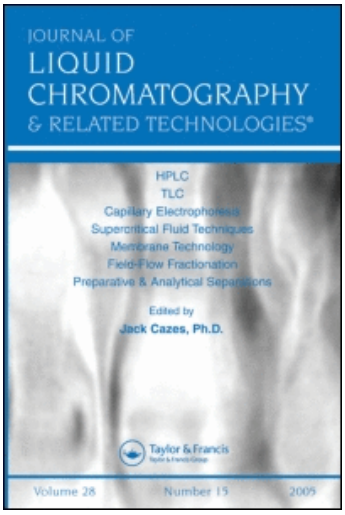

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QUANTITATIVE DETERMINATION OF METHYL RED ADSORPTION ON STATIONARY PHASES USED IN LIQUID CHROMATOGRAPHY

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ABSTRACT

The method described is a modification of the dye adsorption method, first published by Shapiro and Kolthoff. It is reproducible and allows the estimation of the relative amount of the reachable free silanol groups on silica gels and reversed phase materials which are frequently used as stationary phases in liquid chromatography. Results obtained with commercially available thin layer and high performance liquid chromatographic materials, as well as with self-made reversed phase materials, are reported.

INTRODUCTION

Shapiro and Kolthoff (1) used adsorption of methyl red to determine the modification of the specific surface of silica gel by thermal aging. Dye adsorption was also used for the measurement of surface areas of alumina-silica and silica gel (2). More recently, methyl red adsorption was used as a simple limit test for unreacted silanol groups after preparation of reversed phases from silica gel (3,4,5). Possible coloration of the reversed phase, due to adsorption of methyl red (MR), was detected visually. The method has now been modified in order to determine the amount of

MR adsorbed on reversed phase materials used in high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). The results allow comparison of the degree of coverage of reversed phase materials. Difficulties concerning the application of the MR adsorption test such as the influence of the possible presence of adsorbed acids and bases, the necessity for working with well dried samples, the dependence on MR concentration and on particle and pore size, have been mentioned in the literature (6,7).

EXPERIMENTAL

Samples - Reagents. The samples used throughout the experiments were : Kieselgel H (Typ 60) TLC, Kieselgel 60 H 15µ TLC, Kieselgel 60 H silanisiert TLC, LiChrosorb SI 60 10µ, LiChrosorb SI 100 10µ, LiChrosorb DIOL 10µ, LiChrosorb RP 18 10µ, LiChrosorb RP 8 5µ and 10µ, LiChrosorb RP 2 10µ, from E. Merck, Darmstadt, GFR; µ Bondapak 10µ, µ Bondapak phenyl 10µ and µ Bondapak NH_2 10µ from Waters Associates, Milford, U.S.A.; RSiL C 18 LL 10µ and RSiL C 18 HL 30µ from Alltech Europe, Eke, Belgium; Polygosil C 8 10µ from Macherey-Nagel, Düren, GFR; Zorbax C 8 7µ from Du Pont, Wilmington, U.S.A.; SAS-Hypersil 5µ from Shandon, Cheshire, U.K.; RP I, II and III are home-made reversed phases prepared with trimethylchlorosilane (TMCS) according to Little et al. (8).

The following reagents were used : methyl red, benzene reinst, sodium hydroxide reinst and acetic acid p.a., E. Merck, Darmstadt, GFR; Methanol 99+%, acetone 99+% and dichloromethane ACS from Aldrich Europe, Beerse, Belgium. The benzene was dried on sodium and glass-distilled before use. Methanol 99+% was glass distilled.

Purification of Methyl Red. Dissolve 25 g methyl red in 500 ml dichloromethane-acetone 80:20. Purify by chromatography over a column of 50 mm ID filled with 250 g Kieselgel 60 Merck (230-400 mesh) in dichloromethane. Elute first with dichloromethane,

then with dichloromethane-acetone 80:20. Follow the elution by means of TLC on silica gel plates (ready-made plates, E. Merck) with dichloromethane-acetone 9:1 v/v as the mobile phase. Evaporate the fractions containing methyl red (Rf 0.63) and dry the residue at 120°C for 2 hours.

Preparation of the Methyl Red Stock Solution. Weigh accurately about 400.0 mg methyl red, dried for 2 hours at 120°C, dissolve in dry benzene, and adjust to a final volume of 100.0 ml with the same solvent. Dissolution can be accelerated by the use of ultrasonics.

Preparation of the Methanolic Acetate Buffer. Dilute a mixture of 18.0 ml NaOH 1 N and 100.0 ml HOAc 1 N to 500.0 ml with water (pH 4.0). Dilute 25.0 ml of this solution with methanol to 500.0 ml.

Apparatus. All measurements were carried out with a Beckman model 25 spectrophotometer, using 1 mm cells.

METHOD

Dry the sample for three hours at 120°C. Allow to cool over phosphorous pentoxide in a dessicator. Weigh quickly and accurately about 50.0 mg of the sample in a dried tube with normalized stopper. Add immediately 5.0 ml methyl red (MR) stock solution, shake for 2 minutes on a vortex, centrifuge for 3 minutes at 4000 rpm and dilute 1.0 ml of the supernatant solution to 50.0 ml with the methanolic acetate buffer. Prepare, following the same procedure, a blanc solution for the determination of the specific absorbance. Measure the absorption at 494 nm, using 1 mm cells, and calculate the results as mg adsorbed methyl red per g substrate.

RESULTS

All measurements were carried out twice. The mean values are given in Table 1.

Results obtained with a stock solution of 200 mg MR/100 ml are also reported in Table 1. The reproducibility of the method was checked by carrying out a series of twelve completely independent measurements on the same batch of partially covered silica, resulting in a standard deviation of 2.95 and a coefficient of variance of 2.32 for a mean value of 127 mg/g. The standard deviation increases as the methyl red adsorption values decrease. This means that values close to zero are not significant, even small negative results can be obtained.

TABLE 1

Methyl Red Adsorption Values

Sample	Amount of methyl red adsorbed in mg/g substrate (MRAV)	
	Stock 400 mg MR/100 ml	Stock 200 mg MR/100 ml
Kieselgel H Typ 60	205	150
Kieselgel 60 H 15µ TLC	203	148
LiChrosorb SI 60	198	145
LiChrosorb SI 100	141	115
µ Bondapak NH_2	123	105
RP I	91	69
RSiL C 18 LL 10µ	-	66
RP II	55	43
RP III	52	43
RSiL C 18 HL 30µ	34	30
Kieselgel 60 H sil TLC	31	22
Polygosil C 8	27	20
µ Bondapak phenyl	7	1
LiChrosorb RP 2	5	2
SAS-Hypersil	4	1
LiChrosorb DIOL	4	2
Zorbax C 8	3	0
LiChrosorb RP 18	2	- 1
LiChrosorb RP 8 5µ	2	2
LiChrosorb RP 8 10µ	2	0
µ Bondapak C 18	1	- 1

DISCUSSION

The influence of different parameters on the methyl red adsorption values (MRAV) has been checked.

Necessity of Purification of MR. It is necessary to purify the methyl red since it contains polar impurities which dissolve only partially in benzene. Moreover, some of these polar impurities may dissolve during the preparation of the stock solution in benzene and precipitate again upon storage. During purification by column chromatography as described above, the more polar impurities are not eluted. One impurity is eluted close to the methyl red band; this impurity was isolated and identified by mass spectrometry as the 2-[[4-(methylamino)phenyl] azo]-benzoic acid, methyl red being the corresponding dimethylamino analogue. The two products are clearly separated by TLC in the conditions as described above, having Rf-values of 0.46 and 0.63 respectively. Several minor apolar impurities move with the front. Methyl red samples from Aldrich Europe, Beerse, Belgium and Fluka AG, Buchs, Switzerland were also examined by TLC, both showed chromatograms comparable with that obtained with the Merck product. Attempts to purify methyl red by other methods such as crystallization or Soxhlet extraction gave unsatisfactory results.

Instability of Absorbance. Carrying out the method as described by Shapiro and Kolthoff (1), using benzene as the solvent for the preparation of the dilutions, stability problems were observed. The absorbance started to raise from the moment the cell was brought into the spectrophotometer and stabilised only after about half an hour. This is represented in figure 1. Thermal expansion of the solution is certainly not an acceptable explanation for the phenomenon since the absorbance increases. Closed cells were used, excluding evaporation. On the other hand,

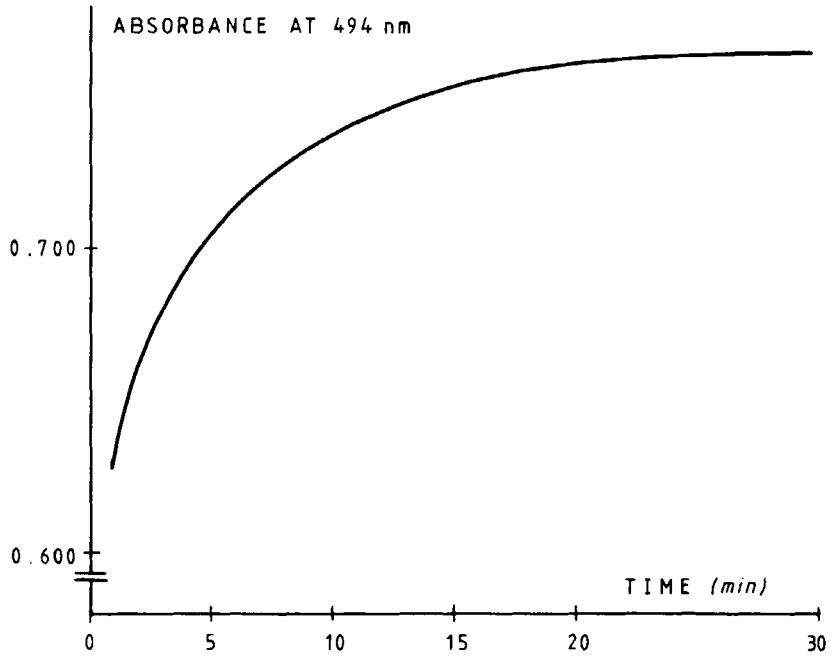

Figure 1 - Influence of Residence Time in the Spectrophotometer on the Absorbance of a 0.55 mg/100 ml Solution of Methyl Red in Benzene using 1 cm cells.

stable readings were obtained with a solution of iodine in benzene, measured in the same conditions. This indicates that the instability was due to the methyl red. In the literature photochemical decarboxylation of azobenzene-2-carboxylic acids has been reported (9), as well as photochemical isomerization of methyl red and analogues (10,11). The exact cause for the instability was not investigated yet, but probably photochemical trans-cis isomerization is the best explanation. Wildes (10) reports that cis-trans back-isomerization is dependent upon the polarity of the solvent; apolar solvents (hexane, benzene) giving very low rates, and polar solvents (isopropyl alcohol, acetone, dimethylsulphoxide) giving high rates of back-isomerization. The use of polar solvents thus, causes the isomerization

phenomenon to be invisible, unless special techniques such as flash spectroscopy are applied. Indeed, very stable results were obtained, when after adsorption on the substrate, further dilutions were prepared with methanol instead of benzene. The adsorption process itself has to take place in an apolar solvent in order to avoid competition of the solvent with the methyl red for adsorption on the active sites of the silica (1).

With the introduction, however, of methanol as the solvent for dilution, another problem arose. Although the results were stable, they were not or badly reproducible, which was thought to be due to the influence of the pH-variation on the indicator MR, this influence being more important in a polar solvent than in apolar benzene. Therefore methanolic acetate buffer, pH 4, was used, whereafter stable and well reproducible results were obtained. Measured in the conditions mentioned, MR shows a broad absorption band with maximum at 494 nm. The specific absorbance at this wavelength is 942. The absorbances can be measured even several days after the preparation of the dilutions. The use of an acid buffer is also a supplementary guarantee that cis-trans back-isomerization is very fast. Indeed, Lovrien (11) reports that even in water the relaxation is sufficiently slow to observe photochromism, provided the pH is alcaline.

Linearity of the Method. The linearity of the method was also checked. The results are represented in figure 2. It shows that the amount of benzene present in the dilution, has an influence on the absorbance. As a consequence this amount of benzene has to be kept constant in a series of experiments, otherwise a deviation of linearity occurs.

Dependence on MR-Concentration. Shapiro and Kolthoff (1) report that a final methyl red concentration of at least 0.4 mg/ml solution, in equilibrium with the sample, is needed in order to obtain

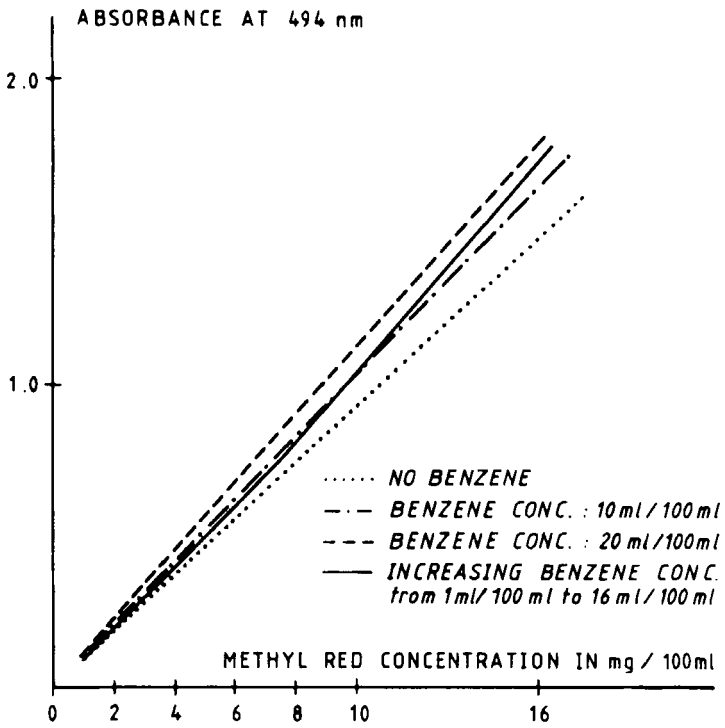

Figure 2 - Influence of the Amount of Benzene on the Absorbance of a Methyl Red Solution with Methanolic Acetate Buffer as the main solvent. To obtain linearity the benzene concentration has to be constant.

reproducible results; at higher concentrations, the final amount of adsorbed MR is no more influenced by the equilibrium concentration. In our laboratory, experiments with Kieselgel H and RP II, a self-made reversed phase material, were carried out to check these data. Stock solutions of 100 mg, 200 mg, 300 mg and 400 mg MR per 100 ml were used, corresponding to respectively 0.11, 0.56, 1.24 and 2.06 mg MR/ml at equilibrium for Kieselgel H and to respectively 0.68, 1.58, 2.45 and 3.45 mg MR/ml at equilibrium for RP II. Data at intermediate equilibrium concentrations were obtained by adding volumes , greater than 5 ml, of the stock solutions. The results are represented in figures 3 and 4 respectively. These figures

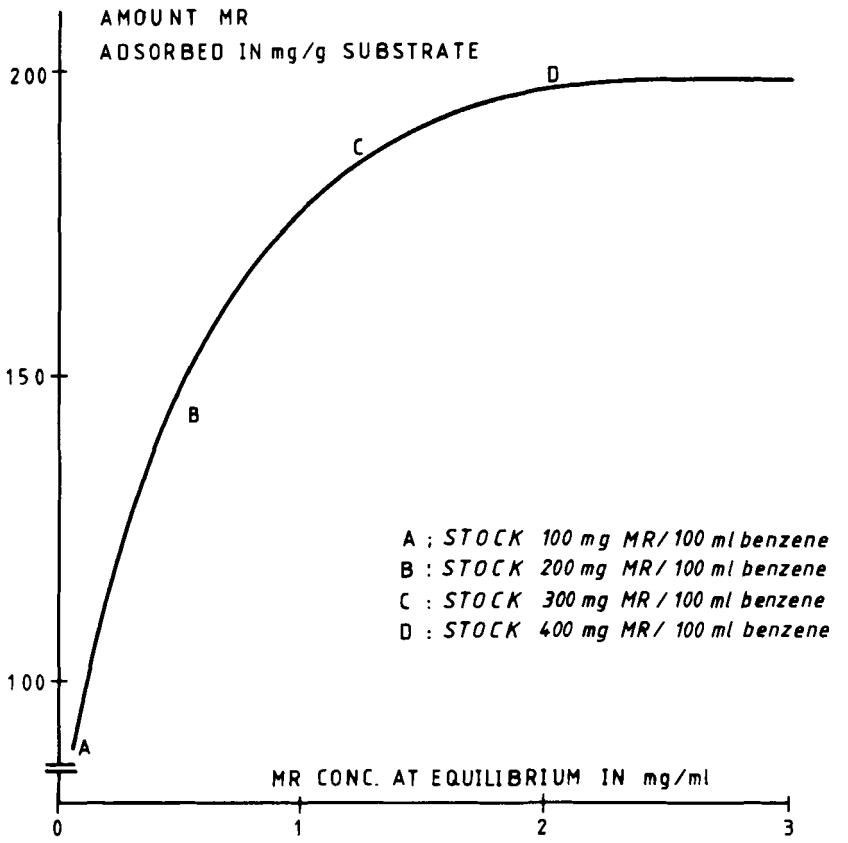

Figure 3 - Influence of the Methyl Red Concentration at Equilibrium on the Amount of Methyl Red adsorbed on Kieselgel H.

clearly show that the influence of the MR concentration is more important for silica gel than for reversed phase materials. Obviously the use of conditions giving equilibrium concentrations of 2 mg/ml (Kieselgel H) and 3.5 mg/ml (RP II) seems to be indicated in order to obtain reproducible results. This explains the choice of a 400 mg MR/100 ml benzene stock solution for the proposed method. The choice of the concentration is also limited by the solubility of MR in benzene, which is about 530 mg/100 ml at about 20°C. These results clearly indicate that interlaboratory results can only be

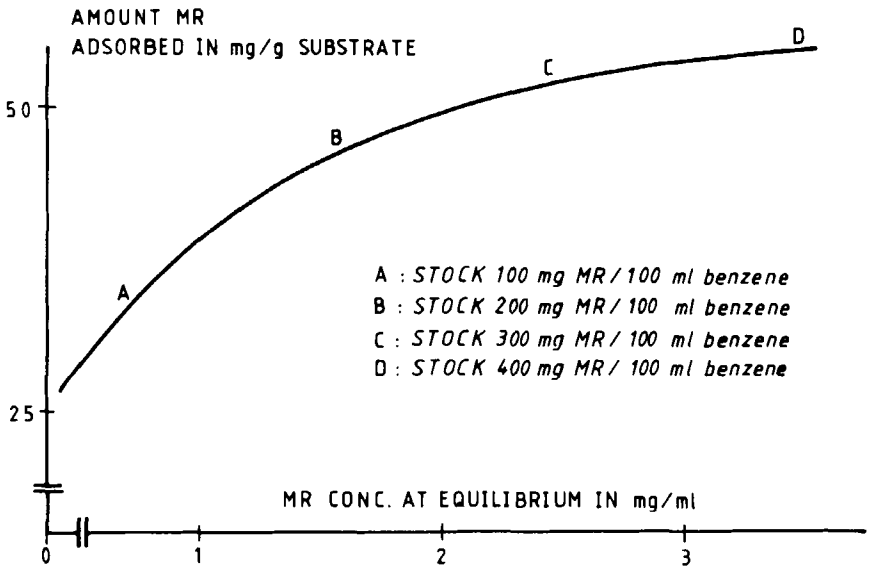

Figure 4 - Influence of the Methyl Red Concentration at Equilibrium on the Amount of Methyl Red adsorbed on RP II.

compared provided that the stock solutions used are of nearly the same concentration and are sufficiently concentrated (about 400 mg/100 ml) to minimalize the influence on the results.

The Influence of Drying Temperature and -Time. Unger (6) mentions the necessity for the sample being completely in its dehydrated state since residual water is the cause of too low results. Shapiro and Kolthoff (1) report that the presence of small amounts of water (up to 15 %) does not greatly hinder the adsorption of MR. Higher amounts, however, cause a pronounced decrease in dye adsorption. For an experiment with silica samples containing 14.6 % and 5 % water respectively, they report MRAV of about 105 mg/g and 114 mg/g. In our laboratory, an experiment was carried out in which a comparison was made between unheated silica (Kieselgel H) and the same silica heated for 3 h at 120°C or at 180°C. The MRAV were

respectively 143, 154 and 152 mg/g, which means that the presence of small amounts of adsorbed water is less important than expected.

No significant differences were obtained when the sample was dried at 120°C during periods going from 30 min up to 15 h. Since it is not recommended to dry the reversed phase materials at too high temperatures, a drying time of 3 h at 120°C was finally chosen.

Influence of the Shaking Period. The influence of the time during which the samples were shaken on the vortex apparatus was briefly investigated . No significant differences were noticed when the samples were shaken for periods from 1 to 30 min.

Discussion of the Results. With the method described a large range of methyl red adsorption values (MRAV) is obtained for the samples examined : from zero to about 200 mg/g for respectively highly covered reversed phase materials and silica gel (Table 1). Most commercial HPLC reversed phase materials give values close to zero. Uncompletely covered HPLC materials are Polygosil C 8, RSiL C 18 HL 30μ (HL for high loading), which is a material used for preparative work, and RSiL C 18 LL 10μ (LL for low loading). The latter material is intended to be only partially covered and is said to behave as a C 8 reversed phase material. The RP I, II and III samples are obtained by reaction of silica with trimethylchlorosilane at room temperature according to Little et al. (8). Obviously this method is not suitable to prepare highly covered reversed phases. A TLC material such as Kieselgel 60 H silanisiert has higher MRAV than most of the commercial HPLC materials.

The MRAV obtained for μ Bondapak NH_2 is higher than expected, which is an indication for adsorption of MR by the amino groups. This means that the methyl red adsorption test is not useful for reversed phase materials containing groups showing acid-base interaction with MR. Hennion et al. (5) report that the methyl

red adsorption test is even not applicable to reversed phases in which very weakly acid groups such as diol-functions are introduced. Our results for LiChrosorb DIOL are not in agreement with this statement.

The close relationship between the MRAV obtained for Kieselgel H, Kieselgel H 15μ and LiChrosorb SI 60 (10μ) proves that the adsorption is independent on particle size or particle size distribution, since both parameters decrease from the first, a common TLC material, to the last. The difference in MRAV between this group of silica gels, having all a pore size of 6 nm, and LiChrosorb SI 100, having a pore size of 10 nm, clearly demonstrates that the MRAV depends upon pore size and thus upon specific surface. From the ratio of the surfaces claimed for the LiChrosorb SI 60 and SI 100 (500 m^2/g and 300 m^2/g, ratio 1.66) one would expect an even more important difference in MRAV (198 mg/g and 141 mg/g, ratio 1.4). This could be an indication for partial exclusion of methyl red from smaller pores, occuring more frequently with LiChrosorb SI 60 than with LiChrosorb SI 100. The relationship between MRAV and precise BET specific surface area remains to be investigated.

The method as proposed seems to be useful to distinguish between highly and lowly covered reversed phase materials. Less covered materials can be classified depending on their degree of coverage whereas little difference can be seen between well covered materials.

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