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Green, fast and cheap paper-based method for estimating equivalence ratio of cationic carriers to DNA in gene delivery formulations

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ABSTRACT

To achieve efficient and safe cationic carrier-mediated gene delivery for gene therapy, the optimal ratio of carrier to DNA in formulations is a key factor and it is usually determined prior to transfection experiments. In this work, a simplified drop-and-read assay was developed for the first time using paper as a platform to estimate the equivalence ratio of cationic carriers to negatively charged DNA. By spotting a series of complexes containing varied ratios of carrier to DNA on filter paper, then allowing them to dry and finally dropping yellowish-green anionic 2′,7′-dichlorofluorescein dye solution on top of the complexes, the equivalence point was detectable by the instant formation of stable pink spots as a result of the dye adsorption onto the positively charged complexes and free carriers. The method gave the same results as those determined by gel retardation assay and zeta potential measurement, however it allowed more rapid reporting of results in 5 min and required no tedious steps, harmful reagents or expensive instruments. By using paper instead of microcentrifuge tubes and omitting centrifugation, plasticware and electrical energy were no longer consumed and disposal of this degradable material was more environmentally friendly. With respect to analytical performance, filter paper inherently holding negative charge helped to trap and concentrate the complexes on the white background, enabling greater visibility of the colored spots and a lower required amount of DNA used for the assay. The method was successfully applied to estimate the equivalence ratios in a variety of gene delivery formulations containing different types of cationic carriers, i.e. polymers, dendrimers, liposomes and niosomes.

1. Introduction

Lab-on-paper is a powerful and attractive way to develop budget, user- and environmentally friendly assays since methods performed in this format are mainly designed for convenience, equipment-free operation and low consumption of samples/reagents as well as electrical power (Dou et al., 2015; Hu et al., 2014; Li et al., 2012; Sharma et al., 2018). From a general viewpoint, paper is an inexpensive, disposal and degradable material. As an analytical device, paper made typically from cellulose fibers possesses a high porosity and surface-to-volume ratio, thereby being capable of adsorbing and allowing liquids to penetrate by capillary flow without the need for external pieces of equipment such as pumps. In addition, the white background of paper enables obvious readouts of colored results with the naked eye. These advantages have highlighted paper as one of the most popular platforms for assays of various types of analytes including small molecules and biomolecules (Cate et al., 2015; Chen et al., 2012; Dungchai et al., 2010). For nucleic acid related work, paper-based tests and devices have been widely developed and used for the extraction, amplification and detection of the DNA/RNA of infectious pathogens (Magro et al., 2017; Rodriguez et al., 2015; Teengam et al., 2017; Zhang et al., 2014) and for the differentiation of DNA from different sources (Araújo et al., 2012). These devices are ideally suited for point-of-care diagnostics and meet the World Health Organization ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end users) criteria (Lee et al., 2010; Martinez et al., 2010).

Apart from the use of nucleic acid for diagnostic purposes, the delivery of exogenous DNA or RNA into a patient's cells in order to correct gene expression – so-called “gene therapy” – is currently a potential strategy in medicine for the treatment of certain diseases e.g. genetic abnormalities, autoimmune disorders and cancers (Foldvari et al., 2016; Ibraheem et al., 2014). To achieve successful gene transfer and

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therapeutic outcomes, meaning that the transfection efficiency is high and no apparent toxicity is obtained, proper kinds and amounts of transfection reagents must be used for incorporation with the delivered genes. In gene transfection mediated by non-viral, cationic carriers, e.g. cationic polymers and cationic lipids where the carriers form small and compact complexes with negatively charged DNA via electrostatic interactions and help to protect the DNA from degradation and facilitate its cellular uptake and intracellular traffic into the nucleus, the charge ratio of the carriers to DNA has been found to correlate with transfection efficiency (Huang et al., 2013; Jin et al., 2014; Reed et al., 2006). At low carrier to DNA ratios, DNA condenses poorly resulting in large particles with the remaining negative charges entering cells inefficiently. Meanwhile, a ratio of carrier to DNA which is too high leads to the formation of extremely positively charged complexes as well as excessive free cationic carriers in the formulation which interact with negatively charged components of the cell membranes and unfavorably cause membrane disruption (Hunter, 2006; Lv et al., 2006). Practically, a particular ratio which produces a slightly positive net charge on the surface of complexes is desirable (Mansouri et al., 2006; Zhao et al., 2009) and therefore determined by physicochemical methods and optimized prior to transfection and biological evaluation.

For the estimation of the ratio, gel retardation assay and zeta potential analysis are the two most commonly used methods (Amin et al., 2016; He et al., 2013; Honary and Zahir, 2013; Plianwong et al., 2013a). The first one relies on the electrophoresis of complexes at varying carrier to DNA ratios. At a ratio where the carriers form equivalent complexes by charge with DNA resulting in neutral particles, no migration of DNA to the gel is observed. Despite being regarded as an accustomed technique in many laboratories, a gel retardation assay consists of many time-consuming steps, i.e. gel casting, sample running and final staining. Moreover, ethidium bromide which is used as a DNA dye is carcinogenic and harmful to the environment (Singer et al., 1999). In zeta potential analysis, the surface charges of complexes are measured, usually by dynamic light scattering optical devices. The equivalence ratio is indicated by a zero or slightly positive zeta potential measurement value. This method is convenient and capable of reporting quantitative results, however the major drawback is the high-cost of the equipment involved. Previously, we reported a new method based on dye adsorption (Plianwong et al., 2013b). In this assay, yellowish-green, anionic 2′,7′-dichlorofluorescein dye is added to the carrier/DNA complexes in microcentrifuge tubes. After centrifugation of the mixture to sediment, the equivalence ratio is indicated by the formation of pink pellets as a result of the dye's color change from green to pink once it is adsorbed onto the positively charged complexes. This method was shown to give accurate results with a rapid analysis time of less than 10 min when tested with a cationic polymer, namely polyethyleneimine (PEI), without the use of expensive zetasizer, tedious gel electrophoresis or harmful dye. Though promising, some weak points, i.e. consumption of electrical energy in the centrifugation step and release of plastic tube waste after the assay, were identified when considering the green analytical chemistry metrics (Gałuszka et al., 2013; Tobiszewski, 2016). In addition, the observation of the faint pink color of the pellets or smears through the walls of the tubes was sometimes unclear.

In the work reported here, we detail the further development of a greener version of the 2′,7′-dichlorofluorescein-based method by performing the assay on filter paper, not only to exclude the use of plastic tubes and the centrifugation step, but also to facilitate the readout of results. Moreover, besides the cationic polymer PEI, other types of gene carriers including cationic dendrimers, cationic liposomes and cationic niosomes were used to assess the applicability of the proposed method.

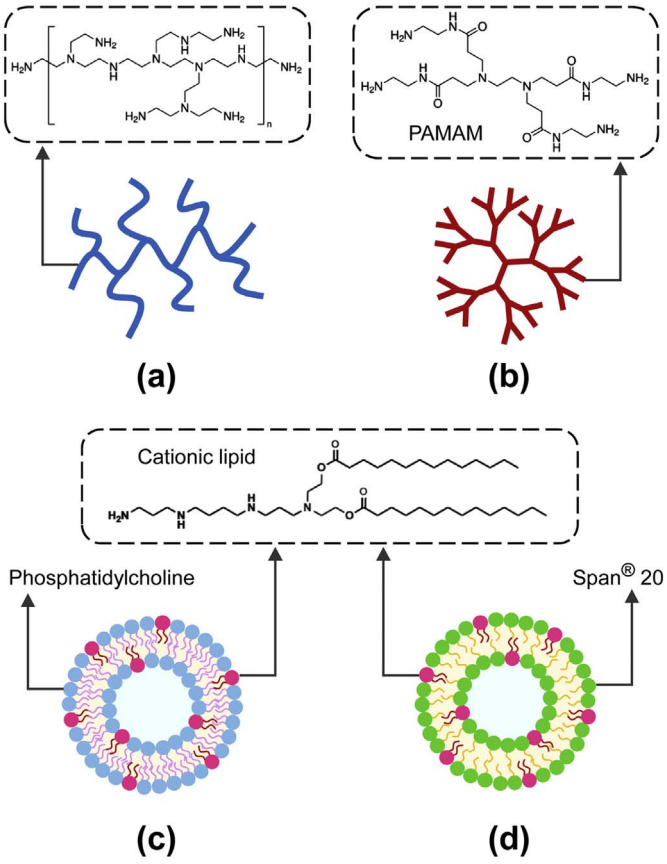


Fig. 1. Cationic carriers for gene delivery used in this study: (a) PEI cationic polymer, (b) SuperFect® cationic dendrimer, (c) cationic liposome, and (d) cationic niosome.

2. Experimental

2.1. Materials

Filter papers were purchased from Whatman International Ltd. (Maidstone, England). 2′,7′-Dichlorofluorescein was obtained from Fluka (Buchs, Switzerland). Branched PEI (molecular weight of 25 kDa) cationic polymer was purchased from Sigma-Aldrich (Munich, Germany) and SuperFect™ cationic dendrimer was commercially obtained from Qiagen (Hilden, Germany). The cationic lipid (Fig. 1) used for the preparation of cationic liposomes and niosomes was kindly provided by Dr. Boon-ek Yingyongnarongkul, Faculty of Science, Ramkhamhaeng University, Thailand.

Plasmid DNA encoding enhanced green fluorescent protein (pEGFP-C2) was cloned in *Escherichia coli* and purified using plasmid midi kits (Qiagen, Hilden, Germany). After purification, the size (4.7 kbp) was verified by digestion by the *Eco*RI restriction enzyme. The quality and quantity of plasmid were assessed by its optical density at 260 nm and 280 nm. The purified plasmid was kept in a Tris-EDTA buffer (pH 7.5) at 4 °C.

2.2. Preparation of cationic carriers and formulation of carrier/DNA complexes

Four types of cationic carriers were used in this study (Fig. 1). PEI (25 kDa), which is a highly branched polymer with primary, secondary and tertiary amine groups and is widely accepted as the gold standard in gene delivery (Patnaik and Gupta, 2013), was employed as the representative cationic polymer. SuperFect® was used as the cationic dendrimer. This transfection reagent is fractured or activated generation 6, poly(amidoamine) (PAMAM) dendrimer with a tree-like

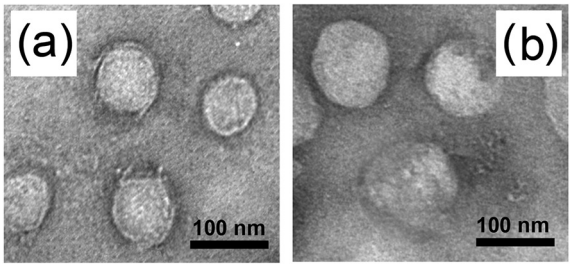


Fig. 2. Transmission electron microscopic images of (a) cationic liposomes and (b) cationic niosomes prepared in this study.

spherical architecture with branches radiating from a central core and terminating at charged amine groups. Cationic liposomes and cationic niosomes were prepared in the laboratory using a slightly modified thin film hydration with sonication method, as described by Paecharoenchai et al. (Paecharoenchai et al., 2014). These two types of lipid-based carriers contained cationic lipid (molecular weight of 711), which is composed of spermine, as the cationic moiety, and the di(oxyethyl) amino group as the central core structure (Fig. 1). Briefly, the liposomes were prepared in a glass tube by adding a solution of cationic lipid to a solution of phosphatidylcholine and cholesterol to obtain a mixture of phosphatidylcholine/cholesterol/cationic lipid in a molar ratio of 5:5:1. The organic solvents were then removed by evaporation under nitrogen to produce a dry thin film at the bottom of the tube. Finally, the film was hydrated by adding Tris buffer (pH 7.4) and subjected to probe-sonication on ice for 30 min. In the case of cationic niosomes, the preparation protocol was the same as that of the liposomes except that a non-ionic surfactant, namely sorbitan monolaurate (Span® 20), was used instead of phosphatidylcholine. Both liposomes and niosomes were spherical in shape as seen under a transmission electron microscope (TEM) (Fig. 2). An analysis using a zetasizer revealed that the liposomes and niosomes had diameters of 92 ± 7 nm and 120 ± 9, and they exhibited positive surface charges of +41.9 ± 2.3 mV and +43.0 ± 4.2 mV respectively.

To formulate the complexes of carrier to DNA at varying ratios, different amounts of cationic carrier solution were added to fixed 1 μg solutions of DNA. The solutions were mixed by being gently pipetted up and down for 3–5 s and then left at room temperature for 30 min. For the SuperFect® dendrimer, the incubation time was 10 min as recommended by the manufacturer's handbook.

2.3. Estimation of the equivalence ratio of cationic carriers to DNA

2.3.1. Paper-based method

The determination of the optimal ratio at which the cationic carriers formed equivalent complexes by charge with DNA was carried out by dropping 10 μL of carrier/DNA complex solutions in a series in order of increasing carrier to DNA ratios onto filter paper with the aid of a micropipette. The positive and negative controls, i.e. carrier solutions in the amounts corresponding to tested complexes but without DNA, and a solution containing 1 μg DNA, were spotted in parallel to a line of carrier/DNA complex spots. After the spots were briefly allowed to dry in the air or with the aid of a hair dryer, 3 μL of 2′,7′-dichlorofluorescein solution was dropped on top of the spots and the formation of pink spots occurred immediately and was observable with the naked eye. The equivalence ratio of carrier to DNA was indicated by the first pink spot in the series of the carrier/DNA complexes tested. To validate the visibility of the pink color, at least one point before the optimal ratio in a set of the positive controls had to be pink. In a negative control, the spot had to be yellowish-green. The experiments were done in triplicate.

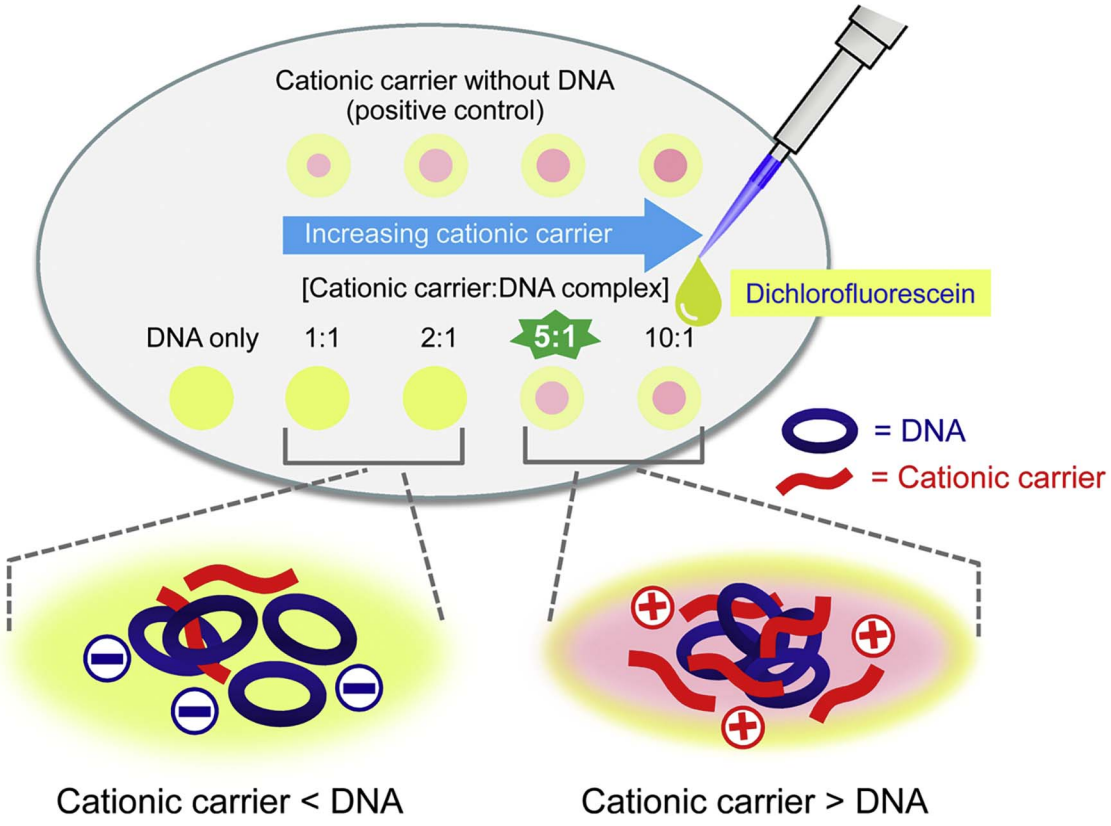


Fig. 3. Paper-based method for estimation of the equivalence ratio of cationic carriers to DNA. This point (carrier ≥ DNA) is marked by the first pink spot formed (for example, a ratio of 5:1 in the figure) when the yellow-green dye, namely 2′,7′-dichlorofluorescein, is attracted to adsorb onto the positively charged complexes and excess of carriers fixed on the filter paper. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

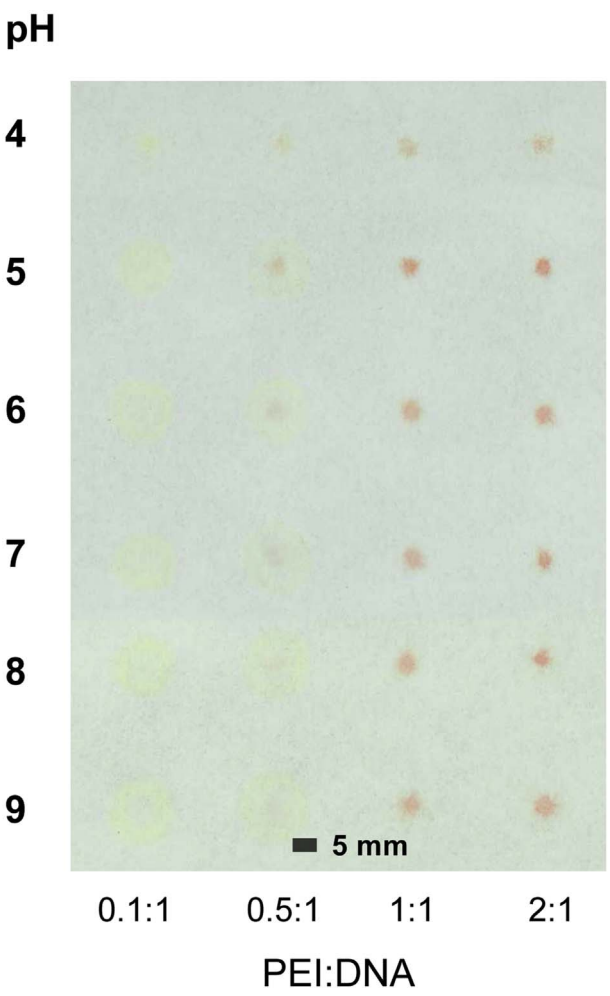


Fig. 4. Effect of pH of dye solution on the formation of pink spots. Here, PEI/DNA complexes were tested on Whatman® filter paper no. 1 and 0.075 mg/mL 2′,7′-dichlorofluorescein solutions at varied pHs were used. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3.2. In-tube dye adsorption method

The in-tube method followed the procedures described previously (Plianwong et al., 2013b). Five microliters of 0.075 mg/mL 2′,7′-dichlorofluorescein solution (pH 8) was added to each of a set of microcentrifuge tubes containing 30 μL of the solutions of complexes composed of various carrier to DNA ratios. The solutions were then mixed and centrifuged at 20,000 rpm for 5 min to settle the complexes. To discard the excess and unadsorbed dye, pellets were washed with sterile water and centrifuged and the supernatant was finally removed. The optimal carrier to DNA ratio for complex formation was determined by visual observation of the first tube with a pink smear or with pink pellets at the bottom of the tube. The experiments were done in triplicate.

2.3.3. Gel retardation assay

The gel retardation assay was conducted by loading free DNA (pEGFP-C2) and carrier/DNA complexes at various ratios onto 0.8% agarose gel and subjecting it to electrophoresis in a 1X Tris-acetate-EDTA buffer under a constant electric field (100 V) at room temperature for 45 min. Subsequently, the gel was stained with 0.5 mg/mL ethidium bromide. The bands were visualized and photographed by a UV transilluminator using a GelDoc system. The experiments were done in triplicate.

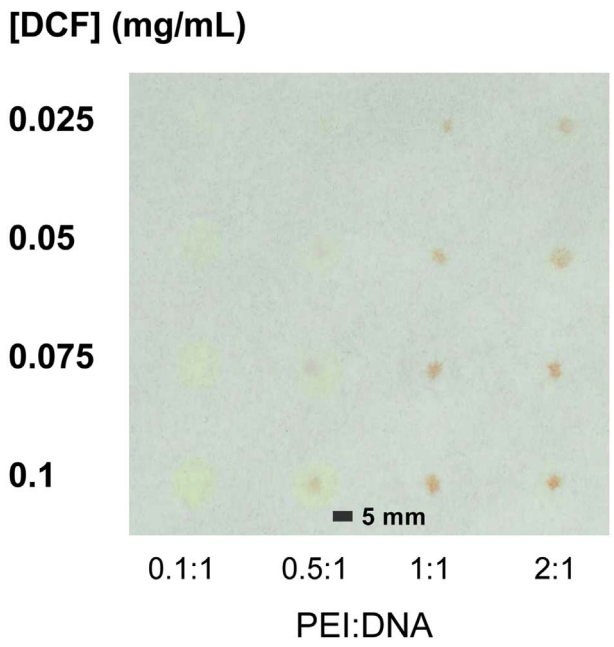


Fig. 5. Effect of concentration of dye solution on the formation of pink spots. Here, PEI/DNA complexes were tested on Whatman® filter paper no. 1 using 2′,7′-dichlorofluorescein solutions at pH 5, but of different concentrations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

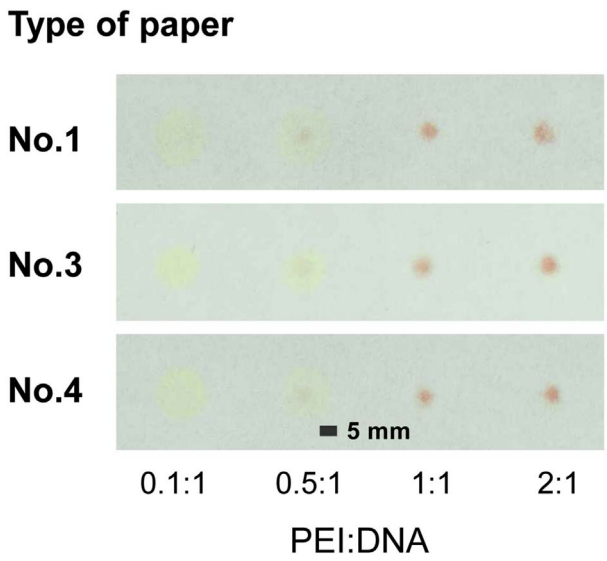


Fig. 6. Effect of types of filter paper on the formation of pink spots. Here, PEI/DNA complexes were tested using 0.075 mg/mL 2′,7′-dichlorofluorescein solution, pH 5. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3.4. Zeta potential analysis

The surface charges of the carrier/DNA complexes were measured using electrophoretic light scattering as zeta potential values at 25 °C using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). All the samples were diluted with water before analysis and measurements were performed three times for each replicate. Zeta potential values were reported as mean ± standard deviation.

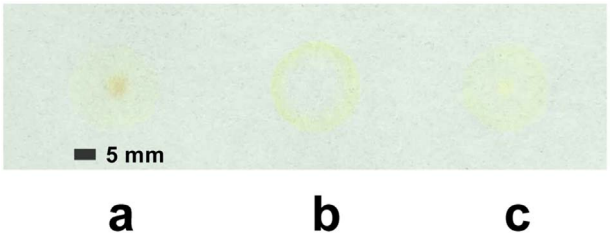


Fig. 7. Effect of order-of-dropping on the formation of pink spots: (a) complexes then dye, (b) dye then complexes, (c) complexes pre-mixed with dye. Here, PEI/DNA complexes were tested on Whatman® filter paper No. 1 using 0.075 mg/mL 2′,7′-dichlorofluorescein, at a pH of 5. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

3.1. Principles of the paper-based method

Even though the previously reported dye adsorption-based method, performed in microcentrifuge tubes, offers several advantages for estimating the equivalence ratio of cationic carriers to DNA in formulations aimed for gene delivery (Araújo et al., 2012), it can be further refined to be greener and faster as well as having more user-friendly and cost-saving features. This improvement is achieved by changing from in-tube testing to a paper platform (Fig. 3). Using this approach, small volumes of carrier/DNA complex solutions at varied ratios are dropped onto filter paper. As paper consists of cellulose fiber networks which are negatively charged due to the presence of acidic groups, e.g. hydroxyl or carboxyl groups that either originate from cell wall constituents or are introduced during pulping, it acts as an adsorbent for positively charged molecules (Dagaonkar and Majumdar, 2012; Sood et al., 2010). In agreement with the results of our experiments, Whatman® filter papers showed a negative surface charge with a zeta potential value of about −30 mV. This material therefore helps not only to dry the drops of liquid using its blotting ability, but also to trap the positively charged carrier/DNA complexes and free cationic carriers densely in a confined area by charge interaction. This demonstrates the role of cellulosic paper in helping to concentrate charged samples on this material which has never before been reported of any paper analytical devices. Once a yellowish-green dye solution is applied to the dried tested spots, dichlorofluoresceinate ions are attracted to adhere to the positively charged molecules retained on the paper, leading to an immediate change of the green color to pink which can be easily observed. This phenomenon relies on the same basis which is used for the detection of the endpoint in Fajan's argentometric titration (Fajans and Hassel, 1929; Kolthoff et al., 1929). To avoid false negative results caused by the limited capability of the human eye when a faint pink color is formed due to the low amount of carrier used, another set of carrier-only spots in the same varied amounts are run in parallel as positive controls. To obtain valid results, the equivalence ratio is read from the first pink spot in the carrier/DNA series and at least one point before the optimal ratio in the control set must also be pink. In the assay, a drop of DNA only is also included as a negative control to ensure that this negatively charged molecule does not turn pink with the dye.

3.2. Optimization of method

3.2.1. pH of dye solution

Since pH plays an important role, not only in the dissociation of 2′,7′-dichlorofluorescein, which is a weak acid (pKa ≈ 8.1) resulting in the availability of the dichlorofluoresceinate ion, but also the ionization of the carriers which may affect the electrostatic attraction of the dye to adsorb onto their surface, the optimal pH of the dye solution was investigated. As shown in Fig. 4, a pH range of 5 to 6 was found to be the most appropriate since it produced distinctly colored results. At a too low pH value (pH 4), the dichlorofluoresceinate ion was present in the solution at a low concentration due to the poor dissociation of the acid. Conversely, at too high pH levels (pH 8 and 9), OH^- in the dye solution might compete with the dichlorofluoresceinate ion to adsorb onto the carrier/DNA complexes and free carriers fixed on the paper. Therefore, 2′,7′-dichlorofluorescein solution with a pH value of 5 was chosen for use.

3.2.2. Concentration of dye solution

The amount of DNA in the complexes was fixed at 1 μg, and varied concentrations of 2′,7′-dichlorofluorescein solution at pH 5 were tested, the results being shown in Fig. 5. The use of 2′,7′-dichlorofluorescein at 0.025 mg/mL resulted in a pink spot which was too pale at the optimal ratio and might have led to misinterpretation of the results. Increasing the dye concentrations enhanced the intensity of the pink spots as well as the yellow backgrounds. For the optimal concentration which was adequate for the formation of obvious pink spots, and at the same time was not too high to produce an interfering background and wastage of reagents, 0.075 mg/mL of dye was used.

3.2.3. Type of filter paper

A variety of Whatman® filter papers with different characteristics are available and it was possible that each type could have specific performance characteristics which would be better suited for the proposed experiment. Three types of paper were therefore selected for the comparison. Whatman® filter paper No. 1 was chosen because it is a general-purpose paper and is typically available in laboratories. No. 3 is unique for its thickness and high loading capacity, and No. 4 allows fluid to flow through at a greater flow rate. The results (Fig. 6) show that there was no significant difference between these three types of paper in terms of drying speed of liquid drops and appearance of colored spots. Typically, the size of pink spots was about 3–5 mm. Any of the three Whatman® filter papers could have been used as effective supporting materials with comparable performances, however in this study Whatman® filter paper No.1 was chosen for use due to its ease of availability.

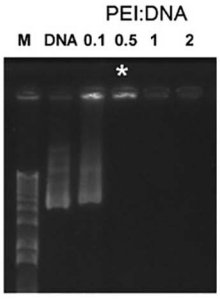
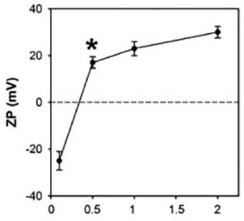
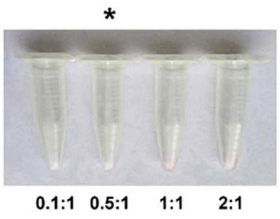
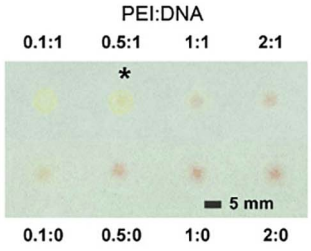
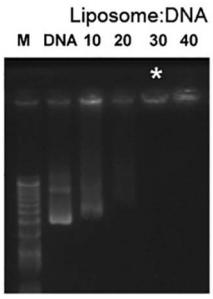
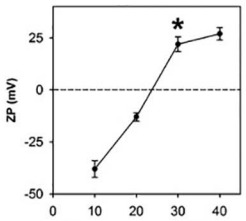
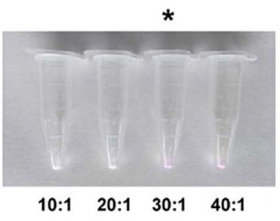
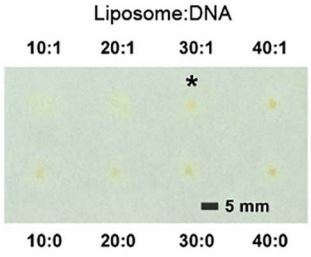
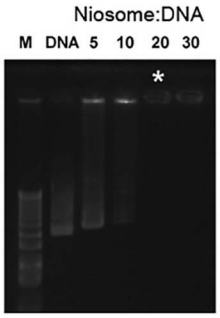
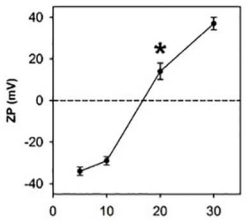
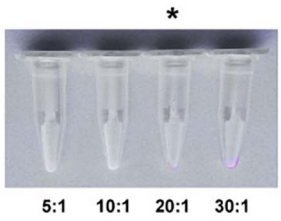
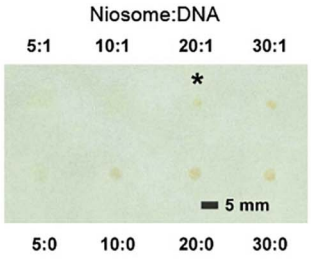
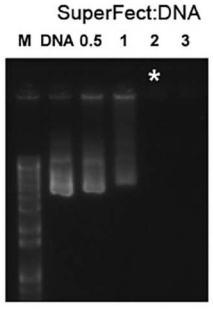
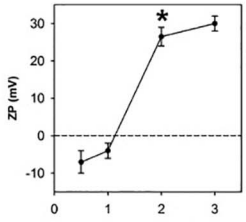
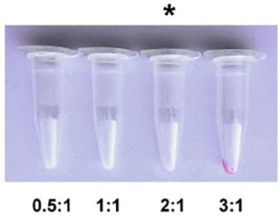
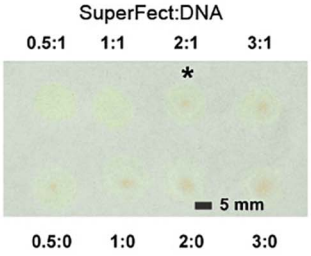
3.2.4. Order of mixing/dropping

The effect of order-of-reagent mixing/dropping was studied and the results revealed that spots obtained from protocols with different orders were apparently different in color intensity and sharpness. As shown in Fig. 7, obvious pink spots were seen in the protocols performed by dropping the solutions of carrier/DNA complexes onto the paper first, then allowing the drops to dry and finally overlaying the spots with dye solution. In protocols where the order is reversed, i.e. initially spotting and drying dye solution onto the paper, and then applying the complex solutions on top of the dye spots, no pink spot was formed because the dye was previously fixed on the paper and thus there was a lack of free dichlorofluoresceinate ions to adsorb onto the complexes. In a final attempt, the solutions of 2′,7′-dichlorofluorescein and the complexes were mixed together before being dropped onto the paper. Using this method, only pale pink spots were obtained since the complexes and cationic carriers were likely to be neutralized by the dichlorofluoresceinate ions in the solution, so they were not concentrated by the negatively charged filter paper. These results show that the most efficient protocol was to drop the test complexes onto the paper prior to the application of the dye.

3.2.5. Minimal amount of DNA required for the assay

To avoid the misleading interpretation of results occurring when the pink spots were too faint, the effect of the amount of DNA on the detection limit was examined. By varying the amounts of DNA from 0.1 to 2 μg for the preparation of complexes with carriers and then testing with 0.075 mg/mL 2′,7′-dichlorofluorescein solution, it was found that too pale a pink color was obtained when DNA of less than 0.25 μg was used, rendering an ambiguous result interpretation. Therefore, it is

Table 1
Estimation of the equivalence ratios of cationic carriers to pEGFP-C2 by different methods. (For interpretation of the references to color in this table, the reader is referred to the web version of this article.)

Cationic carrier	Method for estimation of equivalence ratio of cationic carrier to DNA			
	Gel retardation assay	Zeta potential measurement	In-tube dye adsorption method	Paper-based dye adsorption method**
PEI				
*Equivalence ratio	0.5:1	0.5:1	0.5:1	0.5:1
Liposome				
*Equivalence ratio	30:1	30:1	30:1	30:1
Niosome				
*Equivalence ratio	20:1	20:1	20:1	20:1
SuperFect				
*Equivalence ratio	2:1	2:1	2:1	2:1

** No pink spot was obtained in the negative control in which DNA without the carriers was tested with DCF.

Table 2
Comparison of the proposed paper-based method for estimation of the equivalence ratio of cationic carriers to DNA to other methods.

Characteristics	Method for estimation of equivalence ratio			
	Paper-based dye adsorption method	In-tube dye adsorption method	Gel retardation assay	Zeta potential measurement
Time of analysis[a]	5 min	10 min	1 h	1 h
Amount of DNA required (μg)	0.25	0.50	0.25	1
Instruments required	No	Centrifuge	- Gel electrophoresis - UV transilluminator	Zetasizer
Consumption of electricity	No	Yes	Yes	Yes
Use of harmful reagents	No	No	Yes, ethidium bromide (carcinogen)	No
Waste release	Papers (easily disposed of and degradable)	Plastic microcentrifuge tubes	Ethidium bromide contaminated agarose gel	Plastic cells used for zetasizer

[a] Based on the analysis of five ratios of carrier to DNA.

recommended that at least 0.25 μg DNA should be used for the complex formation with the carriers and then tested with 0.075 mg/mL 2′,7′-dichlorofluorescein. It was found that the required amount of DNA for accurate result reading using a paper-based method was lower than that needed for the in-tube method in which the detection limit was 0.50 μg DNA (Plianwong et al., 2013a, 2013b). This enhanced sensitivity was probably due to the fact that the pink color formed in the paper-based method was the result of a combination of the carrier/DNA complexes and the cationic carriers left in the formulations whereas that formed in the tube-based method was only from the complexes. In addition, the capability of filter paper to concentrate the positively charged components as well as having a white background helped to improve the color detectability.

3.3. Applicability and comparison to other methods

After the optimal protocol was established, the method was applied to the testing of various types of gene carriers including cationic polymers, cationic dendrimers, cationic liposomes and cationic niosomes. The results of the equivalence ratios of carrier to DNA estimated from the proposed paper-based assay were compared to those determined by a gel retardation assay, zeta potential analysis and in-tube dye adsorption method. As seen in Table 1, the results were in excellent agreement with the other methods for all types of gene carriers. Moreover, the proposed method gave reproducible results according to within-day and between-days repeatability evaluations. The paper-based dye adsorption method is therefore an accurate, precise and time-saving assay suitable for use in the development of gene delivery formulation as well as for the rapid and high-throughput evaluation of the DNA complexation ability of newly synthesized cationic polymers, e.g. biodegradable or ligand-conjugated polymers, as well as new types of cationic lipid-based carriers.

As shown in Table 2, a dye-adsorption method performed either in-tube or on paper platforms offers several advantages over gel retardation assay and zeta potential analysis since it requires no harmful DNA stain, tedious gel electrophoresis or expensive instruments such as a zetasizer. In comparison to the in-tube method, the paper-based assay is easier to perform, as it only requires drop-and-read steps, omitting reagent mixing, centrifugation or separation of supernatant. After the complexes are prepared, the results can be reported in 5 min by visually assessing the pink spots which are formed immediately once the reagent is added. In comparison with the reading of the results through the wall of microcentrifuge tubes, doing this onto a white paper background is more obvious and easy. Furthermore, since the paper efficiently traps the positively charged molecules, it helps to concentrate the complexes and carriers in a confined zone, thereby enhancing the color intensity and detectability. In our experience, the pink color of the dry spots on filter paper is stable for several months. With respect to greenness, the replacement of plastic tubes with paper made of cellulose, the most common and abundant biopolymers in nature, conformed to the principles of green chemistry regarding the use of renewable and biodegradable resources (Gałuszka et al., 2013). Furthermore, this assay is energy-saving since centrifugation, electrophoresis and zeta potential measurement are omitted, and electricity is therefore no longer consumed. These features not only benefit the analyst and the environment but also reduce the costs of analysis.

4. Conclusion

The first paper-based method was developed using a green approach for the determination of the equivalence ratio of cationic carriers to DNA, relying on dye adsorption. The method was greener, easier, faster and cheaper than the current commonly used methods. Furthermore, it was effectively applied to the testing of various formulations comprising different types of cationic carriers. The proposed method was therefore not only a suitable test prior to transfection, but also a paradigm of the convergence of pharmaceutical sciences, green analytical chemistry and molecular medicines.

Conflicts of interest

None.

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