Bull. Pharm. Sci. Assiut University
Vol. 3, PP. 41- 58

STUDIES ON THE SOLUBILITY OF RIFAMPICIN
II Effect of Some Aromatic Monocarboxylic Acids Sodium Salts, Nicotinamide and Isoniazid on the Water-Solubility of Rifampicin.

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The effect of some salts of aromatic monocarboxylic acids namely, sodium benzoate, sodium p-aminobenzoate, sodium salicylate and sodium p-aminosalicyl - ate as well as nicotinamide and isoniazid on the water solubility of rifampicin was investigated. The apparent water-solubility of rifampicin is augmented markedly in presence of these agents. The solubilizing power of these agents towards rifampicin is highly dependent on the specific type and in most cases proportional to the concentration of the agent. Of the salts of the aromatic monocarboxylic acids, sodium p-aminosalicylate showed the highest solubilizing power followed in order by sodium salicylate, sodium p-aminobenzoate and finally sodium benzoate with the least solubilizing power.

Nicotinamide showed higher solubilizing power than that of isoniazid but much less than that of sodium p-aminosalicylate and sodium salicylate. The solubilizing power of isoniazid is greater than that of sodium benzoate and sodium p-aminobenzoate. The spectral pattern of rifampicin showed a change in optical density in presence of these agents which is proportional to the agent used, suggesting a role of molecular interaction between these agents and rifampicin in the solubilization process.

In many instances, limited or poor water-solubility of drugs presents a formidable problem in formulation of acceptable dosage forms. So, augmenting the water-solubility represents an important goal for the formulator dealing with such type of drugs. Various approaches can be adopted for attaining this goal[1-3]. Hydrotropy as described by Neuberg[4] has been used as an approach to increase the water-solubility of many pharmaceutical substances[3,5-7].

As a class of hydrotropic agents, sodium salts of aromatic monocarboxylic acids represent one of the most effecient class of hydrotropes [4,7-11]. Nicotinamide and isoniazid being pyridine derivatives have been reported to be active in complex formation[12] and by virtue of this they can act as good solubilizers[12,13] while the solubilization of many water-insoluble drugs by these agents has received much attention[5-15]. Consideration of using these agents to augment the water-solubility of the very slightly water-soluble antibacterial drug, rifampicin,[16] has not been attempted.

The work hereby presented deals with the evaluation of the effect of sodium salts of aromatic monocarboxylic acids, nicotinamide and isoniazid on the water-solubility of rifampicin . The salts evaluated are namely sodium benzoate, sodium p-aminobenzoate, sodium salicylate and sodium p-aminosalicylate. The possibility of molecular interaction between rifampicin and the tested agents was also traced using differential visible spectrophotometry to throw some light on the mechanism of solubilization of rifampicin by these agents.

EXPERIMENTAL

Materials:

Pharmaceutical or pure grades of rifampicin , nicotinamide, isoniazid , sodium benzoate, sodium p-aminobenzoate sodium salicylate, sodium p-aminosalicylate.

Equipment:

Rotating bottle apparatus with constant temperature water bath ($\pm$ 0.1^{o}C) .

Spectophotometer (Spektromom 204).

Solubility Measurment :

Excess amounts of medicament were placed in a series of glass stoppered tubes (50 ;ml. capacity). Five ml of the appropriate solution of the solubilizer were added to each tube. The tubes were tightly closed and then rotated at 45 r.p.m. in a constant temperature water bath at 20^{o}C. After equilibrium was attained (90 minutes), the contents of the tubes were filtered

rapidly and the extent of rifampicin dissolved was determined in an aliquot of the filtrate using direct spectrophotometry at 485 nm after appropriate dilution with methanol, comparing its absorbance to that of a freshly prepared standard methanolic solution .

RESULTS AND DISCUSSION

Fig. 1 illustrates the effect of sodium benzoate, sodium p-aminobenzoate, sodium salicylate, and sodium p-aminosalicylate on the equilibrium solubility of rifampicin. Table I compares the solubility augmenting capacity of the various concentrations of these agents relative to the original solubility of rifampicin. It is quite evident from Fig. 1 and Table I that all the tested salts promote the water-solubility of rifampicin. However, this effect is quite dependent on the specific type of the salt used and increased with the concentration. Highest rifampicin-solubility augmenting capacity is observed with sodium p-aminosalicylate followed in order by sodium salicylate, sodium p-aminobenzoate and finally sodium benzoate which have the least augmenting capacity

These results point to the relationship between the solubility augmenting capacity of salts and their chemical structures. Upon comparing the solubility augmenting capacity of sodium benzoate to that of sodium salicylate and that of sodium p-aminobenzoate to that of p-aminosalicylate, one can safely conclude that, introduction of a hydroxyl group, ortho to the carboxylic group strongly potentiates the solubilizing capacity of the respective anion towards rifampicin. On the other hand, upon comparing the solubility augmenting capacity of sodium benzoate to that of sodium p-aminobenzoate and that of sodium salicylate to that of sodium p-aminosalicylate leads to the conclusion that; introduction of an amino group para to the carboxylic function, slightly potentiates the solubilizing effect of the respective anion. Presence of both groups, that is the ortho hydroxyl and the para amino produces a cumulative potentiating effect, hence the solubility augmenting capacity of the p-aminosalicylate is the highest of all the tested salts.

Molecular interaction was thought to be at least partly re-

sponsible for the solubilizing action of the tested salt on rifampicin. To trace the possiblity of these molecular interaction between these salts and rifampicin, differential spectrophotometery of rifampicin in presence of the tested salts, was adopted, Figures 2-5 illustrate the spectral pattern of rifampicin in presence of various concentrations of each of these salts compared to that of rifampicin in water. It is quite obvious from these Figures that the spectral pattern of rifampicin undergoes a change involing the optical density in presence of all the tested salt, an effect which is dependent on the concentration of the salt. In addition, the spectra of rifampicin in presence of sodium salicylate and sodium p-aminosalicylate show isosbestic points at about 505 and 510 nm respectively. These spectral behavior of rifampicin in presence of aromatic acids salts is an evidence of molecular interaction between these salts and rifampicin.

So, the solubilization of rifampicin by these salts could be postulated to be a consequence of the formation of water-soluble association products through molecular interaction involving the anionic species of the salt and dipolar groups which are abundant in the rifampicin molecule[16], the result of such interaction is the creation of ion-dipolar attraction forces between anionic moieity of the salt and rifampicin leading to association and solubilization. The observed solubilization potentiating effect of substituent groups namely the ortho hydroxy or/and para amino groups, on the anionic species of the salt could be attributed to one or more of the following effects,:(a) these groups, through the balance of inductive and mesomeric effects, could have a strengthing and/or atabilizing effect on the electric charge an effect which is reflected by more stronger attraction forces between the anionic species and rifampicin, thus the formed association product would have lower tendency to dissociate, (b) both the hydroxyl groups and the amino groups are themselves dipolar capable of interacting with dipolar groups or induced polarized centers on rifampicin molecule, thus creating dipole-dipole or dipole-induced dipole attraction forces of which hydrogen bonding represents a high probability, a situation which results in greater association tendency between rifam-

picin and the anionic species, (c) the presence of the polar hydroxy and/or amino group on the anionic species might result in increased hydration of the formed association product of rifampicin and the anionic species thus resulting in higher solubilizing tendency.

However, the solubility of rifampicin obtained with the tested salts could not be entirely interpretted on the basis of molecular interection and formation of water-soluble association products. If this is the only mechanism responsible for the solubilization, it should proceed stoichiometricaly. Table II presents the molecular solubilizing power of these salts towards rifampicin, calculated as moles of rifampicin solubilized per mole of salt. It is obvious from this table that in addition to the dependence of the solubilizing power on the type of the salt, it is also dependent on the concentration of the specific salt used specially in cases of sodium salicylate and sodium p-aminosalicylate, where the molar solubilizing power at one molar concentration exceeds five folds that of 0.2 molar concentration . The change among the solubilizing powers according to the concentration of the salt could be attributed to that at higher concentration , extra mechanisms other than stoichiometric molecular interaction work for solubilization of rifampicin. These could be postulated as; at higher salt concentration the anionic species tend to associate to form aggregates in a manner similar to the formation of micelles of surfactants, and in these aggregates rifampicin is highly solubilized, and/or at higher concentration the salt causes disrubtion of the structuring of water and facilitating the mixing and interaction of rifampicin and water molecules thus leading to promotion of rifampicin.

Fig. 6 depicts the effect of nicotinamide and isoniazid on the apparent water solubility of rifampicin. Table III compares the solubility augmenting capacity on rifampicin solubility relative to its original solubility, produced by nicotinamide and isoniazid at various concentration levels. On the bases of the presented results, it is quite apparent that both agents augment to different extents the water-solubility of rifampicin. The solubi-

lity augmenting effect is increased with the concentration of the agent used. Although, that both, nicotinamide and isoniazid are pyridine derivatives, it is quite evident from the solubility data that nicotinamide has more solubilizing efficiency compared to isoniazid thus projecting the contribution of the ultrafine structure of the agent i.e. the type and position of the substituent in detrmening the solubilizing efficiency.

Differential spectrophotometry was applied to trace the possibility of molecular interaction between rifampicin and each of nicotinamide and isoniazid to throw some light on the mechanism of their solubilizing action. Figures 7 and 8 present the spectra of rifampicin determined in water and aqueous solution of these agents. The spectral behavior of rifampicin presented in these figures gives an evidence for the possiblity of moleculer interaction which could play a part in the solubilization process. The difference of the solubilizing efficiency of the two agents might be attributedm to the difference in the position and structure of the side radicle attached to the pyridine ring thus determining the ultimate polarity of the molecule and hence the intermolecular attraction forces that could be formed between the specific molecule and rifampicin.

Table IV compares the molecular solubilizing power of nicotinamide and isoniazid towards rifampicin. It is again obvious from this table that nicotinamide has much higher solubilizing power towards rifampicin compared to isoniazid. It is also quite apparent from this table that the solubilizing power of nicotinamide can be considered constant all over the range of concentration tested while that of isoniazid is continually increasing with the concentration. This behavior of isoniazid could be interpretted on similar basis as previously mentioned with sodium salicylate and sodium p-aminosalicylate.

Finally, the solubilizing power of all the tested hydrotropic agents towards rifampicin can be arranged in the decreasing order as follow, sodium p-aminosalicylate, sodium salicylate, nicotinamide, isoniazid, sodium p-aminobenzoate and sodium benzoate. It is worthy to note that the proven solubilizing efficiency of both sodium p-aminosalicylate and isoniazid towards rifampicin could be of clinical importance in the formulation of antitubercular drug combinations.

Table I : Effect of Sodium Benzoate, Sodium p-aminobenzoate, Sodium Salicylate and Sodium p-aminosalicylate on the Water-Solubility of Rifampicin

Concentration of the solubilizer. (mol/l)	*Solubility Augmenting Capacity* $\frac{S}{S_o}$ *of*			
	Sodium benzoate	*Sodium p-aminobenzoate*	*Sodium salicylate*	*Sodium p-aminosalicylate*
0.2	2.57	2.73	4.09	5.30
0.4	4.00	4.24	12.72	14.53
0.6	4.80	6.38	42.49	61.03
0.8	6.87	9.69	68.15	80.09
1.0	10.56	15.14	84.64	105.77

S Is the apparent water-solubility of rifampicin in presence of the solubilizing agent (mol/l)

S_o Is the original water-solubility of rifampicin (mol/l).

Table II : Molecular Solubilizing Power of Sodium Benzoate Sodium p-aminobenzoate, Sodium salicylate and Sodium p-aminosalicylate towards Rifampicin.

Concentration of the solubilizer. (mol/l)	*Molecular Solubilizing Power* $\frac{S-S_o)^{++}}{C}$ *of*			
	Sodium benzoate	*Sodium p-aminobenzoate*	*Sodium salicylate*	*Sodium p-aminosalicylate*
0.2	20.35×10^{-3}	22.35×10^{3}	39.97×10^{-3}	55.65×10^{-3}
0.4	19.40×10^{-3}	20.95×10^{-3}	75.84×10^{-3}	87.56×10^{-3}
0.6	16.41×10^{-3}	23.09×10^{-3}	178.95×10^{-3}	258.94×10^{-3}
0.8	18.97×10^{-3}	28.10×10^{-3}	217.14×10^{-3}	255.86×10^{-3}
1.0	24.77×10^{-3}	36.59×10^{-3}	216.48×10^{-3}	271.60×10^{-3}

^{++}S Is the apparent water-solubility of rifampicin in presence of the solubilizing agent (mol/l)

So Is the original water-solubility of rifampicin (mol/l)

C Is the molar concentration of the salt used.

Table III : Effect of Nicotinamide and Isoniazid on the Water-Solubility of Rifampicin .

Concentration of the solubilizer.(mol/l)	Solubility Augmenting Capacity $\frac{S}{SO}^{X}$ of	
	Nicotinamide	Isoniazid
0.2	5.81	2.92
0.4	11.91	5.75
0.6	14.97	9.37
0.8	18.83	12.74
1.0	24.23	---

^{x}S and S_o as indicated under Table I .

Table IV : Molecular Solubilizing Power of Nicotinamide and Isoniazid towards Rifampicin .

Concentration of the solubilizer (mol/l)	Molecular Solubilizing Power ($\frac{S-S_o}{C}$)^{xx} of	
	Nicotinamide	Isoniazid
0.2	$62.80X10^{-3}$	$29.22X10^{-3}$
0.4	$71.20X10^{-3}$	$33.65X10^{-3}$
0.6	$60.75X10^{-3}$	$41.89X10^{-3}$
0.8	$58.23X10^{-3}$	$44.06X10^{-3}$
1.0	$60.69X10^{-3}$	

^{xx}S , So and C as indicated under Table II .

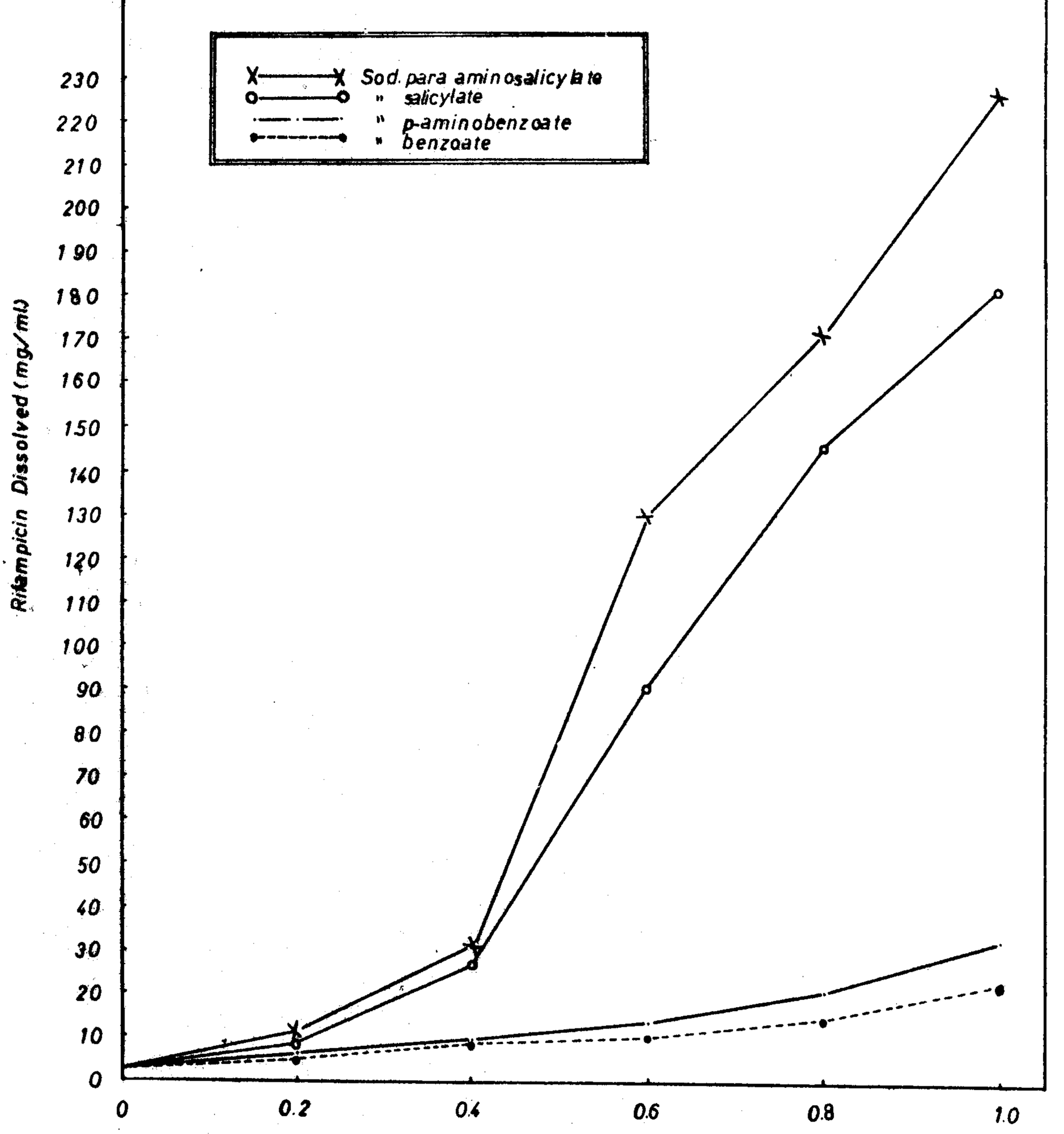

Fig (1) EFFECT OF SODIUM SALTS OF MONOCARBOXYLIC AROMATIC ACIDS ON THE SOLUBILITY OF RIFAMPICIN AT 20°.

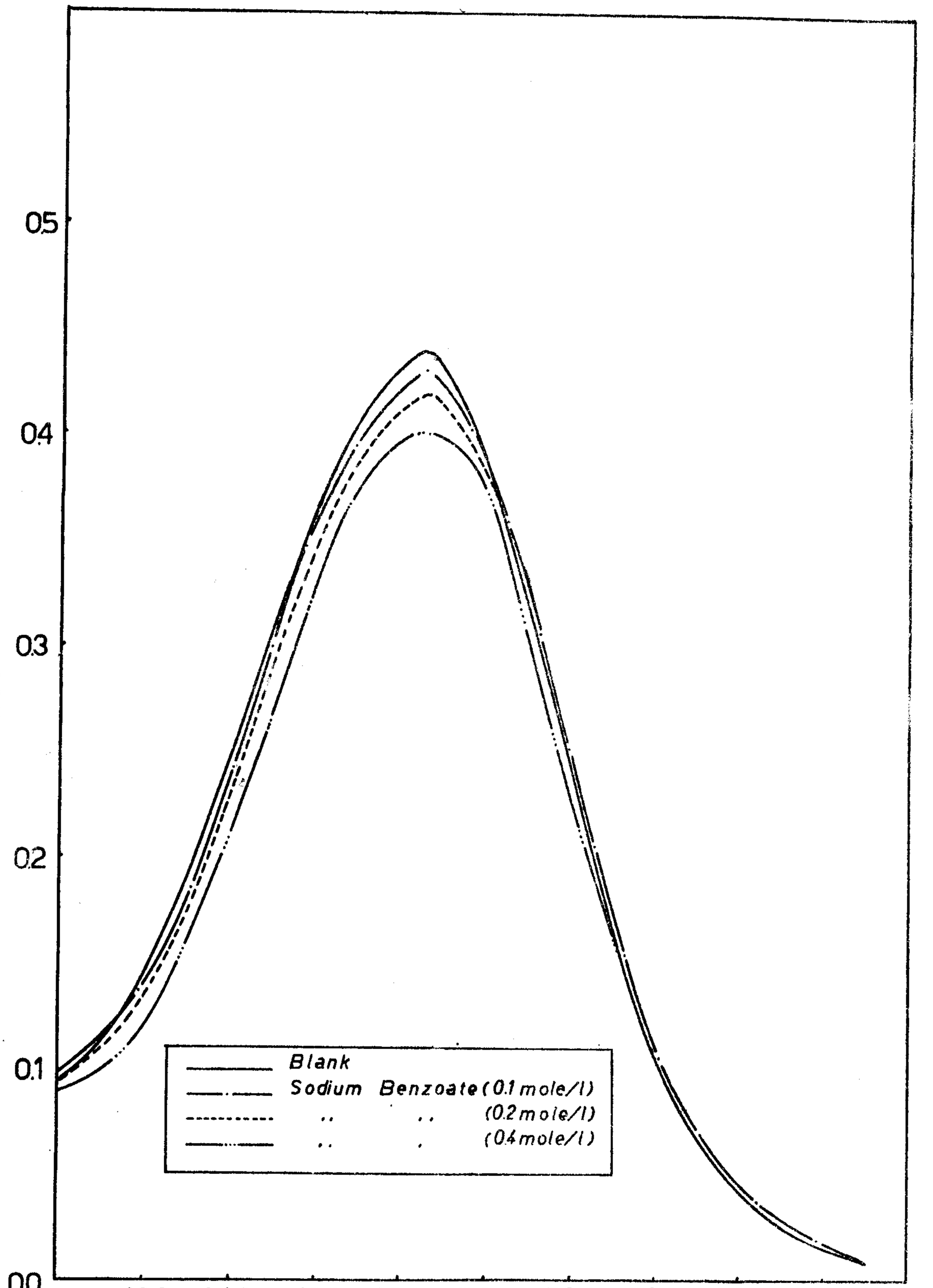

Fig.(2) DIFFERENTIAL VISIBLE SPECTRUM OF RIFAMPICIN IN PRESENCE OF DIFFERENT CONCENTRATIONS OF SODIUM BENZOATE.

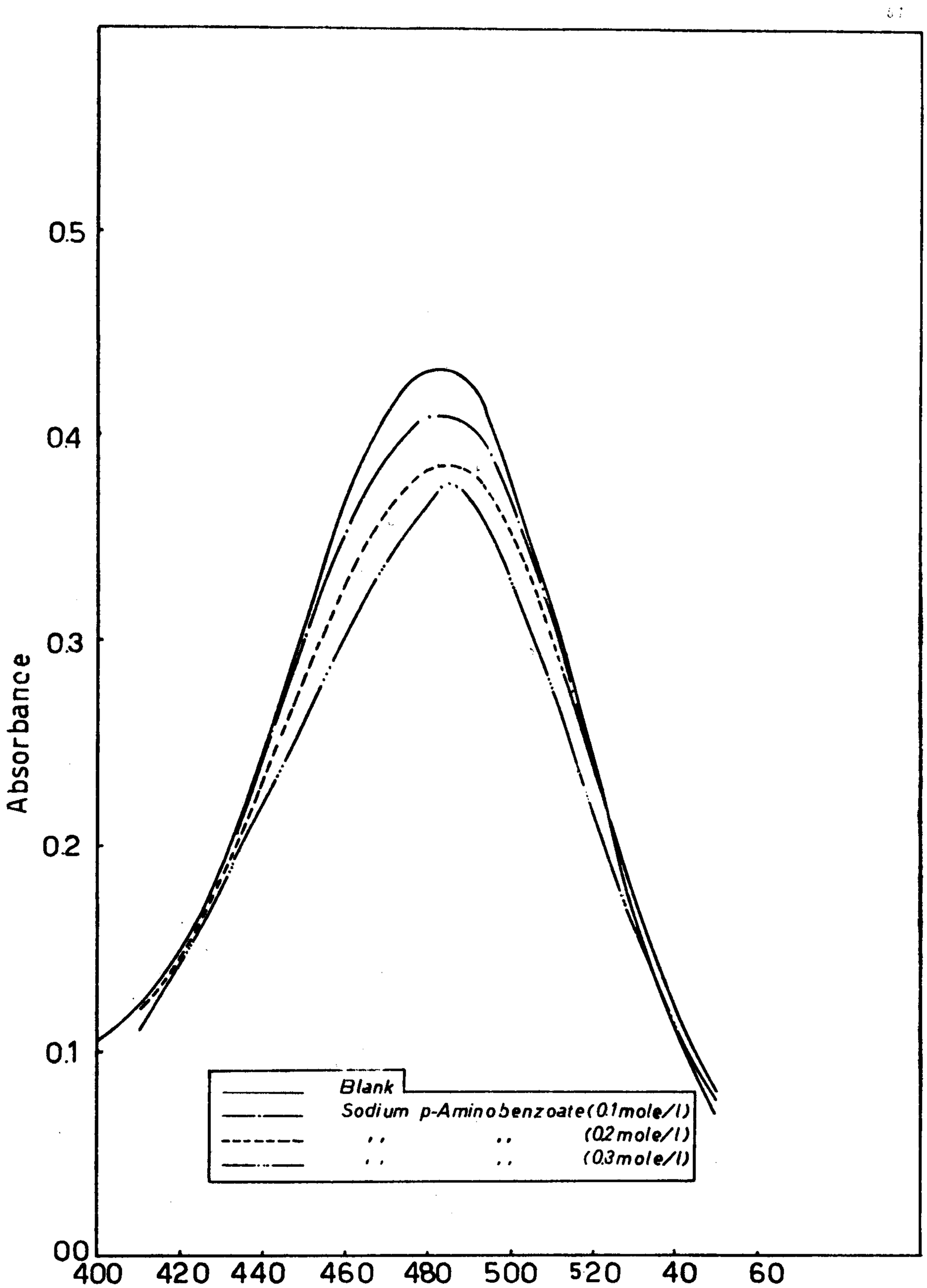

Fig.(3) DIFFERENTIAL VISIBLE SPECTRUM OF RIFAMPICIN IN PRESENCE OF DIFFERENT CONCENTRATIONS OF SODIUM p-AMINOBENZOATE.

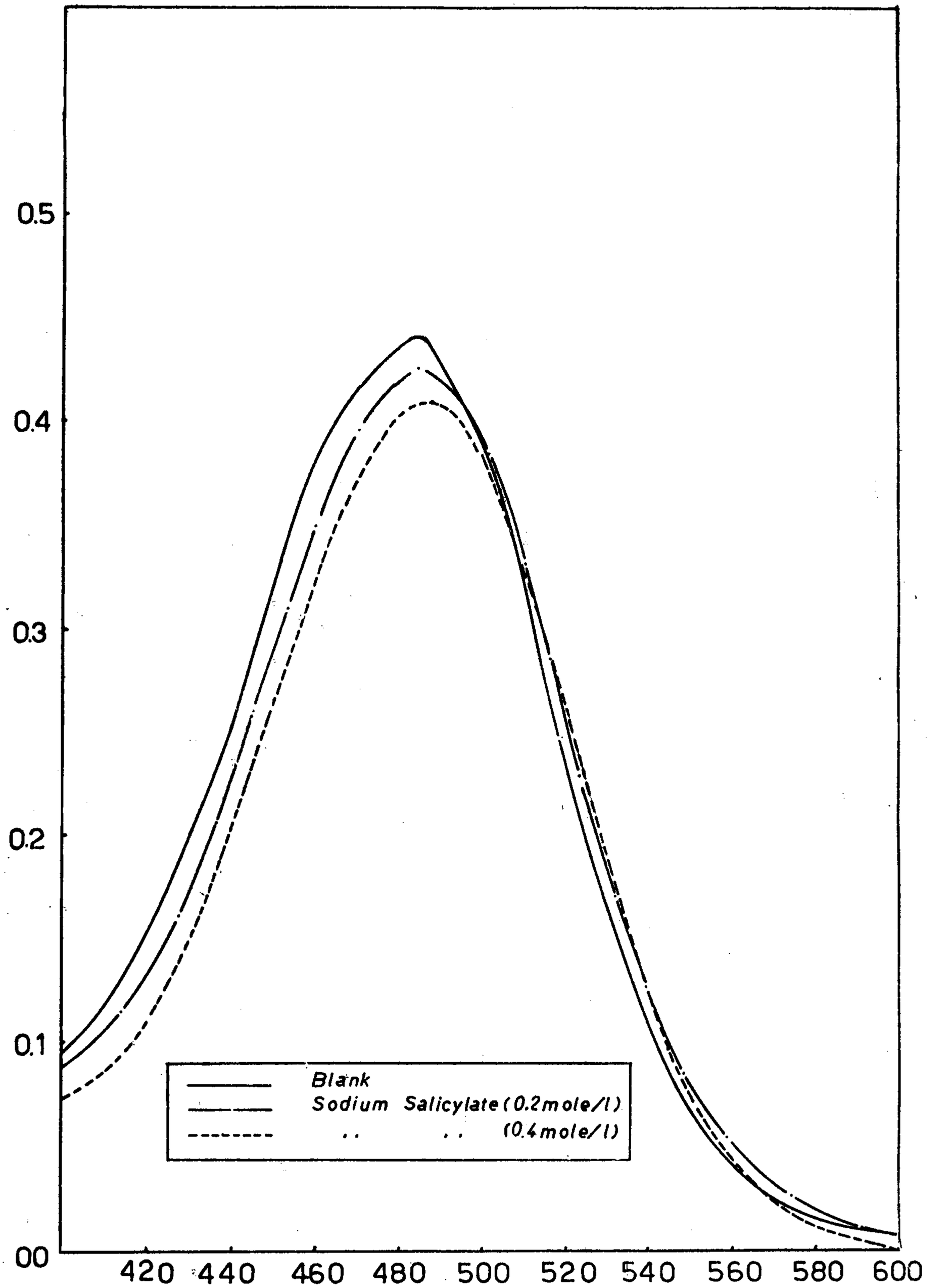

Fig.(4) DIFFERENTIAL VISIBLE SPECTRUM OF RIFAMPICIN IN PRESENCE OF DIFFERENT CONCENTRATIONS OF SODIUM SALICYLATE.

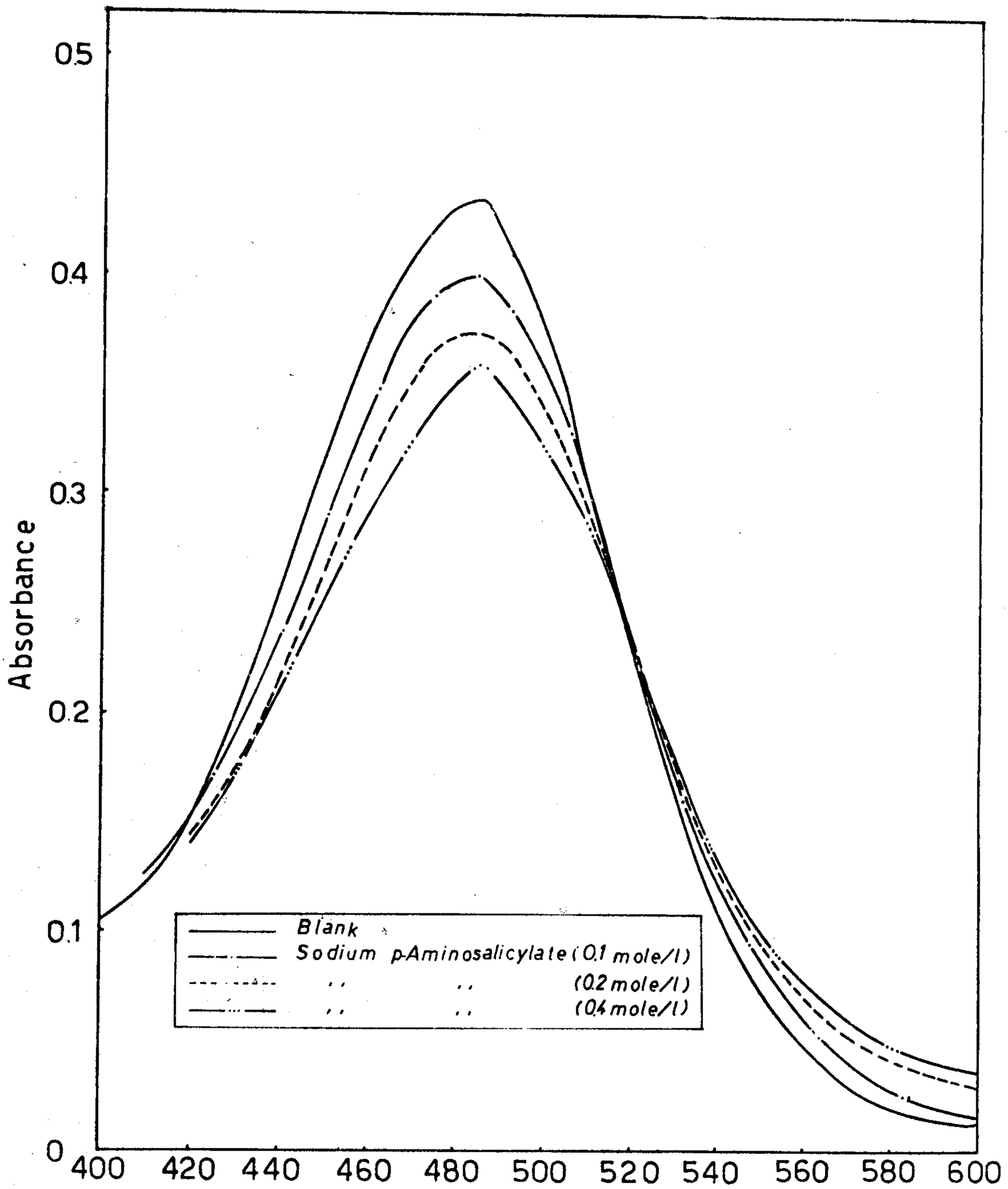

Fig. (5) DIFFERENTIAL VISIBLE SPECTRUM OF RIFAMPICIN IN PRESENCE OF DIFFERENT CONCENTRATIONS OF SODIUM p-AMINOSALICYLATE.

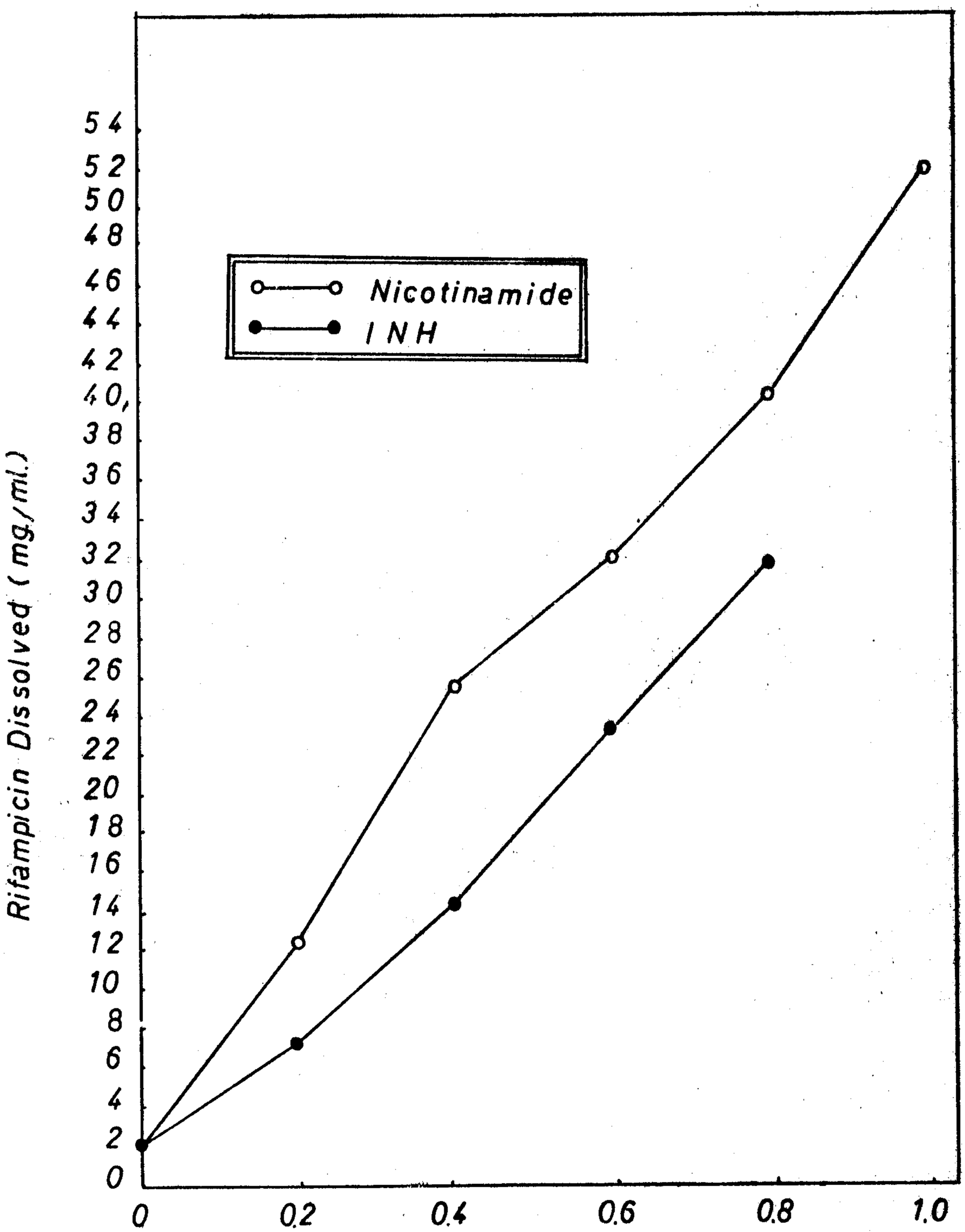

Fig. 6: Effect of Nicotinamide and Isoniazid (INH) on Water Solubility of Rifampicin at 20°.

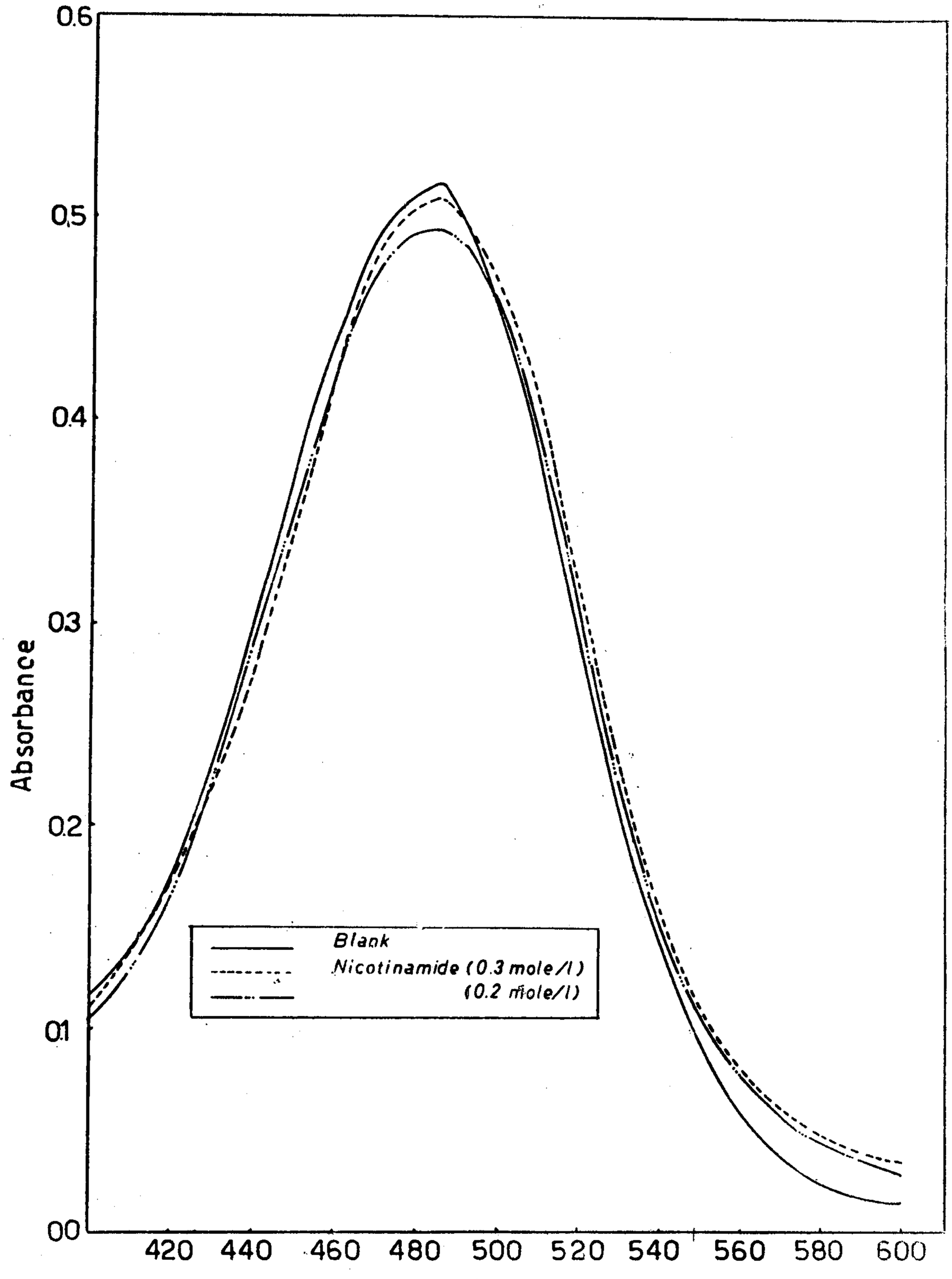

Fig.(7) DIFFERENTIAL VISIBLE SPECTRUM OF RIFAMPICIN IN PRESENCE OF DIFFERENT CONCENTRATIONS OF NICOTINAMIDE.

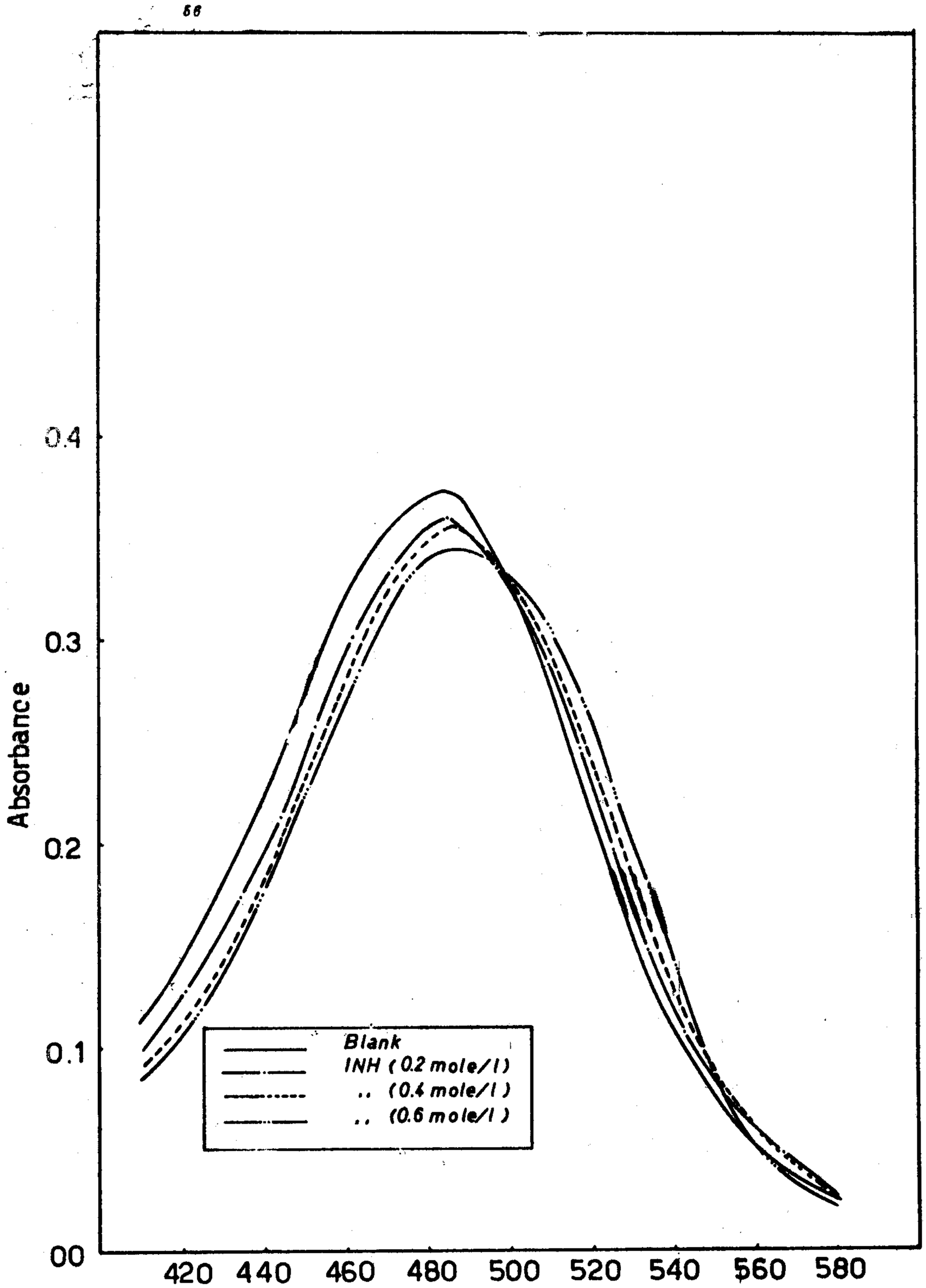

Fig.(8) DIFFERENTIAL VISIBLE SPECTRUM OF RIFAMPICIN IN PRESENCE OF DIFFERENT CONCENTRATIONS OF ISONIAZID (I N H).

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دراسات على ذوبان الريفاميسين

٢ـ تأثير أملاح الصوديوم لبعض الاحماض العطرية وحيدة الكربوكسليك

النيكوتناميد ، الايزونيازيد على الذوبان المائى للريفاميسين

السيد على ابراهيم ـ على على قاسم ـ اسماعيل عطيه ـ سيد اسماعيل محمد

تمت دراسة تاثير املاح الصوديوم لبعض الاحماض العطرية وحيدة الكاربوكسليك وهى جاوات الصوديوم مقابل امينوجاوات الصوديوم ، سالسيلات الصوديوم ومقابل امينو سالسيلات الصوديوم وكذلك النيكوتيناميد والايزونيازيد على الذوبان المائى للريفاميسين

وقد اظهرت النتائج ان الذوبان المائى الظاهرى للريفاميسين يزداد فى وجود هذه المواد وكانت القوه لاذابه لهذه المواد ذات ارتباط وثيق مع نوعيه المادة المستعملة حيث اظهر مقابل امينو سالسيلات اكبر قوة اذابية يتبعها تنازليا سالسيلات الصوديوم ، مقابل امينو جاوات الصوديوم ثم فى النهاية جاوات الصوديوم الذى له اقل قوة اذابية .

وقد اظهر النيكوتناميد قوة اذابية تفوق التى اظهرها الايزونيازيد ولكنها فى نفس الوقت اقل من القوة الاذابية لمقابل امينو سالسيلات والسالسيلات فى حين ان القوة الاذابية للايزونيازيد كانت اعلى من القوة الاذابية لجاوات الصوديوم ، ومقابل امينوجاوات الصوديوم

واوضح النمط الطيفى للريفاميسين تغييرا فى الكثافة الضوئية فى وجود المواد الذى ينبىء بدور التفاعل الجزيئ بين هذه المواد والريفاميسين فى عملية الاذابة .