UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF STARCH/CHITOSAN NANOPARTICLE NANOCOMPOSITE FILMS WITH ANTIBACTERIAL PROPERTIES

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DEVELOPMENT OF STARCH/CHITOSAN NANOPARTICLE NANOCOMPOSITE FILMS WITH ANTIBACTERIAL PROPERTIES

By

RUZANNA BINTI AHMAD SHAPI'I

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the requirement for the Degree of Master of Science

June 2018

This thesis is dedicated to

My mom and siblings for their great sacrifice

Al-Fatihah for my late father who encouraged me to further study in master

With love, respect and a bunch of memories
Indeed, we belong to Allah and indeed to Him we will return.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chairman : Siti Hajar Othman, PhD
Faculty : Engineering

The application of biopolymers as food packaging material are limited due to the poor desired properties. Incorporation of chitosan nanoparticle (CNP) into biopolymer films can improve the desired properties of the films which include mechanical, thermal, barrier, and antibacterial properties. Thus, this work is directed towards developing starch/CNP films with improved properties. CNP was first synthesized using different concentrations of chitosan 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, and 2.1% w/v) and the average particle size of CNP was measured via DLS and TEM. DLS analysis revealed that the particle size of CNP increased from 46.76 to 225.33 nm (4-fold increment) with the increase in initial concentration of chitosan. The average particle size of 0.9% w/v CNP measured via DLS was 106.34 nm while that measured via TEM analysis was 53.93 nm. Note that, TEM provides the actual size of CNP in dehydrated state whereas DLS provide hydrodynamic size in hydrated state hence TEM was more accurate to measure the size of the produced CNP. Starch/CNP films were produced using solution casting method with varying concentration of CNP (5, 10, 15, 20, 25, 30, 35% w/w of solid starch). The mechanical, thermal, barrier, and optical properties of the starch/CNP films (average thickness: 0.07 mm) were characterized. Addition of 15% w/w CNP into starch films improved the tensile strength from 1.12 to 10.03 MPa (7.96-fold increment) and elongation at break from 67 to 90.77% (0.35-fold increment). This was due to the good interfacial interaction formed between starch and CNP thus produced a compact and rigid structure of the film. Improvement in the structure of the films led to the enhancement in the thermal stability of the film whereby residue of starch film increased from 7.91% to 23.48% (2-fold increment). Furthermore, reduction in the water vapor permeability from 1.1 x 10^{-11} g/Pa h m to 0.63 x 10^{-11} g/Pa h m (4-fold increment) and oxygen permeability from 7.38 x 10^{-3} cm^3/Pa.day.m to 3.59 x 10^{-3} cm^3/m.day.Pa (0.51-fold increment) of the film was observed. Meanwhile, addition of CNP slightly increased the opacity of 15% w/w starch/CNP films from 8.07 to 14.67 due to the presence of CNP within the starch matrix that hinder the light transmission pass through the film. The antibacterial properties of starch/CNP films were evaluated via disc-diffusion analysis to observe the inhibition zone of the films. Starch/CNP films incorporated with 15% and 20% w/w CNP exhibited clearer inhibitory zone for gram-positive bacteria (*B. cereus*, *S.*

aureus) compared to gram-negative bacteria (*E. coli, S. typhi)*. The application of starch/CNP films was demonstrated on the cherry tomatoes whereby the microbial growth in cherry tomatoes was evaluated during 10 days storage period. It was found that the 15% w/w starch/CNP film was more efficient to inhibit the microbial growth in cherry tomatoes (7 x 10^2 cfu/mL) compared to starch film (2.15 x 10^3 cfu/mL), thus confirmed the antibacterial properties of the starch/CNP film. In conclusion, starch/CNP film developed in this study exhibit improved properties and has the potential to be used as food packaging material.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

PENGHASILAN FILEM BIOKOMPOSIT KANJI/KITOSAN NANOPARTIKEL BERSIFAT ANTIBAKTERIA

Oleh

RUZANNA AHMAD SHAPI'I

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Penggunaan biopolimer sebagai bahan pembungkusan makanan adalah sangat terhad kerana kekurangan ciri-cirinya. Penambahan nanopartikel kitosan (CNP) ke dalam filem biopolimer dapat meningkatkan ciri-ciri yang diperlukan oleh pembungkus makanan seperti sifat mekanikal, termal, perlindungan, dan antibakteria. Oleh itu, kajian ini bertujuan untuk menghasilkan filem kanji/CNP dengan ciri-ciri yang lebih baik. CNP dihasilkan dengan menggunakan kepekatan kitosan yang berbeza iaitu 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, dan 2.1% w/v dan purata saiz zarah CNP diukur menggunakan DLS dan TEM. Analisis DLS mendapati bahawa saiz zarah CNP meningkat dari 46.76 hingga 225.33 nm (peningkatan 4 kali ganda) apabila kepekatan kitosan meningkat. Purata saiz zarah bagi 0.9% w/v CNP yang diukur menggunakan DLS adalah 106.34 nm manakala yang diukur melalui analisis TEM adalah 53.93 nm. Secara teorinya, TEM mengukur saiz sebenar CNP dalam keadaan kering tetapi DLS pula mengukur saiz hidrodinamik CNP dalam keadaan terhidrat. Jadi, TEM lebih tepat untuk mengukur saiz CNP berbanding DLS. Filem kanji/CNP dihasilkan dengan kepekatan CNP yang berbeza (5, 10, 15, 20, 25, 30, 35% w/w kanji pepejal) menggunakan kaedah "solvent casting". Ciri mekanikal, haba, perlindungan, dan optik filem kanji/CNP (purata ketebalan: 0.07 mm) diukur dan dianalisis. Penambahan 15% w/w CNP ke dalam filem kanji meningkatkan "tensile strength" dari 1.12 hingga 10.03 MPa (peningkatan 7.96 kali ganda) dan "elongation at break" dari 67 hingga 90.77% (peningkatan 0.35 kali ganda). Ini disebabkan oleh interaksi yang baik di antara antara kanji dan CNP menghasilkan struktur filem yang kompak dan teguh. Penambahbaikan dalam struktur filem menyebabkan peningkatan dalam kestabilan terma filem di mana sisa filem kanji yang mengandungi 15% w/w CNP meningkat daripada 7.91% kepada 23.48% (peningkatan 2 kali ganda). Bagi ciri perlindungan pula, penambahan CNP ke dalam filem kanji juga mengurangkan kebolehtelapan wap air dari 1.1 x 10^{-11} g/Pa.h.m kepada 0.63 x 10-11 g/Pa.h.m (peningkatan 4 kali ganda) dan kebolehtelapan oksigen dari 7.38 x 10^{-3} cm^3/Pa.day.m ke 3.59 x 10^{-3} cm^3/m.day.Pa (peningkatan 0.51 kali ganda,). Selain itu, penambahan 15% w/w CNP meningkatkan sedikit ciri kelegapan filem kanji dari 8.07 hingga 14.67. Hal ini kerana kehadiran CNP dalam matriks kanji yang menghalang laluan cahaya melalui filem. Ciri-ciri antibakteria filem kanji/CNP dinilai melalui analisis "disc diffusion" untuk melihat zon perencatan bakteria. Filem kanji/CNP yang mengandungi 15% dan 20% w/w CNP menunjukkan zon perencatan

bakteria gram-positif (B. cereus, S. aureus) yang lebih jelas berbanding bakteria gram-negatif (E. coli, S. typhi). Filem kanji/CNP digunakan untuk membungkus tomato ceri bagi mengkaji pertumbuhan bakteria dalam tomato yang disimpan selama 10 hari. Kajian mendapati bahawa filem kanji/CNP lebih efisien untuk menghalang pertumbuhan bakteria dalam tomato ceri (7 x 10^2 cfu/mL) berbanding dengan filem kanji (2.15 x 10^3 cfu/mL). Kesimpulannya, penambahan CNP di dalam filem kanji menghasilkam filem yang lebih baik dan mempunyai potensi untuk digunakan sebagai bahan pembungkusan makanan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ASTM	-	American standard testing material
CH	-	Bulk chitosan
CMC	-	Carboxymethyl cellulose
CNP	-	Chitosan nanoparticle
DLS	-	Dynamic light scattering
DSC	-	Differential scanning calorimetry
DTA	-	Differential thermal analysis
EAB	-	Elongation at break
FTIR	-	Fourier transform infrared spectroscopy
HDPE	-	High-density polyethylene
HNT	-	Halloysite nanotube
JIS	-	Japan industrial standard
LDPE	-	Low-density polyethylene
MMT	-	Montmorillonite
Mw	-	Molecular weight
NaOH	-	Sodium hydroxide
OP	-	Oxygen permeability
OTR	-	Oxygen transmission rate
PBS	-	Polybutylene succinate
PCL	-	Polycaprolactone
PE	-	Polyethylene
PET	-	Polyethylene terephthalate
PGA	-	Polyglycolic acid
PLA	-	Polylactic acid
PP	-	Polypropylene
PS	-	Polystyrene
PVA	-	Polyvinyl alcohol
SEM	-	Scanning electron microscope
TA	-	Texture analyzer
TEM	-	Transmission electron microscope
TGA	-	Thermogravimetric analysis
TMA	-	Thermomechanical analysis
TPP	-	Sodium tripolyphosphate
TS	-	Tensile strength
WVP	-	Water vapor permeability
WVTR	-	Water vapor transmission rate
YM	-	Young modulus

CHAPTER 1

INTRODUCTION

1.1 Background

Over the years, there is growing development of food packaging materials and technology due to the increase in market demands for the food products. Packaging materials such as glass, metal, paper, and plastic are usually used to preserve and lengthen the shelf life of food product. Among the materials, plastics such as polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS) are widely use as food packaging material due to their excellent properties (Shin and Selke, 2014). However, conventional plastic is usually made up from petroleum and non-degradable, thus increases the municipal solid waste in the landfill which can lead to serious earth pollution (Singh and Sharma, 2016). According to Tang et al. (2012), packaging materials resulted to the highest contribution of the total solid waste at 31.2% and food packaging alone accounts for almost two-thirds of total packaging solid waste. This severe environmental impact lead to the global concern of developing biodegradable plastic from alternative materials that are sustainable and non-pollutant.

Biopolymer materials have great potential to replace the conventional plastics due to its non-toxicity, non-pollutant, biodegradable and availability (Dutta et al., 2009; Pereda et al., 2012; Mendes et al., 2016). Biopolymers can be categorized into synthetic biopolymers and natural biopolymers. Advance technology in biopolymers field lead to the development of synthetic biopolymers such as polylactic acid (PLA), polycaprolactone (PCL), polyglycolic acid (PGA), polyvinyl alcohol (PVA), and polybutylene succinate (PBS) (Rhim et al., 2013). The advantages of material produced from synthetic biopolymer include transparent, durable, and exhibits flexible properties which are nearly comparable with the conventional plastic. Considering the high cost and complicated method to synthesis synthetic biopolymer, application of synthetic biopolymer as food packaging material is limited thus packaging materials from natural biopolymer is a good alternative (Imre and Pukanszky, 2015). Natural biopolymers are usually derived from polysaccharide (starch, cellulose, chitosan, and agar) and protein (gelatin, gluten, alginate, whey protein, and collagen) which are abundant and can be naturally found on earth (Malhotra et al., 2015). Natural and non-toxic properties of natural biopolymers have been another advantages for natural biopolymer to be used as food packaging material because they are promising in terms of food safety.

Of all the mentioned natural biopolymers, starch-based biopolymers such as maize, rice, corn, yam, potato, and tapioca can be used to produce thin film (Jimenez et al., 2012). Furthermore, the process of producing thin film from starch does not involves any synthetic chemicals, thus produces non-toxic and edible film which is favorable to be used as food packaging materials. The present work used tapioca starch as the main biopolymer matrix to produce thin film because of its good film-forming properties, abundant availability, and inexpensive sources (Jimenez et al., 2012; Kowalczyk et al., 2015). Tapioca starch is produced from the cassava plant tuber (*Manihot Esculenta*) which is easy to be cultivated in tropical climate country, thus provides continuous supply of high quantity and quality starch sources in Malaysia (Piyachomkwan, 2011). It is worth to be noted that in Malaysia, cassava plant is cultivated for the tapioca starch production while other starches such as corn and rice are cultivated as the main food supplies for human and animal (Hanif et al., 2016). Thin film that is produced from tapioca starch is generally odorless, tasteless, colorless, non-toxic and biodegradable thus its application is suitable as food packaging material (Flores et al., 2007). However, the mechanical properties of the biopolymer material plastics are still not satisfied compared to conventional plastic which limit their application as food packaging materials (Davis and Song, 2006; Rhim et al., 2013). Incorporation of additives such as plasticizer, filler, antibacterial agent, and antioxidant agent can improve the properties of biopolymer films. Addition of plasticizer such as glycerol, sorbitol, xylitol, and glucose into biopolymer matrix can enhance the elasticity of the film (Vieira et al., 2011). The starch film also can be blended with other natural biopolymers such as chitosan to improve these drawbacks (Xu et al., 2005; Shen et al., 2010; Mei et al., 2013).

Chitosan ($C_6H_{11}NO_4$) is a linear polysaccharide and the second most abundant polysaccharide found in nature after cellulose (Dutta et al., 2009). Furthermore, chitosan (CH) is approved as a food additive in Japan, Italy, and Finland due to its biocompatibility properties with living tissue (Lima et al., 2010). Advancement of nanotechnology reveals that incorporation of nanoparticles (1 to 100 nm) into the matrix of biopolymer is more effective to improve the properties of biopolymer compared to bulk material counterparts (Hannon et al., 2015). Chitosan nanoparticle (CNP) exhibits good antibacterial properties and has been used in food packaging (Antoniou et al., 2015), food coating (Pilon et al., 2015), textiles industry (Ali et al., 2011), water treatment (Sivakami et al., 2013) and others. Addition of CNP into thin film may produce the antibacterial film, which is able to inhibit the microbial growth and extend the shelf life of food (Shen et al., 2010).CNP can be synthesized using various methods such as reverse emulsion (Brunel et al., 2008), precipitation and polyelectrolyte complexation (Nishimura et al., 2004), and ionic gelation (Calvo et al., 1997). Among the mentioned methods, ionic gelation is the most promising method to produce CNP due to its non-toxicity (Al-Qadi et al., 2012; Rampino et al., 2013). During ionic gelation process, the cations in CH were cross-linked with the polyanions in sodium tripolyphosphate (TPP) thus formed the CNP spontaneously (Gokce et al., 2014). Note that it is very important to produce small size of CNP with no agglomeration as agglomeration would affect the properties of the film. This can be achieved by determination of optimum parameters of ionic gelation process such as initial concentration of chitosan, concentration of TPP, initial pH of chitosan, and ratio of TPP to chitosan.

CNP exhibit different properties compared with CH due to the different shapes, size and chemical structure, thus exhibit different mechanism to reinforce the biopolymer film (Hosseini et al., 2015). Regular spherical shape and tiny size of CNP provides large surface area of CNP, thus increase the compatibility of CNP to interact with the starch matrix. Good interaction between starch and CNP molecules improve the properties of starch film efficiently compared to CH. Parallel with the promising properties of CNP, incorporation of CNP into several biopolymers such as potato starch (Chang et al., 2010), fish gelatin (Hosseini et al., 2015), and tara gum (Antoniou et al., 2015) has been found in previous literature. A comparative study between tara gum films incorporated with chitosan nanoparticle (CNP) and bulk chitosan (CH) has been done by Antoniou et al. (2015). They found that incorporation of CNP into tara gum matrix was more efficient to improve the properties of tara gum film compared with CH. Although the previous study demonstrated that CNP filler was more efficient to improve the properties of tara gum film compared to bulk filler, to the best of our knowledge, there is no comparison study was done for other types of biopolymers.

The present work is directed to develop tapioca starch-based film incorporated with CNP (starch/CNP film) as the antibacterial film with the improved mechanical, thermal, and barrier properties. Although potato starch/CNP film has been produced by Chang et al. (2010), it is worth to be noted that they used CNP in powder form which was more difficult to handle and easily agglomerated (Gokce et al., 2014). A better approach to enhance the performance of CNP as the filler can be achieved by using freshly prepared CNP in suspension form. It is also worth to determine the suitable concentration of CNP in the starch/CNP film because it is an imperative parameter that will affect the mechanical, thermal, barrier, and antibacterial properties of the film.

1.2 Problem Statement

Biopolymer film particularly starch film generally exhibits weak and brittle structure thus limit the application of the film as food packaging material. It is very important to produce strong and flexible film so that the film is able to wrap the food properly. Glycerol is usually added in the starch film to improve the elasticity and flexibility properties of the film. However, interruption of glycerol molecules in between starch chain can reduce the strength of the film, thus the film is easy to tear and shrink. Furthermore, starch film also exhibits poor barrier properties due to its natural hydrophilic properties and porous structure of the starch matrix. Addition of glycerol also contributes to the increase in hydrophilic properties of the film. This limitation allows the water vapor and oxygen to permeate through the film and enter to the food product. Excess water vapor and oxygen that are expose to the food accelerates deterioration of the food, thus can shorten the shelf life of food. It is very important to improve all the mentioned limitation of starch film in order to ensure the performance of starch film as food packaging material is comparable to the conventional plastic.

Addition of nanofiller particularly CNP has a great potential to increase the strength of the film and other properties including barrier properties and thermal stability. CNP acts as the reinforcing agent and tends to form good interfacial interaction with the starch chain and fill the empty spaces in the starch matrix, thus improve the structure and properties of the film. Although some studies have synthesized CNP, most of the previous studies produced CNP with the size larger than 100 nm which was not in the range of the nanoparticle size. Only a study by Gokce et al. (2014) who investigated the effects of pH, chitosan to TPP weight ratio, and ultrasonication time managed to produce CNP in the size range of 40 to 110 nm. However, Gokce et al. (2014) did not investigated the effect of initial concentration of chitosan, which is an imperative parameter on the particle size of CNP. Therefore, this study is directed to investigate the effect of initial concentration of CH on the particle size of CNP while the remaining parameters being fixed according to optimum parameters found by Gokce et al. (2014).

The efficiency of CNP as filler is greatly dependent on the dispersion of CNP in the film matrix. Most of the studies that utilize CNP as the filler in food packaging materials dealt with the CNP in powder form. The problem with using CNP in powder form is that the powder is easy to agglomerate thus, reducing the efficiency of the CNP as reinforcing agent. The agglomeration is due to the dehydration stress that occurred during the freeze-drying process (Rampino et al., 2013; Gokce et al., 2014). The use of CNP in suspension form can reduce the potential of CNP to form agglomerate in the matrix compared with the use of dried CNP in powder form. To the best of our knowledge, no studies have utilized CNP in suspension form in starch film formulation. Thus, this study is directed towards producing and utilizing CNP in suspension form, which is a novel formulation of starch/CNP film.

Despite previous literature has revealed the advantages of CNP as filler, only Chang et al. (2010) have investigated the effect of concentration of CNP in starch film particularly potato starch film. It is worth to be noted that they used CNP in powder form which tends to agglomerate when high concentration of CNP was added into the film. No detail literature has been reported on the real application of CNP suspension as filler in tapioca starch film. Moreover, no work has been done to investigate the potential application of the starch/CNP film as antibacterial food packaging. Although CNP has been proven as an antibacterial agent in the literature, most of the published research work only carried out in-vitro. In-vivo evaluation of antibacterial properties of starch/CNP film on the real food product should be carried out to demonstrate the potential application of the film as antibacterial packaging. Thus, the aim of this work has been directed towards developing starch/CNP films as potential antibacterial food packaging material. The optimum concentration of CNP that could inhibit microbial growth was investigated using disc diffusion method. Then, the real application of starch/CNP film was demonstrated on the cherry tomatoes and the microbial growth in cherry tomatoes was evaluated during 10 days of storage period.

1.3 Objectives and Scope of Work

This thesis reports a comprehensive study of the preparation, characterization, and application of starch/CNP films. There are two primary objectives of the present work:

1. To produce and characterize starch and starch/CNP films (morphological, mechanical, thermal, barrier, and optical).

2. To investigate the bacterial inhibition zone of the starch film and starch/CNP films and total plate count of cherry tomatoes wrapped in starch film and starch/CNP films.

The scope of work for the first objective has been directed to synthesize and characterize the particle size of CNP. The effect of different initial concentrations of CH (0 to 2.1% w/w) on the particle size of CNP was investigated using dynamic light scattering technique (DLS) and transmission electron microscope (TEM). In order to develop starch/CNP film, the effect of various parameters such as concentration of glycerol (0 to 25% w/w), types of filler (CH and CNP), and concentration of CNP (0 to 35% w/w) on the characteristic of the film were investigated. The characteristics of the films produced were characterized in term of morphological (scanning electron microscope (SEM), TEM), mechanical (texture analyzer (TA)), barrier (water vapor permeability cup, oxygen permeability analyzer), optical (color spectrophotometer), and thermal properties (thermogravimetric analysis (TGA)). The molecular bonding between starch and CNP (Fourier transform infrared spectroscopy (FTIR)) was also investigated.

The scope of work for the second objective has been directed towards determining the sufficient concentration of CNP (0 – 20% w/w) that could inhibit the growth of gram-positive bacteria (*B. cereus*, *S. aureus*) and gram-negative bacteria (*E. coli*, *S. typhi*) via disc diffusion method. Then, the real application of starch/CNP film was also demonstrated on cherry tomatoes and the total plate count was evaluated during 10 days of storage period.

1.4 Research Contributions

This work illustrates the continuing improvement on the synthesis of CNP for nanocomposite film application whereby in this work, CNP is produced in suspension form which brings high potential to improve dispersion of CNP in the film matrix. Incorporation of CNP into the biopolymer matrix particularly starch will contribute towards improvement of the mechanical, thermal, barrier, and antibacterial properties of the starch/CNP film. Determination of suitable concentration of glycerol and CNP in the starch/CNP film could produce efficient film for food packaging application. The application of starch/CNP film as the antibacterial food packaging will hopefully open the window towards industrial application.

1.5 Thesis Outline

This thesis consists of five chapters. Chapter 1 is an introduction which begins with an overview of the food packaging materials, biopolymer, filler, synthesis of CNP, and potential application of nanocomposite film. Problem statements regarding development of starch/CNP film and its application as food packaging is highlighted followed by the objectives and scope of work as well as the research contributions.

Chapter 2 covers the literature review that consists of critical review of previous works that are related to food packaging, biopolymer, nanofiller, and ionic gelation process. The properties of bionanocomposite film such as morphological, mechanical, thermal, barrier, optical, and antibacterial are also explained in this chapter. The potential application of bionanocomposite film in previous work such as antibacterial film is also discussed in this chapter.

Chapter 3 reports a detailed description of preparation, characterization of starch/CNP film, and application of starch/CNP film as antibacterial food packaging film. This chapter also covers the materials and method used to synthesize the CNP. The details methods and equipments used to produce and characterize the starch/CNP film are also included. The methodology of antibacterial properties of starch/CNP film is also discussed in this chapter.

Chapter 4 presents the results and discussion on the main findings of the present work including the analysis and interpretation of the results. The results and discussion presented in Chapter 4 are arranged according to the objectives of present work. Section 4.1 to Section 4.4 cover the first objective while Section 4.5 cover the second objective of the present work.

Chapter 5 presents the overall conclusion from the results discussed in Chapter 4 as well as some recommendations for potential future work of the research area.

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