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The methodology for determination of 1-methylcyclopropene in gas-air mixtures after release from supramolecular complexes

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1-Methylcyclopropene is used as an agrochemical in the form of supramolecular complexes with various receptors to extend the shelf life of fruits and vegetables. In the present work we studied and compared three different preparations: supramolecular complexes of 1-methylcyclopropene with cucurbit[6]uril and α-cyclodextrin, as well as a newly developed multicomponent preparation based on supramolecular complex of 1-methylcyclopropene with α-cyclodextrin. To control the content of 1-methylcyclopropene in vegetable storage air, a gas chromatography method was adapted from the literature for determination of 1-methylcyclopropene in gas-air mixtures. A comprehensive procedure has been developed that includes sampling of gas-air mixtures, their transportation, and gas chromatographic analysis, ensuring reliable quantitative determination of 1-methylcyclopropene in the air of vegetable storages. Laboratory tests and experiments under real conditions of use were carried out, and the concentrations of 1-methylcyclopropene at the maximum level were shown to be quite close for these three preparations, which indicates the comparable efficiency thereof.

Keywords: 1-methylcyclopropene, α-cyclodextrin, cucurbit[6]uril, supramolecular complex, gas chromatography.

Методологія кількісного визначення 1-метилциклопропену в газо-повітряних сумішах після вивільнення із супрамолекулярних комплексів. *Д.Ю.Мясніков а, Н.О.Пінчукова, Г.С.Власенко, І.О.Зінченко, О.І.Збруєв, Є.В.Євтушенко, Т.М.Гуріна, О.С.Прокопчук, В.А.Чебанов*

1-Метилциклопропен використовується в агропрепаратах у формі супрамолекулярних комплексів з різними рецепторами для подовження терміну зберігання фруктів та овочів. У даній роботі було досліджено та порівняно три різні препарати: супрамолекулярні комплекси 1-метилциклопропену з кукурбіт[6]урилом і α-циклодекстрином, а також експериментальний багатокомпонентний препарат на основі супрамолекулярного комплексу 1-метилциклопропену з α-циклодекстрином. Для контролю вмісту 1-метил-

циклопропену в повітрі овочесховищ метод газової хроматографії було адаптовано з літератури для кількісного визначення 1-метилциклопропену в газоповітряних сумішах. Розроблена комплексна процедура, що включає пробовідбір газо-повітряних сумішей, їх транспортування та аналіз методом газової хроматографії, що забезпечує надійне кількісне визначення 1-метилциклопропену в повітрі овочесховищ. Для трьох різних препаратів були проведені лабораторні випробування та експерименти в реальних умовах використання, в результаті яких було встановлено, що концентрації 1-метилциклопропену в повітрі, при досягненні максимального значення є близькими, що свідчить про порівнянну ефективність трьох препаратів.

1. Introduction

Fruits and vegetables provide energy, vitamins, minerals, fiber, and other phytochemicals, contributing to a high-quality and balanced diet. Fruits are stored after harvest to maintain their freshness quality and extend their shelf life to reduce the loss of commercial value associated with high metabolic rates and disease susceptibility. Both qualitative and quantitative losses occur between harvest and consumption. On average, one-third of edible products worldwide are lost after harvest [1]. For some fruits, especially apples, the main reason is rapid ripening and fruit wilting under the influence of the plant hormone ethylene [2]. Inhibiting the action of ethylene is the most effective way to slow down these processes [1]. In this direction, the discovery and development of methods for the synthesis of 1-methylcyclopropene (1-MCP) (Fig. 1), inhibitor of the action of ethylene, became very important [3]. 1-MCP was patented in 1996 and then rapidly registered and commercialized due to its non-toxic mode of action and effectiveness at low concentrations. When fruit and vegetable products are treated with gaseous 1-MCP, it binds strongly to the receptors of the plant hormone ethylene and blocks its action, leading first to ripening and then to spoilage of the products. Under the influence of 1-MCP, these processes slow down dramatically. This compound is currently used to extend the shelf life of many fruits, vegetables, and flowers [4–7].

1-MCP is a colorless gas that is extremely unstable during storage due to its high reactivity and tendency to isomerize and polymerize. Since the compound is unstable, special efforts are required for its storage and administration. 1-MCP is stored in commercial agrochemicals in the form of supramolecular complexes in which it is entrapped in the cavities of supramolecular receptors. The world's leading postharvest treatment preparation based on 1-MCP is "Smartfresh", manufactured by AgroFresh (USA) [8]. The product is produced in the form of a supramolecular complex of 1-MCP with α-cyclodextrin (α-CD) (Fig. 1). In Ukraine, another postharvest treatment preparation based on 1-MCP was developed and produced, registered under the trademark "Oberig^{pro}", where the supramolecular receptor is cucurbit[6]uril (CB[6]) (Fig. 1) [9]. Considering the high demand of the agro-industrial market of Ukraine for preparations for prolongation of shelf lives of fruits, we have developed a new multicomponent preparation based on the complex of 1-MCP with α-cyclodextrin, which differs from the known preparations in convenience of application, namely, greater solubility in pure water at room temperature and without the need for stirring. The newly developed experimental multicomponent preparation was also studied.

The aim of the work is to compare the efficiency of three preparations for postharvest processing of apples based on supramolecular complexes of 1-MCP in terms of percentage of the released 1-MCP after dissolution, the release rate, and the maximal gas concentration in the air of storage. To achieve this goal, the following tasks were solved: the method of analysis by gas chromatography (GC) of gas-air mixtures of 1-MCP was improved in order to control its content in the air of vegetable storages during use, i.e., the method of air sampling was adopted and chromatography conditions were clarified; both laboratory experiments and field trials under conditions close to the real use conditions were performed to compare three preparations based on supramolecular 1-MCP complexes; the release rate of 1-MCP from the complexes was also studied.

2. Experimental

The complex 1-MCP with CB [6] was synthesized according to the literature method [9].

The synthesis of the complex 1-MCP with α-CD was carried out according to the following procedure: a 10 l round-bottomed flask, on a magnetic stirrer, was loaded with a solution of 1000 g of α-CD in 8 liters of water, air was pumped out of the

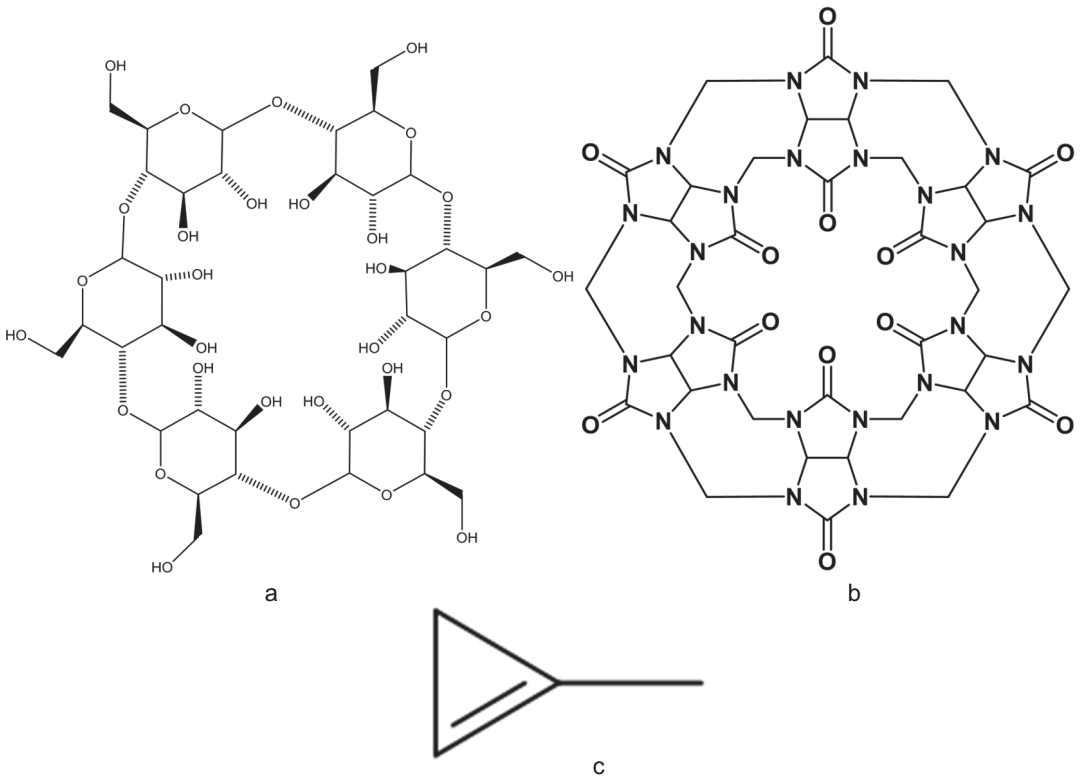

Fig. 1. Structural formulas: a: α-cyclodextrin; b: cucurbit[6]uril; c: 1-MCP.

flask, and 40 g of gaseous 1-MCP obtained by evaporation of liquid 1-MCP in a 100 ml round-bottomed flask connected to the reaction flask with a tube was introduced into the reaction mixture within 20 minutes at 20°C under intensive stirring. The precipitate formed was filtered, washed with 500 ml of water, and dried at 30°C first for 12 hours in the air, then under vacuum. The yield was 842 g (80 %).

The newly developed experimental multicomponent preparation was prepared using the complex of 1-MCP with α-CD, sodium dodecyl sulfate, citric acid, and sodium hydrogencarbonate, taken in appropriate proportions.

Analysis of the complex of 1-MCP and CB[6] using high performance liquid chromatography (HPLC) was carried out according to the literature method [10]. For the complex of 1-MCP with α-CD, pure water was used as a solvent instead of 20 % sodium acetate in the derivatization reaction, due to the different solubility of the complex.

Determination of 1-MCP content in gas-air mixtures by the gas chromatography (GC) method was adapted from the literature [11]. The following analysis conditions were applied: Zebron ZB-624plus (60 m·250 μm·1.4 μm) chromatographic column; flow rate was 1 ml/min; nitrogen was used as carrier gas; thermostat temperature at 60°C; flame ionization detector; analysis time was 10 min. To build the calibration graph, gas-air mixtures were prepared within the expected 1-MCP content range by injecting the calculated amount of pure gaseous 1-MCP into a hermetically sealed 10 l flask with a gas-tight syringe.

Laboratory testing of various preparations based on 1-MCP complexes.

The amount of the complex, calculated to produce the appropriate concentration (100, 20, and 10 ppm), and a magnetic stirring bar were placed in a 10 ml vial, which was then immersed into a 10 l flask (or a 60 l chemical reactor), 1 ml of the appropriate solution facilitating the release of 1-MCP from the complex (a mixture of 20 % sodium acetate and ethanol in a ratio of 10:1 for the complex of 1-MCP with CB [6], a mixture of water and ethanol in a ratio of 10:1 for the complex of 1-MCP with α-CD, and pure water was used for the new preparation) was quickly added into the vial by pipette, the flask (reactor) was hermetically sealed with a stopper, and was placed on a magnetic stirrer (in reactor experiments magnetic stirrer was attached to the bottom of the reactor from outside). A hand-made fan was previously placed in the flask at the mid-height of the vessel for even distribu-

tion of 1-MCP in the volume of the flask. After 15 min the gas-air mixture was sampled with a syringe and analyzed by GC. The mass fraction of 1-MCP released from the complex was calculated using the formula 1.

$$w = \frac{m(1-MCP)}{m(compl)} \cdot 100\% = \\ = \frac{Ccal \cdot 10^{-6} \cdot V_f}{m(compl) \cdot 452} \cdot 100\% \quad (1)$$

where: m(1-MCP) is the mass of released 1-MCP, g; $m(compl)$ is the weight of the complex, g; $Ccal$ is the concentration of 1-MCP, determined according to the calibration graph, ppm; 10^{-6} is the recalculation of concentration from ppm to ml; V_f is the flask volume, l; 452 is the volume occupied by 1 g of 1-MCP, ml/g at 101 kPa.

Testing of 1-MCP preparations in the storage.

Based on the results of HPLC measurements the amount of the complex was calculated to produce the concentration of 1-MCP in the room air at the level of 6.7 ppm. The following solutions were used as solvents: a mixture of 20 % sodium acetate and ethanol in a ratio of 10:1 for the complex of 1-MCP with CB [6], a mixture of water and ethanol in a ratio of 10:1 for the complex of 1-MCP with α-CD, and pure water for the new preparation. The preparations were dissolved by stirring on a magnetic stirrer. The new composition was not stirred during the experiments, since it was one of the main requirements to the preparation. Air sampling for GC analysis was carried out as follows: two needles from a syringe were inserted through a septum into a hermetically closed 20 ml vial at different depths, one of the needles was connected to a tube leading out of the storage, the other one to a syringe with a capacity of 100 ml. Using a syringe, the air was pumped out of the vial, which was replaced by the air from the storage through another needle. The vial was thus blown with 300 ml of storage air, after which the needles were removed, leaving the air sample inside. Air samples from the storage were taken at certain time intervals, namely the first sample after 30 minutes after the start of dissolution of the complex, the second after 60 minutes, and the third after 24 hours.

3. Results and discussion

The efficiency of three different preparations based on supramolecular complexes of 1-MCP was estimated using three criteria:

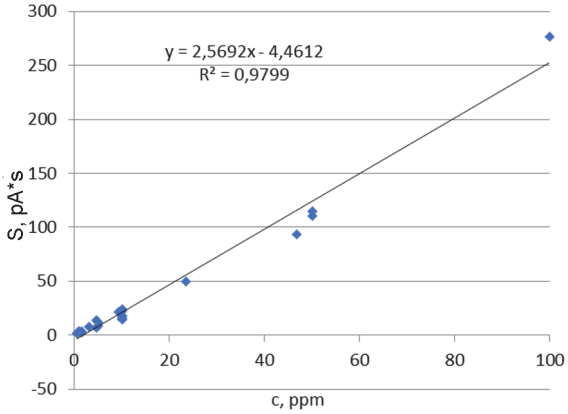

Fig. 2. Calibration graph for determining the concentration of 1-MCP in gas-air mixtures.

the first one was the amount (percentage) of gas released from the complex under experimental conditions, the maximum concentration achieved, and the third criterion was the gas release rate, or the time required to achieve the maximum concentration in the air. For the accurate dosage of the preparations, the mass fraction of 1-MCP in the studied complexes was determined by the HPLC method [10] with prior derivatization of 1-MCP, as described in the experimental section. Thus, the content of 1-MCP was 2.8 wt% in the complex with α-CD and 3.1 wt% in a complex with CB [6]. In general, the procedure for treatment of fruits in the storages involves dissolution of the preparation taken in the necessary amount to create a concentration at the level of 1 ppm in the hermetically sealed room of the store, usually under stirring, and further exposure of the fruits within 24 hours. However, the exact concentration of 1-MCP in the air is not controlled usually because of the lack of specified measuring devices for express field analysis of the air, and its concentration is generally calculated based on the supposition that the gas is completely released from the preparation solution. However, our previous experience shows that some part of the gas may be retained by the solution and the actual concentration of 1-MCP may be lower than supposed. Thus, to ensure the efficiency of the preparation used for postharvest treatment of fruits, it is important to directly control 1-MCP content in the air of the storage after release.

In our experiments for 1-MCP determination in the air, the GC method was adapted using a flame ionization detector; the conditions are given in the experimental section.

Table 1. Results of determining the mass fraction of 1-MCP released from the complex with cucurbit[6]uril (3.6 wt% MCP content) by GC method

Flask (reactor) volume, l	Concentration of 1-MCP, (calculated), ppm	Concentration of 1-MCP, (found), ppm	The mass fraction of released 1-MCP, wt%
10	100	69.2	2.1
10	100	60.2	1.8
60	10	7.1	2.1

A calibration graph was prepared to quantify 1-MCP in the gas-air mixtures (Fig. 2).

To test the methodology, several laboratory tests and experiments under real conditions of use of the preparations were performed to determine the mass fraction of 1-MCP released from the complex with CB [6]. A laboratory reactor with a full volume of 60 l was used (to simulate real condition) as a closed volume in which the samples were dissolved and 1-MCP was released. Since at the initial stage of the experiments it was important to elaborate the GC methodology and to study the reproducibility of the results, the concentrations were higher than 1 ppm. In the first experiments, the concentration of 1-MCP was set at 100 ppm, and then it was reduced to 10 ppm which is closer to the real conditions for the use of the preparations. The results obtained are shown in Table 1. It can be seen from the Table that the concentrations of 1-MCP calculated from the calibration graph are lower than expected, which can be explained by the incomplete release of 1-MCP from the complex, as was said above. However, the GC results of the parallel studies are reproducible. The representative chromatogram of the gas mixture sample in the reactor obtained during the release of 1-MCP from the complex suspension is shown in Fig. 3.

Similarly, reactor tests were performed for a new multicomponent preparation based on the complex of 1-MCP with α-CD. The weight of the complex was calculated so that the concentration of 1-MCP in the gas-air mixture was 20 ppm. The experiments were performed both at room temperature and at 5°C, the average temperature in the storage. To simplify the calculations, the difference in the specific volume of 1-MCP with temperature and pressure variations was neglected in the experiments. At room temperature, the concentration of 1-MCP was 28.6 ppm, while at 5°C it was 15.8 ppm. As we can see, the concentration of 1-MCP at 5°C is much lower than at room temperature, which can be explained by incomplete dissolution of the complex

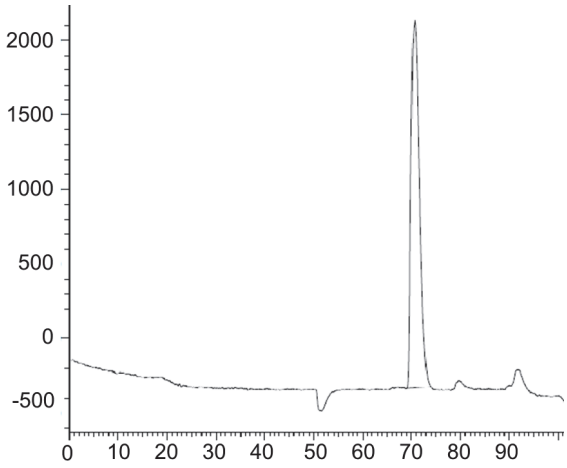

Fig. 3. Chromatogram of a sample of the gas-air mixture, where the peak at 7.2 min. corresponds to 1-MCP.

due to poorer solubility at lower temperatures, resulting in incomplete release of 1-MCP.

In further experiments, three samples of the studied preparations were tested in the empty storage. The tests were carried out with the aim of elaborating the procedure for dissolving the preparations, sampling, transporting and analyzing the samples. Moreover, the change of 1-MCP concentration depending on time was also monitored to determine the optimal treatment time for different preparations. The results obtained are presented in Table 2. As we can see from the Table, the amount of 1-MCP released in all tests is slightly lower than expected (6.7 ppm), which could be due to incomplete dissolution of the complexes or incomplete release of 1-MCP from the resulting solutions. However, it is important to note that the results obtained for the different preparations are quite close when used under the same conditions.

The next day, the gas concentration either did not change or even decreased, which could be due to a loss of the gas for undetermined reasons. The results obtained show that all three preparations can be con-

Table 2. Results of GC analysis of preparations based on 1-MCP in the air of the storage

	Concentration of 1-MCP for the complex with CB[6], ppm		Concentration of 1-MCP for the complex with α-CD, ppm		Concentration of 1-MCP for the multicomponent preparation, ppm	
t, min	*T* = 11°C	*T* = 16°C	*T* = 11°C	*T* = 14°C	*T* = 11°C	*T* = 18°C
30	8.1	8.4	5.9	6.3	4.3	5.6
60	7.5	8.9	7.7	5.9	4.1	5.8
Next day	5.7	6.1	5.1	4.7	4.9	5.7

sidered equal in terms of their efficiency in postharvest fruit treatment. However, the new multicomponent preparation can be recommended as advantageous due to its ease of use, as it dissolves well in water and does not require stirring equipment, while showing comparable results to the known commercial counterparts.

From Table 2, it can be seen that temperature has an effect on the amount of 1-MCP released. At lower temperatures, we found a tendency for the concentrations to decrease for all preparations.

4. Conclusions

The methodology for determining the quantitative content of 1-MCP in gas-air mixtures after the decomposition of complexes and the release of 1-MCP was adapted and the methods of air sampling and chromatography conditions were chosen. Three preparations based on supramolecular complexes of 1-MCP were tested in the laboratory with different scaling and in a storage. It was found that all three preparations are characterized by comparable efficiency and the choice of the 1-MCP preparation for postharvest treatment of apples can be done by the customers based on their preferences and commercial availability of the products. On the basis of the conducted experiments, it was established that the GC method makes it possible to reliably determine the concentration of 1-MCP, at the level expected in the air of storages during real use of the preparations.

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