Characterization of Polymer Blends as Mucoadhesive Materials for Gastroretentive Drug Delivery System

Krit Suknuntha

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Pharmaceutical Sciences

Prince of Songkla University

2012

Thesis Title	Characterization of Polymer Blends as Mucoadhesive Materials for Gastroretentive Drug Delivery System
Author	Mr. Krit Suknuntha
Major Program	Pharmaceutical Sciences

Major Advisor:

……
(Prof. Dr. Vimon Tantishaiyakul)

Co-advisor:

……
(Assoc. Prof. Dr. Nimit Worakul)

……
(Dr. Wirach Taweepreda)

Examining Committee:

……Chairperson
(Assoc. Prof. Dr. Ruedeekorn Wiwattanapatapee)

……
(Prof. Dr. Vimon Tantishaiyakul)

……
(Assoc. Prof. Dr. Satit Puttipipatkhachorn)

……
(Assoc. Prof. Dr. Nimit Worakul)

……
(Assoc. Prof. Dr. Sanae Kaewnopparat)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Pharmaceutical Sciences

……
(Prof. Dr. Amornrat Phongdara)
Dean of Graduate School

ชื่อวิทยานิพนธ์ คุณลักษณะของพอลิเมอร์ผสมเพื่อใช้เป็นสารเกาะติดเยื่อเมือกสำหรับระบบนำส่งยาคงค้างในกระเพาะอาหาร

ผู้เขียน นายกฤษณ์ สุขนันทร์ธะ

สาขาวิชา เภสัชศาสตร์

ปีการศึกษา 2554

บทคัดย่อ

วัตถุประสงค์ของวิทยานิพนธ์นี้คือพัฒนา ประเมินคุณลักษณะและประเมินคุณสมบัติในการยึดเกาะเยื่อเมือกของไคโตซาน พอลิไวนิลไพโรลิโดน หรือ พีวีพี เจลาตินชนิดเอ เจลาตินชนิดบีและพอลิเมอร์ผสมกับไคโตซานในอัตราส่วนของปริมาตรต่างๆ โดยเน้นการประเมินคุณสมบัติการยึดเกาะเยื่อเมือกและการยึดเกาะกับเซลล์เป็นสำคัญ สำหรับในส่วนของพอลิเมอร์ที่มีคุณสมบัติของการยึดเกาะเยื่อเมือกและการยึดเกาะกับเซลล์ที่ดีจะถูกเลือกนำมาเป็นสารเคลือบในสูตรตำรับที่เป็นยาอะม็อกซีซิลินในรูปแบบของเม็ดบีดของแคลเซียมอัลจิเนต จากการศึกษาโดยการวัดความหนืดของสารละลาย การศึกษาด้วยเครื่องวัดผิวสัมผัสและการศึกษาการยึดเกาะของเซลล์เอชทียี่สิบเก้าจะพบว่าสารพอลิเมอร์ผสมของไคโตซานและพีวีพีแสดงให้เห็นว่ามีคุณสมบัติในการยึดเกาะเยื่อเมือกและคุณสมบัติในการยึดเกาะกับเซลล์ที่ดี อีกทั้งยังดีกว่าสารพอลิเมอร์ผสมของไคโตซานและเจลาติน ดังนั้นสารพอลิเมอร์ผสมของไคโตซานและพีวีพีจึงถูกเลือกมาใช้เพื่อเป็นสารเคลือบยาอะม็อกซีซิลินในรูปแบบของเม็ดบีด นอกจากนี้จากการศึกษายังพบว่าสารพอลิ

เมอร์ผสมของไคโตซานและพีวีพีในอัตราส่วนของปริมาตรที่เท่ากับ 5:5 ให้ผลในการยึดเกาะเยื่อเมือกและคุณสมบัติในการยึดเกาะกับเซลล์ที่ดีมากกว่าสารพอลิเมอร์เดี่ยวและสารพอลิเมอร์ผสมของไคโตซานและพีวีพีในอัตราส่วนอื่นๆ จากการศึกษาการล้างออกเพื่อประเมินคุณสมบัติในการยึดเกาะเยื่อเมือกของเม็ดบีดที่เคลือบพบว่าให้ค่าที่ดีกว่าแบบเม็ดบีดที่ไม่เคลือบอย่างมีนัยสำคัญ การศึกษาทางด้านสเปกโทรสโคปีด้วยเทคนิคดิฟฟิวรีเฟลกแตนซ์อินฟราเรดสเปกโทรสโคปีเพื่อดูอันตรกิริยาของพอลิเมอร์ พอลิเมอร์ผสมและพอลิเมอร์ผสมกับมิวซินสามารถบ่งชี้ถึงอันตรกิริยาซึ่งเป็นปัจจัยหนึ่งของคุณสมบัติในการยึดเกาะเยื่อเมือก จากการศึกษาด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดพบว่าพื้นผิวของเม็ดบีดที่มีการเคลือบจะมีลักษณะที่เรียบกว่าแบบเม็ดบีดที่ไม่มีการเคลือบ นอกจากนี้เม็ดบีดที่มีการเคลือบยังพบว่ามีคุณสมบัติในการควบคุมการปลดปล่อยตัวยาได้ และกลไกในการปลดปล่อยตัวยาจะเป็นแบบซุปเปอร์เคสสองซึ่งเป็นผลมาจากการพองตัวอย่างมากและรวดเร็วของเม็ดบีดของอัลจิเนต รูปแบบของยาอะม็อกซีซิลินในรูปแบบของเม็ดบีดที่มีการเคลือบด้วยพอลิเมอร์ที่มีคุณสมบัติที่ดีในการยึดเกาะเยื่อเมือกและยึดเกาะกับเซลล์มีศักยภาพในการที่จะนำไปพัฒนาในรูปแบบยาที่ใช้ในการรักษาการติดเชื้อเฮลิโคแบคเตอร์ไพโรไลได้

Thesis Title	Characterization of Polymer Blends as Mucoadhesive Materials for Gastroretentive Drug Delivery System
Author	Mr. Krit Suknuntha
Major Program	Pharmaceutical Sciences
Academic Year	2011

ABSTRACT

The purposes of this thesis were to develop, characterize and evaluate mucoadhesive polymer of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A, gelatin type B and their blends with chitosan at various volume ratio with an emphasis on assessing their muco- and bioadhesive properties. These materials with good muco- and bioadhesive properties were used to coat calcium alginate beads containing amoxicillin (AMX). Viscosity measurements, texture analysis, and HT29 cell adhesion evaluation of these materials demonstrated that C/PVP blends showed a good mucoadhesive and bioadhesive properties when compared to chitosan/gelatin blends and these blends were selected for an AMX bead coating materials. Moreover, the C/PVP at a volume ratio of 5/5 had optimum muco/bioadhesive properties when compared to chitosan, PVP and blends at other ratios. Wash-off tests indicated that mucoadhesive property of coated AMX alginate beads was significantly higher than uncoated beads. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was used to study the interactions of all polymer or polymer blends with mucin, since such interactions may be important factors that cause mucoadhesion. Scanning electron microscopy (SEM) revealed that the surfaces of coated beads were

smoother than those of uncoated beads. All the coated AMX alginate beads were able to afford a controlled release of AMX. The release mechanism for AMX from these beads exhibited super case II transport, probably as a result of rapid and extensive swelling of the alginate beads. These coated AMX alginate beads show potential for development of appropriate formulations which exhibit high gastroretention and also possibly useful for the treatment of *Helicobacter pylori* infections.

CONTENTS

	Page
บทคัดย่อ	iii
ABSTRACT	v
ACKNOWLEDGEMENT	vii
CONTENTS	viii
LIST OF TABLES	xv
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS AND SYMBOLS	xxvi
CHAPTER 1: INTRODUCTION	1
1.1 Physiology of the stomach	1
1.2 Mucosal surface and mucin	3
1.3 *Helicobacter pylori*	5
1.4 Treatment of *Helicobacter pylori*	9
1.5 Strategy of drug delivery for treatment of *Helicobacter pylori*	12
1.6 Bioadhesive/mucoadhesive drug delivery system	15
1.6.1 Mechanisms and mucoadhesion theories	17
1.6.1.1 Wetting theory of mucoadhesion	18
1.6.1.2 Diffusion theory of mucoadhesion	18
1.6.1.3 Adsorption theory of mucoadhesion	19
1.6.1.4 Electrical theory of mucoadhesion	20
1.6.1.5 Fracture theory of mucoadhesion	20

CONTENTS (CONTINUED)

		Page
1.7	Polymers suitable for mucoadhesive drug delivery system	22
	1.7.1 Poly(vinyl pyrrolidone)	24
	1.7.2 Chitosan	27
	1.7.3 Gelatin	29
	1.7.4 Sodium Alginate	30
1.8	Evaluation of Mucoadhesion	32
	1.8.1 Direct methods	33
	1.8.1.1 Tensile strength assay	33
	1.8.1.2 Dynamic assay under shear forces	35
	1.8.1.3 Cell adhesion assay	36
	1.8.2 Indirect methods	37
	1.8.2.1 Viscosity or rheology measurement	37
	1.8.2.2 Spectroscopic method	38
1.9	Study and aims for developing mucoadhesive drugs for eradication of *Helicobacter pylori*	38
1.10	The aim of this study	40
CHAPTER 2: EVALUATION OF MUCOADHESIVE POLYMERS USING VISCOSITY MEASUREMENTS		41
2.1	Introduction and objectives	41
2.2	Experimental methods	43
	2.2.1 Materials	43

CONTENTS (CONTINUED)

	Page
2.2.2 Sample preparations	43
2.2.3 Viscosity measurement	44
2.2.4 Statistical analysis	46
2.3 Results and discussion	46
2.3.1 Viscosity characterization	46
2.3.2 Effect of mucin on viscosity enhancement	51
2.3.3 Force of mucoadhesion	54
2.4 Conclusions	57
CHAPTER 3: EVALUATION OF THE INTERACTIONS BETWEEN POLYMER - POLYMER AND POLYMER - MUCIN USING DIFFUSE REFLECTANCE INFRARED FOURIER TRANSFORM SPECTROSCOPY (DRIFTS)	58
3.1 Introduction and objectives	58
3.2 Experimental methods	61
3.2.1 Materials	61
3.2.2 Sample preparations	62
3.2.3 DRIFTS measurement	62
3.3 Results and discussion	63
3.3.1 DRIFTS of single polymer characterization	63
3.3.2 DRIFTS of polymer blends characterization	67

CONTENTS (CONTINUED)

	Page
3.3.3 DRIFTS of the combination systems of polymer with mucin characterization	73
3.4 Conclusions	97
CHAPTER 4: AN *IN VITRO* EVALUATION OF MUCOADHESIVE POLYMER USING THE TENSILE STRENGTH TEST METHOD	99
4.1 Introduction and objectives	99
4.2 Experimental methods	102
4.2.1 Materials	102
4.2.2 Sample preparations	102
4.2.3 Texture analyzer measurement	102
4.2.4 Statistical analysis	106
4.3 Results and discussion	106
4.3.1 Mucoadhesion of a single polymer	106
4.3.2 Mucoadhesion of polymer blends	108
4.4 Conclusions	111
CHAPTER 5: AN *IN VITRO* CELL ADHESION ASSAY TO MEASURE BIOADHESION OF MUCOADHESIVE POLYMER	112
5.1 Introduction and objectives	112
5.2 Experimental methods	113
5.2.1 Materials	113

CONTENTS (CONTINUED)

Page

5.2.2 Cell culture 114

5.2.3 Cell adhesion assay 114

5.2.4 Statistical analysis 116

5.3 Results and discussion 117

5.3.1 Bioadhesion of single polymers 117

5.3.2 Bioadhesion of polymer blends 118

5.4 Conclusions 121

CHAPTER 6: AMOXICILLIN MUCOADHESIVE BEAD PREPARATION AND PROPERTIES 122

6.1 Introduction and objectives 122

6.2 Experimental methods 124

6.2.1 Materials 124

6.2.2 Mucoadhesive bead preparations 124

6.2.3 Drug loading capacity 126

6.2.4 Scanning electron microscope (SEM) 127

6.2.5 Bead size analysis 127

6.2.6 Swelling study of dried beads 128

6.2.7 Mucoadhesive properties of AMX beads using wash-off 128

6.2.8 Statistical analysis 129

6.3 Results and discussion 130

6.3.1 Drug loading capacity 130

CONTENTS (CONTINUED)

Page

6.3.2 Uncoated and coated amoxicillin bead morphology 133

6.3.3 Swelling of dried bead 138

6.3.4 Mucoadhesive properties of AMX beads using wash-off 142

6.4 Conclusions 148

CHAPTER 7: DRUG RELEASE STUDY OF AMOXICILLIN FROM ALGINATE BEADS 149

7.1 Introduction and objectives 149

7.2 Experimental methods 151

7.2.1 Materials 151

7.2.2 Drug release study 152

7.2.3 Statistical analysis 153

7.3 Results and discussion 154

7.3.1 Drug release 154

7.4 Conclusions 162

CHAPTER 8: SUMMARY AND CONCLUSIONS 163

REFERENCES 170

APPENDIX A: Statistical analysis results of evaluation of mucoadhesive polymers using viscosity measurements 199

APPENDIX B: Statistical Analysis Results of an *in vitro* evaluation of mucoadhesive polymer using the tensile strength test method 214

CONTENTS (CONTINUED)

	Page
APPENDIX C: Statistical Analysis Results of an *in vitro* cell adhesion assay to measure bioadhesion of mucoadhesive polymer	222
APPENDIX D: Statistical analysis results of amoxicillin mucoadhesive bead preparation and properties	230
APPENDIX E: Statistical analysis results of drug release study of amoxicillin from alginate beads	252
APPENDIX F: United States Pharmacopeia and National Formulary (USP33-NF28) of amoxicillin	277
VITAE	283

LIST OF TABLES

Table	Running title	Page
1.1	The regimens for *Helicobacter pylori* therapy 2011 to 2012	11
1.2	Approximate molecular weights for different grades of PVP	25
2.1	Flow behavior index (n) and consistency index (K_c) derived from the power law model of the chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA), gelatin type B (GB) and their blend at different volume ratio with and without mucin, n = 3	50
6.1	Statistical results of time and the time x group interaction effect obtained from the MANOVA of swelling of the amoxicillin (AMX) beads	140
6.2	Statistical results of the percentage weight change at each time level obtained from ANOVA	141
6.3	Statistical results of time and time x group interaction effects obtained from the MANOVA of the amoxicillin (AMX) bead wash-off profiles	144
6.4	Statistical results of percent bead remaining at each time level obtained from ANOVA	146
7.1	Mathematical models of release kinetic	150
7.2	Statistical results of time and the time x group interaction effect obtained from MANOVA of the dissolution profiles of the amoxicillin (AMX) beads	156

LIST OF TABLES (CONTINUED)

Table	Running title	Page
7.3	Statistical results of percentage release at each time level of the uncoated bead, C/PVP 9/1 and poly(vinylpyrrolidone) (PVP) obtained from ANOVA	157
7.4	Kinetic analysis of the release data of amoxicillin derived from several kinetic equation models	161

LIST OF FIGURES

Figure	Running title	Page
1.1	Physiologic anatomy of the stomach	2
1.2	Structure of the stomach wall	3
1.3	Diagram of mucin molecules. Several subunits are bound through cysteine-rich domains (S) that form disulfide bridges (S-S). Thick bars represent the highly glycosylated domains	5
1.4	Schematic diagrams of Helicobacter pylori infection	6
1.5	The mobility patterns of the stomach in fasted state	14
1.6	Schematic representation of the diffusion theory of mucoadhesion before contact (A), upon contact (B) and after contact for a period of time (C)	19
1.7	Schematic diagrams showing the progression of mucoadhesive fracture	21
1.8	Schematic diagram of the interrelations between mucoadhesive theories (red circle) and properties of mucoadhesive material (blue circle)	22
1.9	Chemical structure of poly(vinyl pyrrolidone)	25
1.10	Chemical structure of chitin (poly (N-acetyl-β-D-glucosamine)) (a), chitosan (poly (D-glucosamine)) (b) repeat units and structure of partially acetylated chitosan (c)	28
1.11	Chemical structure of alginate block types consisting of D-mannuronic acid (M) and L-guluronic acid (G)	32

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
1.12	Mucoadhesive testing system utilizing the texture analyzer equipment	34
1.13	Schematic illustration of the experiment set up used for the continuous flow assay	36
2.1	Schematic of viscosity measurement using the Brookfield viscometer with SC4-18 spindle and a small sample adaptor	45
2.2	Apparent viscosity of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) and their blends at different volume ratio at various shear rates	48
2.3	Apparent viscosity of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin and their blend at different volume ratio with mucin at various shear rates	49
2.4	Viscosity enhancement ($\eta_{enhance}$) of chitosan (C), poly(vinyl-pyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin (n = 3)	52
2.5	Viscosity enhancement ($\eta_{enhance}$) of chitosan (C), poly(vinyl-pyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin and their blend at different volume ratio with mucin (n = 3)	53

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
2.6	Force of mucoadhesion of the combination system of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin (n = 3)	55
2.7	Force of mucoadhesion of the combination system of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin and their blend at different volume ratio with mucin (n = 3)	56
3.1	The projection of the spectrometer beam into the sample of diffuse reflectance mode	59
3.2	Abrasive disc for DRIFTS measurement	61
3.3	DRIFTS spectrum of chitosan	63
3.4	DRIFTS spectrum of poly(vinylpyrrolidone) (PVP)	64
3.5	DRIFTS spectrum of gelatin type A (GA)	65
3.6	DRIFTS spectrum of gelatin type B (GB)	66
3.7	DRIFTS spectrum of mucin	67
3.8	DRIFTS spectra of polymer blends of chitosan (C) and poly(vinyl-pyrrolidone) (PVP) at various volume ratios	69
3.9	DRIFTS spectra of polymer blends of chitosan (C) and gelatin type A (GA) at various volume ratios	71
3.10	DRIFTS spectra of polymer blends of chitosan (C) and gelatin type B (GB) at various volume ratios	72

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
3.11	DRIFTS spectra of combination system of chitosan (C) with mucin at various volume ratios	74
3.12	DRIFTS spectra of combination system of poly(vinylpyrrolidone) (PVP) with mucin at various volume ratios	75
3.13	DRIFTS spectra of combination system of gelatin type A (GA) with mucin at various volume ratios	77
3.14	DRIFTS spectra of combination system of gelatin type B (GB) with mucin at various volume ratios	78
3.15	Schematic interaction diagram of polymer blends of chitosan and poly(vinylpyrrolidone) (PVP) with mucin (A) and polymer blends of chitosan and gelatin with mucin (B)	80
3.16	DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (9/1), binary mixtures of C/mucin (9/5) and PVP/mucin (1/5) and ternary mixture of C/PVP/mucin at 9/1/5 volume ratio	81
3.17	DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (7/3), binary mixtures of C/mucin (7/5) and PVP/mucin (3/5) and ternary mixture of C/PVP/mucin at 7/3/5 volume ratio	82

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
3.18	DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (5/5), binary mixtures of C/mucin (5/5) and PVP/mucin (5/5) and ternary mixture of C/PVP/mucin at 5/5/5 volume ratio	83
3.19	DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (3/7), binary mixtures of C/mucin (3/5) and PVP/mucin (7/5) and ternary mixture of C/PVP/mucin at 3/7/5 volume ratio	84
3.20	DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (1/9), binary mixtures of C/mucin (1/5) and PVP/mucin (9/5) and ternary mixture of C/PVP/mucin at 1/9/5 volume ratio	85
3.21	DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (9/1), binary mixtures of C/mucin (9/5) and GA/mucin (1/5) and ternary mixture of C/GA/mucin at 9/1/5 volume ratio	87
3.22	DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (7/3), binary mixtures of C/mucin (7/5) and GA/mucin (3/5) and ternary mixture of C/GA/mucin at 7/3/5 volume ratio	88

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
3.23	DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (5/5), binary mixtures of C/mucin (5/5) and GA/mucin (5/5) and ternary mixture of C/GA/mucin at 5/5/5 volume ratio	89
3.24	DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (3/7), binary mixtures of C/mucin (3/5) and GA/mucin (7/5) and ternary mixture of C/GA/mucin at 3/7/5 volume ratio	90
3.25	DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (1/9), binary mixtures of C/mucin (1/5) and GA/mucin (9/5) and ternary mixture of C/GA/mucin at 1/9/5 volume ratio	91
3.26	DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (9/1), binary mixtures of C/mucin (9/5) and GB/mucin (1/5) and ternary mixture of C/GB/mucin at 9/1/5 volume ratio	92
3.27	DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (7/3), binary mixtures of C/mucin (7/5) and GB/mucin (3/5) and ternary mixture of C/GB/mucin at 7/3/5 volume ratio	93

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
3.28	DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (5/5), binary mixtures of C/mucin (5/5) and GB/mucin (5/5) and ternary mixture of C/GB/mucin at 5/5/5 volume ratio	94
3.29	DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (3/7), binary mixtures of C/mucin (3/5) and GB/mucin (7/5) and ternary mixture of C/GB/mucin at 3/7/5 volume ratio	95
3.30	DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (1/9), binary mixtures of C/mucin (1/5) and GB/mucin (9/5) and ternary mixture of C/GB/mucin at 1/9/5 volume ratio	96
4.1	Schematic mucoadhesion mechanism of polymer film on mucosal tissue	100
4.2	The mucoadhesive rig accessory and schematic of the mucoadhesive force measurement using the texture analyzer: upper cylindrical probe (1), polymer film (2), porcine stomach tissue (3), mucoadhesive rig (4)	103
4.3	Stages for determination of the mucoadhesive interaction using the texture analyzer	104

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
4.4	Typical plot of force versus distance data from the texture analyzer and area under curve is represented work of adhesion	105
4.5	The work of adhesion of a single polymer of chitosan, poly(vinylpyrro-lidone) (PVP), gelatin type A (GA) and gelatin type B (GB) on porcine gastric tissue	107
4.6	The work of adhesion of chitosan (C) and poly(vinylpyrrolidone) (PVP) and their blend at various volume ratio on porcine stomach tissue (n = 6 – 10)	109
4.7	The work of adhesion of chitosan (C) and gelatin type A (GA) and their blend at various volume ratio on porcine stomach tissue (n = 6 – 10)	109
4.8	The work of adhesion of chitosan (C) and gelatin type B (GB) and their blend at various volume ratio on porcine stomach tissue (n = 6 – 10)	110
5.1	Method procedures for cell adhesion analysis	115
5.2	Schematic reaction of formazan formation by mitochondria reductase from living cells	116
5.3	Percent relative HT29 cell attachment on single polymer films of chitosan, poly(vinylpyrrolidone) (PVP), gelatin type A (GA) and gelatin type B (GB) compared to the control (n=4) $*P < 0.05$	118

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
5.4	Percent relative HT29 cell attachment on chitosan (C), poly(vinylpyrro-lidone) (PVP) and their blends of C/PVP film compared with control (n=4) $*P < 0.05$	119
5.5	Percent relative HT29 cell attachment on chitosan (C), gelatin type A (GA) and their blends of C/GA film compared with control (n=4) $*P < 0.05$	120
5.6	Percent relative HT29 cell attachment on chitosan (C), gelatin type B (GB) and their blends of C/GB film compared with control (n=4) $*P < 0.05$	121
6.1	Chemical structure of amoxicillin	123
6.2	Preparation procedures for amoxicillin (AMX) loaded muco-adhesive beads	126
6.3	The standard curve of amoxicillin solution obtained from UV-visible spectrophotometer (A) and high performance liquid chroma-tography (HPLC) (B)	131
6.4	The UV spectra of standard (A) and sample (B) of amoxicillin	132
6.5	Chromatograms of standard (A) and sample (B) of amoxicillin at different concentration	133
6.6	Schematic of calcium-alginate bead formation	134

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
6.7	Scanning electron micrographs of amoxicillin (AMX) uncoated bead with slighly oval shape of AMX bead (X60) (a) and surface of AMX uncoated bead (X750) (b)	136
6.8	Scanning electron micrographs of amoxicillin (AMX) coated bead with polymer (X750) of chitosan (C) (a), poly(vinylpyrrolidone) (PVP) (g) and their blends of 9/1 (b), 7/3 (c), 5/5 (d), 3/7 (e) and 1/9 (f)	137
6.9	Schematic of the binding mechanism of coated amoxicillin beads	138
6.10	Swelling behavior of coated amoxicillin (AMX) beads with chitosan (C), poly(vinylpyrrolidone) (PVP) and their blends at various volume ratios compared with the control of uncoated AMX bead(mean ± S.D., n=3)	139
6.11	The in vitro wash-off test for uncoated and coated amoxicillin (AMX) beads with chitosan (C), PVP (P) and their blends at various volume ratios (mean ± S.D., n=3)	143
6.12	Schematic interaction mechanism of coated amoxicillin (AMX) bead with mucin	147
7.1	Dissolution profiles of amoxicillin (AMX) powder and AMX from uncoated and coated beads with chitosan (C), poly(vinylpyrrolidone) (PVP) and their blends of C/PVP at various volume ratios at pH 4 (mean ± S.D., n=3)	155

LIST OF FIGURES (CONTINUED)

Figure	Running title	Page
7.2	Polymeric coating disruption of amoxicillin (AMX) coated bead in dissolution medium	158
7.3	An idealized release system controlled by swelling	162

LIST OF TABLES

Table	Running title	Page
1.1	The regimens for *Helicobacter pylori* therapy 2011 to 2012	11
1.2	Approximate molecular weights for different grades of PVP	25
2.1	Flow behavior index (n) and consistency index (K_c) derived from the power law model of the chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA), gelatin type B (GB) and their blend at different volume ratio with and without mucin, n = 3	50
6.1	Statistical results of time and the time x group interaction effect obtained from the MANOVA of swelling of the amoxicillin (AMX) beads	140
6.2	Statistical results of the percentage weight change at each time level obtained from ANOVA	141
6.3	Statistical results of time and time x group interaction effects obtained from the MANOVA of the amoxicillin (AMX) bead wash-off profiles	144
6.4	Statistical results of percent bead remaining at each time level obtained from ANOVA	146
7.1	Mathematical models of release kinetic	150
7.2	Statistical results of time and the time x group interaction effect obtained from MANOVA of the dissolution profiles of the amoxicillin (AMX) beads	156

LIST OF TABLES (CONTINUED)

Table	Running title	Page
7.3	Statistical results of percentage release at each time level of the uncoated bead, C/PVP 9/1 and poly(vinylpyrrolidone) (PVP) obtained from ANOVA	157
7.4	Kinetic analysis of the release data of amoxicillin derived from several kinetic equation models	161

LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
γ	Shear rate
$\eta_{enhance}$	Viscosity enhancement value
η_m	Viscosity value of mucin
η_p	Viscosity value of polymer or polymer blend
η_t	Viscosity value of polymer/mucin or polymer blend/mucin system
τ	Shear stress
AMX	Amoxicillin
ANOVA	Analysis of variance
C	Chitosan
C/GA	Polymer blend of chitosan and gelatin type A
C/GB	Polymer blend of chitosan and gelatin type B
C/PVP	Polymer blend of chitosan and poly(vinylpyrrolidone)
DMEM	Dulbecco's Modified Eagles Medium
DMSO	Dimethyl sulfoxide
DRIFTS	Diffuse reflectance infrared Fourier transform spectroscopy
Eq.	Equation
G	L-guluronic acid
GA	Gelatin type A
GB	Gelatin type B

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

h	Hour(s)
HPLC	High performance liquid chromatography
K_c	The consistency index
kV	kilo volt
M	D-mannuronic acid (Chapter 1)
M	Molar (Chapter 6 and 7)
MANOVA	Multi-variate analysis of variance
mg	milligrams
min	Minute(s)
mm	millimetre
MTT	3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
mL	millilitre
n	non-Newtonian index (Chapter 2)
n	Release exponent (Chapter 7)
PVP	Poly(vinylpyrrolidone)
s	Second(s)
SEM	Scanning electron microscope
t	Time (minute)
v/v	Volume by volume
w/v	Weight by volume

CHAPTER 1

INTRODUCTION

1.1 Physiology of the stomach

The stomach has its main role as a food reservoir, where ingested food is processed into a chyme in the presence of HCl and excreted pepsin begins digestion The stomach is divided into three anatomical regions. The uppermost part is the fundus region, which produces slow contractions. The largest part is the body, which acts as a reservoir for ingested food, and liquids and the lowest part of the stomach is the antrum (Figure 1.1). The mid- to upper portion of the stomach wall produces weak peristaltic constrictor waves that move toward the antrum about once every 15 to 20 seconds. The propagation of these constrictor waves becomes more intense and provide a powerful peristaltic action potential, that forces the antral content toward the small intestine. The stomach has a gastric emptying time of about 2 h then, after stomach emptying for several hours or more, another type of intense contraction occurs, called hunger contractions. They are rhythmical peristaltic contractions in the body of the stomach [1, 2].

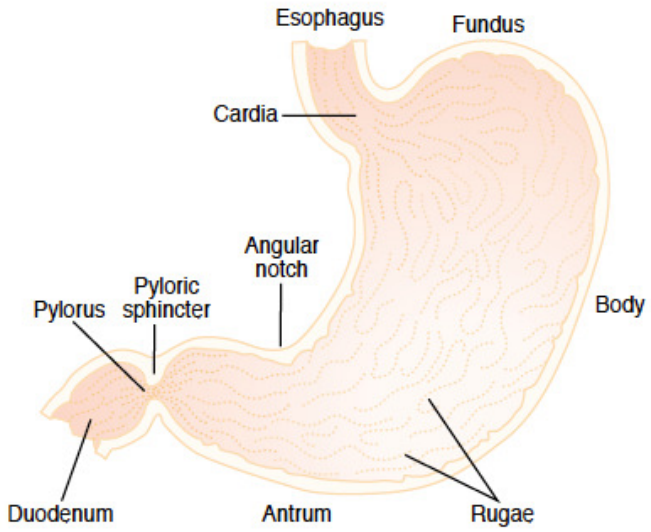


Figure 1.1 Physiologic anatomy of the stomach [2]

The structure of stomach wall (Figure 1.2) consists of 3 parts, mucosa layer, submucosa layer, and muscular layers that include both circular and longitudinal muscle layers. The mucosa of the stomach is thick and has many gastric glands. The human stomach secretes between 1.0 and 1.5 L of gastric juice per day. This gastric juice is highly acidic because of its hydrochloric acid content and is rich in enzymes, thus the gastric pH is about 1.5 [2]. The mucosa layer is covered with a thick tenacious mucus that is secreted by the columnar cells of the epithelium. Gastric mucus is a glycoprotein, that lubricates food masses, facilitates movement within the stomach, and its major role is to form a protective layer against gastric acid and proteolytic enzymes. The submucosa layer of the stomach contains many lymphocytes that help to protect against invading agents. The muscular layer is the

powerful layer that produces the wave of peristaltic contractions responsible for gastric emptying [2].

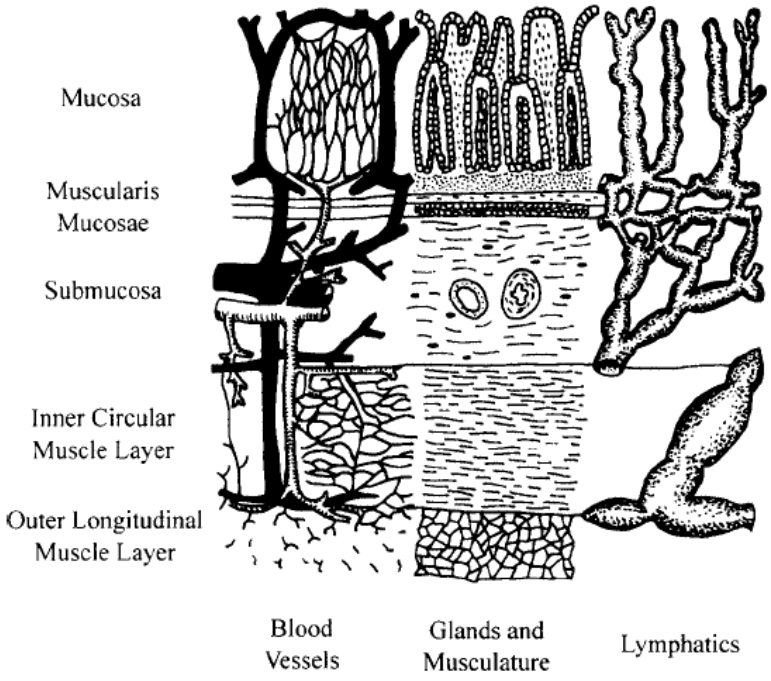


Figure 1.2 Structure of the stomach wall [2]

1.2 Mucosal surface and mucin

The mucosa or the mucus membrane is the surface tissue that lines the stomach wall and provides protection and acts as a lubricating gel of sticky viscous fluid throughout the gastrointestinal tract. This viscous fluid called mucus is secreted by the goblet cells in the epithelium or by special exocrine glands. The mucus forms

a diffusion barrier between the luminal and the cell surface that could bind bacteria, parasites, and viruses and plays an important role by interacting with and modulating the immune response, inflammation, and for protecting the mucosa from the external environment [3]. The gel forming properties of mucus is due to the presence of macromolecular components that are responsible for the viscous and elastic gel-like properties called mucin. Mucin possesses a linear protein core with a high serine and threonine content that are highly glycosylated by oligosaccharide side chains. These oligosaccharide side chains consist primarily of N-acetylgalactosamine, N-acetylglucosamine, fucose, galactose, sialic acid (N-acetyl neuraminic acid) and traces of mannose and sulfate [3]. The typical structure of a mucin molecule consists of several subunits connected by disulfide bridges. The oligosaccharide chains consisting of 5-15 monomers exhibit branching and are attached to the protein core by O-glycosidic bonds to the hydroxyl side chains of serine and threonine and are arranged in a “bottle brush” configuration around the protein core as shown in Figure 1.3 [4]. Other terminal residues in the oligosaccharide side chains are sialic acid, which has an axial carboxyl group. Mucus is negatively charged at neutral pH and uncharged at acidic pH. Numerous hydroxyl and carboxyl groups on mucin molecules can form hydrogen bonding with other polymer molecules.

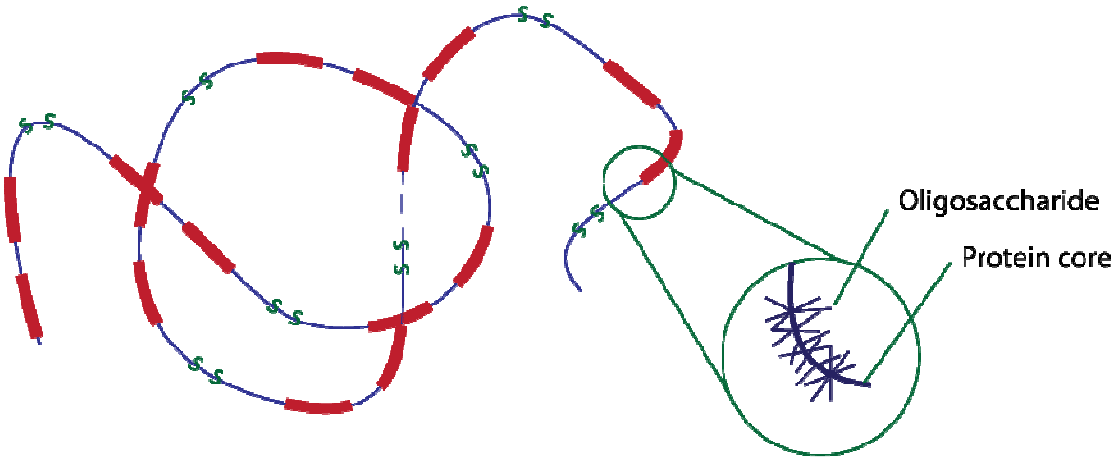


Figure 1.3 Diagram of mucin molecules. Several subunits are bound through cysteine-rich domains (S) that form disulfide bridges (S-S). Thick bars represent the highly glycosylated domains

1.3 *Helicobacter pylori*

In 1984 Warren and Marshall found a small curved and S-shaped bacillus in gastric biopsy specimens. The bacterium was closely associated with the surface epithelium, both within and between the gastric pits. These bacteria were often found in chronic gastritis and active chronic gastritis but these curved bacilli were often present in large numbers and found growing between the cells of the surface epithelium. These bacilli survived in the surface of the stomach in spite of the pH gradient from acid in the gastric lumen to near neutral in the mucosal vessels as shown in Figure 1.4. The bacteria grew in close contact with the epithelium, presumably near the neutral end of this gradient, and were being partly protected by

the overlying mucus. Initially these bacteria were named *Campylobacter pylori* because of their shape [5, 6].

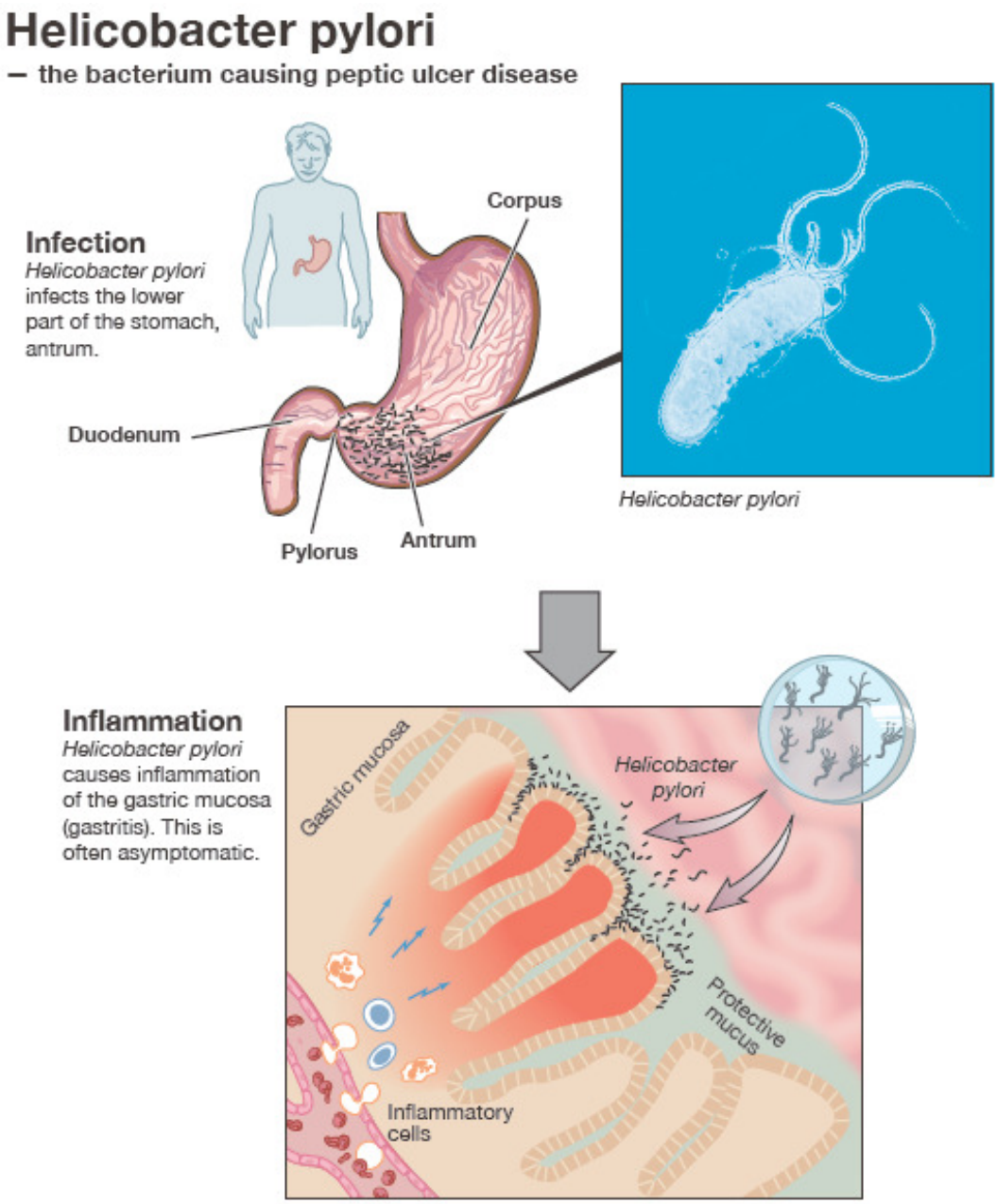


Figure 1.4 Schematic diagrams of *Helicobacter pylori* infection [7]

Nowadays, *Campylobacter pylori* has been reclassified as *Helicobacter pylori.* *H. pylori* is a Gram-negative spiral-shaped bacteria and is estimated to infect more than half of the world's population, predominantly in developing countries. *H. pylori* has a long latent period of subclinical infection during which it causes gastric mucosal inflammation and progressive mucosal damage. This bacteria is now well established as the cause of the gastritis-associated gastrointestinal diseases such as gastric ulcer, duodenal ulcer, gastric cancer and gastric mucosal-associated lymphoid tissue (MALT) lymphoma (MALToma) [8]. The incidence of *H. pylori* infections in developing countries has been reported since 1995 and continues to be high at between 3% and 10% per year [9].

Peptic ulcer disease is a problem of the gastrointestinal tract characterized by mucosal damage due to an increase of pepsin and gastric acid secretion. These problems usually occur in the stomach and proximal duodenum and rarely occur in the lower esophagus, the distal duodenum or the jejunum [10]. This disease has been a major threat to the world's population over the past two centuries with a high morbidity and substantial mortality [11]. The symptoms of peptic ulcer disease include epigastric discomfort, especially for pain relieved by food intake or antacids and pain that causes awakening at night or that occurs between meals, and results in a loss of appetite and weight [10]. Several environmental and host factors contribute to the formation of peptic ulcer such as increasing gastric acid secretion and weakening of the mucosal barrier. Environmental factors can help to induce peptic ulcer such as smoking, excessive alcohol use and drug use especially for non-steroidal anti-inflammatory drugs (NSAID). Emotional stress and psychosocial factors are the host factors most frequently identified as important contributors to

ulcer pathogenesis [11]. Moreover, a bacterial infection by *H. pylori* can induce peptic ulcer disease. The uses of NSAID and infection by *H. pylori* are the predominant causes of peptic ulcer disease, however more than 50% of the world's population has a chronic *H. pylori* infection of the gastric mucosa, yet only 5-10% of those infected develop ulcers [11]. The epidemiology of peptic ulcer disease in Thailand was reported during 1981 to 1988 with the prevalence rate remained fairly constant at around 111 to 112 per 100,000 populations during that period based on hospitalized case. The death rates of peptic ulcer cases based on national data between 1977 and 1987 fell slightly from 3.4 to 1 per 100,000 populations and deaths were higher in males than in female [12].

Although *H. pylori* is associated with gastric ulcer, most infected people (> 70%) are asymptomatic [13]. Thus an accurate diagnostic of H. pylori infection are important to identify the gastric ulcer with *H. pylori* infection for choosing the appropriate treatment. The diagnostic procedures for *H. pylori* are classified into two methods, an invasive test and non-invasive test. The invasive test is based on biopsy or endoscopy techniques such as histological examination, culture and a rapid urease test and the non-invasive techniques include serology, the urea breath test, urine/blood or detection of *H. pylori* antigen in stool specimens. Both of these techniques seem to be accurate for the diagnosis of *H. pylori*. However, a single test (with the exception of culture) is not sufficient to provide a diagnosis of infection thus at least two different tests are used for confirmation [14]. In addition both techniques of diagnosis have been reported to have the same degree of accuracy for detecting *H. pylori* [15, 16].

1.4 Treatment of *Helicobacter pylori*

The guidelines for treating *H. pylori* infection are triple line drug therapy that is a combination of an anti-acid secreting agent and antibiotic drugs. Many antibacterial agents have a very low minimum inhibitory concentration (MIC) against *H. pylori* in culture yet no single agent is effective *in vivo* when administered alone. The two main causes for drug ineffectiveness include the instability of some antibiotics at the low pH of the gastric acid and because the antibiotic never reaches an effective inhibitory concentration at the site of *H. pylori* colonized in the stomach epithelium because of it being protected by the mucus and the short residence time of the antibiotic in the stomach [17]. Even with the correct use of the triple drug combination, infection cannot be eradicated in up to 23% of patients [18]. The empirical regimens for treatment *H. pylori* infection are classified into two types of drug administration, sequential therapy and concomitant therapy. The sequential therapy does involve changing the number or type of drugs during the therapy period. The concomitant therapy all drugs are administered for the entire duration of the therapy. The recommended primary therapies for *H. pylori* infection for a concomitant therapy include a proton pump inhibitor (PPI), clarithromycin, and amoxicillin, or metronidazole (clarithromycin based triple therapy) for 14 days or a PPI or H_2 antagonist agent, bismuth, metronidazole and tetracycline (bismuth quadruple therapy) for 10 – 14 days. Or a sequential therapy consists of a PPI and amoxicillin for 5 days followed by a PPI, clarithromycin, and tinidazole for an additional 5 days may provide an alternative to clarithromycin-based triple or bismuth quadruple therapy [19]. Moreover the several effective regimens for treatment of *H.*

pylori published as clinical treatment guidelines are summarized in Table 1.1 [20]. These regimens are generally effective except in areas with a high prevalence (≥20 – 30%) of clarithromycin resistance, or dual clarithromycin and metronidazole resistance and have a more than 95% rate of treatment success in patients infected with susceptible strains and should still achieve a high eradication rate (>85%) in patients infected with antimicrobial-resistant strains [20].

Numerous studies have shown that the risk of ulcer recurrence is markedly reduced after successful eradication of *H. pylori*. After successful *H. pylori* eradication, recrudescence or re-infection may happen. In Thailand the risk of *H. pylori* re-infection is low only about 3% although in some developing countries there is a much higher prevalence of *H. pylori* infection [21].

Table 1.1 The regimens for *Helicobacter pylori* therapy 2011 to 2012 [20]

Treatment	**Drugs, dosages and duration**
Concomitant therapy	Amoxicillin (1 g), clarithromycin (500 mg), and tinidazole (500 mg) or metronidazole (500 mg) plus a PPI all given twice daily for 10–14 days
Sequential therapy	Amoxicillin (1 g) plus a PPI twice daily for 5 days, followed by clarithromycin (500 mg) and tinidazole (500 mg) or metronidazole (500 mg) plus a PPI all twice daily for a further 5 days (total 10 days)
Sequential–concomitant therapy	Amoxicillin (1 g) plus a PPI twice daily for 7 days, followed by amoxicillin (1 g), clarithromycin (500 mg) and tinidazole (500 mg) or metronidazole (500 mg) for a further 7 days (total 14 days)
Bismuth quadruple therapy	Bismuth subsalicylate or bismuth subcitrate and tetracycline hydrochloride (500 mg) both four times daily with meals and at bedtime plus metronidazole (500 mg) or tinidazole (500 mg) three times daily with meals and a PPI twice daily for 10 days, or preferably 14 days.

1.5 Strategy of drug delivery for treatment of *Helicobacter pylori*

Treatment guidelines for *H. pylori* infections explain that levels of clarithromycin in gastric juice, mucosa and serum have been found that are above the minimum inhibitory concentration (MIC) for up to six hours following oral dosing with a triple therapy regime (omeprazole, clarithromycin, amoxicillin) but only for two hours in the gastric mucosa following a 1 g dose of amoxicillin [22]. Strategies to increase amoxicillin effectiveness include administration of high oral doses (up to 1 g three times daily) in order to attain high local concentrations of antibiotic in the stomach [23]. Another reason for an ineffective treatment with antibiotics is the instability of the antibiotics such as amoxicillin and clarithromycin in gastric acid [24]. The minimum inhibitory concentration of amoxicillin against *H. pylori* including sensitive and resistance strains is from 8 - 256 μg/L and implies that if a successful local delivery were achieved, then lower doses of antibiotic may be effective [25]. The essential reason for unsuccessful eradication is the lack of residence time of the antimicrobial agents in the stomach so that an effective concentration of antibiotics at the site of infection cannot be achieved in the gastric mucous layer or epithelial cell surfaces where the *H. pylori* exist, [26, 27]. Conventional tablets or capsules are, in general, used for eradication therapy but these preparations do not remain in the stomach for long. Therefore, it is difficult to reach the minimum inhibitory concentrations within the gastric mucus in which the *H. pylori* has colonized [28, 29]. It has been proposed that a local delivery could increase drug levels in the gastric mucus and mucosa to allow for effective bactericidal levels and extend the contact time of the drugs with the organism. This

has been demonstrated with a 1 h topical treatment of antibiotic, which was delivered directly to the stomach via a naso-gastric tube and achieved a high cure rate of 96% [30]. This reason leads to the need to develop an oral dosage form with a prolonged gastric residence time sufficient to eradicate *H. pyroli*.

There is a need to develop gastric retentive systems that can overcoming the challengers of the physiological barriers present in the human gastrointestinal tract. In addition to the thick protective mucus layer, gastrointestinal motility patterns are another factor to overcome fo effective drug delivery to the stomach. In the fasting state following the digestive phase of approximately 2 h, when the last of the digestible food has left the stomach there are so called housekeeper contractions that occur with strong contractions to ensure removal of all indigestive residues through an open pylorus. All the mobility patterns of the stomach in the fasting state are shown in Figure 1.5 [2]. Thus a drug delivery system must be able to resist the forces of the fasting state mobility for an extended period. In the fed state, the stomach turns the food into sizes of less than 1 mm, which is then emptied to the duodenum. The composition of the food determines its residence time in the stomach with liquids emptying rapidly and solids much more slowly. Small particles (<1 mm) may pass rapidly into the small intestine with the liquid component whereas larger particles will take longer to reach this size. Gastric residence time is generally much longer in the fed rather than in the fasting state [2].

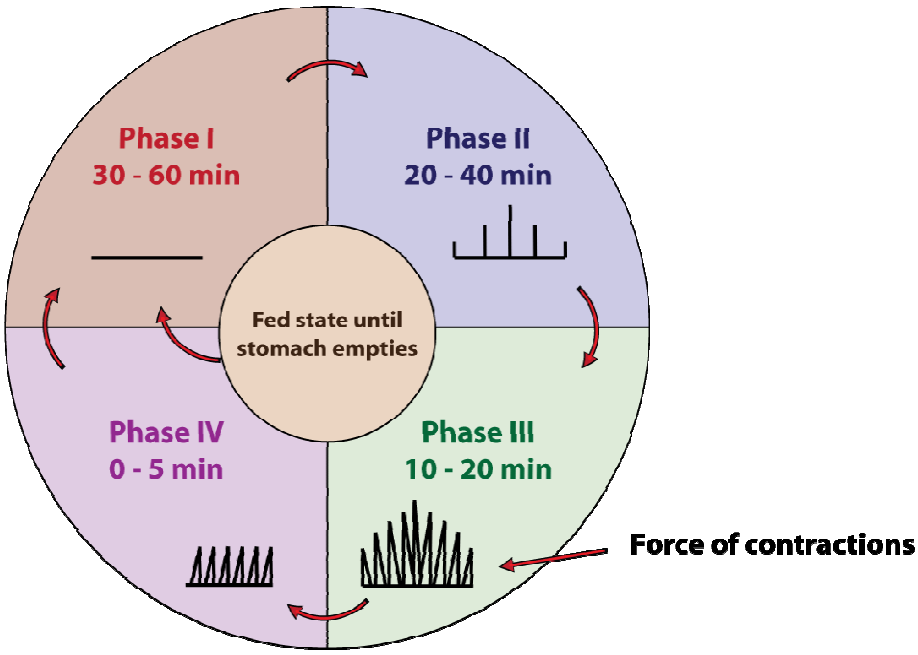


Figure 1.5 The mobility patterns of the stomach in fasted state

Another challenging factor is the natural turnover of mucus at the mucosal site that is possibly the biggest barrier for mucoadhesive drug delivery systems. The turnover time for mucus limits the residence time of the mucoadhesive polymers on the mucus layer. Mucoadhesive polymers become detached from the surface during turnover. The turnover rate may be different in the presence of mucoadhesive systems. Mucus turnover does leave a substantial amount of soluble mucin molecules on the epithelial membrane that can interact with a mucoadhesive polymer. The mucin turnover time is between 47 - 270 min [31]. The gastric residence time of a dosage form is also influenced by posture, age, gender, disease status and concomitant medication such as omeprazole [32]. Several drug delivery formulations have been explored to increase the gastric retention time including high

density and magnetic systems, but the three main systems are floating systems, bio/mucoadhesive systems and swelling systems [33].

1.6 Bioadhesive/mucoadhesive drug delivery system

The term bioadhesion has been used to describe the ability of some synthetic and biological macromolecules and hydrocolloids to adhere to a biological tissues or surface [34]. Whereas the interactions that occur primarily with the mucus layer and materials is referred as mucoadhesion. For drug delivery purposes, the term bioadhesion implies attachment of a drug carrier system to a specified biological location or defined as a substance that is capable of interacting with biological materials and being retained on them or holding them together for extended periods of time [35]. Leung and Robinson [36] described mucoadhesion as the interaction between a mucin surface and a synthetic or natural polymer. Mucoadhesion should not be confused with bioadhesion; in bioadhesion, the polymer is attached to a biological membrane and if the substrate is a mucus membrane then the term mucoadhesion is used.

Bioadhesives are classified into three types based on the presence or absence of non-biological (artificial) materials in the adhesion process rather than on the mechanisms of bioadhesion. Type I refers to the bioadhesion of two biological substrates such as cell fusion and cell aggregation, type II refers to the bioadhesion of cells onto an artificial material such as culture dishes or adhesion to a variety of substances including metals, woods and type III refers to the adhesion of artificial

substances to biological substrates such as the adhesion of polymers to a mucosal epithelium, skin or soft tissues [35]. The type III bioadhesion has been intensively investigated by several research groups and has led to the development of mucoadhesive drugs. The systems containing mucoadhesive water-soluble polymers, that become adhesive on hydration, can be used for targeting a drug to a particular region of the body for extended periods of time. Several types of mucosal layers line the regions of the body including gastrointestinal tract, urogenital tract, airways, ear, nose and eyes. Thus, the mucoadhesive drug delivery systems could be designed specifically for buccal, oral, vaginal, rectal, nasal and ocular routes of administration [37].

The mucoadhesive drug delivery systems have three distinct advantages when compared to conventional dosage forms [38]. Firstly, the mucoadhesive systems, which are readily localized in the region to which they have been applied, can improve and enhance the bioavailability of several drugs such as amoxicillin [28], clarithromycin [39] or even a large molecules such as calcitonin [40] or insulin [41]. Secondly, these dosage forms can facilitate an intimate contact with an underlying absorption surface resulting in better absorption. Lastly, they can prolong the residence time at the site of application to permit a once or twice a day dosing.

1.6.1 Mechanisms and mucoadhesion theories

Mechanisms for mucoadhesion were first proposed in 1988 by Ducheˇne and co-worker [42]. The first stage involves an intimate contact between a mucoadhesive compound and a mucus surface that results from a good wetting of the bioadhesive surface or from the swelling of the mucoadhesive polymer. In the second stage, after contact is established, penetration of the mucoadhesive polymer chain into the tissue surface or interpenetration of the chains of the mucoadhesive polymer and the mucus occurred. Then, weak chemical bonds could be formed during the final stage.

The theories developed to try to understand and explain the adhesive performance of adhesives have been adapted to gain an understanding of bio/mucoadhesion. The five main theories proposed to explain the mucoadhesion phenomena are the wetting, adsorption, diffusion, electrical and fracture theories. None of these theories can explain mucoadhesion on its own for all the different pharmaceutical formulations but several of these theories can be combined to obtain a unified picture of the mucoadhesive process [37].

1.6.1.1 Wetting theory of mucoadhesion

The wetting theory is perhaps the oldest established theory of adhesion. The wetting theory is predominantly applicable to liquid bioadhesive systems. It analyzes adhesive and contact behavior in terms of the ability of a liquid or paste to spread over a biological system. It is best applied to liquid or low-viscosity bioadhesives. The wetting theory calculates the work of adhesion as expressed in terms of surface and interfacial tensions [35, 43].

1.6.1.2 Diffusion theory of mucoadhesion

The concept of interpenetration and entanglement of mucoadhesive polymer chains and mucus chains producing semi-permanent adhesive bonds is supported by the diffusion theory. It is believed that bond strength increases with the interpenetration of the polymer chains into the mucin chains and reaches a sufficient depth. Penetration of polymer chains into the mucus network depended on the concentration gradients and diffusion coefficient. The existence of concentration gradients will drive the polymer chains of the mucoadhesive polymer into the mucus network and the mucin chains into the mucoadhesive matrix until an equilibrium penetration depth is achieved as shown in Figure 1.6 [42]. In this theory, the mucoadhesive polymer and mucus should have a similar chemical structure in which to have good solubility and give the strongest mucoadhesive bond [44].

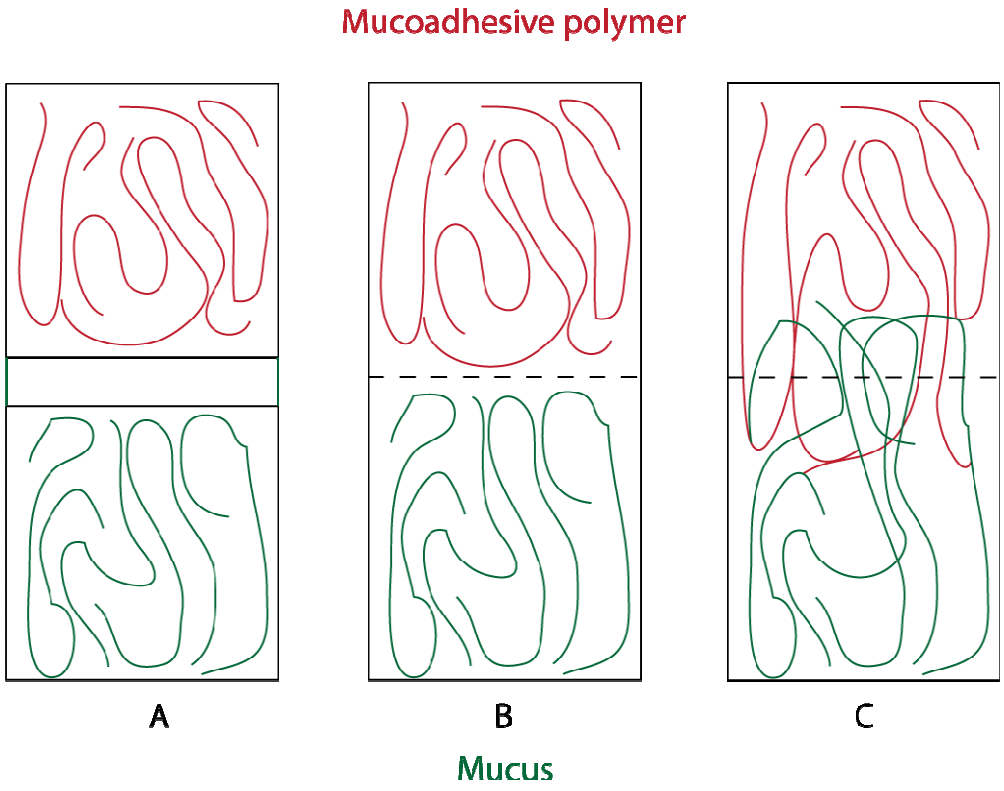


Figure 1.6 Schematic representation of the diffusion theory of mucoadhesion before contact (A), upon contact (B) and after contact for a period of time (C)

1.6.1.3 Adsorption theory of mucoadhesion

According to the adsorption theory, after an initial contact between two surfaces, the material adhered because of surface forces between molecules at the two surfaces. Two types of chemical bonds resulting from these forces can be distinguished. Firstly, primary chemical bonds of a covalent nature are undesirable in mucoadhesion because their high strength may result in permanent bonds. Secondly, secondary chemical bonds that have many different forces of attraction, including

electrostatic force, van der Waals force, hydrogen bonds and hydrophobic interactions [35].

1.6.1.4 Electrical theory of mucoadhesion

The electrical theory hypothesis relies on the assumption that the mucoadhesive material and the target biological material have different electrical structures. An electron transfer develops from the contact between the two materials because of the differences in their electronic structures. The bioadhesive force is believed to be due to the forces of attraction across this electrical double layer. The system is charged when the adhesive and substrate are in contact and discharged when they are separated [3].

1.6.1.5 Fracture theory of mucoadhesion

The most applicable theory for studying mucoadhesion through mechanical measurements is the fracture theory. This theory is related to the separation of two surfaces after adhesion and the fracture strength is regarded as equal to the adhesive strength. The fracture theory deals only with analyzes of the forces required to separate the two surfaces after adhesion, it does not assume or require entanglement, diffusion or interpenetration for polymer chains [3]. Thus, it is appropriate for being mainly used for calculation of the adhesive bonds for a rigid or

semi-rigid formulation [37]. A schematic diagram of the fracture theory of mucoadhesion is illustrated in Figure 1.7 [3].

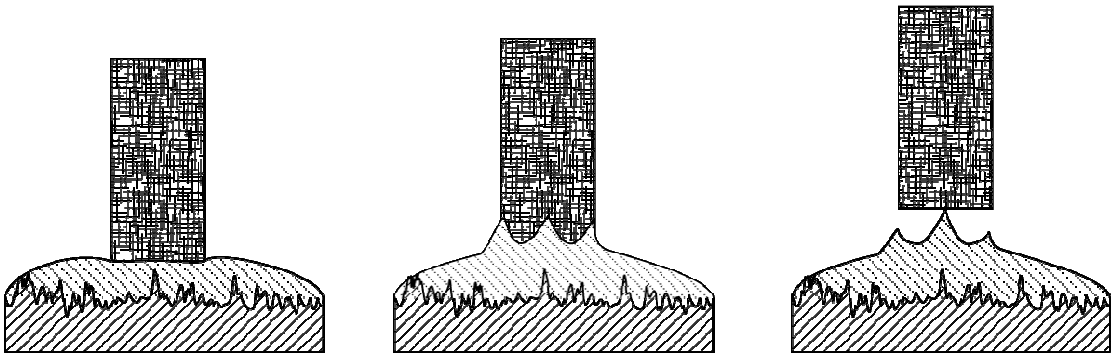


Figure 1.7 Schematic diagrams showing the progression of mucoadhesive fracture

The interrelation between the mucoadhesive theories of mucoadhesive and properties of mucoadhesive materials is illustrated in Figure 1.8. The overlapping areas between the circles indicate how and to what extent the mucoadhesive theories are connected to the material properties [45]. As visualized in Figure 1.8, first the mucoadhesive swells (wetting theory) and then molecular bonding (electronic and adsorption theories) occurs due to the formation of non-covalent bonds within the mucus–mucoadhesive interface. Next spatial conformation (diffusion theory) is introduced to achieve an interpenetration between the mucus and mucoadhesive layers. Then molecular bonding continues with the formation of new non-covalent and covalent bonds inside the mucus–mucoadhesive interface. The rheological

properties are an indication of the extent of covalent molecular bonding and spatial conformation [45].

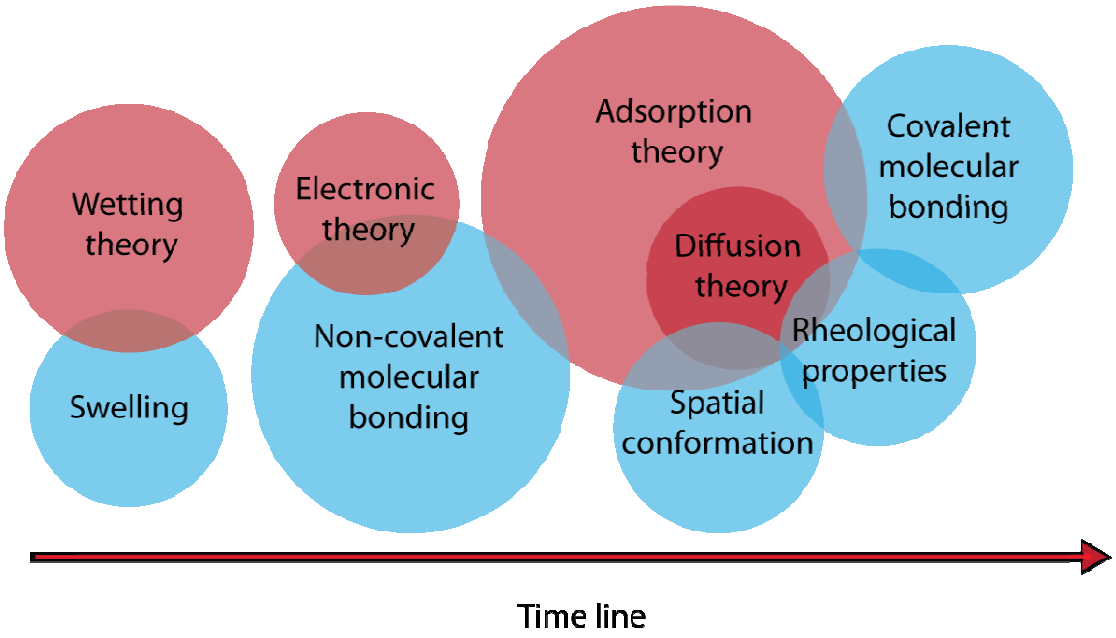


Figure 1.8 Schematic diagram of the interrelations between mucoadhesive theories (red circle) and properties of mucoadhesive materials (blue circle)

1.7 Polymers suitable for mucoadhesive drug delivery system

Three major categories of polymers have been used with some success as mucoadhesive agents: hydroxyl-containing, carboxyl-containing and other polymers mostly with charged species. These characteristics will lead to forming strong hydrogen bond interactions or strong ionic interactions between the

mucoadhesive polymer and mucin [34]. To overcome the relatively short GI residence time and improve the localization for a controlled or sustained release of drug delivery system, mucoadhesive polymers that can adhere to the mucin/epithelial surface are effective and lead to significant improvement in oral drug delivery [35]. Polymers that adhere to a mucin-epithelial surface can be classified into three broad categories: (i) polymers that become sticky when placed in water and owe their mucoadhesion or stickiness; (ii) polymers that adhere through nonspecific, non-covalent interactions which are primarily electrostatic in nature (although hydrogen and hydrophobic bonding may be significant) and (iii) polymers that bind to specific receptor sites on the cell surface. All three polymer types can be used for improving drug delivery [35].

An ideal polymer for mucoadhesive drug delivery systems should have the following characteristics: (i) the polymer and its degradation products should be nontoxic, compatible with the environment, be non-irritant and non-absorbable from the GI tract, (ii) it should be easily to administer, (iii) it possesses a high drug/polymer ratio, (iv) it should be easy and inexpensive to fabricate, (v) it has a good mechanical strength, (vi) it should preferably form a strong non-covalent bond with the mucus or epithelial cell surface, (vii) it should adhere quickly to moist tissue and possess some site specificity and (viii) the polymer must not decompose on storage or during the shelf life of the dosage form [43, 46]. Furthermore, it can be concluded that the most appropriate mucoadhesive polymer, should be a cationic and anionic polymers that will bind more effectively than neutral polymer. Polyanionic polymers were better than polycationic ones in terms of binding/potential toxicity and water-insoluble polymers that give a greater flexibility in dosage form design compared to rapidly or

slowly dissolving water-soluble polymers. Anionic polymers with sulfate groups bound more effectively than those with carboxylic groups. The degree of binding was proportional to the charge density on the polymer and polymers with high binding included carboxymethyl cellulose, gelatin, hyaluronic acid, carbopol and polycarbophil [35]. In addition, carboxylated polyanions appear to be better than sulfated polyanions when both mucoadhesiveness and toxicity are considered [47].

Polymers used for mucoadhesion can be classified by the source; synthetic and natural polymers. Some of the commonly used polymers for modern mucoadhesive drug delivery systems are briefly described below.

1.7.1 Poly(vinyl pyrrolidone)

Poly(vinyl pyrrolidone) or PVP is a synthetic neutral polymer consisting essentially of linear 1-vinyl-2-pyrrolidinone groups, the differing degree of polymerization of which results in polymers of various molecular weights. The chemical structure of PVP is shown in Figure 1.9 [48]. It is characterized by its viscosity in aqueous solution, relative to that of water, expressed as a K-value, in the range of 10–120. The K-value is calculated using the Fikentscher's equation (Eq. 1.1) [49]:

$$\log z = c\left(\frac{75k^2}{1+1.5kc}\right)+k \qquad (1.1)$$

where z is the relative viscosity of the solution of concentration c (in %w/v) and k is the K-value x 10^{-3}. The approximate molecular weights for different PVP grades are shown in Table 1.2 [48].

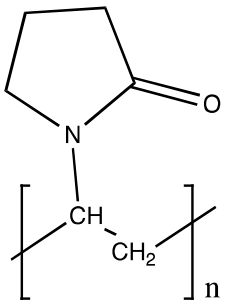


Figure 1.9 Chemical structure of poly(vinyl pyrrolidone)

Table 1.2 Approximate molecular weights for different grades of PVP

K-value	**Approximate molecular weight**
12	2,500
15	8,000
17	10,000
25	30,000
30	50,000
60	400,000
90	1,000,000
120	3,000,000

PVP has been used in pharmaceutical formulations for many years, primarily in solid dosage forms. In tableting, PVP solutions are used as binders in wet granulation processes. PVP is used as a solubilizing agent for oral and parenteral formulations, and has been shown to enhance dissolution of poorly soluble drugs from solid dosage forms as a solid dispersion technique [50, 51]. PVP solutions may also be used as coating agents, binders or film forming agent when coating active pharmaceutical ingredients on a support such as sugar beads. Moreover, PVP is additionally used as a suspending, stabilizing, or viscosity-increasing agent in a number of topical and oral suspensions and solutions [48]. For the safety of PVP after being consumed orally, PVP may be regarded as essentially nontoxic since it is not absorbed from the GI tract or mucous membranes. PVP additionally has no irritant effect on the skin and causes no sensitization [48].

PVP has been widely used in mucoadhesive drug development such as mucoadhesive hydrogels, mucoadhesive microsphere, mucoadhesive gels or buccal patches [52-54]. Due to its properties, PVP has been used as a viscosity-increasing agent that can alter the rheology characteristics of hydrogels to extend the residence time of hydrogel adherence to mucus membranes [55], improve drug delivery carriers such as microspheres by forming a complex with other polymer molecule with the carbonyl groups of PVP via strong hydrogen bonds [52, 56] and utilize the films formed for mucoadhesive buccal patches [54]. The use of PVP can enhance both of the mucoadhesive properties and dissolution of poorly soluble drugs [57]. The addition of the hydrophilic polymer PVP promotes faster dissolution by increasing the surface wettability and consequently water penetration within the matrix leading to an

increase of the water-soluble content [58]. Moreover, PVP has also shown some bioadhesive properties with human epithelial cell cultures [47].

1.7.2 Chitosan

Chitosan is the *N*-deacetylated product of the polysaccharide chitin found in a wide range of natural sources such as crustaceans, fungi, insects and some algae. It is a polymer of poly (*N*-acetyl-β-D-glucosamine) as shown in Figure 1.10 [59]. Manufactured chitosan is usually obtained from crustaceans (crab, krill and crayfish), especially because a large amount of their exoskeleton is available as a byproduct of food processing [60].

When the degree of deacetylation of chitin reaches about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media and is called chitosan. The solubilization occurs by protonation of the NH_2 function of the D-glucosamine repeat unit, whereby the polysaccharide is converted to a polyelectrolyte in an acidic medium [59]. Chitosan is insoluble in water, alkali and organic solvents, but soluble in most solutions of organic acids when the pH of the solution is less than 6. Acetic and formic acids are two of the most widely used acids for dissolving chitosan. Some dilute inorganic acids, such as nitric acid, hydrochloric acid, perchloric acid, and H_3PO_4, can also be used to prepare a chitosan solution, but only after prolonged stirring and warming [61]. Chitosan is of importance in the pharmaceutical field due to its unique polymeric cationic characteristics, good biocompatibility, nontoxicity and its biodegradability. Chitosan has been proposed as

a useful excipient for either the sustained release of water-soluble drugs and for enhancing the bioavailability of poorly water-soluble compounds [62, 63].

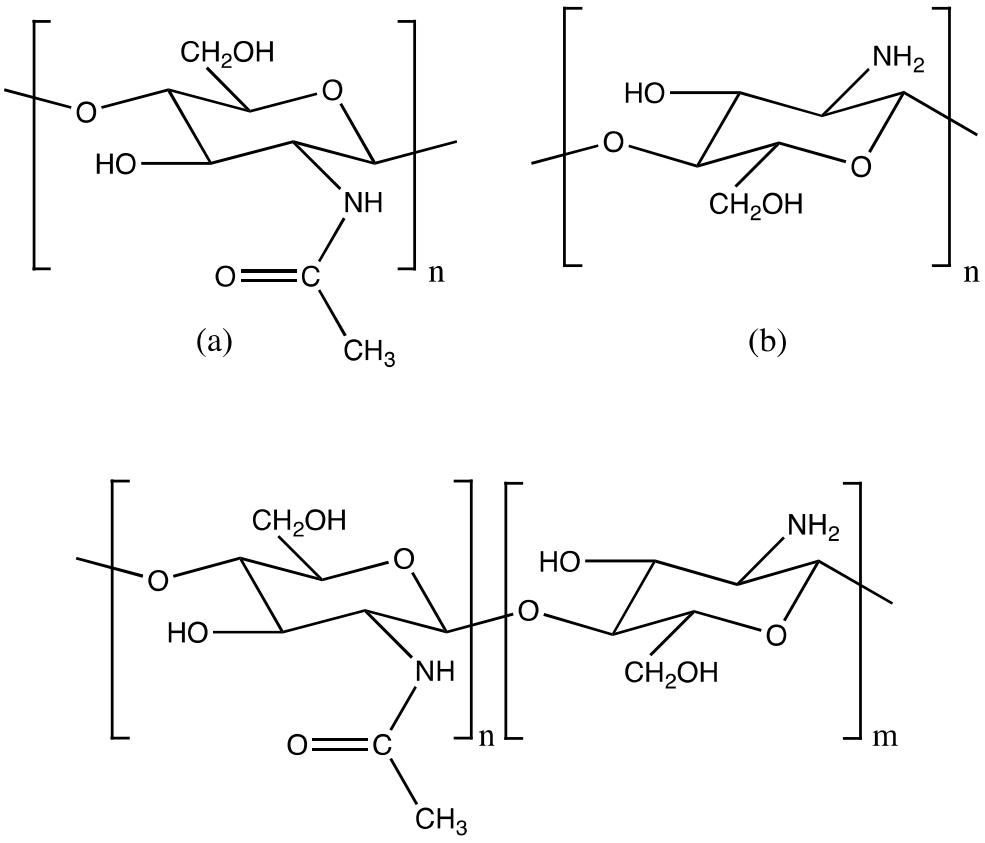


Figure 1.10 Chemical structure of chitin (poly (*N*-acetyl-β-D-glucosamine)) (a), chitosan (poly (D-glucosamine)) (b) repeat units and structure of partially acetylated chitosan (c)

Chitosan exhibits strong mucoadhesive properties due to the formation of hydrogen and ionic bonds between the positively charged amino groups of chitosan and the negatively charged sialic acid residues of the mucin glycoproteins [64]. Furthermore, chitosan enhanced drug or peptides permeability through the mucosal surface [65, 66]. Thus, mucoadhesive formulations using chitosan have been intensively studied for prolonging the residence times of the delivery system such as a mucoadhesive microsphere [67], mucoadhesive gel [68], mucoadhesive film [69] or mucoadhesive bead [70]. It has been reported that the higher the molecular weight of chitosan the better is its ability to promote its mucoadhesive properties [71]. Furthermore, chitosan also has antibacterial activity and acts to enhance absorption, thus chitosan is a good candidate polymer for drug delivery [72, 73].

1.7.3 Gelatin

Gelatin is a natural polymer that is derived from collagen, and commonly used for pharmaceutical and medical applications because of its biodegradability and biocompatibility in physiological environments [74]. Two different types of gelatin can be produced depending on the method of collagen pretreatment prior to the extraction process. In the preparation of gelatin from animal skins (gelatin type B), it is necessary to remove the hairs attached to the pelt, and this is usually accomplished by treatment with aqueous alkali-lime water [75]. This extraction process targets the amide groups of asparagine and glutamine and hydrolyses them into carboxyl groups, thus converting many of these residues to

aspartate and glutamate [74]. The treatment of bones for the preparation of gelatin (gelatin type A) may involve the removal of mineral salts by treatment with dilute acid [75]. This dilute acidic extraction process, in contrast to the acidic pre-treatment does little to affect the amide groups present [74]. The two extraction processes of gelatin result in electrically different gelatin molecules This is because the alkaline processed gelatin possesses a greater proportion of carboxyl groups rendering it negatively charged, whereas the acidic process produces a positively charged [74]. Gelatin A with an isoelectric point of between 7 and 9, whereas gelatin B obtained by alkaline hydrolysis has an isoelectric point of between 4.7 and 5.3 [76].

Gelatin has been reported to have bioadhesive [77] and mucoadhesive properties that can prolong the residence time of any delivery system [76]. The bioadhesive or mucoadhesive properties of native gelatin are quite low, thus blending of gelatin with other polymer or modified gelatin have been reported to improve these properties for enhancing residence times [78]. Several mucoadhesive formulations using gelatin have been developed such as microsphere [76, 78], mucoadhesive tablets [79] and mucoadhesive films [80].

1.7.4 Sodium Alginate

Alginates are natural polysaccharide polymers isolated from brown seaweed. The seaweed is extracted with a dilute alkaline solution that solubilizes the alginic acid present [81]. The alginic acid can then be converted to a salt of which sodium alginate is the major form currently in use. Alginic acid is a linear polymer

consisting of two sugar monomers D-mannuronic acid (M block) and L-guluronic acid (G block) residues that are arranged in the polymer chain in blocks as shown in figure 1.11 [81]. Alginate forms gel or precipitates with divalent cations and multivalent cations especially with Ca^{2+} ions. The mechanism of gel formation of an alginate will be discussed later in Chapter 6. Due to the properties of an alginate that allows it to form gels in the presence of calcium ions, alginate has been used extensively for drug carrier systems. The polymer matrix of an alginate gel can play a significant role in the design of a controlled release product based on the diffusion mechanism [81].

Alginate also shows mucoadhesive properties and is used as a drug carrier for mucoadhesive drug delivery systems such as an alginate bead [82-85]. Alginate beads have the advantages of being nontoxic with high biocompatibility [86]. Another advantageous property is their inability to re-swell in acidic environment while they easily re-swell in an alkaline environment, so acid-sensitive drugs incorporated into the beads would be protected from the gastric juice [87]. Thus, alginate beads have been used as an entrapment matrix for drugs, cells or proteins. For mucoadhesive drug delivery systems, several alginate bead formulations have been modified to enhance the mucoadhesive properties by coating with another mucoadhesive polymer [85] or by forming a complex with another polymer during bead formation [88].

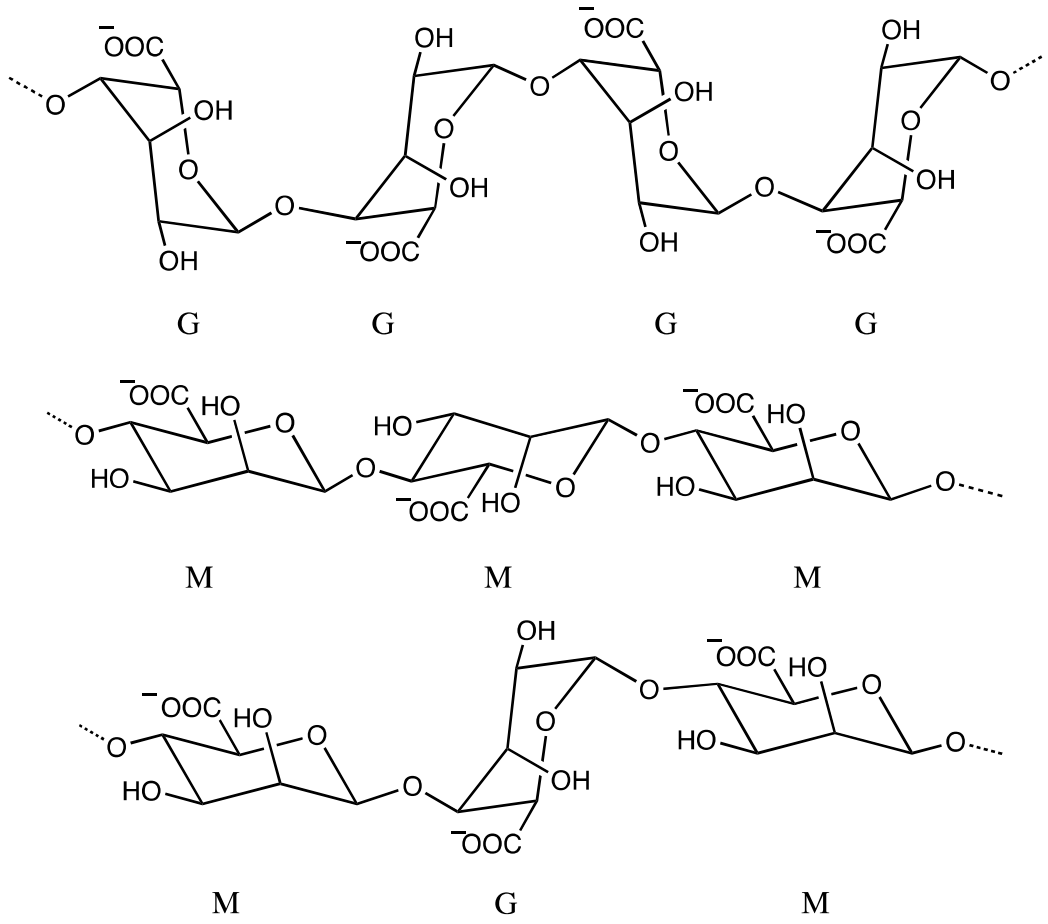


Figure 1.11 Chemical structure of alginate block types consisting of D-mannuronic acid (M) and L-guluronic acid (G)

1.8 Evaluation of Mucoadhesion

Several test methods for studying mucoadhesion have been reported. These tests are necessary not only to screen a large number of candidate mucoadhesive materials, but also to study their mechanisms. The test methods can be classified into two major categories; (i) direct methods and (ii) indirect methods [89].

1.8.1 Direct methods

A direct determination of the mucoadhesion ability may involve a quantitative determination of the force required to detach the mucoadhesive from the surface. An alternative approach is the determination of another quantitative parameter such as the time required to detach the mucoadhesive from the surface when the polymer is subject to a constant applied force [89]. The direct methods for mucoadhesion evaluation are described as follow.

1.8.1.1 Tensile strength assay

The detachment force in tensile strength assays is determined using either a commercially available instrument such as a materials testing machine or a texture analyzer as depicted in Figure 1.12 [90]. Methods using tensile strength have usually examined the force necessary to separate the two surfaces after a mucoadhesive bond has been established. Briefly, the mucoadhesive material under examination is attached to a surface of a solid support. The test material was then lowered onto the biological substrate and left for a certain period to allow interaction between the material and the biological tissue. After this period, the solid support is raised at a constant rate until total detachment occur. The results are usually presented as the maximum detachment force (MDF) or the area under the force – distance curve representing the total work of adhesion [89]. This technique was widely used to measure the force of mucoadhesion for several formulated systems

such as a mucoadhesive patch [90], mucoadhesive gel [91], mucoadhesive tablet [92] and mucoadhesive film [93].

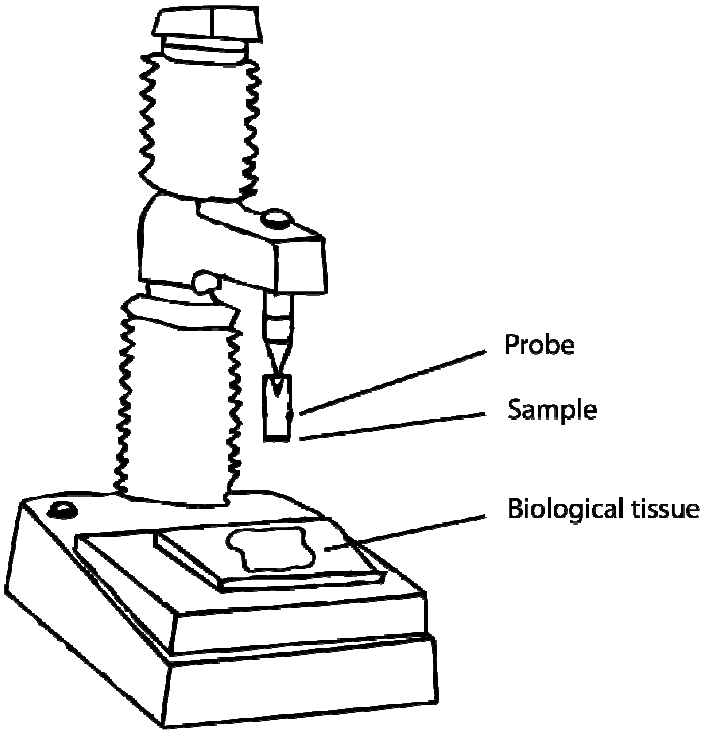


Figure 1.12 Mucoadhesive testing system utilizing the texture analyzer equipment

1.8.1.2 Dynamic assay under shear forces

The dynamic assay using shear force was used to determine the residence time of the mucoadhesive system on the biological tissue while applying a shear force such as movement, rotating of the solid support or wash off method [65, 71, 94, 95]. For this technique the shear forces were applied by the movement of the solid support with the biological tissue attached with a mucoadhesive formulation. The quantitative measured parameter is the time until detachment, disintegration and/or erosion of the tablets is observed. Another dynamic assay is the continuous flow assay that quantifies the ability of a polymer to maintain binding with the mucosal surface under shear forces subjected as a continuous flow. This method was first introduced by using glass spheres coated with the tested polymer and a known amount of particles was placed on fresh mucus attached to the floor of the flow cell for a fixed time in a humid environment to allow hydration of the polymer and prevent drying of the tissue. The experiments were performed by washing the mucosal surface with a flowing phosphate buffer or dilute hydrochloric acid solution at a constant rate for a fixed time. The percentage of beads washed away was determined by weighing the wash solution after drying, and the results were expressed as an index of mucoadhesion calculated from the percentage of particles retained on the tissue [95]. A schematic diagram of the continuous flow assay is shown illustrated in Figure 1.13.

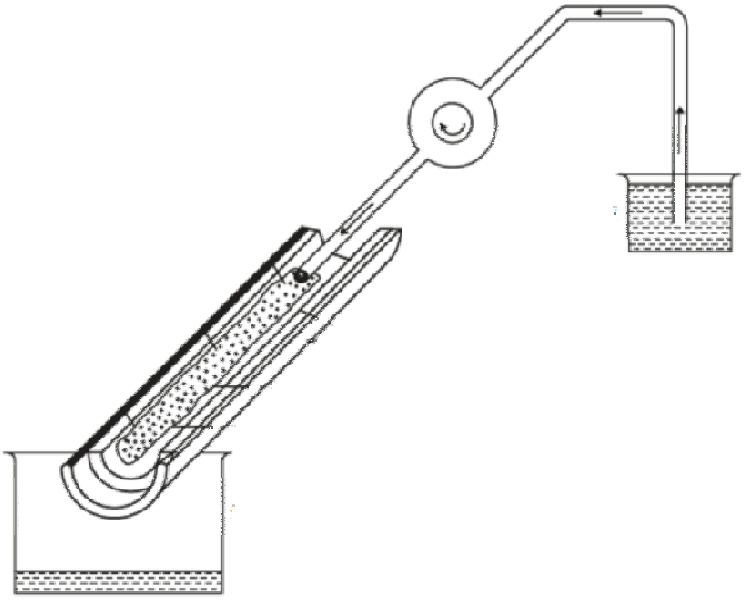


Figure 1.13 Schematic illustration of the experiment set up used for the continuous flow assay

1.8.1.3 Cell adhesion assay

The cell adhesion assay has been report for mucoadhesion evaluation by using cell culture techniques [96, 97]. Several cell culture types were used to determine the mucoadhesive properties of polymer film such as human intestinal epithelium cell line (HT29) [96], mouse embryo fibroblast and chondrocyte cell line [97]. For this technique, cell line was seed on the polymer film then incubated for allow cell to attach for a period of time then quantified the amount of cell attach on the polymer surface. The quantitative measured parameter for this technique is the amount of cell attachment on mucoadhesive materials.

1.8.2 Indirect methods

An indirect determination of the mucoadhesive properties is used to characterize the physicochemical properties or studies the interactions between the mucoadhesive and the mucin. Some results from these techniques are correlated with the force of mucoadhesion. The indirect methods for mucoadhesion evaluation were described as follow.

1.8.2.1 Viscosity or rheology measurement

The use of viscosity or rheology measurements is the technique for the study of flow and deformation of materials, and offers a straightforward means to monitor the strength of the interaction and to predict the mucoadhesion ability [89]. The interaction between mucin and the mucoadhesive polymer that leads to enhance the total resistance to the flow exerted by chain entanglement, non-covalent bonds such as hydrogen, electrostatic and hydrophobic bonding interactions [98]. These interactions can be monitored by viscosity or rheology changes. The synergistic effect of the polymer – mucin system appears to be an outcome of molecular interactions and can be converted to the force of mucoadhesion [98]. This technique is useful for screening various polymers [89]. Therefore, this technique should not be used as a standalone method for detecting mucoadhesive properties of polymer – mucin systems.

1.8.2.2 Spectroscopic method

In a similar way to rheology measurements spectroscopic methods detect mucus – polymer interactions at the molecular level. Analysing a Fourier transform infrared spectroscopy (FTIR) trace is a useful technique used to identify interactions between polymers and mucin. Changes in the spectrum will be observed as a result of H-bonding between the mucin and the polymer. This technique provides the mechanism of an interaction between the polymer and mucin at the molecular level. Other spectroscopic or microscopic method have been reported for observing mucoadhesive properties such as X-ray photoelectron spectroscopy (XPS) [99], atomic force microscopy (AFM) [100], fluorescence techniques [96] and confocal laser microscopy [101].

1.9 Study and aims for developing mucoadhesive drugs for eradication of *Helicobacter pylori*

The development of mucoadhesive drug delivery systems has been intensively studied for several years since the guidelines for *H. pylori* eradication has failed due to physiological barriers or resistance to antimicrobial agents, a short residence time of antimicrobial agents in the stomach or even to the stability of antimicrobial agents in the acidic conditions in the stomach [102]. To overcome these problems, a high dose of antibiotics is necessary or devising a way to increase the residence time of an antimicrobial agent in the stomach. After the report of a

complete eradication of *H. pylori* due to an extended gastric residence time of the antimicrobial agent, several researchers have focused on increasing the residence time of the delivery system for eradication of *H. pylori* [30].

Several mucoadhesive formulations for *H. pylori* eradication were developed for delivering antimicrobial agents to the stomach with an extended residence time. For example, mucoadhesive microspheres of amoxicillin prepared by the spray-chilling method demonstrated a greater anti *H. pylori* activity than conventional suspensions by increasing the residence time. This result established that a topical action of amoxicillin on the gastric mucus played an important role in the clearance of *H. pylori* [28]. Another research work using mucoadhesive microspheres of amoxicillin using carbopol 934P as the mucoadhesive material and ethyl cellulose as carrier system showed high mucoadhesion properties, high drug entrapment efficiency up to 56%, exhibited sustained release properties and was more effective against *H. pylori* [103]. A floating mucoadhesive microsphere of clarithromycin was made from ethyl cellulose and carbopol 934P. This system showed both mucoadhesive and floating properties and provided a sustained release and a better eradication of *H. pylori* [104]. A mucoadhesive bead of calcium alginate coated with chitosan used as a drug carrier demonstrated high mucoadhesive properties and prolonged the residence time of the delivery system compared with uncoated bead [85]. This formulation also showed a sustained release characteristic by the diffusion mechanism [105]. All of these mucoadhesive formulations may provide therapeutic concentration at a much lower dose, and may also reduce adverse effects.

1.10 The aim of this study

The aims of this study are to find the most suitable polymers with mucoadhesive properties including chitosan, gelatin and PVP and their blends. These polymers have been previously reported to have good mucoadhesive or bioadhesive properties and these polymer blends may provide the synergistic effect of mucoadhesion. Several techniques are used to determine the mucoadhesive or bioadhesive properties of the polymer and polymer blends such as viscosity measurement, spectroscopic techniques, texture analysis, cell culture techniques, wash-off technique. A bead formulation is also selected for study due to the high entrapment efficiency up to 90% and use of amoxicillin as a model drug [106]. From the literature reviews, there have been no reports for mucoadhesive beads coated with polymer blends thus the amoxicillin bead formulation coated with suitable polymer blend may provide better mucoadhesive properties than a single polymer coated bead. All amoxicillin mucoadhesive beads are studied for their gastric residence time efficiency for eradication and drug release.

References

1. Ahn, J., Choi, H., Cho, C. (2001) A novel mucoadhesive polymer prepared by template polymerization of acrylic acid in the presence of chitosan. *Biomaterials* 22, 923-8.
2. Washington, N., Washington, C., Wilson, C. Physiological pharmaceutics : barriers to drug absorption. 2nd ed. London ; New York: Taylor & Francis; 2001.
3. Mathiowitz, E., Chickering, D., Lehr, C. Bioadhesive drug delivery systems : fundamentals, novel approaches, and development. New York: Marcel Dekker; 1999.
4. Watanabe, Y., Inoko, Y. (2007) Small-angle light and X-ray scattering measurements of a protein-oligosaccharide complex mucin in solution. *J. Appl. Crystallogr.* 40, s209-s12.
5. Warren, R., Marshall, B. (1983) Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1, 1273-5.
6. Marshall, B., Warren, J. (1984) Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1, 1311-5.
7. Nobelprize.org. Press Release: The 2005 Nobel Prize in Physiology or Medicine. http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/press.html2005.
8. Vilaichone, R., Mahachai, V., Graham, D. (2006) Helicobacter pylori diagnosis and management. *Gastroenterol. Clin. North Am.* 35, 229-47.
9. Parsonnet, J. (1995) The incidence of Helicobacter pylori infection. *Aliment. Pharmacol. Ther.* 9 Suppl 2, 45-51.
10. Ramakrishnan, K., Salinas, R. (2007) Peptic ulcer disease. *Am. Fam. Physician* 76, 1005-12.
11. Chan, F., Leung, W. (2002) Peptic-ulcer disease. *Lancet* 360, 933-41.
12. Wilairatana, S., Kladchareon, N., Israsena, S., Wilairatana, P. (1991) Epidemiology of peptic ulcer disease in Thailand. *Gastroenterol. Jpn.* 26 Suppl 3, 265-6.
13. Logan, R., Walker, M. (2001) ABC of the upper gastrointestinal tract: Epidemiology and diagnosis of Helicobacter pylori infection. *BMJ* 323, 920-2.
14. Ricci, C., Holton, J., Vaira, D. (2007) Diagnosis of Helicobacter pylori: invasive and non-invasive tests. *Best practice & research. Clinical gastroenterology* 21, 299-313.
15. Cutler, A., Havstad, S., Ma, C. K., Blaser, M., Perez-Perez, G., Schubert, T. (1995) Accuracy of invasive and noninvasive tests to diagnose Helicobacter pylori infection. *Gastroenterology* 109, 136-41.
16. McColl, K., Murray, L., Gillen, D., Walker, A., Wirz, A., Fletcher, J., Mowat, C., Henry, E., Kelman, A., Dickson, A. (2002) Randomised trial of endoscopy with testing for Helicobacter pylori compared with non-invasive H pylori testing alone in the management of dyspepsia. *BMJ* 324, 999-1002.
17. Shah, S., Qaqish, R., Patel, V., Amiji, M. (1999) Evaluation of the factors influencing stomach-specific delivery of antibacterial agents for Helicobacter pylori infection. *J. Pharm. Pharmacol.* 51, 667-72.
18. Cianci, R., Montalto, M., Pandolfi, F., Gasbarrini, G., Cammarota, G. (2006) Third-line rescue therapy for Helicobacter pylori infection. *World J Gastroenterol* 12, 2313-9.

19. Chey, W., Wong, B. (2007) American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *Am. J. Gastroenterol.* 102, 1808-25.
20. Rimbara, E., Fischbach, L., Graham, D. (2011) Optimal therapy for Helicobacter pylori infections. *Nature reviews. Gastroenterology & hepatology* 8, 79-88.
21. Thong-Ngam, D., Mahachai, V., Kullavanijaya, P. (2007) Incidence of Helicobacter pylori Recurrent Infection and Associated Factors in Thailand. *J. Med. Assoc. Thai.* 90, 1406-10.
22. Nakamura, M., Spiller, R., Barrett, D., Wibawa, J., Kumagai, N., Tsuchimoto, K., Tanaka, T. (2003) Gastric Juice, Gastric Tissue and Blood Antibiotic Concentrations Following Omeprazole, Amoxicillin and Clarithromycin Triple Therapy. *Helicobacter* 8, 294-9.
23. Graham, D., Abudayyeh, S., El-Zimaity, H., Hoffman, J., Reddy, R., Opekun, A. (2006) Sequential therapy using high-dose esomeprazole-amoxicillin followed by gatifloxacin for Helicobacter pylori infection. *Aliment. Pharmacol. Ther.* 24, 845-50.
24. Erah, P., Goddard, A., Barrett, D., Shaw, P., Spiller, R. (1997) The stability of amoxycillin, clarithromycin and metronidazole in gastric juice: relevance to the treatment of Helicobacter pylori infection. *J. Antimicrob. Chemother.* 39, 5-12.
25. Han, S., Bhakdi, S., Maeurer, M., Schneider, T., Gehring, S. (1999) Stable and Unstable Amoxicillin Resistance in Helicobacter pylori: Should Antibiotic Resistance Testing Be Performed Prior to Eradication Therapy? *J. Clin. Microbiol.* 37, 2740-1.
26. Liu, Z., Lu, W., Qian, L., Zhang, X., Zeng, P., Pan, J. (2005) In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. *J Control Release* 102, 135-44.
27. Megraud, F., Lamouliatte, H. (2003) Review article: the treatment of refractory Helicobacter pylori infection. *Aliment. Pharmacol. Ther.* 17, 1333-43.
28. Nagahara, N., Akiyama, Y., Nakao, M., Tada, M., Kitano, M., Ogawa, Y. (1998) Mucoadhesive microspheres containing amoxicillin for clearance of Helicobacter pylori. *Antimicrob. Agents Chemother.* 42, 2492-4.
29. Umamaheswari, R., Jain, S., Tripathi, P., Agrawal, G., Jain, N. (2002) Floating-bioadhesive microspheres containing acetohydroxamic acid for clearance of Helicobacter pylori. *Drug delivery* 9, 223-31.
30. Kimura, K., Ido, K., Saifuku, K., Taniguchi, Y., Kihira, K., Satoh, K., Takimoto, T., Yoshida, Y. (1995) A 1-h topical therapy for the treatment of Helicobacter pylori infection. *Am. J. Gastroenterol.* 90, 60-3.
31. Lehr, C., Poelma, F. G., Junginger, H., Tukker, J. (1991) An estimate of turnover time of intestinal mucus gel layer in the rat in situ loop. *Int. J. Pharm.* 70, 235-40.
32. Cucchiara, S., Minella, R., Campanozzi, A., Salvia, G., Borrelli, O., Ciccimarra, E., Emiliano, M. (1997) Effects of omeprazole on mechanisms of gastroesophageal reflux in childhood. *Dig. Dis. Sci.* 42, 293-9.
33. Conway, B. (2005) Drug delivery strategies for the treatment of Helicobacter pylori infections. *Curr. Pharm. Des.* 11, 775-90.
34. Peppas, N., Buri, P. (1985) Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. *J. Controlled Release* 2, 257-75.

35. Ahuja, A., Khar, R., Ali, J. (1997) Mucoadhesive Drug Delivery Systems. *Drug Dev. Ind. Pharm.* 23, 489-515.
36. Sau, L., Robinson, J. (1988) The contribution of anionic polymer structural features to mucoadhesion. *J. Controlled Release* 5, 223-31.
37. Edsman, K., Hagerstrom, H. (2005) Pharmaceutical applications of mucoadhesion for the non-oral routes. *J. Pharm. Pharmacol.* 57, 3-22.
38. Chowdary, K., Rao, Y. (2004) Mucoadhesive microspheres for controlled drug delivery. *Biol. Pharm. Bull.* 27, 1717-24.
39. Majithiya, R., Murthy, R. (2005) Chitosan-Based Mucoadhesive Microspheres of Clarithromycin as A Delivery System for Antibiotic to Stomach. *Current Drug Delivery* 2, 235-42.
40. Thirawong, N., Thongborisute, J., Takeuchi, H., Sriamornsak, P. (2008) Improved intestinal absorption of calcitonin by mucoadhesive delivery of novel pectin–liposome nanocomplexes. *J. Controlled Release* 125, 236-45.
41. Pluta, J., Haznar, D., Suszka, A., Ryszka, F. (2008) Insulin availability from mucoadhesive tablets. *Pharmazie* 63, 650-3.
42. Ducheˇne, D., Touchard, F., Peppas, N. (1988) Pharmaceutical and Medical Aspects of Bioadhesive Systems for Drug Administration. *Drug Dev. Ind. Pharm.* 14, 283-318.
43. Shaikh, R., Raj, T., Garland, M., Woolfson, A., Donnelly, R. (2011) Mucoadhesive drug delivery systems. *Journal of pharmacy & bioallied sciences* 3, 89-100.
44. Kinloch, A. (1980) The science of adhesion. *Journal of Materials Science* 15, 2141-66.
45. Dodou, D., Breedveld, P., Wieringa, P. (2005) Mucoadhesives in the gastrointestinal tract: revisiting the literature for novel applications. *Eur. J. Pharm. Biopharm.* 60, 1-16.
46. Langer, R., Peppas, N. (1981) Present and future applications of biomaterials in controlled drug delivery systems. *Biomaterials* 2, 201-14.
47. Park, K., Robinson, J. (1984) Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion. *Int. J. Pharm.* 19, 107-27.
48. Rowe, R., Sheskey, P., Quinn, M., American Pharmacists Association. Handbook of pharmaceutical excipients. 6th ed. London ; Chicago
Washington, DC: Pharmaceutical Press ;
American Pharmacists Association; 2009.
49. Swei, J., Talbot, J. (2003) Viscosity correlation for aqueous polyvinylpyrrolidone (PVP) solutions. *J. Appl. Polym. Sci.* 90, 1153-5.
50. Kim, E., Chun, M., Jang, J., Lee, I., Lee, K., Choi, H. (2006) Preparation of a solid dispersion of felodipine using a solvent wetting method. *Eur. J. Pharm. Biopharm.* 64, 200-5.
51. Karavas, E., Ktistis, G., Xenakis, A., Georgarakis, E. (2005) Miscibility Behavior and Formation Mechanism of Stabilized Felodipine-Polyvinylpyrrolidone Amorphous Solid Dispersions. *Drug Dev. Ind. Pharm.* 31, 473-89.
52. Chun, M., Cho, C., Choi, H. (2005) Mucoadhesive microspheres prepared by interpolymer complexation and solvent diffusion method. *Int. J. Pharm.* 288, 295-303.

53. Choi, G., Jung, H., Ryu, M., Yoon, J., Oh, K., Kim, K. (1998) Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. *Int. J. Pharm.* 165, 33-44.
54. Perioli, L., Ambrogi, V., Angelici, F., Ricci, M., Giovagnoli, S., Capuccella, M., Rossi, C. (2004) Development of mucoadhesive patches for buccal administration of ibuprofen. *J. Controlled Release* 99, 73-82.
55. Alsarra, I., Hamed, A., Alanazi, F., Neau, S. (2011) Rheological and mucoadhesive characterization of poly(vinylpyrrolidone) hydrogels designed for nasal mucosal drug delivery. *Arch. Pharmacal Res.* 34, 573-82.
56. Chun, M., Cho, C., Choi, H. (2002) Mucoadhesive drug carrier based on interpolymer complex of poly(vinyl pyrrolidone) and poly(acrylic acid) prepared by template polymerization. *J. Controlled Release* 81, 327-34.
57. Karavas, E., Georgarakis, E., Bikiaris, D. (2006) Application of PVP/HPMC miscible blends with enhanced mucoadhesive properties for adjusting drug release in predictable pulsatile chronotherapeutics. *Eur. J. Pharm. Biopharm.* 64, 115-26.
58. Jain, S., Jain, A., Gupta, Y., Kharya, A. (2008) Design and development of a mucoadhesive buccal film bearing progesterone. *Pharmazie* 63, 129-35.
59. Marguerite, R. (2006) Chitin and chitosan: Properties and applications. *Prog. Polym. Sci.* 31, 603-32.
60. Galed, G., Miralles, B., Paños, I., Santiago, A., Heras, Á. (2005) N-Deacetylation and depolymerization reactions of chitin/chitosan: Influence of the source of chitin. *Carbohydr. Polym.* 62, 316-20.
61. Li, Q., Dunn, E., Grandmaison, E., Goosen, M. (1992) Applications and Properties of Chitosan. *Journal of Bioactive and Compatible Polymers* 7, 370-97.
62. Sahasathian, T., Kerdcholpetch, T., Chanweroch, A., Praphairaksit, N., Suwonjandee, N., Muangsin, N. (2007) Sustained release of amoxicillin from chitosan tablets. *Arch. Pharmacal Res.* 30, 526-31.
63. Orienti, I., Cerchiara, T., Luppi, B., Bigucci, F., Zuccari, G., Zecchi, V. (2002) Influence of different chitosan salts on the release of sodium diclofenac in colon-specific delivery. *Int. J. Pharm.* 238, 51-9.
64. Rossi, S., Ferrari, F., Bonferoni, M., Caramella, C. (2000) Characterization of chitosan hydrochloride-mucin interaction by means of viscosimetric and turbidimetric measurements. *Eur. J. Pharm. Sci.* 10, 251-7.
65. Bernkop-Schnurch, A. (2000) Chitosan and its derivatives: potential excipients for peroral peptide delivery systems. *Int. J. Pharm.* 194, 1-13.
66. Illum, L., Farraj, N., Davis, S. (1994) Chitosan as a Novel Nasal Delivery System for Peptide Drugs. *Pharm. Res.* 11, 1186-9.
67. Cho, S., Choi, H. (2005) Preparation of mucoadhesive chitosan-poly(acrylic acid) microspheres by interpolymer complexation and solvent evaporation method II. *Arch. Pharmacal Res.* 28, 612-8.
68. Gratieri, T., Gelfuso, G., Rocha, E., Sarmento, V., de Freitas, O., Lopez, R. (2010) A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. *Eur. J. Pharm. Biopharm.* 75, 186-93.
69. Aksungur, P., Sungur, A., Unal, S., Iskit, A., Squier, C., Senel, S. (2004) Chitosan delivery systems for the treatment of oral mucositis: in vitro and in vivo studies. *J Control Release* 98, 269-79.

70. Elzatahry, A., Eldin, M. S., Soliman, E., Hassan, E. (2009) Evaluation of Alginate-Chitosan Bioadhesive Beads as a Drug Delivery System for the Controlled Release of Theophylline. *J. Appl. Polym. Sci.* 111, 2452-9.
71. Lehr, C., Bouwstra, J., Schacht, E., Junginger, H. (1992) In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int. J. Pharm.* 78, 43-8.
72. Qi, L., Xu, Z., Jiang, X., Hu, C., Zou, X. (2004) Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydr. Res.* 339, 2693-700.
73. No, H., Young, N., Ho, S., Meyers, S. (2002) Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int. J. Food Microbiol.* 74, 65-72.
74. Young, S., Wong, M., Tabata, Y., Mikos, A. (2005) Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *J. Controlled Release* 109, 256-74.
75. Hunter, E., Turner, A. (1940) The iso-electric point of gelatin. *Transactions of the Faraday Society* 36, 835-9.
76. Bonferoni, M., Chetoni, P., Giunchedi, P., Rossi, S., Ferrari, F., Burgalassi, S., Caramella, C. (2004) Carrageenan-gelatin mucoadhesive systems for ion-exchange based ophthalmic delivery: in vitro and preliminary in vivo studies. *Eur. J. Pharm. Biopharm.* 57, 465-72.
77. Matsuda, S., Iwata, H., Se, N., Ikada, Y. (1999) Bioadhesion of gelatin films crosslinked with glutaraldehyde. *J. Biomed. Mater. Res.* 45, 20-7.
78. Wang, J., Tauchi, Y., Deguchi, Y., Morimoto, K., Tabata, Y., Ikada, Y. (2000) Positively Charged Gelatin Microspheres as Gastric Mucoadhesive Drug Delivery System for Eradication of H. pylori. *Drug delivery* 7, 237-43.
79. Kotagale, N., Patel, C., Parkhe, A., Khandelwal, H., Taksande, J., Umekar, M. (2010) Carbopol 934-Sodium Alginate-Gelatin Mucoadhesive Ondansetron Tablets for Buccal Delivery: Effect of pH Modifiers. *Indian journal of pharmaceutical sciences* 72, 471-9.
80. Abruzzo, A., Bigucci, F., Cerchiara, T., Cruciani, F., Vitali, B., Luppi, B. (2012) Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. *Carbohydr. Polym.* 87, 581-8.
81. Tonnesen, H., Karlsen, J. (2002) Alginate in drug delivery systems. *Drug Dev. Ind. Pharm.* 28, 621-30.
82. Taylor, C., Pearson, J., Draget, K., Dettmar, P., Smidsrid, O. (2005) Rheological characterisation of mixed gels of mucin and alginate. *Carbohydr. Polym.* 59, 189-95.
83. Das, M., Maurya, D. (2008) Evaluation of diltiazem hydrochloride-loaded mucoadhesive microspheres prepared by emulsification-internal gelation technique. *Acta Pol. Pharm.* 65, 249-59.
84. Murata, Y., Toniwa, S., Miyamoto, E., Kawashima, S. (1999) Preparation of alginate gel beads containing chitosan salt and their function. *Int. J. Pharm.* 176, 265-8.
85. Gåserød, O., Jolliffe, I., Hampson, F., Dettmar, P., Skjåk-Bræk, G. (1998) The enhancement of the bioadhesive properties of calcium alginate gel beads by coating with chitosan. *Int. J. Pharm.* 175, 237-46.

86. Sankalia, M., Mashru, R., Sankalia, J., Sutariya, V. (2005) Papain entrapment in alginate beads for stability improvement and site-specific delivery: Physicochemical characterization and factorial optimization using neural network modeling. *AAPS PharmSciTech* 6, E209-E22.
87. TOSHIHISA, Y., TSUNEO, O., TAKAFUMI, O., KEN, I. (1987) Calcium-Induced Gelation of Alginic Acid and pH-Sensitive Reswelling of Dried Gels(Pharmaceutical). *Chem. Pharm. Bull.* 35, 1555-63.
88. Gonzalez-Rodriguez, M., Holgado, M., Sanchez-Lafuente, C., Rabasco, A., Fini, A. (2002) Alginate/chitosan particulate systems for sodium diclofenac release. *Int. J. Pharm.* 232, 225-34.
89. Davidovich-Pinhas, M., Bianco-Peled, H. (2010) Mucoadhesion: a review of characterization techniques. *Expert opinion on drug delivery* 7, 259-71.
90. Wong, C., Yuen, K., Peh, K. (1999) Formulation and evaluation of controlled release Eudragit buccal patches. *Int. J. Pharm.* 178, 11-22.
91. Gratieri, T., Gelfuso, G., Rocha, E., Sarmento, V., de Freitas, O., Lopez, R. (2010) A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. *Eur. J. Pharm. Biopharm.* 75, 186-93.
92. Alam, M., Ahmad, F., Khan, Z., Khar, R., Ali, M. (2007) Development and evaluation of acid-buffering bioadhesive vaginal tablet for mixed vaginal infections. *AAPS PharmSciTech* 8, E109.
93. Peh, K., Khan, T., Ching, H. (2000) Mechanical, bioadhesive strength and biological evaluations of chitosan films for wound dressing. *Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques* 3, 303-11.
94. Wittaya-areekul, S., Kruenate, J., Prahsarn, C. (2006) Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. *Int. J. Pharm.* 312, 113-8.
95. Rao, K., Buri, P. (1989) A novel in situ method to test polymers and coated microparticles for bioadhesion. *Int. J. Pharm.* 52, 265-70.
96. Keely, S., Rullay, A., Wilson, C., Carmichael, A., Carrington, S., Corfield, A., Haddleton, D., Brayden, D. (2005) In vitro and ex vivo intestinal tissue models to measure mucoadhesion of poly (methacrylate) and N-trimethylated chitosan polymers. *Pharm. Res.* 22, 38-49.
97. Lim, S., Song, D., Cho, K., Oh, S., Lee-Yoon, D., Bae, E., Lee, J. Cell Adhesion and Degradation Behaviors of Acetylated Chitosan Films. 3rd Kuala Lumpur International Conference on Biomedical Engineering 2006. 183082007. p. 94-7.
98. Hassan, E., Gallo, J. (1990) A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. *Pharm. Res.* 7, 491-5.
99. Lawrie, G., Keen, I., Drew, B., Chandler, A., Rintoul, L., Fredericks, P., Grøndahl, L. (2007) Interactions between Alginate and Chitosan Biopolymers Characterized Using FTIR and XPS. *Biomacromolecules* 8, 2533-41.
100. Li, D., Yamamoto, H., Takeuchi, H., Kawashima, Y. (2010) A novel method for modifying AFM probe to investigate the interaction between biomaterial polymers (Chitosan-coated PLGA) and mucin film. *Eur. J. Pharm. Biopharm.* 75, 277-83.
101. Liu, L., Fishman, M., Hicks, K., Kende, M. (2005) Interaction of various pectin formulations with porcine colonic tissues. *Biomaterials* 26, 5907-16.

102. el-Omar, E., Penman, I., Ardill, J., Chittajallu, R., Howie, C., McColl, K. (1995) Helicobacter pylori infection and abnormalities of acid secretion in patients with duodenal ulcer disease. *Gastroenterology* 109, 681-91.
103. Patel, J., Chavda, J. (2009) Formulation and evaluation of stomach-specific amoxicillin-loaded carbopol-934P mucoadhesive microspheres for anti-Helicobacter pylori therapy. *J. Microencapsulation* 26, 365-76.
104. Rajinikanth, P., Karunagaran, L., Balasubramaniam, J., Mishra, B. (2008) Formulation and evaluation of clarithromycin microspheres for eradication of Helicobacter pylori. *Chem. Pharm. Bull.* 56, 1658-64.
105. Huguet, M., Dellacherie, E. (1996) Calcium alginate beads coated with chitosan: Effect of the structure of encapsulated materials on their release. *Process Biochem.* 31, 745-51.
106. Whitehead, L., Collett, J., Fell, J. (2000) Amoxycillin release from a floating dosage form based on alginates. *Int. J. Pharm.* 210, 45-9.

CHAPTER 2

Evaluation of mucoadhesive polymers using viscosity measurements

2.1 Introduction and objectives

An evaluation of mucoadhesion by using viscosity measurement is a simple rheological method for the *in vitro* assessment of mucoadhesive bond strengths. Interactions of mucin and polymer produce physical chain entanglements and non-covalent intermolecular interactions including electrostatic, hydrogen bondings and hydrophobic interactions. These interaction forces are identical to those involved in the process of mucin – polymer adhesion that produce a resistance to an exerted flow, that can be monitored by measuring the change of viscosity in a mucin – polymer system. Several authors have suggested that rheological synergism between polymer and mucin can be used as an *in vitro* parameter to determine the mucoadhesive properties of the material [107-110]. Rheological data from these reports have been interpreted on a molecular basis involving physical and chemical interactions. In fact, both physical and chemical bond energies in mucin – polymer interactions can be transformed into mechanical energy or work and this work causes changes in the shape or arrangement of macromolecules and is the basis for the changes in viscosity. A simple procedure to assess the force of mucoadhesion was

proposed by Hassan and Gallo. They proposed the viscosity component of mucoadhesion ($\eta_{enhance}$) that could be obtained by Eq. 2.1 [111]:

$$\eta_t = \eta_m + \eta_p + \eta_{enhance} \quad (2.1)$$

where η_t is the viscosity of the system, η_m and η_p are the individual viscosities of mucin and polymer, respectively. All parameters, η_t, η_m, and η_p are measured at the same concentration, temperature, time, and shear rate. Consequently, the force of mucoadhesion (F) represent the additional intermolecular friction force per unit area and was determined by Eq. 2.2 [111]:

$$F = \eta_{enhance}\sigma \quad (2.2)$$

where σ is the shear rate (s^{-1}) and $\eta_{enhance}$ is based on the experimentally measured value.

The aim of this study was to investigate the mucoadhesion of mucin with various polymers including chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA), gelatin type B (GB), and polymer blends of C/PVP, C/GA, C/GB at various volume ratios through viscosity measurements performed on the polymers alone and on their mixtures with mucin.

2.2 Experimental methods

2.2.1 Materials

Chitosan with an average viscosity, molecular weight of 300,000 – 500,000 Da with a 75% – 85% degree of deacetylation was obtained from Fluka (GmbH, Buchs). Gelatin type A from porcine skin with a bloom strength of 225 and gelatin type B from bovine skin with a bloom strength of 300 were from Sigma (St. Louis, MO). PVP K-90 (Kollidon 90), with an average molecular weight (M_w) of 1,100,000, was kindly supplied by BASF, Thailand. Mucin Type 2 from porcine stomach was from Sigma (St. Louis, MO). All other reagents were of analytical grade.

2.2.2 Sample preparations

The chitosan (C) stock solution (2% w/v) was prepared by dissolving chitosan (2.0 g) in 0.05 M hydrochloric acid solution (100 mL). poly(vinylpyrrolidone) (PVP), gelatin type A (GA), and gelatin type B (GB) (2.0 g) were dissolved in water (100 mL) to obtain stock solutions with a final concentration of 2%w/v. Mucin solutions at concentrations of 15% w/v were prepared by dispersing the dried mucin (15.0 g) in water (100 mL). All polymers, and mucin solutions were continually stirred for 4 h at room temperature until completely

dissolved. Polymer blends of C/PVP, C/GA, and C/GB were prepared by mixing the 2% w/v polymer stock solutions in the volume ratio of 1/9, 3/7, 5/5, 7/3, and 9/1. All polymer blends were gently mixed using a reciprocating shaker until homogeneous. The mixtures of polymer and mucin were prepared by blending 5 mL of mucin solution (15%w/v) with 2 mL of polymer or polymer blend solutions. The final volume of the mixtures was adjusted with water to 8 mL. Subsequently, the mixture was mixed using a reciprocating shaker until a homogeneous dispersion was obtained. The final concentrations of polymers, polymer blends and mucin solutions were 0.5, 0.5 and 9.375% w/v, respectively. These mixtures of mucin-polymers and mucin-polymer blends were equilibrated at 25.0 ± 0.1 °C for 1 h before analysis. All sample were freshly prepared for viscosity measurement.

2.2.3 Viscosity measurement

All viscosity measurements were performed using a Brookfield model DV-III Ultra programmable viscometer (Brookfield Engineering Laboratories, Inc., USA) with SC4-18 spindle and a small sample adaptor, at 25.0 ± 0.1 °C. A schematic of the instrument setup is shown in Figure 2.1. Samples were allowed to equilibrate for 1 minute before the test. The apparent viscosity at a shear rate of 15.84 s^{-1} was selected for comparison. All viscosity measurements were performed in triplicate. The flow behavior of the polymer or polymer blend with and without mucin is described by the consistency index (K_c) or non-Newtonian index (n) using the power law (Eq. 2.3):

$$\tau = K_c\gamma^n \qquad (2.3)$$

where τ is the shear stress, and γ is the shear rate. The consistency index (K_c) or non-Newtonian index (n) was obtained directly from the Rheocal version 3.1 software package.

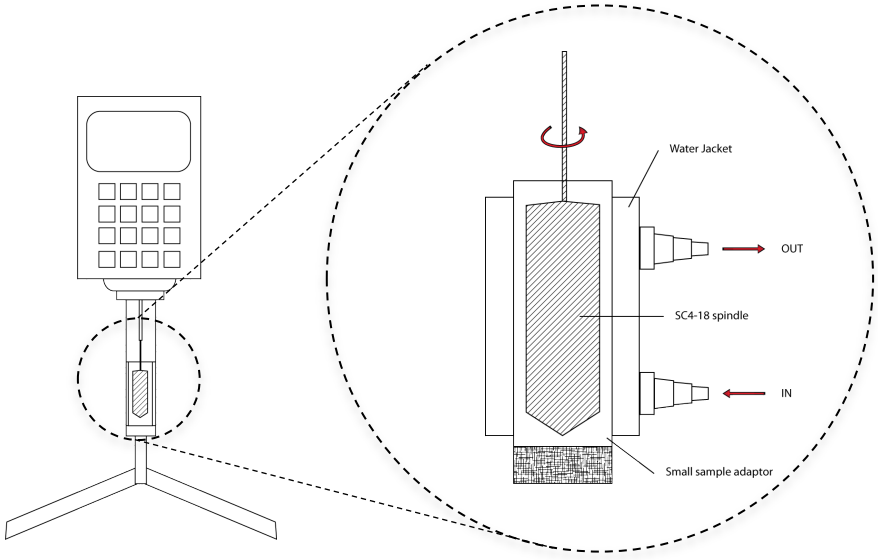


Figure 2.1 Schematic of viscosity measurement using the Brookfield viscometer with SC4-18 spindle and a small sample adaptor

2.2.4 Statistical analysis

Analysis of variance (ANOVA) was performed using the SPSS version 10.0 for Windows (SPSS Inc., USA). *Post hoc* testing ($p<0.05$) of the multiple comparisons was performed by Tukey's test.

2.3 Results and discussion

2.3.1 Viscosity characterization

The fluid flow behavior of polymer, polymer blends, mucin and combinations of polymer and mucin was evaluated. The plots of apparent viscosity of polymer, polymer blends or mucin alone and a combination of polymer and mucin versus shear rate are shown in Figure 2.2 and 2.3, respectively. For a Newtonian liquid, viscosity remains constant over a wide range of shear rates, whereas the liquids in which viscosity varies with shear rate are called non-Newtonian liquids. A pseudoplastic fluid is a non-Newtonian liquid that demonstrates a decrease in viscosity as the shear rate increases. All combination systems of polymer or polymer blends and mucin also displayed a shear thinning or pseudoplastic flow behavior where viscosity decreased with an increasing rate of shear. During the increasing rate of shear, the polymer chains become disentangled and the hydrogen bonds may be broken resulting in a reduction in viscosity.

The flow behavior index (n) and the consistency index (K_c) were calculated from the plots of shear stress versus shear rate (Eq. 2.3). The exponent n from the power law model is an indication of the departure from Newtonian behavior. For pseudoplastic fluids, $0 < n < 1$, and for dilatant fluids, $n > 1$. As n approaches 1, flow becomes less shear dependent, and $n = 1$ for a Newtonian flow. The flow behavior and consistency index of the polymer, polymer blends, mucin alone and its combination with mucin are shown in Table 2.1. The flow behavior index of the polymer, polymer blends and combination systems corresponded to the shear thinning flow behavior (pseudoplastic flow). The shear thinning behavior of the polymer, polymer blend, and combinations of polymer blend and mucin solutions can be rationalized in terms of polymer entanglements, where (under shear) the rate of disentanglement exceeded the rate at which the new entanglements formed, and led to a reduction in the crosslinking density and, as a consequence, the viscosity decreased [112]. The trend of exponent n of the polymer and polymer blends was demonstrated to increase after mixing with mucin, and seemed to be more Newtonian in behavior. The viscosity decreased when increasing a shear rate indicated that the combination of polymer or polymer blend and mucin increased the chain flexibility of the polymer and possibly promoted interactions between the polymer and mucin molecules [113, 114].

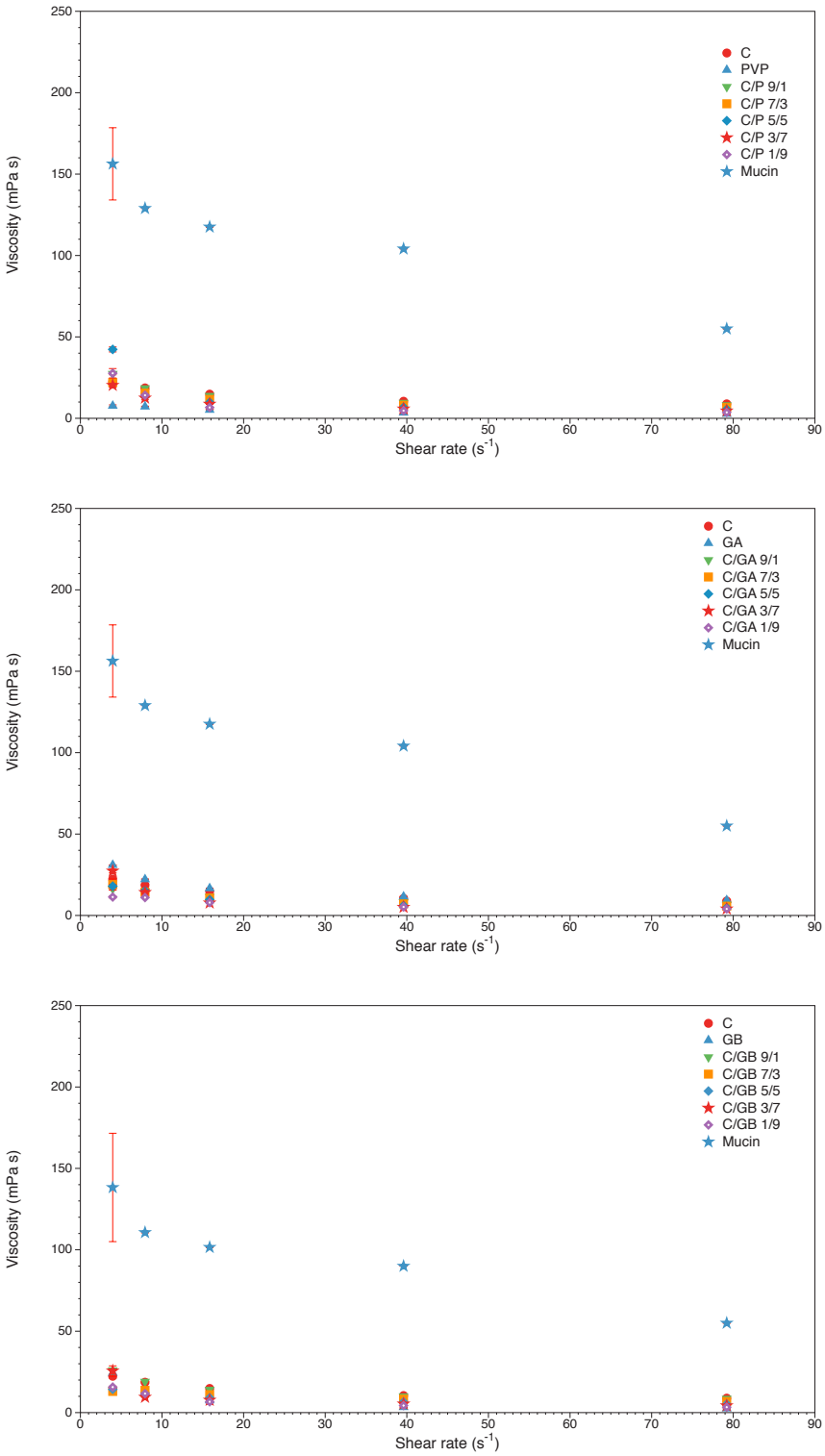


Figure 2.2 Apparent viscosity of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) and their blends at different volume ratio at various shear rates

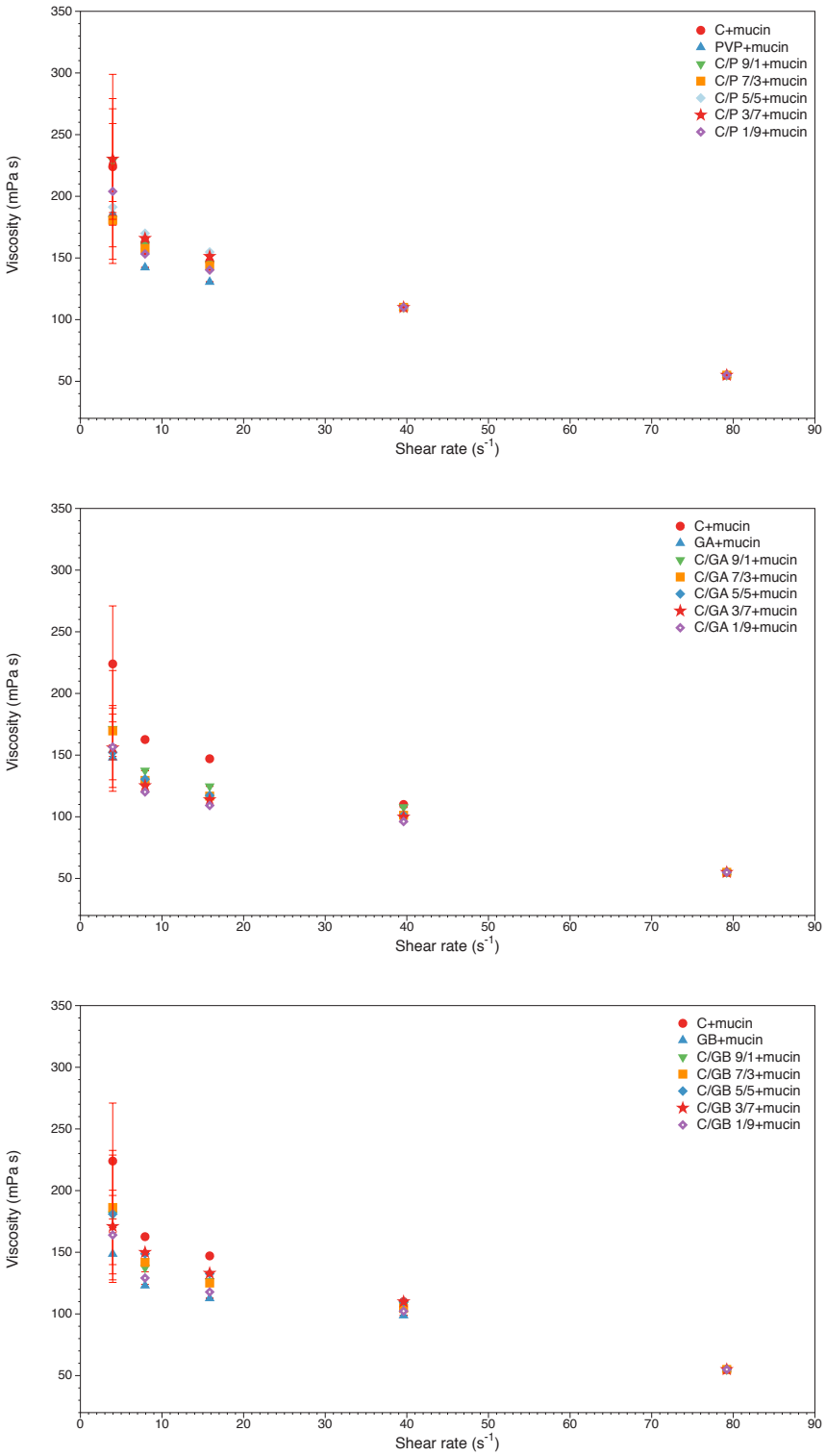


Figure 2.3 Apparent viscosity of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin and their blend at different volume ratio with mucin at various shear rates

Table 2.1 Flow behavior index (n) and consistency index (K_c) derived from the power law model of the chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA), gelatin type B (GB) and their blend at different volume ratio with and without mucin, n = 3

Sample	Flow behavior index (n)		Consistency index (K_c)	
	Polymer	Polymer + mucin	Polymer	Polymer + mucin
Chitosan	0.68 ± 0.01	0.58 ± 0.05	35.32 ± 1.29	419.19 ± 81.90
C/PVP 9/1	0.59 ± 0.01	0.58 ± 0.08	44.61 ± 0.98	423.28 ± 120.80
C/PVP 7/3	0.60 ± 0.01	0.64 ± 0.01	36.44 ± 1.62	335.65 ± 7.29
C/PVP 5/5	0.38 ± 0.01	0.61 ± 0.01	66.86 ± 3.18	376.68 ± 8.30
C/PVP 3/7	0.51 ± 0.02	0.57 ± 0.05	36.57 ± 3.17	438.38 ± 86.77
C/PVP 1/9	0.33 ± 0.02	0.62 ± 0.07	57.82 ± 4.04	366.78 ± 91.74
PVP	0.63 ± 0.02	0.65 ± 0.05	13.95 ± 0.69	316.44 ± 63.34
C/GA 9/1	0.69 ± 0.01	0.67 ± 0.03	26.33 ± 0.99	286.05 ± 28.96
C/GA 7/3	0.59 ± 0.05	0.68 ± 0.07	33.26 ± 5.98	272.32 ± 71.83
C/GA 5/5	0.53 ± 0.02	0.70 ± 0.01	36.49 ± 2.61	248.51 ± 9.10
C/GA 3/7	0.37 ± 0.01	0.70 ± 0.05	55.37 ± 3.74	246.63 ± 46.47
C/GA 1/9	0.63 ± 0.01	0.70 ± 0.05	21.19 ± 0.84	241.02 ± 37.75
GA	0.60 ± 0.00	0.71 ± 0.00	51.90 ± 0.57	239.91 ± 1.45
C/GB 9/1	0.63 ± 0.00	0.66 ± 0.06	41.64 ± 0.05	300.76 ± 69.22
C/GB 7/3	0.76 ± 0.02	0.65 ± 0.06	20.13 ± 1.59	316.61 ± 71.93
C/GB 5/5	0.67 ± 0.01	0.65 ± 0.02	21.93 ± 1.15	318.71 ± 24.53
C/GB 3/7	0.46 ± 0.03	0.66 ± 0.00	39.50 ± 4.64	304.06 ± 1.67
C/GB 1/9	0.47 ± 0.01	0.69 ± 0.05	32.38 ± 0.72	263.84 ± 53.76
GB	0.22 ± 0.06	0.71 ± 0.04	68.43 ± 17.21	233.29 ± 32.39

2.3.2 Effect of mucin on viscosity enhancement

Enhancement of the viscosity ($\eta_{enhance}$) of a single polymer with mucin is shown in Figure 2.4. For a single polymer, chitosan had the highest $\eta_{enhance}$ value. The high values of viscosity enhancement indicated that the system demonstrated good viscosity synergism and interactions between polymers and mucin. The increased $\eta_{enhance}$ values of the combination systems of polymer or polymer blends with mucin are shown in Figure 2.5. The $\eta_{enhance}$ values of the polymer blend of C/PVP, C/GB at a volume ratio of 5/5 and 3/7 were significantly higher than the other blends and the single polymer whereas C/GA at a volume ratio of 9/1 was significantly higher than for other blends but lower than for chitosan.

Two different types of gelatin can be produced depending on the method by which collagen was pretreated, prior to the extraction process. The alkaline processed gelatin, (type B), has the amide group of asparagine and glutamine converted to aspartate and glutamate, respectively thus rendering it negatively charged with an isoelectric point of 5.0. In contrast, the acidic processed gelatin (type A) does not significantly affect the amide groups present so it is positively charged with an isoelectric point of 8.8 [115-117]. The gelatin containing carboxylic acid (-COOH) and amino ($-NH_2$) groups has been observed for the strong interactions with chitosan which replace the macromolecular chain-water interactions [118]. The GB containing the abundant of carboxylic acid (-COOH) groups may strongly interacted with amino ($-NH_2$) group on the chitosan molecules, whereas, the GA containing a lot of amino ($-NH_2$) groups resulting to weak interaction with chitosan. Thus, the mixture of these polymer blends with mucin solution showed the viscosity

enhancement of C/GB blends that is higher than for the C/GA blends can be ascribed to the strong interaction between GB and chitosan and leading to resistance to flow and to deformation when apply the shear force than the C/GA does. However, both of the C/GA and C/GB also has an interaction with the mucin molecules [119]. For C/PVP blends system, PVP is a neutral soluble polymer and as the amide groups of PVP remain unchanged at various pH values this indicated that the interactions might be by hydrogen bonding produced between the hydroxyl or amino groups of chitosan and the amide groups of PVP.

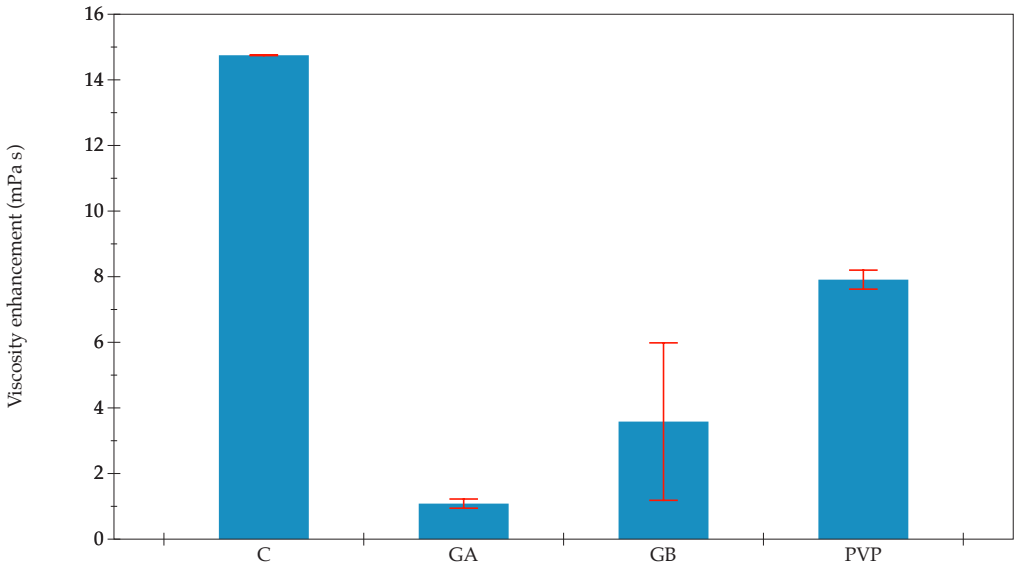


Figure 2.4 Viscosity enhancement ($\eta_{enhance}$) of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin (n = 3)

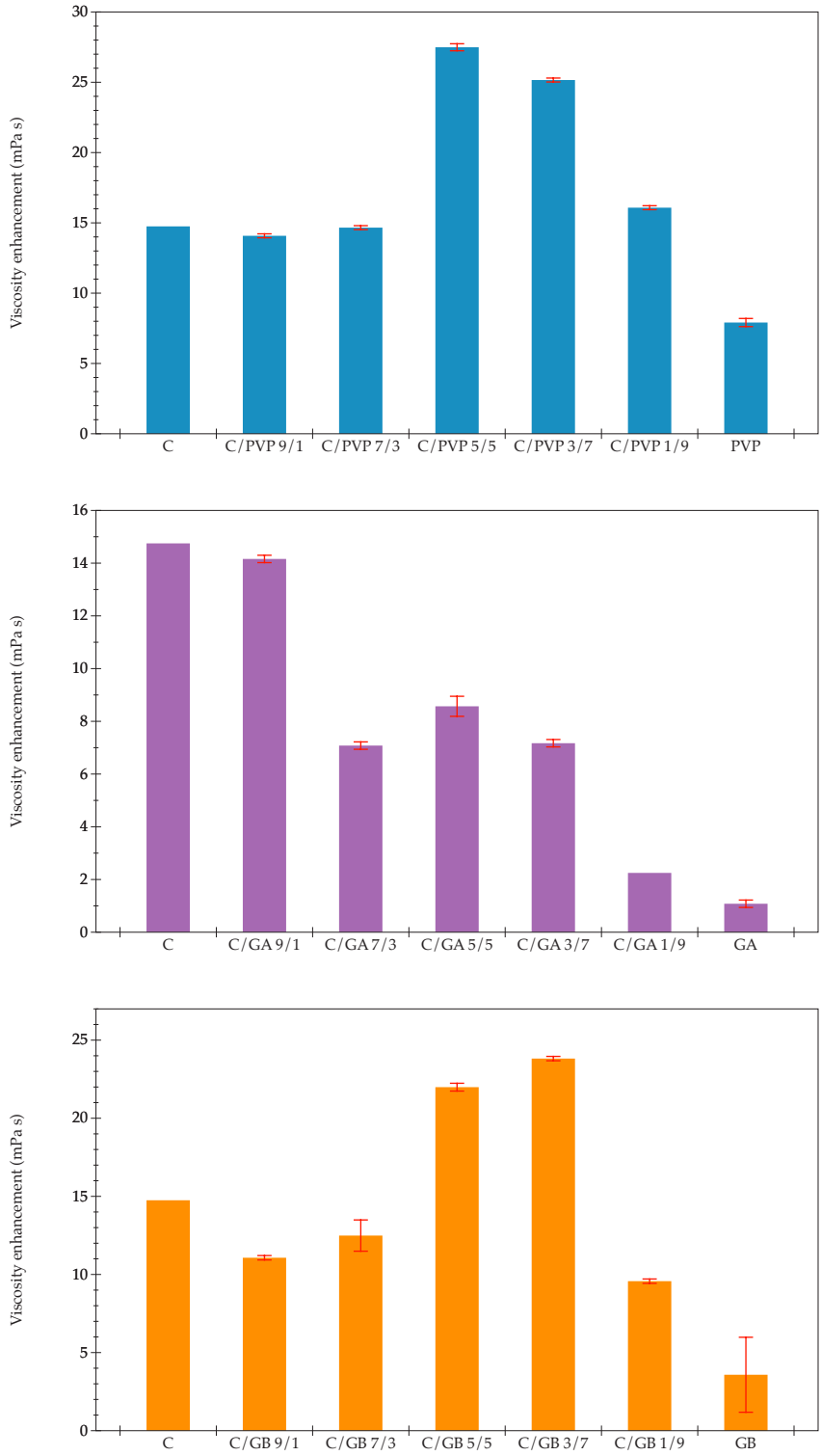


Figure 2.5 Viscosity enhancement ($\eta_{enhance}$) of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin and their blend at different volume ratio with mucin (n = 3)

2.3.3 Force of mucoadhesion

A mucoadhesive force is required between a drug device and a mucosal surface to successfully retain the device and retard the natural clearance processes. The force of mucoadhesion was calculated from the equation expressed in Eq. 2.2 using a single shear rate of 15.84 s^{-1} to compare the mucoadhesion force, because the viscosity at this shear rate could be determined for all samples. Figure 2.6 shows the force of mucoadhesion of a single polymer with mucin. According to the highest viscosity enhancement value of chitosan, the highest mucoadhesion force was obtained from chitosan with mucin. This result indicated that chitosan was a better mucoadhesive polymer than PVP, GA and GB. The mucoadhesion force of the polymer blends with mucin are shown in Figure 2.7, the mucoadhesion force in the polymer blends of C/PVP at a volume ratio of 5/5 were the highest, whereas the polymer blends of C/GB at a volume ratios of 5/5 and 3/7 were significantly higher than other blends and a single polymer. The strong interaction or high mucoadhesion force of the polymer blends was dependent on the interaction between the polymer and mucin molecules and polymer chain flexibility [120]. Chitosan and PVP are linear molecules and can more easily interpenetrate the mucin random coil than can gelatin, which is a branched macromolecule. In the combination system of C/PVP, both of chitosan and PVP can be formed a good polymer network and there might be a strong synergistic mucoadhesive interaction of the polymer blend and mucin. For C/GA and C/GB blends, the polymer network forming of C/GB is better than the polymer network of C/GA due to their interaction between chitosan and gelatin described in Section 2.3.2. Thus the polymer blends of C/GB show a synergistic

effect of mucoadhesion. The ratios of polymer blends of C/PVP and C/GB at 5/5 and 3/7 show a force of mucoadhesion higher than other blend can be described by the optimum functional groups that provide a high amount of an interaction between polymer blend and mucin. These results indicate that the chitosan blend with PVP or GB at these ratios was able to interact strongly with mucin.

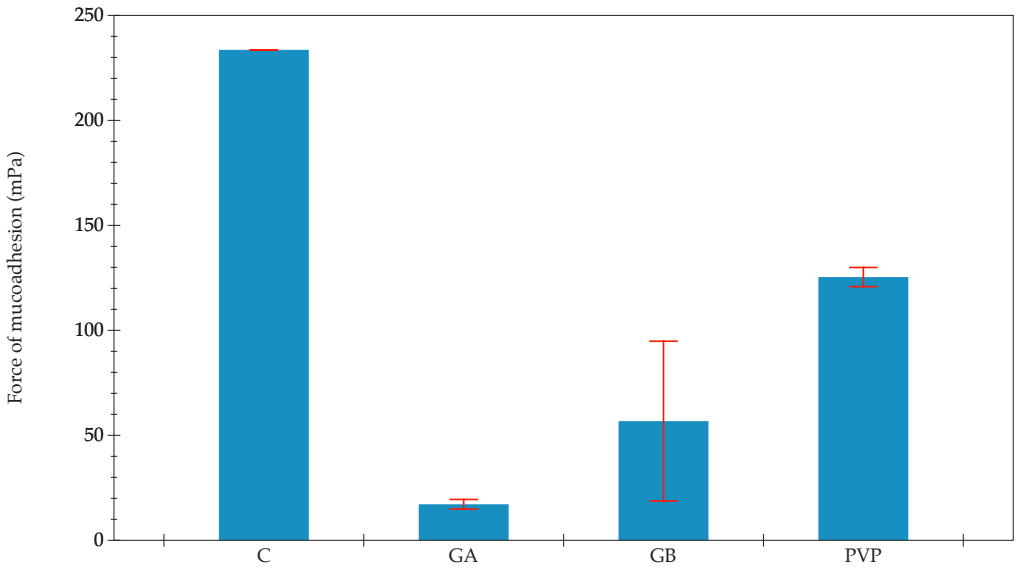


Figure 2.6 Force of mucoadhesion of the combination system of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin (n = 3)

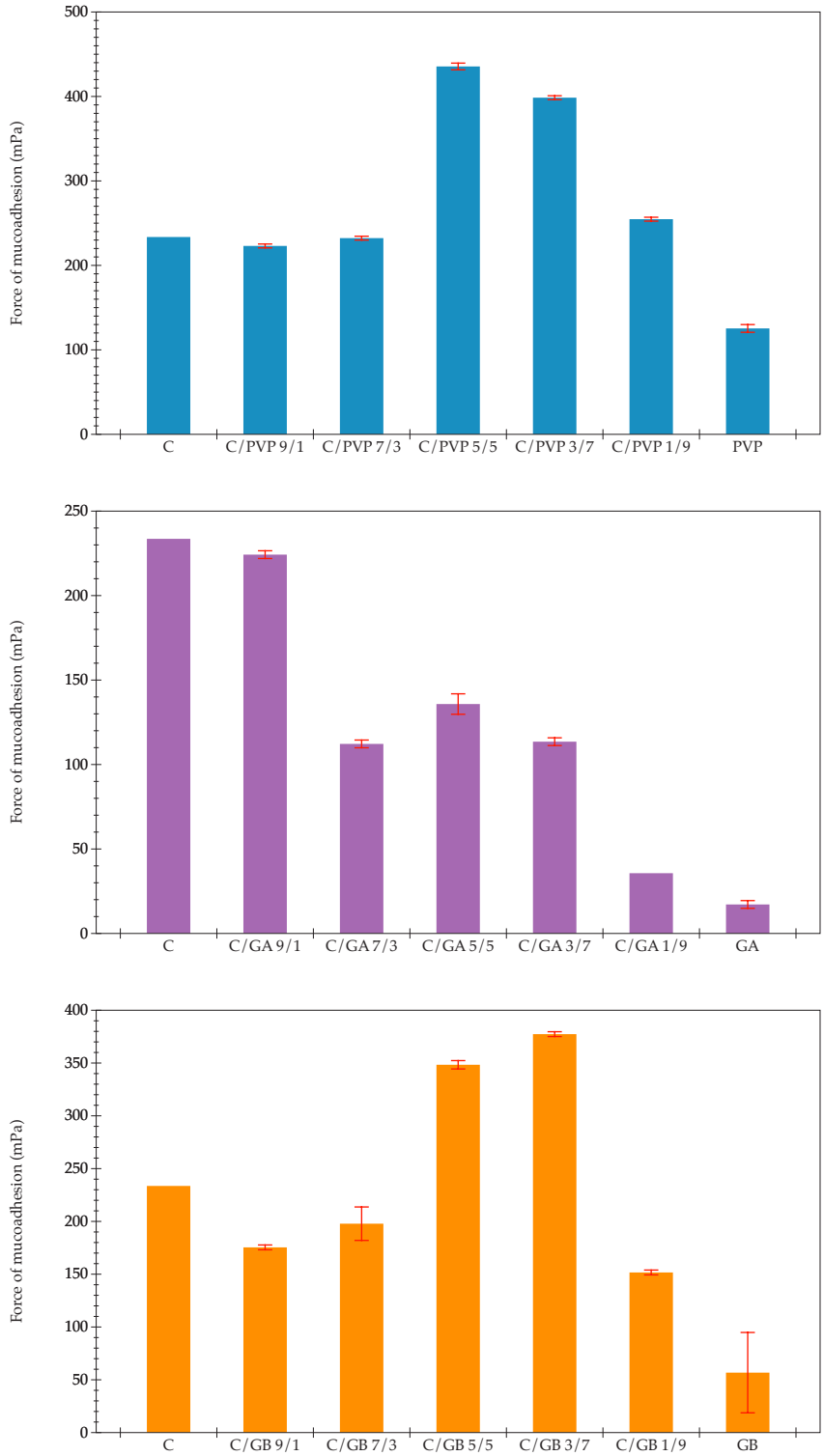


Figure 2.7 Force of mucoadhesion of the combination system of chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA) or gelatin type B (GB) with mucin and their blend at different volume ratio with mucin (n = 3)

2.4 Conclusions

In this investigation, chitosan, PVP, GA and GB all showed some interaction with mucin. Polymer blends of chitosan and PVP had a strong interaction with mucin, especially for the blend of a 5/5 volume ratio that had the highest mucoadhesive force. The mucoadhesive interaction between the polymer or polymer blends and mucin was investigated by using viscosity measurements to study the chain interpenetrations of the mucoadhesive polymers with mucin. The viscosity measurement was a simple method for evaluation of mucoadhesion but this technique unambiguously measures only the mechanical properties, that is, the resistance to flow and to deformation, of the polymers and how the resistance changes in the presence of mucin. In some cases, especially for a strong gel polymer such as carbopol show a negative values for the synergism parameters, and the positive values of the synergism parameters are only seen with weak gels [121]. Furthermore, it does not give any direct information on what happens at the interface [122], Hagerstrom and co-worker [121, 123] concluded that rheological method should not be used as a stand-alone method for determining the mucoadhesive properties of polymer-mucin mixtures so other techniques including spectroscopy study, texture analyzer etc. were performed to confirm the mucoadhesion of the polymers.

References

107. Rossi, S., Ferrari, F., Bonferoni, M., Caramella, C. (2001) Characterization of chitosan hydrochloride-mucin rheological interaction: influence of polymer concentration and polymer:mucin weight ratio. *Eur. J. Pharm. Sci.* 12, 479-85.
108. Chun, M., Kwak, B., Choi, H. (2003) Preparation of buccal patch composed of carbopol, poloxamer and hydroxypropyl methylcellulose. *Arch. Pharmacal Res.* 26, 973-8.
109. Chun, M., Cho, C., Choi, H. (2001) A novel mucoadhesive polymer prepared by template polymerization of acrylic acid in the presence of poloxamer. *J. Appl. Polym. Sci.* 79, 1525-30.
110. Kim, T., Ahn, J., Choi, H., Choi, Y., Cho, C. (2007) A Novel Mucoadhesive Polymer Film Composed of Carbopol, Poloxamer and Hydroxypropylmethylcellulose. *Arch. Pharmacal Res.* 30, 381-6.
111. Hassan, E., Gallo, J. (1990) A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. *Pharm. Res.* 7, 491-5.
112. Chan, C. K., Whitehouse, C., Gao, P., Chai, C. K. (2001) Flow induced chain alignment and disentanglement as the viscosity reduction mechanism within TLCP/HDPE blends. *Polymer* 42, 7847-56.
113. Hao, J., Chan, L., Shen, Z., Heng, P. (2005) Complexation Between PVP and Gantrez Polymer and Its Effect on Release and Bioadhesive Properties of the Composite PVP/Gantrez Films. *Pharm. Dev. Technol.* 9, 379 - 86.
114. Riley, R., Smart, J., Tsibouklis, J., Dettmar, P., Hampson, F., Davis, J., Kelly, G., Wilber, W. (2001) An investigation of mucus/polymer rheological synergism using synthesised and characterised poly(acrylic acid)s. *Int. J. Pharm.* 217, 87-100.
115. Rossi, S., Ferrari, F., Bonferoni, M., Caramella, C. (2000) Characterization of chitosan hydrochloride-mucin interaction by means of viscosimetric and turbidimetric measurements. *Eur. J. Pharm. Sci.* 10, 251-7.
116. Sriamornsak, P., Wattanakorn, N. (2008) Rheological synergy in aqueous mixtures of pectin and mucin. *Carbohydr. Polym.* 74, 474-81.
117. Young, S., Wong, M., Tabata, Y., Mikos, A. (2005) Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *J. Controlled Release* 109, 256-74.
118. Yin, Y., Yao, K., Cheng, G., Ma, J. (1999) Properties of polyelectrolyte complex films of chitosan and gelatin. *Polym. Int.* 48, 429-32.
119. Abruzzo, A., Bigucci, F., Cerchiara, T., Cruciani, F., Vitali, B., Luppi, B. (2012) Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. *Carbohydr. Polym.* 87, 581-8.
120. Khutoryanskiy, V. (2011) Advances in mucoadhesion and mucoadhesive polymers. *Macromol Biosci* 11, 748-64.
121. Hagerstrom, H., Edsman, K. (2003) Limitations of the rheological mucoadhesion method: the effect of the choice of conditions and the rheological synergism parameter. *Eur. J. Pharm. Sci.* 18, 349-57.
122. Caramella, C., Rossi, S., Bonferoni, M. A rheological approach to explain the mucoadhesive behavior of polymer hydrogels. In: Mathiowitz E, Chickering III D,

Lehr C-M, editors. Bioadhesive drug delivery systems: Fundamentals, novel approaches, and development. New York: Marcel Dekker, Inc.; 1999. p. 25 - 65.
123. Hagerstrom, H., Paulsson, M., Edsman, K. (2000) Evaluation of mucoadhesion for two polyelectrolyte gels in simulated physiological conditions using a rheological method. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* 9, 301-9.

CHAPTER 3

Evaluation of the interactions between polymer - polymer and polymer - mucin using Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

3.1 Introduction and objectives

An evaluation of mucoadhesion through a study of the intermolecular interactions between polymer and mucin is useful for describing the qualitative bond strength of their molecules. Spectroscopic investigation of a mucoadhesive polymer is used in several techniques such as x-ray photoelectron spectroscopy, ion scattering spectroscopy, infrared spectroscopy, etc. and several research articles have discussed only the interaction between the polymer blends [124-127], a single polymer with mucin [128], and surface analysis of the mucoadhesive polymer and the mucin [129, 130]. Spectroscopic studies of the interactions of the polymer blend and mucin are useful for describing the mechanisms of mucoadhesion synergism of polymer blends with mucin using a viscosity study. The use of spectroscopic techniques may provide further insights into the mechanism of mucoadhesion of molecules at the molecular level.

A study of the interaction of polymer – polymer and polymer – mucin was evaluated using FT-IR with the diffuse reflectance mode using an abrasive pad because chitosan film is difficult to grind to a powder and prepared as a KBr disc. The diffuse reflectance is a good sampling tool for powdered or crystalline materials in the mid-IR and NIR spectral ranges which the main advantages are an informative and both qualitative and quantitative within a seconds of measurement [131]. Diffuse reflectance relies upon the focused projection of the spectrometer beam into the sample where it is reflected, scattered and transmitted through the sample material. The back reflected, diffusely scattered light (some of which is absorbed by the sample) is then collected by the accessory and directed to the detector optics [132] as shown in Figure 3.1.

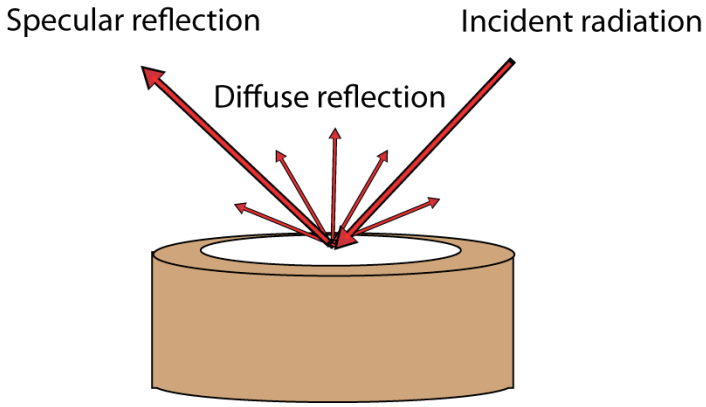


Figure 3.1 The projection of the spectrometer beam into the sample of diffuse reflectance mode

The raw diffuse reflectance spectra will appear different from its transmission equivalent (stronger than expected absorption from weak IR bands), thus, the theory to correlation of reflection and transmission data to the scattering and absorption properties of a material will be used. The Kubelka – Munk conversion can be applied to a diffuse reflectance spectrum to compensate for these differences. This conversion is available in most FTIR software packages. The Kubelka – Munk equation is expressed as Equation 1:

$$f(R) = \frac{(1-R)^2}{2R} = \frac{k}{s} \quad (1)$$

where R is the absolute reflectance of the sampled layer, K is the molar absorption coefficient and s is the scattering coefficient. A small amount of the sample can be collected by abrasion on a diamond or silicon carbide (SiC) abrasion disk as shown in Figure 3.2 and analyzed immediately using the diffuse reflectance accessory [132].

The aim of this study was to investigate the interaction between polymer – polymer of the polymer blends of C/PVP, C/GA, C/GB at various volume ratios and polymer – mucin of C/mucin, PVP/mucin, GA/mucin and GB/mucin.

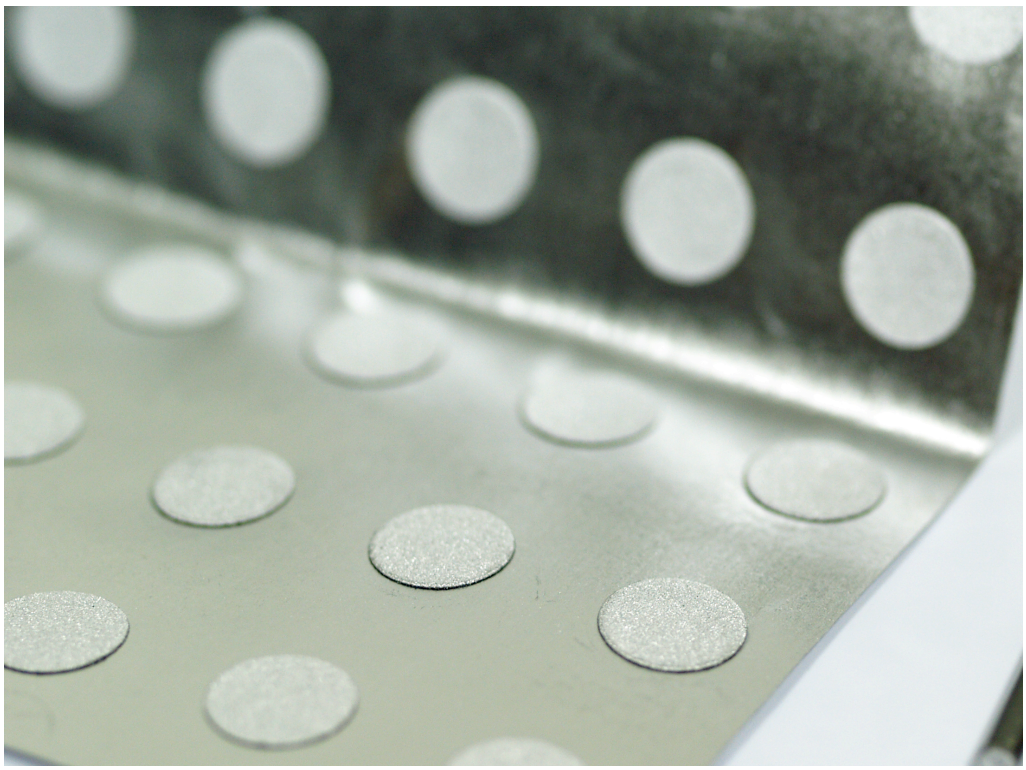


Figure 3.2 Abrasive disc for DRIFTS measurement

3.2 Experimental methods

3.2.1 Materials

All materials and chemical reagents were use as same as described in section 2.2.1.

3.2.2 Sample preparations

All polymer stock solutions and polymer blends were prepared as same as described in section 2.2.2. For DRIFTS study the mucin solutions were prepared at the concentrations of 2% w/v by dispersing the dried mucin (2.0 g) in water (100 mL). The mixture of polymer and mucin were prepared by mixing equal volumes of a stock solution of polymer or polymer blend with a stock solution of mucin. All samples were cast on a polystyrene disc and dried in an oven at 60 °C for 8 h and kept in desiccators before measurement.

3.2.3 DRIFTS measurement

The study of interactions between mucin and polymer or mucin and polymer blend were performed on a Spectrum One FTIR spectrometer (Perkin-Elmer, Massachusetts, USA), using the diffuse reflectance accessory with a supplied sample cup holder. All samples were prepared using an abrasive disc to abrasion on a surface of polymer film and measured using DRIFTS technique. In the attempt to obtain an acceptable signal/noise ratio, the spectra were recorded from 4400 to 450 cm^{-1} by averaging 64 scans at 4 cm^{-1} of resolution. All reflectance spectra were converted to the Kubelka – Munk (KM) unit and analyzed using the PerkinElmer Spectrum for Windows version 5.02 software package.

3.3 Results and discussion

3.3.1 DRIFTS of single polymer characterization

Chitosan, (1-4)-2-amino-2-deoxy-β-D-glucan, contains amino and hydroxyl groups on its backbone, and both can be served as a proton donors/proton acceptors in hydrogen bonding interactions between chitosan molecules or between chitosan and other polymers. The infrared spectrum of chitosan shows an amino band (NH bending) at 1517 cm^{-1} and a small carbonyl (C=O) stretching peak at 1615 cm^{-1} according to some residue of an acetyl group in chitosan. In addition, chitosan displays a broad peak at around 3400 cm^{-1} resulting from the N-H and O-H vibrations, as shown in Figure 3.3.

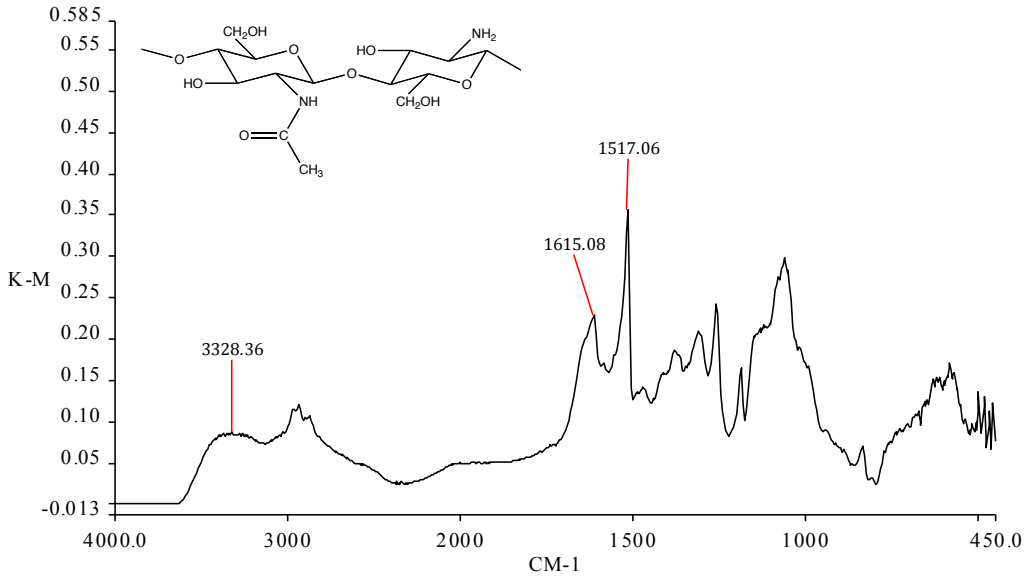


Figure 3.3 DRIFTS spectrum of chitosan

The molecule of PVP contains a proton acceptor group of an amide carbonyl with a strong characteristic peak of an amide carbonyl (C=O) at 1698 cm^{-1} and a C-N stretching peak at 1288 cm^{-1} (Figure 3.4). PVP is a very hydrophilic polymer with a large amount of trace moisture in the molecule that results in an infrared spectrum of PVP with a broad peak of O-H stretching from moisture in the molecule at around 3400 – 3100 cm^{-1}.

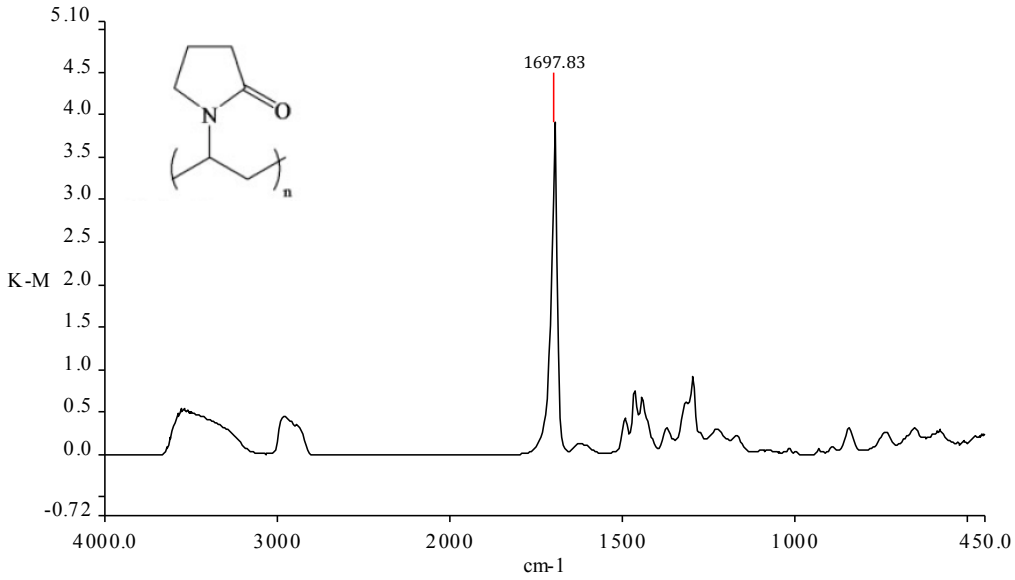


Figure 3.4 DRIFTS spectrum of poly(vinylpyrrolidone) (PVP)

Figures 3.5 and 3.6 show the infrared spectra of GA and GB, respectively. Gelatin may be defined as a protein made soluble by hydrolysis of collagen derived from the skin, the white connective tissue and bones of animals. The amino acid composition of gelatin has a high contents of glycine, proline, hydroxyproline and alanine [133]. Gelatin molecules contain a proton donor at their

amine and hydroxyl groups with a broad peak at 3400 cm^{-1} being attributed to N-H and O-H stretching, the peaks at 1697 - 1702 and 1566 - 1570 cm^{-1} are assigned to carbonyl and amide band, respectively [134]. Furthermore, the two different types of gelatin show similar infrared spectra.

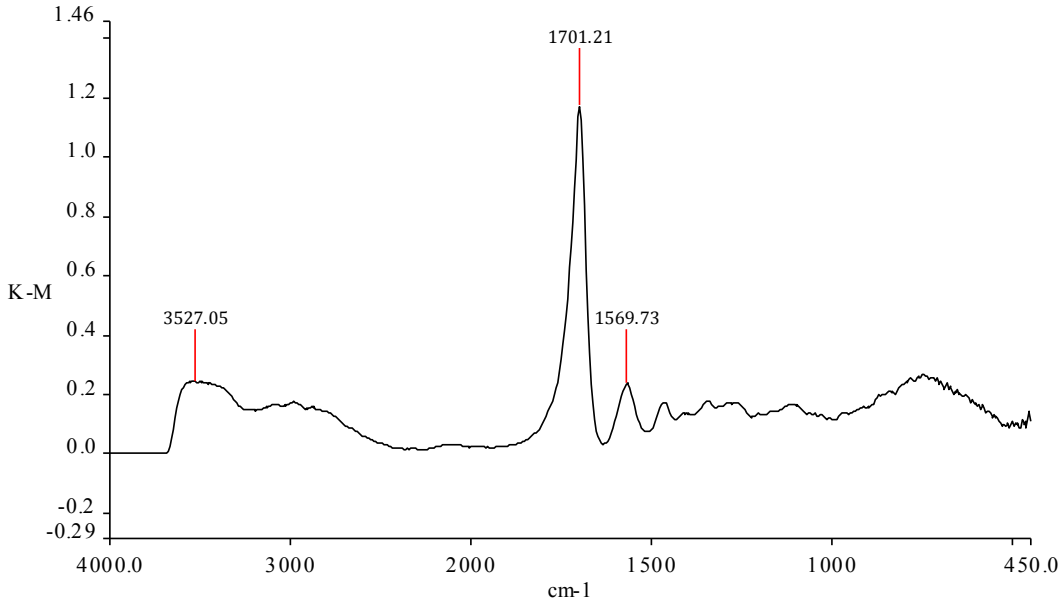


Figure 3.5 DRIFTS spectrum of gelatin type A (GA)

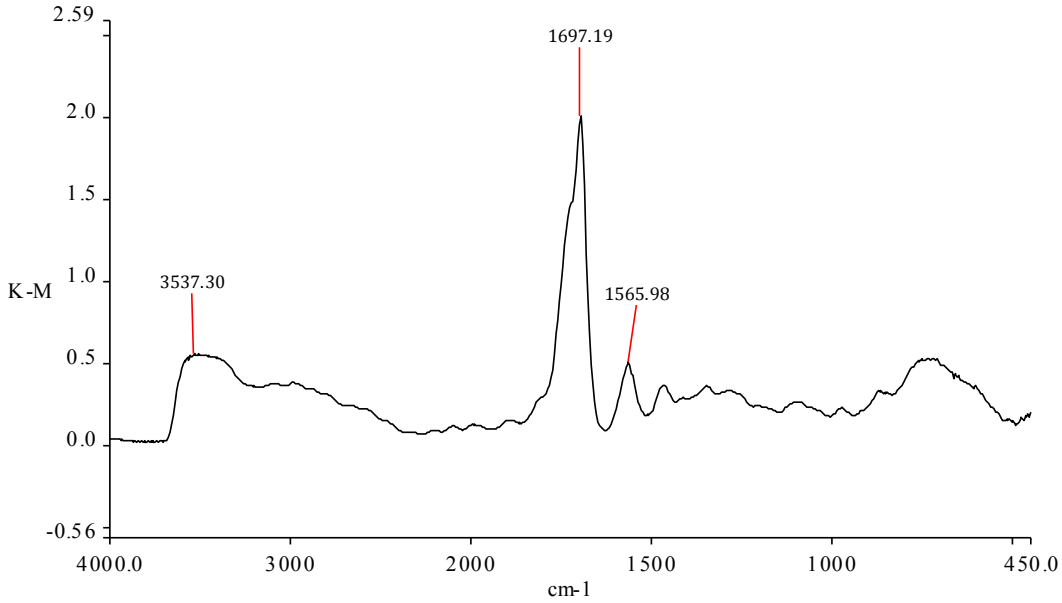


Figure 3.6 DRIFTS spectrum of gelatin type B (GB)

Mucin is characterized by dense O-linked glycans that arise from *N*-acetyl galactosamine conjugated to serine or threonine residues on the amino acid backbone [135]. The mucin molecules contains several amino and carboxyl groups, depicted as a proton donor and proton acceptor group, respectively. Sialic acid was found to be an integral part of many mucin molecules [136]. A mucin spectrum is shown in Figure 3.7 with the broad band at 3400-3100 cm^{-1} masking many stretching vibrations of an amino N-H and amide N-H and the primary amine deformation (expected 1600 cm^{-1}) is masked by the stronger carbonyl stretching peak at 1692 cm^{-1} attributable to carboxylic groups from the sialic acid side chain, and an amide band at 1562 cm^{-1} [128]. The narrow bands at 1076 and 1038 cm^{-1} are assigned to the C-N stretching vibrations for the primary and secondary α-carbons of the primary amines.

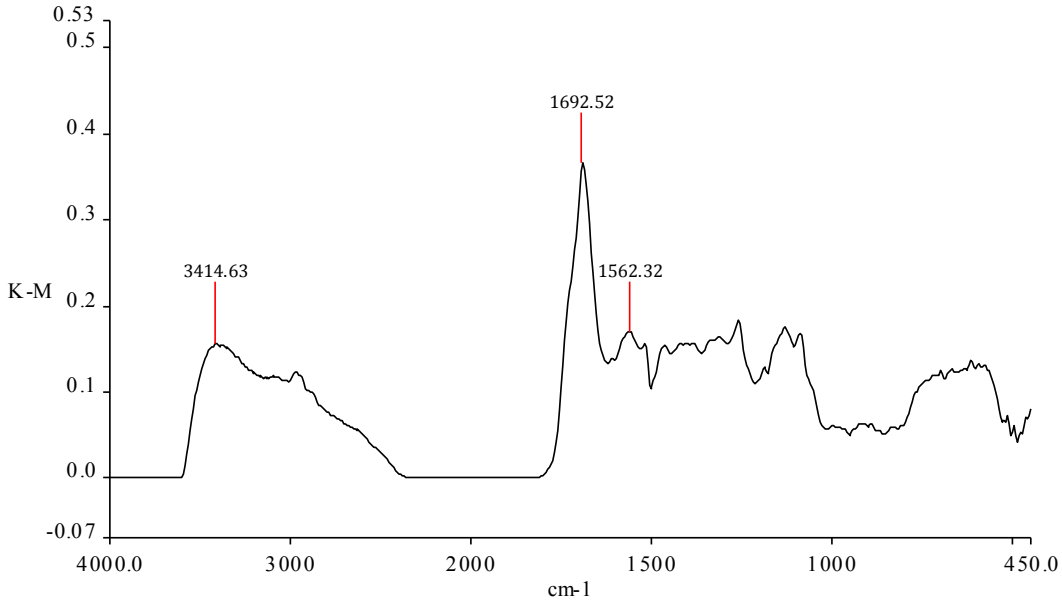


Figure 3.7 DRIFTS spectrum of mucin

3.3.2 DRIFTS of polymer blends characterization

The polymer interactions of C/PVP, C/GA, and C/GB were observed as a peak shift of C=O stretching peak in the polymer blends. According to the molecular structure of chitosan and PVP, the hydrogen bonding interaction between chitosan and PVP could be formed at both hydroxyl and amine functional groups of chitosan and with the amide groups of PVP. Chitosan also forms hydrogen bonding interactions with gelatin between hydroxyl and amine groups of chitosan with amide groups of gelatin.

Figure 3.8 shows the spectra of a polymer blend of C/PVP with various ratios. Although the peak corresponding to the N-H stretching of chitosan was interfered with by the broad band of O-H stretching, the C=O stretching (carbonyl

peak) of polymer blends of C/PVP were observed. The carbonyl peak is mostly shifted to the lower wavenumber of 1674-1694 cm^{-1} for C/PVP blends to indicate an interaction between PVP and chitosan. This result indicates that the amide carbonyl of PVP was involved in hydrogen bonding with the amine or hydroxyl group of chitosan and shifts the carbonyl peak position of PVP to a lower wavenumber. According to the previous work, the interaction between chitosan and PVP also observed between amine or hydroxyl group of chitosan and carbonyl group of PVP and hydrogen bonding interaction prefer to form between the proton acceptor (C=O) of PVP and the proton donor group (C_6-OH) group of chitosan [137]. Furthermore, several composition ratios of C/PVP show a similar in their spectra of polymer blends.

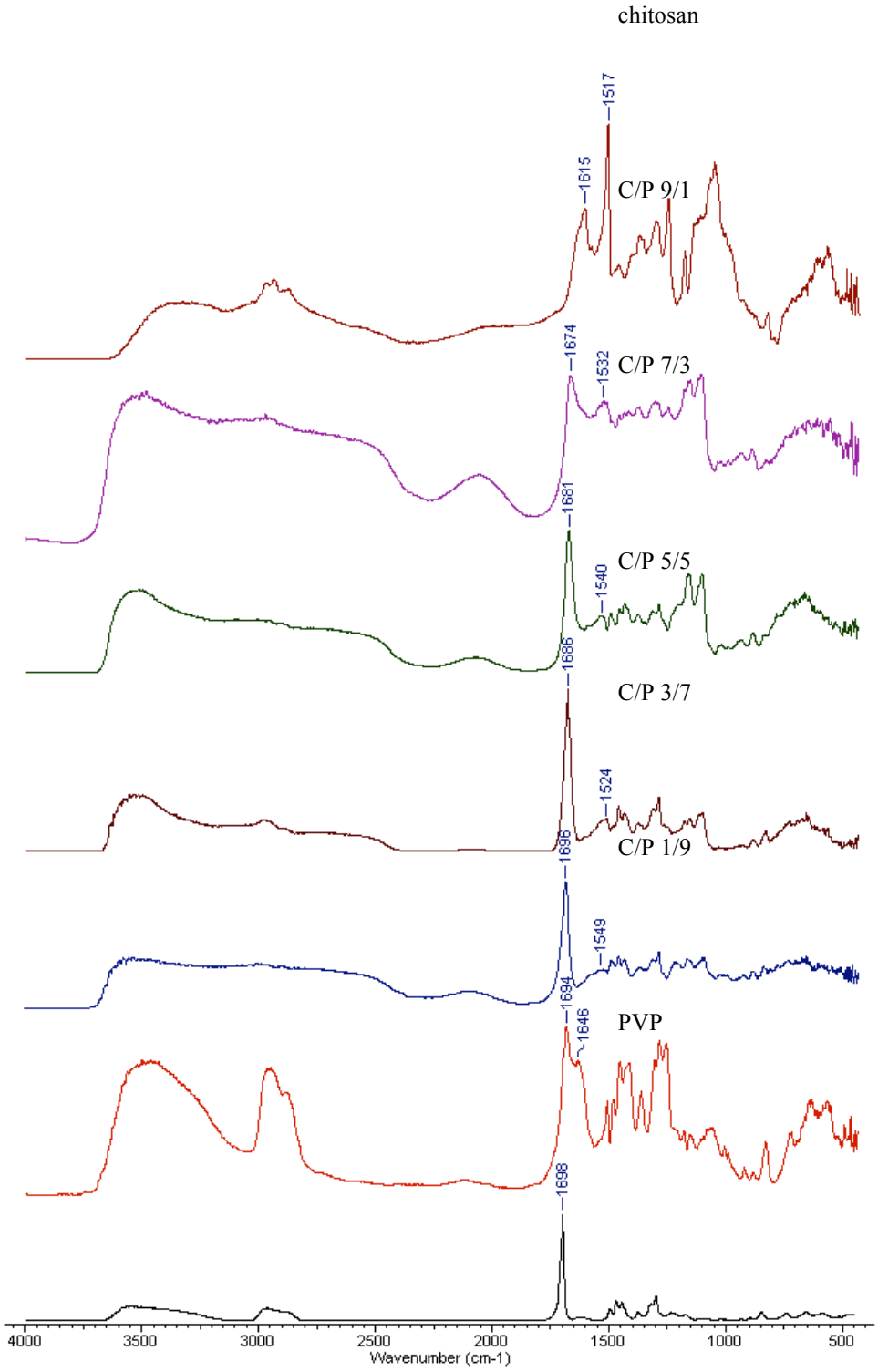


Figure 3.8 DRIFTS spectra of polymer blends of chitosan (C) and poly(vinyl-pyrrolidone) (PVP) at various volume ratios

The DRIFTS spectra of C/GA, and C/GB are shown in Figures 3.9, and 3.10, respectively. The carbonyl peaks in the spectra of the polymer blends of C/GA, and C/GB were observed with slightly shifted to a lower wavenumber due to the hydrogen bonding interaction between the amide of gelatin with an amine or hydroxyl group of chitosan. The carbonyl peak shift in the polymer blend of C/GB was shifted more than that for the polymer blends of C/GA. This indicated a higher interaction of C/GB than C/GA. Different composition ratios of chitosan/gelatin also show the similar spectra.

The carbonyl stretching observed from C/PVP blends showed the highest shifts and this could be due to a higher interaction between chitosan and PVP compared to that between the chitosan and gelatin. These molecular interaction results obtained by the DRIFTS studies substantiate the view that the polymer blends of C/PVP show higher interactions than the C/GA and C/GB. And the rank order of polymer interaction is C/PVP > C/GB > C/GA blends.

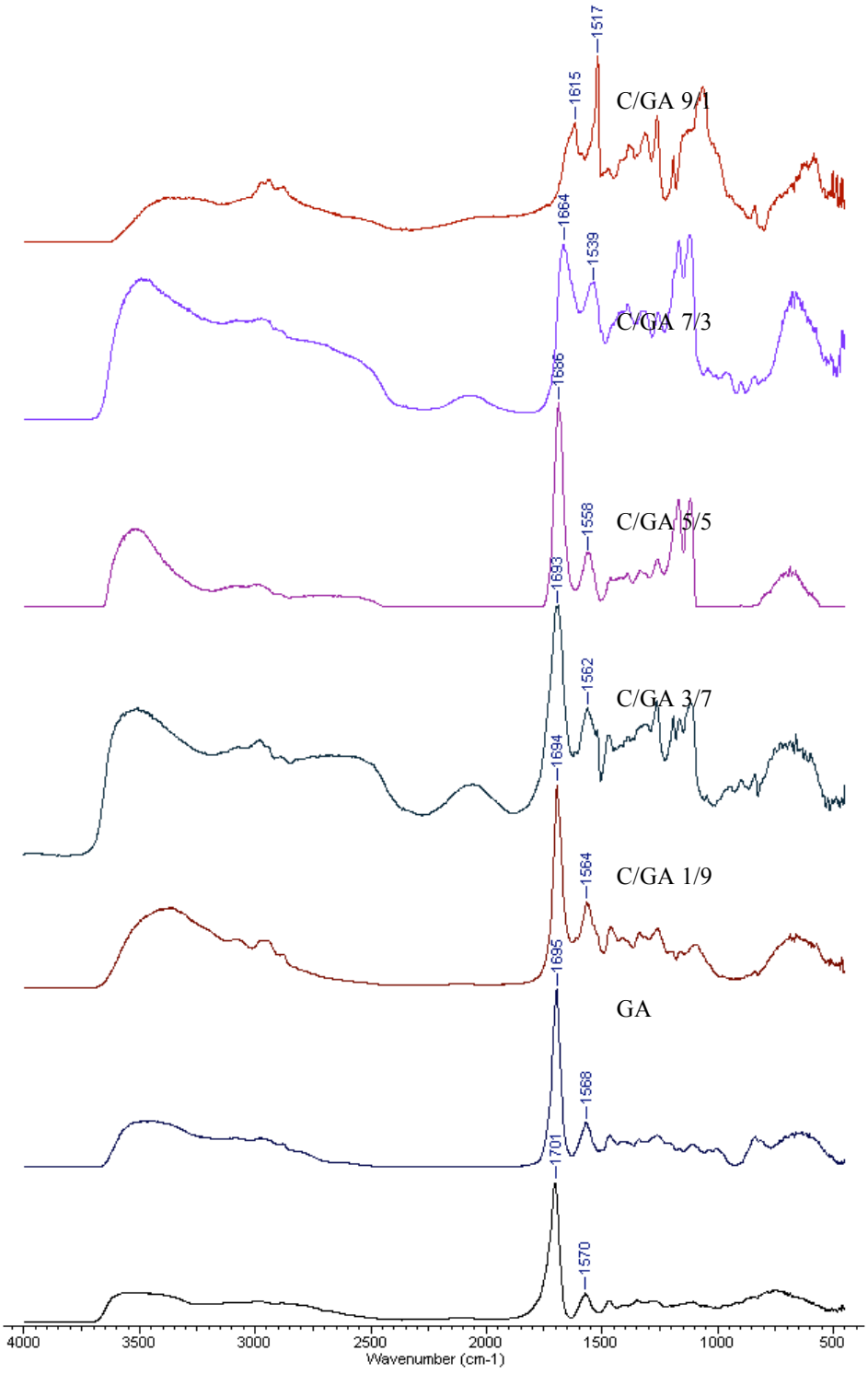


Figure 3.9 DRIFTS spectra of polymer blends of chitosan (C) and gelatin type A (GA) at various volume ratios

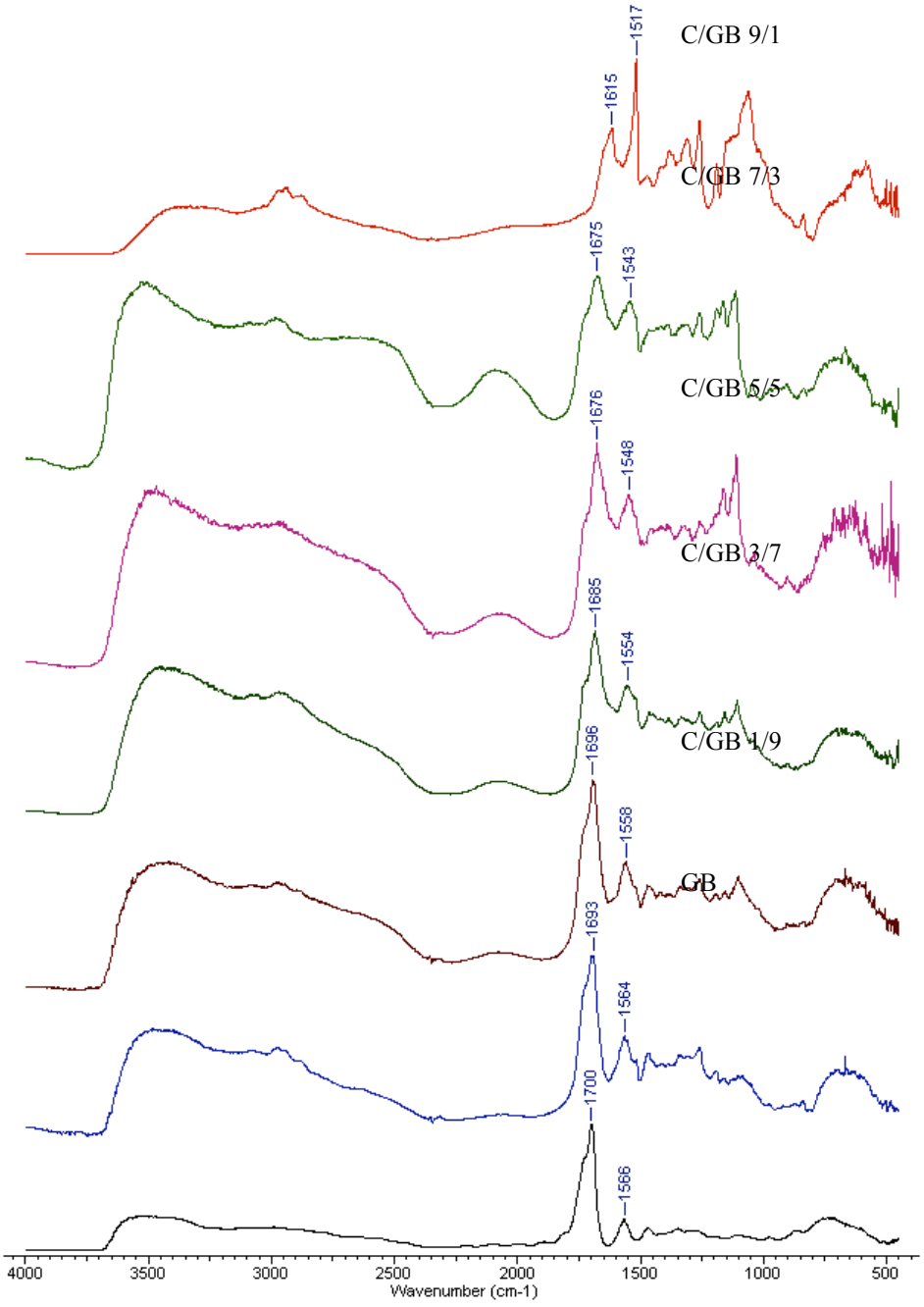


Figure 3.10 DRIFTS spectra of polymer blends of chitosan (C) and gelatin type B (GB) at various volume ratios

3.3.3 DRIFTS of the combination systems of polymer with mucin characterization

Polymers/mucin systems of C/mucin, PVP/mucin, GA/mucin, and GB/mucin were investigated for any hydrogen bonding interactions. The interaction between polymers and mucin was also observed in the combination system of polymer and mucin.

DRIFTS spectra of the combination system of C/mucin and PVP/mucin are shown in Figures 3.11 and 3.12, respectively. The peak position of the amine group of all C/mucin and PVP/mucin combination systems could not be observed due to the broad band of O-H stretching. However, the carbonyl peak of all C/mucin combination system was observed to shift to a lower wavenumber from the 1692 cm^{-1} of sialic acid from mucin to 1690 – 1683 cm^{-1}. These results indicated that hydrogen bonding interactions were occurring between the proton acceptor, C=O, of sialic acid with the amine or hydroxyl groups of chitosan. All PVP/mucin combination systems showed a carbonyl peak with a slightly shift to a lower wavenumber from 1698 cm^{-1} (assigned to C=O of PVP) to 1696 – 1692 cm^{-1}. This result indicated that the C=O of PVP could be formed hydrogen bonding with the amine groups of mucin. The carbonyl peak shift of the C/mucin system seemed to be higher than PVP/mucin system that are probably due to the high proton donor group, amine and hydroxyl groups of chitosan, thus, chitosan having more hydrogen bonding interaction sites with mucin.

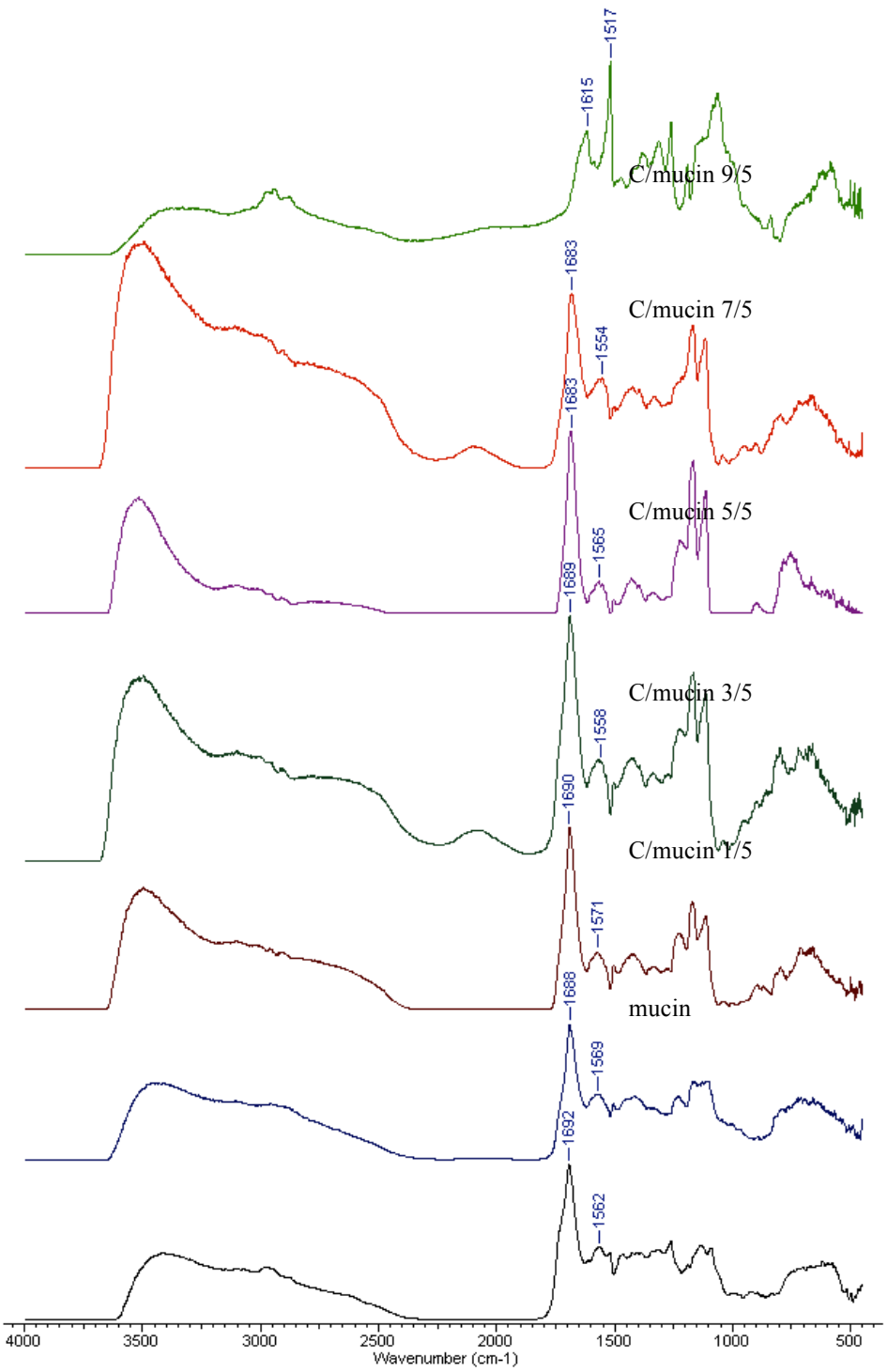


Figure 3.11 DRIFTS spectra of combination system of chitosan (C) with mucin at various volume ratios

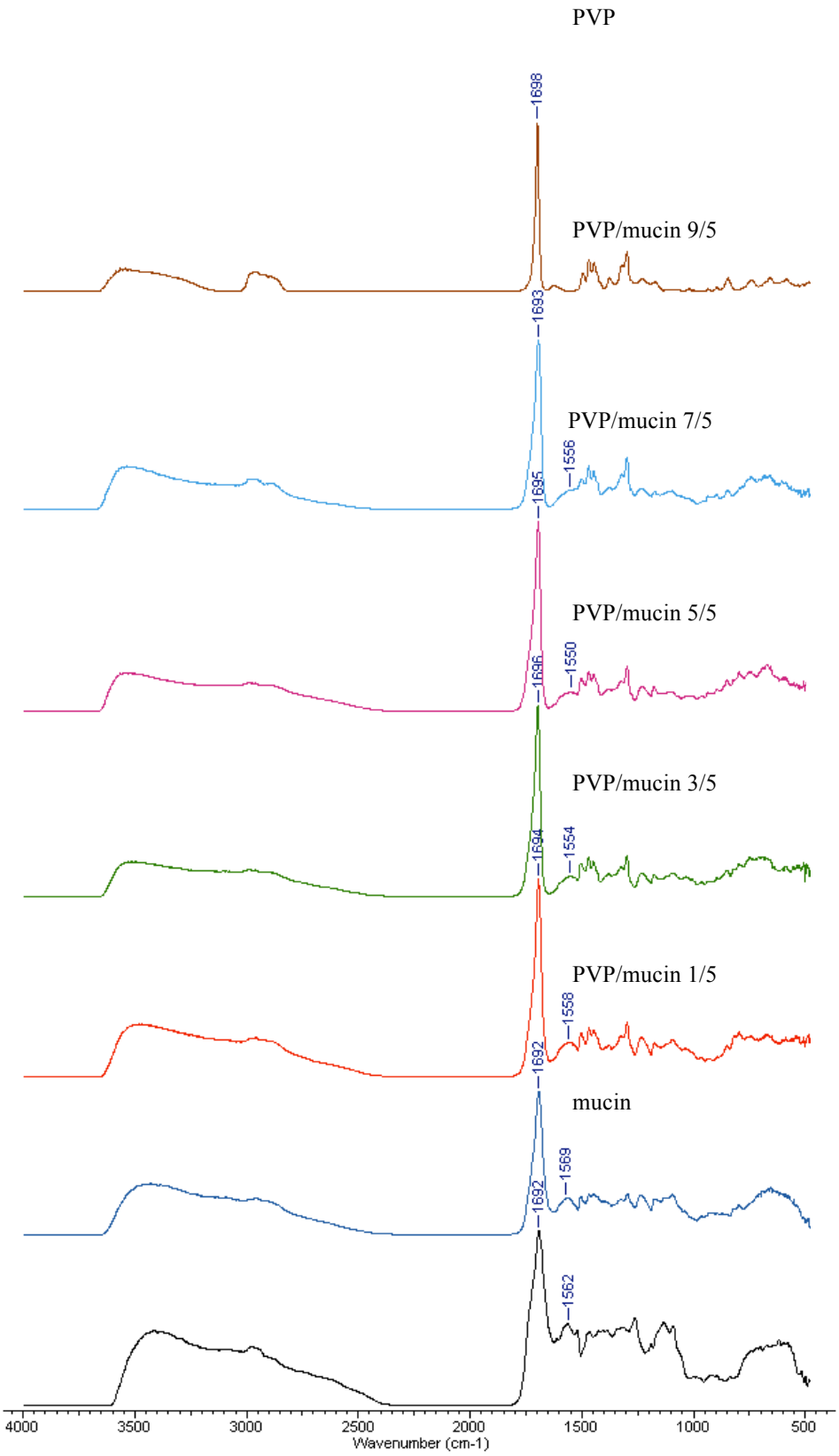


Figure 3.12 DRIFTS spectra of combination system of poly(vinylpyrrolidone) (PVP) with mucin at various volume ratios

Figures 3.13 and 3.14 show the DRIFTS spectra of the combination system of GA/mucin and GB/mucin, respectively. The carbonyl peak of all gelatin/mucin systems has a slightly shift to a lower wavenumber. Gelatin contains amino and amide groups on the molecules that play an important role by becoming involved in the hydrogen bonding interaction with the sialic acid of mucin. Furthermore, gelatin with a high content of primary amino groups shows good characteristics for producing gastric mucoadhesive properties *in vitro* [138].

The carbonyl peak position of all polymer/mucin combination systems showed the highest peak shift with C/mucin. The interactions observed from the carbonyl peak shift of the gelatin/mucin systems seems to be similar for GA and GB, however, the peak shift of the PVP/mucin combination systems are higher than the gelatin/mucin systems. This result indicated that chitosan has a stronger interaction with mucin than the other polymers. The rank order of interaction with mucin is chitosan > PVP > GA or GB.

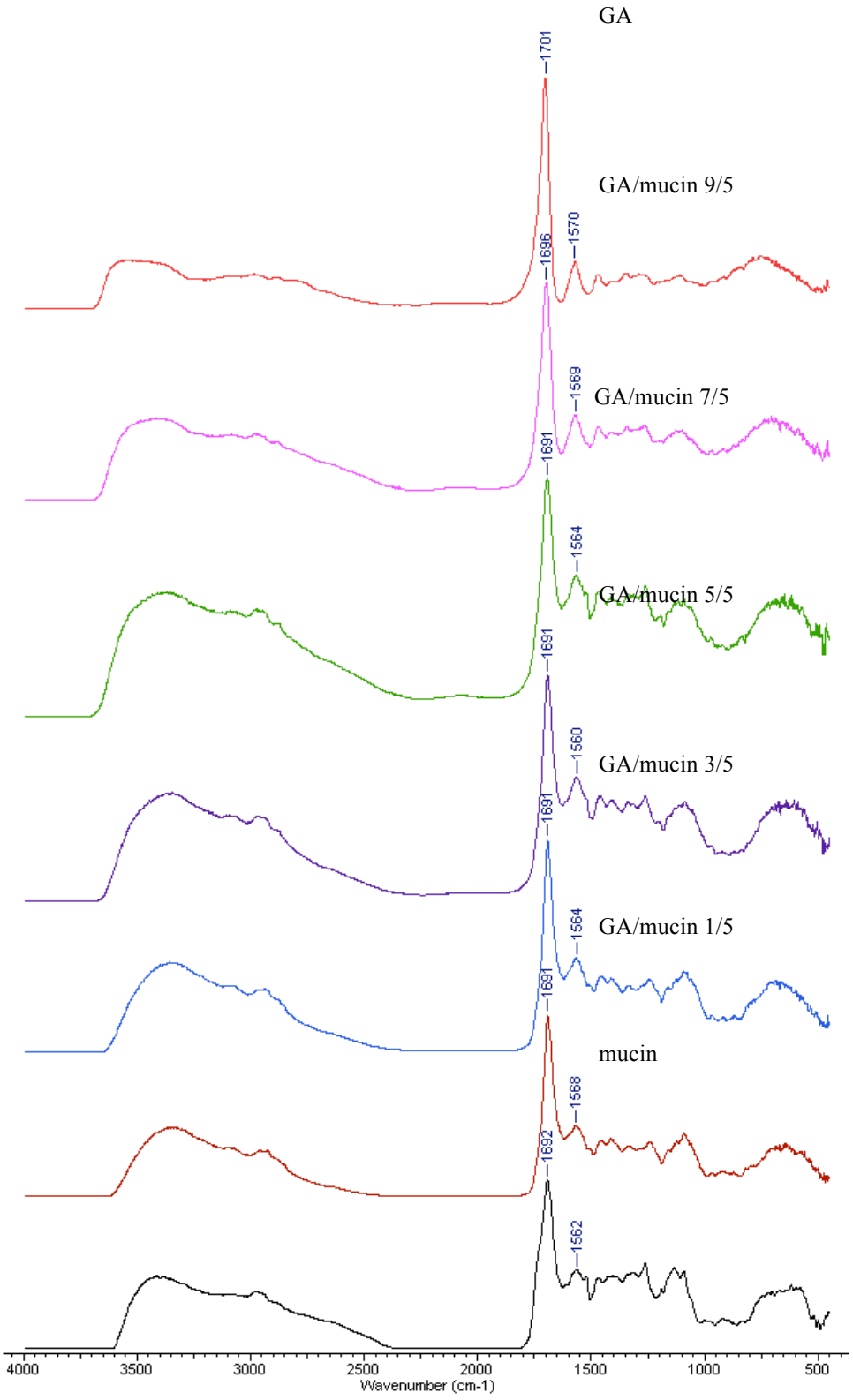


Figure 3.13 DRIFTS spectra of combination system of gelatin type A (GA) with mucin at various volume ratios

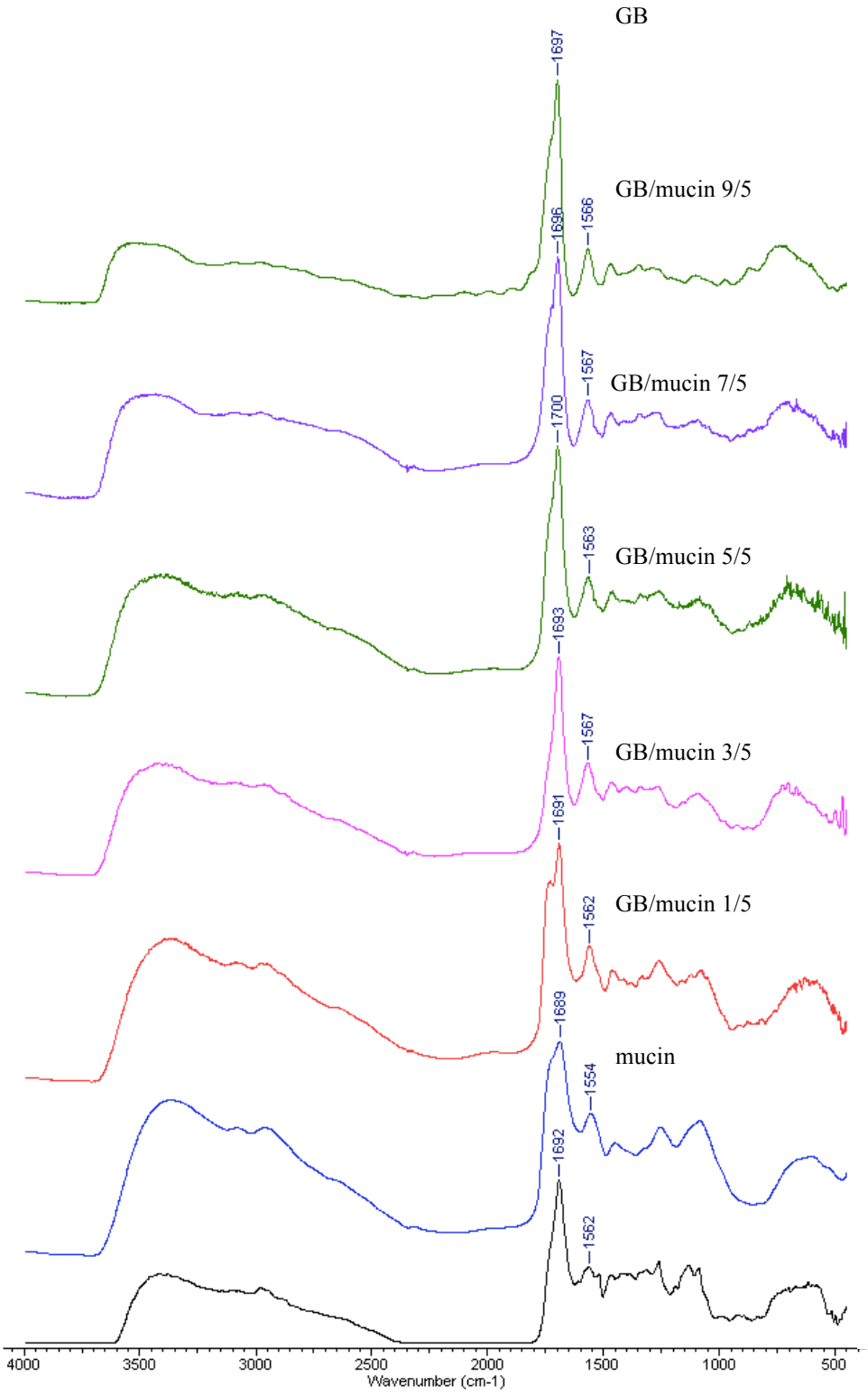


Figure 3.14 DRIFTS spectra of combination system of gelatin type B (GB) with mucin at various volume ratios

The DRIFTS spectra of all polymer/polymer and polymer/mucin combination systems show an intermolecular interaction via hydrogen bonding. The C/PVP blends show strong interactions between chitosan and PVP due to the high carbonyl peak shift and each polymer also has high interaction with mucin as depicted in Figure 3.15(A). Although, GA and GB have a weak interaction with mucin due to there being observed only a slight shift of the carbonyl peak in gelatin/mucin combination systems as shown in Figure 3.15(B), GB has a greater interaction with chitosan than GA due to a higher carbonyl peak shift in the C/GB blends. Furthermore, the carbonyl stretching of mixtures of the polymer blend of C/PVP has a larger shift than the C/GA and the C/GB.

Moreover the spectra of ternary mixture of polymer blend of C/PVP, C/GA and C/GB at various volume ratios with mucin also investigated. Polymer blends of C/PVP with mucin at volume ratio of C/PVP/mucin of 9/1/5, 7/3/5, 5/5/5, 3/7/5 and 1/9/5 are shown in Figure 3.16, 3.17, 3.18, 3.19 and 3.20, respectively. The possibility of hydrogen bonding interaction in ternary mixture can be formed between (1) carbonyl of PVP with amine or hydroxyl groups of chitosan, (2) carbonyl of PVP with amine groups of mucin or (3) carboxylic groups of mucin with amine or hydroxyl groups of chitosan. Thus, the complication of hydrogen bonding interaction in ternary mixture of C/PVP/mucin cannot be identified from the DRIFTS spectra of ternary mixture and the peak of N-H stretching cannot be observed due to the broad of O-H stretching peak. Most of ternary mixtures of C/PVP/mucin were observed the carbonyl peak shifted to the lower wavenumber of about 1695 cm^{-1} when compare to carbonyl peak observed from PVP. These carbonyl peak positions were observed at higher wavenumber than that observed from C/PVP and C/mucin and can be

suggested by the interaction of three components system of C/PVP/mucin may deviate from the two components system due to the interfering of hydrogen bonding interaction from the third component. The DRIFTS spectra of ternary mixture of C/PVP/mucin can describe the hydrogen bonding interactions in the three components system.

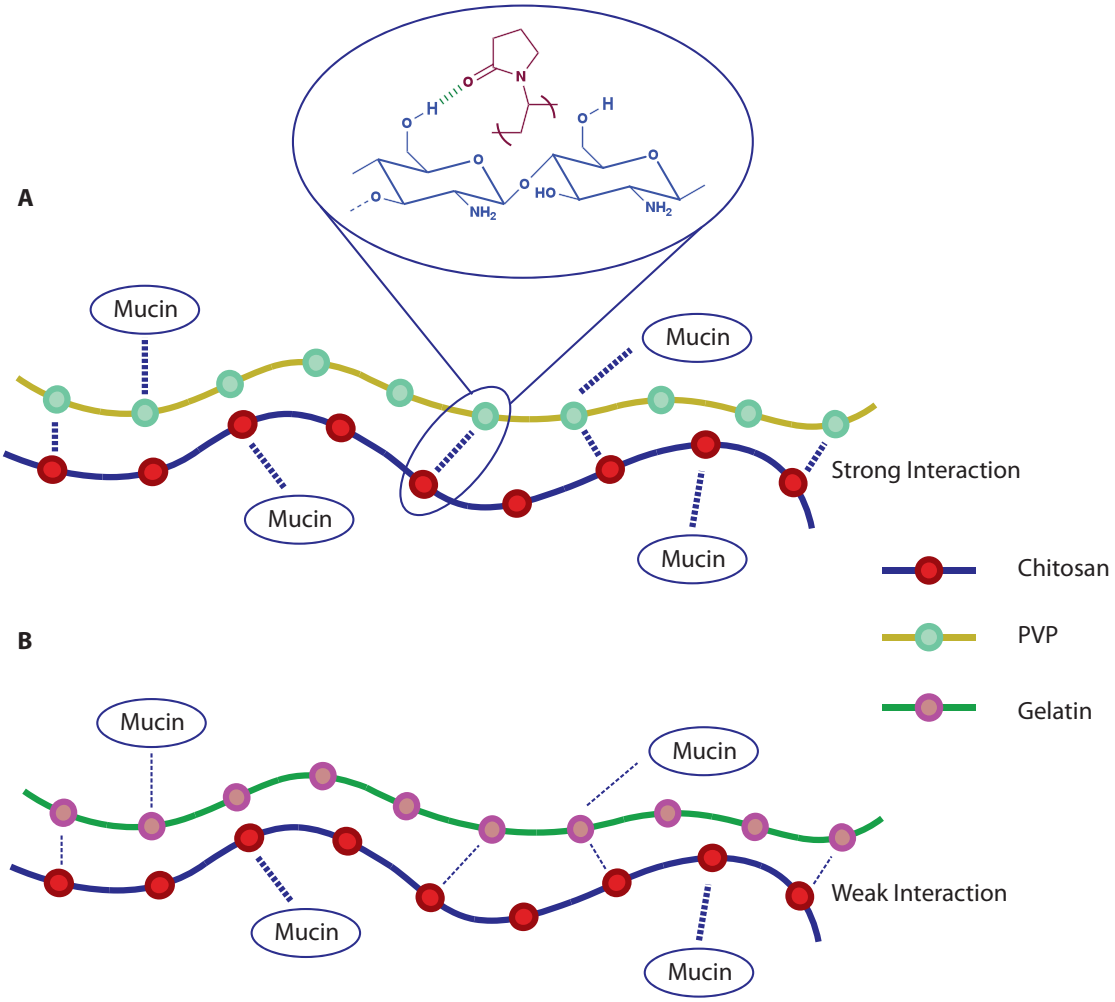


Figure 3.15 Schematic interaction diagram of polymer blends of chitosan and poly(vinylpyrrolidone) (PVP) with mucin (A) and polymer blends of chitosan and gelatin with mucin (B)

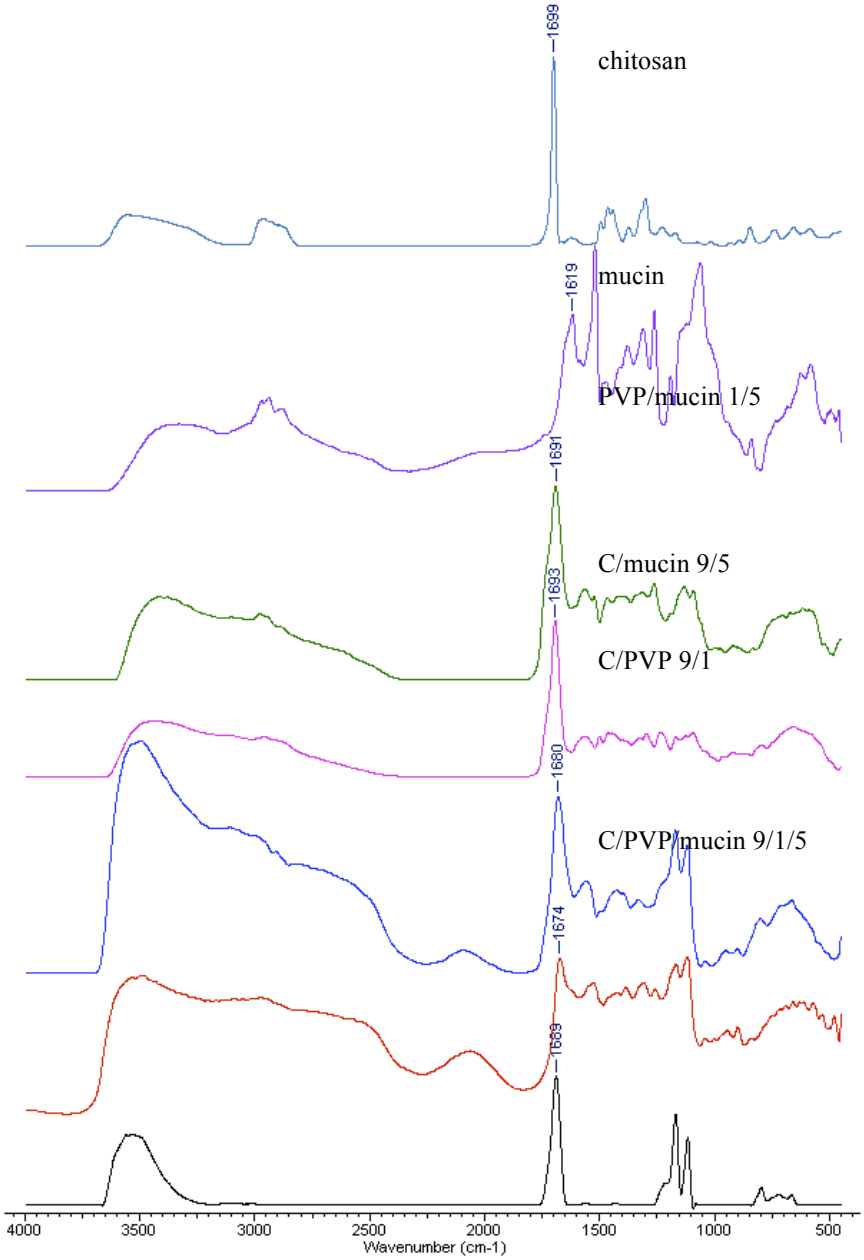


Figure 3.16 DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (9/1), binary mixtures of C/mucin (9/5) and PVP/mucin (1/5) and ternary mixture of C/PVP/mucin at 9/1/5 volume ratio

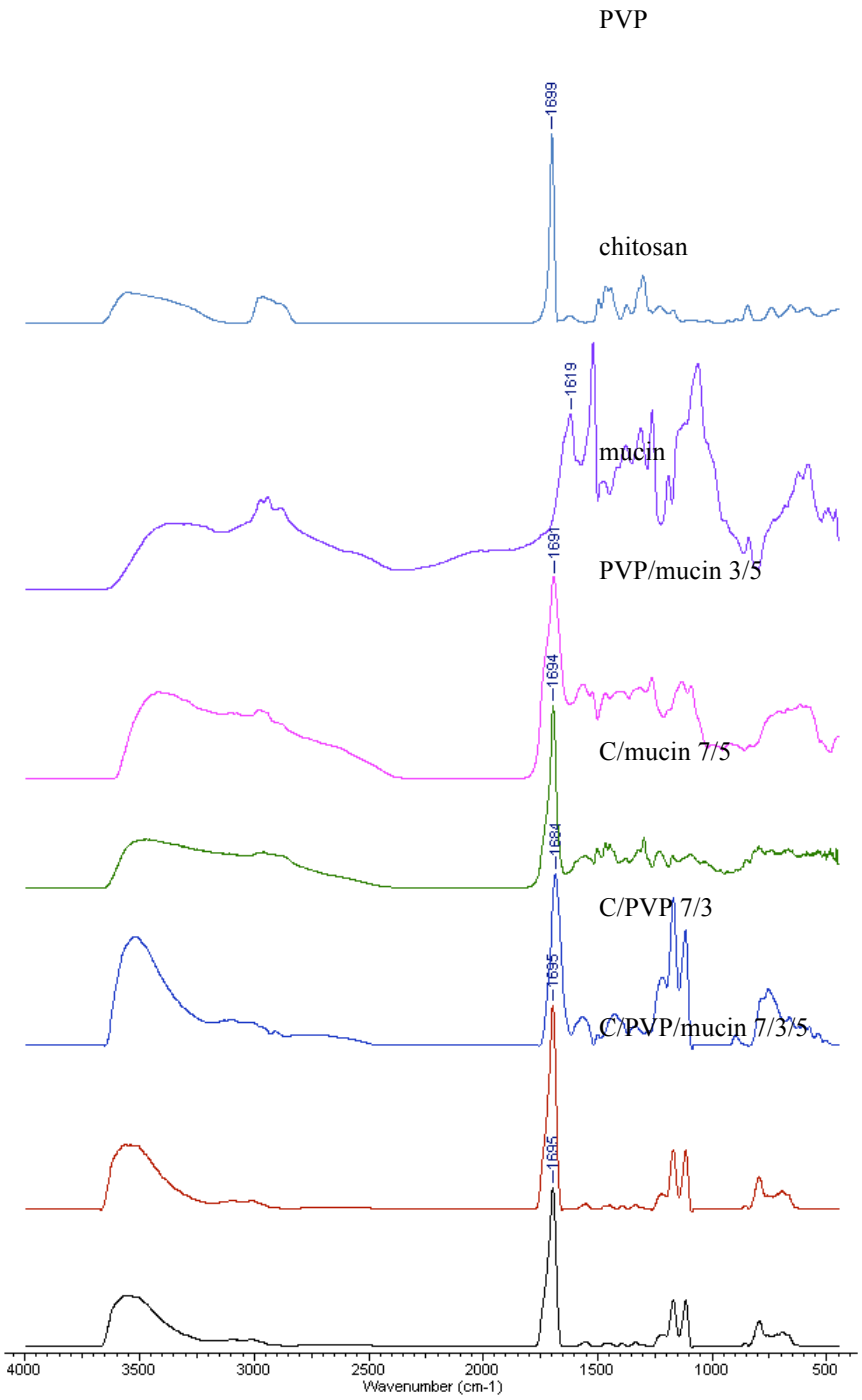


Figure 3.17 DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (7/3), binary mixtures of C/mucin (7/5) and PVP/mucin (3/5) and ternary mixture of C/PVP/mucin at 7/3/5 volume ratio

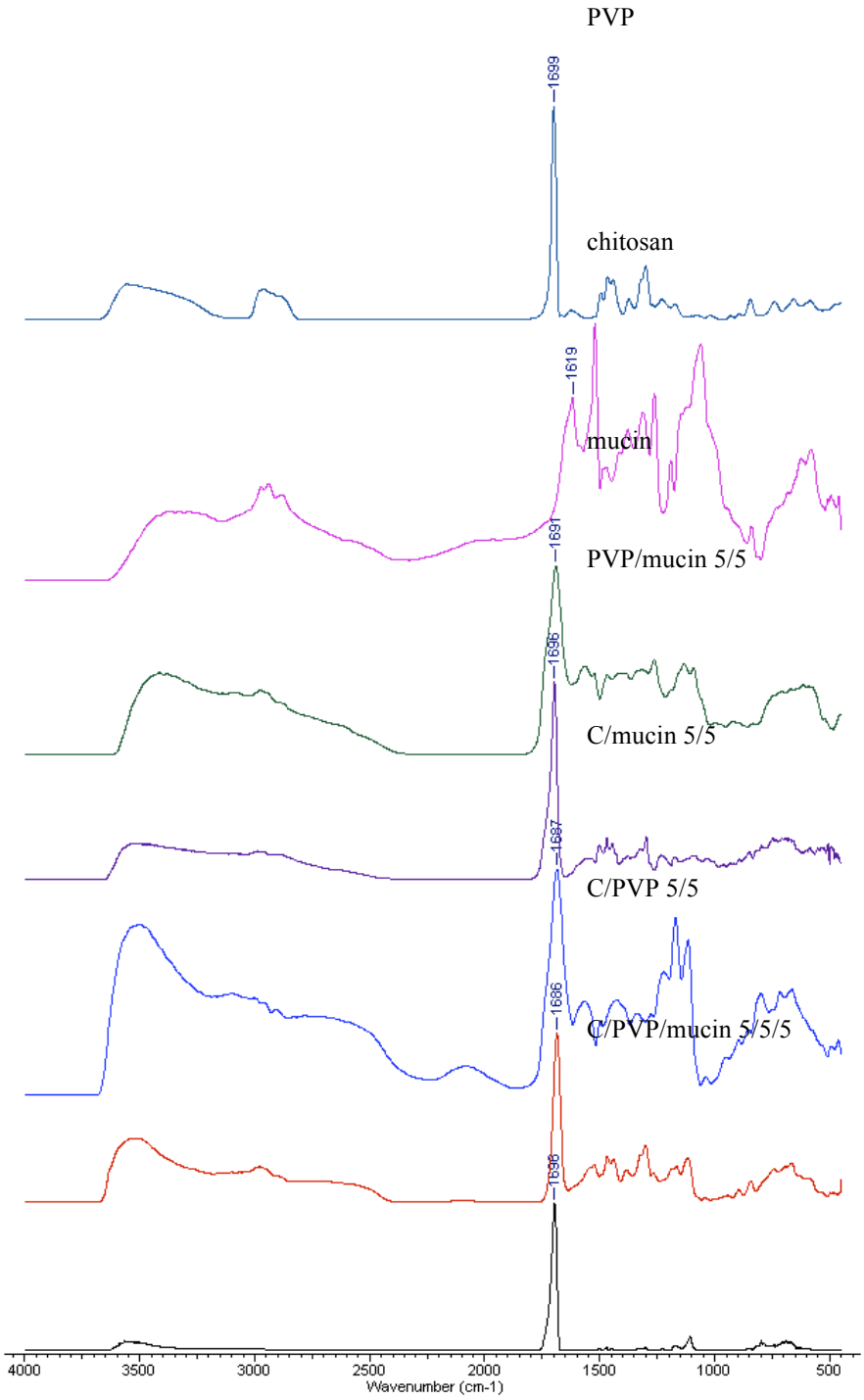


Figure 3.18 DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (5/5), binary mixtures of C/mucin (5/5) and PVP/mucin (5/5) and ternary mixture of C/PVP/mucin at 5/5/5 volume ratio

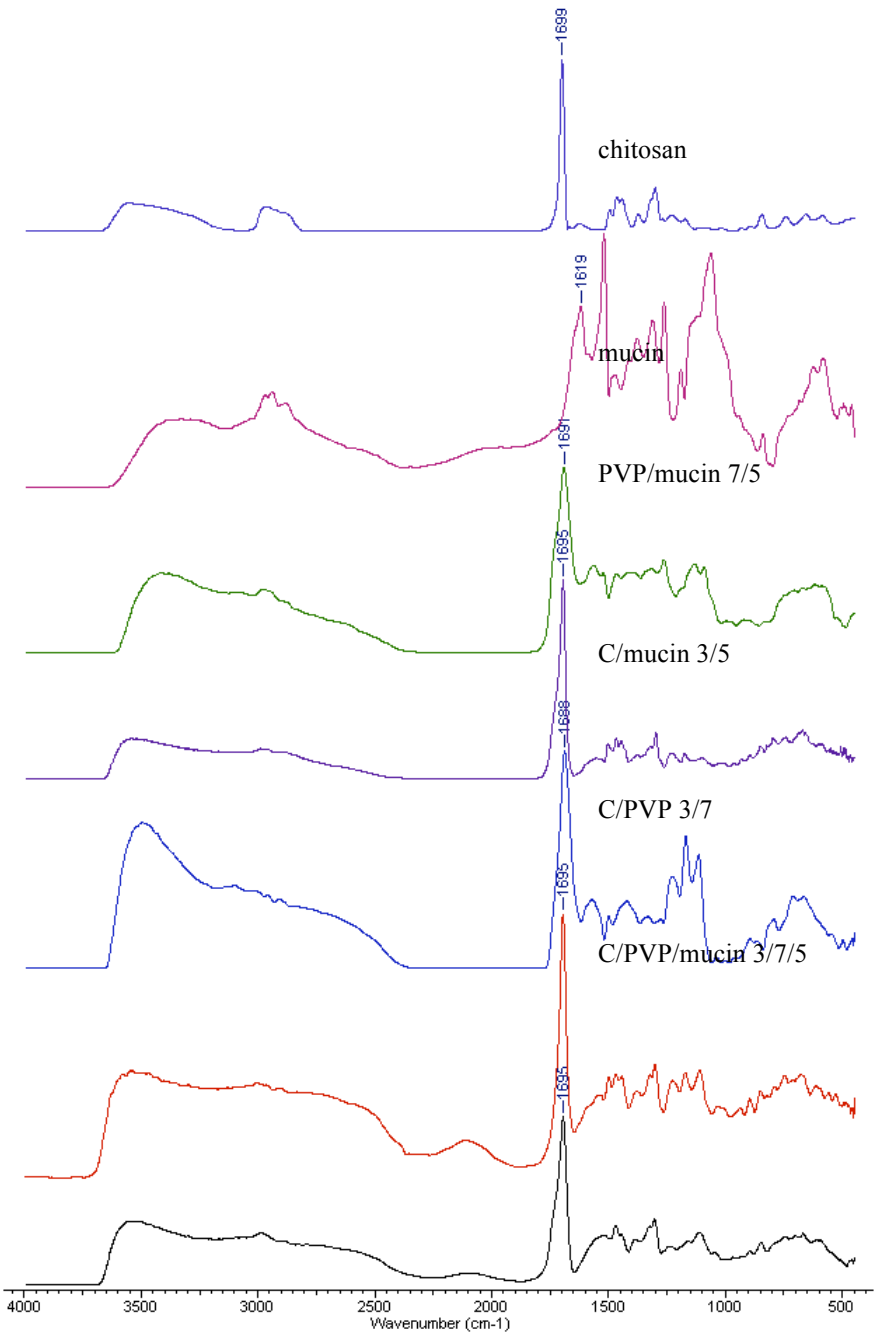


Figure 3.19 DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (3/7), binary mixtures of C/mucin (3/5) and PVP/mucin (7/5) and ternary mixture of C/PVP/mucin at 3/7/5 volume ratio

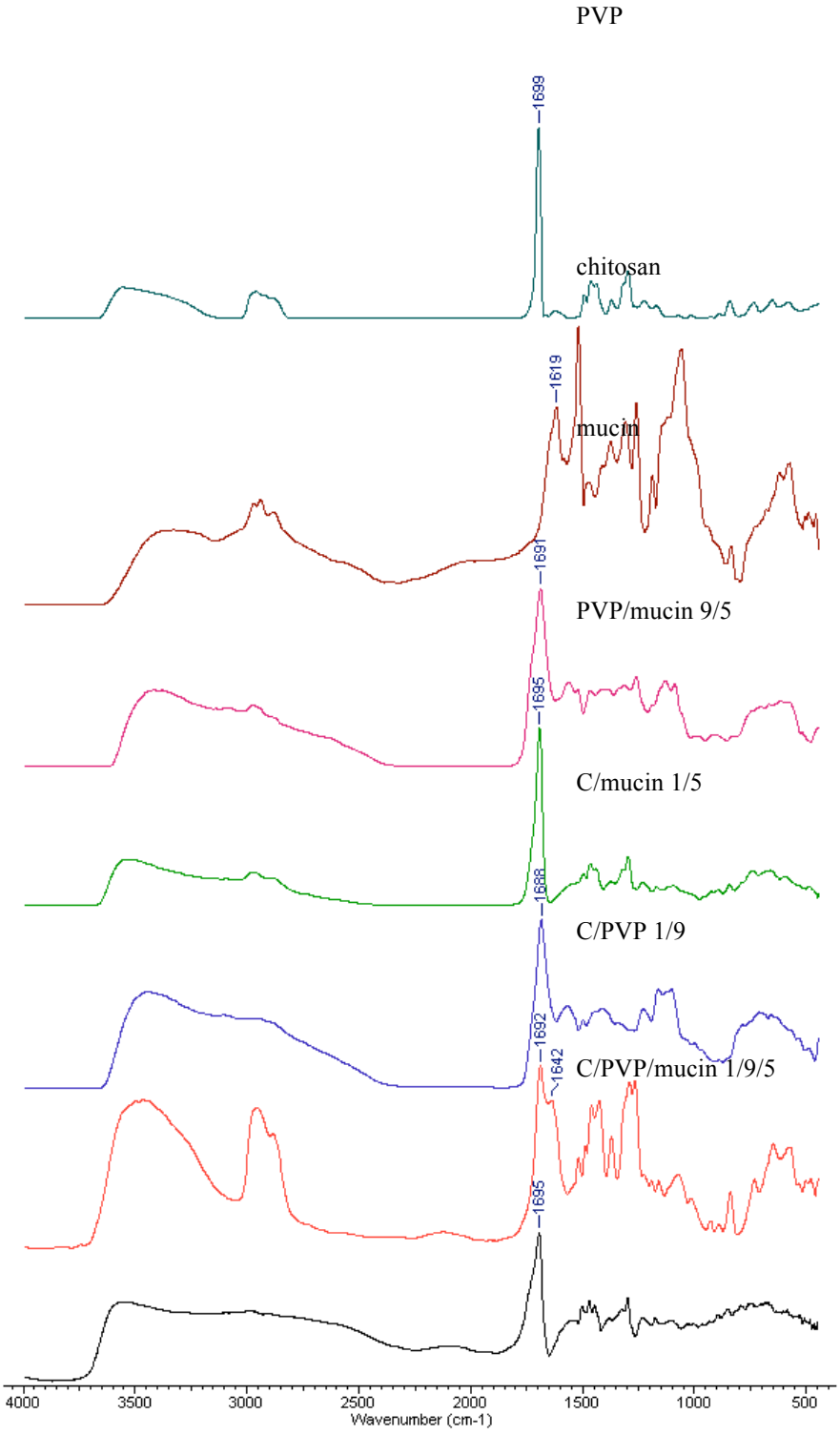


Figure 3.20 DRIFTS spectra of chitosan (C), poly(vinylpyrrolidone) (PVP), mucin, polymer blend of C/PVP (1/9), binary mixtures of C/mucin (1/5) and PVP/mucin (9/5) and ternary mixture of C/PVP/mucin at 1/9/5 volume ratio

The ternary components systems of C/GA/mucin and C/GB/mucin at different volume ratios are shown in Figure 3.21 – 3.25 and Figure 3.26 – 3.30, respectively. For the three components system of chitosan/gelatin/mucin, the hydrogen bonding interaction can be formed between (1) carboxylic groups of gelatin with amine or hydroxyl groups of chitosan, (2) carboxylic group of mucin with amine or hydroxyl groups of chitosan, (3) carboxylic group of mucin with amine groups of gelatin or (4) amine group of mucin with carboxylic group of gelatin. The DRIFTS spectra of chitosan/gelatin/mucin system cannot observe the N-H stretching peak due to the broad of O-H stretching peak, thus, only carbonyl peak shifted was observed from ternary components system. Because the carbonyl peaks of mucin and gelatin are close together so these peak shifted observed in ternary components system cannot be identified the hydrogen bonding occurred at whether carboxylic group of mucin or gelatin. The carbonyl peak of C/GA/mucin and C/GB/mucin were shifted to lower wavenumber of 1697 – 1685 cm^{-1} and 1698 – 1674 cm^{-1} when compare to carbonyl peak observed from GA or GB, respectively. Some of these carbonyl peak positions were observed at higher wavenumber than that observed from C/GA and GA/mucin or C/GB and GB/mucin and these results can concluded that with additional the third compound may be interfere the hydrogen bonding interaction of the binary components system.

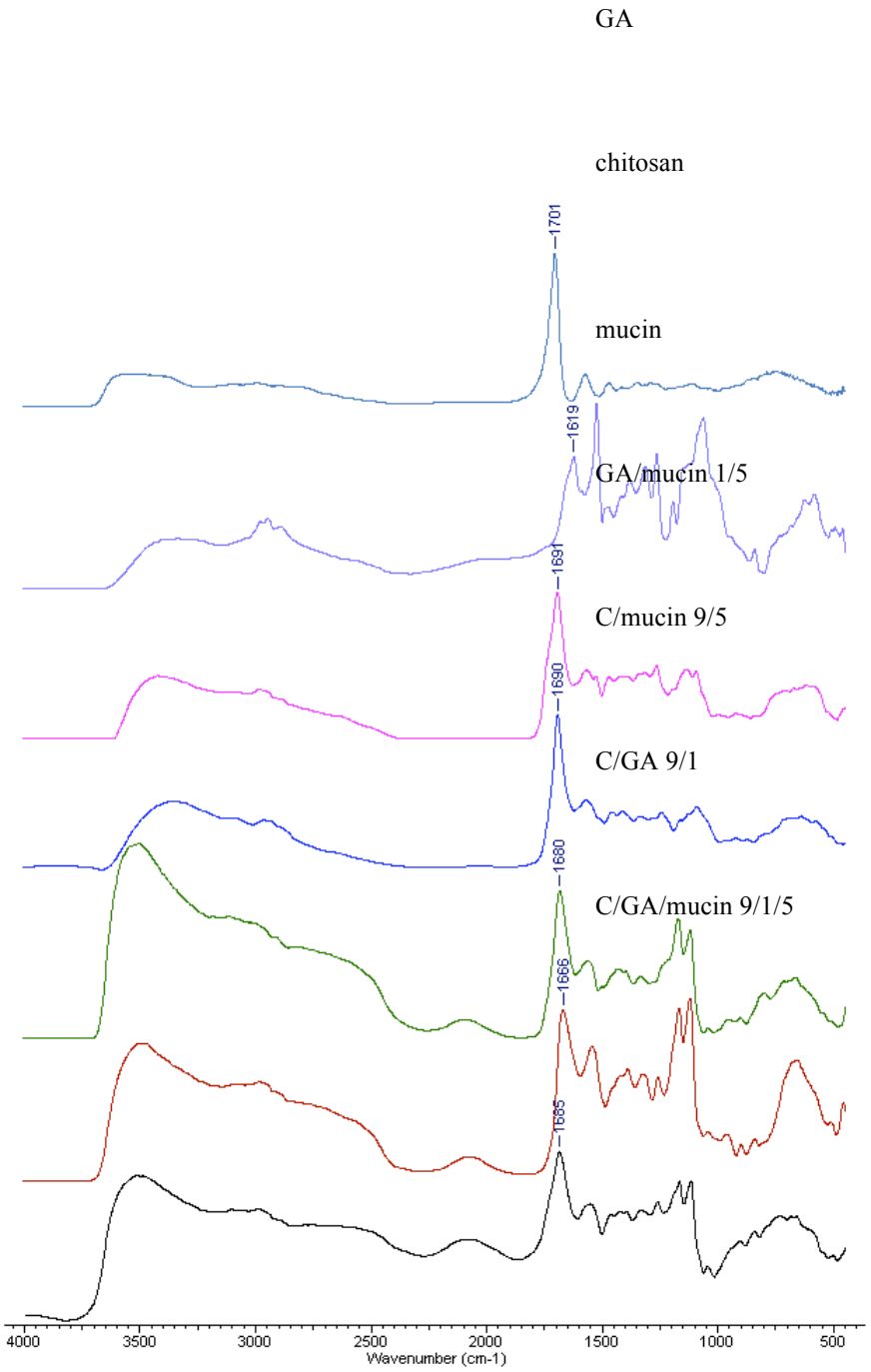


Figure 3.21 DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (9/1), binary mixtures of C/mucin (9/5) and GA/mucin (1/5) and ternary mixture of C/GA/mucin at 9/1/5 volume ratio

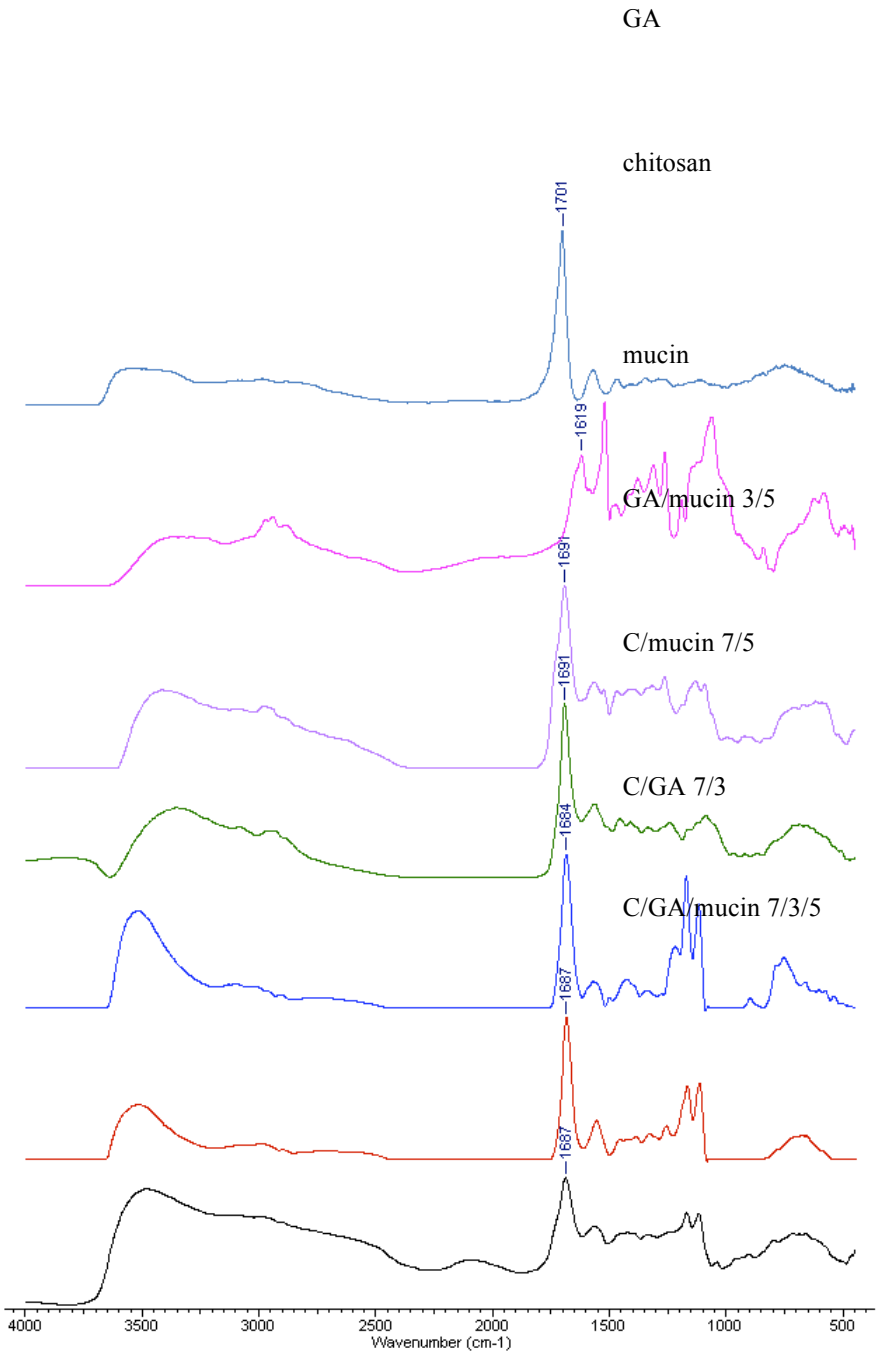


Figure 3.22 DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (7/3), binary mixtures of C/mucin (7/5) and GA/mucin (3/5) and ternary mixture of C/GA/mucin at 7/3/5 volume ratio

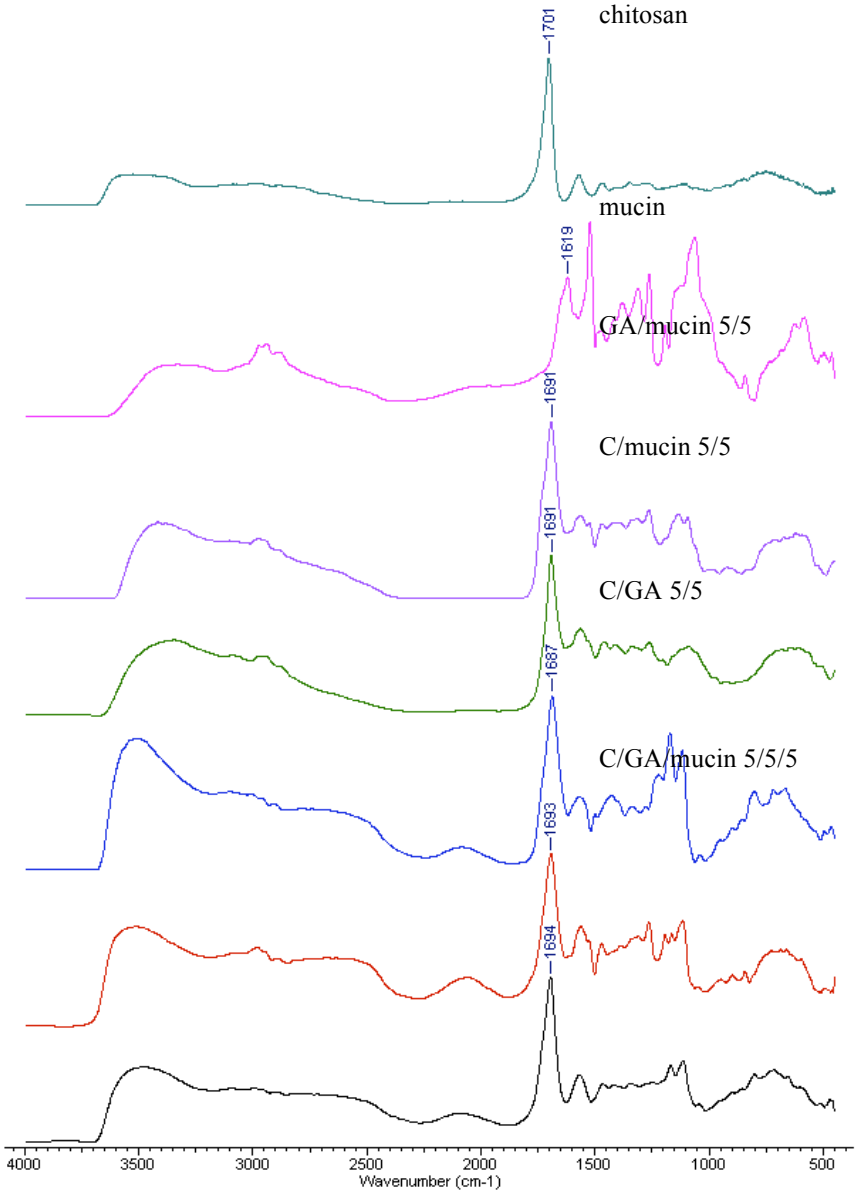


Figure 3.23 DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (5/5), binary mixtures of C/mucin (5/5) and GA/mucin (5/5) and ternary mixture of C/GA/mucin at 5/5/5 volume ratio

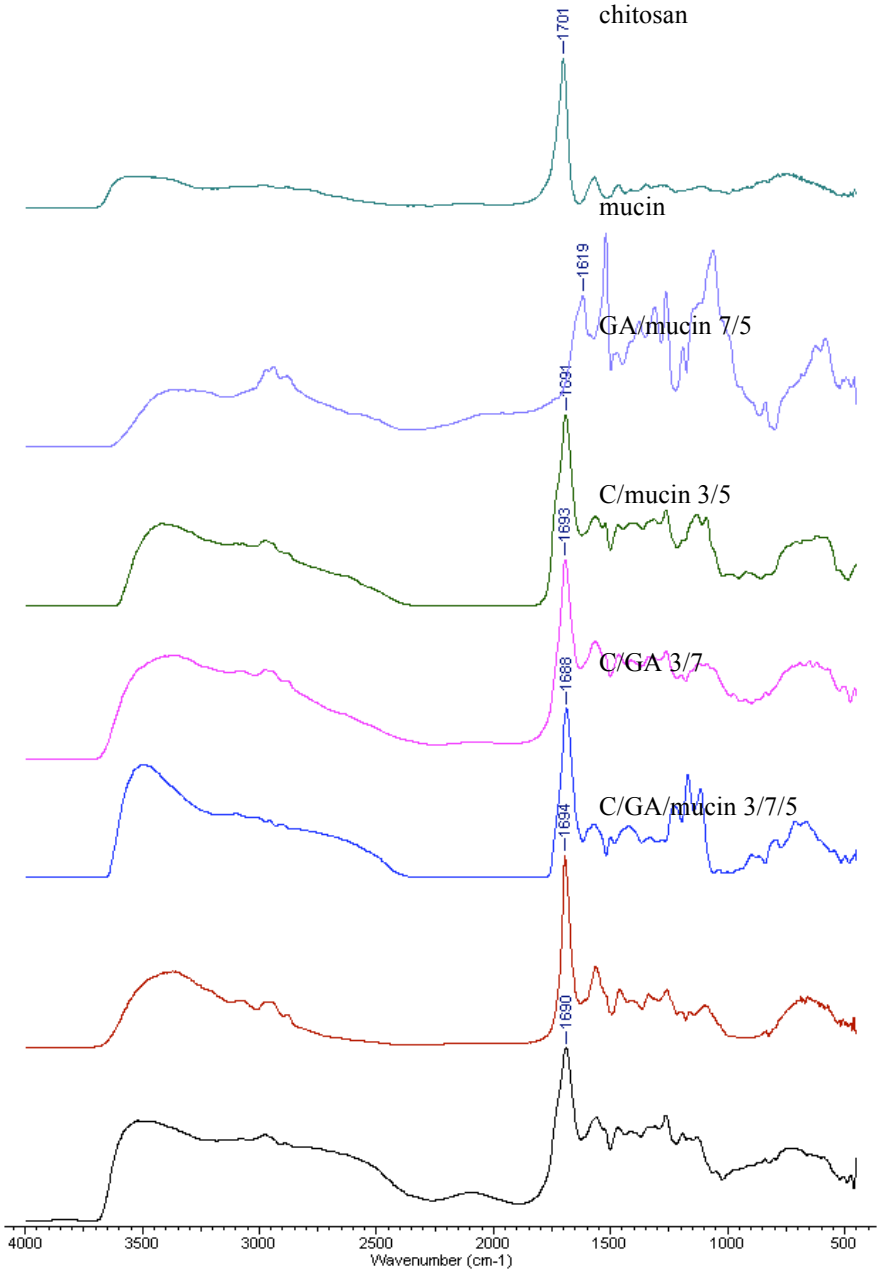


Figure 3.24 DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (3/7), binary mixtures of C/mucin (3/5) and GA/mucin (7/5) and ternary mixture of C/GA/mucin at 3/7/5 volume ratio

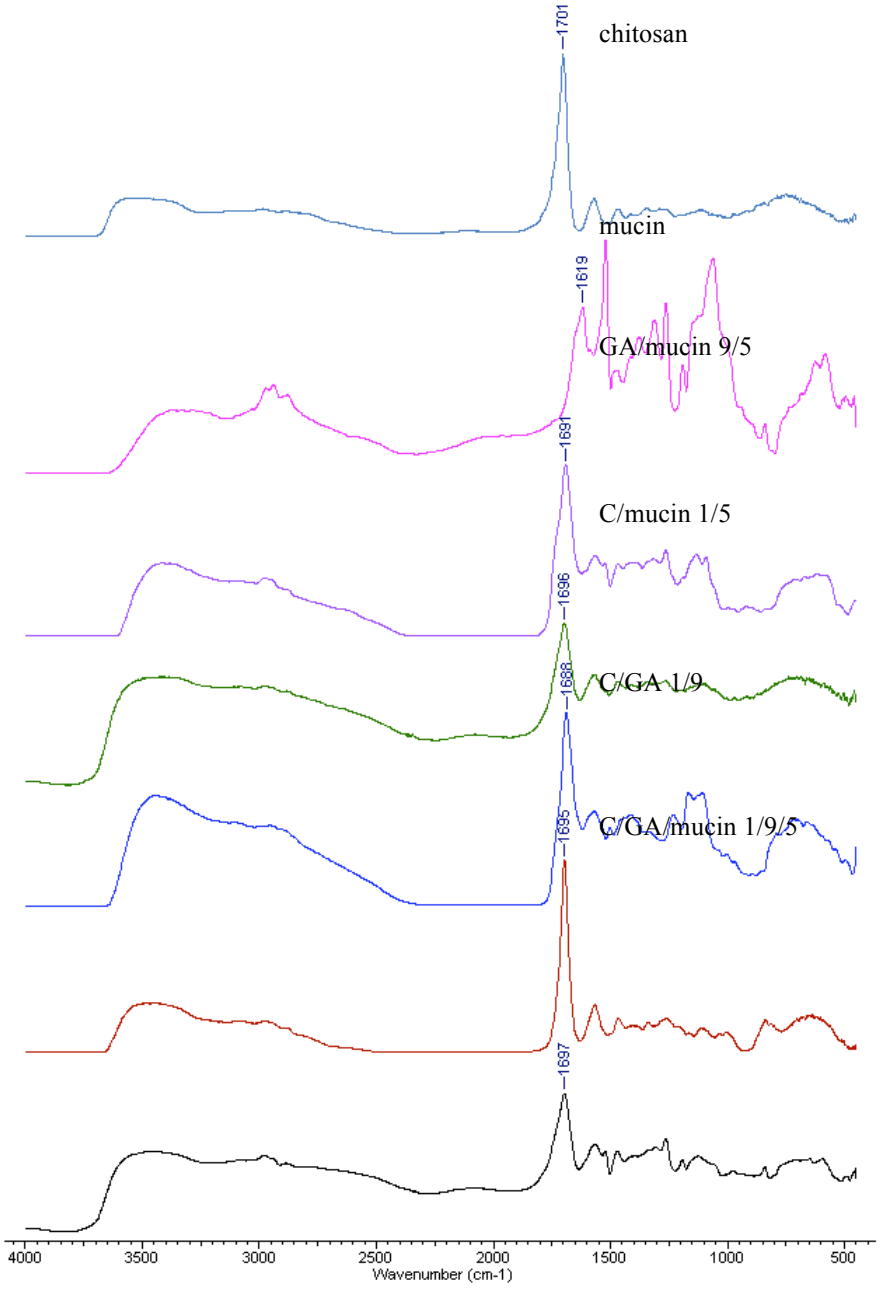


Figure 3.25 DRIFTS spectra of chitosan (C), gelatin type A (GA), mucin, polymer blend of C/GA (1/9), binary mixtures of C/mucin (1/5) and GA/mucin (9/5) and ternary mixture of C/GA/mucin at 1/9/5 volume ratio

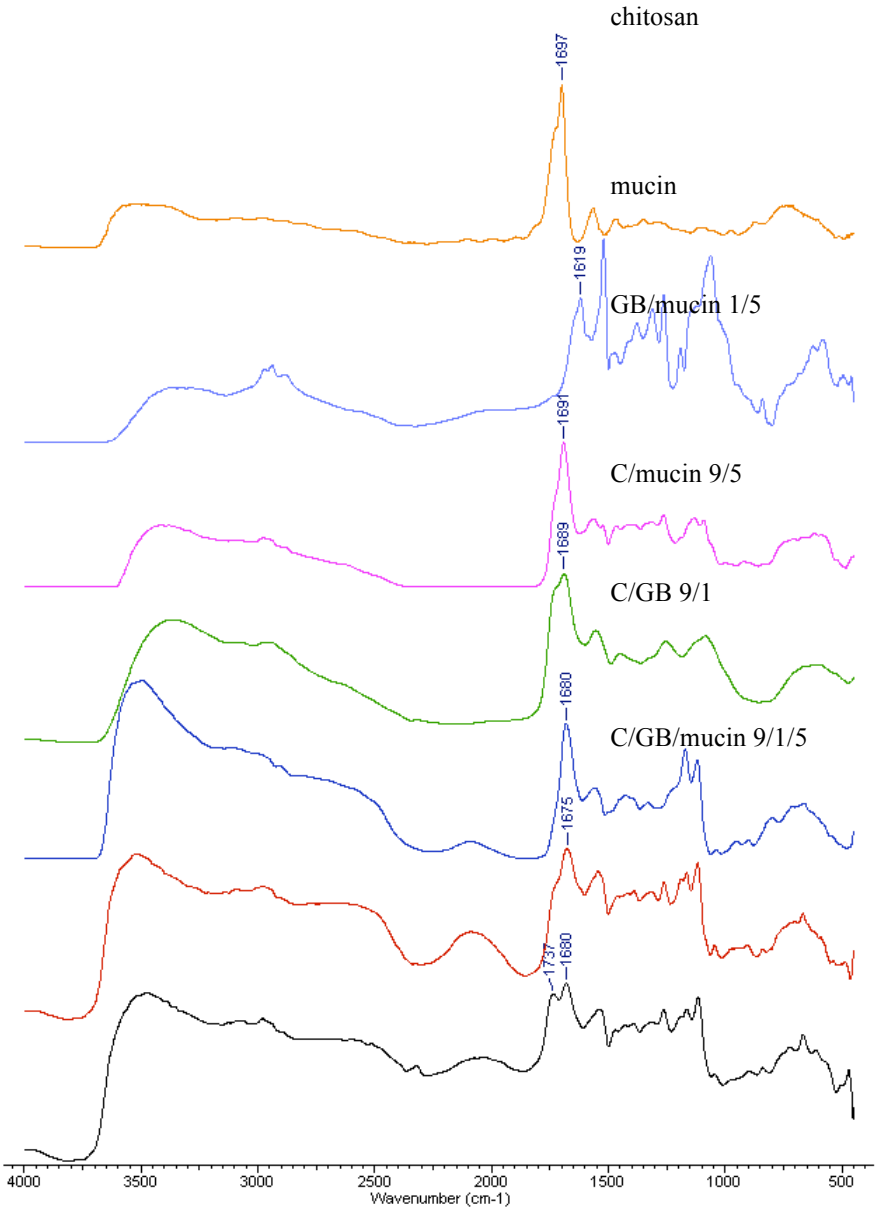


Figure 3.26 DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (9/1), binary mixtures of C/mucin (9/5) and GB/mucin (1/5) and ternary mixture of C/GB/mucin at 9/1/5 volume ratio

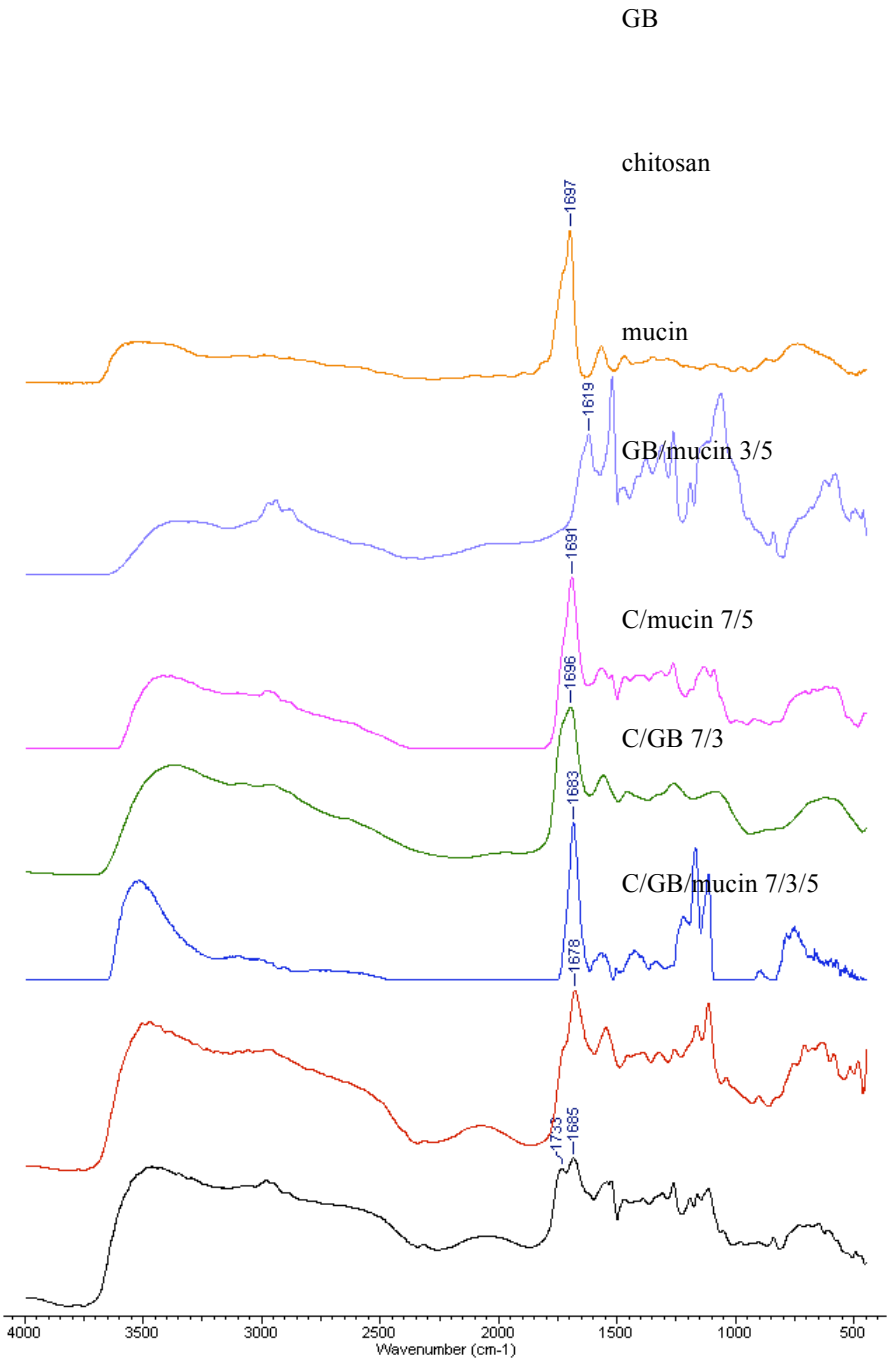


Figure 3.27 DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (7/3), binary mixtures of C/mucin (7/5) and GB/mucin (3/5) and ternary mixture of C/GB/mucin at 7/3/5 volume ratio

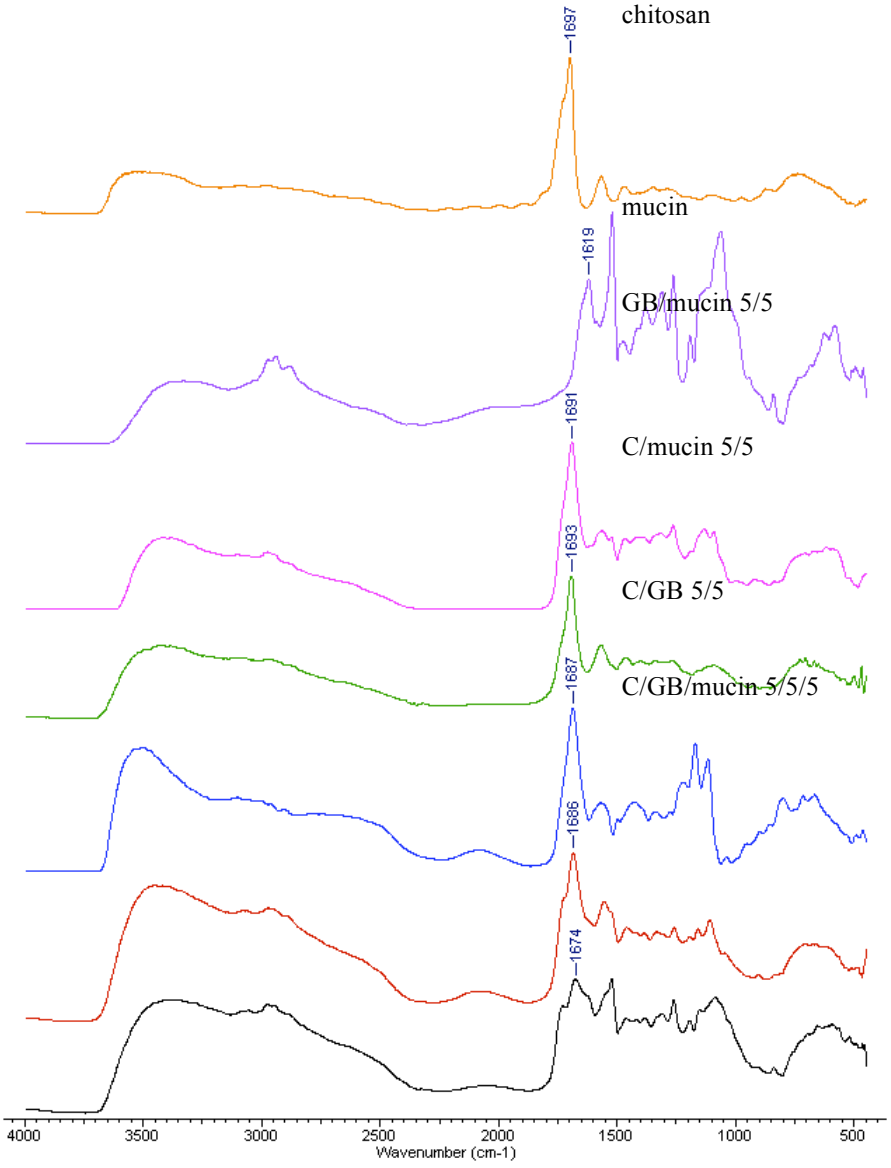


Figure 3.28 DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (5/5), binary mixtures of C/mucin (5/5) and GB/mucin (5/5) and ternary mixture of C/GB/mucin at 5/5/5 volume ratio

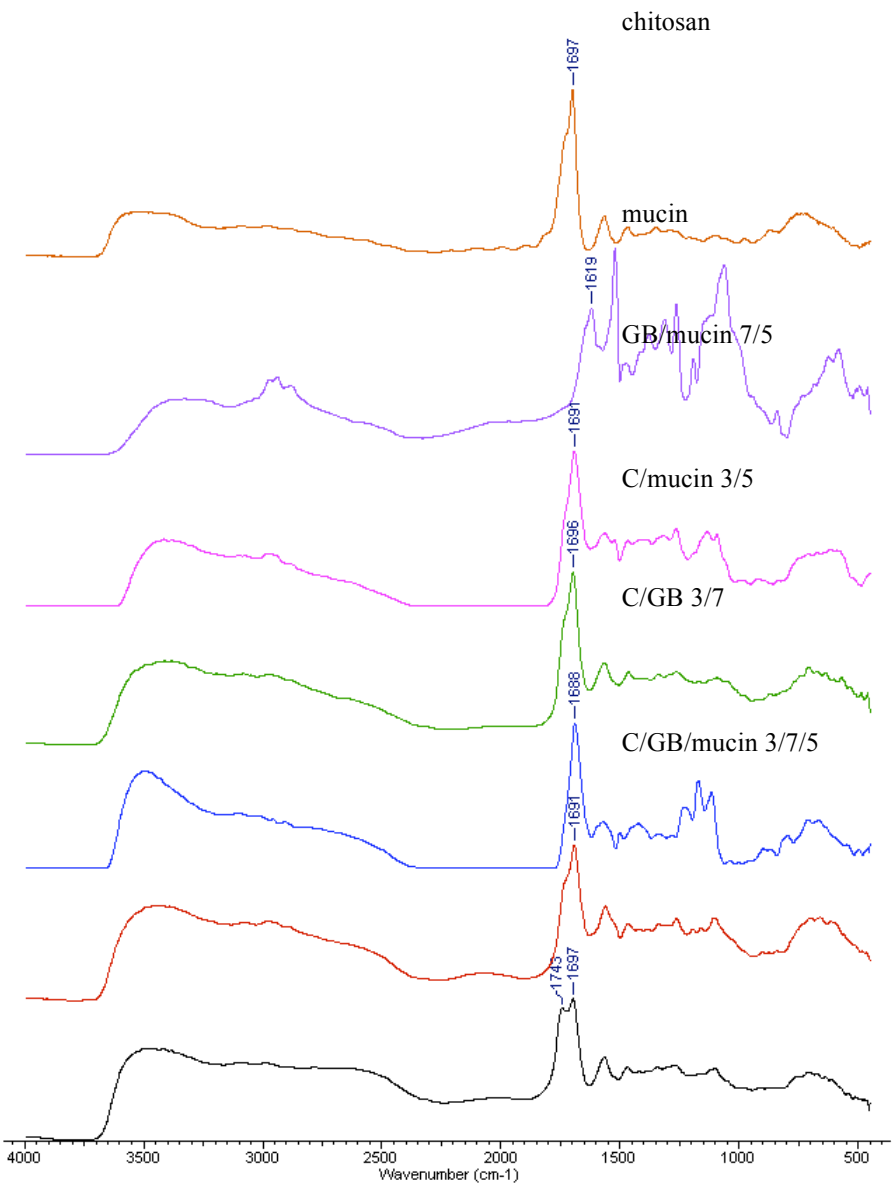


Figure 3.29 DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (3/7), binary mixtures of C/mucin (3/5) and GB/mucin (7/5) and ternary mixture of C/GB/mucin at 3/7/5 volume ratio

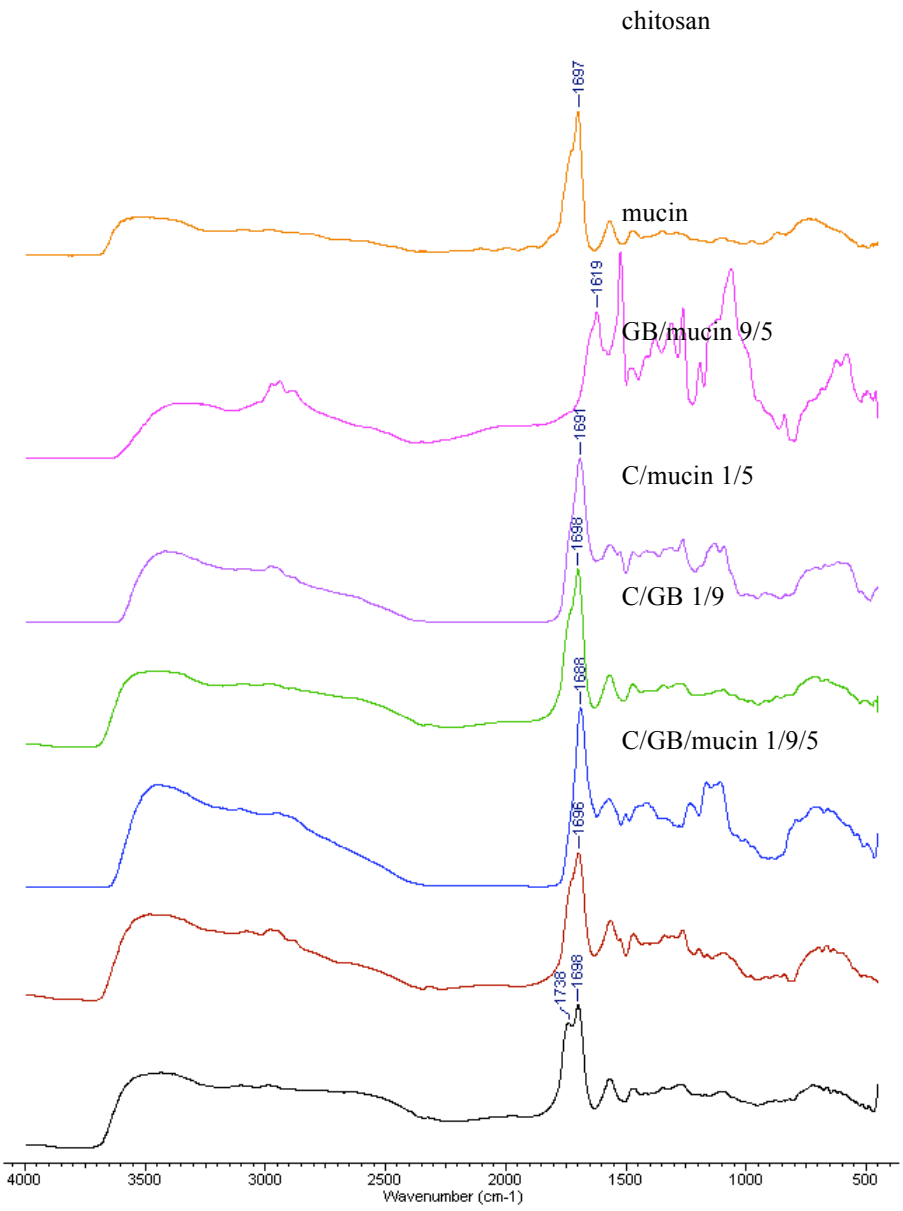


Figure 3.30 DRIFTS spectra of chitosan (C), gelatin type B (GB), mucin, polymer blend of C/GB (1/9), binary mixtures of C/mucin (1/5) and GB/mucin (9/5) and ternary mixture of C/GB/mucin at 1/9/5 volume ratio

In summary, of the binary mixture systems of polymer blends and polymer blends with mucin, C/PVP, C/mucin and PVP/mucin show the highest intermolecular interaction according to the high polymer interaction and the high interaction between polymer and mucin. Binary mixture systems of GB show intermolecular interactions that are higher than for the binary mixture systems of GA. These interaction results can help to describe the mechanism of mucoadhesion of the polymer blend and polymer/mucin systems and could be referred to the viscosity phenomenon of polymer blend with mucin. The rank order of interaction of polymer blends with mucin is C/PVP > C/GB > C/GA, and these results agree with the viscosity study in Chapter 2. Although the DRIFTS spectra of ternary mixture cannot identified the specific hydrogen bonding interaction due to the combination and complication of hydrogen bonding formation in ternary components system, the DRIFTS spectra of ternary mixture show an interfering of the third component on the carbonyl peak position in binary mixture and can be summarized that all three components can form a hydrogen bonding in the ternary mixture.

3.4 Conclusions

In this study, the mucoadhesive interactions of polymer blends and polymer/mucin systems were investigated using a spectroscopic technique. All polymer blends and polymers/mucin systems show intermolecular interaction via hydrogen bonding.

Polymer blends of C/PVP show high intermolecular interactions and

also represent high interaction with mucin. Gelatins have weak interactions in the polymer blend and in binary mixtures with mucin, however, interaction of the polymer blends and polymer/mucin of the GB seem to be higher than the polymer blends of the GA. The infrared spectra of polymer blends and polymer/mucin systems can provide information of molecular interaction mechanisms in ternary mixtures of polymer blend/mucin systems. These results explain that intermolecular bonding such as hydrogen bonding and van der Waals interaction play key roles in the attraction forces between mucin and polymer. The strong mucoadhesion of polymer blends is described well by intermolecular hydrogen bonding and is in agreement with the viscosity study of the polymer or polymer blends with mucin in Chapter 2. The results obtained from this study support the diffusion theory of mucoadhesion mechanisms.

References

124. Taylor, C., Pearson, J., Draget, K., Dettmar, P., Smidsrid, O. (2005) Rheological characterisation of mixed gels of mucin and alginate. *Carbohydr. Polym.* 59, 189-95.
125. Karavas, E., Georgarakis, E., Bikiaris, D. (2006) Application of PVP/HPMC miscible blends with enhanced mucoadhesive properties for adjusting drug release in predictable pulsatile chronotherapeutics. *Eur. J. Pharm. Biopharm.* 64, 115-26.
126. Mortazavi, S. (1995) An in vitro assessment of mucus/mucoadhesive interactions. *Int. J. Pharm.* 124, 173-82.
127. Vishnu, Y., Chandrasekhar, K., Ramesh, G., Rao, Y. (2007) Development of mucoadhesive patches for buccal administration of carvedilol. *Curr Drug Deliv* 4, 27-39.
128. Patel, M., Smart, J., Nevell, T., Ewen, R., Eaton, P., Tsibouklis, J. (2003) Mucin/Poly(acrylic acid) Interactions: A Spectroscopic Investigation of Mucoadhesion. *Biomacromolecules* 4, 1184-90.
129. Peppas, N. A., Buri, P. A. (1985) Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. *J. Controlled Release* 2, 257-75.
130. Jabbari, E., Wisniewski, N., Peppas, N. (1993) Evidence of mucoadhesion by chain interpenetration at a poly (acrylic acid)/mucin interface using ATR-FTIR spectroscopy. *J. Controlled Release* 26, 99-108.
131. Berntsson, O., Burger, T., Folestad, S., Danielsson, L., Kuhn, J., Fricke, J. (1999) Effective Sample Size in Diffuse Reflectance Near-IR Spectrometry. *Anal. Chem.* 71, 617-23.
132. PIKE Technologies. Diffuse Reflectance – Theory and Applications. 2009.
133. Nijenhuis, K. Thermoreversible networks : viscoelastic properties and structure of gels. Advances in polymer science,. Berlin ; New York: Springer; 1997. p. xx, 267 p.
134. Tang, Q., Wu, J., Lin, J., Fan, S., Hu, D. (2009) A multifunctional poly(acrylic acid)/gelatin hydrogel. *J Mater Res* 24, 1653-61.
135. Parthasarathy, R., Rabuka, D., Bertozzi, C., Groves, J. (2007) Molecular orientation of membrane-anchored mucin glycoprotein mimics. *J Phys Chem B* 111, 12133-5.
136. Bettelheim, F. (1963) Physical chemistry of mucins. *Ann. N.Y. Acad. Sci.* 106, 247-58.
137. Suknuntha, K., Tantishaiyakul, V., Vao-Soongnern, V., Espidel, Y., Cosgrove, T. (2008) Molecular modeling simulation and experimental measurements to characterize chitosan and poly(vinyl pyrrolidone) blend interactions. *J Polym Sci Pol Phys* 46, 1258-64.
138. Wang, J., Tabata, Y., Bi, D., Morimoto, K. (2001) Evaluation of gastric mucoadhesive properties of aminated gelatin microspheres. *Journal of controlled release : official journal of the Controlled Release Society* 73, 223-31.

CHAPTER 4

An *in vitro* evaluation of mucoadhesive polymer using the tensile strength test method

4.1 Introduction and objectives

The issue of maintaining a proper dosage of a drug over an extended period of time on a mucous tissue by adhesive interactions has attracted the attention of many investigators. In the pharmaceutical field, these efforts have been mainly dedicated to improve or control drug absorption or for a local effect by targeting specific mucosal tissues of the human body. Theories for the interpretation of the interaction between polymeric materials and the surface of a mucosal tissue have been investigated using several methods. Several techniques for the *in vitro* determination of the mucoadhesion have been reported and most of them have been based on the tensile strength measurement [139-141]. The mechanisms of mucoadhesion at an interface are comprised of two stages. Initially, wetting of the mucoadhesive surface forms an intimate contact with the mucosal gel. The second stage is the penetration of the mucoadhesive molecules into the mucus gel network, followed by the formation of secondary chemical bonds between the mucus and the mucoadhesive polymer [142].

Although, there are several theories that can explain the mucoadhesion mechanisms, in isolation, none of these theories can explain mucoadhesion by the many and varied pharmaceutical formulations that have been developed. Indeed, mucoadhesion probably results from a combination of several mechanisms. Consequently, some researchers prefer to divide the adhesion process into sequential phases, each of which is associated with a different mechanism as shown in Figure 4.1. First, the dosage form wets and swells (wetting theory), after which non-covalent (physical) bonds are created within the mucus/polymer interface (electronic and adsorption theories). Then, the polymer and protein chains interpenetrate (diffusion theory) and entangle together, to form further non-covalent (physical) and covalent (chemical) bonds (electronic and adsorption theories) [143].

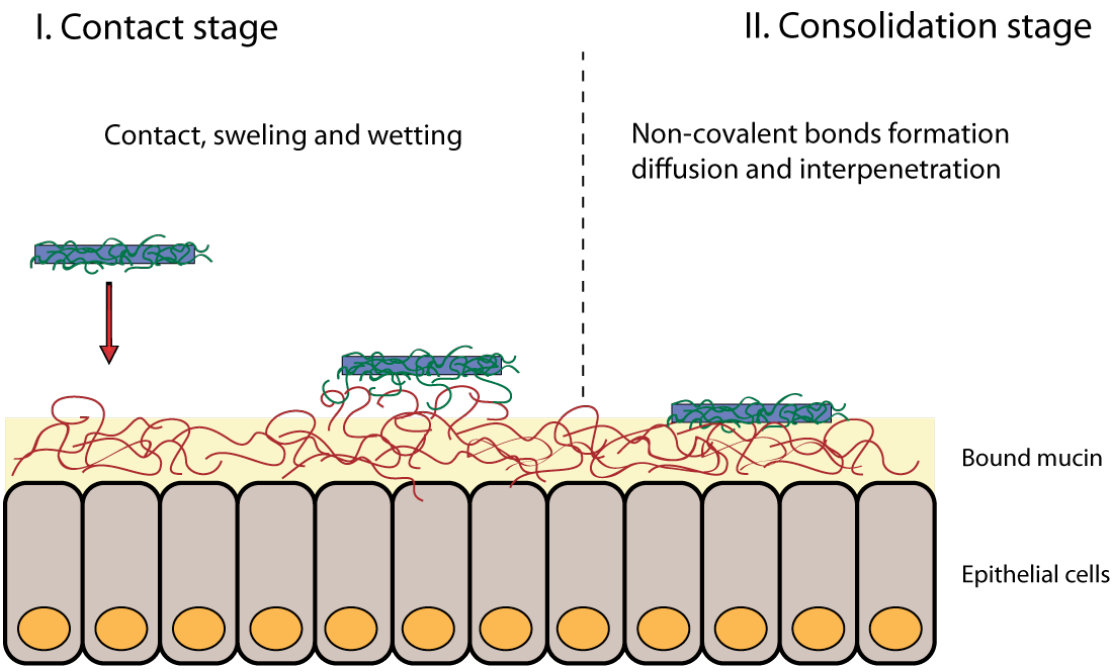


Figure 4.1 Schematic mucoadhesion mechanism of polymer film on mucosal tissue

An evaluation of the mucoadhesion using the tensile strength test usually examines the force necessary to separate two surfaces after mucoadhesive bonding has occurred. The tensile strength test using a texture analyzer has been reported for studying the mechanical characteristics of the mucoadhesion of the polymers and the other dosage forms such as tablet [144], pellet [145], film [146], gel [146], dry particle [147] etc. The strength of the mucoadhesion using this technique was evaluated through the measure of the maximum force required to separate the polymer or dosage form from the surface of substrate after contact at a specified time and force, and the work of adhesion is calculated. Many parameters such as test speed of the probe, contact time and trigger force affect the result. However, some research workers have reported that validation of the test parameters under porcine gastric stomach using texture analyzer are useful and these parameter were used in this study [146].

The objective of this study was to investigate the mucoadhesive force using mucoadhesion to the porcine stomach tissue by various polymer films including chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA), gelatin type B (GB), and polymer blends of C/PVP, C/GA, C/GB at various volume ratios.

4.2 Experimental methods

4.2.1 Materials

All materials and chemical reagents in this experiment were used as the same as described in Section 2.2.1.

4.2.2 Sample preparations

All polymer and polymer blends solution were prepared with the same method that described in Section 2.2.2. Polymers and polymer blends films were prepared by casting fully mixed polymer or polymer blend solutions on a polystyrene plate then dried in an oven at 60 °C for 8 h. These samples were kept in desiccator and used for a texture analyzer measurement.

4.2.3 Texture analyzer measurement

A texture analyzer, TA-XT2 (Stable Micro System, Haslemere, UK), equipped with a 5 kg load cell and a mucoadhesive rig was used for all tensile strength measurement. A schematic of the mucoadhesive force determination using the texture analyzer is shown in Figure 4.2. All polymer blend films were cut into a circle shape with a diameter of 1 cm and fixed onto the upper cylindrical probe of 1

cm diameter of the instrument using double sided adhesive tape. A porcine stomach tissue was obtained from the animal immediately after slaughter at a local slaughter house (Faculty of Natural Resources, Prince of Songkla University). The tissue was washed with deionized water to remove undigested food, kept in 4 °C and used within 6 h. The underlying connective tissues were subsequently removed to isolate the mucosal membrane. Stomach tissue was cut into a square shape and fixed to a mucoadhesive rig on the instrument stage. The film attached to the upper probe was wet with 50 µL of deionized water and then lowered to contact the tissue with a constant speed of 1 mm/s. The film made contact with the tissue at a constant force of 0.08 N for 2 min. After a definite time of contact, the film was slowly withdrawn upward at a constant speed of 1 mm/s until contact between the surfaces ceased.

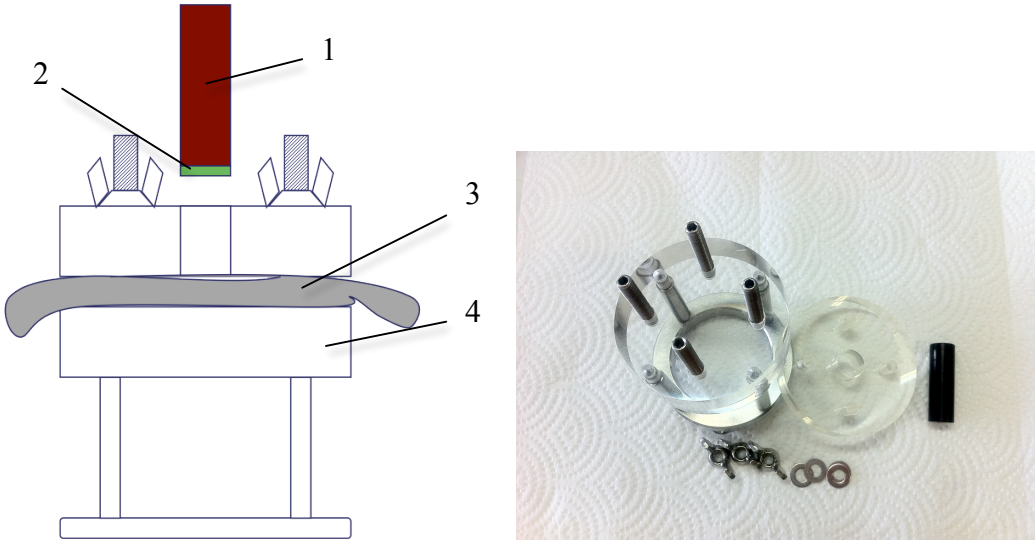


Figure 4.2 The mucoadhesive rig accessory and schematic of the mucoadhesive force measurement using the texture analyzer: upper cylindrical probe (1), polymer film (2), porcine stomach tissue (3), mucoadhesive rig (4)

The detailed information required for determination of the mucoadhesion using this instrument consisted of three basic steps as shown in Figure 4.3 [148]. The first step was the move to contact, when the upper cylindrical probe with the attached polymer film sample was moved in the direction of the porcine stomach tissue at a constant speed (1 mm/s). At contact, the movement was stopped when the pressure of the cylindrical probe reached 0.08 N. After a definite time of contact, the cylindrical probe began to move in the opposite direction at a constant velocity (1 mm/s) and stopped when the cylindrical probe became completely detached from the porcine stomach tissue.

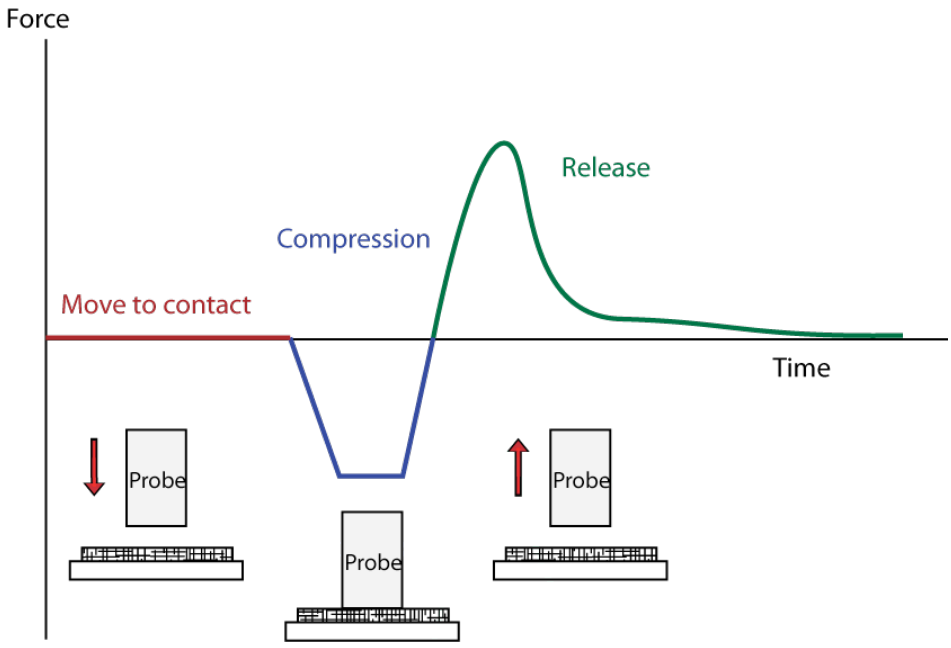


Figure 4.3 Stages for determination of the mucoadhesive interaction using the texture analyzer

The force – distance curve during the upward movement of the film were obtained directly from the Texture Expert software. The area under the force – distance curve was calculated as being due to the work of mucoadhesion (Figure 4.4). In order to confirm reproducibility and validity of the data obtained, the determination of samples was performed 6 to 10 times, and the contact area of the porcine stomach tissue was changed for each sample film.

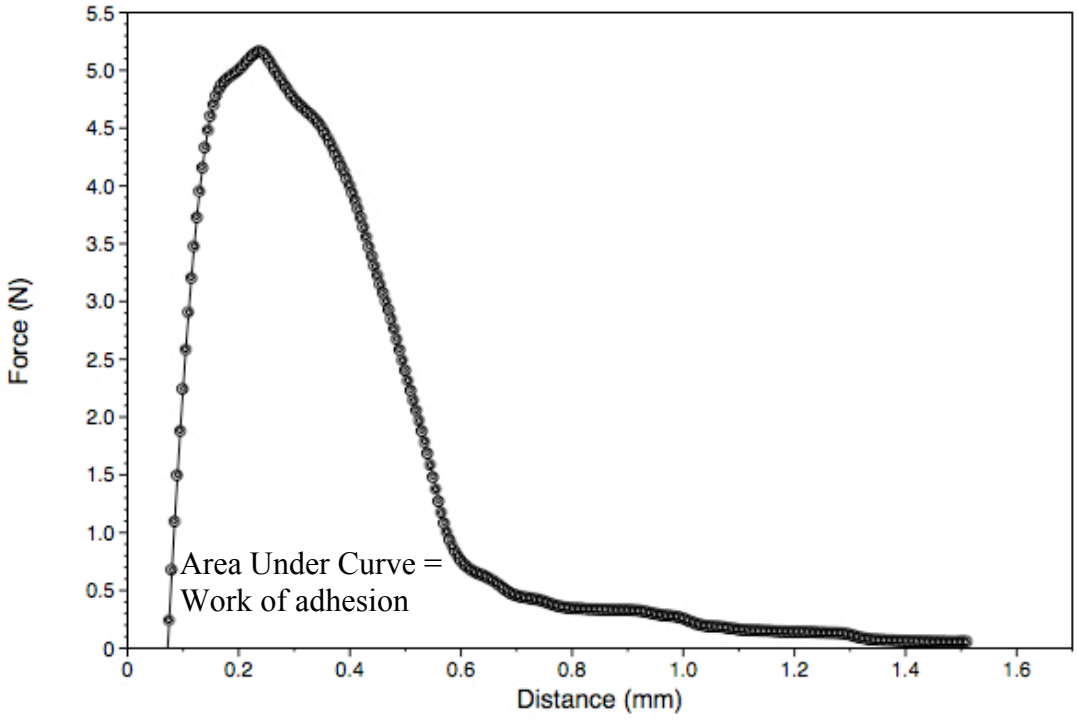


Figure 4.4 Typical plot of force versus distance data from the texture analyzer and area under curve is represented work of adhesion

4.2.4 Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS for Windows version 10.0 (SPSS Inc., USA). Post hoc testing ($p<0.05$) of the multiple comparisons was performed by Turkey's test.

4.3 Results and discussion

4.3.1 Mucoadhesion of a single polymer

The work of adhesion of C, PVP, GA, and GB is shown in Figure 4.5. The work of mucoadhesion of chitosan is significantly the highest and GB also shows better mucoadhesion than GA. This is in agreement with results from viscosity study in Chapter 2. The hydrated state with a sufficient amount of water will promote a mucoadhesive network and exposes available adhesive sites for bond formation and enhances polymer chain mobilization for interpenetration, thus polymer behavior seems to be similar to the viscosity study [149]. Although PVP shows a better mucoadhesion than gelatin in the viscosity study, the work of mucoadhesion on porcine gastric tissue seem to be lower and this result maybe explained by the characteristic of PVP. A polymer film of PVP is water soluble, while chitosan, gelatin A, and gelatins B are insoluble during an ambient test (25 °C) with the texture analyzer. Thus PVP becomes soluble and slippery from mucin, resulting in its

adhesive properties being lost since the polymer dissolves in the available water [150, 151]. Another factor is the water movement between the mucoadhesive polymer and mucus at the interface. Water transfer may be associated with the mucoadhesive properties; rapid water movement increases the adhesive properties [142]. Yoshioka et al. [152] represented water mobility as indicated by the water activity of gelatin being higher than for PVP.

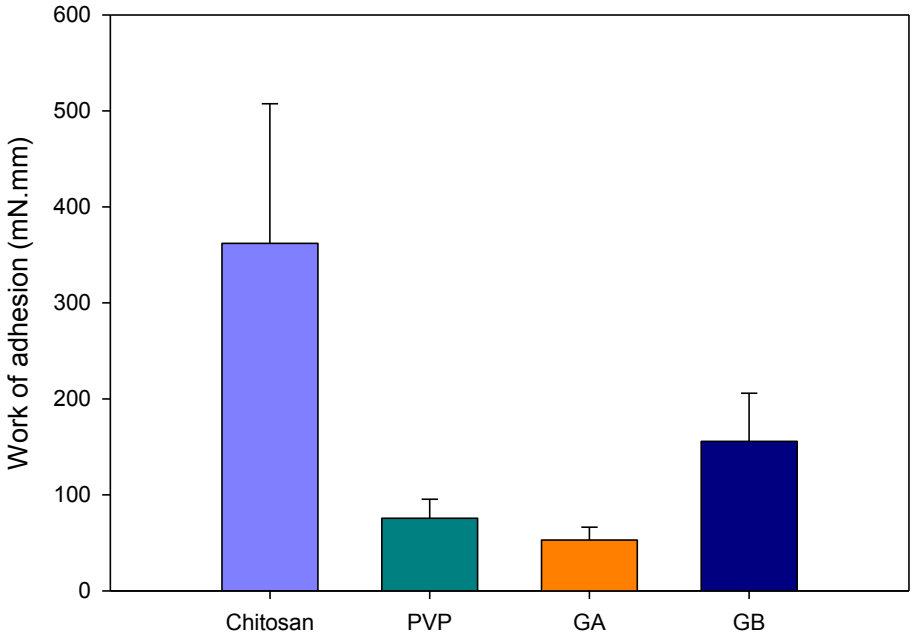


Figure 4.5 The work of adhesion of a single polymer of chitosan, poly(vinylpyrrolidone) (PVP), gelatin type A (GA) and gelatin type B (GB) on porcine gastric tissue

4.3.2 Mucoadhesion of polymer blends

The work of mucoadhesion of the polymer blend of C/PVP, C/GA, and C/GB are shown in Figures 4.6, 4.7, and 4.8, respectively. The work of mucoadhesion of polymer blend of C/PVP is higher than that for chitosan or PVP, especially for the C/PVP blend at a volume ratio of 5/5 is statistical significantly. The work of mucoadhesion of the polymer blends of chitosan and gelatin seem to be lower than that for chitosan. The work of mucoadhesion of the polymer blend of C/PVP is higher than that for the blends of chitosan with GA or GB. Hydrogen bonds between the polymers and the polymers with mucin may cause the high mucoadhesion of C/PVP. According to the previous studies in Chapter 3, hydrogen bond formation occurred after polymer chain interpenetration at the initial stage of an intimate contact between the polymer and mucosal surface and 2 min of contact time of the polymer and mucin is enough to form secondary chemical bonds [142, 146, 153].

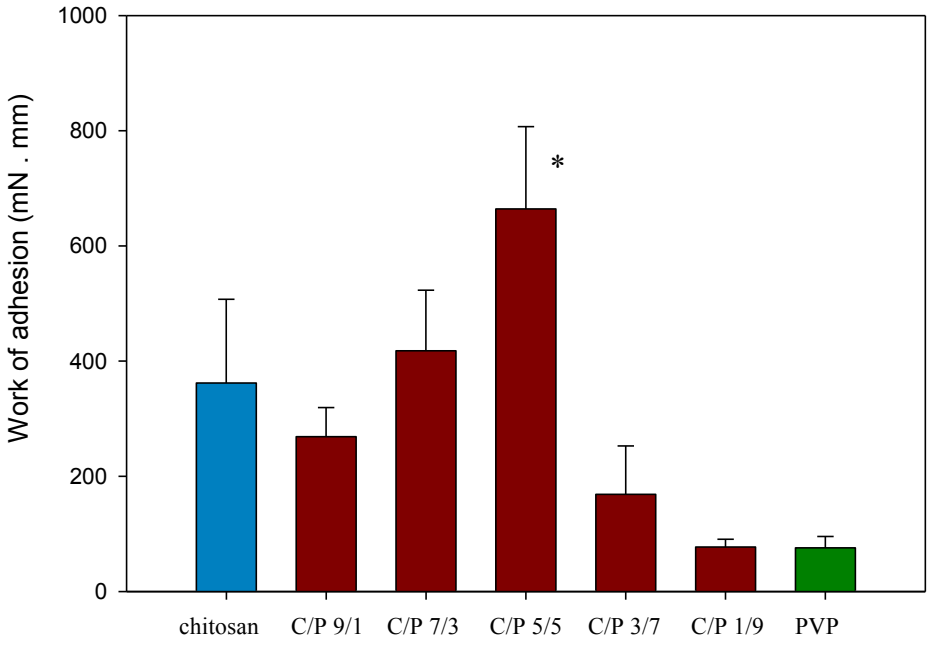


Figure 4.6 The work of adhesion of chitosan (C) and poly(vinylpyrrolidone) (PVP) and their blend at various volume ratio on porcine stomach tissue (n = 6 – 10)

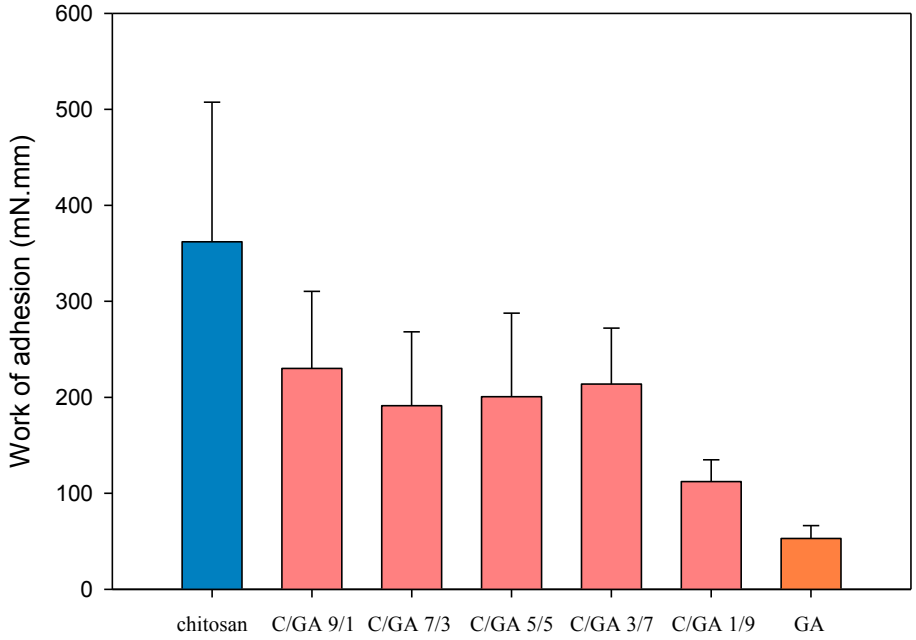


Figure 4.7 The work of adhesion of chitosan (C) and gelatin type A (GA) and their blend at various volume ratio on porcine stomach tissue (n = 6 – 10)

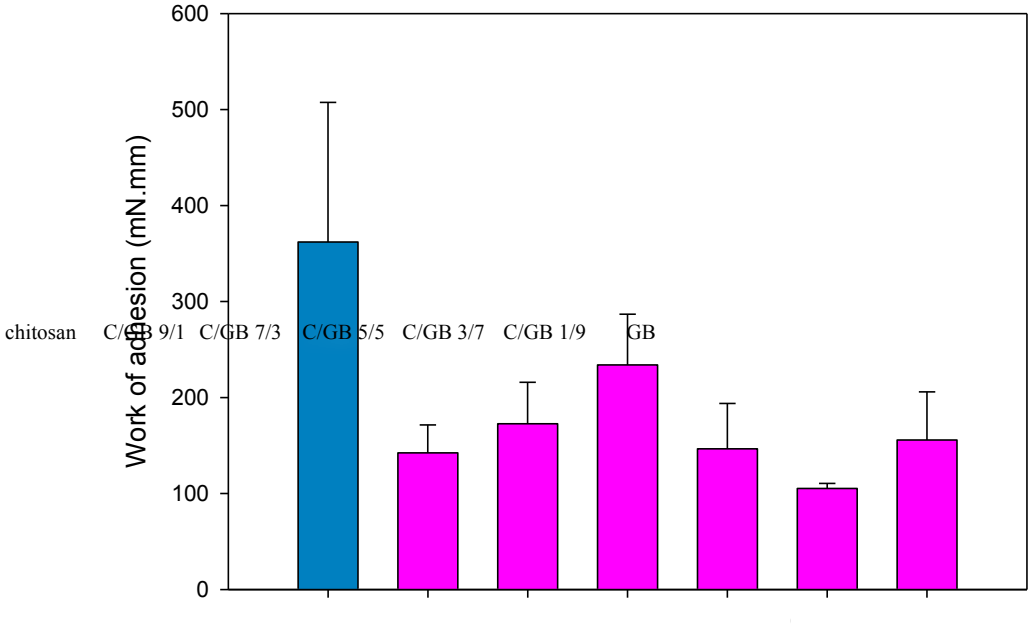


Figure 4.8 The work of adhesion of chitosan (C) and gelatin type B (GB) and their blend at various volume ratio on porcine stomach tissue (n = 6 – 10)

The mucoadhesive properties of polymer blends of chitosan and PVP are in agreement with the viscosity measurements, the DRIFTS study, and the texture analyzer study. The results from the viscosity study and the texture analyzer seem to be different for the polymer blend of C/GB, that is, the mucoadhesion of the polymer blend from the viscosity result is higher than from the texture analyzer when compared with the single polymer. These results can be described with the intermolecular interaction study (Chapter 2) that the interaction between chitosan and gelatin show a weak interaction and in between gelatin and mucin also show a weak interaction, thus, polymer blend of C/GA or C/GB do not show any synergistic effects on texture analysis study. There are several mechanisms and theories that might explain the described mucoadhesion and those most commonly presented in

conjunction with mucoadhesion are the absorption, diffusion, electronic, fracture and wetting theories. In some situations, the viscosity measurement is not described well for mucoadhesion especially by the electronic theory. The negative interaction due to a strong interaction does not produce a strengthening of the macroscopic rheological behavior [154]. The texture analyzer is based on measuring the force or work required to detach the formulation from the tissue that are quite similar to the in *vivo* situation [155]. Due to the fact that the viscosity measurement (Chapter 2) and spectroscopy study (Chapter 3) have demonstrated a significant high mucoadhesion and high interaction of the C/PVP blend at a 5/5 ratio, the texture analyzer techniques results of C/PVP blend at these ratio also show the high mucoadhesive.

4.4 Conclusions

The texture analysis method is a suitable method for comparing new mucoadhesive polymers. This technique provides a direct measure of the force of a mucoadhesive polymer on a mucus tissue that is similar to the in *vivo* gastric situation. In this study, the mucoadhesive properties of the polymer and polymer blends are related to their intermolecular interaction and physicochemical properties. The polymer blends of C/PVP show a potential for development in applications of mucoadhesive drug delivery systems.

References

139. Hagerstrom, H., Edsman, K. (2001) Interpretation of mucoadhesive properties of polymer gel preparations using a tensile strength method. *J. Pharm. Pharmacol.* 53, 1589-99.
140. Thirawong, N., Nunthanid, J., Puttipipatkhachorn, S., Sriamornsak, P. (2007) Mucoadhesive properties of various pectins on gastrointestinal mucosa: an in vitro evaluation using texture analyzer. *Eur. J. Pharm. Biopharm.* 67, 132-40.
141. Fransen, N., Bjork, E., Edsman, K. (2008) Changes in the mucoadhesion of powder formulations after drug application investigated with a simplified method. *J. Pharm. Sci.* 97, 3855-64.
142. Vishnu, Y., Chandrasekhar, K., Ramesh, G., Rao, Y. (2007) Development of mucoadhesive patches for buccal administration of carvedilol. *Curr Drug Deliv* 4, 27-39.
143. Khutoryanskiy, V. (2011) Advances in mucoadhesion and mucoadhesive polymers. *Macromol Biosci* 11, 748-64.
144. Park, C. R., Munday, D. L. (2004) Evaluation of selected polysaccharide excipients in buccoadhesive tablets for sustained release of nicotine. *Drug Dev Ind Pharm* 30, 609-17.
145. Piao, J., Lee, J. E., Weon, K. Y., Kim, D. W., Lee, J. S., Park, J. D., Nishiyama, Y., Fukui, I., Kim, J. S. (2009) Development of novel mucoadhesive pellets of metformin hydrochloride. *Arch Pharm Res* 32, 391-7.
146. Hagesaether, E., Hiorth, M., Sande, S. A. (2009) Mucoadhesion and drug permeability of free mixed films of pectin and chitosan: an in vitro and ex vivo study. *Eur J Pharm Biopharm* 71, 325-31.
147. Fransen, N., Bjork, E., Edsman, K. (2008) Changes in the mucoadhesion of powder formulations after drug application investigated with a simplified method. *J Pharm Sci* 97, 3855-64.
148. Kharenko, E., Larionova, N., Demina, N. (2008) Mucoadhesive Drug Delivery Systems: Quantitative Assessment of Interaction Between Synthetic and Natural Polymer Films and Mucosa. *Pharm. Chem. J.* 42, 392-9.
149. Kurskaya, E., Vainerman, E., Timofeeva, G., Rogozhin, S. (1980) A study of soluble complexes of acid-process and alkaline-process gelatins by turbidimetric titration. *Colloid & Polymer Science* 258, 1086-91.
150. Mathiowitz, E., Chickering, D., Lehr, C. Bioadhesive drug delivery systems : fundamentals, novel approaches, and development. New York: Marcel Dekker; 1999.
151. Dodou, D., Breedveld, P., Wieringa, P. (2005) Mucoadhesives in the gastrointestinal tract: revisiting the literature for novel applications. *Eur. J. Pharm. Biopharm.* 60, 1-16.
152. Yoshioka, S., Aso, Y., Otsuka, T., Kojima, S. (1995) Water mobility in poly(ethylene glycol)†-†, poly(vinylpyrrolidone)†-†, and gelatin†-†water systems, as indicated by dielectric relaxation time, spin†-†latice relaxation time, and water activity. *J. Pharm. Sci.* 84, 1072-7.

153. Thirawong, N., Nunthanid, J., Puttipipatkhachorn, S., Sriamornsak, P. (2007) Mucoadhesive properties of various pectins on gastrointestinal mucosa: An in vitro evaluation using texture analyzer. *Eur. J. Pharm. Biopharm.* 67, 132-40.
154. Rodríguez, R., Alvarez-Lorenzo, C., Concheiro, A. (2001) Rheological evaluation of the interactions between cationic celluloses and Carbopol 974P in water. *Biomacromolecules* 2, 886-93.
155. Edsman, K., Hagerstrom, H. (2005) Pharmaceutical applications of mucoadhesion for the non-oral routes. *J. Pharm. Pharmacol.* 57, 3-22.

CHAPTER 5

An *in vitro* cell adhesion assay to measure bioadhesion of mucoadhesive polymer

5.1 Introduction and objectives

The use of polymers as bioadhesives (adhering to epithelium) and mucoadhesive (adhering to mucus) offer significant potential for a prolonged and local drug release [156]. There are several intestinal models for a mucoadhesion study that adequately provide a mucus gel lining in the gastro intestinal tract to which adherence can be measured such as goat [157], pig [158], rabbit [159], and rat stomach [160]. For a bioadhesion study, samples were assessed by their binding to a plastic plate [161-164], and a silicone elastomer [165]. However adhesion to these preparations occurred in sub-optimal physiological conditions. To overcome this problem, cell adhesion assays have been introduced to measure bioadhesion. The cell adhesion properties of chitosan were intensively studied using several cell type such as fibroblast cells [166, 167], primary chick dorsal root ganglion cells [168] and human osteosarcoma cells [169]. The covering of a culture plate surface with gelatin was used to facilitate attachment of a variety of cell types for use in binding assays [170]. Cell culture techniques are useful for studies on bioadhesion and the method

described here can allow for testing in conditions that are similar to those in normal physiological condition with the cell monolayer represented the gastrointestinal environment. The physicochemical properties of biomaterials are important for cell adhesion. Modification of polymer architecture to bring about more specific properties relevant to wider range of application is a challenge for drug delivery systems.

In this study a cell culture technique was used to measure bioadhesion of a polymer and polymer blends using HT29 monolayers. HT29 monolayers are used as an *in vitro* cell culture model of the human intestinal epithelium, as these monolayers are not covered with a mucus layer, and therefore, adherence of the polymers to these monolayers can be considered as "bioadhesion". The objective of this study was to investigate the bioadhesion of polymers through the relative cell adhesion assay of HT29 cells on various polymers film including chitosan (C), poly(vinylpyrrolidone) (PVP), gelatin type A (GA), gelatin type B (GB), and polymer blends of C/PVP, C/GA, C/GB at various volume ratios.

5.2 Experimental methods

5.2.1 Materials

All materials and chemical reagents used were the same as those described in Section 2.2.1. All tissue culture reagents were from Gibco (Biosciences,

Dublin, Ireland). The ultra low attachment 96 well plate was from Corning Costar (Cat. #3474).

5.2.2 Cell culture

HT29 cells (passage 121–128) were obtained from the American Type Culture Collection (ATCC, VA, USA). The cells were grown and sub-cultured in the Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal calf serum, 1% non-essential amino acids and 1% L-glutamine at 5% CO_2, 95% O_2 at 37 °C.

5.2.3 Cell adhesion assay

All polymer and polymer blend solutions were prepared by the same method as in Section 2.2.2. All single polymers and polymer blend solutions (100 μL) were cast on an ultra low attachment 96 well plate (Corning Costar Cat. #3474). The plate was then dried in an oven at 40 °C for 8 h then neutralized with 100 μL of 0.05N NaOH and dried again in an oven at 40 °C for 2 h. Subsequently, 100 μL of trypsinize HT29 cell suspension was added in each well. The plates were incubated for 3 h to allow for cell attachment on the polymer. Afterwards, the cells were washed with phosphate buffered saline three times to remove all non-attached cells. Attached cells on the polymer were quantified using 3-(4,5-dimethyltiazol-2-yl)-2,5-

diphenyl-tetrazolium bromide (MTT) test. The procedures used are illustrated in Figure 5.1.

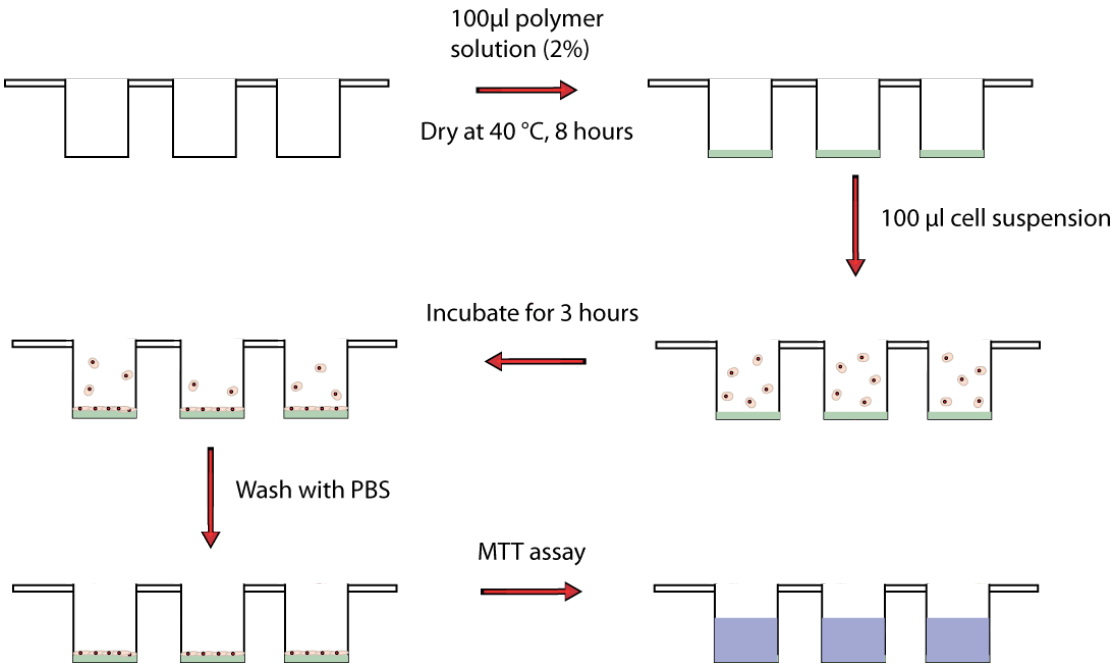


Figure 5.1 Method procedures for cell adhesion analysis

For the quantification assay of cell attachment, in brief, the MTT solution (100 µL) was added to each well and the plate was incubated for 3 h. The formation of a purple formazan from MTT by the mitochondrial reductase in a living cell was dissolved with dimethyl sulfoxide (DMSO) (100 µL) and quantified using the DTX-880 multimode detection microplate reader (Beckman Coulter, Fullerton, USA) at 570 nm. The schematic reaction for the formation of formazan is depicted in Figure 5.2. The percent relative cell attachment of HT29 cells onto a polymer film was calculated by subtracting the formazan values obtained using non-polymer coated

plates and comparing this with the formazan values obtained prior to washing that represented 100% cell attachment.

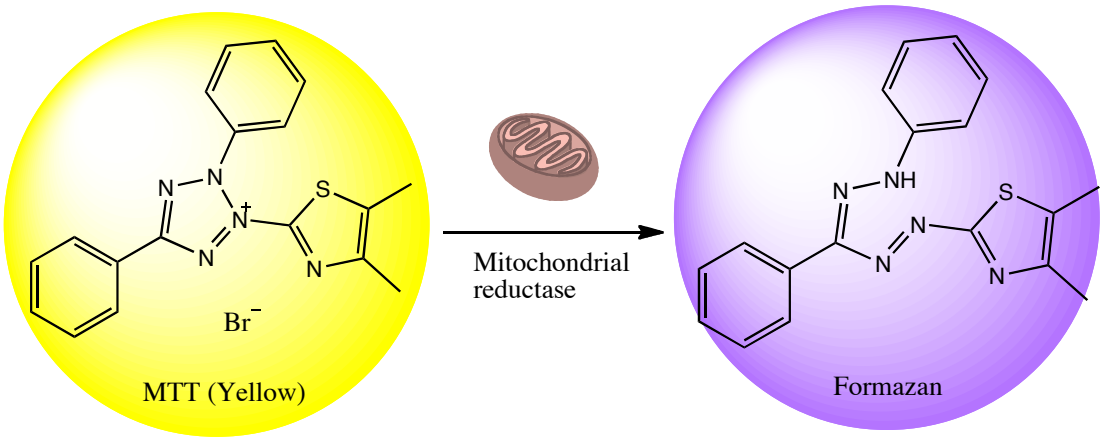


Figure 5.2 Schematic reaction of formazan formation by mitochondria reductase from living cells

5.2.4 Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS for Windows version 10.0 (SPSS Inc., USA). Post hoc testing ($p<0.05$) of the multiple comparisons was performed by Turkey's test.

5.3 Results and discussion

5.3.1 Bioadhesion of single polymers

The relative HT29 cell adhesion to chitosan, PVP, gelatin A, and gelatin B compared with control group (with no film) is shown in Figure 5.3. HT29 cells adhesion is higher for chitosan film than for PVP, and gelatin. Chitosan has been reported to support cell adhesion so it is not surprising that chitosan shows the highest cell adhesion [171, 172]. Although PVP has not been reported to have bioadhesive properties, this result demonstrated some bioadhesion to PVP. PVP is also widely used in mucoadhesive formulations however it allows for only poor attachment of cells to a surface film [173, 174]. PVP films provide a poor surface for binding of HT29 cells possibly due to it properties. PVP film is more easily soluble and swollen with water than chitosan and gelatin thus HT29 cell interactions with PVP may be lower. Gelatin possesses some bioadhesive characteristic and there have been some reports that cell adhesion on unmodified gelatin film is weak [175]. Two types of gelatin used (Type A and B) produced similar results for adhesion of HT29 cell and these were in agreement with results observed for adhesion of fibroblast cells on a gelatin scaffold [176]. However bioadhesive materials with unmodified physicochemical properties can probably interact with a cell or a soft tissue through hydrogen bonding between their functional groups of biomaterials and amino or hydroxyl group of the cell molecules [175]. The result in this study indicated that for

the single unmodified bioadhesive polymer, the rank orders of the relative HT29 cell adhesion were chitosan > gelatin type B or gelatin type A > PVP.*

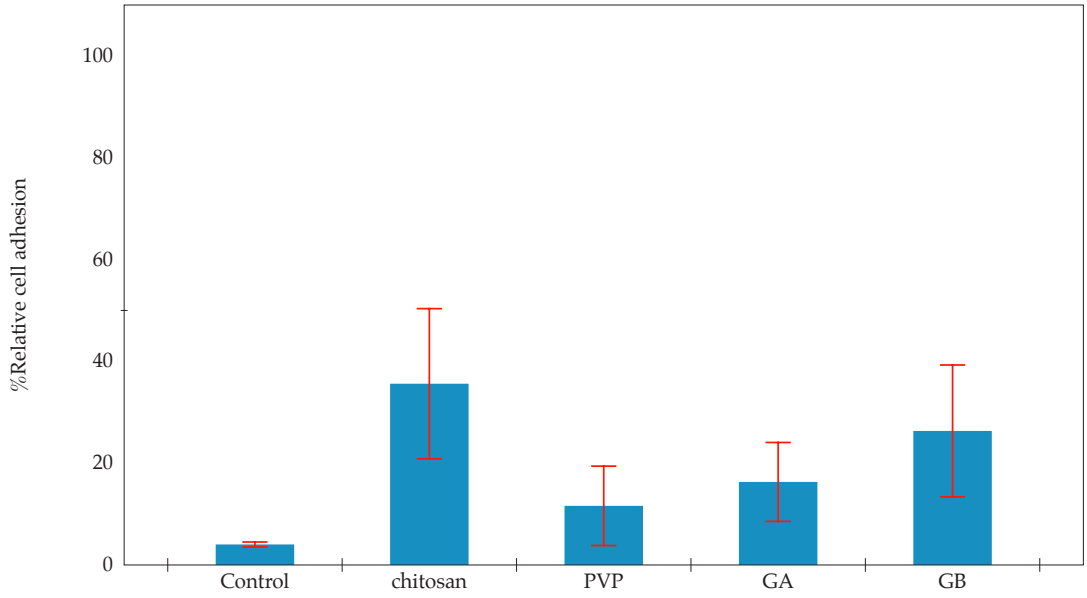


Figure 5.3 Percent relative HT29 cell attachment on single polymer films of chitosan, poly(vinylpyrrolidone) (PVP), gelatin type A (GA) and gelatin type B (GB) compared to the control (n=4) *$P < 0.05$

5.3.2 Bioadhesion of polymer blends

The relative cell adhesion to the polymer blends of C/PVP, C/GA, and C/GB are shown in Figure 5.4, 5.5, and 5.6, respectively. Some of the polymer blends had more cells attached than for the single polymers. The relative cell adhesion of HT29 on polymer blend films of C/PVP, C/GA, and C/GB were similar. The C/PVP blends at a volume ratio of 5/5 produce the highest bioadhesion. From the results of

the viscosity study (Chapter 2) and texture analyzer study (Chapter 4), chitosan and PVP at volume ratio of 5/5 also produced excellent mucoadhesion. In addition, PVP had a mucoadhesion property and the mucoadhesion was enhanced when it was blended with other polymers [177, 178]. In this case, PVP blended with chitosan also had enhanced bioadhesion.

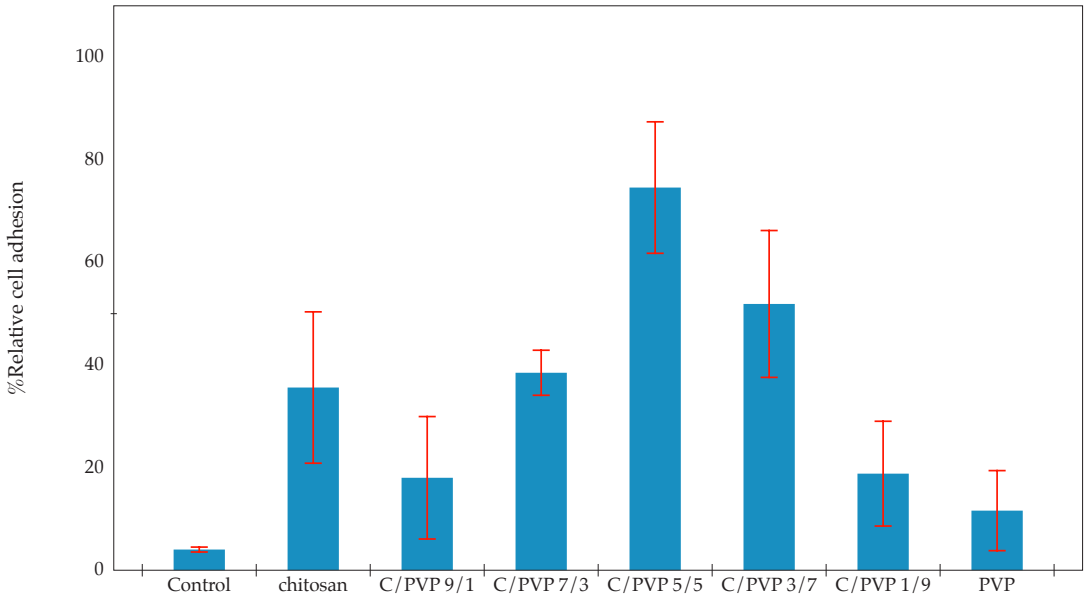


Figure 5.4 Percent relative HT29 cell attachment on chitosan (C), poly(vinylpyrro-lidone) (PVP) and their blends of C/PVP film compared with control (n=4) *$P < 0.05$

C/GA and C/GB blends at a volume ratio of 3/7 produced the highest cell attachment. Although C/GB at a volume ratio of 3/7 produced the highest cell adhesion but did not represent any significant difference compared to the single polymer, this result demonstrated the potential of using polymer blend for enhancing

*

bioadhesive properties. This result indicated that polymer blends of chitosan and gelatin promote cell adhesion in term of “bioadhesion", although, they have weak adhesion to mucus. These results of the ability of polymer blends of C/GA or C/GB to enhance cell attachment have been previously reported [179]. The improving of biological activity or enhancement of cell adhesion by chitosan blended with gelatin may be due to the fact that gelatin containing Arg – Gly – Asp (RGD)-like sequences can promotes cell adhesion and migration by forming a polyelectrolyte complex [179]. Furthermore, chitosan and gelatin blends also have been reported to have excellent biocompatibility with osteblastic cell cultures [180].

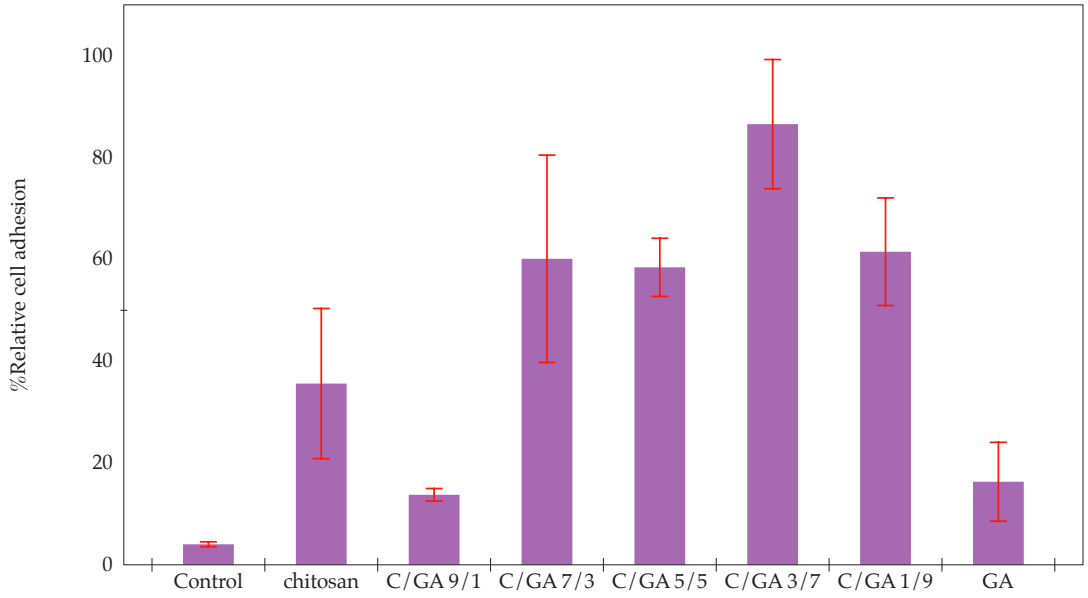


Figure 5.5 Percent relative HT29 cell attachment on chitosan (C), gelatin type A (GA) and their blends of C/GA film compared with control (n=4) *$P < 0.05$

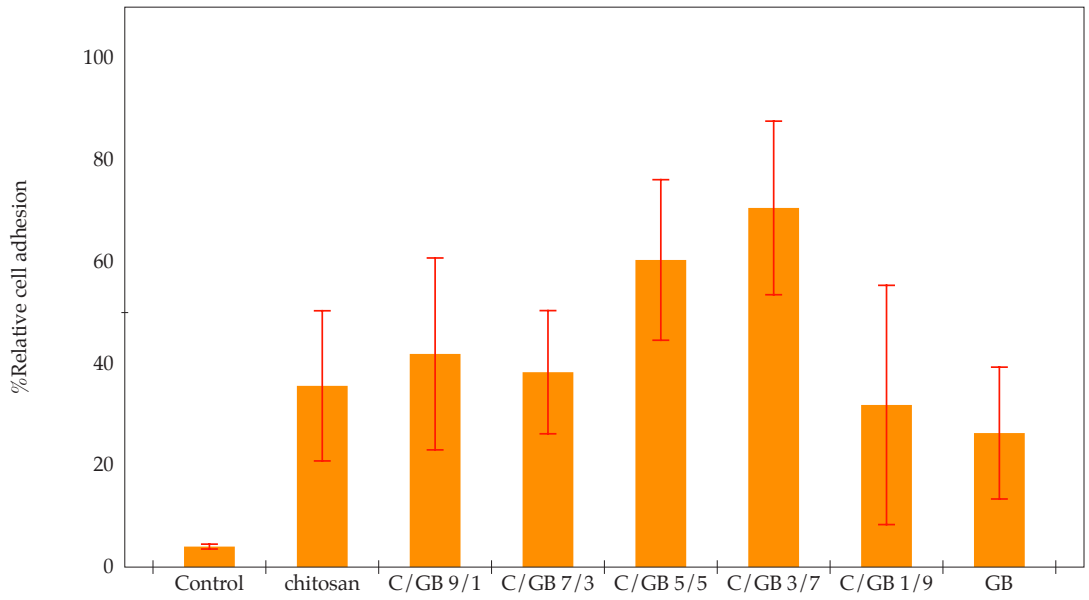


Figure 5.6 Percent relative HT29 cell attachment on chitosan (C), gelatin type B (GB) and their blends of C/GB film compared with control (n=4) *$P < 0.05$

5.4 Conclusions

The modified bioadhesive polymers by blending with other polymer can enhance cell adhesion when compare with the single polymer [181-183]. Although polymer blends of chitosan and gelatin show excellent bioadhesive properties, C/PVP blends are excellent for improving both mucoadhesive and bioadhesive properties and have been selected for further studies.

References

156. Keely, S., Rullay, A., Wilson, C., Carmichael, A., Carrington, S., Corfield, A., Haddleton, D., Brayden, D. (2005) In vitro and ex vivo intestinal tissue models to measure mucoadhesion of poly (methacrylate) and N-trimethylated chitosan polymers. *Pharm. Res.* 22, 38-49.
157. Badhan, A., Mashru, R., Shah, P. P., Thakkar, A., Dobaria, N. (2009) Development and evaluation of sustained release gastroretentive minimatrices for effective treatment of H. pylori infection. *AAPS PharmSciTech* 10, 459-67.
158. Riley, R., Smart, J., Tsibouklis, J., Young, S., Hampson, F., Davis, A., Kelly, G., Dettmar, P., Wilber, W. (2002) An in vitro model for investigating the gastric mucosal retention of 14C-labelled poly(acrylic acid) dispersions. *Int. J. Pharm.* 236, 87-96.
159. Mahrag, K., Ch'ng, H. (1998) Evaluation of possible mechanism(s) of bioadhesion. *Int. J. Pharm.* 160, 61-74.
160. Liu, Z., Lu, W., Qian, L., Zhang, X., Zeng, P., Pan, J. (2005) In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. *J. Controlled Release* 102, 135-44.
161. Ahn, J.-S., Choi, H.-K., Chun, M.-K., Ryu, J.-M., Jung, J.-H., Kim, Y.-U., Cho, C.-S. (2002) Release of triamcinolone acetonide from mucoadhesive polymer composed of chitosan and poly(acrylic acid) in vitro. *Biomaterials* 23, 1411-6.
162. Chun, M., Cho, C., Choi, H. (2005) Mucoadhesive microspheres prepared by interpolymer complexation and solvent diffusion method. *Int. J. Pharm.* 288, 295-303.
163. Chun, M., Cho, C., Choi, H. (2001) A novel mucoadhesive polymer prepared by template polymerization of acrylic acid in the presence of poloxamer. *J. Appl. Polym. Sci.* 79, 1525-30.
164. Kim, T., Ahn, J., Choi, H., Choi, Y., Cho, C. (2007) A Novel Mucoadhesive Polymer Film Composed of Carbopol, Poloxamer and Hydroxypropylmethylcellulose. *Arch. Pharmacal Res.* 30, 381-6.
165. Hao, J., Chan, L., Shen, Z., Heng, P. (2005) Complexation Between PVP and Gantrez Polymer and Its Effect on Release and Bioadhesive Properties of the Composite PVP/Gantrez Films. *Pharm. Dev. Technol.* 9, 379 - 86.
166. Zhu, A., Wang, S., Yuan, Y., Shen, J. (2002) Cell adhesion behavior of chitosan surface modified by bonding 2-methacryloyloxyethyl phosphorylcholine. *Journal of biomaterials science. Polymer edition* 13, 501-10.
167. Bettini, R., Romani, A. A., Morganti, M. M., Borghetti, A. F. (2008) Physicochemical and cell adhesion properties of chitosan films prepared from sugar and phosphate-containing solutions. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* 68, 74-81.
168. Freier, T., Koh, H., Kazazian, K., Shoichet, M. (2005) Controlling cell adhesion and degradation of chitosan films by N-acetylation. *Biomaterials* 26, 5872-8.
169. Sailaja, G., Ramesh, P., Kumary, T., Varma, H. (2006) Human osteosarcoma cell adhesion behaviour on hydroxyapatite integrated chitosan-poly(acrylic acid) polyelectrolyte complex. *Acta Biomater* 2, 651-7.

170. Sigma-Aldrich. Attachment and Matrix Factors. BioFiles. 2008:4 - 13.
171. Freier, T., Koh, H., Kazazian, K., Shoichet, M. (2005) Controlling cell adhesion and degradation of chitosan films by N-acetylation. *Biomaterials* 26, 5872-8.
172. Chatelet, C., Damour, O., Domard, A. (2001) Influence of the degree of acetylation on some biological properties of chitosan films. *Biomaterials* 22, 261-8.
173. Jones, D., Lawlor, M., Woolfson, A. (2003) Rheological and mucoadhesive characterization of polymeric systems composed of poly(methylvinylether-co-maleic anhydride) and poly(vinylpyrrolidone), designed as platforms for topical drug delivery. *J. Pharm. Sci.* 92, 995-1007.
174. Bruschi, M., Jones, D., Panzeri, H., Gremião, M., de Freitas, O., Lara, E. (2007) Semisolid systems containing propolis for the treatment of periodontal disease: In Vitro release kinetics, syringeability, rheological, textural, and mucoadhesive properties. *J. Pharm. Sci.* 96, 2074-89.
175. Matsuda, S., Iwata, H., Se, N., Ikada, Y. (1999) Bioadhesion of gelatin films crosslinked with glutaraldehyde. *J. Biomed. Mater. Res.* 45, 20-7.
176. Ratanavaraporn, J., Damrongsakkul, S., Sanchavanakit, N., Banaprasert, T., Kanokpanont, S. (2006) Comparison of Gelatin and Collagen Scaffolds for Fibroblast Cell Culture. *Journal of Metals, Materials and Minerals.* 16, 31 - 6.
177. Karavas, E., Georgarakis, E., Bikiaris, D. (2006) Application of PVP/HPMC miscible blends with enhanced mucoadhesive properties for adjusting drug release in predictable pulsatile chronotherapeutics. *Eur. J. Pharm. Biopharm.* 64, 115-26.
178. Jones, D., Woolfson, A., Brown, A., Coulter, W., McClelland, C., Irwin, C. (2000) Design, characterisation and preliminary clinical evaluation of a novel mucoadhesive topical formulation containing tetracycline for the treatment of periodontal disease. *J Control Release* 67, 357-68.
179. Huang, Y., Onyeri, S., Siewe, M., Moshfeghian, A., Madihally, S. (2005) In vitro characterization of chitosan-gelatin scaffolds for tissue engineering. *Biomaterials* 26, 7616-27.
180. Nagahama, H., Maeda, H., Kashiki, T., Jayakumar, R., Furuike, T., Tamura, H. (2009) Preparation and characterization of novel chitosan/gelatin membranes using chitosan hydrogel. *Carbohydr. Polym.* 76, 255-60.
181. Zhu, A., Wang, S., Yuan, Y., Shen, J. (2002) Cell adhesion behavior of chitosan surface modified by bonding 2-methacryloyloxyethyl phosphorylcholine. *J. Biomater. Sci. Polym. Ed.* 13, 501-10.
182. Bettini, R., Romani, A., Morganti, M., Borghetti, A. (2008) Physicochemical and cell adhesion properties of chitosan films prepared from sugar and phosphate-containing solutions. *Eur. J. Pharm. Biopharm.* 68, 74-81.
183. Pulieri, E., Chiono, V., Ciardelli, G., Vozzi, G., Ahluwalia, A., Domenici, C., Vozzi, F., Giusti, P. (2008) Chitosan/gelatin blends for biomedical applications. *J Biomed Mater Res A* 86, 311-22.

CHAPTER 6

Amoxicillin mucoadhesive bead preparation and properties

6.1 Introduction and objectives

Amoxicillin is an antibacterial drug used against *Helicobacter pylori* in triple-line drug therapy. Due to the two main causes for drug ineffectiveness include the instability of some antibiotics at the low pH of the gastric acid and short residence time of the antibiotic in the stomach [184]. In order to improve the efficacy of anti *H. pylori* agents, the residence time of the drug in the stomach should be extended. Kimura *et al.* successfully treated patients to eliminate *H. pylori* infection by using a balloon catheter for retention of the drugs by the stomach for up to 1 h [185]. Several researchers have proposed and developed a local drug delivery in the stomach such as a floating-bioadhesive formulation [186, 187], bioadhesive microspheres [188, 189], mucoadhesive beads [190]. These drug delivery systems can increase the gastric residence time of anti *H. pylori* agents and allow more contact time of the drugs so they can penetrate through the gastric mucus layer and act locally at the infectious sites. Mucoadhesive beads can be used to increase the gastro-retentive time of the drugs and consequently they may be able to improve the efficacy against *H. pylori*. In addition in an attempt to increase the stability of amoxicillin, concomitant use of a proton pump inhibitor or H_2-receptor antagonist such as omeprazole, lanzoprazole,

cimetidine or ranitidine according to the triple-line drug therapy are required. These agents can raise the gastric pH to 3 – 5, hence, the buffer solution used in this study was at pH 4.

Amoxicillin is the β−lactam antibiotic that is useful and frequently prescribed antimicrobial agents with the mechanism of action: inhibition of synthesis of the bacterial peptidoglycan cell wall [191]. The structure of amoxicillin is shown in Figure 6.1. Amoxicillin is off-white crystalline powder with water solubility of 4 mg/ml, instable in the acidic pH and most stable in aqueous solutions of pH 4 – 7 [192, 193].

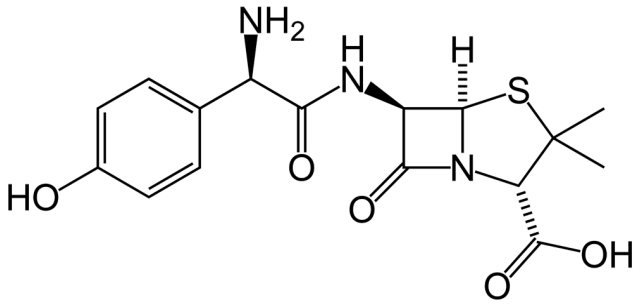


Figure 6.1 Chemical structure of amoxicillin

In this study mucoadhesive beads were prepared using alginate as the matrix, followed by coating them with chitosan, PVP, and a polymer blend of C/PVP. In this study amoxicillin was used as the model drug. The drug loading capacity was investigated, and morphology was determined using a scanning electron microscope (SEM).

6.2 Experimental methods

6.2.1 Materials

All materials and chemical reagents used were the same as those described in Section 2.2.1. Alginic acid sodium salt from brown algae with a viscosity (2% solution at 25°C) of 250 cps, and amoxicillin trihydrate (AMX) were purchased from Sigma (St. Louis, MO, USA). All reagents were of analytical grade.

6.2.2 Mucoadhesive bead preparations

Coating solutions of chitosan, PVP, and chitosan – PVP blends at a 1.5% w/v were prepared by dissolving 1.5 g of chitosan in 0.05 M hydrochloric acid solution to 100 mL. An amount of 1.5 g of PVP was dissolved in 100 mL of water to obtain a final concentration of 1.5% w/v. Chitosan and PVP solutions were continually stirred for 4 h until completely dissolved. Polymer blends of C/PVP were prepared by mixing the 1.5% w/v of polymer solutions in the volume ratios of 1/9, 3/7, 5/5, 7/3, and 9/1. All polymer blends were gently mixed using a reciprocating shaker until homogeneous.

Alginate beads were prepared as previously described with some modifications [190]. In brief, the alginate solution was prepared by stirring the mixture of alginate (2.0 g) and water (100 mL) for 4 h. AMX (5.0 g) was then dispersed in the alginate solution and the mixture was continually stirred for 20 min.

Subsequently, the mixture was dropped into a gently agitated 2% w/v solution of calcium chloride using a syringe with the needle gauge number of 23. After continuous stirring for 10 min, the beads were separated by filtration and washed with water and dried in an oven at 40 °C for 8 h. Afterward, the dried alginate beads were coated with 1.5% w/v of chitosan, PVP or C/PVP blend solutions by immersing dried bead into these solutions for 10 min. Subsequently, the coated beads were again dried in the oven at 40 °C for 8 h. A schematic of the preparation procedure for the amoxicillin loaded mucoadhesive bead is depicted in Figure 6.2. All of AMX beads were kept in desiccator before further study.

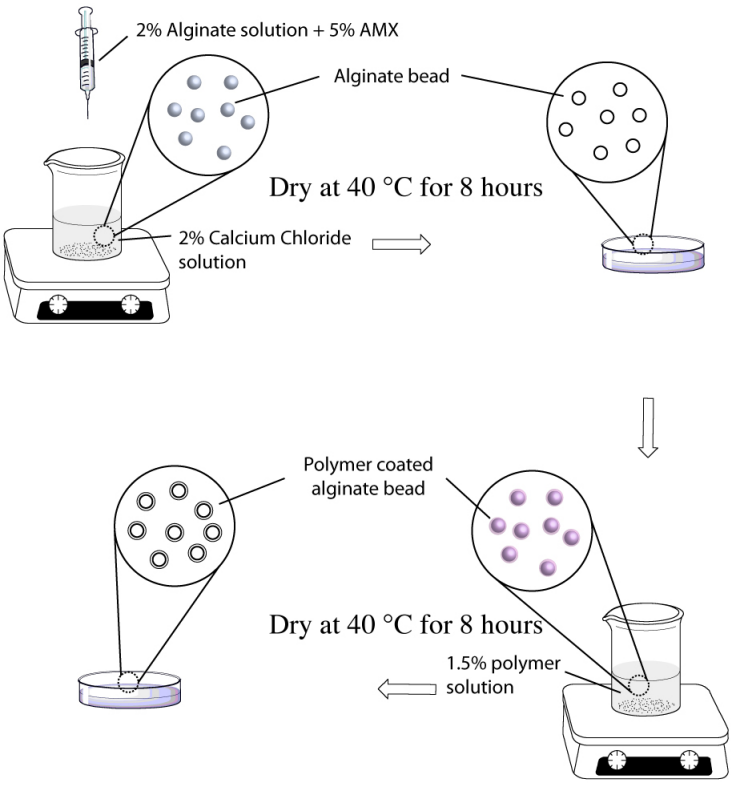


Figure 6.2 Preparation procedures for amoxicillin (AMX) loaded mucoadhesive beads

6.2.3 Drug loading capacity

The AMX loading capacity in the alginate bead was quantitatively determined by immersing 0.015 g of dried beads in 80 mL of pH 4 phosphate buffer prepared with 0.1 M KH_2PO_4, using either KOH or phosphoric acid to adjust the pH to 4.0, to dissolve the AMX dispersed inside the bead. AMX beads were continually stirred using a magnetic stirrer for 5 h then sonicated for 20 min. The magnetic bar was removed, thoroughly rinsed, and the phosphate buffer was added to 100 mL. The solution was collected and the AMX content was analyzed using 8452A HP Diode Array spectrophotometer (Hewlett Packard, California, USA) at 230 nm. Moreover high performance liquid chromatography (HPLC) was also used to check the degradation products of AMX. HPLC analyse was perform on Jasco PU-2080 single pump equipped with Jasco UV-1575 photodiode array detector using Ascentis C18 HPLC column (15cm x 4.6 mm, 5 μm). All chromatogram were obtained using Water 740 Data Module integrator system. The mobile phase for HPLC system was prepared in according to the United State Pharmacopeia (USP33) [194], in brief, 6.8 g/L of monobasic potassium phosphate in water at pH 5 was mixed with acetonitrile to volume ratio of 24:1. AMX was detected using a maximum wavelength of 230 nm with flow rate of 1.0 mL/min and injection volume of 20 μL. Standard solutions of AMX for analyse with UV and HPLC techniques were prepared in the concentration range of 0.4 – 200 μg/mL and covered all of samples concentration. All the experiments were carried out in triplicate. The percentage of drug loading was calculated using the following equation.

$$\%\,Drug\ loading = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} \times 100 \quad (1)$$

6.2.4 Scanning electron microscope (SEM)

The morphology of the uncoated and coated AMX beads was observed using a JSM-6400 scanning electron microscope (Jeol, Tokyo, Japan) at an accelerated voltage of 20 kV. The samples were mounted on metal stubs using a double-sided adhesive tape. All samples were coated with gold using a direct current sputtering technique.

6.2.5 Bead size analysis

Uncoated AMX beads were dispersed in methanol and the diameter of uncoated AMX beads were measured with a Beckman Coulter LS230 equipped with a Small-Volume Module Plus and Beckman Coulter Particle Characterization software version 3.29 (USA). All experiment was performed in triplicate.

6.2.6 Swelling study of dried beads

Swelling studies of uncoated and coated AMX dried beads were performed in 0.1M phosphate buffer, pH 4, at 37.0 ± 0.5 °C. An accurate weight of the beads of about 15.2 – 16.40 g was immersed in a buffer solution with slight agitation with a shaker. The beads were removed periodically from the solution, blotted to remove excess liquid, and weighed on an electronic balance. The swelling ratio or percentage of weight change was determined using the following equation [195, 196]:

$$\%\ weight\ change = \frac{W_t - W_d}{W_t} \times 100 \qquad (2)$$

where W_t is the weight of the swollen beads at time *t*, and W_d is the weight of dried beads. The experiments were performed in triplicate.

6.2.7 Mucoadhesive properties of AMX beads using wash-off method

An in vitro evaluation of the mucoadhesive properties of the AMX coated bead was carried out using the wash-off method from porcine stomach tissue. The stomach tissue from a freshly slaughtered pig was washed with physiological saline and attached to a microscopic slide. Thirty AMX coated beads were spread in contact with the stomach using a pressure of 25 g on the microscopic slide for 2 min

[197]. The mucoadhesive property of the polymer coated bead was measured by connecting the prepared slide with the arm of a QC-21 disintegration test system (Hanson Research, Chatsworth, USA). AMX coated beads were forced to wash off under the reciprocating motion of the disintegration apparatus in 8000 mL phosphate buffer, pH 4.0, at 37 ± 0.5 °C. This test was performed for 3 h and the number of AMX coated bead remaining attached to the porcine stomach was counted every 30 min.

6.2.8 Statistical analysis

Analysis of variance (ANOVA) was performed using the SPSS version 10 for Windows (SPSS Inc., USA). *Post hoc* testing ($p<0.05$) of the multiple comparisons was performed by Tukey's test. A profile analysis of the bead swelling and wash-off was analyzed using multivariate ANOVA (MANOVA) with repeated measurements. In these models, the percentage weight change and percentage beads remaining were dependent variables, different groups were the independent variable and time was the repeated factor. Firstly, MANOVA was applied when the hypothesis on groups was tested from the means or level of the curve profiles and the hypothesis on time x groups interaction was interpreted as parallelism or shape of the curve profiles. The Wilks lambda statistic was preferred to obtain *p-values* in the MANOVA test [198-200]. Subsequently, Tukey's *post hoc* multiple comparison tests were performed for the average across the levels of the within-subject factors. For multivariations, the *post hoc* tests were performed for each dependent variable

separately [201]. For the second step, if there were any significantly differences shown from the MANOVA, ANOVA was applied to test separately at each time point to see the differences between the groups.

6.3 Results and discussion

6.3.1 Drug loading capacity

The stability of amoxicillin has been reported at several pH values of buffered aqueous solutions including gastric juice. Amoxicillin was most stable at a pH of between 4 – 7 with half-lives of more than 153.1 h [192]. Thus the analysis of amoxicillin after extraction in pH 4 phosphate buffer for 5 h was performed by UV-visible spectrophotometry. The standard curves of AMX for UV and HPLC system are shown in Figure 6.3(A) and 6.3(B), respectively. The UV spectra of standard and sample AMX solutions were shown in Figure 6.4(A) and 6.4(B), respectively. In addition the chromatogram of AMX solution did not show any degradation peak of AMX as shown in Figure 6.5. Drug loading of the AMX bead, calculated based on the uncoated beads, was 76.49%.

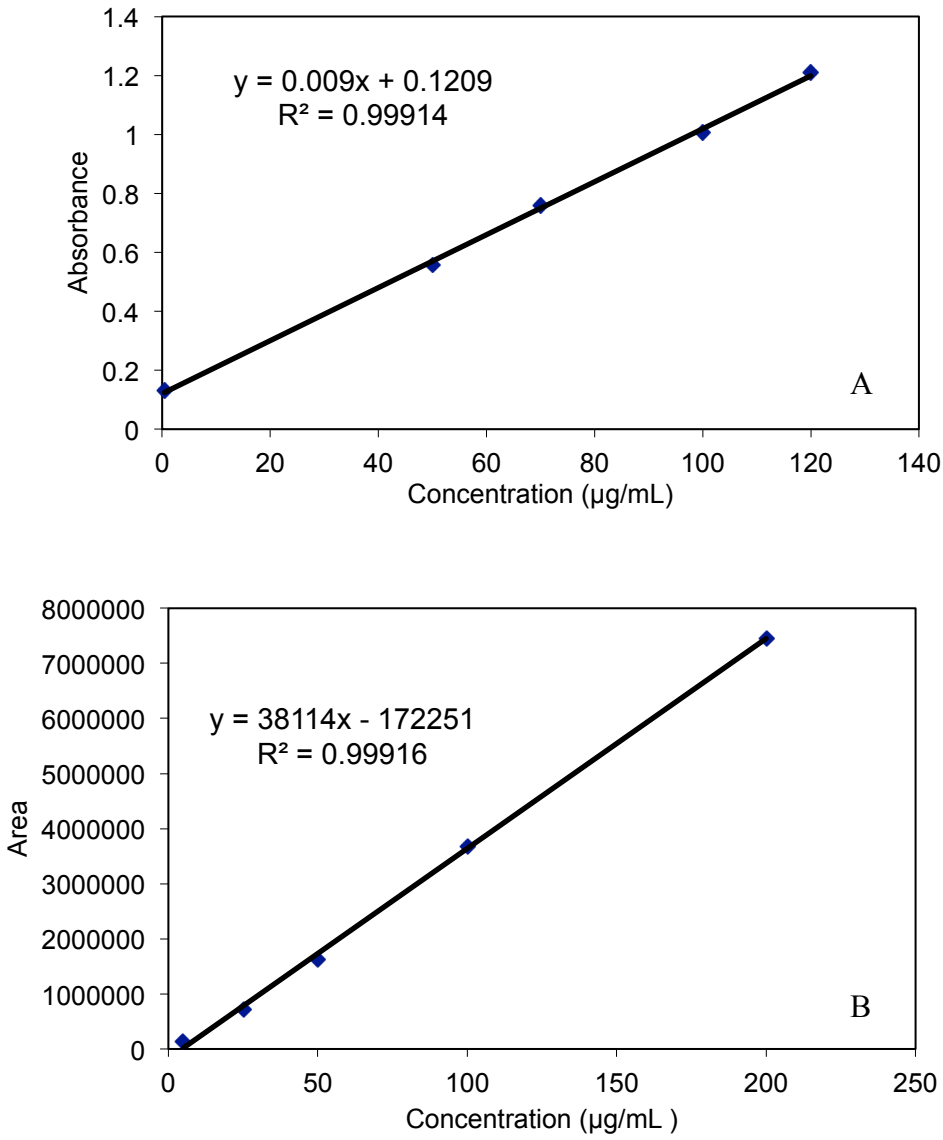


Figure 6.3 The standard curve of amoxicillin solution obtained from UV-visible spectrophotometer (A) and high performance liquid chromatography (HPLC) (B)

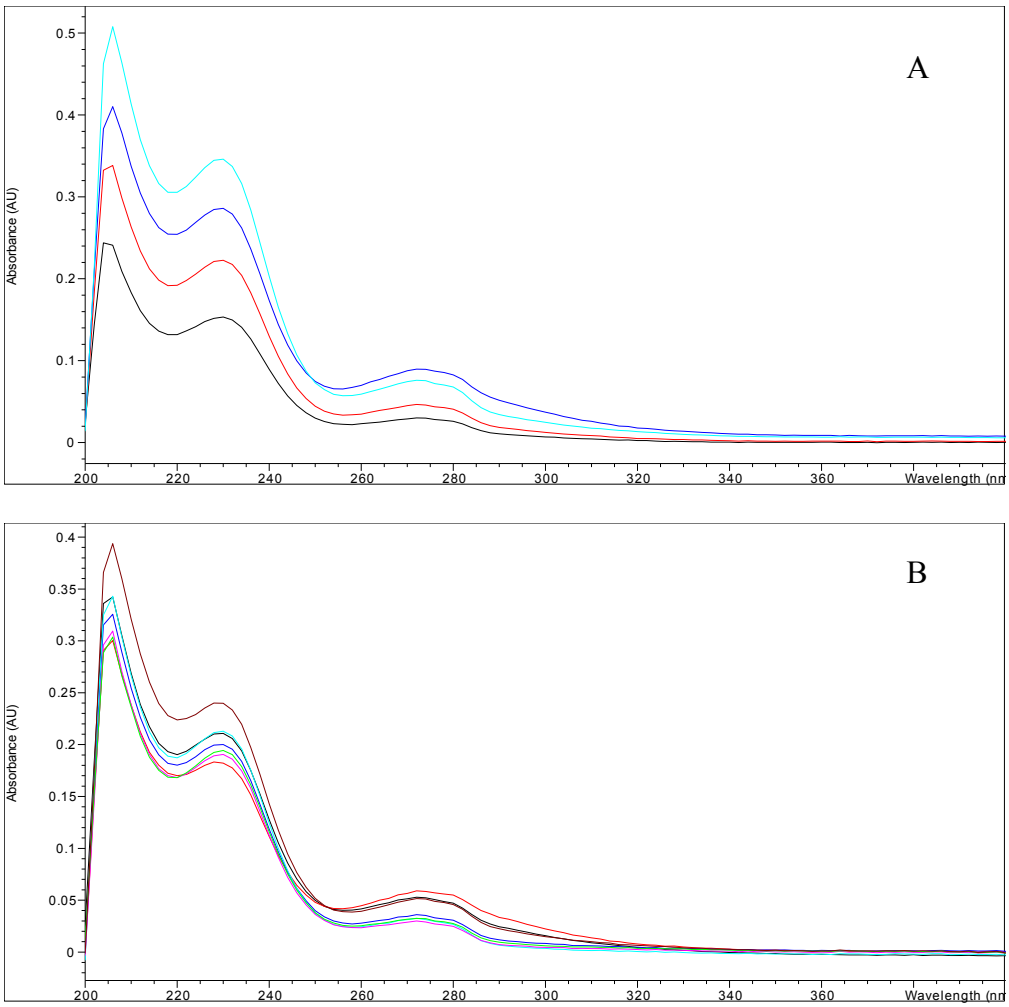


Figure 6.4 The UV spectra of standard (A) and sample (B) of amoxicillin

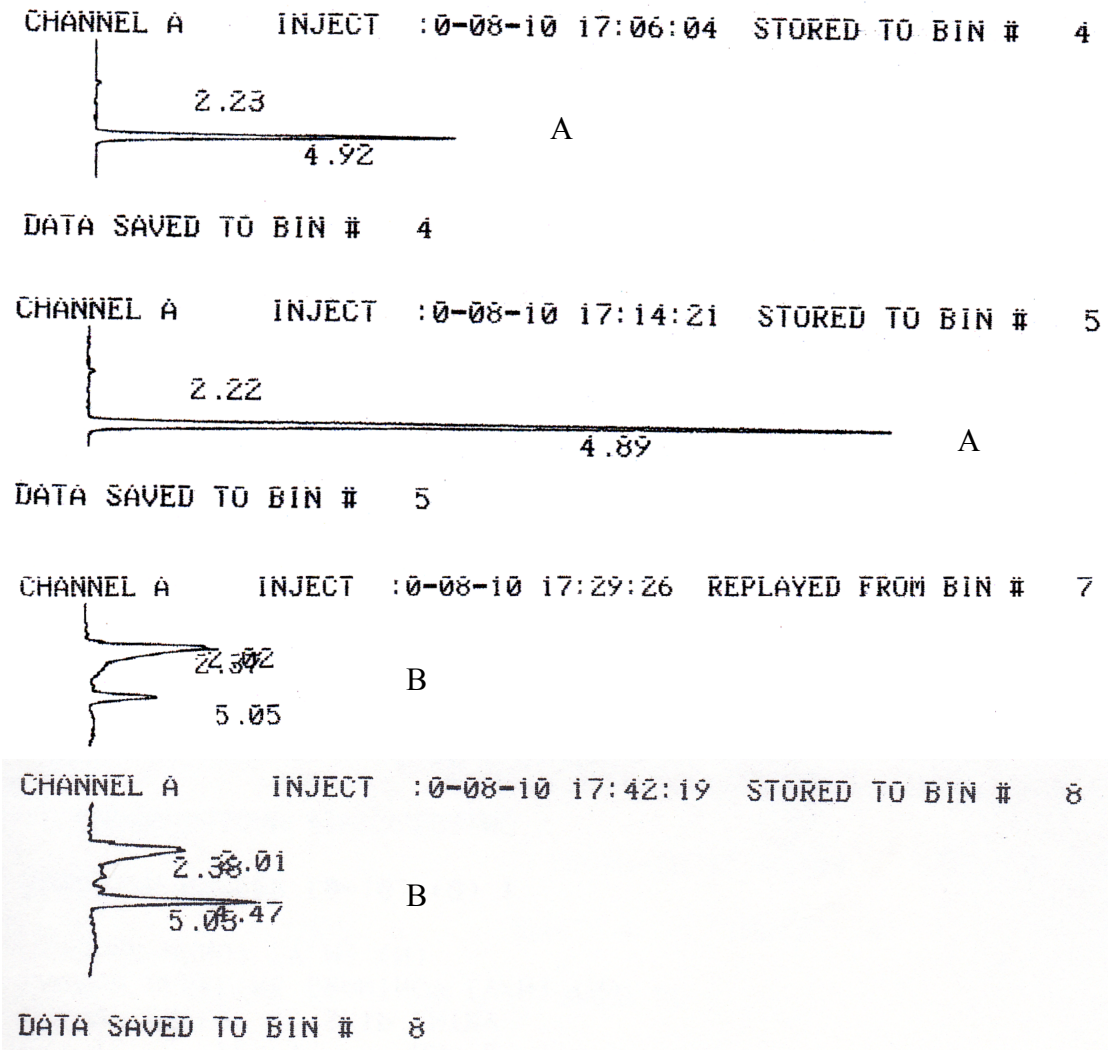


Figure 6.5 Chromatograms of standard (A) and sample (B) of amoxicillin at different concentration

6.3.2 Uncoated and coated amoxicillin bead morphology

Alginate beads were formed in the presence of calcium ions (Ca^{2+}) at junctions in the G-G sequence rich chain region, which is called the "egg box" junction as depicted in Figure 6.6. As a result, the calcium-alginate beads shrank in

size when dried [202]. The AMX wet beads just after preparation were found to be spherical in shape, but upon drying in air at room temperature their spherical nature was lost, as is evident from the SEM in Figure 6.7.

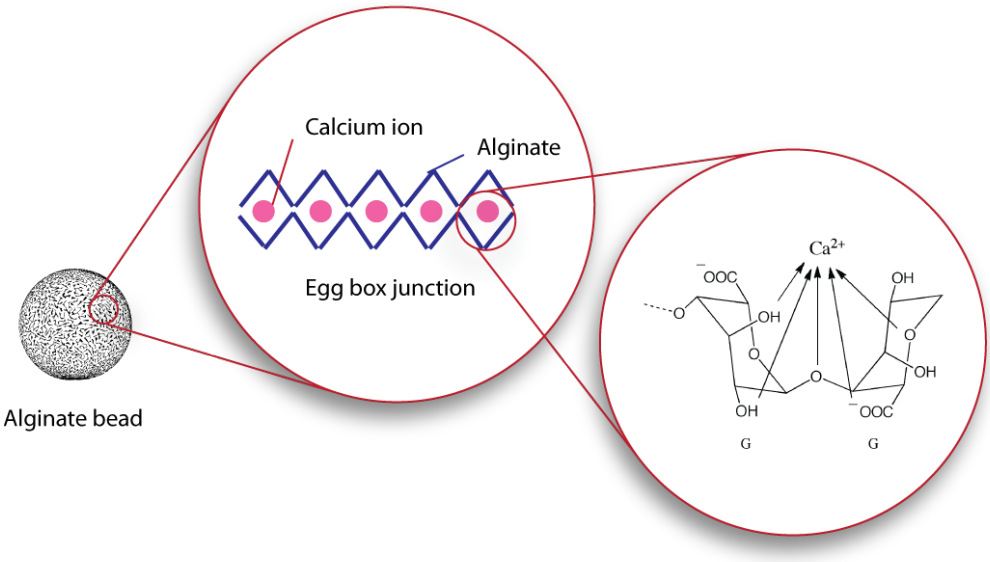


Figure 6.6 Schematic of calcium-alginate bead formation

The diameter of the uncoated AMX beads were measured with a Beckman Coulter LS230 equipped with a Small-Volume Module Plus and Beckman Coulter Particle Characterization software version 3.29 (USA) was 1.23 ± 0.25 mm. SEM micrographs of uncoated and coated beads are shown in Figure 6.7 and Figure 6.8, respectively. The uncoated beads had a rough surface (Figure 6.7), whereas, the surfaces of coated beads with C/PVP blend, chitosan, and PVP were smooth (Figs 6.8 a-g). The smooth surface is evidence for the presence of a polymer film that was coated on the surface of the alginate bead. Furthermore, there is no visible porous

characterisic for either uncoated or coated beads as viewed by SEM micrographs. This result is in agreement with Elzatahry *et al.* [190], where C - PVP coated alginate beads showed a smoother surface than non-coated beads.

The poly-cationic nature of the chitosan molecule leads to a strong interaction with negatively charged alginate. The electrostatic interaction of the carboxylic acid groups of alginate with the amine group of chitosan form when the alginate is dropped into the chitosan solution and results in the formation of a membrane. PVP also has a hydrogen bonding interaction with chitosan and alginate thus the interaction of the coating polymer solution on the AMX beads can be formed by ionic interactions between the anionic alginate and the cationic chitosan and hydrogen bonding between the alginate and PVP. The proposed binding mechanism of coated AMX beads can be described by the schematic interactions between alginate and chitosan or PVP at the interphasic membrane as shown in Figure 6.9 [203].

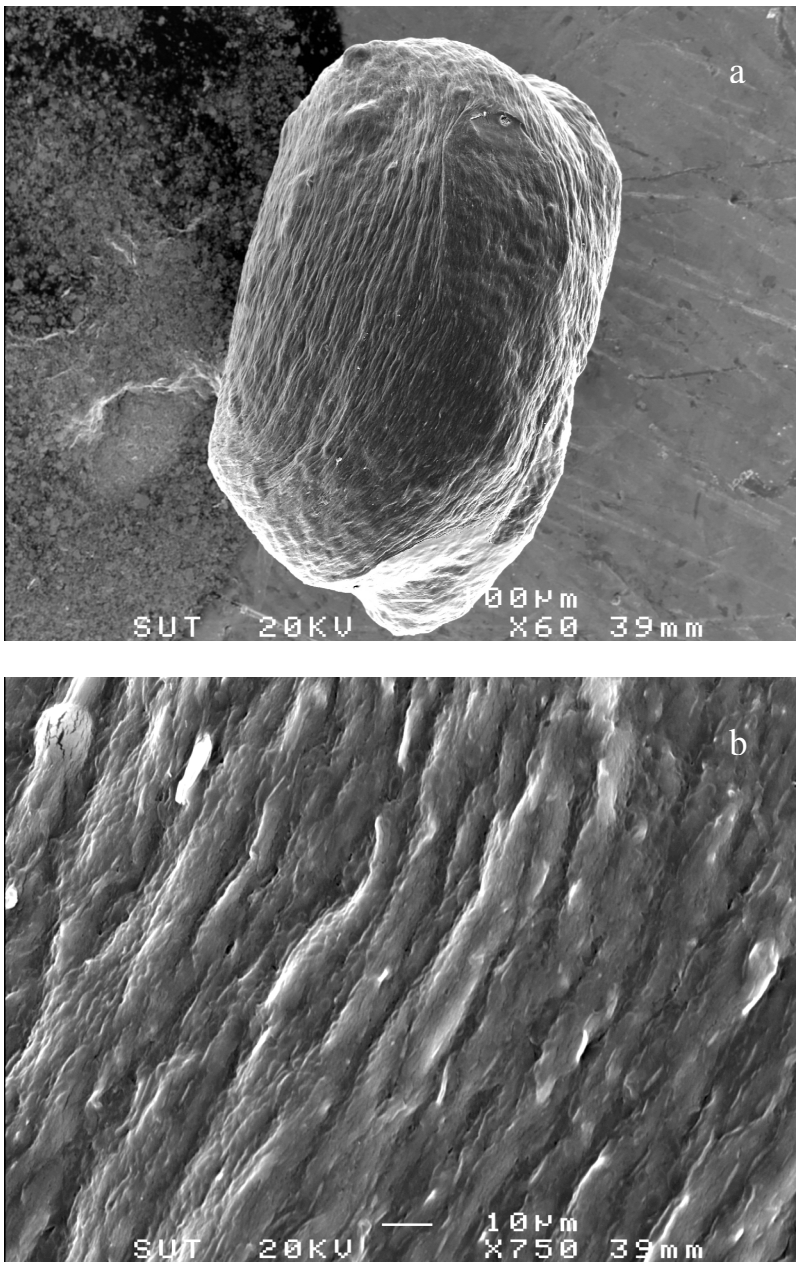


Figure 6.7 Scanning electron micrographs of amoxicillin (AMX) uncoated bead with slighly oval shape of AMX bead (X60) (a) and surface of AMX uncoated bead (X750) (b)

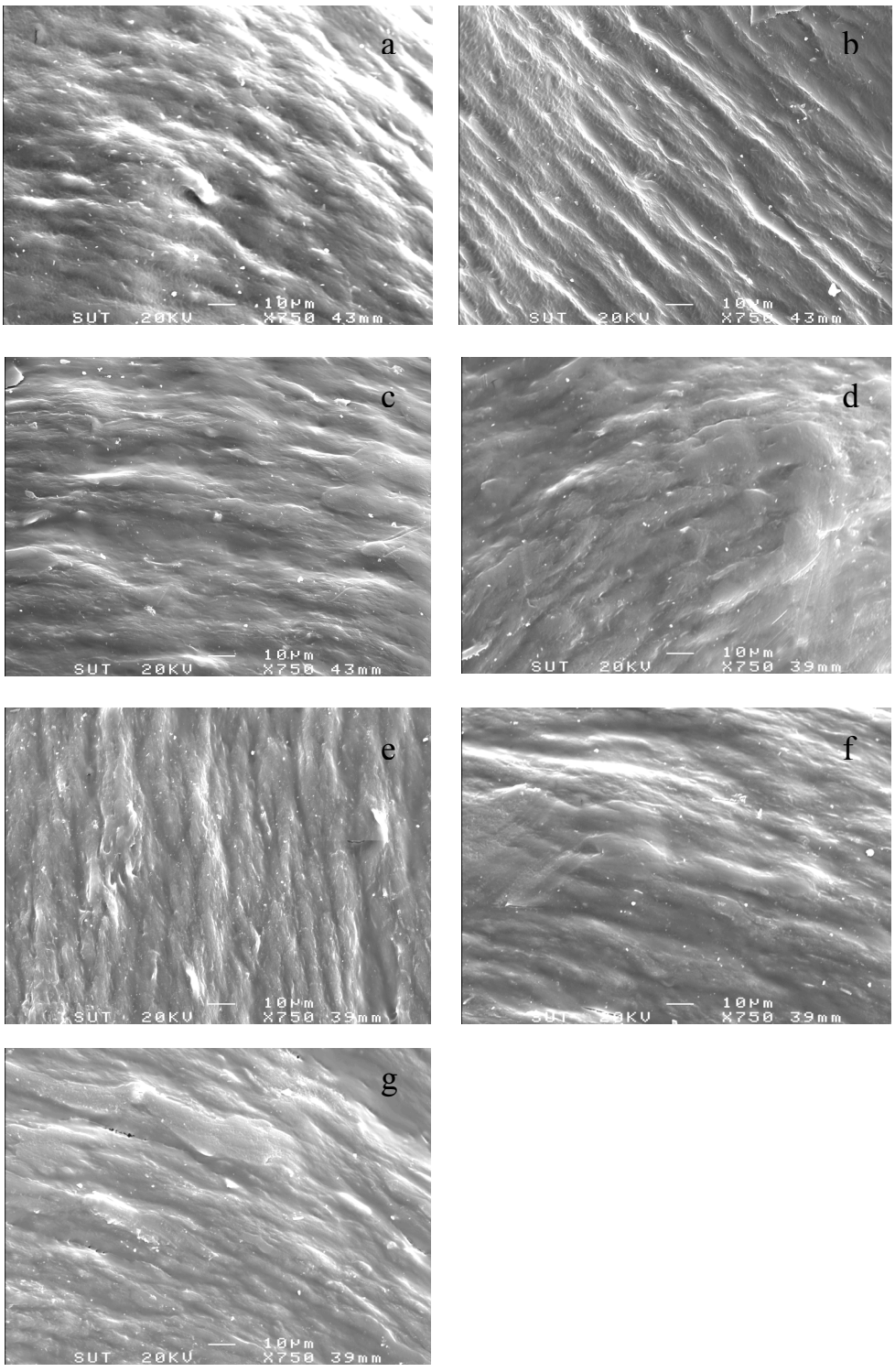


Figure 6.8 Scanning electron micrographs of amoxicillin (AMX) coated bead with polymer (X750) of chitosan (C) (a), poly(vinylpyrrolidone) (PVP) (g) and their blends of 9/1 (b), 7/3 (c), 5/5 (d), 3/7 (e) and 1/9 (f)

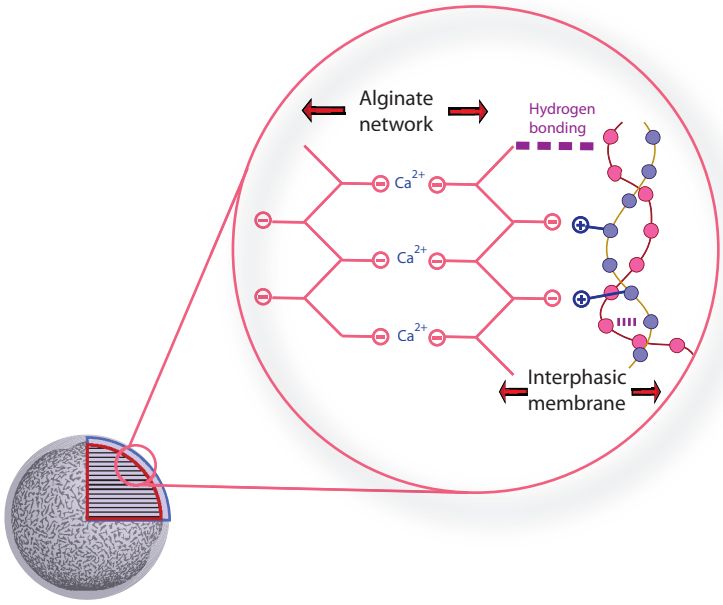


Figure 6.9 Schematic of the binding mechanism of coated amoxicillin beads

6.3.3 Swelling of dried bead

The swelling behavior of the uncoated and coated AMX beads in pH 4 phosphate buffer is displayed in Figure 6.10. The swelling of dry beads is mainly attributed to the hydration of the hydrophilic group of alginate, chitosan and PVP. In this case free water penetrates inside the beads in order to fill the inert pores among the polymer chains, so contributes to a greater degree of swelling [203].

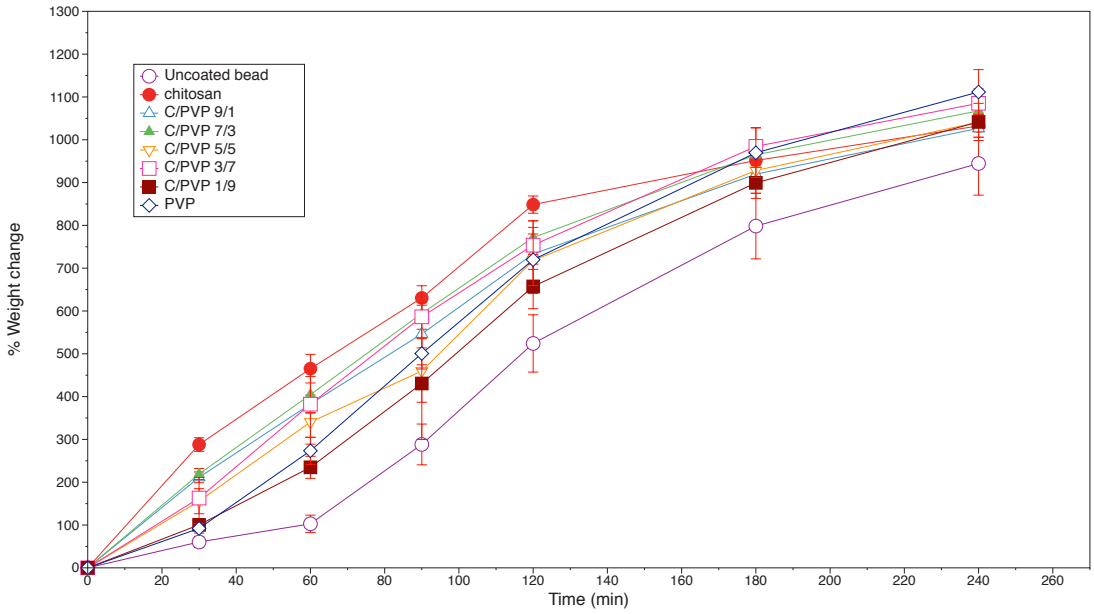


Figure 6.10 Swelling behavior of coated amoxicillin (AMX) beads with chitosan (C), poly(vinylpyrrolidone) (PVP) and their blends at various volume ratios compared with the control of uncoated AMX bead(mean ± S.D., n=3)

The MANOVA analysis of the level and shape after swelling of the AMX bead profiles is shown in Table 6.1. The MANOVA analysis observed test statistic for level, Wilk's Lambda test for time, leads to rejection of the hypothesis of homogeneity of the group means, thus the level of all profiles are different ($P<0.05$). The hypothesis on the effect of the time x group interaction, which is interpreted as parallelism or the shape of the profiles, indicated that the different percentage weight change is constant at any two points of time considered, was rejected, i.e., the swelling profiles were not parallel. The Wilk's Lambda values indicated significant differences between the shapes of the swelling profiles of the AMX beads. Furthermore, *post hoc* analyses of multiple comparison of swelling profiles were

pairwise compared and indicated significant differences between the uncoated and coated AMX beads ($P<0.05$). The result from the MANOVA, level and shape comparisons, can be summarized in that the coated AMX bead group showed similar swelling profiles ($P>0.05$). Subsequently, ANOVA with the Dunnett's test was performed to see the different percentage weight change at each time level as reported in Table 6.2.

Table 6.1 Statistical results of time and the time x group interaction effect obtained from the MANOVA of swelling of the amoxicillin (AMX) beads

Effect	**Wilks' Lambda**	***p***
time	0.001	<0.001
time x group	0.001	<0.001

Table 6.2 Statistical results of the percentage weight change at each time level obtained from ANOVA

Time (min)	Comparison (Dunnett's test)		Difference	*p*
30	Chitosan	Bead	227.53333	<0.001
	C/PVP 9/1	Bead	150.92667	<0.001
	C/PVP 7/3	Bead	158.70000	<0.001
	C/PVP 5/5	Bead	95.28000	0.001
	C/PVP 3/7	Bead	102.60333	<0.001
	C/PVP 1/9	Bead	40.00333	0.197
	PVP	Bead	31.74333	0.395
60	Chitosan	Bead	362.39667	<0.001
	C/PVP 9/1	Bead	279.78000	<0.001
	C/PVP 7/3	Bead	301.84333	<0.001
	C/PVP 5/5	Bead	237.55667	<0.001
	C/PVP 3/7	Bead	279.39333	<0.001
	C/PVP 1/9	Bead	131.96000	0.008
	PVP	Bead	170.63000	0.001
90	Chitosan	Bead	342.30333	<0.001
	C/PVP 9/1	Bead	258.07000	0.002
	C/PVP 7/3	Bead	305.90000	<0.001
	C/PVP 5/5	Bead	171.41667	0.036
	C/PVP 3/7	Bead	298.14333	<0.001
	C/PVP 1/9	Bead	142.33333	0.098
	PVP	Bead	212.43333	0.008
120	Chitosan	Bead	324.35333	<0.001
	C/PVP 9/1	Bead	208.96667	0.001
	C/PVP 7/3	Bead	247.30000	<0.001
	C/PVP 5/5	Bead	193.81333	0.002
	C/PVP 3/7	Bead	229.63667	<0.001
	C/PVP 1/9	Bead	133.19667	0.035
	PVP	Bead	195.61000	0.002
180	Chitosan	Bead	153.24333	0.005
	C/PVP 9/1	Bead	121.26667	0.026
	C/PVP 7/3	Bead	166.39333	0.002
	C/PVP 5/5	Bead	129.81667	0.017
	C/PVP 3/7	Bead	186.19667	0.001
	C/PVP 1/9	Bead	100.94000	0.076
	PVP	Bead	171.22333	0.002
240	Chitosan	Bead	87.81333	0.085
	C/PVP 9/1	Bead	82.18333	0.116
	C/PVP 7/3	Bead	123.00667	0.011
	C/PVP 5/5	Bead	96.65000	0.052
	C/PVP 3/7	Bead	140.61667	0.004
	C/PVP 1/9	Bead	97.09667	0.050
	PVP	Bead	167.05667	0.001

Almost all the coated AMX beads with different ratios of chitosan-PVP show significantly different weight changes from the uncoated AMX beads ($P<0.05$). The percentage weight change of the coated AMX bead in pH 4 phosphate buffer is higher than the uncoated bead over the first 120 min then after 180 min, some coated beads show a similar weight change to the uncoated bead. The swelling of the coated beads was higher than for the uncoated beads. The hydration of the hydrophilic groups of chitosan and PVP may induce the higher swelling of the coated beads compared to the uncoated beads, another important factor that influences their swelling behavior at pH 4 is that protonization of the amino groups of chitosan creates a repulsive force between the polymer chain of chitosan and causes a swelling of the chitosan membrane. Thus, coated alginate beads swell more than the uncoated beads as previously observed for chitosan coated dried beads [203].

6.3.4 Mucoadhesive properties of AMX beads using wash-off

The up-down movement of the arm of the disintegration equipment in pH 4 phosphate buffer is simulated to a stomach movement. All AMX beads were counted every 30 min and reported as percentage attached to the porcine stomach tissue. The percentage of uncoated and coated AMX beads attached to the mucosa at pH 4.0 is presented in Figure 6.11.

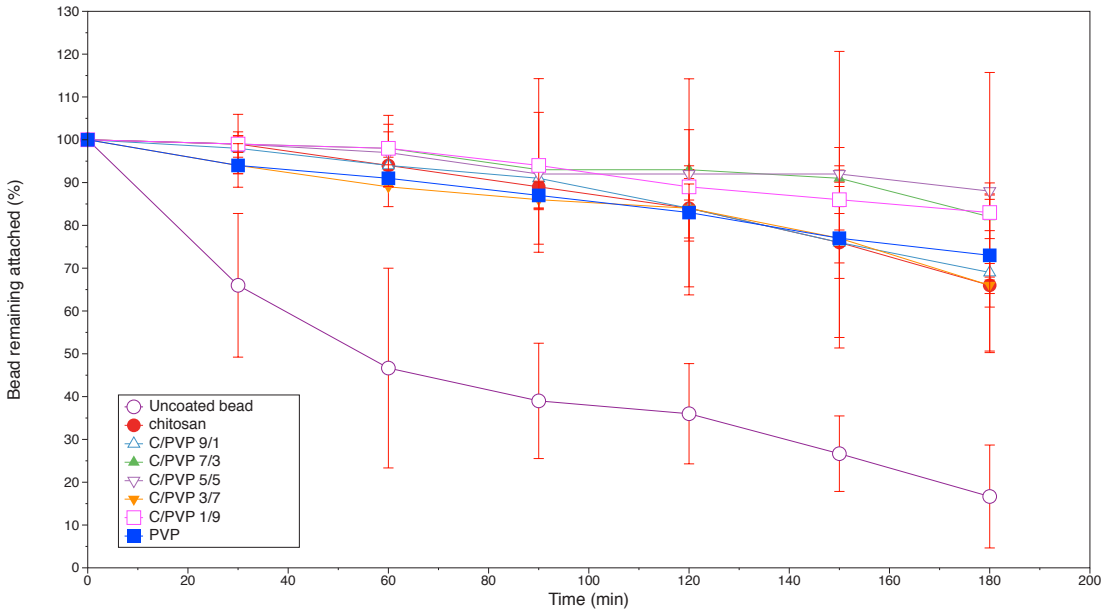


Figure 6.11 The in vitro wash-off test for uncoated and coated amoxicillin (AMX) beads with chitosan (C), PVP (P) and their blends at various volume ratios (mean ± S.D., n=3).

Uncoated AMX beads show the lowest attachment against porcine stomach tissue at 3 h. The MANOVA analysis of the level and shape result of AMX wash-off profiles is shown in Table 6.3. The MANOVA analysis observed test statistic for level, Wilk's Lambda test for time, show the different levels of the swelling profiles ($P<0.05$). The parallelism or shape in accordance to the time x group interaction effect indicated that the wash-off profiles were not parallel. Wilk's Lambda values indicated significant differences between the shapes of the wash-off profiles of the AMX beads. Furthermore, *post hoc* analyses of the multiple comparisons of wash off profiles were pairwise compared and indicated significant differences between the coated and uncoated AMX bead profiles ($P<0.05$). Wash-off

profiles of all coated AMX beads with difference chitosan-PVP ratios had the same level and shape. Thus, ANOVA with Dunnett's test was performed to see if there were differences of the percentage bead remaining at each time level of coated beads comparing to the uncoated beads as reported in Table 6.4.

Table 6.3 Statistical results of time and time x group interaction effects obtained from the MANOVA of the amoxicillin (AMX) bead wash-off profiles

Effect	**Wilks' Lambda**	***p***
time	0.046	<0.001
time x group	0.017	0.018

The percentage of beads remaining for all coated beads was significantly higher than for the uncoated beads after 30 min. The remaining uncoated beads decreased rapidly after 30 to 180 min. Even though not statistically significant for the coated AMX bead, the coated beads with C/PVP at 5/5 volume ratio exhibited the slowest wash off after 3 h. An interesting property was that the coated beads remained intact during the 180 min at pH 4 even though the swelling ratio was very high, while the uncoated beads only maintained their integrity for about 30 min. The results of the wash-off test indicated that chitosan, PVP, and their blends demonstrated a good mucoadhesive property. C/PVP blends at 5/5 volume ratio produced the highest mucoadhesion as determined from the results from the viscosity

measurement, texture analyzer, HT29 cell adhesion, and wash off method. The coating polymers on the AMX beads can enhance the retention time of the drug formulation at the stomach surface.

Table 6.4 Statistical results of percent bead remaining at each time level obtained from ANOVA

Time (min)	Comparison (Dunnett's test)		Difference	*p*
30	Chitosan	Bead	33.33333	<0.001
	C/PVP 9/1	Bead	32.22222	<0.001
	C/PVP 7/3	Bead	33.33333	<0.001
	C/PVP 5/5	Bead	33.33333	<0.001
	C/PVP 3/7	Bead	28.88889	<0.001
	C/PVP 1/9	Bead	33.33333	<0.001
	PVP	Bead	28.88889	<0.001
60	Chitosan	Bead	47.77778	<0.001
	C/PVP 9/1	Bead	47.77778	<0.001
	C/PVP 7/3	Bead	51.11111	<0.001
	C/PVP 5/5	Bead	50.00000	<0.001
	C/PVP 3/7	Bead	42.22222	<0.001
	C/PVP 1/9	Bead	51.11111	<0.001
	PVP	Bead	44.44444	<0.001
90	Chitosan	Bead	50.00000	<0.001
	C/PVP 9/1	Bead	52.22222	<0.001
	C/PVP 7/3	Bead	54.44444	<0.001
	C/PVP 5/5	Bead	53.33333	<0.001
	C/PVP 3/7	Bead	46.66667	<0.001
	C/PVP 1/9	Bead	55.55556	<0.001
	PVP	Bead	47.77778	<0.001
120	Chitosan	Bead	48.88889	<0.001
	C/PVP 9/1	Bead	48.88889	<0.001
	C/PVP 7/3	Bead	57.77778	<0.001
	C/PVP 5/5	Bead	56.66667	<0.001
	C/PVP 3/7	Bead	48.88889	<0.001
	C/PVP 1/9	Bead	53.33333	<0.001
	PVP	Bead	47.77778	<0.001
150	Chitosan	Bead	48.88889	<0.001
	C/PVP 9/1	Bead	48.88889	<0.001
	C/PVP 7/3	Bead	64.44444	<0.001
	C/PVP 5/5	Bead	65.55556	<0.001
	C/PVP 3/7	Bead	50.00000	<0.001
	C/PVP 1/9	Bead	58.88889	<0.001
	PVP	Bead	50.00000	<0.001
180	Chitosan	Bead	48.88889	<0.001
	C/PVP 9/1	Bead	52.22222	<0.001
	C/PVP 7/3	Bead	65.55556	<0.001
	C/PVP 5/5	Bead	71.11111	<0.001
	C/PVP 3/7	Bead	48.88889	<0.001
	C/PVP 1/9	Bead	66.66667	<0.001
	PVP	Bead	56.66667	<0.001

The schematic interaction of coated beads with mucin is depicted in Figure 6.12. During the coated AMX beads became attached to porcine stomach tissue, swelling of the beads resulted in polymer chain entanglement and interpenetration into the mucin thus forming hydrogen bonding between the polymer and mucin and leading to drug retention at the stomach wall. In addition, coated beads using these single polymers and their blends may be able to provide gastro-retention, which would facilitate local drug delivery to the stomach wall.

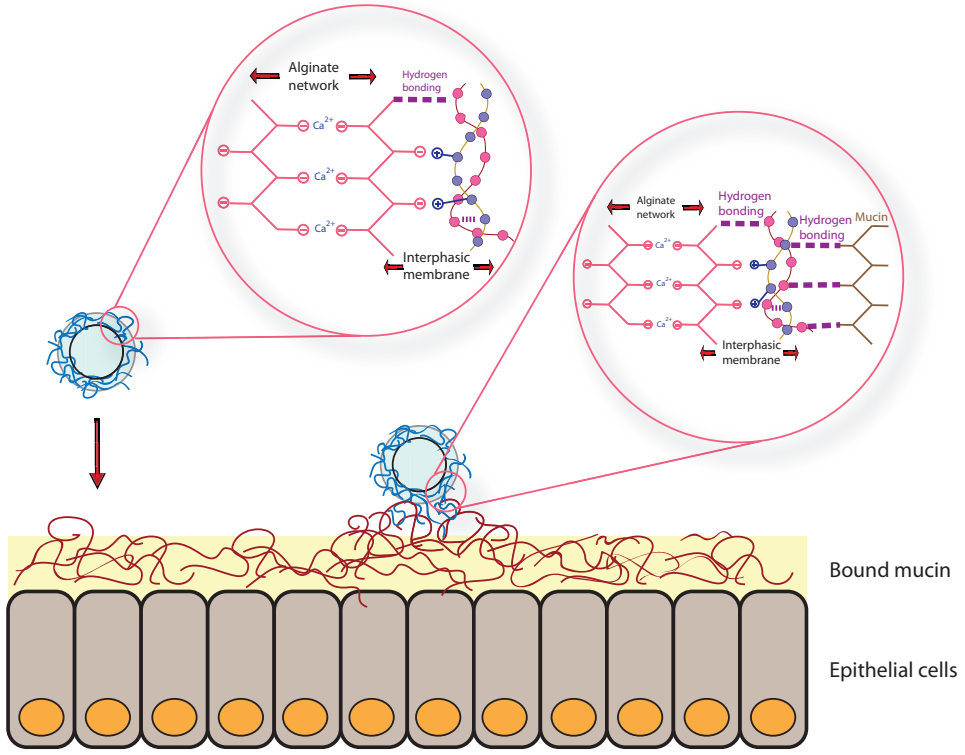


Figure 6.12 Schematic interaction mechanism of coated amoxicillin (AMX) bead with mucin

6.4 Conclusions

The coating of AMX beads with polymer blends of C/PVP exhibit much improved adhesion to the mucus compared to the uncoated beads. The alginate beads coated with these materials have a high potential for their utilization to increase the gastro-retentive times of various drugs via mucoadhesion. Coated AMX beads have a potential to remain intact and attached to the stomach for more than 3 h. Coated AMX-alginate beads may be useful for a more effective eradication of *H. pylori* infections, especially since it has previously been demonstrated that a 1 h contact with antibiotics can effectively remove these microorganisms [185].

References

184. Shah, S., Qaqish, R., Patel, V., Amiji, M. (1999) Evaluation of the factors influencing stomach-specific delivery of antibacterial agents for Helicobacter pylori infection. *J. Pharm. Pharmacol.* 51, 667-72.
185. Kimura, K., Ido, K., Saifuku, K., Taniguchi, Y., Kihira, K., Satoh, K., Takimoto, T., Yoshida, Y. (1995) A 1-h topical therapy for the treatment of Helicobacter pylori infection. *Am. J. Gastroenterol.* 90, 60-3.
186. Jianhua, Z., Chaowu, L., Decai, B., Yanjun, Z., Xiaojun, M. (2006) Preparation and evaluation of floating-bioadhesive microparticles containing clarithromycin for the eradication of *Helicobacter pylori*. *J. Appl. Polym. Sci.* 102, 2226-32.
187. Ishak, R., Awad, G., Mortada, N., Nour, S. (2007) Preparation, in vitro and in vivo evaluation of stomach-specific metronidazole-loaded alginate beads as local anti-Helicobacter pylori therapy. *J Control Release* 119, 207-14.
188. Govender, S., Pillay, V., Chetty, D., Essack, S., Dangor, C., Govender, T. (2005) Optimisation and characterisation of bioadhesive controlled release tetracycline microspheres. *Int. J. Pharm.* 306, 24-40.
189. Liu, Z., Lu, W., Qian, L., Zhang, X., Zeng, P., Pan, J. (2005) In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. *J Control Release* 102, 135-44.
190. Elzatahry, A., Eldin, M. S., Soliman, E., Hassan, E. (2009) Evaluation of Alginate-Chitosan Bioadhesive Beads as a Drug Delivery System for the Controlled Release of Theophylline. *J. Appl. Polym. Sci.* 111, 2452-9.
191. Goodman, L. S., Brunton, L. L., Blumenthal, D. K., Murri, N., Hilal-Dandan, R. Goodman & Gilman's The pharmacological basis of therapeutics. New York: McGraw-Hill Medical; 2011. Available from: http://www.accessmedicine.com/resourceTOC.aspx?resourceID=651.
192. Erah, P., Goddard, A., Barrett, D., Shaw, N., Spiller, R. (1995) Effect of pH on the Stability of Amoxycillin in Buffered Aqueous Solutions and in Gastric Juice*. *Pharm. Pharmacol. Commun.* 1, 597-600.
193. O'Neil, M. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 14th ed: Merck; 2006.
194. United States Pharmacopeia and National Formulary (USP 33-NF 28): The United States Pharmacopeial Convention; 2010.
195. Wang, K., He, Z. (2002) Alginate–konjac glucomannan–chitosan beads as controlled release matrix. *Int. J. Pharm.* 244, 117-26.
196. Dong, Z., Wang, Q., Du, Y. (2006) Alginate/gelatin blend films and their properties for drug controlled release. *Journal of Membrane Science* 280, 37-44.
197. Wittaya-areekul, S., Kruenate, J., Prahsarn, C. (2006) Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. *Int. J. Pharm.* 312, 113-8.
198. Mauger, J., Chilko, D., Howard, S. (1986) On the Analysis of Dissolution Data. *Drug Dev. Ind. Pharm.* 12, 969-92.
199. Hurtado, M., Vargas, Y., Dominguez-Ramirez, A., Cortes Arroyo, A. (2003) Comparison of dissolution profiles for albendazole tablets using USP apparatus 2 and 4. *Drug Dev. Ind. Pharm.* 29, 777-84.

200. Yuksel, N., Kanik, A., Baykara, T. (2000) Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and -independent methods. *Int. J. Pharm.* 209, 57-67.
201. SPSS Advanced Statistics 17.0: SPSS Inc.; 2007.
202. Lee, O., Ha, B., Park, S., Lee, Y. (1997) Studies on the pH-dependent swelling properties and morphologies of chitosan/calcium-alginate complexed beads. *Macromol. Chem. Phys.* 198, 2971-6.
203. Pasparakis, G., Bouropoulos, N. (2006) Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate-chitosan beads. *Int. J. Pharm.* 323, 34-42.

CHAPTER 7

Drug release study of amoxicillin from alginate beads

7.1 Introduction and objectives

The formulations of mucoadhesive drug agents are interesting because they are designed as drug delivery systems that can prolong the residence time of the drug at the site of absorption and facilitate an intimate contact with the underlying absorptive surface to enhance the drugs bioavailability [204]. Mucoadhesive drug delivery systems such as the monolithic (or matrix) type and the reservoir type (or membrane bound) have drug release characteristics that control the rate of drug release and transport [205]. Bead formulation using an alginate polymer matrix show a more sustained release of the drug compared to a pure drug [206]. The release kinetics of the drug from the matrices may be affected by several factors such as swelling of the polymer, drug diffusion from the polymer matrices, polymer erosion and geometry of the matrices [207]. Kinetic models of the drug release from alginate matrices can be estimated using several kinetic models such as zero order, first order, Higuchi, Korsmeyer-Peppas, Hixson-Crowell and Baker-Lonsdale models (Higuchi's model for spherical matrices) [208]. The mathematical models for all release kinetics are shown in Table 7.1 [209-211].

Table 7.1 Mathematical models of release kinetic

Function	***Equation***
Zero order	$\%diss = kt$
Korsmeyer-Peppas	$\%diss = kt^n$
First order	$\%diss = 100(1-e^{-kt})$
Higuchi	$\%diss = kt^{0.5}$
Hixson-Crowell	$\%diss = 100\left[1-\left(1-\frac{kt}{4.6416}\right)^3\right]$
Baker-Lonsdale	$\frac{2}{3}\left[1-\left(1-\frac{\%diss}{100}\right)^{\frac{2}{3}}\right]-\left(\frac{\%diss}{100}\right)=kt$

Several applied methods were used to compare the dissolution profiles of drug release such as those ANOVA-based, model-dependent and model-independent methods. All the methods are useful and capable of comparing dissolution profiles. The ANOVA-based method and model-dependent methods have more detail and are represented by the curve profile but for the model-independent methods, the difference factor, f_1, and similarity factor, f_2, were calculated to compare all dissolution profiles. Although the model-independent method seem to be easier to apply and interpret, this method may not adequately represent the curve. The evaluation of model-independent methods is complicated and requires an acceptable model approach including statistical analysis of the curve fitting parameters [209, 212]. An applied method to compare the dissolution profiles for this study has been

based on the analysis of variance (ANOVA) methods. Multivariate ANOVA (MANOVA) was performed for the level and shape approach. From MANOVA, the hypothesis of group means was represented by the level and the hypothesis on the time x group interaction effect is referred to as parallelism or the shape of the profiles [212]. The dissolution profiles were tested for their difference levels and shape of the profile using ANOVA-based methods and also provided informative of the dissolution profile behavior.

In this study the characteristics of the release of amoxicillin from mucoadhesive in pH 4 phosphate buffer. The release profiles of the amoxicillin were fitted to various kinetic models and the dissolution profiles were compared using the ANOVA based method.

7.2 Experimental methods

7.2.1 Materials

Potassium dihydrogenphosphate (KH_2PO_4) was from Univar (Australia). Potassium hydroxide and phosphoric acid were from RCI Labscan (Thailand). All other reagents were of analytical grade.

7.2.2 Drug release study

The releases of AMX from uncoated and coated beads with various ratios of chitosan-PVP were selected for this study due to their good mucoadhesive properties. All drug release study was determined using a VK 7000 dissolution tester (Vankel Industries, Edison, NJ, USA) with a USP27 apparatus 2 at pH 4. Phosphate buffer (pH 4), consisting of 0.1 M KH_2PO_4, using either KOH or phosphoric acid to adjust the pH to 4.0, was prepared as the dissolution medium. This medium (200 mL) was maintained at 37.0 °C ± 0.5 °C during the test. Approximately 20 mg of beads were used in each experiment. Samples (5 mL) were taken at 6, 12, 18, 24, 30, 40, 60, 90, 120, and 180 min and replaced with 5 mL of fresh medium. Amoxicillin was most stable at pH 4 – 7 with a half-life of more than 153.1 h [213]. The amount of AMX released from in the samples at pH 4 was determined using an 8452A HP Diode Array spectrophotometer (Hewlett Packard) at 230 nm. High performance liquid chromatography (HPLC) was also performed to check the possibility of degraded products of AMX, which might interfere with the analysis of AMX using the simple UV assay. The analysis method for AMX using UV spectrophotometer and HPLC were described in Section 6.2.3. However, no degradation peaks were detected for samples at pH 4. Thus, at this pH the more simple UV spectrometric method was used for the analysis of all dissolution samples. These dissolution tests were performed in triplicate.

7.2.3 Statistical analysis

All drug release results were fitted to several mathematical release models using the Sigmaplot for Windows version 11 (Systat Software Inc., USA). Analysis of variance (ANOVA) was performed using SPSS version 10 for Windows (SPSS Inc., USA). *Post hoc* testing ($p<0.05$) of the multiple comparisons was performed by Tukey's test. Profile analysis of the drug release was analyzed using multivariate ANOVA (MANOVA) with repeated measurements. In these models, the percentage of drug released was a dependent variable, groups were independently variable and time was the repeated factor. First, MANOVA was applied with the hypothesis on groups being tested from their means or the level of the profiles and the hypothesis on the time x groups interaction was interpreted as parallelism or from the shape of the profiles. The Wilks lambda statistic was preferred to obtain *p-values* in MANOVA [209, 212, 214]. Subsequently, Tukey's *post hoc* multiple comparison tests were performed for the average across the levels of the within-subjects factors. For multivariate analysis, the *post hoc* tests were performed for each dependent variable separately [215]. For the second step, if there were any significantly differences from MANOVA, ANOVA with *post hoc* analysis using *Dunnet's* or *Turkey's* test was applied to test separately at each time point to recognise the differences between groups.

7.3 Results and discussion

7.3.1 Drug release

The triple line drug therapy guideline for treatment of gastric ulcer with a *Helicobacter pylori* infection used a proton pump inhibitor or H_2 receptor antagonists in a combination with antibiotics for eradication of *H. pylori*. These agents can raise the gastric pH to 3-5 and can therefore improve amoxicillin stability, hence, the drug release studies were performed at a pH of 4 [216, 217]. AMX released from the uncoated and coated beads were compared with an AMX powder as shown in Figure 7.1. The AMX release profiles from the beads showed a sustained release characteristic when compared with the release from the AMX powder. The AMX powder showed high solubility at the initial time and seemed to be completely dissolved in about 30 min whereas the release of AMX from the coated and uncoated beads was complete in about 2.5 h. Kimura et al. [218] have reported that at least 1 h of local amoxicillin dissolved was enough for the treatment of *H. pylori* infection, thus, the bead formulation has a potential for *H. pylori* eradication.

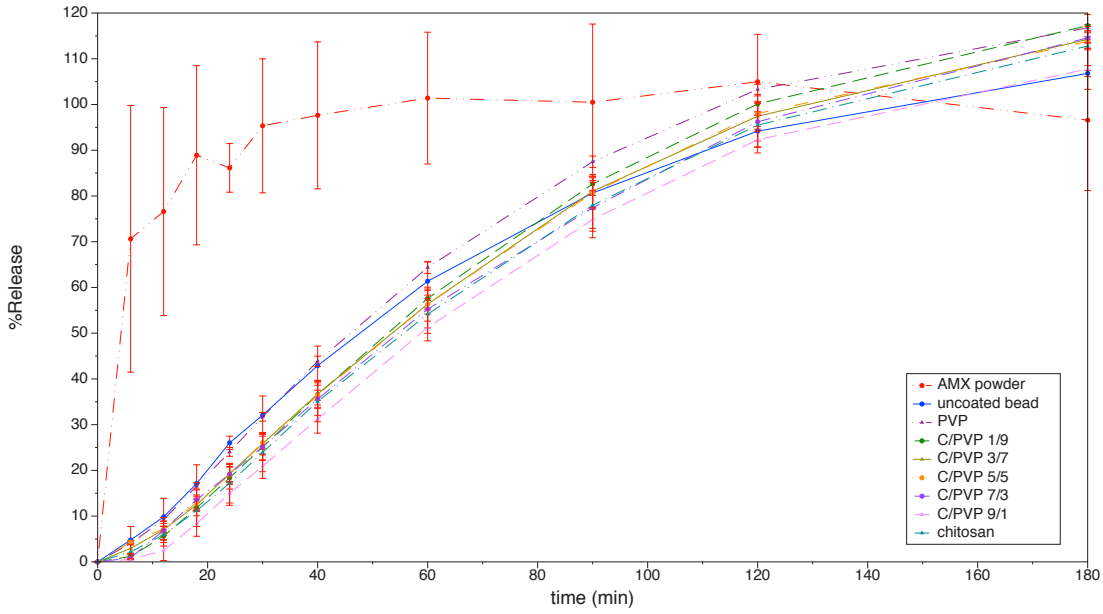


Figure 7.1 Dissolution profiles of amoxicillin (AMX) powder and AMX from uncoated and coated beads with chitosan (C), poly(vinylpyrrolidone) (PVP) and their blends of C/PVP at various volume ratios at pH 4 (mean ± S.D., n=3)

The MANOVA analyses of the level and shape results of the AMX dissolution profiles are shown in Table 7.2. The observed MANOVA analysis test statistic for the level or the Wilk's Lambda test for time led to rejection of the hypothesis of homogeneity of the group means, thus the level of all dissolution profiles are different ($P<0.05$). The hypothesis on the time x group interaction effect, which is interpreted as parallelism or the shape of the profiles, indicated that the different percentage release change is constant at any two points of time considered, was also rejected, i.e., the release profiles were not parallel. The Wilk's Lambda values indicated significant differences between the shapes of dissolution profiles of the AMX beads. Furthermore, *post hoc* analyses of the multiple comparisons of the

release profiles were pairwise compared and indicated significant differences between uncoated and coated AMX beads ($P<0.05$). The Turkey's *post hoc* analysis of the MANOVA analysis shows significant differences ($P<0.05$) in the level and shape between coated, C/PVP 9/1, and uncoated beads. Furthermore, pairwise comparisons of the beads coated with C/PVP 9/1 and PVP also showed significant differences between the level and shape approaches. Subsequently, ANOVA together with the *Turkey's* test was performed to see the differences of the percentage release at each time level of the uncoated bead, C/PVP 9/1 and PVP (Table 7.3).

Table 7.2 Statistical results of time and the time x group interaction effect obtained from MANOVA of the dissolution profiles of the amoxicillin (AMX) beads

Effect	**Wilks' Lambda**	***p***
time	0.001	<0.001
time x group	0.001	0.007

Table 7.3 Statistical results of percentage release at each time level of the uncoated bead, C/PVP 9/1 and poly(vinylpyrrolidone) (PVP) obtained from ANOVA

Time (min)	Comparison (Turkey's test)		Difference	*p*
6	C/PVP 9/1	Uncoated bead	4.25673	0.013
	C/PVP 9/1	PVP	3.51394	0.052
12	C/PVP 9/1	Uncoated bead	7.33628	0.010
	C/PVP 9/1	PVP	6.75608	0.019
18	C/PVP 9/1	Uncoated bead	8.74835	0.010
	C/PVP 9/1	PVP	8.17208	0.017
24	C/PVP 9/1	Uncoated bead	11.05225	0.001
	C/PVP 9/1	PVP	9.06775	0.006
30	C/PVP 9/1	Uncoated bead	11.15690	0.005
	C/PVP 9/1	PVP	10.79330	0.006
40	C/PVP 9/1	Uncoated bead	11.68512	0.007
	C/PVP 9/1	PVP	12.62811	0.003
60	C/PVP 9/1	Uncoated bead	10.10668	0.023
	C/PVP 9/1	PVP	13.10415	0.002
90	C/PVP 9/1	Uncoated bead	5.80311	0.537
	C/PVP 9/1	PVP	12.64147	0.011
120	C/PVP 9/1	Uncoated bead	1.87294	0.994
	C/PVP 9/1	PVP	11.00991	0.009
180	C/PVP 9/1	Uncoated bead	0. .96162	1.000
	C/PVP 9/1	PVP	8.99965	0.008

The AMX coated bead C/PVP 9/1 showed a lower level of its dissolution profile than the uncoated bead during the first 60 min due to the polymer coating film on the bead that retarded the drug release from the alginate bead. The bead coating with PVP showed a higher level of dissolution profile than the C/PVP 9/1 coated bead presumably due to the water solubility of the PVP that induced water absorption and increased the swelling of the bead. The dissolution profiles of most of the coated bead showed a similar level and shape of their dissolution profiles and these results can be ascribed to the high swelling of the bead in the dissolution medium that exceeded the strength of the coating polymer and led to the disruption of the polymeric coating on the bead as depicted in Figure 7.2 [219].

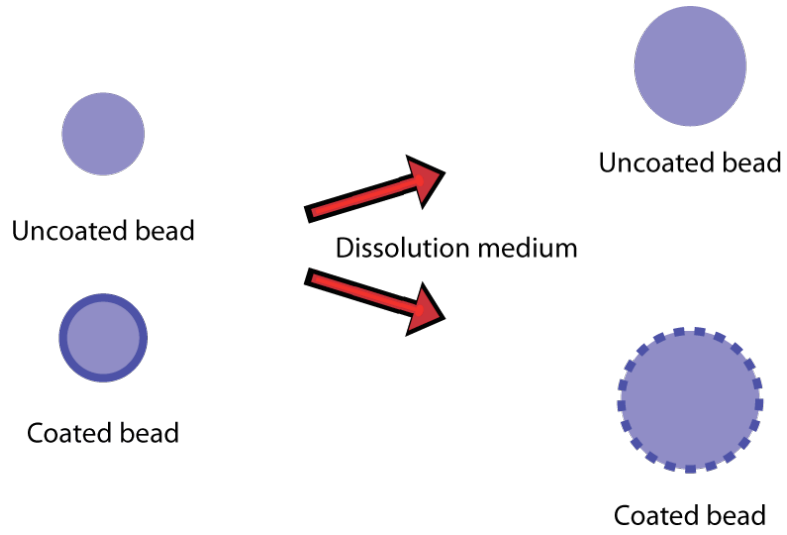


Figure 7.2 Polymeric coating disruption of amoxicillin (AMX) coated bead in dissolution medium

The AMX release behavior from AMX beads were fitted to several kinetic models. The data of AMX release kinetics for the Korsmeyer-Peppas equation model were used for examining only the first 60% of the release curves [220]. The statistical curve fitting results are reported in Table 7.4. Although uncoated beads were well fitted to the Hixon-Crowell equation model, most of the release kinetic profiles of the AMX beads best fitted with the Korsmeyer-Peppas equation model. The uncoated bead release kinetics indicated that the surface erosion or changes in the surface area of the bead relative to the drug diffusion inside the bead was predominantly best fitted to the Hixson-Crowell model [221, 222]. Moreover, the Korsmeyer-Peppas release kinetic model has been observed for alginate beads and the similar release profiles for the AMX from the result of the MANOVA analysis, thus, the Korsmeyer-Peppas model was used for discussion [223]. The Korsmeyer-Peppas or power law equation model can classify the mechanism of drug transport based on the diffusional release exponent (n) value as Fickian diffusion (Case I) ($n = 0.5$), non-Fickian (anomalous) diffusion ($0.5 < n < 1.0$), Case II transport ($n = 1.0$) and Super Case II transport ($n > 1.0$) [224]. As listed in Table 7.4, the n values were higher than 1 indicating that the release process is by Super Case II transport. This transport mechanism is the system controlled by swelling of the polymer that releases the drug. In these systems the drug was dissolved or dispersed in the polymer solution then the solvent was removed leaving the drug dispersed in the polymer matrix. In this state there is no drug diffusion in the solid phase until the dissolution medium penetrated into the polymer matrix. Therefore, the swollen polymer allowed the drug to diffuse outward as depicted in Figure 7.3 [225]. As a result of the high swelling of these

uncoated or coated dried alginate beads, the coated materials have not much influence on the release of AMX from the beads. Thus for these dried beads, the release profiles of the uncoated or coated beads were similar. These phenomena have been previously observed for coated dried beads [226, 227].

Table 7.4 Kinetic analysis of the release data of amoxicillin derived from several kinetic equation models

Sample	Release kinetics										
	First order		Higuchi		Hixson-Crowell		Baker-Lonsdale		Korsmeyer-Peppas		
	r^2	k	r^2	k	r^2	k	r^2	k	r^2	k	n
Uncoated bead	0.9602	0.0156	0.8920	7.1962	0.9826	0.0044	0.8380	0.0014	0.9739	0.9501	1.0231
Chitosan	0.9167	0.0137	0.8502	7.2912	0.9512	0.0039	0.7658	0.0012	0.9708	0.3203	1.2575
C/PVP 9/1	0.9170	0.0125	0.8285	6.8875	0.9505	0.0036	0.7508	0.0010	0.9789	0.1617	1.4115
C/PVP 7/3	0.9208	0.0140	0.8617	7.4036	0.9549	0.0040	0.7751	0.0012	0.9816	0.4182	1.1964
C/PVP 5/5	0.9250	0.0145	0.8696	7.5157	0.9596	0.0041	0.7822	0.0013	0.9952	0.4553	1.1795
C/PVP 3/7	0.9220	0.0145	0.8655	7.5135	0.9566	0.0041	0.7781	0.0013	0.9878	0.4233	1.1985
C/PVP 1/9	0.9083	0.0147	0.8530	7.6397	0.9453	0.0042	0.7619	0.0013	0.9875	0.3068	1.2830
PVP	0.9290	0.0169	0.8961	8.1165	0.9617	0.0048	0.8043	0.0016	0.9953	0.7111	1.1051

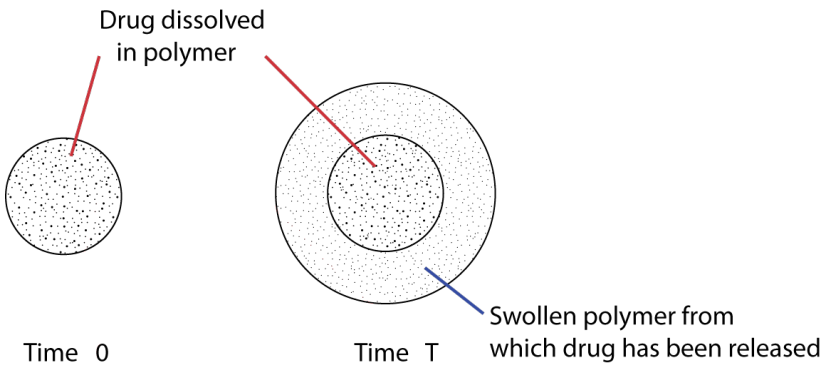


Figure 7.3 An idealized release system controlled by swelling

7.4 Conclusions

The bead formulation using an alginate produces a sustained release of amoxicillin for local eradication of *H. pylori*. The complete release of the drug was obtained after about 2.5 h and that is sufficient delay for treatment of *H. pylori* infections [218]. The release of amoxicillin from the bead was described by the diffusion of the drug from the swelling polymer matrix by a Super Case II transport mechanism. This amoxicillin mucoadhesive bead formulation has the potential for development as a mucoadhesive drug delivery system due to the long retention time in the stomach with a sustained release.

CHAPTER 8

Summary and conclusions

Mucoadhesive/bioadhesive drug delivery systems have been described for use in several applications and sites of action such as buccal, vaginal, eye, stomach, colon etc. Several polymers have been selected for these delivery systems especially natural polymers, due to their biocompatibility, biodegradability and safety for humans. Chitosan has often been used for mucoadhesive/bioadhesive drug delivery systems because of its antibacterial activity and ability to improve drug absorption by its ability to provide a tight binding to cells. Gelatin has also been reported to increase cell adhesion and has the potential for forming a mucoadhesive/bioadhesive drug delivery system. Although poly(vinylpyrrolidone) (PVP) is a synthetic polymer, it has been used for several pharmaceutical applications as a film forming agent or binder for formulations of medicinal tablets. Use of these polymer blends of chitosan, gelatin and PVP may enhance or provide a much more effective mucoadhesion when compared with single polymers.

There are multiple theories to explain the processes of mucoadhesion/bioadhesion; electronic, adsorption, wetting, fracture and diffusion theories. In isolation, none of these can fully explain the mucoadhesion properties of several pharmaceutical formulations. Several analytical techniques have been developed to evaluate mucoadhesion in pharmaceutical products. The analytical

techniques can be classified into two categories, indirect and direct methods for evaluation of mucoadhesion. In this thesis the indirect method for studying mucoadhesion was used to assess the interactions between the mucoadhesive materials and mucin layers using viscosity studies (Chapter 2) and FT-IR studies (DRIFTS) (Chapter 3). The direct method is to measure the force that is required to detach the mucoadhesive formulation from a mucosal surface using a texture analyzer and a cell culture technique (Chapter 4 and Chapter 5) respectively.

The indirect techniques for evaluation of mucoadhesion were based on polymer entanglement, penetration, chain diffusion and chemical interactions that are the key elements for the mucoadhesion process. At the start of the mucoadhesion process the polymer chain and the mucin are interpenetrated and this can be monitored using spectroscopic techniques. The DRIFTS analysis provided useful information for polymer and mucin interactions at the molecular level and this technique is also use for a qualitative and a quantitative method. Although a viscosity study is helpful for screening the mucoadhesive polymer due to its rapid measurement, simplicity and ability to be completed within an hour, this technique has limitations because of the large variations of results obtained and cannot be used for a strong gel polymer. Furthermore, the latter technique is not recommended to use as a stand alone method for detecting mucoadhesive properties of polymer-mucin mixtures.

The cell culture technique, texture analyzer and wash-off technique are direct techniques that determine a real situation of polymer attachment to the cell or mucus layers. The texture analyzer may involve a quantitative determination of the force required to detach the mucoadhesive material from the surface. The work of

mucoadhesion was calculated from the area under the force – distance curve and is reported in terms of the mucoadhesion properties. For the cell culture technique the HT29 cells were attached directly onto the polymer surface and binding was quantified by measuring the amount of cell adhesion and represented in a terms of bioadhesion. Although these two techniques did directly measure mucoadhesion and bioadhesion phenomena, the time required for these measurements of mucoadhesion must also be taken into consideration. The wash-off technique is a direct technique to determine the mucoadhesion time under a shear force until the material became detached from the polymer. Both direct and indirect techniques were used in this work to assess the development of a novel mucoadhesive polymer. The advantages and disadvantages of several techniques used in this study for mucoadhesion evaluation can be summarized in Table 8.1.

Table 8.1 Summarize techniques for mucoadhesion evaluation used in this study

Method	Category	Detail	Advantages/Disadvantages
Viscosity measurement	Indirect method	Study the polymer chain interpenetration and entanglement of mucoadhesive polymer and mucus	Advantages: Quick and easy for screening the polymers with mucoadhesive properties Disadvantages: The large variation of results due to differences in mucin type and concentration and different measurement configurations
Spectroscopic method	Indirect method	Study the interaction of mucoadhesive polymer and mucin at molecular level	Advantages: Provide the detail of mechanisms or molecular interaction between mucoadhesive polymer and mucin Disadvantages: This technique does not provide the force of mucoadhesion

Table 8.1 Summarize techniques for mucoadhesion evaluation used in this study (Continue)

Method	Category	Detail	Advantages/Disadvantages
Texture analyzer	Direct method	Study the force necessary to separate the mucoadhesive formulation and the mucosal surface	Advantages: Provide a direct measurement force that is an appropriate results for mucoadhesion evaluation Disadvantages: The large variation of results due to differences stomach tissue, thus, several repeat measurements are required and instrument configurations are needed to be adjusted
Cell culture	Direct method	Study the muco- or bioadhesive properties of polymer using *in vitro* cell culture	Advantages: Represent the muco- or bioadhesive properties of polymer that depend on the cell culture types and this test is closed to gastrointestinal system Disadvantages: This technique is quite complicate and needs several reagents and instruments

Table 8.1 Summarize techniques for mucoadhesion evaluation used in this study (Continue)

Method	Category	Detail	Advantages/Disadvantages
Wash-off method	Direct method	Study the retention time of muco-adhesive formulation against the mucosal surface	Advantages: Provide a direct measurement of retention time of mucoadhesive formulation against mucosal surface under shear movement Disadvantages: The large variation of results due to differences stomach tissue, thus, several repeat measure-ments are required

From this results of the work reported in this thesis the polymer blend of C/PVP at a ratio of 5/5 showed good mucoadhesive and bioadhesive properties due to the high intermolecular interaction between chitosan and PVP, C/mucin and PVP/mucin that was observed using the DRIFTS technique. Several techniques such as viscosity measurements, texture analysis, cell culture technique and wash-off technique were also used to determine and evaluate the mucoadhesive and bioadhesive properties of this polymer blend. Moreover, the results from several techniques also demonstrated good mucoadhesive and bioadhesive properties of the C/PVP at a 5/5 ratio. A bead formulation with this polymer can also sustain the release of amoxicillin when compared with amoxicillin powder and can prolong the drug release for about 2.5 h. This may be enough for eradication of *H. pylori* infections.

This study has clearly demonstrated the possibility of using chitosan and PVP as a mucoadhesive material for oral drug delivery. The polymer blend of chitosan and PVP has a synergistic effect because of its mucoadhesion properties and has the potential for development of mucoadhesive drug delivery vehicle.

REFERENCES

1. Ahn, J., Choi, H., Cho, C. (2001) A novel mucoadhesive polymer prepared by template polymerization of acrylic acid in the presence of chitosan. Biomaterials 22, 923-8.
2. Washington, N., Washington, C., Wilson, C. Physiological pharmaceutics: barriers to drug absorption. 2nd ed. London ; New York: Taylor & Francis; 2001.
3. Mathiowitz, E., Chickering, D., Lehr, C. Bioadhesive drug delivery systems : fundamentals, novel approaches, and development. New York: Marcel Dekker; 1999.
4. Watanabe, Y., Inoko, Y. (2007) Small-angle light and X-ray scattering measurements of a protein-oligosaccharide complex mucin in solution. J. Appl. Crystallogr. 40, s209-s12.
5. Warren, R., Marshall, B. (1983) Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1, 1273-5.
6. Marshall, B., Warren, J. (1984) Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1, 1311-5.
7. Nobelprize.org. Press Release: The 2005 Nobel Prize in Physiology or Medicine. http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/press.html2005.
8. Vilaichone, R., Mahachai, V., Graham, D. (2006) *Helicobacter* diagnosis and management. Gastroenterol. Clin. North Am. 35, 229-47.

9. Parsonnet, J. (1995) The incidence of *Helicobacter pylori* infection. Aliment. Pharmacol. Ther. 9 Suppl 2, 45-51.

10. Ramakrishnan, K., Salinas, R. (2007) Peptic ulcer disease. Am. Fam. Physician 76, 1005-12.

11. Chan, F., Leung, W. (2002) Peptic-ulcer disease. Lancet 360, 933-41.

12. Wilairatana, S., Kladchareon, N., Israsena, S., Wilairatana, P. (1991) Epidemiology of peptic ulcer disease in Thailand. Gastroenterol. Jpn. 26 Suppl 3, 265-6.

13. Logan, R., Walker, M. (2001) ABC of the upper gastrointestinal tract: Epidemiology and diagnosis of *Helicobacter pylori* infection. BMJ 323, 920-2.

14. Ricci, C., Holton, J., Vaira, D. (2007) Diagnosis of *Helicobacter pylori*: invasive and non-invasive tests. Best practice & research. Clinical gastroenterology 21, 299-313.

15. Cutler, A., Havstad, S., Ma, C. K., Blaser, M., Perez-Perez, G., Schubert, T. (1995) Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. Gastroenterology 109, 136-41.

16. McColl, K., Murray, L., Gillen, D., Walker, A., Wirz, A., Fletcher, J., Mowat, C., Henry, E., Kelman, A., Dickson, A. (2002) Randomised trial of endoscopy with testing for *Helicobacter pylori* compared with non-invasive *H. pylori.* testing alone in the management of dyspepsia. BMJ 324, 999-1002.

17. Shah, S., Qaqish, R., Patel, V., Amiji, M. (1999) Evaluation of the factors influencing stomach-specific delivery of antibacterial agents for *Helicobacter pylori* infection. J. Pharm. Pharmacol. 51, 667-72.

18. Cianci, R., Montalto, M., Pandolfi, F., Gasbarrini, G., Cammarota, G. (2006) Third-line rescue therapy for *Helicobacter pylori* infection. World J Gastroenterol 12, 2313-9.

19. Chey, W., Wong, B. (2007) American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. Am. J. Gastroenterol. 102, 1808-25.

20. Rimbara, E., Fischbach, L., Graham, D. (2011) Optimal therapy for *Helicobacter pylori* infections. Nature reviews. Gastroenterology & hepatology 8, 79-88.

21. Thong-Ngam, D., Mahachai, V., Kullavanijaya, P. (2007) Incidence of *Helicobacter pylori* Recurrent Infection and Associated Factors in Thailand. J. Med. Assoc. Thai. 90, 1406-10.

22. Nakamura, M., Spiller, R., Barrett, D., Wibawa, J., Kumagai, N., Tsuchimoto, K., Tanaka, T. (2003) Gastric juice, gastric tissue and blood antibiotic concentrations following omeprazole, amoxicillin and clarithromycin triple therapy. Helicobacter 8, 294-9.

23. Graham, D., Abudayyeh, S., El-Zimaity, H., Hoffman, J., Reddy, R., Opekun, A. (2006) Sequential therapy using high-dose esomeprazole-amoxicillin followed by gatifloxacin for *Helicobacter pylori* infection. Aliment. Pharmacol. Ther. 24, 845-50.

24. Erah, P., Goddard, A., Barrett, D., Shaw, P., Spiller, R. (1997) The stability of amoxycillin, clarithromycin and metronidazole in gastric juice: relevance to the treatment of *Helicobacter pylori* infection. J. Antimicrob. Chemother. 39, 5-12.

25. Han, S., Bhakdi, S., Maeurer, M., Schneider, T., Gehring, S. (1999) Stable and Unstable Amoxicillin Resistance in *Helicobacter pylori*: Should Antibiotic Resistance Testing Be Performed Prior to Eradication Therapy? J. Clin. Microbiol. 37, 2740-1.

26. Liu, Z., Lu, W., Qian, L., Zhang, X., Zeng, P., Pan, J. (2005) In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. J Control Release 102, 135-44.

27. Megraud, F., Lamouliatte, H. (2003) Review article: the treatment of refractory *Helicobacter pylori* infection. Aliment. Pharmacol. Ther. 17, 1333-43.

28. Nagahara, N., Akiyama, Y., Nakao, M., Tada, M., Kitano, M., Ogawa, Y. (1998) Mucoadhesive microspheres containing amoxicillin for clearance of *Helicobacter pylori*. Antimicrob. Agents Chemother. 42, 2492-4.

29. Umamaheswari, R., Jain, S., Tripathi, P., Agrawal, G., Jain, N. (2002) Floating-bioadhesive microspheres containing acetohydroxamic acid for clearance of Helicobacter pylori. Drug delivery 9, 223-31.

30. Kimura, K., Ido, K., Saifuku, K., Taniguchi, Y., Kihira, K., Satoh, K., Takimoto, T., Yoshida, Y. (1995) A 1-h topical therapy for the treatment of *Helicobacter pylori* infection. Am. J. Gastroenterol. 90, 60-3.

31. Lehr, C., Poelma, F. G., Junginger, H., Tukker, J. (1991) An estimate of turnover time of intestinal mucus gel layer in the rat in situ loop. Int. J. Pharm. 70, 235-40.

32. Cucchiara, S., Minella, R., Campanozzi, A., Salvia, G., Borrelli, O., Ciccimarra, E., Emiliano, M. (1997) Effects of omeprazole on mechanisms of gastroesophageal reflux in childhood. Dig. Dis. Sci. 42, 293-9.

33. Conway, B. (2005) Drug delivery strategies for the treatment of *Helicobacter pylori* infections. Curr. Pharm. Des. 11, 775-90.

34. Peppas, N., Buri, P. (1985) Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. J. Controlled Release 2, 257-75.

35. Ahuja, A., Khar, R., Ali, J. (1997) Mucoadhesive Drug Delivery Systems. Drug Dev. Ind. Pharm. 23, 489-515.

36. Sau, L., Robinson, J. (1988) The contribution of anionic polymer structural features to mucoadhesion. J. Controlled Release 5, 223-31.

37. Edsman, K., Hagerstrom, H. (2005) Pharmaceutical applications of mucoadhesion for the non-oral routes. J. Pharm. Pharmacol. 57, 3-22.

38. Chowdary, K., Rao, Y. (2004) Mucoadhesive microspheres for controlled drug delivery. Biol. Pharm. Bull. 27, 1717-24.

39. Majithiya, R., Murthy, R. (2005) Chitosan-based mucoadhesive microspheres of clarithromycin as a delivery system for antibiotic to stomach. Current Drug Delivery 2, 235-42.

40. Thirawong, N., Thongborisute, J., Takeuchi, H., Sriamornsak, P. (2008) Improved intestinal absorption of calcitonin by mucoadhesive delivery of novel pectin–liposome nanocomplexes. J. Controlled Release 125, 236-45.

41. Pluta, J., Haznar, D., Suszka, A., Ryszka, F. (2008) Insulin availability from mucoadhesive tablets. Pharmazie 63, 650-3.

42. Ducheˇne, D., Touchard, F., Peppas, N. (1988) Pharmaceutical and medical aspects of bioadhesive systems for drug administration. Drug Dev. Ind. Pharm. 14, 283-318.

43. Shaikh, R., Raj, T., Garland, M., Woolfson, A., Donnelly, R. (2011) Mucoadhesive drug delivery systems. Journal of pharmacy & bioallied sciences 3, 89-100.

44. Kinloch, A. (1980) The science of adhesion. Journal of Materials Science 15, 2141-66.

45. Dodou, D., Breedveld, P., Wieringa, P. (2005) Mucoadhesives in the gastrointestinal tract: revisiting the literature for novel applications. Eur. J. Pharm. Biopharm. 60, 1-16.

46. Langer, R., Peppas, N. (1981) Present and future applications of biomaterials in controlled drug delivery systems. Biomaterials 2, 201-14.

47. Park, K., Robinson, J. (1984) Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion. Int. J. Pharm. 19, 107-27.

48. Rowe, R., Sheskey, P., Quinn, M., American Pharmacists Association. Handbook of pharmaceutical excipients. 6th ed. London; Chicago Washington, DC: Pharmaceutical Press; American Pharmacists Association; 2009.

49. Swei, J., Talbot, J. (2003) Viscosity correlation for aqueous polyvinylpyrrolidone (PVP) solutions. J. Appl. Polym. Sci. 90, 1153-5.

50. Kim, E., Chun, M., Jang, J., Lee, I., Lee, K., Choi, H. (2006) Preparation of a solid dispersion of felodipine using a solvent wetting method. Eur. J. Pharm. Biopharm. 64, 200-5.

51. Karavas, E., Ktistis, G., Xenakis, A., Georgarakis, E. (2005) Miscibility behavior and formation mechanism of stabilized felodipine-polyvinylpyrrolidone amorphous solid dispersions. Drug Dev. Ind. Pharm. 31, 473-89.

52. Chun, M., Cho, C., Choi, H. (2005) Mucoadhesive microspheres prepared by interpolymer complexation and solvent diffusion method. Int. J. Pharm. 288, 295-303.

53. Choi, G., Jung, H., Ryu, M., Yoon, J., Oh, K., Kim, K. (1998) Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. Int. J. Pharm. 165, 33-44.

54. Perioli, L., Ambrogi, V., Angelici, F., Ricci, M., Giovagnoli, S., Capuccella, M., Rossi, C. (2004) Development of mucoadhesive patches for buccal administration of ibuprofen. J. Controlled Release 99, 73-82.

55. Alsarra, I., Hamed, A., Alanazi, F., Neau, S. (2011) Rheological and mucoadhesive characterization of poly(vinylpyrrolidone) hydrogels designed for nasal mucosal drug delivery. Arch. Pharmacal Res. 34, 573-82.

56. Chun, M., Cho, C., Choi, H. (2002) Mucoadhesive drug carrier based on interpolymer complex of poly(vinyl pyrrolidone) and poly(acrylic acid) prepared by template polymerization. J. Controlled Release 81, 327-34.

57. Karavas, E., Georgarakis, E., Bikiaris, D. (2006) Application of PVP/HPMC miscible blends with enhanced mucoadhesive properties for adjusting drug

release in predictable pulsatile chronotherapeutics. Eur. J. Pharm. Biopharm. 64, 115-26.

58. Jain, S., Jain, A., Gupta, Y., Kharya, A. (2008) Design and development of a mucoadhesive buccal film bearing progesterone. Pharmazie 63, 129-35.

59. Marguerite, R. (2006) Chitin and chitosan: Properties and applications. Prog. Polym. Sci. 31, 603-32.

60. Galed, G., Miralles, B., Paños, I., Santiago, A., Heras, Á. (2005) N-Deacetylation and depolymerization reactions of chitin/chitosan: Influence of the source of chitin. Carbohydr. Polym. 62, 316-20.

61. Li, Q., Dunn, E., Grandmaison, E., Goosen, M. (1992) Applications and Properties of Chitosan. Journal of Bioactive and Compatible Polymers 7, 370-97.

62. Sahasathian, T., Kerdcholpetch, T., Chanweroch, A., Praphairaksit, N., Suwonjandee, N., Muangsin, N. (2007) Sustained release of amoxicillin from chitosan tablets. Arch. Pharmacal Res. 30, 526-31.

63. Orienti, I., Cerchiara, T., Luppi, B., Bigucci, F., Zuccari, G., Zecchi, V. (2002) Influence of different chitosan salts on the release of sodium diclofenac in colon-specific delivery. Int. J. Pharm. 238, 51-9.

64. Rossi, S., Ferrari, F., Bonferoni, M., Caramella, C. (2000) Characterization of chitosan hydrochloride-mucin interaction by means of viscosimetric and turbidimetric measurements. Eur. J. Pharm. Sci. 10, 251-7.

65. Bernkop-Schnurch, A. (2000) Chitosan and its derivatives: potential excipients for peroral peptide delivery systems. Int. J. Pharm. 194, 1-13.

66. Illum, L., Farraj, N., Davis, S. (1994) Chitosan as a novel nasal delivery system for peptide drugs. Pharm. Res. 11, 1186-9.

67. Cho, S., Choi, H. (2005) Preparation of mucoadhesive chitosan-poly(acrylic acid) microspheres by interpolymer complexation and solvent evaporation method II. Arch. Pharmacal Res. 28, 612-8.

68. Gratieri, T., Gelfuso, G., Rocha, E., Sarmento, V., de Freitas, O., Lopez, R. (2010) A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. Eur. J. Pharm. Biopharm. 75, 186-93.

69. Aksungur, P., Sungur, A., Unal, S., Iskit, A., Squier, C., Senel, S. (2004) Chitosan delivery systems for the treatment of oral mucositis: in vitro and in vivo studies. J Control Release 98, 269-79.

70. Elzatahry, A., Eldin, M. S., Soliman, E., Hassan, E. (2009) Evaluation of alginate-chitosan bioadhesive beads as a drug delivery system for the controlled release of theophylline. J. Appl. Polym. Sci. 111, 2452-9.

71. Lehr, C., Bouwstra, J., Schacht, E., Junginger, H. (1992) In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int. J. Pharm. 78, 43-8.

72. Qi, L., Xu, Z., Jiang, X., Hu, C., Zou, X. (2004) Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr. Res. 339, 2693-700.

73. No, H., Young, N., Ho, S., Meyers, S. (2002) Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int. J. Food Microbiol. 74, 65-72.

74. Young, S., Wong, M., Tabata, Y., Mikos, A. (2005) Gelatin as a delivery vehicle for the controlled release of bioactive molecules. J. Controlled Release 109, 256-74.

75. Hunter, E., Turner, A. (1940) The iso-electric point of gelatin. Transactions of the Faraday Society 36, 835-9.

76. Bonferoni, M., Chetoni, P., Giunchedi, P., Rossi, S., Ferrari, F., Burgalassi, S., Caramella, C. (2004) Carrageenan-gelatin mucoadhesive systems for ion-exchange based ophthalmic delivery: in vitro and preliminary in vivo studies. Eur. J. Pharm. Biopharm. 57, 465-72.

77. Matsuda, S., Iwata, H., Se, N., Ikada, Y. (1999) Bioadhesion of gelatin films crosslinked with glutaraldehyde. J. Biomed. Mater. Res. 45, 20-7.

78. Wang, J., Tauchi, Y., Deguchi, Y., Morimoto, K., Tabata, Y., Ikada, Y. (2000) Positively charged gelatin microspheres as gastric mucoadhesive drug delivery system for eradication of *H. pylori.* Drug delivery 7, 237-43.

79. Kotagale, N., Patel, C., Parkhe, A., Khandelwal, H., Taksande, J., Umekar, M. (2010) Carbopol 934-sodium alginate-gelatin mucoadhesive ondansetron tablets for buccal delivery: Effect of pH modifiers. Indian journal of pharmaceutical sciences 72, 471-9.

80. Abruzzo, A., Bigucci, F., Cerchiara, T., Cruciani, F., Vitali, B., Luppi, B. (2012) Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. Carbohydr. Polym. 87, 581-8.

81. Tonnesen, H., Karlsen, J. (2002) Alginate in drug delivery systems. Drug Dev. Ind. Pharm. 28, 621-30.

82. Taylor, C., Pearson, J., Draget, K., Dettmar, P., Smidsrid, O. (2005) Rheological characterisation of mixed gels of mucin and alginate. Carbohydr. Polym. 59, 189-95.

83. Das, M., Maurya, D. (2008) Evaluation of diltiazem hydrochloride-loaded mucoadhesive microspheres prepared by emulsification-internal gelation technique. Acta Pol. Pharm. 65, 249-59.

84. Murata, Y., Toniwa, S., Miyamoto, E., Kawashima, S. (1999) Preparation of alginate gel beads containing chitosan salt and their function. Int. J. Pharm. 176, 265-8.

85. Gåserød, O., Jolliffe, I., Hampson, F., Dettmar, P., Skjåk-Bræk, G. (1998) The enhancement of the bioadhesive properties of calcium alginate gel beads by coating with chitosan. Int. J. Pharm. 175, 237-46.

86. Sankalia, M., Mashru, R., Sankalia, J., Sutariya, V. (2005) Papain entrapment in alginate beads for stability improvement and site-specific delivery: Physicochemical characterization and factorial optimization using neural network modeling. AAPS PharmSciTech 6, E209-E22.

87. Toshihisa, Y., Tsuneo, O., Takafumi, O., Ken, I. (1987) Calcium-induced gelation of alginic acid and ph-sensitive reswelling of dried gels (pharmaceutical). Chem. Pharm. Bull. 35, 1555-63.

88. Gonzalez-Rodriguez, M., Holgado, M., Sanchez-Lafuente, C., Rabasco, A., Fini, A. (2002) Alginate/chitosan particulate systems for sodium diclofenac release. Int. J. Pharm. 232, 225-34.

89. Davidovich-Pinhas, M., Bianco-Peled, H. (2010) Mucoadhesion: a review of characterization techniques. Expert opinion on drug delivery 7, 259-71.

90. Wong, C., Yuen, K., Peh, K. (1999) Formulation and evaluation of controlled release Eudragit buccal patches. Int. J. Pharm. 178, 11-22.

91. Gratieri, T., Gelfuso, G., Rocha, E., Sarmento, V., de Freitas, O., Lopez, R. (2010) A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. Eur. J. Pharm. Biopharm. 75, 186-93.

92. Alam, M., Ahmad, F., Khan, Z., Khar, R., Ali, M. (2007) Development and evaluation of acid-buffering bioadhesive vaginal tablet for mixed vaginal infections. AAPS PharmSciTech 8, E109.

93. Peh, K., Khan, T., Ching, H. (2000) Mechanical, bioadhesive strength and biological evaluations of chitosan films for wound dressing. Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques 3, 303-11.

94. Wittaya-areekul, S., Kruenate, J., Prahsarn, C. (2006) Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. Int. J. Pharm. 312, 113-8.

95. Rao, K., Buri, P. (1989) A novel in situ method to test polymers and coated microparticles for bioadhesion. Int. J. Pharm. 52, 265-70.

96. Keely, S., Rullay, A., Wilson, C., Carmichael, A., Carrington, S., Corfield, A., Haddleton, D., Brayden, D. (2005) In vitro and ex vivo intestinal tissue models to measure mucoadhesion of poly (methacrylate) and N-trimethylated chitosan polymers. Pharm. Res. 22, 38-49.

97. Lim, S., Song, D., Cho, K., Oh, S., Lee-Yoon, D., Bae, E., Lee, J. Cell adhesion and degradation behaviors of acetylated chitosan films. 3rd Kuala

Lumpur International Conference on Biomedical Engineering 2006. 183082007. p. 94-7.

98. Hassan, E., Gallo, J. (1990) A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. Pharm. Res. 7, 491-5.

99. Lawrie, G., Keen, I., Drew, B., Chandler, A., Rintoul, L., Fredericks, P., Grøndahl, L. (2007) Interactions between alginate and chitosan biopolymers characterized using FTIR and XPS. Biomacromolecules 8, 2533-41.

100. Li, D., Yamamoto, H., Takeuchi, H., Kawashima, Y. (2010) A novel method for modifying AFM probe to investigate the interaction between biomaterial polymers (Chitosan-coated PLGA) and mucin film. Eur. J. Pharm. Biopharm. 75, 277-83.

101. Liu, L., Fishman, M., Hicks, K., Kende, M. (2005) Interaction of various pectin formulations with porcine colonic tissues. Biomaterials 26, 5907-16.

102. el-Omar, E., Penman, I., Ardill, J., Chittajallu, R., Howie, C., McColl, K. (1995) *Helicobacter pylori* infection and abnormalities of acid secretion in patients with duodenal ulcer disease. Gastroenterology 109, 681-91.

103. Patel, J., Chavda, J. (2009) Formulation and evaluation of stomach-specific amoxicillin-loaded carbopol-934P mucoadhesive microspheres for anti-*Helicobacter pylori* therapy. J. Microencapsulation 26, 365-76.

104. Rajinikanth, P., Karunagaran, L., Balasubramaniam, J., Mishra, B. (2008) Formulation and evaluation of clarithromycin microspheres for eradication of *Helicobacter pylori*. Chem. Pharm. Bull. 56, 1658-64.

105. Huguet, M., Dellacherie, E. (1996) Calcium alginate beads coated with chitosan: Effect of the structure of encapsulated materials on their release. Process Biochem. 31, 745-51.

106. Whitehead, L., Collett, J., Fell, J. (2000) Amoxycillin release from a floating dosage form based on alginates. Int. J. Pharm. 210, 45-9.

107. Rossi, S., Ferrari, F., Bonferoni, M., Caramella, C. (2001) Characterization of chitosan hydrochloride-mucin rheological interaction: influence of polymer concentration and polymer:mucin weight ratio. Eur. J. Pharm. Sci. 12, 479-85.

108. Chun, M., Kwak, B., Choi, H. (2003) Preparation of buccal patch composed of carbopol, poloxamer and hydroxypropyl methylcellulose. Arch. Pharmacal Res. 26, 973-8.

109. Chun, M., Cho, C., Choi, H. (2001) A novel mucoadhesive polymer prepared by template polymerization of acrylic acid in the presence of poloxamer. J. Appl. Polym. Sci. 79, 1525-30.

110. Kim, T., Ahn, J., Choi, H., Choi, Y., Cho, C. (2007) A Novel Mucoadhesive Polymer Film Composed of Carbopol, Poloxamer and hydroxypropylmethylcellulose. Arch. Pharmacal Res. 30, 381-6.

111. Hassan, E., Gallo, J. (1990) A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. Pharm. Res. 7, 491-5.

112. Chan, C. K., Whitehouse, C., Gao, P., Chai, C. K. (2001) Flow induced chain alignment and disentanglement as the viscosity reduction mechanism within TLCP/HDPE blends. Polymer 42, 7847-56.

113. Hao, J., Chan, L., Shen, Z., Heng, P. (2005) Complexation between pvp and gantrez polymer and its effect on release and bioadhesive properties of the composite pvp/gantrez films. Pharm. Dev. Technol. 9, 379 - 86.

114. Riley, R., Smart, J., Tsibouklis, J., Dettmar, P., Hampson, F., Davis, J., Kelly, G., Wilber, W. (2001) An investigation of mucus/polymer rheological synergism using synthesised and characterised poly(acrylic acid)s. Int. J. Pharm. 217, 87-100.

115. Rossi, S., Ferrari, F., Bonferoni, M., Caramella, C. (2000) Characterization of chitosan hydrochloride-mucin interaction by means of viscosimetric and turbidimetric measurements. Eur. J. Pharm. Sci. 10, 251-7.

116. Sriamornsak, P., Wattanakorn, N. (2008) Rheological synergy in aqueous mixtures of pectin and mucin. Carbohydr. Polym. 74, 474-81.

117. Young, S., Wong, M., Tabata, Y., Mikos, A. (2005) Gelatin as a delivery vehicle for the controlled release of bioactive molecules. J. Controlled Release 109, 256-74.

118. Yin, Y., Yao, K., Cheng, G., Ma, J. (1999) Properties of polyelectrolyte complex films of chitosan and gelatin. Polym. Int. 48, 429-32.

119. Abruzzo, A., Bigucci, F., Cerchiara, T., Cruciani, F., Vitali, B., Luppi, B. (2012) Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. Carbohydr. Polym. 87, 581-8.

120. Khutoryanskiy, V. (2011) Advances in mucoadhesion and mucoadhesive polymers. Macromol Biosci 11, 748-64.

121. Hagerstrom, H., Edsman, K. (2003) Limitations of the rheological mucoadhesion method: the effect of the choice of conditions and the rheological synergism parameter. Eur. J. Pharm. Sci. 18, 349-57.

122. Caramella, C., Rossi, S., Bonferoni, M. A rheological approach to explain the mucoadhesive behavior of polymer hydrogels. In: Mathiowitz E, Chickering III D, Lehr C-M, editors. Bioadhesive drug delivery systems: Fundamentals, novel approaches, and development. New York: Marcel Dekker, Inc.; 1999. p. 25 - 65.

123. Hagerstrom, H., Paulsson, M., Edsman, K. (2000) Evaluation of mucoadhesion for two polyelectrolyte gels in simulated physiological conditions using a rheological method. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 9, 301-9.

124. Taylor, C., Pearson, J., Draget, K., Dettmar, P., Smidsrid, O. (2005) Rheological characterisation of mixed gels of mucin and alginate. Carbohydr. Polym. 59, 189-95.

125. Karavas, E., Georgarakis, E., Bikiaris, D. (2006) Application of PVP/HPMC miscible blends with enhanced mucoadhesive properties for adjusting drug release in predictable pulsatile chronotherapeutics. Eur. J. Pharm. Biopharm. 64, 115-26.

126. Mortazavi, S. (1995) An in vitro assessment of mucus/mucoadhesive interactions. Int. J. Pharm. 124, 173-82.

127. Vishnu, Y., Chandrasekhar, K., Ramesh, G., Rao, Y. (2007) Development of mucoadhesive patches for buccal administration of carvedilol. Curr Drug Deliv 4, 27-39.

128. Patel, M., Smart, J., Nevell, T., Ewen, R., Eaton, P., Tsibouklis, J. (2003) Mucin/Poly(acrylic acid) interactions: A spectroscopic investigation of mucoadhesion. Biomacromolecules 4, 1184-90.

129. Peppas, N. A., Buri, P. A. (1985) Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. J. Controlled Release 2, 257-75.

130. Jabbari, E., Wisniewski, N., Peppas, N. (1993) Evidence of mucoadhesion by chain interpenetration at a poly (acrylic acid)/mucin interface using ATR-FTIR spectroscopy. J. Controlled Release 26, 99-108.

131. Berntsson, O., Burger, T., Folestad, S., Danielsson, L., Kuhn, J., Fricke, J. (1999) Effective sample size in diffuse reflectance near-IR spectrometry. Anal. Chem. 71, 617-23.

132. PIKE Technologies. Diffuse reflectance – theory and applications. 2009.

133. Nijenhuis, K. Thermoreversible networks : viscoelastic properties and structure of gels. Advances in polymer science,. Berlin ; New York: Springer; 1997. p. xx, 267 p.

134. Tang, Q., Wu, J., Lin, J., Fan, S., Hu, D. (2009) A multifunctional poly(acrylic acid)/gelatin hydrogel. J Mater Res 24, 1653-61.

135. Parthasarathy, R., Rabuka, D., Bertozzi, C., Groves, J. (2007) Molecular orientation of membrane-anchored mucin glycoprotein mimics. J Phys Chem B 111, 12133-5.

136. Bettelheim, F. (1963) Physical chemistry of mucins. Ann. N.Y. Acad. Sci. 106, 247-58.

137. Suknuntha, K., Tantishaiyakul, V., Vao-Soongnern, V., Espidel, Y., Cosgrove, T. (2008) Molecular modeling simulation and experimental measurements to characterize chitosan and poly(vinyl pyrrolidone) blend interactions. J Polym Sci Pol Phys 46, 1258-64.

138. Wang, J., Tabata, Y., Bi, D., Morimoto, K. (2001) Evaluation of gastric mucoadhesive properties of aminated gelatin microspheres. Journal of controlled release: official journal of the Controlled Release Society 73, 223-31.

139. Hagerstrom, H., Edsman, K. (2001) Interpretation of mucoadhesive properties of polymer gel preparations using a tensile strength method. J. Pharm. Pharmacol. 53, 1589-99.

140. Thirawong, N., Nunthanid, J., Puttipipatkhachorn, S., Sriamornsak, P. (2007) Mucoadhesive properties of various pectins on gastrointestinal mucosa: an in vitro evaluation using texture analyzer. Eur. J. Pharm. Biopharm. 67, 132-40.

141. Fransen, N., Bjork, E., Edsman, K. (2008) Changes in the mucoadhesion of powder formulations after drug application investigated with a simplified method. J. Pharm. Sci. 97, 3855-64.

142. Vishnu, Y., Chandrasekhar, K., Ramesh, G., Rao, Y. (2007) Development of mucoadhesive patches for buccal administration of carvedilol. Curr Drug Deliv 4, 27-39.

143. Khutoryanskiy, V. (2011) Advances in mucoadhesion and mucoadhesive polymers. Macromol Biosci 11, 748-64.

144. Park, C. R., Munday, D. L. (2004) Evaluation of selected polysaccharide excipients in buccoadhesive tablets for sustained release of nicotine. Drug Dev Ind Pharm 30, 609-17.

145. Piao, J., Lee, J. E., Weon, K. Y., Kim, D. W., Lee, J. S., Park, J. D., Nishiyama, Y., Fukui, I., Kim, J. S. (2009) Development of novel muco-adhesive pellets of metformin hydrochloride. Arch Pharm Res 32, 391-7.

146. Hagesaether, E., Hiorth, M., Sande, S. A. (2009) Mucoadhesion and drug permeability of free mixed films of pectin and chitosan: an in vitro and ex vivo study. Eur J Pharm Biopharm 71, 325-31.

147. Fransen, N., Bjork, E., Edsman, K. (2008) Changes in the mucoadhesion of powder formulations after drug application investigated with a simplified method. J Pharm Sci 97, 3855-64.

148. Kharenko, E., Larionova, N., Demina, N. (2008) Mucoadhesive Drug Delivery Systems: Quantitative Assessment of Interaction Between Synthetic and Natural Polymer Films and Mucosa. Pharm. Chem. J. 42, 392-9.

149. Kurskaya, E., Vainerman, E., Timofeeva, G., Rogozhin, S. (1980) A study of soluble complexes of acid-process and alkaline-process gelatins by turbidimetric titration. Colloid & Polymer Science 258, 1086-91.

150. Mathiowitz, E., Chickering, D., Lehr, C. Bioadhesive drug delivery systems: fundamentals, novel approaches, and development. New York: Marcel Dekker; 1999.

151. Dodou, D., Breedveld, P., Wieringa, P. (2005) Mucoadhesives in the gastrointestinal tract: revisiting the literature for novel applications. Eur. J. Pharm. Biopharm. 60, 1-16.

152. Yoshioka, S., Aso, Y., Otsuka, T., Kojima, S. (1995) Water mobility in poly(ethylene glycol), poly(vinylpyrrolidone), and gelatin-water systems, as indicated by dielectric relaxation time, spin-latice relaxation time, and water activity. J. Pharm. Sci. 84, 1072-7.

153. Thirawong, N., Nunthanid, J., Puttipipatkhachorn, S., Sriamornsak, P. (2007) Mucoadhesive properties of various pectins on gastrointestinal mucosa: An in vitro evaluation using texture analyzer. Eur. J. Pharm. Biopharm. 67, 132-40.

154. Rodríguez, R., Alvarez-Lorenzo, C., Concheiro, A. (2001) Rheological evaluation of the interactions between cationic celluloses and Carbopol 974P in water. Biomacromolecules 2, 886-93.

155. Edsman, K., Hagerstrom, H. (2005) Pharmaceutical applications of mucoadhesion for the non-oral routes. J. Pharm. Pharmacol. 57, 3-22.

156. Keely, S., Rullay, A., Wilson, C., Carmichael, A., Carrington, S., Corfield, A., Haddleton, D., Brayden, D. (2005) In vitro and ex vivo intestinal tissue models to measure mucoadhesion of poly (methacrylate) and N-trimethylated chitosan polymers. Pharm. Res. 22, 38-49.

157. Badhan, A., Mashru, R., Shah, P. P., Thakkar, A., Dobaria, N. (2009) Development and evaluation of sustained release gastroretentive minimatrices for effective treatment of *H. pylori* infection. AAPS PharmSciTech 10, 459-67.

158. Riley, R., Smart, J., Tsibouklis, J., Young, S., Hampson, F., Davis, A., Kelly, G., Dettmar, P., Wilber, W. (2002) An *in vitro* model for investigating the gastric mucosal retention of 14C-labelled poly(acrylic acid) dispersions. Int. J. Pharm. 236, 87-96.

159. Mahrag, K., Ch'ng, H. (1998) Evaluation of possible mechanism(s) of bioadhesion. Int. J. Pharm. 160, 61-74.

160. Liu, Z., Lu, W., Qian, L., Zhang, X., Zeng, P., Pan, J. (2005) *In vitro* and in vivo studies on mucoadhesive microspheres of amoxicillin. J. Controlled Release 102, 135-44.

161. Ahn, J.-S., Choi, H.-K., Chun, M.-K., Ryu, J.-M., Jung, J.-H., Kim, Y.-U., Cho, C.-S. (2002) Release of triamcinolone acetonide from mucoadhesive polymer composed of chitosan and poly(acrylic acid) in vitro. Biomaterials 23, 1411-6.

162. Chun, M., Cho, C., Choi, H. (2005) Mucoadhesive microspheres prepared by interpolymer complexation and solvent diffusion method. Int. J. Pharm. 288, 295-303.

163. Chun, M., Cho, C., Choi, H. (2001) A novel mucoadhesive polymer prepared by template polymerization of acrylic acid in the presence of poloxamer. J. Appl. Polym. Sci. 79, 1525-30.

164. Kim, T., Ahn, J., Choi, H., Choi, Y., Cho, C. (2007) A novel mucoadhesive polymer film composed of carbopol, poloxamer and hydroxypropyl-methylcellulose. Arch. Pharmacal Res. 30, 381-6.

165. Hao, J., Chan, L., Shen, Z., Heng, P. (2005) Complexation between pvp and gantrez polymer and its effect on release and bioadhesive properties of the composite pvp/gantrez films. Pharm. Dev. Technol. 9, 379 - 86.

166. Zhu, A., Wang, S., Yuan, Y., Shen, J. (2002) Cell adhesion behavior of chitosan surface modified by bonding 2-methacryloyloxyethyl phosphoryl-choline. Journal of biomaterials science. Polymer edition 13, 501-10.

167. Bettini, R., Romani, A. A., Morganti, M. M., Borghetti, A. F. (2008) Physicochemical and cell adhesion properties of chitosan films prepared from sugar and phosphate-containing solutions. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 68, 74-81.

168. Freier, T., Koh, H., Kazazian, K., Shoichet, M. (2005) Controlling cell adhesion and degradation of chitosan films by N-acetylation. Biomaterials 26, 5872-8.

169. Sailaja, G., Ramesh, P., Kumary, T., Varma, H. (2006) Human osteosarcoma cell adhesion behaviour on hydroxyapatite integrated chitosan-poly(acrylic acid) polyelectrolyte complex. Acta Biomater 2, 651-7.

170. Sigma-Aldrich. Attachment and matrix factors. BioFiles. 2008:4 - 13.

171. Freier, T., Koh, H., Kazazian, K., Shoichet, M. (2005) Controlling cell adhesion and degradation of chitosan films by N-acetylation. Biomaterials 26, 5872-8.

172. Chatelet, C., Damour, O., Domard, A. (2001) Influence of the degree of acetylation on some biological properties of chitosan films. Biomaterials 22, 261-8.

173. Jones, D., Lawlor, M., Woolfson, A. (2003) Rheological and mucoadhesive characterization of polymeric systems composed of poly(methylvinylether-co-maleic anhydride) and poly(vinylpyrrolidone), designed as platforms for topical drug delivery. J. Pharm. Sci. 92, 995-1007.

174. Bruschi, M., Jones, D., Panzeri, H., Gremião, M., de Freitas, O., Lara, E. (2007) Semisolid systems containing propolis for the treatment of periodontal

disease: *In vitro* release kinetics, syringeability, rheological, textural, and mucoadhesive properties. J. Pharm. Sci. 96, 2074-89.

175. Matsuda, S., Iwata, H., Se, N., Ikada, Y. (1999) Bioadhesion of gelatin films crosslinked with glutaraldehyde. J. Biomed. Mater. Res. 45, 20-7.

176. Ratanavaraporn, J., Damrongsakkul, S., Sanchavanakit, N., Banaprasert, T., Kanokpanont, S. (2006) Comparison of gelatin and collagen scaffolds for fibroblast cell culture. Journal of Metals, Materials and Minerals. 16, 31 - 6.

177. Karavas, E., Georgarakis, E., Bikiaris, D. (2006) Application of PVP/HPMC miscible blends with enhanced mucoadhesive properties for adjusting drug release in predictable pulsatile chronotherapeutics. Eur. J. Pharm. Biopharm. 64, 115-26.

178. Jones, D., Woolfson, A., Brown, A., Coulter, W., McClelland, C., Irwin, C. (2000) Design, characterisation and preliminary clinical evaluation of a novel mucoadhesive topical formulation containing tetracycline for the treatment of periodontal disease. J Control Release 67, 357-68.

179. Huang, Y., Onyeri, S., Siewe, M., Moshfeghian, A., Madihally, S. (2005) In vitro characterization of chitosan-gelatin scaffolds for tissue engineering. Biomaterials 26, 7616-27.

180. Nagahama, H., Maeda, H., Kashiki, T., Jayakumar, R., Furuike, T., Tamura, H. (2009) Preparation and characterization of novel chitosan/gelatin membranes using chitosan hydrogel. Carbohydr. Polym. 76, 255-60.

181. Zhu, A., Wang, S., Yuan, Y., Shen, J. (2002) Cell adhesion behavior of chitosan surface modified by bonding 2-methacryloyloxyethyl phosphorylcholine. J. Biomater. Sci. Polym. Ed. 13, 501-10.

182. Bettini, R., Romani, A., Morganti, M., Borghetti, A. (2008) Physicochemical and cell adhesion properties of chitosan films prepared from sugar and phosphate-containing solutions. Eur. J. Pharm. Biopharm. 68, 74-81.

183. Pulieri, E., Chiono, V., Ciardelli, G., Vozzi, G., Ahluwalia, A., Domenici, C., Vozzi, F., Giusti, P. (2008) Chitosan/gelatin blends for biomedical applications. J Biomed Mater Res A 86, 311-22.

184. Shah, S., Qaqish, R., Patel, V., Amiji, M. (1999) Evaluation of the factors influencing stomach-specific delivery of antibacterial agents for *Helicobacter pylori* infection. J. Pharm. Pharmacol. 51, 667-72.

185. Kimura, K., Ido, K., Saifuku, K., Taniguchi, Y., Kihira, K., Satoh, K., Takimoto, T., Yoshida, Y. (1995) A 1-h topical therapy for the treatment of *Helicobacter pylori* infection. Am. J. Gastroenterol. 90, 60-3.

186. Jianhua, Z., Chaowu, L., Decai, B., Yanjun, Z., Xiaojun, M. (2006) Preparation and evaluation of floating-bioadhesive microparticles containing clarithromycin for the eradication of Helicobacter pylori. J. Appl. Polym. Sci. 102, 2226-32.

187. Ishak, R., Awad, G., Mortada, N., Nour, S. (2007) Preparation, in vitro and in vivo evaluation of stomach-specific metronidazole-loaded alginate beads as local anti-*Helicobacter pylori* therapy. J Control Release 119, 207-14.

188. Govender, S., Pillay, V., Chetty, D., Essack, S., Dangor, C., Govender, T. (2005) Optimisation and characterisation of bioadhesive controlled release tetracycline microspheres. Int. J. Pharm. 306, 24-40.

189. Liu, Z., Lu, W., Qian, L., Zhang, X., Zeng, P., Pan, J. (2005) In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. J Control Release 102, 135-44.

190. Elzatahry, A., Eldin, M. S., Soliman, E., Hassan, E. (2009) Evaluation of alginate-chitosan bioadhesive beads as a drug delivery system for the controlled release of theophylline. J. Appl. Polym. Sci. 111, 2452-9.

191. Goodman, L. S., Brunton, L. L., Blumenthal, D. K., Murri, N., Hilal-Dandan, R. Goodman & Gilman's The pharmacological basis of therapeutics. New York: McGraw-Hill Medical; 2011. Available from: http://www.accessmedicine.com/resourceTOC.aspx?resourceID=651.

192. Erah, P., Goddard, A., Barrett, D., Shaw, N., Spiller, R. (1995) Effect of pH on the stability of amoxycillin in buffered aqueous solutions and in gastric juice. Pharm. Pharmacol. Commun. 1, 597-600.

193. O'Neil, M. The Merck Index: An encyclopedia of chemicals, drugs, and biologicals. 14th ed: Merck; 2006.

194. United States Pharmacopeia and National Formulary (USP 33-NF 28): The United States Pharmacopeial Convention; 2010.

195. Wang, K., He, Z. (2002) Alginate–konjac glucomannan–chitosan beads as controlled release matrix. Int. J. Pharm. 244, 117-26.

196. Dong, Z., Wang, Q., Du, Y. (2006) Alginate/gelatin blend films and their properties for drug controlled release. Journal of Membrane Science 280, 37-44.

197. Wittaya-areekul, S., Kruenate, J., Prahsarn, C. (2006) Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. Int. J. Pharm. 312, 113-8.

198. Mauger, J., Chilko, D., Howard, S. (1986) On the analysis of dissolution data. Drug Dev. Ind. Pharm. 12, 969-92.

199. Hurtado, M., Vargas, Y., Dominguez-Ramirez, A., Cortes Arroyo, A. (2003) Comparison of dissolution profiles for albendazole tablets using USP apparatus 2 and 4. Drug Dev. Ind. Pharm. 29, 777-84.

200. Yuksel, N., Kanik, A., Baykara, T. (2000) Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and -independent methods. Int. J. Pharm. 209, 57-67.

201. SPSS advanced statistics 17.0: SPSS Inc.; 2007.

202. Lee, O., Ha, B., Park, S., Lee, Y. (1997) Studies on the pH-dependent swelling properties and morphologies of chitosan/calcium-alginate complexed beads. Macromol. Chem. Phys. 198, 2971-6.

203. Pasparakis, G., Bouropoulos, N. (2006) Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate-chitosan beads. Int. J. Pharm. 323, 34-42.

204. Nayak, A., Hasnain, M., Beg, S., Alam, M. (2010) Mucoadhesive beads of gliclazide: Design, development, and evaluation. ScienceAsia 36, 319-25.

205. Jasti, B., Li, X., Cleary, G. (2003) Recent advances in mucoadhesive drug delivery systems. Business Briefing Pharmatech 194-6.

206. Almeida, F., Almeida, A. (2004) Cross-linked alginate-gelatine beads: a new matrix for controlled release of pindolol. J Control Release 97, 431-9.

207. Grassi, M., Grassi, G. (2005) Mathematical modelling and controlled drug delivery: matrix systems. Curr Drug Deliv 2, 97-116.

208. Nochos, A., Douroumis, D., Bouropoulos, N. (2008) *In vitro* release of bovine serum albumin from alginate/HPMC hydrogel beads. Carbohydr. Polym. 74, 451-7.

209. Yuksel, N., Kanik, A., Baykara, T. (2000) Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and -independent methods. Int. J. Pharm. 209, 57-67.

210. Polli, J., Rekhi, G., Augsburger, L., Shah, V. (1997) Methods to compare dissolution profiles and a rationale for wide dissolution specifications for metoprolol tartrate tablets. J. Pharm. Sci. 86, 690-700.

211. Lu, D., Abu, K., Mao, F. (1996) Nonlinear data fitting for controlled release devices: An integrated computer program. Int. J. Pharm. 129, 243-51.

212. Mauger, J., Chilko, D., Howard, S. (1986) On the analysis of dissolution data. Drug Dev. Ind. Pharm. 12, 969-92.

213. Erah, P., Goddard, A., Barrett, D., Shaw, N., Spiller, R. (1995) Effect of pH on the stability of amoxycillin in buffered aqueous solutions and in gastric juice. Pharm. Pharmacol. Commun. 1, 597-600.

214. Hurtado, M., Vargas, Y., Dominguez-Ramirez, A., Cortes Arroyo, A. (2003) Comparison of dissolution profiles for albendazole tablets using USP apparatus 2 and 4. Drug Dev. Ind. Pharm. 29, 777-84.

215. SPSS advanced statistics 17.0: SPSS Inc.; 2007.

216. Blum, R., Shi, H., Karol, M., Greski-Rose, P., Hunt, R. (1997) The comparative effects of lansoprazole, omeprazole, and ranitidine in suppressing gastric acid secretion. Clin. Ther. 19, 1013-23.

217. Gouda, B., Lydon, A., Badhe, A., Shorten, G. (2004) A comparison of the effects of ranitidine and omeprazole on volume and pH of gastric contents in elective surgical patients. Eur. J. Anaesthesiol. 21, 260-4.

218. Kimura, K., Ido, K., Saifuku, K., Taniguchi, Y., Kihira, K., Satoh, K., Takimoto, T., Yoshida, Y. (1995) A 1-h topical therapy for the treatment of *Helicobacter pylori* infection. Am. J. Gastroenterol. 90, 60-3.

219. Lin, S., Ayres, J. (1992) Calcium Alginate Beads as Core Carriers of 5-Aminosalicylic Acid. Pharm. Res. 9, 1128-31.

220. Ritger, P., Peppas, N. (1987) A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J. Controlled Release 5, 37-42.

221. Kim, M., Park, G., Jun, S., Lee, S., Park, J., Hwang, S. (2005) Controlled release tamsulosin hydrochloride from alginate beads with waxy materials. J. Pharm. Pharmacol. 57, 1521-8.

222. Hixson, A., Crowell, J. (1931) Dependence of reaction velocity upon surface and agitation. Industrial & Engineering Chemistry 23, 923-31.

223. Setty, C., Sahoo, S., Sa, B. (2005) Alginate-coated alginate-polyethyleneimine beads for prolonged release of furosemide in simulated intestinal fluid. Drug Dev. Ind. Pharm. 31, 435-46.

224. Korsmeyer, R., Gurny, R., Doelker, E., Buri, P., Peppas, N. (1983) Mechanisms of solute release from porous hydrophilic polymers. Int. J. Pharm. 15, 25-35.

225. Langer, R., Peppas, N. (1981) Present and future applications of biomaterials in controlled drug delivery systems. Biomaterials 2, 201-14.

226. Elzatahry, A., Eldin, M. S., Soliman, E., Hassan, E. (2009) Evaluation of alginate-chitosan bioadhesive beads as a drug delivery system for the controlled release of theophylline. J. Appl. Polym. Sci. 111, 2452-9.

227. Pasparakis, G., Bouropoulos, N. (2006) Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate-chitosan beads. Int. J. Pharm. 323, 34-42.

Appendix A

Statistical analysis results of evaluation of mucoadhesive polymers using viscosity measurements

Oneway ANOVA of viscosity enhancement ($\eta_{enhance}$) of single polymers with mucin

ANOVA

nb

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	322.258	3	107.419	73.137	.000
Within Groups	11.750	8	1.469		
Total	334.008	11			

Multiple Comparisons

nb

Tukey HSD

					95% Confidence Interval	
(I) group4	(J) group4	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Chitosan	PVP	6.83197*	.98953	.001	3.6632	10.0008
	GA	13.66357*	.98953	.000	10.4948	16.8324
	GB	11.16357*	.98953	.000	7.9948	14.3324
PVP	Chitosan	-6.83197*	.98953	.001	-10.0008	-3.6632
	GA	6.83160*	.98953	.001	3.6628	10.0004
	GB	4.33160*	.98953	.010	1.1628	7.5004
GA	Chitosan	-13.66357*	.98953	.000	-16.8324	-10.4948
	PVP	-6.83160*	.98953	.001	-10.0004	-3.6628
	GB	-2.50000	.98953	.130	-5.6688	.6688
GB	Chitosan	-11.16357*	.98953	.000	-14.3324	-7.9948
	PVP	-4.33160*	.98953	.010	-7.5004	-1.1628
	GA	2.50000	.98953	.130	-.6688	5.6688

*. The mean difference is significant at the 0.05 level.

Oneway ANOVA of viscosity enhancement ($\eta_{enhance}$) of combination systems of polymer or polymer blends with mucin

1. C/PVP

ANOVA

nb1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	836.978	6	139.496	4262.681	.000
Within Groups	.458	14	.033		
Total	837.436	20			

Multiple Comparisons

nb1

Tukey HSD

					95% Confidence Interval	
(I) group1	(J) group1	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
C	C/P 9/1	.66657*	.14770	.007	.1622	1.1709
	C/P 7/3	.08333	.14770	.997	-.4210	.5877
	C/P 5/5	-12.74723*	.14770	.000	-13.2516	-12.2429
	C/P 3/7	-10.41440*	.14770	.000	-10.9188	-9.9100
	C/P 1/9	-1.33300*	.14770	.000	-1.8374	-.8286
	PVP	6.83197*	.14770	.000	6.3276	7.3363
C/P 9/1	C	-.66657*	.14770	.007	-1.1709	-.1622
	C/P 7/3	-.58323*	.14770	.019	-1.0876	-.0789
	C/P 5/5	-13.41380*	.14770	.000	-13.9182	-12.9094
	C/P 3/7	-11.08097*	.14770	.000	-11.5853	-10.5766
	C/P 1/9	-1.99957*	.14770	.000	-2.5039	-1.4952
	PVP	6.16540*	.14770	.000	5.6610	6.6698
C/P 7/3	C	-.08333	.14770	.997	-.5877	.4210
	C/P 9/1	.58323*	.14770	.019	.0789	1.0876
	C/P 5/5	-12.83057*	.14770	.000	-13.3349	-12.3262
	C/P 3/7	-10.49773*	.14770	.000	-11.0021	-9.9934
	C/P 1/9	-1.41633*	.14770	.000	-1.9207	-.9120
	PVP	6.74863*	.14770	.000	6.2443	7.2530
C/P 5/5	C	12.74723*	.14770	.000	12.2429	13.2516
	C/P 9/1	13.41380*	.14770	.000	12.9094	13.9182

	C/P 7/3	12.83057*	.14770	.000	12.3262	13.3349
	C/P 3/7	2.33283*	.14770	.000	1.8285	2.8372
	C/P 1/9	11.41423*	.14770	.000	10.9099	11.9186
	PVP	19.57920*	.14770	.000	19.0748	20.0836
C/P 3/7	C	10.41440*	.14770	.000	9.9100	10.9188
	C/P 9/1	11.08097*	.14770	.000	10.5766	11.5853
	C/P 7/3	10.49773*	.14770	.000	9.9934	11.0021
	C/P 5/5	-2.33283*	.14770	.000	-2.8372	-1.8285
	C/P 1/9	9.08140*	.14770	.000	8.5770	9.5858
	PVP	17.24637*	.14770	.000	16.7420	17.7507
C/P 1/9	C	1.33300*	.14770	.000	.8286	1.8374
	C/P 9/1	1.99957*	.14770	.000	1.4952	2.5039
	C/P 7/3	1.41633*	.14770	.000	.9120	1.9207
	C/P 5/5	-11.41423*	.14770	.000	-11.9186	-10.9099
	C/P 3/7	-9.08140*	.14770	.000	-9.5858	-8.5770
	PVP	8.16497*	.14770	.000	7.6606	8.6693
PVP	C	-6.83197*	.14770	.000	-7.3363	-6.3276
	C/P 9/1	-6.16540*	.14770	.000	-6.6698	-5.6610
	C/P 7/3	-6.74863*	.14770	.000	-7.2530	-6.2443
	C/P 5/5	-19.57920*	.14770	.000	-20.0836	-19.0748
	C/P 3/7	-17.24637*	.14770	.000	-17.7507	-16.7420
	C/P 1/9	-8.16497*	.14770	.000	-8.6693	-7.6606

*. The mean difference is significant at the 0.05 level.

2. C/GA

ANOVA

nb3

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	498.196	6	83.033	2536.269	.000
Within Groups	.458	14	.033		
Total	498.654	20			

Multiple Comparisons

nb3

Tukey HSD

(I) group3	(J) group3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C	C/GA 9/1	.59023*	.14773	.018	.0858	1.0947
	C/GA 7/3	7.66357*	.14773	.000	7.1591	8.1680
	C/GA 5/5	6.17357*	.14773	.000	5.6691	6.6780
	C/GA 3/7	7.58023*	.14773	.000	7.0758	8.0847
	C/GA 1/9	12.49690*	.14773	.000	11.9924	13.0014
	GA	13.66357*	.14773	.000	13.1591	14.1680
C/GA 9/1	C	-.59023*	.14773	.018	-1.0947	-.0858
	C/GA 7/3	7.07333*	.14773	.000	6.5689	7.5778
	C/GA 5/5	5.58333*	.14773	.000	5.0789	6.0878
	C/GA 3/7	6.99000*	.14773	.000	6.4855	7.4945
	C/GA 1/9	11.90667*	.14773	.000	11.4022	12.4111
	GA	13.07333*	.14773	.000	12.5689	13.5778
C/GA 7/3	C	-7.66357*	.14773	.000	-8.1680	-7.1591
	C/GA 9/1	-7.07333*	.14773	.000	-7.5778	-6.5689
	C/GA 5/5	-1.49000*	.14773	.000	-1.9945	-.9855
	C/GA 3/7	-.08333	.14773	.997	-.5878	.4211
	C/GA 1/9	4.83333*	.14773	.000	4.3289	5.3378
	GA	6.00000*	.14773	.000	5.4955	6.5045
C/GA 5/5	C	-6.17357*	.14773	.000	-6.6780	-5.6691
	C/GA 9/1	-5.58333*	.14773	.000	-6.0878	-5.0789
	C/GA 7/3	1.49000*	.14773	.000	.9855	1.9945
	C/GA 3/7	1.40667*	.14773	.000	.9022	1.9111
	C/GA 1/9	6.32333*	.14773	.000	5.8189	6.8278
	GA	7.49000*	.14773	.000	6.9855	7.9945

C/GA 3/7	C	-7.58023*	.14773	.000	-8.0847	-7.0758
	C/GA 9/1	-6.99000*	.14773	.000	-7.4945	-6.4855
	C/GA 7/3	.08333	.14773	.997	-.4211	.5878
	C/GA 5/5	-1.40667*	.14773	.000	-1.9111	-.9022
	C/GA 1/9	4.91667*	.14773	.000	4.4122	5.4211
	GA	6.08333*	.14773	.000	5.5789	6.5878
C/GA 1/9	C	-12.49690*	.14773	.000	-13.0014	-11.9924
	C/GA 9/1	-11.90667*	.14773	.000	-12.4111	-11.4022
	C/GA 7/3	-4.83333*	.14773	.000	-5.3378	-4.3289
	C/GA 5/5	-6.32333*	.14773	.000	-6.8278	-5.8189
	C/GA 3/7	-4.91667*	.14773	.000	-5.4211	-4.4122
	GA	1.16667*	.14773	.000	.6622	1.6711
GA	C	-13.66357*	.14773	.000	-14.1680	-13.1591
	C/GA 9/1	-13.07333*	.14773	.000	-13.5778	-12.5689
	C/GA 7/3	-6.00000*	.14773	.000	-6.5045	-5.4955
	C/GA 5/5	-7.49000*	.14773	.000	-7.9945	-6.9855
	C/GA 3/7	-6.08333*	.14773	.000	-6.5878	-5.5789
	C/GA 1/9	-1.16667*	.14773	.000	-1.6711	-.6622

*. The mean difference is significant at the 0.05 level.

3. C/GB

ANOVA

nb2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	899.309	6	149.885	152.149	.000
Within Groups	13.792	14	.985		
Total	913.101	20			

Multiple Comparisons

nb2

Tukey HSD

					95% Confidence Interval	
(I) group2	(J) group2	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
C	C/GB 9/1	3.67357*	.81040	.007	.9064	6.4407
	C/GB 7/3	2.25690	.81040	.147	-.5103	5.0241
	C/GB 5/5	-7.24310*	.81040	.000	-10.0103	-4.4759
	C/GB 3/7	-9.07643*	.81040	.000	-11.8436	-6.3093
	C/GB 1/9	5.17357*	.81040	.000	2.4064	7.9407
	GB	11.16357*	.81040	.000	8.3964	13.9307
C/GB 9/1	C	-3.67357*	.81040	.007	-6.4407	-.9064
	C/GB 7/3	-1.41667	.81040	.599	-4.1838	1.3505
	C/GB 5/5	-10.91667*	.81040	.000	-13.6838	-8.1495
	C/GB 3/7	-12.75000*	.81040	.000	-15.5172	-9.9828
	C/GB 1/9	1.50000	.81040	.539	-1.2672	4.2672
	GB	7.49000*	.81040	.000	4.7228	10.2572
C/GB 7/3	C	-2.25690	.81040	.147	-5.0241	.5103
	C/GB 9/1	1.41667	.81040	.599	-1.3505	4.1838
	C/GB 5/5	-9.50000*	.81040	.000	-12.2672	-6.7328
	C/GB 3/7	-11.33333*	.81040	.000	-14.1005	-8.5662
	C/GB 1/9	2.91667*	.81040	.036	.1495	5.6838
	GB	8.90667*	.81040	.000	6.1395	11.6738
C/GB 5/5	C	7.24310*	.81040	.000	4.4759	10.0103
	C/GB 9/1	10.91667*	.81040	.000	8.1495	13.6838
	C/GB 7/3	9.50000*	.81040	.000	6.7328	12.2672
	C/GB 3/7	-1.83333	.81040	.325	-4.6005	.9338
	C/GB 1/9	12.41667*	.81040	.000	9.6495	15.1838
	GB	18.40667*	.81040	.000	15.6395	21.1738

C/GB 3/7	C	9.07643*	.81040	.000	6.3093	11.8436
	C/GB 9/1	12.75000*	.81040	.000	9.9828	15.5172
	C/GB 7/3	11.33333*	.81040	.000	8.5662	14.1005
	C/GB 5/5	1.83333	.81040	.325	-.9338	4.6005
	C/GB 1/9	14.25000*	.81040	.000	11.4828	17.0172
	GB	20.24000*	.81040	.000	17.4728	23.0072
C/GB 1/9	C	-5.17357*	.81040	.000	-7.9407	-2.4064
	C/GB 9/1	-1.50000	.81040	.539	-4.2672	1.2672
	C/GB 7/3	-2.91667*	.81040	.036	-5.6838	-.1495
	C/GB 5/5	-12.41667*	.81040	.000	-15.1838	-9.6495
	C/GB 3/7	-14.25000*	.81040	.000	-17.0172	-11.4828
	GB	5.99000*	.81040	.000	3.2228	8.7572
GB	C	-11.16357*	.81040	.000	-13.9307	-8.3964
	C/GB 9/1	-7.49000*	.81040	.000	-10.2572	-4.7228
	C/GB 7/3	-8.90667*	.81040	.000	-11.6738	-6.1395
	C/GB 5/5	-18.40667*	.81040	.000	-21.1738	-15.6395
	C/GB 3/7	-20.24000*	.81040	.000	-23.0072	-17.4728
	C/GB 1/9	-5.99000*	.81040	.000	-8.7572	-3.2228

*. The mean difference is significant at the 0.05 level.

Oneway ANOVA of the mucoadhesive force of a single polymer with mucin

ANOVA

force4

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	80855.444	3	26951.815	73.136	.000
Within Groups	2948.141	8	368.518		
Total	83803.585	11			

Multiple Comparisons

force4

Tukey HSD

					95% Confidence Interval	
(I) group4	(J) group4	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Chitosan	PVP	108.22000*	15.67413	.001	58.0259	158.4141
	GA	216.43000*	15.67413	.000	166.2359	266.6241
	GB	176.83000*	15.67413	.000	126.6359	227.0241
PVP	Chitosan	-108.22000*	15.67413	.001	-158.4141	-58.0259
	GA	108.21000*	15.67413	.001	58.0159	158.4041
	GB	68.61000*	15.67413	.010	18.4159	118.8041
GA	Chitosan	-216.43000*	15.67413	.000	-266.6241	-166.2359
	PVP	-108.21000*	15.67413	.001	-158.4041	-58.0159
	GB	-39.60000	15.67413	.130	-89.7941	10.5941
GB	Chitosan	-176.83000*	15.67413	.000	-227.0241	-126.6359
	PVP	-68.61000*	15.67413	.010	-118.8041	-18.4159
	GA	39.60000	15.67413	.130	-10.5941	89.7941

*. The mean difference is significant at the 0.05 level.

Oneway ANOVA of the mucoadhesive force of the polymer and polymer blends with mucin

1. C/PVP

ANOVA

force1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	210006.887	6	35001.148	4263.023	.000
Within Groups	114.946	14	8.210		
Total	210121.833	20			

Multiple Comparisons

force1

Tukey HSD

					95% Confidence Interval	
(I) group1	(J) group1	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
C	C/P 9/1	10.56000*	2.33957	.007	2.5713	18.5487
	C/P 7/3	1.32000	2.33957	.997	-6.6687	9.3087
	C/P 5/5	-201.92000*	2.33957	.000	-209.9087	-193.9313
	C/P 3/7	-164.96333*	2.33957	.000	-172.9520	-156.9747
	C/P 1/9	-21.12000*	2.33957	.000	-29.1087	-13.1313
	PVP	108.22000*	2.33957	.000	100.2313	116.2087
C/P 9/1	C	-10.56000*	2.33957	.007	-18.5487	-2.5713
	C/P 7/3	-9.24000*	2.33957	.019	-17.2287	-1.2513
	C/P 5/5	-212.48000*	2.33957	.000	-220.4687	-204.4913
	C/P 3/7	-175.52333*	2.33957	.000	-183.5120	-167.5347
	C/P 1/9	-31.68000*	2.33957	.000	-39.6687	-23.6913
	PVP	97.66000*	2.33957	.000	89.6713	105.6487
C/P 7/3	C	-1.32000	2.33957	.997	-9.3087	6.6687
	C/P 9/1	9.24000*	2.33957	.019	1.2513	17.2287
	C/P 5/5	-203.24000*	2.33957	.000	-211.2287	-195.2513
	C/P 3/7	-166.28333*	2.33957	.000	-174.2720	-158.2947
	C/P 1/9	-22.44000*	2.33957	.000	-30.4287	-14.4513
	PVP	106.90000*	2.33957	.000	98.9113	114.8887
C/P 5/5	C	201.92000*	2.33957	.000	193.9313	209.9087
	C/P 9/1	212.48000*	2.33957	.000	204.4913	220.4687
	C/P 7/3	203.24000*	2.33957	.000	195.2513	211.2287

	C/P 3/7	36.95667*	2.33957	.000	28.9680	44.9453
	C/P 1/9	180.80000*	2.33957	.000	172.8113	188.7887
	PVP	310.14000*	2.33957	.000	302.1513	318.1287
C/P 3/7	C	164.96333*	2.33957	.000	156.9747	172.9520
	C/P 9/1	175.52333*	2.33957	.000	167.5347	183.5120
	C/P 7/3	166.28333*	2.33957	.000	158.2947	174.2720
	C/P 5/5	-36.95667*	2.33957	.000	-44.9453	-28.9680
	C/P 1/9	143.84333*	2.33957	.000	135.8547	151.8320
	PVP	273.18333*	2.33957	.000	265.1947	281.1720
C/P 1/9	C	21.12000*	2.33957	.000	13.1313	29.1087
	C/P 9/1	31.68000*	2.33957	.000	23.6913	39.6687
	C/P 7/3	22.44000*	2.33957	.000	14.4513	30.4287
	C/P 5/5	-180.80000*	2.33957	.000	-188.7887	-172.8113
	C/P 3/7	-143.84333*	2.33957	.000	-151.8320	-135.8547
	PVP	129.34000*	2.33957	.000	121.3513	137.3287
PVP	C	-108.22000*	2.33957	.000	-116.2087	-100.2313
	C/P 9/1	-97.66000*	2.33957	.000	-105.6487	-89.6713
	C/P 7/3	-106.90000*	2.33957	.000	-114.8887	-98.9113
	C/P 5/5	-310.14000*	2.33957	.000	-318.1287	-302.1513
	C/P 3/7	-273.18333*	2.33957	.000	-281.1720	-265.1947
	C/P 1/9	-129.34000*	2.33957	.000	-137.3287	-121.3513

*. The mean difference is significant at the 0.05 level.

2. C/GA

ANOVA

force3

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	124982.444	6	20830.407	2535.911	.000
Within Groups	114.998	14	8.214		
Total	125097.442	20			

Multiple Comparisons

force3

Tukey HSD

(I) group3	(J) group3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C	C/GA 9/1	9.35000*	2.34011	.017	1.3595	17.3405
	C/GA 7/3	121.39000*	2.34011	.000	113.3995	129.3805
	C/GA 5/5	97.79000*	2.34011	.000	89.7995	105.7805
	C/GA 3/7	120.07000*	2.34011	.000	112.0795	128.0605
	C/GA 1/9	197.92000*	2.34011	.000	189.9295	205.9105
	GA	216.43000*	2.34011	.000	208.4395	224.4205
C/GA 9/1	C	-9.35000*	2.34011	.017	-17.3405	-1.3595
	C/GA 7/3	112.04000*	2.34011	.000	104.0495	120.0305
	C/GA 5/5	88.44000*	2.34011	.000	80.4495	96.4305
	C/GA 3/7	110.72000*	2.34011	.000	102.7295	118.7105
	C/GA 1/9	188.57000*	2.34011	.000	180.5795	196.5605
	GA	207.08000*	2.34011	.000	199.0895	215.0705
C/GA 7/3	C	-121.39000*	2.34011	.000	-129.3805	-113.3995
	C/GA 9/1	-112.04000*	2.34011	.000	-120.0305	-104.0495
	C/GA 5/5	-23.60000*	2.34011	.000	-31.5905	-15.6095
	C/GA 3/7	-1.32000	2.34011	.997	-9.3105	6.6705
	C/GA 1/9	76.53000*	2.34011	.000	68.5395	84.5205
	GA	95.04000*	2.34011	.000	87.0495	103.0305
C/GA 5/5	C	-97.79000*	2.34011	.000	-105.7805	-89.7995
	C/GA 9/1	-88.44000*	2.34011	.000	-96.4305	-80.4495
	C/GA 7/3	23.60000*	2.34011	.000	15.6095	31.5905
	C/GA 3/7	22.28000*	2.34011	.000	14.2895	30.2705
	C/GA 1/9	100.13000*	2.34011	.000	92.1395	108.1205
	GA	118.64000*	2.34011	.000	110.6495	126.6305

C/GA 3/7	C	-120.07000*	2.34011	.000	-128.0605	-112.0795
	C/GA 9/1	-110.72000*	2.34011	.000	-118.7105	-102.7295
	C/GA 7/3	1.32000	2.34011	.997	-6.6705	9.3105
	C/GA 5/5	-22.28000*	2.34011	.000	-30.2705	-14.2895
	C/GA 1/9	77.85000*	2.34011	.000	69.8595	85.8405
	GA	96.36000*	2.34011	.000	88.3695	104.3505
C/GA 1/9	C	-197.92000*	2.34011	.000	-205.9105	-189.9295
	C/GA 9/1	-188.57000*	2.34011	.000	-196.5605	-180.5795
	C/GA 7/3	-76.53000*	2.34011	.000	-84.5205	-68.5395
	C/GA 5/5	-100.13000*	2.34011	.000	-108.1205	-92.1395
	C/GA 3/7	-77.85000*	2.34011	.000	-85.8405	-69.8595
	GA	18.51000*	2.34011	.000	10.5195	26.5005
GA	C	-216.43000*	2.34011	.000	-224.4205	-208.4395
	C/GA 9/1	-207.08000*	2.34011	.000	-215.0705	-199.0895
	C/GA 7/3	-95.04000*	2.34011	.000	-103.0305	-87.0495
	C/GA 5/5	-118.64000*	2.34011	.000	-126.6305	-110.6495
	C/GA 3/7	-96.36000*	2.34011	.000	-104.3505	-88.3695
	C/GA 1/9	-18.51000*	2.34011	.000	-26.5005	-10.5195

*. The mean difference is significant at the 0.05 level.

3. C/GB

ANOVA

force2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	225663.200	6	37610.533	152.163	.000
Within Groups	3460.406	14	247.172		
Total	229123.607	20			

Multiple Comparisons

force2

Tukey HSD

					95% Confidence Interval	
(I) group2	(J) group2	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
C	C/GB 9/1	58.19000*	12.83672	.007	14.3579	102.0221
	C/GB 7/3	35.75000	12.83672	.147	-8.0821	79.5821
	C/GB 5/5	-114.76000*	12.83672	.000	-158.5921	-70.9279
	C/GB 3/7	-143.77000*	12.83672	.000	-187.6021	-99.9379
	C/GB 1/9	81.95000*	12.83672	.000	38.1179	125.7821
	GB	176.83000*	12.83672	.000	132.9979	220.6621
C/GB 9/1	C	-58.19000*	12.83672	.007	-102.0221	-14.3579
	C/GB 7/3	-22.44000	12.83672	.599	-66.2721	21.3921
	C/GB 5/5	-172.95000*	12.83672	.000	-216.7821	-129.1179
	C/GB 3/7	-201.96000*	12.83672	.000	-245.7921	-158.1279
	C/GB 1/9	23.76000	12.83672	.539	-20.0721	67.5921
	GB	118.64000*	12.83672	.000	74.8079	162.4721
C/GB 7/3	C	-35.75000	12.83672	.147	-79.5821	8.0821
	C/GB 9/1	22.44000	12.83672	.599	-21.3921	66.2721
	C/GB 5/5	-150.51000*	12.83672	.000	-194.3421	-106.6779
	C/GB 3/7	-179.52000*	12.83672	.000	-223.3521	-135.6879
	C/GB 1/9	46.20000*	12.83672	.036	2.3679	90.0321
	GB	141.08000*	12.83672	.000	97.2479	184.9121
C/GB 5/5	C	114.76000*	12.83672	.000	70.9279	158.5921
	C/GB 9/1	172.95000*	12.83672	.000	129.1179	216.7821
	C/GB 7/3	150.51000*	12.83672	.000	106.6779	194.3421
	C/GB 3/7	-29.01000	12.83672	.326	-72.8421	14.8221
	C/GB 1/9	196.71000*	12.83672	.000	152.8779	240.5421
	GB	291.59000*	12.83672	.000	247.7579	335.4221

C/GB 3/7	C	143.77000*	12.83672	.000	99.9379	187.6021
	C/GB 9/1	201.96000*	12.83672	.000	158.1279	245.7921
	C/GB 7/3	179.52000*	12.83672	.000	135.6879	223.3521
	C/GB 5/5	29.01000	12.83672	.326	-14.8221	72.8421
	C/GB 1/9	225.72000*	12.83672	.000	181.8879	269.5521
	GB	320.60000*	12.83672	.000	276.7679	364.4321
C/GB 1/9	C	-81.95000*	12.83672	.000	-125.7821	-38.1179
	C/GB 9/1	-23.76000	12.83672	.539	-67.5921	20.0721
	C/GB 7/3	-46.20000*	12.83672	.036	-90.0321	-2.3679
	C/GB 5/5	-196.71000*	12.83672	.000	-240.5421	-152.8779
	C/GB 3/7	-225.72000*	12.83672	.000	-269.5521	-181.8879
	GB	94.88000*	12.83672	.000	51.0479	138.7121
GB	C	-176.83000*	12.83672	.000	-220.6621	-132.9979
	C/GB 9/1	-118.64000*	12.83672	.000	-162.4721	-74.8079
	C/GB 7/3	-141.08000*	12.83672	.000	-184.9121	-97.2479
	C/GB 5/5	-291.59000*	12.83672	.000	-335.4221	-247.7579
	C/GB 3/7	-320.60000*	12.83672	.000	-364.4321	-276.7679
	C/GB 1/9	-94.88000*	12.83672	.000	-138.7121	-51.0479

*. The mean difference is significant at the 0.05 level.

Appendix B

Statistical Analysis Results of an *in vitro* evaluation of mucoadhesive polymer using the tensile strength test method

Oneway ANOVA of the work of adhesion of a single polymer

1. Single polymer

ANOVA

work4

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.612	3	.204	208.458	.000
Within Groups	.019	19	.001		
Total	.630	22			

Multiple Comparisons

Dependent Variable: work4

Tukey HSD

(I) group4	(J) group4	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	PVP	.3406262*	.0173984	.000	.291704	.389548
	GA	.3633453*	.0189365	.000	.310099	.416592
	GB	.4083926*	.0189365	.000	.355146	.461639
PVP	Chitosan	-.3406262*	.0173984	.000	-.389548	-.291704
	GA	.0227191	.0183113	.610	-.028769	.074208
	GB	.0677664*	.0183113	.008	.016278	.119255
GA	Chitosan	-.3633453*	.0189365	.000	-.416592	-.310099
	PVP	-.0227191	.0183113	.610	-.074208	.028769
	GB	.0450473	.0197785	.139	-.010567	.100661
GB	Chitosan	-.4083926*	.0189365	.000	-.461639	-.355146
	PVP	-.0677664*	.0183113	.008	-.119255	-.016278
	GA	-.0450473	.0197785	.139	-.100661	.010567

*. The mean difference is significant at the 0.05 level.

Oneway ANOVA of the work of adhesion of a combination system of polymer or polymer blends

1. C/PVP

ANOVA

work

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.501	6	.250	40.865	.000
Within Groups	.178	29	.006		
Total	1.679	35			

Multiple Comparisons

Dependent Variable: work
Tukey HSD

(I) group1	(J) group1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	C/PVP 9/1	.1475500	.0553323	.143	-.027527	.322627
	C/PVP 7/3	-.0016167	.0473837	1.000	-.151544	.148311
	C/PVP 5/5	-.2481167(*)	.0473837	.000	-.398044	-.098189
	C/PVP 3/7	.2476353(*)	.0473837	.000	.097708	.397563
	C/PVP 1/9	.3391133(*)	.0473837	.000	.189186	.489041
	PVP	.3406262(*)	.0435352	.000	.202876	.478376
C/PVP 9/1	Chitosan	-.1475500	.0553323	.143	-.322627	.027527
	C/PVP 7/3	-.1491667	.0571469	.160	-.329986	.031653
	C/PVP 5/5	-.3956667(*)	.0571469	.000	-.576486	-.214847
	C/PVP 3/7	.1000853	.0571469	.589	-.080734	.280905
	C/PVP 1/9	.1915633(*)	.0571469	.032	.010744	.372383
	PVP	.1930762(*)	.0539988	.019	.022218	.363934
C/PVP 7/3	Chitosan	.0016167	.0473837	1.000	-.148311	.151544
	C/PVP 9/1	.1491667	.0571469	.160	-.031653	.329986
	C/PVP 5/5	-.2465000(*)	.0494907	.000	-.403094	-.089906
	C/PVP 3/7	.2492520(*)	.0494907	.000	.092658	.405846
	C/PVP 1/9	.3407300(*)	.0494907	.000	.184136	.497324
	PVP	.3422429(*)	.0458195	.000	.197265	.487221
C/PVP 5/5	Chitosan	.2481167(*)	.0473837	.000	.098189	.398044
	C/PVP 9/1	.3956667(*)	.0571469	.000	.214847	.576486
	C/PVP 7/3	.2465000(*)	.0494907	.000	.089906	.403094
	C/PVP 3/7	.4957520(*)	.0494907	.000	.339158	.652346
	C/PVP 1/9	.5872300(*)	.0494907	.000	.430636	.743824
	PVP	.5887429(*)	.0458195	.000	.443765	.733721
C/PVP 3/7	Chitosan	-.2476353(*)	.0473837	.000	-.397563	-.097708
	C/PVP 9/1	-.1000853	.0571469	.589	-.280905	.080734
	C/PVP 7/3	-.2492520(*)	.0494907	.000	-.405846	-.092658
	C/PVP 5/5	-.4957520(*)	.0494907	.000	-.652346	-.339158

	C/PVP 1/9	.0914780	.0494907	.528	-.065116	.248072
	PVP	.0929909	.0458195	.419	-.051987	.237969
C/PVP 1/9	Chitosan	-.3391133(*)	.0473837	.000	-.489041	-.189186
	C/PVP 9/1	-.1915633(*)	.0571469	.032	-.372383	-.010744
	C/PVP 7/3	-.3407300(*)	.0494907	.000	-.497324	-.184136
	C/PVP 5/5	-.5872300(*)	.0494907	.000	-.743824	-.430636
	C/PVP 3/7	-.0914780	.0494907	.528	-.248072	.065116
	PVP	.0015129	.0458195	1.000	-.143465	.146491
PVP	Chitosan	-.3406262(*)	.0435352	.000	-.478376	-.202876
	C/PVP 9/1	-.1930762(*)	.0539988	.019	-.363934	-.022218
	C/PVP 7/3	-.3422429(*)	.0458195	.000	-.487221	-.197265
	C/PVP 5/5	-.5887429(*)	.0458195	.000	-.733721	-.443765
	C/PVP 3/7	-.0929909	.0458195	.419	-.237969	.051987
	C/PVP 1/9	-.0015129	.0458195	1.000	-.146491	.143465

* The mean difference is significant at the .05 level.

2. C/GA

ANOVA

work2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.431	6	.072	18.667	.000
Within Groups	.112	29	.004		
Total	.542	35			

Multiple Comparisons

Dependent Variable: work2
Tukey HSD

(I) group1	(J) group1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	C/GA 9/1	.1862433(*)	.0375496	.001	.067432	.305054
	C/GA 7/3	.2250233(*)	.0375496	.000	.106212	.343834
	C/GA 5/5	.2157093(*)	.0375496	.000	.096898	.334520
	C/GA 3/7	.2025233(*)	.0375496	.000	.083712	.321334
	C/GA 1/9	.3040973(*)	.0375496	.000	.185286	.422908
	GA	.3633453(*)	.0375496	.000	.244534	.482156
C/GA 9/1	Chitosan	-.1862433(*)	.0375496	.001	-.305054	-.067432
	C/GA 7/3	.0387800	.0392193	.952	-.085314	.162874
	C/GA 5/5	.0294660	.0392193	.988	-.094628	.153560
	C/GA 3/7	.0162800	.0392193	1.000	-.107814	.140374
	C/GA 1/9	.1178540	.0392193	.071	-.006240	.241948
	GA	.1771020(*)	.0392193	.002	.053008	.301196
C/GA 7/3	Chitosan	-.2250233(*)	.0375496	.000	-.343834	-.106212
	C/GA 9/1	-.0387800	.0392193	.952	-.162874	.085314
	C/GA 5/5	-.0093140	.0392193	1.000	-.133408	.114780
	C/GA 3/7	-.0225000	.0392193	.997	-.146594	.101594
	C/GA 1/9	.0790740	.0392193	.427	-.045020	.203168
	GA	.1383220(*)	.0392193	.021	.014228	.262416
C/GA 5/5	Chitosan	-.2157093(*)	.0375496	.000	-.334520	-.096898
	C/GA 9/1	-.0294660	.0392193	.988	-.153560	.094628
	C/GA 7/3	.0093140	.0392193	1.000	-.114780	.133408
	C/GA 3/7	-.0131860	.0392193	1.000	-.137280	.110908
	C/GA 1/9	.0883880	.0392193	.300	-.035706	.212482
	GA	.1476360(*)	.0392193	.012	.023542	.271730
C/GA 3/7	Chitosan	-.2025233(*)	.0375496	.000	-.321334	-.083712
	C/GA 9/1	-.0162800	.0392193	1.000	-.140374	.107814
	C/GA 7/3	.0225000	.0392193	.997	-.101594	.146594
	C/GA 5/5	.0131860	.0392193	1.000	-.110908	.137280
	C/GA 1/9	.1015740	.0392193	.166	-.022520	.225668
	GA	.1608220(*)	.0392193	.005	.036728	.284916
C/GA 1/9	Chitosan	-.3040973(*)	.0375496	.000	-.422908	-.185286

	C/GA 9/1	-.1178540	.0392193	.071	-.241948	.006240
	C/GA 7/3	-.0790740	.0392193	.427	-.203168	.045020
	C/GA 5/5	-.0883880	.0392193	.300	-.212482	.035706
	C/GA 3/7	-.1015740	.0392193	.166	-.225668	.022520
GA	GA	.0592480	.0392193	.736	-.064846	.183342
	Chitosan	-.3633453(*)	.0375496	.000	-.482156	-.244534
	C/GA 9/1	-.1771020(*)	.0392193	.002	-.301196	-.053008
	C/GA 7/3	-.1383220(*)	.0392193	.021	-.262416	-.014228
	C/GA 5/5	-.1476360(*)	.0392193	.012	-.271730	-.023542
	C/GA 3/7	-.1608220(*)	.0392193	.005	-.284916	-.036728
	C/GA 1/9	-.0592480	.0392193	.736	-.183342	.064846

* The mean difference is significant at the .05 level.

3. C/GB

ANOVA

work3

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.614	6	.102	304.551	.000
Within Groups	.008	25	.000		
Total	.623	31			

Multiple Comparisons

Dependent Variable: work3
Tukey HSD

(I) group1	(J) group1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	C/GB 9/1	.4218204(*)	.0122996	.000	.382459	.461182
	C/GB 7/3	.4197224(*)	.0122996	.000	.380361	.459084
	C/GB 5/5	.4153010(*)	.0118353	.000	.377426	.453176
	C/GB 3/7	.4184019(*)	.0129649	.000	.376911	.459892
	C/GB 1/9	.4199386(*)	.0140037	.000	.375124	.464753
	GB	.4192593(*)	.0122996	.000	.379898	.458621
C/GB 9/1	Chitosan	-.4218204(*)	.0122996	.000	-.461182	-.382459
	C/GB 7/3	-.0020980	.0115962	1.000	-.039208	.035012
	C/GB 5/5	-.0065195	.0111025	.997	-.042050	.029011
	C/GB 3/7	-.0034185	.0122996	1.000	-.042780	.035943
	C/GB 1/9	-.0018819	.0133901	1.000	-.044733	.040969
	GB	-.0025612	.0115962	1.000	-.039671	.034549
C/GB 7/3	Chitosan	-.4197224(*)	.0122996	.000	-.459084	-.380361
	C/GB 9/1	.0020980	.0115962	1.000	-.035012	.039208
	C/GB 5/5	-.0044214	.0111025	1.000	-.039952	.031109
	C/GB 3/7	-.0013205	.0122996	1.000	-.040682	.038041
	C/GB 1/9	.0002161	.0133901	1.000	-.042635	.043067
	GB	-.0004632	.0115962	1.000	-.037573	.036647
C/GB 5/5	Chitosan	-.4153010(*)	.0118353	.000	-.453176	-.377426
	C/GB 9/1	.0065195	.0111025	.997	-.029011	.042050
	C/GB 7/3	.0044214	.0111025	1.000	-.031109	.039952
	C/GB 3/7	.0031009	.0118353	1.000	-.034775	.040976
	C/GB 1/9	.0046376	.0129649	1.000	-.036853	.046128
	GB	.0039583	.0111025	1.000	-.031572	.039489
C/GB 3/7	Chitosan	-.4184019(*)	.0129649	.000	-.459892	-.376911
	C/GB 9/1	.0034185	.0122996	1.000	-.035943	.042780
	C/GB 7/3	.0013205	.0122996	1.000	-.038041	.040682
	C/GB 5/5	-.0031009	.0118353	1.000	-.040976	.034775
	C/GB 1/9	.0015366	.0140037	1.000	-.043278	.046351
	GB	.0008573	.0122996	1.000	-.038504	.040219
C/GB 1/9	Chitosan	-.4199386(*)	.0140037	.000	-.464753	-.375124

	C/GB 9/1	.0018819	.0133901	1.000	-.040969	.044733
	C/GB 7/3	-.0002161	.0133901	1.000	-.043067	.042635
	C/GB 5/5	-.0046376	.0129649	1.000	-.046128	.036853
	C/GB 3/7	-.0015366	.0140037	1.000	-.046351	.043278
	GB	-.0006793	.0133901	1.000	-.043530	.042172
GB	Chitosan	-.4192593(*)	.0122996	.000	-.458621	-.379898
	C/GB 9/1	.0025612	.0115962	1.000	-.034549	.039671
	C/GB 7/3	.0004632	.0115962	1.000	-.036647	.037573
	C/GB 5/5	-.0039583	.0111025	1.000	-.039489	.031572
	C/GB 3/7	-.0008573	.0122996	1.000	-.040219	.038504
	C/GB 1/9	.0006793	.0133901	1.000	-.042172	.043530

* The mean difference is significant at the .05 level.

Appendix C

Statistical Analysis Results of an *in vitro* cell adhesion assay to measure bioadhesion of mucoadhesive polymer

Oneway ANOVA of the relative cell adhesion of a single polymer

1. Single polymer

ANOVA

r4

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2460.374	4	615.093	6.071	.004
Within Groups	1519.774	15	101.318		
Total	3980.148	19			

Multiple Comparisons

r4
Tukey HSD

(I) g4	(J) g4	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	chitosan	-31.5745214*	7.1175225	.004	-53.552894	-9.596149
	PVP	-7.5831365	7.1175225	.821	-29.561509	14.395236
	GA	-12.2821234	7.1175225	.449	-34.260496	9.696249
	GB	-22.3006365*	7.1175225	.046	-44.279009	-.322264
chitosan	control	31.5745214*	7.1175225	.004	9.596149	53.552894
	PVP	23.9913849*	7.1175225	.029	2.013013	45.969757
	GA	19.2923980	7.1175225	.099	-2.685974	41.270770
	GB	9.2738849	7.1175225	.694	-12.704487	31.252257
PVP	control	7.5831365	7.1175225	.821	-14.395236	29.561509
	chitosan	-23.9913849*	7.1175225	.029	-45.969757	-2.013013
	GA	-4.6989869	7.1175225	.962	-26.677359	17.279385
	GB	-14.7175000	7.1175225	.283	-36.695872	7.260872
GA	control	12.2821234	7.1175225	.449	-9.696249	34.260496
	chitosan	-19.2923980	7.1175225	.099	-41.270770	2.685974
	PVP	4.6989869	7.1175225	.962	-17.279385	26.677359
	GB	-10.0185131	7.1175225	.632	-31.996885	11.959859
GB	control	22.3006365*	7.1175225	.046	.322264	44.279009
	chitosan	-9.2738849	7.1175225	.694	-31.252257	12.704487
	PVP	14.7175000	7.1175225	.283	-7.260872	36.695872
	GA	10.0185131	7.1175225	.632	-11.959859	31.996885

*. The mean difference is significant at the 0.05 level.

Oneway ANOVA of the relative cell adhesion of a polymer blends

1. C/PVP

ANOVA

r1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15318.351	7	2188.336	19.164	.000
Within Groups	2740.545	24	114.189		
Total	18058.895	31			

Multiple Comparisons

r1
Tukey HSD

(I) g1	(J) g1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	chitosan	-31.5745214*	7.5561023	.007	-56.599674	-6.549369
	C/P 1/9	-14.7911443	7.5561023	.528	-39.816297	10.234008
	C/P 3/7	-47.8631365*	7.5561023	.000	-72.888289	-22.837984
	C/P 5/5	-70.5461440*	7.5561023	.000	-95.571296	-45.520992
	C/P 7/3	-34.4531365*	7.5561023	.003	-59.478289	-9.427984
	C/P 9/1	-13.9853832	7.5561023	.594	-39.010536	11.039769
	PVP	-7.5831365	7.5561023	.969	-32.608289	17.442016
chitosan	control	31.5745214*	7.5561023	.007	6.549369	56.599674
	C/P 1/9	16.7833772	7.5561023	.375	-8.241775	41.808530
	C/P 3/7	-16.2886151	7.5561023	.411	-41.313768	8.736537
	C/P 5/5	-38.9716225*	7.5561023	.001	-63.996775	-13.946470
	C/P 7/3	-2.8786151	7.5561023	1.000	-27.903768	22.146537
	C/P 9/1	17.5891383	7.5561023	.320	-7.436014	42.614291
	PVP	23.9913849	7.5561023	.067	-1.033768	49.016537
C/P 1/9	control	14.7911443	7.5561023	.528	-10.234008	39.816297
	chitosan	-16.7833772	7.5561023	.375	-41.808530	8.241775
	C/P 3/7	-33.0719922*	7.5561023	.004	-58.097145	-8.046840
	C/P 5/5	-55.7549997*	7.5561023	.000	-80.780152	-30.729847
	C/P 7/3	-19.6619922	7.5561023	.203	-44.687145	5.363160
	C/P 9/1	.8057611	7.5561023	1.000	-24.219391	25.830914
	PVP	7.2080078	7.5561023	.977	-17.817145	32.233160
C/P 3/7	control	47.8631365*	7.5561023	.000	22.837984	72.888289
	chitosan	16.2886151	7.5561023	.411	-8.736537	41.313768
	C/P 1/9	33.0719922*	7.5561023	.004	8.046840	58.097145
	C/P 5/5	-22.6830075	7.5561023	.096	-47.708160	2.342145
	C/P 7/3	13.4100000	7.5561023	.642	-11.615152	38.435152
	C/P 9/1	33.8777534*	7.5561023	.003	8.852601	58.902906

	PVP	40.2800000*	7.5561023	.000	15.254848	65.305152
C/P 5/5	control	70.5461440*	7.5561023	.000	45.520992	95.571296
	chitosan	38.9716225*	7.5561023	.001	13.946470	63.996775
	C/P 1/9	55.7549997*	7.5561023	.000	30.729847	80.780152
	C/P 3/7	22.6830075	7.5561023	.096	-2.342145	47.708160
	C/P 7/3	36.0930075*	7.5561023	.002	11.067855	61.118160
	C/P 9/1	56.5607608*	7.5561023	.000	31.535608	81.585913
	PVP	62.9630075*	7.5561023	.000	37.937855	87.988160
C/P 7/3	control	34.4531365*	7.5561023	.003	9.427984	59.478289
	chitosan	2.8786151	7.5561023	1.000	-22.146537	27.903768
	C/P 1/9	19.6619922	7.5561023	.203	-5.363160	44.687145
	C/P 3/7	-13.4100000	7.5561023	.642	-38.435152	11.615152
	C/P 5/5	-36.0930075*	7.5561023	.002	-61.118160	-11.067855
	C/P 9/1	20.4677534	7.5561023	.168	-4.557399	45.492906
	PVP	26.8700000*	7.5561023	.029	1.844848	51.895152
C/P 9/1	control	13.9853832	7.5561023	.594	-11.039769	39.010536
	chitosan	-17.5891383	7.5561023	.320	-42.614291	7.436014
	C/P 1/9	-.8057611	7.5561023	1.000	-25.830914	24.219391
	C/P 3/7	-33.8777534*	7.5561023	.003	-58.902906	-8.852601
	C/P 5/5	-56.5607608*	7.5561023	.000	-81.585913	-31.535608
	C/P 7/3	-20.4677534	7.5561023	.168	-45.492906	4.557399
	PVP	6.4022466	7.5561023	.988	-18.622906	31.427399
PVP	control	7.5831365	7.5561023	.969	-17.442016	32.608289
	chitosan	-23.9913849	7.5561023	.067	-49.016537	1.033768
	C/P 1/9	-7.2080078	7.5561023	.977	-32.233160	17.817145
	C/P 3/7	-40.2800000*	7.5561023	.000	-65.305152	-15.254848
	C/P 5/5	-62.9630075*	7.5561023	.000	-87.988160	-37.937855
	C/P 7/3	-26.8700000*	7.5561023	.029	-51.895152	-1.844848
	C/P 9/1	-6.4022466	7.5561023	.988	-31.427399	18.622906

*. The mean difference is significant at the 0.05 level.

2. C/GA

ANOVA

r2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23620.781	7	3374.397	26.997	.000
Within Groups	2999.773	24	124.991		
Total	26620.553	31			

Multiple Comparisons

r2
Tukey HSD

					95% Confidence Interval	
(I) g2	(J) g2	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
control	chitosan	-31.5745214*	7.9053947	.011	-57.756500	-5.392543
	C/GA 1/9	-57.4681365*	7.9053947	.000	-83.650115	-31.286158
	C/GA 3/7	-82.5392942*	7.9053947	.000	-108.721273	-56.357316
	C/GA 5/5	-54.4081365*	7.9053947	.000	-80.590115	-28.226158
	C/GA 7/3	-56.0852547*	7.9053947	.000	-82.267233	-29.903276
	C/GA 9/1	-9.7256365	7.9053947	.915	-35.907615	16.456342
	GA	-12.2821234	7.9053947	.772	-38.464102	13.899855
chitosan	control	31.5745214*	7.9053947	.011	5.392543	57.756500
	C/GA 1/9	-25.8936151	7.9053947	.054	-52.075594	.288363
	C/GA 3/7	-50.9647728*	7.9053947	.000	-77.146751	-24.782794
	C/GA 5/5	-22.8336151	7.9053947	.120	-49.015594	3.348363
	C/GA 7/3	-24.5107333	7.9053947	.078	-50.692712	1.671245
	C/GA 9/1	21.8488849	7.9053947	.152	-4.333094	48.030863
	GA	19.2923980	7.9053947	.268	-6.889581	45.474377
C/GA 1/9	control	57.4681365*	7.9053947	.000	31.286158	83.650115
	chitosan	25.8936151	7.9053947	.054	-.288363	52.075594
	C/GA 3/7	-25.0711577	7.9053947	.067	-51.253136	1.110821
	C/GA 5/5	3.0600000	7.9053947	1.000	-23.121979	29.241979
	C/GA 7/3	1.3828818	7.9053947	1.000	-24.799097	27.564860
	C/GA 9/1	47.7425000*	7.9053947	.000	21.560521	73.924479
	GA	45.1860131*	7.9053947	.000	19.004035	71.367992
C/GA 3/7	control	82.5392942*	7.9053947	.000	56.357316	108.721273
	chitosan	50.9647728*	7.9053947	.000	24.782794	77.146751
	C/GA 1/9	25.0711577	7.9053947	.067	-1.110821	51.253136
	C/GA 5/5	28.1311577*	7.9053947	.029	1.949179	54.313136
	C/GA 7/3	26.4540395*	7.9053947	.046	.272061	52.636018
	C/GA 9/1	72.8136577*	7.9053947	.000	46.631679	98.995636
	GA	70.2571708*	7.9053947	.000	44.075192	96.439149
C/GA 5/5	control	54.4081365*	7.9053947	.000	28.226158	80.590115
	chitosan	22.8336151	7.9053947	.120	-3.348363	49.015594

	C/GA 1/9	-3.0600000	7.9053947	1.000	-29.241979	23.121979
	C/GA 3/7	-28.1311577*	7.9053947	.029	-54.313136	-1.949179
	C/GA 7/3	-1.6771182	7.9053947	1.000	-27.859097	24.504860
	C/GA 9/1	44.6825000*	7.9053947	.000	18.500521	70.864479
	GA	42.1260131*	7.9053947	.000	15.944035	68.307992
C/GA 7/3	control	56.0852547*	7.9053947	.000	29.903276	82.267233
	chitosan	24.5107333	7.9053947	.078	-1.671245	50.692712
	C/GA 1/9	-1.3828818	7.9053947	1.000	-27.564860	24.799097
	C/GA 3/7	-26.4540395*	7.9053947	.046	-52.636018	-.272061
	C/GA 5/5	1.6771182	7.9053947	1.000	-24.504860	27.859097
	C/GA 9/1	46.3596182*	7.9053947	.000	20.177640	72.541597
	GA	43.8031313*	7.9053947	.000	17.621153	69.985110
C/GA 9/1	control	9.7256365	7.9053947	.915	-16.456342	35.907615
	chitosan	-21.8488849	7.9053947	.152	-48.030863	4.333094
	C/GA 1/9	-47.7425000*	7.9053947	.000	-73.924479	-21.560521
	C/GA 3/7	-72.8136577*	7.9053947	.000	-98.995636	-46.631679
	C/GA 5/5	-44.6825000*	7.9053947	.000	-70.864479	-18.500521
	C/GA 7/3	-46.3596182*	7.9053947	.000	-72.541597	-20.177640
	GA	-2.5564869	7.9053947	1.000	-28.738465	23.625492
GA	control	12.2821234	7.9053947	.772	-13.899855	38.464102
	chitosan	-19.2923980	7.9053947	.268	-45.474377	6.889581
	C/GA 1/9	-45.1860131*	7.9053947	.000	-71.367992	-19.004035
	C/GA 3/7	-70.2571708*	7.9053947	.000	-96.439149	-44.075192
	C/GA 5/5	-42.1260131*	7.9053947	.000	-68.307992	-15.944035
	C/GA 7/3	-43.8031313*	7.9053947	.000	-69.985110	-17.621153
	C/GA 9/1	2.5564869	7.9053947	1.000	-23.625492	28.738465

*. The mean difference is significant at the 0.05 level.

3. C/GB

ANOVA

r3

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11618.275	7	1659.754	6.710	.000
Within Groups	5936.427	24	247.351		
Total	17554.703	31			

Multiple Comparisons

r3
Tukey HSD

					95% Confidence Interval	
(I) g3	(J) g3	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
control	chitosan	-31.5745214	1.1120952	.132	-68.406145	5.257102
	C/GB 1/9	-27.8209284	1.1120952	.242	-64.652552	9.010695
	C/GB 3/7	-66.5256365*	1.1120952	.000	-103.357260	-29.694013
	C/GB 5/5	-56.3054247*	1.1120952	.001	-93.137048	-19.473801
	C/GB 7/3	-34.2556365	1.1120952	.081	-71.087260	2.575987
	C/GB 9/1	-37.8463091*	1.1120952	.041	-74.677932	-1.014686
	GB	-22.3006365	1.1120952	.499	-59.132260	14.530987
chitosan	control	31.5745214	1.1120952	.132	-5.257102	68.406145
	C/GB 1/9	3.7535931	1.1120952	1.000	-33.078030	40.585216
	C/GB 3/7	-34.9511151	1.1120952	.072	-71.782738	1.880508
	C/GB 5/5	-24.7309033	1.1120952	.373	-61.562527	12.100720
	C/GB 7/3	-2.6811151	1.1120952	1.000	-39.512738	34.150508
	C/GB 9/1	-6.2717877	1.1120952	.999	-43.103411	30.559836
	GB	9.2738849	1.1120952	.989	-27.557738	46.105508
C/GB 1/9	control	27.8209284	1.1120952	.242	-9.010695	64.652552
	chitosan	-3.7535931	1.1120952	1.000	-40.585216	33.078030
	C/GB 3/7	-38.7047081*	1.1120952	.035	-75.536332	-1.873085
	C/GB 5/5	-28.4844964	1.1120952	.218	-65.316120	8.347127
	C/GB 7/3	-6.4347081	1.1120952	.999	-43.266332	30.396915
	C/GB 9/1	-10.0253807	1.1120952	.983	-46.857004	26.806243
	GB	5.5202919	1.1120952	1.000	-31.311332	42.351915
C/GB 3/7	control	66.5256365*	1.1120952	.000	29.694013	103.357260
	chitosan	34.9511151	1.1120952	.072	-1.880508	71.782738
	C/GB 1/9	38.7047081*	1.1120952	.035	1.873085	75.536332
	C/GB 5/5	10.2202118	1.1120952	.981	-26.611412	47.051835
	C/GB 7/3	32.2700000	1.1120952	.117	-4.561623	69.101623
	C/GB 9/1	28.6793274	1.1120952	.212	-8.152296	65.510951
	GB	44.2250000*	1.1120952	.011	7.393377	81.056623
C/GB 5/5	control	56.3054247*	1.1120952	.001	19.473801	93.137048
	chitosan	24.7309033	1.1120952	.373	-12.100720	61.562527

	C/GB 1/9	28.4844964	1.1120952	.218	-8.347127	65.316120
	C/GB 3/7	-10.2202118	1.1120952	.981	-47.051835	26.611412
	C/GB 7/3	22.0497882	1.1120952	.513	-14.781835	58.881412
	C/GB 9/1	18.4591157	1.1120952	.711	-18.372508	55.290739
	GB	34.0047882	1.1120952	.085	-2.826835	70.836412
C/GB 7/3	control	34.2556365	1.1120952	.081	-2.575987	71.087260
	chitosan	2.6811151	1.1120952	1.000	-34.150508	39.512738
	C/GB 1/9	6.4347081	1.1120952	.999	-30.396915	43.266332
	C/GB 3/7	-32.2700000	1.1120952	.117	-69.101623	4.561623
	C/GB 5/5	-22.0497882	1.1120952	.513	-58.881412	14.781835
	C/GB 9/1	-3.5906726	1.1120952	1.000	-40.422296	33.240951
	GB	11.9550000	1.1120952	.956	-24.876623	48.786623
C/GB 9/1	control	37.8463091*	1.1120952	.041	1.014686	74.677932
	chitosan	6.2717877	1.1120952	.999	-30.559836	43.103411
	C/GB 1/9	10.0253807	1.1120952	.983	-26.806243	46.857004
	C/GB 3/7	-28.6793274	1.1120952	.212	-65.510951	8.152296
	C/GB 5/5	-18.4591157	1.1120952	.711	-55.290739	18.372508
	C/GB 7/3	3.5906726	1.1120952	1.000	-33.240951	40.422296
	GB	15.5456726	1.1120952	.849	-21.285951	52.377296
GB	control	22.3006365	1.1120952	.499	-14.530987	59.132260
	chitosan	-9.2738849	1.1120952	.989	-46.105508	27.557738
	C/GB 1/9	-5.5202919	1.1120952	1.000	-42.351915	31.311332
	C/GB 3/7	-44.2250000*	1.1120952	.011	-81.056623	-7.393377
	C/GB 5/5	-34.0047882	1.1120952	.085	-70.836412	2.826835
	C/GB 7/3	-11.9550000	1.1120952	.956	-48.786623	24.876623
	C/GB 9/1	-15.5456726	1.1120952	.849	-52.377296	21.285951

*. The mean difference is significant at the 0.05 level.

Appendix D

Statistical analysis results of amoxicillin mucoadhesive bead preparation and properties

MANOVA

1. MANOVA analysis of the swelling of dry beads

General Linear Model

Within-Subjects Factors

Measure:MEASURE_1

time	Dependent Variable
1	t0
2	t30
3	t60
4	t90
5	t120
6	t180
7	t240

Between-Subjects Factors

		Value Label	N
group	1	Bead	3
	2	Chitosan	3
	3	C/PVP 9/1	3
	4	C/PVP 7/3	3
	5	C/PVP 5/5	3
	6	C/PVP 3/7	3
	7	C/PVP 1/9	3
	8	PVP	3

Multivariate Tests[c]

Effect		Value	F	Hypothesis df	Error df	Sig.
time	Pillai's Trace	.999	2725.062[a]	6.000	11.000	.000
	Wilks' Lambda	.001	2725.062[a]	6.000	11.000	.000
	Hotelling's Trace	1486.398	2725.062[a]	6.000	11.000	.000
	Roy's Largest Root	1486.398	2725.062[a]	6.000	11.000	.000
time * group	Pillai's Trace	2.947	2.206	42.000	96.000	.001
	Wilks' Lambda	.001	3.949	42.000	55.047	.000
	Hotelling's Trace	29.041	6.454	42.000	56.000	.000
	Roy's Largest Root	21.415	48.948[b]	7.000	16.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

c. Design: Intercept + group

Within Subjects Design: time

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.008	66.344	20	.000	.462	.813	.167

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b. Design: Intercept + group
Within Subjects Design: time

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	21989045.901	6	3664840.984	4253.401	.000
	Greenhouse-Geisser	21989045.901	2.773	7930000.168	4253.401	.000
	Huynh-Feldt	21989045.901	4.880	4505870.760	4253.401	.000
	Lower-bound	21989045.901	1.000	21989045.901	4253.401	.000
time * group	Sphericity Assumed	319884.916	42	7616.308	8.839	.000
	Greenhouse-Geisser	319884.916	19.410	16480.202	8.839	.000
	Huynh-Feldt	319884.916	34.161	9364.144	8.839	.000
	Lower-bound	319884.916	7.000	45697.845	8.839	.000
Error(time)	Sphericity Assumed	82716.105	96	861.626		
	Greenhouse-Geisser	82716.105	44.366	1864.391		
	Huynh-Feldt	82716.105	78.081	1059.357		
	Lower-bound	82716.105	16.000	5169.757		

Tests of Within-Subjects Contrasts

Measure:MEASURE_1

Source	time	Type III Sum of Squares	df	Mean Square	F	Sig.
time	Linear	21906369.827	1	21906369.827	13441.387	.000
	Quadratic	1992.111	1	1992.111	1.148	.300
	Cubic	52301.784	1	52301.784	167.165	.000
	Order 4	27458.573	1	27458.573	59.057	.000
	Order 5	921.431	1	921.431	5.498	.032
	Order 6	2.174	1	2.174	.003	.960
time * group	Linear	53063.356	7	7580.479	4.651	.005
	Quadratic	217918.725	7	31131.246	17.934	.000
	Cubic	22771.546	7	3253.078	10.397	.000
	Order 4	8064.101	7	1152.014	2.478	.063
	Order 5	9045.431	7	1292.204	7.711	.000
	Order 6	9021.756	7	1288.822	1.501	.236
Error(time)	Linear	26076.320	16	1629.770		
	Quadratic	27774.391	16	1735.899		

	Cubic	5006.013	16	312.876		
	Order 4	7439.238	16	464.952		
	Order 5	2681.408	16	167.588		
	Order 6	13738.733	16	858.671		

Tests of Between-Subjects Effects

Measure:MEASURE_1
Transformed Variable:Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	46325571.784	1	46325571.784	5640.474	.000
group	653596.318	7	93370.903	11.369	.000
Error	131409.027	16	8213.064		

Post Hoc Tests

group

Multiple Comparisons

MEASURE_1

Tukey HSD

					95% Confidence Interval	
(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Bead	Chitosan	-213.9490*	27.96778	.000	-310.7777	-117.1204
	C/PVP 9/1	-157.3133*	27.96778	.001	-254.1420	-60.4847
	C/PVP 7/3	-186.1633*	27.96778	.000	-282.9920	-89.3347
	C/PVP 5/5	-132.0762*	27.96778	.004	-228.9049	-35.2475
	C/PVP 3/7	-176.6557*	27.96778	.000	-273.4844	-79.8271
	C/PVP 1/9	-92.2186	27.96778	.068	-189.0472	4.6101
	PVP	-135.5281*	27.96778	.003	-232.3568	-38.6994
Chitosan	Bead	213.9490*	27.96778	.000	117.1204	310.7777
	C/PVP 9/1	56.6357	27.96778	.496	-40.1929	153.4644
	C/PVP 7/3	27.7857	27.96778	.969	-69.0429	124.6144
	C/PVP 5/5	81.8729	27.96778	.131	-14.9558	178.7015
	C/PVP 3/7	37.2933	27.96778	.873	-59.5353	134.1220
	C/PVP 1/9	121.7305*	27.96778	.009	24.9018	218.5591
	PVP	78.4210	27.96778	.162	-18.4077	175.2496
C/PVP 9/1	Bead	157.3133*	27.96778	.001	60.4847	254.1420

	Chitosan	-56.6357	27.96778	.496	-153.4644	40.1929
	C/PVP 7/3	-28.8500	27.96778	.962	-125.6787	67.9787
	C/PVP 5/5	25.2371	27.96778	.981	-71.5915	122.0658
	C/PVP 3/7	-19.3424	27.96778	.996	-116.1710	77.4863
	C/PVP 1/9	65.0948	27.96778	.337	-31.7339	161.9234
	PVP	21.7852	27.96778	.992	-75.0434	118.6139
C/PVP 7/3	Bead	186.1633*	27.96778	.000	89.3347	282.9920
	Chitosan	-27.7857	27.96778	.969	-124.6144	69.0429
	C/PVP 9/1	28.8500	27.96778	.962	-67.9787	125.6787
	C/PVP 5/5	54.0871	27.96778	.549	-42.7415	150.9158
	C/PVP 3/7	9.5076	27.96778	1.000	-87.3210	106.3363
	C/PVP 1/9	93.9448	27.96778	.061	-2.8839	190.7734
	PVP	50.6352	27.96778	.623	-46.1934	147.4639
C/PVP 5/5	Bead	132.0762*	27.96778	.004	35.2475	228.9049
	Chitosan	-81.8729	27.96778	.131	-178.7015	14.9558
	C/PVP 9/1	-25.2371	27.96778	.981	-122.0658	71.5915
	C/PVP 7/3	-54.0871	27.96778	.549	-150.9158	42.7415
	C/PVP 3/7	-44.5795	27.96778	.747	-141.4082	52.2491
	C/PVP 1/9	39.8576	27.96778	.833	-56.9710	136.6863
	PVP	-3.4519	27.96778	1.000	-100.2806	93.3768
C/PVP 3/7	Bead	176.6557*	27.96778	.000	79.8271	273.4844
	Chitosan	-37.2933	27.96778	.873	-134.1220	59.5353
	C/PVP 9/1	19.3424	27.96778	.996	-77.4863	116.1710
	C/PVP 7/3	-9.5076	27.96778	1.000	-106.3363	87.3210
	C/PVP 5/5	44.5795	27.96778	.747	-52.2491	141.4082
	C/PVP 1/9	84.4371	27.96778	.112	-12.3915	181.2658
	PVP	41.1276	27.96778	.812	-55.7010	137.9563
C/PVP 1/9	Bead	92.2186	27.96778	.068	-4.6101	189.0472
	Chitosan	-121.7305*	27.96778	.009	-218.5591	-24.9018
	C/PVP 9/1	-65.0948	27.96778	.337	-161.9234	31.7339
	C/PVP 7/3	-93.9448	27.96778	.061	-190.7734	2.8839
	C/PVP 5/5	-39.8576	27.96778	.833	-136.6863	56.9710
	C/PVP 3/7	-84.4371	27.96778	.112	-181.2658	12.3915
	PVP	-43.3095	27.96778	.772	-140.1382	53.5191
PVP	Bead	135.5281*	27.96778	.003	38.6994	232.3568
	Chitosan	-78.4210	27.96778	.162	-175.2496	18.4077
	C/PVP 9/1	-21.7852	27.96778	.992	-118.6139	75.0434
	C/PVP 7/3	-50.6352	27.96778	.623	-147.4639	46.1934

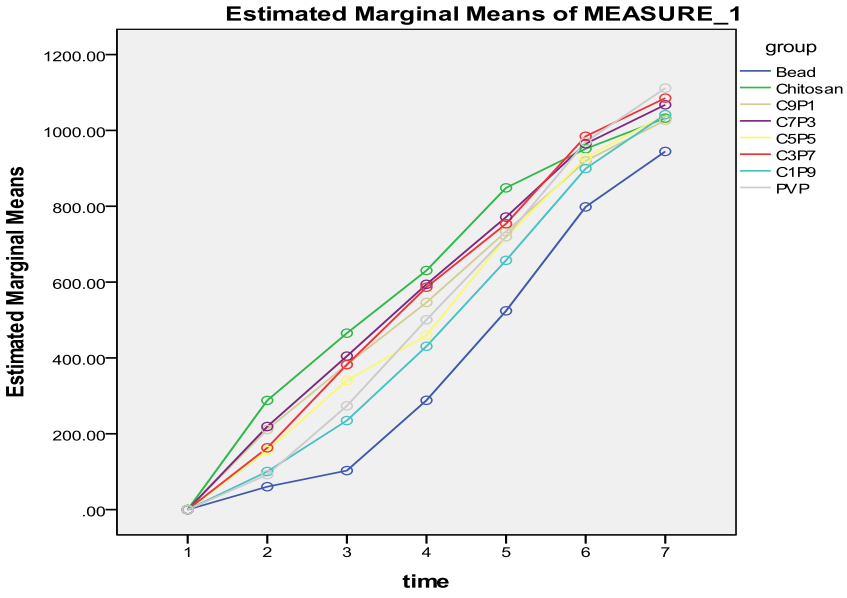
	C/PVP 5/5	3.4519	27.96778	1.000	-93.3768	100.2806
	C/PVP 3/7	-41.1276	27.96778	.812	-137.9563	55.7010
	C/PVP 1/9	43.3095	27.96778	.772	-53.5191	140.1382

Based on observed means.

The error term is Mean Square(Error) = 1173.295.

*. The mean difference is significant at the .05 level.

Profile Plots



2. MANOVA analysis of the wash-off method

General Linear Model

Within-Subjects Factors

Measure:MEASURE_1

time	Dependent Variable
1	t0
2	t30
3	t60
4	t90
5	t120
6	t150
7	t180

Between-Subjects Factors

		Value Label	N
group	1	Bead	3
	2	Chitosan	3
	3	C/PVP 9/1	3
	4	C/PVP 7/3	3
	5	C/PVP 5/5	3
	6	C/PVP 3/7	3
	7	C/PVP 1/9	3
	8	PVP	3

Multivariate Tests[c]

Effect		Value	F	Hypothesis df	Error df	Sig.
time	Pillai's Trace	.954	38.173[a]	6.000	11.000	.000
	Wilks' Lambda	.046	38.173[a]	6.000	11.000	.000
	Hotelling's Trace	20.822	38.173[a]	6.000	11.000	.000
	Roy's Largest Root	20.822	38.173[a]	6.000	11.000	.000
time * group	Pillai's Trace	2.092	1.224	42.000	96.000	.208
	Wilks' Lambda	.017	1.823	42.000	55.047	.018
	Hotelling's Trace	12.047	2.677	42.000	56.000	.000
	Roy's Largest Root	9.019	20.615[b]	7.000	16.000	.000

a. Exact statistic
b. The statistic is an upper bound on F that yields a lower bound on the significance level.
c. Design: Intercept + group
Within Subjects Design: time

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.014	58.954	20	.000	.495	.885	.167

Tests for the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.014	58.954	20	.000	.495	.885	.167

Tests for the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b. Design: Intercept + group
Within Subjects Design: time

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	17161.905	6	2860.317	105.184	.000
	Greenhouse-Geisser	17161.905	2.968	5782.458	105.184	.000
	Huynh-Feldt	17161.905	5.312	3230.601	105.184	.000
	Lower-bound	17161.905	1.000	17161.905	105.184	.000
time * group	Sphericity Assumed	8056.085	42	191.812	7.054	.000
	Greenhouse-Geisser	8056.085	20.775	387.769	7.054	.000
	Huynh-Feldt	8056.085	37.186	216.643	7.054	.000
	Lower-bound	8056.085	7.000	1150.869	7.054	.001
Error(time)	Sphericity Assumed	2610.582	96	27.194		
	Greenhouse-Geisser	2610.582	47.487	54.975		
	Huynh-Feldt	2610.582	84.997	30.714		
	Lower-bound	2610.582	16.000	163.161		

Tests of Within-Subjects Contrasts

Measure:MEASURE_1

Source	time	Type III Sum of Squares	df	Mean Square	F	Sig.
time	Linear	17000.595	1	17000.595	228.437	.000
	Quadratic	4.321	1	4.321	.125	.728
	Cubic	142.670	1	142.670	4.488	.050
	Order 4	1.326	1	1.326	.135	.718
	Order 5	3.726	1	3.726	.401	.536
	Order 6	9.267	1	9.267	2.748	.117
time * group	Linear	5895.966	7	842.281	11.318	.000
	Quadratic	1534.392	7	219.199	6.356	.001
	Cubic	520.910	7	74.416	2.341	.075
	Order 4	69.165	7	9.881	1.009	.461
	Order 5	26.874	7	3.839	.413	.880
	Order 6	8.778	7	1.254	.372	.906
Error(time)	Linear	1190.741	16	74.421		
	Quadratic	551.764	16	34.485		
	Cubic	508.642	16	31.790		
	Order 4	156.710	16	9.794		
	Order 5	148.765	16	9.298		
	Order 6	53.960	16	3.373		

Tests of Between-Subjects Effects

Measure:MEASURE_1

Transformed Variable:Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1191800.595	1	1191800.595	3706.299	.000
group	34925.860	7	4989.409	15.516	.000
Error	5144.974	16	321.561		

Post Hoc Tests

Multiple Comparisons

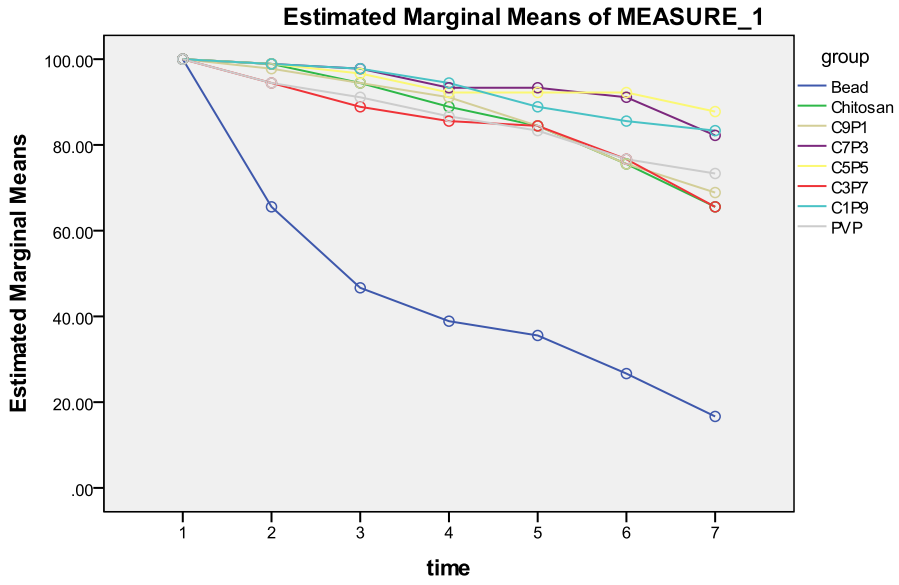
MEASURE_1

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	-39.6825*	5.53397	.000	-58.8420	-20.5231
	C/PVP 9/1	-40.3175*	5.53397	.000	-59.4769	-21.1580
	C/PVP 7/3	-46.6667*	5.53397	.000	-65.8261	-27.5072
	C/PVP 5/5	-47.1429*	5.53397	.000	-66.3023	-27.9834
	C/PVP 3/7	-37.9365*	5.53397	.000	-57.0960	-18.7771
	C/PVP 1/9	-45.5556*	5.53397	.000	-64.7150	-26.3961
	PVP	-39.3651*	5.53397	.000	-58.5245	-20.2056
Chitosan	Bead	39.6825*	5.53397	.000	20.5231	58.8420
	C/PVP 9/1	-.6349	5.53397	1.000	-19.7944	18.5245
	C/PVP 7/3	-6.9841	5.53397	.900	-26.1436	12.1753
	C/PVP 5/5	-7.4603	5.53397	.867	-26.6198	11.6991
	C/PVP 3/7	1.7460	5.53397	1.000	-17.4134	20.9055
	C/PVP 1/9	-5.8730	5.53397	.956	-25.0325	13.2864
	PVP	.3175	5.53397	1.000	-18.8420	19.4769
C/PVP 9/1	Bead	40.3175*	5.53397	.000	21.1580	59.4769
	Chitosan	.6349	5.53397	1.000	-18.5245	19.7944
	C/PVP 7/3	-6.3492	5.53397	.936	-25.5087	12.8102
	C/PVP 5/5	-6.8254	5.53397	.910	-25.9848	12.3340
	C/PVP 3/7	2.3810	5.53397	1.000	-16.7785	21.5404
	C/PVP 1/9	-5.2381	5.53397	.976	-24.3975	13.9213
	PVP	.9524	5.53397	1.000	-18.2071	20.1118
C/PVP 7/3	Bead	46.6667*	5.53397	.000	27.5072	65.8261
	Chitosan	6.9841	5.53397	.900	-12.1753	26.1436
	C/PVP 9/1	6.3492	5.53397	.936	-12.8102	25.5087
	C/PVP 5/5	-.4762	5.53397	1.000	-19.6356	18.6833
	C/PVP 3/7	8.7302	5.53397	.756	-10.4293	27.8896
	C/PVP 1/9	1.1111	5.53397	1.000	-18.0483	20.2706
	PVP	7.3016	5.53397	.879	-11.8579	26.4610
C/PVP 5/5	Bead	47.1429*	5.53397	.000	27.9834	66.3023
	Chitosan	7.4603	5.53397	.867	-11.6991	26.6198
	C/PVP 9/1	6.8254	5.53397	.910	-12.3340	25.9848
	C/PVP 7/3	.4762	5.53397	1.000	-18.6833	19.6356
	C/PVP 3/7	9.2063	5.53397	.708	-9.9531	28.3658
	C/PVP 1/9	1.5873	5.53397	1.000	-17.5721	20.7467
	PVP	7.7778	5.53397	.842	-11.3817	26.9372
C/PVP 3/7	Bead	37.9365*	5.53397	.000	18.7771	57.0960
	Chitosan	-1.7460	5.53397	1.000	-20.9055	17.4134
	C/PVP 9/1	-2.3810	5.53397	1.000	-21.5404	16.7785
	C/PVP 7/3	-8.7302	5.53397	.756	-27.8896	10.4293
	C/PVP 5/5	-9.2063	5.53397	.708	-28.3658	9.9531

	C/PVP 1/9	-7.6190	5.53397	.855	-26.7785	11.5404
	PVP	-1.4286	5.53397	1.000	-20.5880	17.7309
C/PVP 1/9	Bead	45.5556*	5.53397	.000	26.3961	64.7150
	Chitosan	5.8730	5.53397	.956	-13.2864	25.0325
	C/PVP 9/1	5.2381	5.53397	.976	-13.9213	24.3975
	C/PVP 7/3	-1.1111	5.53397	1.000	-20.2706	18.0483
	C/PVP 5/5	-1.5873	5.53397	1.000	-20.7467	17.5721
	C/PVP 3/7	7.6190	5.53397	.855	-11.5404	26.7785
	PVP	6.1905	5.53397	.943	-12.9690	25.3499
PVP	Bead	39.3651*	5.53397	.000	20.2056	58.5245
	Chitosan	-.3175	5.53397	1.000	-19.4769	18.8420
	C/PVP 9/1	-.9524	5.53397	1.000	-20.1118	18.2071
	C/PVP 7/3	-7.3016	5.53397	.879	-26.4610	11.8579
	C/PVP 5/5	-7.7778	5.53397	.842	-26.9372	11.3817
	C/PVP 3/7	1.4286	5.53397	1.000	-17.7309	20.5880
	C/PVP 1/9	-6.1905	5.53397	.943	-25.3499	12.9690

Based on observed means.
The error term is Mean Square(Error) = 45.937.
*. The mean difference is significant at the .05 level.



ANOVA

1. ANOVA analysis of the percentage weight change of the AMX beads at t=30 minutes

Oneway

ANOVA

t30

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	121,757.495	7	17,393.928	34.206	.000
Within Groups	8,136.084	16	508.505		
Total	129,893.579	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t30

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	227.53333*	18.41205	.000	173.6999	281.3668
C/PVP 9/1	Bead	150.92667*	18.41205	.000	97.0932	204.7601
C/PVP 7/3	Bead	158.70000*	18.41205	.000	104.8666	212.5334
C/PVP 5/5	Bead	95.28000*	18.41205	.001	41.4466	149.1134
C/PVP 3/7	Bead	102.60333*	18.41205	.000	48.7699	156.4368
C/PVP 1/9	Bead	40.00333	18.41205	.197	-13.8301	93.8368
PVP	Bead	31.74333	18.41205	.395	-22.0901	85.5768

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

2. ANOVA analysis of the percentage weight change of the AMX beads at t=60 minutes

Oneway

ANOVA
t60

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	278,914.348	7	39,844.907	22.660	.000
Within Groups	28,134.374	16	1,758.398		
Total	307,048.721	23			

Post Hoc Tests
Multiple Comparisons
Dependent Variable: t60
Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	362.39667*	34.23836	.000	262.2900	462.5033
C/PVP 9/1	Bead	279.78000*	34.23836	.000	179.6733	379.8867
C/PVP 7/3	Bead	301.84333*	34.23836	.000	201.7367	401.9500
C/PVP 5/5	Bead	237.55667*	34.23836	.000	137.4500	337.6633
C/PVP 3/7	Bead	279.39333*	34.23836	.000	179.2867	379.5000
C/PVP 1/9	Bead	131.96000*	34.23836	.008	31.8533	232.0667
PVP	Bead	170.63000*	34.23836	.001	70.5233	270.7367

*. The mean difference is significant at the 0.05 level.
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

3. ANOVA analysis of the percentage weight change of the AMX beads at t=90 minutes

Oneway

ANOVA

t90

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	259,902.845	7	37,128.978	8.062	.000
Within Groups	73,691.388	16	4,605.712		
Total	333,594.233	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t90

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	342.30333*	55.41186	.000	180.2892	504.3174
C/PVP 9/1	Bead	258.07000*	55.41186	.002	96.0559	420.0841
C/PVP 7/3	Bead	305.90000*	55.41186	.000	143.8859	467.9141
C/PVP 5/5	Bead	171.41667*	55.41186	.036	9.4026	333.4308
C/PVP 3/7	Bead	298.14333*	55.41186	.000	136.1292	460.1574
C/PVP 1/9	Bead	142.33333	55.41186	.098	-19.6808	304.3474
PVP	Bead	212.43333*	55.41186	.008	50.4192	374.4474

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

4. ANOVA analysis of the percentage weight change of AMX beads at t=120 minutes

Oneway

ANOVA

t120

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	187,850.443	7	26,835.778	9.702	.000
Within Groups	44,258.196	16	2,766.137		
Total	232,108.638	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t120

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	324.35333*	42.94289	.000	198.7962	449.9104
C/PVP 9/1	Bead	208.96667*	42.94289	.001	83.4096	334.5238
C/PVP 7/3	Bead	247.30000*	42.94289	.000	121.7429	372.8571
C/PVP 5/5	Bead	193.81333*	42.94289	.002	68.2562	319.3704
C/PVP 3/7	Bead	229.63667*	42.94289	.000	104.0796	355.1938
C/PVP 1/9	Bead	133.19667*	42.94289	.035	7.6396	258.7538
PVP	Bead	195.61000*	42.94289	.002	70.0529	321.1671

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

5. ANOVA analysis of the percentage weight change of the AMX beads at t=180 minutes

Oneway

ANOVA
t180

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	73,584.115	7	10,512.016	5.035	.004
Within Groups	33,401.392	16	2,087.587		
Total	106,985.507	23			

Post Hoc Tests
Multiple Comparisons
Dependent Variable: t180
Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	153.24333*	37.30583	.005	44.1680	262.3187
C/PVP 9/1	Bead	121.26667*	37.30583	.026	12.1913	230.3420
C/PVP 7/3	Bead	166.39333*	37.30583	.002	57.3180	275.4687
C/PVP 5/5	Bead	129.81667*	37.30583	.017	20.7413	238.8920
C/PVP 3/7	Bead	186.19667*	37.30583	.001	77.1213	295.2720
C/PVP 1/9	Bead	100.94000	37.30583	.076	-8.1354	210.0154
PVP	Bead	171.22333*	37.30583	.002	62.1480	280.2987

*. The mean difference is significant at the 0.05 level.
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

6. ANOVA analysis of the percentage weight change of the AMX beads at t=240 minutes

Oneway

ANOVA

t240

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51,471.989	7	7,353.141	4.439	.006
Within Groups	26,503.698	16	1,656.481		
Total	77,975.687	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t240

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	87.81333	33.23132	.085	-9.3489	184.9756
C/PVP 9/1	Bead	82.18333	33.23132	.116	-14.9789	179.3456
C/PVP 7/3	Bead	123.00667*	33.23132	.011	25.8444	220.1689
C/PVP 5/5	Bead	96.65000	33.23132	.052	-.5123	193.8123
C/PVP 3/7	Bead	140.61667*	33.23132	.004	43.4544	237.7789
C/PVP 1/9	Bead	97.09667	33.23132	.050	-.0656	194.2589
PVP	Bead	167.05667*	33.23132	.001	69.8944	264.2189

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

7. ANOVA analysis of the percentage beads remaining of the AMX bead at t=30 minutes

Oneway

ANOVA

t30

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2,751.389	7	393.056	8.663	.000
Within Groups	725.926	16	45.370		
Total	3,477.315	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t30

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	33.33333*	5.49972	.000	17.2532	49.4135
C/PVP 9/1	Bead	32.22222*	5.49972	.000	16.1421	48.3024
C/PVP 7/3	Bead	33.33333*	5.49972	.000	17.2532	49.4135
C/PVP 5/5	Bead	33.33333*	5.49972	.000	17.2532	49.4135
C/PVP 3/7	Bead	28.88889*	5.49972	.000	12.8087	44.9691
C/PVP 1/9	Bead	33.33333*	5.49972	.000	17.2532	49.4135
PVP	Bead	28.88889*	5.49972	.000	12.8087	44.9691

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

8. ANOVA analysis of the percentage beads remaining of the AMX bead at t=60 minutes

Oneway

ANOVA

t60

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6,199.537	7	885.648	10.285	.000
Within Groups	1,377.778	16	86.111		
Total	7,577.315	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t60

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	47.77778*	7.57677	.000	25.6247	69.9309
C/PVP 9/1	Bead	47.77778*	7.57677	.000	25.6247	69.9309
C/PVP 7/3	Bead	51.11111*	7.57677	.000	28.9580	73.2642
C/PVP 5/5	Bead	50.00000*	7.57677	.000	27.8469	72.1531
C/PVP 3/7	Bead	42.22222*	7.57677	.000	20.0691	64.3753
C/PVP 1/9	Bead	51.11111*	7.57677	.000	28.9580	73.2642
PVP	Bead	44.44444*	7.57677	.000	22.2914	66.5975

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

9. ANOVA analysis of percentage beads remaining of the AMX beads at t=90 minutes

Oneway

ANOVA

t90

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7,148.148	7	1,021.164	15.983	.000
Within Groups	1,022.222	16	63.889		
Total	8,170.370	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t90

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	50.00000*	6.52630	.000	30.9183	69.0817
C/PVP 9/1	Bead	52.22222*	6.52630	.000	33.1405	71.3039
C/PVP 7/3	Bead	54.44444*	6.52630	.000	35.3627	73.5261
C/PVP 5/5	Bead	53.33333*	6.52630	.000	34.2516	72.4150
C/PVP 3/7	Bead	46.66667*	6.52630	.000	27.5850	65.7484
C/PVP 1/9	Bead	55.55556*	6.52630	.000	36.4739	74.6373
PVP	Bead	47.77778*	6.52630	.000	28.6961	66.8595

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

10. ANOVA analysis of the percentage beads remaining of the AMX beads at t=120 minutes

Oneway

ANOVA

t120

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7,338.889	7	1,048.413	13.015	.000
Within Groups	1,288.889	16	80.556		
Total	8,627.778	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t120

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	48.88889*	7.32828	.000	27.4623	70.3154
C/PVP 9/1	Bead	48.88889*	7.32828	.000	27.4623	70.3154
C/PVP 7/3	Bead	57.77778*	7.32828	.000	36.3512	79.2043
C/PVP 5/5	Bead	56.66667*	7.32828	.000	35.2401	78.0932
C/PVP 3/7	Bead	48.88889*	7.32828	.000	27.4623	70.3154
C/PVP 1/9	Bead	53.33333*	7.32828	.000	31.9068	74.7599
PVP	Bead	47.77778*	7.32828	.000	26.3512	69.2043

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

11. ANOVA analysis of the percentage beads remaining of the AMX beads at t=150 minutes

Oneway

ANOVA

t150

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9,029.630	7	1,289.947	11.101	.000
Within Groups	1,859.259	16	116.204		
Total	10,888.889	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t150

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	48.88889*	8.80166	.000	23.1545	74.6233
C/PVP 9/1	Bead	48.88889*	8.80166	.000	23.1545	74.6233
C/PVP 7/3	Bead	64.44444*	8.80166	.000	38.7100	90.1789
C/PVP 5/5	Bead	65.55556*	8.80166	.000	39.8211	91.2900
C/PVP 3/7	Bead	50.00000*	8.80166	.000	24.2656	75.7344
C/PVP 1/9	Bead	58.88889*	8.80166	.000	33.1545	84.6233
PVP	Bead	50.00000*	8.80166	.000	24.2656	75.7344

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

12. ANOVA analysis of percentage bead remaining of AMX bead at t=180 minutes

Oneway

ANOVA

t180

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10,514.352	7	1,502.050	16.222	.000
Within Groups	1,481.481	16	92.593		
Total	11,995.833	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t180

Dunnett t (2-sided)

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Chitosan	Bead	48.88889*	7.85674	.000	25.9172	71.8606
C/PVP 9/1	Bead	52.22222*	7.85674	.000	29.2506	75.1939
C/PVP 7/3	Bead	65.55556*	7.85674	.000	42.5839	88.5272
C/PVP 5/5	Bead	71.11111*	7.85674	.000	48.1394	94.0828
C/PVP 3/7	Bead	48.88889*	7.85674	.000	25.9172	71.8606
C/PVP 1/9	Bead	66.66667*	7.85674	.000	43.6950	89.6383
PVP	Bead	56.66667*	7.85674	.000	33.6950	79.6383

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix E

Statistical analysis results of drug release study of amoxicillin from alginate beads

MANOVA

1. MANOVA analysis of AMX bead dissolution profiles

General Linear Model

Within-Subjects Factors
Measure: MEASURE_1

factor1	Dependent Variable
1	t0
2	t6
3	t12
4	t18
5	t24
6	t30
7	t40
8	t60
9	t90
10	t120
11	t180

Between-Subjects Factors

		Value Label	N
group	1	Bead	3
	2	Chitosan	3
	3	C/PVP 9/1	3
	4	C/PVP 7/3	3
	5	C/PVP 5/5	3
	6	C/PVP 3/7	3
	7	C/PVP 1/9	3
	8	PVP	3

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
factor1	Pillai's Trace	1.000	4,120.402[b]	10.000	7.000	.000
	Wilks' Lambda	.000	4,120.402[b]	10.000	7.000	.000
	Hotelling's Trace	5,886.288	4,120.402[b]	10.000	7.000	.000
	Roy's Largest Root	5,886.288	4,120.402[b]	10.000	7.000	.000
factor1 * group	Pillai's Trace	3.852	1.591	70.000	91.000	.019
	Wilks' Lambda	.000	1.987	70.000	47.633	.007
	Hotelling's Trace	26.548	2.005	70.000	37.000	.011
	Roy's Largest Root	12.377	16.091[c]	10.000	13.000	.000

a. Design: Intercept + group
Within Subjects Design: factor1
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
factor1	.000	170.720	54	.000	.282	.499	.100

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + group
Within Subjects Design: factor1

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
factor1	Sphericity Assumed	382323.159	10	38232.316	15,457.643	.000
	Greenhouse-Geisser	382323.159	2.823	135448.771	15,457.643	.000
	Huynh-Feldt	382323.159	4.989	76631.453	15,457.643	.000
	Lower-bound	382323.159	1.000	382323.159	15,457.643	.000
factor1 * group	Sphericity Assumed	970.080	70	13.858	5.603	.000
	Greenhouse-Geisser	970.080	19.758	49.097	5.603	.000
	Huynh-Feldt	970.080	34.924	27.777	5.603	.000
	Lower-bound	970.080	7.000	138.583	5.603	.002
Error(factor1)	Sphericity Assumed	395.738	160	2.473		
	Greenhouse-Geisser	395.738	45.162	8.763		
	Huynh-Feldt	395.738	79.826	4.958		
	Lower-bound	395.738	16.000	24.734		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	factor1	Type III Sum of Squares	df	Mean Square	F	Sig.
factor1	Linear	351617.633	1	351617.633	36,930.766	.000
	Quadratic	28465.988	1	28465.988	3,982.015	.000
	Cubic	16.795	1	16.795	5.804	.028
	Order 4	1351.382	1	1351.382	773.267	.000
	Order 5	471.880	1	471.880	688.293	.000
	Order 6	101.629	1	101.629	268.824	.000
	Order 7	283.058	1	283.058	326.447	.000
	Order 8	11.266	1	11.266	24.107	.000
	Order 9	3.397	1	3.397	7.170	.017
	Order 10	.132	1	.132	.239	.632
factor1 * group	Linear	339.425	7	48.489	5.093	.003
	Quadratic	528.520	7	75.503	10.562	.000
	Cubic	49.641	7	7.092	2.451	.065
	Order 4	21.347	7	3.050	1.745	.169
	Order 5	9.074	7	1.296	1.891	.138
	Order 6	7.633	7	1.090	2.884	.038
	Order 7	5.961	7	.852	.982	.477
	Order 8	4.219	7	.603	1.290	.316
	Order 9	2.723	7	.389	.821	.584
	Order 10	1.537	7	.220	.399	.889
Error(factor1)	Linear	152.336	16	9.521		

	Quadratic	114.378	16	7.149	
	Cubic	46.303	16	2.894	
	Order 4	27.962	16	1.748	
	Order 5	10.969	16	.686	
	Order 6	6.049	16	.378	
	Order 7	13.873	16	.867	
	Order 8	7.477	16	.467	
	Order 9	7.580	16	.474	
	Order 10	8.809	16	.551	

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	448384.054	1	448384.054	8,796.682	.000
group	1517.515	7	216.788	4.253	.008
Error	815.551	16	50.972		

Post Hoc Tests
group

Multiple Comparisons

Measure: MEASURE_1

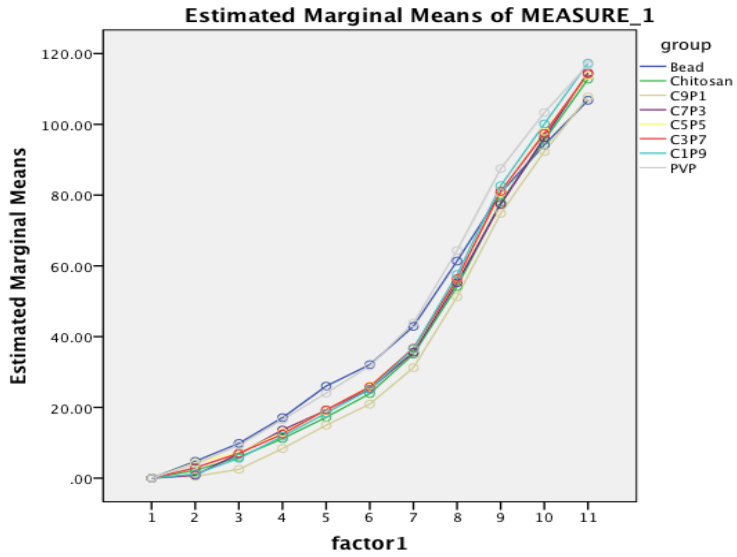
Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	3.6202	1.75761	.477	-2.4649	9.7053
	C/PVP 9/1	6.4597*	1.75761	.033	.3746	12.5448
	C/PVP 7/3	2.8173	1.75761	.743	-3.2678	8.9024
	C/PVP 5/5	1.8986	1.75761	.952	-4.1865	7.9838
	C/PVP 3/7	2.0353	1.75761	.933	-4.0498	8.1205
	C/PVP 1/9	1.7713	1.75761	.966	-4.3139	7.8564
	PVP	-2.3300	1.75761	.876	-8.4151	3.7552
Chitosan	Bead	-3.6202	1.75761	.477	-9.7053	2.4649
	C/PVP 9/1	2.8395	1.75761	.736	-3.2456	8.9246
	C/PVP 7/3	-.8029	1.75761	1.000	-6.8880	5.2822
	C/PVP 5/5	-1.7216	1.75761	.971	-7.8067	4.3635
	C/PVP 3/7	-1.5849	1.75761	.982	-7.6700	4.5002
	C/PVP 1/9	-1.8489	1.75761	.958	-7.9341	4.2362
	PVP	-5.9502	1.75761	.058	-12.0353	.1349
C/PVP 9/1	Bead	-6.4597*	1.75761	.033	-12.5448	-.3746
	Chitosan	-2.8395	1.75761	.736	-8.9246	3.2456
	C/PVP 7/3	-3.6424	1.75761	.469	-9.7275	2.4427
	C/PVP 5/5	-4.5611	1.75761	.227	-10.6462	1.5241
	C/PVP 3/7	-4.4244	1.75761	.256	-10.5095	1.6608
	C/PVP 1/9	-4.6884	1.75761	.202	-10.7736	1.3967
	PVP	-8.7897*	1.75761	.003	-14.8748	-2.7045
C/PVP 7/3	Bead	-2.8173	1.75761	.743	-8.9024	3.2678
	Chitosan	.8029	1.75761	1.000	-5.2822	6.8880
	C/PVP 9/1	3.6424	1.75761	.469	-2.4427	9.7275
	C/PVP 5/5	-.9187	1.75761	.999	-7.0038	5.1664
	C/PVP 3/7	-.7820	1.75761	1.000	-6.8671	5.3031
	C/PVP 1/9	-1.0460	1.75761	.998	-7.1312	5.0391

	PVP	-5.1473	1.75761	.131	-11.2324	.9378
C/PVP 5/5	Bead	-1.8986	1.75761	.952	-7.9838	4.1865
	Chitosan	1.7216	1.75761	.971	-4.3635	7.8067
	C/PVP 9/1	4.5611	1.75761	.227	-1.5241	10.6462
	C/PVP 7/3	.9187	1.75761	.999	-5.1664	7.0038
	C/PVP 3/7	.1367	1.75761	1.000	-5.9484	6.2218
	C/PVP 1/9	-.1274	1.75761	1.000	-6.2125	5.9578
	PVP	-4.2286	1.75761	.302	-10.3137	1.8565
C/PVP 3/7	Bead	-2.0353	1.75761	.933	-8.1205	4.0498
	Chitosan	1.5849	1.75761	.982	-4.5002	7.6700
	C/PVP 9/1	4.4244	1.75761	.256	-1.6608	10.5095
	C/PVP 7/3	.7820	1.75761	1.000	-5.3031	6.8671
	C/PVP 5/5	-.1367	1.75761	1.000	-6.2218	5.9484
	C/PVP 1/9	-.2641	1.75761	1.000	-6.3492	5.8211
	PVP	-4.3653	1.75761	.269	-10.4504	1.7198
C/PVP 1/9	Bead	-1.7713	1.75761	.966	-7.8564	4.3139
	Chitosan	1.8489	1.75761	.958	-4.2362	7.9341
	C/PVP 9/1	4.6884	1.75761	.202	-1.3967	10.7736
	C/PVP 7/3	1.0460	1.75761	.998	-5.0391	7.1312
	C/PVP 5/5	.1274	1.75761	1.000	-5.9578	6.2125
	C/PVP 3/7	.2641	1.75761	1.000	-5.8211	6.3492
	PVP	-4.1012	1.75761	.334	-10.1864	1.9839
PVP	Bead	2.3300	1.75761	.876	-3.7552	8.4151
	Chitosan	5.9502	1.75761	.058	-.1349	12.0353
	C/PVP 9/1	8.7897*	1.75761	.003	2.7045	14.8748
	C/PVP 7/3	5.1473	1.75761	.131	-.9378	11.2324
	C/PVP 5/5	4.2286	1.75761	.302	-1.8565	10.3137
	C/PVP 3/7	4.3653	1.75761	.269	-1.7198	10.4504
	C/PVP 1/9	4.1012	1.75761	.334	-1.9839	10.1864

Based on observed means.
The error term is Mean Square(Error) = 4.634.
*. The mean difference is significant at the

Profile Plots



ANOVA

1. ANOVA analysis of the percentage release from the AMX bead at t = 6 minutes

Oneway

ANOVA

t6

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	58.190	7	8.313	5.299	.003
Within Groups	25.100	16	1.569		
Total	83.289	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t6
Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	2.58596	1.02265	.251	-.9546	6.1265
	C/PVP 9/1	4.25673*	1.02265	.013	.7162	7.7973
	C/PVP 7/3	3.92497*	1.02265	.024	.3844	7.4656
	C/PVP 5/5	.46964	1.02265	1.000	-3.0709	4.0102
	C/PVP 3/7	1.83449	1.02265	.632	-1.7061	5.3751
	C/PVP 1/9	3.59824*	1.02265	.045	.0577	7.1388
	PVP	.74279	1.02265	.995	-2.7978	4.2834
Chitosan	Bead	-2.58596	1.02265	.251	-6.1265	.9546
	C/PVP 9/1	1.67077	1.02265	.725	-1.8698	5.2113
	C/PVP 7/3	1.33902	1.02265	.883	-2.2016	4.8796
	C/PVP 5/5	-2.11632	1.02265	.471	-5.6569	1.4243
	C/PVP 3/7	-.75146	1.02265	.994	-4.2920	2.7891
	C/PVP 1/9	1.01228	1.02265	.969	-2.5283	4.5529
	PVP	-1.84317	1.02265	.627	-5.3837	1.6974
C/PVP 9/1	Bead	-4.25673*	1.02265	.013	-7.7973	-.7162
	Chitosan	-1.67077	1.02265	.725	-5.2113	1.8698
	C/PVP 7/3	-.33176	1.02265	1.000	-3.8723	3.2088
	C/PVP 5/5	-3.78709*	1.02265	.032	-7.3277	-.2465
	C/PVP 3/7	-2.42224	1.02265	.318	-5.9628	1.1183
	C/PVP 1/9	-.65849	1.02265	.997	-4.1991	2.8821
	PVP	-3.51394	1.02265	.052	-7.0545	.0266
C/PVP 7/3	Bead	-3.92497*	1.02265	.024	-7.4656	-.3844
	Chitosan	-1.33902	1.02265	.883	-4.8796	2.2016
	C/PVP 9/1	.33176	1.02265	1.000	-3.2088	3.8723
	C/PVP 5/5	-3.45534	1.02265	.058	-6.9959	.0852
	C/PVP 3/7	-2.09048	1.02265	.485	-5.6311	1.4501
	C/PVP 1/9	-.32674	1.02265	1.000	-3.8673	3.2138
	PVP	-3.18219	1.02265	.095	-6.7228	.3584
C/PVP 5/5	Bead	-.46964	1.02265	1.000	-4.0102	3.0709
	Chitosan	2.11632	1.02265	.471	-1.4243	5.6569

	C/PVP 9/1	3.78709*	1.02265	.032	.2465	7.3277
	C/PVP 7/3	3.45534	1.02265	.058	-.0852	6.9959
	C/PVP 3/7	1.36486	1.02265	.873	-2.1757	4.9054
	C/PVP 1/9	3.12860	1.02265	.104	-.4120	6.6692
	PVP	.27315	1.02265	1.000	-3.2674	3.8137
C/PVP 3/7	Bead	-1.83449	1.02265	.632	-5.3751	1.7061
	Chitosan	.75146	1.02265	.994	-2.7891	4.2920
	C/PVP 9/1	2.42224	1.02265	.318	-1.1183	5.9628
	C/PVP 7/3	2.09048	1.02265	.485	-1.4501	5.6311
	C/PVP 5/5	-1.36486	1.02265	.873	-4.9054	2.1757
	C/PVP 1/9	1.76374	1.02265	.673	-1.7768	5.3043
	PVP	-1.09171	1.02265	.955	-4.6323	2.4489
C/PVP 1/9	Bead	-3.59824*	1.02265	.045	-7.1388	-.0577
	Chitosan	-1.01228	1.02265	.969	-4.5529	2.5283
	C/PVP 9/1	.65849	1.02265	.997	-2.8821	4.1991
	C/PVP 7/3	.32674	1.02265	1.000	-3.2138	3.8673
	C/PVP 5/5	-3.12860	1.02265	.104	-6.6692	.4120
	C/PVP 3/7	-1.76374	1.02265	.673	-5.3043	1.7768
	PVP	-2.85545	1.02265	.165	-6.3960	.6851
PVP	Bead	-.74279	1.02265	.995	-4.2834	2.7978
	Chitosan	1.84317	1.02265	.627	-1.6974	5.3837
	C/PVP 9/1	3.51394	1.02265	.052	-.0266	7.0545
	C/PVP 7/3	3.18219	1.02265	.095	-.3584	6.7228
	C/PVP 5/5	-.27315	1.02265	1.000	-3.8137	3.2674
	C/PVP 3/7	1.09171	1.02265	.955	-2.4489	4.6323
	C/PVP 1/9	2.85545	1.02265	.165	-.6851	6.3960

*. The mean difference is significant at the 0.05 level.

2. ANOVA analysis of the percentage percentage release from the AMX bead at t =12 minutes

Oneway

ANOVA

t12

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	108.436	7	15.491	3.576	.016
Within Groups	69.311	16	4.332		
Total	177.747	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t12

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	3.90566	1.69940	.351	-1.9779	9.7892
	C/PVP 9/1	7.33628*	1.69940	.010	1.4527	13.2198
	C/PVP 7/3	2.94954	1.69940	.667	-2.9340	8.8331
	C/PVP 5/5	2.66492	1.69940	.761	-3.2187	8.5485
	C/PVP 3/7	2.80656	1.69940	.715	-3.0770	8.6901
	C/PVP 1/9	4.24244	1.69940	.264	-1.6411	10.1260
	PVP	.58019	1.69940	1.000	-5.3034	6.4638
Chitosan	Bead	-3.90566	1.69940	.351	-9.7892	1.9779
	C/PVP 9/1	3.43062	1.69940	.500	-2.4530	9.3142
	C/PVP 7/3	-.95612	1.69940	.999	-6.8397	4.9275
	C/PVP 5/5	-1.24074	1.69940	.995	-7.1243	4.6428
	C/PVP 3/7	-1.09910	1.69940	.997	-6.9827	4.7845
	C/PVP 1/9	.33678	1.69940	1.000	-5.5468	6.2204
	PVP	-3.32546	1.69940	.536	-9.2090	2.5581
C/PVP 9/1	Bead	-7.33628*	1.69940	.010	-13.2198	-1.4527
	Chitosan	-3.43062	1.69940	.500	-9.3142	2.4530
	C/PVP 7/3	-4.38674	1.69940	.232	-10.2703	1.4968
	C/PVP 5/5	-4.67136	1.69940	.177	-10.5549	1.2122
	C/PVP 3/7	-4.52972	1.69940	.203	-10.4133	1.3539
	C/PVP 1/9	-3.09384	1.69940	.617	-8.9774	2.7897
	PVP	-6.75608*	1.69940	.019	-12.6397	-.8725
C/PVP 7/3	Bead	-2.94954	1.69940	.667	-8.8331	2.9340
	Chitosan	.95612	1.69940	.999	-4.9275	6.8397
	C/PVP 9/1	4.38674	1.69940	.232	-1.4968	10.2703
	C/PVP 5/5	-.28462	1.69940	1.000	-6.1682	5.5989
	C/PVP 3/7	-.14298	1.69940	1.000	-6.0265	5.7406
	C/PVP 1/9	1.29290	1.69940	.993	-4.5907	7.1765
	PVP	-2.36934	1.69940	.847	-8.2529	3.5142
C/PVP 5/5	Bead	-2.66492	1.69940	.761	-8.5485	3.2187
	Chitosan	1.24074	1.69940	.995	-4.6428	7.1243
	C/PVP 9/1	4.67136	1.69940	.177	-1.2122	10.5549
	C/PVP 7/3	.28462	1.69940	1.000	-5.5989	6.1682
	C/PVP 3/7	.14164	1.69940	1.000	-5.7419	6.0252
	C/PVP 1/9	1.57752	1.69940	.978	-4.3060	7.4611
	PVP	-2.08472	1.69940	.912	-7.9683	3.7988
C/PVP 3/7	Bead	-2.80656	1.69940	.715	-8.6901	3.0770

	Chitosan	1.09910	1.69940	.997	-4.7845	6.9827
	C/PVP 9/1	4.52972	1.69940	.203	-1.3539	10.4133
	C/PVP 7/3	.14298	1.69940	1.000	-5.7406	6.0265
	C/PVP 5/5	-.14164	1.69940	1.000	-6.0252	5.7419
	C/PVP 1/9	1.43588	1.69940	.987	-4.4477	7.3195
	PVP	-2.22637	1.69940	.882	-8.1099	3.6572
C/PVP 1/9	Bead	-4.24244	1.69940	.264	-10.1260	1.6411
	Chitosan	-.33678	1.69940	1.000	-6.2204	5.5468
	C/PVP 9/1	3.09384	1.69940	.617	-2.7897	8.9774
	C/PVP 7/3	-1.29290	1.69940	.993	-7.1765	4.5907
	C/PVP 5/5	-1.57752	1.69940	.978	-7.4611	4.3060
	C/PVP 3/7	-1.43588	1.69940	.987	-7.3195	4.4477
	PVP	-3.66225	1.69940	.424	-9.5458	2.2213
PVP	Bead	-.58019	1.69940	1.000	-6.4638	5.3034
	Chitosan	3.32546	1.69940	.536	-2.5581	9.2090
	C/PVP 9/1	6.75608*	1.69940	.019	.8725	12.6397
	C/PVP 7/3	2.36934	1.69940	.847	-3.5142	8.2529
	C/PVP 5/5	2.08472	1.69940	.912	-3.7988	7.9683
	C/PVP 3/7	2.22637	1.69940	.882	-3.6572	8.1099
	C/PVP 1/9	3.66225	1.69940	.424	-2.2213	9.5458

*. The mean difference is significant at the 0.05 level.

3. ANOVA analysis of the percentage release from the AMX bead at t = 18 minutes

Oneway

ANOVA

t18

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	168.788	7	24.113	3.893	.012
Within Groups	99.109	16	6.194		
Total	267.897	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t18

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	5.93799	2.03213	.132	-1.0976	12.9735
	C/PVP 9/1	8.74835*	2.03213	.010	1.7128	15.7839
	C/PVP 7/3	3.51005	2.03213	.672	-3.5255	10.5456
	C/PVP 5/5	4.16263	2.03213	.483	-2.8729	11.1982
	C/PVP 3/7	4.64058	2.03213	.358	-2.3950	11.6761
	C/PVP 1/9	5.28611	2.03213	.225	-1.7494	12.3217
	PVP	.57627	2.03213	1.000	-6.4593	7.6118
Chitosan	Bead	-5.93799	2.03213	.132	-12.9735	1.0976
	C/PVP 9/1	2.81036	2.03213	.852	-4.2252	9.8459
	C/PVP 7/3	-2.42794	2.03213	.922	-9.4635	4.6076
	C/PVP 5/5	-1.77536	2.03213	.985	-8.8109	5.2602
	C/PVP 3/7	-1.29741	2.03213	.998	-8.3330	5.7381
	C/PVP 1/9	-.65188	2.03213	1.000	-7.6874	6.3837
	PVP	-5.36172	2.03213	.212	-12.3973	1.6738
C/PVP 9/1	Bead	-8.74835*	2.03213	.010	-15.7839	-1.7128
	Chitosan	-2.81036	2.03213	.852	-9.8459	4.2252
	C/PVP 7/3	-5.23830	2.03213	.233	-12.2738	1.7972
	C/PVP 5/5	-4.58573	2.03213	.371	-11.6213	2.4498
	C/PVP 3/7	-4.10777	2.03213	.498	-11.1433	2.9278
	C/PVP 1/9	-3.46224	2.03213	.685	-10.4978	3.5733
	PVP	-8.17208*	2.03213	.017	-15.2076	-1.1365
C/PVP 7/3	Bead	-3.51005	2.03213	.672	-10.5456	3.5255
	Chitosan	2.42794	2.03213	.922	-4.6076	9.4635
	C/PVP 9/1	5.23830	2.03213	.233	-1.7972	12.2738
	C/PVP 5/5	.65258	2.03213	1.000	-6.3830	7.6881
	C/PVP 3/7	1.13053	2.03213	.999	-5.9050	8.1661
	C/PVP 1/9	1.77606	2.03213	.985	-5.2595	8.8116
	PVP	-2.93378	2.03213	.825	-9.9693	4.1018
C/PVP 5/5	Bead	-4.16263	2.03213	.483	-11.1982	2.8729
	Chitosan	1.77536	2.03213	.985	-5.2602	8.8109
	C/PVP 9/1	4.58573	2.03213	.371	-2.4498	11.6213
	C/PVP 7/3	-.65258	2.03213	1.000	-7.6881	6.3830
	C/PVP 3/7	.47795	2.03213	1.000	-6.5576	7.5135
	C/PVP 1/9	1.12349	2.03213	.999	-5.9121	8.1590
	PVP	-3.58636	2.03213	.650	-10.6219	3.4492

C/PVP 3/7	Bead	-4.64058	2.03213	.358	-11.6761	2.3950
	Chitosan	1.29741	2.03213	.998	-5.7381	8.3330
	C/PVP 9/1	4.10777	2.03213	.498	-2.9278	11.1433
	C/PVP 7/3	-1.13053	2.03213	.999	-8.1661	5.9050
	C/PVP 5/5	-.47795	2.03213	1.000	-7.5135	6.5576
	C/PVP 1/9	.64553	2.03213	1.000	-6.3900	7.6811
	PVP	-4.06431	2.03213	.511	-11.0999	2.9712
C/PVP 1/9	Bead	-5.28611	2.03213	.225	-12.3217	1.7494
	Chitosan	.65188	2.03213	1.000	-6.3837	7.6874
	C/PVP 9/1	3.46224	2.03213	.685	-3.5733	10.4978
	C/PVP 7/3	-1.77606	2.03213	.985	-8.8116	5.2595
	C/PVP 5/5	-1.12349	2.03213	.999	-8.1590	5.9121
	C/PVP 3/7	-.64553	2.03213	1.000	-7.6811	6.3900
	PVP	-4.70984	2.03213	.342	-11.7454	2.3257
PVP	Bead	-.57627	2.03213	1.000	-7.6118	6.4593
	Chitosan	5.36172	2.03213	.212	-1.6738	12.3973
	C/PVP 9/1	8.17208*	2.03213	.017	1.1365	15.2076
	C/PVP 7/3	2.93378	2.03213	.825	-4.1018	9.9693
	C/PVP 5/5	3.58636	2.03213	.650	-3.4492	10.6219
	C/PVP 3/7	4.06431	2.03213	.511	-2.9712	11.0999
	C/PVP 1/9	4.70984	2.03213	.342	-2.3257	11.7454

*. The mean difference is significant at the 0.05 level.

4. ANOVA analysis of the percentage release from the AMX bead at t = 24 minutes

Oneway

ANOVA

t24

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	269.827	7	38.547	6.611	.001
Within Groups	93.292	16	5.831		
Total	363.119	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t24

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	8.81406*	1.97159	.007	1.9881	15.6400
	C/PVP 9/1	11.05225*	1.97159	.001	4.2263	17.8782
	C/PVP 7/3	6.86095*	1.97159	.048	.0350	13.6869
	C/PVP 5/5	6.95037*	1.97159	.044	.1244	13.7763
	C/PVP 3/7	6.73817	1.97159	.054	-.0878	13.5641
	C/PVP 1/9	7.64547*	1.97159	.023	.8195	14.4714
	PVP	1.98450	1.97159	.967	-4.8414	8.8104
Chitosan	Bead	-8.81406*	1.97159	.007	-15.6400	-1.9881
	C/PVP 9/1	2.23819	1.97159	.939	-4.5878	9.0641
	C/PVP 7/3	-1.95311	1.97159	.969	-8.7791	4.8728
	C/PVP 5/5	-1.86369	1.97159	.976	-8.6896	4.9623
	C/PVP 3/7	-2.07589	1.97159	.958	-8.9018	4.7501
	C/PVP 1/9	-1.16859	1.97159	.998	-7.9945	5.6574
	PVP	-6.82957*	1.97159	.050	-13.6555	-.0036
C/PVP 9/1	Bead	-11.05225*	1.97159	.001	-17.8782	-4.2263
	Chitosan	-2.23819	1.97159	.939	-9.0641	4.5878
	C/PVP 7/3	-4.19130	1.97159	.440	-11.0172	2.6346
	C/PVP 5/5	-4.10188	1.97159	.465	-10.9278	2.7241
	C/PVP 3/7	-4.31408	1.97159	.406	-11.1400	2.5119
	C/PVP 1/9	-3.40678	1.97159	.671	-10.2327	3.4192
	PVP	-9.06775*	1.97159	.006	-15.8937	-2.2418
C/PVP 7/3	Bead	-6.86095*	1.97159	.048	-13.6869	-.0350
	Chitosan	1.95311	1.97159	.969	-4.8728	8.7791
	C/PVP 9/1	4.19130	1.97159	.440	-2.6346	11.0172
	C/PVP 5/5	.08942	1.97159	1.000	-6.7365	6.9154
	C/PVP 3/7	-.12278	1.97159	1.000	-6.9487	6.7032
	C/PVP 1/9	.78452	1.97159	1.000	-6.0414	7.6105
	PVP	-4.87646	1.97159	.273	-11.7024	1.9495
C/PVP 5/5	Bead	-6.95037*	1.97159	.044	-13.7763	-.1244
	Chitosan	1.86369	1.97159	.976	-4.9623	8.6896
	C/PVP 9/1	4.10188	1.97159	.465	-2.7241	10.9278
	C/PVP 7/3	-.08942	1.97159	1.000	-6.9154	6.7365
	C/PVP 3/7	-.21220	1.97159	1.000	-7.0381	6.6137
	C/PVP 1/9	.69510	1.97159	1.000	-6.1308	7.5210
	PVP	-4.96588	1.97159	.255	-11.7918	1.8601

C/PVP 3/7	Bead	-6.73817	1.97159	.054	-13.5641	.0878
	Chitosan	2.07589	1.97159	.958	-4.7501	8.9018
	C/PVP 9/1	4.31408	1.97159	.406	-2.5119	11.1400
	C/PVP 7/3	.12278	1.97159	1.000	-6.7032	6.9487
	C/PVP 5/5	.21220	1.97159	1.000	-6.6137	7.0381
	C/PVP 1/9	.90730	1.97159	1.000	-5.9186	7.7332
	PVP	-4.75368	1.97159	.299	-11.5796	2.0723
C/PVP 1/9	Bead	-7.64547*	1.97159	.023	-14.4714	-.8195
	Chitosan	1.16859	1.97159	.998	-5.6574	7.9945
	C/PVP 9/1	3.40678	1.97159	.671	-3.4192	10.2327
	C/PVP 7/3	-.78452	1.97159	1.000	-7.6105	6.0414
	C/PVP 5/5	-.69510	1.97159	1.000	-7.5210	6.1308
	C/PVP 3/7	-.90730	1.97159	1.000	-7.7332	5.9186
	PVP	-5.66098	1.97159	.144	-12.4869	1.1650
PVP	Bead	-1.98450	1.97159	.967	-8.8104	4.8414
	Chitosan	6.82957*	1.97159	.050	.0036	13.6555
	C/PVP 9/1	9.06775*	1.97159	.006	2.2418	15.8937
	C/PVP 7/3	4.87646	1.97159	.273	-1.9495	11.7024
	C/PVP 5/5	4.96588	1.97159	.255	-1.8601	11.7918
	C/PVP 3/7	4.75368	1.97159	.299	-2.0723	11.5796
	C/PVP 1/9	5.66098	1.97159	.144	-1.1650	12.4869

*. The mean difference is significant at the 0.05 level.

5. ANOVA analysis of percentage release of AMX bead at t=30 minutes

Oneway

ANOVA

t30

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	300.809	7	42.973	5.063	.003
Within Groups	135.803	16	8.488		
Total	436.612	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t30

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	8.15451	2.37875	.053	-.0811	16.3901
	C/PVP 9/1	11.15690*	2.37875	.005	2.9213	19.3925
	C/PVP 7/3	6.90761	2.37875	.137	-1.3280	15.1432
	C/PVP 5/5	5.99028	2.37875	.255	-2.2453	14.2259
	C/PVP 3/7	6.24484	2.37875	.216	-1.9907	14.4804
	C/PVP 1/9	6.99082	2.37875	.129	-1.2448	15.2264
	PVP	.36361	2.37875	1.000	-7.8720	8.5992
Chitosan	Bead	-8.15451	2.37875	.053	-16.3901	.0811
	C/PVP 9/1	3.00240	2.37875	.900	-5.2332	11.2380
	C/PVP 7/3	-1.24690	2.37875	.999	-9.4825	6.9887
	C/PVP 5/5	-2.16423	2.37875	.981	-10.3998	6.0714
	C/PVP 3/7	-1.90966	2.37875	.990	-10.1453	6.3259
	C/PVP 1/9	-1.16369	2.37875	1.000	-9.3993	7.0719
	PVP	-7.79090	2.37875	.071	-16.0265	.4447
C/PVP 9/1	Bead	-11.15690*	2.37875	.005	-19.3925	-2.9213
	Chitosan	-3.00240	2.37875	.900	-11.2380	5.2332
	C/PVP 7/3	-4.24930	2.37875	.637	-12.4849	3.9863
	C/PVP 5/5	-5.16662	2.37875	.415	-13.4022	3.0690
	C/PVP 3/7	-4.91206	2.37875	.474	-13.1477	3.3235
	C/PVP 1/9	-4.16609	2.37875	.658	-12.4017	4.0695
	PVP	-10.79330*	2.37875	.006	-19.0289	-2.5577
C/PVP 7/3	Bead	-6.90761	2.37875	.137	-15.1432	1.3280
	Chitosan	1.24690	2.37875	.999	-6.9887	9.4825
	C/PVP 9/1	4.24930	2.37875	.637	-3.9863	12.4849
	C/PVP 5/5	-.91733	2.37875	1.000	-9.1529	7.3183
	C/PVP 3/7	-.66276	2.37875	1.000	-8.8984	7.5728
	C/PVP 1/9	.08321	2.37875	1.000	-8.1524	8.3188
	PVP	-6.54400	2.37875	.177	-14.7796	1.6916
C/PVP 5/5	Bead	-5.99028	2.37875	.255	-14.2259	2.2453
	Chitosan	2.16423	2.37875	.981	-6.0714	10.3998
	C/PVP 9/1	5.16662	2.37875	.415	-3.0690	13.4022
	C/PVP 7/3	.91733	2.37875	1.000	-7.3183	9.1529
	C/PVP 3/7	.25456	2.37875	1.000	-7.9810	8.4902
	C/PVP 1/9	1.00054	2.37875	1.000	-7.2351	9.2361
	PVP	-5.62667	2.37875	.320	-13.8623	2.6089

C/PVP 3/7	Bead	-6.24484	2.37875	.216	-14.4804	1.9907
	Chitosan	1.90966	2.37875	.990	-6.3259	10.1453
	C/PVP 9/1	4.91206	2.37875	.474	-3.3235	13.1477
	C/PVP 7/3	.66276	2.37875	1.000	-7.5728	8.8984
	C/PVP 5/5	-.25456	2.37875	1.000	-8.4902	7.9810
	C/PVP 1/9	.74597	2.37875	1.000	-7.4896	8.9816
	PVP	-5.88124	2.37875	.273	-14.1168	2.3544
C/PVP 1/9	Bead	-6.99082	2.37875	.129	-15.2264	1.2448
	Chitosan	1.16369	2.37875	1.000	-7.0719	9.3993
	C/PVP 9/1	4.16609	2.37875	.658	-4.0695	12.4017
	C/PVP 7/3	-.08321	2.37875	1.000	-8.3188	8.1524
	C/PVP 5/5	-1.00054	2.37875	1.000	-9.2361	7.2351
	C/PVP 3/7	-.74597	2.37875	1.000	-8.9816	7.4896
	PVP	-6.62721	2.37875	.167	-14.8628	1.6084
PVP	Bead	-.36361	2.37875	1.000	-8.5992	7.8720
	Chitosan	7.79090	2.37875	.071	-.4447	16.0265
	C/PVP 9/1	10.79330*	2.37875	.006	2.5577	19.0289
	C/PVP 7/3	6.54400	2.37875	.177	-1.6916	14.7796
	C/PVP 5/5	5.62667	2.37875	.320	-2.6089	13.8623
	C/PVP 3/7	5.88124	2.37875	.273	-2.3544	14.1168
	C/PVP 1/9	6.62721	2.37875	.167	-1.6084	14.8628

*. The mean difference is significant at the 0.05 level.

6. ANOVA analysis of percentage release of AMX bead at t=40 minutes

Oneway

ANOVA

t40

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	362.536	7	51.791	5.149	.003
Within Groups	160.944	16	10.059		
Total	523.480	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t40

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	7.84805	2.58960	.110	-1.1175	16.8136
	C/PVP 9/1	11.68512*	2.58960	.007	2.7195	20.6507
	C/PVP 7/3	7.31493	2.58960	.156	-1.6506	16.2805
	C/PVP 5/5	6.47423	2.58960	.262	-2.4913	15.4398
	C/PVP 3/7	6.19906	2.58960	.307	-2.7665	15.1646
	C/PVP 1/9	6.32761	2.58960	.285	-2.6380	15.2932
	PVP	-.94299	2.58960	1.000	-9.9086	8.0226
Chitosan	Bead	-7.84805	2.58960	.110	-16.8136	1.1175
	C/PVP 9/1	3.83707	2.58960	.806	-5.1285	12.8026
	C/PVP 7/3	-.53312	2.58960	1.000	-9.4987	8.4325
	C/PVP 5/5	-1.37382	2.58960	.999	-10.3394	7.5918
	C/PVP 3/7	-1.64899	2.58960	.998	-10.6146	7.3166
	C/PVP 1/9	-1.52044	2.58960	.999	-10.4860	7.4451
	PVP	-8.79103	2.58960	.057	-17.7566	.1745
C/PVP 9/1	Bead	-11.68512*	2.58960	.007	-20.6507	-2.7195
	Chitosan	-3.83707	2.58960	.806	-12.8026	5.1285
	C/PVP 7/3	-4.37019	2.58960	.695	-13.3358	4.5954
	C/PVP 5/5	-5.21089	2.58960	.504	-14.1765	3.7547
	C/PVP 3/7	-5.48606	2.58960	.444	-14.4516	3.4795
	C/PVP 1/9	-5.35752	2.58960	.471	-14.3231	3.6081
	PVP	-12.62811*	2.58960	.003	-21.5937	-3.6625
C/PVP 7/3	Bead	-7.31493	2.58960	.156	-16.2805	1.6506
	Chitosan	.53312	2.58960	1.000	-8.4325	9.4987
	C/PVP 9/1	4.37019	2.58960	.695	-4.5954	13.3358
	C/PVP 5/5	-.84070	2.58960	1.000	-9.8063	8.1249
	C/PVP 3/7	-1.11587	2.58960	1.000	-10.0814	7.8497
	C/PVP 1/9	-.98733	2.58960	1.000	-9.9529	7.9782
	PVP	-8.25792	2.58960	.083	-17.2235	.7077
C/PVP 5/5	Bead	-6.47423	2.58960	.262	-15.4398	2.4913
	Chitosan	1.37382	2.58960	.999	-7.5918	10.3394
	C/PVP 9/1	5.21089	2.58960	.504	-3.7547	14.1765
	C/PVP 7/3	.84070	2.58960	1.000	-8.1249	9.8063
	C/PVP 3/7	-.27518	2.58960	1.000	-9.2407	8.6904
	C/PVP 1/9	-.14663	2.58960	1.000	-9.1122	8.8189
	PVP	-7.41722	2.58960	.146	-16.3828	1.5484
C/PVP 3/7	Bead	-6.19906	2.58960	.307	-15.1646	2.7665
	Chitosan	1.64899	2.58960	.998	-7.3166	10.6146
	C/PVP 9/1	5.48606	2.58960	.444	-3.4795	14.4516
	C/PVP 7/3	1.11587	2.58960	1.000	-7.8497	10.0814

	C/PVP 5/5	.27518	2.58960	1.000	-8.6904	9.2407
	C/PVP 1/9	.12855	2.58960	1.000	-8.8370	9.0941
	PVP	-7.14204	2.58960	.175	-16.1076	1.8235
C/PVP 1/9	Bead	-6.32761	2.58960	.285	-15.2932	2.6380
	Chitosan	1.52044	2.58960	.999	-7.4451	10.4860
	C/PVP 9/1	5.35752	2.58960	.471	-3.6081	14.3231
	C/PVP 7/3	.98733	2.58960	1.000	-7.9782	9.9529
	C/PVP 5/5	.14663	2.58960	1.000	-8.8189	9.1122
	C/PVP 3/7	-.12855	2.58960	1.000	-9.0941	8.8370
	PVP	-7.27059	2.58960	.161	-16.2362	1.6950
PVP	Bead	.94299	2.58960	1.000	-8.0226	9.9086
	Chitosan	8.79103	2.58960	.057	-.1745	17.7566
	C/PVP 9/1	12.62811*	2.58960	.003	3.6625	21.5937
	C/PVP 7/3	8.25792	2.58960	.083	-.7077	17.2235
	C/PVP 5/5	7.41722	2.58960	.146	-1.5484	16.3828
	C/PVP 3/7	7.14204	2.58960	.175	-1.8235	16.1076
	C/PVP 1/9	7.27059	2.58960	.161	-1.6950	16.2362

*. The mean difference is significant at the 0.05 level.

7. ANOVA analysis of the percentage release from the AMX bead at t = 60 minutes

Oneway

ANOVA

t60

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	355.658	7	50.808	4.977	.004
Within Groups	163.353	16	10.210		
Total	519.011	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t60

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	7.21426	2.60890	.172	-1.8182	16.2467
	C/PVP 9/1	10.10668*	2.60890	.023	1.0743	19.1391
	C/PVP 7/3	6.10685	2.60890	.331	-2.9256	15.1393
	C/PVP 5/5	5.04296	2.60890	.550	-3.9895	14.0754
	C/PVP 3/7	5.01330	2.60890	.557	-4.0191	14.0457
	C/PVP 1/9	3.77455	2.60890	.823	-5.2579	12.8070
	PVP	-2.99747	2.60890	.935	-12.0299	6.0349
Chitosan	Bead	-7.21426	2.60890	.172	-16.2467	1.8182
	C/PVP 9/1	2.89242	2.60890	.946	-6.1400	11.9248
	C/PVP 7/3	-1.10740	2.60890	1.000	-10.1398	7.9250
	C/PVP 5/5	-2.17129	2.60890	.988	-11.2037	6.8611
	C/PVP 3/7	-2.20096	2.60890	.987	-11.2334	6.8315
	C/PVP 1/9	-3.43971	2.60890	.879	-12.4721	5.5927
	PVP	-10.21173*	2.60890	.021	-19.2441	-1.1793
C/PVP 9/1	Bead	-10.10668*	2.60890	.023	-19.1391	-1.0743
	Chitosan	-2.89242	2.60890	.946	-11.9248	6.1400
	C/PVP 7/3	-3.99982	2.60890	.780	-13.0322	5.0326
	C/PVP 5/5	-5.06372	2.60890	.545	-14.0961	3.9687
	C/PVP 3/7	-5.09338	2.60890	.538	-14.1258	3.9390
	C/PVP 1/9	-6.33213	2.60890	.292	-15.3646	2.7003
	PVP	-13.10415*	2.60890	.002	-22.1366	-4.0717
C/PVP 7/3	Bead	-6.10685	2.60890	.331	-15.1393	2.9256
	Chitosan	1.10740	2.60890	1.000	-7.9250	10.1398
	C/PVP 9/1	3.99982	2.60890	.780	-5.0326	13.0322
	C/PVP 5/5	-1.06389	2.60890	1.000	-10.0963	7.9685
	C/PVP 3/7	-1.09356	2.60890	1.000	-10.1260	7.9389
	C/PVP 1/9	-2.33231	2.60890	.982	-11.3647	6.7001
	PVP	-9.10432*	2.60890	.047	-18.1367	-.0719
C/PVP 5/5	Bead	-5.04296	2.60890	.550	-14.0754	3.9895
	Chitosan	2.17129	2.60890	.988	-6.8611	11.2037
	C/PVP 9/1	5.06372	2.60890	.545	-3.9687	14.0961
	C/PVP 7/3	1.06389	2.60890	1.000	-7.9685	10.0963
	C/PVP 3/7	-.02967	2.60890	1.000	-9.0621	9.0028
	C/PVP 1/9	-1.26842	2.60890	1.000	-10.3008	7.7640
	PVP	-8.04043	2.60890	.100	-17.0729	.9920
C/PVP 3/7	Bead	-5.01330	2.60890	.557	-14.0457	4.0191
	Chitosan	2.20096	2.60890	.987	-6.8315	11.2334

	C/PVP 9/1	5.09338	2.60890	.538	-3.9390	14.1258
	C/PVP 7/3	1.09356	2.60890	1.000	-7.9389	10.1260
	C/PVP 5/5	.02967	2.60890	1.000	-9.0028	9.0621
	C/PVP 1/9	-1.23875	2.60890	1.000	-10.2712	7.7937
	PVP	-8.01077	2.60890	.102	-17.0432	1.0217
C/PVP 1/9	Bead	-3.77455	2.60890	.823	-12.8070	5.2579
	Chitosan	3.43971	2.60890	.879	-5.5927	12.4721
	C/PVP 9/1	6.33213	2.60890	.292	-2.7003	15.3646
	C/PVP 7/3	2.33231	2.60890	.982	-6.7001	11.3647
	C/PVP 5/5	1.26842	2.60890	1.000	-7.7640	10.3008
	C/PVP 3/7	1.23875	2.60890	1.000	-7.7937	10.2712
	PVP	-6.77201	2.60890	.226	-15.8044	2.2604
PVP	Bead	2.99747	2.60890	.935	-6.0349	12.0299
	Chitosan	10.21173*	2.60890	.021	1.1793	19.2441
	C/PVP 9/1	13.10415*	2.60890	.002	4.0717	22.1366
	C/PVP 7/3	9.10432*	2.60890	.047	.0719	18.1367
	C/PVP 5/5	8.04043	2.60890	.100	-.9920	17.0729
	C/PVP 3/7	8.01077	2.60890	.102	-1.0217	17.0432
	C/PVP 1/9	6.77201	2.60890	.226	-2.2604	15.8044

*. The mean difference is significant at the 0.05 level.

8. ANOVA analysis of the percentage release from the AMX bead at t = 90 minutes

Oneway

ANOVA

t90

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	304.228	7	43.461	3.291	.023
Within Groups	211.314	16	13.207		
Total	515.541	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t90

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	2.68113	2.96728	.981	-7.5920	12.9543
	C/PVP 9/1	5.80311	2.96728	.537	-4.4701	16.0763
	C/PVP 7/3	3.23416	2.96728	.950	-7.0390	13.5073
	C/PVP 5/5	-.02013	2.96728	1.000	-10.2933	10.2530
	C/PVP 3/7	-.39240	2.96728	1.000	-10.6656	9.8808
	C/PVP 1/9	-1.98444	2.96728	.997	-12.2576	8.2887
	PVP	-6.83836	2.96728	.348	-17.1115	3.4348
Chitosan	Bead	-2.68113	2.96728	.981	-12.9543	7.5920
	C/PVP 9/1	3.12197	2.96728	.958	-7.1512	13.3951
	C/PVP 7/3	.55303	2.96728	1.000	-9.7201	10.8262
	C/PVP 5/5	-2.70127	2.96728	.981	-12.9744	7.5719
	C/PVP 3/7	-3.07354	2.96728	.961	-13.3467	7.1996
	C/PVP 1/9	-4.66557	2.96728	.759	-14.9387	5.6076
	PVP	-9.51950	2.96728	.080	-19.7927	.7537
C/PVP 9/1	Bead	-5.80311	2.96728	.537	-16.0763	4.4701
	Chitosan	-3.12197	2.96728	.958	-13.3951	7.1512
	C/PVP 7/3	-2.56895	2.96728	.985	-12.8421	7.7042
	C/PVP 5/5	-5.82324	2.96728	.533	-16.0964	4.4499
	C/PVP 3/7	-6.19551	2.96728	.461	-16.4687	4.0777
	C/PVP 1/9	-7.78755	2.96728	.216	-18.0607	2.4856
	PVP	-12.64147*	2.96728	.011	-22.9146	-2.3683
C/PVP 7/3	Bead	-3.23416	2.96728	.950	-13.5073	7.0390
	Chitosan	-.55303	2.96728	1.000	-10.8262	9.7201
	C/PVP 9/1	2.56895	2.96728	.985	-7.7042	12.8421
	C/PVP 5/5	-3.25429	2.96728	.948	-13.5275	7.0189
	C/PVP 3/7	-3.62656	2.96728	.914	-13.8997	6.6466
	C/PVP 1/9	-5.21860	2.96728	.653	-15.4918	5.0546
	PVP	-10.07252	2.96728	.057	-20.3457	.2006
C/PVP 5/5	Bead	.02013	2.96728	1.000	-10.2530	10.2933
	Chitosan	2.70127	2.96728	.981	-7.5719	12.9744
	C/PVP 9/1	5.82324	2.96728	.533	-4.4499	16.0964
	C/PVP 7/3	3.25429	2.96728	.948	-7.0189	13.5275
	C/PVP 3/7	-.37227	2.96728	1.000	-10.6454	9.9009
	C/PVP 1/9	-1.96431	2.96728	.997	-12.2375	8.3089
	PVP	-6.81823	2.96728	.351	-17.0914	3.4549

C/PVP 3/7	Bead	.39240	2.96728	1.000	-9.8808	10.6656
	Chitosan	3.07354	2.96728	.961	-7.1996	13.3467
	C/PVP 9/1	6.19551	2.96728	.461	-4.0777	16.4687
	C/PVP 7/3	3.62656	2.96728	.914	-6.6466	13.8997
	C/PVP 5/5	.37227	2.96728	1.000	-9.9009	10.6454
	C/PVP 1/9	-1.59203	2.96728	.999	-11.8652	8.6811
	PVP	-6.44596	2.96728	.415	-16.7191	3.8272
C/PVP 1/9	Bead	1.98444	2.96728	.997	-8.2887	12.2576
	Chitosan	4.66557	2.96728	.759	-5.6076	14.9387
	C/PVP 9/1	7.78755	2.96728	.216	-2.4856	18.0607
	C/PVP 7/3	5.21860	2.96728	.653	-5.0546	15.4918
	C/PVP 5/5	1.96431	2.96728	.997	-8.3089	12.2375
	C/PVP 3/7	1.59203	2.96728	.999	-8.6811	11.8652
	PVP	-4.85393	2.96728	.724	-15.1271	5.4192
PVP	Bead	6.83836	2.96728	.348	-3.4348	17.1115
	Chitosan	9.51950	2.96728	.080	-.7537	19.7927
	C/PVP 9/1	12.64147*	2.96728	.011	2.3683	22.9146
	C/PVP 7/3	10.07252	2.96728	.057	-.2006	20.3457
	C/PVP 5/5	6.81823	2.96728	.351	-3.4549	17.0914
	C/PVP 3/7	6.44596	2.96728	.415	-3.8272	16.7191
	C/PVP 1/9	4.85393	2.96728	.724	-5.4192	15.1271

*. The mean difference is significant at the 0.05 level.

9. ANOVA analysis of the percentage release from the AMX bead at t = 120 minutes

Oneway

ANOVA

t120

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	250.642	7	35.806	3.760	.013
Within Groups	152.372	16	9.523		
Total	403.014	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t120

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	-1.32815	2.51969	.999	-10.0517	7.3954
	C/PVP 9/1	1.87294	2.51969	.994	-6.8506	10.5965
	C/PVP 7/3	-2.04421	2.51969	.990	-10.7678	6.6793
	C/PVP 5/5	-3.84841	2.51969	.783	-12.5720	4.8751
	C/PVP 3/7	-3.25748	2.51969	.889	-11.9810	5.4661
	C/PVP 1/9	-5.94227	2.51969	.323	-14.6658	2.7813
	PVP	-9.13697*	2.51969	.037	-17.8605	-.4134
Chitosan	Bead	1.32815	2.51969	.999	-7.3954	10.0517
	C/PVP 9/1	3.20110	2.51969	.897	-5.5225	11.9247
	C/PVP 7/3	-.71606	2.51969	1.000	-9.4396	8.0075
	C/PVP 5/5	-2.52026	2.51969	.968	-11.2438	6.2033
	C/PVP 3/7	-1.92933	2.51969	.993	-10.6529	6.7942
	C/PVP 1/9	-4.61412	2.51969	.610	-13.3377	4.1094
	PVP	-7.80882	2.51969	.097	-16.5324	.9147
C/PVP 9/1	Bead	-1.87294	2.51969	.994	-10.5965	6.8506
	Chitosan	-3.20110	2.51969	.897	-11.9247	5.5225
	C/PVP 7/3	-3.91715	2.51969	.769	-12.6407	4.8064
	C/PVP 5/5	-5.72135	2.51969	.364	-14.4449	3.0022
	C/PVP 3/7	-5.13042	2.51969	.490	-13.8540	3.5931
	C/PVP 1/9	-7.81521	2.51969	.097	-16.5388	.9083
	PVP	-11.00991*	2.51969	.009	-19.7335	-2.2864
C/PVP 7/3	Bead	2.04421	2.51969	.990	-6.6793	10.7678
	Chitosan	.71606	2.51969	1.000	-8.0075	9.4396
	C/PVP 9/1	3.91715	2.51969	.769	-4.8064	12.6407
	C/PVP 5/5	-1.80420	2.51969	.995	-10.5278	6.9194
	C/PVP 3/7	-1.21327	2.51969	1.000	-9.9368	7.5103
	C/PVP 1/9	-3.89806	2.51969	.773	-12.6216	4.8255
	PVP	-7.09276	2.51969	.159	-15.8163	1.6308
C/PVP 5/5	Bead	3.84841	2.51969	.783	-4.8751	12.5720
	Chitosan	2.52026	2.51969	.968	-6.2033	11.2438
	C/PVP 9/1	5.72135	2.51969	.364	-3.0022	14.4449
	C/PVP 7/3	1.80420	2.51969	.995	-6.9194	10.5278
	C/PVP 3/7	.59093	2.51969	1.000	-8.1326	9.3145
	C/PVP 1/9	-2.09386	2.51969	.988	-10.8174	6.6297
	PVP	-5.28856	2.51969	.455	-14.0121	3.4350
C/PVP 3/7	Bead	3.25748	2.51969	.889	-5.4661	11.9810

	Chitosan	1.92933	2.51969	.993	-6.7942	10.6529
	C/PVP 9/1	5.13042	2.51969	.490	-3.5931	13.8540
	C/PVP 7/3	1.21327	2.51969	1.000	-7.5103	9.9368
	C/PVP 5/5	-.59093	2.51969	1.000	-9.3145	8.1326
	C/PVP 1/9	-2.68479	2.51969	.955	-11.4083	6.0388
	PVP	-5.87949	2.51969	.334	-14.6030	2.8441
C/PVP 1/9	Bead	5.94227	2.51969	.323	-2.7813	14.6658
	Chitosan	4.61412	2.51969	.610	-4.1094	13.3377
	C/PVP 9/1	7.81521	2.51969	.097	-.9083	16.5388
	C/PVP 7/3	3.89806	2.51969	.773	-4.8255	12.6216
	C/PVP 5/5	2.09386	2.51969	.988	-6.6297	10.8174
	C/PVP 3/7	2.68479	2.51969	.955	-6.0388	11.4083
	PVP	-3.19470	2.51969	.898	-11.9183	5.5289
PVP	Bead	9.13697*	2.51969	.037	.4134	17.8605
	Chitosan	7.80882	2.51969	.097	-.9147	16.5324
	C/PVP 9/1	11.00991*	2.51969	.009	2.2864	19.7335
	C/PVP 7/3	7.09276	2.51969	.159	-1.6308	15.8163
	C/PVP 5/5	5.28856	2.51969	.455	-3.4350	14.0121
	C/PVP 3/7	5.87949	2.51969	.334	-2.8441	14.6030
	C/PVP 1/9	3.19470	2.51969	.898	-5.5289	11.9183

*. The mean difference is significant at the 0.05 level.

10. ANOVA analysis of the percentage release from the AMX bead at t = 180 minutes

Oneway

ANOVA

t180

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	308.482	7	44.069	7.003	.001
Within Groups	100.691	16	6.293		
Total	409.173	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: t180

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bead	Chitosan	-5.99104	2.04828	.132	-13.0825	1.1004
	C/PVP 9/1	-.96162	2.04828	1.000	-8.0531	6.1298
	C/PVP 7/3	-7.77443*	2.04828	.027	-14.8659	-.6830
	C/PVP 5/5	-7.00152	2.04828	.054	-14.0930	.0899
	C/PVP 3/7	-7.43844*	2.04828	.036	-14.5299	-.3470
	C/PVP 1/9	-10.45453*	2.04828	.002	-17.5460	-3.3631
	PVP	-9.96126*	2.04828	.003	-17.0527	-2.8698
Chitosan	Bead	5.99104	2.04828	.132	-1.1004	13.0825
	C/PVP 9/1	5.02942	2.04828	.280	-2.0620	12.1209
	C/PVP 7/3	-1.78339	2.04828	.985	-8.8748	5.3081
	C/PVP 5/5	-1.01048	2.04828	1.000	-8.1019	6.0810
	C/PVP 3/7	-1.44740	2.04828	.996	-8.5389	5.6440
	C/PVP 1/9	-4.46350	2.04828	.411	-11.5549	2.6280
	PVP	-3.97022	2.04828	.547	-11.0617	3.1212
C/PVP 9/1	Bead	.96162	2.04828	1.000	-6.1298	8.0531
	Chitosan	-5.02942	2.04828	.280	-12.1209	2.0620
	C/PVP 7/3	-6.81281	2.04828	.064	-13.9043	.2786
	C/PVP 5/5	-6.03990	2.04828	.126	-13.1313	1.0515
	C/PVP 3/7	-6.47683	2.04828	.087	-13.5683	.6146
	C/PVP 1/9	-9.49292*	2.04828	.005	-16.5844	-2.4015
	PVP	-8.99965*	2.04828	.008	-16.0911	-1.9082
C/PVP 7/3	Bead	7.77443*	2.04828	.027	.6830	14.8659
	Chitosan	1.78339	2.04828	.985	-5.3081	8.8748
	C/PVP 9/1	6.81281	2.04828	.064	-.2786	13.9043
	C/PVP 5/5	.77291	2.04828	1.000	-6.3185	7.8644
	C/PVP 3/7	.33598	2.04828	1.000	-6.7555	7.4274
	C/PVP 1/9	-2.68011	2.04828	.883	-9.7716	4.4113
	PVP	-2.18684	2.04828	.955	-9.2783	4.9046
C/PVP 5/5	Bead	7.00152	2.04828	.054	-.0899	14.0930
	Chitosan	1.01048	2.04828	1.000	-6.0810	8.1019
	C/PVP 9/1	6.03990	2.04828	.126	-1.0515	13.1313
	C/PVP 7/3	-.77291	2.04828	1.000	-7.8644	6.3185
	C/PVP 3/7	-.43693	2.04828	1.000	-7.5284	6.6545
	C/PVP 1/9	-3.45302	2.04828	.696	-10.5445	3.6384
	PVP	-2.95975	2.04828	.824	-10.0512	4.1317
C/PVP 3/7	Bead	7.43844*	2.04828	.036	.3470	14.5299

	Chitosan	1.44740	2.04828	.996	-5.6440	8.5389
	C/PVP 9/1	6.47683	2.04828	.087	-.6146	13.5683
	C/PVP 7/3	-.33598	2.04828	1.000	-7.4274	6.7555
	C/PVP 5/5	.43693	2.04828	1.000	-6.6545	7.5284
	C/PVP 1/9	-3.01609	2.04828	.811	-10.1075	4.0754
	PVP	-2.52282	2.04828	.910	-9.6143	4.5686
C/PVP 1/9	Bead	10.45453*	2.04828	.002	3.3631	17.5460
	Chitosan	4.46350	2.04828	.411	-2.6280	11.5549
	C/PVP 9/1	9.49292*	2.04828	.005	2.4015	16.5844
	C/PVP 7/3	2.68011	2.04828	.883	-4.4113	9.7716
	C/PVP 5/5	3.45302	2.04828	.696	-3.6384	10.5445
	C/PVP 3/7	3.01609	2.04828	.811	-4.0754	10.1075
	PVP	.49327	2.04828	1.000	-6.5982	7.5847
PVP	Bead	9.96126*	2.04828	.003	2.8698	17.0527
	Chitosan	3.97022	2.04828	.547	-3.1212	11.0617
	C/PVP 9/1	8.99965*	2.04828	.008	1.9082	16.0911
	C/PVP 7/3	2.18684	2.04828	.955	-4.9046	9.2783
	C/PVP 5/5	2.95975	2.04828	.824	-4.1317	10.0512
	C/PVP 3/7	2.52282	2.04828	.910	-4.5686	9.6143
	C/PVP 1/9	-.49327	2.04828	1.000	-7.5847	6.5982

*. The mean difference is significant at the 0.05 level.

Appendix F

United States Pharmacopeia and National Formulary (USP33-NF28) of amoxicillin

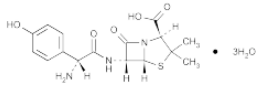
1. Amoxicillin

r_U = peak response of amoxapine from the *Sample solution*
r_S = peak response of amoxapine from the *Standard solution*
C_S = concentration of USP Amoxapine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amoxapine in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Simulated gastric fluid (without enzyme); 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Standard solution: USP Amoxapine RS in *Medium*
  Sample solution: Sample per *Dissolution* ⟨711⟩.
  Spectrometric conditions
  Analytical wavelength: 294 nm
  Analysis: Determine the amount of $C_{17}H_{16}ClN_3O$ dissolved from UV absorbances of filtered portions of the *Sample solution*, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Amoxapine RS.
  Tolerances: NLT 80% (Q) of the labeled amount of $C_{17}H_{16}ClN_3O$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxapine RS

Amoxicillin



$C_{16}H_{19}N_3O_5S \cdot 3H_2O$ 419.45
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[amino(4-hydroxyphenyl)acetyl]amino-3,3-dimethyl-7-oxo-, trihydrate [2*S*-[2α,5α,6β(*S**)]]-;
(2*S*,5*R*,6*R*)-6-[(*R*)-(-)-2-Amino-2-(*p*-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate [61336-70-7].
Anhydrous 365.41
[26787-78-0].

DEFINITION
Amoxicillin contains NLT 900 µg and NMT 1050 µg of $C_{16}H_{19}N_3O_5S$ per mg, calculated on the anhydrous basis.

IDENTIFICATION
- **INFRARED ABSORPTION** ⟨197K⟩

ASSAY
- **PROCEDURE**
  Diluent: 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1.
  Mobile phase: Acetonitrile and *Diluent* (1:24). Decrease the acetonitrile concentration to increase the retention time of amoxicillin.
  Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. [NOTE—Use this solution within 6 h.]
  Sample solution: 1.2 mg/mL of Amoxicillin in *Diluent*. [NOTE—Use this solution within 6 h.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Column: 4-mm × 25-cm; packing L1
  Flow rate: 1.5 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Capacity factor: 1.1–2.8
  Column efficiency: NLT 1700 theoretical plates
  Tailing factor: NMT 2.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the quantity, in µg, of $C_{16}H_{19}N_3O_5S$/mg taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = concentration of *Sample solution* (mg/mL)
P = stated amoxicillin content of USP Amoxicillin RS (µg/mg)
Acceptance criteria: 900–1050 µg of $C_{16}H_{19}N_3O_5S$ per mg on the anhydrous basis

SPECIFIC TESTS
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **DIMETHYLANILINE** ⟨223⟩: Meets the requirement
- **PH** ⟨791⟩: 3.5–6.0
  Sample solution: 2 mg/ml
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 11.5%–14.5%
- **STERILITY TESTS** ⟨71⟩: Where the label states that Amoxicillin is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium,* except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, to use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, and to shake the tubes once daily.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Amoxicillin is sterile or Amoxicillin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.25 Endotoxin Unit/mg of amoxicillin.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is intended for veterinary use only and that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Label all other Amoxicillin to indicate that it is to be used in the manufacture of nonparenteral drugs only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxicillin RS
  USP Endotoxin RS

Amoxicillin Boluses

DEFINITION
Amoxicillin Boluses contain NLT 90.0% and NMT 110.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

2. Amoxicillin capsule

IDENTIFICATION

• **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Standard solution: 4 mg/mL of USP Amoxicillin RS in 0.1 N hydrochloric acid. [NOTE—Use within 10 min of preparation.]

Sample solution: 4 mg/mL of amoxicillin, from powdered Boluses in 0.1 N hydrochloric acid

Application volume: 5 µL

Developing solvent system: Methanol, chloroform, pyridine, and water (9:8:1:3)

Spray reagent: 3 mg/mL of ninhydrin in alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, and dry with warm air for 10 min. Locate the spots on the plate by spraying lightly with *Spray reagent*, and dry at 110° for 15 min.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Diluent: 6.8 mg/mL of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1.

Mobile phase: Acetonitrile and *Diluent* (1:24). Decrease the acetonitrile concentration to increase the retention time of amoxicillin.

Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. [NOTE—Use this solution within 6 h.]

Sample solution: Transfer an equivalent to 250 mg of amoxicillin, from finely powdered Boluses (NLT 5), to a 250-mL volumetric flask, add *Diluent* to volume, and mix. Sonicate if necessary to ensure complete dissolution of the amoxicillin. Pass a portion of this solution through a filter of 1-µm or finer porosity. [NOTE—Use this solution within 6 h.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: 1.1–2.8

Column efficiency: NLT 1700 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{19}N_3O_5S$ in the portion of Boluses taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of amoxicillin from the *Sample solution*

r_S = peak response of amoxicillin from the *Standard solution*

C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

P = stated content of USP Amoxicillin RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Medium: Simulated gastric fluid being used instead of water

Time: 30 min

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 7.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **LABELING:** Label Boluses to indicate that they are for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Amoxicillin RS

Amoxicillin Capsules

DEFINITION

Amoxicillin Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

IDENTIFICATION

• **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**

Standard solution: 4 mg/mL of USP Amoxicillin RS in 0.1 N hydrochloric acid

[NOTE—Use within 10 min after preparation.]

Sample solution: Equivalent to 4 mg/mL of amoxicillin, from Capsule contents in 0.1 N hydrochloric acid. [NOTE—Use within 10 min.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Methanol, chloroform, pyridine, and water (9:8:1:3)

Spray reagent: 3 mg/mL of ninhydrin in alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, and dry with warm air for 10 min. Locate the spots on the plate by spraying lightly with *Spray reagent* and dry at 110° for 15 min.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Diluent: Dissolve 6.8 g/L of monobasic potassium phosphate in water, and adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1.

Mobile phase: Acetonitrile and *Diluent* (1:24). Decrease the acetonitrile concentration to increase the retention time of amoxicillin.

Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. [NOTE—Use this solution within 6 h.]

Sample solution: Remove, as completely as possible, the contents of NLT 20 Capsules, mix the combined contents, and transfer a quantity, equivalent to 200 mg of anhydrous amoxicillin, to a 200-mL volumetric flask, and add *Diluent* to volume. Sonicate if necessary to ensure complete dissolution. Pass a portion of this solution through a suitable filter having a 1-µm or finer porosity, and use the filtrate. [NOTE—Use this solution within 6 h.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: 1.1–2.8
Column efficiency: NLT 1700 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{19}N_3O_5S$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)
P = stated amoxicillin content of USP Amoxicillin RS (mg/mg)
Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩:
Test 1
Medium: Water; 900 mL
Apparatus 1: 100 rpm, for Capsules containing 250 mg
Apparatus 2: 75 rpm, for Capsules containing 500 mg
Time: 60 min
Analytical wavelength: UV 272 nm
Standard solution: USP Amoxicillin RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to *Standard solution*.
Tolerances: NLT 80% (Q) of the labeled amount of $C_{16}H_{19}N_3O_5S$ is dissolved.
Test 2 (If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.)
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 90 min
Analytical wavelength: UV 272 nm
Standard solution: USP Amoxicillin RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to concentration that is similar to *Standard solution*.
Tolerances: NLT 80% (Q) of the labeled amount of $C_{16}H_{19}N_3O_5S$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 14.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amoxicillin RS

Amoxicillin Intramammary Infusion

DEFINITION
Amoxicillin Intramammary Infusion is a suspension of Amoxicillin in a suitable vegetable oil vehicle. It contains NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$). It contains a suitable dispersing agent and preservative.

IDENTIFICATION
• **THIN-LAYER CHROMATOGRAPHY**
Standard solution: 4 mg/mL of USP Amoxicillin RS in 0.1 N hydrochloric acid. [NOTE—Use within 10 min after preparation.]
Sample solution: Transfer a quantity of Intramammary Infusion, equivalent to 60 mg of amoxicillin, to a 50-mL centrifuge tube. Add 25 mL of toluene, and centrifuge. Decant and discard the toluene. Wash the residue with four 25-mL portions of toluene, sonicating for 30 s after each addition of toluene. Dry the residue in a vacuum over silica gel. Add 15 mL of 0.1 N hydrochloric acid to the residue.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Methanol, chloroform, pyridine, water, and (9:8:1:3)
Spray reagent: 3 mg/mL of ninhydrin in alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, and dry with warm air for 10 min. Locate the spots on the plate by spraying lightly with *Spray reagent* and dry at 110° for 15 min.
Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.

ASSAY
• **PROCEDURE**
Analysis: Proceed as directed for amoxicillin under *Antibiotics—Microbial Assays* ⟨81⟩. Expel the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer No. 3* and 1.0 mL of polysorbate 80, and blend for 3–5 min. Allow to stand for 10 min, and dilute a measured volume of the aqueous phase quantitatively and stepwise with *Buffer No. 3* to obtain a *Sample Dilution* having a concentration assumed to be equal to the median dose level of the Standard.
Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed disposable syringes.
• **LABELING:** Label it to indicate that it is intended for veterinary use only.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amoxicillin RS

3. Amoxicillin tablet

Mode: LC
Detector: UV 230 nm
Column: 4-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: 1.1–2.8
Column efficiency: NLT 1700 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{19}N_3O_5S$ in each portion of the constituted Amoxicillin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anhydrous amoxicillin in the *Sample solution* (mg/mL)
P = stated amoxicillin content of USP Amoxicillin RS (µg/mg)
Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For solids packaged in single-unit containers: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS
• **PH** ⟨791⟩**:** 5.0–7.5, in the suspension constituted as directed in the labeling
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 3.0%, except where it is labeled as containing 80 mg of amoxicillin per mL after constitution, the limit is NMT 4.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amoxicillin RS

Amoxicillin Tablets

DEFINITION
Amoxicillin Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

IDENTIFICATION
• **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 4 mg/mL of USP Amoxicillin RS in 0.1 N hydrochloric acid [NOTE—Use within 10 min.]
Sample solution: 4 mg/mL, from powdered Tablets in 0.1 N hydrochloric acid. [NOTE—Use within 10 min after preparation.]
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Methanol, chloroform, pyridine, and water (9:8:1:3)
Spray reagent: 3 mg/mL of ninhydrin in alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Dry the plate with the aid of a current of warm air for 10 min. Locate the spots on the plate by spraying lightly with *Spray reagent*, and dry at 110° for 15 min.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*

ASSAY
• **PROCEDURE**
Diluent: 6.8 g/L of monobasic potassium phosphate in water, and adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1
Mobile phase: Acetonitrile and *Diluent* (1:24). Decrease the acetonitrile concentration to increase the retention time of amoxicillin.
Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Diluent.* [NOTE—Use this solution within 6 h.]
Sample solution: Place NLT 5 Tablets in a high-speed glass blender jar containing *Diluent* sufficient to yield a concentration of 1 mg of anhydrous amoxicillin/mL, blend for 4 ± 1 min, allow to stand for 5 min, and centrifuge a portion of the mixture. [NOTE—Where the volume of *Diluent* required would exceed 500 mL, place 5 Tablets in a volumetric flask of such capacity that when finally diluted to volume, a concentration of 1 mg of anhydrous amoxicillin per mL would be obtained. Add a volume of *Diluent* equivalent to three-fourths of the capacity of the volumetric flask, and sonicate for 5 min. Dilute with *Diluent* to volume, add a magnetic stirring bar, and stir for 30 min. Centrifuge a portion of this solution.]
Pass a portion of the clear supernatant through a suitable filter having a 1-µm or finer porosity, and use the filtrate. [NOTE—Use this solution within 6 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 230 nm
Column: 4-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: 1.1–2.8
Column efficiency: NLT 1700 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{19}N_3O_5S$ in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of amoxicillin from the *Sample solution*
r_S = peak response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)
P = stated content of USP Amoxicillin RS (mg/mg)
Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Determine the amount of $C_{16}H_{19}N_3O_5S$ dissolved by employing the following method.
pH 5.0 Buffer: 27.2 g of monobasic potassium phosphate in 3 L of water, adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1, and dilute with water to obtain 4 L of solution
Mobile phase: Acetonitrile and *pH 5.0 Buffer* (1:39), and pass through a filter having a 0.5-µm or finer porosity

Standard solution: 0.05 mg/mL of USP Amoxicillin RS in *pH 5.0 Buffer*. [NOTE—Use this solution within 6 h.]
Sample solution: Pass a portion of the sample through a filter having a 0.5-µm or finer porosity. Quantitatively dilute a volume of the filtrate with water to obtain a concentration of 0.045 mg/mL of amoxicillin.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column
Analytical: 3.9-mm × 30-cm; packing L1
Guard: 2-mm × 2-cm; packing L2
Temperature: Analytical column is maintained at a constant temperature of 40 ± 1°
Flow rate: 0.7 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: 1.1–2.8
Column efficiency: NLT 1700 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{19}N_3O_5S$ dissolved by the formula:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V \times P \times (100/L)$$

r_U = peak response of amoxicillin from the *Sample solution*
r_S = peak response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
V = volume of the dissolution medium, 900 mL
D = dilution factor for the *Sample solution*
P = stated content of USP Amoxicillin RS (mg/mg)
L = label claim (mg/Tablet)

Time: 30 min
Tolerances: NLT 75% (Q) of the labeled amount of $C_{16}H_{19}N_3O_5S$ is dissolved.
For products labeled as chewable tablets: Proceed as directed above.
For chewable tablets labeled to contain 200 mg or 400 mg
Time: 20 min
Tolerances: NLT 70% (Q) of the labeled amount of $C_{16}H_{19}N_3O_5S$ is dissolved.
For chewable tablets labeled to contain 125 mg or 250 mg
Time: 90 min
Tolerances: NLT 70% (Q) of the labeled amount of $C_{16}H_{19}N_3O_5S$ is dissolved.
For veterinary products: Proceed as directed above, except to use *Apparatus 2* at 100 rpm.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label chewable Tablets to indicate that they are to be chewed before swallowing. Tablets intended solely for veterinary use are so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Amoxicillin RS

Amoxicillin Tablets for Oral Suspension

DEFINITION

Amoxicillin Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

IDENTIFICATION

- **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 4 mg/mL of USP Amoxicillin RS in 0.1 N hydrochloric acid
Sample solution: An aqueous dispersion of Amoxicillin Tablets for Oral Suspension in 0.1 N hydrochloric acid containing 4 mg/mL of amoxicillin. Use within 10 min of preparation.
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Methanol, chloroform, pyridine, and water (9:8:1:3)
Spray reagent: 3 mg/mL of ninhydrin in alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Dry the plate with the aid of a current of warm air for 10 min. Locate the spots on the plate by spraying lightly with *Spray reagent*, and dry at 110° for 15 min.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

- **PROCEDURE**
Diluent: 6.8 g/L of monobasic potassium phosphate in water, and adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1
Mobile phase: Acetonitrile and *Diluent* (1:24). Decrease the acetonitrile concentration to increase the retention time of amoxicillin.
Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. [NOTE—Use this solution within 6 h.]
Sample solution: Prepare a dispersion of 20 Tablets for Oral Suspension using a measured volume of water. Quantitatively dilute a portion of the dispersion with *Diluent* to obtain a solution containing 1.2 mg/mL of amoxicillin. Pass a portion of the solution through a filter having a 1-µm or finer porosity, and use the filtrate. [NOTE—Use this solution within 6 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: 1.1–2.8
Column efficiency: NLT 1700 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{19}N_3O_5S$ in each Amoxicillin Tablet for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of amoxicillin from the *Sample solution*
r_S = peak response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

VITAE

Name Mr. Krit Suknuntha

Student ID 4853001

Educational Attainment

Degree	Name of Institution	Year of Graduation
B.Pharm.	Prince of Songkla University	2004

Scholarship Awards during Enrolment

The scholarship support from the Thailand Research Fund through the Royal Golden Jubilee PhD program grant number PHD/0170/2547.

List of Publication and Proceeding

Suknuntha, K., Tantishaiyakul, V., Worakul, N., Taweepreda, W. (2011) Characterization of muco- and bioadhesive properties of chitosan, PVP, and chitosan/PVP blends and release of amoxicillin from alginate beads coated with chitosan/PVP. *Drug Dev. Ind. Pharm.* 37, 408-18.

Suknuntha, K., Tantishaiyakul, V., Vao-Soongnern, V., Espidel, Y., Cosgrove, T. (2008) Molecular modeling simulation and experimental measurements to characterize chitosan and poly(vinyl pyrrolidone) blend interactions. *J. Polym. Sci., Part B: Polym. Phys.* 46, 1258-64.

List of abstracts and proceedings

Oral presentation

Krit Suknuntha and Vimon Tantishaiyakul. Viscosity studies of mucoadhesion of chitosan - poly(vinylpyrrolidone) and chitosan - gelatin blends. The Fourth International Workshop for Far East Asian Young Rheologists (IWFEAYR-4), Suranaree University of Technology, Nakornrachasrima, Thailand, 21st – 23rd January 2009.