Multiphysics model development to characterize fundamental mechanism of bio-responsive hydrogels

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**MULTIPHYSICS MODEL DEVELOPMENT
TO CHARACTERIZE FUNDAMENTAL
MECHANISM OF BIO-RESPONSIVE
HYDROGELS**

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Abstract

Environmentally bio-responsive hydrogel is of tremendous interest in plethora of advanced engineering applications, such as hydrogel-based urease-loaded dialysis membranes and hydrogel-based hemoglobin-mediated oxygen carrier. However, literature reviews reveal that the coupled bio-chemo-electro-mechanical responses of these hydrogels remain poorly understood, due to lack of accurate mathematical models to numerically characterize the environmental-induced hydrogel synergistic performances. Therefore, the present research work focuses on the development of multiphysics models to obtain a deeper understanding of such materials, especially when operating at extreme of physiological environmental conditions.

The first academic achievement made in this present work is the development of a multiphysics model for describing the coupled bio-chemo-electro-mechanical responses of urease-loaded hydrogel. By coupling the multiphysics interactions occurring in the hydrogel together, the model consists of three governing mass, momentum and energy conservation equations, and also four sets of constitutive relations, namely mass flux, fixed charge equation, and nonlinear mechanical equation. A novel rate of reaction is also incorporated into the model to describe the urease activity as a function of ambient temperature coupled with environmental solution pH, capturing the environmental-sensitive urease ionization and denaturation states. For model validation, the multiphysics model is examined with the comparison between present numerical results and published experimental observations, where good agreements are achieved, especially for temperature-, pH- and urease-induced swelling deformations and urease catalytic activity of the polyelectrolyte hydrogels. The result shows that the urease catalytic activity patterns differ in

anionic and cationic urease-loaded hydrogels by increasing the environmental concentration of sodium chloride at a relatively higher environmental concentration of urea, whereas the urease catalytic activity remains almost unchanged when the environmental pH increases above the acid-base dissociation constant pK_a of the polyacidic hydrogel. The result also shows that the osmotic pressure response of urease-loaded hydrogel enlarges linearly by increasing physiological urea concentration making it biocompatible for healthcare diagnostic applications.

The second academic achievement is the development of another multiphysics model to elucidate the coupled-stimulated responses of hemoglobin-loaded polyelectrolyte hydrogels. A developed constitutive relation is integrated into the model to capture immobile hemoglobin bioactivity as a function of ambient oxygen coupled with environmental pH. After validation against the reported experimental observations, it is taken that the multiphysics model can effectively characterize the hemoglobin saturation with oxygen for (1) neonatal and (2) adult hemoglobins, and also the pH-induced swelling deformation of hemoglobin-loaded polyelectrolyte hydrogels. The result demonstrates that the hydration-induced swelling deformation of the polyampholytic hydrogel changes in a bowl-shaped fashion by increasing the environmental pH value, in which the pH-induced swelling deformation of initially balanced polyampholytic hydrogel changes from a “bowl” to “V”-shaped like pattern with decrease of immobile acidic and basic components ionization strength. In addition, the result demonstrates that the strength increase of both the immobile acidic and basic components in the initially balanced polyampholytic hydrogel causes the hydrogel to exhibit isoelectric point behavior at wider environmental pH range, whereas the initially

unbalanced polyampholytic hydrogel collapses at the environmental pH coinciding with dissociation constant of the dominant immobile charge group, if the initial dominant immobile charge group concentration is twice that of the counter one.

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Table 4.1 Input data for the numerical simulation of the multiphysics model for hemoglobin-loaded hydrogel.

Chapter 1 Introduction

In this chapter, the background and motivation, objective and scopes and the layout of this thesis are presented.

1.1 Background and Motivation

Environmentally responsive hydrogels are the go-to choice of material in the field of medicine for two main reasons: (1) low in toxicity and (2) excellent biocompatibility [1]. It is well-established that the hydrogel exhibits a variety of practical electromechanical properties which include super absorption capacity, great swelling/de-swelling capability, high counter-ion permeability and excellent catalytic behavior [2], leading to the exploitation of the hydrogel in numerous bioengineering applications, such as surgical actuators, dialysis membranes and drugs. One of the earliest application of the hydrogel in medicine is current contact lens, as proposed by Wichterle and Lim [3], replacing plastic-based first-generation contact lens which was usually associated with high material toxicity. The environmentally responsive hydrogels are usually functionalized with stimuli-reactive neutral/charged monomers [4] which results in the hydrogel to exhibit coupled bio-chemo-electro-mechanical responses, when subjected to environmental cues, including, but not limited to, ion concentration, ionic strength, light intensity, pressure, magnetic or ultrasound [5-7].

A major advancement in the research front of environmentally-responsive hydrogels is the development of bio-responsive hydrogel where researchers integrated bioactive agents into the hydrogel, which initiates a new branch of (bio) analyte-specific environmental-responsive hydrogels. Thereby, the bio-responsive hydrogel usually consists of two main functional components: (i)

sensor and (ii) actuator. The immobilized bioactive agent in the hydrogel acts as the biosensor component due to its highly selective chemical reactions. Once the bio-responsive hydrogel encounters the relevant stimulus, it generates a chemical reaction in the hydrogels. This triggers a mechanical response by the polymeric network chains, which behave as the actuator component of the system. Literature surveys revealed that the bio-responsive hydrogels include, but not limited to, glucose oxidase-, urease-, lipase-, hemoglobin-, antigen- or antibody-loaded hydrogels [2, 8-11]. These exciting new biomaterials show tremendous application potentials in a broad range of bioengineering medical devices which explains the emergence of new bio-responsive hydrogel-related works in academic databases. Hence, it is worthwhile and necessary to examine the fundamental mechanism of these bio-responsive hydrogels to address paucity of information in the literature.

In the quest to investigate the newly developed material, scientists employed costly and time-consuming trial-and-error experimental technique conducted to investigate the behaviors of the hydrogels under varying environmental conditions. As a result of the trial-and-error experiment, researchers were often led to off-tangent research directions [4]. Interestingly, multiphysics modeling is a viable alternative option for obtaining a further understanding into the reactive behaviors of the bio-responsive hydrogel. However, the published mathematical models in open literature are incapable of predicting the coupled bio-chemo-electro-mechanical responses of the hydrogels [10, 12]. It is also noted that the published mathematical models failed to take into account the impacts of the environmental circumstances on the performance of the bio-reactive hydrogels, especially pH, temperature or

ionic strength. In order to overcome these shortcomings, it is worthwhile to develop multiphysics models for the simulation of the bio-responsive hydrogels for predicting the coupled multi-domain responses of the bio-responsive hydrogels in response to specific stimulus under varying environmental conditions. The models will be able to elucidate the fundamental mechanism and key parameters of the hydrogels and then to achieve an optimization of the hydrogel-based machines, such as sensors/actuators and bio-micro-fluidic valves [13]. Therefore, due to the huge diversity in applications of these bio-responsive hydrogels, it is worthwhile and necessary to develop a detailed theoretical model for describing and getting a greater understanding into the responsive performance of the hydrogels.

1.2 Objective and Scope

The aim of this present research work is to develop two multiphysics models for simulation of the behavior of urease- and hemoglobin-loaded hydrogels by coupling the electrical, chemical, and mechanical fields together to obtain a greater insight into such materials. The specific objectives are detailed as follows.

- ❖ Model development. Two multiphysics models are developed to describe the fundamental mechanism of urease-loaded and hemoglobin-incorporated hydrogels by merging chemo-electro-mechanical domains. In the model, a correlation between the functional group (urease/hemoglobin) and environmental conditions is integrated into the model, capturing the bioactivity of the system.
- ❖ Model validation. In order to develop a robust mathematical model, the present numerical results obtained from the model are validated against the

experimental observations, where good agreements are achieved for both models.

- ❖ Model exploration. The model is numerically solved, where the present numerical results are utilized to investigate qualitatively and quantitatively the performance of the hydrogels in variation of biochemical, electrical, and mechanical fields.

(i) *Hydrogel performance in the biochemical field.* The present work improves the understanding of the bioactivity of fixed urease and hemoglobin when operating in charged polymeric systems. In particular, the effects of environmental conditions on chemical reaction of urease/hemoglobin are also elucidated, including environmental solution pH, ionic strength and temperature.

(ii) *Hydrogel performance in the electrical field.* The current work describes the influence of environmental conditions on the electrical performances of the hydrogels, especially its electrical potential acting over hydrogel-solution interface. In addition, the effects of initial immobile charge concentration are also examined on the hydrogel electrical behaviors.

(iii) *Hydrogel performance in the mechanical field.* The present work characterizes the coupled biochemical-electrical effects on the responsive hydration-induced mechanical deformation of the hydrogel. As such, the environmental-induced mechanical deformation is also described when responding to the extreme of physiological conditions.

1.3 Outline

This thesis comprises of five chapters, as outlined below.

Chapter 1 explains the motivation of investigating the fundamental mechanism of urease-loaded and hemoglobin-enriched hydrogels followed by the objective and scope and outline of the present thesis.

Chapter 2 examines the current literature on the experimental and theoretical works on reactive performances of the bio-responsive hydrogels, especially the urease-loaded and hemoglobin-incorporated hydrogels. In addition, the modeling works are also examined to unveil the limitation of the published mathematical model.

Chapter 3 formulates a multiphysics model to investigate the reactive performance of urease-loaded hydrogel by incorporating bio-electrochemical interactions between environmental urea-rich salt solution and functional groups within the hydrogel. The model is directly validated by comparing against published experimental results, where it reasonably captures the environmental-induced reactive performances of urease-loaded hydrogel.

Chapter 4 describes a multiphysics model to examine the coupled responses of hemoglobin-loaded polyelectrolyte hydrogel by accounting bio-electrochemical interactions between environmental oxygen-rich salt solution and functional groups within the hydrogel. The model is directly validated by comparison with published experimental data, where it rationally characterizes the dual oxygen-pH coupled stimuli behaviors of the hydrogel.

Chapter 5 entails the present theoretical efforts with recommendation for future work.

Chapter 2 Literature Review

This chapter deals with literature surveys of environmentally bio-responsive hydrogels, especially protein-enriched hydrogels. After that, literature surveys are also conducted for the modeling of hydrogel and protein equilibrium responses.

2.1 Experimental Investigation on Bio-responsive Hydrogels

The word hydrogel is derived from the Latin prefix hydro where “hydro” means water and “gel” referring to a solid structure [14]. As such, hydrogel is a three-dimensional (3D) structure consisting of highly cross-linked polymeric network chains [15-17], where the hydrogel is able to imbibe and retain large fluid quantity in the system due to hydrophilic and elastic nature of the polymeric network chains. Thereby, the polymeric system consists of large fraction of water with a small polymer counterpart [3, 18], resulting in the hydrogel to demonstrate mixed solid-liquid characteristics. The ingestion of fluid into the hydrogel stretches its polymeric network chains, up till to a certain extent, where the elastic-nature of the crosslinked polymeric network chains prevents the dissolution of the polymeric system during hydration [19]. It is well-established that the random-nature cross-linkage of the polymeric network chains creates a porous structure, where the increase of hydrogel hydration promotes the movement of mobile environmental cues into its system.

Interestingly, environmentally responsive hydrogel is capable of converting environmental cues into an electromechanical response, when the polymeric network chains encounter the environmental cues, such as glucose [20], urea [21], salt [22], temperature [23], light [24] and pH [25]. Literature survey revealed that the focus of investigations into environmentally responsive

hydrogels is usually pointed to pH-responsive hydrogel, where it is synthesized by incorporating weakly acidic or basic group. As such, the ionization state of the immobile charge group can be tuned by manipulating the environmental pH, where the increase of pH-governed ionized immobile charge component concentration usually induces the hydration of the polymeric system. The polymeric system usually contains anionic and/or cationic components [26-28].

The microscopic structure of an ionic hydrogel in which is functionalized with immobile charged components, constructing the hydrogel. It is commonly known that hydrogel is able to swell up to 1000-fold of its initial size, due to the electrostatic interactions of fixed-fixed and fixed-mobile and mobile-mobile charge components in the polymeric system [29, 30]. Interestingly, the mechanical performance of the polymeric hydrogel can be tuned with the manipulation of the electrostatic interactions of the immobile and mobile charge components via changing the environmental solution salt concentration [31].

On the one hand, the anionic hydrogel consists of weakly acidic group on the polymeric network chains, which forms cation mobile solutes and anionic immobile charge group when subjected to environmental solution with specific pH values, including poly(methacrylicacid)-, poly(acrylic-acid)-, poly(N-isopropylacrylamide-co-acrylic-acid)- poly(methacrylic-acid-ethylene-glycol)-, poly(methacrylic-acid-co-methacryloxyethyl-glucoside)-, and poly(N,N-dimethylacrylamide-co-2-acrylamido-2-methylpropane sulfonic acid)-based hydrogel [32-49]. It is known that the fixed acidic group of the hydrogel remains un-ionized, when subjected to environmental $\text{pH} < \text{acid-base dissociation constant } \text{pK}_a$ which makes the hydrogel to behave like a neutral hydrogel [50]. On the other hand, the cationic hydrogel composes of weakly

basic group attached on the polymeric network chains which ionizes into anion mobile solutes and cationic immobile charge group in response to environmental solution, where the weakly basic group, such as amine, are ionized by accepting positive charge mobile ions from the environment solution [51]. This includes poly (2-diethylaminoethyl-methacrylate)-, poly (ethylene oxide)- and poly (ethyleneimine)-based cationic hydrogels. It is well-established that fixed basic group tends to remain unionized, when it is immersed in environmental $\text{pH} > \text{acid-base dissociation constant } \text{pK}_a$ of the basic group. The ionization of fixed acidic/basic group of the hydrogel causes the increase hydration-induced swelling deformation of the hydrogel [50], due to greater electrostatic repulsion between fixed-fixed charges coupled with larger imbalance of counter-ion concentration over the hydrogel-solution interface [52].

We now ask: What is bio-responsive hydrogel? In order to answer this question, literature surveys are conducted in which they reveal that the bio-responsive hydrogel is usually developed by incorporating biological components onto the acidic/basic-loaded crosslinked polymeric network chains (environmentally responsive hydrogel) via physical entrapment or UV radiation [53, 54]. This enhances the specificity of hydrogel, where it only exhibits a response upon stimulation by certain environmental bio-cues [19]. Due to the interesting characteristics of bio-responsive hydrogel, such as specific catalytic behaviors, excellent ion-sorption ability and high swelling capability, it is thus exhibits great potential as a material platform for developing soft actuator, dialysis membrane, gas carrier and artificial muscle, consequently requiring greater attention amongst research to address paucity of information of such

materials in the literature, where this thesis focuses on the investigation of both urease-loaded and hemoglobin-incorporated hydrogels.

2.1.1 Urease Immobilized Hydrogel.

It is well-established that urease is a naturally occurring group of enzymes found in bacteria, fungi, algae, invertebrates and plant. A literature surveys unveil that majority of the published investigation aimed on the urease derivation from different bacteria and various plants. Thereby, the plant-based ureases were usually obtained from jack bean [55], soybean [56] and pigeon pea [57], while the bacterial-origin ureases were typically extracted from *Bacillus pasteurii* [58, 59], *Proteus mirabilis* [60] and *Providencia rettgeri* [56]. To improve the yield of the enzyme, urease was typically purified, increasing the urease catalytic activity by either: (i) crystallization [61] or (ii) chromatographic [62]. In order to synthesize the urea-sensitive urease-loaded hydrogel, researchers employed several techniques for the immobilization of urease in the hydrogel, such as: (i) covalent binding [63], (ii) adsorption [64], (iii) encapsulation [65], and (iv) entrapment [66]. Since the hydrogel retains large amount of water inside its polymeric network chains, which mimics the biological tissues to some extent, the urease immobilization in the hydrogel provides a suitable microenvironment for the operation of the urease, improving the stabilization of urease towards environmentally induced denaturation such that it is physically more robust to free urease in a free solution [67]. Bayramoglu and Arica [53, 68-71], Baysal *et al.*, [72-74] and Chen and Chiu [63, 75-77] made significant contribution into the experimental investigations of urease immobilized hydrogels.

Bayramoglu and Arica developed urease-loaded hydrogels via irradiation of UV or gamma rays on a series of various co-polymers, such as NVP, NIPAM, HEMA and PEG with urease [53, 68-71]. They reported that improvement of pH- and temperature-induced urease catalytic activity, and the urease immobilized in poly(N-isopropylacrylamide-co-poly(ethyleneglycol)-methacrylate) hydrogel retained almost ~100.0 % of the enzymatic activity after eight times of use [53], demonstrating consistency with experimental observation presented by Krajewska *et al.* where the immobilized urease in polysulphone membrane was almost independent of the number of reuse after 17 times of usage [78].

Furthermore, Chen and Chiu proposed a polymeric system-based catalytic reactor, where anion-exchange membrane and urease-loaded hydrogel were clamped together to divide feed and stripping solutions [63, 75-77]. The five-compartment electro-dialyzer employing urease functionalized with polymeric system to remove urea from aqueous solution. Besides that, Lee *et al.* [79] and Moynihan *et al.* [80] also developed hydrogel-based electro-dialyzers with the protected urease for the regeneration of the dialysate solution. Interestingly, Baysal *et al.* integrated urease in PEG-based hydrogel, where it was then encapsulated within the living red blood cells [72-74] as an injectable urea dialyzer for transport of reactive urease in the vasculature system.

Over the past decades, there were great efforts to immobilize urease in hydrogels for potential applications as bioreactor, biosensor and bioseparator [63, 80-82], in which the hydrophilic polymeric network chains: (i) provided support; (ii) improved stability towards variation of pH, temperature and ionic strength; (iii) increased shelf life for the immobilized urease [83-87].

Unfortunately, the fundamental understanding of urease-loaded charged hydrogel [85] as a biofunctional membrane is still preliminary. Therefore, the urease catalytic performance of the urease-loaded anionic and cationic hydrogel is necessarily investigated, when subjected to variation of environmental cues, such as pH, temperature, urea and sodium chloride.

A literature search reveals that the effect of environmental sodium chloride concentration [88] still remains unclear on the performance of urease catalytic in the charged hydrogel [89]. Usually the change in the environmental sodium chloride concentration alters the fixed group density of the urease-loaded charged hydrogel [90], forming a new microenvironment with different ionic concentration and pH values in the hydrogel. This perhaps leads to the change of urease catalytic activity due to the modification of the environmental sodium chloride concentration. As such, the behavioral patterns of urease catalytic activity in the anionic or cationic hydrogel are necessarily examined as a function of the environment sodium chloride concentration. The literature survey also reveals that, the previous investigations aimed only on the impact of environmental pH and temperature on the catalytic activity in the charged hydrogel [89, 91, 92]. Therefore, in-depth research on the urease catalytic behaviors in the charged anionic or cationic hydrogel are necessarily elucidated as a function environmental sodium chloride concentration to understand the way sodium chloride modifies the reactive performances of the urease-loaded hydrogels.

To our best knowledge, no study was involved in the urease catalytic behaviors in the charged hydrogel with variation of environmental urea concentrations at different catalytic and Michaelis constants. For a nonspecific

immobilized enzyme in a charged polymeric environment, several interesting experimental observations were reported on its catalytic behaviors [93], including (1) the dilution of the enzymatic activity due to hydrophilic nature of the polymeric system [50], (2) the ionization of the enzyme due to the acidic or basic nature of the polymeric system [94], and (3) the alteration of optimum operating conditions of the enzyme [94]. However, some experimental observations seem to be conflicting each other, in which few of them demonstrated that the enzyme activity improves when operating in a charged polymeric environment [89], while another experiment showed that the immobilization of enzyme into a charged hydrogel decreases its enzymatic activity [95]. As such, it is worthwhile to develop a multiphysics model to elucidate the urease catalytic activity in a charged hydrogel in response to urea cue with variation of catalytic and Michaelis constants to systematically tailor such materials.

2.1.2 Hemoglobin-loaded Hydrogel.

In general, free hemoglobin consists of two components, the organic and inorganic parts [96-98], where the inorganic heme iron complex of hemoglobin binds with oxygen O_2 molecules [99-101], forming oxygen-heme iron complexes [102]. Interestingly, the association of oxygen O_2 with the iron complex leads to a tense-to-relax structural change in the hemoglobin, altering oxygen O_2 affinity of the free hemoglobin [103-106]. In addition, formation of the free oxygen-hemoglobin complex is also influenced by environmental pH, where the affinity of hemoglobin towards oxygen O_2 is decreased, when acidity of the environmental solution increases. It is commonly known that despite the attractive capability of the hemoglobin for sensing and capturing oxygen O_2 in

salt solution [107], a few crucial electrochemical reactions influence performance of free hemoglobin in biological fluids including: (1) the environmental pH-induced reduction of hemoglobin-oxygen activity [108] and (2) the oxidization of oxygen-binding group (heme) in the hemoglobin [109]. Therefore, researchers began immobilizing hemoglobin into hydrogels as a plausible strategy to enhance the performance of the hemoglobin for sensing and storing of oxygen O₂ in salt solution [108, 110-114]

Literature surveys unveil that there were tremendous investigation to realize the possibility of integrating hemoglobin in the polymeric system, especially by Chang *et al.* [115-117] and Palmer *et al.* [108, 112, 118, 119]. The focus of the investigations of hemoglobin-hydrogel system were pointed to the: (i) pH-induced swelling behaviors of hemoglobin-loaded hydrogel [2], (ii) hemoglobin adsorption into hydrogel reported by Shirahama *et al.* [120], Vidal *et al.* [121], Uysal *et al.* [122], and Dessy *et al.* [123], (iii) hemoglobin isolation from human blood via hydrogel-based bio-selector, (iv) molecularly imprinted method for developing hemoglobin immobilized hydrogels conducted by Xia *et al.* [124], Uysal *et al.* [122] and Gou *et al.* [125].

In terms of application, there were great efforts to incorporate hemoglobin into the hydrogels for developing bio-engineering devices, such as: (i) biosensors by Sun *et al.* [126], Shan *et al.* [127], Chen and Lu [128], Zheng *et al.* [129], Reddy *et al.* [130], and Zhong *et al.* [131] and (ii) oxygen carriers reported by Philips *et al.* [132], Eike and Palmer [118], and Li *et al.* [133]. Unfortunately, literature reviews unveil that the reactive performances of hemoglobin-loaded hydrogel remain poorly understood, in which no efforts were conducted experimentally and theoretically to investigate the performance

of hemoglobin-loaded polyampholyte hydrogel as a function of ambient oxygen O_2 coupled with environmental pH.

It is well known that the oxygen O_2 and pH are two critical biomarkers in the human system, which facilitate: (i) the detection of diseases, such as breast and liver cancers [134] and (ii) the navigation of gas transport in hemoglobin-rich blood [135]. As a result, it is important for an advanced biosensor to detect and measure these two important biomarkers in biological fluids, simultaneously. In addition, in terms of the hydrogel-based biofuel cell [136], particularly the enzyme mediates pH-driven one [137], their performances are usually limited by the oxygen O_2 concentration in the microenvironment [138], due to poor solubility of the physiological gas in the water-loaded polymeric system. Therefore, it is necessary to incorporate the hemoglobin into the polyampholyte hydrogel for developing and characterizing the oxygen-pH stimuli coupled biosensing or oxygen-rich biofuel material platform [108], in which the hemoglobin is inherently associated with the attractive oxygen O_2 sensing and capturing properties [139], while the acidic-basic groups in the hydrogel undergo ionization/deionization in response to environmental pH. In other words, incorporation of both the hemoglobin and immobile charge component with the polymeric network chains give rise to its dual oxygen- and pH-reactive behaviors. Therefore, it is worthwhile and necessary to elucidate the reactive performances of the hemoglobin-loaded hydrogel, especially via theoretical modelling for systematically tailoring such materials as the biosensing and biofuel cell platforms [140, 141].

2.1.3 Other Bio-responsive Hydrogels

On the one hand, lipase enzyme can be integrated with the hydrogel for catalyzing the esterification of lipid. It is commonly known in biology that lipid is a group of organic groups where it is insoluble in water, including hormones, fats and oils. Literature searches reveal that majority of the published investigation aimed on the extraction of lipase from *Candida rugose* which is a group of fungi or *Pseudomonas aeruginosa* which is a type of microbial [142-147]. The focus of the investigations of lipase-loaded system was usually aim to: (i) the stabilities of the immobilized lipase [148-151], (ii) the enhancement of lipase catalytic activity by improving accessibility of the lipid into the composite hydrogels [152-160], (iii) the lipase leaching and activity in lipase-alginate/chitosan hydrogel as reported by Betigeri and Neau [161], (iv) the coupled effects of pH and temperature on the immobilized lipase catalytic performance [9, 162-165], (v) the mechanical performance of the hydrogel when subjected to different environmental solvents [166, 167]. In terms of application, the lipase-loaded hydrogels were usually explored as bioreactors and super-absorbent [168, 169].

On the other hand, enzyme-sensitive biomaterial can be integrated with the polymeric network chains for developing enzyme-induced biodegradable polymeric systems. It is well-reported that the polymer biodegradation involves the dissolution of its crosslinked polymeric network chains, which is facilitated by enzymatic biological agents. Literature searches unveil that majority of the published investigation aimed on the enzyme such as: (i) esterase, proteases, collagenase and bovine serum albumin [170, 171]. In terms of application, the

lipase-loaded hydrogels appear to be attractive in the field of medical, bioreactor, and biomedical devices.

2.2 Modeling the Equilibrium of Hydrogel Responses and Protein Activities

The continuum mathematical models in open-literature were developed for capturing the environmentally responsive behavior of hydrogel and protein, responding to different stimuli. Here, a good starting point for developing the new mathematical model will be the mass, energy and momentum conservation laws.

2.2.1 Transport Model

The transport model was firstly developed by De *et al.* [172] for investigating the mechanical responses of the hydrogel, in which the model was extended by later researchers [173-176]. The model was derived from the mass conservation law, or so-called the diffusion equation, where the ionic transport is caused by its concentration gradient over the hydrogel-solution interface. In order to consider the migration of ion transport in the system, the model was extended to include the electrical potential effect on the mobile ion concentration in the hydrogel, coupling the effect of diffusion-governed with the migration-administered ionic transport [177], given as follows

$$-\nabla \cdot \left(\frac{D_k}{RT} c_k \left[RT \frac{1}{c_k} \nabla c_k + z_k F \nabla \Phi \right] \right) + v_k r_k = 0 \quad (k = 1, 2, 3, \dots, N) \quad (2.1)$$

where D_k , c_k , R and T indicate the diffusivity tensor (m^2/s), concentration (mM), universal gas constant (8.314 J/mol K) and absolute temperature (K). z_k , F and Φ indicate the valence number, Faraday constant ($96,487 \text{ C/mol}$) and

electrical potential (mV). v_k represents coefficient of the reaction rate and r_k refers to the source term (mM/s).

The electrical potential is modelled by the Poisson equation, which correlated the electrical potential acting over the hydrogel-solution with total charge concentration of the system

$$\nabla^2 \Phi = -\frac{F}{\varepsilon_r \varepsilon_0} \left(\sum_{k=1}^N z_k c_k + z_f c_f \right) \quad (2.2)$$

where ε_r is the relative dielectric constant of the surrounding medium, ε_0 the vacuum permittivity ($8.85418 \times 10^{-12} \text{ C}^2/\text{N m}^2$), z_f valence of the fixed charge concentration and c_f density of the immobile charge groups within the hydrogel, which is given as [178]

$$c_f = \frac{c_{m0}^s}{H} \frac{c_{H^+}}{K_a + c_{H^+}} \quad (2.3)$$

where c_{m0}^s the density of the total ionizable immobile charge groups within the dry gel, H the hydration of the hydrogel and K_a acid-base dissociation constant of the immobile charge groups attached with the hydrogel.

The mechanical deformation problem for hydrogel was characterized using the conservation of linear momentum law, which can be written as

$$\nabla \cdot \sigma = 0 \quad (2.4)$$

where σ is the stress tensor. The transport model suggested that the hydrogel deformation was driven solely by imbalance of ionic distribution the hydrogel-solution, given as

$$p = RT \sum_k c_k - \bar{c}_k \quad (2.5)$$

where p is osmotic pressure acting over the interface of hydrogel-solution and $\bar{c}_k$ is concentration of external solution. The Poisson-Nernst-Planck equations are integrated with the non-linear mechanical equation to complete model formulation.

The second transport model found in the literature is termed the Stefan-Maxwell model, or termed the multicomponent diffusion equation, it was earlier-established by Bisschop *et al.* [179], and followed by other subsequent researchers [178-182]. The model was derived based on the relationship between driving-friction forces, given as

$$F_{dr,k} = F_{fr,k} \quad (2.6)$$

The relationship was further re-termed into the following

$$-\frac{d}{dr} \left[\ln 1 - \phi_p + \phi_p + \chi \phi_p^2 + v \left(\frac{\rho_n}{M_c} \left(1 - \frac{2}{Q} \right) \left(\frac{\phi_p}{\phi_{p0}} \right)^{1/3} \right) \right] = \left(\frac{1}{D_{eff}} \right) \phi_p \mathbf{v}_s - \mathbf{v}_p \quad (2.7)$$

where the volume fraction of polymer is given as ϕ_p , χ is Flory-Huggins parameter, v is partial molar volume of the solvent ρ_n is density of the polymer network in dry solid state, and M_c is average molecular weight between two junctions of the polymeric network chain. The network functionality Q at a cross-link usually has a value of 3 or 4, depending on the type of polymer and cross-linking agent. On the right side of the equation, D_{eff} is effective diffusion coefficient, $\mathbf{v}_s$ and $\mathbf{v}_p$ are of solvent and polymer permeation flow linear velocities.

As such, the model postulated that the hydration of the hydrogel was caused by (i) the mixing of solvent and polymer, (ii) the interaction between hydrogel-

environmental solution and (iii) the elastic forces exhibited by the hydrogel. It is noted that, one major concern associated with this model is the increase effort for determining the transport parameter i.e. the effective diffusion coefficients, especially when dealing with multi-components ion systems.

2.2.2 Multiphasic Mixture Model

As the name suggested, the multiphasic mixture model assumed that the hydrogel consisted of three phases namely, solid, water and ion, in which each phase is associated with its governing equation. The degree of hydration-induced deformation of the hydrogel is governed by electrochemical potential gradient of water and mobile ions between the hydrogel-solution, in which the deformation of the hydrogel is limited to (1) the elastic contractive forces exerted by the hydrogel and (2) the frictional forces between the different phases and solvent.

Interestingly, Feng *et al.* coupled the multiphase model with the transport model, mentioned above [183], where the conservation law of momentum was employed for each phases, given as

$$\begin{aligned} \nabla \sigma_p - \phi_p \nabla p - \phi_w c_f F \nabla \Phi + f_{pw} \mathbf{v}_w - \mathbf{v}_p &= 0 \\ -\phi_w \left(\nabla p - RT \sum_k \nabla c_k \right) + f_{wp} \mathbf{v}_p - \mathbf{v}_w + f_{wk} \mathbf{v}_k - \mathbf{v}_w &= 0 \\ -\phi_w \left(RT \sum_k \nabla c_k + F z_k c_k \nabla \Phi \right) + f_{iw} \mathbf{v}_w - \mathbf{v}_i &= 0 \end{aligned} \quad (2.8)$$

where equation 2.8 can be simplified into

$$\nabla \sigma_p - p = 0 \quad (2.9)$$

and thus, the flow of water relative to polymer network can be written as

$$\mathbf{v}_w - \mathbf{v}_p = -\frac{\phi_w}{f_{wp}} \nabla p + F z_k c_k \nabla \Phi \quad (2.10)$$

The Nernst-Planck equation is used to describe the ionic and molecular transports of the hydrogel, given as

$$\frac{\partial(\phi_w c_k)}{\partial t} = \nabla \cdot \left(\frac{D_k \phi_w}{RT} c_k \left[RT \frac{1}{c_k} \nabla c_k + z_k F \nabla \Phi \right] \right) \quad (2.11)$$

The hydration-induced deformation of the hydrogel is taken to be a small deformation-like behavior, given as

$$\sigma_p = \lambda \text{tr } \varepsilon \mathbf{1} + 2\mu \varepsilon \quad (2.12)$$

where λ and μ are Lamé coefficients of the polymeric network chains, and ε is strain tensor of the hydrogel. The multiphasic model considered the multi-body interaction of the different phases, which was ignored in the transport model. However, this increases the complexity of the model with the introduction of effective diffusion coefficients into the mathematical formulation. On a side note, it is known that the molecular dynamics model investigates the behavior of the hydrogels in microscopic scale. However, when dealing millions of solutes movements between hydrogel-solution system, it is often much more practical to work at a macroscopic level with continuum models.

2.2.3 Urease Enzymatic Activity

Michaelis and Menten developed a general continuum mathematical model to characterize the binding of enzyme with its substrate [184], where it describes the enzyme kinetics as a function of its substrate concentration, given as follows

where E_{act} is active enzyme, S is substrate of the enzyme, ES_{act} is the enzyme-substrate complex, and P is product of the enzyme-substrate reaction. K_M is the Michaelis constant where it measures the affinity enzyme to the substrate, while k_{cat} is enzyme rate of reaction which it indicates how fast the enzyme perform after the formation of the enzyme-subtract complex. Briggs and Haldane established a relationship between the Michaelis constant and the rate of equations, given as [185]

$$K_M = \frac{k_2 + k_{cat}}{k_1} \quad (2.14)$$

where k_1 and k_2 are the forward and backward reactions rate for the formulation of ES_{act} complex. The reaction velocity V_{max} is given as the function substrate, where the equilibrium dissociation constant for the ES_{act} complex is given as follows

$$K_S = \frac{E_{act} S}{ES_{act}} \quad (2.15)$$

and

$$E_0 = E_{act} + ES_{act} \quad (2.16)$$

where Equations 2.15 and 2.16 is coupled to give

$$\frac{E_{Total}}{ES_{act}} = \left(\frac{K_S}{S} + 1 \right) \quad (2.17)$$

Thus the reaction velocity is given as [177]

$$V = \frac{V_{max} S}{K_S + S} \quad (2.18)$$

Unfortunately, the Michaelis-Menten equation fails to account for the environmental-induced, especially environmental temperature. Enzyme starts to denature at temperature above the physiological temperature where it changes the enzyme conformation, in which the non-reversible denaturation process reduced its catalytic activity [186]

The equilibrium model was proposed to account for the denaturation of enzyme as a function of environmental temperature, given as [187-190]. For this purpose, a three-state (active, inactive and denatured states) mathematical model is formulated which describes the enzyme activity via

where two folded forms of enzyme are used, one being catalytically active denoted by E_{act} and the other one is catalytically inactive but not denaturized denoted by E_{inact} and X being the denatured form of the enzyme. K_{eq} is the equilibrium constant and k_{inact} is the enzyme denaturation rate constant.

The enzyme velocity is thus given as

$$V_{max} = \frac{k_{cat} E_0}{1 + K_{eq}} \exp \frac{-k_{inact} K_{eq} t}{1 + K_{eq}}. \quad (2.20)$$

It is reported by [190] that the simulated data obtained from the equilibrium model is able to capture the experimental data more accurately than the conventional model [190].

2.2.4 Reaction of Hemoglobin with Ligands

It is well-established that the formation of oxyhemoglobin on the polymeric network chains is governed by equilibrium reactions between hemoglobin and oxygen O_2 , as shown below

where Hb/HbH^+ and $\text{O}_2\text{Hb} / \text{O}_2\text{HbH}^+$ refer to reduced/protonated reduced and oxygenated/protonated oxygenated hemoglobins, while K_{O_2} denotes association constant of oxygen O_2 from the hemoglobin.

The immobile hemoglobin consists of ionizable functional components, where the ionization of the hemoglobin is determined by the equilibrium electrochemical reactions for the binding/unbinding of hydrogen H^+ ion from the hydrogel

where K_1 and K_2 represent association constants for reduced and oxygenated hemoglobins, and they can be written as $K_1 = [\text{HbH}^+] / [\text{H}^+][\text{Hb}]$ and $K_2 = [\text{O}_2\text{HbH}^+] / [\text{H}^+][\text{O}_2\text{Hb}]$. We assume that these reactions are assumed to be in equilibrium locally due to faster reaction rate, in comparison with progression of hydrogel hydration.

The total hemoglobin concentration $[\text{Hb}]^T$ in the hydrogel is can written as

$$[\text{Hb}]^T = [\text{Hb}] + [\text{O}_2\text{Hb}] + [\text{HbH}^+] + [\text{O}_2\text{HbH}^+] \quad (2.23)$$

where it consists of reduced and protonated reduced hemoglobins, and oxygenated and protonated oxygenated hemoglobins.

The saturation of hemoglobin S with the oxygen O_2 is given as

$$S = \frac{[\text{HbO}_2]}{[\text{Hb}]^T} \quad (2.24)$$

where the oxygenated hemoglobin concentration $[\text{HbO}_2]$ can be described as follows

$$\begin{aligned} [\text{HbO}_2] &= [\text{O}_2\text{Hb}] + [\text{O}_2\text{HbH}^+] \\ &= K_{O_2} [\text{O}_2][\text{Hb}] (1 + K_2 [\text{H}^+]) \end{aligned} \quad (2.25)$$

while the total hemoglobin concentration $[\text{Hb}]^T$ can be written as

$$\begin{aligned} \text{Hb}^T &= \text{Hb} + \text{HbH}^+ + [\text{HbO}_2] \\ &= \text{Hb} \left(1 + K_1 [\text{H}^+] + K_{O_2} [\text{O}_2] (1 + K_2 [\text{H}^+]) \right) \end{aligned} \quad (2.26)$$

2.3 Remarks

The literature surveys unveil that previously published experimental works remain insufficient to characterize the reactive performances of such materials including:

For urease-loaded hydrogel:

- how does the immobilized urease concentration influence reactive performances of the hydrogel?
- how does different polymer monomers used to construct urease-loaded hydrogel influence its urea-sensitive responses?
- osmotic pressure of the urease-loaded hydrogel at different:
 - environmental conditions.
 - urease kinetic properties.
 - initial immobile charge component density.
- urease catalytic behavior of the hydrogel as a function of:
 - urea concentration
 - salt concentration

The hemoglobin-enriched hydrogel reactive behaviors as a function of:

- hemoglobin loading
- immobile charge component density
- ambient oxygen level
- environmental pH value
- coupled environmental salt- and oxygen-stimuli concentration

Therefore, these studies will be conducted and discussed in Chapter 3 and 4.

Chapter 3 Multiphysics Model Development to Investigate the Reactive Characteristics of Urease-loaded Hydrogel

3.1 Introduction

This chapter deals with the multiphysics model development for investigating the coupled bio-electro-chemo-mechanical reactive performances of urea-sensitive hydrogel, incorporating the multi-physical interactions between the immobilized urease and the environmental urea-rich salt solution. The model includes Poisson-Nernst-Planck (PNP) equation for the transport of mobile species between its microenvironment and the environmental solution coupled with a nonlinear mechanical equation to account for the conversion of biochemical and electric energies into its mechanical counterpart. In order to capture the urea-induced behaviors of the hydrogel, two correlations are also integrated into the multiphysics model to establish the relationship: (i) between the urease reaction rate and diffusive concentration of urea, and also (ii) between the fixed charge group density and diffusive hydrogen ionic concentration. As such, the original contribution of Chapter 3 is that a novel reaction rate equation is proposed to characterize the urease ionization and denaturation states, capturing the urease activity as a function of pH coupled with temperature. The model is directly validated by comparison with published experimental data. This chapter is organized as follows. After the introduction presented in Section 3.1, the multiphysics model is developed in Section 3.2,

followed by Section 3.3 and 3.4 for validation and results discussions, respectively. Finally, several remarks are drawn in Section 3.5.

3.2 Model Formulation

For developing the model, five items are necessarily described mathematically including: (a) the mass transport between the hydrogel polymeric system and environmental solution; (b) the urease-induced urea reaction in the polymeric system; (c) the interplay of mobile-immobile charges in the polymeric system; (d) the mechanical pressure arising from the urea-urease biochemical interaction and (f) the volumetric behaviors of the polymeric system, as visualized in Fig. 3.1.

The multiphysics model is developed with below premises:

- (i) The hydrogel operates at constant temperature, where the urea hydrolysis rate is assumed a constant in the polymeric system.
- (ii) The enzyme is distributed and decorated homogenously on the polymeric network chains.
- (iii) The optimal temperature T_{op} of the enzyme is assumed the midpoint temperature of its transition between active and inactive forms T_{eq} , namely $\Delta H_{eq} \approx 2\Delta G_{cat}$, in which ΔH_{eq} is the enthalpy change due to the transition of active enzyme into its inactive counterpart, and ΔG_{cat} is the enzymatic reaction activation energy [204].
- (iv) The polymeric system is associated with a macroporous nature, where the mass diffusivity coefficients D_k remain the same, in either the polymeric system or the environmental solution.
- (v) The environmental solution is assumed unstirred.

3.2.1 Conservation of Mass

In the model, the Nernst-Planck equation is employed for characterizing the mass transport in the system, as shown below [205]

$$\nabla \cdot \left(\mathbf{C}^{-1} D_k \left[\nabla \cdot C_k + \frac{z_k F}{RT} C_k \nabla \cdot \Phi \right] \right) = v_k r_k \left(k = H^+, OH^-, NH_4^+, HCO_3^-, cation, anion \right) \quad (3.1)$$

where $\mathbf{C}^{-1}$ the inverse of the right Cauchy-Green tensor and $\mathbf{C}^{-1} = \mathbf{F}^{-1} \mathbf{F}^{-T}$. D_k , C_k , z_k , v_k and r_k refer to diffusivity coefficient (m^2/s), mass concentration (mM), charge number, chemical reaction stoichiometric coefficient, and catalytic reaction rate. R , T , F , and Φ denote the universal gas constant (8.314 J/mol K), environmental temperature (K), Faraday constant (96,487 C/mol), and electrical potential of the polymeric system.

3.2.2 Conservation of Momentum

The hydrolysis of urea reaction in the polymeric system effects the reactive swelling behaviors of the system due to changes of ionic concentration in the hydrogel. It is well-established that the polymeric system achieves the equilibrium swelling state, only if the osmotic swelling forces are balanced by the restrictive forces display by the polymeric network chains. The momentum conservation law can be utilized to describe the equilibrium swelling state

$$\nabla \cdot \mathbf{P} = \mathbf{0} \quad (3.2)$$

given that $\mathbf{P}$ is the Piola stress.

3.2.3 Free Energy Imbalance Inequality

For formulating the mechanical constitutive equations for the polymeric system at constant environmental temperature, the local free energy imbalance inequality is used, given as follows [206]

$$\dot{W} - (u_1 + u_2 + u_3) \leq 0 \quad (3.3)$$

where $\dot{W}$ is temporal Helmholtz free energy density of the polymeric system. On the other hand, u_1, u_2 and u_3 refer to the work per volume rate incorporated into the polymeric system through the mechanical, electric and chemical domains.

The free energy of the polymeric system are described by summing: [207]:

(i) the mechanical stretching free energy density of the elastic-nature polymeric network chains W_1 , (ii) the mixing free energy density due to polymeric network chains- environmental solution interaction W_2 , (iii) the mixing free energy density due mobile charges-environmental solution interaction W_3 , and (iv) the electric field free energy density due to mobile-immobile ions interactions W_4

$$W = W_1 + W_2 + W_3 + W_4 \quad (3.4)$$

The elastic hydrogel stretching of free energy density, is given as [208]

$$W_1 = \frac{1}{2} N k_B T \left[\text{tr}(\mathbf{F}^T \mathbf{F}) - 2 \ln(\det \mathbf{F}) - 3 \right] \quad (3.5)$$

where $N k_B T$ is the ground-state shear modulus G , N is the polymeric chains per volume of the dry polymer number ($2.43 \times 10^{25} \text{ m}^{-3}$), and k_B is the Boltzmann constant ($1.381 \times 10^{-23} \text{ J/K}$). By employing the method suggested by Chester *et al.*, the right Cauchy-Green tensor is written as $\mathbf{C} = (\lambda_s)^2 \mathbf{C}_e$ [209], where λ_s is the swelling stretch and $\mathbf{C}_e$ refers to the elastic right Cauchy-Green tensor. Thereby, W_1 is rewritten as

$$W_1 = \frac{1}{2} N k_B T \left[(\lambda_s)^2 \text{tr}(\mathbf{C}_e) - 2 \ln(\det \mathbf{F}) - 3 \right] \quad (3.6)$$

The mixing free energy density of hydrogel-environmental solution is given as [209, 210]

$$W_2 = RTC \left(\ln(Cv) - \ln(1 + Cv) + \frac{\chi}{1 + Cv} \right) \quad (3.7)$$

where C is the number of water molecules in the environmental solution, v is the volume of a mole of fluid molecules and χ is the polymer-solvent interaction parameter.

The mixing free energy density of environmental solution-mobile charges, written as [211]

$$W_3 = RT \sum_k C_k (\ln C_k - \ln C - 1) \quad (3.8)$$

The free energy density of the electric field formed due to mobile-immobile charges interaction can be written as [208]

$$W_4 = \frac{1}{2\epsilon_r \epsilon_0 J} (\mathbf{H} \cdot \mathbf{C} \cdot \mathbf{H}) \quad (3.9)$$

where $J = (\lambda_s)^3$, $\mathbf{H}$ is the dielectric displacement, and $\epsilon_r \epsilon_0$ is the relative dielectric constant. The hydrogel volumetric change is caused the hydration-induced swelling, where the W_4 can rewritten [209]

$$W_4 = \frac{1}{2\epsilon_r \epsilon_0 (1 + Cv)^{1/3}} (\mathbf{H} \cdot \mathbf{C}_e \cdot \mathbf{H}) \quad (3.10)$$

The mechanical power per volume can be written as [212, 213]

$$\mathbf{u}_1 = J\sigma : \mathbf{L} \quad (3.11)$$

where σ is the symmetric Cauchy stress tensor and $\mathbf{L}$ is the velocity, and $\mathbf{u}_1$ can be expressed as [209, 213]

$$\mathbf{u}_1 = (\mathbf{P}\mathbf{F}_s^T + Jp\mathbf{F}_e^{-T}) : \dot{\mathbf{F}}_e - j_s p \quad (3.12)$$

where $\mathbf{F}_s$ denotes the deformation gradient for the hydrogel swelling, while $\mathbf{F}_e$ denotes the deformation gradient of the hydrogel elastic deformation.

The rate of work done in electric field per volume can be expressed as [206]

$$\mathbf{u}_2 = \mathbf{E} \cdot \dot{\mathbf{H}} - F\Phi \sum_k z_k \dot{C}_k \quad (3.13)$$

The rate of work per volume caused by the movement of solution and mobile solutes is written as [214]

$$\mathbf{u}_3 = -\left(\mu \nabla \cdot \mathbf{J} + \sum_k \mu_k \nabla \cdot \mathbf{J}_k\right) - \left(\mathbf{J} \nabla \mu + \sum_k \mathbf{J}_k \nabla \mu_k\right) \quad (3.14)$$

in which μ and μ_k refer to chemical potentials of environmental solution and mobile charge and $\mathbf{J}$ and $\mathbf{J}_k$ denotes the mass fluxes of environmental solution and mobile charge. As such, the free energy imbalance inequality equation to describe the reactive mechanical behaviors of the urease-loaded hydrogels is written as

$$\begin{aligned} & \frac{1}{(1+Cv)^{1/3}} \left(Nk_B T (1+Cv) \mathbf{F}_e + \frac{1}{\varepsilon} (\mathbf{F}_e (\mathbf{H} \otimes \mathbf{H})) - (1+Cv)^{4/3} (\sigma \mathbf{F}_e^{-T} + p \mathbf{F}_e^{-T}) \right) : \dot{\mathbf{F}}_e \\ & + \dot{C} \left(\mu + Nk_B T \left[\frac{v}{(1+Cv)^{1/3}} \left(\frac{1}{3} \text{tr}(\mathbf{C}_e) - \frac{1}{(1+Cv)^{2/3}} \right) \right] + RT \left(\ln \frac{Cv}{1+Cv} + \frac{1}{1+Cv} + \frac{\chi}{(1+Cv)^2} \right) \right) \\ & - \dot{C} \left(-pv + RT \sum_k \left(\frac{C_k}{C} \right) + \frac{1}{6\varepsilon_r \varepsilon_0} \frac{v}{(1+Cv)^{4/3}} (\mathbf{H} \cdot \mathbf{C}_e \cdot \mathbf{H}) \right) + \dot{C}_k \left(RT \ln \frac{C_k}{C} + z_k F\Phi - \mu_k \right) \\ & + \left(\mathbf{J} \nabla \mu + \sum_k \mathbf{J}_k \nabla \mu_k \right) \leq 0 \end{aligned}$$

3.2.4 Biochemistry for Constitutive Equation

For utilizing equation 3.1, the conventional reaction rate r_k which describes the urease-mediated urea hydrolysis is expressed as [215]

$$r_k = k_{cat} \frac{C_{E_{act}} C_{urea}}{K_M} \quad (3.16)$$

where $C_{E_{act}}$ and C_{urea} are the active urease and urea concentrations, respectively.

k_{cat} is the constant enzyme reaction rate and K_M is the Michaelis constant. It is well-established that the equation 3.16 neglected the environmental impacts on the urease activity, especially environmental pH and temperature conditions. Thereby, equation 3.16 can be rewritten as follow for considering the effect of temperature on urease catalytic behaviors [190]

$$r_k = V_{max} C_{urea} \frac{1 + K_{EQ}}{C_{urea} + K_M K_{EQ}} \quad (3.17)$$

in which V_{max} is the urease maximum reaction velocity, and $V_{max} = \frac{k_{cat} C_{E_{act}}}{1 + K_{EQ}}$

[216], $K_{EQ} = \exp \left[\frac{\Delta H_{eq}}{R} \left(\frac{1}{T_{eq}} - \frac{1}{T} \right) \right]$ is the equilibrium constant between active

and inactive urease, and C_{E_0} is the initial urease concentration.

The pH-induced urease denaturation is described mathematically by [217]

where $C_{EH^+_{inact}}$ and $C_{EOH^-_{inact}}$ are the ionized ureases concentrations and C_X

refers to the denatured urease concentration [217, 218]. K_{EOH} and K_{EH} are the equilibrium constant for the urease hydroxylation and protonation, and K_w is the water dissociation constant. Thereby, the initial and denatured urease concentrations difference can be expressed as

$$C_{E_0} - C_X = C_{E_{act}} \left(1 + \frac{K_W}{K_{EOH} C_{H^+}} + \frac{C_{H^+}}{K_{EH}} \right) \quad (3.20)$$

Last, the chemical reaction rate r_k for including the coupled temperature-pH impacts on urease catalytic activity can be written as

$$r_k = V_{\max} \frac{C_{urea}}{K_M} \left(\frac{C_{urea}}{K_M} + K_{EQ} \right) \left(1 + \frac{K_{EOH} C_{H^+}}{K_W} + \frac{K_{EH}}{C_{H^+}} \right) \quad (3.21)$$

3.2.5 Electrical Field for Constitutive Equation

To incorporate the coupled impact of the electrical potential Φ of polymeric system and mobile charges transport in the model, the Poisson equation is utilized [205]

$$\nabla \cdot (\epsilon_r \epsilon_0 J \mathbf{C}^{-1} \nabla \Phi) = -F \left(\sum_k z_k C_k + z_f C_f \right) \quad (3.22)$$

in which immobile ion concentration C_f of anionic or cationic hydrogel [178]

$$C_f = \frac{C_f^0}{H} \frac{K_a}{K_a + C_{H^+}} \quad \text{or} \quad C_f = \frac{C_f^0}{H} \frac{C_{H^+}}{K_a + C_{H^+}} \quad (3.23)$$

where C_f^0 is the initial immobile charges in the hydrogel, H is the ratio of final to the initial volume of the hydrogel, and K_a is the acid-base ionization constant of the immobile ionizable components in the polymeric system.

3.2.6 Mechanical Field for Constitutive Equation

By employing equation 3.15, at equilibrium, the first bracket is equal to 0, in which Piola stress $\mathbf{P}$ can be written as [208]

$$\begin{aligned} \mathbf{P} = & - (1 + Cv)^{2/3} p \mathbf{F}_e^{-T} + N k_B T (1 + Cv)^{1/3} \mathbf{F}_e \\ & + (1 + Cv)^{-2/3} \mathbf{F}_e \frac{1}{\mathcal{E}} (\mathbf{H} \otimes \mathbf{H}) \end{aligned} \quad (3.24)$$

where the Piola stress $\mathbf{P}$ is summation of hydrostatic pressure p , elastic polymeric network chains stress, and Maxwell stress. The Maxwell stress is neglectable at mechanical equilibrium [206] , and we can write

$$\mathbf{P} = -(\lambda_s)^2 p \mathbf{F}_e^{-T} + Nk_B T (\lambda_s) \mathbf{F}_e \quad (3.25)$$

Again from equation 3.15, at steady-state, the chemical potentials of the environmental and microenvironmental solutions are [219]

$$\mu = RT \frac{\sum_k C_k}{C} - RT \left(\ln \frac{Cv}{(1+Cv)} + \frac{1}{(1+Cv)} + \frac{\chi}{(1+Cv)^2} \right) \quad (3.26)$$

$$- Nk_B T \left[\frac{v}{(1+Cv)^{1/3}} \left(\frac{1}{3} \text{tr}(\mathbf{C}_e) - \frac{1}{(1+Cv)^{2/3}} \right) \right] - v p$$

$$\bar{\mu} = RT \frac{\sum_k \bar{C}_k}{\bar{C}} \quad (3.27)$$

At Donnan equilibrium, the chemical potentials in the environmental and microenvironmental solutions matches at the hydrogel-solution interface, consequently the hydrostatic pressure p is expressed as [207]

$$p = RT \sum_k \left(\frac{C_k}{J-1} - \bar{C}_k \right) - \frac{RT}{v} \left(\ln \frac{Cv}{(1+Cv)} + \frac{1}{(1+Cv)} + \frac{\chi}{(1+Cv)^2} \right) \quad (3.28)$$

$$- Nk_B T \left[\frac{1}{(1+Cv)^{1/3}} \left(\frac{1}{3} \text{tr}(\mathbf{C}_e) - \frac{1}{(1+Cv)^{2/3}} \right) \right]$$

As seen in equation 3.28, the mechanical pressure on the interface between hydrogel-solution consists of a few multi-physical components, including polymer-solution, polymer-polymer, and mobile-immobile ions synergistic [220, 221].

3.2.7 Boundary Conditions

In this section, the steady-state simulations are conducted, where the responses of cylindrical urease-loaded hydrogels are examined one dimensionally. The mechanical swelling of the hydrogels is assumed at radial direction, where center ($X = 0$) of the hydrogel is under Neumann boundary conditions, whereas the solution is subjected to Dirichlet boundary conditions [222].

For continuity, the Neumann boundary condition is assumed at the cylindrical hydrogel ($X = 0$), given as follows

$$\frac{\partial C_k}{\partial X} = 0, \quad \frac{\partial \Phi}{\partial X} = 0 \quad (k = H^+, OH^-, \dots, N) \text{ at } X = 0 \quad (3.29)$$

whereas the Dirichlet boundary conditions are applied at the environment solution

$$C_k = \bar{C}_k, \quad \Phi = 0 \quad (k = H^+, OH^-, \dots, N) \quad (3.30)$$

Furthermore, equation 3.25 can be rewritten as

$$\mathbf{P} = -(\lambda_s)^2 p(1) + Nk_B T (\lambda_s) \left(1 + \frac{\partial u_e}{\partial X} \right) \quad (3.31)$$

in which the stretch λ_s is given as

$$\lambda_s = \frac{\partial x_s}{\partial X} = 1 + \frac{\partial u_s}{\partial X} = \frac{L_{gel}}{L_{gel}^o} \quad (3.32)$$

where u_s is the swelling displacement and L_{gel} is the hydrogel radius.

By equation 3.32, the Piola stress $\mathbf{P}$ is again recast

$$\mathbf{P} = -\left(1 + \frac{\partial u_s}{\partial X} \right)^2 p(1) + Nk_B T \left(1 + \frac{\partial u_s}{\partial X} \right) \quad (3.33)$$

and by substituting equation 3.33 into the momentum equation 3.2 we obtain

$$\frac{\partial}{\partial X} \left[-\left(1 + \frac{\partial u_s}{\partial X} \right)^2 p + Nk_B T \left(1 + \frac{\partial u_s}{\partial X} \right) \right] = 0 \quad (3.34)$$

Last, the swelling stretch λ_s acting over the hydrogel-solution interface

$$\lambda_s = \frac{Nk_B T}{p}, \quad X = L_{gel} \quad (3.35)$$

For simulating the reactive performance of the hydrogel, COMSOL Multiphysics 5.1 software is used to execute the multiphysics model, due to its ability to solve coupled governing and constitutive equations. First, the immobile ion density equation 3.23 is solved for getting the immobile charge concentration C_f associating to applied input parameter and conditions. Next, the Nernst-Planck equation 3.1 which is coupled to the Poisson equation 3.22 are computed numerically to obtain the converged solution of mobile mass concentration C_k and electric potential Φ . Then, the C_k is inserted inside equation 3.25 to obtain the λ_s . Lastly, above mentioned steps are repeated till all independent variables achieve convergence, as visualized in Fig. 3.2.

3.3 Validation of Model

To probe the accuracy of the model to capture the responsive characteristics of urease-loaded hydrogel, the current numerical finding is necessarily examined with published experimental result, especially for: (i) the mechanical behaviors of the hydrogel and (ii) the biochemical activities of the hydrogel under varying environmental conditions.

3.3.1 Mechanical Behaviors of the Hydrogel

For examining the multiphysics model, the equilibrium urea-mediated mechanical behaviors of the polymeric system are investigated against the published experimental observation. In the experimental study performed by Ogawa and Kokufuta [10], the urease-loaded p(NIPAM)-based polymeric system is submersed in maleate-buffer of 5 mM, consisting urea concentration

of 1 mM, with pH = 4 and temperature of 35 °C, where urease concentration C_{E_0} of 0.2 to 8.0 mg/mL is functionalized in the hydrogel. The input needed to execute the model are: $L = 2500 \mu\text{m}$, $z_k = 1$ (cationic hydrogel), $C_f^0 = 0.775$ mmol/g, $K_a = 10^{-5.57}$, and the rest are tabulated in Table 3.1.

Fig. 3.3 demonstrates that the swelling of the hydrogel is invariant at concentration of immobilized urease C_{E_0} larger than 0.5 mg/mL, where the immobile enzyme strengthens the mechanical behaviors of the hydrogel, limiting the swelling performance of the hydrogel. For hydrogel loaded with smaller urease concentration C_{E_0} (e.g. < 0.5 mg/mL), the current numerical results overpredicted the experimental observations. Fortunately, the difference between numerical and experimental results are still within the experimental error of $\pm 13 \mu\text{m}$, as visualized in Fig. 3.3.

Apart from that, Ogawa and Kokufuta also examine the impact of hydrogel initial radius on its hydration-induced swelling deformation as function of the immobile urease concentration, as visualized in Fig. 3.4 [10]. Therefore, the current multiphysics model is re-performed, revealing that the increase of the urease enzymatic activity in the hydrogel enhances the deionization of the cationic immobile charge group. This depletes the ionic concentration difference between the hydrogel and its environmental solution, which leads to swelling-to-collapse volumetric behavior of the hydrogel. In addition, the swelling of the hydrogel seems to be almost independent of the urease concentration above 1.0 mg/mL. This is perhaps due to the limiting concentration of urea in the hydrogel, which explains the invariant swelling deformation of the hydrogel. It is noted that from Fig. 3.4, the current numerical results usually underestimate the experimental equilibrium swelling behaviour of the hydrogel as a function of urease concentration.

Fortunately, the errors of the present numerical simulations are usually within the reported experimental error of $\pm 13 \mu\text{m}$, where it can be concluded that the numerical result agrees well with the experimental findings. Hence, as seen in Figs 3.3 and 3.4, the present numerical results seem to agree well with the published experimental data, achieving a difference of $\sim 3.0\%$.

It is commonly known that the volumetric response of urease-loaded hydrogel is a function of mechanical pressure acting over the hydrogel-solution interface, such that lower hydrostatic pressure indicates larger swelling deformation of the hydrogel. This phenomenon is theoretically characterized by the nonlinear mechanical equation 3.35, which establishes a relationship between the numerically obtained pressure acting over the hydrogel-solution interface and the swelling deformation of the hydrogel. Consequently, the ability of the present multiphysics model to accurately capture the pressure acting over the hydrogel-solution interface reasonably quantifies the hydration-induced swelling of the hydrogel. Unfortunately, the latest literature review clearly reveals that no studies were made in both theoretical and experimental aspects of the urea-actuated osmotic pressure response of urease-loaded hydrogels. As an alternative, the capability of the multiphysics model is examined to capture the hydration-induced swelling deformation of the hydrogels for model validation.

The pH-induced reactive performances of urease-loaded hydrogel are investigated by both the present multiphysics model and the published experimental data, as illustrated in Fig. 3.5(a). In the published experimental work [10], urea-sensitive p (NIPAM)-based hydrogel was immersed in maleate buffer of 5 mM, consisting 1 mM of urea, with environmental temperature of

35 °C, at environmental $\overline{\text{pH}} = 4 \sim 7$, and the concentration of immobilized urease 0.2 ~ 2 mg/mL. Several other inputs are needed by the present model to investigate urease-loaded hydrogel, such as $L = 2500 \text{ } \mu\text{m}$, $L_{gel}^0 = 250 \text{ } \mu\text{m}$, $z_f = 1$ (cationic hydrogel), $C_f^0 = 0.775 \text{ mmol/g}$ and $K_a = 10^{-5.57} \text{ (M)}$, and the rest are provided in Table. 3.1.

Fig. 3.5(a) presents the pH-induced swelling behaviors of the urea-sensitive hydrogel by both the present numerical results and the experimental data [10]. As seen in Fig. 3.5(a), the hydrogel collapses with the increase of environmental $\overline{\text{pH}}$ from 4 to 7, where the calculated error between the current numerical results and experimental data is ~3.3 %. This observation indicates the ability of the multiphysics model for describing the swelling behaviors of the urease-loaded hydrogel in response to environmental $\overline{\text{pH}}$ with a good degree of accuracy. The swelling transition behavior is explained through ionization state of the immobile charge group of the hydrogel. The vinylimidazole immobile charge group of the urea-sensitive hydrogel remains mainly unionized if environmental solution $\overline{\text{pH}} > \text{pK}_a=5.6$ (dissociation constant of the fixed charge group), while the immobile charge group becomes mostly ionized if the hydrogel is immersed in environmental solution $\overline{\text{pH}} < \text{pK}_a=5.6$. As environmental $\overline{\text{pH}}$ exceeds pK_a value of the immobile charge group [49], the interaction between the dominantly unionized and the minorly ionized immobile charge group, causing the shrinkage of the hydrogel [223]. Therefore, the hydrogel remains at collapse state, until the immobile charge group becomes dominantly ionized at solution $\overline{\text{pH}} < \text{pK}_a=5.6$ [50].

In addition, Fig. 3.5(b) visualizes the thermally performance of the urea-sensitive hydrogel by the present numerical results and experimental findings.

In the published experimental work [53], the urease-loaded p(NIPAM)-based hydrogel was immersed in the phosphate environmental solution(PBS) of 0.1 M, comprising of urea concentration $\bar{C}_{urea} = 10$ mM, at environmental $\bar{pH} = 7.0$ and environmental temperature $15 \sim 45$ °C with the concentration of urease 2 mg/mL. The inputs needed to execute the multiphysics model: $L = 2500$ μm , $L_{gel}^0 = 50$ μm , $C_f^0 = 1800$ mM, $z_k = -1$ (anionic hydrogel), $K_a = 10^{-5.0}$ (M), and the other input parameters are given in Table. 3.1. A global error of 2.11% is achieved between the present numerical results and experimental data, demonstrating the capability of the multiphysics model to accurately characterize the performance of thermal-induced behaviors of the urea-sensitive hydrogel. In general, the polymeric network chains become less hydrophilic by increasing the environmental temperature, collapsing the polymeric system [224].

3.3.2 Biochemical Activities of the Hydrogel

It was reported by Pozniak *et al.* [78] that the immobilization of urease in the cationic hydrogel enhanced the stability of the pH-induced urease enzymatic activity, showing a wider pH optimum range from environmental pH 5.0 to 6.5, but they did not fully explain the reason for the enhancement of urease activity in the cationic hydrogel. As such, the experimental observation remains poorly comprehended, such that the multiphysics model is necessarily performed to get the further insights into the fundamental mechanism of enhancement of urease activity in the cationic hydrogel. In the experimental work conducted by Pozniak *et al.* [78], the urease-loaded hydrogels were immersed in the phosphate environmental solution(PBS) of 22 mM, containing the

concentration of urea $\bar{C}_{urea}=10 \text{ g/dm}^3$ with temperature of $25 \text{ }^\circ\text{C}$, where the urease concentration of 1.25 mg/cm^3 was functionalized in the polymeric system. Few other inputs are needed to perform the multiphysics model: $L=250 \text{ }\mu\text{m}$, $L_{gel}^0=50 \text{ }\mu\text{m}$, $z_k=1$, $C_f^0=0.15 \text{ mmol/g}$, $T_{op}=62 \text{ }^\circ\text{C}$, $K_a=10^{-8.0}$ (M), $K_M=22.1 \text{ mM}$, and $k_{cat}=1.5 \times 10^3 \text{ 1/s}$, whereas the rests are tabulated in Table 3.1. Therefore, several interesting numerical results are worked out by the present multiphysics model, and they unveil that, when the environmental pH is lower than the acid-base dissociation of the fixed cationic group (such as environmental $\text{pH} < \text{pK}_a=8.0$), the hydrogel remains highly swollen with water, as seen in Fig. 3.6. This is why the immobilized urease behaves optimally at the wider pH optimum range from environmental pH 5.0 to 6.5. Furthermore, when environmental pH is higher than the acid-base dissociation of the fixed cationic charge group (such as environmental $\text{pH} > \text{pK}_a=8.0$), the fixed cationic group is dominantly deionized, leading to the dehydration-driven shrinkage of the hydrogel, as seen in Fig. 3.6. This explains the decrease of the urease enzymatic activity in the cationic hydrogel. Therefore, the swelling deformation of the hydrogel significant influences the urease catalytic activity in the cationic hydrogel, where the immobilized urease catalytic activity is improved with the increase of hydration-induced swelling deformation of the hydrogel. Hence, an error less than $\sim 3.0 \%$ is achieved between the present numerical results and the experimental data, demonstrating the capability of the multiphysics model to characterize well the performance of pH-induced urease catalytic activity in cationic hydrogel.

Kutcherlapati *et al.* [81] reported that the immobilization of urease in the anionic hydrogel displaced the pH optimum of the urease from a physiological

pH to a non-physiological pH value (such as from environmental pH 7.5 to 8.5), but they also did not entirely explain the reason for the displacement of the urease pH optimum to more basic conditions, when operating in anionic hydrogel. Since the experimental data are still not-well understood, the multiphysics model is necessarily performed to get the further insights into the fundamental mechanism of the immobilized urease, when operating in the anionic hydrogel. In the experimental work conducted by Kutcherlapati *et al.* [81], the urease-loaded hydrogels were immersed in 100 mM, with either the acetate buffer or the phosphate buffer or the tris-HCL buffer, containing the concentration of urea $\bar{C}_{urea}=3.5$ mM with temperature 27 °C, where the concentration of urease 18 μM was functionalized in the polymeric system. Few inputs are needed for performing the multiphysics model: $L=250\ \mu\text{m}$, $L_{gel}^0=50\ \mu\text{m}$, $z_k=-1$, $C_f^0=100$ mM, $K_a=10^{-4.5}$ (M), $T_{op}=50\ ^\circ\text{C}$, $K_M=0.26$ mM, and $k_{cat}=1.5\times 10^3$ 1/s and the rests are tabulated in Table 3.1. As such, several intriguing numerical results are worked out by the multiphysics model, and they reveal that, the increase of environmental pH to 8.5 causes the microenvironment to equilibrate at the pH, coinciding with the pH optimum of free urease ~ 7.5 [81], as seen in Figs. 3.7(a) and 3.7(b). This explains why the immobilized urease performs optimally at environmental $\bar{\text{pH}}=8.5$, when operating in the anionic hydrogel. In addition, if the environmental $\bar{\text{pH}}$ is larger than the pH optimum of the immobilized urease in the anionic hydrogel (such as environmental $\bar{\text{pH}}>8.5$), the microenvironment pH shifts further away from pH optimum of free urease (such as pH=7.5), as seen in Figs. 3.7(a) and 3.7(b). This typically results in the increase of the ionization and denaturation of the

urease, which clarifies the drastic decrease of the urease enzymatic activity, with the further increase of environmental $\overline{\text{pH}}$ higher than 8.5. Therefore, it is demonstrated that, the urease catalytic activity in the anionic hydrogel is largely governed by microenvironment pH, where the immobilized urease catalytic activity is enhanced, when the microenvironment approaches the pH optimum of free urease. Hence, an error less than ~4.0 % is achieved between the present numerical results and the experimental data, showing the ability of the multiphysics model to capture the performance of pH-induced urease catalytic activity in anionic hydrogel.

Although Arica *et al.* [53] unveiled that the decrease of the environmental temperature improved the urease catalytic activity in the ion-exchange hydrogel, but they didn't thoroughly explain the reason why the urease activity is enhanced in the temperature-sensitive ion-exchange hydrogel, with the decrease of environmental temperature, such that the experimental data still remains incompletely understood. Therefore, the present multiphysics model is necessarily performed to get the further insights into the fundamental mechanism of the urease activity, operating in the ion-exchange hydrogel subjected to variation of environmental temperature, as visualized in Fig. 3.8. In the published experimental work [53], the urease-loaded p(NIPAM)-based hydrogel was immersed in the phosphate environmental solution(PBS) of 0.1 M, comprising of urea concentration $\overline{C}_{urea} = 10$ mM, at environmental pH= 7.0 and environmental temperature 15 ~ 45 °C with the concentration of urease 2 mg/mL. The inputs needed to perform the model: $L = 250$ μm , $L_{gel}^0 = 50$ μm , $C_f^0 = 2800$ and 3200mM, $T_{op} = 50$ °C, $z_k = -1$, $K_a = 10^{-5.0}$ (M), $K_M = 27.0$ mM, $k_{cat} =$

1.5×10^3 1/s, and the other inputs are provided in Table. 3.1. Several interesting numerical results are thus obtained by the multiphysics model, and they reveal that the rise of environmental temperature causes the increase of acidity in the microenvironment, as seen in Fig. 3.8(b). This is due to the increase of the fixed anionic charge group concentration by increasing the environmental temperature, resulting in the decrease of pH in the microenvironment [225]. Furthermore, the increase of environmental temperature also decreases the hydration-induced swelling deformation of the urease-loaded ion-exchange hydrogel, due to the enlargement of contractive hydrostatic pressure acting on the hydrogel-solution interface, consequently reducing the urea-loaded water intake into the hydrogel. As such, when the environmental temperature is increased, the increase of acidity of the microenvironment coupled with the decrease of the hydration-induced swelling deformation, both resulting in the decrease of urease catalytic activity in the temperature-sensitive hydrogel, as illustrated in Fig. 3.8. It is noted that from Fig. 3.8, the present numerical results usually overestimate the temperature-induced urease activity of published experimental data, in which the error between them is $\sim 5\%$. The model neglects the effect of urease inhibitors that usually exists in the environmental solution, consequently lowering the urease activity. However, the model still captures well the temperature-induced urease activity, qualitatively and quantitatively.

3.4 Results and Discussion

This section explores the impact of (i) urease loading and property, (ii) polymeric monomer and (iii) environmental conditions on the responsive behaviors of the hydrogels.

3.4.1 How Does the Immobilized Urease Concentration Influence Reactive Performances of the Hydrogel?

For examining the optimal loading of urease in the hydrogel, the urease catalytic activity in the hydrogel is examined as a function of the immobilized urease concentration [78] when subjected to the urea concentration of 30 mM. This critical concentration value was measured from the tear film and bloodstream of renal patients [226] when the renal disease further develops into the advanced stage. That is why urea level of 30 mM is chosen to examine the optimal loading of the urease-loaded hydrogel. Thereby, the multiphysics model is necessarily performed to get an insight into optimal loading of the urease when operating inside the hydrogel immersed in salt solution of 143 mM with pH=7.0 and environmental temperature 37 °C, in which the material inputs needed to execute model: $L = 250 \text{ } \mu\text{m}$, $L_{gel}^0 = 50 \text{ } \mu\text{m}$, $C_f^0 = 1800 \text{ mM}$, $z_f = -1$, $K_a = 10^{-5.0} \text{ (M)}$, $K_M = 27 \text{ mM}$, and $k_{cat} = 1.5 \times 10^3 \text{ 1/s}$, and the remaining inputs are provided in Table. 3.1.

Several intriguing numerical results are worked out by the model, as visualized in Fig. 3.9(a), revealing that: (i) initially the urease activity increases linearly until the urease concentration at 4.5 mg/mL; (ii) the urease activity then enlarges nonlinearly with successive increase of the urease concentration, up till $C_E = 7.0 \text{ mg/mL}$; and (iii) finally, the urease activity achieves the stabilized maximum with the further increase of the immobilized urease concentration. Therefore, the optimal loading of urease in the hydrogel is achieved at $C_E = 7.0 \text{ mg/mL}$, corresponding to maximum urease activity in the hydrogel, as seen in Fig. 3.9(a), when the hydrogel is subjected to urea concentration of 30 mM.

This is because the initial increase of immobilized urease concentration accelerates chemical reaction rate of the urease-urea complexes, where the further increase of the urease concentration in the hydrogel may cause the urea to be a limiting factor in the microenvironment, and thus the urease activity approaches stabilization, when the urease concentration in the hydrogel becomes higher than 5.5 mg/mL.

Fig. 3.9(b) illustrates the swelling actuation of the hydrogel as a function of immobilized urease concentration when subjected to urea concentration of 30 mM. As seen in Fig. 3.9(b), the hydration-induced swelling deformation of the hydrogel reduces in a sigmoid-like pattern with increase of the immobilized urease concentration, where the hydrogel swelling deformation becomes independent of immobilized urease concentration, when the urease concentration is higher than 6.0 mg/mL. The increase of immobilized urease concentration significantly enlarges contractive hydrostatic pressure acting on the hydrogel-solution interface, which then decreases hydration of the hydrogel, limiting ingestion of urea into the hydrogel. This explains why urea becomes a limiting factor for the immobilized urease-urea reaction when the concentration of immobilized urease is higher than 6.0 mg/mL, as seen in Figs. 3.9(a) and 3.9(b).

3.4.2 How Does Different Polymer Monomers used to Construct Urease-loaded Hydrogel Influence its Urea-sensitive Responses?

The effect of immobilized urease concentration is examined particularly on the urea-induced mechanical reactive performances of commonly employed urease-loaded hydrogels. For example, acrylates- or methacrylates-based hydrogel, the polymeric systems, poly(2-hydroxyethyl-methacrylate) (PHEMA) [68], poly(N-

isopropylacrylamide) (PNIPAM) [10] and poly(acrylamide) (PAAM) [57] hydrogels are often associated with different hydrophilicities [227, 228], but the urea-induced responsive behavior of these different hydrogels as a function of immobilized urease concentration still remains unclear in current literature. Therefore, the present multiphysics model needs to be performed to elucidate the urea-induced reactive performances of urease-loaded PHEMA, PNIPAM and PAAM hydrogels, respectively, where the hydrophilicity of the polymeric network chains [229] is captured by the polymer-solvent interaction parameter χ , or called the chi-parameter, in the multiphysics model [230], in which larger chi-parameter χ leads to higher hydrophobicity of the polymeric network chains. The chi-parameter χ was measured 0.59, 0.52 and 0.50, respectively, for PHEMA, PNIPAM and PAAM hydrogels [224, 231], whereas other material inputs to perform the model: $L = 250 \mu\text{m}$, $L_{gel}^0 = 50 \mu\text{m}$, $C_f^0 = 1800 \text{ mM}$, $C_E = 7.0 \text{ mg/mL}$, $z_f = -1$, $K_a = 10^{-5.0} \text{ (M)}$, $K_M = 27 \text{ mM}$, and $k_{cat} = 1.5 \times 10^3 \text{ 1/s}$, and the rest of the inputs are given in Table. 3.1.

Several interesting numerical results are worked out by the multiphysics model, and they unveil that the hydration-induced swelling deformation of the hydrogel reduces in a sigmoid-like pattern with increase of the immobilized urease concentration, as illustrated in Fig. 3.10(a). The higher urease concentration causes enlargement of urea hydrolysis in the hydrogel, resulting in the greater deionization of the immobile charge group in the hydrogel, which then decreases the hydration-induced swelling deformation of the hydrogel. It is also observed in Fig. 3.10(a) that the PAAM hydrogel seems to exhibit the higher hydration-induced swelling deformation, which is expected as the

polymer network chains of PAAM hydrogel is more hydrophilic than both PNIPAM and PHEMA hydrogels, as quantified by chi-parameter χ .

Fig. 3.10(b) illustrates the osmotic pressure response Δp of urease-loaded hydrogel versus immobilized urease concentration of PHEMA, PNIPAM and PAAM hydrogels. As seen in Fig. 3.10(b), the larger concentration of urease in the hydrogel results in bigger shrinkage of the hydrogel, presumably due to greater urea-induced deionization of the immobile charge group. This consequently increases the osmotic pressure response Δp of the hydrogel in a sigmoid-like pattern with higher urease concentration in the hydrogel. In addition, the urease-loaded hydrogel exhibits larger osmotic pressure response Δp , if the hydrogel is incorporated with a more hydrophobic polymeric network chains. This perhaps because the lowered solubility of the system in the solvent, causing the enlargement of contractive hydrostatic forces between hydrogel-bulk solution interface, thus exhibits higher osmotic pressure response Δp [232].

Fig. 3.11 shows the osmotic pressure response Δp of urease-loaded hydrogels versus environmental urea concentration $\bar{C}_{urea}$ for different urease-loaded PHEMA, PNIPAM and PAAM hydrogels, with immobilized urease concentration of 7.0 mg/mL. It is seen that, when the concentration of urea $\bar{C}_{urea} = 90$ mM, the osmotic pressure response Δp enlarges from 8.9 to 10.2 kPa (the increase by ~14.6 %), when the hydrogel changes from PAAM to PHEMA hydrogel. In addition, the change to a more hydrophobic hydrogel from PAAM to PHEMA hydrogel also enlarges the slope of the linear urea response from 0.099 to 0.113 kPa/mM. Consequently, the more hydrophobic hydrogel enhances capability of the hydrogel in detecting small changes of

concentration of urea $\bar{C}_{urea}$ in salt solution by exhibiting greater osmotic pressure response Δp .

3.4.3 Osmotic Pressure of the Urease-loaded Hydrogel

The subsection below investigates the impacts of (i) environmental conditions, (ii) urease kinetics properties and (iii) initial fixed charge density on the osmotic pressure of the hydrogel.

3.4.3.1 Impacts of Environmental Conditions

In order to explore urease-loaded hydrogel as a material platform for continuous quantification of urea concentration, the osmotic pressure response Δp of the hydrogel is necessarily quantified, responding to variation of urea concentrations. For this aim, the multiphysics model is performed to establish the relationship between the environmental urea concentration in salt solution and the osmotic pressure of urease-loaded hydrogel. The material inputs needed to perform the model: $L = 250 \text{ } \mu\text{m}$, $L_{gel}^0 = 50 \text{ } \mu\text{m}$, $C_f^0 = 1800 \text{ mM}$, $z_f = -1$, M , $K_a = 10^{-5.0} \text{ (M)}$, $K_M = 27 \text{ mM}$, and $k_{cat} = 1.5 \times 10^3 \text{ 1/s}$ with concentration of immobilized urease of 0.5 mg/mL , and the remaining inputs are provided in Table. 3.1.

In this work, the osmotic pressure response Δp of the hydrogel is the differential osmotic pressure between the hydrogel immersed in urea-loaded solution and the hydrogel immersed in reference solution with $\bar{C}_{urea} = 0$, given as

$$\Delta p = p(\bar{C}_{urea}) - p(\bar{C}_{urea} = 0) \quad (3.36)$$

For the current examination, unless specified otherwise, the urease-loaded polymeric system is submersed in an environmental solution, containing concentration of urea $\bar{C}_{urea}$ from 0 to 400 mM at $\bar{pH}=7.0$ and temperature 37 °C with sodium chloride concentration of 143 mM.

Fig. 3.12(a) is plotted to examine the osmotic pressure response Δp of urea-sensitive hydrogel as a function of environmental urea concentration $\bar{C}_{urea}$ under varying environmental temperatures. It is seen that the osmotic pressure response Δp is categorized into two stages, as shown in Fig. 3.12(a): (i) the osmotic pressure response Δp increases linearly up to concentration of urea $\bar{C}_{urea} = 25$ mM and (ii) the osmotic pressure response Δp remains relatively unchanged with increasing the urea level further, presumably due to urease active-site saturation with urea. When immobilized urease concentration is set at $C_E = 0.5$ mg/mL, the linear urea-induced osmotic pressure response range of the hydrogel encompasses urea level of human tear, which demonstrates its potential as physiological-responsive osmotic urea biosensors.

It was previously reported in literature that the increase of environmental temperature leads to higher ionized immobile charge group density C_f in the charged hydrogel [225]. Since the pH of the hydrogel is regulated by the ionized immobile charge component density C_f , the larger fixed negatively charged group density C_f results in the smaller pH value in the hydrogel. Indeed, as the environmental temperature increases from 37 to 40 °C, the hydrogel becomes more acidic (further from the urease optimum pH=7.4). As seen in Fig. 3.12(a), the increase in acidity reduces the osmotic pressure

responsiveness of the urease-loaded hydrogel in detecting the environmental urea due to the increase both inactivation and denaturation of the mobilized urease.

In addition, Fig. 3.12(b) visualizes effect of the environmental $\overline{\text{pH}}$ on the osmotic pressure response Δp of the urease-loaded hydrogel as a function of concentration of urea $\overline{C}_{urea}$. At the concentration of urea $\overline{C}_{urea} = 25$ mM, the osmotic pressure Δp response reduces from 6.12 to 0.49 kPa as the environmental $\overline{\text{pH}}$ moves from 7 to 5. The decrease of the environmental $\overline{\text{pH}}$ leads to the increase of pH-induced ionization of the immobilized urease, and thus significantly reduces the urea-induced osmotic activity in the hydrogel. Thereby, the osmotic pressure response Δp of the urease-loaded hydrogel is highly sensitive to the environmental $\overline{\text{pH}}$, and relatively insensitive to the environmental temperature, as seen in Fig. 3.12. To the best of our knowledge, urease-loaded hydrogel is a very few examples of biomaterial which exhibits both the physiological urea- and pH-reactive performances, and consequently this creates an opportunity to develop a robust biosensor for detection of both excess concentration of urea $\overline{C}_{urea}$ and abnormal pH level in biological fluids, since CKD patients are also usually associated with an abnormal blood pH.

3.4.3.2 Impacts of Urease Kinetic Properties

Fig. 3.13(a) illustrates the osmotic pressure response Δp of the urease-loaded hydrogels versus the environmental concentration of urea $\overline{C}_{urea}$ for different immobilized urease catalytic constants k_{cat} . The catalytic constant k_{cat} of the urease indicates how fast the urease catalyzes the urea after the formation of the urease-urea complex, where the higher k_{cat} value refers to the larger number of

urea hydrolyses into its products by a single urease in a given time. When the concentration of urea $\bar{C}_{urea} = 25$ mM, the osmotic pressure response Δp enlarges from 2.4 to 6.1 kPa (increase by ~154.2 %) as k_{cat} increases from 0.5×10^3 to 1.0×10^3 s⁻¹. In addition, the increase of k_{cat} from 1.0×10^3 to 1.50×10^3 s⁻¹ also enlarges the slope of linear urea response from 0.14 to 0.25 kPa/mM. Consequently, the higher k_{cat} value may enhance the capability of the hydrogel in detecting small changes of urea concentration $\bar{C}_{urea}$ in salt solution.

Moreover, Fig. 3.13(b) presents the osmotic pressure response Δp of the hydrogel as a function of urea concentration $\bar{C}_{urea}$ at different urease Michaelis constant K_M values, such as 0.26 [81], 22.1 [78] and 27.0 mM [53]. The Michaelis constant K_M typically measures how efficiently the immobilized urease selects and hydrolyses urea into ammonium NH_4^+ and bicarbonate HCO_3^- ions, where lower K_M value corresponds to greater affinity of the urease active sites toward urea. As such, when concentration of urea $\bar{C}_{urea} = 100$ mM, the decrease of K_M from 27 to 0.26 mM increases the osmotic pressure response Δp of the hydrogel from 9.1 to 11.7 kPa (a rise by 28.6 %), as seen in Fig. 3.13(b). Moreover, the decrease of K_M from 27 to 0.26 mM also increases slope of the linear urea response from 0.28 and 0.73 kPa/mM, which results in a higher performance hydrogel for detecting urea in the environmental solution. Therefore, another possible strategy to enhance the ability of the hydrogel for sensing a change in the extremely low physiological concentration of urea $\bar{C}_{urea}$, is to employ urease which inherently associated with lower K_M value, such as jack bean-derived ureases [81].

3.4.3.3 Impacts of Initial Fixed Charge Group Density

In order to achieve an electroneutrality state, the fixed ionized charge group in the hydrogel attracts a significant number of counter-ions from its environmental solution into the hydrogel for balancing excess charges in the polymeric system. This phenomenon increases the imbalance of ionic concentration over the hydrogel-solution interface, promoting the hydration-induced swelling deformation of the hydrogel. Thereby, the immobile charge group concentration plays a crucial role in the swelling deformation of urease-loaded hydrogel, especially when it is immersed in salt solution. From literature reviews, it was found that the effect of initial fixed charge concentration especially on the osmotic pressure of urea-responsive urease-loaded hydrogel is not sufficiently investigated and remains unclear. For this aim, the multiphysics model is performed, in which the inputs needed by the model: $L = 250 \mu\text{m}$, $L_{gel}^0 = 50 \mu\text{m}$, $z = -1$, $K_a = 10^{-5.0}$ (M), $K_M = 27 \text{ mM}$, and $k_{cat} = 1.5 \times 10^3 \text{ 1/s}$ with the immobilized urease concentration of 0.5 mg/mL in the hydrogel, whereas rest of the inputs are tabulated in Table 3.1.

Fig. 3.14 illustrates the osmotic pressure response Δp of urease-loaded hydrogels immersed in salt concentration of 143 mM (human blood plasma) as a function of concentration of urea $\bar{C}_{urea}$ for systems with different initial immobile charge group densities C_f^0 . It is visualized that the osmotic pressure response Δp of the hydrogel enlarges with initial concentration of immobile charge group C_f^0 . The hydrogel with higher initial concentration of immobile charge group C_f^0 generates larger urea-induced response of the osmotic pressure

because of the greater difference of ionic distribution over the polymeric system-environmental solution interface. This consequently improves the sensing capability of the hydrogel, for detecting larger concentration of urea $\bar{C}_{urea}$, as visualized in Fig. 3.14. Thereby, the current urease-loaded hydrogel shows application potential in biosensor technology, exhibiting the physiological urea-responsive behaviors, as seen in Figs. 3.10-3.14. Hence, the urease-loaded hydrogel could provide an innovative means for monitoring urea level in bloodstream—in real-time—via urea osmotic biosensor.

3.4.4 Urease Catalytic Behavior

The subsection below investigates the impacts of (i) urea and (ii) salt concentrations on the urease catalytic behaviors of the hydrogel.

3.4.4.1 Influences of Urea Concentration

The urease-loaded charged hydrogel consists of the urea-sensitive functional group bonded to their polymeric network chains. As previously revealed, the urea-sensitive hydrogel exhibits the swelling deformation response with the changes of environmental urea concentration [233]. The hydrogel swelling equilibrium is achieved, when the multiphysics interactions within the hydrogel are at a steady-state, including ion-ion, ion-solvent and polymer-solvent interactions. However, the final swelling equilibrium state not only depends on the concentration of urea, but also the urease enzymatic properties. Therefore, it is necessary to elucidate the responsive behavior of the urease-loaded charged hydrogel for an insight into the urease catalytic performance, when subjected to variation of urea concentrations.

For this aim, the multiphysics model is performed, where the inputs needed to execute the model: $L = 250 \mu\text{m}$, $L_{gel}^0 = 50 \mu\text{m}$, $z = -1$, $K_M = 32 \text{ mM}$, and $k_{cat} = 1.5 \times 10^3 \text{ 1/s}$, $K_a = 10^{-5.0} \text{ (M)}$, $C_f^0 = 1000 \text{ mM}$ with the urease concentration of 0.5 mg/mL immobilized in the hydrogel, and the rest are provided in Table 3.1. For the current work, the polymeric system is submersed in an environmental solution, where the environmental urea concentration $\bar{C}_{urea}$ is taken from 1 to 1000 mM at environmental $\overline{\text{pH}} = 7.0$ and environmental temperature 37°C with environmental sodium chloride concentration $\bar{C}_{NaCl} = 100 \text{ mM}$.

By the numerical simulation, Fig. 3.15(a) visualizes the effect environmental temperature on the urease activity in the charged hydrogel in response to changes of environmental urea concentration $\bar{C}_{urea}$ at environmental sodium chloride concentration $\bar{C}_{NaCl} = 100 \text{ mM}$. It is visualized that the urease activity in the charged hydrogel enlarges with the enlargement of urea concentration $\bar{C}_{urea}$. Interestingly, the urease activity of the charged polymeric system further increases by decreasing the environmental temperature, which increases the hydration of the polymeric system [233]. The increase of swelling deformation enlarges the pore size, and thus enhances the urea transport into the hydrogel, which results in the increase of the urease activity with the lower environmental temperature. Notably, renal patients suffer from blood temperature drops, due to hypothermia. Hence, the thermo-responsive urease-loaded charged hydrogel is a viable option for a high-performance dialysis system, since the urease catalytic activity increases, when

the environmental temperature drops from 37 (normal person) to 35 °C (renal patient), as seen in Fig. 3.15(a).

In addition, Fig. 3.15(b) demonstrates the effect environmental $\overline{\text{pH}}$ on the urease activity in the charged hydrogel, responding to the changes of environmental urea concentration $\overline{C}_{urea}$ at environmental sodium chloride concentration $\overline{C}_{NaCl} = 100$ mM. Usually the urease operates optimally at a narrow range of pH values. If the environmental $\overline{\text{pH}}$ is closer to the urease optimum such as pH of ~ 7 , a higher urease activity is expected, resulting in the increase of urea degradation rate. Surprisingly, the urease activity almost coincides with each other for the urease-loaded charged hydrogel subjected to $\overline{\text{pH}} = 7$ and 9. When $\overline{\text{pH}} > \text{pK}_a = 5$, the immobile charge group in the system is dominantly ionized, such that the increase of $\overline{\text{pH}}$ from 7 to 9 is not accompanied by any significant change of the immobile charge component density, and thus the microenvironment pH remains almost unchanged. Consequently, the urease-loaded charged hydrogel demonstrates a similar urease activity behavior, when subjected to $\overline{\text{pH}} > 7$. Therefore, the immobilized urease into a charged hydrogel provides an opportunity to stabilize the pH-induced urease catalytic reaction, which allows the optimization of the urease-induced urea hydrolysis with respect to environmental $\overline{\text{pH}}$, if environmental $\overline{\text{pH}} > \text{pK}_a$.

Furthermore, Fig. 3.16(a) shows the influence of urease catalytic constants k_{cat} on the equilibrated swelling deformation of the charged hydrogel as a function of urea concentration $\overline{C}_{urea}$ at environmental sodium chloride concentration $\overline{C}_{NaCl} = 100$ mM. When $k_{cat} = 1.5 \times 10^3 \text{ s}^{-1}$, the charged hydrogel begins to shrink at $\overline{C}_{urea} = 0.1$ mM, whereas at $k_{cat} = 0.5 \times 10^3 \text{ s}^{-1}$, the system only

begins to collapse when $\bar{C}_{urea}$ is larger than 1 mM. Thereby, two conclusions are drawn from the visualization Fig. 3.16(a): (1) the hydrogel is more sensitive towards lower $\bar{C}_{urea}$ values, if the charged hydrogel is immobilized with urease of higher k_{cat} values, otherwise a higher $\bar{C}_{urea}$ is required before the hydrogel starts to collapse, and (2) the hydrogel is more responsive towards a wider range of $\bar{C}_{urea}$ concentrations with larger urease k_{cat} values. Therefore, the urease-loaded charged hydrogel system is able to detect and also demonstrate an effective catalytic effect from the abnormally low to the extremely high physiological urea level for a high-performance dialysis charged membrane, with larger urease k_{cat} values.

Fig. 3.16(b) is plotted to investigate the urease enzymatic activity in the charged hydrogel as a function of environmental urea concentration $\bar{C}_{urea}$ with variation of urease catalytic constants k_{cat} at environmental sodium chloride concentration $\bar{C}_{NaCl} = 100$ mM. As observed from Fig. 3.16(a), the urease enzymatic activity enlarges in proportion to the increase of lower $\bar{C}_{urea}$ values. However, the urease enzymatic activity seems to achieve a saturation state at large $\bar{C}_{urea}$, since majority of the urease active sites are saturated with urea. As seen in Fig. 3.16(a), the hydrogel undergoes dehydration with the increase of $\bar{C}_{urea}$, causing the polymeric network chains to collapse, and this consequently hinders the transport of urea into the hydrogel. Therefore, a further increase of $\bar{C}_{urea}$ (e.g., > 100 mM) is not accompanied by a significant increase of urease catalytic behavior. A possible strategy to increase the urease activity in the hydrogel, subjected to higher $\bar{C}_{urea}$ value, is to enhance the urea transport into the charged hydrogel. This perhaps is achievable if the charged hydrogel is

subjected to the lower environmental temperatures, in which the polymeric network chains become more hydrophilic, and thus are able to imbibe larger quantity of water molecules into the hydrogel [233]. This could enhance the urea transport into the hydrogel [234], and perhaps enlarge the urease enzymatic activity in the hydrogel, particularly at higher $\bar{C}_{urea}$ values.

In addition, Fig. 3.17(a) illustrates the influence of urease Michaelis constants K_M on the equilibrated swelling deformation of the charged hydrogel as a function of urea concentration $\bar{C}_{urea}$ at environmental sodium chloride concentration $\bar{C}_{NaCl} = 100$ mM. As well known, K_M corresponds to the $\bar{C}_{urea}$ value, where the rate of urease reaction in the charged environment is equal to halve maximum value of urease reaction. A lower K_M value corresponds to a higher affinity of the immobilized urease active site towards the mobile urea solute. It is also seen from Fig. 3.17(a) that, with the decrease of K_M value, the hydrogel collapses at the smaller $\bar{C}_{urea}$ value. By decreasing K_M value from 36 to 27 mM, the swelling-to-collapse transition of the system occurs at a lower $\bar{C}_{urea}$ value, such as from 2.5 to 0.15 mM. Moreover, the linear concentration range of urea $\bar{C}_{urea}$ also enlarges for the urease-loaded charged hydrogel system, when the hydrogel is immobilized with urease of lower K_M values, leading to a more responsive system in sensing lower $\bar{C}_{urea}$ values.

Fig. 3.17(b) presents the urease activity of the charged hydrogel at various urease Michaelis constants K_M at environmental concentration of sodium chloride concentration $\bar{C}_{NaCl} = 100$ mM. For example, at $\bar{C}_{urea} = 20$ mM, the urease activity increases by ~4%, when the urease K_M value increases from 2 to 50 K_M . At $\bar{C}_{urea} = 200$ mM, however, the urease activity only increases by ~0.4

%, a 10-fold reduction. These results are consistent with the previously published work [235], where a higher value of enzymatic activity is expected, when $\bar{C}_{urea}$ is significantly lower than urease Michaelis constant K_M value ($\bar{C}_{urea} \ll K_M$) [236]. Apparently, the enhanced affinity of the urease towards its substrate could limit the urease catalytic behavior, due to the excessive urea adsorption by urease [237], as seen in Fig. 3.17(b). Consequently, another possible strategy to optimize the urease enzymatic activity in the hydrogel is to incorporate urease with $K_M > 100$ mM, since urea levels in renal patients usually exceeds 71 mM. Interestingly, the hydrogel collapses at smaller $\bar{C}_{urea}$ value with the decrease of K_M value, but due to excessive urea adsorption by the urease, its activity decreases with the reduction of K_M value, as seen in Fig. 3.17(b) and 3.4(b).

Fig. 3.18(a) visualizes the effect of urease concentration on the equilibrated swelling deformation of the urease-loaded charged hydrogel as a function of environmental urea concentrations $\bar{C}_{urea}$ at environmental sodium chloride concentration $\bar{C}_{NaCl} = 100$ mM. By increasing the concentration of immobilized urease in the hydrogel, the swelling deformation decreases, presumably due to the increase of the hydrophobicity of the polymeric network chains, and thereby reduces the water ingestion into the hydrogel [205]. Additionally, the increase of urease activity with the higher urease concentration perhaps reduces the ionized fixed charge concentration in the hydrogel, which in turn decreases the osmotic ionic swelling pressure of the charged hydrogel by reducing the ionic concentration difference over the hydrogel-bulk interface, resulting in the shrinkage of the hydrogel.

Importantly, Fig. 3.18(b) shows the urease activity in the charged hydrogel, when subjected to different urea concentrations $\bar{C}_{urea}$ at various urease concentrations at environmental sodium chloride concentration $\bar{C}_{NaCl} = 100$ mM. The increase of urease concentration enlarges the number of enzyme active sites for the hydrolysis of urea, leading to a higher urease enzymatic activity in the charged hydrogel system. When $\bar{C}_{urea} = 10$ mM, the urease activity increases by ~31% in going from urease concentration 0.3 to 0.5 mg/mL. Likewise, when $\bar{C}_{urea} = 100$ mM, the urease activity enlarges by ~27% in going from urease concentration 0.3 to 0.5 mg/mL. However, as the urease concentration increases, the charged hydrogel becomes relatively denser which decreases its stretching capability, as seen in Fig. 3.18(a).

3.4.4.2 Urease Catalytic Behavior Induced by Salt Concentration

For a typical charged hydrogel, the initial increase of the environmental sodium chloride causes the enlargement of the immobile charge component density in the hydrogel [90], in which the charged hydrogel attracts a significant amount of counter-ions from its environmental solution to electrically balance the immobile charge group and co-ions in the charged hydrogel, to achieve the electroneutrality state. This promotes the swelling deformation of the charged hydrogel. The further increase of environmental sodium chloride concentration leads to the deswelling of the charged hydrogel, when the environmental sodium chloride concentration is larger than the fixed charge concentration of the hydrogel, due to suppression of the hydrogel swelling by the largely concentration of co-ions in the environmental solution.

For examining the responses of the urease-loaded anionic or cationic hydrogels subject to variation of environmental sodium chloride concentration,

the multiphysics model is performed, where the inputs needed to execute the model: $L = 250 \mu\text{m}$, $L_{gel}^0 = 50 \mu\text{m}$, $C_f^0 = 1000 \text{ mM}$, $K_a = 10^{-5.0} \text{ (M)}$, $K_M = 32 \text{ mM}$, and $k_{cat} = 1.5 \times 10^3 \text{ 1/s}$ with the urease concentration of 0.5 mg/mL immobilized in the hydrogel, while the rest are tabulated in Table 3.1. In addition, the hydrogel for the present simulation is immersed in environmental solution, where its salt concentration $\bar{C}_{NaCl}$ is taken from 1 to 1000 mM at environmental $\bar{pH} = 7.0$ and temperature 37°C .

By the numerical simulation, Fig. 3.19 visualizes the swelling behavior of the urease-loaded charged hydrogels, when subjected to variation environmental sodium chloride concentrations $\bar{C}_{NaCl}$ at different urea concentrations. It is demonstrated, for the cationic-based urease-loaded hydrogel, the increase of environmental sodium chloride concentration $\bar{C}_{NaCl}$ make the system undergoes a swelling-to-collapse volume transition, due to the decrease of the osmotic swelling pressure in the hydrogel, which is consistent with the published experimental observation [90]. For anionic-based urease-loaded hydrogel, however, when environmental urea concentration $\bar{C}_{urea} = 100 \text{ mM}$, the salt-induced behavior of the hydrogel deviates from the cationic-based urease-loaded hydrogel, where a swelling transition is found at a higher $\bar{C}_{NaCl}$, such as $\bar{C}_{NaCl} = 100 \text{ mM}$. This behavior is perhaps due to the increase of acid generation (counter-ions) by both the urea hydrolysis and dissociation of the immobile charge group, causing the ion exchange between the sodium Na^+ ions in the environmental solution and the hydrogen H^+ ions in the anionic hydrogel, and thus the hydrogel swells [238]. This behavior is not observed in cationic-based urease-loaded hydrogel, as the hydrogen H^+ ions (co-ions) are electrically

repel by the immobile charge group of the hydrogel to maintain the electroneutrality state, such that the ion exchange between Na^+ and H^+ mentioned above is absent, causing the cationic hydrogel to collapse with the increase of $\bar{C}_{\text{NaCl}}$.

Importantly, Fig. 3.20 quantifies the urease activity of the charged urease-loaded hydrogels by changing the environmental sodium chloride concentration $\bar{C}_{\text{NaCl}}$. For the cationic-based hydrogel, the urease activity increases, when $\bar{C}_{\text{NaCl}}$ enlarges, as seen in Fig. 3.20. Apparently, the dehydration of the cationic hydrogel, due to the increase of $\bar{C}_{\text{NaCl}}$ could create a microenvironment, favoring higher urease catalytic performances. However, for anionic-based hydrogel, when $\bar{C}_{\text{urea}}=100$ mM, the urease activity decreases, when $\bar{C}_{\text{NaCl}}$ enlarges beyond 100 mM, as the hydration of the hydrogel dilutes its enzymatic activity, which in turn decreases the urease catalytic behavior, as seen in both Figs. 3.16(b) and 3.17(b). As such, the anionic and cationic hydrogels exhibit a different urease catalytic activity behavioral pattern in response to the increase of environment sodium chloride concentration at a relatively higher urea concentration $\bar{C}_{\text{urea}}$, such as 100 mM.

As well known, the equilibrium partitioning of co- and counter-ions between the hydrogel and the environmental solution is governed by the ionized fixed charged group density. Due to the immobile charge group, the number of counter-ions greatly exceeds that of co-ions in the hydrogel, in order to achieve the electrical balance between the immobile charge group and co-ions in the microenvironment. The imbalance ionic concentration between the hydrogel and the environmental solution causes to a Donnan potential acting between them. Thereby, the electrical potential difference at the hydrogel-solution

interface, consequently prevents the co-ions from moving into the hydrogel. This behavior is usually referred to as the Donnan exclusion [239]. Therefore, the ion exchange behavior (ion-selectivity) of the hydrogel is highly dependent on the strength of the electrical potential Φ at the hydrogel-solution interface.

Fig. 3.21 illustrates the electrical potential Φ of both the urease-loaded charged polymeric system and environmental solution at different environmental sodium chloride concentrations $\bar{C}_{NaCl}$. It is observed that the electrical potential Φ is ~ 0 in the environmental solution due to electroneutrality condition, while Φ in the hydrogels are almost a constant. This observation is consistent with the published experimental work [239]. It is also shown from Fig. 10 that the increase of $\bar{C}_{NaCl}$ decreases the electrical potential Φ of the charged urease-loaded hydrogel, resulting in the hydrogel to behave more like a neutral hydrogel. For cationic hydrogel, the magnitude of the electrical potential Φ decreases by $\sim 84\%$, when $\bar{C}_{NaCl}$ increases from 10 to 1000 mM. For the anionic hydrogel however, the electrical potential Φ decreases by $\sim 95\%$, when $\bar{C}_{NaCl}$ increases from 10 to 1000 mM. Therefore, the ion exchange behavior (ion-selectivity) of the hydrogel is significantly lowered with the decrease of the strength electrical potential in the hydrogel-solution interface, when the $\bar{C}_{NaCl}$ increases for both the anionic and cationic urease-loaded hydrogels.

3.5 Remarks

Herein, a multi-physical model is formulated for characterizing the urea-reactive performances of urease-loaded polymeric system, incorporating the interactions between the immobilized urease and the environmental urea-rich salt solution. The model includes the Poisson-Nernst-Planck (PNP) equation for

the transport of mobile solutes between its microenvironment and the environmental solution coupled with a nonlinear mechanical equation to account for the conversion of biochemical and electric energies into its mechanical counterpart. In order to capture the urea-induced behaviors of the hydrogel, two correlations are also integrated into the multiphysics model to establish the relationship: (i) between the urease reaction rate and diffusive concentration of urea, and also (ii) between the immobile-mobile ions. Interestingly, a novel urease rate of reaction equation is then proposed, and incorporated into the PNP equation to characterize the transport problem between the polymer-solution interface.

As discussed in Section 3.3, the multiphysics model accurately captures the experimental observations reported in the literature by providing a deeper understanding into the behaviors of urease-loaded hydrogel. Furthermore, the model permits systematic investigation of the polymeric system, where the relationships between the osmotic pressure of the urea-responsive hydrogel and the environmental concentration of urea are elucidated by varying environmental conditions and urease enzymatic properties. Therefore, not only that the multiphysics model is an important tool to obtain a greater understanding of the urease-loaded hydrogel, but it is also an important computational tool for designing and optimizing such materials to develop high-performance osmotic urea biosensor.

Table 3.1 Input for the multiphysics model for urease-loaded hydrogel.

Parameter	Value
Diffusion coefficients D_{H^+} [240]	$9.3 \times 10^{-9} \text{ m}^2/\text{s}$
Diffusion coefficients D_{OH^-} [240]	$5.2 \times 10^{-9} \text{ m}^2/\text{s}$
Diffusion coefficients $D_{NH_4^+}$ [240]	$2.0 \times 10^{-9} \text{ m}^2/\text{s}$
Diffusion coefficients $D_{HCO_3^-}$ [240]	$1.2 \times 10^{-9} \text{ m}^2/\text{s}$
Flory interaction parameter χ [224]	0.5090-0.9975
Permittivity $\epsilon_r \epsilon_0$ (water)	$7.1 \times 10^{-10} \text{ A s/V.m}$
Urease optimum temperature T_{op} [241]	323.15 K
Equilibrium constant K_W	10^{-14}
Equilibrium constant K_H [218]	$7.6 \times 10^{-4} \text{ mol/ m}^3$
Equilibrium constant K_{OH} [218]	$1.3 \times 10^{-5} \text{ mol/ m}^3$
Enthalpy change for transition from the active to inactive enzyme ΔH_{eq} [63, 190]	89594 J/mol

Fig. 3.1 A general scheme for the systematic investigation of the urease-loaded charged hydrogel-based system.

Fig. 3.2 Computational diagram of the multiphysics model to characterize the system when subjected to environmental stimuli.

Fig. 3.3 Observation of swelling performance of the hydrogel between the numerical results by the model and Ogawa and Kokufuta's correlation, and their experimental data [10] at initial radius $145.0 \mu m$.

Fig. 3.4 Observation of swelling performance of the hydrogel between the numerical results by the present multiphysics model and the experimental data [10] at different initial radii: (a) $235.5 \mu m$ and (b) $318.5 \mu m$.

Fig. 3.5 Validation of the multiphysics model, capturing the urea-responsive characteristics subject to environmental conditions: (a) The pH-induced reactive behavior of the urease-loaded hydrogel subject to environmental pH [34] (b) Thermally reactive performance of the hydrogel from environmental 15 to 45 [38].

Fig. 3.6. (a) The comparison between the published experimental observation [78] and the present numerical result for characterizing the pH-induced urease catalytic activity in cationic hydrogel. (b) The hydration-driven swelling performance of the hydrogel under varying environmental pH.

Fig. 3.7. (a) The comparison between the published experimental observation [81] and the present numerical result for the pH-induced urease catalytic activity in anionic hydrogel. (b) The pH of the hydrogel as a function of the environmental pH.

Fig. 3.8. (a) The comparison between the published experimental observation [53] and the present numerically simulating result for the temperature-induced urease catalytic activity in anionic hydrogel. (b) The pH of the hydrogel as a function of the environmental temperature. (c) The comparison between the published experimental observation [53] and the present numerical result for the temperature-induced swelling hydration deformation of the urease-loaded hydrogel. (d) The contractive hydrostatic pressure acting on the hydrogel as a function of the environmental temperature.

Fig. 3.9. (a) The activity of the immobilized urease and (b) the swelling ratio of urease-loaded hydrogel as a function of urease concentration in the hydrogel, when subjected to urea concentration of 30 mM with environmental pH=7.0 and temperature 37 °C.

Fig. 3.10. (a) The urea-actuated hydration-induced swelling deformation of the hydrogel as a function of concentration of urease, when subjected to urea concentration 30 mM; and (b) The urea-actuated osmotic pressure response Δp of the hydrogel as a function of concentration of urease, when subjected to urea concentration 30 mM, with environmental pH=7.0 and room temperature. The osmotic pressure response Δp of the hydrogel is the differential osmotic pressure between the urease-loaded hydrogel and its bare counterpart $\Delta p = p(C_E) - p(C_E = 0)$.

Fig. 3.11. The osmotic pressure response Δp of urease-loaded hydrogel versus concentration of urea $\bar{C}_{urea}$ for PHEMA, PNIPAM and PAAM hydrogels, with environmental pH=7.0 and room temperature. The osmotic pressure response Δp is the differential osmotic pressure between the hydrogel immersed in urea-filled solution and the hydrogel immersed in reference solution with $\bar{C}_{urea} = 0$.

Fig. 3.12 The osmotic pressure response Δp subjected to variation concentrations of urea $\bar{C}_{urea}$, with environmental pH=7.0 and temperature 37 °C at: (a) different environmental temperatures and (b) variation of environmental pH. The osmotic pressure response Δp is the differential osmotic pressure between the hydrogel immersed in urea-filled solution and the hydrogel immersed in reference solution with $\bar{C}_{urea} = 0$.

Fig. 3.13 The osmotic pressure response Δp subjected to variation concentrations of urea $\bar{C}_{urea}$, with environmental pH=7.0 and temperature 37 °C for: (a) different urease catalytic constants k_{cat} and (b) variation urease Michaelis constants K_M , such as 0.26 [81], 22.1 [78] and 27.0 mM [53]. The osmotic pressure response Δp is the differential osmotic pressure between the hydrogel immersed in urea-filled solution and the hydrogel immersed in reference solution with $\bar{C}_{urea} = 0$.

Fig. 3.14 The osmotic pressure response Δp of hydrogels loaded with urease versus concentration of urea $\bar{C}_{urea}$ for different concentrations of initial immobile charge group within the hydrogel, with environmental pH=7.0 and temperature 37 °C. The osmotic pressure response Δp is the differential osmotic pressure between the hydrogel immersed in urea-filled solution and the hydrogel immersed in reference solution with $\bar{C}_{urea} = 0$.

Fig. 3.15 The urease activity of the anionic charged hydrogel as a function of urea concentration $\bar{C}_{urea}$ at different environmental: (a) temperature and (b) pH.

Fig. 3.16. (a) The equilibrated responsive hydration and (b) its corresponding urease activity of the anionic charged polymeric system as a function of urea concentration $\bar{C}_{urea}$ at different urease catalytic constants k_{cat} .

Fig. 3.17. (a) The equilibrated responsive hydration of the anionic charged polymeric system as a function of urea concentration $\bar{C}_{urea}$ at different Michaelis constants K_M and (b) the urease activity in the charged hydrogel as a function of Michaelis constant K_M at $\bar{C}_{urea} = 20$ and 200 mM, respectively.

Fig. 3.18. (a) The equilibrated responsive hydration and (b) its corresponding urease activity of the anionic charged polymeric system as a function of urea concentration $\bar{C}_{urea}$ at different urease concentrations.

Fig. 3.19 The swelling deformation of the urea-sensitive charged hydrogel as a function of environmental sodium chloride concentration $\bar{C}_{NaCl}$ at different $\bar{C}_{urea}$ values for: (a) cationic and (b) anionic urease-loaded hydrogels.

Fig. 3.20 The urease activity of the charged hydrogel as a function of environmental sodium chloride concentration $\bar{C}_{NaCl}$ values at different environmental urea concentrations $\bar{C}_{urea}$ values for: (a) cationic and (b) anionic urease-loaded hydrogels.

Fig. 3.21 Influence of the environmental sodium chloride concentration $\bar{C}_{NaCl}$ on the distributive profile of the electrical potential Φ at $\bar{C}_{urea} = 10$ mM for: (a) cationic and (b) anionic charged urease-loaded hydrogels.

Chapter 4 Development of a Multiphysics Model to Examine the Stimulated Responses of Hemoglobin-loaded Polyelectrolyte Hydrogels

4.1 Introduction

For fully understanding the impact of pH-, oxygen- and salt-stimuli on coupled chemo-electro-mechanical responses of hemoglobin-loaded polyelectrolyte hydrogel, a multiphysics model is developed in this chapter for elucidating the multiphysical interaction between immobile functional components bounded onto polymeric network chains of the hydrogel and hydrogen ion-oxygen-enriched environmental solution. Two constitutive relationships are incorporated into the model to capture: (1) ionization of fixed charge group as a function of its ionization strength coupled with diffusive ion concentration, and (2) bioactivity of hemoglobin as a function of both its ionization and saturation states, where a reaction rate equation is developed for capturing the bioactivity of the hemoglobin as a function pH coupled with oxygen levels. The equation is then incorporated into the model for executing the Poisson-Nernst-Planck (PNP) equation, permitting the model to characterize the immobile hemoglobin activity as a function of environmental conditions. As such, the main contribution of Chapter 4 is the coupling of the developed hemoglobin reaction rate equation with the PNP equation to characterize the transport problem between the polymer-solution interface. The multiphysics model is then verified by comparing with experimental observations in open-literature for capturing

the oxygen-induced hemoglobin saturation, the pH-actuated deformation and the salt-mediated electrical behaviors of hemoglobin-loaded polyelectrolyte hydrogels. Thereby, this chapter is organized as follows. After the introduction is presented in Section 4.1, the multiphysics model is developed in Section 4.2, followed by Sections 4.3, 4.4 and 4.5 for model implementation, model validation, and results and discussions, respectively. Finally, several remarks are drawn in Section 4.6.

4.2 Development of Model

For examining the reactive performances of the hemoglobin-loaded polyampholyte hydrogel, a multiphysics model is formulated to characterize: (a) the movement (diffusion and migration) of ions and oxygen O_2 between the microenvironment of polyampholyte hydrogel and the environmental solution; (b) the biochemical binding/unbinding reaction between the mobile solutes (oxygen and ions) and the immobilized functional groups (hemoglobin and charge group); (c) the electrical interactions between the mobile (both co- and counter-) ions in the microenvironment and the immobilized charge groups (both anionic and cationic) in the hydrogel; and (d) the oxygen- and pH-responsive actuation behaviors of the hydrogels.

The present multiphysics model is developed based on the premise of:

- (i) The ambient oxygen O_2 dissolves in environmental solution, such that the concentration of oxygen O_2 in the environmental solution is a function of ambient oxygen O_2 level, namely $[O_2] = \alpha pO_2$, where α is the solubility of oxygen O_2 in the water and pO_2 is the ambient oxygen O_2 level (mmHg).
- (ii) The oxygen O_2 reversibly binds to the immobilized hemoglobin in the hydrogel, and thus the oxygen O_2 concentration in the hydrogel is

summation of mobile (dissolved in microenvironment) and fixed (oxygen-hemoglobin complex) oxygen O_2 concentrations.

(iii) The net charge concentration of the hemoglobin-loaded polyampholyte hydrogel is contributed by both the fixed (acidic and basic groups) and mobile (co and counter ions) charges, where the ionizable group density on the immobilized hemoglobin is neglected.

(iv) The anti-polyelectrolyte behavior of polyampholyte hydrogel is accounted into the model via the Debye-Huckel theory, in which a correction term is incorporated into the mechanical equation, accounting for the electrostatic interactions between the acidic and basic groups.

4.2.1 Hemoglobin reaction mechanism

In this work, the hemoglobin-loaded hydrogel is subjected to variation of ambient oxygen O_2 levels, where the oxygen-hemoglobin interactions is given as

where Hm is the heme group located in the immobilized hemoglobin Hb, O_2 is the dissolved oxygen in the hydrogel, O_2Hm is the immobilized oxygenated heme site, and the association constant K_{O_2} is characterized by the ratio of its forward k_{OxyHb} rate to the backward k_{Oxy} one.

Furthermore, the hydrogel is also immersed in a solution with varying pH values, influencing the ionization of both the reduced Hm and oxygenated O_2Hm heme sites, where the equilibrium position of these phenomena are described by

where HmH^+ and O_2HmH^+ are both the protonated reduced and oxygenated heme sites, respectively. k_{F1} and k_{F2} are the forward rates, and k_{B1} and k_{B2} are the backward rates (where subscript 1 refers to the reduced heme sites and subscript 2 denotes the oxygenated heme sites).

Therefore, the total hemoglobin concentration $[\text{Hb}]$ in the hydrogel is thus written as

$$[\text{Hb}] = [\text{Hm}] + [\text{O}_2\text{Hm}] + [\text{HmH}^+] + [\text{O}_2\text{HmH}^+] \quad (4.4)$$

where the immobilized hemoglobin involves in two important chemical reactions: (i) the oxygenation/deoxygenation of heme group and (ii) both the protonization of reduced and oxygenated heme groups.

The saturation of hemoglobin S with the oxygen O_2 is given as

$$S = \frac{[\text{HbO}_2]}{[\text{Hb}]} \quad (4.5)$$

where the oxygenated hemoglobin concentration $[\text{HbO}_2]$ can be described as follows

$$\begin{aligned} [\text{HbO}_2] &= [\text{O}_2\text{Hm}] + [\text{O}_2\text{HmH}^+] \\ &= K_{O_2} [\text{O}_2][\text{Hm}] + K_2 [\text{H}^+][\text{O}_2\text{Hm}] \\ &= K_{O_2} [\text{O}_2][\text{Hm}] (1 + K_2 [\text{H}^+]) \end{aligned} \quad (4.6)$$

in which K_{O_2} and K_2 are defined by the ratio of its forward and backward rates and can be given as $K_{O_2} = [O_2Hm]/[O_2][Hm]$ and $K_2 = [O_2HmH^+]/[H^+][O_2Hm]$, and the total hemoglobin concentration $[Hb]$ can be expressed as

$$\begin{aligned} Hb &= Hm + HmH^+ + [HbO_2] \\ &= Hm + K_1 [H^+][Hm] + K_{O_2} [O_2][Hm] (1 + K_2 [H^+]) \\ &= Hm (1 + K_1 [H^+] + K_{O_2} [O_2] (1 + K_2 [H^+])) \end{aligned} \quad (4.7)$$

while K_1 is expressed by the ratio of $[HmH^+]/[H^+][Hm]$

The rate of oxygenation of hydrogel can be characterized mathematically by

$$r_{O_2} = k_{OxyHb} (1-S)[O_2][Hb] + k_{Oxy} S[Hb] \quad (4.8)$$

where the first term on the right characterizes the increase of concentration of the fixed oxygen O_2 in the form of oxygen-hemoglobin complex and the second term on the right describes the increase of dissociation of mobile oxygen O_2 from the fixed hemoglobin, which both enrich the microenvironment with oxygen O_2 . There is no consumption of oxygen O_2 in the system, explaining the absent of an oxygen O_2 sink term in equation 4.8.

4.2.2 Acid-base reaction mechanism

It is commonly known that the ionization of weakly acidic and basic groups bounded onto polymeric network chains of the hydrogel is determined by the electrochemical reactions for association/dissociation of hydrogen ion with the ionizable functional components

where A and B are fixed acidic and basic groups, and K_A and K_B are dissociation constants for the Eq. 4.9, in which they can be written as $K_A = [\text{A}^-][\text{H}^+]/[\text{HA}]$ and $K_B = [\text{B}][\text{H}^+]/[\text{HB}^+]$.

The initial ionizable dry fixed acidic $C_A^{o,D}$ and basic group $C_B^{o,D}$ concentrations can be written as $C_A^{o,D} = [\text{A}^-] + [\text{HA}]$ and $C_B^{o,D} = [\text{HB}^+] + [\text{B}]$ where by employing the dissociation constants described above, we can re-write equation 9 into equation 10, in which the initial ionizable dry fixed acidic $C_A^{o,D}$ and basic group $C_B^{o,D}$ concentrations can be transformed into its counterpart hydrated state by $C_A^{o,D} = \frac{C_A^o}{H}$ and $C_B^{o,D} = \frac{C_B^o}{H}$, where H is the hydration of the membrane and is defined as ratio of final volume V_f to initial volume V_o of the membrane [178].

$$\begin{aligned} [\text{A}^- / C_A] &= C_A^{o,D} \frac{K_A}{K_A + [\text{H}^+]} \text{ and} \\ [\text{HB}^+ / C_B] &= C_B^{o,D} \left(\frac{[\text{H}^+]}{K_B + [\text{H}^+]} \right) \end{aligned} \quad (4.10)$$

4.2.3 Formulation of the multiphysics model

For the development of the model, five mobile solutes are present oxygen (O_2), hydrogen ion (H^+), hydroxide ion (OH^-), a cation and an anion solutes. For

this, reaction-migration-diffusion equation is utilized to described the mass transport between microenvironment-environment [242]

$$\nabla \cdot \mathbf{N}_k + r_k = 0 \quad (k = O_2, H^+, OH^-, \text{anion}, \text{cation}) \quad (4.11)$$

where $\mathbf{N}_k$ indicates molar flux (mM/s) and r_k refers to rate of chemical reaction for k th species. For charged polymeric system, under the influence of chemical concentration gradient coupled with electrical field, the ionic and molecular transports between the hydrogel and its environmental solution can be described mathematically by a Nernst-Planck equation [233]

$$\begin{aligned} \nabla \cdot \mathbf{C}^{-1} D_k [\nabla C_k + F \mu_k z_k C_k \nabla \Phi] + r_k &= 0 \\ (k = O_2, H^+, OH^-, \text{anion}, \text{cation}) \end{aligned} \quad (4.12)$$

in which D_k , C_k , z_k , μ_k and r_k refer to diffusion coefficient (m^2/s), mobile solutes concentration (mM), valence number, ion mobility, and rate of chemical reaction. R , T , F , and Φ are the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), environmental temperature (K), Faraday constant ($96,487 \text{ Cmol}^{-1}$), and electrical potential of the hydrogel. $\mathbf{C}^{-1}$ is inverse of the right Cauchy-Green tensor.

The ion mobility for k th species can be defined by Nernst-Einstein relationship, establishing a relationship between the ion mobility and its diffusion coefficient, given as

$$\mu_k = \frac{D_k}{RT} \quad (4.13)$$

In order to capture impacts of electrical potential strength on the mobile ions concentrations in the hydrogel, equation 4.14 is incorporated into the

model which gives the relationship between the polyampholytic polymeric system electrical potential and the system net charge concentration [205, 233]

$$\nabla \cdot (\varepsilon_r \varepsilon_0 \mathbf{C}^{-1} \nabla \Phi) = -\frac{F}{(1 + C\nu)} \left(\sum_k z_k C_k - C_A + C_B \right) \quad (4.14)$$

where ε_r is relative dielectric constant of environmental solution and ε_0 is permittivity of vacuum, and C is number of moles of water molecule and ν is volume of a mole of water molecules. C_A and C_B are concentration of the fixed acidic and basic charge groups, respectively.

Surface conductivity characterizes the ionic transport of the hydrogel as function transferable counter- and co-ion concentrations coupled with their diffusion kinetics, where the surface conductivity of the hydrogel can be written as [243, 244]

$$K^\sigma = \frac{F^2}{RT} \left(\sum_k D_k C_k \right) \quad (4.15)$$

where K^σ is surface conductivity (S/m) of the hydrogel, where it is a function of counter- or co-ion concentration coupled with its respective diffusion coefficients.

In terms of hydration-induced swelling deformation of the hydrogel, the mechanical swelling pressure acting over the hydrogel-solution interface arises due to: (i) the elastic nature of the polymeric network chains, (ii) the ionic concentration difference between the hydrogel and environmental solution, (iii) the mixing between the polymeric system and the environmental solution, and (iv) the electrical interactions between the immobile charge groups and the mobile ions, which accounts for the anti-polyelectrolyte behaviors [245]. At

swelling equilibrium, the hydrostatic pressure p over the hydrogel-solution interface is defined as the sum of the osmotic pressure, given as [233]

$$p = RT \sum_k \left(\frac{C_k}{H} - \frac{\kappa^3}{24\pi} \sigma \right) - RT \sum_k \left(\bar{C}_k - \frac{\bar{\kappa}^3}{24\pi} \bar{\sigma} \right) - N_k k_B T \left[\frac{1}{(1+Cv)^{1/3}} \left(\frac{1}{3} \text{tr}(\mathbf{C}_e) - \frac{1}{(1+Cv)^{2/3}} \right) \right] - \frac{RT}{v} \left(\ln \frac{Cv}{(1+Cv)} + \frac{1}{(1+Cv)} + \frac{\chi}{(1+Cv)^2} \right) \quad (4.16)$$

where κ is inverse screening length, N_k is the number of polymeric network chains per volume ($2.43 \times 10^{25} \text{ m}^{-3}$), $k_B T$ is the absolute temperature in unit of energy and χ is polymer-solvent interaction parameter which indicates the disaffinity between hydrogel-solution. It is seen that the mechanical pressure on the interface between hydrogel-solution consists of a few multi-physical components, including polymer-solution, polymer-polymer, and mobile-immobile ions synergistic interactions.

The inverse screening length κ can be written as [245]

$$\kappa = \sqrt{\frac{F^2}{\epsilon_r \epsilon_0 RT} \sum (C_k z_k^2 + C_f z^2)} \quad (4.17)$$

and σ is given as [246]

$$\sigma_k(\kappa a) = 1 - \frac{3}{2} \kappa a + \frac{9}{5} (\kappa a)^2 \quad (4.18)$$

where κ and a refer to inverse screening length and ion radius

It is well-established that, at swelling equilibrium condition, the hydration-induced deformation of the hydrogel progresses, until it is balanced by the elastic forces exerted by the polymeric network chains to achieve an equilibrium hydration state, where the swelling deformation λ_s of the free surface between hydrogel-solution interface can be given as [233]

$$\lambda_s = \frac{N_k k_B T}{p} = \frac{L_{gel}}{L_{gel}^0} \quad (4.19)$$

where $N_k k_B T$ is ground shear modulus, p is hydrostatic pressure, and L_{gel} and L_{gel}^0 are final and initial lengths of the hydrogel, respectively.

4.3 Numerical Implementation

In this chapter, the multiphysics model is solved in steady-state 1D via COMSOL for examining the responsive characteristics of a cylindrical hemoglobin polyelectrolyte hydrogels in response to environmental stimuli.

The swelling of the polymeric system is limited to only the radial direction, where Neumann boundary conditions are set at $X=0$,

$\frac{\partial C_k}{\partial X} = 0$, $\frac{\partial \Phi}{\partial X} = 0$ ($k = H^+, OH^-, \dots, N$) while Dirichlet boundary conditions

are assumed at the environmental solution $C_k = \bar{C}_k$, $\Phi = 0$.

4.4 Model Validation with Experimental Observations

In order to validate the multiphysics model, the examination between the present numerical results and the published experimental observation is performed for: (i) the oxygen O_2 association with hemoglobin at different ambient levels and (ii) the pH-actuated swelling hydration deformation of the hemoglobin-loaded hydrogel.

4.4.1 Saturation of Hemoglobin with Oxygen

Fig. 4.1(a) visualizes the saturation of neonatal hemoglobin with the oxygen O_2 by the present numerical results and the published experimental observation [247], responding to the changes of ambient oxygen O_2 level. In the experimental work conducted by Shiao and Ou (2007), the association of oxygen O_2 with the neonatal hemoglobin is measured from the ambient oxygen

O₂ level 30 to 100 mmHg at environmental pH=7.4, whereas the inputs needed by the model to characterize numerically the oxygen saturation of the hemoglobin are provided in Table. 4.1. As observed in Fig. 4.1, the results show that the saturation of hemoglobin with the oxygen O₂ increases in a sigmoid-like pattern with the enlargement of the ambient oxygen O₂ level. Thereby, the present numerical results are in a good agreement with the published experimental data, with a difference of $\sim 1.3\%$, and thus the multiphysics model is able to capture well the oxygen-reactive performances of the hemoglobin.

Fig. 4.2 compares the saturation of newborn and adult hemoglobins with oxygen O₂ via present numerical results and published experimental observation [248, 249] when responding to changes of ambient oxygen O₂ level at environmental pH of 7.40. As illustrated in Fig. 1, the saturation of hemoglobin with oxygen O₂ monotonously enlarges with increase of ambient oxygen O₂ level, where the model reasonably captured the oxyhemoglobin saturation of newborn hemoglobin from 0 to 100 mmHg, while the model managed to quantify well the oxyhemoglobin saturation of adult hemoglobin from oxygen level 50 to 100 mmHg but overestimating the adult oxyhemoglobin saturation from 0 to 50 mmHg. This is because the model neglected the effect of 2,3-diphosphoglycerate (2,3-DPG) on the oxygen-sensitive behaviors of hemoglobin which exists mainly in adult hemoglobin, consequently lowering its oxygen affinity at lower physiological oxygen levels [135]. It is well established that newborn hemoglobin is not associated with 2,3-DPG, leading to the greater oxygen affinity in newborn hemoglobin as compared to its adult counterpart, especially from oxygen level 0 to 70 mmHg. Therefore, the model is able to characterize reasonably well the saturation of

newborn and adult hemoglobins, when subjected to physiological ambient level at environmental pH of 7.40.

4.4.2 Hydration-induced Deformation Responses of the Hydrogel

In addition, Fig. 4.1(b) probes the pH-induced responsive swelling characteristics of the hemoglobin-loaded polyacidic hydrogel via both the present numerical results and published experimental observation [2], where the material inputs needed by the model: $L = 250 \text{ } \mu\text{m}$, $L_{gel}^0 = 50 \text{ } \mu\text{m}$, $K_A = 10^{-5} \text{ (M)}$, $C_A^0 = 180 \text{ mM}$, with concentration of immobilized hemoglobin 0.01 mM , while the remaining inputs are provided in Table. 4.1. The polymeric system is submersed in a 10 mM salt solution, where the environmental pH is taken from 1 to 13 and ambient oxygen O_2 level 160 mmHg , with temperature $37 \text{ }^\circ\text{C}$. The pH-induced volume transition behaviors of the hemoglobin-loaded hydrogel are divided into four stages: (i) the hydrogel is at a collapse state with the increment of the environmental pH lesser than 3, as majority of the immobile charge group still remains unionized, (ii) the hydrogel undergoes a collapse-to-swelling transition behavior from environmental pH 3 to 7, due to increase of the concentration of ionized immobile charge group, (iii) the hydrogel remains at the swelling state from environmental pH 7 to 9, as the fixed charge is still dominantly ionized, and (iv) the hydrogel collapses by further increasing the environmental pH, due to suppression of the osmotic swelling by the largely concentration of counter-ions in the environment solution.

Fig. 4.3(a) examines the impact of environmental pH on reactive behaviors of polyampholyte hydrogel at temperature $25 \text{ }^\circ\text{C}$ with $\chi = 0.45$ and $Nk_B T = 0.03 \text{ MPa}$ [250, 251], in which the present numerical result is in a good agreement with published experimental observation [250, 251], consequently

showing the capability of the model to reasonably capture the reactive performances of polyampholyte hydrogel under varying pH levels. As illustrated in Fig. 4.3(a), the hydrogel undergoes swelling-to-collapse-to-swelling-to-collapse actuation behaviors by increasing environmental pH, where the hydrogel experiences the swelling-to-collapse volume transition behavior from pH 3 to 4, while it subsequently undergoes the collapse-to-swelling actuation behavior with further increase of environmental pH value from 8 to 9. A comparison between Fig. 4.5 and Fig. 4.3(a) reveals that when ambient oxygen level is higher than 100 mmHg, the neutral pH-induced collapse state reduces solubility of the hydrogel in the environmental solution, consequently resulting in higher oxygen O₂ activity in the system due to greater solvent dehydration tendency, which offsets oxygenation effect in the hydrogel. Interestingly, the deformation of the hydrogel remains unchanged from environmental pH 5 to 7, exhibiting independence between environmental pH and actuation behavior of the hydrogel. This provides an avenue for independent assessment of external osmotic active analyte and environmental pH, especially when operating at physiological conditions (such as from pH 5 to 7).

Fig. 4.3(b) is plotted to investigate how incorporation of hemoglobin onto the polymeric network chains of polyampholyte hydrogel influences its pH-actuated volumetric response when subjected to low or high physiological oxygen O₂ level. Similarly, as observed in Fig. 4.3(a), Fig 4.3(b) also demonstrates that typical and hemoglobin-loaded polyampholyte hydrogels experience a bowl-shaped like swelling deformation pattern with increase of environmental pH. The higher ionic concentration of hydrogen or hydroxide ion

(such as $\text{pH} < 3$ or $\text{pH} > 10$) suppresses swelling deformation of typical polyampholyte hydrogel, consequently leading to its shrinkage at the extreme pH levels. Interestingly, this phenomenon is absent in hemoglobin-loaded polyampholyte hydrogel because the oxygen-rich microenvironment in the hydrogel consequently offsets the effect of high hydrogen or hydroxide ion concentration (such as $\text{pH} < 3$ or $\text{pH} > 10$) in the environmental solution, especially on its deformation performance. Furthermore, as observed in the inset of Fig. 4.3(b), the osmotic pressure response of the present hydrogel exhibits a similar bowl-shaped like behavioral pattern by increasing environmental pH when operating at high or low ambient O_2 level, pointing to independence between ambient O_2 and pH-induced hydrogel osmotic pressure responses.

4.5 Results and Discussions

This section explores the impact of (i) hemoglobin and immobile charge group loadings, (ii) ambient oxygen level, (iii) environmental pH value and (iv) environmental salt- and oxygen-coupled stimuli on the responsive behaviors of the hydrogels.

4.5.1 Effects of Hemoglobin and Immobile Charge Group Loading

In general, the oxygen O_2 is stored in the hemoglobin by forming the oxygen-hemoglobin complex (oxyhemoglobin), where typically the concentration of oxyhemoglobin in a free hemoglobin solution enlarges in the sigmoid-like fashion with increase of ambient oxygen O_2 level. However, the effect of ambient oxygen O_2 coupled with the environmental pH on the reactive performances of the hydrogel remains unclear in the current literature. In order to address the paucity of information, the multiphysics model is necessarily

performed, where the material inputs needed by the model for the pNIPAM-based hydrogel are obtained from referenced experimental and numerical investigations, given as $L = 250 \text{ } \mu\text{m}$, $L_{gel}^0 = 50 \text{ } \mu\text{m}$, $K_A = 10^{-6} \text{ (M)}$, $K_B = 10^{-8} \text{ (M)}$, $C_A^0 = 0.1 \text{ M}$ and $C_B^0 = 0.1 \text{ M}$ with concentration of immobilized hemoglobin 1 mM [10, 251-253], while the remaining inputs are provided in Table. 4.1. For the current examination, unless specified otherwise, the polymeric system is submersed in the salt solution of $C_{NaCl} = 100 \text{ mM}$ at temperature $37 \text{ }^\circ\text{C}$, where the environmental pH is taken from 1 to 13 and ambient oxygen O_2 from 0.1 to 1000 mmHg.

Fig. 4.4 investigates the loading influence of the functional group on the dual oxygen- and pH-reactive performances of the hemoglobin-loaded polyampholyte hydrogel. Fig. 4.4(a) visualizes the impact of the hemoglobin concentration on the oxygen-driven swelling deformation of polyampholyte hydrogel at environmental pH=7.4 (blood plasma pH), in which the hydrogel demonstrates the swelling response for the oxygen O_2 , ranging from 1 to 100 mmHg, even for the hydrogels loaded with different hemoglobin concentrations. This suggests the independence between the hydrogel linear swelling deformation response and the hemoglobin concentration in the hydrogel. Fig. 4.4(b) examines the effect of immobile charge component density on the pH-actuated swelling deformation of the hydrogel at the oxygen O_2 concentration of 160 mmHg (atmospheric level), where the increase of immobile charge group concentration causes the enlargement of the swelling deformation of the hydrogel. However, its pH-driven swelling deformation pattern remains unchanged for hydrogel incorporated with different immobile charge group concentrations. Therefore, Fig. 4.4 unveils that the linear swelling

deformation response of the present hydrogel is independent of the functional component loading, including the immobile charge component density and the immobilized hemoglobin concentration.

4.5.2 Effects of Ambient Oxygen Level

In the following part, the reactive performances of typical and hemoglobin-loaded N-isopropylacrylamide (NIPAM)-based polyampholyte hydrogels are examined by investigating the impacts of dual pH-O₂ stimuli on the hydrogel reactive performances. The aim of this subsection is achieved by performing the multiphysics model, where the material inputs needed by the model are given as $Nk_B T = 0.1 \text{ MPa}$, $L = 250 \text{ } \mu\text{m}$, $L_{gel}^0 = 50 \text{ } \mu\text{m}$ with concentration of immobilized hemoglobin 1 mM ($1 \times 10^{-6} \text{ mol/cm}^3$), while the rest of the inputs are provided in Table. 4.1. Unless specified otherwise, the hydrogel is immersed in the salt solution of 100 mM at temperature $37 \text{ }^\circ\text{C}$ with environmental pH from 1 to 13 and ambient oxygen O₂ from 0.1 to 1000 mmHg . In this work, the osmotic pressure response Δp of the hydrogel is the differential osmotic pressure between the present hydrogel and the hydrogel at isoelectric point pI (collapse-state), namely $\Delta p = p - p(\text{pI})$ [254].

Fig. 4.5(a) investigates the influence of environmental pH on the saturation of hemoglobin operating in polyampholyte hydrogel with oxygen O₂, when responding to changes of ambient oxygen O₂ level. As seen in Fig. 4.5(a), the hemoglobin saturates bi-linearly with oxygen O₂ by increasing ambient level, where oxygen affinity of the hemoglobin decreases with increase of environmental acidity. These behaviors are consistent with hemoglobin operating in physiological environment, in which the drop of blood pH causes

the decrease of hemoglobin oxygen affinity, consequently releasing the oxygen into bloodstream. Due to the cooperative nature of oxygen O₂ binding with hemoglobin, the oxyhemoglobin saturation drastically increases from ambient oxygen level 1 to 100 mmHg, coinciding with the physiological conditions in the bloodstream. This makes hemoglobin-loaded polyampholyte hydrogel a biocompatible oxygen-sensing biomaterial platform, where it is highly responsive to changes of physiological oxygen level.

Fig. 4.5(b) examines the impact of environmental pH on hemoglobin-mediated oxygen activity in the hydrogel as a function of ambient oxygen level, when operating in salt-loaded environmental solution. For hydrogel subjected to increasing ambient oxygen level from 1 to 30 mmHg, the increase of environmental pH from 2 to 7 (5 pH units) enlarges the linear slope of oxygen O₂ uptake rate from 1.45 to 1.51 mM s⁻¹/ mmHg (enhances by 4.14 %), while the subsequent increase of environmental pH from 7 to 12 (5 pH units) only increases the linear slope by a mere 0.66 %. This shows that the oxygen-sensing performance of the hydrogel is enhanced with decrease of environmental alkalinity. In addition, at higher ambient oxygen level (*i.e.*, >100 mmHg), the pH neutral condition accelerates the oxygen activity in the hydrogel, presumably due to influence of hydrogel dehydration state, and this hypothesis is further explored below.

4.5.3 Effects of Environmental pH

In order to further investigate the influences of environment pH on hemoglobin-loaded polyampholyte hydrogel, Fig. 4.6(a) is plotted to visualize the effect of fixed acidic and basic groups strength on swelling deformation of the hydrogel as a function of environment pH, when operating at ambient oxygen O₂ level of

160 mmHg. As illustrate in Fig. 4.6(a), the collapse-to-swelling transitional performance of the hydrogel is caused by the pH-driven increase of excess charges in the hydrogel [90], whereas the swelling-to-collapse characteristics of the hydrogel is provoked by the increase of electrostatic attraction between the fixed acidic and the basic groups [255]. In addition, as observed in Fig. 4.6(a), the strength increase of both the fixed acidic and basic groups makes the swelling deformation of the hydrogel to be independent of the wider environment pH range. The strength increase of fixed acidic and basic charge groups causes them to remain dominantly ionized at the wider pH range, electrically neutralizing each other, and consequently decreasing the tendency of the hydrogel to hydrate itself. Therefore, as visualized in Fig. 4.6(a), the swelling deformation pattern of the hydrogel changes from a “V-” to a “bowl”-shaped like swelling deformation pattern with the strength increase of fixed acidic and basic groups.

Fig. 4.6(b) visualizes the osmotic pressure response of hemoglobin-loaded polyampholyte hydrogel as a function of environmental pH with different strengths of fixed acidic and basic groups at ambient oxygen O₂ level of 160 mmHg. It is unveiled in Fig. 4.6(b) that the pH-actuated osmotic pressure response of the hydrogel is categorized into three stages: (i) the osmotic pressure response decreases with the initial increment of the environment pH, (ii) the osmotic pressure response remains unchanged with subsequent rise of environmental pH, showing independence between environmental pH and hydration-induced swelling deformation of the hydrogel, and (iii) the osmotic pressure response enlarges with further alkalinity increment of environmental solution. As such, Fig. 4.6(b) establishes the relationship between

environmental pH and the pressure acting over hydrogel-solution interface, where osmotic swelling pressure response of the hydrogel changes in a bowl-like pattern by increasing environmental pH, due to the modification of pH-sensitive acidic-basic electrical interactions in the hydrogel.

Fig. 4.7(a) demonstrates the pH-responsive immobile charge group ionization degree of hemoglobin-loaded polyampholyte hydrogels with several strengths of fixed acidic and basic groups at ambient oxygen O₂ level of 160 mmHg. As visualized in Fig. 4.7(a), the hydrogel turns from positively to neutral, and then negatively charged, with increase of environmental pH from acidic to neutral, and then to basic one. In addition, the strength increase of fixed acidic and basic groups makes the hydrogel to exhibit electrically neutral-state at a wider environmental pH. A comparison between Fig. 4.6(a) and Fig. 4.7(a) reveals that the electrically neutral-state of the hydrogel directly corresponds to its collapse-state, where the hydrogel is at a collapse-state when the electrostatic attraction between the fixed acidic and the basic groups is at a maximum.

Fig. 4.7(b) investigates the effect of environmental pH on electrical potential of hemoglobin-loaded polyampholyte hydrogel with different strengths of fixed acidic and basic groups. It is known that, when both the fixed and mobile charges are electrically balanced, the hydrogel exhibits isoelectric point behaviors if the Donnan potential of the system is ~0 mV. As reported in the literature, the isoelectric point of polyampholyte hydrogel is usually determined by the density of ionized immobile charge groups [250, 256], but net charge concentration of the entire polymeric system is contributed by both fixed and mobile charges. Thereby, the equilibrated electrical potential of the

hydrogel should be employed to determine the isoelectric point of polyampholyte hydrogel [257], instead of the immobile charge component density in the system, since the electrical potential is a function of net charge concentration contributed by both the mobile and fixed charges in the hydrogel. Thereby Fig. 4.7(b) shows that the hydrogel undergoes positive-to-neutral-to-negative electrical potential transitional behaviors by increasing the environmental pH. Interestingly, the hydrogel also tends to remain at the neutral charged-state at a wider environmental pH range, when the strength of the fixed acidic and basic groups is increased.

Fig. 4.8 investigates the loading influence of fixed acidic and basic groups, where the initial concentration of dominant immobile charge group is twice that of its counter one, where Fig. 4.8(a) examines the responsive behavior of the hydrogel loaded with $C_A^0 = 200$ mM and $C_B^0 = 100$ mM, while Fig. 4.8(b) probes the responsive behavior of the hydrogel loaded with $C_A^0 = 100$ mM and $C_B^0 = 200$ mM. A comparison between Fig. 4.8(a) and Fig. 4.8(b) reveals that both are mirror images of each other, and when initial concentration of dominant immobile charge group is twice that of its counterpart, both collapse state and isoelectric point performance of the hydrogel are observed at environmental pH coinciding with the acid-base dissociation constant of the dominant charge group [258]. Moreover, when the strength of fixed acidic and basic groups increases, the collapse state of the hydrogel seems to shift to the environmental pH which favors ionization of the initially minority immobile charge group. If $C_A^0 = 200$ mM and $C_B^0 = 100$ mM, the increase of acidic and basic groups strength shifts the hydrogel collapse-state towards the acidic scale,

promoting the ionization/deionization of the basic/acidic group, as visualized in Fig. 4.8(a).

Fig. 4.9 presents the effect of environmental pH on electrical potential of the initially unbalanced hemoglobin-loaded polyampholyte hydrogel, with different strengths of fixed acidic and basic groups. As seen in Fig. 4.9 that the pH-responsive electrical potential of initially unbalanced polyampholyte hydrogel is categorized into three stages: (i) the electrical potential of the hydrogel decreases with the initial increment of the environment pH, (ii) the electrical potential of the hydrogel seems to be unchanged with the subsequent rise of the environment pH, showing the pH-insensitive mechanical behaviors and (iii) the electrical potential of the hydrogel strengthen with the further increment of environmental alkalinity. In addition, the strength decrease of fixed acidic and basic groups consequently enlarges the slope of the linear response, particularly at physiological pH values. Thereby, the present initially unbalanced polyampholyte hydrogel shows application potential in area of biosensor technology for detecting change in the physiological pH in biological fluids.

4.5.4 Effects of Environmental Salt- and Oxygen-stimuli

In order to examine the influences of environment salt- and oxygen-coupled stimuli on hemoglobin-loaded polyelectrolyte hydrogel, Fig. 4.10(a) is plotted to investigate the deformation of polybasic membrane as a function of environmental salt concentration $7C_{NaCl}$ for system with different initial fixed charge densities. The material inputs needed by the model are given as $L=15\text{ mm}$, $L_{gel}^0=1.5\text{ mm}$, $K_B=10^{-10}\text{ (M)}$, $\chi=0.3$, $C_B^{0,A}=175\text{ mM}$ / $C_B^{0,B}=810\text{ mM}$ (for A and B simulation inputs, respectively) [259, 260], while the

remaining inputs are provided in Table. 4.1. For the current examination, the membrane is submersed in environmental pH of 7.0 and ambient oxygen O₂ level 160 mmHg, with temperature 37 °C. As observed in Fig. 4.10(a), the results show that the swelling deformation of the membrane decreases in a sigmoid-like pattern with the enlargement of environmental salt concentration, due to weakening of osmotic swelling pressure by increasing salt concentration. On the other hand, Fig. 4.10 (b) probes the deformation of polyampholytic membrane as a function of environmental salt concentration for system with different initial fixed charge densities. The material inputs needed by the model are given as $L=1000 \mu\text{m}$, $L_{gel}^0 = 252.5 \mu\text{m}$, $K_A = 10^{-1.5} \text{ (M)}$, $K_B = 10^{-11.5} \text{ (M)}$, $\chi = 0.3$, $C_A^0 + C_B^0 = 700 \text{ mM}$, where $C_B^{0,C} - C_A^{0,C} = -2 \text{ mM}$ or $C_B^{0,D} - C_A^{0,D} = -14 \text{ mM}$ [261], while the remaining inputs are provided in Table.4.1. The polyampholytic membrane undergoes collapse-to-swelling behaviors, or so-called the anti-polyelectrolyte phenomena, due to high diffusion of mobile solutes into the membrane which promotes its swelling behaviors. Overall, the present numerical results are in a good agreement with the published experimental observations, consequently further validating the model.

We now examine the surface conductivity of anion- and cation-charged polymers via present numerical results and published experimental observation [262] via Fig. 4.11. The material inputs needed by the model are given as $L=900 \mu\text{m}$, $L_{gel}^0 = 90 \mu\text{m}$ [262], $\chi=0.1$ (highly hydrated system) [229], fully ionized initial immobile charge component density $C_A^{o,CMX} = 7620 \text{ mM}$ / $C_B^{o,AMX} = 7930 \text{ mM}$ [243] (for CMX and AMX simulation inputs, respectively), while the remaining inputs are provided in Table. 4.1. As seen in Fig. 4.11, the surface conductivity of the membrane increases monotonously by increasing the

environmental salt concentration where the surface conductivity reaches an almost convergence state when the salt concentration is increased larger than ~ 0.1 M, due to the diminishing electrical potential gradient between membrane-solution interface. The higher membrane solubility (*i.e.* smaller χ values) causes the greater stretching of the polymeric network chains, diluting the counter- and co-concentrations in the polymer and reducing the immobile charge component density in the membrane which decreases the Donnan potential strength acting over the membrane-solution interface. These coupled effects lead to the plateauing of the polymeric system surface conductivity with increase of salt concentration, as seen in Fig. 4.11. As such, the more hydrophilic polymeric system is associated with smaller surface conductivity level, given that the environmental salt and initial immobile charge component concentrations of the polymeric systems are the same. After comparison against the experimental observations, the multiphysics model can reasonably describes the coupled bio-chemo-electro-mechanical responses of hemoglobin-loaded membrane, and thus the model is validated.

For examining the effects of environmental salt concentration coupled with ambient oxygen level on surface conductivity and Donnan potential of hemoglobin-loaded pNIPAM-based polyelectrolyte membranes, the multiphysics is necessarily performed, in which the material inputs needed by the model are given as $L = 100 \mu\text{m}$, $L_{gel}^0 = 50 \mu\text{m}$, $K_A = 10^{-5} / K_B = 10^{-9}$ (M), $\chi = 0.91$, $C_A^0 / C_B^0 = 100$ mM [224], and the remaining inputs are provided in Table. 4.1. For the current examination, unless specified otherwise, the membrane is submersed in an environmental solution of pH 7.40 with ambient temperature of 37 °C.

Fig. 4.12(a) examines the effects of salt concentration C_{NaCl} on oxygen-induced deformation of hemoglobin-loaded polyacidic membrane with initial fixed charge concentration $C_f^o = 100$ mM [263]. Fig. 4.12(a) illustrates that the membrane hydrates in a sigmoid-shaped fashion with increase of ambient oxygen O_2 level, where the membrane linear swelling deformation response ranges from oxygen O_2 level of 1 to 100 mmHg. When $C_f^o \geq C_{NaCl}$, the increase of environmental salt concentration C_{NaCl} enlarges screening effects on the immobile charge group which reduces the ionic osmotic swelling pressure in the membrane, leading to its shrinkage. Surprisingly, when $C_{NaCl} > C_f^o$, the increase of environmental salt concentration C_{NaCl} causes the membrane to significantly hydrate itself. For example, at ambient oxygen O_2 level of 100 mmHg, the membrane de-hydrates by 0.2 % if salt concentration C_{NaCl} increases from 10 to 100 mM, but the hydration of the membrane increases by 2.7 % when salt concentration C_{NaCl} increases from 100 to 1000 mM. However, the tendency of the membrane to swell at higher salt concentration (*i.e.* when $C_{NaCl} > C_f^o$) known as the anti-polyelectrolyte behavior, requires further investigation as this phenomenon remains incompletely understood in current literature, as visualized in Figs. 4.12-4.15.

Fig. 4.12(b) illustrates the influence of salt concentration C_{NaCl} on oxygen O_2 loaded in the polyacidic membrane as function ambient oxygen O_2 level. It appears that oxygen O_2 concentration in the system enlarges in a sigmoid-shaped characteristic by increasing ambient oxygen O_2 level, where the loading of oxygen O_2 in the membrane linearly increases only from ~ 1 to 100 mmHg.

This is because the active-sites of immobile hemoglobin are saturated with oxygen O_2 when ambient level is increased beyond 100 mmHg, showing independence between oxygen O_2 loading and ambient level. In addition, Fig. 3(b) also reveals that the oxygen O_2 loading in the membrane decreases with increase of environmental salt concentration C_{NaCl} . This result is consistent with published experimental observation where hemoglobin oxygen O_2 affinity decreases with increase of salt concentration C_{NaCl} [135].

The loading of counter-ion (*i.e.* sodium Na^+ ion) and co-ion (*i.e.* chloride Cl^- ion) in the present polyacidic membrane is visualized in Fig. 4.12(c) and Fig. 4.12(d), respectively, as a function of ambient oxygen O_2 level when subjected to variation of environmental salt concentrations C_{NaCl} . From Figs. 4.12(c) and 4.12(a), it is seen that the increase of hydration-induced swelling deformation of the membrane, by enlarging ambient oxygen O_2 level, decreases the counter-ion concentration in the system. This is because the increase of hydration reduces immobile charge component density in the membrane. Consequently, its ability to electrically attract counter-ion into the polymeric system is weakened. For example, when at constant ambient oxygen O_2 level of 100 mmHg, the ratio of counter-ion concentration in the system to its environmental solution decreases with increase of environmental salt concentration C_{NaCl} , such that the ratio changes from 1.85 to 1.06 when salt concentration C_{NaCl} is enlarged from 100 to 1000 mM, demonstrating the decrease of membrane Donnan potential strength [264]. On the other hand, Fig. 4.12(d) shows that the loading of co-ion is enlarged in a sigmoid-shaped manner with decrease of oxygen-induced Donnan potential strength. But when

subjected to lower environmental salt concentration C_{NaCl} of 10 mM, the co-ion concentration in the system appears to be unchanged with increase of ambient oxygen O_2 level due to relatively stronger Donnan potential strength.

Fig. 4.12(e) examines the influences of environmental salt concentration C_{NaCl} on Donnan potential of hemoglobin-loaded polyacidic membrane as a function of ambient oxygen O_2 level. Fig. 4.12(e) shows that the Donnan potential strength of the membrane decreases in a sigmoid-like shaped by increasing ambient oxygen O_2 level, in which the Donnan potential strength is further weakened with increase of environmental salt concentration C_{NaCl} . The increase of ambient oxygen O_2 level enlarges swelling deformation of the membrane which reduces immobile charge group concentration, consequently weakening Donnan potential strength of the membrane. Moreover, the increase of environmental salt concentration C_{NaCl} also reduces Donnan potential strength of the membrane, in which the greater number of mobile ions enhances the screening of immobile charge group in the membrane, electrically neutralizing the membrane. Although, it appears that the ambient oxygen O_2 level influences the Donnan potential strength of the membrane, however, how ambient oxygen O_2 impacts the surface conductivity of the membrane remains unclear in the open literature.

Fig. 4.12(f) is plotted to investigate the impacts of ambient oxygen O_2 level on surface conductivity of the membrane when subjected to variation of environmental salt concentrations C_{NaCl} . A comparison between Fig. 4.12(e) and Fig. 4.12(f) shows that although the Donnan potential strength of the membrane decreases in a sigmoid-like shaped by increasing ambient oxygen O_2

level. However, the surface conductivity of the membrane remains almost unchanged with enlargement of ambient oxygen O_2 level. This is because, by increasing ambient oxygen O_2 level, the increase of hydration-induced swelling deformation promotes counter- and co-ions transport in the membrane [265], weakening the membrane Donnan potential strength. This coupled effects cause the surface conductivity of the membrane to remain almost the same with increasing ambient oxygen O_2 level. From Fig. 4.12(e), it is established that the increase environmental salt concentration C_{NaCl} weakens Donnan potential strength of the membrane which then promotes both counter- and co-ions transport between the interfaces over membrane-solution, enhancing the surface conductivity of the system. For example, when at ambient oxygen O_2 level of 100mmHg, the increase of environmental salt concentration C_{NaCl} from 10 to 100 mM or from 100 to 1000 mM enhances surface conductivity of the membrane by 2.23- or 9.33-fold, respectively. As seen in Fig. 4.12(a), the increase of environmental salt concentration C_{NaCl} from 100 to 1000 mM leads to increase hydration-induced swelling deformation coupled with decrease of Donnan potential strength, perhaps explaining the improvement of surface conductivity by 9.33, instead of 2.23, times when environmental salt concentration C_{NaCl} is further increased by 10-fold.

Fig. 4.13(a) investigates the salt-induced volumetric behaviors of hemoglobin-loaded polyelectrolyte membrane with initial fixed charge concentration $C_f^o=100$ mM when subjected to variation of ambient oxygen O_2 levels, such as 1, 10 and 100 mmHg. These critical levels are chosen because hemoglobin-mediated oxygen transport in the membrane is only reactive from

ambient oxygen O_2 level of 1 to 100 mmHg, as shown in Figs. 4.13. As observed in Fig. 4.13(a), the salt-induced volumetric behaviors of the membrane are divided into three stages: (1) when $C_{NaCl} < 0.1 C_f^o$, the initial increase of salt concentration C_{NaCl} provokes the ionization of the immobile charge group, leading to a collapse-to-swelling volumetric transitional behavior [266], (ii) when $0.1 C_f^o \leq C_{NaCl} < C_f^o$, the following increase of salt concentration C_{NaCl} causes the membrane to undergo a swelling-to-collapse transitional characteristics due to increase of screening effects by mobile ions on the immobile charge group, limiting the swelling capability of the membrane, and (iii) when $C_{NaCl} \geq C_f^o$, the membrane significantly rehydrates itself with the further increase of environmental salt concentration C_{NaCl} due to a large electrochemical potential difference between the membrane and its environmental solution which overwhelms the immobile charge group-mediated migration of ion transport, consequently driving the diffusion of environmental mobile ions down its concentration gradient into the membrane. The in-depth examination of Fig. 4.13 (a) reveals that the initial immobile charge concentration C_f^o of the polymeric system plays a pivotal role in its hydration-induced swelling deformation characteristics, which warrants further investigation of initial immobile charge group effects on the membrane in the later part, as plotted from Figs. 4.14.

Fig. 4.13(b) visualizes the oxygen O_2 loading in the membrane as a function environmental salt concentration C_{NaCl} at different ambient oxygen O_2 levels. As shown in Fig. 4.13(b), it appears that a reciprocal relationship is observed

between oxygen O_2 concentration and hydration-induced deformation of the membrane. For example, the collapse-to-swelling volume transition behaviors causes the decrease of oxygen O_2 concentration in the system, while the swelling-to-collapse volume transition characteristics leads to increase of oxygen O_2 concentration in the system. Furthermore, the increase ambient oxygen O_2 level enlarges oxygen-hemoglobin saturation which promotes the oxygen O_2 loading in the membrane. For example, when subjected to environmental salt concentration C_{NaCl} of 100 mM, the increase of ambient oxygen level O_2 from 1 to 10 mmHg or from 10 to 100 mmHg enlarges the oxygen O_2 concentration in the system by 3.70- or 1.34-fold, respectively. It is well-established that the oxygen-hemoglobin saturation increases bi-linearly with increase of ambient oxygen level O_2 [267], in which the linear hemoglobin saturation curve slope is relatively larger if the ambient level is increased from 1 to 10 mmHg as compared to the slope when ambient level is increased from 10 to 100 mmHg. This explains the greater increase of oxygen O_2 concentration in the system associated with the increment of lower ambient level, such as from 1 to 10 mmHg.

The counter-ion (*i.e.* sodium ion) and co-ion (*i.e.* chloride ion) loaded in the present hemoglobin-enriched polyacidic membrane is illustrated in Fig. 4(c) and Fig. 4(d), respectively, as a function of environmental salt concentration C_{NaCl} when subjected to variation of ambient oxygen O_2 levels. From Fig. 4.13(c), it is demonstrated that the counter-ion concentration increases bi-linearly with increase of environmental salt concentration C_{NaCl} : (1) when $C_{NaCl} \leq C_f^o$ (*i.e.* 100mM) the initial increase of salt concentration C_{NaCl}

promotes counter-ion migration, where the counter-ion concentration in the system is much greater than that of environmental solution and (2) when $C_{NaCl} > C_f^o$, the further increase of environmental salt concentration C_{NaCl} significantly weakens the Donnan potential strength of the membrane in which the dominant driving force for ionic transport in the system arises due to concentration gradient, almost diminishing the imbalance of counter-ion concentration between the membrane and its environmental solution. On the other hand, Fig. 4.13(d) shows that the co-ion concentration is tri-linearly enlarged by increasing environmental salt concentration C_{NaCl} : (1) when $C_{NaCl} \leq 0.01 C_f^o$ (*i.e.* 1mM), the co-ion concentration is apparently invariant with initial increase of salt concentration due to strong Donnan potential in the membrane, (ii) when $0.01 C_f^o < C_{NaCl} \leq C_f^o$, the subsequent increase of environmental salt concentration C_{NaCl} starts to weaken the Donnan potential strength in the membrane, however, its co-ion concentration is still much lesser than that of environmental solution and (iii) when $C_{NaCl} > C_f^o$, the Donnan potential strength in the membrane is depleted, in which the difference of counter-ion concentration between membrane and environmental solution is minuscule.

Fig. 4.13(e) inspects the Donnan potential of hemoglobin-loaded membrane as a function of environmental salt concentration, when subjected to variation of ambient oxygen levels. As visualized in Fig. 4.13(e), the Donnan potential strength of the membrane monotonously decreases with increase of salt concentration, where it approaches ~ 0 mV if $C_{NaCl} \geq C_f^o$. It is well established that Donnan potential measures capability of membrane to exclude co-ions into

its polymeric system, where the co/counter-ions concentration in the system is decreased/increased with stronger electrical potential strength in the membrane.

Fig. 4.13(f) investigates the surface conductivity of the membrane as a function of environmental salt concentration C_{NaCl} when subjected to variation of ambient oxygen O_2 levels. Fig. 4.13(f) shows that the surface conductivity of the membrane is enhanced bi-linearly by increasing the salt concentration C_{NaCl} , in which, when $C_{NaCl} \geq C_f^o$, the surface conductivity begins to converge into a single surface conductivity value at ambient oxygen level of 1, 10 or 100 mmHg, given that the membrane is operating at a constant environmental salt concentration C_{NaCl} . In addition, the surface conductivity of the membrane is almost independent of ambient oxygen O_2 level, especially at two salt concentration C_{NaCl} regions, low ($C_{NaCl} \leq \sim 1$) and high ($C_{NaCl} \geq \sim 1000 \text{ mM}$) environmental salt concentrations. On the one hand, at high environmental salt concentration, the dominantly diffusion-governed ion transport into the membrane cancels the effects of increasing ambient oxygen O_2 level on surface conductivity of the membrane. On the other hand, at low environmental salt concentration C_{NaCl} , the principally migration-administered counter-ion transport into the membrane, due to relatively strong Donnan potential strength, leads to the independence between ambient oxygen O_2 level and surface conductivity of the membrane.

We now answer the question of how initial immobile charge component density C_f^o influences salt-induced behaviors of hemoglobin-loaded polyelectrolyte membranes [90]. Fig. 4.14(a) examines the impacts of initial

immobile charge component density C_f^o on deformation behaviors of the membrane as a function of environmental salt concentration C_{NaCl} . Fig. 4.14(a) demonstrates that the salt-induced volumetric behaviors of the membrane are divided into three stages: (i) when $C_{NaCl} < 0.1 C_f^o$, the initial increment of environmental salt concentration C_{NaCl} promotes ionization of immobile charge group, resulting in the swelling of the membrane [266], (ii) when $0.1 C_f^o \leq C_{NaCl} \leq C_f^o$, the subsequent increment of salt concentration C_{NaCl} causes the enlargement immobile charge group screening by the mobile ions, making the membrane to exhibit a swelling-to-collapse behaviors and (iii) when $C_{NaCl} > C_f^o$, the membrane significantly imbibes environmental solvent due to entrance of counter- and co-ions with the further increase of environmental salt concentration C_{NaCl} , where the mobile ions diffuse down its concentration gradient into the polymeric system.

Fig. 4.14(b) visualizes the impacts of initial immobile charge component density on oxygen O_2 loading in the membrane as a function environmental salt concentration C_{NaCl} . As shown in Figs. 4.14(a) and 4.14(b), an inverse relationship is observed between oxygen O_2 concentration in the system and hydration-induced deformation of the membrane, where the increase of swelling deformation of the membrane decreases oxygen O_2 concentration in the polymeric system. Furthermore, the increase initial immobile charge component density C_f^o enlarges swelling deformation of the membrane by diluting oxygen O_2 concentration in the system. For example, when subjected to environmental salt concentration C_{NaCl} of 100 mM, the increase of initial

immobile charge component density C_f^o from 10 to 100 mM or from 100 to 1000 mM enlarges the hydration of the membrane which decreases oxygen O_2 concentration in the system by 3.54 or 17.5 %, respectively.

The counter-ion (*i.e.* sodium ion) and co-ion (*i.e.* chloride ion) concentration in the present hemoglobin-enriched polyacidic membrane is illustrated in Fig. 4.14(c) and Fig. 4.14(d), respectively, as a function of environmental salt concentration C_{NaCl} , when operating at varying densities of initial immobile charge group C_f^o . As seen in Fig. 4.14(c), the imbalance of counter-ion concentration between the membrane and environmental solution diminishes with increasing environmental salt concentration C_{NaCl} , in which: (i) when $C_{NaCl} \leq C_f^o$, the initial increase of salt concentration C_{NaCl} decreases the ratio of counter-ion concentration in the system to its environmental solution due to the greater screening effects of the fixed charge group by mobile ions, weakening the Donnan inclusion of counter-ions into the membrane and (ii) when $C_{NaCl} > C_f^o$, the further increase of environmental salt concentration significantly weakens the Donnan potential strength in which the ratio of counter-ion concentration in the system to its environmental solution is ~ 1 . On the other hand, Fig. 4.14(d) shows that the co-ion concentration is generally enlarged by increasing environmental salt concentration. When $C_{NaCl} \leq 0.1 C_f^o$, the initial increase of salt concentration C_{NaCl} decreases the ratio of co-ion concentration in the system to environmental solution, in which the stronger Donnan potential causes co-ion concentration in the system to remain unchanged with the initial increase of salt concentration C_{NaCl} . When $C_{NaCl} >$

C_f^o , the further increase of environmental salt concentration C_{NaCl} completely vanishes Donnan potential in membrane, in which the ratio of co-ion concentration in the system to its environmental solution is ~ 1 .

Fig. 4.14(e) evaluates the Donnan potential of hemoglobin-loaded membrane as a function of environmental salt concentration C_{NaCl} for membrane loaded with different initial fixed charge densities C_f^o . As visualized in Fig. 4.14(e), the Donnan potential strength of the membrane monotonously decreases with increase of salt concentration C_{NaCl} [268], where it approaches ~ 0 mV when $C_{NaCl} \geq 0.1C_f^o$. On the other hand, Fig. 4.14(f) visualizes the effects initial fixed charge concentration C_f^o on surface conductivity of the membrane as a function of environmental salt concentration C_{NaCl} . When operating at a constant environmental salt concentration C_{NaCl} , the increase of immobile charge component density from 10 to 100 mM enhances the migration of counter-ions into the membrane, resultantly improving the surface conductivity of the membrane. When environmental salt concentration C_{NaCl} is greater than 100 mM, the surface conductivity of the membrane appears to remain unchanged with increase of immobile charge component density C_f^o from 10 to 100 mM, due to weakened Donnan potential strength of the membrane in which ion transport is dominantly diffusion-governed, suggesting a strong dependence between surface conductivity of the membrane and salt-rich environmental solution.

We now examine the salt-induced volumetric behaviors of hemoglobin-loaded polyacidic, polybasic and polyampholytic membranes where the total

initial fixed charge concentration C_f^o of respective membranes is set at 100 mM. As visualized in Fig. 4.15(a), the polyacidic or polybasic experiences a collapse-to-swelling-collapse-swelling volumetric behaviors by increasing environmental salt concentration C_{NaCl} , where the swelling deformation of polyacidic or polybasic almost coincides with each other. It is established by *English et al.* that when operating at zero excess charges (Donnan potential strength of ~ 0 mV) in the membrane, the decrease of environmental salt concentration C_{NaCl} increases the electrical attraction between immobile acidic and basic charge groups in the polyampholytic membrane which collapses its polymeric system [261]. This observation is consistent with our present numerical result, such that when $C_{NaCl} < C_A^o / C_B^o$, the present polyampholytic membrane undergoes a swelling-to-collapse volumetric transitional behaviors by decreasing environmental salt concentration C_{NaCl} , and it remains at a collapse-state with further decrease of the environmental salt concentration C_{NaCl} due to maximum electrical attraction between acidic-basic charge group.

On the one hand, Fig. 4.15(b) visualizes the oxygen O_2 loading in hemoglobin-loaded polyacidic, polybasic and polyampholytic membranes as a function environmental salt concentration C_{NaCl} . As seen in Fig. 4.15(b), the oxygen O_2 concentration is decreased in the membrane by increasing environmental salt concentration C_{NaCl} , consistent with Figs. 4.12(b) and 4.13(b). As expected, the relatively smaller degree hydration of polyampholytic membrane enlarges the oxygen concentration in the polymeric system. On the other hand, Figs. 4.15(c) and 4.15(d) illustrate the sodium Na^+ and chloride Cl^-

ion concentration, respectively, in the membranes as a function of environmental salt concentration C_{NaCl} . In general, sodium Na^+ and chloride Cl^- ion concentration enlarges bi-linearly with increase of environmental salt concentration, where the ratio of sodium/chloride-ion concentration in the polyampholytic membrane to environmental solution is constantly ~ 1 . This is because when the membrane is operating at charge balanced conditions, where the Donnan potential strength of the polyampholytic membrane is extremely weakened to approximately ~ 0 mV, which causes the ion transport to be dominantly diffusion-governed. As such, it is imperative to investigate the Donnan potential strength to elucidate this assumption, as shown in Fig. 4.15(e).

Fig. 4.15(e) inspects the Donnan potential of hemoglobin-loaded polyacidic, polybasic and polyampholytic membranes as a function of environmental salt concentration C_{NaCl} when operating at pH neutral conditions. As visualized in Fig. 4.15(e), the Donnan electrical potential strength of polyacidic/basic membrane bi-linearly decreases with increase of environmental salt concentration C_{NaCl} , where the Donnan potential approaches ~ 0 mV if salt concentration $C_{NaCl} \geq$ initial fixed charge concentration C_f^o . On the other hand, for present polyampholytic membrane at neutral pH conditions, its Donnan potential is almost independent of environmental salt concentration C_{NaCl} . When the polyampholytic membrane is operating at environmental pH coinciding with its isoelectric point—average value of dissociation constants for acidic and basic charge groups, such as $(pK_a + pK_b)/2 =$ isoelectric point—the Donnan potential of the membrane remains a constant value of ~ 0 mV, as

illustrated from Fig. 4.15(e), suggesting maximum electrical attraction between the fixed acidic-basic groups which causes the Donnan potential of the membrane to be unresponsive toward changes of environmental salt concentration C_{NaCl} .

Fig. 4.15(f) investigates the surface conductivity of hemoglobin-loaded polyacidic, polybasic and polyampholytic membranes as a function of environmental salt concentration C_{NaCl} . The surface conductivity pattern of the polyacidic and polybasic membrane almost coincide with each other when operating at environmental salt concentration C_{NaCl} ranging from 0.1 to 1000 mM, as illustrated in Fig. 4.15(f). However, the polybasic membrane is observed to have greater surface conductivity level due to higher ion mobility associated with its counter-ion [269], whereas the surface conductivity of present polyampholytic membrane increases linearly by increasing environmental salt concentration C_{NaCl} . Since the Donnan potential of present polyampholytic membrane is insignificant when operating at pH neutral condition, as shown in Fig. 4.15(f), the increase of environmental salt concentration C_{NaCl} enlarges its concentration gradient between membrane-solution, promoting the ion transport in the system via diffusion-governed mechanism.

4.6 Remarks

To the best of our knowledge, this is the first work to investigate the reactive behaviours of the hemoglobin-loaded hydrogel theoretically. In this chapter, the multiphysics model is formulated to characterize osmotic pressure and electrical potential responses of hemoglobin-loaded polyelectrolyte hydrogel, accounting

the multi-physical interactions between immobile functional groups in the hydrogel and environmental salt-loaded solution. In order to capture the dual pH- and oxygen-reactive behaviors of the hydrogel, two correlations are also integrated into the multiphysics model to capture: (i) ionization of the immobile charge groups as a function of its ionization strength coupled with hydrogen ion concentration, and (ii) bioactivity of the hemoglobin as a function of both its ionization and saturation states. The model is also validated by examining the current numerical data against published experimental observations, successfully obtaining a good similarity.

As described in section 4.4 and 4.5, the model reasonably captures the pH-actuated swelling deformation pattern of polyampholyte hydrogel, where the swelling deformation pattern of the initially balanced hydrogel changes from a “V-” to a “bowl”-shaped like swelling deformation pattern with the strength increase of fixed acidic and basic groups. Furthermore, the model also allows systematic investigation of the impact of pH- and oxygen-coupled stimuli on osmotic pressure and electrical potential responses of hemoglobin-loaded polyampholyte hydrogel, establishing a relationship between electromechanical responses of the hydrogel and its oxygen-rich environmental salt solution. The result unveils that the oxygen-actuated osmotic pressure response of hemoglobin-loaded hydrogel is independent of the environmental pH, while the electrical potential response of the hydrogel is relatively unresponsive towards the changes of ambient oxygen O_2 level at pH neutral condition. Therefore, the multiphysics model is credible tool to obtain a greater understanding into hemoglobin-loaded polyelectrolyte hydrogel, which permits systematic studies of such materials under varying physiological conditions.

Table 4.1 Input data for the numerical simulation of the multiphysics model for hemoglobin-loaded polyelectrolyte hydrogel.

Parameter	Value
Diffusion coefficients D_{H^+} [240]	$9.3 \times 10^{-9} \text{ m}^2/\text{s}$
Diffusion coefficients D_{OH^-} [240]	$5.2 \times 10^{-9} \text{ m}^2/\text{s}$
Flory interaction parameter χ for pNIPAM [224]	0.906
Permittivity $\epsilon_r \epsilon_0$ (water)	$7.1 \times 10^{-10} \text{ A s/V.m}$
Forward rate k_{OxyHb} ; Backward rate k_{Oxy} [270]	$3.3 \times 10^3 \text{ mM}^{-1} \text{ s}^{-1}$; 50 s^{-1}
Association constant: K_1 ; K_2 [271]	$\frac{1}{10^{-7.58}}$; $\frac{1}{10^{-7.72}}$
Ion radius a [245]	$3 \text{ \AA}$
Solubility of oxygen O_2 α	$1.34 \text{ }\mu\text{M/mm Hg}$

Fig. 4.1 The comparison between the present numerical result and the published experimental observations [2, 247]: (a) the oxygen saturation with neonatal hemoglobin as a function of ambient oxygen levels; (b) the pH-induced swelling deformation of the hemoglobin-loaded hydrogel.

Fig. 4.2 The saturation of (a) newborn and (b) adult oxyhemoglobins as a function of ambient oxygen level with environmental pH of 7.4 via published experimental observation [248, 249] and present numerical result. For newborn hemoglobin: $k_{OxyHb}/k_{Oxy} = 170 \text{ mM}^{-1}$, and for adult hemoglobin: $k_{OxyHb}/k_{Oxy} = 50 \text{ mM}^{-1}$ [272].

Fig. 4.3 (a) A comparison between present numerical result and published experimental observations [250, 251] for the pH-induced swelling deformation behaviors of a typical polyampholyte hydrogel at environmental temperature 25 °C. (b) The pH-induced swelling deformation behaviors of hemoglobin-loaded polyampholyte hydrogel, while inset of Fig.3(b) shows the pH-actuated osmotic pressure response of hemoglobin-loaded hydrogel. The osmotic pressure response Δp of the hydrogel is the differential osmotic pressure between the hydrogel and the hydrogel at isoelectric point.

Fig. 4.4 (a) The impact of hemoglobin loading on the oxygen-induced swelling deformation of the hemoglobin-loaded polyampholyte hydrogel. (b) The influence of fixed acidic and basic groups densities on the pH-driven reactive performances of the hemoglobin-loaded polyampholyte hydrogel.

Fig. 4.5 The impact of environmental pH on (a) newborn hemoglobin saturation with oxygen and (b) hemoglobin-mediated oxygen activity in the hydrogel as a function of ambient oxygen level.

Fig. 4.6 (a) The pH-induced swelling deformation behaviors and (b) the pH-actuated osmotic pressure response of hemoglobin-enriched polyampholyte hydrogel loaded with different strengths of fixed acidic and basic groups at ambient oxygen O_2 level of 160 mmHg. The osmotic pressure response Δp of the hydrogel is the differential osmotic pressure between the present hydrogel and the hydrogel at isoelectric point.

Fig. 4.7 The pH-induced (a) net charge concentration and (b) electrical potential response of hemoglobin-enriched polyampholyte hydrogel loaded with different strengths of fixed acidic and basic groups at ambient oxygen O_2 level of 160 mmHg.

Fig. 4.8 The pH-actuated osmotic pressure response of hemoglobin-enriched polyampholyte hydrogel loaded with different strengths of fixed acidic and basic groups at ambient oxygen O_2 level of 160 mmHg for: $C_A^0 = 200$ mM and $C_B^0 = 100$ mM and (b) $C_A^0 = 100$ mM and $C_B^0 = 200$ mM. The osmotic pressure response Δp of the hydrogel is the differential osmotic pressure between the present hydrogel and the hydrogel at isoelectric point. The insets of (a) and (b) are its corresponding pH-actuated swelling deformation behaviors.

Fig. 4.9 The pH-actuated electrical potential of hemoglobin-enriched polyampholyte hydrogel loaded with different strengths of fixed acidic and basic groups at ambient oxygen O_2 level of 160 mmHg for: (a) $C_A^0 = 200$ mM and $C_B^0 = 100$ mM and (b) $C_A^0 = 100$ mM and $C_B^0 = 200$ mM.

Fig. 4.10. Comparison of membrane swelling deformation as a function of environmental salt concentration via present numerical simulation and published experimental observation for: (a) polybasic [259, 260] and (b) polyampholytic membranes [261].

Fig. 4.11. Comparison of membrane surface conductivity as a function of environmental salt concentration via present numerical simulation and published experimental observation for AMX and CMX charged polymeric system [262].

Fig. 4.12. The (a) swelling deformation, (b) oxygen loading, (c) counter-concentration, (d) co-ion concentration, (e) electrical potential and (f) surface conductivity of the hemoglobin-loaded polyacidic membrane with initial fixed charge concentration of 100 mM as a function of ambient oxygen level, when operating at environmental pH of 7.40 and temperature of 37 °C.

Fig. 4.13. The (a) swelling deformation, (b) oxygen loading, (c) counter-concentration, (d) co-ion concentration, (e) electrical potential and (f) surface conductivity of the hemoglobin-loaded polyacidic membrane with initial fixed charge concentration of 100 mM as a function of environmental salt concentration, when operating at environmental pH of 7.40 and temperature of 37 °C.

Fig. 4.14. The effect of initial fixed charge concentration on (a) swelling deformation, (b) oxygen loading, (c) counter- concentration, (d) co-ion concentration, (e) electrical potential and (f) surface conductivity of the hemoglobin-loaded polyacidic membrane with initial fixed charge concentration of 100 mM as a function of environmental salt concentration, when operating at environmental pH of 7.40 and temperature of 37 °C.

Fig. 4.15. The (a) swelling deformation, (b) oxygen loading, (c) counter-concentration, (d) co-ion concentration, (e) electrical potential and (f) surface conductivity of the hemoglobin-loaded polyacrylic, polybasic and polyampholytic membranes with initial fixed charge concentration of 100 mM as a function of environmental salt concentration, when operating at environmental pH of 7.40 and temperature of 37 °C.

Chapter 5 Conclusions and Recommendations

In this chapter, the main conclusion of current works are presented, followed by suggestions for the future research work.

5.1 Conclusions from the Present Work

The present research work deals with the development and simulation of multiphysics models to investigate the responsive behaviours of bio-responsive hydrogels by coupling the biological, chemical, electrical and mechanical fields together. The model is presented to establish the relationships between analyte-loaded environmental solution and hydrogel mechano-electrochemical responses. Subsequently, the model is directly validated by comparison with published experimental data, where it can capture well the environmental-induced responsive behaviours of the hydrogels. Finally, the effects of extreme environmental circumstances on the reactive performances of the hydrogel are elucidated via the multiphysics model.

The main conclusions of this thesis are briefly described in the following two subsections, respectively.

5.1.1 Urease-loaded Hydrogel

The first academic achievement is the novel multiphysics model development for examining the reactive performances of urease-loaded polymeric system.

- A multiphysics model is formulated to characterize the coupled bio-chemo-electro-mechanical responses of urease-loaded hydrogel, where a novel kinetic reaction rate equation is incorporated into the model to

describe the urease activity as a function of ambient temperature coupled with environmental solution pH.

- For model validation, the present numerical result is examined with published experimental data, where good agreements are achieved, especially for temperature-, pH- and urease-induced swelling deformation and urease catalytic activity of the polyacidic and polybasic hydrogels.
- The result illustrates that the urease catalytic activity patterns differ in anionic and cationic urease-loaded hydrogels by increasing environmental sodium chloride at a relatively larger environmental urea concentration, whereas the urease catalytic activity remains almost independent of environmental pH, if acid-base dissociation constant pK the of the polyacidic hydrogel is smaller than its environmental pH. The result also shows that the osmotic pressure response of urease-loaded hydrogel enlarges linearly with increase of physiological urea concentration, pointing to a biocompatible sensory membrane.

5.1.2 Hemoglobin-loaded Hydrogel

The second of major achievement made in this thesis is the a novel multiphysics model formulation for describing the reactive performances of hemoglobin-loaded hydrogel.

- A multiphysics model is presented to elucidate the coupled pH- and oxygen-stimulated responses of hemoglobin-loaded polyelectrolyte hydrogels, where a developed constitutive relation is integrated into the model to capture the hemoglobin bioactivity as a function of ambient oxygen coupled with environmental pH.

- Subsequently, after examination with the published experimental observation in open literature, it is concluded that the model can effectively capture the hemoglobin saturation with oxygen for (1) neonatal and (2) adult hemoglobins.
- The result shows that the hydration of the polyampholytic hydrogel modifies in a bowl-shaped fashion by increasing the environmental pH value. In addition, the result demonstrates that the strength increase of both the fixed acidic and basic groups in the initially balanced hydrogel causes the hydrogel to exhibit isoelectric point behavior at wider environmental pH range, whereas the initially unbalanced polyampholytic hydrogel collapses at the environmental pH coinciding with dissociation constant of the dominant immobile charge group, if the initial concentration of dominant immobile charge group is twice that of the counter one.

5.2 Recommendations for the Future Work

The current work achieves a greater understanding of the characteristics of urease- and hemoglobin-loaded hydrogels under varying environmental conditions. A few future research directions are recommended below for gaining a further insight into such materials.

- It would be interesting to solve the multiphysics model in multiple dimensions for a more accurate and precise characterization of the hydrogel, although the present 1D numerical solution is capable of capturing well the experimental observations.
- In the biochemical field, the present work has simulated the performance of a single bio-functional component-loaded hydrogel. However, a high-performance bio-responsive hydrogel is usually incorporated with two or more bio-functional components on the

polymeric network chains. Therefore, it is recommended to investigate urease-hemoglobin-loaded hydrogel under varying physiological conditions.

- In the electrical field, the present work assumed that the immobile charge group is homogenously decorated on the polymeric network chains. However, in practice the immobile charge group is bounded non-homogenously on the polymeric network chains. Therefore, it would be interesting to examine the performance of the polymeric network chains, involving both homogenous and non-homogenous immobile charge group arrangement in the hydrogels.
- In the mechanical field, the present work has numerically investigated the osmotic pressure response of the hydrogel. Therefore, it is definitely worthwhile to conduct experimental investigation with the assistance of the model for further examining the current multiphysics model.

Appendix

As the representative cases, the response of the hydrogel is investigated as a function of mesh size to ensure the numerical solution is invariant of the mesh size, for achieving solution convergence

Fig. A1 The numerical solution is examined as a function of mesh size for case in Fig. 3.3 at variation of urease concentrations.

Fig. A1 examines the numerical solution, for cases in Fig. 3.3, as a function of the mesh size. It is clearly demonstrated that the numerical solutions appear to be invariant of the mesh size if it is $\leq \sim 50$ nm. Therefore, the optimal mesh size for Fig. 3.3 cases is chosen at 50 nm. To further ensure mesh independent solution, for cases in Fig. 3.3, around 10,000 default-shaped quadratic elements, amounting to $\sim 10^5$ degree of freedom is employed which requires ~ 30 minutes for each case via the Newton's method, subjecting to tolerance factor of 0.1.

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Publications Associated to this Thesis

The list of publications in peer reviewed journals with content related to this thesis is listed below, where majority of the text and figure which comprises of this thesis is obtained from the following journal papers.

Journal

1. **K.B. Goh**, Hua Li & K. Y. Lam (2017). Development of a multiphysics model to characterize the responsive behavior of urea-sensitive hydrogel as biosensor. *Biosensors and Bioelectronics*, 91, 673-679.
2. **K.B. Goh**, Hua Li & K. Y. Lam (2017). Characterization of dual urea- and pH-induced behaviors of smart hydrogel at human physiological conditions. *Procedia Engineering*, 214, 86-92
3. **K.B. Goh**, Hua Li & K. Y. Lam (2018). Urease catalytic behaviors induced by both urea and salt concentrations in charged hydrogels as dialysis membranes. *Reactive and Functional Polymers*, 127, 74-84.
4. **K.B. Goh**, Hua Li & K. Y. Lam (2018). Modeling the Urea-Actuated Osmotic Pressure Response of Urease-Loaded Hydrogel for Osmotic Urea Biosensor. *Sensors and Actuators B: Chemical*, 268, 465-474.
5. **K.B. Goh**, Hua Li & K. Y. Lam (2018). Modeling the impact of pH- and oxygen-coupled stimuli on osmotic pressure and electrical potential responses of hemoglobin-loaded polyampholyte hydrogel *ACS Applied Biomaterials (In Press)*
6. **K.B. Goh**, Hua Li & K. Y. Lam (2018). Dual oxygen- and pH-reactive performances of hemoglobin-loaded polyelectrolyte hydrogel for oxygen-pH biosensor and biofuel cell platform. *Under review*

7. **K.B. Goh**, Hua Li & K. Y. Lam (2018). Effects of salt- and oxygen-coupled stimuli on Donnan potential and surface conductivity of hemoglobin-loaded polyelectrolyte hydrogels *Under review*

Conference

1. **K.B. Goh**, Hua Li & K. Y. Lam (July 2017). Characterization of reactive performances of enzyme-loaded ionic hydrogel as biosensor and therapeutic agent. Society of Engineering Science 2017 Meeting, Boston, MA. (Oral)
2. **K.B. Goh**, Hua Li & K. Y. Lam (July 2017). Multiphysics Modelling the Urea-Induced Responsive Behavior of the Urea-Sensitive Hydrogel. 14th U.S. National Congress for Computational Mechanics, Montreal, Quebec. (Oral & Poster)
3. **K.B. Goh**, Hua Li & K. Y. Lam (June 2017). Development of a Multiphysics Model to Characterize the Responsive Behavior of Urea-Sensitive Hydrogel. 9th International Conference on Materials for Advanced Technologies, Singapore. (Oral & Session chair)