Spectrophotometric method for the determination of Glyphosate in relation to its environmental and toxicological analysis

D.K. Sharma*[1], Atul Gupta[2], Rajinder Kashyap[1], and Naresh Kumar[1]

[1]*Department of Chemistry, Himachal Pradesh University, Shimla -171005, India*
[2]*Department of Chemistry, S.V.G.C. Ghumarwin-174021, India*
*To whom correspondences should be addressed
E-mail: dksharma_dk@rediffmail.com

Abstract

Glyphosate is a broad spectrum herbicide and its behavior in soil is an important consideration for the evaluation of its environmental toxicity. Therefore, the present study deals with the adsorption of glyphosate herbicide on three Indian soils by using batch equilibrium method. To carry out the adsorption study, a new simple, sensitive and rapid method was developed, based on the transformation of amino group of glyphosate in aqueous acetonitrile to corresponding dithiocarbamate derivative and reaction of later with copper(I) perchlorate to form yellowish green colored complex, showing λ_{max} at 392 nm. The method is quite sensitive and the molar absorptivity (ε) and Sandell's sensitivity were found to be 1.85×10^3 L mol^{-1} cm^{-1} and 0.091 µg cm^{-2} respectively. The leaching potential of this herbicide vis-à-vis associated environmental pollution risk was evaluated by Groundwater Ubiquity Score (GUS) model. Its value has been observed < 1.8 which classifies glyphosate as non-leacher pesticide in terms of leaching behaviour.

1. Introduction

Glyphosate (N-(phosphonomethyl)-glycine) is a broad-spectrum, non-selective, post-emergence type of organophosphorous herbicide, widely used to control annual and perennial plants [1]. The worldwide use of this herbicide requires the special concerns with its ill effects on environment and human beings. Various adverse effects of glyphosate including teratogenesis, carcenogenesis, mutagenesis and other developmental effects are well reported in literature [2-7]. In view of wide spread use of glyphosate and potential hazards associated with its use, it is worthwhile to consider the final fate of glyphosate herbicide in environment.

In general the fate of pesticide is mostly played out in the soil as the later is ultimate reservoir for pesticides irrespective of their application target. These chemicals enter the soil in different ways i.e. with washing of remaining deposits on crops by rain or irrigation water and incorporated with crop residues. In soil environment, the pesticides get fractionated between soil solution phase (in free form) and soil solid phase through adsorption on clay and organic fractions (in bounded form) [8]. The adsorption of pesticides affects the various processes like bioactivity, mobility, persistence, toxicity, volatilization and bioaccumulation because all these phenomena are operative only on the unadsorbed fractions of pesticide [9]. All these processes influence the extent of surface and ground water contaminations. Therefore, from environmental point of view, thorough understanding of adsorption of this pesticide on soil is paramount for the prediction of its movement in soils and aquifers. Of no less importance is the determination of this herbicide in residues on environmental samples for the purpose of monitoring pollution and health hazards.

To accomplish above objectives, it is therefore, inevitable to find a simple, sensitive and reliable technique of wide applicability for the analysis of glyphosate herbicide. Most of the reported analytical methods for its determination are based on the chromatographic methods coupled with other techniques [10-13], chemiluminiscence [14] and polarographic [15]. These methods need separation and derivatisation into stable products which is not easily achievable. Moreover spectrophometric methods [16-18] reported in literature require coloured reagents and chromophore groups. Our efforts in this direction have led to the

development of a new, simple and rapid spectrophotometric method for the determination of glyphosate using copper(I) perchlorate in aqueous acetonitrile. The method is based on the transformation of amino group of glyphosate in aqueous acetonitrile to corresponding dithiocarbamate derivative and reaction of later with copper(I) perchlorate to form yellowish green colored complex showing maximum absorbance at 392 nm. The copper(I) reagent forms a colorless solution in acetonitrile which is extremely stable. That copper(I) is stabilized by coordination with acetonitrile is well established [19,20]. The proposed spectrophotometric method was successfully applied to the analysis of glyphosate in commercial herbicide formulation (for the purpose of quality control) and its recovery from water samples (to monitor health hazards). The present method has also been validated to study the adsorption of glyphostate herbicide on three Indian soils of different soil characteristics. The various adsorption parameters viz. distribution coefficient or soil-adsorption coefficient (K_d), soil organic carbon partition coefficient (K_{oc}) and Gibb's free energy ($\Delta G°$) have been calculated. The pesticide leaching is an important process with respect to contamination risk to aquatic environment and the risk of leaching has also been evaluated for glyphosate in terms of Groundwater Ubiquity Score (*GUS*).

2. Materials and Methods

The analytical standard of glyphosate (Sigma, 95%) was used. The purity was checked by a reported method [18]. Acetonitrile, carbon disulphide, disodium salt of ethylenediamminetetra acetate (EDTA), sodium bicarbonate and perchloric acid 70% were obtained from Merck-Schuchardt, Germany. A standard solution of copper(I) perchlorate, 0.01 M in acetonitrile was prepared by the method as described earlier [19]. Domestic microwave oven, Samsung was used. All the spectrophotometric measurements were made with carry100Bio UV visible spectrophotometer. The soils used in the adsorption study were collected from Solan district of Himachal Pradesh, India.

2.1. Preparation of Calibration Graph for Pure Compound

Aliquots (0.2-2 ml) of standard solution of glyphosate (0.001 M in water) were taken in 10 ml measuring flasks and volume made to 3 ml with water. To each solution, 1 ml EDTA (0.05 M in water) was added to avoid any interference from the metal ions present in different substrates. The mixture solution was then mixed with carbon disulphide (1 ml, 1% in acetonitrile) and 1 ml of 2% aqueous sodium bicarbonate solution and then kept in microwave oven for 50 s. Each solution was mixed with copper(I) perchlorate (1 ml, 0.01 M in acetonitrile) and perchloric acid (1 ml, 2% solution in acetonitrile) and final volume was made up to the mark with acetonitrile. The absorbance of yellowish green colour was measured at 392 nm against a reagent blank and the absorbance values were plotted against concentration of pure compound used and a calibration curve was prepared (Fig. 1). The calibration characteristics viz. Beer's law range, molar absorptivity, Sandell's sensitivity, slope and intercept values and correlation coefficient were calculated from calibration curve and are given in Table 1.

Table 1. Spectrophotometric determination of glyphosate as colored copper-dithiocarbamate complex: Calibration characteristics

| Optical characteristics	Corresponding value
λ_{max}	392 (nm)
Beer's law range	3.38-33.8 ($\mu g \ ml^{-1}$)
Molar absorptivity (ε)	1.85×10^3 ($L \ mol^{-1} \ cm^{-1}$)
Sandell's sensitivity	0.091 $\mu g \ cm^{-2}$
Stability of color	90 (min)
Slope	0.0135
Intercept	-0.0512
Correlation coefficient (*r*)	0.99432

2.2. Formulation Analysis

A soluble granule formulation, Glyfos Daker, containing 71% active ingredient, procured from local market was used. A single large sample of formulation equivalent to 16.9 mg active ingredient was dissolved in water, and final volume was made to 100 ml with the same solvent. Suitable aliquots of the above solution were taken and processed for analysis as described above for the pure compound. Assay results are given in Table 2.

Figure 1. Spectrophotometric method for the determination of glyphosate:

(a) Absorption spectra of yellowish green colored copper(I)-dithiocarbamate complex
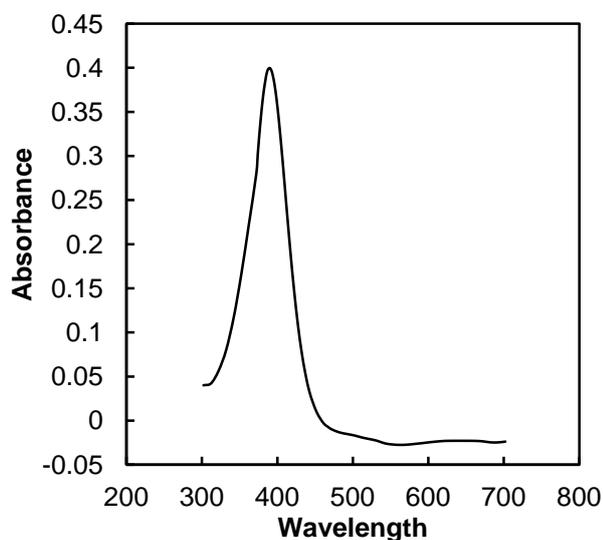
(b) Relationship between absorbance and concentration (calibration graph)
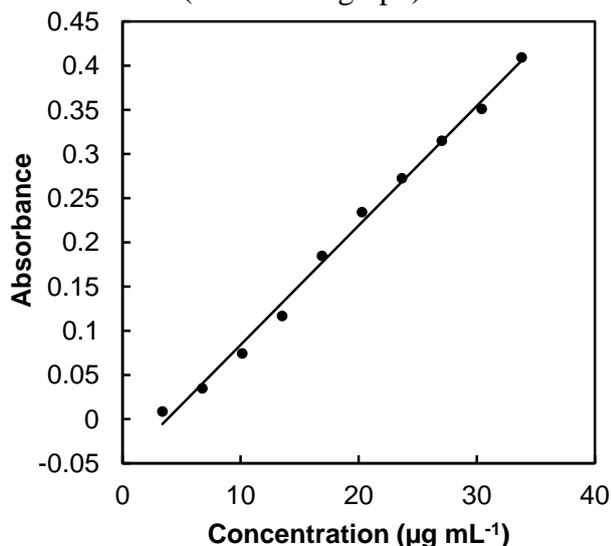
Table 2. Assay of a commercial formulation of glyphosate containing 71% active ingredient

Amount taken (µg)	Recovery (%)[a]	
	Present method	Comparison method[b]
33.8	92.4 ± 0.81	87.4 ± 0.95
50.7	90.3 ± 1.02	88.0 ± 0.92
67.6	93.2 ± 0.50	90.9 ± 1.08
84.5	96.5 ± 0.95	91.7 ± 0.89

[a]values are mean ± standard deviation for 5 determinations; [b]reference [18].

2.3. Determination in Spiked Water Samples

The water samples (4 ml) were spiked with different concentrations of glyphosate in the ranges from 33.8 to 84.5 µg. The spiked water samples were then processed for analysing of glyphosate concentration using the procedure described above for pure compound. The results are given in the Table 3.

Table 3. Recovery of glyphosate from water samples

Amount taken (µg)	Recovery (%)[a]	
	Present method	Comparison method[b]
33.8	94.2 ± 0.88	87.4 ± 0.95
50.7	96.3 ± 0.91	91.0 ± 0.72
67.6	99.1 ± 0.85	93.9 ± 1.08
84.5	97.9 ± 0.58	95.7 ± 1.02

[a]values are mean ± standard deviation for 5 determinations; [b]reference [18]

Table 4. Characteristics of the different Indian soils used in the adsorption study of glyphosate

Soil sample	pH	Clay (%)	Organic carbon (%)	Cation exchange capacity (meq/100g)
I	6.8	9.8	0.64	11.5
II	6.9	10.2	0.82	12.8
III	7.6	14.1	0.69	11.6

2.4. Soil Adsorption Study

Glyphosate adsorption isotherm on three Indian soils of different soil characteristics (Table 4) were obtained by the batch equilibration technique using 50 ml conical flask [21]. Triplicate soil samples (2 g) were equilibrated with glyphosate solutions in the concentration range from 8.46-25.36 µg ml^{-1} by shaking mechanically at room temperature (24±1°C) for 24 h equilibrium time. After equilibration, the suspensions were centrifuged and the equilibrium concentrations (C_e) were determined in supernatants by the method described above. Adsorption isotherms were evaluated by using Freundlich's adsorption equation which is written as:

$$X = K_f C_e^{n_f} \tag{1}$$

where X is the amount of pesticide adsorbed mg Kg^{-1} of the adsorbent; C_e is the equilibrium solution concentration (mg L^{-1}); K_f and n_f are adsorption coefficients which are calculated from the least square methods applied to the

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linear form of the Freundlich's adsorption equation

$$\log X = \log K_f + n_f \log C_e \qquad (2)$$

Another parameters for the adsorption process viz. distribution coefficient or soil-adsorption coefficient (K_d), Gibb's free energy (ΔG°), soil organic carbon partition coefficient (K_{oc}) and Groundwater Ubiquity Score (GUS) have been calculated by using equations 3-6 respectively [22-24].

$$K_d = \frac{X}{C_e} \qquad (3)$$

$$\Delta G^\circ = -RT \ln K_d \qquad (4)$$

$$K_{oc} = K_d \times \left(\frac{100}{\% O.C.} \right) \qquad (5)$$

$$GUS = \log t_{1/2} \left[4 - \log(K_{oc}) \right] \qquad (6)$$

where R = gas constant, T = absolute temperature, $t_{1/2}$ = pesticide persistence (half life), OC = organic carbon content of soil. GUS score is used to study the leaching behaviour of pesticides and these can be classified as leacher ($GUS > 2.8$), transition ($2.8 > GUS < 1.8$) and non-leacher ($GUS < 1.8$) [24]. All these parameters for the adsorption of glyphosate on three Indian soils were calculated and the results are shown in Table 5.

Table 5. Adsorption parameters for the adsorption of glyphosate on three Indian soils

Soil sample	K_f	n_f	K_d	K_{oc}	ΔG° (KJ mol^{-1})	GUS	Adsorption isotherm
I	35.48	0.43	4.77	749.4	-11.88	1.67	L-type
II	23.44	0.56	4.96	604.5	-12.28	1.80	L-type
III	20.89	0.57	4.34	620.5	-10.76	1.78	L-type

3. Results and Discussions

The present spectrophotometric method is based on the transformation of amino group of glyphosate in aqueous acetonitrile to corresponding dithiocarbamate derivative and reaction of later with copper(I) perchlorate to form yellowish green colored complex showing maximum absorbance at 392 nm. The smooth and quantitative transformation of herbicide to yellowish green colored copper(I)-dithiocarbamate derivative was brought about rapidly by microwave heating. It is well known that microwave heating mode is quite different from conventional heating as it dissipates heat inside the medium and raises the energy of the molecules rapidly. Therefore, under microwave assisted heating, more molecules become energized, and this usually results in higher reaction rates and greater selectivity [25]. Effect of time of microwave heating on the development, stability and sensitivity of the color, vis-à-vis development of the proposed method was studied before applying it to the analysis of glyphosate. It has been observed that maximum color intensity and its stability was obtained corresponding to 60s in microwave. The most plausible course of reaction is:
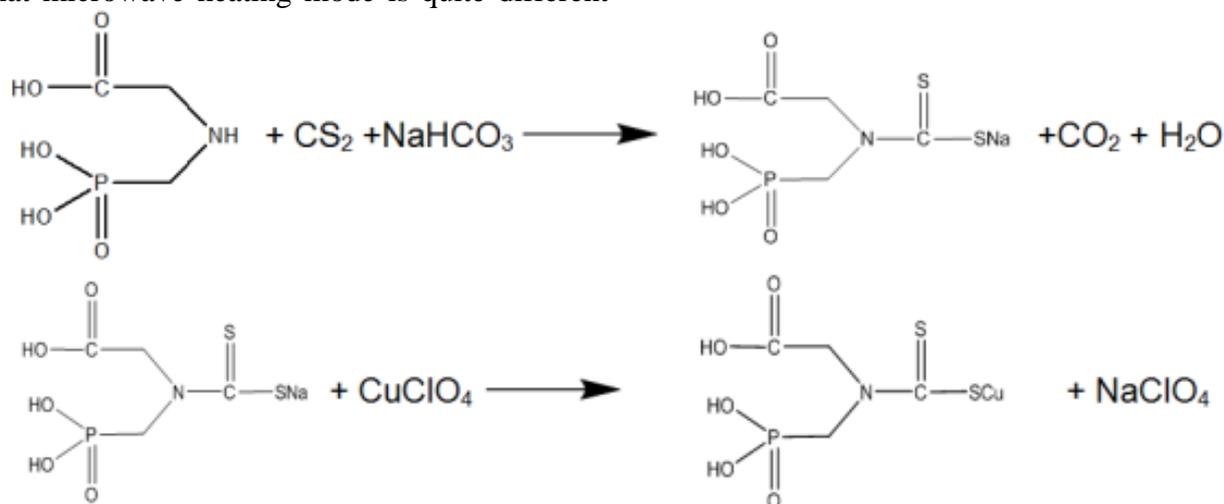
Copper(I)-dithiocarbamate complex (I)

That the reaction proceeds in 1:1 molar ratio has also been established by photometric titration of glyphosate with copper(I) perchlorate in aqueous acetonitrile. In this titration performed at 392nm (λ_{max} of coloured complex) the absorbance increases till glyphosate to copper(I) molar ratio 1:1 is achieved indicating the formation of coloured complex (I) and thereafter it attains almost constant values indicating no more formation of coloured complex. An inverted L-shaped titration curve is obtained (Fig. 2).

Figure 2. Photometric titration curve of glyphosate (as dithiocarbamate) with copper (I) perchlorate
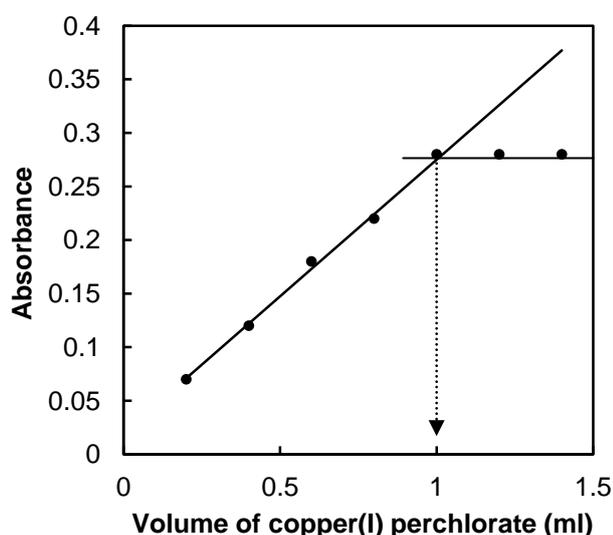

The kinetic stability of colored complex was studied with time and the color is found to be stable for at least 90 mins. The proposed spectrophotometric method obeys Beer's law in the range of 3.38-33.8 µg ml^{-1} of glyphosate. The method is quite sensitive and the molar absorptivity (ε) and Sandell's sensitivity were found to be 1.85×10^3 L mol^{-1} cm^{-1} and 0.091 µg cm^{-2} respectively at 392 nm. Sandell's sensitivity represents the number of micrograms of the determinand per ml of a solution having absorbance of 0.001 for a path length of 1 cm [26]. The method has successfully been applied to the analysis of a commercial formulation of glyphosate for its active ingredient content. The recoveries were in the range 90.3-96.5%, with *RSD*s in the range 0.5-1.02% (Table 2). The formulation analysis is essential not only to ensure the quality of marketed products of the pesticide but also to get reliable residue and adsorption data. The method has also been applied with success to the determination of this herbicide on water samples for the purpose of monitoring pollution and health hazards. Recoveries of the herbicide from water samples were good, ranging from 94.2-99.1%, with *RSD*s ranging from 0.58-0.91% (Table 3) show good precision and accuracy of the method. The results have, however, been compared with an independent method [18].

Figure 3. Adsorption isotherm of glyphosate on soil I at (24±1℃). (Error bars represent the standard deviation of three replicates).
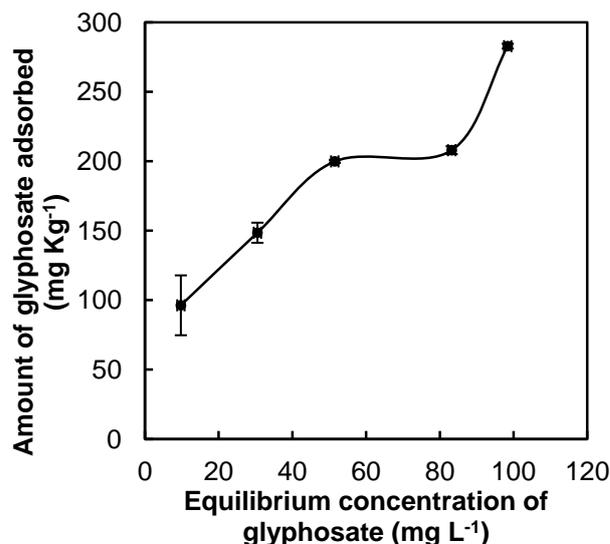
Figure 4. Adsorption isotherm of glyphosate on soil II at (24±1℃). (Error bars represent the standard deviation of three replicates)
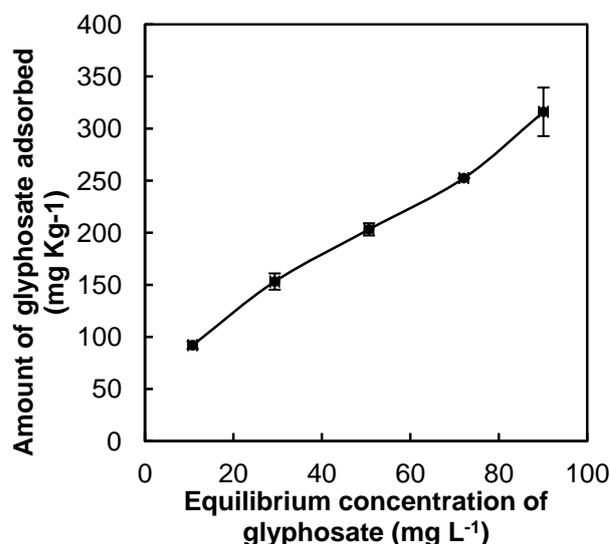

The adsorption isotherms of glyphosate herbicide on three Indian soils of different soil characteristics (Table 4) are shown in Figs. 3-5 respectively. According to the initial portion of the curve, these isotherms may be classified as L-type of Gile's classification [27] (Table 5).

The L-types of isotherms represent a system where the solid surface has an average affinity for the pesticide and the solvent is relatively inert: there is no strong competition from the solvent for adsorption sites [28]. The adsorption coefficients K_f and n_f were calculated for three soil samples from Freundlich's adsorption equation and results are presented in Table 5. The adsorption coefficient K_f represents the amount of pesticide adsorbed at an equilibrium concentration of 1 mg L^{-1} and n_f represents the variation in adsorption with varying concentration of pesticide [9]. The observed value of n_f was less than 1 in all three cases indicating that with the increase in the concentration of pesticide, the percentage adsorption of the pesticide by the soil decreased. This might be due to the fact that at higher concentration, there is an increased difficulty to access the adsorption site [29]. Other parameters such as the soil-adsorption coefficient (K_d), soil organic carbon partition coefficient (K_{oc}) and Groundwater Ubiquity Score (GUS) for the adsorption of glyphosate were also calculated and are presented in Table 5. The soil-adsorption coefficient (K_d) values for glyphosate were observed 4.77, 4.96 and 4.34 for soil I, soil II and soil III respectively. Generally, the higher the K_d value, the greater is the pesticide adsorption [9]. From Table 5 it can be inferred that glyphosate is adsorbed maximally in case of soil II (with higher K_d). The K_d for a pesticide is soil-specific and varies with soil texture and its organic matter content but the soil organic carbon partition coefficient (K_{oc}) is less soil specific [30] and is calculated by normalizing adsorption coefficient (K_d) with the organic carbon (OC) content of the soil. The value of K_{oc} for glyphosate adsorption on three soil substrates decreased with increased fraction of organic content, indicating that adsorption is not only due to organic matter content of soil but also influenced through some other factors [9]. The organic matter content of soils affects the adsorption of pesticide: the higher the organic matter contents, the higher the adsorption [31]. The cation exchange capacity (CEC) is another parameter that influences the adsorption of pesticide. The value of CEC is directly proportional to the hydrophobic nature of adsorbent: the greater the value of CEC of soil, the more hydrophobic its surface. The soils with

higher value of CEC have a lower adsorption affinity for hydrophilic (water soluble) pesticides and vice versa [32]. The value of Gibb's free energy (ΔG^{o}) for the adsorption of glyphosate herbicide was observed negative in all the cases suggesting the energetically favourable adsorption process. In view of the fact that glyphosate has pronounced adsorption in all three tested soils and consequently inhibit the penetration of this herbicide into ground water. A number of models are available to evaluate the leaching potential of pesticide vis-à-vis associated environmental pollution risk. Groundwater Ubiquity Score (GUS) is the most commonly used model which relate pesticide persistence (half life) and adsorption in soil (K_{oc}). The leaching potential of herbicide in terms of GUS index was determined by using experimentally observed K_{oc} value for each soil sample and literature reported half life of glyphosate [24]. The GUS score for glyphosate has been observed below 1.8 which classifies it as a non leacher herbicide.

Figure 5. Adsorption isotherm of glyphosate on soil III at (24±1℃). (Error bars represent the standard deviation of three replicates)
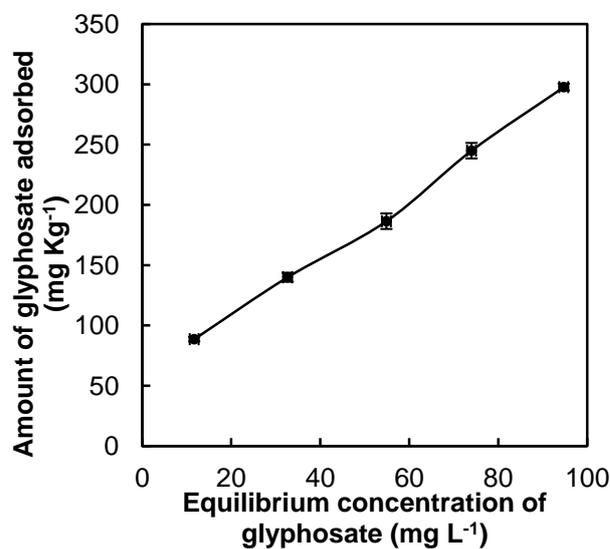
4. Conclusion

The proposed spectrophotometric method for the determination of glyphosate possesses advantages over the commonly used spectrophotometric method in terms of the instantaneous development of the colour with a colorless copper(I) reagent, sufficient stability of the colour as well as reagent solution, non-extraction of the coloured product (a time

consuming feature, common in colorimetric analysis), well established stoichiometry of the colour reaction and above all simplicity and rapidity of the procedures. From the soil-adsorption study, the values of soil organic carbon partition coefficient (K_{oc}) suggest greater adsorption in soil types and consequently inhibit its penetration into water sources. The leaching behaviour of the herbicide in terms of ground ubiquity score (*GUS*) was below 1.8, classifying glyphosate as non-leacher pesticide and hence it does not represent hazard to ground water contamination. It is further concluded that the toxicity due to this herbicide can also be reduced by adjusting the application dose according to soil properties. The soils with high organic carbon content increase its adsorption and consequently reduce its leaching losses.

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