

Uma experiência laboratorial simples para introduzir o conceito de poluição disseminada a estudantes das áreas do ambiente e agricultura

A simple laboratory project for introducing nonpoint source pollution concept to students of environmental and agricultural related courses

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RESUMO

Este trabalho descreve uma experiência laboratorial simples para introduzir o conceito de poluição disseminada (nonpoint source pollution) a estudantes do Ensino Secundário e Universitário das áreas de Ambiente e de Agricultura. O objectivo deste trabalho é introduzir aos estudantes conceitos e técnicas, tais como os sistemas de libertação controlada, a teoria da difusão (1ª lei de Fick) e a análise espectrofotométrica. Este projecto laboratorial inclui três módulos experimentais a serem efectuados durante 3 semanas de aulas. Neste trabalho serão descritos os conteúdos programáticos deste projecto laboratorial.

Os estudantes devem ser alertados para o problema da disseminação da poluição de nutrientes e pesticidas que ocorre devido à lixiviação. A contaminação de águas de superfície e de lençóis de água contribui para o alastramento da poluição a áreas circundantes. Para demonstrar a disseminação de tais poluentes, escolheu-se o fósforo inorgânico (P) como exemplo de um agroquímico comum. Os estudantes são convidados a

seguir o eventual movimento do P inorgânico até aos lençóis de água. Com este propósito, foram preparados géis de gelatina contendo P inorgânico para se obter uma libertação contínua do P e a uma velocidade controlada. A libertação lenta do P permite uma diminuição do número de aplicações e da quantidade do ingrediente activo, prevenindo a lixiviação com a consequente redução da contaminação dos lençóis de água. Após a realização da experiência, os estudantes estão aptos para comparar as vantagens da libertação controlada de P inorgânico vs. a sua aplicação por métodos convencionais.

ABSTRACT

This paper reports a simple laboratory project to introduce students to the nonpoint source pollution, which may be an issue of great interest to both undergraduate and graduate students of environmental or agricultural chemistry courses. The aim of this work is to introduce to the students the concepts and techniques such as the polymeric-based controlled release system of an agro-

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chemical, theory of diffusion (first Fick law) and spectrophotometric analysis. Thus, this laboratory project includes three experimental modules to be conducted during three weeks. Programmatic contents are described in this proceeding.

Students must be aware that dissemination of nutrients and pesticides is prone to occur by both surface runoff and groundwater leaching, causing damages on all neighboring land. To demonstrate dissemination of such pollutants, we have chosen inorganic phosphorus as example of a common agrochemical. Students are invited to follow the eventual movement of the inorganic P into the groundwater. With this purpose, gelatin gels containing inorganic P were prepared to obtain a continuous release of inorganic P at a controlled rate. The slow release of P allows fewer applications and less active ingredient needed, helping to prevent leaching, with consequent reduction of groundwater contamination. At this point, students are able to compare the advantages of slow release inorganic P vs. its application by conventional methods.

INTRODUCTION

Due to the intensive agricultural system and technological progress, the use of chemicals, such as pesticides and fertilizers, is an accepted practice today. Simultaneously, the impact of pesticides and fertilizers leaching from soils on surface water and groundwater quality is becoming a matter of concern (Pretty, 1995; Campbell & Edwards, 2001). Agrochemicals may be carried along with the water that moves downward from the surface, until they eventually reach the groundwater (leaching).

The slow-release formulations are excellent alternatives to the conventional soluble formulations, because active substances are

released at a slow and constant rate over an extended period of time. Consequently, less frequent application is required and plants are able to take up the substances without waste by leaching (Bajpai & Giri, 2002). As an academic example, a gelatin hydrogel containing inorganic phosphorus was prepared.

The aim of this work is introduce to the students the concepts and techniques such as the polymeric-based controlled release system of an agrochemical, theory of diffusion (first Fick law) and spectrophotometric analysis. Thus, this laboratory project includes three experimental modules to be conducted during three weeks (12-15 hours) for a maximum number of students in the laboratory class of 12 students divided in 6 groups. Programmatic contents are described in Table 1. For the different experimental activities we suggest the material and reagents as listed in the same table.

LESSON 1:

Aim 1. Preparation of gelatin matrix formulations containing inorganic P

Gelatin gels containing phosphorus (GelP) were prepared from a 20% (w/v) aqueous solution of gelatin (Gelatin powder food grade DAD, Ph Eur, BP, NF, from Merck, CAS [9000-70-8], 80-120 bloom number). Gels were obtained on the bottom of 50 mL glass beakers (3.8 cm diameter) by dissolving different quantities of KH_2PO_4 (from Merck, CAS [7778-77-0]) in 10 mL of the gelatin solution at 40 °C. Gels were partially dried and stored at 4 °C, during 7 days. They were ground in a mortar with a pestle immediately before use. Gelatin gels of two different KH_2PO_4 quantities were obtained containing either 100 mg (GelP100) or 400 mg (GelP400) of P. The

TABLE 1- Programmatic contents, material and reagents suggested for a simple laboratory project including three experimental modules to be conducted during three lessons (12-15 hours)

Experimental activity	Material and reagents/group
Lesson 1	
<ul style="list-style-type: none"> ✓ Preparation of gelatin matrix formula-tions contain-ing inorganic P; ✓ Spectrophotometric determination of P by va-nadomolybdophosphoric acid method: Calibration Curve. 	<ul style="list-style-type: none"> • 50 mL beaker (2) • 50 mL volumetric flask (9) • 1 L volumetric flask (2) • 10 mL volumetric pipet (2) • 1, 2, 5, 10, 15, 20 and 25 mL volumetric pipets • NH_4VO_3, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$, KH_2PO_4, HCl con., gelatin • Spectrophotometer UV/Vis • Plastic cuvettes
Lesson 2	
<ul style="list-style-type: none"> ✓ Controlled release of inorganic P from gels against distilled water; ✓ Mathematical analysis of results (M_t/M_0 vs. $t^{1/2}$). 	<ul style="list-style-type: none"> • Cheesecloth • 200 mL beaker (2) • Magnetic stirrer • 200 μL micropipet • 1 and 4 mL volumetric pipets • Spectrophotometer UV/Vis • Plastic cuvettes
Lesson 3	
<ul style="list-style-type: none"> ✓ Soil-column/water release test of unen-capsulated P and from gels; ✓ Mathematical analysis of results (% P release vs. irri-gation water). 	<ul style="list-style-type: none"> • 2 glass soil-column (i.d 5.0 cm, height 15.5 cm) • Absorbent cotton • 100 mL collector (2) • 100 g of soil • 0.45 μm GF/C • 200 μL micropipet • 1 and 4 mL volumetric pipets • 50 mL volumetric flask • Spectrophotometer UV/Vis • Plastic cuvettes

water weight lost ratio ΔW (%) was calculated according to the formula

$$\Delta W (\%) = \frac{W_0 - W_7}{W_0} \times 100,$$

where W_0 is the initial gel weight and W_7 is the gel weight after storage time. For both GelP100 and GelP400 the water weight lost obtained was 25-28%

Aim 2. Spectrophotometric determination of P by vanadomolybdophosphoric acid method

Inorganic P was monitored by vanadomolybdophosphoric acid colorimetric

method (Clesceri, Greenberg & Eaton, 1998; Harris, 1998). The absorbance measurements were converted to inorganic P concentrations using a P calibration curve, as showed in Figure 1.

LESSON 2:

Aim 1. Controlled release of inorganic P from gels against distilled water; Determination of inorganic P release from gelatin gels

Ground GelP400 and GelP100 formula-tions were wrapped in a cheesecloth and immersed in fresh distilled water

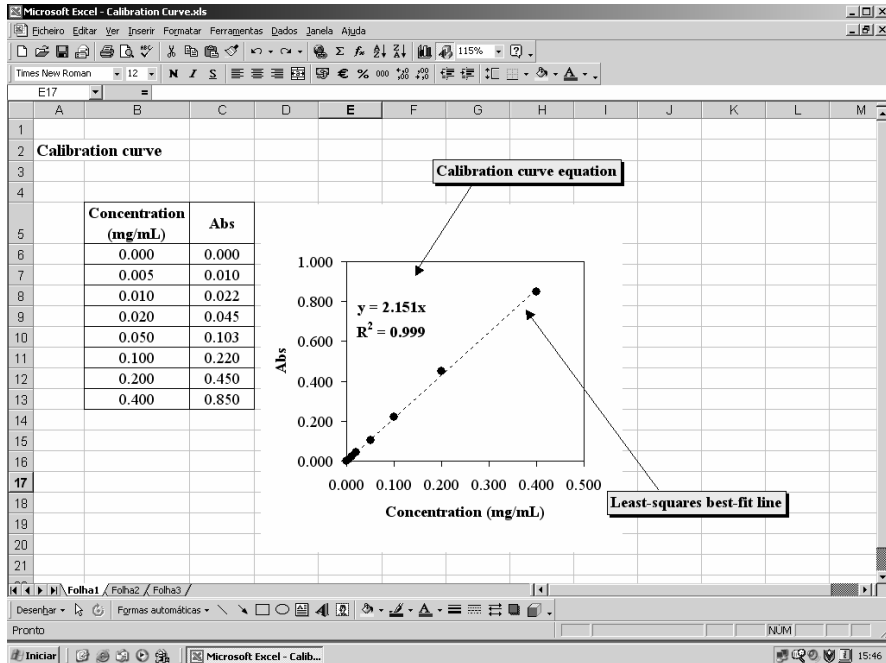


Figure 1 – Typical Excel spreadsheet showed a calibration curve plot.

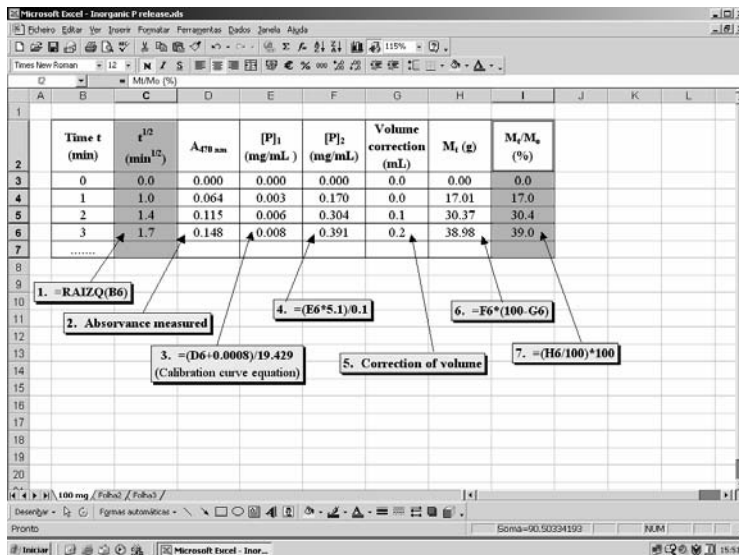


Figure 2 – Typical layout of an Excel spreadsheet for finding the different values of fractional release (M_t/M_0) of inorganic P.

(100 mL) with magnetic stirring for 120 min at room temperature. Released inorganic P was monitored by vanadomolybdophosphoric acid colorimetric method (Clesceri Greenberg & Eaton, 1998; Harris, 1998). 100 μ L fractions of the release solution were collected at regular time intervals and added to 1.00 mL of the vanadate-molybdate reagent and 4.00 mL of distilled water in a test tube (total volume = 5.10 mL). After 10 min, the absorbance of each solution was measured at 470 nm. The absorbance measurements were converted to inorganic P concentrations using the P calibration curve (Figure 1) and the total amount of P released was calculated. For these mathematical calculations, an Excel spreadsheet was proposed with the various steps involved in finding the different values of fractional release (M_t/M_o) of inorganic P from GelP (Figure 2). After students make the Excel spreadsheet, they must represent M_t/M_o vs. $t^{1/2}$ plot (column I vs. column C, Figure 2).

Aim 2. Mathematical analysis of results (M_t/M_o vs. $t^{1/2}$)

In this lesson students have to prepare a controlled release system to obtain a continuous release, at a controlled rate, of inorganic P. Thus, gelatin matrix formulations of inorganic P (GelP formulations, containing two different amounts of P) were tested and release of inorganic P from gels was evaluated against distilled water. A simple analysis of the inorganic P release was provided by Sinclair and Peppas (1984)

$$\frac{M_t}{M_o} = kt^n \quad \text{Equation 1,}$$

Where M_t is the mass of inorganic P re-

leased at time t , M_o is the total mass of encapsulated P, k is the diffusion kinetic constant and n is the diffusion exponent. The value of the exponent is a good indication of the release mechanism. When fractional release, M_t/M_o , is linear with the square root of time, the release-curve profiles match Fick's law. Figure 3 shows the profiles of inorganic P release obtained with both GelP400 and GelP100. As shown in this figure, both experimental release-curves can be considered linear up to 10-15 min ($3.2 < t^{1/2} < 3.9$), corresponding to a fractional release of 70 %. After this time interval, there is a release rate decrease for both gels. Moreover, total release for GelP400 was obtained up to 120 min while for GelP100 this is obtained earlier (up to 40 min). Based on this observation we can assure a mechanism of the release being controlled by diffusion, indicating a Fickian behavior during the first 15 minutes, allowing us to apply the equation 1. After this period of

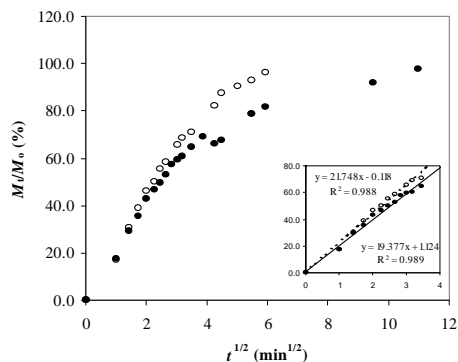


Figure 3 – Fractional release (M_t/M_o) of P vs. $t^{1/2}$ from GelP into 100 mL of distilled water with magnetic stirring at room temperature. (○) - GelP100 (●) - GelP400.

time the P release is controlled by gelatin dissolution. In fact, such tendency to dissolution in aqueous solution is typical of gela-

tin gels prepared without any kind of further crosslinking, though, a relationship between inorganic P quantities and gel mechanical properties can be observed.

LESSON 3:

Aim 1. Soil-column/water release test of unencapsulated P and from gels

The soil-water system studies of P from gels and unencapsulated P were performed in a glass column (inner diameter 5.0 cm, height 15.5 cm) containing soil. Figure 4 shows the scheme used in the release tests. Both ground GelP and unencapsulated P were mixed separately with 50 g of soil. A measured amount of distilled water (50 mL) was then added to the top of column. The water passing through the column was

collected, filtered by a 0.45 μm GF/C membrane filter and analyzed for released P by spectrophotometry. Four irrigations were simulated. All the water passing through the column was collecting before the next irrigation and volume of each irrigation was measured.

After collected the irrigation water the students have to prepare the sample for spectrophotometry analysis. Thus, 100 μL fractions of the irrigation water were collected and added to 1.00 mL of the vanadate-molybdate reagent and 4.00 mL of distilled water in a test tube (total volume = 5.10 mL). After 10 min, the absorbance of each solution was measured at 470 nm.

For calculate the total amount of P released from either unencapsulated or encapsulated P an Excel spreadsheet was proposed in Figure 5.

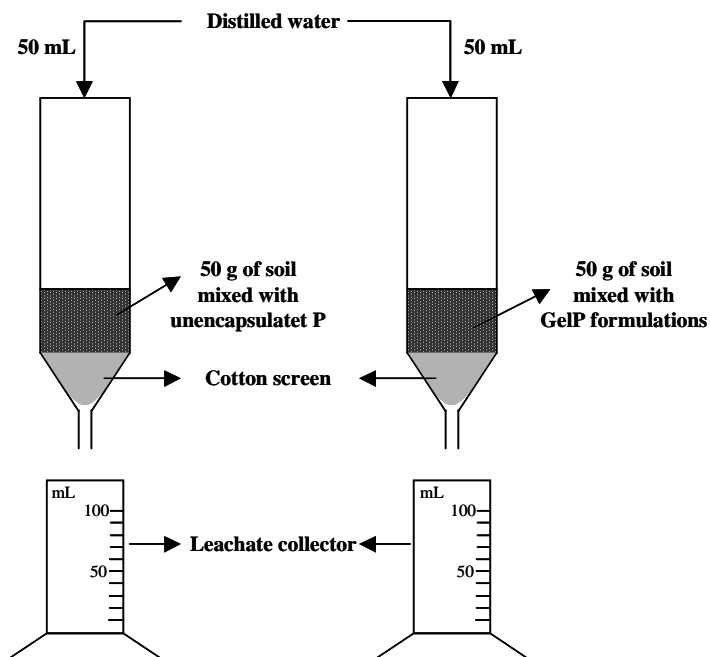


Figure 4 – Scheme of the soil-column/water release tests (not to scale).

Irrigation water	A _{470 nm}	[P] ₁ (mg/mL)	[P] ₂ (mg/mL)	Volume collected (mL)	M ₁ (g)	M ₁ /M ₀ (%)
1	0.143	0.007	0.377	19.00	7.17	7.2
2	0.252	0.013	0.664	46.00	30.52	30.5
3	0.242	0.012	0.637	49.00	31.23	31.2
4	0.175	0.009	0.460	49.00	22.55	22.5

Figure 5 – Typical layout of an Excel spreadsheet for finding percentage of P released from either GelP formulations or unencapsulated P (M_1/M_0) in the soil-column/water release tests.

After making the Excel spreadsheet, students must represent the percentage of P released from unencapsulated P and GelP formulations (M_1/M_0) vs. irrigation water plot (column H vs. column B, Figure 5).

Aim 2. Mathematical analysis of results (% P release vs. irrigation water)

For both 100 mg and 400 mg quantities of unencapsulated P similar profiles were obtained and total leaching P loss is under 100 %, which is consistent with P fixation capacity of soil (Campbell *et al.*, 2001; Xia *et al.*, 2003). Results related to 400 mg of unencapsulated P are shown in Figure 6 (curve —●—).

Comparing the levels of leached unencapsulated P, among all the irrigation waters, we observe that they are significant only over the first two irrigation waters.

To maintain P levels in soil, for a longer period of time, a new P application is necessary following the second irrigation water. Thus, an application of P more often and in smaller doses would be more convenient.

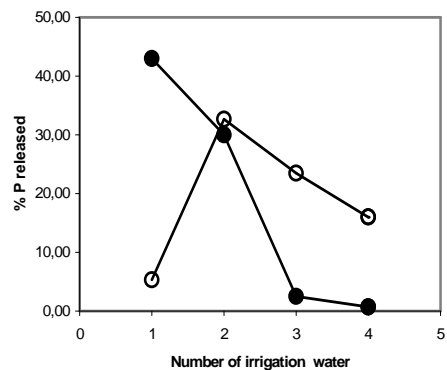


Figure 6 –Percentage of P released over four irrigation waters: (○) GelP400 formulation and (●) 400 mg of unencapsulated P.

As alternative, we have used a controlled release formulation, which would not release the active ingredient all at once. GelP400 and GelP100 formulations, prepared as described in the previous section, were used as controlled release systems and the same soil-water system was used to indicate the leachability of these formulations for comparison with unencapsulated P. Since, similar profiles were obtained, only results related to GelP 400 mg were shown in Figure 2 (curve —o—). For the first irrigation the quantity of P leached is quite less for GelP formulations when compared with corresponding quantities of unencapsulated P. According to the results obtained in kinetic studies and since, similar free water in the soil column had not drained out, difference on P leaching between unencapsulated P and GelP can be assigned to a controlled release ruled by diffusion mechanism. Over the next three irrigation waters controlled P release is ruled by the gelatin dissolution, showing quantities of leached P always above 10 %. Production of a more long-term delivery system (over a period of 1 to 3 moths) would require gelatin stabilization by using crosslinking procedures delaying in time the gelatin dissolution process. Chemical crosslinking of gelatin is accomplished traditionally with dilute solutions of glutaraldehyde or formaldehyde, but these crosslinking agents have been shown to be toxic. Therefore other crosslinking methods have been studied using natural molecules, such as glyceraldehyde, genipin, dextran and alginate (Kosmala, Henthorn & Peppas, 2000; Yao, Liu, Chang, Hsu & Chen, 2004). Research work has been done focused on establishing suitable, non-toxic crosslinking methods to produce stable gels.

CONCLUSIONS

Gelatin gel containing inorganic P provides controlled release of an active ingredient. Release-curves against distilled water show a linear relationship between release and square root of time, according to laws of diffusion. The relatively low cost, the ease of preparation and the use of biodegradable gels are ideal to be employed as controlled release systems by students who have their first contact with this kind of devices. The availability of inorganic P to the leaching medium was more uniform, lasting over a larger number of irrigation waters, than when applied by conventional methods. Students must realize that applications in fewer and larger doses are inefficient since they provide concentrations beyond crop needs. These high concentrations are prone to escape from soil into water sources, causing serious health risks and environmental pollution. On the other hand, application to soil of an active ingredient immobilized in a polymeric matrix, result in its continuous release, at a controlled rate, which is really advantageous because it improves nutrient uptake and absorption and reduce pollution effects.

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