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Laboratory Experiment

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Molecular Recognition: Detection of Colorless Compounds Based On Color Change

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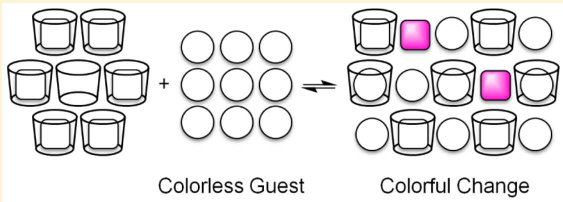
Supporting Information

ABSTRACT: A laboratory experiment is described in which students measure the amount of cetirizine in allergy-treatment tablets based on molecular recognition. The basis of recognition is competition of cetirizine with phenolphthalein to form an inclusion complex with β-cyclodextrin. Phenolphthalein is pinkish under basic condition, whereas it's complex form with β-cyclodextrin is colorless. Addition of the cetirizine leads to release of phenolphthalein from the phenolphthalein-β-cyclodextrin inclusion complex that alters the solution color under basic conditions. The intensity of the color change is proportional to the cetirizine concentration. This simple experiment provides an opportunity for students to become familiar with the concepts of inclusion complexes and molecular recognition. Students also gain hands-on experience with a spectrophotometer and engage in plotting a calibration curve based on absorbance or color changes and determination of cetirizine.



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The term molecular recognition refers to the specific interaction between two or more molecules through noncovalent interactions.[1] The host−guest interaction that is a simple class of molecular recognition consists of the fit between the host cavity and a guest molecule.[2] The best-known example of compounds acting as molecular hosts are cyclodextrins (CDs).[3,4] These molecules exhibit a truncated-cone structure (Figure 1) with a hydrophilic outer side and a hydrophobic inner cavity that can accommodate different organic molecules depending on the size of both the chosen cyclodextrin and the guest compound.[5] The compound in which the guest compound is located in cyclodextrin cavity known as inclusion complex. Owing to their facile host−guest interactions, mostly in aqueous solutions, β-CD and substituted β-CD have found extensive applications in many fields including pharmaceutical technology.[6] Because the hydrophobic cavity of β-CD is different compared to the aqueous phase, changes in physicochemical properties of guest molecules can be observed on formation of the inclusion complex.[7]

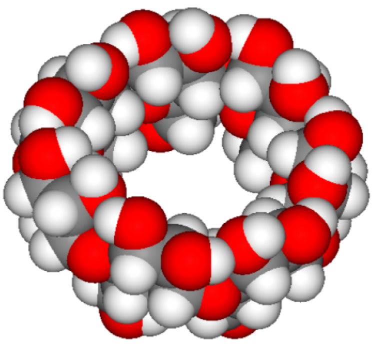

Figure 1. Doughnut-shaped structure of β-CD.

For example, the spectra of free and β-CD surrounded form of some color indicators are different in aqueous solutions.[8,9] Displacement of indicators in β-CD cavity by any other molecule results in significant color changes (spectral changes) that provide a basis for detection of many colorless molecules.[10] Although this phenomenon occurs in a much smaller world than the one we are habituated to, information about it can be conveniently transmitted to us via simple color change. Detection of colorless molecules based on such a reversible and fast color changes is an example of "molecular recognition" because it strongly depends on molecular shapes and structure.[11] Therefore, it is useful and appropriate to provide an introduction and simple experiment to the concept of molecular recognition and its application in detection of pharmaceuticals. This would be particularly suitable for an undergraduate analytical chemistry class.

EXPERIMENTAL OVERVIEW

In the first part of the experiment, students study the color and spectral changes of phenolphthalein (PHP) in the presence of β-CD. PHP, a typical acid−base indicator, is colorless in acidic and neutral solutions; the color change of PHP occurs at pH of around 9 (Scheme 1).[12]

By addition of β-CD to pinkish basic solution of PHP, the solution's color fades due to formation of colorless 1:1 inclusion complex between PHP and β-CD (Scheme 2).[13]

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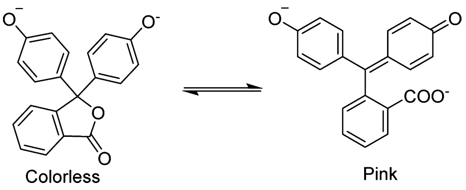
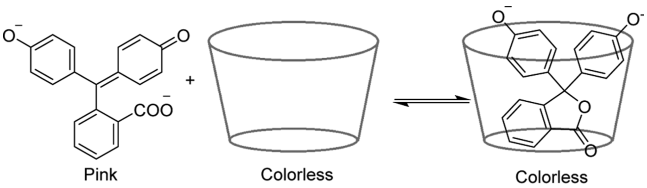
Scheme 1. Acid−Base Equilibrium and Color Change of Phenolphthalein



Decoloration of the solution is proportional to the concentration of β-CD, whereas the pH of solution does not change; a clear visualization of inclusion complexation.

Scheme 2. Mechanism of Color Change by Inclusion Complex Formation



The second part of the experiment is the quantitative measurement of cetirizine using visible absorption or color changes. Cetirizine is one of the most widespread antihistamine drugs that is commonly used for the relief of allergy.[14] Cetirizine has affinity to form an inclusion complex with β-CD.[15] The formation constants for PHP-β-CD (2.4×10^4 M^{-1}) and cetirizine-β-CD (4.1×10^3 M^{-1}) determine the amounts of each inclusion complex formed in a solution that contains β-CD, PHP, and cetirizine. Competition of cetirizine with PHP for complex formation with β-CD causes the breakdown of some PHP-β-CD complexes due to the consumption of some free β-CD to form cetirizine-β-CD complex. In other word consumption of β-CD by cetirizine shifts the dissociation equilibrium of PHP-β-CD to the production of free β-CD and PHP based on Le Châtelier's principle. The released PHPs in aqueous basic solution change to their colorful forms and make the solution pinkish. The enhancement of the pink color of the solution is proportional to the cetirizine concentration and makes a basis for its measurement (Scheme 3).

This experiment was accomplished in an introductory analytical chemistry laboratory course having 10 students. The laboratory was equipped with four spectrophotometers, and students worked in four groups of two and three. The first group of students completed the experiment in 1 h and 40 min, and the last group was done in 2 h and 20 min.

EXPERIMENTAL PROCEDURES

Students begin this experiment by preparing a carbonate buffer solution with pH equal to 10.5 and the stock solutions of PHP, β-CD, and cetirizine. Students then measure the absorbance of standard buffered PHP solutions, at 554 nm, before and after addition of the desired aliquot of β-CD. They then measure the absorbance of a series of solutions with the same pH, same concentrations of PHP and β-CD, and different concentrations of cetirizine to construct an absorbance changes versus concentration calibration curve. Finally, students determine the total mass of cetirizine contained in the tablet using their calibration curve and derived equations.

In the experiments, students used a Unico Spectrophotometer, which has a grating-based optical system with wavelength range of 325−1100 nm (visible range). Cetirizine was obtained from Osveh Pharmacy Company and the other chemical reagents were purchased from Sigma-Aldrich and used as received. The stock solution of cetirizine was prepared by dissolving cetirizine in 5 mL of mixture of ethanol and water (50/50) and diluting to the mark in a 20 mL volumetric flask with distilled water. A stock solution of β-CD was prepared in distilled water. A weighed amount of PHP was first dissolved in a small amount of NaOH solution and the resultant mixture then diluted with water to 50 mL. Sample dilutions were carried out by taking the appropriate aliquots from the stock solutions followed by dilution with carbonate buffer (pH = 10.5). The total concentrations of prepared buffers were 0.1 M. The experimental details are given in the Supporting Information.

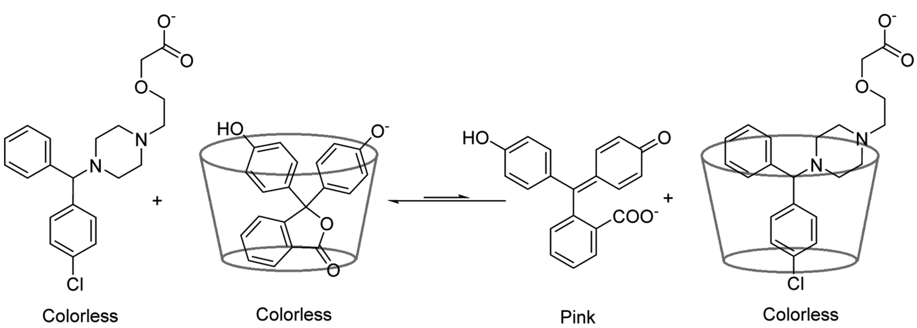
HAZARDS

Pharmaceuticals used in laboratory experiments should never be consumed, inside or outside the laboratory. Approved safety goggles and proper laboratory clothing and gloves must be used in a laboratory. Sodium hydroxide is a highly corrosive chemical and contact can severely irritate and burn the skin and eyes with possible eye damage. Laboratory chemicals including sodium carbonate, sodium hydrogen carbonate, and phenolphthalein can cause eye and skin irritation. These chemicals must be handled with proper safety. Flush eyes or hands with plenty of water for at least 15 min in the case of eye or skin contacts.

RESULTS AND DISCUSSION

The basic solution of PHP shows an absorption band with maximum absorbance at 554 nm (Figure 2A curve A_{PHP}). By addition of β-CD to the PHP solution, the absorption of PHP solution decreases gradually (Figure 2A curve $A_{PHP\text{-}CD}$). Addition of cetirizine to PHP-β-CD solution causes an increase in the color and absorbance of the solution (Figure 2B).

Scheme 3. Mechanism of Color Change by Competitive Inclusion Complex Formation[16]



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J. Chem. Educ. XXXX, XXX, XXX−XXX

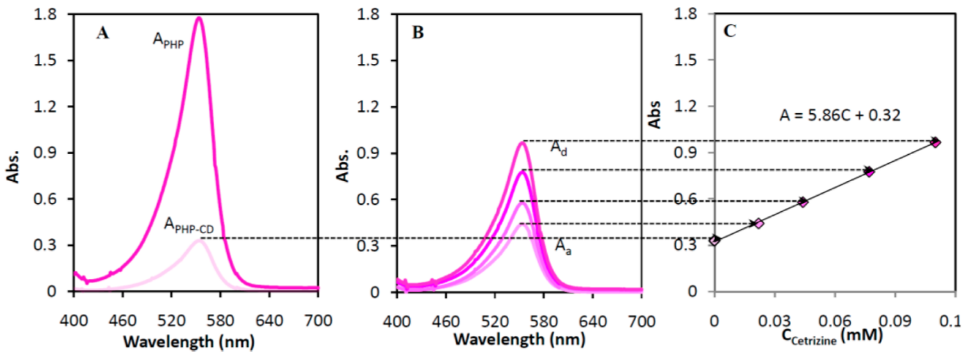

Figure 2. (A) Absorption spectra of 6.3×10^{-5} M of PHP (A_{PHP}) at pH 10.5 in the absence and presence of 1.5×10^{-4} M of β-CD (A_{PHP-CD}) and (B) 2.2×10^{-5}, 4.4×10^{-5}, 7.7×10^{-5}, and 1.1×10^{-4} M of cetirizine; (A_a) to (A_d) respectively and (C) the representative calibration curve obtained by one group of students.

Plotting the difference of absorption of PHP-β-CD solutions before and after addition of cetirizine gives a linear calibration plot (Figure 2C).

Measuring the absorbance change of PHP-β-CD solution by addition of a sample prepared by dissolution of a 10 mg of cetirizine tablet yielded a total mass of 9.3 mg based on the constructed calibration curve, corresponding to 93% of the expected cetirizine value. The results of the mass of cetirizine from the different groups give the average value of 9.6 ± 0.5 mg.

■ CONCLUSION

This lab experiment allows the students to deal with the concepts of inclusion complex and molecular recognition by either observed absorbance change or even visual color changes. This experiment provides students with a working example of how a competitive host−guest interaction can be used for detection and quantification of pharmaceuticals. The spectrophometric method gives experience in working with spectrophotometers that how an indirect absorbance or color changes can be used for the quantitative analysis. The students in this experiment also deal with analyses of data and calibration curve and measurements of concentration for both known and unknown samples. This experiment could be extended for recognition and analysis of other pharmaceuticals and comparing their interaction with β-CD.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00232.

Experimental details, experimental handout for students and notes for the instructor. (PDF)

Experimental details, experimental handout for students and notes for the instructor. (DOCX)

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Notes

The authors declare no competing financial interest.

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J. Chem. Educ. XXXX, XXX, XXX−XXX