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SOLUBILIZING PROPERTIES OF VOLATILE SOLIDS SHALL REPLACE HAZARDOUS ORGANIC SOLVENTS IN THIN LAYER CHROMATOGRAPHY STUDIES

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Abstract: The pharmaceutical assays and other tests are largely validated by using organic solvents viz butanol, methanol, chloroform, acetone, carbon tetrachloride, chloromethane, benzene, cyclohexane, ether, etc. These above-listed organic solvents also have their applications extended to thin-layer chromatography and other techniques of analysis. However, most of these organic solvents have proved to affect the research personnel in more than one way. They lead to numerous adversities that include headaches, drowsiness, nausea, eye irritation on single exposure and long-term exposure may lead to chronic renal failure, liver damage, neurosis, etc. Also, they are costly and their unsafe disposal might lead to environmental pollution. Hence, they aren't considered safe for frequent usage. On the basis of various experiments on solubilization, it is believed that all substances whether liquids, gasses or solids possess solubilizing powers. With this approach, we have augmented the solubilizing power of the ethanol (comparatively less harmful organic solvent) by preparing the blends of ethanol with co-solvents like Thymol, Menthol and Camphor (organic solids that possess solubilizing power and are less harmful). Later, these blends were used as a mobile phase to perform Thin Layer Chromatography so as to obviate the use of previously used harmful organic solvents. The selected drugs were Nalidixic acid, Nicotinic acid, Metronidazole, Paracetamol and Guaifenesin to carry out the investigation. The spotting of these drugs was done on the TLC plates. Later, these spots were run by using different blends as mobile phases.
The mixed solvency concept is cost-effective and also prevents working with harmful organic solvents. Thus, the introduction of the novel method of solubilization can revolutionize the pharmaceutical industry.

Keywords: Thin Layer Chromatography, Organic solvents, Mixed solvency concept, Solubilizers

1. INTRODUCTION:

All substances whether solids, liquids or gases possess solubilizing power (1). A solution made by using various solubilizers in small concentrations would show collegial or additive enhancement in solubility in substitute of using a large concentration of one solubilizer (1,2). This mixed solvency concept may be utilized to prepare the concentrated solutions using organic solids (thymol, menthol, and camphor) as solubilizers in ethanol. The present experiment demonstrates the application of the mixed solvency concept for Thin Layer Chromatographic analysis of drugs like Guaiphenesin, Metronidazole, Nalidixic acid, Nicotinic acid and Paracetamol. By the mixed solvency concept, ethanol has been converted into a stronger solvent, employing various concentrations of the above-mentioned organic solids precluding the use of originally used harmful organic solvents. By using the mixed-solvency concept, attempts can be made to make ethanol, which is a quite safe organic solvent, a strong solvent by choosing appropriate non-toxic solubilizers. Therefore, the use of toxic organic solvents for various analyses like thin layer chromatography, titrimetric analysis, UV spectrophotometric analysis and HPTLC can be discouraged to a greater extent.

1.1 HYDROTROPIC SOLUBILIZATION AND MIXED SOLVENCY CONCEPT:

The term hydrotropic agent was first introduced by Neuberg in 1916 to delegate anionic organic salts which at relatively high concentrations in water can substantially increase the aqueous solubility of solutes that are poorly water-soluble. Hydrotropy is a phenomenon of solubilization wherein the addition of a large amount of another solute results in an increment in the water solubility of the first solute.

According to Maheshwari's mixed solvency concept, all substances in the universe have solubilizing power which means all solids, liquids and gases have solubilizing properties. (1,2) For gases and solids to possess solubilizing power, they must be present in a liquid state for the formation of hydrogen bonds and Van der Waals forces.

The gas molecules can be converted into a liquid state by liquefaction of gas or by dissolution of gas in a solvent. Such molecules of gases in the liquid state are capable of hydrogen bonds and weak Vander Waals forces with molecules of solutes for their dissolution.

The solid molecules can be converted into liquid state by melting, dissolution in a solvent or by eutectic mixture formation. The solubilities of a large number of poorly water-soluble drugs have been enhanced by hydrotropy, mixed hydrotropy and mixed solvency concepts. (1-27)

In the present research work, camphor, menthol and thymol were dissolved in ethanol to have their molecules in a liquid state to take part in hydrogen bonding and Vander Waals forces. Such molecules shall play a role in the solubility phenomenon. Camphor, Menthol and Thymol are volatile materials and shall be evaporated out from TLC plates by slight heating together with ethanol.

1.2 THIN LAYER CHROMATOGRAPHY:

Thin Layer Chromatography (TLC) is a quick, efficient and sensitive method used to resolve the components of a mixture, record the progress of a reaction, analyze samples and monitor the purity of drugs.

TLC involves a dynamic equilibrium of particles between the mobile and stationary phases.

The mobile phase (solvent system consists of a single component or a mixture of up to five components). The prepared mobile phase must be a homogenous system and must not have any cloudiness. The desired solvent must provide the greatest solubility for the sample. There must be a balance between the sample affinity for the mobile and stationary phases to attain separation.

1.3 RETENTION FACTOR:

The distance travelled by each component of a mixture can be quantified by using Retention factors (Rf). It is the ratio of the distance travelled by the solute to that of the distance travelled by the solvent front. Its value lies in the range of zero to one.

Rf value= distance travelled by solute/distance travelled by solvent front.

1.4 APPARATUS REQUIRED:

Beakers-200ml, Volumetric flask-10ml, Glass slides, Mortar and Pestle, Weighing balance, and Spatula.

1.5 MATERIALS REQUIRED:

Guaifenesin, Metronidazole, Nalidixic acid, Nicotinic acid, Paracetamol, Thymol, Menthol, Camphor, Ethanol, Silica Gel G-254 and Distilled water.

1.6 METHOD OF PREPARATION OF TLC PLATES:

Silica gel GF 254 was used for preparing the TLC plates. Suspension of the Silica gel GF 254 was prepared and was evenly spread on the glass slides of approx. 7.0-7.5 cm long. Then, the plates were heated and dried in a hot air oven at 70-80 degrees Celsius for 60 mins.

Table 1 describes the pharmacopeial methods to carry out the TLC of the selected drugs. However, such methods use harmful organic solvents.

Table:1 Pharmacopeial methods for TLC of the selected drugs

DRUGS	REGULATORY BOOK	TLC methods	
		Mobile phase	**Test solution**
1. Guaifenesin	IP	A mixture of 80 volumes of carbon tetrachloride and 20 volumes of ethanol (95 percent).	a. Dissolve 0.2 g of the substance under examination in 10 ml of dichloromethane. b. Dilute 5 ml of the test solution (a) to 50 ml with dichloromethane
2. Metronidazole	IP	A mixture of 80 volumes of chloroform, 10 volumes of diethylamine, 10 volumes of ethanol (95 per cent) and 1 volume of water	Dissolve 0.1 g of the substance under examination in 10 ml of acetone.
3. Nalidixic acid	IP	A mixture of 70 volumes of ethanol (95 per cent), 20 volumes of dichloromethane and 10 volumes of 5 M ammonia.	a. Dissolve 0.2 gm of the substance under examination in 10 ml of dichloromethane. b. A 0.1 per cent w/v solution of the substance under examination in dichloromethane.
4. Nicotinic acid	IP	A mixture of 85 volumes of 1-propanol, 10 volumes of anhydrous formic acid and 5 volumes of water.	Dissolve 0.2 gm of the substance under examination in 10 ml of water. Warm slightly, if necessary.
5. Paracetamol	IP	A mixture of 65 volumes of chloroform 25 volumes of acetone and 10 volumes of toluene.	a. Transfer 1gm of the substance under examination, finely powdered, to a ground-glass stoppered 15-ml centrifuge tube, add 5 ml of peroxide-free ether, shake mechanically for 30 mins and centrifuge at 1000 rpm for 15 mins or until a clear supernatant liquid is obtained. b. Dilute 1 ml of the test solution to 10 ml with ethanol (95 percent).

1.7 METHOD OF PREPARATION OF SAMPLE SOLUTIONS:
The drug solutions were prepared by dissolving the known quantity of the drug in 10 ml of the suitable solvent.

Table 2: solutions of drugs employed for putting the spots on TLC plates.

Drug Sample	Solvent	Solution concentration
1. Guaifenesin	Water	2%
2. Metronidazole	Water	0.5%
3. Nalidixic acid	Ethanol	1%
4. Nicotinic acid	Water	2%
5. Paracetamol	Ethanol	2%

1.8 METHOD OF PREPARATION OF MOBILE PHASES:
Mobile phases were prepared by dissolving the suitable Organic solids in ethanol. Thus, making their ethanolic solutions. For eg: Blend 1 was made by dissolving 3 gm of Thymol in a minimum amount of ethanol. Later, the volume of the solution was made up to 10 ml. Similarly, other blends were prepared as mentioned in Table no: (TLC studies).

Table 3: Composition of various mobile phases (ethanolic solutions)

Blend-1	30%T
Blend-2	20%T + 10%C
Blend-3	10%T + 10%M + 10%C
Blend-4	20%C
Blend-5	15%C
Blend-6	20%T + 5%M
Blend-7	20%T + 5%M + 5%C

(T: Thymol, M: Menthol, C: Camphor)

TLC STUDIES
Thin layer chromatography (TLC) of the drugs was carried out using glass slides. The slides were precoated using a thin layer of Silica gel GF 254. Capillaries were fused and then used to apply small spots of sample drugs on the TLC plate. After, the TLC plates were kept in a hot air oven at 100°C for 1 hour. The plates were viewed under the UV chamber for the drug sample spot.

TLC was then carried out using different prepared blends as mobile phases. Again, the plates were kept in a hot air oven at 100°C for 1 hour for the solvent (mobile phase) to evaporate from the plates. The drug spots were viewed in a UV (ultraviolet) chamber. The R_f (Retention factor) values of the drug spots were calculated using the following formula:

R_f = Distance traveled by solute / Distance traveled by solvent front

RESULTS AND DISCUSSION:
Results of TLC studies are reported in Table 4. Only selected mobile phases for good spots without tailing effects are mentioned in table 4.

Table 4: Results of TLC studies of selected drugs

S.no.	Drug	Blend	Rf value
1.	Guaiphenesin (28)	Blend 3 (10%T.+10%M.+10%C.)	0.76
2.	Metronidazole (29)	Blend 3 (10%T.+10%M.+10%C.)	0.70
		Blend 4 (20%C.)	0.87
		Blend 6 (20%T.+5%M.)	0.93
		Blend 7 (20%T.+5%M.+5%C.)	0.85
3.	Nalidixic acid (30)	Blend 2 (20%T. +10%C.)	0.50
4.	Nicotinic acid (31)	Blend 1 (30%T.)	0.55
		Blend 2 (20%T.+10%C)	0.70
		Blend 3 (10%T.+10%M.+10%C.)	0.60
		Blend 4 (20%C.)	0.70
5.	Paracetamol (32)	Blend 1 (30%T.)	0.90
		Blend 2 (20%T.+10%C.)	0.91
		Blend 3 (10%T.+10%M.+10%C.)	0.83
		Blend 7 (20%T.+5%M.+5%C.)	0.89

(T: Thymol, M: Menthol, C: Camphor)

In **the** case of Guaiphenesin Blend 3 was found suitable and the spot was free from tailing effect. Similarly, Blends 3,4,6 and 7 for Metronidazole, Blend 2 for Nalidixic acid, Blend 1,2,3 and 4 for Nicotinic acid and Blend 1,2,3 and 7 for Paracetamol were found suitable and gave results without tailing effect.

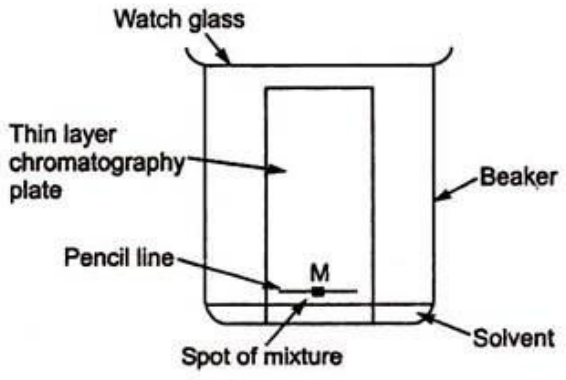

Fig 1: Chamber for thin layer chromatography (33)

CONCLUSION:

It is, therefore concluded that the Rf values obtained using the suggested methods of mixed solvency concept are satisfactory. The use of Thin Layer Chromatography is quite prominent in many industries and various fields of research including the pharmaceutical industry, clinical analysis, toxicology, analytical laboratory, etc. These techniques employ the use of harmful

organic solvents like carbon tetrachloride, chloroform, 1,2 dichloroethane, benzene, toluene, trichloroethylene, methyl alcohol, acetonitrile, etc. Some of these solvents are carcinogenic and a large number of organic solvents are harmful to the human health and the environment. The employment of organic solids as solvents deters the use of these hazardous organic solvents.

Thus, it is concluded that the suggested TLC methods by the mixed solvency concept that employ the use of organic solids as solvents are novel, simple, non-carcinogenic and environment-friendly. The prime advantage of this technique is the exclusion of harmful organic solvents in TLC analysis.

This technique can be employed for the analysis of other drugs too. The mixed solvency concept can be adopted as a novel method for TLC analysis. Like camphor, menthol, thymol, and other volatile solids shall also be helpful in TLC replacing hazardous organic solvents.

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