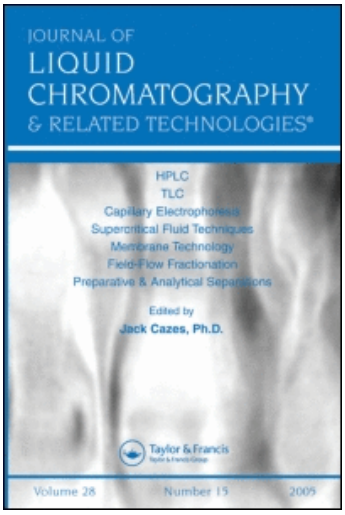

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IMPROVED SEPARATION OF VITAMIN B COMPLEX AND FOLIC ACID USING SOME NEW SOLVENT SYSTEMS AND IMPREGNATED TLC

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IMPROVED SEPARATION OF VITAMIN B COMPLEX AND FOLIC ACID USING SOME NEW SOLVENT SYSTEMS AND IMPREGNATED TLC

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ABSTRACT

TLC separation of vitamins of the 'B' complex group and folic acid has been achieved on plates impregnated with different transition metal ions. The metal ions used were Mn^{++}, Fe^{++}, Co^{++}, Ni^{++}, Cu^{++}, Zn^{++} and Hg^{++}. hR_f values for all the vitamins, using six new solvent systems, worked out for the purpose, for each of the four different concentrations of each metal ion have been reported. The results have been discussed for each metal ion compared and the best conditions of separation have been identified.

INTRODUCTION

The water soluble group of vitamins plays a very important role in the action of certain enzymes, acting as coenzymes, along with the prevention of some diseases. Trace analysis of vitamin-B complex becomes indispensable as it is the monitor of the water soluble group.[1] Also, the vitamins of the B complex group are responsible for the healthy functioning of the muscles, nerves, gastro-intestinal system, skin, and blood. Deficiency of these may lead to diseases such as beri-beri, macrocyclic anaemia, chellosis, and pernicious anaemia. There are many reports using HPLC,[2,3] GC,[4] HPCE,[5] and CZE[6] for the separation of water soluble vitamins.

TLC separation of mixtures of vitamin-B complex,[7] acid labile cobalamin,[8] vitamin B6,[9] vitamin B_2,[10] and folic acid[11] have also been reported. Impregnating reagents have been reported to improve the separation of a number of compounds.[12] TLC resolution of constituents of vitamin-B complex[13-15] on impregnated plates have also been described by some workers. Nevertheless, less work has been done on separation of vitamins using impregnated TLC.

Simultaneous analysis of vitamin B_1, B_2, B_6, B_{12}, and folic acid is very important for quality control of multivitamin preparations. So, studies on the use of impregnated plates for the separation of these vitamins were made and six new, improved solvent systems were worked out; the results obtained are presented in this paper.

EXPERIMENTAL

Vitamin samples analyzed were B_1, B_2, B_6, B_{12}, and folic acid. These samples were purchased from BDH (India), Glaxo (India), and Cyanamid (India). All the impregnating reagents of AR Grade were from E. Merck (Bombay). The silica gel G (E. Merck (India) Ltd., Bombay) with $CaSO_4$ (13%), iron, chloride (0.03% each), and giving pH 7 in an aqueous suspension (10%) was used, and solvents used were also from E. Merck (Bombay).

Vitamin samples were extracted from commercial tablets, capsules, and injection vials available for individual components. The tablets (containing 200 mg of vitamin) were ground to a fine powder and extracted with absolute ethanol (20 mL) thrice. The mother liquor was decanted and the crystals were washed with a little ether. The purity of each vitamin was confirmed by m.p. and by recording UV spectra.[16] The yield was about 75%. The solutions of vitamins (10^{-3}M) were prepared in 70% ethanol.

Thin layer plates (20cm X 20cm X 0.5 mm) were prepared by spreading a slurry of silica gel G in distilled water in a ratio of 1:2 with the help of Stahl type applicator. The plates were then dried overnight at 50 ± 2 ^{o}C in an oven. For impregnated plates, the slurry was prepared in aqueous solutions of different metal ions, the ions used were Mn^{++}, Fe^{++}, Co^{++}, Ni^{++}, Cu^{++}, Cd^{++}, Zn^{++}, or Hg^{++} and 0.1, 0.2, 0.3, 0.4% of each of these ions was used.

Samples were applied at the 500 ng level with the help of 100μL Hamilton syringe. The chromatograms were developed during 70 minutes for a 10 cm run in all the solvent systems. The plates were air dried after development. Vitamin B_{12} remained as a natural bright red spot. Spots of vitamin B_2, B_6, and folic acid appeared yellow. However, vitamin B_1 was located by exposing the plates to iodine vapors.

RESULTS AND DISCUSSION

Systematic studies were made to find out effective solvent systems for the separation of vitamins and the following six systems were found to be successful for this purpose:

A_1: Chloroform-n-butanol-acetic acid-ammonia (4:7:5:1;v/v);

A_2: Chloroform-n-butanol-water-acetic acid-ammonia (3:5:0.5:5:0.5;v/v);

A_3: Benzene-butylacetate-n-propanol-acetic acid-ammonia (1:4:1:5:1;v/v);

A_4: Carbon tetrachloride-butylacetate-propionic acid-ammonia (3:7:9:3;v/v);

A_5: Carbon tetrachloride-butylacetate-methanol-ammonia (1.5:4.5:7:0.5;v/v);

A_6: Carbon tetrachloride-butylacetate-propionic acid-methanol-water (2:3:1:0.5:3;v/v).

In order to improve the separation of vitamins, four different concentrations viz. 0.1%, 0.2%, 0.3% and 0.4% of each metal ion (Mg^{++}, Fe^{++}, Co^{++}, Ni^{++}, Cu^{++}, Zn^{++}, Cd^{++}, and Hg^{++}) were tried. Some of the best results, showing the influence of metal ions on chromatographic behaviour of vitamins, are shown in Tables 1-8. The results shown in each of the tables have been discussed in paragraphs and compared with those on plates without any impregnating reagents.

The hR_f values were affected by the concentration of impregnating reagent in all the solvent systems. Each reported hR_f value is the average of at least three or more identical runs. The spots were more compact on impregnated layers than on plain silica gel layer. The resolution possibilities of vitamins were calculated by dividing the distance between two spot centres by the sum of the two spot radii and a value of 1.50 or more was considered as a measure of complete resolution. The variation in hR_f values with different transition metal ions can be attributed to complex formation and variation in solubilities of complexes in different solvent systems or their different adsorption coefficients during the development of the chromatogram. The effect of each metal ion at four different concentrations in the six solvent systems is discussed below.

Impregnation with $MnSO_4$

There was a general decrease in R_f values of vitamins on plates impregnated with $MnSO_4$ in all the nine solvent systems, in comparison to plain plates. On going from 0.1% to 0.2% impregnation, hR_f values increased mostly

Table 1

HR_f Values of Vitamins on Plates Impregnated with Different Concentrations of $MnSO_4$

Sample	A1 (%)				A2 (%)				A3 (%)				A4 (%)				A5 (%)				A6 (%)			
	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3
No.	**0.4**				**0.4**				**0.4**				**0.4**				**0.4**				**0.4**			
1	40	24	28	30	38	14	28	29	40	23	30	38	50	37	48	47	10	20	15	16	10	07	07	08
	32				28				39				48				14				07			
2	65	55	54	56	65	53	60	63	68	52	47	50	63	58	59	55	62	60	60	61	70	58	57	70
	55				62				53				53				62				68			
3	62	51	57	59	62	49	58	59	66	55	56	58	60	54	56	59	69	65	65	64	35	33	31	33
	59				58				58				60				65				31			
4	68	65	62	64	64	62	54	54	73	58	60	64	65	62	64	62	73	71	75	76	52	49	48	51
	64				51				63				64				74				50			
5	60	32	55	58	50	24	50	38	54[T]	32	48[T]	50*	51	40	42	42	12	15	10	11	38[T]	33	41	43
	56				36				52				45				19				41			

* Slight tailing; T, Tailing. Sample No. 1, vitamin B_1; 2, vitamin B_2; 3, vitamin B_6; 4, folic acid; 5, vitamin B_{12}.

A_1: chloroform-n-butanol-acetic acid-ammonia (4:7:5:1, v/v); A_2: chloroform-n-butanol-water-acetic acid-ammonia (3:5:0.5:5:0.5, v/v); A_3: benzene-Butylacetate-n-propanol-acetic acid-ammonia (1:4:1:5:1, v/v); A_4: carbon tetrachloride-butylacetate-propionic acid-ammonia (3:7:9:3, v/v); A_5: carbon tetrachloride-butylacetate-methanol-ammonia (1.5:4.5:7:0.5, v/v); A_6: carbon tetrachloride-butylacetate-propionic acid-methanol-water (2:3:1:0.5:3, v/v).

Table 2

HR_f Values of Vitamins on Plates Impregnated with Different Concentrations of $FeSO_4$

Sample	A1 (%)				A2 (%)				A3 (%)				A4 (%)				A5 (%)				A6 (%)			
	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3
No.	**0.4**				**0.4**				**0.4**				**0.4**				**0.4**				**0.4**			
1	40 34	27	32	35	38 21	30	21	23	40 42	15	21	41	50 43	35	34	32	10 15	15	18	17	10 08	10	22	26
2	65 65	38	66	69	65 55	48	56	57	68 54	25	50	54	63 56	55	53	52	62 63	48	62	63	70 66	75	78	67
3	62 63	45	60	65	62 51	41	53	54	66 55	31	53	57	60 59	58	57	56	69 58	53	50	55	35 68	55	69	70
4	68 69	49	70	70	64 47	45	61	63	73 61	35	60	63	65 65	63	61	61	73 76	57	78	77	52 71	71	71	73
5	60 50	23	45	51	50 30	36	29^T	31	54 51	19	42	50	51 48	45	40	39	12 19	17	20	21	38^T 49	39	48	51

T, Tailing. Sample No. 1, vitamin B_1; 2, vitamin B_2; 3, vitamin B_6; 4, folic acid; 5, vitamin B_{12}.

A_1: chloroform-n-butanol-acetic acid-ammonia (4:7:5:1, v/v); A_2: chloroform-n-butanol-water-acetic acid-ammonia (3:5:0.5:5:0.5, v/v); A_3: benzene-Butylacetate-n-propanol-acetic acid-ammonia (1:4:1:5:1, v/v); A_4: carbon tetrachloride-butylacetate-propionic acid-ammonia (3:7:9:3, v/v); A_5: carbon tetrachloride-butylacetate-methanol-ammonia (1.5:4.5:7:0.5, v/v); A_6: carbon tetrachloride-butylacetate-propionic acid-methanol-water (2:3:1:0.5:3, v/v).

Table 3

HR_f Values of Vitamins on Plates Impregnated with Different Concentrations of $CoSO_4$

Sample	A1 (%)				A2 (%)				A3 (%)				A4 (%)				A5 (%)				A6 (%)			
	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3
No.	**0.4**				**0.4**				**0.4**				**0.4**				**0.4**				**0.4**			
1	40 22	38	20	24	38 25	24	25	26	40 42	46	22	43	50 42	42	35	33	10 18	12	20	19	10 16	18	15	18
2	65 57	64	55	59	65 58	60	61	61	68 50	73	49	51	63 54	55	39	36	62 60	59	62	61	70 68	71	68	70
3	62 55	61	57	58	62 53	56	56	58	66 56	73	51	55	60 50	59	58	50	69 59	63	69	60	35 60	61	65	66
4	68 65	65	65	67	64 48	68	69	61	73 61	76	72	60	65 64	64	64	63	73 75	67	71	73	52 65	67	72	74
5	60 43	54	34	45	50 40	35	40^T	41	54^T 46	56	42^T	49	51 49	47	40	40	12 26	30	23^T	24	38^T 35	39	34	36

T, Tailing. Sample No. 1, vitamin B_1; 2, vitamin B_2; 3, vitamin B_6; 4, folic acid; 5, vitamin B_{12}.

A_1: chloroform-n-butanol-acetic acid-ammonia (4:7:5:1, v/v); A_2: chloroform-n-butanol-water-acetic acid-ammonia (3:5:0.5:5:0.5, v/v); A_3: benzene-Butylacetate-n-propanol-acetic acid-ammonia (1:4:1:5:1, v/v); A_4: carbon tetrachloride-butylacetate-propionic acid-ammonia (3:7:9:3, v/v); A_5: carbon tetrachloride-butylacetate-methanol-ammonia (1.5:4.5:7:0.5, v/v); A_6: carbon tetrachloride-butylacetate-propionic acid-methanol-water (2:3:1:0.5:3, v/v).

Table 4

HR_f Values of Vitamins on Plates Impregnated with Different Concentrations of $NiSO_4$

Sample	A1 (%)				A2 (%)				A3 (%)				A4 (%)				A5 (%)				A6 (%)			
	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3
No.	**0.4**				**0.4**				**0.4**				**0.4**				**0.4**				**0.4**			
1	40	22	28	30	38	06	24	25	40	31	38	36	50	40	32	31	10	15	28	24	10	23	15	19
	27				24				42				28				26				16			
2	65	47	55	58	65	63	55	57	68	34	64	60	63	52	38	46	62	52	60	61	70	69	65	66
	56				56				59				42				63				65			
3	62	52	52	55	62	52	56	55	66	15	65	60	60	59	50	49	69	65	67	65	35	63	66	68
	54				53				63				47				67				60			
4	68	65	53	62	64	59	58	59	73	30	64	63	65	64	51	55	73	72	73	73	52	75	73	76
	60				47				66				52				74				61			

T, Tailing. Sample No. 1, vitamin B_1; 2, vitamin B_2; 3, vitamin B_6; 4, folic acid; 5, vitamin B_{12}.
A_1: chloroform-n-butanol-acetic acid-ammonia (4:7:5:1, v/v); A_2: chloroform-n-butanol-water-acetic acid-ammonia (3:5:0.5:5:0.5, v/v); A_3: benzene-Butylacetate-n-propanol-acetic acid-ammonia (1:4:1:5:1, v/v); A_4: carbon tetrachloride-butylacetate-propionic acid-ammonia (3:7:9:3, v/v); A_5: carbon Tetrachloride-butylacetate-methanol-ammonia (1.5:4.5:7:0.5, v/v); A_6: carbon tetrachloride-butylacetate-propionic acid-methanol-water (2:3:1:0.5:3, v/v).

Table 5

HR_f Values of Vitamins on Plates Impregnated with Different Concentrations of $CuSO_4$

Sample	A1 (%)				A2 (%)				A3 (%)				A4 (%)				A5 (%)				A6 (%)			
	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3
No.	**0.4**				**0.4**				**0.4**				**0.4**				**0.4**				**0.4**			
1	40	31	39	41	38	22^T	20	31	40	35	40	44	50	52	42	35	10	20	29	27	10	24	18	21
	39				29				41				36				30				19			
2	65	64	65	66	65	63	60	61	68	60	66	67	63	59	56	53	62	64	63	64	70	75	70	73
	64				59				57				55				64				71			
3	62	60	69	75	62	60	59	58	66	61	68	70	60	63	61	50	69	74	75	76	35	72	66	67
	71				55				60				59				67				68			
4	68	68	57	61	64	64	63	64	73	71	64	71	65	69	52	46	73	78	80	76	52	79	79	82
	60				58				63				62				75				77			
5	60	42	40	49	50	39	38	35	54^T	43	45	47	51	48	36	38	12	24	26	26	38^T	33	25	27
	46				34				46				40				25				25			

T, Tailing. Sample No. 1, vitamin B_1; 2, vitamin B_2; 3, vitamin B_6; 4, folic acid; 5, vitamin B_{12}.

A_1: chloroform-n-butanol-acetic acid-ammonia (4:7:5:1, v/v); A_2: chloroform-n-butanol-water-acetic acid-ammonia (3:5:0.5:5:0.5, v/v); A_3: benzene-Butylacetate-n-propanol-acetic acid-ammonia (1:4:1:5:1, v/v); A_4: carbon tetrachloride-butylacetate-propionic acid-ammonia (3:7:9:3, v/v); A_5: carbon Tetrachloride-butylacetate-methanol-ammonia (1.5:4.5:7:0.5, v/v); A_6: carbon tetrachloride-butylacetate-propionic acid-methanol-water (2:3:1:0.5:3, v/v).

Table 6

HR_f Values of Vitamins on Plates Impregnated with Different Concentrations of $ZnSO_4$

Sample	A1 (%)				A2 (%)				A3 (%)				A4 (%)				A5 (%)				A6 (%)			
	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3
No.	0.4				0.4				0.4				0.4				0.4				0.4			
1	40 36	39	38	40	38 23	18[T]	21	25	40 39	22	42	41	50 26	30	35	32	10 27	18	27	26	10 10	34	12	14
2	65 62	60	61	63	65 50	65	49	53	68 57	40	67	61	63 44	44	48	51	62 63	58	61	62	70 69	78	55	61
3	62 69	57	66	72	62 63	55	57	64	66 59	43	68	58	60 31	48	55	47	69 58	64	70	65	35 64	56	60	67
4	68 52	66	53	55	64 58	60	60	61	73 60	63	65	65	65 52	54	55	54	73 70	66	71	73	52 58	60	71	79
5	60 41	47	32	43	50 27	32	33	29	54[T] 45	37	48	46	51 34	34	39	36	12 23	22	24	25	38[T] 18	42	21	22

T, Tailing. Sample No. 1, vitamin B_1; 2, vitamin B_2; 3, vitamin B_6; 4, folic acid; 5, vitamin B_{12}.
A_1: chloroform-n-butanol-acetic acid-ammonia (4:7:5:1, v/v); A_2: chloroform-n-butanol-water-acetic acid-ammonia (3:5:0.5:5:0.5, v/v); A_3: benzene-Butylacetate-n-propanol-acetic acid-ammonia (1:4:1:5:1, v/v); A_4: carbon tetrachloride-butylacetate-propionic acid-ammonia (3:7:9:3, v/v); A_5: carbon tetrachloride-butylacetate-methanol-ammonia (1.5:4.5:7:0.5, v/v); A_6: carbon tetrachloride-butylacetate-propionic acid-methanol-water (2:3:1:0.5:3, v/v).

Table 7

HR_f Values of Vitamins on Plates Impregnated with Different Concentrations of $CdSO_4$

Sample	A1 (%)				A2 (%)				A3 (%)				A4 (%)				A5 (%)				A6 (%)			
	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3
No.	**0.4**				**0.4**				**0.4**				**0.4**				**0.4**				**0.4**			
1	40	17	21	25	38	11	13	15	40	17	42	41	50	36	36	30	10	23	31	30	10	24	11	15
	21				14				37				26				29				11			
2	65	45	46	49	65	53	48	47	68	48	66	50	63	55	47	49	62	47	65	64	70	70	59	67
	47				43				51				47				66				65			
3	62	49	49	51	62	43	49	53	66	59	65	51	60	58	53	42	59	52	72	64	35	63	55	61
	50				49				53				40				63				54			
4	68	67	41	69	64	50	50	57	73	68	69	61	65	65	48	46	73	60	76	76	52	65	70	72
	41				55				58				44				74				60			
5	60	23	26	33	50	14	26	25	54^T	14	46	43	51	31	35	35	12	39	24	24	38^T	30	17	19
	30				24				45				33				19				18			

T, Tailing. Sample No. 1, vitamin B_1; 2, vitamin B_2; 3, vitamin B_6; 4, folic acid; 5, vitamin B_{12}.

A_1: chloroform-n-butanol-acetic acid-ammonia (4:7:5:1, v/v); A_2: chloroform-n-butanol-water-acetic acid-ammonia (3:5:0.5:5:0.5, v/v); A_3: benzene-Butylacetate-n-propanol-acetic acid-ammonia (1:4:1:5:1, v/v); A_4: carbon tetrachloride-butylacetate-propionic acid-ammonia (3:7:9:3, v/v); A_5: carbon tetrachloride-butylacetate-methanol-ammonia (1.5:4.5:7:0.5, v/v); A_6: carbon tetrachloride-butylacetate-propionic acid-methanol-water (2:3:1:0.5:3, v/v).

Table 8

HR_f Values of Vitamins on Plates Impregnated with Different Concentrations of $HgSO_4$

Sample	A1 (%)				A2 (%)				A3 (%)				A4 (%)				A5 (%)				A6 (%)			
	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3	Plain	0.1	0.2	0.3
No.	0.4				0.4				0.4				0.4				0.4				0.4			
1	40 19	16	21	24	38 17	15	17	19	40 37	32	40	39	50 30	36	34	30	10 19	12	15	17	10 11	15	10	11
2	65 44	25	42	46	65 41	31	40	44	68 50	52	55	49	63 43	52	48	48	62 54	50	45	61	70 61	71	57	60
3	62 50	37	47	50	62 52	35	49	55	66 51	50	69	52	60 38	56	55	41	69 61	60	65	60	35 47	47	49	50
4	68 41	45	40	45	64 50	41	53	52	73 57	56	66	59	69 50	59	51	52	73 73	64	69	71	52 49	36	66	67
5	60 29	22	24	31	50 38	27	36^T	40	54^T 43	42	49^T	43*	51 33	39	37	34	12 21	19	10	20	38^T 16	20	10	12

* Slight tailing; T, Tailing. Sample No. 1, vitamin B_1; 2, vitamin B_2; 3, vitamin B_6; 4, folic acid; 5, vitamin B_{12}.
A_1: chloroform-n-butanol-acetic acid-ammonia (4:7:5:1, v/v); A_2: chloroform-n-butanol-water-acetic acid-ammonia (3:5:0.5:5:0.5, v/v); A_3: benzene-Butylacetate-n-propanol-acetic acid-ammonia (1:4:1:5:1, v/v); A_4: carbon tetrachloride-butylacetate-propionic acid-ammonia (3:7:9:3, v/v); A_5: carbon tetrachloride-butylacetate-methanol-ammonia (1.5:4.5:7:0.5, v/v); A_6: carbon tetrachloride-butylacetate-propionic acid-methanol-water (2:3:1:0.5:3, v/v).

in solvent systems A_1, A_2, A_3, and A_4, while these values decreased in solvent A_6. No definite trend was observed with solvent system A_5. Varying the concentration of $MnSO_4$ from 0.2% to 0.3%, hR_f values, in general, increased in all the solvent systems except in solvent system A_4, where these values decreased. Further, on increasing the concentration to 0.4%, an increase in hR_f values was observed in solvent systems A_3, A_4, and A_5.

It is evident from Table 1 that vitamins which were not resolved on plain plates in different solvent systems are resolved after impregnation with $MnSO_4$. These are vitamin B_6 and B_{12} in solvent system A_1 (at 0.1%), in solvent system A_6 (at 0.2%, 0.3% and 0.4%), vitamin B_2, B_6, and B_{12} in solvent system A_2 (at 0.1%, 0.3% and 0.4%); vitamin B_2 and B_6 in solvent system A_3 (at 0.1% to 0.4%); vitamin B_1 and B_{12} in solvent system A_5 (at 0.1% to 0.4%) and vitamin B_1, B_2, B_6, folic acid and B_{12} in solvent system A_4 (at 0.1% to 0.4%). Best results were achieved at 0.3% of $MnSO_4$ with all the solvent systems except A_1.

Impregnation with $FeSO_4$

The hR_f values were generally decreased with $FeSO_4$ impregnation in comparison to plain plates (Table 2) in all the solvent systems except solvent system A_6 where these values generally increased. An increase of concentration of impregnating reagent from 0.1% to 0.2% resulted into a general decrease in R_f values in solvent system A_4, while an increase in these values was observed in solvent systems A_1, A_2, A_3, A_5, and A_6. On going from 0.2% to 0.3%, these values generally decreased in solvent system A_4 only, in all the other solvent systems these values increased subsequently. While, from 0.3% to 0.4%, hR_f values decreased in all the solvent systems except in solvent system where these values increased (in contrast 0.3%).

Table 2 shows that impregnation of $FeSO_4$ resulted into separation of following vitamins in comparison to plain plates: vitamin B_6 and B_{12} in solvent systems A_1 and A_6 (at 0.1% to 0.4%); vitamin B_2, B_6, and folic acid in solvent system A_2 (at 0.1% to 0.4%); vitamin B_2 and B_6 in solvent system A_3 (at 0.1% to 0.4%); vitamin B_1, B_2, B_6, folic acid, and B_{12} (at 0.1% to 0.4%) in solvent system A_4 (at 0.1% to 0.4%) and vitamin B_1 and B_{12} in solvent system (at 0.3%). The best results were obtained at 0.1% impregnation of $FeSO_4$ in all the solvent systems except solvent system A_5.

Impregnation with $CoSO_4$

For most of the vitamins, hR_f values decreased in comparison to plain plates when $CoSO_4$ was used as impregnating reagent, in comparison to plain plates (Table 3) except that these were increased with solvent system A_6. On

comparing the effect of different concentrations of $CoSO_4$, it was found that at 0.1% to 0.2%, hR_f values increased in solvent systems A_2 and A_5, decreased in solvent systems A_1, A_3, A_4, and A_6. On increasing the concentration to 0.3%, hR_f values mostly increased in every solvent system except solvent systems A_4 and A_5, where a decrease in these values was observed. Further increasing the concentration to 0.4%, hR_f values increased only in solvent system A_4 and decreased mostly in all the other solvent systems.

Successfully resolved vitamins which were not resolved on plain plates (Table 3) are: vitamin B_6 and B_{12} in solvent systems in A_1 and A_6 (at 0.1% to 0.4%); vitamin B_2, B_6 and folic acid in solvent system A_2 (at 0.1%, 0.2% and 0.4%); vitamin B_2 and B_6 in solvent system A_3 (at 0.3% and 0.4%); vitamin B_1, B_2, B_6, folic acid, B_{12} in solvent system A_4 (at 0.1%, 0.3%, 0.4%) and vitamin B_1 and B_{12} in solvent system A_5 (at 0.1% to 0.4%). Each vitamin was resolved at 0.1% in solvent systems A_2, A_4, A_5, and A_6.

Impregnation with $NiSO_4$

Decreases in hR_f values were observed, in general, using $NiSO_4$ as impregnating reagent with all the solvent systems in comparison to plain plates (Table 4) except for solvent systems A_5 and A_6 where these values generally increased in most of the cases. On changing the concentration from 0.1% to 0.2%, hR_f values generally increased in solvent systems A_2, A_4, A_5, and generally decreased in all the other solvent systems, i.e., solvent systems A_3 and A_6. No regular trend was observed with solvent system A_1. Varying the concentration from 0.2% to 0.3%, hR_f values decreased with solvent systems A_3, A_4, and A_5, and increased with rest of the solvent systems. Whereas, from 0.3% to 0.4%, hR_f values increased in solvent systems A_3, A_5 and decreased with other solvent systems. It was found that using solvent system A_4, there was a general decrease in hR_f values from 0.1% to 0.4%.

Table 4 shows the resolved vitamins on impregnated plates with $NiSO_4$ which were not resolved earlier on plain plates. These vitamins are vitamin B_6 and B_{12} in solvent systems A_1 and A_6. (at 0.1% to 0.4%); vitamin B_2, B_6 and folic acid in solvent system A_2 (at 0.1% to 0.4%); vitamin B_2, and folic acid in solvent system A_3 (at 0.1% to 0.4%); all the vitamins in solvent system A_4 (at 0.1%, 0.2% and 0.3%); vitamin B_1 and B_{12} in solvent system A_5 (at 0.1%). Best results were achieved at 0.1% with all the solvent systems except A_3.

Impregnation with $CuSO_4$

Impregnation with $CuSO_4$ resulted in a general decrease of hR_f values in comparison to plain plates (Table 5) in all the solvent systems except solvent

systems A_5 and A_6. On going from 0.1% to 0.2%, hR_f values, in general, increased in solvent systems A_1, A_3, and A_5. These values decreased in all the other solvent systems. From 0.2% to 0.3%, hR_f values generally decreased in solvent systems A_2, A_4, and A_5. Increase in these values was found in solvent systems A_3 and A_6. Further, from 0.3% to 0.4%, hR_f values in general decreased in all the solvent systems except in solvent system A_4 where these values increased.

Table 5 shows the vitamins which resolved with $CuSO_4$ impregnation and had not resolved with plain plates in the same solvent system. These are vitamin B_6 and B_{12} in solvent systems A_1 and A_6 (at 0.1% to 0.4%); vitamin B_2, B_6, and folic acid in solvent system A_2 (at 0.3%); vitamin B_2 and B_6 in solvent system A_3 (at 0.3% and 0.4%); vitamin B_2, B_6, folic acid, and B_{12} in solvent system A_4 (at 0.1% to 0.4%); vitamin B_1 and B_{12} in solvent system A_5 (at 0.1%, 0.2% and 0.4%). Best results were obtained at 0.4% in all the solvent systems except A_2.

Impregnation with $ZnSO_4$

hR_f values decreased in most of the cases with $ZnSO_4$ impregnation in comparison to plain plates (Table 6) in all the developed systems except solvent system A_6 where these values increased mostly. While studying the effect of different concentrations of $ZnSO_4$ it was found that, on going from 0.1% to 0.2%, hR_f values increased mostly in solvent systems A_2, A_3, A_4, and A_5. These values decreased mostly in solvent systems A_1 and A_6. Varying the concentration from 0.2% to 0.3%, a decrease in hR_f values was observed mostly in solvent systems A_3 and A_4. These values increased in all the other solvent systems. Further increasing the concentration to 0.4%, hR_f values decreased in all the solvent systems.

It is evident, from Table 6, that vitamins which were not resolved on plain plates in various solvent systems are resolved after impregnation with $ZnSO_4$. These are vitamin B_6 and B_{12} in solvent systems A_1 and A_6 (0.1% to 0.4%); vitamin B_2, B_6, and folic acid in solvent system A_2 (0.1% to 0.4%); vitamin B_2 and B_6 in solvent system A_3 (at 0.1% and 0.3%); vitamin B_1, B_2, B_6, folic acid, and B_{12} in solvent system A_4 (at 0.1%, 0.3% and 0.4%); vitamin B_1 and B_{12} in solvent system A_5 (at 0.1%, 0.2% and 0.4%). Best results were obtained at 0.3% impregnation in solvent systems A_1, A_2, A_3, A_4, and A_6.

Impregnation with $CdSO_4$

There was a general decrease in hR_f values with $CdSO_4$ impregnation in all the solvent systems in comparison to plain plates (Table 7). On going from 0.1% to 0.2%, hR_f values, in general, decreased in solvent systems A_4 and A_6

while general increase in hR_f values was observed in solvent systems A_2, A_3, and A_5. On increasing the concentration from 0.2% to 0.3%, hR_f values increased in solvent system A_2 and A_6 and decreased in solvent systems A_3, A_4, and A_5. Further changing the concentration from 0.3% to 0.4% hR_f values decreased in all the solvent systems except A_3 where an increase in hR_f values was observed.

Table 7 shows the vitamins which where not resolved earlier on plain plates. These are vitamins B_6 and B_{12} in solvent systems A_1 and A_6 (at 0.1% to 0.2%); vitamin B_2, B_6, and folic acid in solvent system A_2 (at 0.1%, 0.3% and 0.4%); vitamin B_2 and B_6 in solvent system A_3 (at 0.1% and 0.2%); vitamin B_1, B_2, B_6, folic acid, and B_{12} in solvent system A_4 (at 0.1%, 0.3% and 0.4%); vitamin B_1 and B_{12} in solvent system A_5 (at 0.1% to 0.4%). It was interesting to find out that 0.1% to 0.4%, hR_f values decreased in solvent system A_4. Best results were achieved at 0.4% in all solvent systems except solvent system A_3.

Impregnation with $HgSO_4$

It was observed, in general, that use of $HgSO_4$ as impregnating reagent resulted in decrease in hR_f values with all the solvent systems in comparison to plain plates (Table 8), except solvent A_6 where hR_f values increased in general. Increasing the concentration from 0.1% to 0.2%, hR_f values increased in solvent systems A_3, A_4, and A_5. On going from 0.2% to 0.3%, these values decreased in solvent systems A_3 and A_4. A general increase was found in solvent systems A_1, A_2, A_5, and A_6. Further, varying the concentration to 0.4%, a decrease in hR_f values was observed in solvent system A_5 where these values were increased. A decrease in hR_f values from 0.1% to 0.4% was found in solvent system A_4.

It is clear from Table 8 that vitamins which were not resolved on plain plates in different solvent systems are resolved after impregnation with $HgSO_4$. These are vitamins B_6 and B_{12} in solvent systems A_1 and A_6 (at 0.1% to 0.4%); vitamin B_2, B_6, and folic acid in solvent system A_2 (at 0.1%, 0.2% and 0.3%); vitamin B_2 and B_6 in solvent system A_3 (at 0.1%, 0.2% and 0.3%); vitamin B_1, B_2, B_6, B_{12}, and folic acid in solvent system A_4 (at 0.1% to 0.4%); vitamin B_1 and B_{12} in solvent system A_5 (at 0.1%, 0.2% and 0.3%). Best results were obtained at 0.1% impregnation with solvent systems A_1, A_2, A_4, and A_6.

Better resolution, with disappearance of tailing in most of the cases, with general decrease in hR_f values, was observed on impregnated plates as compared to untreated ones in all the developed solvent systems. All the concentrations of $CoSO_4$ in solvent system A_1 were unsuccessful as there was incomplete/poor resolution of vitamins under study, either due to tailing or close hR_f values.

Sometimes diffused spots were also obtained. 0.2% Impregnation of each metal ion in all the six solvent systems was proved to be relatively poor in resolving vitamins on thin silica plates.

It can be assumed that, at these concentrations, metal ions are influencing the chromatographic behavior by complex formation. The complex formed changed the adsorption/partition characteristics during the development of chromatograms and better resolution of vitamin-B complex and folic acid was achieved. It can also be assumed that weak electron donation from N, O, or S atoms or π electron donation from the aromatic ring of the vitamins to the metal ion affected the chromatographic behavior. Vitamin B_{12} is a porphyrin derivative and porphyrins are known to form metallic complexes with metals such as manganese, iron, copper, and zinc.[16]

On the basis of observed results, it was inferred that $CuSO_4$ at 0.4% impregnation in all the employed solvent systems (except solvent system A_2) resulted in the simultaneous resolution of constituents of vitamin-B complex and folic acid with appreciable difference in hR_f values. Very sharp and compact spots were obtained with $CuSO_4$, providing improved resolution. Different concentrations of metal ions providing successful/improved resolution in the developed solvent systems have been shown in Table 5.

Constituents of vitamin-B complex and folic acid can be separated and identified in pharmaceutical and multivitamin preparations with less running time as reported earlier.[15] By using any of the above developed solvent systems, vitamins which were not resolved on the untreated plates were resolved with most of the impregnating reagents.

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