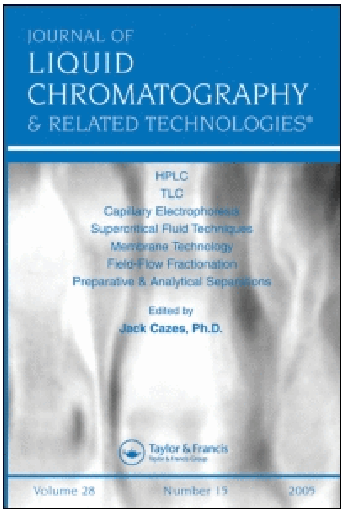

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INTERACTION OF DIQUAT AND PARAQUAT WITH GLUTATHIONE STUDIED BY MEANS OF CHARGE-TRANSFER CHROMATOGRAPHY

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ABSTRACT

The interaction of reduced and oxidized glutathiones with the herbicides diquat and paraquat was studied by charge-transfer chromatography carried out on unimpregnated cellulose layers. Diquat and paraquat interact both with reduced and oxidized glutathiones forming charge-transfer complexes. The interaction is of hydrophilic character in both cases. The formation of covalently bonded conjugates was not observed. The strength of interaction decreases in ionic (LiCl, NaCl, KCl, $MgCl_2$ and $CaCl_2$) environment in the case of oxidized glutathione. The charge of cation had a higher impact on the strength of interaction than that of the hydrated ion radii.

INTRODUCTION

Paraquat (1,1'-dimethyl-4,4'-bipyridylium ion), normally applied in the form of the dichloride salt, and diquat (1,1'-ethylene-2,2'-bipyridylium ion), normally applied as its dibromide salt, are non-selective, contact herbicides extensively used in the agrochemical practice. In green plant, bipyridylium herbicides compete for electrons from the primary electron acceptor of photosystem I. The free radicals formed are reoxidized by molecular oxygen giving rise to superoxide radical anions (1), which can be either a potent oxidant by forming hydroxyl radicals or a reductant by forming singlet oxygen (2). Paraquat causes wilting of leaves due to the light dependent degradation of biological membranes (3) and induces lipid peroxidation and the oxidation of cellular components (4). These latter effects are also present in nonphotosynthesizing organisms. The physiological basis for paraquat tolerance is not well understood. It does not lie in the differences in the oxygen radical scavenging system, in whole plant uptake, or in an inducible stress response. It is possible that the tolerant mutants sequester paraquat either within the cell or in the cell wall (5,6).

Recent research indicates that amino acids (7,8) and various peptides (9,10) are able to prevent paraquat toxicity. As glutathione plays a preponderant role in many detoxification processes (11), the importance of glutathione and glutathione-dependent enzymes in paraquat resistance has been extensively demonstrated (12-14). According to our knowledge, the direct interaction between the bipyridylium herbicides and glutathiones has never been studied in detail.

Charge transfer reversed-phase thin-layer chromatography has been extensively applied to study the interaction between bioactive compounds of low molecular weight such as diquat and paraquat - dibasic and dicarboxilic amino acids (15), auxin - dibasic amino acids (16) etc.

The objectives of our investigation were to study the interaction of the bipyridylium herbicides with reduced and oxidized glutathione and to determine the effect of ionic environment on the strength of interaction. The study of the effect of ionic environment was motivated by the observation that salts may increase paraquat tolerance (17).

MATERIAL AND METHODS

Reduced and oxidized glutathiones were purchased from REANAL Fine Chemicals (Budapest, Hungary). Diquat and paraquat were the products of ICI (Haslemere, England). DC-Alufolien Cellulose F_{254} (Merck, Darmstadt, Germany) were used without any pretreatment. The herbicides were separately dissolved (2 mg ml^{-1}) in methanol; 5 µl of these solutions were spotted onto the plates. Aqueous solutions of glutathiones in the concentration range of 0 - 12 mM were used as eluents. After development and drying the spots were detected under UV light.

To study the effect of ionic environment on the strength of interaction, the interactive strengths between herbicides and glutathiones was determined in the presence of 0 - 8 mM LiCl, NaCl, KCl, $MgCl_2$ and $CaCl_2$. Each experiment was run in quadruplicate.
The R_M values characterizing molecular lipophylicity were calculated: $R_M = \log(1/R_f - 1)$. The strength of interaction and the effect of the physicochemical parameters of ions on the interactive strength were calculated by stepwise regression analysis. The R_M values were taken as dependent variables, the linear and quadratic forms of glutathione and salt concentration, the charge and the hydrated radii of cations as

well as the glutathione concentration multiplied with salt concentration, with cation charge and with cation radii were taken as independent variables. The inclusion of the last three combined variables in the calculation was motivated by the supposition that the various physicochemical parameters of salts may modify the interacting capacity of glutathiones. The calculation was separately carried out for each herbicide-glutathione pair. The number of accepted variables was not limited, the acceptance level of the individual independent variables was set to 99% significance level.

RESULTS AND DISCUSSION

Only one UV active spot was observed on each chromatogram markedly below the glutathione front and the spot position strongly depended on the concentration of glutathione in the eluent. As the mobility of a covalently bonded new compound had to be independent of the glutathione concentration, this finding suggests that the interaction does not result in the formation of covalently bonded conjugates during the analysis time (maximum 4 hours at room temperature). We have to draw the attention to the fact that our data do not exclude the possibility of conjugate formation after longer interacting time.

Both glutathione derivatives decreased the lipophilicity (R_M value) of both bipyridylium herbicides (Figs 1 - 4). This finding indicates that glutathiones interact with the herbicides and the interaction is of hydrophilic character. We assume that electrostatic forces between the acidic groups of glutathiones and the polar substructures of herbicides account for the interaction. The relationship between the R_M value of

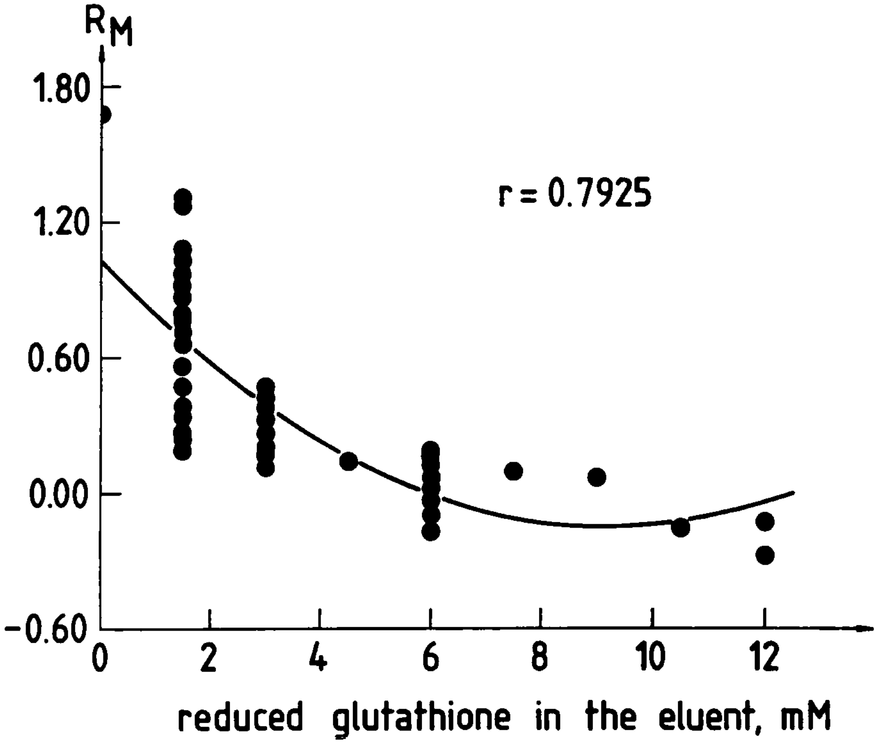

Figure 1. Dependence of R_M value of diquat on the concent ration of reduced glutathione in the eluent. Salt free and salt containing eluents are equally included in the Figure.

herbicides and the glutathione concentration in the eluent is significant also in the case when each (salt free and salt containing) eluent system is included in the calculation. This results suggests that the environmental salt concentration is of secondary importance in the herbicide - glutathione interaction. The dependence of the R_M value of herbicides on the glutathione concentration is similar in each case. This observation suggests that the free sulfhydryl group of

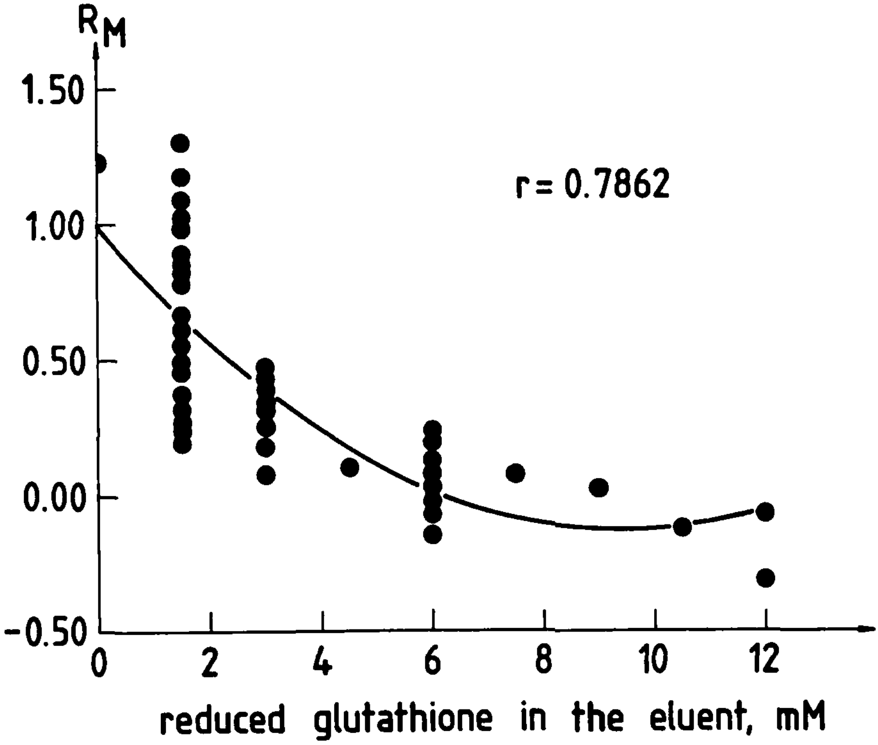

Figure 2. Dependence of R_M value of paraquat on the concentration of reduced glutathione in the eluent. Salt free and salt containing eluents are equally included in the Figure.

reduced glutathione has a negligible impact on the herbicide - glutathione interaction. As glutathiones exhibit lower lipophilicity than the herbicides under the experimental conditions, it is apparent that the glutathione - herbicide complex is less lipophilic than the herbicides themselves. Consequently, glutathiones can influence the distribution of herbicides between the hydrophilic and lipophilic compartments of plant

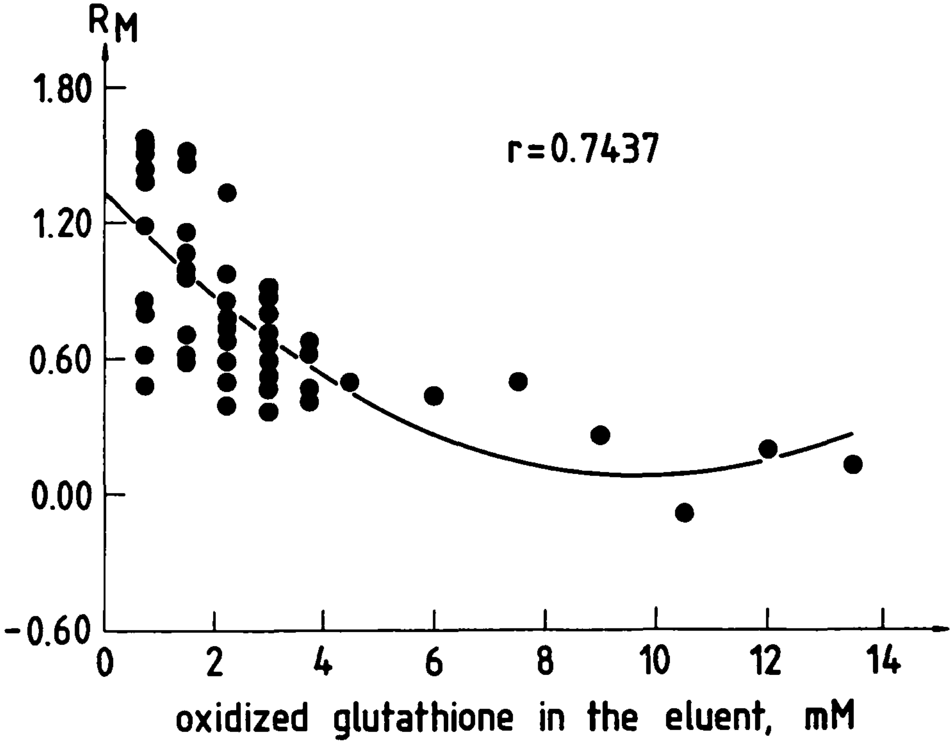

Figure 3. Dependence of R_M value of diquat on the concentration of oxidized glutathione in the eluent. Salt free and salt containing eluents are equally included in the Figure.

tissues, modifying in this way their mobility, availability and toxicity. The correlation between the lipophilicity of herbicides and the concentration of glutathione is markedly nonlinear. This result is somewhat unexpected, because in many cases linear correlations were found between the concentration of the interacting agent and the R_M value of the other molecules (18-20). This nonlinear relationship may be due to the changing stoichiometry of the herbicide -

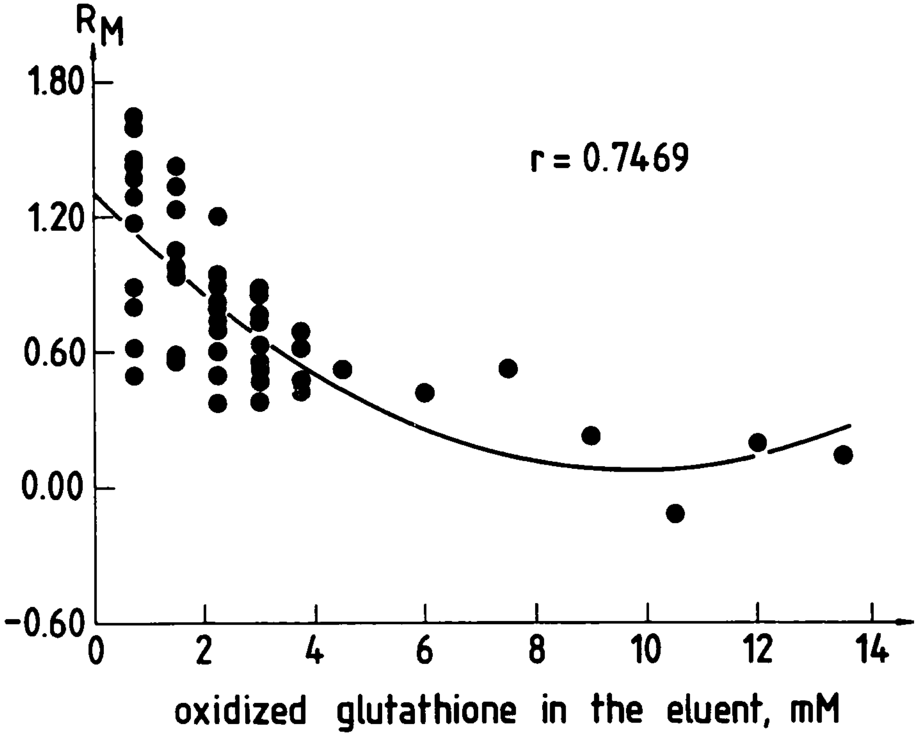

Figure 4. Dependence of R_M value of paraquat on the concentration of oxidized glutathione in the eluent. Salt free and salt containing eluents are equally included in the Figure.

glutathione complex: at lower glutathione concentrations the complex contains less glutathione (i.e. glutathione - herbicide 1:1 mol.ratio) than at higher concentrations (i.e. glutathione - herbicide 2:1). Unfortunately, charge transfer chromatography does not give information about the exact stoichiometry of the complex, therefore this explanation is only hypothetical. The ions (salts are probably entirely dissoci-

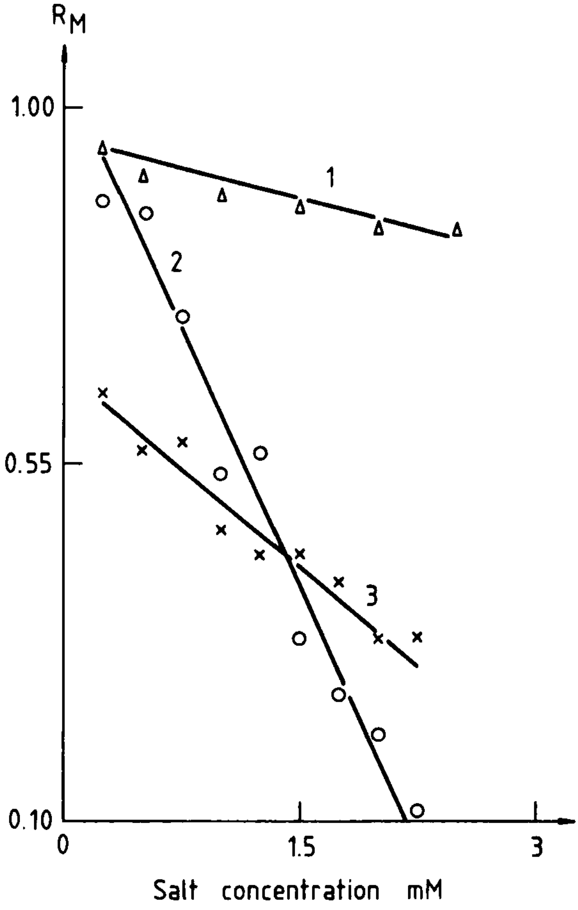

Figure 5. Dependence of R_M value of diquat on the concentration of salts in the eluent. 1.5 mM reduced glutathione.

1. KCl 2. $CaCl_2$ 3. MgC_12

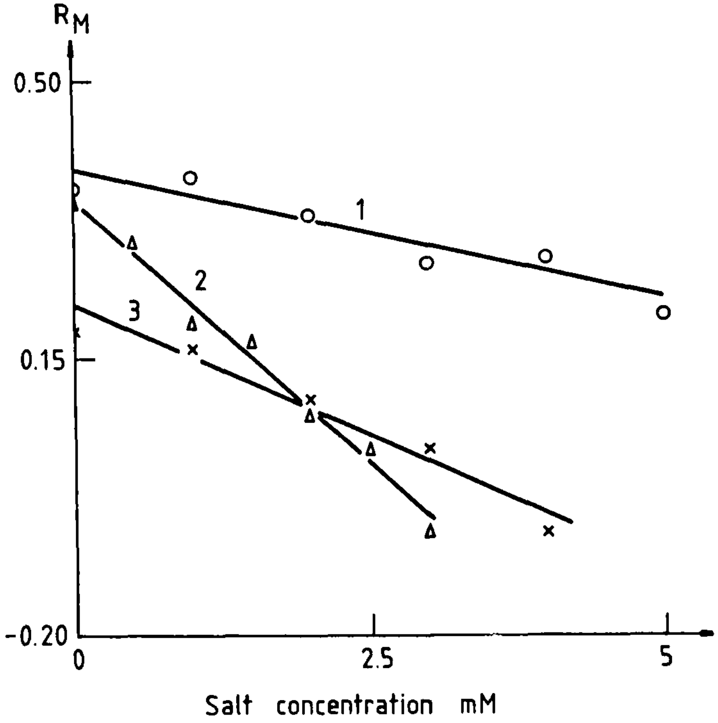

Figure 6. Dependence of R_M value of paraquat on the concentration of salts in the eluent.

1. KCl, 3 mM reduced glutathione
2. $CaCl_2$, 3 mM reduced glutathione
3. KCl, 6 mM reduced glutathione

ated in the aqueous solution at these low concentrations) also decrease the lipophilicity of herbicides (Figs 5 and 6), the effect of divalent cations being stronger than that of monovalent ones. These data can be explained by the supposition that the ions suppress the dissociation of the polar substructures in the herbicides making them less liable for the electro-

TABLE 1.

Dependence of the Lipophilicity of Diquat on the Eluent Composition. Reduced Glutathione. The Results of Stepwise Regression Analysis (n = 80).

$$R_M = a + b_1.x_1 + b_2.x_2 + b_3.(x_1)^2$$

Independent variable	Parameters b	s_b	Path coefficient%
x_1	-27.75	2.89	57.33
x_2	-23.31	3.93	12.07
$(x_1)^2$	1.33	0.26	30.60

a = 139.96 F = 74.32 r^2 = 0.7458

x_1 = concentration of reduced glutathione in the eluent (mM),
x_2 = cation charge

static interactions presumably accounting for the complex formation.

The results of stepwise regression analysis are compiled in Tables 1-4.

In each case the equation selected by the stepwise regression analysis fits well to the experimental data, the significance level being over 99.9% (see F values). The change of independent variables accounts for about 66 - 80% of the change of lipophilicity of herbicides (see r^2 values). The calculations prove the existence of quadratic correlation between the lipophilicity of bipyridylium herbicides and the glutathione concentration, this interaction explains the overwhelming majority of the retention change of herbicides see path coefficient values).

The physicochemical parameters of cations play a negligible, however, significant role in the interaction. The outstanding influence of cation charge on the

TABLE 2.

Dependence of the Lipophilicity of Diquat on the Eluent Composition. Oxidized Glutathione. The Results of Stepwise Regression Analysis (n = 63).

$$R_M = a + b_1.x_1 + b_2.x_1.x_2 + b_3.x_3$$

Independent variable	Parameters b	s_b	Path coefficient%
x_1	-8.18	1.52	39.79
$x_1.x_2$	-10.92	1.75	38.38
x_3	30.19	11.18	21.83

a = 103.64 F = 38.73 r^2 = 0.6632

x_1 = concentration of oxidized glutathione in the eluent (mM),
x_2 = cation charge,
x_3 = cation radii

TABLE 3.

Dependence of the Lipophilicity of Paraquat on the Eluent Composition. Reduced Glutathione. The Results of Stepwise Regression Analysis (n = 80).

$$R_M = a + b_1.x_1 + b_2.x_2 + b_3.(x_1)^2$$

Independent variable	Parameters b	s_b	Path coefficient%
x_1	-24.95	3.03	58.81
x_2	-16.86	4.12	9.96
$(x_1)^2$	1.19	0.27	31.23

a = 124.67 F = 55.64 r^2 = 0.6871

x_1 = concentration of reduced glutathione in the eluent (mM),
x_2 = cation charge

TABLE 4.

Dependence of the Lipophilicity of Paraquat on the Eluent Composition. Oxidized Glutathione. The Results of Stepwise Regression Analysis (n = 63).

$$R_M = a + b_1.x_1 + b_2.x_2 + b_3.(x_1)^2 + b_4.x_1.x_2$$

Independent variable	Parameters b	s_b	Path coefficient%
x_1	-40.24	4.81	47.56
x_2	-52.66	8.39	15.51
$(x_1)^2$	2.01	0.35	30.10
$x_1.x_2$	7.99	2.83	6.83

a = 204.42 F = 57.09 r^2 = 0.7974

x_1 = concentration of oxidized glutathione in the eluent (mM),
x_2 = cation charge

interaction makes it probable that the divalent cations may influence the complex formation by promoting the ternary complex formation of herbicide-Ca-glutathione stoichiometry.

Summarizing our results, we have established that diquat and paraquat can directly interact with reduced and oxidized glutathiones. This interaction may have some role in the beneficial effect of glutathione to paraquat toxicity. The free sulfhydryl group is not a prerequisite for the interaction and the influence of the environmental salt concentration is of secondary importance.

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