Advances in Colloid and Interface Science 290 (2021) 102365

Contents lists available at ScienceDirect

Advances in Colloid and Interface Science

journal homepage: www.elsevier.com/locate/cis

Historical Perspective

Applications of serum albumins in delivery systems: Differences in interfacial behaviour and interacting abilities with polysaccharides

Aixa Aguilera-Garrido [a,1], Teresa del Castillo-Santaella [a,1], Yan Yang [a], Francisco Galisteo-González [a], María José Gálvez-Ruiz [a,b], José Antonio Molina-Bolívar [b,c], Juan Antonio Holgado-Terriza [d], Miguel Ángel Cabrerizo-Vílchez [a,*], Julia Maldonado-Valderrama [a,b]

[a] Department of Applied Physics, University of Granada, Avenida de Fuente Nueva, s/n, C.P. 18071 Granada, Spain
[b] Excellence Research Unit "Modeling Nature" (MNat), University of Granada, Avda. del Hospicio, s/n, C.P. 18010 Granada, Spain
[c] Department of Applied Physics II, Engineering School, University of Málaga, 29071 Málaga, Spain
[d] Department of Software Engineering, University of Granada, C/Periodista Daniel Saucedo Aranda, s/n, 18071 Granada, Spain

ARTICLE INFO

Article history:
Received 31 October 2020
Received in revised form 13 January 2021
Accepted 13 January 2021
Available online 20 January 2021

Keywords:
Albumin
Hyaluronic acid
Emulsion
Interfacial tension
Polysaccharide
ζ-Potential
Interaction

ABSTRACT

One of the major applications of Serum Albumins is their use as delivery systems for lipophilic compounds in biomedicine. Their biomedical application is based on the similarity with Human Serum Albumin (HSA), as a fully biocompatible protein. In general, Bovine Serum Albumin (BSA) is treated as comparable to its human homologue and used as a model protein for fundamental studies since it is available in high amounts and well understood. This protein can act as a carrier for lipophilic compounds or as protective shell in an emulsion-based vehicle. Polysaccharides are generally included in these formulations in order to increase the stability and/or applicability of the carrier. In this review, the main biomedical applications of Albumins as drug delivery systems are first presented. Secondly, the differences between BSA and HSA are highlighted, exploring the similarities and differences between these proteins and their interaction with polysaccharides, both in solution and adsorbed at interfaces. Finally, the use of Albumins as emulsifiers for emulsion-based delivery systems, concretely as Liquid Lipid Nanocapsules (LLNs), is revised and discussed in terms of the differences encountered in the molecular structure and in the interfacial properties. The specific case of Hyaluronic Acid is considered as a promising additive with important applications in biomedicine. The literature works are thoroughly discussed highlighting similarities and differences between BSA and HSA and their interaction with polysaccharides encountered at different structural levels, hence providing routes to control the optimal design of delivery systems.

Contents

* Corresponding author.
E-mail address: mcabre@ugr.es (M.Á. Cabrerizo-Vílchez).
[1] Shared first authorship; equal contribution to the work.

https://doi.org/10.1016/j.cis.2021.102365
0001-8686/

Abreviations

HSA	Human Serum Albumin
BSA	Bovine Serum Albumin
LLN	Liquid Lipid Nanocapsule
HA	Hyaluronic Acid
cBSA	Cationic Bovine Serum Albumin
FcRn	neonatal FC receptor
RSA	Rat Serum Albumin
SDS	sodium dodecyl sulphate
ATAB	alkyltrimethylammonium bromide

1. Biomedical applications of serum albumins (human and bovine)

Serum Albumins are one of the most abundant proteins present in the blood stream which act as carrier for a wide range of endogenous and exogenous molecules [1]. In addition to their role in transport, they present different physiological functions as maintaining the osmotic pressure and pH [2,3]. Serum Albumins are very interesting proteins from a medical point of view, since they are non-toxic, biocompatible, biodegradable, and non-immunogenic [2], and they have attracted great attention in drug delivery owing to several facts. On the one hand, their long half-life in blood (on average 19–21 days in humans) [1]. This long half-life is related to the binding of Serum Albumins to neonatal FC receptor (FcRn), which avoids the intracellular degradation and contributes to the recycling of the protein [1,4]. On the other hand, the coating of nanoparticles with Albumins contributes to increase their circulation time in the blood thanks to their ability to avoid opsonins adsorption on the surface of nanoparticles, reducing the macrophage attraction and the subsequent phagocytosis [5–7]. Another interesting aspect of Albumins in cancer research is its accumulation in malignant tissue, which brings the possibility to carry a higher amount of active antitumour compounds to the tumour site via Albumin association [8]. The accumulation of Albumin on tumours is related with the high metabolic rate of tumour cells and the source of nutrients that Albumins and the compounds that they carry provide. The Enhanced Permeation and Retention Effect (EPR), provoked by leaky-capillaries on the tumour environment in combination with a defective lymphatic drainage system, also contributes to a higher affluence of Albumin to the tumour site. However, the receptors involved in the transport of Albumin into tumour cells are not clear, although an overexpression of FcRn receptor seems to play an important role on that transport [8].

Accordingly, the use of Serum Albumins in the clinical area is overwhelmingly widespread [9]. Human Serum Albumin (HSA), in particular, is a biomarker of many diseases, including cancer, ischemia, postmenopausal obesity, rheumatoid arthritis, as well as different diseases needing monitoring of the glycaemic control [10–12]. It is also widely used to treat several diseases, including shock, burns, hypovolemia, trauma, surgical blood loss, haemorrhage, acute respiratory distress syndrome, cardiopulmonary bypass, acute liver failure, haemodialysis, chronic liver disease, nutrition support, and hypoalbuminemia [13–18]. Other biotechnological applications of Serum Albumins include implantable biomaterials and the development of suitable scaffolds that supports three-dimensional tissue formation [19,20], biochromatography, surgical ligand trapping, adhesives and sealants, and fusion proteins [21–23]. In the bionanotechnology field, Albumin-based nanocarriers have been recognized to be one of the most promising and effective vehicles for the delivery of drugs or nutraceuticals [24,25]. Albumin nanoparticles are preferred in comparison to other proteins because they have several unique features [26]. For instance, they exhibit a high binding capacity to hydrophobic drugs and possess certain functional groups on the surface suitable for covalent attachment of drugs or cell-targeting agents [27].

The use of emulsions and nanoemulsions as delivery systems presents numerous advantages with respect to the classical administration of lipophilic compounds. It is frequent to find in literature formulations in which the objective is to encapsulate biologically-active and poor water-soluble molecules like curcumin [28], lipid molecules such as carotenoids and polyphenols [29], or anticancer drugs like paclitaxel [30]. Nanoemulsions can protect encapsulated drugs, avoiding its degradation and facilitating its solubility in the oil phase. Furthermore, nanoemulsions can be directed towards specific targets leading to more effective therapies, where smaller drug doses are needed, and with reduced side effects. Liquid lipid nanocapsules (LLNs) are nanoemulsions composed by an oily core and a polymeric shell. LLNs present high efficiency encapsulating lipophilic compounds and protecting them against degradation factors such as pH, light, or enzymes [31,32]. Moreover, the polymeric shell can prevent tissue irritation at the administration site [33]. Finally, nanoemulsions can be produced with a wide range of biocompatible, biodegradable, and non-toxic natural materials, making possible the development of safer therapies for human administration.

Proteins are frequently used as emulsifiers in emulsions and nanoemulsions, and Serum Albumins are among the most commonly used proteins in the food and pharmaceutical industry [34]. The outstanding performance of proteins as emulsifiers comes from their amphiphilic nature and interfacial activity, that contribute to the formation of interfacial viscoelastic films, providing resistance to mechanical stresses as well as electrostatic and/or steric stabilization [34,35]. It is well known that the combination of proteins and polysaccharides improves the stability of emulsions [34,35]. At the interface, polysaccharides can enhance the interfacial activity of proteins reinforcing the interfacial layer and protecting the integrity of oil droplets. In bulk, polysaccharides promote steric repulsions between droplets and increase the viscosity, hence improving the stability of the emulsion [35]. Serum Albumins can interact with polysaccharides, via covalent or non-covalent interactions, providing vehicles for drugs in complexed systems or emulsions [36–39]. The implications of such interactions can alter the solubility, the gelling ability, and the heat stability of the complexes as well as their emulsifying capacity [40–42]. Therefore, there is a need to understand their interactions at different levels in order to achieve the desired improvement.

At present, Hyaluronic acid (HA) is one of the polysaccharides that is gaining increasing interest on cancer research for developing drug delivery systems. HA is a long chain polysaccharide which plays a structural role binding different biomolecules, such as proteins, through specific or non-specific interactions [43]. HA is an extremely interesting molecule in several aspects. It specifically binds to CD44 receptor and

receptor for hyaluronic acid-mediated motility (RHAMM) receptors, both overexpressed in Cancer Stem Cells, a small subpopulation of cells within the tumour responsible of chemotherapy resistance, recurrence, and metastasis [44]. This biomolecule, which is non-surface active, has been safely used as crosslinker in tissue engineering and in viscosupplementation strategies [45], and it is applied to decorate nanoparticles for specific cancer cells targeting [43].

The Serum Albumins most commonly used on the development of drug delivery systems are Human Serum Albumin (HSA) and its bovine analogous (BSA). BSA is a non-expensive and easy-obtainable protein with a high degree of similarity with HSA. It is present in cow milk and meat and is frequently used on medical formulations because of its safety, as BSA allergy is very infrequent in humans [46]. In general, BSA is treated as comparable to its human homologue HSA and used as a model protein for fundamental studies since it is available in high amounts and is well understood. However, there are subtle differences between these two proteins which can be relevant when designing drug delivery systems. In this work, HSA and BSA are thoroughly compared in terms of their molecular structure, their interacting abilities with polysaccharides, their interfacial activity, and their emulsifying ability. This comparison enlightens the behaviour of these two homologous proteins, identifying the parameters that could explain possible differences encountered between HSA and BSA as common emulsifiers in drug delivery systems. Hence, this review provides a help-guide on the rational design of Albumin-based drug delivery systems. The interaction of Albumins with polysaccharides, as well as their use in complexes and LLNs as drug delivery systems, are also analysed considering specially the interaction with HA as a promising bioactive polysaccharide for drug delivery formulations.

2. Molecular structure of Albumins: interactions with polysaccharides

As already stated, the use of BSA as a valid model protein for fundamental studies is widespread owing to its structural similarity to HSA. BSA is available in high amounts and is well understood, though its clinical relevance is rather limited [47]. The amino acid sequences of BSA and HSA were first reported in the seventies by Brown et al. [48] and Meloun et al. [49]. BSA and HSA are globular proteins where the secondary α-helix structure is predominant [50], and the 3D model structures of both proteins are actually very similar (Fig. 1A). Still, there are some relevant differences at the 3D structural model, and also at the amino acid sequence level. The alignment of both sequences shows 436 similar amino acids out of 576 [51]. Ma et al. carried out a deep analysis of the differences in the amino acid sequence between BSA, HSA, and the homologous protein in rats (RSA), and the consequences of such sequence differences on their conformational stability and adsorption properties [52]. Ma et al. identified all the sequence variations after the alignment of the proteins and defined whether the non-coincident amino acids where homologous or had different physicochemical properties (Fig. 1B). The major interest of studying these differences lies in the fact that having amino acids with different characteristics on the same position could modify the intramolecular interactions and produce differences in protein conformation or stability [52]. In general terms, BSA and HSA have a low content of tryptophan and methionine, and the proportion of glycine and isoleucine is also low when compared with the average content of these amino acids in other proteins. Also, they have a high proportion of aspartic acid, glutamic acids, lysine, and arginine, which are charged amino acids [53]. BSA and HSA have 17 disulphide bonds, and the position of the cysteines involved in those bonds produce a nine-loop characteristic structure. Along the sequence of both proteins, there are eight pairs of adjacent cysteines which define the loop structure (Fig. 1C). The nine loops are distributed in three homologous domains, in such a way that the three loops included in each domain follow a large loop-small loop-large loop organization (Fig. 1C) [50,53]. The most remarkable difference between BSA and HSA appears in the amino acids sequence of the hydrophobic binding pockets. BSA presents two tryptophan residues in its sequence, a surface Trp134 and an inner Trp212, whereas HSA has only one tryptophan in its amino acid sequence, Trp214 [50]. Another difference between BSA and HSA is the charge distribution along the protein length (Fig. 1C). At pH 7, the estimated charge for domains I, II and III for BSA is −10, −8 and 0, respectively, while for HSA is −9, −8 and +2, respectively [53]. This correlates with the slightly different isoelectric point reported for both proteins: pH 4.9 for BSA [54] and pH 4.4 for HSA [32].

The highlighted differences between the primary structure of BSA and HSA can be relevant regarding their interacting capacities with

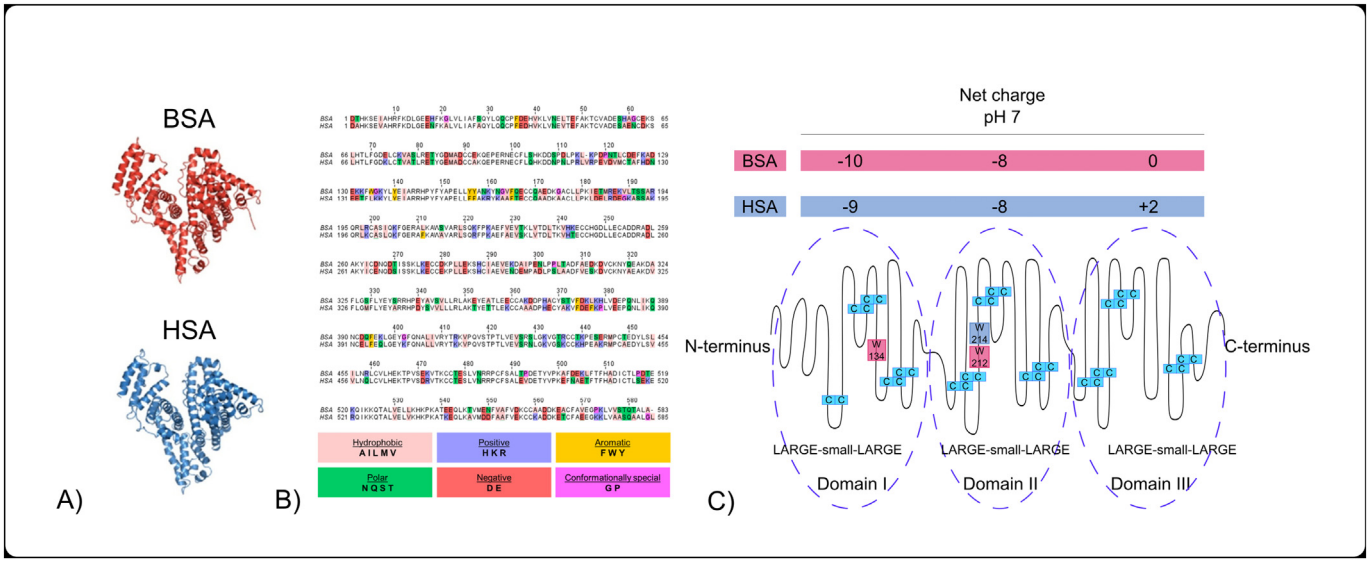

Fig. 1. (A) Modelled 3D structures of BSA and HSA. (B) Alignment of HSA and BSA; the non-coincident amino acids are highlighted with a predetermined colour, depending on their physicochemical properties, according to the Zappo colour scheme [52]. (C) Nine loop structure of HSA and BSA highlighting the location of cysteine groups and the charge distribution along the domains of the protein at pH 7. (A) and (B) adapted from [52] with permission from Elsevier© 2020.

other molecules. The interactions between HSA or BSA with other ligands are generally studied via fluorescence spectroscopy [50,55–59]. This is a sensitive, rapid, and simple technique that provides information about the nature of the binding interaction. Fluorescence spectroscopy is based on the known fluorescence intensity of HSA and BSA from their tryptophan residues when the excitation wavelength is 295 nm. Thus, the interaction between a molecule and a tryptophan from the protein leads to a reduction of the fluorescence intensity and allows studying such interaction. Bourassa et al. studied the interaction of folic acid with BSA and HSA, and they found that folic acid interacted in a stronger way with BSA. They argue that this difference is due to the presence of two tryptophan amino acids on BSA which interact with the folic acid, while there is only one interacting-tryptophan on HSA [50]. Cheng et al. also studied the interaction of indigotin with BSA and HSA, finding out that both were able to form complexes with this dye molecule, via hydrophobic interactions involving tryptophan amino acids. Yet, they reported higher binding constants for HSA and the hydrophobic dye [55]. Our research group at the University of Granada has investigated the mechanisms involved on the interactions of BSA and HSA with maslinic acid and its derivatives, which are bioactive molecules with probed anticancer activity [59–63]. The study of these interactions showed that even small modifications on the drug molecular structure can lead to variations on the strength and nature of the interactions [59,63]. It is known that BSA and HSA have several binding sites for organic and inorganic molecules. Thus, the nature and location of the specific binding site determines the kind of ligands that will bind there, such as ions, hydrophobic or hydrophilic molecules, big or small molecules [53]. Serum Albumins can also interact with polysaccharides forming complexes with improved characteristics regarding food or pharmaceutical applications. The type and strength of the bonding with the protein depends on the chemical nature of the polysaccharide and determines the physicochemical characteristics of the formed complex (Table 1).

2.1. Covalent reactions

The covalent linking of Serum Albumins and polysaccharides provides stable complexes. Covalently linked protein-polysaccharide complexes are generally termed conjugates, and can be achieved through different chemical reactions, such as Maillard reaction, laccase catalysis, carbodiimide reaction or amine reduction. Let us consider these different covalent interactions of Albumins polysaccharides.

The most abundant covalent bonding is achieved by the Maillard reaction, which occurs between amino groups from amino acids and carboxyl groups from reducing sugars. The Maillard reaction takes place during many food processing stages. It gives, as a result, a tanned colour and a characteristic flavour to the food products. This non-enzymatic reaction is driven by high temperatures and can be affected by parameters like pH, ionic strength or protein and polysaccharide concentrations [64,65]. Another parameter that can influence the efficiency of this reaction are the size, shape or the degree of ramification of the own polysaccharide. Dextran is commonly used to bind to proteins via the Maillard reaction [65]. It is a ramified polysaccharide from microbiological origin composed of glucose units which is commonly used in the food, medical, pharmaceutical, and agricultural industry. Jung et al. successfully linked dextran to BSA via the Maillard reaction without inducing major changes on the secondary structure of the protein [65]. However, the binding ratio of BSA to dextran was lower than the binding ratio of BSA to other polysaccharides or samples of dextran with lower molecular weight. Jung et al. suggest that this difference can be due to the steric hindrance set by big-sized polysaccharides which hinders their binding with the protein [65]. The Maillard reaction is usually carried out by heating under a controlled relative humidity [66]. The protein-polysaccharide conjugates are hence natural, non-toxic, and have improved functional properties for food and pharmaceutical issues [65]. The undesirable brown colour resulting from Maillard reaction can be avoided by a pulsed electric field method as an alternative to the heating procedure [64].

A second method for obtaining covalent bonding uses the laccase enzymatic reaction [67]. Laccases are copper-containing enzymes from fungi, plant or bacteria origin, which catalyse the oxidation of phenolic compounds present on aromatic amino acids and in some polysaccharides. Laccases also catalyse the reduction of molecular oxygen (as electron acceptor substrate) to water [67,68]. Similar to the Maillard reaction, the chemical process taking place by laccase is an oxidation-reduction reaction. However, in the Maillard reaction the reducing sugar is oxidated and the amine group provided by the protein is reduced, therefore, both subtracts are involved on the resulting bond. Conversely, the oxidation driven by the laccase produces reactive radicals, which interact with each other, cross-linking the molecules, so

Table 1
Summary of the main types of interactions between BSA/HSA and polysaccharides.

Protein	Saccharide or Polysaccharide	Protein-polysaccharide interaction	Cargo	Reference
BSA	Glucose or mannose	Conjugation/Maillard reaction		[64]
BSA	Dextran	Conjugation/Maillard reaction		[65]
BSA	Hyaluronic acid	Conjugation/Maillard reaction		[88]
BSA	Oligogalacturonate	Conjugation/Reductive amination		[71]
BSA	Sugar Beet Pectin	Conjugation/Maillard reaction/laccase		[67]
BSA	S14PS	Conjugation/Carbodiimide reaction		[70]
BSA	Glucose	Coacervation		[75]
BSA	Whey Protein Isolate-beet pectin	Electrostatic Coacervation/complexation		[85]
BSA	Sugar beet pectin	Complexation/Electrostatic		[80]
BSA	Alginate	Complexation/Electrostatic		[40]
BSA	Dextran	Complexation/Electrostatic		[40]
BSA	Gum arabic	Complexation/Electrostatic		[83]
BSA	Corn starch	Complexation/Electrostatic		[78]
BSA	Chitosan	Complexation/Electrostatic		[57]
BSA	Cellulose	Complexation/Electrostatic		[84]
BSA	Citrus peel pectin	Complexation/Electrostatic	Vitamin C	[42]
BSA	Pectin	Complexation/Electrostatic		[79]
BSA	Low methoxyl pectin	Complexation		[77]
BSA	Pectin/chitosan	Complexation/Electrostatic	Berberine	[37]
BSA/HSA	Cellulose	Complexation/Electrostatic or van der Waals interactions	Cholesterol	[39]
BSA	HA	–		[89]
BSA	HA	–	Brucine	[36]
HSA	HA	–	Erlotinib	[38]

that the linking process is not carried out by the laccase oxidation process itself [68].

Another reaction used to produce covalent bonds in proteins is the carbodiimide-mediated reaction. This is a two-step reaction, which includes the activation of a carboxylic group from the protein by the carbodiimide and the subsequent reaction of the activated carboxyl group with a nucleophilic ligand, like an amine group [69]. This nucleophilic group can belong to a polysaccharide, giving as a result an amide or ester bond. We find an example of this reaction on the covalent linking of BSA and the type 14 capsular polysaccharide from *Streptococcus pneumoniae* [70]. In this case, the aim of the covalent conjugation was the development of more effective immunostimulant conjugates for the production of more effective vaccines, which resulted in a better immunoglobulin G memory response [70]. Reductive amination is another used technique, which we can find, for instance, on the linking of amine groups from BSA to carboxyl groups from artificially-synthesized target oligogalacturonates [71]. The general process consists of the reaction of an amine group with a carboxylic group to form an intermediate imine, which is then reduced to an amine by a reducing agent, and there are different approaches to carry out these processes [72]. The characteristics of the resulting conjugates can differ depending on the Serum Albumin and the polysaccharides involved, but also depending on the linking method used. An example of this will be discussed in detail in Section 4.1, comparing BSA and sugar beet pectin conjugates, linked via the Maillard reaction or via laccase [67].

2.2. Non-covalent interactions

It is possible to take advantage of the interacting forces which drive the spontaneous association of biopolymers in solution to produce protein-polysaccharides complexes via non-covalent interactions [73]. In some cases, this spontaneous complexation leads to the formation of a separated phase, and in this case, the formed complex is named coacervate [73,74]. In the special case of coacervates formed by oppositely charged molecules, the system is a complex coacervate [74]. This process is spontaneous since the Gibbs free energy derived from such interaction is negative and therefore the process is thermodynamically favoured [73]. The analysis of the thermodynamic parameters provides further information about the type of interaction. Zhang et al. propose that the spontaneous interaction between glucose and BSA ($\Delta G < 0$) is mainly driven by hydrogen bonds and van der Waals interaction owing to the negative enthalpy change ($\Delta H < 0$) and independent of the negative entropy change ($\Delta S < 0$) of the process [75].

The formation of complexes and coacervates is affected by changes in pH [57,76], temperature [42,77,78], ions concentration and the nature of such ions [77,79], protein and polysaccharide ratio [76,78], and charge, size, and shape of both biomolecules [65,80]. BSA, HSA and charged polysaccharides are polyelectrolytes, i.e. polymers which possess a net charge at neutral pH [81]. Polyelectrolytes with opposite charge interact owing to electrostatic interactions, to form polyelectrolyte-polyelectrolyte complexes as shown in Table 1 [81]. An example of how polyelectrolyte-polyelectrolyte interactions are modulated through alterations in electrostatic interactions by pH changes is the interaction of BSA and sugar beet pectin reported by Li X. et al. [80]. BSA and pectin form intramolecular soluble complexes when the protein is negative and the polyelectrolyte is positively charged, depending on their isoelectric points. Reducing the charge, by pH alterations, leads to a reduction of the electrostatic repulsion between BSA-pectin complexes. Then, the system evolves to an unstable region where the complexes are insoluble, owing to a further reduction of the pH [80]. This is an interesting work highlighting the impact of the conditions of the surrounding environment on the stability of complexation, which can be used to develop stimuli responsive systems [37,40,82].

The pH changes may also drive denaturation of Albumins promoting new interactions, such as hydrogen bonding or hydrophobic interactions [42,83,84]. This also occurs with temperature changes, which can alter the protein native structure, making 'visible' some inner part of the molecules, and turning them available to build new interactions [42,78]. For instance, inner hydroxyl groups which are exposed owing to changes in molecular conformation, can establish new hydrogen bonding [78]. Furthermore, the denaturation induced by thermal treatment may also promote the formation of intermolecular hydrophobic interactions and even disulphide bonds, leading in this last case to more rigid structures [42]. The ionic strength also affects the interaction between protein and polysaccharides and modulates the formation of a complex, a coacervate, an aggregate, or even the dissociation of the interacting component and the recovery of the individual components [73,74]. Wang et al. argue that at low ionic strength, ions screen some charges on the biopolymers surface reducing the repulsive forces while a further increase on the ionic strength shields the charges that promote both, attractive and repulsive forces between the two biopolymers, reducing their interactions [79]. The protein-polysaccharide ratio is another important factor to consider in the complexation and coacervation processes [85], and it is sometimes linked to other factors [76,77].

Polyelectrolytes can also interact with surfactants, leading to the formation of polyelectrolyte-surfactant complexes. The interaction and the behaviour of the formed complexes are affected now by hydrophobic and electrostatic interactions. The nature of the polyelectrolyte and the surfactant involved will define the contribution of each force to the interaction. Similarly to polyelectrolyte-polyelectrolyte interactions, factors such as temperature, pH or ionic strength can affect the established interaction, as well as the hydrophobicity of the molecules and the solvent and co-solvent [86]. This is nicely illustrated by Chakraborty et al. who compared the interaction of BSA with three different surfactants with the same carbohydrate chain length: anionic sodium dodecyl sulfate (SDS), non-ionic pentaethylene glycol mono-*n*-dodecyl ether ($C_{12}E_5$) and cationic alkyltrimethylammonium bromide (ATAB). At pH 7, where the protein has a negative net charge, electrostatic and hydrophobic interactions were the expected driving forces in the interaction between BSA and cationic-ATAB. Conversely, hydrophobic interaction was expected to be the driving force in the interaction between BSA and non-ionic-$C_{12}E_5$ and anionic-SDS. However, Chakraborty et al. found that the BSA-non-ionic surfactant interaction was weak, while the interaction with the ionic surfactants was stronger and reported different outcomes depending on whether the surfactant was anionic or cationic. On one hand, low concentrations of SDS interacted with the hydrophobic pockets of the globular native protein, and this interaction was reinforced with the interaction of the SDS with the peripheral and positively charged groups from native BSA. SDS was able to interact with the native protein as monomeric or aggregated SDS, and even increased the BSA thermal stability. However, a further increase on the SDS concentration produced unfolding of BSA, and led to the co-existence of SDS-unfolded BSA complexes and SDS micelles. The increased hydrophobicity of the formed complex induced a better interfacial adsorption. On the other hand, cationic-ATAB attained a quick protein unfolding and precipitation, and the increase on the hydrophobicity of the complex was a consequence of charge neutralization. Interestingly, an increase on the length of the carbon chain of the cationic surfactant promoted a stronger interaction with BSA, owing to the role of the hydrophobic effect, which highlights the relevance of this interacting force [87].

2.3. Applications as drug delivery systems

The interaction of Albumins with polysaccharides promotes the formation of structures with improved targeting, transport, or protective characteristics, which can be particularly useful in the drug delivery field. An example of this is the berberine loaded BSA-gelled microcapsules coated by a pectin-chitosan polyelectrolyte multilayer shell for acne treatment [37]. These authors synthesized a calcium

carbonate-BSA template by coprecipitation. Then, the template was coated, following the layer-by-layer technique (Lbl) [90,91], by deposition of alternative layers of calcium cross-linked pectin, and chitosan, leading to the formation of a pectin-chitosan polyelectrolyte complex shell, which provided protection and mucoadhesive properties. The berberine was loaded on the microcapsules, after the coating process and the thermal gelling of the BSA, by a diffusion process and thanks to the electrostatic interaction of the drug with the BSA from the gel core.

In this section, we have introduced different interacting possibilities between proteins and polysaccharides. However, the established interaction between the protein-polysaccharide can define the characteristics and the functionality of the new structure. This is the case of BSA and HSA-coated cellulose nanocrystal, designed as cholesterol-binding carriers to control cholesterol efflux from cell and control cholesterol deposition on arterial walls. Albumins were linked to the cellulose surface by two different methods, namely, physical adsorption and chemical linking by the carbodiimide reaction. Both systems were similarly biocompatible, preserved the integrity and biological function of the Serum Albumin, and were effective as protein delivery systems. However, the covalently linked system provided better results in terms of sustained protein delivery and cholesterol efflux [39].

As mentioned before, HA is an attractive polysaccharide in the design of drug delivery systems. Martins et al. have used this polysaccharide in BSA-HA solid-in-oil nanodispersions synthetised for transdermal delivery of HA [89]. Also, Chen et al. coated with HA preformed brucine-loaded BSA nanoparticles, for specific intraarticular release. The targeting provided by the HA enhanced the uptake by the chondrocytes [36]. Another example is Shen et al. who obtained HSA, HA and Erlotinib nanoparticles by a precipitation method [38]. These authors did not characterize the interaction between the components of the nanocarriers. However, they reported the effective encapsulation of the lipophilic drug on the nanoparticles, as well as an increased cytotoxic effect in vitro compared to non-HA-coated nanoparticles, and a good in vivo performance [38]. The Maillard reaction also promotes covalent binding between BSA and HA. These complexes can encapsulate hydrophobic drugs such as paclitaxel and imidazoacridinones via hydrophobic interaction with BSA. BSA-HA complexes are able to solubilize highly hydrophobic drugs and provide a targeted nanocarrier, which can promote drug accumulation on the specific tumour target [88].

3. Albumins at interfaces: interactions with polysaccharides

3.1. Conformational changes of albumins upon adsorption

Proteins are amphiphilic molecules which adopt structured conformations in aqueous solution, where non-polar groups are congregated inside the structure and polar groups are concentrated at the periphery. In this way, the free energy of the system is minimized by reducing interactions between non-polar groups and water molecules. However, proteins change their structural conformation upon adsorption to non-polar interfaces. The adsorption process of proteins at interfaces depends on factors such as thermodynamic stability, flexibility, amphipathicity, molecular size and charge [92]. The structure adopted by the protein at the interface will provide stabilization to the emulsion against different mechanisms like creaming, flocculation, or coalescence [29]. Globular proteins such as BSA and HSA possibly stabilize emulsions through a combination of electrostatic and steric interactions. The properties of the interfacial film formed at the oil-water interface in emulsified systems are ultimately determined by the protein conformation before adsorption and the conformational changes undergone upon interfacial adsorption [93]. The density, flexibility, cohesion, elasticity, and thickness of the interfacial film will be determined by the stability of the protein conformation and the interaction with the interface.

The structure of BSA changes substantially upon adsorption onto oil-water interfaces as evidenced from a number of literature works with different experimental approaches. Zhai et al. reviewed in detail the current knowledge on unfolding of proteins upon adsorption onto oil droplets in emulsions, as measured with Fourier Transform Infrared Spectroscopy, circular dichroism (far and near UV), and Tryptophan emission fluorescence [92]. These techniques allowed quantitative information about the secondary and tertiary structure of proteins at oil-water interfaces. In general, the conformational changes in BSA seem to be related to the inherit flexibility of the protein, the distribution of hydrophobic/hydrophilic domains within the protein sequence and the hydrophobicity of the oil phase. Castelain and Rampon studied the conformational changes of BSA upon adsorption onto the dodecane-water interface. Interfacial conformational changes in the protein were detected by modifications in the environment of the aromatic amino acids, which were displaced to a more hydrophobic location [94]. Similarly, Husband et al. studied the conformational changes undergone by BSA upon emulsification with tetradecane [95]. The tertiary structure appeared greatly affected by emulsification, as evidenced by changes in the environments of the aromatic residues. Conversely, the secondary structure was barely affected, showing just a reduction in the amount of α-helix content. Later, Rampon et al. demonstrated that the interfacial conformation of BSA depends strongly on the nature of the oil phase [96]. For BSA, dodecane provided the most hydrophobic environment followed by miglyol and sunflower oil. Similarly, Day et al. demonstrated that BSA undergoes larger conformational changes in both secondary and tertiary structures upon adsorption to the hexadecane-water compared to the tricaprin-water interface. They also stated that the reduction in α-helical content of BSA upon emulsification is accompanied by an increase in disordered structures and β-sheet. In fact, BSA experiences extensive conformational changes upon adsorption despite having 17 disulphide bridges, compared to lysozyme, which has only 4 disulphide bridges. Day et al. link this enhanced unfolding to the position of the cysteine residues involved on the disulphide bridge [97]. As shown in Section 2, the disulphide bridge in BSA links residues which are close together in the amino acid sequence (Fig. 1C). This improves the local conformational stability but facilitates the local exposure of hydrophobic regions towards the oil-water interface. In contrast, the disulphide bonds in lysozyme, link residues which are located at the beginning and the end of the amino acid sequence. This contributes to a high internal cohesion of the protein, which prevents exposure of hydrophobic regions onto the oil-water interface. Apparently, this is one of the reasons why BSA is a better emulsifier than lysozyme [97].

Comparison of the amino acids sequence of BSA [98] and HSA [99] (Fig. 1B), shows that the relative position of the disulphide bonds and the amino acids involved are equivalent. This means that the conformational restrictions set by these bonds are the same for both proteins (Fig. 1C). Jorguensen et al. compared specifically the conformations of BSA and HSA upon emulsification with coconut oil, obtaining that the secondary structures of BSA and HSA are almost equally stable. Both Albumins show some aggregate formation and overall secondary structure changes. However, HSA retained slightly more of the overall secondary structure, obtaining 9% change for HSA versus 12% change for BSA. Hence, maybe their conformational stability upon emulsification cannot be directly predicted on the basis of their similar structure [58]. David et al. also compared the unfolding of BSA and HSA upon the reduction of their disulphide bonds, by analysing the unfolding process through Raman spectroscopy [100]. They found out that the reduction of the disulphide bonds from BSA was easier, leading to a less stable conformation under reducing conditions [100]. The comparison of BSA, HSA and RSA carried out by Ma et al. shows that HSA has a higher stability upon temperature-induced oligomerization, resisting better temperature-induced unfolding than BSA [52]. Moreover, BSA formed smaller aggregates than HSA after temperature unfolding and oligomerization. They also studied the adsorption of Albumins onto hydrophilic

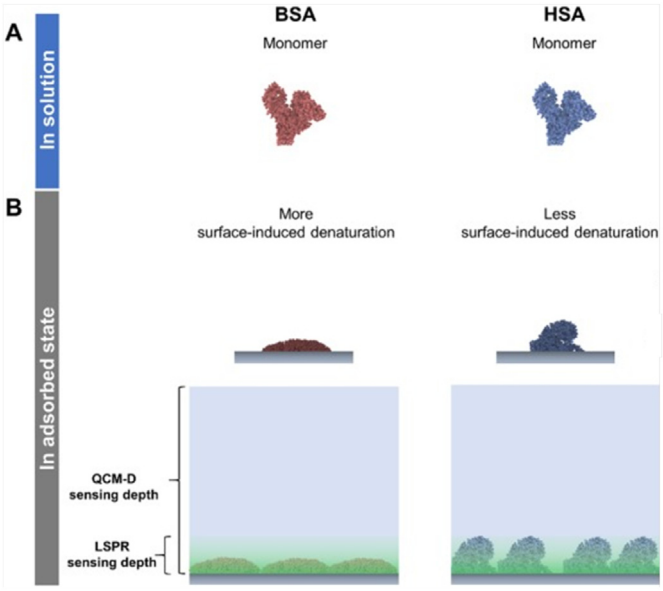

Fig. 2. Schematic illustration of conformations of BSA and HSA in solution and adsorbed at silica surface. BSA shows a lower conformational stability than HSA, which promotes an enhanced surface denaturation.
Adapted from [52], with permission from Elsevier© 2020.

silica surfaces, showing a greater spreading for BSA compared to HSA, and a higher surface-induced denaturation oligomerization [52]. Hence, BSA displayed a lower conformational stability than HSA, with a higher reduction of α-helical percentage than HSA. Therefore, BSA seems to undergo a more extensive surface denaturation upon adsorption and is more expanded than HSA at this interface (Fig. 2). These results show that slight changes on the primary sequence can be responsible for the different conformational stability of these homologue proteins.

3.2. Adsorbed films of albumins and polysaccharides

The conformation of Albumins at fluid interfaces has also been studied by measuring changes in the interfacial tension and interfacial rheology of adsorbed films [101–106]. Interfacial tension provides information of film compactness, the lower the interfacial tension, the more compact the film. Interfacial rheology provides information on the mechanical strength of the film and its resistance to deformation. Albumins, generally provide a compact layer and highly elastic surface film, compared with other proteins, as measured by surface tension and dilatational rheology [105]. The formation of a more cohesive surface layer, compared to random coil proteins like β-casein, is due to the structural stability of BSA as stated by Cascao-Pereira et al. [107]. Similarly, Noskov et al. also attributed the high surface elasticity of BSA films to strong interactions between rigid and compact molecules with the interfacial layer [101]. Changes of pH did not provide significant differences in the surface tension and dilatational elasticity of concentrated interfacial films of BSA in equilibrium, whereas a critical concentration of denaturant did reduce the interfacial elasticity importantly [101,108]. The surface conformation of BSA and BSA derivatives was also investigated by Berthold et al. in terms of surface pressure isotherms and dilatational viscoelasticity [104]. In general, the surface activity of BSA increased owing to chemical modifications, especially acetylated BSA. Again, BSA formed a highly elastic film compared to other proteins. Then, as the chemical modification of BSA induced protein unfolding, the elasticity of the layer was reduced. In agreement to Noskov et al., Berthold et al. attribute the reduced viscoelasticity to the existence of a required threshold value of modification in the protein structure to form observable conformational changes [104].

Douillard studied the adsorption behaviour of BSA at the air-water and benzene-water interfaces, comparing their unfolding degree by application of scaling theory. According to their study, BSA adopts a more expanded conformation at the oil-water interface [109]. Recently, Campana et al. have revisited the different conformations of BSA at air-water and hexadecane-water interfaces by combining interfacial tension and neutron reflectometry [110]. According to these authors, BSA molecules adsorb with the major axis parallel to the water surface, forming a compact layer with a secondary diffuse layer extending towards the aqueous phase. The secondary structure of BSA remains practically unaffected at the air-water interface, while adsorption onto hexadecane interfaces provokes changes in the secondary structure and lead BSA to lose its tertiary structure. Hence, is seems oil-water interfaces promote an improved packing so that the oil-water interface can accommodate more molecules than the air-water interface [110].

Burgess et al. reported back in the 80s a very complete work containing surface tension and shear rheology of BSA under different experimental conditions and in the presence of different polysaccharides, using a Cahn Microbalance connected to a Wilhelmy Plate [106]. According to their study, the interfacial properties of adsorbed BSA depend on ionic strength and pH as the protein conformation is affected by these variables. The interfacial tension of BSA showed the lowest values at the isoelectric point and at high ionic strength, indicative of increased adsorption. The lacking of charges at the isoelectric point and the screened electrostatic repulsion, explained then the formation of a more compact film [106,111]. The differences between HSA and BSA interfacial layers, have been much less studied in the literature, while the majority of the works deal either with BSA or HSA. Del Castillo et al. investigated the microstructure of emulsified HSA providing the interfacial tension values and the digestibility of HSA [112]. Later, Aguilera-Garrido et al. have studied the microstructure of emulsified BSA in a similar work [113]. Fig. 3A compares the interfacial tension of BSA and HSA adsorbed films at the olive oil-water interface after 1 h of adsorption as a function of bulk concentrations. Results show that the final interfacial tension decreased as the concentration of protein increased in the bulk, until it reached a plateau when the interfacial layer saturates for BSA and HSA. However, the interfacial tension of BSA appears significantly lower compared to that of HSA, especially at the lowest concentrations studied. The lower interfacial tension of BSA is possibly associated with an increased interfacial denaturation of BSA upon adsorption, in agreement with findings from Campana et al. [110]. Lu and Su also determined by neutron specular reflection that the surface concentrations of BSA are systematically higher than those of HSA, also indicative of a higher surface activity of BSA [114]. Similarly, the higher interfacial tension of HSA in Fig. 3A reflects a restriction on the conformational change upon adsorption. This agrees with findings from Makievski et al. who state that HSA molecules do not undergo any significant denaturation at the air-water interface by application of a thermodynamic model [115]. Going back to the work of Day et al., they state that freedom on the conformation rearrangement at the interface makes BSA a better emulsifier than lysozyme, which shows restrictions in conformational changes [97]. According to this argument, BSA will possibly be a better emulsifier than HSA, considering the more extensive conformational changes proposed for BSA as suggested by the lower interfacial tension values shown in Fig. 3A.

The presence of polysaccharides can affect the viscoelastic properties of an adsorbed protein film by interacting with the protein in bulk [116]. These interactions generally increase the hydrodynamic thickness of the adsorbed film and the strength of the links between proteins, and both can imply a change in the viscosity and elasticity of the adsorbed layer [116]. Rodríguez-Patino and Pilosof, in a recent review illustrate the different interaction occurring between protein and polysaccharides depending on the existence of favourable or unfavourable

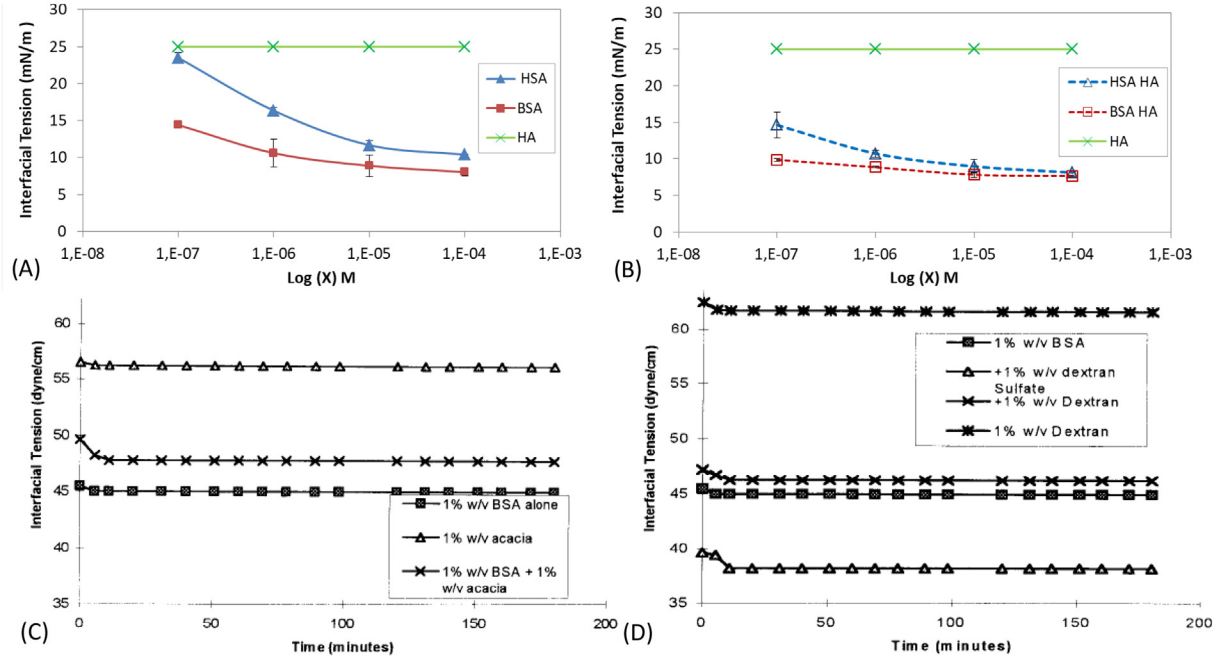

Fig. 3. (A) Interfacial tension of BSA (red, solid squares) and HSA (blue, solid triangles) and HA (green, x) (B) Interfacial tension of mixtures of Albumins with HA, BSA (red, open squares) and HSA (blue, open triangles) and HA (green, x) at the olive oil-water interface. Values plotted as a function of protein concentration in the sample and concentration of HA fixed at $5 \cdot 10^{-7}$ M. Interfacial tension final values reached after 1 h of adsorption at constant interfacial area measured by Pendant Drop Tensiometry (DINATEN®) at the olive oil-water interface in NaH_2PO_4 1.13 mM, pH 7.0 and $T = 37$ °C. Values from HSA taken from reference [112] and values for BSA, taken from reference [113]. Values plotted are mean values ± standard deviations (within the size of the symbols) and lines are a guide for the eye. (C and D) Mixed adsorbed layers at the air-water interface for: (C) BSA-Acacia Gum, and (D) BSA-Dextran and BSA-Dextran Sulphate. Reprinted from [97], with permission from Elsevier 1997.

interactions between them [117]. The interaction with surface active polysaccharides might affect the surface activity of the complex or result in a competitive adsorption process. Conversely, the interaction with non-surface active polysaccharides can promote binding to a pre-formed protein monolayer or adsorption of protein-polysaccharides complexes. Also, the thickening ability of polysaccharides plays a role on emulsion stability. Hence, the interfacial properties of mixed adsorbed films composed of Albumins and polysaccharides are affected by the interaction between these biopolymers and can be determinant in the characteristics of the resulting emulsion. Most of the studieds in the literature deal with the interaction of BSA with polysaccharides.

One of the first works addressing specifically BSA and polysaccharide interactions at the oil-water interface was done by Dickinson and Pawlowsky [118]. Addition of dextran sulphate (anionic, surface active) onto an adsorbed BSA layer doubled its viscoelasticity, which altered the emulsion behaviour by causing bridging flocculation owing to a net attractive electrostatic protein-polysaccharide interaction at the surface of the emulsion droplet [118]. A similar study addressed the interaction of BSA with carrageenan (anionic, non-surface active) [111]. The presence of carrageenan reduced the surface activity of the protein owing to a net attractive interaction between BSA and carrageenan molecules in bulk solution, thereby lowering the effective concentration of free BSA available for adsorption [111]. Burgess et al. addressed the effect of acacia gum, dextran and dextran sulphate on the interfacial tension and interfacial rheology of BSA (Fig. 3C and D). The protein-polysaccharide complexes formed with acacia gum trap BSA, lowering the solubility of the complex protein and increasing slightly the interfacial tension of the mixture (Fig. 3C and D). More recently, Cheng et al. have reported a similar value of the interfacial tension of BSA in the presence of Gum Arabic and have related it to the formation a bilayer [119]. Differently, dextran does not complex with BSA and the increase in the interfacial tension recorded is a consequence of competitive adsorption owing to its surface activity. Similarly, mixtures of BSA with the surface active compound dextran sulphate decreases the interfacial tension of the mixture owing to competitive adsorption [106]. Rodriguez-Niño et al. addressed the interaction of BSA with sucrose [120], reporting a higher surface activity of the mixed system. The authors suggest that the higher viscosity of sucrose solutions prevents the adsorption of BSA, balanced by the lower size of the folded protein molecule in the presence of sucrose [120]. Differently, the presence of Corn Fiber Gum barely changes the interfacial tension of BSA, despite forming a mixed interfacial layer [121]. Kim et al. studied the surface activity of BSA and fucoidan mixtures [122]. Fucoidan is an anionic charged non-surface-active polysaccharide. Fucoidan decreased the surface activity of BSA but acted as an electrolyte, providing a highly negative environment which apparently contributes to stabilizing the emulsion. In some cases, changes in the interfacial tension can be altered by conjugation of BSA with the polysaccharide through different methods (Section 2.1). For example, the interfacial tension of BSA-fucoidan conjugates prepared through Maillard reaction, is lower than that of their mixtures [123]. In contrast, the interfacial tension of peroxidase-treated Corn Fiber Gum-BSA conjugates increased owing to conformational changes induced in the molecule by enzymatic reactions [121].

The interaction of BSA with HA was addressed by Aguilera-Garrido et al. who reported the interfacial tension of BSA and HA as regards emulsification and digestibility given the added functionality of HA [113]. Their results are compared in Fig. 3B to the interaction of HSA with HA under similar experimental conditions. HA is non-surface active anionic polysaccharide [124]. Both proteins (HSA and BSA) improve their adsorption at the oil-water interface in the presence of HA, as the interfacial tension appears lower than that of pure BSA and HSA for all the protein concentrations assayed (Fig. 3A and B). This improvement is more noticeable for HSA, especially at the lowest protein concentrations. Mixed BSA + HA and HSA + HA show very similar interfacial tension; thus, the presence of HA seems to overcome the different surface activity of HSA and BSA, possibly due to the presence of HA within the

interfacial layer. The interaction between Albumins and HA is possibly originated from hydrogen bonding with hydroxyl groups from HA, since both molecules are negatively charged at pH 7. The nature of the interaction and the differences between HSA and BSA cannot be assessed solely by interfacial tension, but require the use of more specialized interfacial characterization techniques. Similarly, differences between HSA and BSA adsorbed layers are still rather unexplored, especially regarding dilatational or shear rheological studies. Moreover, there is a real lack of studies in the literature addressing differences in the interacting abilities of HSA and BSA with polysaccharides at the interface, which hence offers some open questions and plenty of room for new investigations.

3.3. Spread films of albumins and polysaccharides

The lateral interaction between Albumins spread layers can be further assessed by Langmuir Films [125]. This traditional technique provides useful information of the interfacial behaviour of different surface active materials, including biopolymers as proteins, which are involved in fundamental biological processes related with its surface activity [126]. In particular, the Langmuir Film Balance allows further exploring the lateral interactions and packing occurring between surface molecules. In the case of proteins, the interpretation of Surface Pressure-Area isotherms obtained by lateral compression, is usually complex because of the macromolecular conformational flexibility and variety of interfacial architectures encountered [127]. Albumin proteins from different animals have been studied by using the Langmuir technique. Most of the studies deal with Bovine Serum Albumin (BSA), and only few deal with HSA. An extensive review of the behaviour of Albumins by means of Langmuir monolayers was published by Crawford and Leblanc not so long ago [125]. This review includes studies on HSA Langmuir monolayers at different ionic strength and pH of the subphase [128], on BSA Langmuir monolayers and Langmuir-Blodgett films [129], and on lipid-BSA Langmuir monolayers [130,131]. However, these authors do not compare explicitly HSA and BSA but use both proteins indistinctly. More recent studies address the interaction of HSA with lipids [130]. Toimil et al. point out that different parameters such as the subphase pH, the ionic strength, the spreading solvent and the method used for the extension, are important parameters affecting the film stability at the surface by preventing solubilization of the protein into the substrate. Under controlled experimental conditions, they obtain HSA compression isotherms in which the extrapolated area obtained at zero surface pressure was about 1 m^2/mg, which is a typical value for a well spread protein monolayer at the air/water interface [127]. Their compression isotherms showed an inflection point (pseudo-plateau) at surface pressures between 19 and 24 mN/m. This pseudo-plateau can be attributed to a conformational change of the HSA molecules from an unfolded configuration, in which the protein exists as "trains" with all amino acid segments located at the air-water interface, to a coiled configuration due to the folding of the amino acid chains, forming "loops" and "tails" at the interface, with the polar groups of HSA immersed in the subphase and the hydrophobic regions oriented towards the air. When the monolayer is compressed above the plateau, the "looping" of the amino acid residues continues to increase, adopting an "accordion" configuration. The optimization, morphology and durability of HSA spread films at the air-water interface have been studied by means of different techniques, namely ellipsometry, neutron reflectometry, X-ray reflectometry, and Brewster angle microscopy [132]. The authors focus on the spreading method by comparing Gibbs films with Langmuir monolayers, but they do not report compression isotherms as a function of specific area and therefore, the results are not comparable with those displayed in other works. More recently, Schöne et al. have compared literature findings of HSA and BSA monolayers, showing some small discrepancies in their compression states [47].

Additional experimental techniques such as ellipsometry are frequently used in the literature to study the thickness of HSA and BSA monolayers [133–135]. This, being a non-destructive optical technique, allows the thickness of homogeneous monolayers to be measured with great precision, but it is not able to measure or observe structural changes in the monolayer. In this sense, more powerful visualization techniques such as Atomic Force Microscopy (AFM) are used in the literature to obtain a complete topographic characterization of the monolayer [136–142]. However, the AFM does not allow an in situ study of the monolayer in the compression process. On the other hand, Brewster Angle Microscopy (BAM) can study the structure and formation of complexes in the monolayer in the compression process, being an advantage over ellipsometry and AFM. So, for a greater understanding of the lateral interactions of those molecules and to study the possible formation of complex systems with the HA at the interface, Fig. 4 shows the experimental data obtained with a Langmuir Film Balance for BSA, HSA, BSA-HA and HSA-HA monolayers at the air-water interface, in parallel with the Micro-BAM (KSV).

All recorded isotherms show a sigmoidal shape, corresponding to a typical biopolymer Langmuir monolayer, with several regimes of compression [127]. In the first regime, at the beginning of the compression process, the molecules present the behaviour of a non-interacting gas, leading to absence of surface pressure. In the second regime, molecules begin to interact showing a continuous increase of surface pressure between 2 and 17 mN/m, due to the reduction of area per molecule, in an expanded state. Finally, the third regime, with a surface pressure above 17 mN/m, corresponds to a highly interacting regime where molecules are close to each other reaching a condensed state. In the case of

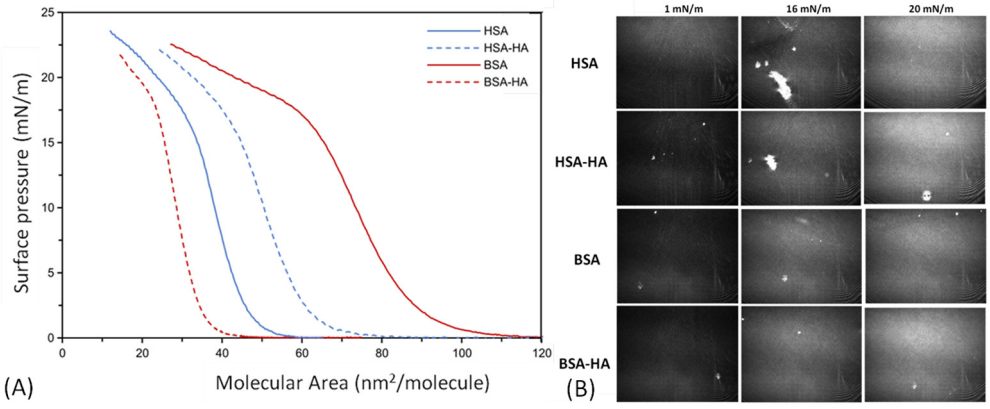

Fig. 4. (A) Compression isotherms of BSA (red solid line), HSA (blue solid line), BSA-HA (red dash line) and HSA-HA (blue dash line). BSA or HSA solutions (0.5 g/L in 0.1% propanol) were spread on subphase buffer pH 7 (NaH_2PO_4 1.13 mM) and 30 mg/L of HA, measured at 25 °C. Mean values plotted with standard deviations <2%. (B) Micro-Brewster Angle Microscopy (Micro-BAM) of the effect of HA on BSA and HSA monolayers at different compression states of the monolayer.

Table 2
Limiting Surface Area and Gibbs Elasticity of BSA, HSA, BSA-HA and HSA-HA monolayers displayed in Fig. 4A.

Sample	Limiting surface area (nm^2/molecule)	Gibbs elasticity (mN/m)
BSA	89 ± 5	52 ± 12
HSA	47 ± 3	50 ± 7
BSA-HA	34.5 ± 1.2	51 ± 16
HSA-HA	61 ± 4	50 ± 8

proteins, the collapse is not clearly attained, and the surface pressure continues increasing. The BSA isotherm depicted in Fig. 4A is similar to those previously reported in [129,140,143], which were measured at pH 7.4. According to Sanchez-González et al., the marked change of slope at about 20 mN/m is due to the α-helices, which can desorb producing a coexistence region between two different conformational regimes. The surface conformation can be studied also by means of the Gibbs elasticity, which is obtained directly from the π-A curve by the following derivative [144]: $\varepsilon_0 = -A\,(d\pi/dA)$. Fig. 4A shows that the shape of the isotherms is similar in all the measured samples, and this result is corroborated with their similar Gibbs elasticity (Table 2) in the range 50–52 mN/m. The limiting surface areas, obtained by extrapolation of the second compression regime to zero surface pressure, are displayed in Table 2 for all the systems evaluated. The value obtained for BSA agrees with that reported by Sanchez-González [129]. The monolayer appears highly expanded at this pH, suggesting the formation of a disordered protein layer possibly due to the vanishing of the existing α-helices of BSA in solution at the air-water interface as reported [47]. HSA displays a significant lower value of the limiting area compared to BSA. This agrees with the restriction on the conformational changes at the interface discussed in Section 2.2, which could result in a more compact conformation, and in agreement with the results from adsorbed films shown in Fig. 3A and B. The Gibbs elasticities of HSA and BSA spread layers are very similar (Table 2). This is related to the inter-molecular interactions which appear similar despite the different conformation of HSA and BSA.

The presence of HA induces drastic changes in both HSA and BSA monolayers, as well as produces contrary effects on both proteins. HA expands the HSA monolayer but compresses the BSA one (Fig. 4A and Table 2). Such a different interaction was certainly unexpected in view of previous results, and deserves to be explored in more detail in future works. Moreover, the interaction occurring within the monolayer appears different to that occurring at the interfacial layer in adsorbed films at the oil-water interface (Fig. 3). In adsorbed films, the Albumins are incubated with HA in bulk prior to adsorption, which possibly leads to the formation of complexes as discussed earlier. In spread films, the Albumins are deposited on a surface containing HA in bulk, which can provide a different scenario. HA seems to promote a stronger interaction with BSA at the air-water interface. BSA is more expanded and unfolded than HSA at this interface and, probably, exposes more binding sites for HA. This again correlates with the less expanded monolayer obtained for BSA + HA, i.e., there are more molecules at the interface. On the contrary, HSA is less unfolded at the interface, preserving part of its secondary and third structure. This surface conformation of HSA prevents interaction with HA, and the compression isotherm appears displaced to higher molecular areas.

Fig. 4B displays a set of images taken by Micro-BAM of the Albumins and Albumin-HA monolayers reported in Fig. 4A. In Micro-BAM images, thicker condensed phases appear brighter (more reflective), while the thinner expanded phase appears darker (less reflective) [144]. Images in Fig. 4B are very homogeneous, in agreement with a single conformational transition at the interface. Similarly, Miao et al. and Raktim et al. described images of BSA and HSA with Atomic Force Microscopy, obtaining homogenous protein films in both cases [138,145]. HSA appears slightly darker, especially at intermediate surface pressures, which agrees with the lower surface activity reported, and suggesting the formation of a thinner film. The presence of HA provides brighter images, mostly at the highest compression values, in agreement with the increased surface activity reported and suggesting the formation of a thicker film. Fig. 4B also shows brighter small spots, which possibly correspond to small surface aggregates. Those brighter areas are more abundant in HSA and HSA-HA monolayer than in the case of BSA and BSA-HA, suggesting that HSA has a higher tendency to form big aggregates than BSA. These results agree with a more stable conformation of BSA [52]. BAM investigations in the literature reveal also exceedingly small bright circular domains at low surface pressures, which grow by compression, forming groups in the condensed region caused by the packing of loop structures. The relative film thickness increases slowly under compression, until a maximum value is attained at the condensed state corresponding to the maximum packing of the protein [47]. However, complex of BSA and HSA with the HA are not observed at the interface with Micro-BAM, so the interaction with HA probably takes place just below the monolayer, within the water subphase. These results also agree with the hypothesis of Sánchez-González et al. who postulated a possible desorption of part of the α-helices at high surface pressures and consequently, with more ability to interact with HA in the subphase [143].

4. Albumins in emulsions: interactions with polysaccharides

4.1. Stability and microstructure of emulsions and nanoemulsions

The application of Albumins and polysaccharides for encapsulating lipophilic compounds in emulsions, nanoemulsions, or LLNs has already been established and can be found in many works [31,112,113]. These delivery systems carry the lipophilic ingredient, or drug, in the oil core, while the Albumin and polysaccharide build up the protective shell. The polysaccharides provide stabilization and add functionality to the delivery system [113].

Table 3 shows a summary of Albumin-polysaccharide-based emulsions and nanoemulsions found in the literature. There is some variability on the colloidal parameters values reported for the different systems. For example, droplet sizes range between 150 nm and a few micrometers, and ζ-potentials range between highly negative values and positive ones (Table 3). This variability reflects the wide range of possibilities that Albumin-polysaccharides emulsified systems offer. Covalent conjugates between Albumins and polysaccharides exemplified in Table 3 are carried out by means of the Maillard reaction as explained in Section 2.1 [66,67,123,146]. However, most of the works found, resort to a non-covalent complexation, which takes advantage mainly of electrostatic interactions (Section 2.2) [82,119,147–149]. Some of these works show modifications of BSA to produce a cationic-BSA (cBSA) that promotes an electrostatic interaction between BSA and anionic polysaccharides, such as HA [82,147,148].

When both compounds are covalently bound, the combination of the polysaccharide and the Albumin basically improves the emulsifying abilities and the emulsion stability, while the mixture of the polysaccharide and the Albumin is non-effective or is less advantageous. In this line, Kim et al. studied BSA-galactomannan conjugates produced with different protein/polysaccharide ratios [146]. The emulsifying activity of the conjugates depended on this ratio, although their performance as emulsifiers was always better than that of BSA alone. Covalent conjugation improved emulsion stability, especially at low pHs, owing to the formation of a viscoelastic layer rather than to the increase in viscosity of the emulsion continuous phase. BSA-galactomannan mixtures were also studied in this work, and they did not improve the stability of the emulsion, corroborating that just an increase in viscosity of the continuous medium was not enough to stabilize the emulsion, and highlighting the advantage provided by covalent linking in the BSA-galactomannan conjugate. Covalently linked galactomannan provided stability by means of steric hindrance, and the authors propose that

Table 3
Albumin and Albumin-polysaccharide emulsions and nanoemulsions (extended information on Fig. 6). The average droplet size and ζ -potential data correspond to the values at pH 7, unless another pH is indicated next to the given value.

Shell	Protein-polysaccharide interaction	Cargo	Size (nm)	ζ –potential (mV)	Reference
HSA	–	Paclitaxel	100–200	−26/9	[151]
HSA	–	–	165	−37.8	[31,32,112]
BSA	–	Curcumin	169	−32.2	[31]
BSA	–	Quercetin	530	–	[152]
BSA and HA	Complexation	–	147	−32.9	[113]
HSA and HA	Complexation	–	152	−42.9	Fig. 6
HA	–	–	176	−40.1	Fig. 6
Cationic BSA and HA	Complexation	DiD, Coumarin-6 or Celastrol	300	−34.2	[82]
Cationic BSA and HA	Complexation	Paclitaxel	324–531	−40	[147]
Cationic BSA and HA	Complexation	All-trans-retinoic acid	180	32.1	[148]
BSA and sugar beet pectin	Conjugation/Maillard reaction	–	3–3.5 10^3	~ −30/−35	[67]
BSA and sugar beet pectin	Complexation	–	~ 200	–	[80]
BSA and Dextran	Conjugation/Maillard reaction	Curcumin	158–255	~ −3	[66]
BSA and Arabic gum	Complexation	β-carotene	221 (pH 4)	−30 (pH 4)	[119]
BSA and Fucoidan	Complexation	–	~ 650	~ −40/−70	[122]
BSA and Fucoidan	Conjugation/Maillard reaction	–	–	–	[123]
BSA and Chondroitin Sulphate	non-covalent complexation	–	450–510 (at pH 3)	~ −42 (at pH 3)	[149]
BSA and Galactomannan	Conjugation/Maillard reaction	–	1140–1650	–	[146]

this mechanism preserves the stability of the system even in environmental conditions were the denatured protein would drive an emulsion destabilization [146]. The comparison of BSA-fucoidan mixtures and conjugates with BSA as emulsifiers [123] comprises another example of the different performance provided by protein and polysaccharides covalent conjugates and mixtures. Fucoidan (non-surface active), did not improve the emulsifying activity index of BSA in a simple mixed system. Conversely, covalent linking of BSA and fucoidan significantly improved the emulsifying activity of the conjugate and promoted interfacial adsorption, as discussed in Section 3.2. Fucoidan also provided higher emulsion stability, avoiding droplets coalescence owing to the steric hindrance set by the branched polysaccharide. This effect was more remarkable in covalent conjugates-stabilized emulsions and reflected in changes in the interfacial tension. However, a slightly improvement of nanoemulsion stability was also observed for BSA-fucoidan complexes, indicating that, in any case, the addition of the polysaccharide was advantageous [123]. In another work, Kim et al. also studied non-covalently linked BSA-fucoidan mixture as emulsifier, and they found out that this non-surface active and strongly negative polysaccharide improved emulsion stability owing to the negative charge provided to the interface, rather than from other effects on interfacial activity as discussed in Section 3.3 [122]. Another example of a better performance of the covalent conjugate, compared with the mixture, is that provided by Chen et al. They reported an improvement on the emulsifying properties and a reduction of the particle size with BSA-sugar beet pectin conjugates and mixtures. However, the stability of the emulsion depended on the type of conjugation. Conjugation by Maillard reaction or laccase enzymatic action, provided different resistance to destabilizing agents such as temperature and pH. These authors propose that the instability of BSA-sugar beet pectin complexes-stabilized emulsions at low pH was owed to the breakdown of the electrostatic interaction between BSA and sugar beet pectin at these pH values [67]. This observation is a relevant issue to consider, since the stability of an emulsion can be tuned by weakening the interaction between non-covalent linked Albumin-polysaccharide mixtures.

4.2. Encapsulation of lipophilic compounds

The control of the loading capacity and release kinetics of drugs encapsulated in emulsions and nanoemulsions is important to achieve an optimal performance. In general, the polysaccharide coating provides an advantage, which can be related to a higher stability of the colloidal system, but also with the loaded-drug protection and release. For example, nanoemulsions prepared with BSA and dextran, covalently linked via Maillard reaction, protect loaded curcumin from degradation and improve curcumin absorption at intestinal level in mice, providing an increased curcumin bioavailability [66]. Also, BSA-Arabic gum coated nanoemulsions are more effective than simple BSA or Arabic gum emulsions towards the protection of loaded β-carotene over time and against different pH, temperature, and radiation conditions. In this case, the BSA-polysaccharide interaction was electrostatic, and both compounds were added together during the emulsification process [119]. On the other hand, the ATRA (all trans retinoic acid) release study carried out by Li et al. revealed no differences between HA-cBSA and BSA nanoemulsions in retaining the compound after 28 days. However, the external HA layer, formed by adsorption of the negatively-charged HA to the positive surface of cBSA-preformed nanoemulsions, furnished the system with an effective targeting against tumour cells assayed in vitro and in vivo [148]. In the case of HA coated cBSA nanoemulsion loaded with paclitaxel, a commonly used chemotherapeutic in clinic, the drug was released faster from these HA coated nanoemulsions [147]. Co-encapsulation of several compounds inside emulsions or nanoemulsions is also possible. Hu et al. produced HA-coated cBSA nanoemulsions with celastrol (hydrophobic) and 1-methyltryptophan (hydrophilic). The release of both compounds was different owing to their different nature. At pH 7, HA-cBSA nanoemulsions showed a slower release than cBSA nanoemulsions, while at pH 4, the former provided a faster release. This release kinetics is considered as an advantage, since the acidic tumour microenvironment would promote the drugs release from the nanoemulsions on the target site [82].

4.3. Targeted delivery: hyaluronic acid

The use of HA for developing delivery systems in the form of nanoparticles is very broad as already stated [38,82,89,147]. However, in most of those works, researchers are mainly interested in the biological interaction between HA-decorated nanoparticles and the target cells, rather than in the physicochemical and interfacial characterization of the nanoparticles, which is usually not very exhaustive [36,82,148]. Therefore, little is known about the interactions between BSA or HSA and HA. The use of BSA and HSA as emulsifiers for the stabilization of nanoemulsions or LLNs, with an olive oil core, has gained a lot of attention lately [31,32,54,112]. LLNs can be synthesized by a solvent-displacement method and have demonstrated a high encapsulation efficiency for hydrophobic drugs, with values over 90% for curcumin, for example [32,54]. These systems included protein cross-linker

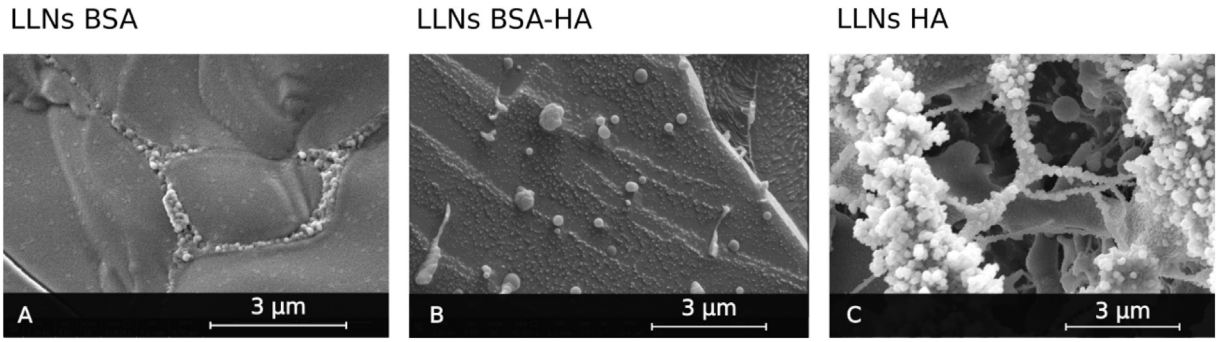

Fig. 5. Cryo-ESEM images of (A) BSA, (B) BSA-HA and (C) HA LLNs. Images taken with FEG-ESEM QUEMSCAN 650F provided with a cryo-preparation system. The sublimation phase was carried out at −90 °C from 2 to 5 min. Then, sample was cut and subjected to three cycles (90s per cycle) of metallization with argon and platinum. The whole process was performed under vacuum conditions and at −90 °C.

glutaraldehyde (GAD) to provide LLNs with a stronger shell and produce more stable particles [112]. The crosslinking process with GAD has been proved to slow down the drug release kinetics from LLNs, while it does not display any toxic effect on MCF-7 breast cancer cellular line [31]. The different morphology of BSA, BSA-HA and HA LLNs has been characterized by cryo-ESEM (cryo-Environmental Scanning Electron Microscopy) and is displayed in Fig. 5. BSA-LLNs adopt a spherical shape and a monodisperse distribution (Fig. 5A). Fig. 5B shows BSA-HA LLNs, in which individual LLNs coexist with small aggregates of several units, and present spherical shape and similar size. Fig. 5C shows HA LLNs with a clearly different organization compared to BSA and BSA-HA LLNs. HA LLNs appear as individual particles but attached to one to another, forming fibrillar structures. This fibrillar organization corresponds to a gel-like organization. In aqueous solution, HA forms a viscous gel owing to intermolecular hydrogen bonds (between the pI of the HA, around pH 2.5, and pH 12) [150]. The gel-forming ability of HA is possibly responsible for the formation of this structure observed with cryo-ESEM, given that HA does not have interfacial activity [124]. Thus, olive oil droplets are entrapped within the HA gel mesh, remaining stable for a short period of time.

Table 3 shows the size distribution of LLNs prepared with HSA and BSA in the presence and absence of HA [31,32,112]. In all cases, LLNs showed a monodisperse distribution, with PDI values between 0.04 and 0.08 (data not shown). Table 3 also shows the characteristics of LLNs stabilized solely by HA. It is noteworthy that HA provides stable LLNs despite its lack of interfacial activity. As stated before, the stabilization of the oil droplets can be related with the high bulk viscosity provided by HA [35] and its gel formation ability (Fig. 5C). However, these LLNs stabilized only with HA displayed low colloidal stability (one week), as compared to the rest of LLNs (over months).

Fig. 6 provides the ζ-potential of LLNs formed by BSA and HSA, and the effect of HA on the microstructure of LLNs. HSA and BSA LLNs display a similar ζ-potential in all the range of pH assayed. Moreover, they present similar isoelectric points (pI≈5), i.e., the pH at which LLNs have no net charge. This value is close to the pI of both proteins, being pH 4.9 for BSA [54] and pH 4.4 for HSA [32]. LLNs show positive charge at pH values below the pI, due to the reduction of the negative charge of carboxylic groups that cannot compensate the positive charge of amino groups, protonated at those low pH values. These groups are titrated at higher pHs, where they release their protons. Thus, as pH rises, carboxylic groups turn to be negatively charged while amino groups offer

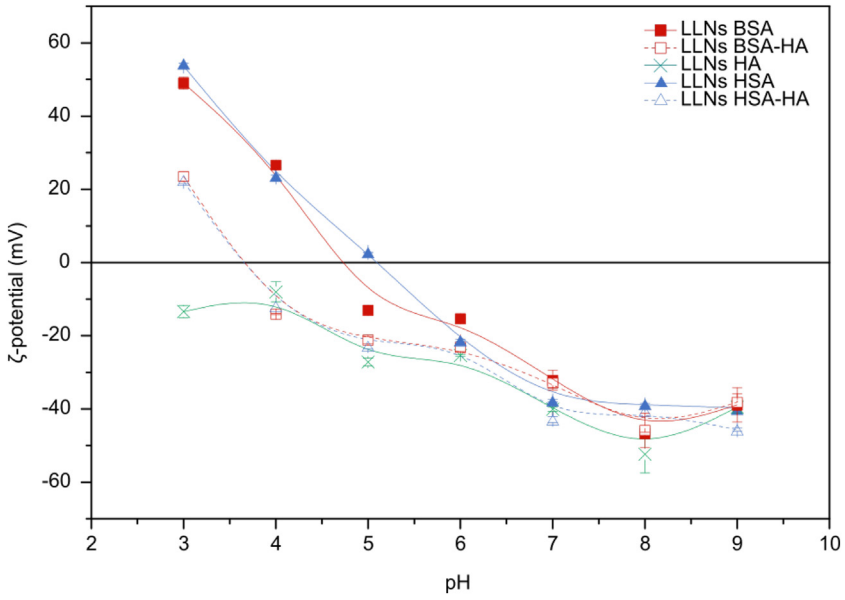

Fig. 6. ζ-potential of BSA (red solid squares), BSA-HA (red hollow squares), HSA (blue solid triangles), HSA-HA (blue hollow triangles) and HA (green crosses) LLNs at different pH values. LLNs were diluted (1:100) and stabilized for 30 min in low ionic strength buffer: acetate buffer at pH 3–5, phosphate buffer pH 6–7 or borate buffer pH 8–11. Colloidal characteristics of LLNs were determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS system (Malvern Instruments, UK). The ζ-potential was calculated according to the Smoluchowsky theory. All measurements were performed in triplicate at 25 °C, and the results plotted are mean value ± standard deviation.

no charge, hence leading to the negative ζ-potential observed on LLNs at more basic pHs. The net negative charge present at the surface of LLNs at pH 7 allows stabilizing the nanoparticles over time, owing to electrostatic forces. The inclusion of polysaccharides on the shell composition leads in most cases to modifications in the colloidal system surface charge, as can be observed in Fig. 6 for LLNs. It is clear that the reduction of the ζ-potential to more negative ζ-potential values on the HA-coated LLNs is provoked by the presence of HA on the surface of LLNs. Moreover, this phenomenon seems to be independent of the synthesis process, i.e., the inclusion of the Albumin and the polysaccharide at the same time on the process, as mixtures or as conjugates, or the coating with the polysaccharide of pre-formed Albumin nanoemulsions [67,119,122,149]. In some works, it has been reported that the modification of ζ-potential values depends on the proportion of polysaccharide [122]

HA LLNs present negative charge at all range of pHs studied (Fig. 6). HA is a long-chain heteropolysaccharide composed of two alternating monosaccharides, *N*-acetylglucosamine and D-glucuronic acid, linked via β-1,3-glycosidic bonds. This configuration leads to a rigid conformation stabilized by hydrogen bonds, where hydrophobic regions are alternated with polar ones [43]. Among those polar regions we found carboxylic groups, which are the responsible of the low pI of HA (pH 2.5) and the negative charge of HA at physiological pH [43,150]. ζ-potential of HA LLNs agrees with the low pI of this molecule, and pH changes are not enough to titrate all carboxylic groups from HA, hence ζ-potential of HA LLNs remains negative along the whole pH range assayed.

There are different works where HA-coated nanoemulsions have been synthesized and characterized [82,121,153–156]. However, many of those formulations include surfactants or HA modifications, in order to achieve the emulsification and stability of the colloidal system [154–156]. For instance, Nars modified a previously optimized synthesis protocol by including HA on the aqueous phase, together with other surfactants (Tween 80 and Cremophor RH 40), to obtain curcumin and resveratrol loaded nanoemulsions as carriers for transnasal brain delivery for neurodegenerative diseases treatment [156]. Another approach is the modification of HA to improve its interfacial properties. In this line, Kong and Park studied the stability of nanoemulsions coated with a modified HA [154]. They increased the amphiphilic character of HA by linking to its carboxylic groups a hydrophobic molecule, glycerol α-monostearate. The increase of the hydrophobic character of modified HA made easier the self-assembly of the molecules. However, they also included low amounts of surfactant on the formulation, achieving sizes on the range of 40–70 nm [154]. A different strategy to include HA in emulsified systems is that of Lui et al., who covalently conjugated β-lactoferrin and HA to stabilize sesamol emulsions [121]. Another possibility to include HA in emulsified delivery systems is the modification of BSA reported in several works [82,147,148]. In these works, the authors modified the BSA to produce a cBSA and produced cBSA nanoemulsions which were later incubated with HA to achieve the surface coating. Hu et al. reported that the BSA modification that they performed increased the isoelectric point of cBSA to pH 9.98, allowing the electrostatic interaction between cBSA-coated NPs and HA [82]. The coating of the cBSA nanoemulsions with HA lead to the reduction of the ζ-potential of the droplets [82,147], in agreement with results from Fig. 6. The interaction occurring between HA and Albumin in Fig. 6, occurs with no conjugation between HA and Albumin nor modification of the protein, which is a great achievement with respect to previous literature works. Concerning the use of HSA or BSA, so far, the physical characterization of LLNs, in terms of droplet size distribution and ζ-potential, does not provide significant differences concerning the interaction of HA with BSA and HSA.

5. Conclusions and future perspectives

BSA and HSA are blood-transport proteins which bind different kinds of molecules. Despite their similarity in structure and function, there are reported references on different affinity of BSA and HSA for the same ligand. Such differences are generally ascribed to variations on their amino acid composition. Here, some of the parameters which could explain the differences encountered between HSA and BSA in their application for drug delivery systems are identified. The interaction with polysaccharides is especially highlighted owing to their common use in the formulations. A detailed comparison of the molecular structure of BSA and HSA is provided focusing on how it can impact their interactions with polysaccharides. A detailed description of the various type of interactions encountered is given. This sets the basis to develop the different interfacial activity and emulsifying capacity as regards encapsulation of lipophilic compounds. The current knowledge on conformational changes upon adsorption of HSA and BSA onto different interfaces is then reviewed and how this relates with their interfacial activity. The interfacial properties of Albumins reflect the limited conformational change undergone by HSA as compared to BSA at the oil-water interface, which can have implications in their functionality in LLNs. Concerning the interaction with polysaccharides, the type of bonding impacts the interfacial activity as does the hydrophobicity of the complex. However, the response of the interfacial activity still offers open questions and challenges, including the correlation with emulsion stability. In general, an improved emulsifying capacity can be linked to a higher surface activity of Albumins, although the correlation with stability is more variable. In fact, no simple correlation is found between interfacial magnitudes and emulsifying capacity of Albumins and polysaccharides, owing to the strong effect in bulk viscosity and variable complexation phenomena involved. Different kinds of interactions taking place at different levels should be carefully analysed to obtain a complete picture of the interaction and the resulting functionality. Observed trends have physicochemical explanations based on interfacial conformations, and can be used as a solid basis for the development of future detailed models on different Albumins. BSA is a valid model protein used for fundamental studies, but HSA is clinically more relevant, and the slight differences should be considered carefully. The differences highlighted along this work for the design of LLNs as drug delivery systems can be a helpful guide to develop reliable systems with realistic application in biomedicine.

Declaration of Competing Interest

None.

Acknowledgements

This work has been funded by the following projects, which are gratefully acknowledged: MAT2017-82182-R and RTI2018-101309-B-C21 (Ministerio de Ciencia e Innovación). The authors also acknowledge "Mancomunidad de los Pueblos de la Alpujarra Granadina" for the funds raised and supplied for this research.

Author contributions

Aixa María Aguilera-Garrido: Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing **Teresa del Castillo-Santaella**: Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing **Yan Yang**: Investigation, Writing - review & editing **Francisco Galisteo-González**: Conceptualization, Funding acquisition, Methodology, Project administration, Writing - review & editing **María José Gálvez-Ruiz**: Conceptualization,

Funding acquisition, Project leader, Writing – review & editing **José Antonio Molina-Bolívar**: Conceptualization, Methodology, Writing – review & editing **Juan Antonio Holgado**: Methodology **Miguel Ángel Cabrerizo-Vílchez**: Conceptualization, methodology, Writing – review **Julia Maldonado-Valderrama**: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Writing - original draft, Writing - review & editing.

References

[1] Knudsen Sand KM, Bern M, Nilsen J, Noordzij HT, Sandlie I, Andersen JT. Unraveling the Interaction between FcRn and Albumin: Opportunities for Design of Albumin-Based Therapeutics, 682; 2015. https://doi.org/10.3389/fimmu.2014.00682.

[2] Karimi M, Bahrami S, Ravari SB, Zangabad PS, Mirshekari H, Bozorgomid M, et al. Albumin nanostructures as advanced drug delivery systems. Expert Opin Drug Deliv. 2017;176:139–48. https://doi.org/10.1016/j.physbeh.2017.03.040.

[3] Lee P, Review Wu X. Modifications of human serum albumin and their binding effect. Curr Pharm Des. 2015;21:1862–5. https://doi.org/10.2174/1381612821666150302115025.

[4] Schmidt EGW, Hvam ML, Antunes F, Cameron J, Viuff D, Andersen B, et al. Direct demonstration of a neonatal Fc receptor (FcRn)-driven endosomal sorting pathway for cellular recycling of albumin. J Biol Chem. 2017;292:13312–22. https://doi.org/10.1074/jbc.M117.794248.

[5] Furumoto K, Yokoe JI, Kichi Ogawara, Amano S, Takaguchi M, Higaki K, et al. Effect of coupling of albumin onto surface of PEG liposome on its in vivo disposition. Int J Pharm. 2007;329:110–6. https://doi.org/10.1016/j.ijpharm.2006.08.026.

[6] Ogawara KI, Furumoto K, Nagayama S, Minato K, Higaki K, Kai T, et al. Pre-coating with serum albumin reduces receptor-mediated hepatic disposition of polystyrene nanosphere: Implications for rational design of nanoparticles. J Control Release. 2004;100:451–5. https://doi.org/10.1016/j.jconrel.2004.07.028.

[7] Thiele L, Diederichs JE, Reszka R, Merkle HP, Walter E. Competitive adsorption of serum proteins at microparticles affects phagocytosis by dendritic cells. Biomaterials. 2003;24:1409–18. https://doi.org/10.1016/S0142-9612(02)00525-2.

[8] Larsen TM, Mandrup OA, Schelde KK, Luo Y, Sørensen KD, Dagnæs-Hansen F, et al. FcRn overexpression in human cancer drives albumin recycling and cell growth; a mechanistic basis for exploitation in targeted albumin-drug designs. J Control Release. 2020;322:53–63. https://doi.org/10.1016/j.jconrel.2020.03.004.

[9] Fanali G, Di Masi A, Trezza V, Marino M, Fasano M, Ascenzi P. Human serum albumin: from bench to bedside. Mol Asp Med. 2012;33:209–90. https://doi.org/10.1016/j.mam.2011.12.002.

[10] Gupta D, Lis CG. Pretreatment serum albumin as a predictor of cancer survival: a systematic review of the epidemiological literature. Nutr J. 2010;9. https://doi.org/10.1186/1475-2891-9-69.

[11] Koga M, Kasayama S. Clinical impact of glycated albumin as another glycemic control marker. Endocr J. 2010;57:751–62. https://doi.org/10.1507/endocrj.k10e-138.

[12] Sbarouni E, Georgiadou P, Voudris V. Ischemia modified albumin changes - Review and clinical implications. Clin Chem Lab Med. 2011;49:177–84. https://doi.org/10.1515/CCLM.2011.037.

[13] Tullis JL. Albumin: 1. Background and use. JAMA. 1977;237:355–60. https://doi.org/10.1001/jama.1977.03270310039005.

[14] Tullis JL. Albumin: 2. Guidelines for clinical use. JAMA. 1977;237:460–3. https://doi.org/10.1001/jama.1977.03270320038019.

[15] Alexander MR, Ambre JJ, Liskow BI, Trost DC. Therapeutic Use of Albumin. JAMA. 1979;241:2527–9. https://doi.org/10.1001/jama.1979.03290490033021.

[16] Erstad BL, Gales BJ, Rappaport WD. The use of albumin in clinical practice. Arch Intern Med. 1991;151:901–11. https://doi.org/10.1001/archinte.1991.00400050051011.

[17] Hastings GE, Wolf PG. The therapeutic use of albumin. Arch Fam Med. 1992;1:281–7. https://doi.org/10.1001/archfami.1.2.281.

[18] Haynes GR, Navickis RJ, Wilkes MM. Albumin administration: what is the evidence of clinical benefit? A systematic review of randomized controlled trials. Eur J Anaesthesiol. 2003;20:771–93. https://doi.org/10.1017/S0265021503001273.

[19] Huang J, Liu L, Yao J. Electrospinning of *Bombyx mori* silk fibroin nanofiber mats reinforced by cellulose nanowhiskers. Fibers Polym. 2011;12:1002–6. https://doi.org/10.1007/s12221-011-1002-7.

[20] Shin EJ, Singh D, Choi SM, Zo SM, Lee YH, Han SS. Functionalizing cellulose scaffold prepared by ionic liquid with bovine serum albumin for biomedical application. Fibers Polym. 2013;14:1965–9. https://doi.org/10.1007/s12221-013-1965-7.

[21] Bertucci C, Pistolozzi M, de Simone A. Circular dichroism in drug discovery and development: an abridged review. Anal Bioanal Chem. 2010;398:155–66. https://doi.org/10.1007/s00216-010-3959-2.

[22] Furukawa M, Tanaka R, Chuang VTG, Ishima Y, Taguchi K, Watanabe H, et al. Human serum albumin-thioredoxin fusion protein with long blood retention property is effective in suppressing lung injury. J Control Release. 2011;154:189–95. https://doi.org/10.1016/j.jconrel.2011.05.013.

[23] Komatsu T, Qu X, Ihara H, Fujihara M, Azuma H, Ikeda H. Virus trap in human serum albumin nanotube. J Am Chem Soc. 2011;133:3246–8. https://doi.org/10.1021/ja1096122.

[24] Kumar S, Dilbaghi N, Rani R, Bhanjana G, Umar A. Novel approaches for enhancement of drug bioavailability. Rev Adv Sci Eng. 2013;2:133–54. https://doi.org/10.1166/rase.2013.1038.

[25] Wu M, Zhong C, Deng Y, Zhang Q, Zhang X, Zhao X. Resveratrol loaded glycyrrhizic acid-conjugated human serum albumin nanoparticles for tail vein injection II: pharmacokinetics, tissue distribution and bioavailability. Drug Deliv. 2020;27:81–90. https://doi.org/10.1080/10717544.2019.1704944.

[26] Elsadek B, Kratz F. Impact of albumin on drug delivery - new applications on the horizon. J Control Release. 2012;157:4–28. https://doi.org/10.1016/j.jconrel.2011.09.069.

[27] Tang QS, Chen DZ, Xue WQ, Xiang JY, Gong YC, Zhang L, et al. Preparation and biodistribution of 188Re-labeled folate conjugated human serum albumin magnetic cisplatin nanoparticles (188Re-folate-CDDP/HSA MNPs) in vivo. Int J Nanomedicine. 2011;6:3077–85. https://doi.org/10.2147/ijn.s24322.

[28] Araiza-Calahorra A, Akhtar M, Sarkar A. Recent advances in emulsion-based delivery approaches for curcumin: from encapsulation to bioaccessibility. Trends Food Sci Technol. 2018;71:155–69. https://doi.org/10.1016/j.tifs.2017.11.009.

[29] McClements DJ, Decker EA, Weiss J. Emulsion-based delivery systems for lipophilic bioactive components. J Food Sci. 2007;72:109–24. https://doi.org/10.1111/j.1750-3841.2007.00507.x.

[30] Piktel E, Niemirowicz K, Wątek M, Wollny T, Deptuła P, Bucki R. Recent insights in nanotechnology - based drugs and formulations designed for effective anti - cancer therapy. J Nanobiotechnol. 2016:1–23. https://doi.org/10.1186/s12951-016-0193-x.

[31] Galisteo-González F, Molina-Bolívar JA, Navarro SA, Boulaiz H, Aguilera-Garrido A, Ramírez A, et al. Albumin-covered lipid nanocapsules exhibit enhanced uptake performance by breast-tumor cells. Colloids Surf B: Biointerfaces. 2018;165:103–10. https://doi.org/10.1016/j.colsurfb.2018.02.024.

[32] Molina-Bolívar JA, Galisteo-González F. Olive-oil nanocapsules stabilized by HSA : influence of processing variables on particle properties. J Nanopart Res. 2015;17. https://doi.org/10.1007/s11051-015-3192-1.

[33] Mora-Huertas CE, Fessi H, Elaissari A. Polymer-based nanocapsules for drug delivery. Int J Pharm. 2010;385:113–42. https://doi.org/10.1016/j.ijpharm.2009.10.018.

[34] Lam RSH, Nickerson MT. Food proteins: a review on their emulsifying properties using a structure-function approach. Food Chem. 2013;141:975–84. https://doi.org/10.1016/j.foodchem.2013.04.038.

[35] Bouyer E, Mekhloufi G, Rosilio V, Grossiord JL, Agnely F. Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: alternatives to synthetic surfactants in the pharmaceutical field? Int J Pharm. 2012;436:359–78. https://doi.org/10.1016/j.ijpharm.2012.06.052.

[36] Chen Z, Chen J, Wu L, Li W, Chen J, Cheng H, et al. Hyaluronic acid-coated bovine serum albumin nanoparticles loaded with brucine as selective nanovectors for intra-articular injection. Int J Nanomedicine. 2013;8:3843–53. https://doi.org/10.2147/IJN.S50721.

[37] Paşcalău V, Bogdan C, Pall E, Matroş L, Pandrea SL, Suciu M, et al. Development of BSA gel/Pectin/Chitosan polyelectrolyte complex microcapsules for Berberine delivery and evaluation of their inhibitory effect on Cutibacterium acnes. React Funct Polym. 2020;147:104457. https://doi.org/10.1016/j.reactfunctpolym.2019.104457.

[38] Shen Y, Li WHA. HSA co-modified erlotinib-albumin nanoparticles for lung cancer treatment. Drug Des Devel Ther. 2018;12:2285–92. https://doi.org/10.2147/DDDT.S169734.

[39] Singla R, Soni S, Padwad YS, Acharya A, Yadav SK. Sustained delivery of BSA/HSA from biocompatible plant cellulose nanocrystals for in vitro cholesterol release from endothelial cells. Int J Biol Macromol. 2017;104:748–57. https://doi.org/10.1016/j.ijbiomac.2017.06.068.

[40] Černocká H, Izadi N, Ostatná V, Strmečki S. BSA-polysaccharide interactions at negatively charged electrode surface. Effects of current density. Electroanalysis. 2019;31:2007–11. https://doi.org/10.1002/elan.201900231.

[41] Lei X, Zhu Y, Wang X, Zhao P, Liu P, Zhang Q, et al. Wine polysaccharides modulating astringency through the interference on interaction of flavan-3-ols and BSA in model wine. Int J Biol Macromol. 2019;139:896–903. https://doi.org/10.1016/j.ijbiomac.2019.08.050.

[42] Peng H, Chen S, Luo M, Ning F, Zhu X, Xiong H. Preparation and self-assembly mechanism of bovine serum albumin-citrus peel pectin conjugated hydrogel: A potential delivery system for Vitamin C. J Agric Food Chem. 2016;64:7377–84. https://doi.org/10.1021/acs.jafc.6b02966.

[43] Fallacara A, Baldini E, Manfredini S, Vertuani S. Hyaluronic acid in the third millennium. Polymers (Basel). 2018;10:701. https://doi.org/10.3390/polym10070701.

[44] Hernández-Camarero P, Jiménez G, López-Ruiz E, Barungia A, Marchal JA, Perán M. Revisiting the dynamic cancer stem cell model: importance of tumour edges. Crit Rev Oncol Hematol. 2018;131:35–45. https://doi.org/10.1016/j.critrevonc.2018.08.004.

[45] Fakhari A, Berkland C. Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler, and in osteoarthritis treatment. Acta Biomater. 2013;9:7081–92. https://doi.org/10.1016/j.actbio.2013.03.005.

[46] Chruszcz M, Mikolajczak K, Mank N, Majorek KA, Porebski PJ, Minor W. Serum albumins - unusual allergens. Biochim Biophys Acta, Gen Subj. 1830;2013:5375–81. https://doi.org/10.1016/j.bbagen.2013.06.016.

[47] Schöne AC, Roch T, Schulz B, Lendlein A. Evaluating polymeric biomaterial-environment interfaces by Langmuir monolayer techniques. J R Soc Interface. 2017;14. https://doi.org/10.1098/rsif.2016.1028.

[48] Brown JR. Serum albumin: amino acid sequence. In: VM Rosenoer, Oratz M, Rothschild Function and Uses MABT-AS, editors. Albumin Struct. Funct. Uses. Pergamon; 1977. p. 27–52b. https://doi.org/10.1016/B978-0-08-019603-9.50009-0.

[49] Meloun B, Morávek L, Kostka V. Complete amino acid sequence of human serum albumin. FEBS Lett. 1975;175:349–55. https://doi.org/10.1016/0014-5793(75)80242-0.

[50] Bourassa P, Hasni I, Tajmir-Riahi HA. Folic acid complexes with human and bovine serum albumins. Food Chem. 2011;129:1148–55. https://doi.org/10.1016/j.foodchem.2011.05.094.
[51] Lakshmanan M, Parthasarathi R, Dhathathreyan A. Do properties of bovine serum albumin at fluid/electrolyte interface follow the Hofmeister series?-An analysis using Langmuir and Langmuir-Blodgett films. Biochim Biophys Acta, Proteins Proteomics. 2006;1764:1767–74. https://doi.org/10.1016/j.bbapap.2006.09.010.
[52] Ma GJ, Ferhan AR, Sut TN, Jackman JA, Cho NJ. Understanding how natural sequence variation in serum albumin proteins affects conformational stability and protein adsorption. Colloids Surf B: Biointerfaces. 2020;194:111194. https://doi.org/10.1016/j.colsurfb.2020.111194.
[53] Peters T. Serum albumin. Adv Protein Chem. 1985;37:161–245. https://doi.org/10.1016/S0065-3233(08)60065-0.
[54] Galisteo-González F, Molina-Bolívar JA. Systematic study on the preparation of BSA nanoparticles. Colloids Surf B: Biointerfaces. 2014;123:286–92. https://doi.org/10.1016/j.colsurfb.2014.09.028.
[55] Cheng ZJ, Zhao HM, Xu QY, Liu R. Investigation of the interaction between indigotin and two serum albumins by spectroscopic approaches. J Pharm Anal. 2013;3:257–69. https://doi.org/10.1016/j.jpha.2013.01.004.
[56] Fan H, Ji N, Zhao M, Xiong L, Sun Q. Interaction of bovine serum albumin with starch nanoparticles prepared by TEMPO-mediated oxidation. Int J Biol Macromol. 2015;78:333–8. https://doi.org/10.1016/j.ijbiomac.2015.04.028.
[57] Li G, Huang J, Chen T, Wang X, Zhang H, Chen Q. Insight into the interaction between chitosan and bovine serum albumin. Carbohydr Polym. 2017;176:75–82. https://doi.org/10.1016/j.carbpol.2017.08.068.
[58] Jorgensen L, Van De Weert M, Vermehren C, Bjerregaard S, Frokjaer S. Probing structural changes of proteins incorporated into water-in-oil emulsions. J Pharm Sci. 2004;93:1847–59. https://doi.org/10.1002/jps.20097.
[59] Molina-Bolívar JA, Galisteo-González F, Carnero Ruiz C, Medina-Ó Donnell M, Parra A. Spectroscopic investigation on the interaction of maslinic acid with bovine serum albumin. J Lumin. 2014. https://doi.org/10.1016/j.jlumin.2014.08.011.
[60] Molina-Bolívar JA, Galisteo-González F, Carnero Ruiz C, Medina O'Donnell M, Parra A. Interaction between the anti-cancer drug diacetyl maslinic acid and bovine serum albumin: a biophysical study. J Mol Liq. 2015. https://doi.org/10.1016/j.molliq.2015.04.050.
[61] Molina-Bolívar JA, Galisteo-González F, Ruiz CC, Medina O'Donnell M, Martínez A, Parra A. Maslinic acid conjugate with 7-amino-4-methylcoumarin as probe to monitor the temperature dependent conformational changes of human serum albumin by FRET. Spectrochim Acta - Part A Mol Biomol Spectrosc. 2019. https://doi.org/10.1016/j.saa.2019.02.014.
[62] Molina-Bolívar JA, Ruiz CC, Galisteo-González F, Medina O'Donnell M, Parra A. Simultaneous presence of dynamic and sphere action component in the fluorescence quenching of human serum albumin by diphthaloylmaslinic acid. J Lumin. 2016. https://doi.org/10.1016/j.jlumin.2016.06.005.
[63] Molina-Bolívar JA, Ruiz CC, Galisteo-González F, O'Donnell MM, Parra A. Energetics of albumin-disuccinylmaslinic acid binding determined by fluorescence spectroscopy. Fluid Phase Equilib. 2015;400:43–52. https://doi.org/10.1016/j.fluid.2015.05.011.
[64] Jian W, Wang L, Wu L, Sun YM. Physicochemical properties of bovine serum albumin-glucose and bovine serum albumin-mannose conjugates prepared by pulsed electric fields treatment. Molecules. 2018;23:570. https://doi.org/10.3390/molecules23030570.
[65] Jung SH, Choi SJ, Kim HJ, Moon TW. Molecular characteristics of bovine serum albumin-dextran conjugates. Biosci Biotechnol Biochem. 2006;70:2064–70. https://doi.org/10.1271/bbb.60026.
[66] Wang C, Liu Z, Xu G, Yin B, Yao P. BSA-dextran emulsion for protection and oral delivery of curcumin. Food Hydrocoll. 2016;61:11–9. https://doi.org/10.1016/j.foodhyd.2016.04.037.
[67] Chen H, Ji A, Qiu S, Liu Y, Zhu Q, Yin L. Covalent conjugation of bovine serum album and sugar beet pectin through Maillard reaction/laccase catalysis to improve the emulsifying properties. Food Hydrocoll. 2018;76:173–83. https://doi.org/10.1016/j.foodhyd.2016.12.004.
[68] Claus H. Laccases: Structure, reactions, distribution. Micron. 2004;35:93–6. https://doi.org/10.1016/j.micron.2003.10.029.
[69] Cammarata CR, Hughes ME, Ofner CM. Carbodiimide induced cross-linking, ligand addition, and degradation in gelatin. Mol Pharm. 2015;12:783–93. https://doi.org/10.1021/mp5006118.
[70] AFM Verheul, Versteeg AA, De Reuver MJ, Jansze M, Snippe H. Modulation of the immune response to pneumococcal type 14 capsular polysaccharide-protein conjugates by the adjuvant Quil A depends on the properties of the conjugates. Infect Immun. 1989;57:1078–83. https://doi.org/10.1128/iai.57.4.1078-1083.1989.
[71] Clausen M, Madsen R. Synthesis of oligogalacturonates conjugated to BSA. Carbohydr Res. 2004;339:2159–69. https://doi.org/10.1016/j.carres.2004.06.012.
[72] Afanasyev OI, Kuchuk E, Usanov DL, Chusov D. Reductive amination in the synthesis of pharmaceuticals. Chem Rev. 2019;119:11857–911. https://doi.org/10.1021/acs.chemrev.9b00383.
[73] Dickinson E. Interfacial structure and stability of food emulsions as affected by protein–polysaccharide interactions. Soft Matter. 2008;4:932–42. https://doi.org/10.1039/B718319D.
[74] de Kruif CG, Weinbreck F, de Vries R. Complex coacervation of proteins and anionic polysaccharides. Curr Opin Colloid Interface Sci. 2004;9:340–9. https://doi.org/10.1016/j.cocis.2004.09.006.
[75] Zhang Q, Ni Y, Kokot S. Competitive interactions between glucose and lactose with BSA: Which sugar is better for children? Analyst. 2016;141:2218–27. https://doi.org/10.1039/c5an02420j.
[76] Li X, Fang Y, Al-Assaf S, Phillips GO, Yao X, Zhang Y, et al. Complexation of bovine serum albumin and sugar beet pectin: Structural transitions and phase diagram. Langmuir. 2012;28:10164–76. https://doi.org/10.1021/la302063u.
[77] Donato L, Garnier C, Novales B, Durand S, Doublier JL. Heat-induced gelation of bovine serum albumin/low-methoxyl pectin systems and the effect of calcium ions. Biomacromolecules. 2005;6:374–85. https://doi.org/10.1021/bm040061f.
[78] Nnyigide OS, Hyun K. The rheological properties and gelation kinetics of corn starch/bovine serum albumin blend. Korea Aust Rheol J. 2020;32:71–8. https://doi.org/10.1007/s13367-020-0008-3.
[79] Wang X, Ruengruglikit C, Wang YW, Huang Q. Interfacial interactions of pectin with bovine serum albumin studied by quartz crystal microbalance with dissipation monitoring: effect of ionic strength. J Agric Food Chem. 2007;55:10425–31. https://doi.org/10.1021/jf071714x.
[80] Li X, Fang Y, Al-Assaf S, Phillips GO, Jiang F. Complexation of bovine serum albumin and sugar beet pectin: Stabilising oil-in-water emulsions. J Colloid Interface Sci. 2012;388:103–11. https://doi.org/10.1016/j.jcis.2012.08.018.
[81] Lankalapalli S, VRM Kolapalli. Polyelectrolyte complexes: a review of their applicability in drug delivery technology. Indian J Pharm Sci. 2009;71:481–7. https://doi.org/10.4103/0250-474X.
[82] Hu Y, Chen X, Xu Y, Han X, Wang M, Gong T, et al. Hierarchical assembly of hyaluronan coated albumin nanoparticles for pancreatic cancer chemoimmunotherapy. Nanoscale. 2019;11:16476–87. https://doi.org/10.1039/c9nr03684a.
[83] Vinayahan T, Williams PA, Phillips GO. Electrostatic interaction and complex formation between gum arabic and bovine serum albumin. Biomacromolecules. 2010;11:3367–74. https://doi.org/10.1021/bm100486p.
[84] Orelma H, Filpponen I, Johansson LS, Laine J, Rojas OJ. Modification of cellulose films by adsorption of cmc and chitosan for controlled attachment of biomolecules. Biomacromolecules. 2011;12:4311–8. https://doi.org/10.1021/bm201236a.
[85] Wang Q, Ren Y, Ding Y, Xu M, Chen B. The influence of pH and enzyme cross-linking on protein delivery properties of WPI-beet pectin complexes. Food Res Int. 2018;105:678–85. https://doi.org/10.1016/j.foodres.2017.11.076.
[86] Khan N, Brettmann B. Intermolecular interactions in polyelectrolyte and surfactant complexes in solution. Polymers. 2019;11:51. https://doi.org/10.3390/polym11010051.
[87] Chakraborty T, Chakraborty I, Moulik SP, Ghosh S. Physicochemical and conformational studies on BSA - surfactant interaction in aqueous medium. Langmuir. 2009;25:3062–74. https://doi.org/10.1021/la803797x.
[88] Edelman R, Assaraf YG, Levitzky I, Shahar T, Livney YD. Hyaluronic acid-serum albumin conjugate-based nanoparticles for targeted cancer therapy. Oncotarget. 2017;8:24337–53.
[89] Martins M, Azoia NG, Shimanovich U, Matamá T, Gomes AC, Silva C, et al. Design of novel BSA/hyaluronic acid nanodispersions for transdermal pharma purposes. Mol Pharm. 2014;11:1479–88. https://doi.org/10.1021/mp400657g.
[90] Sukhorukov GB, Donath E, Lichtenfeld H, Knippel E, Knippel M, Budde A, et al. Layer-by-layer self assembly of polyelectrolytes on colloidal particles. Coll Surf A Physicochem Eng Asp. 1998;137:253–66. https://doi.org/10.1016/S0927-7757(98)00213-1.
[91] Polomska A, Leroux JC, Brambilla D. Layer-by-layer coating of solid drug cores: a versatile method to improve stability, control release and tune surface properties. Macromol Biosci. 2017;17:1–17. https://doi.org/10.1002/mabi.201600228.
[92] Li Zhai J, Day L, Aguilar MI, Wooster TJ. Protein folding at emulsion oil/water interfaces. Curr Opin Colloid Interface Sci. 2013;18:257–71. https://doi.org/10.1016/j.cocis.2013.03.002.
[93] Wilde PJ. Interfaces: their role in foam and emulsion behaviour. Curr Opin Colloid Interface Sci. 2000;5:176–81. https://doi.org/10.1016/S1359-0294(00)00056-X.
[94] Casterlain C, Genot C. Conformational changes of bovine serum albumin upon its adsorption in dodecane-in-water emulsions as revealed by front-face steady-state fluorescence. BBA - Gen Subj. 1994;1199:59–64. https://doi.org/10.1016/0304-4165(94)90096-5.
[95] Husband FA, Garrood MJ, Mackie AR, Burnett GR, Wilde PJ. Adsorbed protein secondary and tertiary structures by circular dichroism and infrared spectroscopy with refractive index matched emulsions. J Agric Food Chem. 2001;49:859–66. https://doi.org/10.1021/jf000688z.
[96] Rampon V, Brossard C, Mouhous-Riou N, Bousseau B, Llamas G, Genot C. The nature of the apolar phase influences the structure of the protein emulsifier in oil-in-water emulsions stabilized by bovine serum albumin. A front-surface fluorescence study. Adv Colloid Interf Sci. 2004;108–109:87–94. https://doi.org/10.1016/j.cis.2003.10.004.
[97] Day L, Zhai J, Xu M, Jones NC, Hoffmann SV, Wooster TJ. Conformational changes of globular proteins adsorbed at oil-in-water emulsion interfaces examined by synchrotron radiation circular dichroism. Food Hydrocoll. 2014;34:78–87. https://doi.org/10.1016/j.foodhyd.2012.12.015.
[98] Bujacz A. Structures of bovine, equine and leporine serum albumin. Acta Crystallogr Sect D Biol Crystallogr. 2012;68:1278–89. https://doi.org/10.1107/S0907444912027047.
[99] Kawai A, VTG Chuang, Kouno Y, Yamasaki K, Miyamoto S, Anraku M, et al. Crystallographic analysis of the ternary complex of octanoate and N-acetyl-L-methionine with human serum albumin reveals the mode of their stabilizing interactions. Biochim Biophys Acta, Proteins Proteomics. 2017;1865:979–84. https://doi.org/10.1016/j.bbapap.2017.04.004.
[100] David C, Foley S, Mavon C, Enescu M. Reductive unfolding of serum albumins uncovered by Raman spectroscopy. Biopolymers. 2008;89:623–34. https://doi.org/10.1002/bip.20972.

[101] Noskov BA, Mikhailovskaya AA, Lin SY, Loglio G, Miller R. Bovine serum albumin unfolding at the air/water interface as studied by dilational surface rheology. Langmuir. 2010;26:17225–31. https://doi.org/10.1021/la103360h.
[102] Noskov BA. Protein conformational transitions at the liquid-gas interface as studied by dilational surface rheology. Adv Colloid Interf Sci. 2014;206:222–38. https://doi.org/10.1016/j.cis.2013.10.024.
[103] Kazakov VN, Fainerman VB, Kondratenko PG, Elin AF, Sinyachenko OV, Miller R. Dilational rheology of serum albumin and blood serum solutions as studied by oscillating drop tensiometry. Colloids Surf B: Biointerfaces. 2008;62:77–82. https://doi.org/10.1016/j.colsurfb.2007.09.015.
[104] Berthold A, Schubert H, Brandes N, Kroh L, Miller R. Behaviour of BSA and of BSA-derivatives at the air/water interface. Coll Surf A Physicochem Eng Asp. 2007;301:16–22. https://doi.org/10.1016/j.colsurfa.2006.11.054.
[105] Bos MA, van Vliet T. Interfacial rheological properties of adsorbed protein layers and surfactants: a review. Adv Colloid Interf Sci. 2001;91:437–71.
[106] Burgess DJ, Sahin NO. Interfacial rheological and tension properties of protein films. J Colloid Interface Sci. 1997;189:74–82. https://doi.org/10.1006/jcis.1997.4803.
[107] Cascão Pereira LG, Théodoly O, Blanch HW, Radke CJ. Dilatational rheology of BSA conformers at the air/water interface. Langmuir. 2003;19:2349–56. https://doi.org/10.1021/la020720e.
[108] Jara FL, Carrera Sánchez C, Rodríguez Patino JM, AMR Pilosof. Competitive adsorption behavior of β-lactoglobulin, α-lactalbumin, bovin serum albumin in presence of hydroxypropylmethylcellulose. Influence of pH. Food Hydrocoll. 2014;35:189–97. https://doi.org/10.1016/j.foodhyd.2013.05.013.
[109] Douillard R. Adsorption of serum albumin at the oil/water interface. Coll Surf. 1994;91:113–9.
[110] Campana M, Hosking SL, Petkov JT, Tucker IM, JRP Webster, Zarbakhsh A, et al. Adsorption of bovine serum albumin (BSA) at the oil/water interface: a neutron reflection study. Langmuir. 2015;31:5614–22. https://doi.org/10.1021/acs.langmuir.5b00646.
[111] Dickinson E, Pawlowsky K. Effect of ι-carrageenan on flocculation, creaming, and rheology of a protein-stabilized emulsion. J Agric Food Chem. 1997;45:3799–806. https://doi.org/10.1021/jf970304d.
[112] del Castillo-Santaella T, Maldonado-Valderrama J, Molina-Bolivar JA, Galisteo-Gonzalez F. Effect of cross-linker glutaraldehyde on gastric digestion of emulsified albumin. Colloids Surf B: Biointerfaces. 2016;145:899–905. https://doi.org/10.1016/j.colsurfb.2016.06.014.
[113] Aguilera-Garrido, A, Del Castillo-Santaella T, Galisteo-Gonzalez F, Gálvez-Ruiz MJ, Maldonado-Valderrama J. Investigating the role of hyaluronic acid in improving curcumin bioaccesibility from nanoemulsions. Fodo Chemistry 2021. Accepted (FOCH_129301).
[114] Lu JR, Su TJ, Penfold J. Adsorption of serum albumins at the air/water interface. Langmuir. 1999;15:6975–83. https://doi.org/10.1021/la990131h.
[115] Makievski AV, Fainerman VB, Bree M, Wüstneck R, Krägel J, Miller R. Adsorption of proteins at the liquid/air interface. J Phys Chem B. 1998;102:417–25. https://doi.org/10.1021/jp9725036.
[116] Murray BS. Rheological properties of protein films. Curr Opin Colloid Interface Sci. 2011;16:27–35. https://doi.org/10.1016/j.cocis.2010.06.005.
[117] Rodriguez Patino JM, Pilosof AMR. Protein-polysaccharide interactions at fluid interfaces. Food Hydrocoll. 2011;25:1925–37. https://doi.org/10.1016/j.foodhyd.2011.02.023.
[118] Dickinson E, Pawlowsky K. Effect of high-pressure treatment of protein on the rheology of flocculated emulsions containing protein and polysaccharide. J Agric Food Chem. 1996;44:2992–3000. https://doi.org/10.1021/jf960188z.
[119] Sheng B, Li L, Zhang X, Jiao W, Zhao D, Wang X, et al. Physicochemical properties and chemical stability of β-carotene bilayer emulsion coated with bovine serum albumin and arabic gum compared to monolayer emulsions. Molecules. 2018;23:1–13. https://doi.org/10.3390/molecules23020495.
[120] Rodríguez Niño MR, Rodríguez Patino JM. Effect of the aqueous phase composition on the adsorption of bovine serum albumin to the air-water interface. Ind Eng Chem Res. 2002;41:1489–95. https://doi.org/10.1021/ie010770z.
[121] Liu Y, Yadav MP, Yin L. Enzymatic catalyzed corn fiber gum-bovine serum albumin conjugates: their interfacial adsorption behaviors in oil-in-water emulsions. Food Hydrocoll. 2018;77:986–94. https://doi.org/10.1016/j.foodhyd.2017.11.048.
[122] Kim DY, Shin WS, Hong WS. The unique behaviors of biopolymers, BSA and fucoidan, in a model emulsion system under different pH circumstances. Macromol Res. 2010;18:1103–8. https://doi.org/10.1007/s13233-010-1101-0.
[123] Kim DY, Shin WS. Functional improvements in bovine serum albumin-fucoidan conjugate through the Maillard reaction. Food Chem. 2016;190:974–81. https://doi.org/10.1016/j.foodchem.2015.06.046.
[124] Georgiev GA, Yokoi N, Ivanova S, Dimitrov T, Andreev K, Krastev R, et al. Surface chemistry study of the interactions of hyaluronic acid and benzalkonium chloride with meibomian and corneal cell lipids. Soft Matter. 2013;9:10841–56. https://doi.org/10.1039/c3sm51849c.
[125] Crawford NF, Leblanc RM. Serum albumin in 2D: a Langmuir monolayer approach. Adv Colloid Interf Sci. 2014;207:131–8. https://doi.org/10.1016/j.cis.2013.10.021.
[126] Giner-Casares JJ, Brezesinski G, Möhwald H. Langmuir monolayers as unique physical models. Curr Opin Colloid Interface Sci. 2014;19:176–82. https://doi.org/10.1016/j.cocis.2013.07.006.
[127] Gálvez-Ruiz MJ. Different approaches to study protein films at air/water interface. Adv Colloid Interf Sci. 2017;247:533–42. https://doi.org/10.1016/j.cis.2017.07.015.
[128] Miñones-Trillo J, Iribarnegaray-Jado E, García-Fernandez S, Sanz-Pedrero S. Monolayers of human serum albumin. Kolloid-Z u Z Polym. 1972;324:318–24.
[129] Sánchez-González J, Ruiz-García J, Gálvez-Ruiz MJ. Langmuir-Blodgett films of biopolymers: a method to obtain protein multilayers. J Colloid Interface Sci. 2003;267:286–93. https://doi.org/10.1016/S0021-9797(03)00754-9.
[130] Toimil P, Prieto G, Miñones J, Trillo JM, Sarmiento F. Monolayer and Brewster angle microscopy study of human serum albumin-Dipalmitoyl phosphatidyl choline mixtures at the air-water interface. Colloids Surf B: Biointerfaces. 2012;92:64–73. https://doi.org/10.1016/j.colsurfb.2011.11.022.
[131] Boury F, Ivanova T, Panaïotov I, Proust JE. Dilatational properties of poly(D,L-lactic acid) and bovine serum albumin monolayers spread at the air/water interface. Langmuir. 1995;11:599–606. https://doi.org/10.1021/la00002a040.
[132] Campbell RA, Ang JC, Sebastiani F, Tummino A, White JW. Spread films of human serum albumin at the air-water interface: optimization, morphology, and durability. Langmuir. 2015;31:13535–42. https://doi.org/10.1021/acs.langmuir.5b03349.
[133] Grigoriev DO, Derkatch S, Kragel J, Miller R. Relationship between structure and rheological properties of mixed BSA/Tween 80 adsorption layers at the air/water interface. Food Hydrocoll. 2007;21:823–30. https://doi.org/10.1016/j.foodhyd.2006.08.018.
[134] Engelhardt K, Rumpel A, Walter J, Dombrowski J, Kulozik U, Braunschweig B, et al. Protein adsorption at the electrified air−water interface: implications on foam stability. Langmuir. 2012;28:7780–7. https://doi.org/10.1021/la301368v.
[135] Benesch J, Askendal A, Tengvall P. Quantification of adsorbed human serum albumin at solid interfaces: a comparison between radioimmunoassay (RIA) and simple null ellipsometry. Colloids Surf B: Biointerfaces. 2000;18:71–81. https://doi.org/10.1016/S0927-7765(99)00136-8.
[136] Zhang F, Pan J, Claesson PM. Electrochimica Acta Electrochemical and AFM studies of mussel adhesive protein (Mefp-1) as corrosion inhibitor for carbon steel. Electrochim Acta. 2011;56:1636–45. https://doi.org/10.1016/j.electacta.2010.10.033.
[137] Ortega-Vinuesa JL, Tengvall P, Lundstrom I. Molecular packing of HSA , IgG , and fibrinogen adsorbed on silicon by AFM imaging. Thin Solid Films. 1998;324:257–73. https://doi.org/10.1016/S0040-6090(98)00363-0.
[138] Sarmah RJ, Sah BK, Kundu S. Monolayer behavior of human serum albumin (HSA) at air-water interface Monolayer Behavior of Human Serum Albumin (HSA) at Air-Water Interface. AIP Conf Proc. 2019;2115:030043.
[139] Gunning AP, Wilde PJ, Clark D, Morris VJ, Parker ML, Gunning PA. Atomic force microscopy of interfacial protein films. J Colloid Interface Sci. 1996;602:600–2.
[140] Furuno T. Atomic force microscopy study on the unfolding of globular proteins in the Langmuir films. Thin Solid Films. 2014;552:170–9. https://doi.org/10.1016/j.tsf.2013.12.052.
[141] Gispert MP, Serro AP, Colac R, Saramago B. Bovine serum albumin adsorption onto 316L stainless steel and alumina : a comparative study using depletion , protein radiolabeling , quartz crystal microbalance and atomic force microscopy. Surf Interface Anal. 2008;40:1529–37. https://doi.org/10.1002/sia.2929.
[142] Chicea D, Chicea R, Chicea LM, et al. Rom Rep Phys. 2013;65:178–85.
[143] Sánchez-González J, Cabrerizo-Vílchez MA, Gálvez-Ruiz MJ, et al. Colloids Surf B: Biointerfaces. 2001;21:19–27. https://doi.org/10.1016/S0927-7765(01)00180-1.
[144] Martín-Molina A, Del Castillo-Santaella T, Yang Y, Maldonado-Valderrama J. Condensation of model lipid films by cholesterol: specific ion effects. Coatings. 2019;9:474. https://doi.org/10.3390/coatings9080474.
[145] Miao S, Leeman H, De Feyter S, Schoonheydt RA. Facile preparation of Langmuir-Blodgett films of water-soluble proteins and hybrid protein-clay films. J Mater Chem. 2010;20:698–705. https://doi.org/10.1039/b913659b.
[146] Kim HJ, Choi SJ, Shin WS, Moon TW. Emulsifying properties of bovine serum albumin-galactomannan conjugates. J Agric Food Chem. 2003;51:1049–56. https://doi.org/10.1021/jf020698v.
[147] Chen X, Wang M, Hu Y, Gong T, Zhang Z-R, Yu R, et al. Low-dose paclitaxel via hyaluronan-functionalized bovine serum albumin nanoparticulate assembly for metastatic melanoma treatment. J Mater Chem B. 2020;8:2139–47. https://doi.org/10.1039/c9tb02780g.
[148] Li Y, Shi S, Ming Y, Wang L, Li C, Luo M, et al. Specific cancer stem cell-therapy by albumin nanoparticles functionalized with CD44-mediated targeting. J Nanobiotechnol. 2018;16:99. https://doi.org/10.1186/s12951-018-0424-4.
[149] Park KY, Kim DY, Shin WS. Roles of chondroitin sulfate in oil-in-water emulsions formulated using bovine serum albumin. Food Sci Biotechnol. 2015;24:1583–9. https://doi.org/10.1007/s10068-015-0204-y.
[150] Gatej I, Popa M, Rinaudo M. Role of the pH on hyaluronan behavior in aqueous solution. Biomacromolecules. 2005;6:61–7. https://doi.org/10.1021/bm040050m.
[151] Lomis N, Westfall S, Farahdel L, Malhotra M, Shum-Tim D, Prakash S. Human serum albumin nanoparticles for use in cancer drug delivery: process optimization and in vitro characterization. Nanomaterials. 2016;6. https://doi.org/10.3390/nano6060116.
[152] Kumar R, Choudhary DK, Debnath M. Development of BSA conjugated on modified surface of quercetin-loaded lipid nanocarriers for breast cancer treatment. Mater Res Express. 2020;7. https://doi.org/10.1088/2053-1591/ab6774.
[153] Cassimjee H, Kumar P, Choonara YE, Pillay V. Proteosaccharide combinations for tissue engineering applications. Carbohydr Polym. 2020;235:115932. https://doi.org/10.1016/j.carbpol.2020.115932.
[154] Kong M, Park HJ. Stability investigation of hyaluronic acid based nanoemulsion and its potential as transdermal carrier. Carbohydr Polym. 2011;83:1303–10. https://doi.org/10.1016/j.carbpol.2010.09.041.
[155] Kim JH, Moon MJ, Kim DY, Heo SH, Jeong YY. Hyaluronic acid-based nanomaterials for cancer therapy. Polymers (Basel). 2018;10:1–15. https://doi.org/10.3390/polym10101133.
[156] Nasr M. Development of an optimized hyaluronic acid- based lipidic nanoemulsion co-encapsulating two polyphenols for nose to brain delivery. Drug Deliv. 2016;7544:1444–52. https://doi.org/10.3109/10717544.2015.1092619.