A non enzymatic glucose biosensor based on ultrasensitive calix[4]arene functionalized boronic acid gold nanoprobe in human blood serum

Alok Pandya, Pinkesh G. Sutariya and Shobhana K. Menon*

Department of Chemistry, School of Sciences, Gujarat University, Ahmedabad, 380009, Gujarat, India.

E-mail: ***shobhanamenon07@gmail.com***

Fax: +91 79 26308545; Tel: +91 79 26302286

1. Synthetic procedure:

1.1 Microwave assisted synthesis of *p*-tert-butylcalix[4]arene,1

The procedure was essentially the same as those developed by Menon et al [Menon et al., 2012] with minor modification .A mixture of p-tert-butyl phenol (4.0 g, 0.33 mM), sodium hydroxide (NaOH)(1 g) and formaldehyde(1.8 ml,0.18 mM) solution was taken in an open vessel and was irradiated with 50 W power in a microwave synthesizer Discover(CEM)by stirring for 3 min. After cooling for 10 min, resulted yellow solid mass. Next, 4 ml of toluene and 30 ml of diphenyl ether was added in this yellow solid, again irradiated with microwave power of 100 W for 5 min with stirring and obtained a dark brown solution. Further, this solution was added in to 75 ml of ethyl acetate and kept for 2 h. Finally, white precipitate was obtained which was filtered and washed with ethyl acetate and finally dried. Yield, 3.5g(96%).Elemental analysis for $C_{44}H_{56}O_4$, Calcd. C;81.44%, H;8.70%, O;9.80%, Found:C;80.11%,H;8.261%,O;9.90%^{1}HNMR: δ_H($CDCl_3$,400MH_Z):1.28(36H,s,tBu),3.81(8H.s,$ArCH_2$ Ar),7.12(8H,s,Ar-H), 9.61(4H,s,Ar-OH). FAB MS(m/z)648 (M+1).

1.2 Synthesis of 25,27-bis(bromoethane)-26,28-dihydroxy-*p-tert*- butyl calix[4]arene,2

A mixture of p-tert-butylcalix[4]arene **1** **(**3.5 g. 0.80 mM), K_2CO_3(1.9 g, 14.0 mM) and Dibromoethane (4 ml, 14.0 mM) in dry acetone (150 ml) was stirred for two days. The solvent was then evaporated under vacuum and the residue taken up with CH_3Cl_2 .The organic phase was washed with 0.1 M HCl up to neutrality and dried over anhydrous Na_2SO_4 . After complete evaporation of the solvent, the resulting crude product was purified by column chromatography (silica gel, hexane 9: ethyl acetate 1);2.9 g, yield(81%). m.p.:223-228^{O}C.Elemental analysis for $C_{46}H_{72}O_8N_4$, Calcd.C;68.3%,H;8.9%,O;15.20%,N;6.9%Found:C;68.1%,H;8.2%,O;14.90%,N;6.3%.^{1}HN MR:δ_H (DMSO,400MH_Z) :,1.20(18H,t-butyl,s,)0.96(18H,t-butyl,s),4.28(4H,-OCH_2,t),3.6(H,OCH_2,t), 3.18 (4H,$ArCH_2$Ar,d),3.97(4H,OCH_2,s),4.30(4H,$ArCH_2$Ar,d),6.42(4H,Ar-H,s),6.85(4H,Ar-H,s), 8.89 (2H, OH,s), ESI-MAAS (m/z) 846 (M+1).

1.3 Synthesis of 25,27-[bis(2-ethoxy)sulfanyl]-26,28-dihydroxy-*p-tert*-butyl calix[4]arene,3

To 50-mL of acetonitrile (Merck) containing 2.9 g of **2** and 0.43 g of thiourea (4.60 mM) was added and the resultant mixture was heated under reflux overnight. Acetonitrile was removed via reduced pressure. The resulting solid product was mixed with 0.47 g of KOH (7.50 mM) and an aliquot of 60 mL of deionised water. The mixture was refluxed for 2h. Compound 25,27-[bis(2-ethoxy)sulfanyl]-26,28-dihydroxy-p-tert-butyl calix[4]arene was extracted with 1 M HCl and CH_2Cl_2, dried with $MgSO_4$(s)and purified with column chromatography (SiO_2 , hexane/EtOAc 1:3),2.5 g:yield:88%, mp 165-168^{0}C. Anal.calc. $C_{48}H_{64}S_2O_4$: C, 74.95; H, 8.39; O, 8.32; S, 8.39%. Found: C, 73.98; H, 8.30; S,

8.12; O, 8.23%. FT-IR (KBr) υ :3200 cm-1(-OH), 2480 cm-1(-SH), 2890 cm-1(Ar-CH),[1]HNMR(DMSO)9.1(2H, OH, s),1.20(18H, t-butyl, s),0.96(18H, t-butyl, s),4.3 (4H, -OCH_2,t),2.85(4H,-OCH_2CH_2SH,q),1.45(2H,-OCH_2CH_2SH,t),3.18(4H, $ArCH_2Ar$, d), 6.87(4H, Ar-H, s),7.05(4H, Ar H, s) ESI -MASS: (m/z) 769(M+K).

1.4 Synthesis of 25,27 diethoxy[4- sulfanyl methy] phenyl boronic acid 26,28-dihydroxy-*p*-*tert*-butyl calix[4]arene,4

A mixture of 25,27-[bis(2-ethoxy)sulfanyl]-26,28-dihydroxy-*p*-tert-butyl calix[4]arene,**3 (**2.5 g. 0.70 mM), K_2CO_3(2.1 g, 14.0 mM) and 4-bromomethyl phenylboronic acid (2.1 g, 11.0 mM) in dry acetone (150 ml) was stirred for 48 h. The reaction mixture was evaporated in vacuum. The residue was dissolved in chloroform, and washed with 1% acetic acid aqueous solution. The organic solution was dried over anhydrous magnesium sulfate, filtered and evaporated. After complete evaporation of the solvent, the resulting crude product was purified by column chromatography (silica gel, hexane 9: ethyl acetate 1);1.8 g, yield:82%. Anal.calc. $C_{62}H_{78}S_2B_2O_8$: C, 71.81; H, 7.58; O, 12.34; S, 6.18, B, 2.08%. Found: C, 71.08; H, 7.40; S, 6.12; O, 12.23, B,1.98%. FT-IR (KBr) υ :3210 cm-1(-OH), 2880 cm-1(Ar-CH),[1]HNMR(DMSO)9.3(2H, OH, s),1.10(18H, t-butyl, s),1.16(18H, t-butyl, s),4.1 (4H, -OCH_2,t),2.85(4H,-OCH_2CH_2SPh,t),3.18(4H, $ArCH_2Ar$, d), 6.87(4H, Ar-H, s),7.05(4H, Ar H, s), 7.31(8H, Ar-H, s), 2.4 (4H,-$B(OH)_2$,s), 3.52(4H,-SCH_2,s) ESI -MASS: (m/z) 1036(M+1).

1.5 *p*-suphonato-25,27 diethoxy[4- sulfanyl methy]l phenyl boronic acid, 26,28-dihydroxy calix[4]arene,5

Compound 25,27- diethoxy[4-sulfanylmethy] phenyl boronic acid 26,28-dihydroxy-*p*-tert-butyl calix[4]arene **4** (1.8 g, 0.75 mM) in 5 mL of sulphuric acid (98%) was stirred at 80^{o}C for 5 h. An aliquot was withdrawn from the solution and poured in to water to determine the progress of the reaction. The reaction was completed when water insoluble material was not detected. After cooling, the precipitate was recovered by filtration. The product was obtained after evaporation of water. The product is dried overnight under high vacuum. Yield,1.4 g (90%), Anal.calc. $C_{46}H_{46}S_6B_2O_{18}$: C, 48.77; H, 4.09; O, 28.25; S, 16.98, B, 1.91%. Found: C, 47.99; H, 4.01; S, 16.72; O, 28.23, B,1.88%. FT-IR (KBr) υ :3200 cm-1(-OH), 2870 cm-1(Ar-CH),[1]HNMR(DMSO)9.1(2H, OH, s),1.10(18H, t-butyl, s),1.16(18H, t-butyl, s),4.1 (4H, -OCH_2,t),2.75(4H,-OCH_2CH_2SPh,t),3.81(4H, $ArCH_2Ar$, d), 6.76(4H, Ar-H, s),7.15(4H, Ar H, s), 7.11(8H, Ar-H, s), 2.4 (4H,-$B(OH)_2$,s),3.65(4H,-SCH_2,s), 9.6(4H,-SO_3H,s) ESI-MASS: (m/z) 1132(M+1).

1.6 Microwave synthesis of gold nanoparticles:

The procedure was essentially the same as those developed by with minor modification[1]. The molar ratio of $HAuCl_4$ to sodium citrate also has been changed accordingly. All glassware was thoroughly cleaned with freshly prepared 3:1 HCl/HNO_3 (aqua regia) and rinsed thoroughly with Milli-Q water prior to use. The synthesis was carried out in a modified CEM Discover microwave using single mode and continuous power at 2.45 GHz. The reactions were carried out in sealed reaction vessel containing 3 ml of 0.35 mM $HAuCl_4$ solution and 2 ml of 13 mM sodium citrate and was heated at 80 ^{0}C at a power up to 100 W for 4 min. The solution changed from pale yellow to burgundy to yield Au nanoparticles of 38 nm.

1.7 *p*-sulphonato-25,27 diethoxy[4- sulfanyl methy] phenyl boronic acid -26,28-dihydroxy calix[4]arene capped gold nanoparticles,6

0.35 mM AuNPs was added to 0.2 mM *p*- sulphonato-25,28 diethoxy[4- sulfanyl methy] phenyl boronic acid-26,28-dihydroxy calix[4]arene, **5** and kept stirring for 1 h, resulting the formation of 5,11,17,23 tetra suphonato-25,28 diethoxy[4- sulfanyl methy] phenyl boronic acid-26,28-dihydroxy calix[4]arene capped gold nanoparticles (*p*SC_4BA-AuNPs).

Fig. S1

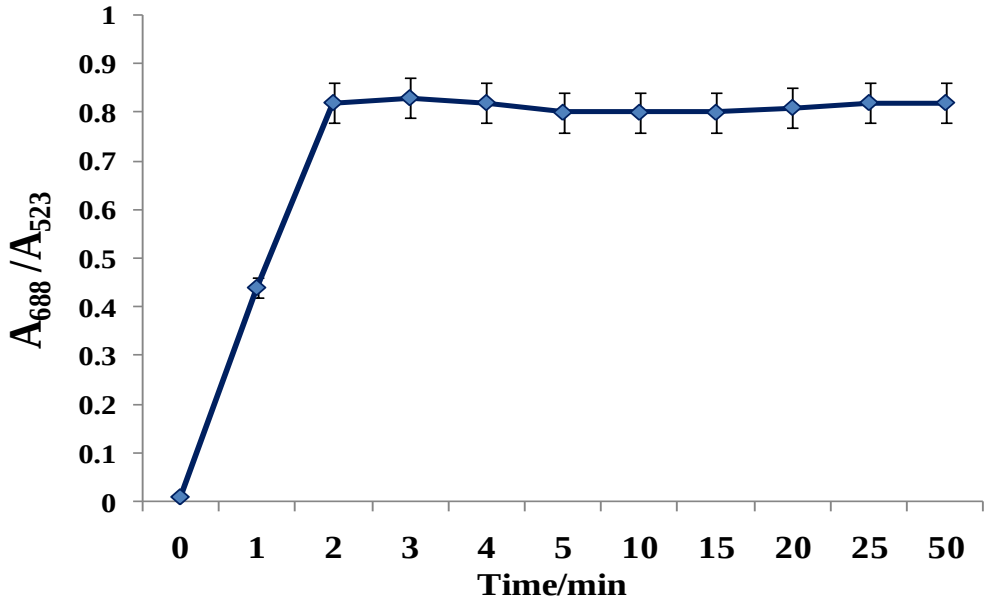

Fig. S1 Shows the response behaviour after addition of 1 µM D-glucose in 0.21 mM *p*SC_4BA- AuNp

Fig S2

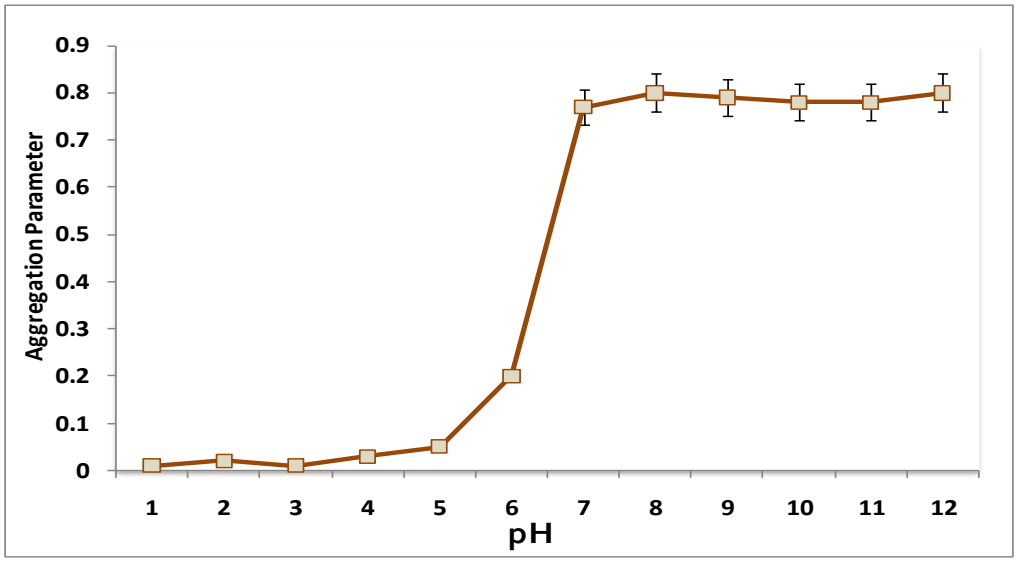

Fig S2 Aggregation parameter of pSC_4BA-AuNp-Glucose at different pH

Solution of pH	**2**	**4**	**6**	**8**	**10**
Stability of pSC_4BA-AuNp	3 h	5h	Weeks	Months	Months

Table S1 Stability of pSC_4BA-AuNp assembly at different pH conditions

Reference:

1. A. Pandya , K.V. Joshi, N.R Modi., Menon S. K.,2012, *Sensors and Actuators:B-Chemical* **168**,54– 61, 2012