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Solubility Determination of Salicylic Acids and its Metabolite

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The solubility action of salicylic acid, acetyl salicylic acid and its metabolite, gentisic acid has been described by spectrophotometry. Drug and metabolite forms 1:1 complex with ferric nitrate. The extinction coefficient of salicylic acid, acetyl salicylic acid and gentisic acid are 1.5403×10^3, 3.8033×10^3 and 2.3856×10^3 L mol^{-1} cm^{-1}, respectively. On the basis of solu-bility ratio of concentrations in aqueous layer to chloroform layer, the drug and metabolite action have been evaluated.

Key Words: Drug action, Spectrophotometry, Metabolite, Salicylic acid, Acetyl salicylic acid, Gentisic acid.

INTRODUCTION

The manner in which any chemical compound is distributed between two phases of water and chloroform shows how it might be distributed between body fatty tissues and water. Generally polar compounds have lower solubility in fats and have greater solubility in aqueous phase. Any compound can be successfully acts as drug if it is effective in penetrating in the fatty tissues, which contains lipids.

Various types of metabolic study have been carried out in literature on acetyl salicylic acid and its subsequent metabolites such as salicylic acid and gentisic acid (2,5-dihydroxybenzoic acid)[1-9].

In present study, a solubility relationship of salicylic acid and acetyl salicylic acid to its metabolite gentisic acid is performed by spectrophotometrically. Further, the drug action of these compounds is also described on the basis of their solubility relationship.

EXPERIMENTAL

All chemicals were of analytical reagent grade and used directly without further purification.

Metal ion solution/salt solution: Ferric nitrate solution of 1×10^{-1} M was prepared by dissolving 4.04 g anhydrous salt of ferric nitrate in 100 mL of double distilled water. From this Fe^{3+} ion solution, the solutions of desired concentration were prepared by appropriate dilutions.

Drug solutions: A stock solution of salicylic acid, acetyl salicylic acid and gentisic acid each of 10×10^{-3} M concentration were prepared by dissolving required amount of salicylic acid, acetyl salicylic acid and gentisic acid, respectively in 0.01 M hydrochloric acid. From these stock solutions different concentration of each drug ranging from 1.0×10^{-3} M to 4.0×10^{-3} M were prepared.

Determination of wavelength of maximum absorption (λ_{max}) for salicylic acid, acetyl salicylic acid and gentisic acid with Fe^{3+} complex: 5.0 mL of salicylic acid of concentrations 1×10^{-3} M and 2×10^{-3} M were transferred to separate tubes. To each tube, 5.0 mL of Fe^{3+} ion solution of 1×10^{-3} M and 2×10^{-3} M was added. The tubes were shaken and allowed to stand for 10 min. Similarly a blank was prepared by taking water in place of drug. The same procedure was repeated for acetyl salicylic acid and gentisic acid.

The optical density measurement of each complex was made in the visible region using Systronic spectrophotometer model no. 118 against reagent blank.

Determination of L:M stoichiometric ratio in the complex formation: Job's continuous variation method was used for the determination of stiochiometric ratio of the complexes of salicylic acid, acetyl salicylic acid and gentisic acid. In this method, 1.0 to 9.0 mL of salicylic acid solution was taken in nine different test tubes. The required volume of Fe^{3+} solution of same concentration was added to each tube so that the total volume of each solution becomes 10 mL. The solution was allowed to stand for 10 min. The optical density of the purple coloured complex formed in each test tube were recorded at wavelength of maximum absorbance (530 nm). The plot of ratio of different salicylic acid concentration to different Fe^{3+} ion concentration was plotted.

Taking acetyl salicylic acid and gentisic acid as a ligand in place of salicylic acid similar determinations were made at their respective λ_{max}.

Verification of Beer-Lambert's law: Different volume of salicylic acid, acetyl salicylic acid and gentisic acid (each 4×10^{-3} M) ranging from 0.25-4.0 mL was taken in various test tubes. To each tube, 1.0 mL of 0.1 M Fe^{3+} was added. The content of each tube was made up to 25.0 mL using 0.01 M hydrochloric acid. After 10 min of interval, optical densities were recorded at the wavelength of maximum absorbance. The plots of optical density *versus* concentration were plotted.

Solubility relationship and drug action of salicylic acid, acetyl salicylic acid and gentisic acid: 25.0 mL of each of salicylic acid, acetyl salicylic acid and gentisic acid of 1×10^{-3} M to 4×10^{-3} M concentration was taken in different separating funnels. To each of the funnel 25.0 mL of distilled chloroform was added. Each funnel was then vigorously and uniformly

shaken for 0.5 h on shaking machine. The two layers were separated and aqueous layer was carefully collected.

1.0 to 5.0 mL of aqueous layer was then taken in different flask and 1.0 mL 0.1 M ferric nitrate solution was added to each flask. The volume of each flask was made to 26.0 mL using 0.1 M hydrochloric acid. The solutions were mixed and the optical density measurement was carried at λ_{max} against reagent blank.

RESULTS AND DISCUSSION

The wavelength of maximum absorption for for salicylic acid, acetyl salicylic acid and gentisic acid complexes were determined by measuring optical densities of complexes of these compounds at various wavelength of visible region. From the plots of optical density *versus* wavelength, the wavelengths of maximum absorption for the complexes are found to be 530, 535 and 410 nm.

The stoichiometric ratios of these complexes were evaluated by taking ratios of different concentrations of ligand to different concentrations of metal ion. It is observed that all the three complexes shows 1:1 stoichiometric ratio (Tables 1-3).

TABLE -1
DETERMINATION OF L:M STOICHIOMETRIC RATIO FOR SALICYLIC ACID AND Fe^{3+} ION
Concentration of salicylic acid solution = 2.0×10^{-3} M; Concentration of ferric nitrate = 2×10^{-3} M; Concentration of HCl = 0.010 M; Total volume of the system used = 10 mL; Maximum wavelength = 530 nm

Volume of SA solution (mL)	Volume of Fe^{3+} solution (mL)	$\frac{[L]}{[M]}$	$\frac{[L]}{[L]+[M]}$	Optical density
1.0	9.0	0.113	0.100	0.313
2.0	8.0	0.254	0.202	0.609
3.0	7.0	0.436	0.303	0.890
4.0	6.0	0.679	0.404	1.144
5.0	5.0	1.018	0.504	1.314
6.0	4.0	1.527	0.604	1.245
7.0	3.0	2.374	0.704	1.003
8.0	2.0	4.071	0.802	0.716
9.0	1.0	9.159	0.902	0.319

Varification of Beer's law: In case of each complex absorbance were recorded at respective λ_{max} value by taking different concentrations of the salicylic acid, acetyl salicylic acid and gentisic acid. These absorbances were plotted *vs.* concentrations. The Beer's law range for the ferric-salicylic acid complex is 8.886-75.9305 μg/mL and for ferric-acetyl salicylic acid complex 17.0241-331.5275 μg/mL while the range of 4.4535-53.5597 μg/mL

TABLE-2
DETERMINATION OF L:M STOICHIOMETRIC RATIO
FOR ACETYL SALICYLIC ACID AND Fe^{3+} ION
Concentration of acetyl salicylic acid solution = 2.012×10^{-3} M; Concentration of ferric nitrate = 2×10^{-3} M; Concentration of HCl = 0.010 M; Total volume of the system used = 10 mL; Maximum wavelength = 535 nm

Volume of SA solution (mL)	Volume of Fe^{3+} solution (mL)	$\frac{[L]}{[M]}$	$\frac{[L]}{[L]+[M]}$	Optical density
1.0	9.0	0.1118	0.1005	0.225
2.0	8.0	0.2115	0.2010	0.426
3.0	7.0	0.4310	0.3012	0.641
4.0	6.0	0.6705	0.4014	0.842
5.0	5.0	1.0058	0.5015	1.177
6.0	4.0	1.5090	0.6014	1.060
7.0	3.0	2.3470	0.7012	0.949
8.0	2.0	4.0240	0.8009	0.651
9.0	1.0	9.0520	0.9005	0.342

TABLE-3
DETERMINATION OF L:M STOICHIOMETRIC RATIO
FOR GENTISIC ACID AND Fe^{3+} ION
Concentration of gentisic acid solution = 2.0724×10^{-3} M; Concentration of ferric nitrate = 2×10^{-3} M; Concentration of HCl = 0.010 M; Total volume of the system used = 10 mL; Maximum wavelength = 410 nm

Volume of GA solution (mL)	Volume of Fe^{3+} solution (mL)	$\frac{[L]}{[M]}$	$\frac{[L]}{[L]+[M]}$	Optical density
1.0	9.0	0.1151	0.1032	0.349
2.0	8.0	0.2591	0.2058	0.593
3.0	7.0	0.4430	0.3075	0.846
4.0	6.0	0.6908	0.4086	1.103
5.0	5.0	1.0362	0.5089	1.243
6.0	4.0	1.5543	0.6085	1.137
7.0	3.0	2.4178	0.7074	0.946
8.0	2.0	4.1443	0.8056	0.737
9.0	1.0	9.3258	0.9032	0.353

is observed for ferric-gentisic acid complex. The corresponding molar extinction coefficients are found to be 1.54034×10^3, 3.8033×10^3 and 2.38564×10^3.

Determination of solubility relationship and drug action of salicylic acid, acetyl salicylic acid with its metabolite gentisic acid: The initial concentrations (g/L) of salicylic acid (S_1), acetylsalicylic acid (A_1) and gentisic acid (G_1) in aqueous layer before mixing with chloroform and concentration (g/L) of salicylic acid (S_2), acetylsalicylic acid (A_2) and gentisic acid (G_2) after mixing with chloroform are presented in Tables 4-6.

TABLE-4
STUDY OF SOLUBILITY RELATIONSHIP AND
DRUG ACTION OF SALICYLIC ACID
Concentration of stock solution of salicylic acid = 4×10^{-3} M; Volume of salicylic acid used for extraction = 25 mL; Volume of chloroform used for extraction = 25 mL; Concentration of ferric nitrate = 0.1 M; Concentration of HCl = 0.01 M; Volume of ferric nitrate (0.1 M) = 1.0 mL; Total volume of system = 26 mL; Maximum wavelength = 530 nm

Conc. of SA (M)	Vol. of SA (mL)	Conc. of SA in aqs. layer		Conc. of SA in $CHCl_3$ layer (S_1-S_2) (g/L) $\times 10^{-3}$	$\frac{(S_2)}{(S_1 - S_2)}$
		Before extraction S_1 (g/L) $\times 10^{-3}$	After extraction S_2 (g/L) $\times 10^{-3}$		
1×10^{-3}	1.0	5.3538	1.3385	4.0154	0.3345
	2.0	10.7077	2.8728	7.8349	0.3667
	3.0	16.0615	4.3958	11.6657	0.3768
	4.0	21.4154	5.8983	15.5171	0.3801
	5.0	26.7692	7.3317	19.4375	0.3772
2×10^{-3}	1.0	10.7077	3.1760	7.5317	0.4217
	2.0	21.4154	5.8090	15.6064	0.3722
	3.0	32.1231	8.5489	23.5742	0.3636
	4.0	42.8308	11.4153	31.4155	0.3634
	5.0	53.5384	14.2942	39.2442	0.3642
3×10^{-3}	1.0	16.0615	4.3499	11.7115	0.3714
	2.0	32.1231	8.9705	23.1526	0.3875
	3.0	48.1846	13.1728	35.0118	0.3762
	4.0	64.2462	17.3465	46.8997	0.3699
	5.0	80.3077	22.5112	57.7965	0.3895

From the above concentrations, the concentration (g/L) of salicylic acid (S_1-S_2), acetyl salicylic acid (A_1-A_2) and gentisic acid (G_1-G_2) in chloro-form layer are found out. These concentrations shows that higher concentrations of salicylic acid and acetyl salicylic acid goes into the chloro-form layer while very low concentration of gentisic acid passes into the chloro-form. Hence, it can be conferred that solubilities of salicylic acid and acetyl salicylic acid is more in chloroform while the solubility of gentisic acid is lower.

Any chemical compound can act as a drug if it is more soluble in lipids while any compounds produced in metabolism can be a good metabolite if it is soluble in water, so that it can be easily excreted in urine. In present study, we have chosen salicylic acid and acetyl salicylic acid as a drug and gentisic acid as metabolite. The solvent used is chloroform because it is lipid like solvent.

In present investigation, it is observed that the solubility of salicylic acid and acetyl salicylic acid is more in chloroform while the ratio of their concentrations in aqueous layer to concentrations in chloroform layer is

TABLE-5
STUDY OF SOLUBILITY RELATIONSHIP AND DRUG ACTION OF ACETYL SALICYLIC ACID

Concentration of stock solution of acetyl salicylic acid = 4×10^{-3} M; Volume of acetyl salicylic acid used for extraction = 25 mL; Volume of chloroform used for extraction = 25 mL; Concentration of ferric nitrate = 0.1 M; Concentration of HCl = 0.01 M; Volume of ferric nitrate (0.1 M) = 1.0 mL; Total volume of system = 26 mL; Maximum wavelength = 535 nm

Conc. of ASA (M)	Vol. of ASA (mL)	Conc. of ASA in aqs. layer		Conc. of ASA in $CHCl_3$ layer (A_1-A_2) (g/L) × 10^{-3}	$\frac{(A_2)}{(A_1 - A_2)}$
		Before extraction A_1 (g/L) × 10^{-3}	After extraction A_2 (g/L) × 10^{-3}		
1×10^{-3}	1.0	6.9230	4.4134	2.5096	1.7586
	2.0	13.8462	8.7981	5.0481	1.7429
	3.0	20.7692	13.2527	7.5165	1.7631
	4.0	27.6923	17.7861	9.9062	1.7955
	5.0	34.6154	22.1539	12.4615	1.7778
2×10^{-3}	1.0	13.8462	8.9011	4.9451	1.7999
	2.0	27.6923	17.7049	9.9874	1.7727
	3.0	41.5385	26.6272	14.9111	1.7857
	4.0	55.3846	35.3585	20.0261	1.7656
	5.0	69.2307	44.2827	24.9480	1.7750
		20.7692	13.2168	7.5524	1.7500
3×10^{-3}	1.0	20.7692	13.2168	7.5524	1.7500
	2.0	41.5385	26.6064	14.9321	1.7818
	3.0	62.3077	39.9618	22.3459	1.7883
	4.0	83.0769	53.1831	29.8938	1.7791
	5.0	103.8426	66.1143	37.7283	1.7524

TABLE-6
STUDY OF SOLUBILITY RELATIONSHIP AND DRUG ACTION OF GENTISIC ACID

Concentration of stock solution of gentisic acid = 4×10^{-3} M; Volume of gentisic acid used for extraction = 25 mL; Volume of chloroform used for extraction = 25 mL; Concentration of ferric nitrate = 0.1 M; Concentration of HCl = 0.01 M; Volume of ferric nitrate (0.1 M) = 1.0 mL; Total volume of system = 26 mL; Maximum wavelength = 410 nm

Conc. of GA (M)	Vol. of GA (mL)	Conc. of GA in aqs. layer		Conc. of GA in $CHCl_3$ layer (G_1-G_2) (g/L) × 10^{-3}	$\frac{(G_2)}{(G_1 - G_2)}$
		Before extraction G_1 (g/L) × 10^{-3}	After extraction G_2 (g/L) × 10^{-3}		
1×10^{-3}	1.0	5.9231	5.5663	0.3563	15.6239
	2.0	11.8461	11.1424	0.7037	15.8340
	3.0	17.7692	16.6667	1.1025	15.1172
	4.0	23.6923	22.2820	1.4103	15.7995
	5.0	29.6154	27.8371	1.7783	15.6538

Conc. of GA (M)	Vol. of GA (mL)	Conc. of GA in aqs. layer		Conc. of GA in $CHCl_3$ layer (G_1-G_2) (g/L) × 10^{-3}	$\frac{(G_2)}{(G_1 - G_2)}$
		Before extraction G_1 (g/L) × 10^{-3}	After extraction G_2 (g/L) × 10^{-3}		
2×10^{-3}	1.0	11.8461	11.1093	0.7368	15.0778
	2.0	23.6923	22.2564	1.4359	15.4999
	3.0	35.5385	33.4048	2.1336	15.6565
	4.0	47.3840	44.5513	2.8327	15.7275
	5.0	59.2307	55.6870	3.5437	15.7144
3×10^{-3}	1.0	17.7692	16.6923	1.0769	15.5000
	2.0	35.5385	33.3866	2.1519	15.5149
	3.0	53.3077	50.0941	3.2136	15.5882
	4.0	71.0769	66.8289	4.2481	15.7315
	5.0	88.8462	83.4756	5.3701	15.5430

lower. Hence salicylic acid and acetyl salicylic acid will acts as a drug. On the other hand, the solubility of gentisic acid is poor in chloroform and greater in water while the ratio of its concentration in aqueous layer to concentration in chloroform layer is very high as compared to ratios of salicylic acid and acetyl salicylic acid. Hence gentisic acid will be a good metabolite.

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