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Research article

Impact of non-covalent interactions on the solvation of ovalbumin in an aqueous environment of different pHs: Thermodynamic and diffusion studies

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ABSTRACT

The solvation behavior of protein is an important factor in protein-based food products. In the present study, the xylitol (XY) - ovalbumin (OVN) interaction in an aqueous solution of different pH conditions is analyzed in two methods. In one method, the thermodynamic parameters Gibbs free energy, free volume, and internal pressure are calculated by using ultrasonic velocity, density, and viscosity in addition the refractive index is also measured. The second method is a theoretical method in which using the Laplace transform technique the diffused amount of protein have been calculated for OVN with and without XY in different pH environment. The addition of XY with OVN makes the system with more free energy and free volume as the internal pressure decreases. This trend shows that preferential interaction occurs between solvent–solute molecules. The diffusivity of OVN is reduced after the addition of XY representing the strength of protein-protein interaction. The effect of pH changes is well reflected in both experimental and theoretical results. The results confirm that acidic pH extremity offers more solvation of OVN compared to alkaline pH extremity.

1. Introduction

Globular proteins play a vital role in food and pharmaceutical applications because of having many functional properties such as gelling, foaming, emulsion, etc [1–4]. These functional properties of proteins can be modified by different types of additives like polysaccharides, sugars, artificial sweeteners, etc. Sugar-based food products have high energy values hence consumption of sugars leads to heavier health risks like obesity and diabetes etc. [5].Hence researchers find alternatives for sugars to avoid health risks such as sugar replacers or sugar alcohols. They have low-calorie values but the same or high sweetness as sugar. Sugar alcohols are classified into seven types namely sorbitol, xylitol (XY), mannitol, maltitol, erythritol, adonitol, and glycerol. Among them, sorbitol, XY, mannitol, and erythritol are commonly used sugar alcohols in various food and nutrient products. In sugar alcohols, XY is widely used

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as a sugar substitute in the form of food additive to improve taste and reduce calories in food [6,7]. Adding polyols such as XY as a cosolvent in food products provides sweetness as well as affects the physical, chemical, and functional properties of food [5,8–10]. When adding cosolvent in a protein-water system, protein solvation is a crucial parameter that alters the interaction between protein and solvent/cosolvent.

Solvation of protein indicates protein interaction with the solvent/cosolvent molecules which leads to the stabilization/destabilization of protein molecules in solution. The solvation process of protein makes the amino acids of a protein involved in the bond formation such as hydrogen bonding, and van der Waals forces with solvent/cosolvent molecules in that way stabilize/denature protein structure in a solution [11]. The nature of the interaction between protein and solvent/cosolvent is categorized into four types namely electrostatic type interactions, preferential exclusion, hydrophobic type interaction, and hydrogen bonding [12]. Such interactions are collectively known as non-covalent interactions. This interaction can be modified based on the food ingredients, especially the type of protein molecule (molecular weight, hydrophobicity, and flexibility), the nature of solvent/cosolvent, the solution profile (pH and ionic strength), and the solution environment (for example temperature and pressure), etc. In this regard, numerous researches [13–17] have been carried out for the ovalbumin (OVN) protein. The pH cum cosolvent inclusion is a simple technique for protein self-assembly, partially unfolding, and stabilization of protein structure in extremely acidic or alkaline conditions through altering the non-covalent interactions inside/outside the protein molecule. Folding and unfolding encourage the self-assembly of proteins with other substances [18]. The deviation in protein structure during pH changes as well as cosolvent inclusion can be well reflected in the physical-chemical properties of protein solution. The physical-chemical properties of protein solutions can be best studied by ultrasonic, volumetric, spectroscopic, diffusion, calorimetric, and thermodynamic studies [13–15]. However, the thermodynamics and diffusion studies are very scarcely attempted [15,17]. The present analysis is aimed at studying the solvation profile of OVN through the analysis of physical-chemical properties in various pH conditions with and without cosolvent XY. This analysis was carried out in both experimental and theoretical aspects. In an experimental aspect, internal pressure, free volume, and change in Gibbs free energy have been carried out. These can be determined by the experimentally measured parameters of ultrasonic velocity, density, viscosity, and refractive index. On the other hand, in the theoretical aspect, the Laplace transform was performed to explore the diffusion coefficient of OVN. Based on these techniques the changes in the non-covalent interactions are analyzed for obtaining the solvation process of OVN.

2. Materials and methods

2.1. Materials

The OVN in powder form was purchased from Sigma Aldrich. Monobasic sodium phosphate (MSP) and dibasic sodium phosphates (DSP) and XY were purchased from NICE Chemicals.OVN solution was prepared in 5 mg/ml concentration.0.2M aqueous sodium phosphates buffers (B)were prepared in the acidic and alkaline ranges (pH 2, 5, 7, 9, 12). In system I, B + OVN was prepared by dissolving OVN in B, in system II, B + XY + OVN was prepared by the addition of 1M of XY.

2.2. Methods

The density (ρ) was measured by a specific gravity bottle of 5 ml at 0.0001 kgm^{-3} range. The ultrasound velocity (u) was measured by an ultrasonic interferometer of 2 MHz frequency (Mittal make F-81 model) at the accuracy of 0.1 ms^{-1}. The viscosity (η) was measured by Ostwald's viscometer (10 ml) with an accuracy of 0.001$mNsm^{-2}$. The surface tension (γ) was measured using the drop weight method. In surface tension measurement an identical drop formation was done by platinum-irridium Du Nouy ring and measurement was done at the accuracy of 0.0001Nm^{-1}. The refractive index (n) was determined by using the Abbe refractometer with an accuracy of ±0.0002.

Internal pressure can be calculated by equation (1)

$$P_i = bRT\ (K\eta/u^{1/2})(\rho^{2/3}\ M_{eff}^{7/6}) \quad (1)$$

Where,

b is the cubic packing factor (b = 2 for all liquids [19])

R – Gas constant (8.314 $JK^{-1}\ mol^{-1}$)

The free volume can be calculated by equation (2)

$$V_f = (M_{eff}u/k\eta)^{3/2} \quad (2)$$

Where $M_{eff} = M_1x_1 + M_2x_2$ where M_1, M_2 are molecular weights and x_1, x_2 are mole fractions of the constituent components.u is ultrasonic velocity and k is a temperature-independent constant ($k = 4.281 \times 10^9$ [20])

The change in the Gibbs free energy was calculated using equation (3) [21].

$$\Delta G = KT\ \ln\ [KT\tau/h] \quad (3)$$

Where,

K – Boltzmann's constant ($1.38 \times 10^{-23}\ JK^{-1}$)

h – Plank's constant (6.63×10^{-34}Js)

T – Temperature
$\boldsymbol{\tau}$ – Relaxation time.
Relaxation time ($\boldsymbol{\tau}$)can be calculated by equation (4)

$$\tau = [4\eta/3u^2\rho]^2 \tag{4}$$

The Laplace transform is one of the familiar mathematical tools for transforming complex differential equations into plain algebraic equations which is to obtain the specific solution more simply [22,23]. If C is the concentration of the given substance in g/cm^3 in a uniform medium with diffusivity constant D measured in cm^{2}s^{-1}, C obeys the equation.

$$\nabla^2 C = \frac{1}{D}\frac{\partial C}{\partial t} \tag{5}$$

The amount of the diffused mass at each point in time t is given by the solution of this equation (5). By taking suitable boundary conditions, the concentration of dissolved solute in a given solvent can be obtained as follows by equation (6)

$$C(x,t) = C_o\left\{1 - \frac{2}{\sqrt{\pi}}\left(\frac{x}{2\sqrt{Dt}} - \frac{\left(x/2\sqrt{Dt}\right)^3}{3|1} + \ldots\right)\right\} \tag{6}$$

This equation reveals that diffusivity D is the most important decisive factor for C(x,t).
Fick's law of diffusion [24] is the best choice for determining diffusivity. However, other parameters such as shape, size, and structure of molecules direct to the [25,26] relation which is represented as

$$D_{AB} = RT \Big/ \eta_B V_A^{1/3} \tag{7}$$

V_A molar volume of the solute,

$$R = 8.2 \times 10^{-8}\left[1 + \left(\frac{3V_B}{V_A}\right)^{2/3}\right] \tag{8}$$

If $V_A < 2.5\ V_B$, then $R = 17.5 \times 10^{-8}$ [19]
The binary diffusivity value of solute A in solvent B can be calculated using equations (7) and (8).
Blanc's law [27] is the best option to approximate the diffusion coefficient in a multi-component system. If x_j is the mole fraction of the jth component and i is a dilute component diffused in a homogenous mixture (protein) can be calculated using equation (9).

$$D_i, mix = \left[\sum_{j=1}^{n}\frac{x_j}{D_{ij}}\right]^{-1} \tag{9}$$

D_{ij} is the diffusivity of component i into j.

3. Result and discussion

3.1. Measured values

The experimental values of density (ρ), ultrasound velocity (u), viscosity (η), and refractive index (n) of OVN in phosphate buffer solution and OVN in xylitol + phosphate buffer solution are given in Table 1. The XY addition influenced the OVN due to the changes in the density, velocity, viscosity values. The increasing values after addition of XY with OVN indicates changes in the protein property [17].The similar results were observed in sucralose interaction with OVN and then the refractive indexes also increases [16].Previously reported the polyols affected the protein properties such as density, viscosity [28]. The XY addition increases the viscosity of the OVN it reflects the foaming property is enhanced. The same results were observed in XY interaction with soy protein isolate [29].

Table 1
Values of experimentally observed parameters.

pH	Density kgm^{-3}		Velocity ms^{-1}		Viscosity mNsm^{-2}		Refractive index	
	B + OVN	B + XY + OVN	B + OVN	B + XY + OVN	B + OVN	B + XY + OVN	B + OVN	B + XY + OVN
2	1023.2	1044.8	1557.5	1593.8	0.8354	0.9409	1.3431	1.3554
5	1021.2	1046.2	1540.1	1570.3	0.9118	0.9816	1.3421	1.3526
7	1017.7	1005.3	1557.2	1521.2	0.7723	0.8178	1.3389	1.3504
9	1019.3	1028.0	1546.0	1566.0	0.7870	0.9091	1.3405	1.3504
12	1027.9	1029.3	1565.5	1572.5	0.8128	0.9694	1.3415	1.3528

3.2. Refractive index results

The refractive index (n) research of protein solution mainly depends on the composition of amino acids of protein and the solvent in which the protein is dissolved. In the earlier refractive research of protein solution, it was believed that n of protein solutions depends on the concentration and the protein amino acid composition and not the fragmentation state of this protein [30]. Later it was improved that estimate the interaction of protein with solvent based on changes observed in 'n' values in accordance to changing the optical properties of the solvent near the protein surface [31]. Sarimov et al. [32] reported a new method of precision interferometry based on measuring 'n' values for proteolysis as well as denaturation of proteins, providing useful information about the conformational state of the protein and its interaction with a solvent. Recently, researchers [33,34] have shown that the refractive index of protein solutions depends on the state of protein fragmentation. Researchers proposed a new model for the refractive index change during the proteolysis reaction. Based on this model, the change of 'n' in a solution largely depends on the local density of water near or inside the protein molecule compared to the average density of water in the bulk volume. Due to the interaction of hydrophilic residues of protein with water, a large density of water accumulates nearer to the protein surface compared to water in the bulk volume. The perusal Table 1 shows that an increase in the refractive index profile irrespective of pH for the B + OVN + XY solution compared to the B + OVN solution. This trend reveals that the local density of water near OVN was increased due to the addition of XY in all pHs. These changes were also well reflected in density changes with XY addition. Further, it was reported that the total surface area of globular protein fragments increases during hydrolysis approximately 2–3.5 times [34]. The larger magnitude of refractive index at acidic pHs compared to neutral and alkaline pHs indicates more solvation of OVN. This trend indicates the addition of XY with OVN leads to increases in the solvation of OVN especially larger in acidic pHs. Hence the trend of 'n' in the present analysis confirms that conformational changes of OVN during pH changes that can be modified by XY addition.

3.3. Internal pressure and free volume results

In thermodynamic analysis of liquid and liquid mixtures, internal pressure and free volume are other important tools [5]. The trend of P_i and V_f for OVN solution of different pHs with and without XY are shown in Figs. 1 and 2.

It can be observed that the reduction of P_i values in addition to XY with OVN irrespective of all pH Fig. 1. This trend can be explained with the help of hydrogen bond interactions. There are three components involved in the present system. Among these three components, OVN has polar and non-polar groups whereas XY and water are polar molecules. Hence there are possibilities of inter and intra-molecular hydrogen bonding between the components OVN-XY system and OVN-Water. According to MD Simulation studies, a large number of hydrogen bonding interactions between OVN – water was noticed compared to OVN –XY [17].

Researchers [13,32] have shown that added solute in protein aqueous solution may be preferential-excluded or preferentially bound from the protein surface hence stabilizing/destabilizing the protein. Further, it was [13,32] reported that when the glucose has been added as a cosolvent inOVN, an aqueous solution makes glucose preferentially-excludedfrom the OVN surface. It was found that due to larger size of glucose molecules compared to water leads to preferential exclusion. Hence it is expected that preferentially exclusion of XY from the OVN surface leads to increases in the compacts of OVN. When the compactness OVN molecule rises the internal pressure of the system tends to increase. Further, it was observed larger magnitude of P_i was in the acidic region compared to alkaline regions. This trend indicates that preferential exclusion is more favorable in the acidic pH compared to the alkaline pH. The protein in an aqueous solution does not occupy all the volume hence there is always some free space between molecules [17]. The free volume (V_f) is defined as the mean volume in which the molecule can have the capability to move freely balancing against the repulsion forces of the nearby molecules. The trend of V_f is shown in Fig. 2 which shows the reverse trend to P_i. More free volumes make the system have less internal pressure whereas less free volume produces the opposite trend. The results of V_f indicate that the XY addition with OVN makes the system with more free volume compared to the normal OVN solution. However, the opposite trend was noticed at pH 5, V_f decreases. Similarly, the larger P_i was observed at pH 5 compared to other pHs. According to MD studies, a lower interaction was noticed between OVN-solvent as well as OVN-XY hence it was a tacit assumption that pH 5 records poor solvation of

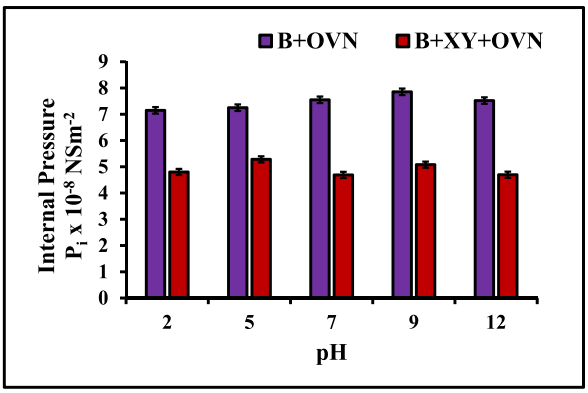

Fig. 1. Internal pressure of OVN systems.

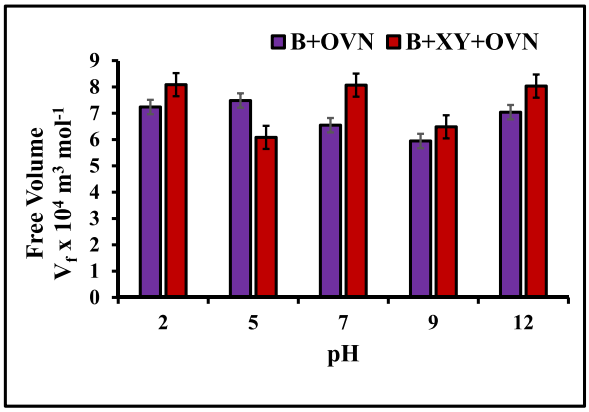

Fig. 2. Free volume of OVN systems.

OVN [17].

3.4. Change in Gibbs free energy results

The fundamental concept behind protein folding is the thermodynamic principle. As concerns protein native structure, the amino acid sequences play a major role. The native structure of any protein has the minimum Gibbs free energy. Gibbs free energy (G) comprises enthalpy and entropy into a single one. The trend of a chemical reaction can be anticipated by measuring the change in Gibbs free energy under a situation where the pressure and temperature should be constant. The present analysis has been obtained at constant temperature (303 K) and atmospheric pressure. Hence the pH variations and addition of cosolventare major portion could control the chemical reaction of OVN in a blended solvent environment. Further, if ΔG is positive then the reaction should be non-spontaneous i.e. an input of external energy can be needed for the reaction. It is a tacit assumption that the supply of external energy can be accomplished through the modification of non-covalent interaction that exists in protein molecules. Further, it is well known that the change in Gibbs free energy leads to the conformation of protein from native to non-native state. There was a proven record that preferential exclusion of a small organic solute from the protein surface results in the arising of Gibbs free energy of protein species [33]. The perusal of Fig. 3 shows the addition of XY with OVN made to raise the ΔGin all pHs. Hence it is confirmed that the preferential exclusion of XY from the OVN surface. However, larger fluctuations were noticed at the acidic pH extremity compared to the alkaline pH extremity.

Further, the lower magnitude of ΔG was recorded at pH 2 compared to pH 12. The observed results indicate that acidic pH extremities exhibit a lesser magnitude of preferential exclusion compared to alkaline pH extremities.

3.5. Diffusion results

The amount of diffused protein (C) has been estimated by the Laplace transform method. As the present system of study has a multi-component nature, the diffusivity can be estimated using Blanc's law [27], two types of systems were analyzed in the current case. System I stand for B + OVN and system II stands for B + XY + OVN. Here, the OVN is known as a solute, phosphate buffer and XY are taken as solvent. As the binary diffusion coefficient D_{AB} of OVN in each solvent component involved in the whole system is the most important decisive factor for C(x,t), it can be estimated and given in Table .2. The individual components' mole fractions, the diffusion

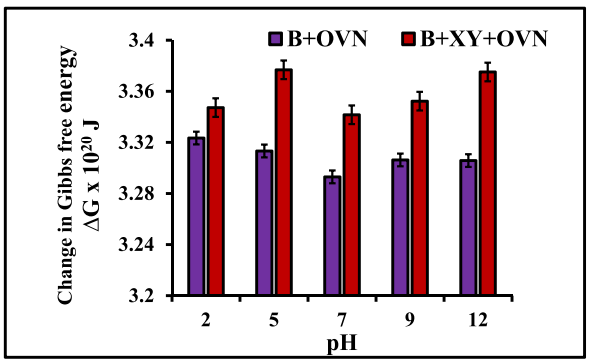

Fig. 3. Change in Gibbs free energy of OVN systems.

Table 2
Estimation of ρ, η, V and D_{AB} of OVN at 303 K

Solvent (B)	ρ kg m^{-3}	η x 10^3 Nsm^{-2}	V x 10^3 m^3	D_{AB} m^2 s^{-1}
Water	997.0	0.8903	0.01854	0.096588
MSP	1700.0	1.5950	0.09176	0.055636
DSP	2066.0	1.8611	0.06873	0.045826
SH	2130.0	0.0011	0.01877	86.032
PA	1834.0	3.8602	0.05343	0.022664
XY	1520.0	1.9152	0.100098	0.033042

SH-Sodium hydroxide PA-Phosphoric acid.

coefficient of a complex component system, and the diffused amount of protein for the B + OVN and B + XY + OVN system are given in Table 3.

The diffusion coefficient of macromolecules plays a crucial role in protein folding. The higher magnitude of diffusion coefficient was recorded at pH 2 compared to pH 12. Further the minimum value of $D_{i,mix}$ was noticed at pH 7. Literature of research [19] revealed that the acidic pH effect on OVN has a lesser magnitude of denaturation compared in alkaline pH. During denaturation, the compact nature of folded proteins has been lost leading to unfolding hence increasing their size. It was reported that [34] the size of the molecule increases leads to a decrease in its diffusion coefficient. As acidic pH has a lower magnitude of denaturation exhibits a higher diffusion coefficient compared to alkaline pH extremity. Further, pH 5 and pH 7 record the intermediate value of $D_{i,\ mix}$represents the native-like state of OVN.

When the cosolvent XY was added, the diffusion coefficient profile of OVN was reduced irrespective of pH. Proteins are macromolecules forming sequences of different amino acids and stabilized by a combined effect of non-covalent interactions. Many amino acids have hydrophobic side chains. They have the minimum force of attraction with water molecules at that same time XY has more interaction with water molecules due to its polar nature. Hence these non-polar side chains tend to enter into the interior of OVN due to the polar environment produced by XY molecules. This trend supports to stabilization of OVN compactness against denaturation of pH extremities.

The diffused amount of OVN is a significant factor since it provides a complete picture of protein-solvent/cosolvent interaction. On observing Tables 3 and it was noticed that pH 2 records a larger diffusion of OVN compared to pH 12 and the minimum diffusion at pH 7. The protein solution usually contains water, buffer, protein, and cosolvent. The notable point is that the cosolvent causes the increase in the solution viscosity by the exclusion volume effect. This trend was reported in the case of sugars and polyols which increase the viscosity of protein solution [35,36]. Electrostatic repulsion and attraction are important factors in the overall protein solution. In an aqueous protein solution, the protein molecule has a positive or negative net charge in the solution depending on its pH. If the medium is acidic the protein has a net positive charge whereas if the medium is alkaline the protein has a net negative charge. Hence the viscosity of OVN increases in both acidic and alkaline pH extremities. Further, the aqueous solutions of XY have a lower dielectric constant than pure water indicating a stronger electrostatic interaction in Water + XY solution than pure water [37,38]. The strong electrostatic interactions surrounding the OVN molecule restrict the mobility of the protein molecule leading to an increase in the solution viscosity. According to the Stokes–Einstein equation, the protein diffusivities have been empirically found to be inversely proportional to solution viscosity [39,40]. Hence the addition of XY suppresses the amount of diffused OVN compared to B + OVN systems irrespective of pH. However, the U-shaped trend in the amount of diffused protein was observed in B + OVN as well as in the B + XY + OVN solution. In summary, the amount of diffused protein in a blended solvent environment of different pHs can be well interpreted by long-range electrostatic interaction.

In summary, both experimental and theoretical studies reveals the same trend, that is acidic pH extremity exhibit more solvation compared to alkaline pH extremity. Further both studies confirm that addition of XY suppress the solvation of OVN irrespective of pH, hence support the OVN stability against pH denaturation.

4. Conclusion

The refractive index of the OVN solution is found to be increased when the cosolvent XY is added irrespective of pH. The reduction of internal pressure and reverse trend in free volume and change in Gibbs free energy were noticed in XY added OVN solution. In diffusion results, it was observed that the addition of XY suppresses the amount of diffusion of OVN compared to B + OVN systems irrespective of pH. However, both thermodynamic as well as diffusion results show clear variation between acidic and alkaline pH regions. Both thermodynamic and diffusion results concluded that acidic pH extremely favors more solvation of OVN compared to alkaline pH extremity.

Data availability statement

There is no supplementary data available.

Table 3
Estimation of $D_{i,mix}$ and C of OVN.

pH	Mole fraction of						Di,mix m^2 s^{-1}	C x 10^{11} kg
	Water	Mono	Dibasic	Phosphoric acid	Sodium Hydroxide	Xylitol		
B + OVN System								
2	0.9779	0.002045	0.001742	0.000016	–	–	980.5041	490.9888
5	0.996249	0.002024	0.001725	0.0000041	–	–	965.7411	490.9202
7	0.996289	0.001763	0.001947	–	–	–	962.5559	490.9052
9	0.994072	0.002136	0.001538	–	0.0022814	–	964.9289	490.9164
12	0.990270	0.002049	0.001486	–	0.006193	–	968.8188	490.9346
B + XY + OVN System								
2	0.972953	0.002432	0.002071	0.000020	–	0.022522	948.3354	490.8372
5	0.973867	0.002351	0.002002	0.0000047	–	0.021773	948.9537	490.8402
7	0.974164	0.002045	0.002259	–	–	0.021531	949.0550	490.8407
9	0.974609	0.002452	0.001778	–	0.002646	0.021160	949.4906	490.8428
12	0.968428	0.002360	0.001711	–	0.007133	0.020366	956.8054	490.8779

CRediT authorship contribution statement

Swenthira K: Writing – original draft, Investigation, Conceptualization, Methodology. **Agalya P:** Data curation. **Sasikumar P:** Resources. **Thafasalijyas Vayalpurayil:** Writing – review & editing. **Mohamed Abbas:** Funding acquisition. **Velusamy V:** Supervision, Writing – original draft.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Thafasalijyas Vayalpurayil reports article publishing charges was provided by King Khalid University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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