

## Transnitrosation reaction between captopril and sodium nitroprusside

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### Abstract

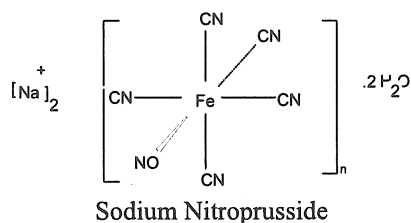
In this method transfer of nitric oxide from Sodium nitroprusside to Captopril has been observed by using UV-visible spectrometric measurements in tris buffer at various pH such as pH=6, pH=7.4 and pH=9. Reaction was studied at various conditions for instance with stirring at room temperature or constant heating with stirring and continuous stirring at different temperatures in various buffer. The reaction was completed in 6 days at pH 7.4, 2 days at pH 6 and 3 days at pH 9.

**Keywords:** Nitric Oxide (NO), sodiumnitroprusside (SNP), tris buffer.

### Introduction

S-nitrosothiols are a group of potent, bioactive compounds that form through the reaction of nitric oxide (NO) with thiols in the presence of oxygen. These compounds are naturally occurring *in-vivo*, stabilize NO and potentiate its biological effects. Transfer of NO to thiols occur very readily in aqueous solution particularly at pH > 8. (Wang et al. 2001, Rucki 1977). The results are consistent with attack by thiolate anion at nitroso nitrogen atom of sodium nitroprusside thiolate anion at SNP.

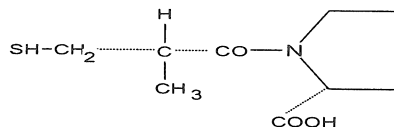
NO transfer from one NO donor, especially S-nitrosothiols, to another thiol has been a focus of interest due to their role in NO transfer *in-vivo*. Mechanistic and kinetic studies on transnitrosation from RSNOs to thiols have been reported previously by Williams, Meyer, Rossi, Means, Whorton and Hogg. As an effort to offer a thermodynamic view of NO transfer *in-vitro*, we reported herewith our investigation on transnitrosation reaction between Captopril and Sodium nitroprusside in different conditions as well as pH (Wang et al. 2001).



Sodium nitroprusside is disodium pentacyanonitrosylferrate (2-) dihydrate. It is also known Sodium nitroferricyanide and Sodium nitroprussiate. It is red-brown, practically odorless, crystals or powder (Moynihan et al. 1994).

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Captopril, an ACE inhibitor with formula  $C_9H_{15}NO_3S$  and also known as Acepril, Capoten, Lapirin. It is a white crystalline powder having a melting point  $106^\circ\text{C}$  and is freely soluble in chloroform, ethanol and methanol (Kerr 1993).



Captopri

## Materials and Methods

A Perkin Elmer EZ-301 UV-visible spectrometer was used for spectral measurement. All the chemicals used were of analytical grade. The purity of synthesized compounds were ascertained by TLC using iodine vapours as visualizing agents. Double distilled water was used for preparation of buffers and drug solutions.

*Nitrosocaptopril preparation:* Captopril was scanned in 0.1 N NaOH and exhibited max wavelength at 237 nm and then a calibration curve was plotted at same wavelength. TLC profile of authentic drug was carried out (Kerr 1993).

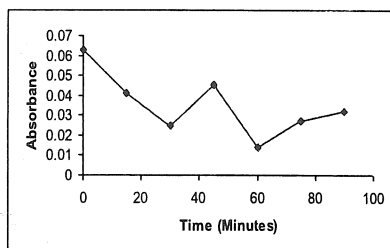
S-nitrosocaptopril was prepared in laboratory by reacting equimolar amounts (29mM) of sodium nitrite and captopril in aqueous solution. The solution was adjusted to pH 2 with 6M HCl and incubated at  $37^\circ\text{C}$  for 5 minutes, in mean time a characteristic orange color developed. Then, sample was neutrallised to pH 7.0 with NaOH. Sample was scanned by using UV spectrophotometer and exhibited max at 331.5 nm, which was different from captopril itself (Ignarro 2003).  $R_f$  value of S-nitrosocaptopril obtained was compared with  $R_f$  value of standard drug i.e. (0.394 for S-nitrosocaptopril and 0.58 for captopril) (Kerr 1993).

S-nitrosocaptopril reacted with Griess reagent and pink color was observed, indicating formation of S-nitroso derivative.

$\lambda_{\text{max}}$  of sodium nitroprusside was observed at 401nm before reacting with Captopril at the conc. of 70mg/100ml in distilled water. Sodium nitroprusside exhibited no fluorescence in acidic, basic or neutral media (Doni Al-Sa' 2002).

*Reaction of captopril (100mcg/mL) with sodium nitroprusside in tris buffer, At pH 7.4 [2, 3]:*  $\lambda_{\text{max}} = 331$  nm obtained at different conditions such as stirring at room temperature or constant heating with stirring and continuous stirring at different temperatures at a interval of 15 minutes.

- a) *Stirring at room temperature:* The reaction mixture was treated with Griess Reagent and no change in color was observed as shown in Graph 1, Table 1.

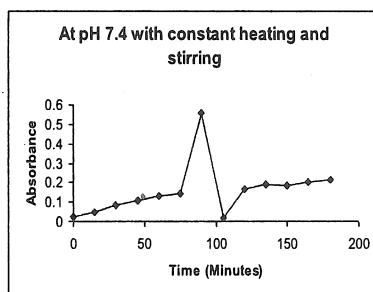


Graph 1. Reaction at pH 7.4

**Table 1. Reaction at pH 7.4**

S.No	Time (min)	Temp (°C)	Abs.
1	0	16	0.026
2	15	20	0.048
3	30	20	0.085
4	45	20	0.109
5	60	20	0.130
6	75	20	0.142
7	90	20	0.56
8	105	20	0.0160
9	120	20	0.167
10	135	20	0.189
11	150	20	0.187
12	165	20	0.201
13	180	20	0.214

- b) *At constant temperature and stirring (first day of reaction):* The reaction mixture was treated with Griess Reagent and purple color was developed within 5-10 minutes as shown in Graph 2, Table 2.

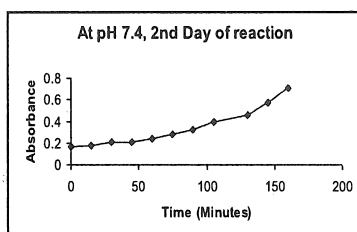


**Graph 2. Reaction at pH 7.4**

**Table 2. Reaction at pH 7.4**

S.No	Time (min)	Abs.
1	0	0.063
2	15	0.041
3	30	0.025
4	45	0.045
5	60	0.014
6	75	0.027
7	90	0.032

On 2<sup>nd</sup> day, the reaction mixture was again treated with Griess Reagent and purple color was observed as shown in Graph 3, Table 3.

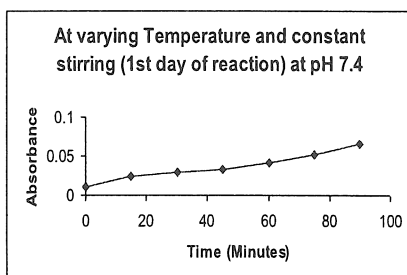


**Graph 3. Reaction at pH 7.4**

**Table 3. Reaction at pH 7.4**

S.No	Time (min)	Temp (°C)	Abs.
1	0	37	0.165
2	15	37	0.174
3	30	37	0.212
4	45	37	0.204
5	60	37	0.240
6	75	37	0.284
7	90	37	0.323
8	105	37	0.395
9	130	37	0.462
10	145	37	0.575
11	160	37	0.704

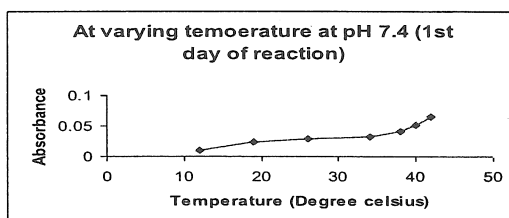
- a) *With continuous stirring at different temperatures (first day of reaction):* The reaction was not increased in starting but after sometime it increases continuously as shown in Graph 4, Table 4.



**Graph 4. Reaction at pH 7.4**

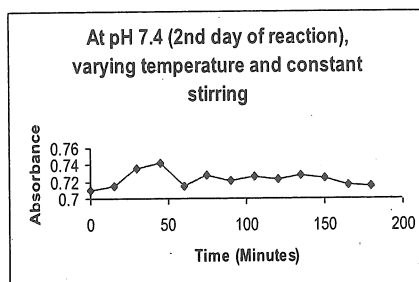
**Table 4. Reaction at pH 7.4**

S.No	Time (min)	Temp (oC)	Abs.
1	0	12	0.010
2	15	19	0.024
3	30	26	0.030
4	45	34	0.033
5	60	38	0.042
6	75	40	0.051
7	90	42	0.066



**Graph 4.1. Reaction at pH 7.4**

*On 2<sup>nd</sup> day of reaction:* The reaction was stopped and color developed upto 2 minutes on treatment with Griess Reagent as shown in Graph 5, Table 5.

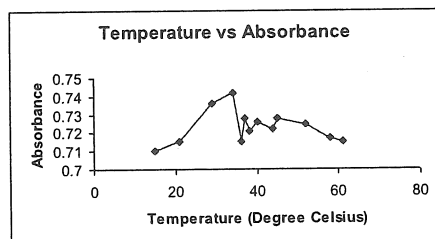


**Graph 5.** Reaction at pH 7.4

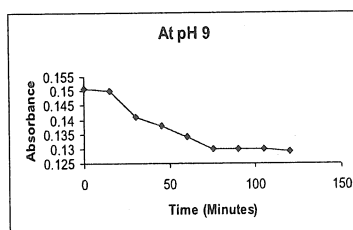
**Table 5.** Reaction at pH 7.4

S.No	Time (min)	Temp (°C)	Abs.
1	0	15	0.710
2	15	21	0.715
3	30	29	0.736
4	45	34	0.742
5	60	36	0.715
6	75	37	0.728
7	90	38	0.721
8	105	40	0.726
9	120	44	0.722
10	135	45	0.728
11	150	52	0.725
12	165	58	0.717
13	180	61	0.715

**Graph 5.1.** Reaction at pH 7.4



*At pH 9, Only with constant stirring at room temperature:* The reaction continuously decreased and developed no color on treatment with Griess Reagent and reaction completed within 2 days as shown in Graph 6, Table 6.



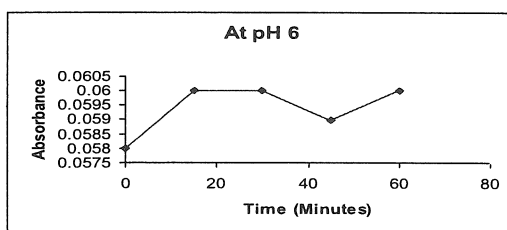
**Graph 6.** Reaction at pH 9

**Table 6. Reaction at pH 9**

S.No	Time min)	Abs.
1	0	0.151
2	15	0.150
3	30	0.141
4	45	0.138
5	60	0.134
6	75	0.130
7	90	0.130
8	105	0.130
9	120	0.129

*At pH 6:*

- a) *Only at constant stirring at room temperature:* No reaction occurred only with stirring as shown in Graph 7, Table 7.

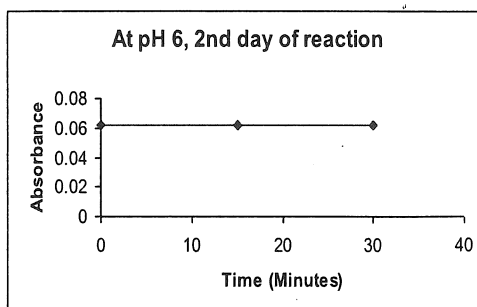


**Graph 7. Reaction at pH 6**

**Table 6. Reaction at pH 6**

S.No	Time (min)	Abs.
1	0	0.058
2	15	0.060
3	30	0.060
4	45	0.059
5	60	0.060

- b) *With constant heating and stirring:* No change in reaction rate was observed as shown in Graph 8, Table 8.

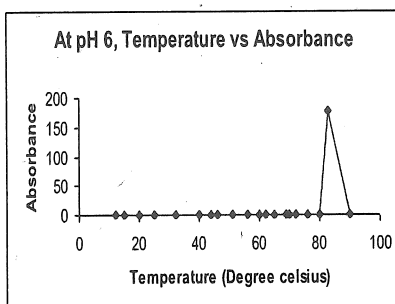


**Graph 8. Reaction at pH 6**

**Table 8. Reaction at pH 6**

S.No	Time (min)	Abs.
1	0	0.062
2	15	0.062
3	30	0.062

- c) With continuous stirring at different temperatures: The reaction first increased and then became constant. The reaction mixture on treatment with Griess Reagent gave purple color as shown in Graph 9, Table 9.

**Graph 9. Reaction at pH 6****Table 9. Reaction at pH 6**

S.No	Time (min)	Temp (°C)	Abs.
1	0	12	0.0988
2	15	15	0.110
3	30	20	0.110
4	45	25	0.116
5	60	32	0.120
6	75	40	0.122
7	90	44	0.121
8	105	46	0.128
9	120	51	0.128
10	135	56	0.133
11	150	60	0.137
12	175	62	0.141
13	190	65	0.157
14	210	69	0.163
15	225	70	0.166
16	240	72	0.177
17	255	76	0.179
18	270	80	0.179
19	285	83	0.177
20	300	90	0.175
21	315	90	0.172

## Results and Discussion

Sodium nitroprusside releases nitric oxide very slowly, when exposed to light; it decomposes to yield Prussian blue sodium salt, HCN and NO. Being slow releaser of NO, it reacts very slowly with thiols on heating. At pH 7.4, the reaction was completed within six days. When stirred at

room temperature no continuous increase in reaction was observed. However, when heated above 37° C, primary thiols such as Captopril, the nitrosothiols formed and were changed to disulphide. But, formation of RSSR' is also indicative of the formation of RSNOs. Hence, temperature plays an important role in reaction of Sodium nitroprusside with captopril.

The reaction completed within two or three days at alkaline or acidic pH such as 9 or 6 respectively, because RSNOs occurs as storage site for NO release only at physiological pH.

### Acknowledgements

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