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A study of the inhibitory effect of some antioxidants and EDTA synergistic effect on the adrenaline oxidation reaction

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ABSTRACT
The antioxidative effect of ($5x10^{-6}$ – $1x10^{-3}$) $mol.L^{-1}$ of some additives on adrenaline (AD) oxidation in a weak acid media (pH =5.7), in the presence of ethylene diamine tetraacetic acid EDTA has been investigated by spectrophotometric method , based on the formation of the colour oxidation product (adrenochrome) in the light of the knowledge of accelerated AD oxidation reaction kinetics with $K_2Cr_2O_7$ using as oxidant. At a very low additives concentration of about ($5x10^{-6}$) $mol.L^{-1}$ there is almost no inhibitory effect has been observed. However, in the same concentration, EDTA increases the inhibitory effect of additives , the increasing are probably due to change in the mechanism of antioxidant oxidation.

INTRODUCTION

One of the important AD reactions is undesirable one , the reaction with atmospheric oxygen (autoxidation) to form oxidation products, wich change it’s biological activity(1) .

Adrenalin (epinephrine) is one of catecholamines with a wide spectrum of biological activity (2-5) frequently employed in therapeutic applications (6-7), and this is reflected by the variety of it’s pharmaceutical dosage forms .

The quantitative AD determination and it’s pharmaceutical preparations storage is complicated by it intrinsic susceptibility to autoxidation , and the need for stabilization with antioxidants (7-10) . Few analytical methods are available for AD determination , for it’s reducing character these methods have been reviewed in the articles(1-15) . Almost all reports have been described the AD biological activity , therapeutic effect , and analysis techniques , whereas little articles were published for AD antioxidants(7-10) .

In this paper kinetic studies of AD oxidation based on the adrenochrome formation were carried out spectrophotometrically , using potassium dichromate($K_2Cr_2O_7$) as an oxidant , in the presence of some sulfur salts and metals as primary antioxidants , and EDTA as synergist at 25°C . This model system , using AD as a basic substratum , has been proposed for testing the antioxidative effect of chemical compounds which can be used in pharmaceutical applications(9) .

EXPERIMENTAL SECTION

Apparatus: Philips PU8620 series.UV/VIS spectrophotometer with special quartz cells (1Cm width and 10 Cm length). pH measurements were recorded by wtw pH DIGT 520 meter fitted with a pH combination electrode .

HAAKE cooling system was used for cooling , and flawil magnetic stirrer , type H-9230/SG was used for stirring .

Preparation of materials

AD stock solution (1000 $mg.L^{-1}$) was prepared by dissolving 0.1 g of analytical reagent grade adrenaline (Searle Chemical) in 100ml of 0.02M HCl solution.

Potassium dichromate solution (0.02M) was prepared by dissolving 0.588 g of $K_2Cr_2O_7$ (BDH Chemicals) in 100 ml distilled water .

EDTA, sulfur salts , and metals salts stock solutions (0.01M) were prepared by dissolving 0.2922, 0.104, 0.2481, 0.741, 0.1901, and 0.2221 g of EDTA, $NaHSO_3$, $NaS_2O_3.6H_2O$, $Na_2S_2O_4$, $Na_2S_2O_5$, and $Na_2S_2O_7$ (BDH Chemicals) respectively, and 0.0989, 0.2496, 0.2033, 0.2361, 0.2116, 0.2442, 0.2875, 0.1333, 0.3921 and 0.4040 g of Cucl, $CuSO_4.5H_2O$. $Mgcl_2.6H_2O$, $Ca(NO_3)_2.4H_2O$, $Sr(NO_3)_2$, $Bacl_2.H_2O$, $ZnSO_4.7H_2O$, $Alcl_3$, $FeSO_4.(NH_4)_2.SO_4.H_2O$, and $Fe(NO_3)_2.H_2O$ respectively in 100 ml distilled water. ($1x10^{-3}$) and ($1x10^{-4}$) $mol.L^{-1}$ solutions were prepared by dilution.

Procedures

Absorption spectra

A 10 ml studying sample was prepared by addition of 0.5 ml AD stock solution to 9 ml distilled water in the quartz cell , after addition of 0.5 ml of $K_2Cr_2O_7$, the adrenochrome colour was developed gradually , and the AD oxidation product (adrenochrome) completely formed after 15 minutes , then the absorbance was at (200-600)nm . After 12 hr. adrenochrome was completely converted to the additional oxidation product (Adrenolutine) , then it's absorption spectrum was recorded (Fig 1).

AD Oxidation Kinetic study

A 10 ml studying sample was prepared as above , but the absorbance was recorded at each minute (A_t) at 490 nm , immediately after the addition of 0.5 ml of $K_2Cr_2O_7$ and the constant absorbance value (A_o) was recorded after 15 minute.

This procedure was repeated at various temperatures between (10-35)°C (Table 1 , Fig 2).

Additives antioxidative effect study

The studied samples consist of 0.5 ml AD stock solution + V_{ml} of additives stock solution to make there concentrations (5×10^{-6} , 5×10^{-5} , 5×10^{-4} and 1×10^{-3}) $mol.L^{-1}$, the volume was completed to 9.5ml with distilled water , and absorbance was recorded after 0.5 ml $K_2Cr_2O_7$ addition for each minute (A_t) at 490 nm , and the constant absorbance value (A_o) was recorded after 15 minute . This procedure was repeated for (5×10^{-6} – 1×10^{-3}) $mol.L^{-1}$ of each additives .

Synergistic effect study

The studied samples consist of 0.5 ml AD stock solution + V_{ml} of additives stock solutions to make there concentrations (5×10^{-6}) $mol.L^{-1}$ + V_{ml} of EDTA stock solution made its concentrations (5×10^{-6} , 5×10^{-5} , 5×10^{-4} and 5×10^{-3}) $mol.L^{-1}$, then the volume was completed to 9.5 ml with distilled water , and the procedure completed as above .

Table (1) : AD oxidation reaction velocity constants values (K_1) at different temperature at (490) nm .

T °C →	10°	25°	28°	30°	32°	35°
Time (min) ↓	K_1(min)					
1	0.0912	0.1321	0.1456	0.1681	0.2035	0.2835
1.5	0.0966	0.1328	0.1473	0.1647	0.2016	0.2745
2	0.0941	0.1333	0.1455	0.1613	0.2074	0.2766
3	0.1005	0.1335	0.1476	0.1707	0.2186	0.2642
3.5	0.1011	0.1388	0.1488	0.1681	0.2162	0.2341
4	0.1021	0.1330	0.1489	0.1662	0.2087	0.2513
Mean	0.0976	0.1330	0.1472	0.1665	0.2093	0.2641

RESULTS AND DISSCUSION

Fig 1 shows the absorption spectrum of AD and its oxidation products at (200 – 600) nm at 25°C .

Fig 2 shows the relation between AD oxidation reaction rate constants (K_1 min^{-1}) and temperature.

Table 1 shows the oxidation rate constants at various temperature between (10-35) °C .
Table 2 shows the rate constants of AD oxidation reaction in the presence of (5×10^{-6} - 1×10^{-3}) $mol.L^{-1}$ of sulfur salts and metals at 25°C and 490 nm .

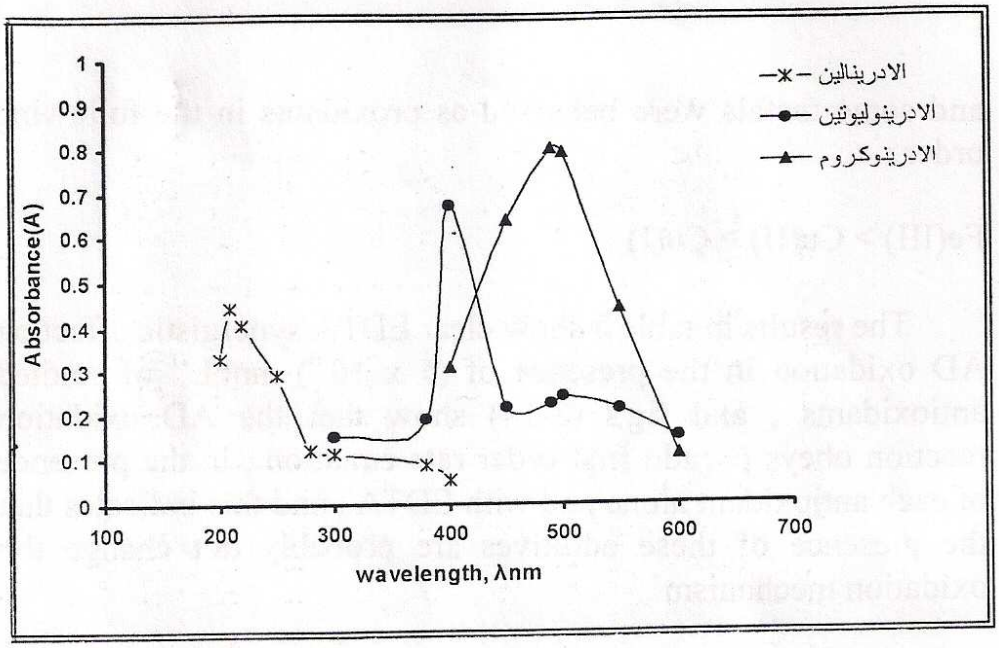

Fig 1 : Absorption spectra of AD and oxidation products at 25°C .

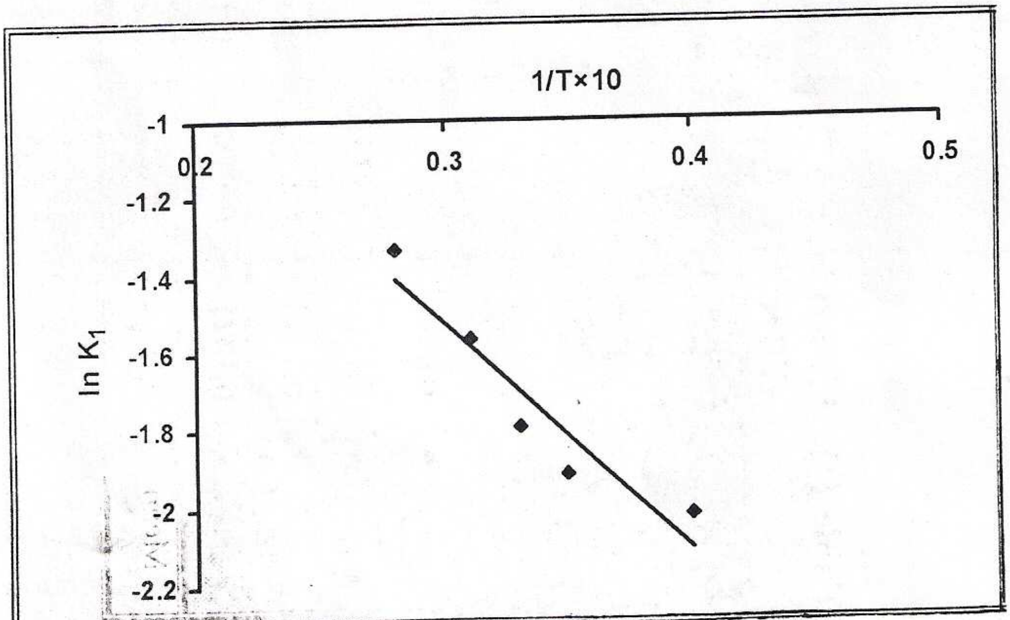

Fig 2 : The relation between rate constants and temperature .

Table (2): Oxidation rate constants mean in the presence of studied additives

A.C →	$5X10^{-6}$	$5X10^{-5}$	$5X10^{-4}$	$1X10^{-3}$
Additives ↓	K_1			
$NaHSO_3$	0.1298	0.1128	0.0569	0.0375
$Na_2S_2O_3$	0.1300	0.0894	$1.049x10^{-3}$	$3.621x10^{-4}$
$Na_2S_2O_4$	0.1098	0.0920	0.0418	$1.663x10^{-3}$
$Na_2S_2O_5$	0.1087	0.0913	0.0296	$2.008x10^{-3}$
Na_2SO_3	0.1114	0.0988	0.0332	$1.047x10^{-2}$
$NaHSO_4$	0.1040	0.1240	0.1610	0.1789
Mg (II)	0.1306	0.1279	0.1241	0.1052
Ca (II)	0.1062	0.1064	0.1028	0.0863
Sr (II)	0.1012	0.1003	0.0773	0.0643
Ba (II)	0.0725	0.0878	0.1121	0.1674
Zn (II)	0.1322	0.1308	0.1300	0.1276
Al (III)	0.0782	0.0710	0.0663	0.0624
Se	0.1072	0.0246	0.0203	$4.92x10^{-3}$
Fe (II)	0.1195	0.0854	-	-

Table (3): AD oxidn. Reaction specific velocities in the presence of ($5x10^{-6}$) $mol.L^{-1}$ additives and EDTA at (490) nm .

EDTA .C →	$5X10^{-6}$	$5X10^{-5}$	$5X10^{-4}$	$1X10^{-3}$
Additives ↓	K_1			
EDTA	0.1330	0.1351	0.1035	0.0823
$NaHSO_3$	0.1158	0.0881	0.0802	0.0610
$Na_2S_2O_3$	0.1329	0.1222	0.0959	0.0797
$Na_2S_2O_4$	0.1138	0.0813	0.0147	$3.303x10^{-3}$
$Na_2S_2O_5$	0.1100	0.0935	0.0701	0.0650
Na_2SO_3	0.1245	0.1089	0.0720	0.0558

$NaHSO_4$	0.0907	0.0870	0.0620	0.0582
Cu(I)	0.1473	0.0952	0.0694	0.0491
Cu(II)	0.1175	0.0986	0.0648	0.0647
Mg (II)	0.1188	0.0987	0.0681	0.0524
Ca (II)	0.1052	0.0812	0.0597	0.0587
Sr (II)	0.0963	0.0813	0.0617	0.0457
Ba (II)	0.0718	0.0629	0.0637	0.0439
Zn (II)	0.1320	0.1268	0.0799	0.0759
Al (III)	0.0823	0.0634	0.0492	0.0391
Se	0.1147	0.1029	0.0870	0.0702
Fe (II)	0.1142	0.1147	0.0757	0.0685
Fe (III)	0.0823	0.0743	0.0448	0.0422

Table 3 shows the oxidation reaction rate constants in the presence of (5×10^{-6}) mol.L^{-1} of studied additives and ($5x10^{-6}$ - $1x10^{-3}$) mol.L^{-1} EDTA at 25°C and 490 nm.

Fig's (3-14) shows linear relation between lnX (X= A_o/A_o-A_t) and the time , according to pseudo first order rate equation $K_1 = 1/t \ln (A_o/A_o-A_t)$ for studied sulfur salts , and the same linear relation was found for studied metals .

CONCLUSION

For AD oxidation reaction , there may be two consecutive steps give two coloured products(9,10) :

AD $\xrightarrow{K_1}$ *Adrenochrome* $\xrightarrow{K_2}$ *Adrenolutine*

The absorption spectra (Fig 1) shows characteristic maxima at 490nm for adrenochrome , whereas adrenolutine at 400 nm.

The AD oxidation reaction kinetic has been studied spectrophotometrically without additives (K_1 = 0.1333min^{-1}) at 25°C and 490 nm , and in the presence of selective sulfur salts and metals .

Table 1 and Fig 2 prove that the oxidation rate (K_1) increases with temperature increasing , and the best temperature for AD storage is 10°C.

The results in table 2 show the comparison of selective sulfur salts inhibitory effect with $NaHSO_3$ effect , these results indicate that the inhibition degree of $Na_2S_2O_n$ salts was more than that of $NaHSO_3$ according to the following order:

$Na_2S_2O_3 > Na_2S_2O_5 \sim Na_2S_2O_4 > Na_2SO_3 > NaHSO_3 > NaHSO_4$

The sulfites , such as $NaHSO_3$ are very commonly used , and a word of caution is in order . In the process of acting as antioxidants , sulfites yield acid sulfates which cause a drop in pH values :

$$2HSO_3^{-} + \frac{3}{2}O_2 \longrightarrow 2SO_4^{2-} + H_2O$$

Sulfites can readily form inactive addition compounds as with , for example ,adrenaline . Also they react with alkenes , alkylhalides, aromatic nitro compounds , and carbonyl compounds , some times , as with thiamine they may cleave molecules . Thus not all antioxidants can be used with all drugs and correct choice is usually based on extensive stability testing , as is done in industrial product development laboratories (1).

Also the studied sulfur salts have less side effects (16) .Thus they can be used instead of $NaHSO_3$ as safty primary antioxidants at very low concentration for storage food and drugs .

$Na_2S_2O_7$ has no reducing character , so it behaives as proxidant increases AD oxidation rate .

The inhibition degree of studied metals was in the following order :

Se > Al (III) > Sr (II) > Ba (II) > Ca (II) > Fe (II) > Mg (II) > Zn (II)

and same metals were behaived as proxidants in the following order :

Fe(III) > Cu(II) > Cu(I)

The results in table 3 show clear EDTA synergistic effect on AD oxidation in the presence of (5×10^{-6}) $mol.L^{-1}$ of studied antioxidants , and fig's (3-14) show that the AD oxidation reaction obeys pseudo first order rate equation , in the presence of each antioxidant alone and with EDTA , and this indicates that the presence of these additives are probably not change the oxidation mechanism .

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