Impregnation Techniques

All of the techniques for impregnating TLC plates described below have been used at one time or another and are illustrated with examples in the subsequent sections on applications.

Spraying

Spraying the TLC plate is a simple and convenient method for applying the reagents used for detection in TLC and indeed is widely used for this type of application. It is, however, much less well suited to the impregnation of TLC plates in order to modify chromatography because of the difficulty of ensuring uniform coverage. It also requires much skill and expertise in order to ensure reproducible results from day to day. Both of these technical difficulties are clearly disadvantages, and spraying is therefore not recommended for routine applications in this area. Spraying is therefore probably best used, if at all, for one-off applications or for screening phases and impregnating reagents for a particular property during method development.

Dipping

Dipping plates in solutions of the impregnating reagent in a suitable (usually volatile) solvent is probably the simplest and most efficient method of ensuring even coverage of the stationary phase with the reagent of choice. Suitable dipping chambers, of the type used for immersing plates in solutions of chromogenic visualization reagents, are available from a number of manufacturers at modest cost. These chambers have relatively small volumes and so are not expensive in reagent. Some automated devices are also available that can be used to control the impregnation time accurately. Dipping, in general, because of the ease of control over parameters such as reagent concentration and impregnation time, should be considered to be the preferred method for impregnating TLC and HPTLC plates.

Pre-development

It is also a relatively easy matter to prepare TLC plates impregnated with the desired reagent by pre-development in a solution of reagent in a suitable solvent. This technique is also referred to as irrigation of the plates. The solvent used for this purpose needs to be sufficiently eluotropic to ensure that any
affinity of the reagent for the stationary phase is overcome or else, at worst, the plate will not be impregnated with the reagent which will stay at or near the origin (or else be distributed as a gradient of decreasing concentration up the plate). Nonmigration of the impregnating reagent is seen with, for example, the ion pair reagent cetrimide if dissolved in water and then used to coat reversed-phase TLC plates in the pre-development method. Because the reagent is relatively nonpolar it has a strong affinity for the stationary phase and remains at the origin. In such cases dipping is to be preferred.

Mixed Phases

Although with the advent of good quality commercial TLC and high performance TLC (HPTLC) products the preparation of TLC plates in the laboratory is no longer widely practised, one method of producing impregnated plates was to produce mixed phases. Thus, the bulk stationary phase (generally silica gel) was mixed with an appropriate amount of the impregnating reagent, a binding agent, slurred in a suitable solvent and spread as a layer on glass plates. After drying and activating (if necessary), the plates were then used as required. Some manufacturers provide a range of mixed phases (e.g. silver nitrate-impregnated plates, buffered layers, ammonium acetate-impregnated layers, etc.).

Specific Examples of Impregnation in TLC

Impregnation to Form Lipophilic Stationary Phases for Reversed-phase Separations

Before the introduction of good-quality bonded phases, the preparation of suitably hydrophobic stationary phases used to be a very popular method for obtaining layers with which to perform reversed-phase separations, and still has many advantages. This is readily achieved using liquid paraffin oil, undecane, n-decane, nitromethane, propylene glycol, silicone oil, decalin, Carbowax 400, formamide, 2-phenoxy- and 2-methoxyethanol, various mineral oils, and substances such as ethyl oleate and similar materials. In addition to modifying chromatography, impregnation with these materials can also be used to enhance or stabilize the fluorescence of suitable analytes, thus improving detection and quantification. However, it is the use of these materials to provide reversed-phase separations that is of interest here.

A typical example of the use of paraffin oil is provided by work on the reversed-phase separation of ecdysteroids (polar, polyhydroxylated steroids found in plants and arthropods). Here silica-gel TLC and HPTLC plates were impregnated with a solution of 7.5% paraffin oil in dichloromethane (v/v) by dipping. The plates were air-dried and chromatography was performed using methanol–water mixtures. In an interesting variation on this general technique of impregnation, a variety of normal-phase separations, for nonsteroidal anti-inflammatory drugs, ecdysteroids, antipyrine and aminophenols, on silica gel were performed with the paraffin added to the mobile phase. Addition of 7.5% (v/v) of paraffin to the normal-phase solvent system did not affect chromatography but did enable the plate to be impregnated so that a subsequent reversed-phase separation in a second dimension could be undertaken immediately that the solvent from the normal-phase separation had evaporated. An example of this for the nonsteroidal anti-inflammatory compounds is shown in Figure 1. It should be noted that, with this type of impregnated plate it is also possible to perform the reversed-phase separation first, remove the nonpolar impregnating reagent with a suitable solvent (i.e. one that does not affect the analytes but does remove the impregnating reagent) and then perform a normal-phase separation in the second dimension. Alternatively, a similar outcome can be achieved by impregnation of that portion of the plate not used for...
the separation in the first dimension, and examples of
a normal-phase separation followed by impregnation
with 2-phenoxethanol or undecane for reversed-
phase chromatography in the second dimension have
been described.

**Impregnation with Silver Nitrate**

Argentation TLC also represents an important meth-
odology and is considered in detail elsewhere in this
work and so will only be briefly described here. The
impregnation of TLC plates with silver nitrate has
been used for many years as a means of improving the
separation of unsaturated compounds, particularly
certain lipids, based on the ability of silver ions to
form charge transfer complexes with the π electrons
of the carbon–carbon double bonds. In general, silica
gel is used: the amount of silver nitrate used varies
from as little as 2% up to 20 or 30% w/w depending
upon the author and application. With silica gel,
impregnation by both spraying (with a 10–20% solu-
tion in either water or methanol) and the preparation
of mixed phases have been described. Following prep-
aration it seems to be good practice either to use the
plates the same day or to store them in a sealed
container in the dark until required. In general, non-
polar solvent systems are employed (e.g. pentane,
hexane-diethyl ether, chloroform-methanol, diethyl
ether, light petroleum, etc.).

Whilst the use of silver nitrate-impregnated plates
has generally been with normal-phase separations on
silica gel, there has been recent work using reversed-
phase (C₈-bonded) layers dipped in solutions contain-
ing between 0.5 and 4% silver nitrate for 10 s. These
plates were investigated for their ability to separate
the cis/trans isomers of capsaicin, and comparison
was made simply using the silver nitrate in the mobile
phase (60 : 40 methanol–water v/v). With impregna-
tion no effects were observed until the 2% (w/v)
impregnating solvent was used, and even with 4% this
was insufficient to provide the required res-
olution. In contrast, when present as a mobile-phase
additive, even as low a concentration as 0.5% w/v
was sufficient to give baseline resolution. It ap-
pears likely from this result that the high solubility of
the silver nitrate in the mobile phase led to its rapid
elution from the impregnated plate, with consequent
loss of effect. Thus, interestingly, it seems from
this work that the use of the silver nitrate in reversed-
phase systems is only practicable when it is present as
a mobile-phase additive.

**Impregnation with Polyl and Sugar Complexing
Reagents**

The ability of borate ions to complex with suitable
polyhydroxylated compounds such as carbohydrates
is well known and has provided the basis of a number
of methods for their separation. TLC with layers
impregnated with sodium arsenite, phosphotungstic,
tungstoarsenate and molybdic acids have also been
shown to have useful properties for the resolution of
mixtures of oligo and monosaccharides.

Various methods have been used to prepare such
layers, including both spraying and the preparation of
mixed layers. Thus, in an early example of the use of
boric acid, sodium borate and sodium arsenite plates
were either sprayed with methanolic or aqueous solu-
tions containing 10–20% of the impregnating reagent
or plates were prepared by mixing 2.8 g of the reagent
in 50 mL of water with 25 g of silica gel G to give a
10% (w/w) mixed layer. These layers were then used
to separate the erythro and threo isomers of a variety of
di- and trihydroxy long chain fatty acid esters.

Similar work on phosphotungstic and molybdic
acid impregnated silica gel TLC plates showed them
to be particularly useful for the separation of
oligosaccharides giving complexes with higher
Rₕ values than the corresponding boric acid com-
plexes under similar conditions. In this case the plates
were made by mixing 35 g of the chromatographic
stationary phase (e.g. silica gel–alumina 1:1 or 3:1
or alumina) with 70 mL of an aqueous solution con-
taining an appropriate amount of the impregnating
reagent and spreading the plates as a 0.4 mm layer on
to glass. The resulting plates were then dried at room
temperature for 24 h, and heated for 1 h at 110°C
before use. In general, the best results were obtained
with TLC plates treated with phosphoric acid–
sodium tungstate or saturated molybdic acid as
impregnating reagents.

**Impregnation with Liquid Ion Exchangers and
Neutral Organophosphorous Compounds
for Metal Ion Separations**

The separation of inorganic ions has been an impor-
tant application of TLC. Another area that has
proved to be of some interest for the use of the
impregnation technique as a means of improving
analyte resolution in TLC has been the employment
of the so-called liquid ion exchangers. This developed
from the widespread use of this type of reagent in
paper and column chromatography. A range of these
liquid ion exchangers were used in early examples of
this type of application. However, extensive experi-
mentation suggested that adogen 464, alamine 336,
amberlite LA1 and primene JM-T were suitable for
this type of application. In these early studies
a 0.1 mol L⁻¹ solution in chloroform was used for
impregnation of the silica gel, with plates prepared
from a suspension of the silica gel in this solution.
Metal ions on TLC have also been resolved on layers
impregnated with neutral organophosphorous compounds such as tri-\( n \)-butyl phosphate and tri-\( n \)-octylphosphine oxide. The bulk of the early literature in this area was collated by Brinkman et al. (see Further Reading).

More recent examples of the use of impregnation for metal ion separations have included further examples of the use of primene JM-T, amberlite LA-1 and LA-2, alamine 336 and aliquat 336, tri-\( n \)-octylamine, tri-\( n \)-butyl phosphate and tri-\( n \)-butyl amine-impregnated silica gel TLC plates.

**Impregnation with Ion Pair Reagents**

The use of ion pair reagents is also discussed in detail elsewhere in this work and will therefore only be briefly described here. A number of workers have shown that ion pair reagents can be used as mobile-phase additives or following impregnation in the stationary phase for the subsequent chromatography of polar organic compounds. The methodology used depends to a large extent on the nature of the reagent, but also to some degree on the stationary phase. So, whilst both silica gel and alkyl-bonded layers can be treated with these reagents, the results are generally better with the bonded phases. In addition, it should be noted that low molecular mass ion pair reagents such as tetrarmethylammonium salts are so soluble that simply impregnating the stationary phase is generally ineffective as, with aqueous solvents, the reagent rapidly dissolves in the mobile phase when chromatography is initiated. This rapidly depletes the amount of reagent available for ion-pairing and results in a generally unsatisfactory chromatographic result. Other reagents, however, typically long chain sulfonic acids or quaternary ammonium compounds (e.g. sodium dodecyl sulfate or cetrimide) are only effective when the plate has been impregnated with the reagent (as a solution in a volatile organic solvent such as chloroform or ethanol) prior to chromatography. We have found that dipping is the most effective means of ensuring an even coating of the layer with these substances.

**Impregnation with Chiral Selectors**

Chiral separations in TLC have been accomplished using chiral stationary phases, chiral mobile-phase additives and by impregnating suitable TLC phases with chiral selectors. Probably the best characterized separation of this type is based on chiral ligand exchange where the chiral selector (2\( S \),4\( R \),2\( \prime \)\( R \)S)-4-hydroxy-1-(2\( \prime \)-hydroxydodecyl)proline/copper [\( \mu \) acetate impregnated into C\( 18 \)-bonded TLC or HPTLC plates. These plates are excellent for the separation of chiral amino acids and related compounds, and substances such as \( \alpha \)-hydroxycarboxylic acids. Plates of this type are commercially available from several manufacturers (as the CHIR and Chiral Plate from Merck and Macherey Nagel, respectively). A typical series of separations on the CHIR HPTLC plate is shown in Figure 2. Alternative systems based on a similar mechanism involve the use of N,N-di-\( n \)-propyl-L-alanine or poly-1-phenylalaninamide copper complexes.

Another example of the impregnation approach to the chiral TLC separations involves the impregnation of diol-bonded HPTLC plates with the chiral ion pair reagent N-benzoxy carbonyl-glycyl-L-proline (ZGP). These plates are prepared by first washing with dichloromethane, then dipping in a 4 mmol L\(^{-1}\) solution of ZGP and 0.4 mmol L\(^{-1}\) ethanolamine. Following drying these plates can be used to separate the enantiomers of \( \beta \)-blockers such as popranolol using chloroform–ethanol solvent systems.

It is also possible to prepare a Pirkle-type stationary phase for chiral separations by dipping amino-propyl-bonded silica gel HPTLC plates into solutions of substances such as (\( R \))-N-(3,5-dinitrobenzoyl)-phenylglycine or (\( L \))-N-(3,5-dinitrobenzoyl)leucine.
activated by allowing them to dry for several hours over desiccant silica gel (activation using heating in an oven gave similar but irreproducible results). This activation was quite critical for the achievement of a good separation, as plates that were too damp gave no resolution. For the TLC of certain acidic mycotoxins, the use of silica gel TLC plates that had been immersed in a 10% solution of oxalic acid in methanol for 2 min and then activated by heating at 110°C for 2 min enabled tailing to be eliminated and resulted in well-defined spots.

**Impregnation with Inorganic Buffer Salts**

The use of phases impregnated with sodium or potassium salts has been described for a number of solutes. Thus, conjugated dihydroxy bile acids were separated on silica gel plates that had been impregnated by dipping in a solution of potassium dihydrogen phosphate. However, a major application of this type of impregnation has been for carbohydrates. As examples, glucose, fructose and sucrose in molasses were resolved on silica gel HPTLC plates dipped in 0.2 mol L⁻¹ aqueous solution of monobasic potassium phosphate. In contrast, when this separation was attempted on nonimpregnated plates it failed. Similar results have been observed by other workers with this type of sample and in addition to potassium-based buffers, plates buffered with 0.15 mol L⁻¹ sodium dihydrogen phosphate (prepared by mixing Kieselghur with the buffer prior to spreading the plates) have also been used to separate sugars (fucose, xylose, ribose, etc). Other workers have also examined impregnation of silica gel, prepared by spreading the adsorbent in a solution of the appropriate salt, with a range of sodium salts (phosphates, acetate, sulfate, phenyl phosphate) for a wide range of carbohydrates, including mono- and oligosaccharides and uronic acids. From these studies the best separations of monosaccharides and uronic acids were obtained with plates impregnated with 0.2–0.3 mol L⁻¹ salt concentrations, whilst oligosaccharides gave the best results with 0.05–0.1 mol L⁻¹ salt solutions. Overall, from the published examples, it seems clear that the TLC separation of carbohydrates does benefit from the use of this type of impregnating reagent.

**Charge Transfer Complexes for the Resolution of Polynuclear Aromatic Hydrocarbons**

A range of compounds have been used to impregnate silica gel TLC plates in order to improve the...
resolution of compounds such as the polynuclear aromatic compounds (fluoranthene, benzopyrine, etc.) based on the formation of charge transfer complexes with different electron acceptors. These reagents have included caffeine, tetracyanoethylene, 1,3,5-trinitrobenzene, picric acid, chloranil, bromanil, benzoquinone and similar compounds, 2,4,7-trinitrofluorenone, teramethyluric acid, urea, pyromellitidianhydride, 9-dicyanomethylene-2,4,7-trinitrofluorenone, sodium desoxycholate, dimethylformamide, styphnic acid and various amino acids and nucleic acid bases. However, although a wide range of reagents have been impregnated into TLC plates in an attempt to enhance the separation of polynuclear aromatic hydrocarbons, not all have been equally effective: in one study picric acid was described as values, whilst styphnic acid showed some effect and trinitrofluorenone (on alumina) proved to be excellent. Some of the reagents listed above also proved to be heat- or light-sensitive further restricting their utility.

A recent example of the use of caffeine, shown by several groups to have a profound effect on the TLC of polynuclear aromatic hydrocarbons, as a means of improving the HPTLC resolution of the polynuclear aromatic hydrocarbons, by charge transfer complex formation, involved preparing a solution of 4 g of the reagent in 96 mL of dichloromethane. Silica gel plates (with a preconcentration zone) were then dipped in this solution for 4 s and dried at 110°C for 30 min. Then, before sample application, the plates were pre-washed by running a blank chromatogram with dichloromethane as the mobile phase with subsequent reactivation at 110°C and then preconditioning the impregnated plate for 30 min at 100% relative humidity prior to sample application and chromatography. Following sample application, development was performed with diisopropyl ether-n-hexane (4:1) as the solvent in an unsaturated TLC tank. Although chromatography could be performed at room temperature, the best results (especially where quantification was to be performed) were obtained when the plates were developed at 22°C. The results of this type of separation, at room temperature and —22°C, are illustrated in Figure 4.

An added benefit of this type of system is that some of the reagents used for charge transfer chromatography also result in enhanced detection of the compounds of interest because of the highly coloured or even fluorescent complexes that are formed with reagents such as chloranil or pyromellitic dianhydride.

**Miscellaneous Impregnation Reagents**

As well as the areas described above, there are a large number of other applications of impregnation to modify the properties of the stationary phase in TLC. Some of these are briefly outlined below in order to give an indication of the extent of this type of work, but the list is by no means exhaustive.

Plates impregnated with ethylenediaminetetraacetic acid have been employed for the chromatography of certain antibiotics (e.g anthracyclines and tetracycline), mycotoxins, citrinin and 8-hydroxyquinolone derivatives, and may confer benefits when the TLC of metal chelating compounds is performed.

TLC plates have also been impregnated with a variety of metal salts for particular compounds or classes of compounds. These include ferric chloride on Kieselguhr (oxine derivatives), zinc salts on silica gel and silanized silica gel (chlorinated anilines, carbamates), cadmium salts on silica gel (aromatic amines), manganese salts on silica gel (aromatic amines), copper sulfate on silica gel (hexosamines, glycosamines, barbiturates), thallium nitrate on silica gel (monoterpene hydrocarbons) and lithium

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**Figure 4** Fluorescence scan of a separation of polynuclear aromatic hydrocarbons (2 ng per spot, except 6 which was 10 ng per spot) on a caffeine-impregnated silica gel layer with chromatography performed at —20°C. 1, Benzo (ghi) perylene; 2, benzo (a) pyrene; 3, benzo (b) fluoranthene; 4, benzo(k)fluoranthene; 6, fluoranthene. Reproduced with permission from Funk W, Gluck V, Schuch B and Donnevert G (1989) Polynuclear aromatic hydrocarbons (PAHs): charge transfer chromatography and fluorimetric determination. *Journal of Planar Chromatography* 2: 28-32.
chloride-impregnated silica gel (pyrrolizidine alkaloids). Magnesium acetate has been used for phospholipids. In addition, lead salts have been employed as impregnating reagents to modify the separations of sugars and polyols. Recently, ammonium cerium (iv) nitrate was used for the separation of aromatic amines.

Other types of impregnation include the use of silica gel with phenol for aliphatic and aromatic amines following impregnation with 2% aqueous solution of the reagent. For the aromatic amines, phenol itself was found to be the most useful reagent, providing a significant improvement in spot shape was noted for a wide range of anilines compared to chromatography on the untreated stationary phase. In the case of aliphatic amines o-chlorophenol gave the best result. For both classes of compound this improvement in chromatography was assumed to be due to hydrogen bond formation between the reagent and the solutes. The chromatography of phenols on cellulose has also been modified by impregnation with 10% polyamide, and on silica gel with 20% polyamide. In addition, aniline-impregnated layers have been used for phenol derivatives whilst sodium nitrite-impregnated silica gel has also been shown to provide separations that could not be achieved on native silica gel. Carbonyl compounds were modified by derivatization to 2,4-dinitrophenylhydrazones on alumina TLC plates impregnated with silver nitrate (44%, w/w). Urea impregnation, described above for polynuclear aromatic hydrocarbons and related compounds, has also been used for the separation of lipid classes on silica gel plates.

Concluding Comments

As indicated in the introduction, impregnation techniques have been employed since the earliest days of TLC and their use greatly extends the versatility of TLC by enabling more selective separations or detection. To some extent the increasing availability of bonded layers has reduced the need for the preparation of silica gel layers impregnated with nonpolar materials such as paraffin oil in order to perform reversed-phase separations. However the usefulness of silver nitrate-impregnated phases for the separation of compounds containing double bonds remains undiminished, and similar observations could be made for many of the impregnation reagents described above. A continued role for impregnated stationary phases in TLC therefore seems likely.

Further Reading


