

LIGHT-STIMULATED LOWERING OF GLUCOSE CONCENTRATION IN A DEXTROSE SOLUTION MEDIATED BY MEROCYANINE MOLECULES

S SVETLOBO STIMULIRANO ZMANJŠANJE VSEBNOSTI GLUKOZE V DEKSTROZNI RAZTOPINI S POMOČJO MEROSIANINSKIH MOLEKUL

Gia Petriashvili,¹ Ketevan Chubinidze,^{1*} Tamara Tatrishvili,² Elene Kalandia,¹
Ana Petriashvili,¹ Mariam Chubinidze³

¹Institute of Cybernetics of Georgian Technical University, Z. Anjaparidze 5, 0186 Tbilisi, Georgia

²Tbilisi State University, Department of Macromolecular Chemistry, I. Chavchavadze Ave.1, 0179 Tbilisi, Georgia

³Jo Ann University Hospital, Ljubljana 35, 0159 Tbilisi, Georgia

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Diabetes mellitus is a chronic metabolic disease characterized by elevated blood glucose levels and has become a global challenge. Currently, the widespread and regular treatment of diabetes mellitus involves the administration of insulin. However, insulin is no longer considered the first choice for type 2 diabetes, and an expanding range of new treatment modalities are emerging as noninsulin-based medications that are promising alternatives to regulate blood glucose levels. In this regard, controlling the glucose level in blood by external stimuli, such as light, offers a new route to governing the blood glucose concentration with the required dose and at the appropriate time. Here, we report on a light-stimulated glucose-lowering method based on spiropyran-merocyanine photoisomerization. We show that upon exposure to violet light (405 nm), the closed isoform of spiropyran molecules inside liquid crystal microspheres transforms into the open merocyanine isoform, which in turn stimulates merocyanine to translocate through the interface of the liquid crystal/dextrose emulsion. Merocyanine readily interacts with glucose molecules and causes a lowering of the emulsion's total glucose concentration by 20 %.

Keywords: diabetes mellitus, dextrose, merocyanine, light-stimulated

Sladkorna bolezen je kronična metabolična bolezen za katero je značilna visoka vsebnost glukoze v človeški krvi in zaradi svoje razširjenosti po svetu postaja globalni izziv. Trenutno obstaja vrsta zakonitih načinov zdravljenja sladkornih bolezni, ki vključujejo uporabo inzulina. Zdravljenje z inzulinom se ne upošteva več kot prva izbira za zdravljenje diabetesa tipa 2. Uvaja se nova razširjena vrsta zdravil, ki ne temeljijo na doziranju inzulina. To so obetajoče alternativne rešitve za reguliranje ravni glukoze v krvi. Pri tem se lahko kontrolira raven glukoze v krvi z zunanjo stimulacijo, kot je na primer svetloba, ki ponuja nov način uravnavanja koncentracije glukoze v krvi z zahtevano dozo in ob pravem času. Opisana je metoda, ki temelji na svetlobno stimuliranem zmanjševanju glukoze s spiropiran-merosianinsko fotoizomerizacijo. Izkazalo se je, da se zaradi izpostavljenosti vijolični svetlobi z valovno dolžino 405 nm, zaprte izofornne spiropiranske molekule znotraj tekoče kristalne mikrosfere pretvorijo v odprto merosianinsko izoformo, ki posledično stimulira premestitev merosianina preko fazne meje tekoči kristal-dekstrozna emulzija. Merosianin tako brez težav reagira z molekulami glukoze in povzroči zmanjšanje celotne emulzijske glukoze za petino.

Ključne besede: sladkorna bolezen, dekstroza, merosianin, svetlobna stimulacija.

1 INTRODUCTION

Diabetes mellitus, more simply called diabetes is a chronic, metabolic disease causing the majority of heart attacks, strokes, kidney failures, blindness and lower limb amputation. Nowadays, the number of people suffering from diabetes and its complications is increasing, in part due to the current worldwide lifestyle: sedentary lifestyle, high-fat diet, obesity and longer life span. Diabetes has become a global health problem since it is the most common clinical disorder, affecting nearly 10 % of the world's population and constantly increasing day by day. The World Health Organization (WHO) reported

that about 1.1 million people die of diabetic complications annually, and the death rate is expected to increase up to 50 % in 2030.¹ The glucose absorbed from the gastrointestinal tract is distributed to tissues via systemic circulation under the control of insulin, a hormone, which causes sugar to move from the bloodstream to the body's cells and is stored and utilized as an energy source. People suffering from type 2 diabetes are resistant to insulin, which keeps a person's blood sugar at high levels.² Although insulin represents a lifesaving therapeutic aid for people suffering from diabetes, at present, it is no longer considered the first choice for type 2 diabetes, and an expanding range of new therapeutic possibilities is emerging. While these may lack the potency of insulin, at a minimum, they allow a major reduction in the intensity of insulin use. Therefore, the

*Corresponding author's e-mail:
chubinidzeketino@yahoo.com (Ketevan Chubinidze)

search for new glucose-lowering drugs with minimal or no side effects is undoubtedly an active challenging area for research and development worldwide.³

Until now, multiple novel mechanisms potentially mediating a reduction in plasma glucose concentrations are being explored, and various antidiabetic drugs have been used to improve the pathological condition in patients with diabetes. Sulfonylurea drugs, glinide, dipeptidyl-peptidase 4 inhibitors, thiazolidinediones, acarbose, metformin, and sodium-glucose cotransporter 2 (SGLT2) inhibitors are used as oral medications for the diabetes treatment. Recently, Japan and South Korea have developed novel glucose-lowering drugs, such as anagliptin, gemigliptin, evogliptin, teneligliptin, telegliptin, gasegliptin, and retagliptin.⁴⁻⁷ Some medications reduce the glucose concentration produced by the liver and slow down the conversion of carbohydrates into sugar. Other medications improve the way insulin works in the body by allowing more glucose to enter into muscles, fat and liver, lowering the blood glucose by delaying the breakdown of carbohydrates and reducing the glucose absorption in the small intestine. However, in any case, it is of great importance to control the intake of antidiabetic drugs to prevent drug overdose. Controlled drug delivery systems have emerged as an alternative to the conventional sort to improve the bioavailability and extent of the drug release, and to maintain drug plasma levels within the therapeutic window with minimal side effects. Among the controlled drug-releasing strategy, the stimuli-responsive method appears as a promising approach to governing and targeting the temporal and spatial presentation of drugs in the body. When these stimuli-responsive drug delivery systems are administered, the drug release is activated and then modulated through some action or external input and facilitated by the energy supplied externally. The stimuli-responsive systems can control drug biodistribution in response to specific stimuli, either exogenous (variations in temperature, magnetic field, ultrasound intensity, light or electric pulses) or endogenous (changes in pH, enzyme concentration or redox gradients).^{8,9}

Light-activated and light-controlled drug delivery systems offer distinct advantages over other stimuli because the light is a fascinating and green stimulus with a micron- or submicron-sized focusing area with controllable wavelength and energy, non-invasive and non-destructive nature, precisely controlled direction and availability that can release a drug at a desired time and place, so it only targets cells and not the surrounding healthy tissues.¹⁰ One of the unique examples of light-controlled molecular switches is the spiropyran (SP), whose closed-ring, hydrophobic isomer transforms into a highly polar, open-ring merocyanine (MC) isoform upon exposure to UV light, whereas the reverse reaction can be induced by visible light or heat. SPs are the most prominent example of light-activated molecules that are far more than just simple photoswitches. The range of stimuli able to induce its reversible isomerization is truly im-

pressive and includes different solvents, metal ions, acids, bases, temperature, redox potential and mechanical force.^{11,12} In this regard, using the photoswitches like SPs, which will control the amount of glucose in the blood upon exposure to light, would be of great importance. In this work, we prepared SP-doped liquid crystal (LC) microspheres, emulsified in a 5 % dextrose (D-glucose) solution, and showed that upon exposure to a light-emitting diode (LED) with violet (405 nm) light, the SP isoforms convert into their MS isoforms, which in turn stimulates the MC isoforms to translocate across the interface of the LC/dextrose solution, and interact with the glucose molecules, leading to a lowering of the total glucose concentration in the solution by 20 %.

2 EXPERIMENTAL PART

In the experiments, to prepare a photochromic mixture, we used the following commercially available materials: as the LC host matrix ZLI-1639 (from Merck), as the photochromic material SP-1',3',3'-Trimethyl-6-nitro-1',3'-dihydrospiro [chromene-2,2'-indole] and macromonomer poly (vinyl alcohol) – (PVA) with an average molecular weight – M_w 55,000–65,000 $g \cdot mol^{-1}$ (both from Sigma-Aldrich). **Figure 1** shows 3D and 2D structures of the SP and MC isoforms controlled by light or temperature.

An SP-doped LC mixture was prepared by doping the LC host substance with the SP material with the following concentration ratio: 99.9 % ZLI-1639 + 0.1 % SP. The prepared SP-LC mixture was stirred in its isotropic phase at about 80 °C for 10 min using a laboratory glass vial. To investigate the absorption behavior of the SP-LC mixture upon exposure to light, we first built a planar optical cell using two glass plates. A 99.4 % water + 0.6 % PVA solution was deposited on the glass substrates by spin-coating and then rubbed to obtain planar alignment of the LC material. The spacing between the plates was set to 24 μm , using Mylar films. The SP-LC mixture was infiltrated by capillarity inside the optical cell. The SP-doped LC microspheres were obtained by emulsifying the SP-LC material in a 5 % dextrose solution, the name of a simple sugar made from corn or wheat that is

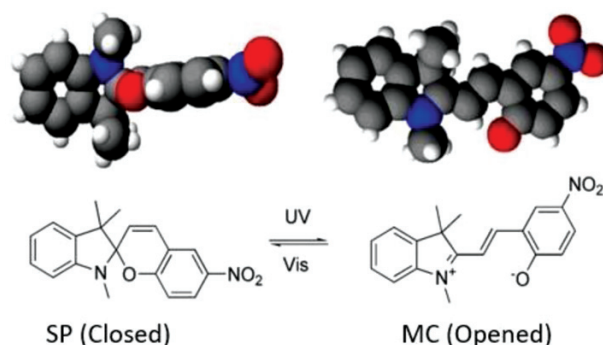


Figure 1: 3D and 2D structures of an SP (closed) and MC (opened) molecule and light-controlled reversible molecular conformations of SP and MC isoforms

chemically identical to glucose or blood sugar. Petri-dish containers (a diameter of 4 cm, height of 2.5 cm) filled with SP-LC/dextrose emulsions, were used to enclose the substances. Emulsions were poured into cuvettes with 1 cm gaps, transparent in the UV/visible ranges of the optical spectrum. To obtain a light-stimulated SP-to-MC conversion, we irradiated samples using an LED, with an emission peak at 405 nm of the optical spectrum. The distance from the LED to the SP-LC/dextrose emulsion was set at 10 cm. The light intensity on the sample surface, measured with an optical power/energy meter, was 0.25 mW/cm². An optical microscope (OM) served for the visualization of the substance at the microscale level. The absorption spectra of the SP-LC mixture and SP-LC/dextrose emulsion were collected using an optical fiber-coupled spectrometer (Ava-Spec AVS-2048-2) having a 1 nm resolution. Experiments were carried out at 37 °C. To precisely measure the volume of the solutions, we used a laboratory pipette with a 0.01 mL accuracy. For the vigorous agitation of the SP-LC/dextrose and SP-LC/water emulsions, we utilized a magnetic stirrer hot plate (MSHP) with variable speed settings ranging from 100 to 2500 min⁻¹, with a controlled mixing speed. The MSHP heats samples with a 0.2 °C accuracy, from ambient temperature to 300 °C. The expanded beam, 40 mm in diameter, is incident on a Petri dish filled with the emulsions. To study the light-induced absorption spectra of the SP-LC mixture, we irradiated an optical cell with an LED. During 12 min of exposure, the maximum absorption of the SP-LC mixture was increased significantly from 0.05 to 2.5 arbitrary units. In **Figure 2**, Curve 1 corresponds to the absorption before the light irradiation. Curves 2, 3, 4 and 5 show the absorption peaks after (2, 6, 10 and 12) s of exposure, respectively.

The experimental set-up for the preparation, irradiation and monitoring of the SP-LC/dextrose and

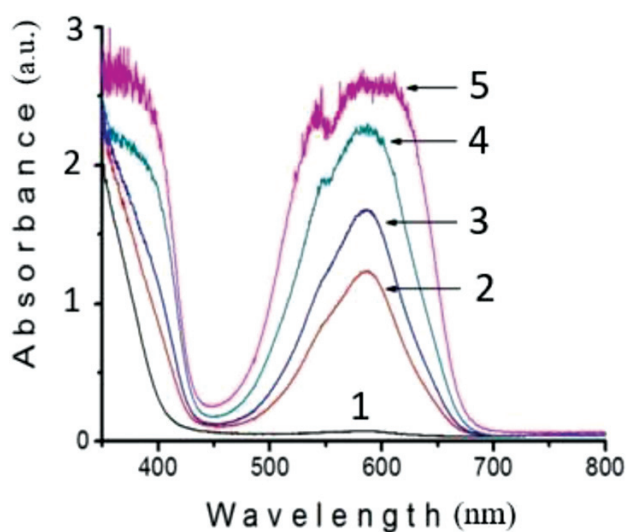


Figure 2: Absorption dependence on the exposure time for mixture 99.1 % ZLI-1639 + 0.1 % SP when irradiated with the LED

SP-LC/water emulsions is depicted in **Figure 3**. The emitted light is directed normally to the emulsions located in a Petri dish container. The backscattering image of the emulsions is detected and captured by the OM coupled with a high-resolution CCD camera. The distance from the LED to the upper surface of the samples is set at 10 cm. To monitor the glucose concentration in the SP-LC/dextrose emulsion, before and after the light irradiation, we used a standard, clinically validated glucose meter (GLM), which accurately measures the current blood glucose using a finger stick blood sample placed on a test strip and inserted into the device.

For the preparation of the SP-LC/dextrose emulsion, 5 mg of SP-LC mixture was extracted from a glass vial and dropped in the Petri dish container filled with 75 mL of 5 % dextrose solution. The emulsion was mixed at about 37 °C for 20 min, at 800 min⁻¹. Since the SP-LC and dextrose solutions are immiscible, this procedure is functional for forming SP-LC microspheres with their sizes depending on the stirring speed and duration. First, the non-irradiated SP-LC/dextrose emulsion was extracted from the Petri dish container and poured into a cuvette with a laboratory pipette; then the light absorption was measured using a spectrometer. Then we carried out the above-described procedures to prepare the SP-LC/dextrose emulsion, but in this case, during the mixing, the SP-LC/dextrose emulsion was irradiated for 12 min with the LED. The obtained emulsion was extracted from the Petri dish container, poured into the cuvette, and the absorption spectrum was measured again using the spectrometer. In **Figure 4**, the left part shows the absorption curves of the SP-LC/dextrose emulsion before (a) and after (b) light exposure, and the right part of the figure shows the cuvettes where the left

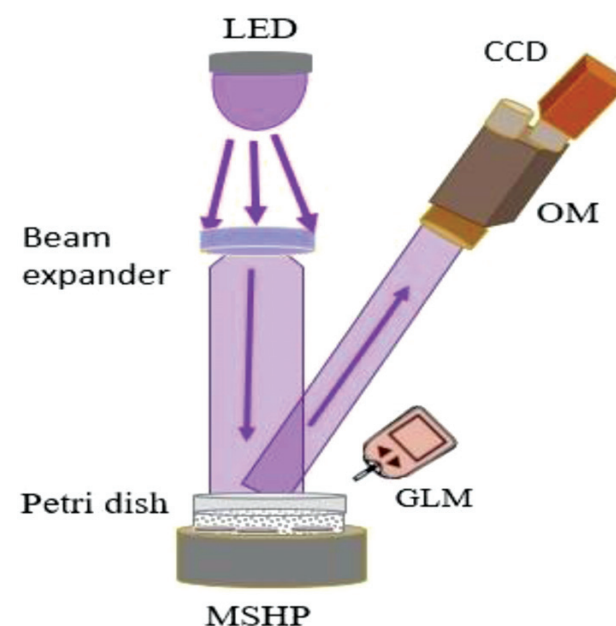


Figure 3: Experimental set-up for the preparation, irradiation and examination of SP-LC/dextrose and SP-LC/water emulsions

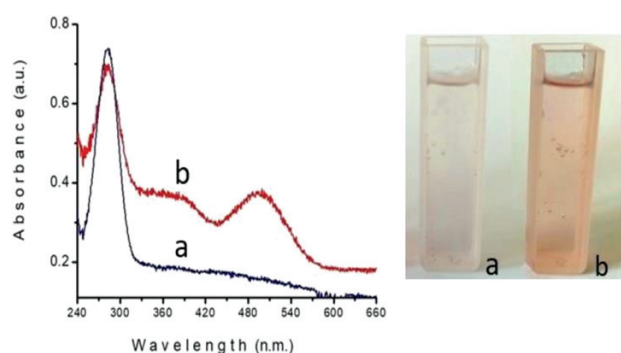


Figure 4: Absorption spectra of the non-irradiated and irradiated emulsions (left part of the picture) and the cuvettes filled with emulsions before and after irradiation with LED (right part of the picture)

cuvette (a) is filled with the SP-LC/dextrose emulsion before exposure to LED, and the right cuvette (b) is filled with the emulsion after exposure to LED. As seen in **Figure 4**, the absorption spectra of the SP-LC/dextrose emulsion without and with irradiation differ significantly. In particular, compared to the non-irradiated sample, there is a significant increase in the light absorption of the irradiated emulsion in the near UV and visible ranges of the optical spectrum with absorption maxima at 380 nm and 495 nm. Here we note that the absorption peak of glucose is located at 285 nm of the optical spectrum.

Next, we compared the absorption spectrum of an SP-LC/dextrose emulsion with the absorption spectrum of an SP-LC/water emulsion. For this purpose, we filled a Petri dish container with distilled water and dropped the SP-LC solution into the water at such a concentration ratio as described above. The obtained solution was mixed at about 37 °C for 20 min, at 800 min⁻¹. The same procedure as in the SP-LC/dextrose emulsion case was repeated and the absorption spectra of the SP-LC/dextrose emulsion (a) and SP-LC/water emulsion (b) were compared. It was found that compared to the absorption spectrum of the SP-LC/dextrose emulsion, the absorption spectrum of the SP-LC/water emulsion further increases

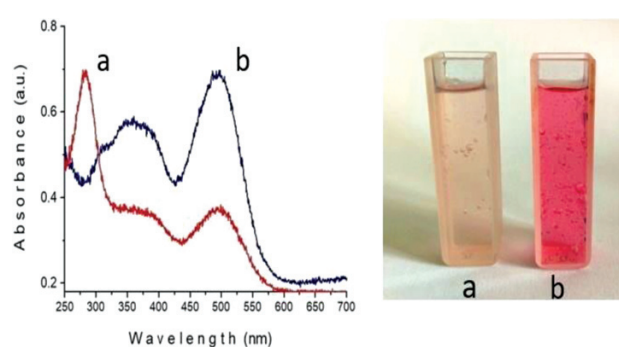


Figure 5: Absorption spectra (the left part of the figure) and the cuvettes filled with irradiated SP-LC/dextrose and SP-LC/water emulsions (the right part of the figure)

in the near UV and visible part of the optical spectrum with absorption maxima at 385 nm and 505 nm, respectively. The left part of **Figure 5** shows the absorption spectra of the SP-LC/dextrose (a) and SP-LC/water (b) emulsions after 12 min of exposure, while the right part of **Figure 5** demonstrates the cuvettes filled with the irradiated SP-LC/dextrose emulsion (a) and irradiated SP-LC/water emulsion (b).

Figure 6 shows the SP-LC/dextrose emulsion under the OM, placed in a Petri dish container before (a) and after (b) the light irradiation. The emulsion before and after the exposure to LED markedly differs. The non-irradiated emulsion is milky in color, while the irradiated emulsion is reddish purple.

Next, we monitored the light-controlled glucose concentration in the SP-LC/dextrose solutions using a portable glucometer based on the enzymatic biosensing technology. Its mechanism relies on the enzymatic electrochemical detection of glucose and involves the sampling of capillary blood from a finger to be analyzed with the use of test strips and a glucometer.¹³ To measure the glucose concentration in the blood, the following standard and popular units are used: mg/dL and mmol/L. Therefore, we used a glucometer to convert the glucose concentration from percent to mg/dL units. First, we

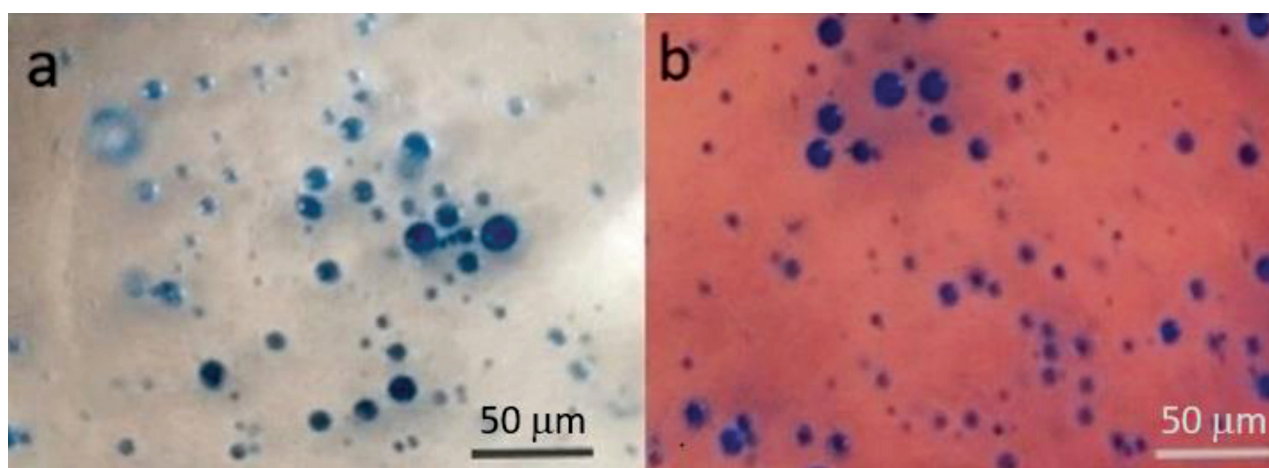


Figure 6: Optical microscope images of SP-LC/dextrose emulsion: (a) before and (b) after exposure to LED

measured the glucose concentration in a 5 % dextrose solution. Then the dextrose solution was diluted several times with distilled water, and the glucose level in the resulting mixture was measured each time. We found that 5 % dextrose corresponds to 500 mg/dL (or 27.78 mmol/L), while 2.5 % dextrose corresponds to 250 mg/dL, etc. We first measured the glucose concentration in a 5 % dextrose solution using a glucometer to study the variation in the glucose concentration as a function of exposure time. After that, we prepared the SP-LC/dextrose emulsion and exposed it to the LED for 1 min, 2 min, and so on for 12 min. After each LED exposure, the SP-LC/dextrose emulsion was extracted from the Petri dish container with a test strip, and the glucose level was measured using the glucometer. The experiments were carried out ten times, and the obtained data were calculated as the arithmetic means according to Equation (1):

$$\frac{\sum_n E(n) \sum_t E(t)}{n} \quad (1)$$

where n is the number of experiments conducted, and t is the exposure time. In our case, the number of experiments is 10, and in each experiment, the exposure time varied from 0 min to 12 min. Accordingly, Equation (1) can be rewritten as follows:

$$\frac{\sum_{n=1}^{10} E(n) \sum_{t=0}^{12} E(t)}{10} \quad (2)$$

Based on Equation (2), we plotted the variation in the glucose concentration in the SP-LC/dextrose emulsion as a function of exposure time. As shown in **Figure 7**, upon exposure to LED, the glucose concentration in the SP-LC/dextrose emulsion gradually decreases by 20 percent over 12 min. Interestingly, the concentration of glucose in the emulsion rapidly decreases at the beginning of irradiation, while further decrease in the glucose concentration slows down during irradiation.

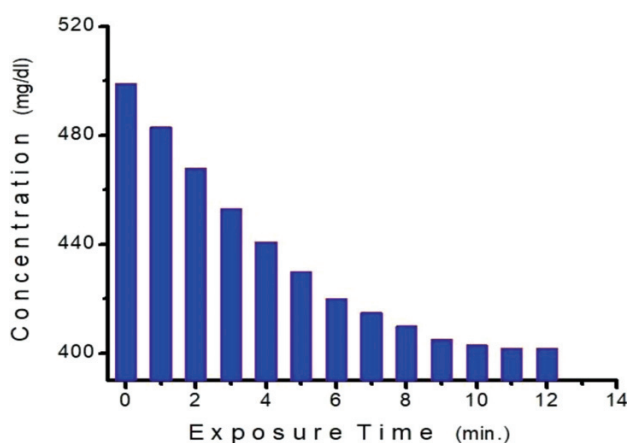


Figure 7: Change in glucose concentration in SP-LC/dextrose emulsion as a function of LED exposure time

We note that after the LED is turned off, the glucose concentration in the SP-LC/dextrose emulsion increases over time. However, this increase is insignificant and never returns to its original level.

3 RESULTS AND DISCUSSION

The photochromism of SPs arises from a light-driven ability to isomerize between the SP and MC forms. The stable state of an SP is a non-colored closed molecular form that can be transformed into its MC state upon irradiation with UV/violet light. More specifically, the carbon–oxygen bond of SP molecules is cleaved when it is transformed into the polar-colored MC form. Ref.¹⁴ shows that upon exposure to UV/violet light, the photochromic molecules inside LC microspheres experience an interconversion from the hydrophobic, oil-soluble, non-polar SP state to the hydrophilic, water-soluble, highly polar MC state. Light-induced photoisomerization destabilizes the LC/water interface, stimulates the translocation of MC molecules across the LC/water barrier, and results in their homogeneous distribution throughout the aqueous environment. Similarly, in our experiments, upon exposure to LED, the SP molecules doped in LC microspheres are photoisomerized from the SP isoforms to the MC ones, which in turn causes MCs to translocate through the LC/dextrose barrier, and spread evenly in the dextrose solution. **Figure 8** shows the emulsion before exposure to LED (a) and after exposure to LED (b).

To describe the interaction of the MC molecules with D-glucose molecules, we used the Chem Draw Ultra software. Based on the calculations, we found that the electronegativity of the oxygen of the OH group of MC is -0.301345 . On the other hand, the electronegativity of the oxygen of the hemiacetal hydroxyl group of the carbon atom (C1) of D-glucose is -0.397001 . Therefore, we guess that due to its high electronegativity, the

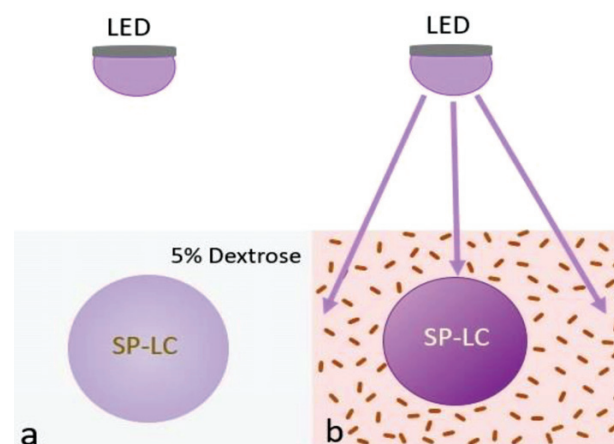


Figure 8: Photoinduced isomerization of an SP-MC molecule, followed by the MC isoform's translocation through the LC/dextrose solution's barrier

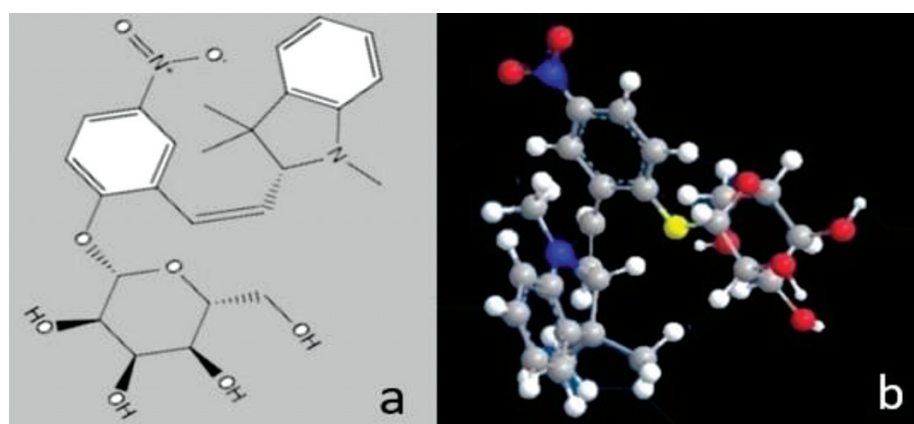


Figure 9: Structural (a) 2D chemical structure and (b) 3D ball-and-stick images of 2-(hydroxymethyl)-6-(4-nitro-2-((Z)-2-((R)-1,3,3-trimethylindolin-2-yl)vinyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triol compound

hemiacetal hydroxyl oxygen of the glucose binds to the carbon of MC. The OH group of MC forms water with the hydrogen of glucose. The interaction between MC and glucose gives the following compound: 2-(hydroxymethyl)-6-(4-nitro-2-((Z)-2-((R)-1,3,3-trimethylindolin-2-yl)vinyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triol, whose structural formula and 3D ball-and-stick model is shown in **Figure 9**.

We assume that MC molecules act as inhibitors of glucose molecules, causing the glucose concentration to lower in the SP-LC/dextrose emulsion. However, despite the obtained results, several issues need to be addressed. The first challenge is the low penetration of violet light into the tissue, and the second challenge is linked to violet light that can photo-damage the tissue. The real solution to these challenges is the two-photon absorption phenomenon stimulating the SP-MC photoisomerization. Compared with single-photon violet-light excitations, the two-photon near-infrared (NIR) excitations can dramatically increase light penetration into the tissue because cellular systems absorb much more weakly in this range of the optical spectrum. Besides, in comparison with violet light, NIR photons have the potential to cause less photodamage to the tissue and living cells. In our experiments, the average size of the obtained LC microspheres was in a range of 5–20 μm . For real applications, the average size of the microspheres must be reduced to submicron dimensions, which is achievable by controlling the stirring speed. On the other hand, we note that an alternative and effective solution to these challenges may be pre-irradiation of the SP-LC mixture poured into a container, followed by administering the activated substance to the human body by injection or orally.

4 CONCLUSIONS

This study investigated a light-stimulated glucose-lowering effect in an SP-LC/dextrose emulsion based on SP-MC photoisomerization phenomena. We have shown that upon exposure to the LED with violet

(405 nm) light, SP isoforms are converted to MS isoforms, followed by their translocation across the LC/dextrose interface and interaction with the glucose molecules. This process causes the lowering of the total glucose concentration in a solution by 20 %. We expect that the proposed modality could provide a possibility to dynamically regulate the concentration of glucose in the blood with the required dose and at the appropriate time to manage and treat type 2 diabetes noninvasively.

The authors confirm that the paper is original and has not been published in this form anywhere else and it is not under consideration for publication elsewhere.

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