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PREFORMULATION STUDIES ON VASICINONE - A BRONCHODILATORY ALKALOID (STUDY OF SOME PHYSICO-CHEMICAL ASPECTS)

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ABSTRACT

Vasicinone, an alkaloid from Adhatoda vasica Nees, having shown potent bronchodilator activity, was investigated for its suitability to formulation. The physico-chemical characteristics of major significance in preformulation studies such as, solubility, intrinsic dissolution rate, pKa and partition coefficient were studied, the results of which are intended to assist the development of a superior product design.

INTRODUCTION

Preformulation studies to determine the physico-chemical parameters of vasicinone were undertaken as a sequel to its isolation from Adhatoda vasica Nees, characterisation and the

pharmacological screening. The investigations have proved its usefulness as a potential bronchodilator, comparable in its activity to aminophylline.[1-4]

Solubility, dissolution rate, pKa and partition coefficient of the compound were studied, the results being intended to provide an objective and rational basis for subsequent design of the drug product and development of a suitable analytical frame work.

No quantitative data being available about the solubility of vasicinone in aqueous or organic solvents, studies were therefore necessiated to ascertain its solubility in water, methanol, ethanol, isopropyl-alcohol, chloroform, ethyl acetate, n-butanol and n-octanol. Experiments were also performed to determine the means of increasing the solubility of the base, such an objective being attempted by formation of hydrochloride of vasicinone, use of solubility enhancing agents and lyophillisation of the hydrochloride salt. The solubility of the base and its hydrochloride salt was also studied in various buffer solutions having pH values ranging from 1.2 to 8.

The dissolution being a function of solubility, it was felt imperative to determine the intrinsic dissolution rate profiles of the base as well as of its hydrochloride. Intrinsic dissolution rates of pellets of vasicinone and its hydrochloride were therefore determined in simulated gastric fluid. The fact that vasicinone hydrochloride has low water solubility and gives a very low pH (1.7) on dissolution, coupled with its anticipated use in asthmatic conditions, may necessitate the presence of buffers in its tablet formulations and it was therefore felt necessary to acquire data relating to the effect of the buffering agents on the dissolution rate of vasicinone hydrochloride in the physiological range of pH.

In addition to data on solubility and dissolution profiles, preformulation studies on a new drug necessitates other related information such as its pKa and partition between aqueous and lipoidal phase, both of which may be of considerable consequence in the development of a superior product design.[5,6]

pKa value which could be utilized in assessing the permeability potential of the compound and in indicating the absorptive areas for the drug was determined by spectrophotometric method.

The oil/water partition ratio forms an important parameter in partition theory. Systems which can measure this process have been developed and studied by Doluisio and Swintosky[7] and by Perrin.[8] Of the different systems reported for the measurement of the oil/water partition correlating to in-vivo drug absorption, a water/octanol system was employed for the determination of this parameter of vasicinone hydrochloride.

EXPERIMENTAL

A. SOLUBILITY STUDIES

i) Solubility of Vasicinone in Various Solvents:

Vasicinone recrystallised from ether-alcohol was powdered and passed through sieve # 60. About 1 g of the drug was shaken with 20 mL each of distilled water, methanol, ethanol, isopropanol, acetone, chloroform, ethyl acetate, n-butanol and n-octanol in quick fit neutral glass stoppered flasks at 25 ± 1°C for a period of 4 hours on a mechanical shaker. Care was taken to ensure the presence of an excess of undissolved drug during shaking with the various solvents. The flasks were then allowed to stand for 1 hour. Samples were withdrawn and the supernatent filtered. In the case of water, the resultant solution was suitably diluted with N/10 HCl and

assayed by determining the absorbance at 233 nm. Measured volumes (5 mL) of all other samples except those in n-octanol and n-butanol were evaporated, the residue from each was extracted with 20 mL of N/10 HCl, suitably diluted and absorbance recorded at 233 nm. A measured quantity (5 mL) of each n-octanol and n-butanol solution was extracted with N/10 HCl, suitably diluted and it's absorbance recorded at 233 nm. The content of vasicinone in each solution calculated by taking 1264 as the value of $E_{1\,cm}^{1\%}$ at max 233 nm of vasicinone in N/10 HCl.

ii) Preparation of Vasicinone Hydrochloride:

Weighed amount of base was dissolved in dry solvent ether in a dry flask and freshly generated moisture free hydrochloric acid gas was bubbled through it. The vasicinone hydrochloride crystals formed (m.p. 227°-228°C) were recovered and recrystallized from alcohol.

iii) Solubility of Vasicinone Hydrochloride in Water - Effect of Polyethylene Glycol 400:

Mixtures of distilled water and polyethylene glycol containing 10, 20 and 40 percent of PEG-400 were prepared. Samples of vasicinone hydrochloride, each weighing about 1 g were added to 20 mL each of water, and water-PEG mixtures prepared as above. The systems were equilibrated as outlined earlier. The samples were filtered and then suitably diluted with distilled water and absorbance of each solution was noted at 225 nm against suitably diluted respective solvent blanks. The concentration of vasicinone hydrochloride in test solution was calculated from standard curve of vasicinone hydrochloride in water.

iv) Freeze drying of Vasicinone Hydrochloride - Effect on Solubility:

A sample of vasicinone hydrochloride of known solubility was dissolved in water and dried in an Edward-lab model

lyophilizer using phosphorous pentoxide as the dessicant. The solubility of the dried samples in distilled water was then determined as described earlier by recording the absorbance of suitably diluted solution at 225 nm.

v) Solubility of Vasicinone and its Hydrochloride in Buffer Solutions:

Excess of vasicinone and its hydrochloride salt were treated separately with buffer solutions* (pH range 1.2 to 8.0) as in earlier experiments. Aliquots were serially diluted with water and the absorbance of solutions noted at 233 nm against a proper blank. The concentration of each drug in test solutions was calculated from their std. curves prepared by noting the absorbance at 233 nm of solutions containing 1 to 10 mcg of the respective drug/mL in water.

*Buffer solutions used were USP XVIII[9] standard buffers except for pH range 3 to 5 for which the following buffers were used. Citric acid buffer pH 3.0 Mcllvaine standard buffer.[10] Acetate buffer pH 4.2 and 5.0 To 50 mL of 0.2M sodium acetate solution was added definite volume of 0.2M acetic acid solution to obtain the desired pH. Volume made to 200 mL with water.

B. DISSOLUTION RATE STUDIES:

Vasicinone and its hydrochloride 40/45 mesh powders were compressed into pellets (Dia = 8 mm, and weight 200 mg) on a Unimek single punch tablet press at a pressure of 2500 kg/cm^2. The dissolution rate of the pellets were determined in an USP XVIII dissolution apparatus.[9] 600 mL simulated gastric fluid USP (without pepsin) pH 1.2 was employed as the dissolution medium and the speed of rotation adjusted to 100 r.p.m., 5 mL of the samples were withdrawn at regular intervals and made the replacement of equal volume after each withdrawl. The samples were filtered and 2 mL of aliquot was diluted to 50 mL in a volumetric flask. The concentration of each drug in test sample was determined as noted earlier by recording the absorbance at 233 nm against an appropriate blank.

3. SPECTROPHOTOMETRIC DETERMINATION OF pKa OF VASICINONE:

Suitably recrystallised and dried drug was dissolved in distilled water to prepare the stock solution (50 mcg/mL). The 0.05M potassium chloride - hydrochloric acid buffer solution of pH values 1.6, 1.8, 2.0, 2.2 and 2.4 were prepared. The stock solution of the drug was then diluted with each buffer solutions to obtain a final concentration of 5 mcg of vasicinone/mL. The exact pH value of diluted solutions was then determined on Elico Digital pH meter (model LI-120). The UV absorption spectra of each solution against a proper blank was recorded on Hitachi made Spectrophotometer (model-200).

The analytical wave length was found to be 222 nm and the readings for the pKa determination were taken at this wave length on a Carl Zeiss model VSU2-P spectrophotometer.

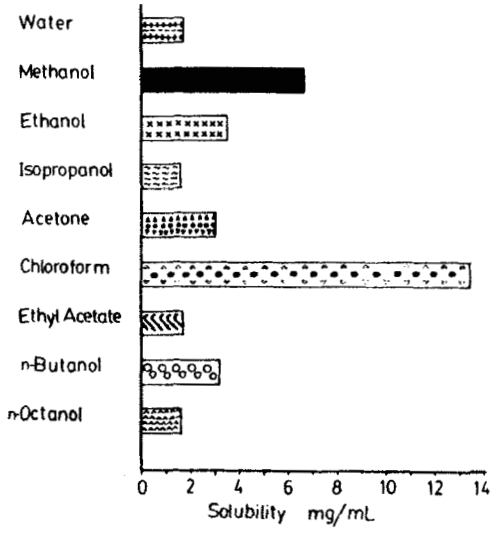

FIGURE - 1

Solubility of Vasicinone in Various Solvents

D. PARTITION COEFFICIENT OF VASICINONE HYDROCHLORIDE:

A saturated solution of drug was prepared in distilled water and assayed spectrophotometrically by noting the absorbance at 225 nm. 10 mL of the solution and 10 mL of n-octanol were then transferred to a separating funnel and the system was agitated by inversion at regular intervals until the system gained equilibrium. The system was then allowed to stand for the separation of the aqueous and n-octanol layers. The drug concentration in the aqueous layer was directly determined by noting the absorbance of the appropriately diluted sample at 225 nm. The drug concentration in n-octanol was determined by extracting 5 mL sample with sufficient 10 mL portions of 0.1N HCl and serially diluting it before noting the absorbance at 233 nm. The partition coefficient of the drug was also determined as a function of pH. The drug was dissolved in an aqueous phase at pH 1.2 and its diffusion through n-octanol to an aqueous phase at pH 7.2 was noted.

RESULTS AND DISCUSSION

The solubility of the vasicinone base in various solvents experimented with (Figure 1) was found to be in the following ascending order with the percent solutions (w/v) formed recorded in parathesis. n-Octanol (0.16), isopropanol (0.16), distilled water (0.16), ethyl acetate (0.17), acetone (0.29), n-butanol (0.31), ethanol (0.34), methanol (0.66) and chloroform (1.33). It is evident that the aqueous solubility of the compound is very poor. Vasicinone hydrochloride was found to have superior aqueous solubility (1.00% in water) which could be further increased on freeze drying the sample to 1.66%. The use of polyethylene glycol-400 to enhance the solubility of the hydrochloride in water yielded positive results (Figure 2). As seen from the Figure, PEG-400 in a 20% concentration gave optimum increase in solubility (1.96%) approximately double its normal aqueous solubility. A 10% solution

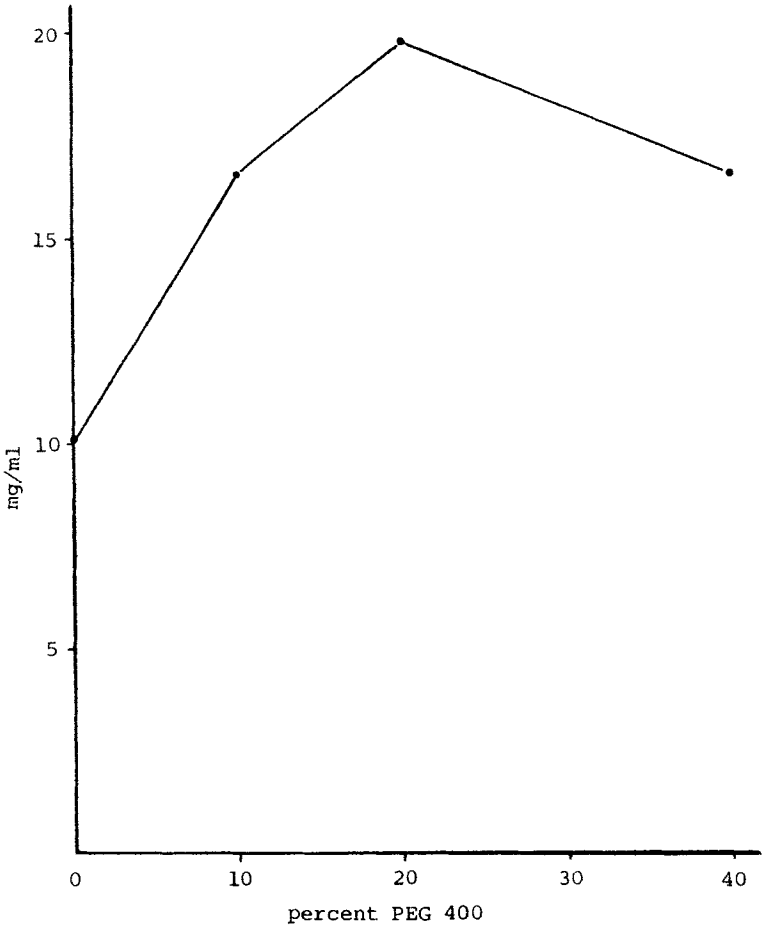

FIGURE - 2

Effect of PEG on Water Solubility of Vasicinone Hydrochloride

of PEG-400 could also appreciably enhance the water solubility to 1.64%. The presence of this co-solvent did not interfere with the assay procedure.

The study of the effect of pH on the solubility of vasicinone and its hydrochloride in different pH buffer solu-

tions as presented graphically (Figure 3) shows a graded increase in solubility with the decrease of pH. These results also show a considerable increase in the water solubility of the vasicinone base and its hydrochloride in the presence of buffer solutions, brought about probably due to *in situ* formation of some very soluble salts.

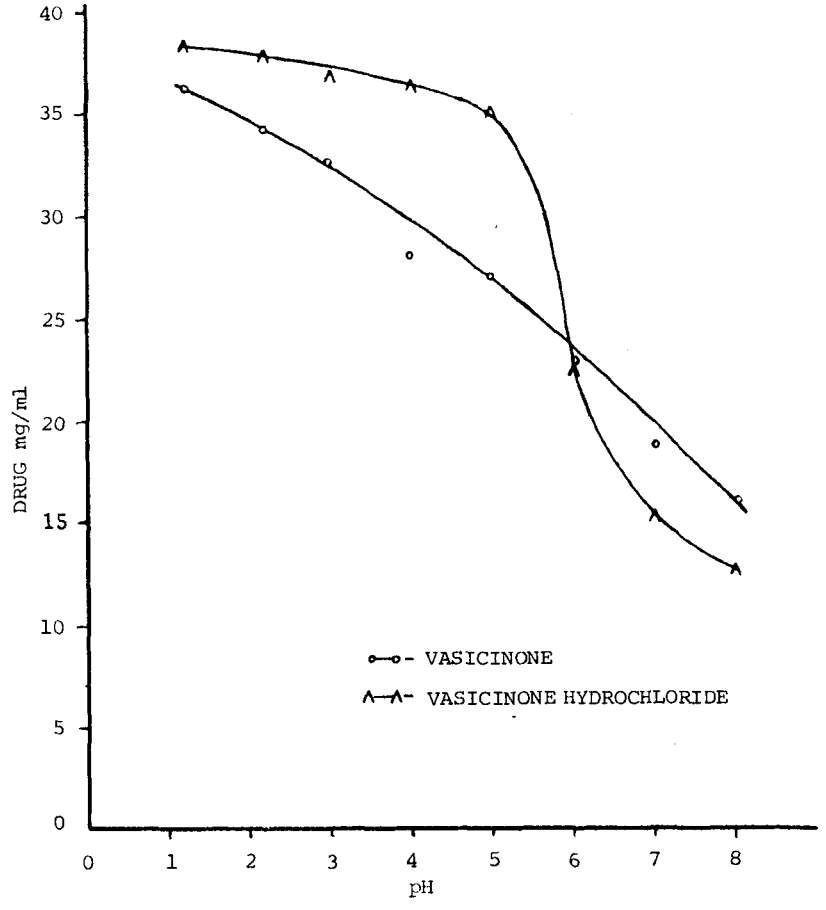

FIGURE - 3

Solubility of Vasicinone and Vasicinone Hydrochloride in Buffer Solutions

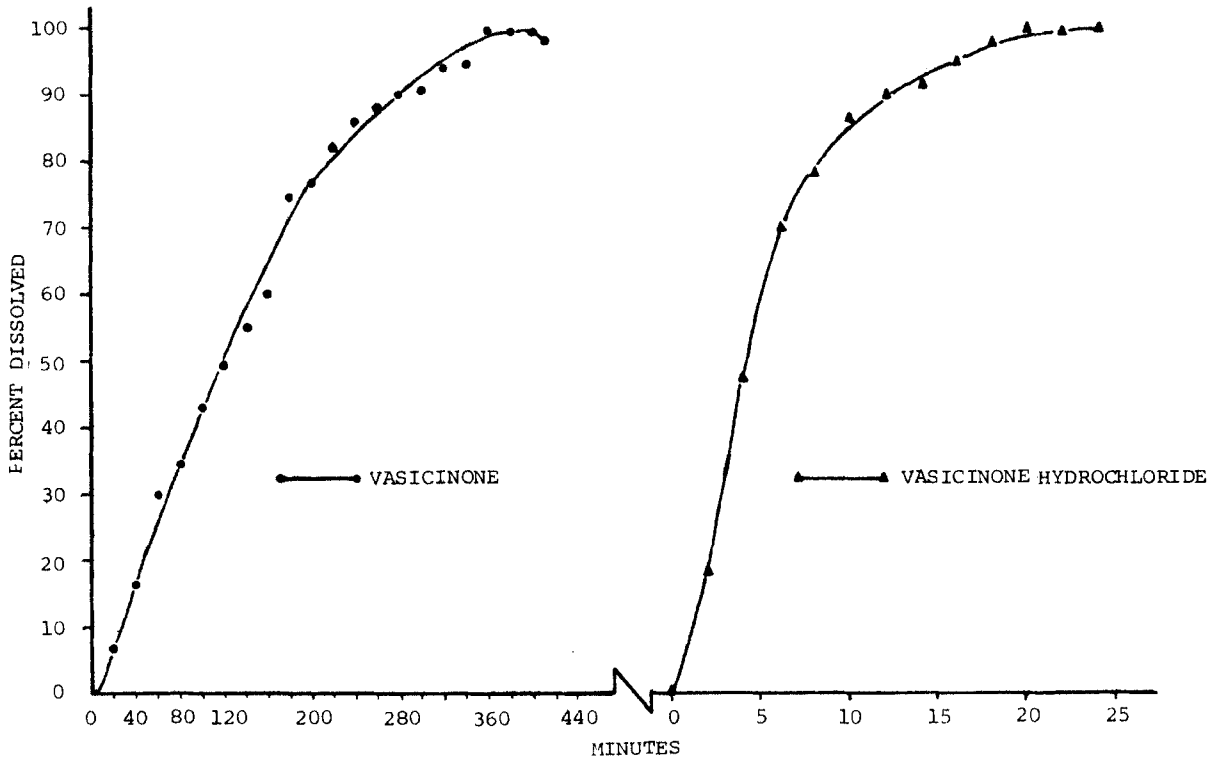

FIGURE - 4

Intrinsic Dissolution Rate of Vasicinone and Vasicinone Hydrochloride in Simulated Gastic Fluid (pH 1.2)

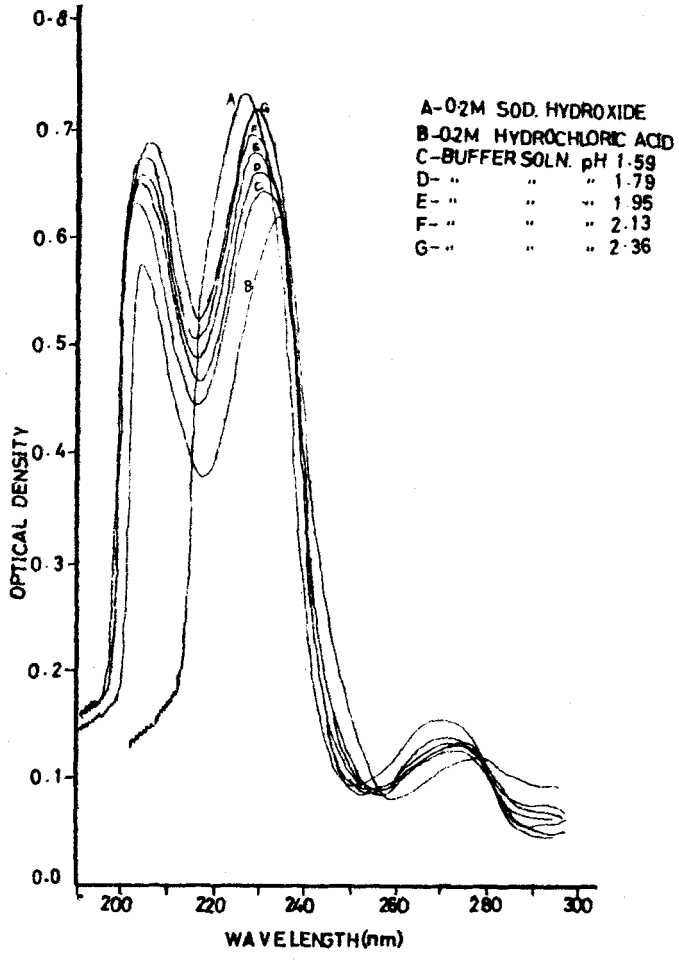

FIGURE - 5

Spectrophotometric Determination of pKa of Vasicinone (Spectras at Different pH to Find Out Analytical Wavelength)

No disintegration of the compressed pellets was observed during the intrinsic dissolution rate studies indicating suitable compression pressure. Further, as expected it was observed that the vasicinone hydrochloride displayed a much faster dissolution rate, 60 per cent of it being dissolved in

TABLE 1

Determination of the Ionization Constant (pKa) of Vasicinone

pH	d^{*}	$d_M^{**} - d$	$d - d_I^{***}$		$\text{Log}\frac{d_M - d}{d - d_I}$	$pKa = pH + \text{Log}\frac{d_M - d}{d - d_I}$	Remarks
2.36	0.661	0.059	0.176	-	0.4746	1.8854	pKa value= 1.91 ± 0.03
2.13	0.635	0.085	0.150	-	0.2467	1.8833	
1.95	0.612	0.108	0.127	-	0.0704	1.8896	
1.79	0.583	0.137	0.098	+	0.1456	1.9356	
1.59	0.558	0.162	0.073	+	0.3461	1.9361	

* Observed optical density (d) of the solution at 222 nm.

** Optical density of neutral molecule (d_M) = 0.72 (0.2M NaOH).

*** Optical density of cation (d_I) = 0.485 (0.2M HCl).

less than 5 minutes in simulated gastric fluid in contrast to vasicinone which required over 2 hours to effect dissolution of 60 per cent of it (Figure 4). Total dissolution of vasicinone hydrochloride and vasicinone pellets was seen to take place in about 25 minutes and 6 hours respectively.

The spectrophotometric method was employed for the determination of pKa, since it is more suitable for sparingly soluble substances and also for work at extremely high and low pH values.[11] 222 nm was shown to be the wave length which shows greatest difference between the absorbance of the two species (Figure 5), 0.2M NaOH (optical density 0.72) and 0.2M HCl (optical density 0.485) were found suitable to give a neutral molecule and cation respectively. The pKa as determined by this method was found to be 1.91 $\pm$ 0.03 (Table 1). The low pKa value found for vasicinone indicates its high ionisation in low pH value and also its superior solubility in acid solution.

Vasicinone in distilled water shows max at 225 nm and in acidic solution at 233 nm. However the absorbance at 233 nm was found to be independent of pH of solution.

In our study for partition coefficient, two aqueous phases employed simulating pH of gut and blood were buffer solutions of pH 1.2 and 7.2 and lipid partitioning was done by n-octanol. The distribution of the drug in the system pH 1.2 buffer : n-octanol : pH 7.2 buffer was found in approximate ratio of 3:3:1, while between water : n-octanol, it was found to be in ratio of 3:2. The results are indicative of there being no permeability limited absorption as it has solubility in both aqueous and lipid phases.

A comparatively high proportion of drug being present in low pH value buffer solution is indicative of higher ionization at lower pH value, the results being in agreement with its low pKa. As a small change in pH can cause a large change in

the ratio of ionised to unionised molecule and consequently a large change in the lipid/water solubilities, the absorption of vasicinone hydrochloride may be expected to improve as it travels from stomach to the duodenum.

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