the results are shown in Figure 4. CV concentration reached a maximum 1.5 h after dosing with a level of 78.91 pg/mL; the lowest measured concentration was 0.80 pg CV/mL, RSD% within ±5% ($n = 5$). The detection results agreed well with the trend found previously (5) and the corresponding Er (%) of CV concentration was within ±5%, indicating the higher accuracy and precision of this method.

Results for a standard addition recovery experiment are listed in Table 5, and these agreed well with those obtained by SF; the recovery rate was 96.4–102% and RSDs were 1.2–1.6%, showing that this method has high accuracy and precision and is suitable for the determination of CV in plasma samples.

Reaction mechanism of sensitizing activated SS–RTP for the determination of CV

In the presence of B-R buffer and SDBS (50°C for 10 min), Amaranth, emitted stable RTP on NCM, but could be oxidized by NaClO and the RTP signal of the system quenched. Amaranth might be oxidized by NaClO to 1,2-dihydroxynaphthalene and 1-amino-2-naphthol and these two materials can not emit RTP

Table 4. Effect of excipients on the determination of CV ($n = 6$)

Coexistent materials	This method		Method of ref. (21)	Method of ref. (22)
	Concentration of coexistent materials (μg/mL)	Er (%)	Concentration of coexistent materials (μg/mL)	Concentration of coexistent materials (μg/mL)
K^+	800	−1.4	500	
Ca^{2+}	800	1.0	100	500
Na^+	800	−2.4	500	500
Mg^{2+}	800	1.3		500
Pb^{2+}	450	−2.2	10	200
Zn^{2+}	800	1.5		500
Ni^{2+}	500	−1.8	50	200
Cu^{2+}	50	2.5	0.1	
Cl^-	800	2.9	500	500
Ac^-	800	1.9	500	
HPO_4^{2-}	800	−1.6	500	500
SO_4^{2-}	800	−1.7	500	500
PO_4^{3-}	800	−1.0		500
HCO_3^-	800	−1.9		500
CO_3^{2-}	800	−2.9		500
NO_3^-	100	−1.4		1.0
Protein	400	−1.1	–	–
Glucose	300	−1.7	–	–
Fat	450	1.0	–	–
Urea	500	2.3	–	–

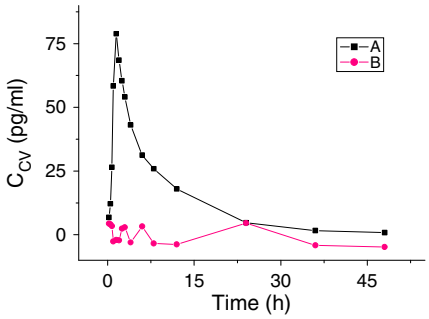

Figure 4. Mean concentration change of CV in plasma of healthy volunteers after 1 mg CV single oral dose; A, CV in plasma; B, corresponding Er (%) of CV concentration; $n = 5$; Time, the time for administration.

signal (23), thus quenching the RTP signal of Amaranth. The reaction is shown in Scheme 1.

If 1000 pg CV was added to the system, NaClO could also oxidize CV and generated chloramine and aromatic ethers (10). The reaction is shown in Scheme 2. The chloramine could further oxidize Amaranth to form naphthalene and 1,2-naphthoquinone (24), accelerating the RTP signal quenching of the Amaranth-NaClO-B-R buffer system (Scheme 3).

However, in the presence of SDBS, the RTP signal of the Amaranth-NaClO-B-R buffer–CV system was intensely quenched (Fig. 1, curve 4, $\Delta I_p = 113.5$) when compared with non-SDBS system ($\Delta I_p = 6.5$), a 17.5-fold increase, demonstrating the strong sensitizing effect of SDBS on CV to activate Amaranth oxidization by NaClO.

In the presence of SDBS, λ_{em}^{max} was blue shifted by 11.1 nm, indicating the formation of a new micellar compound, SDBS-Amaranth, and this led to quenching of RTP providing further evidence of the sensitizing effect of SDBS. SDBS is similar to sodium dodecyl sulphate; Pb^{2+} replaced some sodium ions on the surface of the SDBS-Amaranth micelles and it may increase the probability of intersystem crossing and the population of the triplet state (25).

Conclusion

Sensitizing activated SS–RTP for the determination of CV has been developed and a reaction mechanism is proposed, based on the large quenching of RTP caused by SDBS, sensitizing CV to

Table 5. Analytical results for carvedilol (CV) ($n = 6$)

Sample	Present method (pg/mL)	RSDs (%)	Added (pg/mL)	Obtained (pg/mL)	Recovery (%)	SF (pg/mL)	Er (%)
A	6.54	1.3	1.0	0.983	98.3	6.68	−2.1
B	13.2	1.6	1.0	0.964	96.4	13.4	−1.5
C	22.0	1.3	2.0	2.04	102	21.7	+1.4
D	29.5	1.2	3.0	2.98	99.3	29.8	−1.0
E	37.6	1.3	4.0	4.00	101	37.3	+0.80

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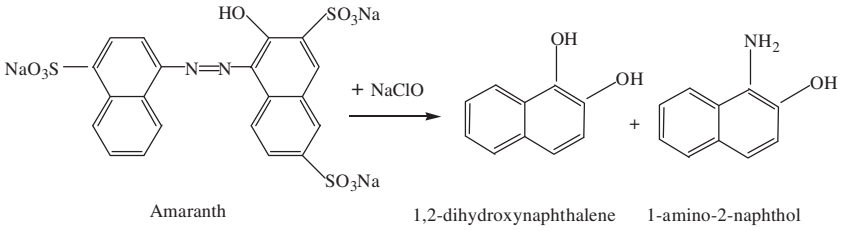
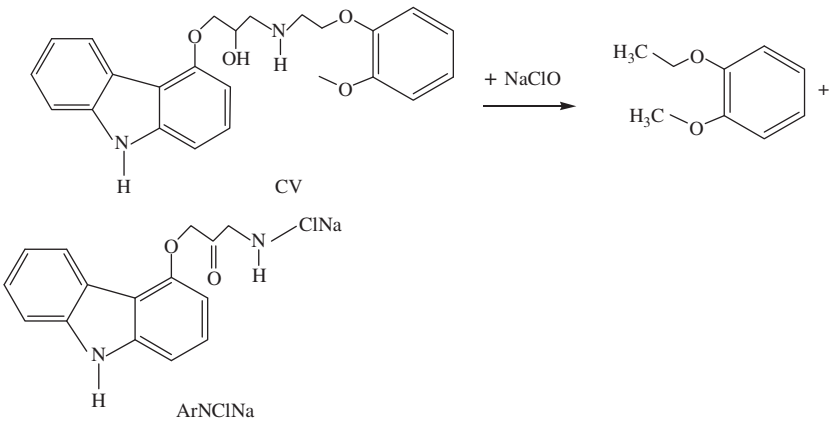
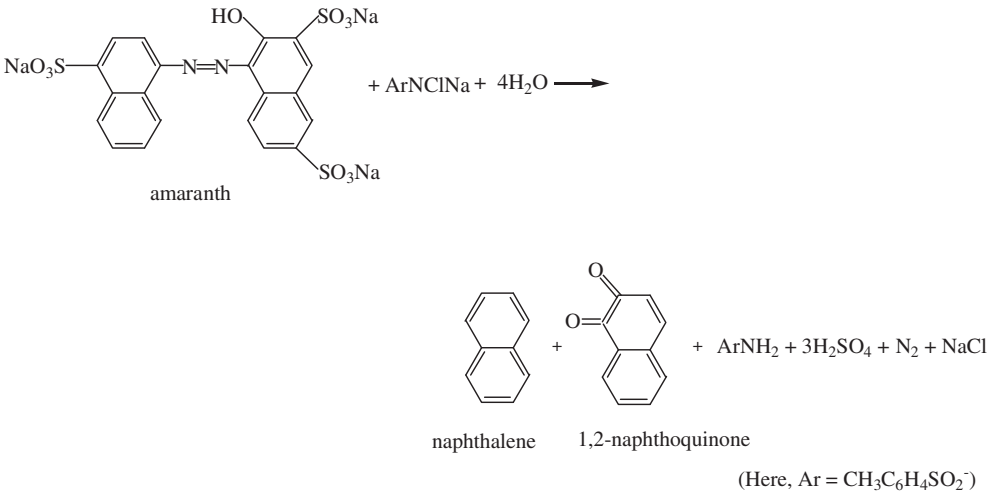


Scheme 1. Redox reaction between Amaranth and NaClO.



Scheme 2. Redox reaction between carvediol (CV) and NaClO.



Scheme 3. Reaction between chloramine and Amaranth.

activate NaClO to oxidize Amaranth. This study has developed a new SS–RTP and CV residue analysis technique, and provided a new method for the detection of doping. It also provides a new way to increase the sensitivity of SS–RTP.

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