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Denaturation process of laccase in various media by refractive index measurements

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ABSTRACT

In this work, we are interested in the denaturation process of a laccase from Tramates versicolor via the determination of the refractive index, the refractive index increment and the specific volume in various media. The measurements were carried out using an Abbe refractometer. We have shown that the refractive index increment values obtained from the slope of the variation of the refractive index vs. Concentration are outside the range refractive index increments of proteins. To correct the results, we have followed the theoretical predictions based on the knowledge of the protein refractive index from its amino acids composition. The denaturation process was studied by calculating the specific volume variation where its determination was related to the Gladstone-Dale and the Lorentz-Lorentz models.

1. Introduction

The characterization of proteins by dynamic, static light scattering and sedimentation is often related to the measurements of the refractive index of solutions where its determination is necessary to calculate several physical parameters [1–7]. For that reason, generally, an experimental compromise was followed to determine the refractive index. The experimental determination of the refractive index of polymer or protein solutions is based on the direct reading of the values obtained with refractometers and the index increments from the slope of the curve representing the variation of the refractive index vs. concentration or temperature. But, the problem is that the direct determination of the refractive index and the refractive index increments of protein allow us to obtain an exact or an approximated value and then an exact or approximated result.

In literature, little works are interested in the investigation of solutions the optical propriety and characterizing biopolymers vs. the knowledge of their refractive index. The refractive index of proteins is a physical parameter serving in various biophysics techniques and optical imaging [8,9]. It represents the consequence of the local polarizability of atoms and chemical groups due to the deformation of electron configurations of protein [10–12]. Therefore, for the best determination of this parameter, Doty et al. [13] have shown the importance of the composition, the density and the environmental factors. In the same way, Adair et al. [14] have considered that the refractive index of proteins or amino acids may be approximately determined from its elementary composition. They have also considered that by means of a good approximation, a consensus value can be used where the amino acids composition represents the determining factor to calculate the exact value of the refractive index increments of proteins [10], knowing that the most index increments of proteins vary between 0.173 and 0.215 ml / g [10]. Likewise, the exact value of the refractive index increments permits the determination of interesting parameter giving information on the protein behaviour in solution is that the specific volume. The specific volume determination of is usually related to several models such as the Gladstone-Dale [15,16] and the Lorentz-Lorentz models [17–20].

Herein, we are interested in the investigation of the optical properties of the laccase from the determination of its real refractive index, its refractive index increment and its specific volume. We have chosen the laccase from fungus Tramates versicolor because the fact that their optical proprieties and denaturation process are rarely investigated even their frequent use in biotechnology where their applications vary from organic synthesis, biosensors and surface treatment.

We have followed the Wiener model [10,21,22] to determine the refractive index increment and Lorentz-Lorentz model to determine the specific volume [17–20]. The values of the refractive index increments and the specific volume of laccase relative to various solutions are obtained from the amino acids composition in the solid state. In our knowledge, there are no subsequent studies who are interested in the determination of the refractive index, the index increment and the variation of the specific volume of laccase for different pH, chemical and organic denaturants and in the presence of ionic liquids.

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Table 1
Samples description.

Chemical name	Source	Purity	Purification method
Laccase	Sigma Aldrich	–	None
Sodium acetate	Sigma Aldrich	99%	None
Phosphate buffer	Sigma Aldrich	99%	None
[pyrr][F]	Synthesized	99%	DURP
[morph][F]	Synthesized	99%	DURP
NaOH	Sigma Aldrich	98%	None
HCl	Sigma Aldrich	37%	None

[pyrr][F]: pyrrolidinium Formate.
[morph][F]: morphilinium Formate.
NaOH: Sodium hydroxide.
HCl: Hydrochloric acid.
DURP: Distillation Under Reduced Pressure.

2. Experimental

All the chemicals and synthesized samples description are given in Table 1. We have chosen a Laccase from fungus Trametes versicolor (EC 1.14.18.1/CASRN ¼ [80498-15-3]) and has a molecular weight of 57 kDa. The laccase is of the highest purity grade available from Sigma-Aldrich and used without further purification. The used laccase is powder and its activity is superior to 0.8 U / mg. This enzyme contains 4 copper atoms distributed in type 1, type 2 and type 3 sites. Ultra-pure water which has a specific conductivity of about 0.65 mS / cm was used to prepare all concentrations. The solution preparations were based on the "Cold Method" technique. The Cold Method was expanded from the preparation polymer aqueous solutions. It consists in the dissolving the enzyme in cold water where the temperature does not exceed 4 °C. All concentrations are prepared from a stock enzyme solution. The solutions were stirred for 3 h. After agitation, the solutions are equilibrated for 24 h at 4 °C.

In order to study the effect of pH, we have used buffer solutions for pH ranging from 2 to 9. To prepare solutions having pH < 6, we have used the Sodium Acetate of 0.1 M. For solutions having pH ranging between 6 and 8, we have used the Phosphate Buffer of 0.1 M.For the solutions having pH ≥ 9, we have used the Tris HCl of 0.1 M. The pH of the solutions was adjusted using the HCl and the NaOH.

The Guanidinuim Chloride (GdmCl) and the urea are used as chemical denaturants for concentrations ranging between 0.5 and 6 M. The phenol, the methanol and the acetonitrile are used as organic denaturants. The volumetric fractions added vary between 10% and 60% of the total volume of the laccase/water solution. All chemical and organic denaturants are Sigma-Aldrich products.

The Pyrrolidinium Formate ($C_5H_{11}NO_2$), and the Morpholinium Formate ($C_5H_{11}NO_3$), represent the ionic liquids used in this work. They are used for volumetric fractions ranging between 20% and 80% of the total volume of the laccase/water solution. The Pyrrolidinium Formate ([Pyrr][F]) and the Morpholinium Formate ([morph][F])are in their liquid state and have a hydrophilic character [23–25].

The Abbe refractometer is the instrument used to determine the refractive index of different solutions studied. The optical system relative to the refractometer is based on the measurement of the refraction limit at the interface between the prism having a refractive index of 1.7 and a liquid having a refractive index less than that of the prism. In this work, the Abbe refractometer has a wavelength of 589 nm. All values are measured with an accuracy of 10^{-4}.

All studied systems are reported at atmospheric pressure. All curves are treated using the software OriginPro (OriginLab/OriginPro 8.5, USA).

3. Theoretical background

To study the effect of solvents on the specific volume of laccase, first we have determined the partial specific volume, the refractivity per gram and the refractive index of the laccase in its solid state from the index data of amino acids related to laccase [26–29]. The refractive index relative to the chemical components is related to the molar refractivity R via the following equality [17]:

$$R = \frac{n^2 - 1}{n^2 + 2}\frac{M}{\rho} \tag{1}$$

For proteins, it was usually considered that $n = n_p$ and $R = R_p$. We recall that n_p is the refractive index of protein in its solid state, R_p is the refraction per gram defined as the weight average of the contribution from the individual amino acid R_a [10,26–30], ρ is the density and M is the molecular weight of the constituent. The refraction per gram R_p is given by:

$$R_p = \frac{\sum_a R_a M_a}{\sum_a M_a} \tag{2}$$

where, $\sum_a$ represents the summation over the number of the same amino acids. McMeekin et al. [26,27] have shown that the refraction per gram can be written as a function of the refractive index of protein in its solid state as:

$$R_p = \frac{n_p^2 - 1}{n_p^2 + 2} v_p \tag{3}$$

From Eq. (3), we can obtain the equation determining the refractive index n_p as a function of R_p and the partial specific volume of the protein v_p:

$$n_p = \sqrt{\frac{2R_p - v_p}{v_p - R_p}} \tag{4}$$

where, v_p is obtained from the amino acids composition using the following relation:

$$v_p = \frac{\sum_a v_a M_a}{\sum_a M_a} \tag{5}$$

Table 2 shows the obtained values of the refractive index of the laccase in its solid state, the refractivity per gram and the partial specific volume.

To determine the specific volume of protein and to follow the denaturation process, we have used the Lorentz-Lorentz model [18–20]. This model consists on the determination of the specific refractivity from the protein refractive index in its solid state. The specific refractivity according to Lorentz-Lorentz is given by:

$$R_{L-L} = \frac{n_p^2 - 1}{n_p^2 + 2} v_{sp} \tag{6}$$

For dilute protein solutions, Wiener [21,22] showed that the refractive index increment is related to the partial specific volume of protein by the equation:

$$\left(\frac{dn}{dc}\right)_{c\to 0} = \frac{3}{2} v_p n_m \frac{n_p^2 - n_m^2}{n_p^2 + 2n_m^2} \tag{7}$$

where, n_m is the refractive index relative to the solvent without laccase. Substituting Eq. (6) in Eq. (7), the refractive index increment is written as a function of the refractivity and the specific volume of the protein

Table 2
Numeric values of the specific volume variation obtained by using the UCSF Chimera 1.8.1 software for different pHs.

n_p	1.6255
R_p	0.2559 ml / g
v_p	0.7233 ml / g

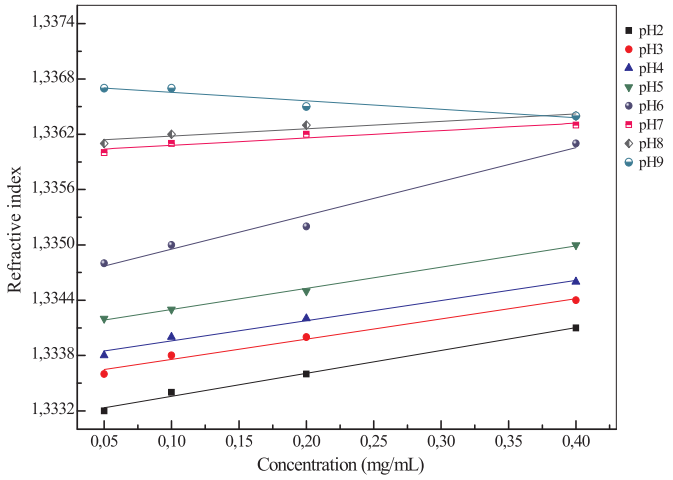

Fig. 1. Refractive index variation for various pH and laccase concentrations.

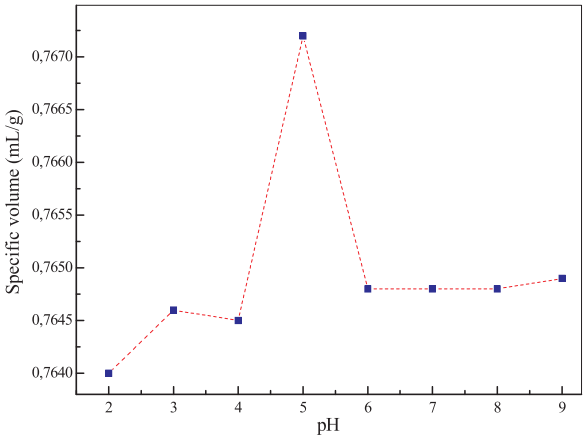

Fig. 2. Specific volume variation of as a function of pH.

solution as follows:

$$\left(\frac{dn}{dc}\right)_{c\to 0} = \frac{(n_m^2-2)^2}{6n_m}\left(R_{L-L} - \frac{n_m^2-1}{n_m^2+2}v_{sp}\right) \tag{8}$$

According to Lorentz-Lorentz [18–20], the specific volume v_{sp} of protein can not be calculated directly. They have shown that the specific volume is the result of a combination between the refraction index of protein in its solid state n_p and the refractive index of solution n_m. The specific volume is then given by the following expression:

$$v_{sp} = \frac{6n_m\left(\frac{dn}{dc}\right)_{c\to 0}}{(n_m^2+2)^2\left(\frac{n_p^2-1}{n_p^2+2} - \frac{n_m^2-1}{n_m^2+2}\right)} \tag{9}$$

4. Results and discussion

4.1. Effect of pH

Fig. 1 illustrates the variation of the refractive index of the laccase for several concentrations and various pH. We note that for a fixed pH, the refractive index increases with increasing concentration. Similarly, for a fixed concentration and increasing pH, the refractive index increases. The curves show also that for pH $\leq$ 5, the slopes of the curves are positive and from pH7 the slopes tendency change where it becomes negative for pH $\geq$ 9.

The experimental index increments obtained from the direct calculation of the slopes of the curves, the theoretical index increments and the refractive index n_m of the solvents values are given in Table 3.

Table 3
Refractive index, refractivity per gram and partial specific volume of the laccase in the solid state.

pH	n_m	$(dn/dc)_{Exp} \times 10^3$(ml/g)	$(dn/dc)_{Theo}$(ml/g)
2	1.3348	0.0024	0.2008
3	1.3338	0.0021	0.2014
4	1.3351	0.0021	0.2006
5	1.3340	0.0023	0.2013
6	1.3345	0.0036	0.2010
7	1.3345	0.0008	0.2010
8	1.3345	0.0008	0.2010
9	1.3350	−0.0009	0.2006

We consider that the change of the slope for pH9 leads to a negative value of the index increments. This negative value is due to the fact that, for increasing concentration the attraction between the solute species. In fact, the laccase the aggregation process becomes favoured and the refractivity of the medium decreases. This process leads to a decrease of the refractive index which explained the negative index increment values.

Fig. 2 illustrates the variation of the specific volume calculated according to the Lorentz-Lorentz model from the data given in Table 3. The curve varies according two regions depending on pH. In the first region, in which the pH of solutions is $\leq$ 4 and $\geq$ 6, the smaller change in the specific volume was explained by the fact that the presence of H^+ and OH^- ions can act on the charged sites of the laccase. The excess H^+ (acid) or OH^- (base) ions disrupt the hydrogen bonds and the disulfide bridges inducing diminution in the functional capacity of the laccase. We consider that in this range of pH a folding process of laccase was produced causing a decrease in the specific volume. This process corresponds probably to the denaturation of the laccase. The solutions having pH $\leq$ 4 and $\geq$ 6 are considered as poor solvents for the laccase.

In the second region, in which the pH varied between 4 and 5, the specific volume increases reach its maximum in the vicinity of pH5. In this range of pH, the electrostatic interactions between the laccase and the solvent become attractive. The H^+ ions in solution interact with the ionizable groups of the laccase, particularly the hydroxyl groups (COOH) of the lateral chains to change the intramolecular interactions by the intermolecular interactions between the laccase and the solvent. The laccase molecules become swollen and the specific volume increases.

It was reported that the laccase from Trametes versicolor has an optimal enzymatic activity for pH values comprised between 4 and 5 [31,32]. Also, the pH induces a change in the ionization of the functional group of the laccase leading to the modification of its structure and a change in the copper fixation sites. Consequently, by correlating the variation of the specific volume to the enzymatic activity, we conclude firstly that the laccase is active only in its swollen conformation and the inverse relationship between specific volume and the refractive index [33] is not checked in the pH range between 4 and 5 and is extremely influenced for pH extremes.

To be sure, we have illustrated in Fig. 3 the variation of the specific volume of the laccase by using the UCSF Chimera 1.8.1 software for different pHs. These illustrations allowed us to follow by modeling the variation of the volume occupied by a chain of the laccase in order to check our results. The volume occupied by the meshs surrounding the laccase represents the variation of the specific volume. We show that the variation of the specific volume of the laccase obtained from the experimental measurements and the Lorentz-Lorentz model are verified. Table 4 below shows the numerical values that confirm the variation of the specific volume.

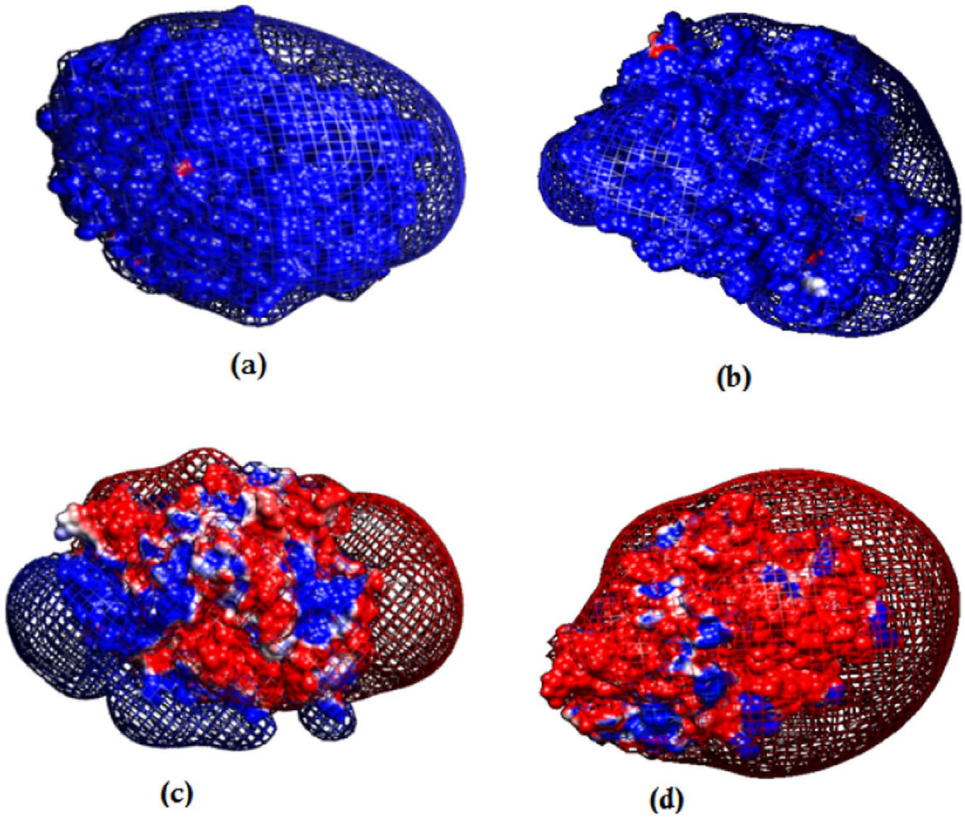

Fig. 3. Variation of the specific volume of the laccase of Trametes versicolor for different pHs illustrated with the software UCSF Chimera 1.8.1. The volume occupied is represented with the meshs surrounding the laccase. (a): pH = 2; (b): pH = 4; (c): pH = 5; (d): pH = 6. The blue color represents the positive charges, the white color represents the neutral charges and the red color represents the negative charges.

Table 4
Solvent refractive index, experimental and theoretical refractive index increments for various laccase concentrations and various pH.

pH	2	4	5	6
Volume occupé par une chaîne de la laccase (10^3 Å^3)	42,07	49,78	63,46	50,11

4.2. Effect of chemical denaturation

Fig. 4a and b illustrate the refractive index versus the laccase concentrations for various urea and GdmCl concentrations. The slopes of the curves allow the determination of the experimental refractive index increments. The calculation of the experimental and theoretical refractive index increments and the refractive index n_m relative to the binary water/denaturant solutions are illustrated in Table 5.

Fig. 5 illustrates the variation of the specific volume of the laccase in the presence of chemical denaturants calculated from the data given in Table 5. We note that the specific volume decreases for increasing chemical denaturant concentrations and the inverse relationship between the specific volume and the refractive index is checked [33]. The curves show also that the specific volume of the laccase decreases rapidly in the presence of GdmCl than this in the presence of urea.

It is worth noting that the addition of chemical denaturants affects the internal structure of laccase by acting on their hydrophobic sites. The hydrophobic interactions cause the compaction of polypeptide chains. The globular native structure of laccase is destabilized and a transition from the folded to the unfolded conformation by exposing their lateral hydrophobic chains in solution is occurring. These structural changes are considered as the transitions of laccase from its native to its denatured state. During these transitions, the hydrodynamic and the optical properties of the laccase are influenced leading to the reduction of its specific volume with increasing denaturant concentrations. Kauzmann et al. [34–36]. reported that it is possible to observe

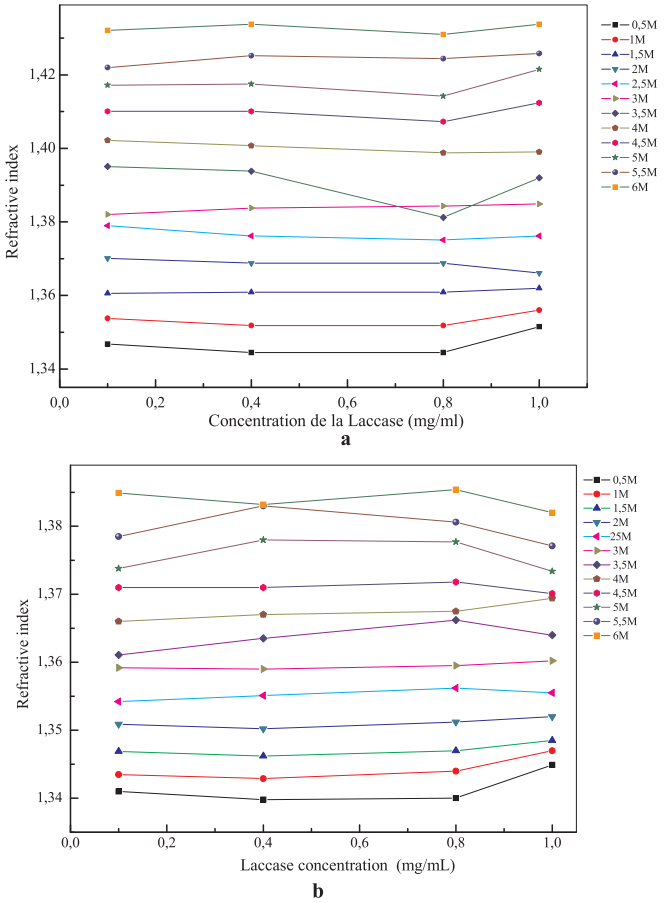

Fig. 4. a: Variation of the refractive index as a function of the concentration of laccase in the presence of GdmCl. **b:** Variation of the refractive index as a function of the laccase concentrations in the presence of Urea.

Table 5
Solvent refractive index, experimental and theoretical refractive index increments for various laccase concentrations in the presence of urea and GdmCl.

Concentration (M)	n_m (water/denaturants)		$(dn/dc)_{Exp} \times 10^3$(ml/g)		$(dn/dc)_{Theo}$(ml/g)	
	Urea	GdmCl	Urea	GdmCl	Urea	GdmCl
0.5	1.3393	1.3420	0.0033	0.0038	0.1979	0.1961
1	1.3439	1.3450	0.0035	0.0017	0.1949	0.1940
1.5	1.3473	1.3520	0.0016	0.0012	0.1927	0.1896
2	1.3510	1.3555	0.0013	−0.0036	0.1903	0.1873
2.5	1.3555	1.3610	0.0017	−0.0032	0.1873	0.1837
3	1.3587	1.3649	0.0010	0.0029	0.1852	0.1812
3.5	1.3630	1.3710	0.0040	−0.0086	0.1824	0.1772
4	1.3670	1.3760	0.0033	−0.0038	0.1798	0.1738
4.5	1.3700	1.3810	−0.0004	0.0007	0.1778	0.1705
5	1.3750	1.3852	−0.0005	0.0022	0.1745	0.1677
5.5	1.3780	1.3910	−0.0018	0.0032	0.1725	0.1632
6	1.3820	1.3951	−0.0016	0.0003	0.1699	0.1622

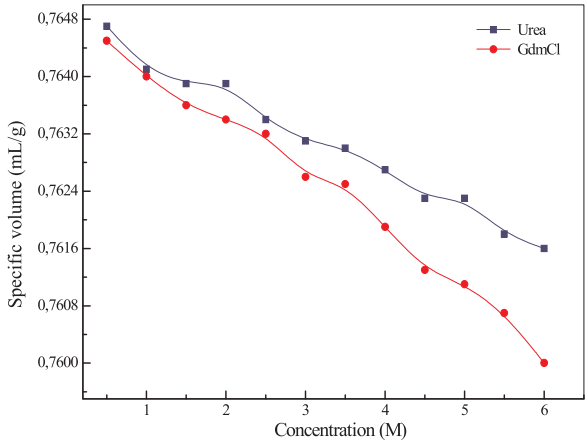

Fig. 5. Specific volume variation for various chemical denaturants concentrations.

small changes in the refractive index by denaturing the protein with urea. They have considered that the changes are due to the denaturation could be accounted by accompanying changes in the specific volume. Kauzmann et al. [34–36] have identified that the denaturation processes are accompanied by a contraction of several hundred cubic centimeters per 100,000 g by proteins.

To explain the difference observed in the specific volume variation of laccase between the GdmCl and urea, we have used the Nandel predictions [37]. Nandel [37] showed that one molecule of urea reacts with seven molecules of water where as one molecule of GdmCl reacts with twelve water molecules. Consequently, the presence of GdmCl increases the hydrophobic interactions in solution than in the presence of urea which leads to the contraction of the laccase explaining the diminution of the specific volume with increasing denaturants concentration.

4.3. Effect of organic solvents

Fig. 6a, b and c illustrate the variation of the refractive index as a function of the laccase concentration for various volumetric fractions of organic solvents. We recall that the pH of the solution was adjusted to 4. Unlike chemical denaturants, the variation of the refractive index is influenced by the presence of organic solvents. The experimental and the theoretical refractive index increments and the refractive index n_m of the binary water/organic solvents in the presence of organic solvents are given in Table 6. We have remarked that for organic solvents percentages ≥10%, the variation of the refractive index increments in the presence of the phenol is lower than those for methanol and acetonitrile.

Fig. 7a and b describe the variation of the specific volume as a function of phenol, methanol and acetonitrile concentrations calculated from the data given in Table 6 respectively. The curves show that the specific volume decreases for all volumetric fraction of phenol whereas it remains constant for percentages of methanol and acetonitrile between 40% and 50% and it increases only for percentages of methanol and acetonitrile > 50%. This variation was probably due to the difference between the action modes of various organic solvents. We remark from Fig. 7a and b that the specific volume variation is more rapid than in the presence of methanol and acetonitrile. We consider that the rapid decay of the specific volume in the presence of phenol was due to the fact that the laccase molecules react via their active sites to degrade the molecules of phenol. For increasing phenol concentration, the laccase flees the solution because it can degrade only one molecule of phenol. The size and the specific volume of the laccase are reduced for increasing phenol concentrations. We note that there is no denaturation process exists in the presence of phenol.

Otherwise, in the presence of various volumetric fractions of methanol and acetonitrile, Fig. 7b, the curves admit a different behaviour to that observed in the presence of phenol; Fig. 7a. We consider that for volumetric fraction ≤ 10%, only a small amount of molecules are able to occupy partially the active sites of laccase. The laccase has not lost its globular conformation which explains the higher value of the specific volume. For volumetric fractions lying between 20% and 40%, other sites of laccase are filled and the native molecules acquire significant conformational changes. The laccase twists and the inner chains having hydrophobic character emerge to interact favourably with the organic solvent. These chains become highly dehydrated and they are protected from contact with the solvent by a film of hydrophilic chains. The laccase loses its native globular conformation and the denaturation process starts. The constancy of the specific volume observed between 40% and 50% indicates that the laccase is totally denatured. Noting that in parallel to the denaturation process and the reduction of the specific volume, a precipitation process starts. The existence of the precipitation process is confirmed for concentrations ≥ 50% where the specific volume of the laccase and the refractivity of the medium increases. This increase is related to the enhancement of the hydrophobic interactions favouring the aggregation process between precipitated chains.

4.4. Effect of ionic liquids

Fig. 8a and b illustrate the variation of the refractive index versus laccase concentrations for various volumetric fractions of Pyrrolinidinuim Formate ([Pyrr][F]) and Morphilinuim Formate ([morph][F]). We recall that the pH of the solution is equal to 4. There is no difference in the refractive index observed according to the variation of the [Pyrr][F]concentration whereas the difference appears in the presence of

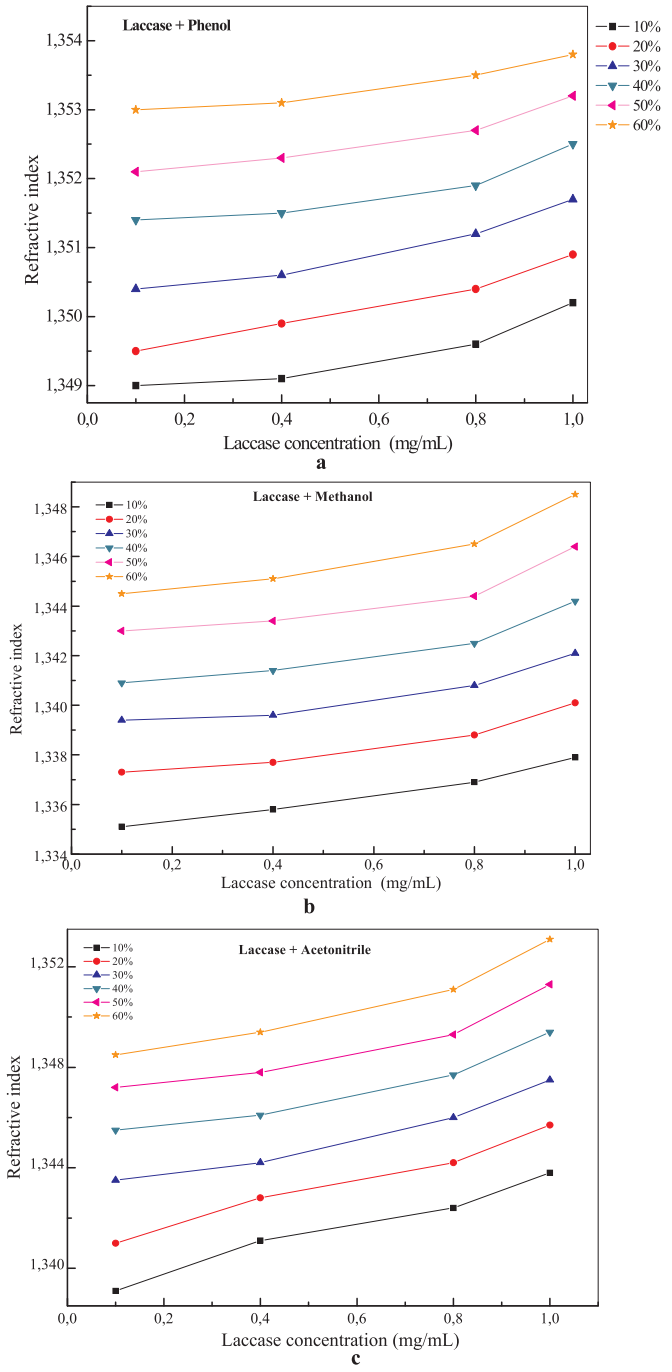

Fig. 6. a: Variation of the refractive index as a function of the laccase concentration in the presence of Phenol. **b:** Variation of the refractive index as a function of the laccase concentrations in the presence of methanol. **c:** Variation of the refractive index as a function of the laccase concentrations in the presence of Acetonitrile.

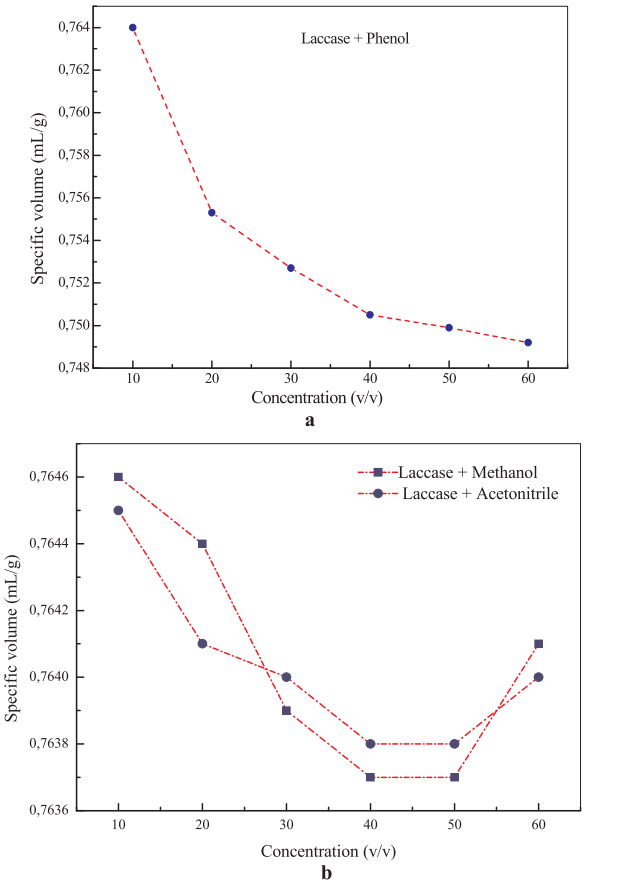

Fig. 7. a: Specific volume variation for various volumetric fractions of phenol. **b:** Specific volume variation for various volumetric fractions of methanol and acetonitile.

[morph][F]. Table 7, illustrates the experimental index increments of laccase solutions for various volumetric fractions of [pyrr][F] and morph][F]. To determine the variation of the specific volume of laccase vs. ionic liquid concentrations, the refractive index n_m of the binary water/ionic liquids and the theoretical refractive index increments are given in Table 7. The obtained values are in the same range of variation of proteins refractive index increments [10]. Otherwise, we remark from Table 7 that the models used permit the calculation of the theoretical values of the refractive index increment whereas we can not determine the experimental value of laccase in pure ionic liquids.

Fig. 9 illustrates the variation of the specific volume for different volume fractions of [pyrr][F] and [morph][F] calculated according to the Lorentz-Lorentz model from the data given in Table 7. We note that the curve associated to [morph][F] is below that associated to [pyrr][F]. This dissimilarity is probably due to the difference in the chemical composition of the ionic liquids where we have considered that the presence of oxygen atom in the aliphatic cycle of [morph] is responsible. We note, also, that the curves admit the same variation as

Table 6
Solvent refractive index, experimental and theoretical refractive index increment for various laccase concentrations in the presence of phenol, methanol and acetonitrile.

Concentration (v/v)	n_m (water/organicsolvent)			$(dn/dc)_{Exp} \times 10^3$(ml/g)			$(dn/dc)_{Theo}$(ml/g)		
	Phenol	Methanol	Acetonitrile	Phenol	Methanol	Acetonitrile	Phenol	Methanol	Acetonitrile
10	1.3490	1.3340	1.3398	0.0012	0.0030	0.0049	0.1916	0.2013	0,1975
20	1.4362	1.3362	1.3405	0.0014	0.0029	0.0049	0.1334	0.1999	0.1971
30	1.4591	1.3385	1.3430	0.0014	0.0029	0.0043	0.1177	0.1983	0.1955
40	1.4765	1.3505	1.3449	0.0011	0.0034	0.0042	0.1057	0.1970	0.1942
50	1.4780	1.3415	1.3450	0.0011	0.0034	0.0043	0.1046	0.1963	0.1942
60	1.4810	1.3422	1.3490	0.0008	0.0041	0.0048	0.1026	0.1960	0.1916

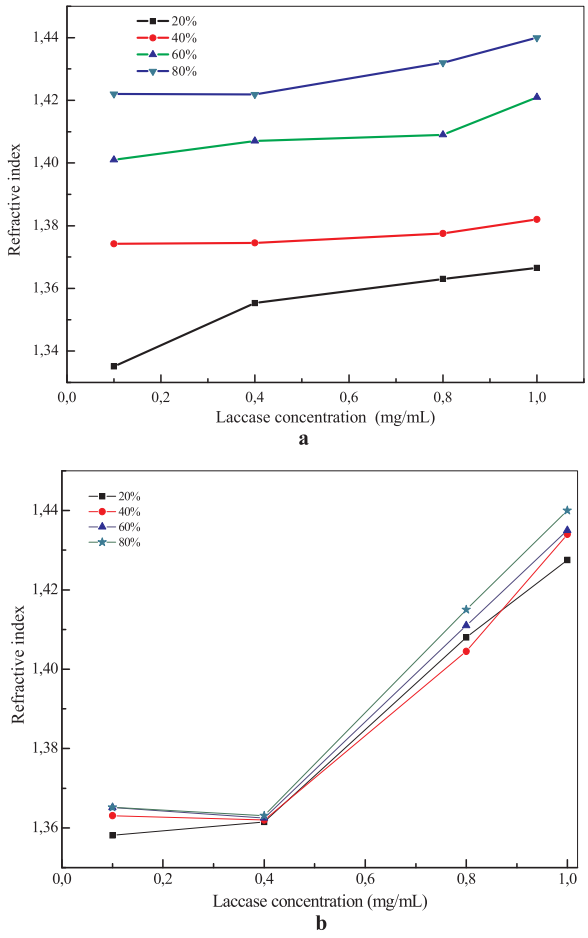

Fig. 8. a: Variation of the refractive index as a function of the laccase concentrations for various volumetric fractions [pyrr][F]. **b:** Variation of the refractive index as a function of the laccase concentrations for various volumetric fractions [morph][F].

those obtained for chemical denaturants. Therefore, we can deduce that the [morph][F] and the [Pyrr][F] ionic liquids act probably as denaturants.

Generally, the presence of ionic liquids in solution affects the stability of the protein due to the chemical interactions between anions and cations and the charged amino acid groups. With increasing ionic liquid concentrations the solubility of the hydrophobic zone decreases. This phenomenon is called the "salting out" [38]. Structurally, the "salting out" increases the hydrophobic interactions within the protein and that by means of a process which stopped the transfer of protons between the copper atoms constituting the active center of the laccase. The Pyrrolinidinuim and the Morphilinuim cations promote the solubility of non-polar amino acids of the laccase and reduce the hydrophobic interactions within the laccase itself. Furthermore, the Formate anions ($HCOO^-$) interact only with the aqueous environment and the polar amino acid of the laccase. This process leads to a non-uniform

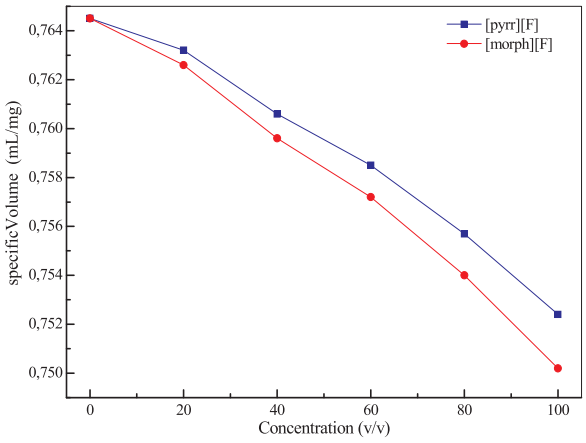

Fig. 9. Specific volume variation for various volumetric fractions of [pyrr][F] and [morph][F].

stretching affecting the globular structure of the laccase. The free energy of unfolding increases and causes a decrease in the specific volume and therefore the distortion of the laccase is activated.

The effect of the ionic liquids is reflected in the structural level of laccase. The inconsistent stretching of the laccase results in a decrease of the volume of the hydrophobic cavities. It leads also to the compaction of the tertiary structure and structural changes in the alpha and beta helix.

5. Conclusion

We recall that in this work we are interested in the investigation of the denaturation process of a laccase from Tramates versicolor via the knowledge of its refractive index, refractive index increment and its specific volume. We have studied firstly the effect of pH where we have shown that the solutions having pH ≤ 4 and pH ≥ 6 are considered as poor solvents and the variation of the specific volume is lower for the pH extreme. Secondly, in the range of pH varying between 4 and 5, the laccase is swollen and its specific volume increases. For pH values between 4 and 5, the solution is considered as good solvents.

During the chemical denaturation, the hydrodynamic and the optical properties of the laccase are influenced where the refractive index of the enzyme acquires small changes which appear in its specific volume variation. In the same way, the denaturation by organic solvents shows that the variation of the refractive index and the specific volume depends largely on laccase and organic solvent concentrations. This fact is the result of the hydrophobic interactions between the laccase and the solvent and in the protein itself.

By studying the effect of the ionic liquids, we have shown that the refractive index increases linearly with increasing volumetric fraction of Pyrrolidinium Formate and Morphilinium Formate. From the comparison between the specific volume variation in the presence of the used ionic liquid and in the presence of urea and GdmCl, we have

Table 7
Solvent refractive index, experimental and theoretical refractive index increments for various laccase concentrations in the presence of [pyrr][F] and [morph][F].

Concentration (v/v)	n_m (water/ionicliquid)		$(dn/dc)_{Exp} \times 10^3$(ml/g)		$(dn/dc)_{Theo}$(ml/g)	
	[pyrr][F]	[morph][F]	[pyrr][F]	[morph][F]	[pyrr][F]	[morph][F]
0	1.3351	1.3351	0.0021	0.0021	0.2006	0.2006
0.2	1.3608	1.3670	0.0813	0.033	0.1839	0.1798
0.4	1.3852	1.3962	0.0830	0.0082	0.1677	0.1604
0.6	1.4120	1.4252	0.0889	0.0189	0.1498	0.1409
0.8	1.4352	1.4439	0.0823	0.0203	0.1341	0.1281
1	1.4571	1.4721	–	–	0.1191	0.1087

concluded that the Pyrrolidinium Formate and the Morphilinium Formate behave as chemical denaturants.

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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2017.05.003.

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