# Phthalein-Pigment as Staining of Slides in Microscope (Uses and application in Health Field)

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**Abstract**
Weigert's elastic stain is a mixture of dyes used in tissue that is useful for identifying elastic fibers. Orsin or a combination of resorcinol and fuchsin are often used for coloring. In order to counteract the rapid nucleation of the cell, red stain or hematoxylin is also used. After being applied to elastic fibers, they appear blue while the cell nuclei become red or blue. A technique used to enhance the contrast in samples, generally at the microscopic level. Staines and dyes are frequently used in histology (the study of tissue under a microscope) and in the medical fields of histopathology, hematology and cytopathology which focus on the study and diagnosis of disease at the microscopic level. Stains can be used to identify biological tissues (highlighting, for example, muscle fibers or connective tissue), cell clusters (classifying various blood cells), or organelles within individual cells. Coloring is not limited to biological materials.

**Keywords**: different, phthaline, staining, pigment.

## Introduction

In biochemistry it involves adding a specific class (DNA, proteins, lipids, carbohydrates) to a substrate to identify or determine the presence of a particular compound. Staining and fluorescent markers can serve similar purposes. Biological staining is also used to differentiate cells in flow cytometry, and to identify proteins or nucleic acids in gel electrophoresis. Phthalein's common use is as an indicator in acid-base titrations. It also serves as a component of universal indicator, together with methyl red, bromothymol blue, and thymol blue[1-3]. Phenolphthalein adopts four different states in aqueous solution: Under very strongly acidic conditions, it exists in protonated form, providing an orange coloration. Between strongly acidic and slightly basic conditions, the lactone form is colorless. The singly deprotonated phenolate form (the anion form of phenol) gives the familiar pink color. In strongly basic solutions, phenolphthalein's pink color undergoes a rather slow fading reaction and becomes completely colorless above 13.0 pH. The rather slow fading reaction that produces the colorless $In(OH)3^{-}$ ion is sometimes used in classes for the study of reaction kinetics. Phenolphthalein's pH sensitivity is exploited in other applications: Concrete has naturally high pH due to the calcium hydroxide formed when Portland cement reacts with water. As the concrete reacts with carbon dioxide in the atmosphere, pH decreases to 8.5-9. When a 1% phenolphthalein solution is applied to normal concrete, it turns bright pink. However, if it remains colorless, it shows that the concrete has undergone carbonation. In a similar application, spackling used to repair holes in drywall contains phenolphthalein. When applied, the basic spackling material retains a pink coloration; when the spackling has cured by reaction with atmospheric carbon dioxide, the pink color fades[4-8]

Phenolphthalein is used in toys, for example as a component of disappearing inks, or disappearing dye on the Hollywood Hair Barbiehair. In the ink, it is mixed with sodium hydroxide, which reacts with carbon dioxide in the air. To develop the hair and "magic" graphical patterns, the ink is sprayed with a solution of hydroxide, which leads to the appearance of the hidden graphics by the same mechanism described above for color change in alkaline solution. The pattern will eventually disappear again because of the reaction with carbon dioxide. Thymolphthalein is used for the same purpose and in the same way, when a blue color is desired.[8-10]

## Experimental

### Materials and physical measurements

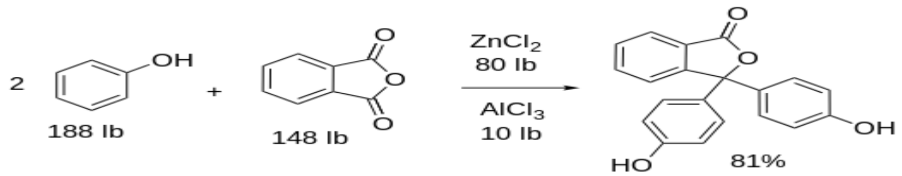


**Fig (1): Preparation of Pigment**

The two most common would be: as a chemical indicator and as a laxative. it turns from colorless to pink in the 8–10 pH range, making it perfect for the titration of strong acids, like hydrochloric(HCl). Over the counter Ex-Lax Gentle is a common laxative that contains phenolphthalein.

### Results and Discussion

Phenolphthalein is an organic compound used as a laboratory reagent and pH indicator. Phenolphthalein exerts laxative effects by stimulating the intestinal mucosa and constricting smooth muscles. However, phenolphthalein is no longer used as a laxative due to the suspected carcinogenicity of this compound.

PHYSICAL DESCRIPTION: White or yellowish-white to pale orange fine crystalline powder. Odorless. Aqueous solution is acidic. Colorless to pH 8.5; pink to deep-red above pH 9. Colorless in presence of large amounts of alkali. Tasteless. (NTP, 1992)**.** Acid - Base indicators (also known as pH indicators) are substances which change colour with pH. They are usually weak acids or bases, which when dissolved in water dissociate slightly and form ions.

Consider an indicator which is a weak acid, with the formula HIn. The acid and its conjugate base have different colours. At low pH values the concentration of $H_3O^+$ is high and so the equilibrium position lies to the left. The equilibrium solution has the colour A. At high pH values, the concentration of $H_3O^+$ is low - the equilibrium position thus lies to the right and the equilibrium solution has colour B. Phenolphthalein is an example of an indicator which establishes this type of equilibrium in aqueous solution.

Phenolphthalein is a colourless, weak acid which dissociates in water forming pink anions. Under acidic conditions, the equilibrium is to the left,and the concentration of the anions is too low for the pink colour to be observed. However, under alkaline conditions, the equilibrium is to the right, and the concentration of the anion becomes sufficient for the pink colour to be observed.We can apply equilibrium law to indicator equilibria - in general for a weak acid indicator: $\mathbf{K_{ln}}$ is known as the **indicator dissociation constant**. The pH of the solution at its turning point is called the $pK_{ln}$ and is the pH at which half of the indicator is in its acid form and the other half in the form of its conjugate base.

**Indicator Range**

At a low pH, a weak acid indicator is almost entirely in the HIn form, the colour of which predominates. As the pH increases - the intensity of the colour of HIn decreases and the equilibrium is pushed to the right. Therefore the intensity of the colour of $In^-$ increases. An indicator is most effective if the colour change is distinct and over a low pH range. For most indicators the range is within ±1 of the $pK_{In}$ value: - please see the table below for examples, to the right is a model of the acid form of each indicator - with the colour of the solution at the turning point. Litmus is a weak acid. It has a seriously complicated molecule which we will simplify to HLit. The "H" is the proton which can be given away to something else. The "Lit" is the rest of the weak acid molecule. There will be an equilibrium established when this acid dissolves in water. Taking the simplified version of this equilibrium:

**Application of Pigments in Appearance of Tissues:**

Most of Pigments were used in appearance of cancer in tissue or Tumors in cells in many studies [13-16]., like :

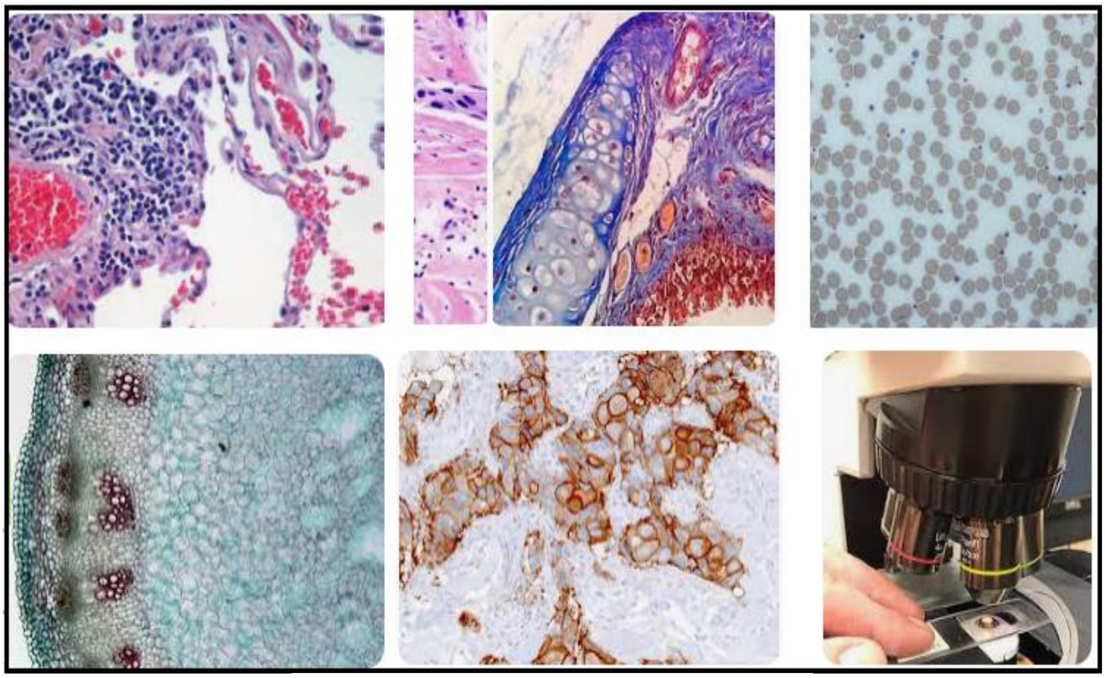

**Photo. 1: Stained Tissue by Pigments**

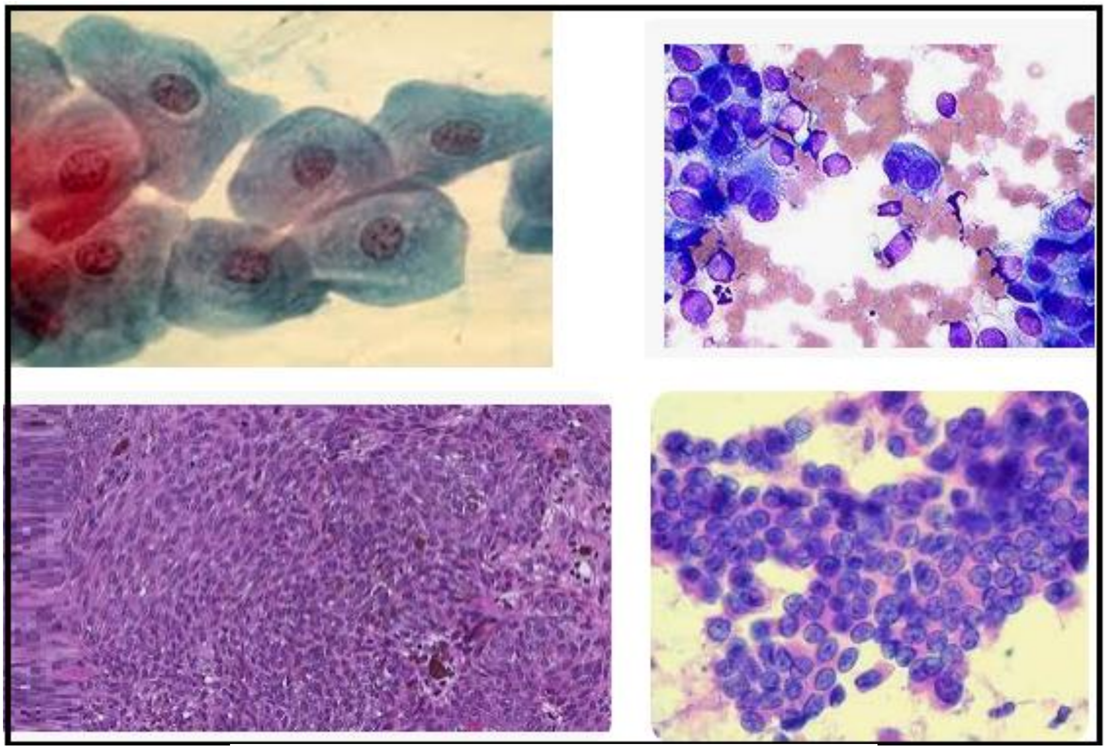

**Photo. 2: Appearance of Tumors by Pigments**

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