Food dyes as an alternative tracking dye for DNA gel electrophoresis

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Abstract:

The chemical, physical and toxicological effects on health of synthetic dyes that used as tracking dye in the electrophoresis requires seriously search about alternative tracking dye. The present study is aimed to find an alternative dye from safe food dyes which commonly used in food coloring. Five dyes were selected depending on their chemical properties and the availability in local market: Brilliant Blue FCF, Tartrazine, Sunset Yellow FCF, Carmoisine, and green traditional, three dyes were chosen to be mixed as loading buffer: Brilliant Blue FCF, Sunset Yellow FCF as a basic because it give the whole range size of most traditional loading buffers that available in market, and adding the Carmoisine as a new indicator for the bands less than 50bp, then mixed with DNA ladder in same percentage used with traditional loading buffers to clarify the effects of dyes on DNA, migrated on 1% agarose with loading buffer promega, results showed more clarity and highly readable separation of dyes and give wide range of size in the food loading mix than promega loading dye, by viewing the gel on UV light the DNA ladder were moved smoothly, bands separated effeminately on gel and in same rate of the DNA ladder that load with promega loading buffer which indicate no interaction between the food dyes and the DNA.Our studies show that the food dye can be used as a tracking dye in place of used synthetic dye. The procedure is found to be easy, practical, safely and reliable.

Key words: food dye, bromophenol blue, gel electrophoresis, tracking dye.

Introduction:

DNA agarose gel electrophoresis is one of the most reliable methods available for the separation of DNA [1]. Bromophenol blue is a tetrabromophenol sulfonaphthalein, widely used as a 'tracking dye' by the scientific community [2]. However traditional product now day available as a mixture of three dyes: Bromophenol blue, Xylene cyanol and orange G, this collection give a highly monitoring efficiency since In 1% agarose gels, Xylene cyanol typically migrates at about the same rate as a 4000 bp, Bromophenol blue migrates at about the same rate as a500bp and Orange G migrates at about the same rate as a 50bp [3].

The three dyes carry a slight negative charge at moderate pH, it will migrate in the same direction as DNA and protein in a gel and thus can be used as a marker ion front [4]. Material Safety Data Sheet (MSDS) of all standard companies providing the dyes are advised that due care must be exercised when handling this material. They may cause irritation with redness and pain when it comes in contact with the skin. In case of accidental inhalation, it may cause irritation to the respiratory tract. Symptoms may include coughing and shortness of breath. Furthermore, it

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may cause pain and irritation in the cornea when it comes in contact with the eye. It is well known that colorants from synthetic sources can be harmful and cause allergies in humans [5]. Therefore, interest in more safety dyes has increased considerably during the last few years [6]. Nowadays, fortunately, there is an increasing awareness among people towards the use of natural products as a substitute for synthetic dyes[7]. Due to their non-toxic property, low pollution and fewer side effects, synthetic food dyes are used more often in food products as well as for other important regular uses. Synthetic food dyes are considered to have fewer side effects, are less toxic, less polluting, less hazardous to health, non carcinogenic and non-poisonous. Of importance is the fact that they are environment-friendly and can be recycled after use [8].

In food technology, nearly five dyes that mostly used in food product, which are: Brilliant Blue FCF, Tartrazine, Sunset Yellow FCF, Carmoisine, and green traditional.

The present study is aimed in a preliminary manner, to find an additional and attractive suitable tracking dye for DNA agarose gel electrophoresis.

Materials and Methods:

Dyes migration and single dye loading buffer preparation: Five dyes were selected depending on their chemical properties and the availability in market: Brilliant Blue FCF, Tartrazine, Sunset Yellow FCF, Carmoisine, and green traditional will take from local markets as 50g of each. Each of these dyes will prepared as a loading buffer by mixing 0.25%g from each dye with 15% glycerol and complete with Tris/Borate/EDTA (TBE) buffer to 10ml, this mixture will give 6X buffer concentration**.** Each dye was mixed with water as 1µl dye: 6µl water, migrated in 1% agarose gel was prepared a according to [9], 0.5g of high pure agarose, promega dissolved in 50 ml 1 X TBE buffer, microwaved for 1 mint, the conditions were 5V/cm for 1hr ,1x TBE solution contains 0.089 M Tris base, 0.089 M Borate and 0.002 M EDTA were used as running buffer [9] with Blue/Orange Loading Dye, 6Xpromega Catalog # G1881 ,This solution is used for loading DNA samples onto gels. The buffer is designed to be used at a 1X final concentration. In a 0.5-1.4% agarose gel (in 0.5X TBE), xylene cyanol FF migrates at approximately 4 kb; bromophenol blue, approximately 300bp; and orange G, approximately 50bp.

Composition of Blue/Orange Loading Dye, 6X: 0.03% bromophenol blue, 0.03% xylene cyanol FF, 0.4% orange G, 15% Ficoll 400, 10mM Tris-HCl (pH 7.5) and 50mM EDTA (pH 8.0).

Mixed Loading buffer preparation: Three food dyes were selected (Brilliant Blue FCF,Sunset Yellow FCF and Carmoisine) to prepare the mixed loading buffer by mixing 0.25%g from each dye with 15% glycerol and complete with TBE buffer to 10ml to give 6X buffer concentration**.**The buffer was mixed with water as 1µl dye: 6µl BenchTop 100bp DNA Ladder, promega, Cat.: # G8291, migrated in 1% agarose gel was prepared a according to [9], the conditions were 5V/cm for 1hr ,1X TBE solution contains 0.089 M Tris base, 0.089 M Borate and 0.002 M EDTA were used as running buffer [9].The mixed buffer was migrate with promega loading buffer that contain Bromophenol blue, Xylene cyanol and orange G,0.25%g from each dye with 15% glycerol and complete with TBE

for comparison. Detection was attempt on white light and UV light using UV transilluminator.

Results and Discussions:

Dyes migration and single dye loading buffer preparation: Dye migrationon 1% agarose with promega loading buffer showed in figure (1): the brilliant blue FCF stopped in between Xylene cyanol and Bromophenol blue and give more bluish color than Bromophenol blue**,** this is obviously expected in regards to the molecular weight (MW)of Bromophenol blue (669.96 g/mol) and brilliant blue FCF (792.84 g/mol) [10].another expected for Xylene cyanol (538.61 g/mol) which has the smallest MW and should be the run faster in gel while it is the slower, may be the reason due to the net charge figure 2 [10, 11].

Sunset yellow give the same range size of orange G and give same color, this due to the Rapprochement in molecular weight (Sunset Yellow FCF452.37 g/mol and Orange G 452.38 g/mol), from figure 2 both has the same molecular structure and same net charge [12]. Tartrazine and Carmoisine, show range size less than orange G (less than 50 bp) and gave yellow color and red respectively [11] and green traditional show it was a mix of the Tartrazine and Brilliant Blue FCF so it can be excluded [12, 13]**.**

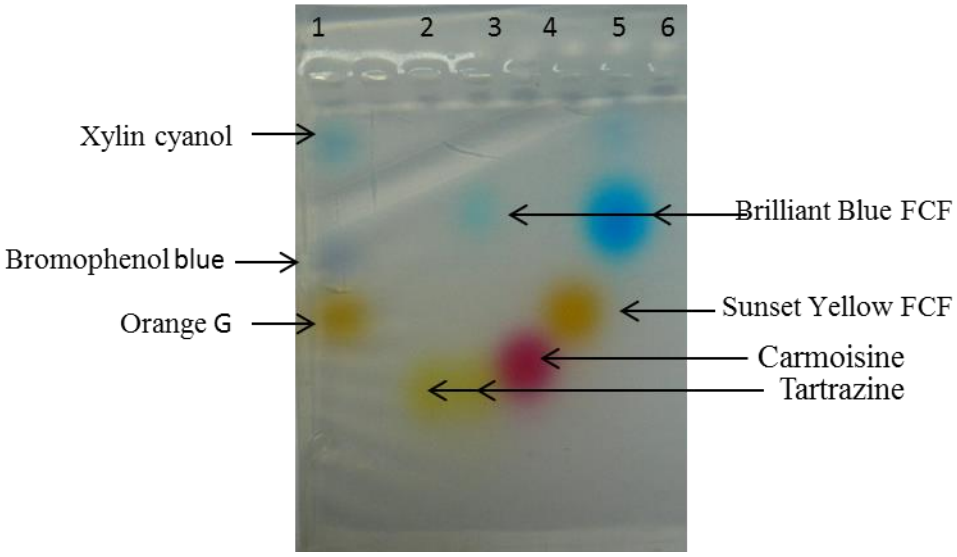

Fig.1: Electrophoreses of food dyes comparing with promega loading buffer. Lane 1: the loading buffer of promega which contain three mixed dyes Xylin cyanol, Bromophenol blue and Orange G, lane 2: Tartrazine, lane 3: green traditional, lane 4: Carmoisine, lane 5: Sunset Yellow FCF and lane 6: Brilliant Blue FCF. Electrophoresis carried on 1% agarose gel, 5V/cm at 1hr.

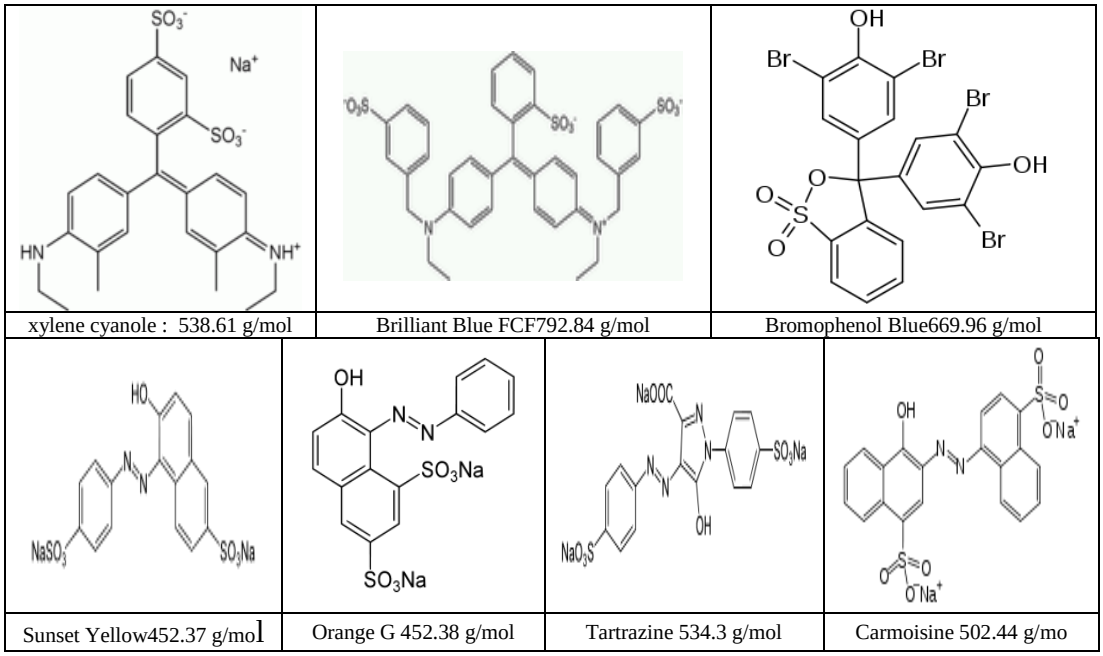

Fig. 2: The chemical structure of synthetic food dyes compare to the dyes that used in commercial kits.

Mixed Loading buffer preparation: Ongoing to drive a new mixed loading buffer depends on food dyes, three dyes were chosen to be mixed: Brilliant Blue FCF, Sunset Yellow FCF as a basic because it give the whole range size of most traditional loading buffers that available in market, and adding the Carmoisine as a new indicator for the bands less than 50bp [14] then mixed with DNA ladder in same percentage used with traditional loading buffers to clarify the effects of dyes on DNA were no effect should be appear on DNA moving on gel, the DNA ladder contains many bands, this will be more useful in detect any error that can be due to the dyes than using single band of DNA sample [15]. After migration on 1% agarose with loading buffer promega, results showed more clarity and highly readable separation of dyes and give very wide range of size range in the food dyes loading mix than promega loading dye figure 3, most of the food dyes is pH insensitive so it used in many foods with deferent ranges of pH, other synthetic dyes (like Bromophenol blue) is pH depended in color so in acidic pH it is red while in basic pH it is blue, this reason may be affect the clarity of color on gel with the progression of the migration and the ionic strength of the buffer will changed[16].

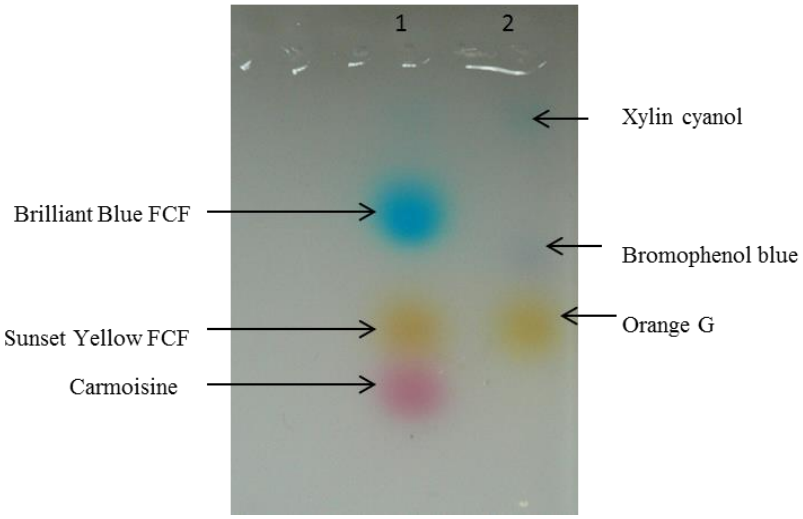

Fig. 3: Electrophoreses of food dyes loading buffer mix comparing with promega loading buffer. Lane 1: mix of three food dyes (Carmoisine, Sunset Yellow FCF and Brilliant Blue FCF), Lane 2: promega loading buffer (Orange G, Bromophenol blue and Xylene cyanol).Electrophoresis carried on 1% agarose gel, 5V/cm at 1hr.

By viewing the gel on UV Light the DNA ladder were moved smoothly, bands separated efficiently on gel and in same rate of the DNA ladder that load with promega loading buffer which indicate no interaction between the food dyes and the DNA figure 4, this expected result because all dyes carries a slight negative charge at moderate pH so they will migrate in the same direction as DNA or protein in a gel, in same time the negative charge on DNA made a repulsion with the negative charge of dyes[17]. Other more stain that attached to DNA should have genotoxic activity such as ethidium bromide which had the ability to insert between the two strands [18], other dyes such as Nile blue which is has no genotoxic activity bond to DNA depending on their positive charge by the attraction. [19]

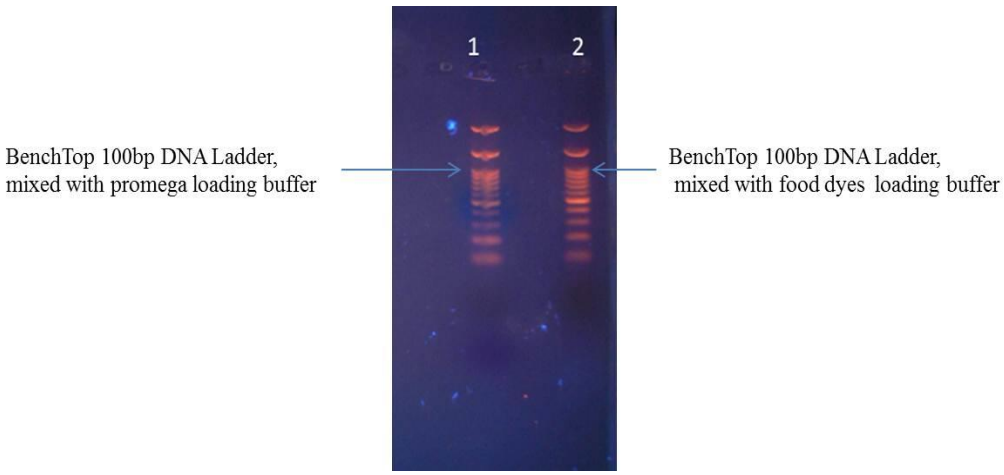

Fig. 4: Electrophoreses of DNA ladder (BenchTop 100bp DNA Ladder, promega, Cat.: # G8291) mixed with promega loading buffer comparing with food dyes loading buffer mix. Lane 1: DNA ladder mixed with promega loading buffer, Lane 2: DNA ladder mixed with food dyes loading buffer mix, Electrophoresis carried on 1% agarose gel, 5V/cm at 1hr.

Also we should refer to the economic feasibility of this study, as the costs of loading solution not less than 36 dollars for each as promega(Cat.: # G8291), while the cost of food dyes does not exceed one U.S. dollar is so cheap and are available in local market, in regards to the safety of food dyes it is enough to use in food industry, so it is often found in daily food. It is also used in soaps, shampoos, mouthwash and other hygiene and cosmetics applications [20].

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استعمال الصبغات الغذائية كبديل عن صبغات التعقب في الترحيل الكهربائي للحامض النووي

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الخلاصة :

التأثيرات الكيمياوية والفيزياوية والسمية للصبغات الصناعية المستعملة في الترحيل الكهربائي يتطلب البحث بشكل جدي عن صبغة بديلة. تهدف هذه الدراسة الى ايجاد صبغات امنة مستعملة في تلوين الاغذية وعليه تم انتقاء خمس صبغات متوفرة في السوق المحلية هي : Brilliant Blue FCF, Tartrazine, Sunset Yellow FCF, carmoisine, and green traditional وبعد ترحيلها تم اختيار ثلاثة منها لمزجها كدارئ تحميل ، فتم مزج Brilliant Blue FCF Sunset Yellow FCF لانها تغطي كل الاحجام الجزيئية المستعملة في دوارئ التحميل التجارية، ثم اضيفت صبغة carmoisine كدليل للحجوم التي هي اقل من 50 زوج قاعدة. حضرت هذه الصبغات كدارئ تحميل ومزجت مع الدليل الحجمي بنفس النسبة المتبعة عند مزج الدنا مع دوارئ التحميل التجارية و ذلك للتأكد من عدم وجود تأ ثيرات سلبية على الدنا عند مزجه . وعند ترحيله على هلام الاكاروز بنسبة 1% مع وجود دارئ ئ التحميل التجاري شركة promega اظهرت النتائج وضوح وفصل عالي ومقروء بشكل جيد لخليط الصبغات الغذائية وبمدى واسع من الاحجام الجزيئية مقارنة بدارئ التحميل التجاري وقد لوحظ انسيابية حركة الدليل الحجمي عند تعريض الهلام للاشعة فوق البنفسجية وانفصلت كما كانت نسبة الفصل بين الحزم مماثلة للدليل الحجمي الممزوج بدارئ التحميل التجاري مما يعني عدم وجود تداخل مابين الصبغات الغذائية المستعملة وحزم الدنا لذا اظهرت هذه الدراسة امكانية استعمال الصبغات الغذائية قيد الدراسة كصبغات تعقب للمسار وان هذه الطريقة تتمثل بانها امنة وسهلة التحضير ومتوفرة بكلف زهيدة .