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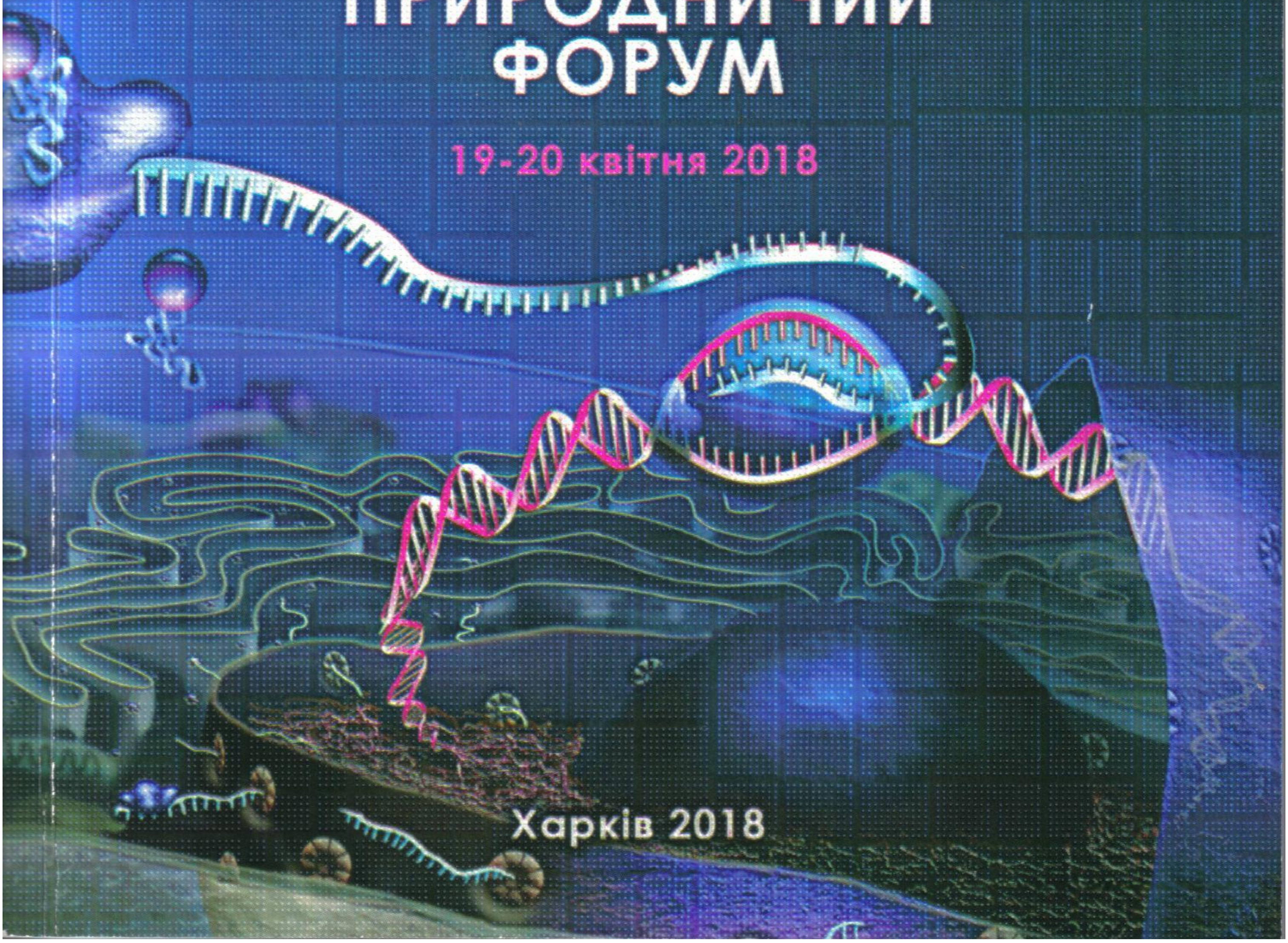

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IMPROVED METHOD OF LIPO-VITAMINS IDENTIFICATION IN BIOLOGICAL FLUIDS

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One way of intensifying educational process at school could be the application of computer-dependent devices and gadgets. It would allow inducing inquisitive activity of school students, considering the students' individual interests and abilities, providing the acting approach to educational process organization, simplifying the processing of obtained information. Computer measurement equipment for school biology and chemistry lessons is produced by Ukrainian industry in insufficient quantities, with poor qualities and a narrow spectrum of selection. Therefore, elaboration of school computer soft- and hardware for laboratory demonstrations, and its innovation in biology and chemistry practical lessons remains to be one of the priority tasks of our educational process (Svechnikova, 2015).

Computer technologies should be not an additional touch, but the inseparable part of the whole educational process, where the former considerably enhances the effectiveness of the latter (Zaytseva, 2015). The issue of a free computer program "Microsoft Visual Studio Express Edition" has given the opportunity to significantly decrease the cost of school software invention and production (Vinnik, 2017).

The H.S. Skovoroda's KNPU Chemistry department staff has been working on a software product for schools under the general name of "ColorKit". The software allows analyzing photo and video files. One of "ColorKit" application directions may be investigation of liquids and fluids optical densities parameters (Vinnik, 2017). This topic is of utmost importance, since the prices of photoelectrocolorimeters are high, and, hence, the majority of secondary schools do not possess such equipment. Besides, the modern research devices are hard to understand in terms of principle of their work for an average school student.

The "ColorKit" software is intended to analyze:

- Photography objects in formats of *. bmp; *. jpg; *. jpeg; *. gif; *. tif, as well as photos obtained by a web-camera directly;
- Video data in formats of *. avi; *. mpg; *. mpeg, *.wmv, as well as real time videos obtained by a web-camera directly.

The given program software allows determining:

- Color in the form of R, G, B sub-pixels magnitudes (R – red, G – green, B – blue); their minimal and maximal indexes, and the magnitudes dispersions;
- Color in the form of H, S, B (H – hue, S – saturation, B – brightness); their minimal and maximal indexes, and the magnitudes dispersions.

For the more effective usage of "ColorKit" program in the school experiment, one has to carefully elaborate the methods of the software application.

The **objective** of the present study is the elaboration of qualitative method to determine concentrations of vitamins A, D, and E in liquids and biological fluids by aid of computer analysis of visual data (with "ColorKit" program), which could be used at school conditions.

The main obstacle in identification and determination of fat-soluble vitamins in solutions is that, these compounds have non-polar molecular structure, whereas the reagents, which are supposed to react with them and form colored complexes in quantitative reactions, are mostly polar, hydrophilic (sulfuric and nitric acids, $FeCl_3$, $FeSO_4$, $SbCl_3$). Therefore, the research primary task was to create a non-polar reagent for each vitamin, so that, the reagent would bind a vitamin and rapidly form a complex of specific color. The intensity of the latter would be proportional to the vitamin concentration, and would be determined by aid of a web-camera and the “ColorKit” program. After careful consideration of the methods on the vitamins determination represented in the scientific literature, and several preliminary experiments to choose the most colorful reactions, the following procedures and reagents were selected. According to a literature source (Yakovleva, 2002), vitamin A is supposed to react with saturated sulfuric acid with the formation of dense blue color, which is to break down to intense yellow dose-dependently. On practice, the reaction never went that way due to high polarity of sulfuric acid. Therefore, the method was improved with preamble mixing saturated sulfuric acid and dichloride-ethane, as a non-polar, inert solvent. The latter absorbed SO_3 from the acid, and was used as the complex qualitative reagent for vitamin A identification. When reacting with the vitamin solution, it formed stable blue color, with dose-dependent intensity, easily registered by the web-camera. In the case of vitamin D and E, dichloride-ethane was mixed with saturated sulfuric and nitric acids, to absorb SO_3 and N_2O_5. Subsequently, the reagent dose-dependently formed the complex of yellow-orange color with the hydrophobic vitamins solutions. The innovation allowed us to operate with minor concentrations of vitamins solutions, due to the high sensitivity of the method.

The research used initial solutions of vitamins (retinol acetate, ergocalciferol, and tocoferol acetate, produced by “Lekchim” company, Uman, Ukraine) for the construction of calibration curves.

The experimental setup included the following units: laboratory palettes or cuvettes with vitamin solutions of different concentrations, which were placed in a box and highlighted with a LED H_4 bulb on the background of white screen. The reaction process was filmed with a web-camera CNR-WCAM820, which was switched on to a mini-laptop Packard Bell ZG6.

The filmed video was processed by the “ColorKit” computer program and the result was represented by calibration curves.

The experiments with vitamin A solutions of different concentrations revealed that, the interaction of retinol acetate with SO_3, dissolved in dichloride-ethane led to the formation of the product of dense blue color, which subsequently turned to yellow. The higher was the concentration of the vitamin in the solution, the slower was the alteration of the color. Thus, in processing the dynamic visual data, the time of the color change can pinpoint the concentration of the vitamin in the investigated solution. Besides, by aid of the software, a colorimetric experiment of vitamin A concentration determination was performed, where a solution of methylenic blue was used, as a standard sample. The experiment showed the colorimetric measurements to be carried out rapidly and directly after the addition of the reagent to the vitamin solution, in order to avoid the breakdown of the blue complex.

The experimental video analysis by aid of “ColorKit” according to RGB-system established logarithmic dependences of the solution color on vitamin A concentration. The correlation coefficients were equal to 0.95-0.99. The experimental video analysis by aid of “ColorKit” according to “Black and White” system revealed linear correlation of dependence of solution black-white color and brightness on the vitamin concentration, although the correlation coefficient was pretty low. The highest magnitude of R was registered for comparison of vitamin A experimental solution color with methylenic blue solution color, which was used as a colorimetric standard for the computer processing of visual data.

All vitamin E experimental solutions were added the elaborated reagent (SO_3 and N_2O_5 dissolved in dichloride-ethane). Thus, each experimental cuvette contained 1 ml alpha-tocoferol acetate solution and 3 ml dichloride-ethane solution with dissolved SO_3 and N_2O_5. The experiment continued to the complete stop of dose dependent color development in all the cuvettes.

The experimental video analysis by aid of “ColorKit” according to RGB-system established logarithmic dependences of the solution color on vitamin E concentration with low correlation coefficients. The same analysis according to “Black-White” system displayed the linear correlation between the solutions concentrations and their color with R magnitude equal to 0.998. The evaluation of vitamin E solutions concentrations dependence upon the samples brightness and saturation by the program could be of high practical usage, as well.

The experimental pattern of vitamin D concentration determination was completely identical to that of vitamin E with the same reagents and solvent. There were four calibrating samples, placed in cuvettes.

The experimental video analysis by aid of “ColorKit” according to “Black-White”-system established a linear correlation in the dependence of solution color intensity on the vitamin concentration, with the high R magnitude, which was equal to 0.94. Of high practical application might be the evaluation of vitamin D solutions concentrations dependence upon the samples brightness.

Conclusions

1. The method of vitamins A, D, E concentrations determination was elaborated by analyzing solutions color with the usage of ColorKit program. Besides, innovation of reagents preparation was proposed, i.e. saturated solution of SO_3 (for vitamin A), or $SO_3 + N_2O_5$ (for vitamin D and E) in dichloride-ethane.
2. Algorithm of processing visual effects of vitamins qualitative colored reactions alterations was established by aid of web-camera CNR-WCAM820 and the ColorKit program.
3. The calibration graphs for the vitamin concentrations determination in solutions were obtained.
4. The elaborated method may be recommended to use at school conditions.

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