swarms of myxobacteria or their fruiting bodies which were luxuri nt and free from contaminants was much easier than from the other two media used. On Krzemieniewski plate[3], fungal contamination was a problem and on Aerobacter plate[4] soil amoebae and amoeboid organisms were the problems. The number of various species of myxobacteria isolated by these three methods are presented in Table I.

TABLE I

Number of fruiting myxobacterial spp. isolated by the three methods

Species of myxobacteria	No. of samples examined	Number of myxobacterial spp. isolated		
		Krzemieniewski plate	Singh and Singh plate	Serratia plate
Myxococcus spp.	119	33	55	55
Chondrococcus spp.	119	18	54	54
Angiococcus spp.	119	23	35	35
Archangium spp.	119	12	30	30
Stolangium spp.	119	3	3	3
Haploangium spp.	119	7	18	18
Polyangium spp.	119	1	2	2
Chondromyces spp.	119	42	38	38
Total	..	139	235	235

The total number of myxobacterial spp. isolated by Krzemieniewski's method gave a slightly different spectrum than 'Aerobacter plate' and 'Serratia plate'. The number of myxobacterial isolates from 'Aerobacter plate' and 'Serratia plate' were equal.

Although the number of myxobacterial species on Aerobacter and Serratia plate are one and the same yet the latter is much better in the sense that fruiting bodies are luxuri nt and mostly free from soil amoebae. The superiority of Aerobacter plate over Krzemieniewski plate[3] was reported earlier and the use of some other eubacterial species for the isolation of myxobacteria was recommended. It has been reported that soil amoebae are very selective in their bacterial food requirements[7]. Bacterial spp. are either readily eaten or partly eaten or not eaten at all by amoebae. *Aerobacter* sp. was one of the best foods for amoebae and amoeboid organism, whereas pigmented species of bacteria were mostly inedible by amoebae[7–8]. It was further reported that myxobacteria too were selective in their food requirements and unpigmented bacteria were more readily eaten than pgimented strains[4]. This was further confirmed by Singh and Yadava[6,9] that most of the pigmented species of bacteria were not lysed by myxobacteria. After screening a large number of eubacterial spp., yeast and yeast like fungi, it was found out that a red pigmented strain of *S. marcescens* was edible by all the myxobacterial species studied and was not readily edible by soil amoebae. Taking these facts into consideration, red pigmented strain of *S. marcescens* was tried for the isolation of myxobacteria on NNA plates with actidion and nystatin. This method has yielded much easier isolation than with Aerobacter plate, hence recommended for the isolation of myxobacteria.

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PROTON-LIGAND STABILITY CONSTANTS OF SALICYLOYL HYDRAZINE IN AQUO-ORGANIC MIXTURES

RECENT studies on the proton-ligand and metal-ligand equilibria in the presence of a co-solvent have revealed that the thermodynamic quantities[1–5] accompanying the chemical reactions are influenced to a greater extent by the solute-solvent interactions than those based on purely electro-static considerations. Further the equilibrium conditions for ligands containing both oxygen and nitrogen donor atoms are affected in different ways by the dielectric constant of the medium and the reports in literature have led to partially contradictory results[2,6–9]. In continuation of our studies on the ligand characteristics of aroyl hydrazines in non-aqueous media[10–11], we have taken up the study fo acido-basic equilibria of a bidentate salicyloyl

hydrazine in the presence of a co-solvent (dioxane, acetonitrile, or methanol) by an electrometric method.

Experimental

Aqueous solution of salicyloyl hydrazine (0·025M) was prepared from Fluka's AG pure sample. Dioxane was refluxed over potassium hydroxide and dired on barium oxide following the procedure of Lind *et al.*[12]. Acrylonitrile, a common impurity in acetonitrile was removed by refluxing with KOH and then the sample was subjected to fractional distillation[13]. B.D.H. Spectroscopic pure methanol was used without further purification. All other chemicals were of AnalaR grade.

In order to obtain the steady and reproducible pH values the electrode was equilibrated in a well strired mixture of aquo-organic medium and tested periodically with aqueous buffers after activating the electrodes with 0·1M HCl. The titration assembly employed is described elsewhere[11]. A constant ionic strengh of 0·1M was maintained with $NaClo_4$.

Procedure

0·02M perchloric acid was titrated with 0·2M sodium hydroxide in presence and in absence of 0·01–0·005M solutions of salicyloyl hydrazine in varying compositions (20–70% vol/vol) of methanol, dioxane or acetonitrile. The hydrogen ion concentration was calculated using the correction factors reported in literature[9].

Results and Discussion

The titration curve of the acid was much lower than that in the presence of ligand in the pH region of 2–5 indicating the proton-ligand association equilibria. The buffer region of the ligand titration curve at 6·9 pH shows the release of protons from the ligand, the inflection and the position in the titration curve indicates the release of only one proton. The average number of protons bound per ligand is calculated from Irving and Rossotti[14] equation and the stepwise formation constants by the method of least squares.

The acido-basic equilibria of the ligand may be represented by eqn. (1) & (2) and from the results recorded in Table I it is clear that the stability constant for the dissociation of the primarilyl electrostatic hydroxyl proton, increases with the increase in the amount of the co-solvent whereas that of covalent amino group proton decreases to a minimum and then begin to increase.

TABLE I

Proton ligand stability constants of salicyloyl hydrazine in aquo-organic media

$\mu = 0·10$; Temperature 28·0 ± 0·2° C

% (vol/vol)	Log K_1^H			% (vol/vol)	Log K_2^H		
	Acetonitrile	Dioxane	Methanol		Acetonitrile	Dioxane	Methanol
20	6·60	7·15	..	20	5·25	2·50	..
40	8·00	8·00	..	40	4·55	2·46	..
50	8·95	8·35	..	50	3·55	2·38	..
55	..	..	5·80	55	..	..	3·95
60	9·60	8·40	..	60	3·63	2·56	..
65	..	..	6·00	65	..	..	2·69
70	10·15	8·65	..	70	3·85	2·64	..
75	..	..	6·45	75	..	..	3·35
80	10·50	..	6·70	80	4·05	..	3·65

Born's treatment[15] of ion-ion interaction in a medium-which is considered as a dielectric continum-predicts an increased ion-ion interaction in solutions with decreasing dielectric constant. This explains the increased stability constant of the first complex ($\log K_1^H$) with decrease in dielectric constant of the medium. The second equilibrium is isoelectric and on considerations similar to that of Born indicate that the stability constant of this equilibrium should be unaffected by the change in the dielectric constant of the medium; or, since the radius of H_2L^+ is greater than that of proton, it may slightly increase.

The observed decrease in the log K_2^H values, which is in contradiction with the Born model, can be explained on consideration of non-electrostatic parameters like the basicity of the solvent[16]. In addition, specific chemical effects and second order interactions also contribute.

Thus the observed change in log K values in media of different dielectric constants is the sum of the

electrostatic and non-electrostatic contributions. Such separation of the medium effects into two categories is supported in the recent literature[9,17,18].

Summary

The acido-basic equilibria of bidentate salicyloyl hydrazine (LH) in aquo-organic media of varying composition (20–80%) is investigated by the pH metric titration technique at $28 \cdot 0 \pm 0 \cdot 2^{\circ}$ C. The terminal amino nitrogen of the ligand associates with a proton (LH_2^+) in the pH region of 2–5, while the ortho hydroxyl proton dissociates above pH of 6·0. The variation of the stability constants with the increase in the co-solvent (acetonitrile, dioxane, or methanol) are explained on the basis of electrostatic and non-electrostatic parameters.

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CORRELATION BETWEEN SUPEROXIDE DISMUTASE AND CATALASE ACTIVITY IN RED BLOOD CELLS OF DIFFERENT ANIMALS

IT has been demonstrated that erythrocuperin prepared from bovine or from human erythrocytes possesses superoxide dismutase activity[1]. The precise physiological role of superoxide dismutase is not clear at present, although its rapid destruction of superoxide anion in cells and tissues would eliminate a probable toxic substance (superoxide anion) by forming molecular oxygen and hydrogen peroxide[2]. The latter in turn could be destroyed rapidly by catalase. Both of these enzymes are present in relatively high concentrations in erythrocytes[3]. Since these enzymes might be expected to function together, their determination in various animals may be very useful for understanding their physiological roles.

The blood was collected from healthy animals in heparinized test tubes. The red blood cells were collected by low speed centrifugation and washed thrice with physiological saline. The cells were hemolysed with water (1 : 10) and then again centrifuged at 5000 rpm to separate the ghosts. The clear supernatant was used as the source of the enzyme. The haemoglobin was determined by cyanomethaemoglobin method[4].

Superoxide disumtase activity was determined by t. e method of Nishikimi *et al.*[5]. The reaction mixture (3 ml) contained 50 μ moles of nitro blue tetrazolium (NBT), 78 μ moles NADH and 3·1 μmoles of phenazine methosulphate (PMS). The reaction was carried out in 0·017 M sodium pyrophosphate buffer pH 8·3 at 25° C and the optical density was recorded at 530 nm. The catalase activity was determined by the method of Sinha[6].

Considerable variations in the level of catalase and superoxide dismutase were noted in the red blood cells of various animals. The results are presented in Table I. The lowest superoxide dismutase activity was observed in frog erythrocytes whereas high level of these was observed in erythrocytes of man, goat and chick. It is apparent that erythrocytes which show high superoxide dismutase activity also have high catalase activity and *vice versa*. However, Hartz *et al.*[7] have shown that there is no relationship between the levels of catalase and superoxide dismutase in human tissue. The results presented here show that in different species, this correlation does exist since