Optimization of Test Placer Composition for Express Colorimetric Determination of Mobile Forms of Phosphorus in Soil and Water Samples

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Abstract. The paper presents the optimized methods of manufacturing test placers for quantitative and visual-bluer colorimetric determination of phosphate ion concentration in a blister cell and a test tube. The test placer contains all the reagents for the analytical reaction. Placers allow the quantitative determination of phosphates in various objects without preliminary preparation of reagent solutions. In a blister cell with a diameter of 6 mm, a much more intense blue color was achieved with a solution layer of no more than 2 mm. The application of reagents to the surface of the silica gel allows them to avoid contact until the chemical analytical reaction takes place. The composition of placers and the conditions for applying reagents are improved. Hydrosulfate ions are able to provide a sufficiently high acidity when adding a few drops of the solution. The reaction proceeds in the volume of the added test solution, which is 2–3 drops.

1. Introduction

Being one of the main elements of nutrition for living organisms and plants, phosphorus is absorbed in the form of salts of orthophosphoric acid, as well as salts of polyphosphoric acids [1, 2, 3, 4, 5]. Thus, in plants, along with organic phosphorus, there are inorganic phosphorus compounds to ensure the growth and vital activity of cells and for the phosphorus reserve. It is in such forms that this element is widely distributed in the water of reservoirs, soils, rocks, in living cells, food products, as well as in many artificially obtained materials.

Table 1 shows the classification of soils by phosphorus content.

The need to apply phosphorus fertilizers with the content of assimilable phosphorus in the amounts of the last three classes is excluded. Surface runoff from the earth primarily causes phosphorus losses. The search for optimal methods for assessing the phosphate regime in soils, as well as other agrochemical indicators, continues to be an urgent task [1-3, 6, 11-16]. In the analyzed solution, phosphorus can be present in the form of three main forms (1, 2, 6). Such forms are: orthophosphate ions, organic phosphorus and condensed phosphorus. Total phosphorus is the sum of these forms. Increased requirements are imposed on such parameters as the detection limit, reproducibility and expressiveness of analysis methods, which stimulate the predominant development of instrumental and automated methods in the analytical chemistry of phosphate ions, as well as in the chemistry of other ions [2, 7, 8, 9]. A lot of attention is paid to test methods [10].
Physical and chemical (covalent fixation or immobilization) these are the two main ways of applying the components of the analytical reaction to the surface of the carrier (17, 18, 19, 20). Such methods are not fixed by GOST standards. When developing chemical test methods, various methods of fixing reagents are offered, and methods of their manufacture are also diverse. Cellulose, polyurethane, inorganic adsorbents and other materials are used as carriers. Thus, test methods can be classified mainly by manufacturing methods.

Various express methods and test systems are given in the literature [1-3, 6, 11-13, 21-24]. In the proposed method, it is possible to achieve higher optical densities of molybdenum-phosphorus blue. At the same time, the volume required for conducting an analytical reaction is also significantly reduced. In standard methods, visual testing at the maximum content of phosphate ions is achieved with an optical layer thickness of about 20 mm [2, 6, 11, 21, 22]. The minimum diameter of the cell is 4–10 mm, and the blue color developed with a layer of solution up to 2 mm [25]. In addition, the upper layer was covered with a half lower layer.

2. Research methods
The blue color in the proposed method develops faster and more intensively and is achieved due to very high concentrations of substances, in particular due to the maximum concentrations of heptamolybdate ions. Heptamolybdate anions \([\text{Mo}_7\text{O}_{24}\text{O}_{6}^6-]\), have a very high negative charge at the initial moment of time. This is associated with a very large ionic strength of the solution and the activity of all ions. The correct quantitative assessment of this effect is very difficult due to the lack of an appropriate theory for concentrated solutions of strong electrolytes [2]. To achieve the required pH level, crystalline substances were selected that provide the necessary acidity (the choice was made empirically).

The active concentrations of substances forming cyan were significantly increased. A search was made for a crystalline and non-hygrosopic substance capable of providing acceptable acidity in the volume of several drops of the solution and a suitable reducing agent. The volume of the added (analyzed) solution is 0.2–0.3 ml [2].

The properties of the proposed placer do not change at room temperature if it is hermetically sealed. It is stored for 3 months. The test placer is suitable for testing aqueous solutions, soil extracts and natural waters, as well as physiological fluids. The range of detectable concentrations is from 0.5 to 100 mg / l in terms of phosphate ions. The results were obtained in a blister cell with an exposure of 3–5–10–20–30 minutes. The course of color changes (shades of blue) is monotonous and almost the same for the concentrations of phosphate ions.

Silica gel alumogel, sorbents of plant origin were studied during the test placers. The paper presents data on silica gel and alumogel measurements. It was important that all the applied chemical reagents (reagents) were on the one hand reliably isolated from each other, on the other hand, at the time of adding the test solution, they quickly and in stoichiometric ratios passed into the solution. Several types of silica gels and other sorbents (carriers) have been tested. Good results were shown by cellulose,

### Table 1. Classification of soils by phosphorus content, mg / 100g of air-dry soil.

| Class | The amount of \(\text{P}_2\text{O}_5\) mg/100g of soil | Content of mobile phosphorus |
|-------|---------------------------------|-----------------------------|
| I     | <1                              | Very low                    |
| II    | 1.0–1.5                         | Low                         |
| III   | 1.5–3.0                         | Medium                      |
| IV    | 3.0–4.5                         | Increased                   |
| V     | 4.5–6.0                         | High                        |
| VI    | >6.0                            | Very high                   |
minced and bleached shover. Studies on silica gels in terms of their modification for the use in various fields are presented [6, 11, 12, 13]. In [21], silica gel was used in tablets of various compositions for the semi-quantitative determination of \( \text{NO}_3^- \) and \( \text{NO}_2^- \). For the application of reagents, the correct choice of temperature, and the time of vacuuming, the time of sorption were considered as important factors. Initially, placers containing paired reagents were investigated. Thus, placers optimized by the composition of reagents and carriers were studied (table. 2) [2, 3].

**Table 2.** The composition of test placers with various sorbents and reagents.

| Components | Designation of the test placer | B-a\(^a\) | B-a | B-\(b\) | B-b |
|------------|--------------------------------|-----------|-----|---------|-----|
| (NH\(_4\))\(_6\)Mo\(_7\)O\(_{24}\)\(\cdot\)4H\(_2\)O | B-a\(^a\) | 1.4-3.4 | 1.0-2.2 | 1.4-4.4 | 4.0-8.0 |
| K\((\text{SbO})\text{C}_4\text{H}_6\text{O}_4\)\(\cdot\)0.5H\(_2\)O | B-a | 0.08-0.2 | 0.08-0.6 | 0.2-0.8 | 0.2-0.6 |
| NH\(_3\)SO\(_2\)OH | B-b | 10-12.0 | 0 | 0 | 6.05-12.0 |
| C\(_6\)H\(_6\)O\(_6\) | B-\(b\) | 0.8-1.25 | 1.2-1.6 | 0.8-16 | 0 |
| C\(_6\)H\(_8\)O\(_6\) | B-a | 0.8-2.0 | 0.8-2.0 | 1.0-4.0 | 0.5-2.0 |
| KH\(_2\)SO\(_4\) | B-a | 0.0 | 8-20.0 | 11.0-16.5 | 8.0-14.2 |

| Sorbents | Silica Gel 40 fr. 63-200 mkm | 60.0 | 65.0 | 0.0 | 52.4 |
|----------|--------------------------------|------|------|-----|------|
| Alumogel (alpha form of aluminum oxide) | 0.0 | 0.0 | 48.2 | 0.0 |

| Deliquescence | low | no | yes | no |
|---------------|-----|----|-----|----|
| Acidity       | 1.2-2.4 | 1.2-2.4 | 1.6-2.2 | 1.2-2.0 |
| Coloring with 3 drops of RO43-, 80mg/l solution | Grey and blue | Grey and blue | Black and blue | Blue |
| Coloring with 3 drops of the test solution | Pale blue | Light blue | Grey and light blue | Light blue |

\(^a\)greyish tint
\(^b\)suspension

The amount of the carrier (sorbent) was selected for each type individually. The colorimetric and spectrophotometric characteristics of the test system were optimized using mass measurements. The composition of the reagents for the photometric reaction is given in table 2. The necessary acidity pH 1.4–2.8 was provided by the introduction of potassium hydrosulfate [2].

We proposed a variant for determining phosphate ions in a solution above the surface of the test system - by means of a placer containing a mixture of reagents [2, 3]. The method is express, that is, the time for the development of color is not more than two minutes. At the same time, both qualitative and semi-quantitative and quantitative determination of phosphates is possible. Due to the absence of differences in the granulometric characteristics and physical properties of various components of the placer with the applied reagents, the homogenization of placers is simplified. The processes of mixing dried components into a single placer are optimized. All reagents were individually applied to silica gel from aqueous solutions, with concentrations that were 85–90% of the saturated ones. The retention time
of solutions in contact with silica gel was optimized. Then the placers based on silica gel and alumogel were dried at a temperature of 40 °C.

To ensure the best analytical qualities of the test system, the amount of the carrier and the content of components were optimized experimentally. The compositions of placers with active substances are given in table 3.

| Table 3. The main variants of placer compositions based on homogenized mixtures. |
|------------------------------------------|---------|---------|---------|---------|
| Components/ Properties                  | B-a1    | B-a2    | B-a6    | B-a6/2  |
| Reagents                                 |         |         |         |         |
| (NH₄)₆Mo₇O₂₄·4H₂O                          | 4.5     | 4.0     | 4.0     | 5.5     |
| K(SbO)C₆H₄O₆·0.5H₂O                        | 0.46    | 0.55    | 0.50    | 0.6     |
| KHSO₄                                      | 14.5    | 12.5    | 18.6    | 18.6    |
| C₆H₄O₆                                    | 1.20    | 1.55    | 0.8     | 0.0     |
| (C₆H₄O₆)                                  | 1.20    | 0.85    | 1.0     | 1.25    |
| KHSO₄                                      | 14.2    | 12.2    | 24.0    | 24.2    |
| Sorbents                                  |         |         |         |         |
| ChemopolL100/400 silica gel               | 64.3    | 66.5    | 58.2    | 52.4    |
| Exposure, day                            | 21      | 21      | 21      | 60      |
| Deliquescence                           | No      | No      | No      | Yes     |
| pH                                       | 1.4-1.6 | 1.4-1.6 | 1.4-1.8 | 1.8-2.1 |
| Observations                             |         |         |         |         |
| 3 drops of the PO43-test solution, 80 mg/l | Grey and blue | Dark grey and blue | Dark blue | Black and blue |
| 3 drops of the PO43-test solution, 0 mg/l  | Light blue | Very pale blue | Light blue | Pale blue |

The priority characteristic of the developed test placer is the constancy of chemical and physical properties, homogeneity and the absence of hygroscopicity. The process of achieving uniformity of micro-volumes in the reagent composition is a mixture of individual placers. Homogenization was carried out after drying. The homogenization was controlled using a microscope. Humidity was controlled using an OHAUS MB25 moisture analyzer.

The test system in the form of a placer should not noticeably change its mass and should not show hygroscopicity under normal conditions. To study the hygroscopicity of combined placers, eight identical samples were produced. The samples were kept under various conditions – in evacuated containers at room temperature, in an environment with an increased moisture content, and also hermetically closed at a temperature of 6–80 °C.

Changes in the physical properties of placers were studied every four days for six weeks. The best results were shown in placers stored in the refrigerator, hermetically sealed. Placers stored at high humidity acquired a grey-blue color and lost their flowability. The shelf life was studied for six months. Both the analytical characteristics of the test placer and their physical properties were tested. Hermetically sealed portions of the placer were opened, external changes were described and a test determination of phosphate ions was performed using a test scale, a digital scale (using a digital color analyzer), the spectral characteristics of placers at various concentrations were studied using a spectrophotometer (figure 1).
Figure 1. The optical density of solutions depending on the concentration of phosphate ions using a placer based on homogenized mixtures.

When using placers stored at room temperature, deviations were observed 4 months after the date of manufacture. Placers stored in the refrigerator reproduced the same results after the expiration of the 6-month experiment. The absence of light was the main condition for storing the test placer. Placers should be stored in dark packages.

“The visual-coloristic reaction should be carried out in conditions when 3 drops of the analyzed solution are added. In this case, the reaction is carried out in a blister cell for the necessary time required for the development of the color of the solution. During the experiments, the results were obtained with different exposures. It can be seen that they significantly depend on the time exposure. The course of changes is monotonous and approximately the same for all concentrations. After constructing and correlating the color scale and standardizing the production of the test placer, we conducted a series of tests of the test method using mass measurements with the involvement of “naive observers” [2, 26].

3. Conclusion
The developed express test method for determining phosphate ions allows to semi-quantitatively (using a scale or a smartphone equipped with a special application) assess the content of phosphate ions in soil extracts and natural reservoirs, food products (uncoloured), physiological fluids. A test placer and a coloristic scale are proposed as test tools. It is also proposed to use a test placer for the quantitative determination of phosphate ions in water samples. For this purpose, it is used as the only reagent introduced into the measuring flask or into the cuvette of the spectrophotometer. The properties of the placer do not change at room temperature if it is hermetically sealed. It is to be stored for 3 months.

The paper offers optimized variants of placers, which have been subjected to a thorough statistical analysis [26]. It was found that it is more reliable to add each component separately to the carrier, both for preparation, and during storage and use of the test system. This makes it possible to exclude the possibility of unwanted contact of reagents and thereby exclude the occurrence of undesirable (side) reactions. Only test placers composed of individual components were studied [2, 3]. In the course of research, a unique method of storing micro-quantities of reagents on the surface of various sorbents, both mineral and vegetable, has also been developed. This opens up wide opportunities for the
unification of the methodology for the manufacture of other test systems, both for chemical and for other types of analysis and testing.

Acknowledgement

The research was carried out with the support and using scientific equipment of the educational and methodological center for collective use “Service Laboratory for Complex Analysis of Chemical Compounds” of the Russian State Agrarian University-Moscow State Agricultural Academy named after K. A. Timiryazev.

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