Monitoring of glucose, salt and pure water in human whole blood: An *in vitro* study

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Abstract: Designing and implementation of non-invasive methods for glucose monitoring in blood is main focus of biomedical scientists to provide a relief from skin puncturing of diabete patient. The objective of this research work is to investigate the shape deformations and the aggregation of red blood cells (RBCs) in the human blood after addition of three different analytes i) (0mM-400mM: Range) of glucose ($C_6H_{12}O_6$), ii) (0mM-400mM: range) of pure salt (NaCl) and iii) (0mM- 350mM: range) of pure water (H_2O). We have observed that the changes in the shape of individual cells from biconcave discs to spherical shapes and eventually the lysis of the cells at optimum concentration of glucose, salts and pure water. This demonstration also provides a base line to facilitate diabetes during partial diagnosis and monitoring of the glucose levels qualitatively both in research laboratories and clinical environment.

Keywords: Whole blood, hyperglycaemia, hypoglycaemia, erythrocytes, hypotonic solutions

INTRODUCTION

A significant development in the medical science has been to combat those diseases, which are always dangerous for the human beings. Diabetes is one of the serious diseases that affect the patient's tissue like blood and consequently, threats the life (Amos *et al*., 1997). The regular monitoring of glucose levels in diabetics assists in determining the right diet and medical treatment (Lahmann *et al*., 1977). Current methods practiced in clinics for measurement of sugar levels in blood requires the puncturing of patient's finger to collect drop of blood. This procedure is painful and harmful to the skin. Thus, patients are not willing for frequent piercing of the skin.

In the last decade, the above invasiveness leaded in the proliferation of many optical methods that don't need the skin contact. These methods are assumed to be more suitable for the purpose of glucose monitoring in blood because of the non-ionizing nature of the optical signals. These techniques to measure both "hyperglycaemic" and "hypoglycemic" condition include near infrared (NIR) scattering spectroscopy (Bruulsema *et al*., 1997), NIR absorption spectroscopy (Pan *et al*., 1996), polarimetry (Cote *et al*., 1992), Raman spectroscopy (Goetz *et al*., 1995), fluorescence detection(Russell *et al*., 1999), and time resolved optoacoustic (Bednov *et al*., 2000), optical coherence tomography (OCT) (Ullah *et al*., 2013), optical diffuse reflectance (Ullah *et al*., 2014), microscopy (Wang, 2013) etc. with their own set of advantages and disadvantages. we have used light microscopy in transmission mode to examine the behaviour of rupturing of red blood cells equipped with camera after admixing of three types of analyte's concentration in blood (*in vitro*) i.e. glucose, salts and pure water. Microscopy has advantage of high spatial resolution in two dimensions mapping than OCT.

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MATERIALS AND METHODS

In the present study, we have investigated three types of analytes glucose ($C_6H_{12}O_6$), salt (NaCl) and distilled water (H_2O) in the whole blood sample. For prevention of clotting of blood, we have taken 9 heparin (complete blood cell) tubes and added anticoagulant (citrate phosphate dextrose adenine (CPDA-1) in each tube (Hirsh *et al*., 2001). we drew 20mL volume of human blood sample from a 29 years old male and stored 2mL in each heparin tube. The blood was drawn after signing a written consent by the donor for the purpose of experimentations. The samples were (Ullah *et al.*, 2014);

Sample set (1)

It consists of (whole blood + nine different concentrations of glucose (0, 50,100, 150, 200, 250, 300, 350, 400mM)) that were admixed in each of heparin tube and shake well gently to mix-up the glucose completely. Here, 0mM glucose means that blood carries inherent glucose but no additional glucose in it. After getting mixed the glucose, a blood smear was prepared within 10 minutes on a glass

slide for all aforementioned concentrations and was kept under a transmission mode microscope with 40X magnification (Ullah *et al*., 2011).

Sample set (2)

It consists of (whole blood + nine different concentrations of salt (0, 50, 100, 150, 200, 250, 300, 350 and 400mM) in each of 2mL whole blood heparin tube that were mixed well gently. Here, 0mM concentration never means that solution is salt free but it conations inherent salt levels in it. The smear was kept under transmission mode microscopic objective with 100X magnification (Ullah *et al.*, 2012b).

Sample set (3)

It consists of (whole blood + eight concentrations of pure water (0, 50, 100, 150, 200, 250, 300 and 350 mM) in each of 2mL whole blood heparin tube. The blood smear was imaged using 100X microscopic light microscope under transmission mode (Ullah *et al.*, 2014). All the experiments were performed at room temperature of 21^{o}C.

RESULTS

Effect of glucose on RBCs

Fig. 1 shows a clear effect of cell's deformations from its natural shape and aggregations as the concentrations of glucose is increased from 0mM to 400mM. The complete burst-up starting from biconcave disc like shape to sphereoidal shape and ultimately damaging of the whole structure has been observed. Fig. 1(a) shows the native structure of erythrocytes i.e. a complete pancake like shape is visible. Fig. 1(b) and 1(c) show the clumping of RBCs tending towards the rouleaux formation. After, the addition of 150-200mM of glucose, the RBCs are deformed and there shape start transition from biconcave discs to more spheroidal shape, getting swellings perhaps due to hyperglycaemic shock and consequently, drastically the burst up of RBCs have been observed as shown in fig. 1(d and e). In fig. 1(f-h), for higher concentration i.e. 250-350mM a very pronounced change of destruction/lyses of the RBCs can be observed. It gets totally deformed in size and shape in drastic shrivelling as shown in fig. 1(i) creating the irreversible situation where RBCs become unable to re-function and die out ultimately. This might be supported by reduction in the optical attenuation due to refractive indices mismatching effect (Ullah *et al.*, 2011, Ullah *et al.*, 2012a).

Effect of pure salt on RBCs

In sample set (2), we have investigated the morphological changes in erythrocytes while discovering the sediment structures, aggregation/destructions, rouleaux formation and changes in the size after addition of different salt concentrations. At higher concentrations of NaCl, hemolyse of RBCs is reached. Figs. 2 (a-i) respectively shows the salt free image of whole RBCs and the shape changes for all concentration (50mM-400mM) that generates the hypertonic environment due to which the water leaves the cell causing the red blood cell to be shrinking and ultimately burst up. Fig. 2 (a) shows the biconcave shape of erythrocytes in the absence of NaCl. Fig. 2(b) depicts the just start-up of the aggregation. It is examined that with the gradual increase of the concentration of salt the size and shape of RBCs is totally deformed and at the optimum level about 300mM, the RBCs have lost their shapes and totally burst up after shrinking. At 150mM (fig. 2(d)) the shape of the erythrocytes starts to become like circles but at 200mM of NaCl (fig. 2(e) the RBCs have been converted into spheres and with 250mM (fig. 2(f)) they are getting start to convert into rod shape. Fig. 2(h) shows the complete conversion into rod shape for 350mM. Fig. 2(i) shows the complete destruction of erythrocytes at 400mM concentration of NaCl. The reason for shape change may include non-preservation of the RBC membrane properties (such as osmotic fragility) that is critical to RBC function and survival due to the use of citrate-phosphate-dextrose (CPD) for anticoagulation at room temperature of blood that changes the metabolism of RBCs (Zimrin *et al.*, 2009, Wilsher *et al.*, 2008). The complete burst up of the RBCs is due to the operation of Cl ion or OH ion in red blood cell that increase the intracellular pH (Veale *et al*., 2011).

Effect of distilled water on RBCs

Fig. 3(a) shows no pronounced change in the absence of pure water. In fig. 3(b) the un-healthy condition of RBCs starts to break up the continuity in erythrocyte's distribution after addition of 50mM concentration of distilled water perhaps due to the fact of high rate of hydration in blood after addition of little amount of water. For higher concentrations of pure water (100 and 150mM), the erythrocyte's shape has started to become aggregate and little swelled resulting in loss of the actual shape (fig. c and d). Fig. 3 (f and g) show the asymmetric irregularities in the shape of the RBCs after absorbing the distilled water (250 and 300mM concentration) by approaching the shape like a sphere and ready to haemolysis. The fact of reduction in haemoglobin in erythrocytes causes its rupturing and consequently, after addition of 300mM of pure water (fig. 3(h)), they lose their original shape completely and get burst. The thrombus formation of blood can be the role of RBCs swelling in the presence of pure water in the relative incorporation of platelets, fibrin, and RBCs (Cadroy *et al.*, 1990).

DISCUSSION

For sample set (1), the effect of sugar is found to be dominant in scattering properties with negligible absorption of light in whole blood. Thus, by losing the immunity, erythrocytes couldn't face the severe

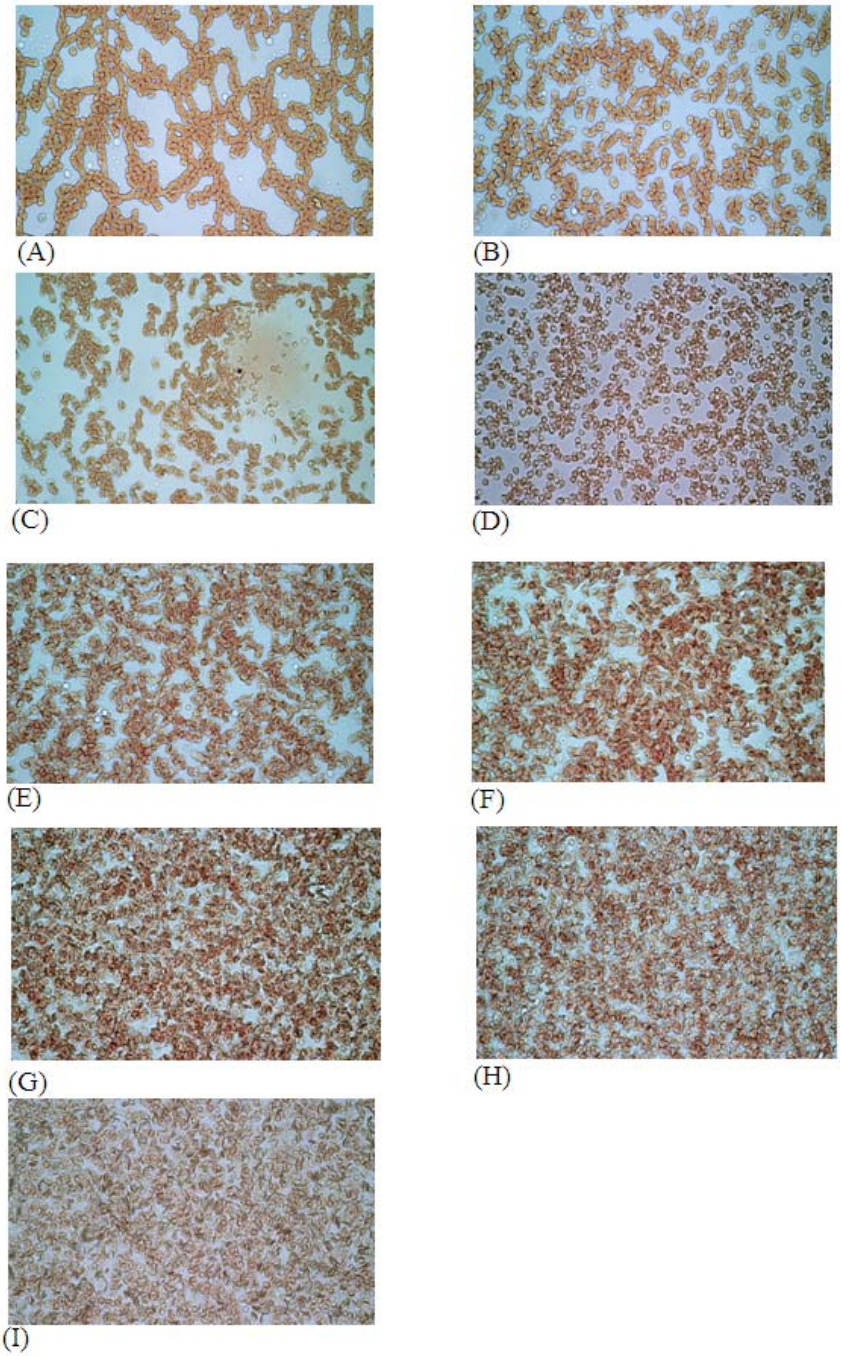

Fig. 1: 2-D images of erythrocytes in whole blood of 19 years old human obtained with light microscope in transmission mode for glucose concentrations of (a) 0mM, (b) 50mM, (c) 100mM, (d) 150mM, (e) 200mM, (f) 250 mM, (g) 300mM, (h) 350mM and (i) 400mM.

hyperglycemic conditions and after swelling the outer membrane were permanently damaged.

For sample set (2), the complete burst up of the RBCs includes the operation of Cl ion or OH ion in a way that Cl from the surrounding medium i.e. plasma shift operates on the premise that in a Cl- free extra cellular medium, intracellular Cl- would efflux from the cell, and in the absence of any other diffusible anion in the extra cellular medium, OH- would enter the cell and increase the intracellular pH. This increase in pH causes the reduction in the diameter of the RBCs and consequently damaging due to shrinking. For sample set (3), the blood flow has been observed having direct influence on thrombus formations for water acting as diffusion analyte with variable RBC's concentration.

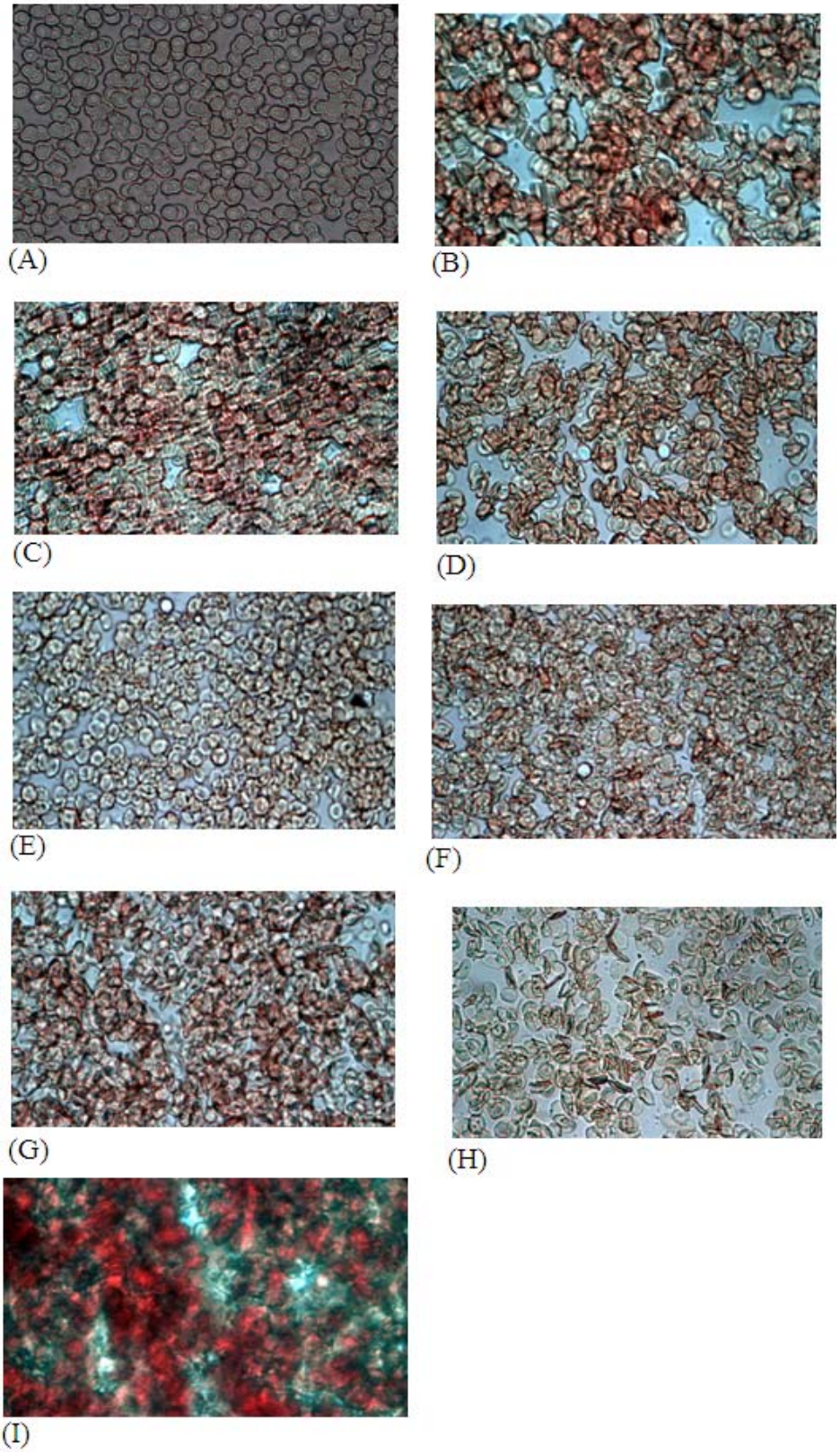

Fig. 2: 2-D images of erythrocytes of human blood with light microscope in transmission mode for the added salt concenrations (a) 0mM, (b) 50mM, (c) 100mM, (d) 150mM, (e) 200mM, (f) 250mM, (g) 300mM, (h) 350mM, and (i) 400mM.

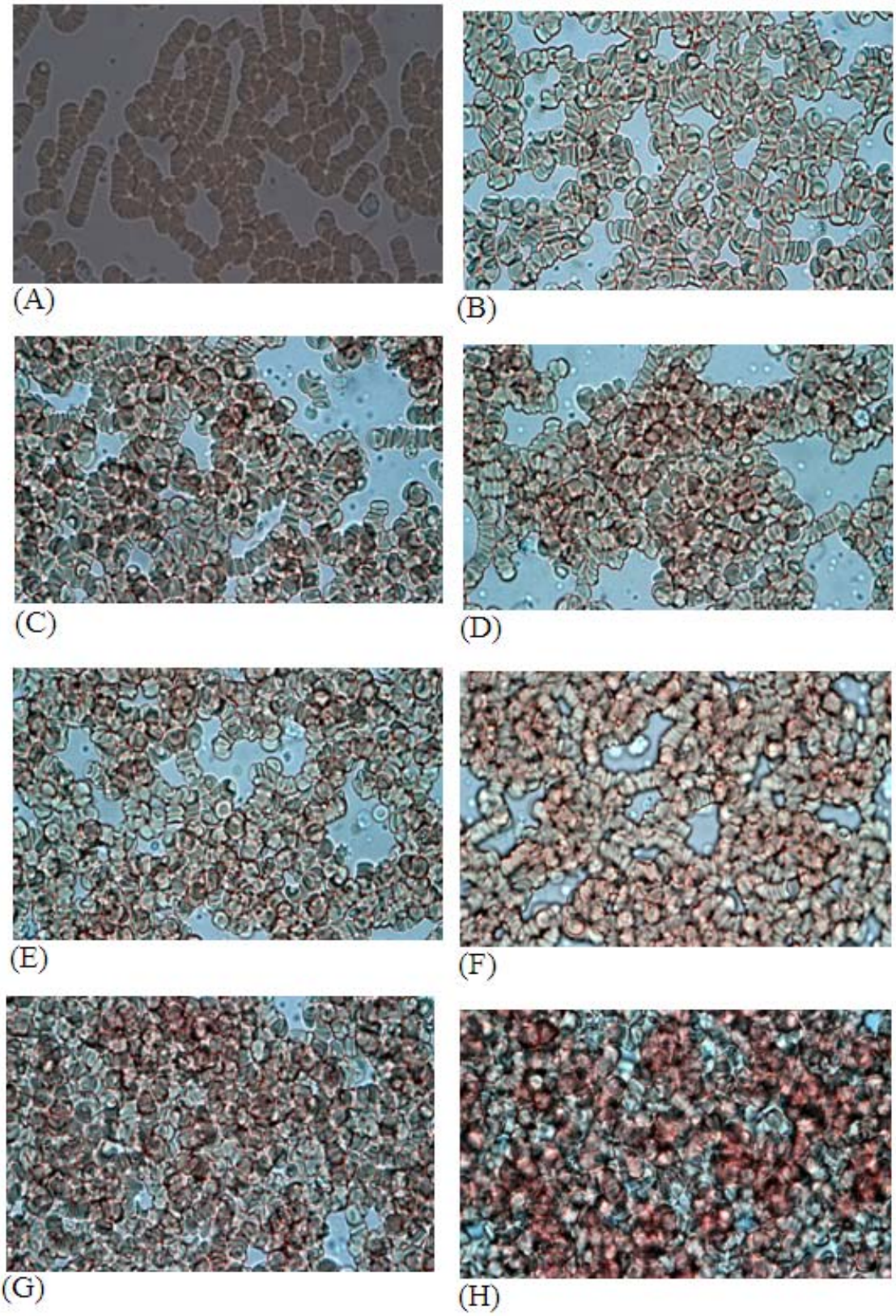

Fig. 3: 2-D images of erythrocytes after addition of pure water in human whole blood with light microscope in transmission mode for concentrations of (a) 0mM, (b) 50mM, (c) 100mM, (d) 150mM, (e) 200mM, (f) 250mM, (g) 300mM and (h) 350mM.

CONCLUSIONS

This comprehensive and qualitative work about the erythrocytes demonstrates that how they behave when human blood was admixed with three different analytes (i.e. glucose, salt and distilled water with different concentrations). From this microscopic work, we have revealed that the shape change of erythrocytes from biconcave disc shape to spheroidal and consequently occurrence of destruction/lysis occurs for higher concentrations. Thus, the quantification of glucose, salt and distilled water has been achieved qualitatively on the basis of the shape changes of erythrocytes.

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