Sains Malaysiana 54(6)(2025): 1559-1568
http://doi.org/10.17576/jsm-2025-5406-11

Molecular Docking-Guided Optimisation of an *Aloe Vera*-Based Buccal Protein Delivery System

(Pengoptimuman Berpandukan Dok Molekul bagi Sistem Penghantaran Protein Bukal Berasaskan *Aloe Vera*)

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Received: 3 January 2025/Accepted: 23 April 2025

ABSTRACT

Proteins play vital roles in the body and are frequently used as therapeutic agents, yet their efficacy is often hindered by issues like stability and poor bioavailability. The buccal drug delivery system offers a promising alternative by directly administering medications through the cheek's mucosal lining, bypassing the digestive tract and enhancing absorption into the bloodstream. In this study, sodium carboxymethyl cellulose (SCMC) and chitosan (CHI) films were prepared for albumin buccal delivery and were characterized for their mechanical strength and later optimized with the help of molecular docking studies. SCMC films exhibited significantly higher albumin release (71.09 ± 8.61 µg/cm^2) compared to CHI films (38.38 ± 5.15 µg/cm^2) and both formulations showed compliance with the Korsemeyer-Peppas model (R^2 approaching ≈ 0.99, n = 0.65) indicating non-Fickian diffusion as a dominant mechanism of drug permeation. Molecular docking studies were instrumental in guiding the design of the optimized formulation for albumin buccal drug delivery, providing insights into molecular interactions and facilitating the rational refinement of albumin-polymer delivery systems. The molecular docking studies showed interactions between albumin and polymers, with stronger hydrogen bonding observed between certain residues of the polymers and albumin, particularly SER-419 and GLU-505 in SCMC and LEU-112, ASN-109, and ASN-111 in chitosan. These findings contribute to understanding the mechanisms underlying drug release and binding interactions, facilitating the development of more effective drug delivery systems, ultimately leading to more efficient and targeted therapeutic interventions.

Keywords: Albumin; buccal films; chitosan; molecular docking; sodium carboxymethyl cellulose

ABSTRAK

Protein memainkan peranan penting dalam tubuh dan sering digunakan sebagai agen terapeutik, namun keberkesanannya sering terhalang oleh isu seperti kestabilan dan bioketersediaan yang rendah. Sistem penghantaran ubat bukal menawarkan alternatif yang menjanjikan dengan memberikan ubat secara langsung melalui lapisan mukosa pipi, memintas saluran pencernaan dan meningkatkan penyerapan ke dalam aliran darah. Dalam kajian ini, filem natrium karboksimetil selulosa (SCMC) dan kitosan (CHI) disediakan untuk penghantaran bukal albumin dan dicirikan untuk kekuatan mekanikal mereka dan kemudian dioptimumkan dengan bantuan kajian pengedokan molekul. Filem natrium karboksimetil selulosa menunjukkan pelepasan albumin yang lebih tinggi (71.09 ± 8.61 µg/cm^2) berbanding filem kitosan (38.38 ± 5.15 µg/cm^2) dan kedua-dua formulasi menunjukkan pematuhan kepada model Korsemeyer-Peppas (r^2 menghampiri ≈ 0.99, n = 0.65) yang menunjukkan penyebaran bukan Fickian sebagai mekanisme dominan penyerapan ubat. Kajian pengedokan molekul memainkan peranan penting dalam membimbing reka bentuk formulasi yang dioptimumkan untuk penghantaran ubat bukal albumin, memberikan gambaran interaksi molekul dan memudahkan penapisan rasional sistem penghantaran albumin-polimer. Kajian pengedokan molekul mendedahkan interaksi antara albumin dan polimer, dengan ikatan hidrogen yang lebih kuat diperhatikan antara residu tertentu polimer dan albumin, terutamanya SER-419 dan GLU-505 dalam SCMC dan LEU-112, ASN-109 dan ASN-111 dalam CHI. Penemuan ini menyumbang kepada pemahaman tentang mekanisme yang mendasari pelepasan ubat dan interaksi pengikatan, memudahkan pembangunan sistem penghantaran ubat yang lebih berkesan, yang akhirnya membawa kepada intervensi terapeutik yang lebih cekap dan tersasar.

Kata kunci: Albumin; filem bukal; kitosan; natrium karboksimetil selulosa; pengedokan molekul

INTRODUCTION

In the field of biomedical research, there have been incredible breakthroughs regarding drug distribution and the targeted drug delivery systems, with the goal of maximizing therapeutic outcomes while avoiding potential side effects. Throughout this progress, protein delivery systems have also been points of interest for facilitating the precise and regulated release of therapeutic proteins. The creation of efficient protein delivery systems is now vital to guaranteeing the biomolecules' stability, bioavailability, and tailored activity (Verma et al. 2021). Proteins are essential macromolecules that perform a variety of roles in the body and are frequently employed as medicinal agents to treat a range of illnesses. However, problems with stability, breakdown in the gastrointestinal system, and low bioavailability when taken by conventional methods might make their usage difficult (Kianfar 2021; Verma et al. 2021). Buccal drug delivery system, a convenient and non-invasive approach, is a specialized technique for delivering drugs or therapeutic agents through the oral cavity's mucosal lining. Because of the presence of extensive network of blood supply in the buccal mucosa, proteins can be directly absorbed into the circulation without going via the digestive system and avoiding problems such as enzymatic degradation, first pass metabolism, and variable absorption (Johnston 2015; Zhang, Zhang & Streisand 2002).

Molecular docking is a computational technique that plays a pivotal role in advancing drug delivery strategies by offering insights into the interactions between therapeutic molecules and their carrier systems, such as polymers or proteins (Ferreira et al. 2015). Molecular docking helps identify appropriate drug molecules, improves their binding affinity to the target, and forecasts how medications will interact with biological systems to help build optimum formulations in the field of drug delivery (Metwally & Hathout 2015). Molecular docking helps with the design and improvement of drug delivery systems by modeling and analyzing these interactions at the molecular level. This eventually results in more effective and focused therapeutic interventions (Casalini 2021; Sahlgren et al. 2017). In the context of this study, molecular docking was not merely used as a screening tool, but rather as a rational approach to elucidate the molecular-level interactions between albumin and the selected polymer matrices. By simulating these interactions, we aim to estimate the three-dimensional configuration and binding affinities of albumin within the polymer matrices, offering mechanistic insights into the nature and strength of hydrogen bonding, electrostatic interactions, and potential binding sites.

Using albumin as a model protein, we have tried to create a buccal medication delivery system for proteins in this work. Due to its biocompatibility, bloodstream circulation, and molecular binding capabilities, the multifunctional protein albumin is a good option for integration into drug delivery systems. It also serves a variety of physiological purposes (Karimi et al. 2016; Kianfar 2021). The goal was to optimize the concentration of polymers in order to create an albumin-delivery system that would work well in the buccal cavity. Mucoadhesive films were created for this purpose by combining chitosan (CHI) or sodium carboxymethyl cellulose (SCMC) with aloe vera gels. Natural polysaccharides with potent mucoadhesive qualities, like acemannan and glucomannans, are found in aloe vera gel (Chelu et al. 2023). Because aloe vera gel is mucoadhesive, it can prolong the duration of contact between the mucosa and the buccal film, which makes it a perfect option for use as a buccal medication delivery system. Aloe vera gel alone, however, could not have strong enough mechanical strength, which could prevent it from being used in some situations where stronger structural qualities are needed (Nabila et al. 2021). Aloe vera gel is frequently mixed with other polymers to improve its structural integrity and produce materials appropriate for medication delivery in order to get around this restriction.

SCMC is a water-soluble derivative and has a special combination of physicochemical characteristics that make it an ideal candidate for improving medication solubility, bioavailability, and controlled release. Another polymer has also shown great promise and versatility as a possible option for drug delivery applications (Gong et al. 2024). The unique characteristics of CHI, such as its biocompatibility, biodegradability, and adaptability, have made it a key component in the creation of novel drug delivery techniques (Desai et al. 2023). These polymers' ability to engage with biological membranes and promote mucoadhesion opens the door for targeted drug delivery systems. With a focus on enhancing drug solubility, stability, and controlled release of proteins, polymeric-based drug delivery systems offer a myriad of opportunities to address challenges in personalized medicine (Mohebbi et al. 2019). In this study, the mechanism behind the drug release of the optimized formulation was also analysed through molecular docking studies which helped to define binding energies and bonding affinity between the albumin and polymers to explain the possible mechanism behind albumin release from the buccal drug delivery systems.

MATERIALS AND METHODS

MATERIALS

Bovine serum albumin, phosphate buffer saline, trifluoroacetic acid HPLC grade, formalin solution, SCMC, and low molecular weight CHI were all purchased commercially from Sigma Aldrich in Germany. We bought aloe vera (*Aloe barbadensis*) leaves from PPA Bio Sdn. Bhd. in Malaysia when they were around a year old. The supplier of glycerine was Bendosen Laboratory Chemicals in Malaysia. Aspartame was sourced from Supelco in Pennsylvania, USA, and Tween 80 was

acquired from R&M Chemicals in Malaysia. Methanol containing hydrochloric acid from Friendemann Schmidt Pty Ltd. in the USA. Every material that was used were of analytical grade or equivalent.

METHODS

PREPARATION OF ALOE VERA GEL

Aloe vera leaves measuring 50-62 cm was first cleaned with tap water and then dried with a lint-free cloth. Next, a knife was used to cut the leaves transversely and remove the outer cuticle. Next, using an Alba heavy duty blender (model no. EBL-A1812G(SS)) (Malaysia), the leaves were homogenized. After removing any generated bubbles, the extract was centrifuged for 30 min at 5,000 rpm and 5 °C using a centrifuge model number, Universal 320 R (Andreas Hettich GmbH & Co. KG, Germany). The supernatant was then meticulously separated and filtered via a Buchner funnel model number FB70155/EUR (Fisher Scientific Inc., USA) using Whatman® filter paper no. 1. After that, the filtrate was gathered and frozen for later use.

PREPARATION OF FILMS

The aloe vera gel was formed into films using solvent casting method. SCMC (1.5 - 3% w/w) was stirred in water until a homogenous mixture was formed while 1.5 - 3% w/w CHI was dissolved in 1% v/v acetic acid aqueous solution owing to its insolubility in water. Different concentrations were tested to optimize the suitable concentration required for the development of most suitable buccal films. Aloe vera gels (50-70% w/w) were also added in the optimized polymeric concentration and again tested for the most suitable concentration of aloe vera gel required for the buccal films. Glycerine (40% of total polymer weight) was added as a plasticizer. To improve buccal permeability, one drop of Tween 80 was added. Mannitol was added at 0.5% w/v as a cooling agent, while aspartame was added at 0.125% w/v as a sweetening agent. The ideal film composition was filled with 0.45 mg of bovine serum albumin (BSA), a model protein. After that, the mixes were let to stand in order to release trapped bubbles. 40 mL of the mixture was put onto an 8.5 cm diameter petri dish and oven-dried for 24 h at 40 °C to create the films. After that, the resulting films were carefully removed from the mold. After that, the film was cut with a sharp knife into squares of 2 cm by 2 cm and kept in a desiccator until needed again. Only samples that were free of nicks, tears, and air bubbles were used for analysis.

PHYSICAL CHARACTERISATION

Physical characterisation was performed after each step of buccal film formulation to observe the changes that take place by addition of all the ingredients and to find out the optimal formulation. Every film that was made was assessed based on its physical attributes, including color, opacity, and smoothness. For additional research, only smooth, flexible, transparent, or translucent films were employed. Thickness measurements were conducted at five separate locations (four corners and one centre). Thickness of films was measured using a digimatic callipers model no. CD-4”CSX (Mitutoyo Corp., Japan) while films were weighed using an analytical balance model no. MS204S (Mettler Toledo International Inc., USA).

MECHANICAL CHARACTERISATION

The buccal films were examined using a universal testing machine (Instron Corp., model 5567, USA) in compliance with the D 882-02 guidelines for films thinner than 1.0 mm from the American Society for Testing and Materials. Before analysis, all samples were conditioned for a minimum of 40 h at 23 ± 2 °C and 50 ± 5% relative humidity. Films were positioned between grips after being cut around a conventional template (dumbbell form) with a gauge length of 30 mm and a width of 5 mm. The films were stretched to their breaking point and the rate of grip separation was set at 12.5 mm/min. For buccal films, the following parameters were calculated: strain, elongation at break point, percentage elongation, tensile strength, and elastic modulus.

ULTRAVIOLET-VISIBLE (UV-VIS) SPECTROSCOPY

To construct the calibration curve of albumin, a stock solution containing 100 mg of BSA dissolved in 100 mL of simulated saliva fluid (SSF) was prepared. This was followed by serial dilutions using SSF to prepare five aliquots of different concentrations (0.04, 0.06, 0.08, 0.12, and 0.16 mg/mL BSA). The aliquots were then analysed using SSF as blank solution in UV-Vis spectrophotometer model UV1800 (Shimadzu Corp., Japan) at the wavelength 279 nm. The test was repeated three times and the resulting absorbance plotted against concentration to plot the calibration curve. The linear regression line equation and the correlation coefficient (r^2) were then determined.

DETERMINATION OF ALBUMIN CONCENTRATION

Release of albumin from films (1 cm × 1 cm) cut out using scissors at random sites of the films was investigated. The films were placed in separate volumetric flasks containing 10 mL of SSF and release was allowed to proceed for 24 h to completion at 37 °C in an orbital shaker shaking at 150 rpm (El Sharawy, Shukr & Elshafeey 2017). The solution was then filtered through a 0.22 µm PVDF filter (Bioflow Lifescience Sdn. Bhd., Malaysia). Albumin concentration was determined using UV-Vis spectroscopy for samples containing albumin by extrapolating from the standard curve.

Ex vivo DETERMINATION OF ALBUMIN PERMEATION THROUGH BUCCAL MUCOSA PREPARATION OF PORCINE BUCCAL MUCOSA

Albumin permeation studies were conducted to determine the release of permeation from the optimized formulations. Normal saline was used to wash porcine buccal mucosa that was procured from a nearby butcher. In the next step, extra fat and connective tissues were meticulously cut from the substrate using a fine scalpel blade. For additional examination, the buccal mucosa was covered in aluminum foil and kept at -20 °C.

Ex vivo MUCOSAL PERMEATION

Albumin released from buccal film was tested for transbuccal permeability using vertical Franz diffusion glass cells (PermeGear Inc., USA) with a diffusional area of 1.0 cm^2 and a receptor volume of 5.0 mL. PBS was added to the receptor compartment and constantly agitated at 130 rpm using a magnetic bar. The donor and receptor compartments were carefully positioned between the porcine buccal membrane and clamped shut. By employing circulating water, the temperature was adjusted to 37 ± 0.5°C, which is very close to the temperature of the human body.

The set-up was equilibrated for half an hour before the start of experiment. To replicate *in vivo* physiological conditions, a mucoadhesive disc with a diameter of 1.15 cm was subsequently placed to the buccal tissue's surface, and 0.5 mL of SSF was introduced to the donor compartment. The receptor arm was sealed during the experiment and the donor top covered with paraffin film to prevent evaporation. Using a plastic syringe, the entire recipient fluid was collected at pre-arranged intervals for six hours (0.5, 1, 1.5, 2, 3, 4, 5, and 6 h). To preserve sink conditions, the sample volume was then replenished with an equivalent volume of pre-warmed PBS at 37 °C. Analysis of albumin-containing samples was done with UV-Vis spectroscopy.

MATHEMATICAL MODELLING

To analyze the release mechanism, the albumin permeation data were fitted to various mathematical models (Korsmeyer-Peppas, Higuchi, Hixson-Crowell, zero order, and first order models). With the exception of Korsmeyer-Peppas equation, which was only fitted for 60% of the drug release, all equations were fitted to the entire release curve. The regression plots were used to calculate the correlation coefficients, and the drug transport mechanism involved in regulated release would be highlighted by this mathematical modeling of release kinetics. The equations of different kinetic release used in this study are given herewith.

$$\text{Zero order equation: } Q=Q_o-_{Kot} \quad (1)$$

$$\text{First order equation: } LogQ=LogQ_o-K_1t \quad (2)$$

$$\text{Higuchi equation: } Q=K_2t^{1/2} \quad (3)$$

$$\text{Hixson-Crowell: } Q_o^{1/3}-Q_t^{1/3}=K_st \quad (4)$$

$$\text{Korsmeyer-Peppas equation: } Q/Q_o=Kt^n \quad (5)$$

The release constants K_o to K_2 are used in the equations, the drug release fraction at time t is represented by Q/Qo, and the diffusion constant n denotes the overall release mechanism. The release mechanisms could be described using the 'n' value from the Korsmeyer-Peppas model's release exponent.

MOLECULAR DOCKING

Molecular docking is a renowned method for determining small molecules' optimal orientation and binding affinity to a receptor, usually a protein (Rehman et al. 2015; Sneha & Doss 2016; Werner et al. 2012; Zulfakar et al. 2018). Albumin and the polymers were molecularly docked using free open-source software, including AutoDock Vina from PyRx Virtual Screening and BIOVIA Discovery Studio 2017 (San Diego, CA). Calculations of energy (kcal/mol) and binding affinity were performed using the AutoDock Vina screening software. The virtual investigation was completed using BIOVIA Discovery Studio 2017. All water molecules and cofactors were omitted from the docking process. A ligand library was created after the three-dimensional (3D) structures were collected from the protein data bank (PDB). Eventually, docking investigations were conducted once Optimisation of all structures had been achieved using energy minimization.

STATISTICAL ANALYSIS

All data are presented as mean ± standard deviation. Statistical analysis using paired t-test was also performed to compare weight and thickness of film, mechanical properties, cumulative amount of drug permeated, flux, and apparent permeability coefficient. Results were considered significant at a p value of < 0.05. Statistical analysis was performed using IBM SPSS Statistic Version 23.0 (IBM Corp., USA).

RESULTS AND DISCUSSION

OPTIMISATION AND CHARACTERISATION OF POLYMER CONCENTRATION

The selection of the type and concentration of polymers, type and concentration of plasticizers, and concentration of *A. vera* was based on two main criteria: organoleptic characterization and mechanical properties, as shown in Figure 1. The selected film formulation should appear translucent or semi-transparent, peelable, homogeneous, and smooth. In addition, the selected buccal film must possess ideal mechanical properties, namely high tensile

strength, percentage elongation at break, and strain values, while having a low elastic modulus. When the mechanical property results were not significant, a secondary test — folding endurance — was used to select the optimal film formulation. To determine the optimal film formulation after albumin was added, different tests were employed, namely albumin content analysis in the film formulation and *in vitro* albumin release across a membrane. The selected albumin film formulation must have the highest drug content and cumulative albumin release.

At the first stage, blank polymeric films were prepared without any addition of plasticizer, Aloe vera and other excipients. SCMC films appeared transparent, whereas CHI films which were translucent and slightly yellow. They were determined for their weight, thickness, and other mechanical features (Table 1). It was observed that as the concentration of the polymer was increased so does the weight and the thickness measurements. Thickness of all films falls below 1 mm, which fulfils the criterion of an ideal buccal film. This specification is critical, as buccal films thicker than 1 mm may compromise flexibility, reduce mucoadhesiveness, and increase discomfort during application. Literature supports that optimal buccal films should typically be less than 1 mm to ensure patient comfort, effective adhesion to the mucosal surface, and overall formulation acceptability (Johnston 2015; Zhang, Zhang & Streisand 2002). This design parameter ensures that the films are sufficiently thin for consistent mucosal contact while maintaining mechanical resilience and user convenience. Moreover, several key mechanical properties such as tensile strength, elongation percentage, breakpoint, modulus of elasticity, strain, and tear resistance were evaluated. It was observed that increasing the concentration of polymers in polymeric films results in a more closely interconnected and robust structure. This interconnectedness leads to improvements in mechanical properties such as tensile strength, elongation percentage, breakpoint, modulus of elasticity, strain, and tear resistance. Therefore, formulations that had 3% polymeric concentrations had strong interactions and entanglements among polymer chains, making these films more resilient and versatile in various applications requiring strength and flexibility. Therefore, 3% concentration of SCMC and CHI was selected as an appropriate amount of polymer that would serve as a backbone for our buccal delivery system. Based on the results, the film concentration at 3% w/w was selected as the optimum concentration for CHI and SCMC film matrix. CHI3 film was found to have the highest tensile strength, although only significant against CHI1.5 ($p < 0.01$) and CHI3 ($p < 0.05$). CHI3 strain values were also found to be higher when compared to lower concentrations, although only significant against CHI1.5 ($p < 0.01$) and CHI2.5 ($p < 0.05$). Film SCMC3 recorded the highest tensile strength, although it was only significant against SCMC1.5 ($p < 0.01$). The SCMC3 film strain value was also observed to be the highest and significant against SCMC1.5 ($p < 0.01$) and SCMC2 ($p < 0.01$).

OPTIMISATION AND CHARACTERISATION OF ALOE VERA GEL CONCENTRATION

In the next step, after the addition of glycerine as a plasticizer, we optimized the concentration of aloe vera gel. The buccal films showed a decrease in rigidity and tensile strength at higher Aloe vera concentrations. The aloe vera gel resulted in a significant decrease in elastic modulus of SCMC formulations, but it was opposite in case of films that were composed of CHI. Furthermore, the percent elongation and the break point were also significantly higher ($p < 0.01$) compared to the lower concentrations of aloe vera gel. Meanwhile CHI formulations again showed the opposite effect as compared to SCMC formulations (Table 2).

An optimal buccal film should possess a relatively high tensile strength, elongation at break, and strain but a low elastic modulus, these parameters were used to select the optimal polymer concentration. The 70% w/w concentration of Aloe vera gel was found to be optimal for the final film formulation for the SCMC and 50% w/w aloe vera concentration was chosen for CHI formulation. The albumin was loaded in the optimized formulations and named CHF (CHI3/GLY40/AVG50) and SCF (SCMC3/GLY40/AVG70). The composition and mechanical characterisation of the optimized formulations is given in Tables 3 and 4. It was observed that SCF buccal film had significantly higher ($p < 0.01$) tear resistance, strain, percentage elongation, breakpoint, modulus elasticity and tensile strength as compared to CHF buccal film.

ANALYSIS OF ALBUMIN CONTENT IN BUCCAL FILM FORMULATIONS

The total percentage of albumin content found in the buccal film formulation was 34.2 ± 11.7% for CHF and 30.0 ± 3.5% for SCF formulations (Table 4). The results showed no significant difference between the drug content in both film formulations.

Ex vivo PERMEATION STUDIES

In vitro albumin release studies were conducted to compare albumin release from CHF and SCF films. The SCF film formulation showed a significantly higher amount of albumin release (71.09 ± 8.61μg/cm^2) ($p < 0.01$) after six hours across the membrane when compared to the CHF film formulation (38.38 ± 5.15 μg/cm^2). The result shows a significantly higher amount of flux ($p < 0.01$) in the SCF film when compared to the CHF film formulation. A gradual release profile can be observed in both the albumin-containing formulation (Figure 1). While the CHI-based films exhibited lower release, such controlled and slower release could be advantageous for sustained buccal delivery where prolonged therapeutic effects are desirable. However, depending on the clinical requirement, the choice of polymer should align with the target release profile.

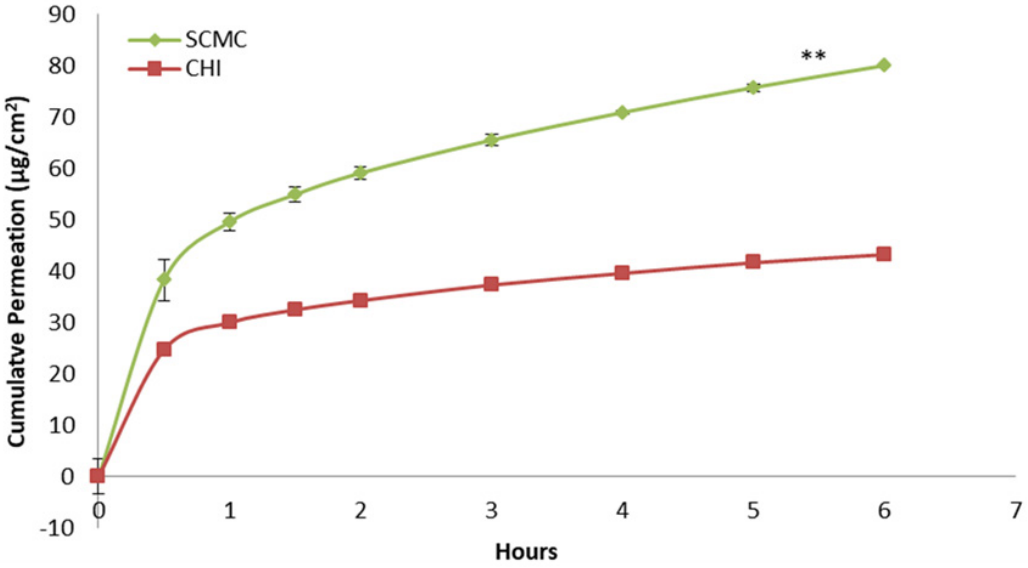

FIGURE 1. *In vitro* albumin release from SCF and CHF film formulations

TABLE 1. Mechanical characterization and optimization of polymer concentration in blank buccal films

Formulation	Weight (mg)	Thickness (mm)	Tensile strength (MPa)	Modulus elasticity (MPa)	Elongation till break point (%mm^{-2})	Percentage elongation (%)	Strain	Tear resistance (N)
CHI 1.5% w/w	38.9 ± 4.33	0.07 ± 0.01	0.08 ± 0.01	2132.26 ± 139.08	0.14 ± 0.02	21.27 ± 3.66	$3.75 \times 10^{-5} \pm 4.19 \times 10^{-6}$	12.01 ± 1.81
CHI 2% w/w	55.86 ± 7.04	0.1 ± 0.01	0.11 ± 0.03	1780.62 ± 623.79	0.17 ± 0.05	25.14 ± 8.08	$6.26 \times 10^{-5} \pm 1.12 \times 10^{-5}$	16.23 ± 4.02
CHI 2.5% w/w	72.21 ± 13.63	0.13 ± 0.02	0.15 ± 0.04	2056.30 ± 408.07	0.13 ± 0.06	19.56 ± 8.55	$7.29 \times 10^{-5} \pm 7.40 \times 10^{-6}$	22.48 ± 5.32
CHI 3% w/w	79.64 ± 12.43	0.15 ± 0.02	0.1681 ± 0.0296	2083.03 ± 418.32	0.15 ± 0.05	22.11 ± 6.92	$8.16 \times 10^{-5} \pm 8.55 \times 10^{-6}$	25.21 ± 4.43
SCMC 1.5% w/w	36.83 ± 5.42	0.07 ± 0.01	0.06 ± 0.02	1625.50 ± 381.17	0.06 ± 0.01	9.17 ± 1.15	$3.58 \times 10^{-5} \pm 2.88 \times 10^{-6}$	8.79 ± 2.39
SCMC 2% w/w	27.47 ± 4.75	0.05 ± 0.02	0.11 ± 0.01	2492.82 ± 268.89	0.05 ± 0.01	8.14 ± 0.78	$4.39 \times 10^{-5} \pm 2.11 \times 10^{-6}$	16.43 ± 2.00
SCMC 2.5% w/w	57.35 ± 3.88	0.10 ± 0.01	0.10 ± 0.03	1922.74 ± 572.58	0.06 ± 0.01	9.68 ± 0.91	$5.02 \times 10^{-5} \pm 4.60 \times 10^{-5}$	14.38 ± 4.17
SCMC 3% w/w	74.87 ± 9.79	0.12 ± 0.02	0.13 ± 0.03	2420.78 ± 651.30	0.06 ± 0.02	8.71 ± 3.47	$5.48 \times 10^{-5} \pm 1.11 \times 10^{-5}$	22.02 ± 6.46

TABLE 2. Mechanical characterization and optimization of blank buccal films with aloe vera

Formulation	Tensile strength (MPa)	Modulus elasticity (MPa)	Elongation till break point (%mm^{-2})	Percentage elongation (%)	Strain	Tear resistance (N)
CHI3/ GLY40/AVG50	0.02 ± 0.01	16.88 ± 6.11	0.29 ± 0.06	43.80 ± 9.21	$1.04 \times 10^{-3} \pm 1.75 \times 10^{-4}$	2.64 ± 0.99
CHI3/ GLY40/AVG60	0.02 ± 0.01	18.79 ± 5.69	0.28 ± 0.04	42.28 ± 5.72	$1.05 \times 10^{-3} \pm 8.98 \times 10^{-5}$	2.91 ± 0.75
CHI3 / GLY40 / AVG70	0.03 ± 0.00	27.16 ± 4.34	0.26 ± 0.03	39.14 ± 4.84	$1.03 \times 10^{-3} \pm 2.20 \times 10^{-4}$	4.11 ± 0.65
SCMC3 / GLY40 / AVG50	0.03 ± 0.01	132.84 ± 62.99	0.32 ± 0.03	47.98 ± 5.15	$2.60 \times 10^{-4} \pm 1.32 \times 10^{-5}$	5.09 ± 2.12
SCMC3 / GLY40 / AVG60	0.02 ± 0.01	99.24 ± 27.81	0.31 ± 0.03	46.95 ± 4.50	$2.32 \times 10^{-4} \pm 7.28 \times 10^{-5}$	3.62 ± 1.81
SCMC3 / GLY40 / AVG70	0.02 ± 0.01	24.76 ± 10.37	0.45 ± 0.03	67.29 ± 4.05	$6.97 \times 10^{-4} \pm 2.75 \times 10^{-4}$	2.55 ± 1.21

TABLE 3. Composition of the optimised SCF and CHF films

Component	SCF	CHF
Polymer	3% w/w Sodium Carboxymethyl Cellulose (SCMC)	3% w/w Chitosan (CHI)
Aloe vera Gel	70% w/w	50% w/w
Plasticizer	Glycerine (40% of total polymer weight)	Glycerine (40% of total polymer weight)
Permeation Enhancer	1 drop of Tween 80	1 drop of Tween 80
Sweetening Agent	0.125% w/v Aspartame	0.125% w/v Aspartame
Cooling Agent	0.5% w/v Mannitol	0.5% w/v Mannitol
Bovine Serum Albumin	0.45 mg	0.45 mg

TABLE 4. Characterization of albumin loaded buccal films

Formulation	Weight (mg)	Thickness (mm)	Tensile strength (MPa)	Modulus elasticity (MPa)	Elongation till break point ($\%mm^{-2}$)	Percentage elongation (%)	Strain	Tear resistance (N)
CHF	78.43 ± 6.05	0.13 ± 0.03	0.01 ± 0.00	9.00 ± 1.22	0.32 ± 0.03	48.14 ± 5.01	$1.34 \times 10^{-3} \pm 1.16 \times 10^{-4}$	1.80 ± 0.29
SCF	107.87 ± 0.49	0.19 ± 0.01	0.02 ± 0.00	11.15 ± 3.36	0.49 ± 0.06	73.83 ± 9.21	$2.08 \times 10^{-3} \pm 8.84 \times 10^{-4}$	3.17 ± 0.70

Formulation	Drug content (%) ± SD	Relative standard deviation (%)	Cumulative permeation ($\mu g/cm^2$)	Drug flux ($\mu g/cm^2/h$)
CHF	34.2 ± 11.7	34.20	38.38 ± 5.15	2.214 ± 0.411
SCF	30.0 ± 3.5	11.54	71.09 ± 8.61	5.195 ± 0.669

The purpose of the permeation study was to identify the mechanism through which the medication crosses the buccal surface and is released. The film swelled dramatically during the permeation trials, creating a channel for the albumin to randomly diffuse out of the formulation. The results show that the structural integrity of buccal films was maintained throughout the release period due to a combination of physical entanglement, intermolecular hydrogen bonding, and optimized polymer concentration. The films were carefully optimized using 3% w/w of either SCMC or CHI, which are known for forming stable, cohesive, and mucoadhesive matrices (Cazorla-Luna et al. 2021; Zhang et al. 2013).

Moreover, the presence of glycerine as a plasticizer and aloe vera gel, which contains naturally occurring polysaccharides like acemannan and glucomannans, further contributed to film flexibility and structural resilience (Pamlényi et al. 2021). This ensured that the films swelled but did not dissolve during the 6-h *ex vivo* permeation studies in simulated saliva fluid (SSF). Instead of disintegrating, the films gradually hydrated and formed a gel-like layer, allowing controlled release of albumin while preserving matrix integrity.

Table 5 presents the kinetic analysis of albumin permeation from all formulations, including the calculated drug release coefficients (R^2) and the diffusion exponent 'n' from the Korsmeyer-Peppas model. Four mathematical models were used to evaluate the drug release kinetics: the first-order model (log cumulative percentage of drug remaining vs. time), the zero-order model (cumulative amount of albumin permeated vs. time), the Higuchi model, and the Korsmeyer–Peppas model (cumulative percentage albumin permeated vs. log time). The release kinetics of albumin from the buccal films followed the Korsmeyer–Peppas model, with an n value of 0.65, indicative of non-Fickian or anomalous transport. This suggests that albumin release is governed by a combination of diffusion through the hydrated polymer matrix and polymer chain relaxation or erosion, characteristic of swellable polymer systems (Siepmann & Peppas 2001). The films formulated with SCMC or CHI exhibited significant swelling behaviour in simulated saliva fluid (SSF), enabling albumin to diffuse through the swollen matrix while maintaining structural integrity. The observed matrix cohesion is likely due to extensive hydrogen bonding and physical entanglement among polymer chains, which slow down disintegration and support controlled release. Furthermore, the inclusion of glycerine as a plasticizer may have enhanced polymer chain mobility, aiding matrix relaxation, while polysaccharides in Aloe vera gel (such as acemannan and glucomannans) reinforced the gel structure and supported sustained hydration (Chelu et al. 2023). This interplay between diffusional and relaxation mechanisms explains the non-Fickian diffusion profile observed in our buccal film system.

MOLECULAR MODELLING

The average molecular binding energy between carboxymethyl cellulose and the albumin was found to be -4.38 ± 0.22 kcal/mol, and -6.46 ± 0.47 kcal/mol for CHI and albumin. In addition, studies were also conducted to propose the binding site, bond length, and interactions between polymers and albumin (Figure 2). It was found that different interactions, such as conventional hydrogen bonds and carbon hydrogen bonds, exist between carboxymethyl cellulose and albumin and CHI and albumin (Table 6). Furthermore, sodium in carboxymethyl cellulose molecule is also responsible for electrostatic attractive charges.

Albumin could interact with sodium carboxy methyl cellulose through SER-419, THR-422, GLU-505, THR-420, and THR-506. Whereas Albumin could also interact with CHI through GLU-505, LEU-112, ASN-111, ASN-109, and PRO-110. In general rule, shorter the bond distance the stronger will be the bond interaction between the two molecules. SER-419 and GLU-505 from SCMC showed the strongest hydrogen bonding interaction with the albumin, whereas for CHI, LEU-112, ASN-109, and ASN-111 had the strongest hydrogen bonding interaction with the albumin. The details of the interaction positioning and the bond length present between each interaction is given in the Table 5. This information proved valuable in guiding the formulation optimization process, supporting our experimental findings related to albumin release kinetics and buccal permeation. Specifically, it helped to explain why SCMC films exhibited superior release and permeation profiles compared to CHI films. Thus, the molecular docking approach not only complemented our experimental data but also provided a mechanistic rationale for the observed drug release behaviour, reinforcing the overall design and functionality of the buccal protein delivery system. Interactions between proteins and the polymer matrix, such as hydrogen bonding or hydrophobic interactions, can influence protein release kinetics. Molecular docking results showed that CHI formed stronger binding interactions with albumin (average binding energy: -6.46 ± 0.47 kcal/mol) compared to sodium CMC (-4.38 ± 0.22 kcal/mol). These stronger interactions likely resulted in greater retention of albumin within the matrix, thereby slowing its release.

By altering the polymer's surface chemistry, these interactions can be controlled. The protein molecules are held in place by a scaffold or matrix made of CHI and SCMC. Diffusion allows the proteins to be released from this matrix. The size and molecular weight of the protein as well as the characteristics of the polymer matrix affect the rate of diffusion. In addition, although molecular docking provided valuable insights into the potential hydrogen bonding and electrostatic interactions between albumin and

TABLE 5. Mathematical kinetic modelling for the buccal films

Formulation	Zero order R^2	First order R^2	Higuchi R^2	Korsemeyer-Peppas R^2	n
CHF	-5.1413	-3.5133	-0.2602	0.9969	0.215
SCF	-2.0599	-0.1400	0.5546	0.9965	0.280

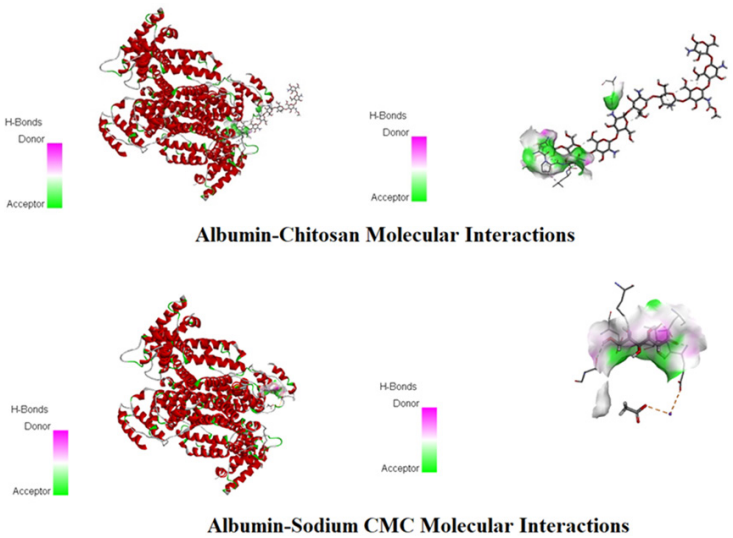

FIGURE 2. Molecular modelling of albumin-chitosan and albumin-sodium CMC

TABLE 6. Interaction details between the polymers and albumin, their category and the bond distance

SCMC-Albumin	Category	Distance A°
CMC:Na1 - A:GLU505:OE1	Electrostatic	4.13724
A:GLU505:N - CMC:O7	Hydrogen Bond	2.19589
CMC:H29 - A:THR420:OG1	Hydrogen Bond	2.50746
CMC:H26 - A:THR506:OG1	Hydrogen Bond	2.69441
A:THR422:N - CMC:O5	Hydrogen Bond	2.53127
CMC:H22 - A:SER419:OG	Hydrogen Bond	3.0897
CMC:H15 - A:SER419:OG	Hydrogen Bond	2.21151
CHI-Albumin	Category	Distance A°
Chitosan:H157 - A:GLU505:OE1	Hydrogen Bond	2.37187
Chitosan:H177 - A:LEU112:O	Hydrogen Bond	1.52722
Chitosan:H134 - A:ASN111:OD1	Hydrogen Bond	2.38968
Chitosan:H135 - A:ASN111:O	Hydrogen Bond	1.98861
Chitosan:H135 - A:ASN111:OD1	Hydrogen Bond	3.06322
Chitosan:H158 - A:ASN109:OD1	Hydrogen Bond	1.78185
Chitosan:H158 - A:PRO110:O	Hydrogen Bond	2.76877
Chitosan:H180 - A:ASN109:OD1	Hydrogen Bond	2.99683
Chitosan:H180 - A:PRO110:O	Hydrogen Bond	2.8924

the polymer matrices, the inclusion of FTIR spectroscopy in future work would offer complementary evidence by directly identifying functional group interactions and confirming structural changes within the films.

CONCLUSION

Because of its wide molecular binding ability, circulatory circulation, and biocompatibility, albumin is an attractive candidate for integration into drug delivery systems. The objective was to optimize the concentration of polymers in order to create a buccal protein delivery system that works well. For this purpose, mucoadhesive films were prepared from Aloe vera gels combined with SCMC or CHI. It was observed that SCMC buccal film had significantly higher ($p < 0.01$) tear resistance, strain, percentage elongation, breakpoint, modulus elasticity and tensile strength as compared to CHI buccal film. SCMC films exhibited significantly higher albumin release ($71.09 \pm 8.61 \mu g/cm^2$) compared to CHI films ($38.38 \pm 5.15 \mu g/cm^2$) and both formulations showed compliance with the Korsemeyer-Peppas model ($r \wedge 2$ approaching ≈ 0.99, $n = 0.65$) indicating non-Fickian showed as a dominant mechanism of drug permeation. The molecular docking studies revealed interactions between albumin and polymers, with stronger hydrogen bonding observed between certain residues of the polymers and albumin, particularly SER-419 and GLU-505 in SCMC and LEU-112, ASN-109, and ASN-111 in CHI. These findings contribute to understanding the mechanisms underlying drug release and binding interactions, facilitating the development of more effective drug delivery systems, ultimately leading to more efficient and targeted therapeutic interventions. Future studies incorporating FTIR analysis are recommended to experimentally validate the molecular interactions predicted through docking, thereby strengthening the mechanistic understanding of albumin–polymer binding within the buccal film matrix.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Education, Malaysia for supporting this research via the Fundamental Research Grant Scheme (FRGS/1/2016/SKK09/UKM/02/4). The authors have no conflict of interest to report.

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