Fluorescence Quenching of β -Casein by Silver Nanoparticles and Copper Nanoparticles in Presence of Sugars: A Quantitative Exploration

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The protein β -casein (BCA) exhibits a strong fluorescence emission, which is sensitive to active quenchers. Metal nanoparticles like copper nanoparticles and silver nanoparticles with enhanced and unique properties are reported to quench protein fluorescence appreciably. In the present work, the fluorescence quenching effects of metal nanoparticles on the protein fluorescence are extensively studied in presence of various sugar molecules. This may play a significant role in the sensing applications of sugar molecular systems in the presence of metal nanoparticles. β -Casein is used as fluorophore and sugar molecular systems like sucrose, maltose, fructose, lactose, galactose and glucose are chosen as quenchers and the protein-sugar interactions are arrived from fluorescence quenching results in the presence of copper nanoparticles and silver nanoparticles. To find the role of life time on the intensity of fluorescence, time resolved fluorescence measurements for the protein-sugar systems in the presence and absence of nanoparticles is measured. The protein-nanoparticles-sugar fluorescence interactions are analyzed by using Stern-Volmer plots and thermodynamic plots using fluorescence intensity quenching data. The trends in the extent of quenching action on protein fluorescence and also the binding interactions among protein sugar systems are quantified in the presence of copper nanoparticles and silver nanoparticles separately, in terms of Stern-Volmer constant values and binding constants values. β -Casein is found to interact strongly with the quenchers in the presence of copper nanoparticle and silver nanoparticle. Silver nanoparticle is read to be a better quenching mediator than copper nanoparticle for the fluorescence intensity in molecular interactions. Order of sugars quenching the protein fluorescence in presence of the nanoparticles is found.

Keywords: β -Casein, Silver nanoparticles, Copper nanoparticles, Sugars, Extended fluorescence quenching, Binding constant.

INTRODUCTION

Invention of non-invasive *in vivo* biosensors is today's necessity in diagnostics, medical analysis and therapy [1,2]. Detection and estimation of sugars in biological systems attract the attention of medical engineers. When protein-carbohydrate interactions are studied and established in presence of silver and copper nanoparticles, new immunotherapeutic strategies may be discovered in the treatment of malignant diseases [3]. In this paper the studies on the interaction of silver nanoparticles (AgNP) and copper nanoparticles (CuNP) with milk protein, β -casein (BCA) is quantitatively described using fluorescence quenching measurements. The interaction studies between the protein and the nanoparticles (Pro-NP) are extensively studied in presence of various monosaccharide and disaccharide sugars like glucose, fructose, galactose, sucrose, maltose and lactose. The protein, nanoparticles and sugar (Pro-NP-Sugar) molecular interaction may lead to the quantitative sensing of various sugars by fluorescence quenching of protein and the nanoparticle corona [4].

Metal nanoparticles are outstanding in bio-sensing due to their SPR, optical properties and reduced size in nanoranges [5-19]. New approach for the sensing of bio-molecules using fluorescence intensity quenching measurements have special advantages like highly sensitive, noninvasive and cost effective [1,20-22]. Metal nanoparticles based sensors are used for highly sensitive biomedical applications now-a-days. Fluorescence based sensing and binding can provide information about the micro environment of the protein, nanoparticles and sugar molecules [23-32]. Time resolved fluorescence measurement is a sophisticated technique to sense even a small change in protein, nanoparticles and sugar molecular interactions.

Fluorescence measurement on the interaction of protein sugars systems in the presence of nanoparticles serves as a tool to indirectly identify sugar molecules like glucose [33]. Therefore, in present study the natural protein β -casein which form a vital part of *in vivo* systems is used as sensitive fluorophores for the quantitative study of interaction of protein, nanoparticles and sugar systems.

EXPERIMENTAL

Synthesis of silver nanoparticle and copper nanoparticle:

Wet chemical method which involves the addition of 5 mL of 1 mM solution of cetyltrimethyl ammonium bromide solution to 20 mL of 1 mM solution of metal salt solution was adopted. The mixture was stirred at 30 °C while drops of freshly prepared sodium borohydride were added. The solution was heated to 40 °C and stirred for 0.5 h. Honey yellow coloured silver nanoparticle and wine red coloured copper nanoparticle which are spherical and highly monodispersed were obtained respectively. The nanoparticles in the suspensions are stored in N₂ for future use and they are characterized using HRTEM, SEM and EDX measurements [34-45].

Fluorescence quenching studies: In the present work, the fluorescence intensity of samples was studied using Cary Eclipse FL120 1M fluorescence spectrophotometer with a xenon flash lamp as a source. All the fluorescence measurements were performed using a standard dimension quartz cuvette with a Teflon stop cock, following the required standardization of the instruments. The quenching of fluorescence emissions of the proteins, keeping the protein as parent molecule and subsequently mixed with the metal nanoparticles followed by the addition of quenchers each separately are studied. The excitation and emission slit width and scan rate were maintained to be constant. The excitation wavelength for β -casein was at 274 nm and the emission range was set to be between 285-600 nm. When the order of addition of protein, nanoparticles and sugars is altered, the decrease in fluorescence intensity was found diminished.

The inner filter effect due to nanoparticles is reduced to minimum by using very low concentrations of the protein and nanoparticles [46-49]. This is confirmed by plotting τ/τ_0 for selected values of protein concentrations. In the protein, nanoparticles and sugar molecular system the effect is excluded as sugar molecules does not have significant absorption in the wavelength range under study [50].

Time resolved fluorescence measurements of β -casein with silver nanoparticle and copper nanoparticle and quenchers: In order to utilize the fluorescence quenching data of various types of functional molecules, the metal nanoparticles interacting with β -casein systems, time resolved fluorescence measurements are carried out to find the role of life time on the intensity of the emitted fluorescence and the sensitivity towards environmental changes [51-54]. These studies are extended for fluorescence quenching by a set of molecules belonging to sugars. This enables to find the binding

interactions of proteins in multi component systems comprising metal nanoparticles and quenching molecules.

The life time measurements of the fluorescence emissions of protein, nanoparticles and sugar systems are studied. For each of the quencher molecular system adopted, the life time measurements are found separately for both in the presence of silver nanoparticle and copper nanoparticle. The average values of the life times measured are presented in Table-1.

Binding constant studies: The intensities of β -casein fluorescence emissions were found to decrease considerably with increase in concentration of quencher solutions. The decrease in intensity of fluorescence data are used to obtain the binding parameter, the binding constant (K_B). The bimolecular quenching constant (K_q) is calculated using Stern Volmer (SV) equation [4,55].

$$\frac{I_0}{I} = 1 + K_{SV}[Q] = 1 + K_1\tau_0[Q]$$

I_0 and I are the steady state fluorescence intensities in the absence and presence of the quencher respectively. $[Q]$ is concentration of quencher and K_{SV} is the Stern-Volmer quenching constant. The fluorescence intensity quenching is analyzed quantitatively by plotting Stern Volmer plots. From the slope values obtained using the plots, the Stern Volmer constants are calculated and subsequently are used to find the bimolecular quenching constant values. The protein and the sugar molecules are considered to be in equilibrium favourably with the protein-sugar association complex. The extent of the stability of the complex is known from the magnitude of the association constant value. The thermodynamic plots are made for analyzing the protein-sugar interaction in terms of binding constant values.

At constant temperature and pressure conditions, since the equilibrium is existing between the molecules, the thermodynamic constant values of the association complex formation (K_B) have been determined. The thermodynamic plots are made by plotting $\log((I_0-I)/I)$ vs. $\log [Q]$. From the linear plots, using the slope and intercept values, the thermodynamic stability constant values are determined [55].

RESULTS AND DISCUSSION

The fluorescence decay life time measurements recorded at 356 nm for pure β -casein are presented in Fig. 1. The life time values for the proteins observed in nanoseconds are listed in Table-2. The average life time τ_0 value for β -casein is found to be 3.28×10^{-9} sec.

TABLE-1
STERN VOLMER CONSTANT VALUES DETERMINED FROM FLUORESCENCE QUENCHING OF β -CASEIN MEDIATED BY SILVER NANOPARTICLE AND COPPER NANOPARTICLE IN THE PRESENCE OF SUGARS AS QUENCHERS

Sugar	Silver nanoparticle			Copper nanoparticle		
	τ (ns)	K_{SV} (M ⁻¹)	K_q (M ⁻¹ ns ⁻¹)	τ (ns)	K_{SV} (M ⁻¹)	K_q (M ⁻¹ ns ⁻¹)
Sucrose	2.71	7.85×10^{-2}	2.90×10^7	2.75	4.49×10^{-2}	1.63×10^7
Maltose	2.76	5.51×10^{-4}	2.00×10^5	2.81	2.63×10^{-2}	9.38×10^6
Fructose	2.81	4.74×10^{-2}	1.69×10^7	2.87	2.22×10^{-2}	7.73×10^6
Lactose	2.86	9.54×10^{-5}	3.34×10^4	2.92	3.48×10^{-4}	1.19×10^5
Galactose	2.90	1.15×10^{-3}	3.96×10^5	3.00	3.25×10^{-3}	1.08×10^6
Glucose	2.95	2.92×10^{-5}	9.90×10^3	3.08	2.86×10^{-5}	9.29×10^3

Fig. 1. Time resolved fluorescence decay of β -casein in the presence of (i) silver nanoparticles and (ii) copper nanoparticles

TABLE-2
FLUORESCENCE DECAY LIFE TIME VALUES
FOR THE PROTEIN IN NANOSECONDS

Protein	Without the presence of nanoparticle	In the presence of silver nanoparticle	In the presence of copper nanoparticle
β -casein	3.28	3.04	3.13

Fluorescence emission spectra of milk protein β -casein, in the absence of any additives, in the presence of copper nanoparticle and each in the presence of six sugar systems of constant composition are recorded separately and are depicted in the form of a super imposed graph in Fig. 2a. The fluorescence emission spectra of β -casein is studied in the presence of silver nanoparticle instead of copper nanoparticle as mentioned above are presented in the Fig. 2b. The emission spectra of β -casein show a strong peak at 356 nm, which upon the addition of the nanoparticles and subsequently the sugars show a gradual decrease in the intensity values.

The influence of sugar concentrations on the emission spectra of β -casein, in the presence and absence of copper nanoparticle and silver nanoparticle are plotted respectively in Fig. 3a and 3b. The values of K_q and K_{SV} determined for

Fig. 2a. Fluorescence spectra of β -casein in the absence of any additives, in the presence of copper nanoparticle and each in the presence of six sugar systems; Excitation: 275 nm; Emission: 356 nm; (1) BCA (2) BCA + CuNP (3) BCA + CuNP + glucose (4) BCA + CuNP + galactose (5) BCA + CuNP + lactose (6) BCA + CuNP + fructose (7) BCA + CuNP + maltose (8) BCA + CuNP + sucrose

Fig. 2b. Fluorescence spectra of β -casein in the absence of any additives, in the presence of silver nanoparticle and each in the presence of six sugar systems; Excitation: 275 nm; Emission: 356 nm; (1) BCA (2) BCA + AgNP (3) BCA + AgNP + glucose (4) BCA + AgNP + galactose (5) BCA + AgNP + lactose (6) BCA + AgNP + maltose (7) BCA + AgNP + fructose (8) BCA + AgNP + sucrose

β -casein protein in presence of the silver nanoparticle and copper nanoparticle as well as in the presence of various sugar systems are listed in the Table-1.

In Fig. 4a and 4b, the thermodynamic plots for the quenching of β -casein protein with copper nanoparticle and silver nanoparticle along with the six sugar systems are given. The thermodynamic constant of the quenching process, which is considered as the binding constant are listed in Table-3 for β -casein protein systems. The K_B values indicate that the binding interactions under experimental conditions adopted here are favourable between the protein and each of the sugar molecules. Comparing the binding interactions of each of the sugar molecular systems, the binding constant values imply that β -casein protein surface is stronger for the binding interactions of the sugars on the surface.

TABLE-3
BINDING CONSTANT VALUES (K_B) AND AVERAGE NUMBER OF BINDING SITES (n) FOR INTERACTION OF β -CASEIN
MEDIATED BY COPPER NANOPARTICLE AND SILVER NANOPARTICLE IN THE PRESENCE OF SUGAR SYSTEMS

Sugar	Without the presence of nanoparticle		In the presence of silver nanoparticle		In the presence of copper nanoparticle	
	K_B	n	K_B	n	K_B	n
Sucrose	8.14×10^2	0.95	9.38×10^2	0.94	8.77×10^2	1.00
Maltose	5.32×10^2	1.02	5.60×10^2	1.51	5.57×10^2	1.01
Fructose	5.18×10^2	1.14	5.28×10^2	0.93	5.15×10^2	1.02
Lactose	5.11×10^2	1.51	9.93×10^1	1.51	8.40×10^1	1.32
Galactose	3.83×10^1	1.42	6.76×10^1	1.14	5.57×10^1	0.98
Glucose	3.81×10^1	1.60	5.01×10^1	1.57	4.30×10^1	1.56

Fig. 3. Fluorescence intensity of β -casein at various quencher concentrations for the six sugar systems in the presence of copper nanoparticle (a), presence of silver nanoparticle (b)

Based on the Stern Volmer plots (Fig. 5), the Stern Volmer constant values are calculated and are considered to qualify the fluorescence quenching property of the sugar systems on the protein. From the Stern Volmer constant values listed in the Table-1, the trend in the K_{SV} values for the six different sugar systems with β -casein protein is found. The inference from Stern Volmer plots agree with those from K_B values observed in the thermodynamic plots.

Time resolved fluorescence measurements of copper nanoparticles and silver nanoparticles with β -casein and quenchers: In the presence of silver nanoparticle and copper nanoparticle in separate experiments the fluorescence lifetime values for β -casein are found to get reduced. The values clearly emphasize the fact that presence of silver nanoparticle and as well as copper nanoparticle reduce the fluorescence life times

Fig. 4. Thermodynamic plots for the quenching of β -casein with (a) copper nanoparticle and (b) silver nanoparticle in the presence of six sugar systems as quenchers

Fig. 5. Stern Volmer plot of β -casein protein with various sugar systems in the presence of copper nanoparticles

of the protein considerably. Silver nanoparticle and copper nanoparticle act as quenchers for the two proteins when Trp fluorescence is considered which can be attributed to binding interactions of the metal nanoparticles with the protein.

The life time measurements are carried out for β -casein in the presence of silver nanoparticle and copper nanoparticle each further studied in presence of quencher molecules from different classes of sugars. The results indicate that the fluorescence decay is mono-exponential in case of β -casein in all the cases. It is found that there is definite binding interaction prevails between β -casein with the quencher molecules.

The silver nanoparticle and copper nanoparticle act as good mediators and enhance the interactions between the quencher molecules chosen and the protein. In fact the presence of metal nanoparticles may stabilize the binding interactions between the pair of protein and quencher molecules. The mechanism of quenching action may be understood by using the following diagram (Fig. 6).

Fig. 6. Mechanism of fluorescence quenching of β -casein in presence of silver nanoparticles and copper nanoparticles

Binding interactions of sugar systems with β -casein in presence of copper nanoparticle and silver nanoparticle:

The results of K_B values indicate that in the presence of silver nanoparticle and copper nanoparticle, the binding interactions are found stronger between β -casein with the six different sugar systems. The K_B values determined and furnished in the table indicate that the free energy change values of the binding interactions are more negative for silver nanoparticle and copper nanoparticle indicating favourable interactions. The values of K_B for all the six sugar molecular systems are higher for silver nanoparticle than the copper nanoparticle for β -casein protein. Therefore based on the overall interactions it is inferred

that the binding interactions set the trend as silver nanoparticle > copper nanoparticle > absence of metal nanoparticles. This is true for the presence of six sugar systems investigated in presence of each of the metal nanoparticle systems.

The trend in the binding interactions of the six sugar systems for the proteins in presence of the nanoparticles is sucrose > maltose > fructose > lactose > galactose > glucose. This effect can be attributed to the mediating behaviour of metal nanoparticles that do not chemically interfere and only influence the hydrogen bonding interactions prevailing between each of sugar molecular system with that of the proteins. The protein surface will be hydrophilic containing mostly $-\text{NH}_3^+$ and $-\text{COO}^-$ ions and the core being hydrophobically compact constituted by the alkyl or aromatic hydrophobic groups of the amino acids present in the proteins. Due to strong hydrogen bond interactions of the hydroxyl group of sugar molecules and the hydrophilic groups on the protein surface, binding interactions are induced [26,56]. The strong hydrophilic-hydrophilic interactions among the sugar molecules with the protein molecules is more for disaccharides than monosaccharide. Such interactions also depend on the closed six membered ring structures of the sugar molecules. The balances between these interactions determine the number of binding sites that will be available to sugar molecules that are possible to collide with the protein.

Conclusion

The molecular protein-nanoparticles-sugar interactions are studied using fluorescence intensity quenching of β -casein protein in the presence of copper nanoparticles and silver nanoparticles and in the presence of various sugars. Quantitative study of fluorescence quenching is done using Stern-Volmer plots and thermodynamic plots and the quenching constant values and the binding parameters are determined. From the quenching constant values, it is found that there is a definite protein-nanoparticle-sugar interaction. Out of the two metal nanoparticles silver nanoparticles is found to be a better mediator than copper nanoparticles for the fluorescence quenching action and hence sugar interactions. Based on the K_q and K_B values, the order of interaction of sugars with the protein in the presence of silver nanoparticles and copper nanoparticles is determined to be sucrose > maltose > fructose > lactose > galactose > glucose.

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REFERENCES

1. K. Yum, T.P. McNicholas, B. Mu and M.S. Strano, *J. Diabetes Sci. Technol.*, **7**, 72 (2013); <https://doi.org/10.1177/193229681300700109>.
2. S. Lalonde, E. Boles, H. Hellmann, L. Barker, J.W. atrick, W.B. Frommer, J.M. Ward, *The Plant Cell Online*, **11**, 707 (1999). <https://doi.org/10.1105/tpc.11.4.707>.
3. J. Holgersson, A. Gustafsson and M.E. Breimer, *Immunol. Cell Biol.*, **83**, 694 (2005); <https://doi.org/10.1111/j.1440-1711.2005.01373.x>.

4. S.K. Vaishnav, J. Korram, R. Nagwanshi, K.K. Ghosh and M.L. Satnami, *J. Fluoresc.*, **26**, 855 (2016); <https://doi.org/10.1007/s10895-016-1773-8>.
5. J. Hesselberth, M.P. Robertson, S. Jhaveri and A.D. Ellington, *Rev. Mol. Biotechnol.*, **74**, 15 (2000); [https://doi.org/10.1016/S1389-0352\(99\)00005-7](https://doi.org/10.1016/S1389-0352(99)00005-7).
6. R.R. Breaker, *Curr. Opin. Biotechnol.*, **13**, 31 (2002); [https://doi.org/10.1016/S0958-1669\(02\)00281-1](https://doi.org/10.1016/S0958-1669(02)00281-1).
7. S. Seetharaman, M. Zivarts, N. Sudarsan and R.R. Breaker, *Nat. Biotechnol.*, **19**, 336 (2001); <https://doi.org/10.1038/86723>.
8. Y. Lu, *Chem. Eur. J.*, **8**, 4588 (2002); [https://doi.org/10.1002/1521-3765\(20021018\)8:20<4588::AID-CHEM4588>3.0.CO;2-Q](https://doi.org/10.1002/1521-3765(20021018)8:20<4588::AID-CHEM4588>3.0.CO;2-Q).
9. S.D. Jayasena, *Clin. Chem.*, **45**, 1628 (1999).
10. K. Haupt and K. Mosbach, *Chem. Rev.*, **100**, 2495 (2000); <https://doi.org/10.1021/cr990099w>.
11. E. Gizeli and C.R. Lowe, *Curr. Opin. Biotechnol.*, **7**, 66 (1996); [https://doi.org/10.1016/S0958-1669\(96\)80097-8](https://doi.org/10.1016/S0958-1669(96)80097-8).
12. A.P.F. Turner, *Science*, **290**, 1315 (2000); <https://doi.org/10.1126/science.290.5495.1315>.
13. D.A. Blake, R.M. Jones, R.C. Blake II, A.R. Pavlov, I.A. Darwish and H. Yu, *Biosens. Bioelectron.*, **16**, 799 (2001); [https://doi.org/10.1016/S0956-5663\(01\)00223-8](https://doi.org/10.1016/S0956-5663(01)00223-8).
14. K. Sokolov, J. Aaron, B. Hsu, D. Nida, A. Gillenwater, M. Follen, C. MacAulay, K. Adler-Storh, B. Korgel, M. Descour, R. Pasqualini, W. Arap, W. Lam and R. Richards-Kortum, *Technol. Cancer Res. Treat.*, **2**, 491 (2003); <https://doi.org/10.1177/153303460300200602>.
15. K. Sokolov, M. Follen, J. Aaron, I. Pavlova, A. Malpica, R. Lotan and R. Richartz-Kortum, *Cancer Res.*, **63**, 1999 (2003).
16. D. Yelin, D. Oron, S. Thiberge, E. Moses and Y. Silberberg, *Opt. Express*, **11**, 1385 (2003); <https://doi.org/10.1364/OE.11.001385>.
17. A.E. Baer, T.A. Laursen, F. Guilak and L.A. Setton, *J. Biomech. Eng.*, **125**, 1 (2003); <https://doi.org/10.1115/1.1532790>.
18. D. Yelin, D. Oron, E. Korkotian, M. Segal and Y. Silberberg, *Appl. Phys. B*, **74**(Suppl.), S97 (2002); <https://doi.org/10.1007/s00340-002-0884-x>.
19. L.R. Hirsch, R.J. Stafford, J.A. Bankson, S.R. Sershen, B. Rivera, R.E. Price, J.D. Hazle, N.J. Halas and J.L. West, *Proc. Natl. Acad. Sci. USA*, **100**, 13549 (2003); <https://doi.org/10.1073/pnas.2232479100>.
20. M.I.J. Stich, L.H. Fischer and O.S. Wolfbeis, *Chem. Soc. Rev.*, **39**, 3102 (2010); <https://doi.org/10.1039/B909635N>.
21. M.A. Arnold, J.T. Olesberg and G.W. Small, eds.: D. D. Cunningham and J. A. Stenken, Near-Infrared Spectroscopy for Noninvasive Glucose Sensing, In: *In Vivo Glucose Sensing*, John Wiley & Sons, Inc., Hoboken, NJ, USA, vol. 174 (2009).
22. M.K. Balaconis, K. Billingsley, J.M. Dubach, K.J. Cash and H.A. Clark, *J. Diabetes Sci. Technol.*, **5**, 68 (2011); <https://doi.org/10.1177/193229681100500110>.
23. E. Alarcón, A.M. Edwards, A. Aspée, C.D. Borsarelli and E.A. Lissi, *Photochem. Photobiol. Sci.*, **8**, 933 (2009); <https://doi.org/10.1039/b901056d>.
24. D.E. Epps, T.J. Raub and F.J. K'ezdy, *Anal. Biochem.*, **227**, 342 (1995); <https://doi.org/10.1006/abio.1995.1290>.
25. D.E. Epps, T.J. Raub, V. Caiolfa, A. Chiari and M. Zamai, *J. Pharm. Pharmacol.*, **51**, 41 (1999); <https://doi.org/10.1211/0022357991772079>.
26. A. Barik, K.I. Priyadarsini and H. Mohan, *Photochem. Photobiol.*, **77**, 597 (2003); [https://doi.org/10.1562/0031-8655\(2003\)077<0597:PSOBOC>2.0.CO;2](https://doi.org/10.1562/0031-8655(2003)077<0597:PSOBOC>2.0.CO;2).
27. E.I. Alarcon, C.J. Bueno-Alejo, C.W. Noel, K.G. Stampelcoskie, N.L. Pacioni, H. Poblete and J.C. Scaiano, *J. Nanopart. Res.*, **15**, 1374 (2013); <https://doi.org/10.1007/s11051-012-1374-7>.
28. U. Kragh-Hansen, *Dan. Med. Bull.*, **37**, 57 (1990).
29. I. Lynch and K.A. Dawson, *Nano Today*, **3**, 40 (2008); [https://doi.org/10.1016/S1748-0132\(08\)70014-8](https://doi.org/10.1016/S1748-0132(08)70014-8).
30. J. Mariam, P.M. Dongre and D.C. Kothari, *J. Fluoresc.*, **21**, 2193 (2011); <https://doi.org/10.1007/s10895-011-0922-3>.
31. M. Moller and A. Denicola, *Biochem. Mol. Biol. Educ.*, **30**, 309 (2002); <https://doi.org/10.1002/bmb.2002.494030050089>.
32. M. Moller and A. Denicola, *Biochem. Mol. Biol. Educ.*, **30**, 175 (2002); <https://doi.org/10.1002/bmb.2002.494030030035>.
33. J.C. Pickup, F. Khan, Z.-L. Zhi, J. Coulter and D.J.S. Birch, *J. Diabetes Sci. Technol.*, **7**, 62 (2013); <https://doi.org/10.1177/193229681300700108>.
34. R. Sierra-Avila, M. Perez-Alvarez, G. Cadenas-Pliego, C.A. Ávila-Orta, R. Betancourt-Galindo, E. Jiménez-Regalado, R.M. Jiménez-Barrera, and J.G. Martínez-Colunga, *J. Nanomater.*, **Article ID 361791** (2014); <https://doi.org/10.1155/2014/361791>.
35. S. Chandra, A. Kumar and P.K. Tomar, *J. Saudi Chem. Soc.*, **18**, 149 (2014); <https://doi.org/10.1016/j.jscs.2011.06.009>.
36. T.M.D. Dang, T.T.T. Le, E. Fribourg-Blanc and M.C. Dang, *Adv. Nat. Sci.: Nanosci. Nanotechnol.*, **2**, 025004 (2011); <https://doi.org/10.1088/2043-6262/2/2/025004>.
37. D. Deng, Y. Jin, Y. Cheng, T. Qi and F. Xiao, *ACS Appl. Mater. Interf.*, **5**, 3839 (2013); <https://doi.org/10.1021/am400480k>.
38. A. Zielinska, E. Skwarek, A. Zaleska, M. Gazda and J. Hupka, *Procedia Chem.*, **1**, 1560 (2009); <https://doi.org/10.1016/j.proche.2009.11.004>.
39. A.W. Orbaek, M.M. McHale and A.R. Barron, *J. Chem. Educ.*, **92**, 339 (2015); <https://doi.org/10.1021/ed500036b>.
40. W. Zhang, X. Qiao and J. Chen, *Chem. Phys.*, **330**, 495 (2006); <https://doi.org/10.1016/j.chemphys.2006.09.029>.
41. B. Khodashenas and H.R. Ghorbani, *J. Arab. Chem.*, (2015); <https://doi.org/10.1016/j.arabjc.2014.12.014>.
42. Z. Khan, S.A. Al-Thabaiti, A.Y. Obaid and A.O. Al-Youbi, *Colloids Surf. B Biointerfaces*, **82**, 513 (2011); <https://doi.org/10.1016/j.colsurfb.2010.10.008>.
43. A.J. Christy and M. Umadevi, *Adv. Nat. Sci.: Nanosci. Nanotechnol.*, **3**, 035013 (2012); <https://doi.org/10.1088/2043-6262/3/3/035013>.
44. J.I. Hussain, S. Kumar, A.A. Hashmi and Z. Khan, *Adv. Mater. Lett.*, **2**, 188 (2011); <https://doi.org/10.5185/amlett.2011.1206>.
45. X. Li, J. Shen, A. Du, Z. Zhang, G. Gao, H. Yang and J. Wu, *Colloids Surf. A Physicochem. Eng. Asp.*, **400**, 73 (2012); <https://doi.org/10.1016/j.colsurfa.2012.03.002>.
46. T. Larsson, M. Wedborg and D. Turner, *Anal. Chim. Acta*, **583**, 357 (2007); <https://doi.org/10.1016/j.aca.2006.09.067>.
47. M. Kubista, R. Sjoback, S. Eriksson and B. Albinsson, *Analyst*, **119**, 417 (1994); <https://doi.org/10.1039/AN9941900417>.
48. M.L. Mertens and J.H.R. Kägi, *Anal. Biochem.*, **96**, 448 (1979); [https://doi.org/10.1016/0003-2697\(79\)90605-5](https://doi.org/10.1016/0003-2697(79)90605-5).
49. X. Luciani, S. Mounier, R. Redon and A. Bois, *Chemomet. Intell. Lab. Syst.*, **96**, 227 (2009); <https://doi.org/10.1016/j.chemolab.2009.02.008>.
50. V.R. Preedy, *Dietary Sugars: Chemistry, Analysis, Function and Effects*, Royal Society of Chemistry, Chap. 17 (2012).
51. N. Tayeh, T. Rungassamy and J.R. Albani, *J. Pharm. Biomed. Anal.*, **50**, 107 (2009); <https://doi.org/10.1016/j.jpba.2009.03.015>.
52. A. Kathiravan, R. Renganathan and S. Anandan, *Polyhedron*, **28**, 157 (2009); <https://doi.org/10.1016/j.poly.2008.09.023>.
53. B. Chakraborty and S. Basu, *J. Lumin.*, **129**, 34 (2009); <https://doi.org/10.1016/j.jlumin.2008.07.012>.
54. D.M. Togashi, A.G. Ryder, D. McMahon, P. Dunne and J. McManus, Vol. 6628 of *Proceedings of SPIE-OSA Biomedical Optics*, Optical Society of America, paper No. 6628-61 (2007); https://doi.org/10.1364/ECBO.2007.6628_61.
55. Y. Teng, R. Liu, S. Yan, X. Pan, P. Zhang and M. Wang, *J. Fluoresc.*, **20**, 381 (2010); <https://doi.org/10.1007/s10895-009-0543-2>.
56. J.S. Gebauer, M. Malissek, S. Simon, S.K. Knauer, M. Maskos, R.H. Stauber, W. Peukert and L. Treuel, *Langmuir*, **28**, 9673 (2012); <https://doi.org/10.1021/la301104a>.