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Fascinating school experiments with fluorescent dyes

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Abstract The world is colourful – at least from our perspective as humans. But where does colour come from? The present paper addresses this question and, in its first part, presents new experiments on additive colour mixing with fluorescent solutions. In addition, further school experiments with impressive fluorescence effects are described.

Keywords: *fluorescence, additive colour mixing, fountain experiment, invisible ink, alginate beads*

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1. Introduction

Experiments with fluorescence effects usually exert a special fascination on their observers. This fascination can be used in chemistry lessons to arouse or strengthen the students' interest in chemical content. Below, fluorescence experiments are presented, which have been developed or expanded by the author of this paper. They mainly cover the topics of additive colour mixing, indicators and acid-base reactions and are characterised, among other things, by their ease of implementation. Several experiments can even be carried out with everyday products, depending on their availability.

2. Additive colour mixing

Additive colour mixing is essential for the sensory perception of the world around us since, as is known. It is exactly the principle our colour vision is based on. The three cone types in the human eye sense either only blue (S cone), green (M cone) or red (L cone). We see mixed colours, such as yellow, only when wavelengths of different lengths are reflected by an object. If all cone types are addressed equally, the colour impression of white is created by our brain. Additive colour mixing is also used in technology, for instance in the colour reproduction of RGB monitors. Red, green and blue light sources are arranged close to each other such that they cannot be perceived individually, but only additively. This concept, however, is much older. The French painter Georges Seurat (1859-1891) invented a painting technique, which was novel in his times, known as pointillism. The whole painting consists of small, closely spaced, regular dabs of pure colour. The overall colour impression in the eye of the beholder only arises at a certain distance from the painting.

In Germany, additive colour mixing is dealt with in all science school subjects. In physics lessons, it is part of optics. In biology, it is included in the explanation of the sense of light and in chemistry lessons it is addressed in the context of dyes. The model experiments described in the textbooks are usually of a physical type (e.g. superimposition of light from red, green and blue light-emitting diodes). The central idea underlying the following experiments is to demonstrate additive colour mixing with the help of fluorescent solutions.

The following two-part experiment can be used as an introduction into the topic. First, a freshly cut chestnut branch is placed or hung in a beaker filled with ethanol in a darkened room and irradiated with UV light (λ = 365 nm).

Note: Also water instead of ethanol can be used for the experiment. However, to facilitate comparison (an ethanolic solution must be used in the second part of the experiment), the first part is described here with ethanol as the solvent.

Figure 1. The bleeding chestnut branch: Starting from the cut surface of a chestnut branch dipped in ethanol, blue fluorescent streaks of aesculin appear under UV light (λ = 365 nm). (Photograph: Ducci)

Blue fluorescent streaks develop from the cut surface and the branch seems to "bleed" [1]. This phenomenon is caused by the fluorescent dye aesculin (structural formula

in Figure 4), which emerges from the chestnut branch (cf. Figure 1). After a short time or after stirring, the entire solution fluoresces blue.

For continuing the experiment, a rolled rim vial (e.g. 30 mL) is required, which is filled with an ethanolic eosin Y solution (dissolve a few crystals of eosin Y in 10 mL ethanol). Under UV light (λ = 365 nm), the eosin Y solution fluoresces yellowish (cf. Figure 2, left). The students are now asked to guess what colour the fluorescence will take on if a freshly cut chestnut branch is also placed in this solution. It can be assumed that an overwhelming majority expects a green fluorescent solution as revealed by surveys of school students in the teaching-learning laboratory of the Institute of Chemistry at the Karlsruhe University of Education and students of the same university. But the ethanolic solution of eosin Y and aesculin fluoresces white (cf. Figure 2, middle and right, respectively)!

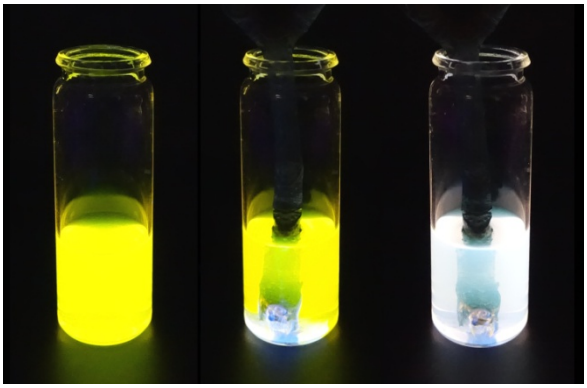

Figure 2. Left: The rolled rim vial contains an ethanolic eosin Y solution. If a freshly cut chestnut branch is placed in the solution (middle), the solution fluoresces white within a few minutes (right). (Photographs: Ducci)

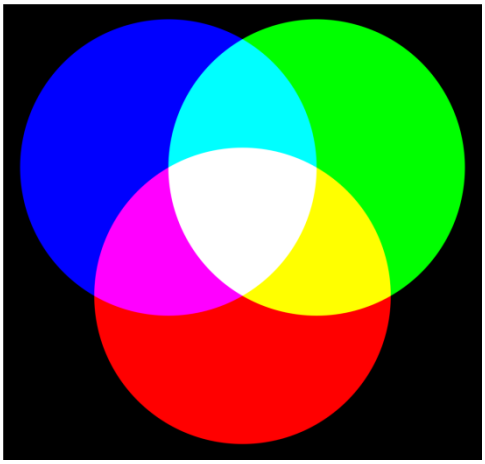

Figure 3. Scheme of additive colour mixing with the primary colours red, green and blue. The background symbolises the colour perception black (no light).

An explanation for the rather frequently observed, false expectation is the fact that school and university students apply the principle of subtractive colour mixing. But here, we have to do with additive colour mixing since the dissolved fluorescent dyes themselves glow under UV light. The three basic colours of additive colour mixing are red, green and blue. The mixed colours are yellow (from red and green), cyan (from green and blue), magenta (from red and blue) and white (from all three primary colours) (cf. Figure 3). The scheme in Figure 3 shows why the mixture of the blue and yellow fluorescent solution fluoresces white in the experiment.

The dissonance between the students' expectations and the actual result should be motivation for the students to carry out further mixing experiments with fluorescent solutions. The scheme shown in Figure 3 can be developed by the students on their own if they are handed out solutions fluorescing in the three primary colours. Suitable solutions are:

(i) Nile red solution (0.005 g in 100 mL ethanol, w = 96%) – red fluorescence

(ii) Fluorescein (disodium salt) solution (0.005 g in 100 mL ethanol, w = 96%) – green fluorescence

(iii) Pyranine solution (0.005 g in 100 mL ethanol, w = 96%) – blue fluorescence

Figure 4 shows the structural formulae of the fluorescent dyes used as well as the structural formula of aesculin. Alternatively, everyday products can also be used for preparing the fluorescent solutions [2].

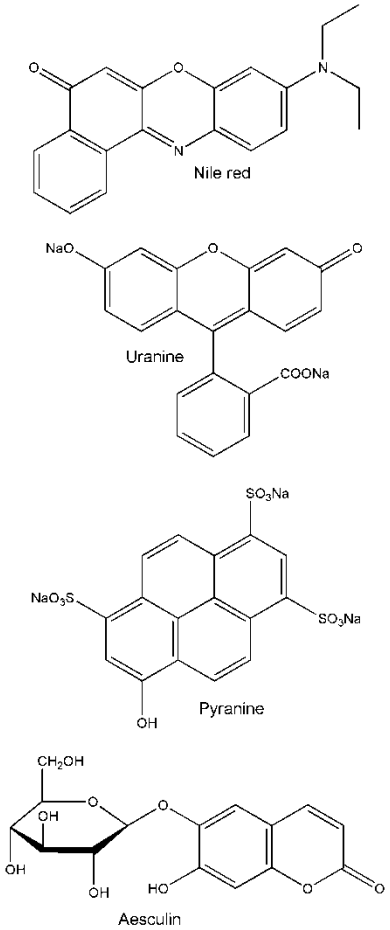

Figure 4. Structural formulae of the fluorescent dyes used.

Procedure:

One of the solutions is placed in a rolled rim vial (e.g. 30 mL). Thereafter, a second solution is added under the light of a UV handlamp (λ = 365 nm) until the mixture fluoresces in the mixed colour. The maximum distance to the UV handlamp should be 5 cm. Table 1 shows the approximate volume ratios and Figure 5 the different solutions or solution mixtures under UV light (λ = 365 nm), sorted in analogy to the scheme in Figure 3.

Table 1. Solution mixtures and fluorescent colours (the solution with figures in bold print is the first to be placed into the vial; the other is added dropwise)

Pyranine-solution	Nile red-solution	Fluorescein-solution	Fluorescent colour
5 mL	approx. 3 mL	-	magenta
5 mL	-	approx. 4 mL	cyan
-	approx. 0.2 mL	5 mL	yellow
approx. 1.5 - 2 mL	0.25 mL	5 mL	white

Figure 5. Demonstration of additive colour mixing with fluorescent solutions. In the Erlenmeyer flasks, the solutions fluoresce in the three primary colours. The rolled rim vials contain the mixtures. (Photograph: Ducci)

3. Fountain experiment with fluorescent dyes

A standard experiment in experimental school chemistry is the so-called fountain experiment [3]. A round-bottomed flask is filled with hydrogen chloride or ammonia and then closed with a pierced stopper into which a glass tube narrowed to the tip is inserted. The tube is inserted into the stopper such that its tip protrudes into the inside of the round-bottomed flask. The other end of the glass tube is dipped into a glass tub filled with water to which an indicator has been added, in a manner that the flask is virtually upside down. The solution in the glass tub slowly rises up in the glass tube. As soon as the first drops enter the flask, a major part of the hydrogen chloride (or ammonia) dissolves in them and is converted to oxonium and chloride ions. In case of ammonia, an equilibrium is established between ammonia and water molecules, on the one hand, and ammonium and hydroxide ions, on the other. The gas dissolving in the flask yields creates a strong negative pressure, causing further solution to shoot into the inside of the flask like a fountain. As a result, the colour of the solution changes since the pH decreases or increases. If, for instance, a universal indicator is used, the colour changes from green to red or blue. In chemistry lessons, this experiment is often performed as part of the "Acidic and alkaline solutions" topic. The fountain experiment can be spectacularly staged by using suitable fluorescent indicators, such as phloxine B or eosin Y.

Procedure:

In a crystallising dish, 2 mL hydrochloric acid, c = 2 mol/L, are added to 400 mL tap water. Subsequently, a spatula tip (approx. 0.02 g) of one of the above fluorescent indicators is added. The solution thus obtained exhibits (almost) no fluorescence. Then, the fountain experiment is carried out under UV light (λ = 365 nm) as described above, using a round-bottomed flask (250 mL) filled with ammonia. The solution rises in the glass tube and fluoresces due to the pH increase. Finally, it flows at high speed into the inside of the round-bottomed flask. The fountain fluoresces orange when using phloxine B (cf. Figure 6, left) and green when using eosin Y.

Figure 6. The ammonia fountain experiment with the fluorescent indicator phloxine B (left), quinine and eosin Y (middle) as well as sodium 4-aminonaphthalene-1-sulfonate and phloxine B (right). (Photographs: Ducci)

Structurally, both dyes are very similar, phloxine B being the tetrachloro derivative of eosin Y (cf. Figure 7). Instead of four chlorine atoms, eosin Y has four hydrogen atoms attached to the carboxyphenyl ring. The reason for the occurrence of fluorescence when the pH is increased lies in the ring opening of the lactone structure of phloxine B (cf. Figure 7) or eosin Y present in the acidic solution.

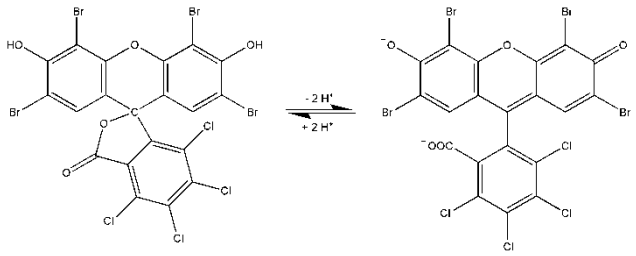

Figure 7. The structural formulae of phloxine B in an acidic (left) and alkaline environment (right). In the alkaline range an aqueous solution of this dye fluoresces orange, whereas in the acidic range the fluorescence is quenched.

Further variations:

(i) If, instead of phloxine B, 0.02 g quinine and 0.02 g eosin Y are dissolved in the saline solution, the liquid in the crystallising dish fluoresces light blue and the fountain in the flask filled with ammonia fluoresces green (cf. Figure 6, middle). Like phloxine B, eosin Y does not exhibit any fluorescence in the acidic range (lactone structure), but only upon opening the ring (cf. Figure 7). In contrast, quinine fluoresces brightly in the acidic range, and the fluorescence is quenched in the alkaline range. Quinine belongs to a group of alkaloids, which occur in

the bark of the cinchona tree. Its structural formula is shown in Figure 8 (left). The fluorescence in the acidic range is attributable to the protonation of the two nitrogen atoms, which are split off again in the alkaline range [4].

(ii) If 0.02 g each of phloxine B and sodium 4-aminonaphthalene-1-sulfonate (cf. Figure 8, right) are dissolved in the hydrochloric acid solution, a blue fluorescence can be observed (cf. Figure 6, right). As soon as the liquid shoots into the round-bottomed flask filled with ammonia, the fluorescence colour changes to magenta (cf. Figure 6, right). The blue fluorescence of the 4-aminonaphthalene-1-sulfonate ions remains, the orange light of phloxine B is added (additive colour mixing).

The students can try out many other variations of this experiment with other fluorescent dyes and indicators. Moreover, it should be mentioned that also redox reactions can be initiated in the fountain experiment by changing the pH of the solution (cf. [6]).

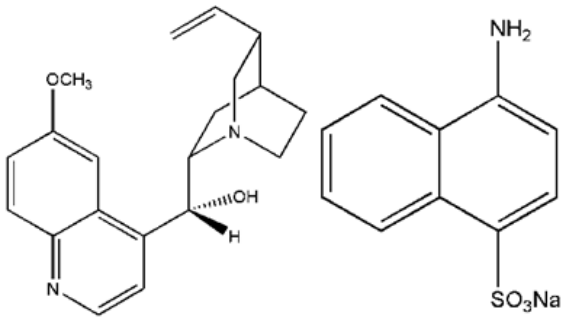

Figure 8. Structural formula of quinine (left, [5]) and of sodium 4-aminonaphthalene-1-sulfonate (right).

4. Invisible fluorescent inks

Writing with colourless invisible inks and, in particular, making them visible is extremely popular with school students. A classic example is lemon juice revealing brownish letters on paper with the help of a candle flame. For several years now, UV pens have also been available in toy shops. They are equipped with a small UV lamp which makes the invisible ink visible. Amazon reviews can be read to explain why these pens are particularly attractive to school students: *"Ideal for cribbing, bright but unobtrusive light, so it's really easy to write down formulae and such invisibly. Really recommended."*

This effect can be achieved with a wide range of fluorescent indicators. It has proven to be expedient for such experiments, which can also be placed within the framework of a lesson unit on "Acidic and alkaline solutions", to use paper containing as few optical brighteners as possible instead of white copy paper. For instance, Parchment A4 writing pad from Dresdner Feinpapier Werkstatt (DFW). The paper is light brown and fluoresces only very weakly under UV light (λ = 365 nm). Alternatively, brown coffee filter paper can be used.

A suitable invisible UV ink is, for instance, an aqueous sodium 4-aminonaphthalene-1-sulfonate solution (dissolve 0.005 g [0.02 g if brown coffee filter paper is used] in 5 mL tap water). The solution is colourless in daylight. When it is applied to the paper with a fine brush (thickness 0), it can only be recognised under UV light (λ = 365 nm) by its blue fluorescence (cf. Figure 9).

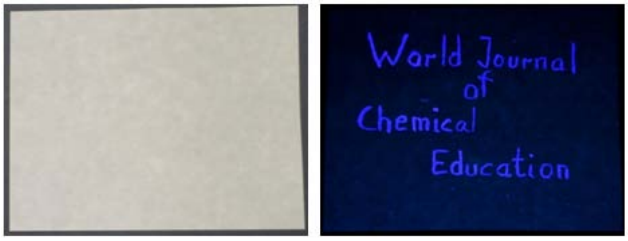

Figure 9. Aqueous sodium 4-aminonaphthalene-1-sulfonate solution as invisible UV ink (left: in daylight, right: under UV light (λ = 365 nm)). (Photographs: Ducci)

For preparing an aqueous sodium 4-aminonaphthalene-1-sulfonate solution, everyday products can also be used. Such products must contain the azo dye azorubine. In the EU, azorubine (CI 14720) is approved as a colour additive for foods and is contained, for instance, in the isotonic drink "Powerade Wild Cherry". This dye can also be found in numerous cosmetic products, such as bubble baths.

When aqueous azorubine solution or Powerade Wild Cherry (or a bubble bath containing azorubine) is mixed with sodium dithionite, the originally red solution decolours and fluoresces blue under UV light (λ = 365 nm) (cf. Figure 10). For this experiment, sodium dithionite can also be replaced by an everyday product. The content of sodium dithionite in colour removers for fabric, for instance, amounts up to 30% by weight. The blue fluorescence is caused by the reductive cleavage of the azo dye, resulting in the formation of 4-aminonaphthalene-1-sulfonate ions (cf. Figure 11). This solution can also be used as an invisible ink on white filter paper (e.g. Whatman™ Filter Papers 1, CAT No. 1001-125).

Figure 10. Reductive cleavage of azorubine in Powerade Wild Cherry using a fabric colour remover containing sodium dithionite. Left: Powerade Wild Cherry, middle: after addition of the colour remover, right: the bottle from the middle under UV light. (Photographs: Ducci)

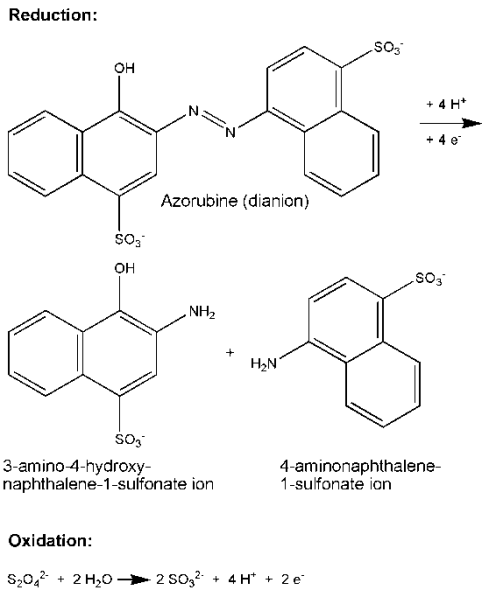

Figure 11. Reaction equation for the reductive cleavage of azorubine with sodium dithionite.

Some yellow highlighters contain the fluorescent dye pyranine (cf. Figure 4), such as those of Pelikan and Faber-Castell make. They can also spectacularly be used in the context of "invisible inks". Such highlighter is used to write on paper which contains as few optical brighteners as possible (cf. above). Under UV light, the writing fluoresces intensively green (cf. Figure 12). A cotton pad is moistened with approx. 1 mL hydrochloric acid, c = 2 mol/L. Then, the writing is dabbed with it (do not wipe), making it invisible in daylight (cf. Figure 12). Under UV light (a cheap UV torch is sufficient to this end), the writing can still be seen in the form of intensely blue fluorescent letters (cf. Figure 12).

Figure 12. The colour solution of a yellow highlighter (top left) containing pyranine fluoresces intensely green under UV light (top right). It can be made invisible by hydrochloric acid (bottom left). Under UV light, the invisible writing fluoresces intensely blue (bottom right). (Photographs: Ducci)

The indicator effect of pyranine is attributable to the bonded hydroxy group. The liquid in the highlighter is alkaline (approx. pH 10), i.e. the equilibrium depicted in Figure 13 is far to the right. When dabbing with acidic solution, it is shifted to the left. In addition, in the strongly acidic range, the sulfonate groups are increasingly protonated.

The invisible writing can be made visible again (daylight) by dabbing with a cotton pad soaked in lye. However, it is more impressive to place the paper in a transparent card game case and add 3 to 4 drops of ammonia solution, w = 9%, on one side next to the paper. The case is quickly closed with its lid and the UV torch is switched on. Ammonia gas evaporates from the drops, which successively sweeps over the writing, causing the fluorescence colour to change to green (cf. Figure 14).

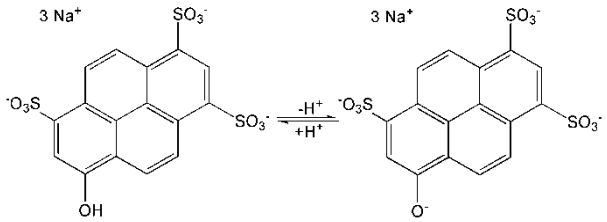

Figure 13. The indicator effect of pyranine is based on the reversible deprotonation of the hydroxy group.

Figure 14. Ammonia gas sweeping from right to left over a writing made of acid pyranine solution, which is invisible in daylight. (Photograph: Ducci)

The experiment shows how a yellow highlighter can easily be checked for its pyranine content: when dabbing with diluted acid, the ink must decolourise. If there is no highlighter with pyranine ink available, the experiment can also be carried out with aqueous pyranine solution (dissolve 0.05 g pyranine in 5 mL tap water).

5. Fluorescent "indicator alginate beads"

In [7], the author of the present paper already reported in detail on how so-called alginate beads can be made useful for chemistry lessons. The central idea of the experiments was to place chemical reactions (redox reactions, etc.) inside alginate beads. Already in [1] it was suggested to introduce acid/alkaline indicators, such as red cabbage juice, into the alginate beads. A new experiment is presented below.

In 5 mL of a sodium alginate solution, w = 1%, 0.001 g each of 5-aminosalicylic acid (5-ASA) and 2-naphthol are dissolved. This mixture is dropped into a calcium chloride solution using a 1 mL dropping pipette (30 to 40 drops are sufficient). Colourless alginate beads are formed as a result. The chemical processes underlying the formation of the alginate beads are described in detail in [7]. The solution containing the beads is poured through a fine tea strainer and the alginate beads remaining in the strainer are rinsed briefly with tap water. Then, the alginate beads are apportioned to three glass vials with snap-on lid under UV light (λ = 365 nm). The first glass vial contains table vinegar (w = 5%), the second tap water and the third ammonia solution, w = 9%. In the first vial, the green fluorescence of the beads is successively extinguished from the outside to the inside. In the tap water, the beads

remain green fluorescent. In the alkaline solution, the fluorescence colour changes from green to blue (cf. Figure 15).

Figure 15. The alginate beads loaded with fluorescent indicators exhibit a different fluorescent behaviour in the acidic (left), neutral (middle) and alkaline (right) solution. The photograph was taken shortly before the fluorescence in the left vial completely extinguished. (Photograph: Ducci)

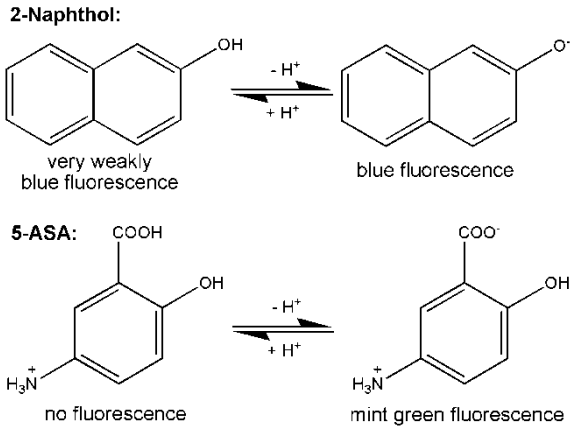

Figure 16. 2-Naphthol and 5-ASA are fluorescent indicators.

Both 5-ASA and 2-naphthol are fluorescent indicators. The mint-green fluorescence of 5-ASA is extinguished below about pH 4. The colour-changing range of 2-Naphthol is between 8.5 and 9.5. Below this range, 2-Naphthol fluoresces only very weakly and above it intensely dark blue. This adds up in the alkaline range with the mint green fluorescence of 5-ASA, resulting in a light blue glow. The equilibria shown in Figure 16 provide an explanation for the fluorescence behaviour of both substances at molecular level.

Conceptual basics as well as further experiments concerning photoluminescence have been published by M. W. Tausch and his coworkers [8,9].

6. Summary

The present paper describes new and modified school experiments with fluorescent dyes and indicators. There are many options for varying the experiments presented, which can be explored by the school students, e.g. in projects or studies for scientific school competitions.

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