Indian Journal of Biotechnology
Vol 13, January 2014, pp 136-139

Short Communications

Azo-based food colours as cost-effective and safe tracking dyes for qualitative electrophoretic analysis of nucleic acids and proteins

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Received 10 November 2012; revised 21 May 2013; accepted 18 July 2013

Synthetic organic colours that contain the azo group (-N=N-) are a major component of dyes widely used in the textile and food processing industries. Given the ready availability of food colours, their modest cost and established safety profiles, we evaluated their suitability as tracking dyes for the gel electrophoresis of nucleic acids and proteins. We have tested 3 azo-based food colours, *viz.*, Orange Red, Lemon Yellow and Bright Green, procured from local grocery stores for this purpose. The results are qualitatively comparable to those obtained using established methods of loading proteins and nucleic acids with the widely used dyes, bromophenol blue and xylene cyanol. This method entails no modification of current protocols for sample loading and gel electrophoresis. Use of these reagents that are 'commonly considered safe' and available at a fraction of the cost of equivalent analytical grade reagents would be advantageous in routine molecular biology procedures, especially in resource-limited contexts.

Keywords: Azo dye, bromophenol blue, food colours, gel electrophoresis, synthetic organic colours, xylene cyanol

Gel electrophoresis is a technique that is routinely and widely used to separate nucleic acids and proteins. The choice of a specific loading dye depends on the molecular mass of the species of interest, in order to ensure that the dye does not obscure species of interest in the gel because of co-migration. Two triarylmethane dyes, xylene cyanol (XC) and bromophenol blue (BB), and an azo dye, Orange G (OG), are commonly used as tracking dyes during the gel electrophoresis of nucleic acids and proteins. XC and BB exhibit an electrophoretic mobility that is consistently less than that of azo dyes, and may co-migrate and obscure higher mol wt bands of interest during the agarose gel electrophoresis of nucleic acids. This is a problem, especially when prior information on the mol wt of the species of interest is not available ahead of gel loading. In contrast, OG migrates more rapidly through gels than either XC or BB and can overcome the problem of co-migration. However, these three dyes are quite expensive, costing at least twenty-four Indian rupees per gram or more, depending on the source (Table 1). Therefore, we examined whether other azo dyes (a category to which OG belongs) used commonly as food colours could substitute for XC, BB and OG in gel electrophoresis.

In the study, three types of food colours were bought from a local grocery store in New Delhi, India. Their detailed compositions and cost comparison are shown in Table 1. The azo dyes present in these food colours are tartrazine, carmoisine, sunset yellow FCF and brilliant blue FCF; sometimes as mixtures as in the case of orange red (OR) and bright green (BG). Stock solutions of loading dye were prepared as follows: 10 mg of the food colour was weighed out into a 1.5 mL Eppendorf tube and suspended in 1 mL of absolute alcohol by briefly vortexing for sterilization, and incubated overnight at room temperature (RT). The dye pellet was recovered the next day by a brief centrifugation at 14,000× g in an Eppendorf centrifuge, washed twice with 70% ethanol and re-centrifuged. The 70% ethanol washes were carried out because food colours typically have considerable amounts of salt (NaCl) (Table 1) that could potentially interfere with gel electrophoresis. The dye pellet was then kept in incubator at 37°C overnight for drying to remove traces of ethanol and incorporated into various loading dyes for different situations as described below. Agarose or SDS-polyacrylamide gel electrophoresis and visualization of samples were carried out as per standard protocols[1].

Loading buffers for DNA were prepared as a 6× concentrate containing Tris-EDTA (TE) buffer, pH 8.0, 30% glycerol and the loading dye of choice (~1 mg/mL). The concentrate was mixed with the appropriate volume of nucleic acid sample (an aliquot of phage λ DNA digested with *Hind*III)

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Competing Interests: The authors have no competing interests to declare.

Table 1—Composition and relative cost of dyes used in loading buffers

Dye	Composition	Chemical category	Source	Cost (INR/g)*
Orange Red (Food colour)	Sodium chloride, Sunset Yellow FCF, Carmoisine 30.2% dye content	Azo	Ajanta Industries Pvt. Ltd., India	2.32
Lemon Yellow (Food colour)	Sodium chloride, Tartrazine 22.2% dye content	Azo	-do-	3.15
Bright Green (Food clour)	Sodium chloride, Tartrazine, Brilliant Blue FCF 17.2% dye content	Azo	-do-	4.07
Bromophenol blue	Bromophenol blue	Triarylmethane	Assorted laboratory suppliers	40-160
Xylene cyanol	Xylene cyanol, Methanol > 46%, Moisture < 10%	Triarylmethane	-do-	110-250
Orange G, sodium salt	$C_{16}H_{10}N_2Na_2O_7S_2$	Azo	-do-	24-80

*Cost is given in Indian rupees per gram (INR/g) after correcting for the amount of NaCl in food colours
Prices given for reagent grade BB, XC and OG are approximate

and loaded on the same gel along with aliquots to which loading dyes containing BB or XC was added. Gel electrophoresis was carried out using both Tris-acetate-EDTA (TAE) and Tris-borate-EDTA (TBE) buffer systems. We find no qualitative differences between the results using food colours and conventional dyes in either buffer system (Figs 1B & C).

After extraction of RNA, it is common practice to run the samples on a non-denaturing agarose gel (TAE or TBE) to get a quick estimate of the quality and quantity of material obtained. RNA extracted from the leaves of *Brassica* spp. by the Trizol® was obtained, and prepared for gel electrophoresis in 83% de-ionized formamide, 10 mM EDTA and the tracking dye of choice (~1 mg/mL). These samples were denatured at 65°C for 10 min, followed by immediate cooling on ice for 3-4 min before being loaded. We found out that the results were qualitatively indistinguishable from those obtained using reagent grade XC and BB (Figs 1D & E), notwithstanding the differences in the amounts loaded in each lane. Moreover, no signs of sample degradation were observable.

In the case of SDS-PAGE of protein, we prepared loading dye (3× concentration) that contained 250 mM Tris-Cl pH 6.8, 6% SDS, 30% glycerol, 16% β-mercaptoethanol and 1 mg/mL of the dye. Purified BSA (Sigma, 1 mg/mL) was the protein sample used. The samples (containing dyes) were denatured at 100°C for 5 min, followed by immediate cooling on ice, and then loaded on the polyacrylamide gel. SDS-PAGE was carried out in a Tris-glycine buffer system. Upon staining the polyacrylamide gel with Coomassie blue, it was found that the results were qualitatively similar to those obtained using reagent grade BB (Figs 2A & B).

The foregoing observations indicate that food colours are viable and highly cost-effective alternatives to conventional tracking dyes. A notable advantage of using these colours is that they co-migrate with only the very lowest mol wt bands during gel electrophoresis as compared to BB and XC (Figs 1A & 2A). In fact, all the three exhibit higher electrophoretic mobilities than BB. This fact allows us to visualize nucleic acid bands over a wider range of mol wt with ultraviolet transillumination, without their being obscured by the tracking dye as often happens in the case of BB and XC (compare lanes 2 & 3 with 4, 5 & 6 in Figs 1B & C). The higher migration rate of food colours also has the effect of shortening the running time required for effective observation and photographing of the gel electrophoresis of nucleic acids. In the case of the SDS-PAGE of proteins, though the problem of obscuring bands of interest is not of concern because of these gels are typically viewed in white light after Coomassie blue staining. Here, the use of food colours saves on the running cost alone. In general, food colours are suitable for the electrophoresis of nucleic acids and proteins in both agarose and SDS-PAGE, respectively. As for nucleic acids, the above protocols are applicable to both TAE- and TBE-based agarose gel electrophoresis.

Systematic investigations of the suitability of alternative loading dyes for electrophoresis are between few and far. However, food colours

(unmixed with nucleic acids or proteins, though) are routinely used for demonstrations of electrophoresis in educational settings (http://biotech.biology.arizona.edu/labs/Electrophoresis_fdclr_teac.html). In

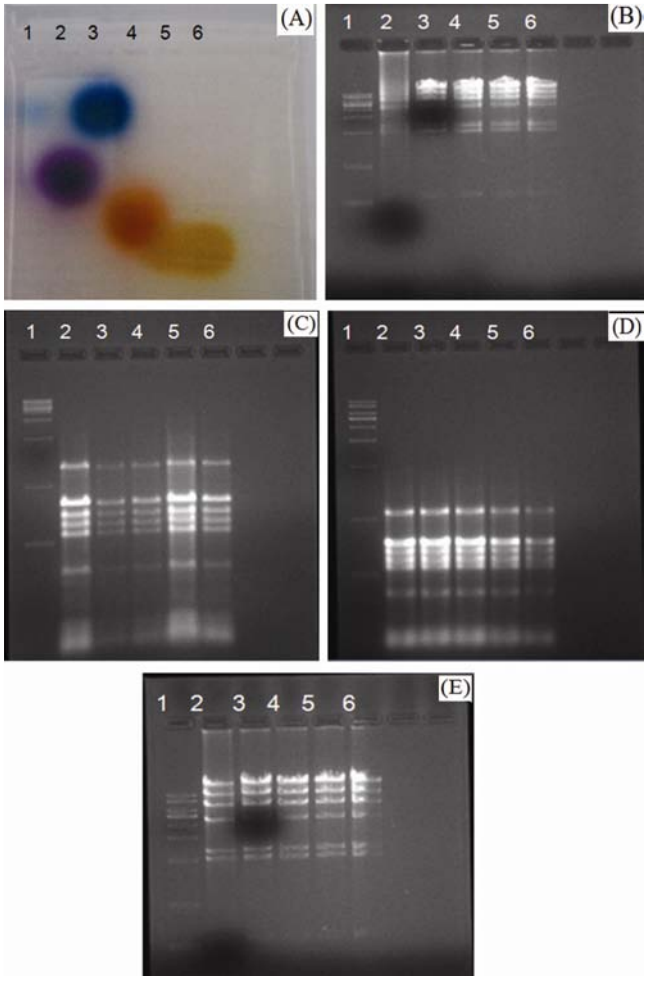

Fig. 1—Electrophoresis of nucleic acids using food colours: (A) Electrophoresis of loading dyes alone on a TAE agarose gel indicating their relative mobilities. [Lane 1, XC and BB; Lane 2, BB; Lane 3, XC; Lane 4, OR; Lane 5, BG; Lane 6, LY. OR, BG, and LY in lanes 4-6 migrate (lane 6) much faster than XC and BB, eliminating the problem of co-migration with higher mol wt species. Bright green and lemon yellow migrate at practically the same rate in agarose gels, resulting in the large spot of colour across lanes 5 and 6.] (B) and (C) *Hind*III digest of λ DNA electrophoresed on 1% agarose gels using 0.5× TBE and 1× TAE buffer systems, respectively. [Lane 1, 1 kb Sharp DNA marker premixed with both XC and BB (Real Biotech Corp.); Lanes 2-6, Aliquots of the *Hind*III digest of λ DNA mixed with BB, XC, OR, BG and LY, respectively.] (D) and (E) *Brassica* spp. RNA electrophoresed on 1% agarose gels using non-denaturing 0.5× TBE and 1× TAE buffer systems, respectively. [Lane 1, 1 kb Sharp DNA marker premixed with both XC and BB (Real Biotech Corp.); Lanes 2-6, Aliquots of *Brassica* spp. RNA mixed with BB, XC, OR, BG and LY respectively.] [Gel A was photographed in white light, and gels (B-E) were photographed under UV transillumination by ethidium bromide fluorescence]

one detailed study that we located, the commonly used annatto dye, a food colourant obtained from pericarps of seeds of the achiote tree (*Bixa orellana* L.), was found to be suitable for SDS-PAGE for both qualitative and subsequent analytical purposes, such as, mass spectrometry[2] .

The present work indicates that at least three commonly available azo-based food colours can be used for routine qualitative experiments, at the very least, after incorporating an easy stock preparation step and involving no modification of existing protocols for electrophoresis or gel loading. The food colours used do not interfere with the migration rates and integrity of nucleic acids or proteins. Moreoever, these dyes are used as food colouring and are considered safe at levels of normal use. The European Food Safety Authority has indicated that the azo dyes used herein have an acceptable safety profile when used as food colours at the level of milligrams per kilogram of the body wt per day. Here, during routine electrophoresis, the dye actually handled amounts to a milligram at the most (in the form of stock solutions)[3-5]. Actual aliquots taken in µL would have only microgram quantities of the dyes that would not translate to any significant risk to the operator. As for BB, XC and OG, there is very little information available on their toxicity to humans or animals. In the material safety data sheets provided with the reagents by various suppliers, we found that OG, BB and XC are classified as irritants for skin and mucous membranes. Incidentally, OG (an azo dye) is metabolized to aniline in humans, which could have potentially harmful effects, indicating that not all azo dyes are necessarily "safe"[6]. However, the choice of

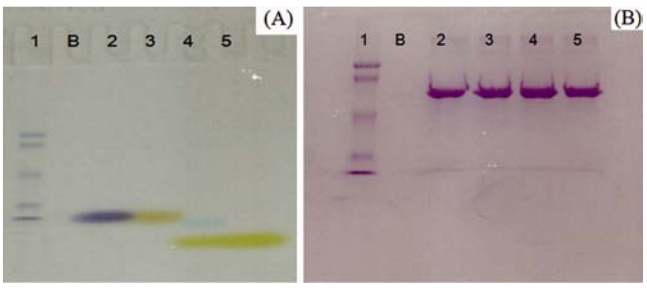

Fig. 2—SDS-PAGE of protein using food colours: (A) Electrophoresis of BSA (1 mg/mL) with BB or food colours in the loading solution, before staining of the gel; (B) Coomassie-staining of the polyacrylamide gel from (A). [Lane 1, Pre-stained marker (BioRad); Lane B, Blank lane without sample, Lanes 2-5, Aliquots of BSA mixed with BB, OR, BG and LY, respectively.]

those azo dyes, which are commonly considered safe for gel electrophoresis, substantially minimizes recurring costs and enhances the safety profile of at least one essential and highly used laboratory reagent. This is especially relevant in the context of resource-limited teaching laboratories in our country.

Acknowledgement

SR was the recipient of a Junior Research Fellowship (DLS/81/48222/LSRB-197/ID/2009) from the Life Sciences Research Board, Defence Research and Development Organization (DRDO), Government of India, New Delhi. Work in our laboratory has been supported by grants from the Life Sciences Research Board, DRDO, New Delhi and the Department of Biotechnology, Government of India, New Delhi. We thank Ms Nisha Sinha, Mr Abhishek Saxena and Mr Shivaraj S M for technical assistance, Dr Anandita Singh for RNA samples and Mr Hari Ram Gupta for consistent logistical support.

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