Supplementary Material

1.1. FTIR spectroscopy of various polydiacetylene based compounds
The distinct peak present at 1693/cm of the polymerized polydiacetylene (blue) is due to the carbonyl bond of strongly hydrogen-bonded carboxylic acid head groups.\textsuperscript{1} On disruption of the polydiacetylene (red), the carbonyl stretching bond can be observed at 1699/cm and is seen shifting to a higher wavelength by 6/cm with slight broadening at 1639/cm, indicating that hydrogen bonding in the head group while reduced, is still persistent. Also, the FTIR spectra shows the scissoring band of the methylene group, which is at 1468/cm and shifts to 1462/cm after disruption.\textsuperscript{1} It is thus evident that there are distinct changes in the polydiacetylene chemistry after disruption that is central to the change in color and emission of fluorescence.

![FTIR spectrum of PCDA, polydiacetylene (crosslinked with UV light, blue) and polydiacetylene destabilized (red)](image)

Figure S1: FTIR spectrum of PCDA, polydiacetylene (crosslinked with UV light, blue) and polydiacetylene destabilized (red)

1.2. Extraction of polar brain lipids from goat brain
As an affordable alternative to the otherwise expensive porcine source of the lipids, a caprine source was explored as a potential source of polar brain lipids (PBL). Brain from domestic goat (\textit{Capra aegagrus hircus}) was obtained from a local abattoir and transported in artificial
cerebrospinal fluid to the laboratory within 30 minutes of sacrificing the animal. The various parts of the brain were separated (cerebellum, cerebrum, midbrain, brain stem). The lipids from the brain were extracted based on a traditional extraction process as outlined by Phillips and Privett.\(^2\) 1 g of cerebral tissue was cut and homogenized using a tissue homogenizer with Teflon tip (Remi, India) in 5 ml of 0.25% acetic acid solution to inactivate enzymes and remove all non-lipid portions. This was followed by standing of the homogenized tissue for 1 minute at room temperature, centrifugation for 5 min at 3000 rpm and removal of the supernatant. The same process of homogenization and centrifugation was repeated until the supernatant was clear. The pellet thus obtained was slurried using a stainless steel spatula and 40ml of chloroform and methanol (1:1 v/v) was added. The mixture was again allowed to stand for 5 minutes and then homogenized for 10 minutes. This was followed by centrifugation at 3000 rpm for 5 minutes. The chloroform: methanol extract obtained as supernatant contains the polar brain lipids. This process was repeated to get a better yield of PBL. The supernatant containing the chloroform: methanol extract was then placed under vacuum in a rotary evaporator for the extraction of lipids. PBL thus obtained was lyophilized and stored at -20°C. A brief schematic of the process has been outlined in the Figure S2.

![Schematic Representation of the process of extraction of brain lipids polar from the goat brain](image)

Figure S2: Schematic Representation of the process of extraction of brain lipids polar from the goat brain
1.3. Optimization of protocol involving the preparation of the nanovesicles using polar brain lipids

As a modification of the solvent injection technique for the preparation of liposomes that traditionally uses ethanol or ether, the solubility of the lipids extracted were compared in various solvents. It was found that the 1 mg of polar brain lipids were better soluble in chloroform as compared to ethanol, methanol, isopropanol or acetone. (Figure S3(A)) For this purpose, chloroform was used as a solvent of choice in the dissolution of the lipids. Polydiacetylene was dissolved in dimethyl sulfoxide (DMSO). Since chloroform and DMSO are miscible, the solvents were used in suitable proportions in the preparation of nanovesicles.

The biomimetic nature of the nanovesicles was retained through the development of an optimized polar brain lipid (PBL): polydiacetylene (PDA) ratio. It was found that after UV crosslinking, the optimal ratio with stable readable color was at PBL: PDA (3:1 w/w). Previous attempts for the testing of drug permeability across the BBB has been established in PAMPA models using phospholipids (DOPC), cholesterol and PBL. The use of PBL in conjunction with PDA gave a better biomimetic model since the phospholipid concentrations (phosphatidylinositol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine), sphingomyelin and other lipid constituents were similar to the brain microvessel lipids. This is of importance, since there is extensive use of BMEC (brain microendothelial cells) in both co-culture models and their miniaturized BBB-on-chip counterparts. After the UV crosslinking of the diacetylene monomers, it was found that while PBL is needed in higher concentrations to maintain biomimetic nature of the nanovesicles, ratio of PBL: PDA equal to or above 4:1 w/w had a very low proportion of PDA which was unfavorable for blue coloration after polymerization and the suspension remained colorless. The suspension 2:1 and 3:1 showed the darkest color and the ratio of 3:1 remained stable for a longer period. (Figure S3, (B, C)) Thus, it was used as the concentration of choice for further studies.
Figure S3(A): Solubility of 1mg PBL in various solvents including ethanol, methanol, isopropanol, chloroform and acetone. (B): Gross images of PBL-PDA nanovesicle suspensions with various ratios of PBL: PDA (w/w) when the PBL was obtained commercially. (C): Gross images of PBL-PDA nanovesicle suspensions with various ratios of PBL: PDA (w/w) when the PBL was extracted in-house from goat brain lipids.

1.4. Algorithm for the detection of hue value for the development of a mobile app

Figure S4: Flowchart representing the steps involved in the development of a mobile application for the detection of BBB permeability through the device developed.

As a part of the steps towards the development of the mobile application, the following standard algorithm was used to obtain the Hue Saturation Value (HSV) from the Red Green Blue (RGB) analysis.
R' = R/255; G' = G/255; B' = B/255; (where R, G and B are the values obtained through RGB analysis)

Max = max(R', G', B');
Min = min(R', G', B');
Change = Max – Min

Calculation of Hue (expressed in degrees):
If Change=0, then Hue (in degrees) =0;
If R' is max and G'≥ B', then Hue (in degrees) = (60/ Change)* (G'-B')
If R' is max and B'>G', then Hue (in degrees) = (60/ Change)* (G'-B') + 360
If G' is max, then Hue (in degrees) = (60/ Change)* (B'-R') + 120
If B' is max, then Hue (in degrees) = (60/ Change)* (R'-G') + 240

Saturation calculation (expressed in percentage):
If Max=0, Saturation=0%;
Or Saturation = Change/Max (expressed as %)
Value Calculation (expressed in percentage):
Value=Max (expressed as %)

1.5. The development of the credit-card sized device.

For this purpose, a mould (86 mm X 55 mm) with pillars of 3 mm diameter and height 3 mm was designed using SolidWorks 2014 (Dassault Systemes SolidWorks Corporation, USA), saved as an .STL file, converted to a .gcode file and fabricated using the thermoplastic polymer acrylonitrile butadiene styrene (ABS) in a Fused Deposition Modelling (FDM) based 3D printer (Protocentre 999, Aha! 3D, India). For the development of the polydimethylsiloxane (PDMS)-based device, PDMS and cross-linker (10:1 w/w) were mixed, degassed and poured into the mould. The mould was then kept at room temperature for the curing of PDMS to give the final device. The wells in the device thus formed were filled with 50 µl of the nanoparticle suspension and used as a miniaturized BBB. (Figure S5)
1.6. Testing of other compounds towards the validation of the Polydiacetylene-based Biomimetic Nanovesicle Platform

There were two types of drugs tested for the validation of the device, those that were permeable across the BBB (BBB+) and those that were not (BBB-). A brief literature review was performed to obtain the details of the drug. The drugs (APIs), novel drug delivery systems as well as colored compounds were tested in various forms at various concentrations were analyzed for changes in hue, absorbance and fluorescence as summarized in Table S1.

Table S1: Testing of the device with suitable compounds

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds with concentration in mM provided in parenthesis</th>
<th>Hue, Absorbance (% blueness) &amp; fluorescence at 560 nm</th>
<th>Inference</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zolpidem (15)</td>
<td>338, 50.3%, 0.26 a.u.</td>
<td>BBB+</td>
<td>Zolpidem that is established as a sedative and hypnotic. While the Brain uptake index (BUI) of zolpidem is 0.67(^7) indicating a rapid brain uptake, the efflux of the drug is equally fast leading to the brain: plasma ratio in rats to be between 0.3-0.5 and thus explaining the short and intense action of the drug.(^8) It has a VolSurf+ descriptor CaCO(_2) value of 1.39 indicating passive permeability (value &gt; - 0.3)</td>
</tr>
<tr>
<td></td>
<td>Compound</td>
<td>Fluorescence (X, Y%, Z a.u.)</td>
<td>BBB Status</td>
<td>Notes</td>
</tr>
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<tr>
<td>2</td>
<td>Dopamine (100)</td>
<td>221, 62.8%, 0.11 a.u.</td>
<td>BBB-</td>
<td>Dopamine is used in vasodilation and not in the treatment of Parkinson’s disease where dopamine is deficient in basal ganglia. This is because the highly polar dopamine does not cross the blood-brain barrier if given into the peripheral circulation has no therapeutic effect in the CNS. Hence levodopa and carbidopa were developed as they could penetrate the BBB and treat Parkinson’s disease.</td>
</tr>
<tr>
<td>3</td>
<td>Diclofenac (125)</td>
<td>224, 67.6%, 0.09 a.u.</td>
<td>BBB-</td>
<td>Diclofenac (NSAID) has limited BBB permeability in most in vitro BBB models.</td>
</tr>
<tr>
<td>4</td>
<td>Temozolomide (200)</td>
<td>234, 38.4%, 0.13 a.u.</td>
<td>BBB-</td>
<td>Temozolomide API which is known to have less permeability across the blood brain barrier of close to 40% and hence the nanovesicle suspension remained blue. However, the testing of soya lecithin: cholesterol liposomes with TPGS turned the suspension red indicating permeability across the BBB. The model can thus be used for the prediction of BBB permeability of novel drug delivery systems too.</td>
</tr>
<tr>
<td>5</td>
<td>Temozolomide Liposomes prepared in-house (eq. temozolomide= 1.5mM)</td>
<td>347, 63.6%, 0.32 a.u.</td>
<td>BBB+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Minocycline (100)</td>
<td>Hue= 56 (due to the yellow color of the compound). Thus, the fluorescence was considered (0.28 a.u.)</td>
<td>BBB+</td>
<td>Minocycline has been known to be a lipophilic molecule which may pass through BBB and accumulate in the CSF and CNS thus finding application in the management of numerous CNS diseases.</td>
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References: