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EFFECTS OF MOLECULAR WEIGHT, POLYDISPERSITY AND SOLUTION VISCOSITY OF CELLULOSE ACETATE BUTYRATE ON PROPERTIES AND RELEASE CHARACTERISTICS OF ASCORBYL PALMITATE MICROCAPSULES

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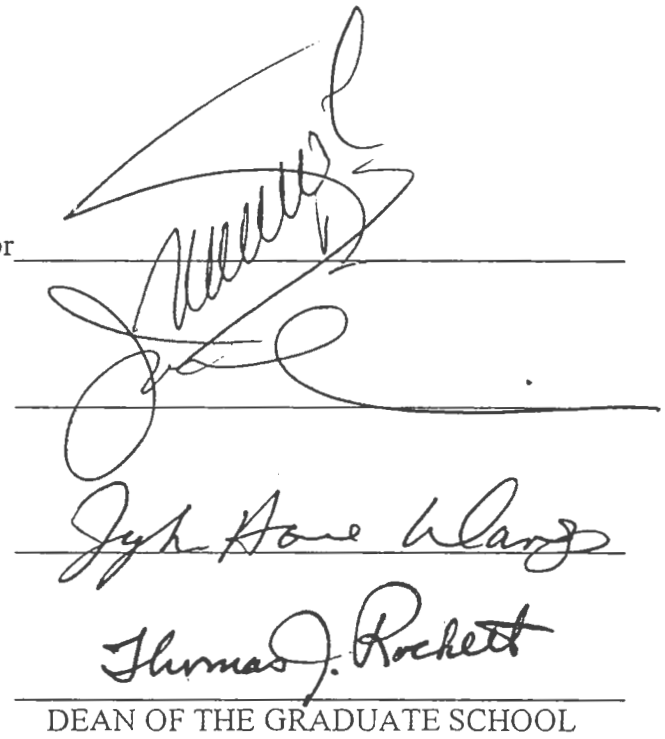

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ABSTRACT

In this study, six different types of cellulose acetate butyrate (CAB) were used to microencapsulate ascorbyl palmitate. Their molecular weight ranged from 53,937 to 264,362. They have varying acetyl, butyryl and hydroxyl contents. By using non-solvent addition microencapsulation method, ascorbyl palmitate microcapsules were prepared with each fraction and their physical chemical properties including yield, mean particle size, size distribution, degree of aggregation, drug loading and release characteristics were evaluated.

The relationship between the formulation variable responses including microcapsule yield, microcapsule size, size distribution, drug loading and degree of aggregation and the polymer molecular weight, viscosity of the polymer solution and polydispersity of the polymer were evaluated by Stepwise Regression Analysis. The effects of molecular weight of cellulose acetate butyrate on the formulation variables but drug loading were significant. The mean particle size and size distribution of microcapsules increased with increasing molecular weight. Solution viscosity and polydispersity of the polymer also affected the mean particle size. The microcapsules made with lower molecular weight polymer caused more aggregation. Drug loading was affected by the solution viscosity and polydispersity. Stepwise Regression Analysis also indicated that viscosity and polydispersity of the polymer were also influenced by polymer molecular weight. Higher molecular weight of the polymer results in the higher viscosity and larger polydispersity.

The burst release was influenced by both the solution viscosity and polydispersity of the cellulose acetate butyrate. Higher viscosity of the polymer solution and smaller degree of polydispersity resulted in the smallest burst effect. The steady state release profiles fitted equally well to both HIGUCHI and Zero Order Equations. However, there is no significant difference between the steady state release rates of the six polymers.

Lower viscosity of the polymer solution resulted in larger amount of release in one and eight hours. Polydispersity also affected the total amount release in one hour. The total release in one hour increased with larger degree of polydispersity.

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PREFACE

Microencapsulation is one of the frequently employed controlled release methods in pharmaceutical and cosmetic technologies. Besides release control it is also used to protect the microencapsulated drug substances from degradation. The release of compounds from these polymer systems is not always predictable, in part due to a lack of understanding on the effects of the properties of the polymer on the final product.

The performance of microcapsules are affected by the properties of polymer, such as molecular weight, viscosity of the polymer solution and polydispersity of the polymer. The effect of polymer molecular weight on the microencapsulation variables was studied and published by Jalil and Nixon[58]. By using poly(L-lactic acid) and phenobarbitone, they demonstrated that the molecular weight differences of poly(L-lactic acid) affected the particle size, degree of aggregation and release rate of the microencapsulated phenobarbitone. The larger molecular weight fraction increased the mean particle size, decreased release rate and aggregation. However, in their study, the possible effects of the corresponding solution viscosity and polydispersity of the polymer were not identified.

In this study, by using different molecular weight fractions of cellulose acetate butyrate with varying viscosity and polydispersity, the effects of molecular weight, solution viscosity and polydispersity of the polymer on the surface characteristics, yield, mean

particle size, particle size distribution, drug loading and degree of aggregation of ascorbyl palmitate microcapsules were determined in addition to release characteristics of ascorbyl palmitate.

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I. INTRODUCTION

1.1 PURPOSES OF MICROENCAPSULATION

Microencapsulation is one of the frequently employed controlled release methods in pharmaceutical and cosmetic technologies. It can be conceived as a technique of trapping active materials in rather stable and protective coatings. The size of microcapsules initially ranges from 5μm to hundred microns and has been getting smaller as the new techniques and new products have been developed. The diameter of nanoparticles are much smaller and of 10 to 1000 nm. Microparticles of different dimensions can be applied to various applications. The micropartcles with the size less than 5um can be used for intravenous therapy and prolong the release of a drug. Numerous studies have been carried out to encapsulate various materials for the purposes of targeted delivery, reducing irritating capacity of drugs[1], controlled release of drugs, improving drug stability and availability[2], isolating drug from tissues, masking unpleasant taste or aiding in storage or handling[3,4]. Some examples are given in Table I. Besides solid microcapsules, non-solid form microcapsules are also available such as liposomes, which are very popular in both pharmaceutical and cosmetic fields.

Microcapsule morphology depends on the process parameters and manufacturing methods[3,37]. Those morphology can be basically categorized to six different types as shown in Figure 1.

Table I : Examples of Microencapsulated Drugs

Purpose of Microencapsulation	Coating Agent	Drug/Active Substance	Reference
Improved Stability	Liposomes	Dexamethasone Sodium Phosphate	2
Reduced Volatility	Acacia	Aromatic Oils	5
Irritation/toxicity reduction	Cellulose Acetate butyrate	Carbamazepine	6
	Cellulose Acetate Propionate	Theophylline	7
	Chitin	6-Mercaptopurine	8,9
	Chitosan	5-Fluorouracil	10
	Ethylcellulose	Theophylline	11
	Eudragit	Indomethacin	12
	Poly(lactic/glycolic) acid	Bupivacaine	13
	"	Lidocaine	13

Table I (continuation)

Purpose of Microencapsulation	Coating Agent	Drug/Active Substance	Reference
Sustained release	Poly(lactic/glycolic) acid	Etidicaine	13
	Cellulose Acetate	Verapamil Hydrochloride	14
	Cellulose Acetate Propionate	Verapamil Hydrochloride	14
	Cellulose Acetate Butyrate	Diltiazem Pectate	15
	"	Ibuprofen	16
	Cellulose Acetate Trimellitate	2,4-dinitrophenyl-hydrazine	17
	"	Tartrazine	18,19
	Ethylene/Vinylacetate Copolymer	Dimethyldidecylamonium Chloride	20
	Cellulose Acetate Butyrate/Polystyrene	Ketoprofen	21
	Ethycellulose	Allopurinol	22
	Eudragit	Acetylsalicylic acid	23

Table I (continuation)

Purpose of Microencapsulation	Coating Agent	Drug/Active Substance	Reference
Sustained Release	Polylactic acid	Quinidine	24
	Poly(lactic/glycolic) acid	AZT	25
	"	Proteins	26
Improved bioavailablity	Alginate	Trypsin	27
	Ethylcellulose	5-Fluorouracil	28
	poly(carboxyphenoxyvaleric acid) and poly(carboxyphenoxyhexane)	p-Nitrophenol, Lysozyme	29
Improved Stability	Polylactic acid	Thymopentin	30
	Poly(lactic/glycolic) acid	Bovine serum albumin	31
	"	Rismorelin porcine	32
	"	Cholecystokinin Derivative Peptide	33

Table I (continuation)

Purpose of Microencapsulation	Coating Agent	Drug/Active Substance	Reference
Taste masking	Cellulose Acetate Propionate	Theophylline	7
	Eudragit	Chloroquine Diphosphate	34
Targeted delivery	Poly(lactic/glycolic) acid	Ciprofloxacin	35
Easy handling	Gelatin/Gum Arabic	Eprazinone	36

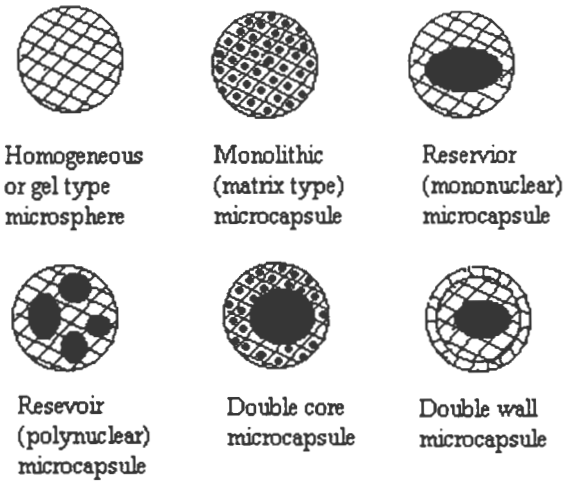


Figure 1 : Schematic Presentation of Different Types of Microspheres and Microcapsules. (From Ref. 3)

1.2 POLYMERS USED FOR MICROENCAPSULATION

Various polymers have been used for microencapsulation. Some of them are listed on Table II. These can be used alone or combined with other polymers to form the coating. Some commonly used polymers include alginates[27,40], cellulose acetate[14], cellulose acetate butyrate[6,14-16,41-44], cellulose acetate phthalate[38,45], cellulose acetate propionate[7,14], cellulose acetate trimellitate[17-19], chitin[8,9], chitosan[10], ethyl cellulose[11,22,28,46-52], Eudragits[12,23,34,53], polylactic acid[13,24,30,31,54-59], polymethyl methacrylate[47], polystyrene[47], polyvinyl chloride[47], gelatin-acacia[60], ethylene/vinylacetate copolymer[20], combination of cellulose acetate butyrate and polystyrene[21], poly(carboxyphenoxyvaleric acid) and poly(carboxyphenoxyhexane)[29], poly(lactic/glycolic acid) copolymer[13,25,26,30-33,35,61]. The list of the polymers and the microencapsulation methods are presented in Table II.

1.3 METHODS WIDELY USED FOR POLYMER BASED MICROENCAPSULATION

1.3.1 Coacervation Technique

"Coacervation" was first introduced by the Dutch scientists Bungenberg de Jong and Kruyt in 1929[62]. The term is meant to describe macromolecular phase separation process caused by partial desolvation of fully solvated macromolecules. For a successful microencapsulation carried out by the coacervation method, the core material and the polymer must be insoluble or poorly soluble in the coacervation medium. There

Table II : Polymers Used for Microencapsulation

Polymer	Degradibility in the Body	Drug Encapsulated	Microencapsulation Method	Reference
Acacia	yes	Aromatic Oils	Spray Drying	5
Alginate	yes	Trypsin	Coacervation Method	27
Cellulose Acetate	no	Verapamil Hydrochloride	Solvent Evaporation Method	14
Cellulose Acetate Butyrate	no	Carbamazepine	Solvent Evaporation Method	6
"	"	Verapamil Hydrochloride	Solvent Evaporation Method	14
"	"	Diltiazem Pectate	Solvent Evaporation Method	15
"	"	Ibuprofen	Solvent Evaporation Method	16
"	"	Propranolol HCl	Solvent Evaporation Method	41
"	"	Paracetamol	a)Solvent Evaporation Method	42
"	"	"	b)Emulsion Non-Solvent Addition Method	42
"	"	Propranolol	Emulsion Non-Solvent Addition Method	43
"	"	Phenylpropanolamine HCl	Solvent Evaporation Method	44

Table II (continuation)

Polymer	Degradibility in the Body	Drug Encapsulated	Microencapsulation Method	Reference
Cellulose Acetate Phthalate	no	Potassium Chloride, Sucrose, Caffeine, Tetracycline HCl, Barium Sulfate, Cornstarch, Sulfasalazine, Hydrochlorothiazide	Emulsion Non-Solvent Addition Method	45
Cellulose Acetate Propionate	no	Theophylline	Solvent Evaporation Method	7
"	"	Verapamil Hydrochloride	Solvent Evaporation Method	14
Cellulose Acetate Trimellitate	no	2,4-dinitrophenyl-hydrazine	Emulsion Non-Solvent Addition Method	17
"	"	Tartrazine	Emulsion Non-Solvent Addition Method	18, 19
Cellulose Acetate Butyrate/Polystyrene	no	Ketoprofen	Complex Emulsion Method	21
Chitin	yes	6-Mercaptopurine	Non-Solvent Addition Method	8, 9

Table II (continuation)

Polymer	Degradibility in the Body	Drug Encapsulated	Microencapsulation Method	Reference
Chitosan	yes	5-Fluorouracil	Emulsion Non-Solvent Addition Method	10
Ethylcellulose	no	Theophylline	Non-Solvent Addition Coacervation Method	11
"	"	Ibuprofen	Solvent Evaporation Method	16
"	"	Allopurinol	Solvent Evaporation Method	22
"	"	5-Fluorouracil	Solvent Evaporation Method	28
"	"	Sodium Diclofenac	Solvent Evaporation Method	46
"	"	Caffeine, Salicylic acid	Fluidized Bed Coating	48
"	"	Acetylsalicylic acid	Non-Solvent Addition Coacervation Method	49
"	"	"	Emulsion Non-Solvent Addition Method	51
"	"	Phenylpropanolamine Hydrochloride	Emulsion Non-Solvent Addition Method	52

Table II (continuation)

Polymer	Degradibility in the Body	Drug Encapsulated	Microencapsulation Method	Reference
Eudragit	yes	Indomethacin	Solvent Evaporation Method	12
"	"	Acetylsalicylic	Non-Solvent Addition Coacervation Method	23
"	"	Chloroquine Diphosphate	Non-Solvent Addition Coacervation Method	34
"	"	Salbutamol	Air Suspension Coating	53
Gelatin/Acacia	yes	Indomethacin	Non-Solvent Addition Coacervation Method	60
poly(carboxyphenoxyvaleric acid) and poly(carboxyphenoxyhexane)	yes	p-Nitrophenol, Lysozyme	Solvent Evaporation Method Emulsion Non-Solvent Addition Method	29
Polystyrene	no	Gelatin	Solvent Evaporation Method	47
Polyvinyl Chloride	no	Gelatin	Solvent Evaporation Method	47
Polymethyl Methacrylate	no	Gelatin	Solvent Evaporation Method	47

Table II (continuation)

Polymer	Degradibility in the Body	Drug Encapsulated	Microencapsulation Method	Reference
Polylactic acid	yes	Quinidine	Solvent Evaporation Method	24
"	"	Thymopentin	Solvent Evaporation Method	30
"	"	Indomethacin, Piroxicam	Centrifugation Method	54
"	"	Phenobarbitone	Solvent Evaporation Method	55-58
Poly(lactic/glycolic) acid	yes	Bupivacaine, Etidicaine, Lidocaine, Mepivacaine	Solvent Evaporation Method	13
"	"	AZT	Solvent Evaporation Method	25
"	"	Proteins	Solvent Evaporation Method	26
"	"	Bovine serum albumin	Solvent Evaporation Method	31
"	"	Rismorelin porcine	Solvent Evaporation Method	32
"	"	Cholecystokinin Derivative Peptide	Solvent Evaporation Method	33

Table II (continuation)

Polymer	Degradibility in the Body	Drug Encapsulated	Microencapsulation Method	Reference
Poly(lactic/glycolic) acid	yes	Ciprofloxacin	Solvent Evaporation Method Evaporation-Extraction Method	35
"	"	Protein	Solvent Evaporation Method	61
Ethylene/Vinylacetate Copolymer	no	Dimethyldidecylamonium Chloride	Solvent Evaporation Method	20

are two types : simple and complex coacervation. Simple coacervation involves using only one polymer solution, then remove the solvent by adding a non-solvent which have a greater affinity for the solvent[11,49, 63-68]. Then the desolvated polymers will precipitate out with surrounding molecules to form the microcapsules.

Wu et al.[11] used ethylcellulose to microencapsulate theophylline by simple coacervation, non-solvent addition, method. Ethylcellulose was dissolved in a solvent (dichloromethane, acetone and ethylacetate), then theophylline was dispersed the polymer solution with stirring. Coacervation was induced by adding a non-solvent (cyclohexane and n-hexane) dropwise to the suspension. Microcapsules were then collected by filtration.

Besides non-solvent addition, heating can also be used to induce phase separation by reducing the solubility of the polymer solution. Paradissis and Parrott[65] used gelatin to microencapsulate several drugs. The drug was dispersed in mineral oil in the aqueous gelatin solution. The phase separation was then achieved by adding isopropanol and reducing the temperature.

Complex coacervation is similar to simple coacervation except that complex coacervation mainly involves the neutralization of the charges on the colloid[49, 68-71]. The charge neutralization is caused by adding two opposite charged polymers together; which results in the reduction of net charge and the loss of solvation of the polymers. This causes the precipitation of the polymers forming a complex coacervate of the

colloids and further forming microcapsules. The negatively charged acacia is the most widely used polymer for positively charged gelatin for complex coacervation[69-72]. Temperature adjustment can also be used to coacervate the polymer solution if the solubility of the polymer changes with the variation in temperature. Jizomoto[72] prepared gelatin-acacia microcapsules containing paraffin oil. The paraffin oil was stirred into the gelatin-acacia solution at 50 - 60°C. Polyethyleneglycol or polyethyleneoxide was added as a desolvating agents, then the mixture was cooled down to form the microcapsules.

1.3.2 Solvent Evaporation Method

Solvent evaporation method is a very popular microencapsulation technique used in many studies[6,7,14,16,22,41,42,44,46,47,55,56,58,68,73]. The origin of the solvent evaporation microencapsulation method can be traced to the late 1960s. An early work has done by Vranken and Claeys[74] to microencapsulate dyes with polystyrene by using solvent evaporation method.

Two basic steps to produce microcapsules by solvent evaporation method are the emulsion formation and solvent removal. The polymer used is first dissolved in a solvent and then the core material is dispersed or dissolved in the polymer solution. The mixture acts as the dispersed phase and is further emulsified into an immiscible liquid which may contain an stabilizer and form an emulsion. The evaporation of the solvent is then achieved with continuous stirring. Microcapsules thus form.

As the solvent evaporates from the emulsion, the viscosity of the dispersed phase keeps increasing that will destabilize the whole system. The highly viscous droplets tend to aggregate during the solvent removal. Therefore a droplet stabilizer usually is needed to form a thin layer around the droplets which results in reducing the extent of collision and coalescence between the droplets. The effect of the stabilizer will be discussed in 1.4.1.2.1.

1.3.3 Pan Coating

The process of pan coating was first described by Blythe[75]. Pan coating for microencapsulation is a highly skilled operation and rather time-consuming. A typical method of coating a drug by pan coating involves roughening the pan first before use by coating with an adhesive solution composed of 10% polyvinylpyrrolidone in isopropanol. Sprinkle with talc when the pan becomes tacky and then pat with a damp cloth to produce a stippled surface upon drying. The core drug should be screened first to remove dust in order to form more regular microcapsules. Then the coating solution is sprayed over the drug which is already in the pan. Coating is continued until the needed wall thickness is obtained. Talc is added intermittently to reduce particle aggregation[4]. Brophy and Deasy[76] used ethylcellulose and hydroxypropylmethylcellulose to pan-coat methylene blue-containing cores. Harris[77] coated potassium chloride with cellulose acetate phthalate by the same method.

1.3.4 Air Suspension Coating

Air suspension coating is a good alternative to pan coating becuase it can provide better and more uniform coating on small core materials irrespective of the size and shape of the core. The process is easier to operate and faster than the pan coating. Air suspension coating was first developed by Wurster, therefore the process is also called Wurster coating[78, 79].

The typical Wurster coating equipment is shown in Figure 2. The solid cores are placed in the coating chamber and fluidized by a high velocity airstream. As a result, the core drugs pass through the coating spray which is from the atomizer nozzle, and the air can rapidly dries the particles as it rises into the coating partition. Then the cores goes up to the expansion chamber where is no airstream to support the particles, therefore the particles will drop down through the annular space where they can be further dried before reentering the coating partition and being further coated again. By this method, the thickness of the wall can be controlled.

1.3.5 Spray Drying

Spray drying is a technique which is used mainly for a wide range of drugs and flavors[35,80-82]. The wall of the microcapsules produced by spray drying tends to be more porous which is suitable to taste masking and other purpose but not controlled release. The instrument and the running cost and are high comparing to other

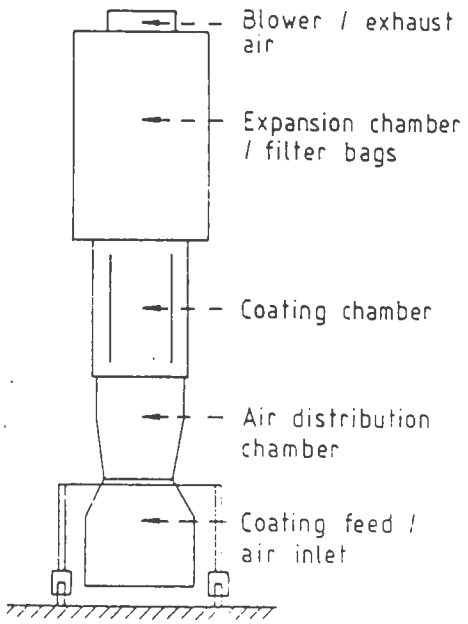


Figure 2(a) : The Diagram of the Wurster Coating Apparatus.

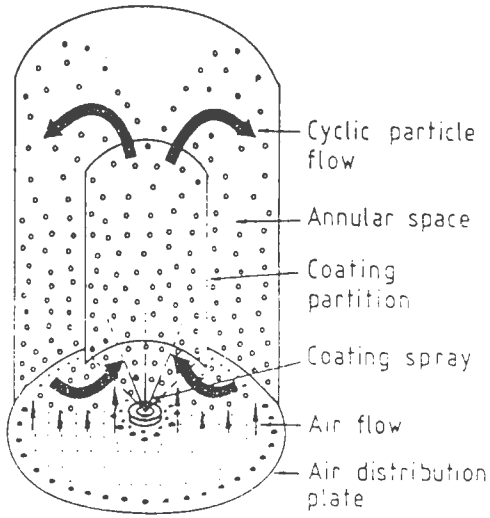


Figure 2(b) : The detailed coating chamber of Wurster Coating Apparatus.

microencapsulation techniques. A diagram of a typical spray dryer is shown in Figure 3.

Spray drying technique involves dispersing the core drug into the polymer-containing solution. The mixture is then atomized and the solvent is spray dried in a spray dryer which results in the encapsulation of the drug. Granatek et al.[80] dispersed dicloxacillin sodium particles in a methylene chloride solution containing ethylcellulose and spermaceti. The mixture was then spray dried to form microcapsules. Many aromatic oils used as flavors were emulsified in a acacia solution and then spray dried to form free-flowing powers with reduced volatility[5].

1.3.6 Emulsion Non-Solvent Addition Method

Emulsion non-solvent addition method is or; similar to coacervation technique which was used in this study. Emulsion non-solvent addition microencapsulation method was first developed by Liard et al.[52] in 1984. They used ethylcellulose as the wall material and aimed to perform the coacervation process by using innocuous or easily removed solvents. Three steps were needed to achieve the emulsion non-solvent addition microencapsulation. First, the organic phase was formed by dissolving polymer in the solvent used in which the core material was either dissolved or suspended. Second, emulsification took place by adding a non-miscible oil phase with stirring. The last step was the addition of a non-solvent which can harden the wall material and thus the microcapsules form. The non-solvent used in emulsion non-solvent addition

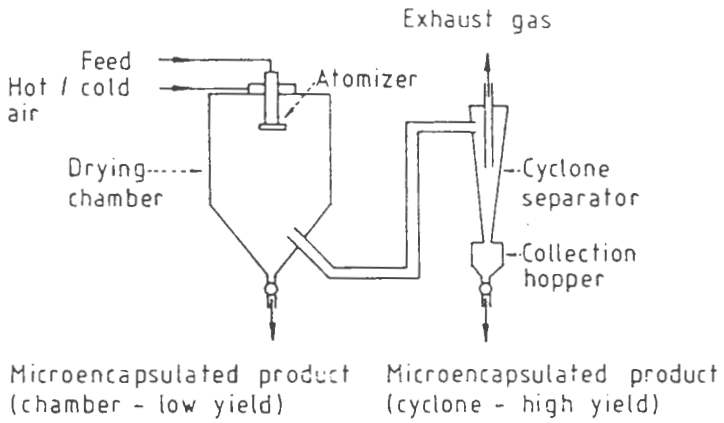


Figure 3 : Schematic Diagram of a Spray Dryer

microencapsulation method should be miscible with the solvent, in which both the polymer and drug remain insoluble. The purpose of the addition of a non-solvent is to compete with the polymer for the solvent affinity. Due to the weak affinity between the polymer and the solvent, polymer would precipitate out and encapsulate the drug. The difference in the solubility parameters of the solvent and the non-solvent would affect the microcapsules formation rate and release rate[11]. The microcapsules formation rate and release rate are faster with smaller solubility parameter difference. The flow chart of the procedure is shown in Figure 4.

In the method used by Liard et al.[52], ethylcellulose was selected as the wall material, acetone as the solvent, hexane as the non-solvent and the mineral oil and petroleum ether as the oil phase.

By using emulsion non-solvent addition technique, the optimal formation of coacervate droplets and reproducible properties can be achieved. Besides, this method has the advantage of carrying out the process at the room temperature. Following Liard et al's study, several studies using cellulose acetate butyrate[42,43], cellulose acetate phthalate[45], cellulose acetate trimellitate[17-19], chitosan[10], ethylcellulose[51,52], and Eudragit[23,34] to perform microencapsulation with the emulsion non-solvent addition method. Both oil-in-water and water-in-oil emulsions can be used in this method. The emulsion non-solvent addition method provides a wide variety for microencapsulation, no matter whether the core material is hydrophilic or hydrophobic, liquid or solid, soluble or insoluble in the polymer solvent.

Flow Chart of Emulsion Non-Solvent Addition Method

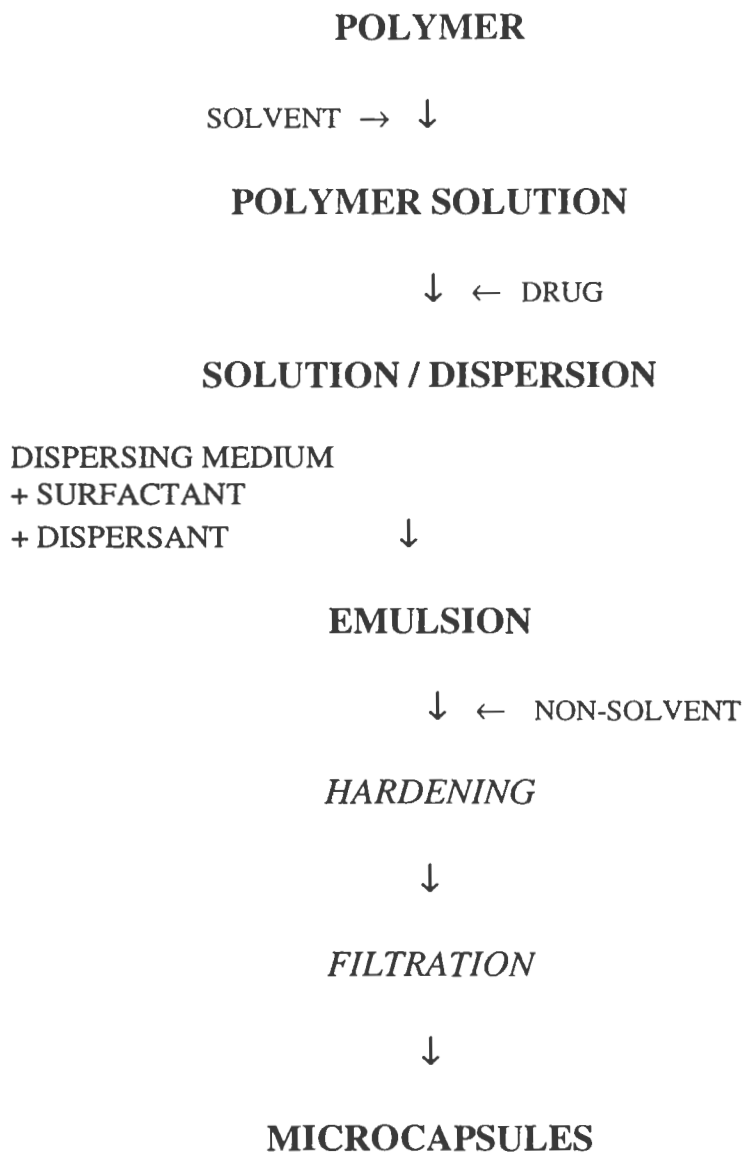


Figure 4 : Flow Chart of Emulsion Non-Solvent Addition Method

In Sprockel and Prapaitrakul's method[42], a model drug, paracetamol, was dispersed in acetone which contained 6% cellulose acetate butyrate as the wall material. The drug dispersion was then emulsified in mineral oil containing 1% Span 80. Hexane was used as a non-solvent and added to the emulsion. The microcapsules thus formed were collected, washed and dried after complete addition of hexane.

In that paper, the objective was to optimize cellulose acetate butyrate encapsulation of paracetamol by choosing the best microencapsulation method. Emulsion non-solvent addition method appeared to be the most practicable method while using cellulose acetate butyrate as the coating polymer.

Effects of drug to polymer ratio and particle size on dissolution characteristics were studied by Chiao and Price[41] by using cellulose acetate butyrate. It was found that smaller particles and slower release rate could be obtained at smaller ratio of drug to polymer (1/2). The same result was also observed by Bhardwaj et al.[14]. Cellulose acetate trimellitate was used to investigate the effects of polymer to solvent concentration and internal phase volume fraction on the particle size distribution. It was found that the lower the polymer to solvent concentration and internal phase volume fraction, the smaller the particles became[9]. Smaller microcapsules could be also obtained by increasing stirring rate during the production[24].

1.4 VARIABLES THAT AFFECT PROPERTIES OF POLYMER BASED MICROCAPSULES

1.4.1 Variables That Affect Particle Size and Particle Size Distribution

The particles size and distribution are associated with the relative rate of separation and coalescence of the dispersed phase droplets. If the emulsion particles are formed faster than they coalesce, then smaller microcapsules can be obtained. Those influencing factors related to microcapsule size include interfacial tension between the dispersed and continuous phases, viscosity of the two phases, volume fraction of the dispersed phase and agitation conditions[20,25,32,37,47,49].

1.4.1.1 effects of process variables

1.4.1.1.1 agitation type and speed

Higher stirring speed provides stronger turbulence force to form the dispersed droplets and increases the separation rate of the droplets. The relationship between the particle size and stirring speed has been widely demonstrated[20,32]. Smaller particles and narrower particle size distribution can be obtained by increasing the stirring speed. Legrand et al.[20] investigated the effect of rotational speed on particle size. Higher stirring velocity, 500rpm, produced the microcapsules with the mean size of 150um, which was smaller than the mean size of microcapsules produced with 250 and 300rpm, whose mean particle size were 350um and 300um. In the same study, the yield and drug loading were also found affected by the stirring speed. Yield was found a parabolic

function of the stirring speed with the highest value obtained at the stirring speed about 300-320rpm. The optimum drug loading efficiency was found as 60% at the stirring speed between 260 and 400 rpm. Drug loading decreased significantly with the stirring speed faster than 400 rpm.

The homogeneity of the turbulence produced also has an effect on particle size distribution. The more uniform turbulence can be achieved by using an impeller with diameter as large as possible to fit in the processing vessel[37]. The variation of sheer force throughout the vessel is then minimized, and the smaller particle size and narrower distribution can be expected.

1.4.1.2 effects of formulation variables

1.4.1.2.1 interfacial tension

In an emulsion, surfactants are used to reduce the interfacial tension of the two phases. The nature of the surfactant and its concentration play an important role in reducing the interfacial tension and stabilizing the dispersed droplets against coalescence. Several works have been done to study the effect of surfactant type and surfactant concentration on the size of microcapsules[16,18,22,32,45,51]. Chen et al.[51] used Tween 20, Tween 60 and Tween 85 to study the effect of surfactant type on the ethylcellulose microcapsules of theophylline. They found that the mean particle size, yield and drug content increased with the decrease in the HLB value of the surfactants used. Different surfactants might lead to the variation in particle size. Beyger and Nairn[45] used Span 80 to produce cellulose acetate phthalate microcapsules and found that the particle size decreased as

the surfactant concentration increased. However, when the concentration of Span 80 exceeded 1%, the mean particle size started increasing and degree of aggregation also increased. Generally, particle sizes were found to be inversely affected by surfactant concentration because the surface energy was reduced more with higher surfactant concentrations which resulted in the easier creation of the new surfaces[16,18,22,32,45,51]. However, the studies concluded that the influence of the surfactant on the particle size could be minimized by the high energy input by stirring into the system which provided the work needed to create new surfaces.

1.4.1.2.2 viscosity and volume fraction of the dispersed phase

A viscous dispersed liquid retards the emulsification rate and forms a relatively coarse emulsion with larger droplet size. A high dispersed phase volume fraction increases the probability of the collision between the droplets since the density of dispersed droplets in the fixed vessel is higher. As a result, the coalescence rate increases and the size of microcapsules increases. This may also result in a wider particle size distribution.

Both the molecular weight[22,56,58] and concentration[12,19,83,84] of the polymer affect the viscosity of the polymer solution. Either increasing polymer concentration or using a polymer with higher molecular weight will result in larger microcapsules. Sanghvi and Nairn[19] had investigated the effects of the concentration of cellulose acetate trimellitate and internal phase volume fractions on the particle size distribution of tartrazine microcapsules, they used solvent evaporation method to control the microcapsule particle size and regulated the ratio of the polymer to solvent concentration and the

volume fraction. Three polymer concentrations were used, 8, 10 and 12%. It was found that the lower the polymer to solvent concentration and internal phase volume fraction, the particles became smaller. In an earlier study, the same authors used cellulose acetate trimellitate to study the effects of the viscosity of both the dispersed and external phase on microcapsule size in an emulsion type of microencapsulation system[17]. Particle size of microcapsules was found to increase in more viscous dispersed phase which were obtained by higher concentration of the polymer dissolved. The opposite effect was observed for the viscosity of the external phase; an increase in the particle size was found when the external phase was changed from light mineral oil to heavy mineral oil.

1.4.1.2.3 drug to polymer ratio

Several studies that appeared in the literature studied the effects of the drug to polymer ratio on the microcapsules size[12,34,41,56,58,85]. Smaller ratio of drug to polymer was found to produce smaller microcapsules and faster release rate. For example, Bhardwaj et al.[14] used cellulose esters to evaluate the effect of drug loading on the particle size and size distribution. The mean particle diameter obviously increased when the drug loading increased from 33.3% to 50%, but the particle size distribution did not vary much. Chiao and Price[41] observed the same results when drug to polymer ratio of Propranolol HCl microcapsules increased from 1/2 to 1/1. The smallest mean particle size was obtained with the lowest drug to polymer ratio(1/2). The phenomenon can be attributed to the relative increase in the total solid content of the internal phase which results in an increase in viscosity of the dispersed phase which results in the increased particle size.

1.4.2 Variables that Affect Release Rate

In diffusion-based release, release of the core drug through a polymer coating involves three possible steps; the first is the penetration of the dissolution medium into the core which is followed by the dissolution of the drug and the removal of the solute to the bulk solution via diffusion. The rate-determining step in this type of release would be the diffusion rate provided that the core drug is soluble in the dissolution medium. However, if the core drug is poorly soluble in the medium, the dissolution of the drug becomes important as the rate-determining step. Therefore, the release rates of the microcapsulated drugs from the polymer walls is chiefly affected by the variation of the permeability of the polymer, the solubility of the encapsulated drug, the molecular weight of the polymer, the ratio of drug to polymer wall and the size of microcapsules.

1.4.2.1 permeability of the polymer

The high pore volume or the surface defects of the microcapsules and the nature of the coating polymer would influence the release rate by facilitating the dissolution medium through the coating and increasing the release from the polymer, since the polymer is more permeable to the dissolution medium. Several studies had been done to determine the two ways of release through the porous polymer[86-88]. Release of the drug through the diffusion process and through the pores formed in the membrane are both important factors for the release of the drug. The porosity of the microcapsules affects the release even more when the encapsulated drug is poorly soluble in the dissolution medium.

1.4.2.2 molecular weight of polymer and drug loading

Release from microcapsules were also influenced by polymer molecular weight and drug loading. Polymers with higher molecular weight tend to lower the release of drugs more than lower molecular weight polymers[7,22,54,57]. Lower drug loading microcapsules had slower release because the ratio of the amount of drug to polymer determines the thickness of the microcapsule wall or polymer path. Thicker walls that the drug molecules should overcome while diffusing to the dissolution medium as matrix which is formed with lower drug to polymer ratios, provides a longer path to penetrate through, therefore slower release rates are obtained[7, 14, 57].

Jalil and Nixon[57] used poly(DL-lactic acid) and phenobarbitone to demonstrate that the molecular weight differences of poly(DL-lactic acid) affected the release rate of the microencapsulated phenobarbitone. Poly(DL-lactic acid) of three different molecular weight were used (5200, 13300 and 20500). The release rate from the microcapsules prepared with the poly(DL-lactic acid) with the highest molecular weight provided the slowest release rate. In the same study, drug to polymer ratio was also demonstrated having an effect on release rate. The quantity of the drug released from the microcapsules decreased when the drug to polymer ratio decreased.

Another study carried out by Shukla and Price[7] using cellulose acetate propionate with three different molecular weights to investigate the effect of polymer molecular weight and different drug loading (40%, 50% and 60%) on the release characteristics. They

demonstrated that the higher molecular weight polymer and higher drug loading greatly prolong the release half-lives.

1.4.2.3 particle size of microcapsules

Particle size of microcapsules plays an important role in the release rates. Release rate increases as the surface area of the microcapsules increases. Therefore with the same concentration of encapsulated drug used in the dissolution medium, decreased particle size microcapsules provide faster release rates.[7,41,44] Arabi et al.[22] studied the effect of the polymer molecular weight and microcapsules size on the release of allopurinol by using ethyl cellulose as the wall material. Smaller size (100um) microcapsules showed slower release compared to the microcapsules with the size of 250um.

1.4.2.4 solvent and non-solvent pairs

In the non-solvent addition microencapsulation method, the choice of solvent and non-solvent could affect the release of the core material. The miscibility of the solvent and non-solvent is higher with smaller solubility parameter differences. The larger differences of the solubility parameters therefore results in slower formation of the microcapsule wall that allow the wall to be more complete and denser, which will slow down the release rate of the drug as it has been observed with ethylcellulose[11,85] and chitin[9] microcapsules in the release of theophylline and 6-mercaptopurine respectively.

II. OBJECTIVES

Cellulose acetate butyrates are hydrophobic cellulose derivatives. They are available in the molecular weight ranges changing from 53,000 to 265,000 with varying acetyl, butyryl and hydroxyl contents. The difference in the molecular weights provides an advantage to study the effects of molecular weight and the substitution groups contents on the general properties of microencapsulated materials.

The objective of this study was to determine the effects of molecular weight, acetyl, butyryl and hydroxyl contents on surface characteristics, yield, mean particle size, particle size distribution, drug loading, degree of aggregation and release characteristics of ascorbyl palmitate microcapsules. The microcapsules prepared with various cellulose acetate butyrates by using three different molecular weight fractions of cellulose acetate butyrate with varying substitution group contents.

III. EXPERIMENTAL

3.1 MATERIALS AND INSTRUMENTS USED

All the chemicals used in this studies are listed in Table III. Ascorbyl palmitate, which is the model drug encapsulated, is described in 3.1.1 and cellulose acetate butyrate in 3.1.2.

3.1.1 Ascorbyl Palmitate

Ascorbyl palmitate is a white crystalline powder. The structure of ascorbyl palmitate is given in Figure 5. Its molecular weight is 414.54 g/mole, and the melting range is between 107-117°C. The bulk density of ascorbyl palmitate is 8.7 lb/ft^3. The specific rotation ($[\alpha]^{20}$) is between +21° and +24°. Ascorbyl Palmitate is soluble in ethanol, benzene, dioxane, ethyleneglycol, 2-ethoxyethanol, propyleneglycol and ethylacetate at 25°C, but very slightly soluble in water[93].

Ascorbic acid is one of the most effective water-soluble antioxidants. For that reason, it quickly oxidizes in water[94]. Ascorbyl palmitate is the palmitic acid ester of ascorbic acid which is a more stable antioxidant than ascorbic acid in the aqueous medium. In cosmetics, ascorbyl palmitate is mainly used as an antioxidant and a skin-lightening agent which inhibits tyrosinase activity during melanin synthesis.

The amount and distribution of melanin produced by melanocytes primarily determines the skin color. Tyrosine is the starting material for melanin formation. Tyrosine

Table III : List of Chemicals

CHEMICALS	LOT NO.	MANUFACTURES
Cellulose Acetate Butyrate 553-0.4	AD-5469B	Eastman Chemical Company Kingsport, TN 37662-5280
Cellulose Acetate Butyrate 381-0.1	BG-7159B	--
Cellulose Acetate Butyrate 531-1	AV-9821B	--
Cellulose Acetate Butyrate 381-2	KR-9587B	--
Cellulose Acetate Butyrate 171-15S	B-23174	--
Cellulose Acetate Butyrate 381-20	C-1675E	--
Butylated Hydroxytoluene (BHT)	60101	--
Ascorbyl Palmitate	5060649	Roche Vitamins & Fine Chemicals Nutley, NJ 07110
Acetone	963777	Fisher Chemical Fair Lawn, NJ 07410
Hexane	965941	--
Magnesium Stearate	EU4149	Witco Corporation Houston, Texas 77053
Mineral Oil	79804	--
Span 60	24588L	ICI Surfactants Wilmington, DE 19850
Span 80	34315	--
Sodium Thiosulfate		Aldrich Chemical Co., Inc. Milwaukee, WI 53233
Polyvinylpyrrolidone	04-0398	BASF Corporation Parsippany, NJ 07054

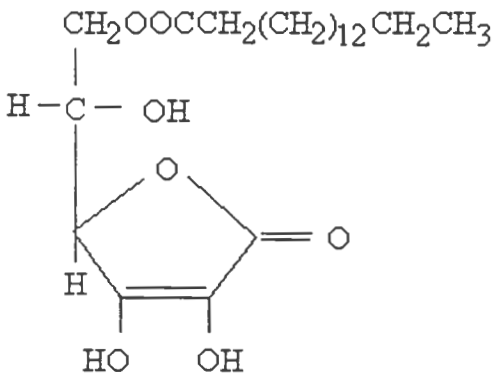


Figure 5 : Chemical Structure of Ascorbyl Palmitate

undergoes an oxidative reaction to form dopaquinone which spontaneously converts to melanin. The conversion process of tyrosine to dopaquinone needs a copper-containing enzyme, tyrosinase, which plays an important role in melanin synthesis[95-98]. This reaction is shown in Figure 6. When tyrosinase activity is inhibited, melanin cannot form. Most cosmetic products claiming skin-lightening effects contain tyrosinase inhibitors, such as hydroquinone, arbutin, placental extract, kojic acid and ascorbic acid derivatives[99,100].

Compared to other tyrosinase inhibitors, ascorbic acid and its derivatives appear to be effective, economical and safe to use. However, the inhibitory effect of ascorbic acid cannot be maximized due to its highly unstable nature. It quickly decomposes to hydroxy ascorbic acid in an aqueous medium, which does not have the same effect as ascorbic acid.

Among several ascorbic acid derivatives, palmitate ester is one of the most widely used tyrosinase inhibitor because, compared to other derivatives of ascorbic acid, it is relatively stable, hydrophobic and acts as a pro-drug. It was found that its skin penetration ability was much higher than ascorbic acid[94]. Therefore it was selected as the model drug for this thesis study.

3.1.2 Cellulose Acetate Butyrate

Cellulose acetate butyrates are hydrophobic cellulose derivatives. Their specific gravity varies between 1.16 to 1.26. Depending on the substitution groups, the viscosity,

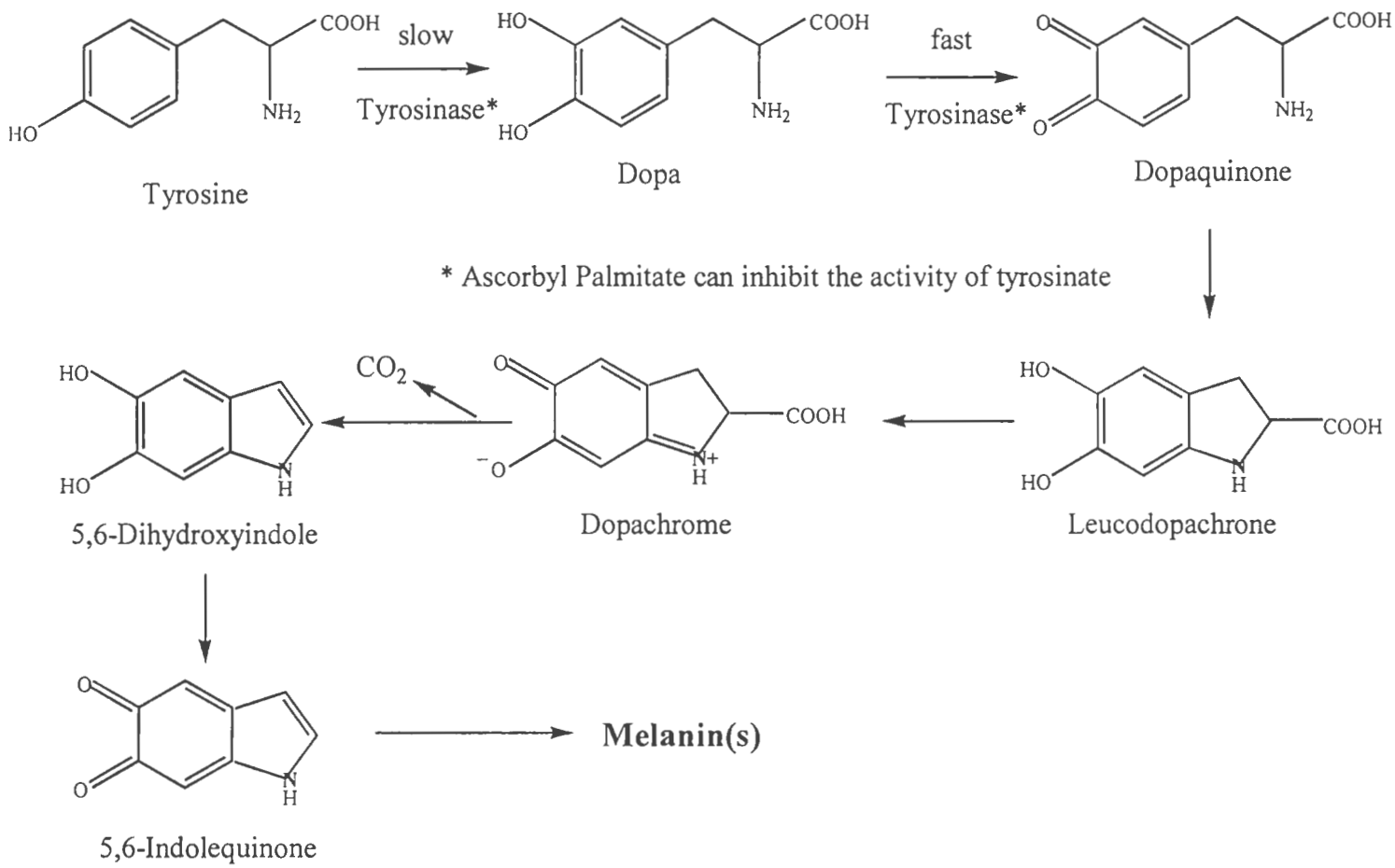


Figure 6 : Chemical Reaction of Melanin Synthesis

glass transition temperature and the melting points of similar molecular weight fractions also vary. Cellulose acetate butyrate dissolves in acetone, ethyl acetate and methylene chloride. It is not soluble in water. The structure of cellulose acetate butyrate is shown in Figure 7. Depending on the acetyl, butyryl and hydroxyl groups content as shown in Table IV, the physical and chemical properties of the cellulose acetate butyrate may vary.

As seen in Table IV, the viscosity of cellulose acetate butyrate in acetone increases as the molecular weight of the polymer increases. Besides polymer molecular weight, the higher hydroxyl content is likely to be influential in the viscosity. The CAB 531-1 and CAB 381-2 have very similar hydroxyl content. In this case, it appears that the higher butyryl content of CAB 531-1 increases the solubility of the polymer in acetone and provides slightly lower viscosity.

The polydispersity of cellulose acetate butyrate fractions used for microencapulation seem to be slightly increasing with the increasing polymer molecular weight. However, the difference between the molecular weight fractions around 60,000 Da and 160,000 Da does not appear to be very different.

3.2 INSTRUMENTS USED

The instruments used in this study are listed in Table V.

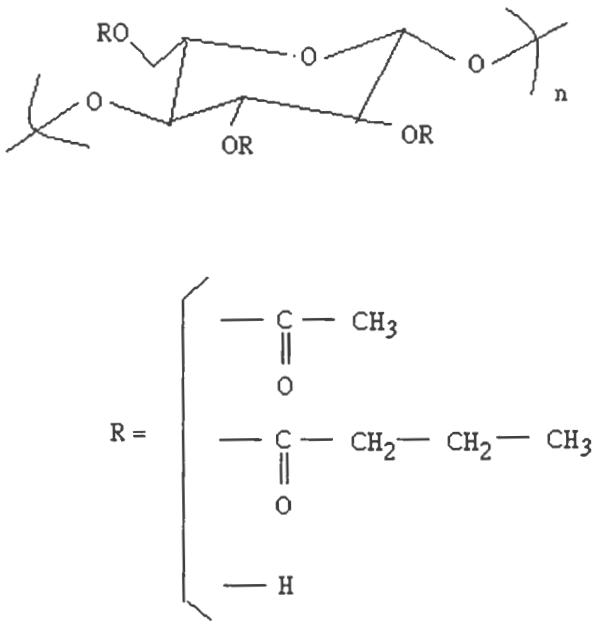


Figure 7 : Chemical Structure of Cellulose Acetate Butyrate

Table IV : Specification of Cellulose Acetate Butyrate

						Substitution Groups (%w/w in overall Mw)		
Polymer Batch No.	**Mw* (Dalton)**	**Viscosity (6%w/w sol.)** (cP)**	**Tg (°C)**	**Tm (°C)**	**Polydispersity**	**Acetyl Content (%)**	**Butyryl Content (%)**	**Hydroxyl Content (%)**
CAB 381-0.1	53937	1.97	123	155-165	3.111	13.5	38.0	1.3
CAB 553-0.4	67840	3.16	136	150-160	3.248	2.0	46.0	4.8
CAB 381-2	155437	11.31	133	171-184	3.208	13.5	38.0	1.3
CAB 531-1	171439	11.19	115	135-150	3.404	3.0	50.0	1.7
CAB 171-15S	216380	49.94	161	230-240	3.844	29.5	17.0	1.1
CAB 381-20	264362	51.45	141	195-205	4.402	13.5	37.0	1.8

* The mean molecular weights shown in Table III were provided by Eastman Chemical Company. The values were obtained by Gel Permeation Chromatography (GPC).

** As measured by a Ubbelholde viscometer as 6% of the polymer in acetone.

- The substitution groups contents were provided as the percentage of the overall molecular weights.

Table V : Instruments Used in This Study.

INSTRUMENTS	MODEL	MANUFACTURES
Olympus Image Analyser	CH507494	Olympus Optical Co., Ltd Tokyo, Japan
Compact Photo Micrographic Equipment	PM-6	Olympus Optical Co., Ltd Tokyo, Japan
PH-meter	811	Orion Research, Inc. Cambridge, Massachusetts
Micromaster Microscope	CK	Fisher Scientific Company Fairlawn, NJ
Sony Trintron Color Video Monitor	PVM-113420	JAVA Video Analysis Software, Jandel Scientific, CA
UV-Visible Spectrophotometer	Hewlettpackard 8451A Diode	Hewlett-Packard Company, Corvallis, Oregon
UV-Visible Spectrophotometer Cell		Perfector Scientific Atoscadero, CA 93423
Scanning Electron Microscope	DX-E30S	Akashi Beam Technology, Tokyo 180, Japan

Table V (continuation)

INSTRUMENTS	MODEL	MANUFACTURES
Mettler Balance	AE240	Northest Blance Service Middletown, Conn. 06457
Electronic Analytical Balance	1601 MP8 Sartorius	Subron-Brinkman Instruments Co. Westbury, NY
Central Scientific Sieve Shaker		Van-Kel Industries, Inc. Chatham, NJ 07228
Ultrasonic Cleanser	T100	National Ultrosonic Corp. Irvington 11, NJ
YAMATO Constant Temperature Shaking Bath	BT-25	YAMATO Scientific Co. Ltd., Japan
Ubbelholde Viscometer Tube		Kimax
Fisher Dyna-Mix	60900285	Fisher Scientific, Pittsburgh, PA 15219
Disposable Scintillation Vials	20mL	Kimble Glass, Inc. Vinelane, NJ 08360

3.3 METHODOLOGY

3.3.1 Determination of the Viscosity of Cellulose Acetate Butyrate Solution

The viscosity of cellulose acetate butyrate in acetone was measured by using Ubbelholde Viscometer Tube (Kimax, J 36, size 350). 60ml cellulose acetate butyrate solution (6%w/w, in acetone) was placed in the viscometer, viscosity was measured by timing the flow time of the liquid between the two marks. Compared to the viscosity of water (1.004 cs, 20°C), the viscosity of the polymer solution was obtained by using Equation (1):

$$\eta = \rho \times (t_p/t_w) \times 1.004 \qquad \text{.....(1)}$$

where η is the viscosity of the polymer solution in centistokes, ρ is the density of the polymer solution (g/cm^3), t_p and t_w are the flow time between the marked points of the viscometer (in seconds) of the polymer solution and water, respectively. The data obtained are listed in Table VI.

3.3.2 Analytical Determination of Ascorbyl Palmitate

Ascorbyl Palmitate is a hydrophobic drug. Its general properties were described in Section 3.1.1. It is poorly soluble in water. Since release studies were to be carried out in the aqueous medium, an analytical method to determine ascorbyl palmitate in water was developed.

Table VI : Viscosity of the 6% w/w Cellulose acetate Butyrate in Acetone.

Polymer Batch No.	Mw (Dalton)	Flow Time (seconds)	Viscosity (cP) (n=3) ($\eta = 0.81 \times (t_p/t_w) \times 1.004$)
381-0.1	53937	23.15	1.97
553-0.4	67840	14.43	3.16
381-2	155437	82.87	11.31
531-1	171439	82.04	11.19
171-15S	216380	363.61	49.94
381-20	264362	376.47	51.45
Water	--	5.95	--

- t_p and t_w are the time required for the polymer solution and water to pass through the two marks on the viscometer tube, respectively.

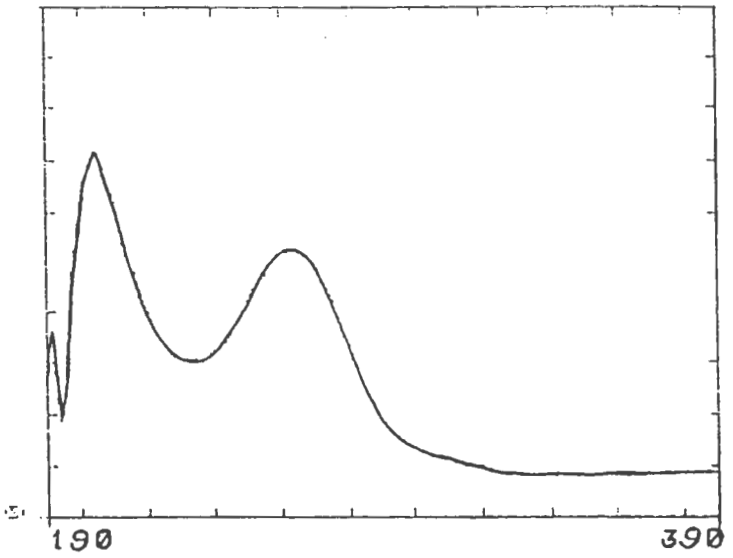
Ascorbyl Palmitate in water shows two characteristic absorption peaks at λ = 204 and 262 nm, Figure 8A. The absorbance at λ = 204 nm would be easily affected by interference from other chemicals used in this study, like cellulose acetate butyrate, and our preliminary experiments demonstrated that it was less sensitive to oxidative changes that takes place in the molecule. The absorbance at λ = 262nm is sensitive to oxidative changes and quickly disappears by oxidation, Figure 8B.

In Figure 8, the ascorbyl palmitate concentration used was 0.1658 g/L. The original intensity of ascorbyl palmitate at this concentration at λ_{262} was 0.5271, it decreased to 0.2875 over 24 hours in deionized water. Therefore $\lambda_2^{nd}{}_{,max}$ was used to follow oxidative stability of ascorbyl palmitate for solubility and release studies. In order to maintain stability during the release rate measurements, 1.5%w/w sodium thiosulfate was added to the solution.

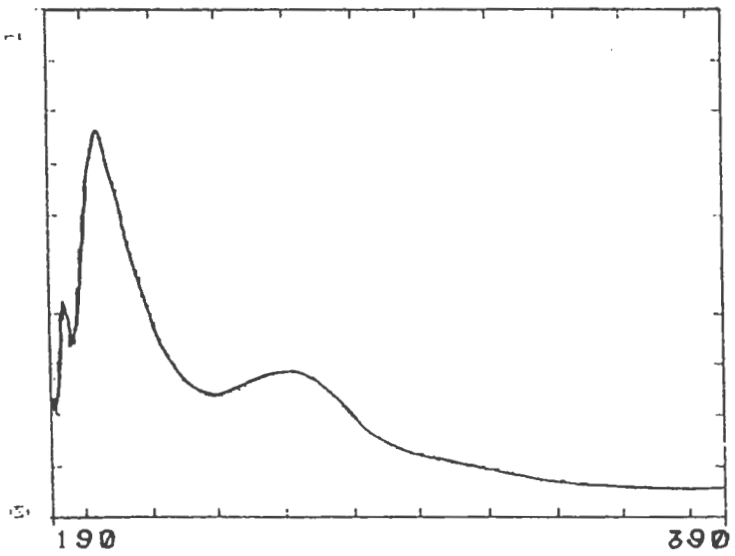
The stability of ascorbyl palmitate was verified by UV analysis. In the presence of sodium thiosulfate, the $\lambda_2^{nd}{}_{,max}$ moved from 265 to 270 nm. The stability of sodium thiosulfate was followed for 72 hours by measuring the intensity of $\lambda_2^{nd}{}_{,max}$, Table VII.

3.3.3 Calibration of Ascorbyl Palmitate for UV Analysis

Ascorbyl palmitate (2 mg) was dissolved in 50 ml water which contained 1.5% sodium thiosulfate. An appropriate volume (1, 2, 3, 4, 5, 6 and 8 ml) of this solution was transferred to seven 10 ml volumetric flasks respectively and made up



(A)



(B)

Figure 8 : UV Profile of Ascorbyl Palmitate in Deionized Water. The Ascorbyl Palmitate Concentration is 0.1658 g/L.
((A): measured right after preparation; (B): measured 24 hours after preparation.)

Table VII : Absorbances of Ascorbyl Palmitate in Deionized Water in the Presence of 1.5% w/w Sodium Thiosulfate Used as an Antioxidant. (λ_{270}, c = 0.1658g/L)

Time (hr)	Batch 1	Batch 2	Average
0	0.354507	0.370128	0.362318
24	0.348857	0.367302	0.358080
48	0.341537	0.368842	0.355190
72	0.365661	0.372044	0.368853

to 10 ml with the dissolution medium. The concentrations thus obtained were 0.004,0.008, 0.012, 0.016, 0.02, 0.024, 0.032 and 0.04 mg/ml. Using the dissolution medium as a blank, the absorbances were measured at 270 nm in four replicate solutions. Figure 9 demonstrates that the results obtained obeyed Beer's Law between 0.005 and 0.040 g/L ascorbyl palmitate concentrations.

3.3.4 Solubility Determination of Ascorbyl Palmitate

To determine the solubility of the ascorbyl palmitate, 1 gm ascorbyl palmitate was added to 50ml deionized water which contained 0.75 gm sodium thiosulfate as an antioxidant. The suspension was shaken for 72 hours in a water bath at 37°±0.5°C. Then the drug was filtered from No.2 filter paper. The supernatant was diluted and analyzed by UV spectrophotometer at the wavelength of 270nm.[24] This procedure was repeated four times. The solubility of ascorbyl palmitate in water containing 1.5%w/w sodium thiosulfate was found as 0.9342 ± 0.0434 g/L. The related data were given in Appendix I, Table I.

3.3.5 Preparation of Microcapsules

The emulsion non-solvent addition method of Sprockel and Prapaitrakul[18] was used for microcapsule preparation. The details of the method and its outcome was discussed in Sec 1.3.6. Based on the findings mentioned in Sec1.3.6, in this study, low polymer to solvent concentration (6%), low drug to polymer ratio (1/2) and high stirring rate (2000rpm) was used to obtain small microcapsules.[31,47]

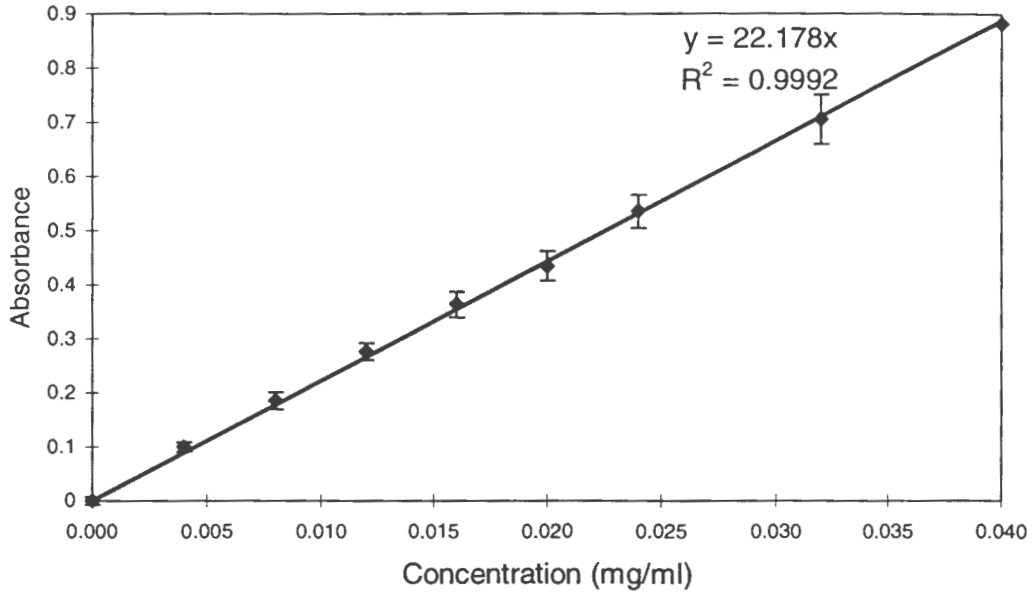


Figure 8, Calibration Curve of Ascorbyl Palmitate

Figure 9.

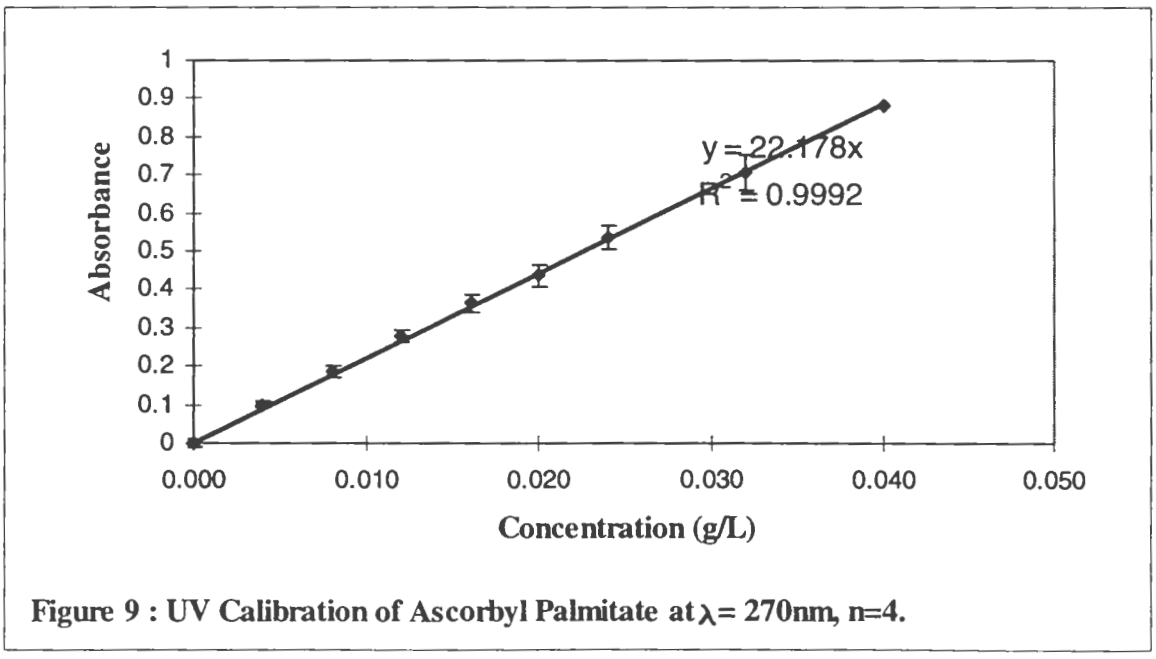


Figure 9 : UV Calibration of Ascorbyl Palmitate at λ= 270nm, n=4.

The total amount of the ingredients added and order of microcapsule formation is described in Figure 10. For microencapsulation formation, the polymer (0.6 g), ascorbyl palmitate (0.3 g) and butylated hydroxytoluene (BHT, 0.01 g) were dissolved in acetone (9.4 g). The oil phase used was 88.1g light mineral oil that contained 0.1g BHT, 0.9g Span and 0.9g magnesium stearate. Selection between Span 60 and Span 80 which were used in 0.2 and 1% concentrations was made earlier by comparing the data obtained with both surfactants by a statistical analysis in order to create the largest differences between the highest and lowest molecular weight coated cellulose acetate butyrate. (Please see Appendix II for details.) Accordingly Span 80 at 1% concentration was selected for microcapsule preparation.

Cellulose acetate butyrate, ascorbyl palmitate and BHT were dissolved in acetone and the mixture was poured into oil phase while being stirred at 2000 rpm with a Fisher Dyna-mixer (Fisher Scientific, Pittsburgh, PA) at ambient temperature. The emulsion formed was stirred at the same speed for another 15 minutes. Hexane (30 mL), which hardened the emulsion droplets, was added to this emulsion drop by drop at the rate of 1.5 ml/min. The particles thus hardened were then filtered and washed by 60 ml of hexane in four portions to remove the remaining mineral oil. They were dried on the filter paper in a dark and cool place. Each batch was prepared in quadruples.

3.3.6 Characterization of the Microcapsules

3.3.6.1 mean particle size, size distribution and degree of aggregation

The mean particle size and the size distribution of the particles were determined and

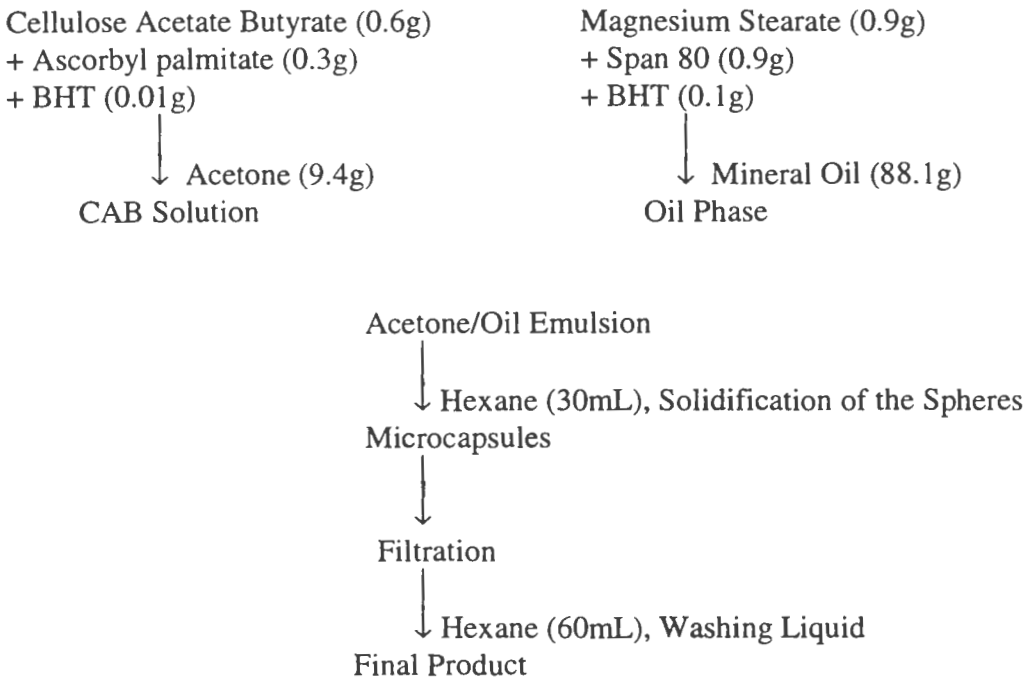


Figure 10 : Flow Chart of Preparation of Ascorbyl Palmitate Microcapsules

characterized by an image analyzer (Olympus Image Analyser, CH507494, Olympus Optical Co., Ltd., Tokyo, Japan). Microcapsules (20 mg) were placed in an 20ml screw-capped glass vial which contained 15ml deionized water and 0.3g polyvinylpyrrolidone which was used as the dispersant. Prior to measurements, the suspension was deaggregated in an ultrasonic bath (Ultrasonic Cleanser, T100, National Ultrosonic Corp., Irvington 11, NJ) for 2 minutes.

This dispersion was used under the microscope. The diameters of at least 740 particles were measured in order that the mean particle size obtained was representative of the batch prepared within 95% confidence level. The means and standard deviation were calculated by using Excel.

The degree of aggregation was calculated by counting the number of aggregated particles that were present in 740 particles and calculating the percentage.

3.3.6.2 microscopical appearance

Surface morphology of the microcapsules were observed by a scanning electron microscope(DX-E30S, ABT Co., Tokyo, Japan). In order to determine the surface morphology of the particles, the microcapsules were first dispersed in alcohol and were transferred to a metallic round disk. After evaporation of the alcohol, the sample was coated with gold in a coating chamber(SC 502, ABT Co., Tokyo, Japan). Scanning electron micrographs were then taken.

3.3.6.3 determination of drug loading

Methylene chloride is a good solvent for cellulose acetate butyrate but does not dissolve ascorbyl palmitate. Therefore ascorbyl palmitate content of the microcapsules was measured by using methylene chloride. For this purpose, 10mg microcapsules was placed in a 100 mL volumetric flask containing 25 mL methylene chloride. The flask was shaken manually to completely dissolve cellulose acetate butyrate, then the ascorbyl palmitate particles that remained in the suspension were filtered. The particles collected on a filter paper were further washed with 25 ml methylene chloride to assure complete dissolution of cellulose acetate butyrate and dried in cool open air in a dark room. The particles left on the filter paper were dissolved in 10 ml dissolution medium (deionized water + 1.5% sodium thiosulfate) and the absorbance of the solution was measured by UV spectrophotometer at 270 nm to determine the ascorbyl palmitate content of the microcapsules.

3.3.6.4 release studies

The microcapsules were sieved by using two standard sieves (75-90um and 90-177um) on a sieve shaker (Central Scientific Sieve Shaker, Van-Kel Industries, Inc. Chatham, NJ) for 5 minutes, then the microcapsules with the diameter ranging from 75 to 90 um were collected and subjected to the release studies. The dissolution medium selected for release studies was composed of deionized water and 1.5% (w/w) sodium thiosulfate. The release characteristics of ascorbyl palmitate was determined by dispersing 5mg

microcapsules (75-90 um) in 50ml dissolution medium which was kept in a 100ml screw-capped glass vial and placed in a water bath shaker (YAMATO Constant Temperature Shaking Bath, BT-25, YAMATO Scientific Co. Ltd., Japan) at 37 ± 0.5°C. Samples (2ml) were withdrawn at 1, 2, 4, 6 and 8 hours time intervals, and were filtered and analyzed by UV spectrophotometer at 270nm. Fresh dissolution medium (2ml) equal to the amount that was withdrawn was immediately introduced into the dissolution vials after each sampling in order to maintain sink conditions.

IV RESULTS AND DISCUSSIONS

4.1 MICROSCOPIC APPEARANCE

The size of microcapsules increased with the increase in polymer molecular weight. The scanning electron microscopic pictures of the microapsules produced with low and high molecular weight polymers (53,937 and 216,380 Daltons) are given in Figure 11. The microcapsules seemed to be round-shaped and the surface appearance do not seem to have obvious difference between them. However, there is substantial surface precipitation.

4.2 THE EFFECTS OF POLYMER PROPERTIES ON MICROCAPSULES PERFORMANCE

The results of polymer viscosity in acetone, yield, mean particle size, size distribution, drug loading and degree of aggregation of the microcapsules produced with the corresponding polymers are listed in Table VIII and the original data are given in Appendix I, Table II. The formulation responses obtained were further drawn against each molecular variable, including molecular weight, solution viscosity and polydispersity of the polymer. Figures 12-14 demonstrate that the yield tends to increases with decreasing polymer molecular weight and solution viscosity. The mean particle size and size distribution of the microcapsules tend to increase with the increasing molecular weight, solution viscosity and polydispersity.

(A)

(B)

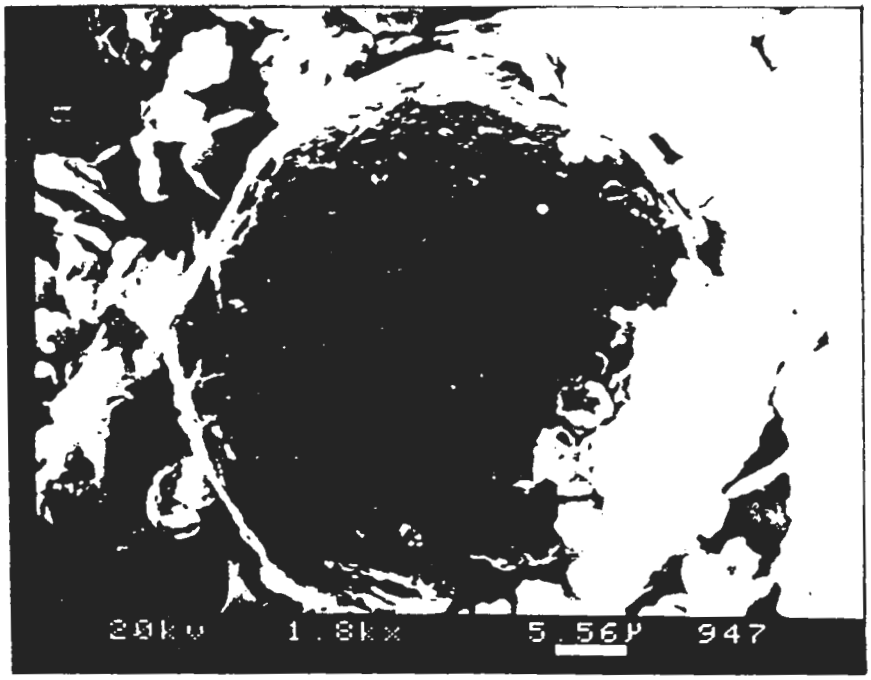


Figure 11 : Scanning Electron Micrographs of Cellulose Acetate Butyrate Microcapsules Containing Ascorbyl Palmitate. (Microcapsules produced with (A) : CAB (53937 Da); (B) : CAB (216380 Da).

Table VIII : Properties of Cellulose Acetate Butyrate Coated Microcapsules.

Polymer Properties						Microcapsules Properties				
Mw (Daltons)	Acetyl Content (%)	Butyryl Content (%)	Hydroxyl Content (%)	Solution Viscosity (cP)	Poly - dispersity (um)	Yield (%)	Mean Particle Size (um)	Size Distribution (um)	Degree of Aggregation (%)	Drug Loading (%)
53937	13.5	38.0	1.3	1.97	3.111	98.17±1.55	24.41±1.90	7.47±0.60	19.16±1.21	11.89±0.96
67840	2.0	46.0	4.8	3.16	3.248	99.03±1.31	24.10±3.36	9.80±0.82	12.50±0.69	12.30±2.25
155437	13.5	38.0	1.3	11.31	3.208	96.25±2.54	49.75±2.28	17.31±2.43	13.34±1.11	12.00±1.42
171439	3.0	50.0	1.7	11.19	3.404	97.03±2.70	38.19±3.44	11.52±1.46	11.02±1.70	11.23±1.13
216380	29.5	17.0	1.1	49.94	3.844	95.14±2.19	57.14±4.67	18.64±1.51	9.34 ±1.40	8.16±1.23
264362	13.5	37.0	1.8	51.45	4.402	94.59±1.66	46.99±2.56	19.85±2.40	9.61±0.91	10.86±1.70

* Each batch was prepared four times.

Figure 12

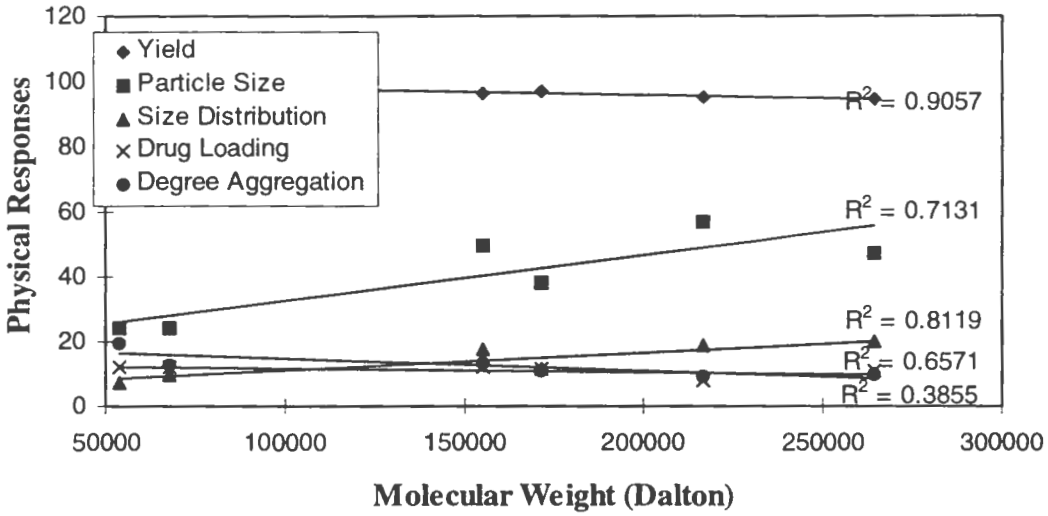


Figure 12 : Effects of Molecular Weight on Physical Responses of the Microcapsules

Figure 13

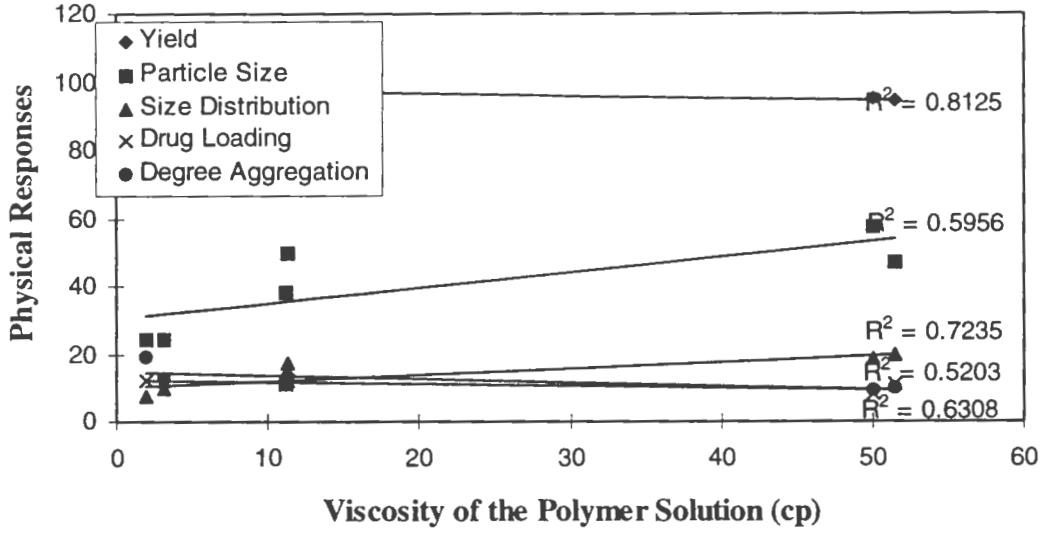


Figure 13 : Effects of the Viscosity of the Polymer Solution on Physical Responses of the Microcapsules

Figure 14

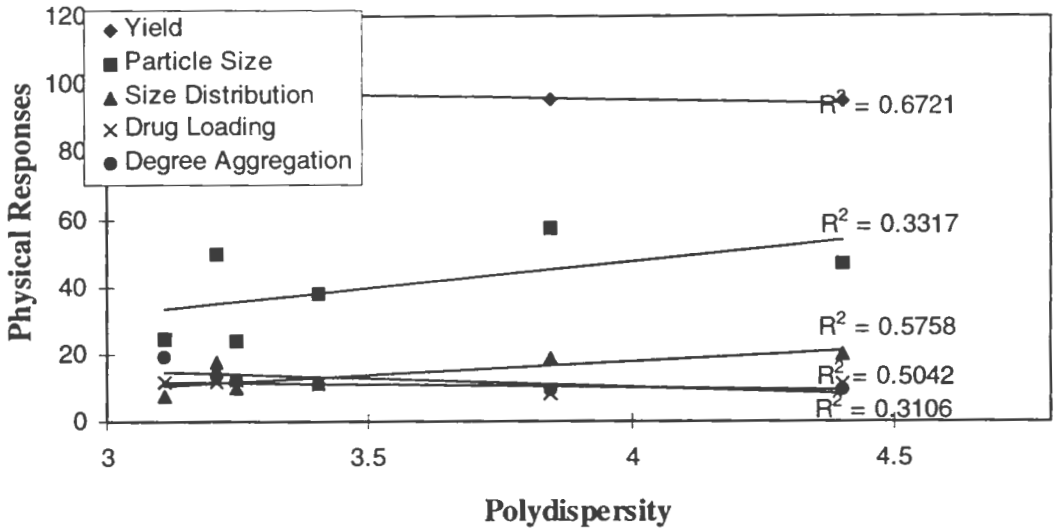


Figure 14 : Effects of the Polydispersity on Physical Responses of the Microcapsules

The degree of aggregation decreases with the increase in polymer molecular weight, solution viscosity and polydispersity.

Although molecular weight stands out as the most significant variable, Figures 12-14 demonstrated that neither the molecular weight nor the solution viscosity and polydispersity was the single cause of the microcapsules characteristics. Therefore the best treatment would be to seek the combined effects of molecular weight, solution viscosity and polydispersity of the polymer via statistical treatments.

The Stepwise Regression Analysis was the statistical method used to determine the significant variables affected the overall properties of the microcapsules. It is based on the comparison of the R-square of each variable. The greater the R-square the variable contribute to the regression, the more important the variable becomes. By using Stepwise Regression Analysis, first the effects of the acetyl, butyryl and hydroxyl groups, molecular weight, viscosity of the polymer solution and polydispersity were sought, Table IX.

From Table IX, the viscosity of the cellulose acetate butyrate solution was found to be affected most significantly by first the polymer molecular weight, then the butyryl content, third; by the hydroxyl content. The viscosity of the polymer increased with increasing molecular weight and hydroxyl content, decreased with the increase in butyryl content. The relationship between the three variables can be described by Equation (2):

Table IX : The Relationships Between the Chemical Structure of Cellulose Acetate Butyrate on the Molecular Viscosity and Polydispersity as Determined by Stepwise Regression Analysis

	POLYMER CHARACTERISTICS			
	Molecular Weight (Dalton)	**Acetyl Content (%)**	**Butyryl Content (%)**	**Hydroxyl Content (%)**
Responses				
Viscosity (cP)	0.000 (1^{st})	NS	0.000 (2^{nd})	0.004 (3^{rd})
Polydispersity	0.000 (1^{st})	NS	NS	0.032 (2^{nd})

The numbers shown in the table were p-values which were only given when the variables were significant.
* The ranking given in the parenthesis were the order of the influence of the polymer properties on the response which were calculated by Minitab Stepwise Regression Program.
NS : Not significant

$$\eta = 15.3 + 0.000222\ Mw - 0.930\ \phi_b + 3.39\ \phi_h \quad \text{.....(2)}$$

$$R^2 = 0.95$$

where η is the viscosity (cP), Mw is the polymer molecular weight (Da), ϕ_b is the butyryl content (%) and ϕ_h is the hydroxyl content (%). (The detailed statistical data obtained are given in Appendix I, Table III.)

On the other hand, the polydispersity of the polymer was affected by only the molecular weight of the polymer and hydroxyl group content, Table IX. The molecular polydispersity increased with increasing polymer molecular weight and hydroxyl content, Appendix I, Table IV. The relationship between the polydispersity, molecular weight and hydroxyl content can be described by Equation (3):

$$\omega = 2.44 + 0.000006\ Mw + 0.0851\ \phi_h \quad \text{.....(3)}$$

$$R^2 = 0.82$$

where ω is polydispersity of cellulose acetate butyrate and ϕ_h is the percent hydroxyl content of the molecule.

Since the substitution groups and the molecular weight significantly affected the solution viscosity and molecular polydispersity and those are the physical chemical factors that are effective during microcapsules formation, the effects of molecular weight, molecular polydispersity and solution viscosity on the formulation variables

such as the yield, mean particle size, size distribution, degree of aggregation and drug loading were sought the next, Table X.

The p-values and R-squares obtained in Table X demonstrated molecular weight of the polymer affected all physical-chemical properties of the microcapsules except drug loading. The acetyl content affects only mean particle size. Butyryl content has the influence over drug loading. Hydroxyl content affects only degree of aggregation.

4.2.1 Effects of Polymer Molecular Weight, Polydispersity and Solution Viscosity on the Yield

The yields of the microcapsules produced with six polymers were shown in Table VIII. Table X demonstrated that the only significant variable which has influence on yield is the polymer molecular weight. Microcapsule yield decreased as the polymer molecular weight increased. Since molecular weight is the only significant factor, its effect on the yield can be shown on a XY graph, Figure 15. The detailed statistical data was given in Appendix I, Table V.

4.2.2 Effects of Polymer Molecular Weight, Polydispersity and Solution Viscosity on the Mean Particle Size, Size Distribution and Degree of Aggregation

Table X also demonstrated that all the three variables have effects on the mean microcapsule size. Polymer molecular weight is the most dominant factor, molecular polydispersity is the second and the solution viscosity is the third. The detailed

Table X : The Significant Relationships Between the Molecular Characteristics of Cellulose Acetate Butyrate and Physical Chemical Properties of the Microcapsules as Determined by Stepwise Regression Analysis

	POLYMER CHARACTERISTICS		
	Molecular Weight (Dalton)	**Solution Viscosity (cp)**	**Molecular Polydispersity**
Responses			
Yield	0.001	NS	NS
Mean Particle Size	0.000 (1^{st})	0.000 (3^{rd})	0.000 (2^{nd})
Size Distribution	0.000	NS	NS
Degree of Aggregation	0.000	NS	NS
Drug Loading	NS	0.003 (1^{st})	0.036 (2^{nd})

The numbers shown in the table were p-values which were only given when the variables were significant.

* The ranking given in the parenthesis were the order of the influence of the polymer properties on the response which were calculated by Minitab Stepwise Regression Program

NS : Not significant

Figure 15: Yield vs Mw

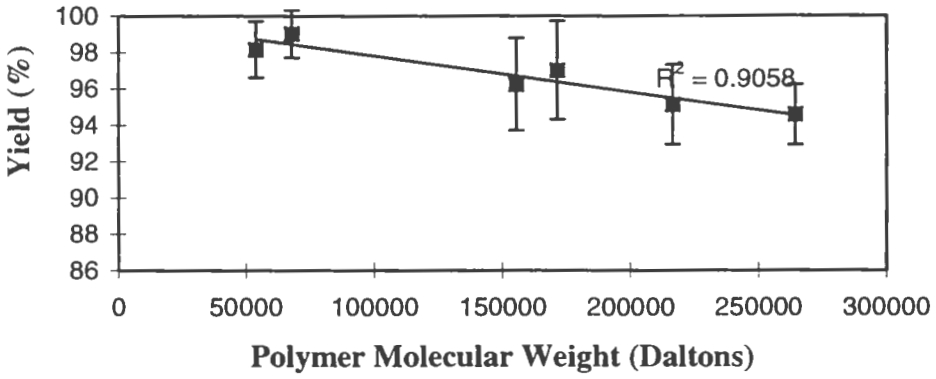


Figure 15 : Effect of Molecular Weight on Yield

statistical analysis data are given in Appendix I, Table VI. Generally the size of microcapsules increased with increasing molecular weight and solution viscosity, but decreased with increasing polydispersity. The relationship between the properties of the polymer and the mean particle size can be described by Equation (4).

$$d = 137 + 0.000185\ Mw - 39.7\omega + 0.668\ \eta \quad \text{.....(4)}$$

$$R^2 = 0.93$$

where d is mean particle size (um), Mw is polymer molecular weight (Da) ω is molecular polydispersity and η is the solution viscosity (cP).

From Table X, molecular weight stood out as the only significant factor that affected particle size distribution of the microcapsules. Details of the analysis was given in Appendix I, Table VII. Higher molecular weight cellulose acetate butyrate produced microcapsules with wider particle size distribution. The effect of polymer molecular weight on size distribution is shown in Figure 16. The relationship of regression can be described by Equation (5).

$$\sigma = 5.02 + 0.000060\ Mw \quad \text{.....(5)}$$

$$R^2 = 0.75$$

where σ is the particle size distribution (um) and Mw is the molecular weight.

Figure 16 : distribution vs Mw

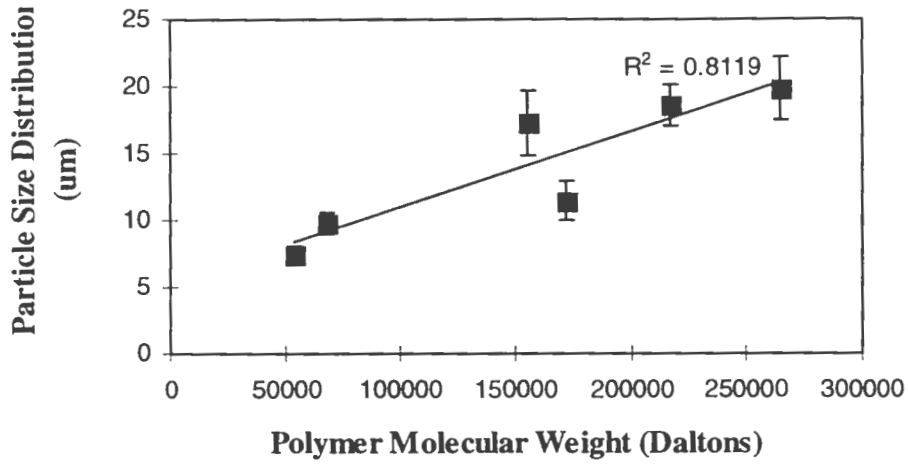


Figure 16 : Effect of Molecular Weight on Particle Size Distribution

Among polymer molecular weight, polydispersity and solution viscosity, only the molecular weight of the polymer significantly affected the degree of aggregation, Table X, Appendix I, Table VIII. The degree of aggregation significantly decreases as the polymer molecular weight increases. The solubility of higher molecular weight polymer in solvent was less than that of lower molecular weight polymer. Therefore, the heavier polymer would precipitate out faster and the molecular chain would be hardened by not allowing the intermolecular interaction of the cellulose acetate butyrate layers around the particles which would cause aggregation. The effect of polymer molecular weight on the degree of aggregation is shown in Figure 17.

4.2.3 Effects of Polymer Molecular Weight, Polydispersity and Solution Viscosity on Drug Loading

Both the solution viscosity and molecular polydispersity significantly affect drug loading, Table X, Appendix I, Table IX. Solution viscosity is the most important factor and the polydispersity is the second. Drug loading increases with lower viscosity of the polymer solution, but decreases with the increasing molecular polydispersity.

4.2.4 Release of Ascorbyl Palmitate from Cellulose Acetate Butyrate Coated Microcapsules

Release profiles of the ascorbyl palmitate from cellulose acetate butyrate microcapsules is shown in Figure 18 where the smallest molecular weight polymer appear to provide

figure 17 : mw vs aggregation

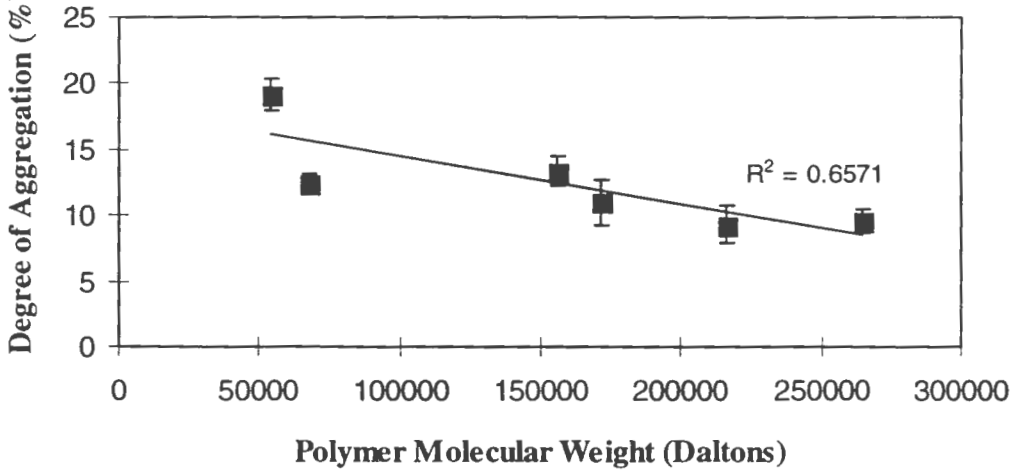


Figure 17 : Effect of Molecular Weight on Degree of Aggregation

figure18 release profiles

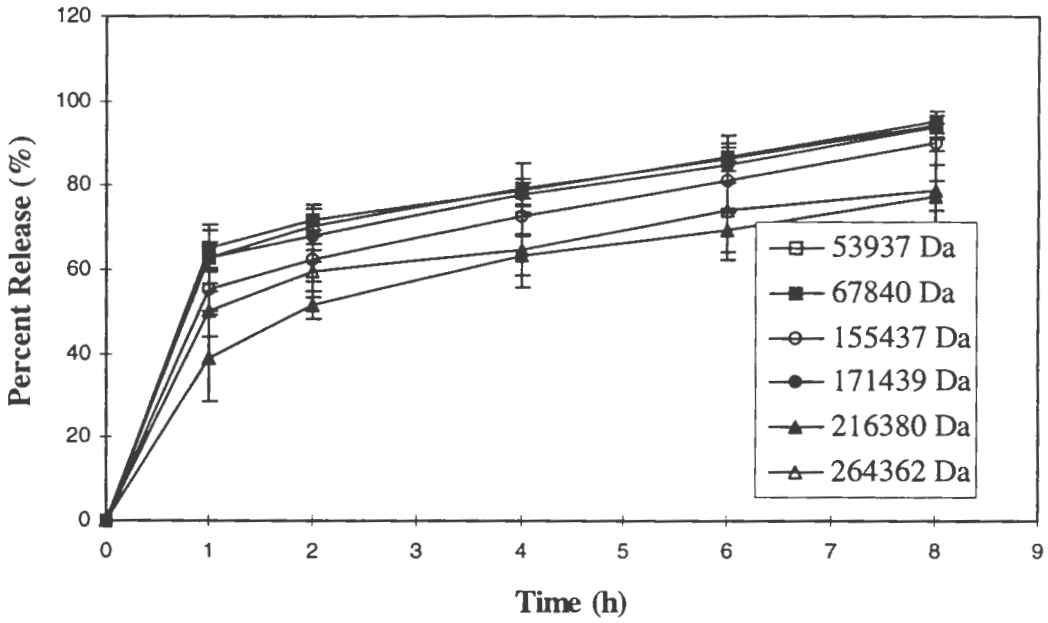


Figure 18 : Release Profiles of Cellulose Acetate Butyrate Coated Microcapsules

the fastest release than the higher molecular weight fractions. From Figure 18, the total amounts released at the end of the first hour and eight hours do not seem to be statistically different from one another.

HIGUCHI Model, Zero Order and First Order release models[105-108] were frequently used models to describe release and dissolution of drugs from microcapsules. The HIGUCHI Equation is shown in Equation (6).

$$M = kt^{1/2} \quad \text{.....(6)}$$

where M is the percent release, k is release rate constant and t is the time of release.

The Zero Order Equation is :

$$M = kt \quad \text{.....(7)}$$

The First Order Equation is :

$$M = 1 - e^{-kt} \quad \text{.....(8)}$$

The graphs given in Figure 18 demonstrated two different release rates for the microcapsules prepared with different molecular weight fractions. Within 0-1 hour a "burst effect" was observed then the release was at a steady state.

"Burst effect" was due to the release of the drug adsorbed on the microcapsule surfaces. From Figure 11, the presence of substantial surface precipitate is apparent. Release studies were carried out without further purification.

Burst release was also reported in several previous publications[57,101]. In the study of Jalil and Nixon's[57], burst effect was observed when phenobarbitone released from polylactic acid microcapsules, and it was not significantly affected by the molecular weight variations of the polylactic acid. Similar profiles were also obtained by Igartua et al.[101] and O'Hagan et al.[102] who both studied the release of albumin from poly(lactide-co-glycolide) microspheres.

The steady state release data obtained were applied to HIGUCHI, Zero Order and First Order Equations. Table XI demonstrates the fit of the data to HIGUCHI, Zero Order and First Order models. From Table XI, it is clearly seen that the steady state release of ascorbyl palmitate from cellulose acetate butyrate mcrocapsules fits both HIGUCHI and Zero Order models equally well. However, microcapsules produced with 216,380 and 264,362 Da polymers began to have some deviation from the Zero Order Release. Therefore, the HIGUCHI model was used to calculate the burst release and steady state release rate. The burst release was determined by the extrapolated values of the HIGUCHI fit at 0 hour. Table XII.

The data of the burst release, steady state release and total release in one and eight hours were analyzed by Stepwise Regression Analysis to find out the significant variables that could affect the release performance. The results are given in Table XIII.

The burst release was demonstrated to be related to first the solution viscosity and then

Table XI : Correlation Coefficient of the HIGUCHI, Zero Order and First Order Fits of the Steady State Release Profiles of the Cellulose Acetate Butyrate Fractions

Batch Name	Molecular Weight (Daltons)	R^2 (HIGUCHI)	R^2 (Zero Order)	R^2 (First Order)
CAB 553-0.4	67,840	0.9877	0.9932	0.9170
CAB 381-0.1	53,937	0.9978	0.9888	0.9675
CAB 531-1	171,439	0.9925	0.9956	0.9378
CAB 381-1	155,437	0.9972	0.9927	0.9750
CAB 171-15S	216,380	0.9843	0.9426	0.9876
CAB 381-20	264,362	0.9826	0.9634	0.9831
Average		0.9904	0.9794	0.9613

Table XII : Total Amount Release in 1 and 8 hours and Steady State Release Rate of Ascorbyl Palmitate Microcapsules

Polymer Properties						Release Properties of Microcapsules			
Mw (Daltons)	Acetyl Content (%)	Butyryl Content (%)	Hydroxyl Content (%)	Solution Viscosity (cP)	Poly - dispersity (um)	Burst Release (α, %)	Total Release at 1 Hour (%)	Total Release in 8 Hours (%)	Steady State Release Rate
53937	13.5	38.0	1.3	1.97	3.111	0.4625	63.02±3.71	94.11±2.64	0.1666
67840	2.0	46.0	4.8	3.16	3.248	0.4881	65.03±4.48	94.96±2.64	0.1580
155437	13.5	38.0	1.3	11.31	3.208	0.3585	55.13±4.73	89.94±4.89	0.1879
171439	3.0	50.0	1.7	11.19	3.404	0.4502	62.74±8.07	93.73±2.82	0.1675
216380	29.5	17.0	1.1	49.94	3.844	0.2082	38.83±10.20	77.42±3.52	0.2025
264362	13.5	37.0	1.8	51.45	4.402	0.3597	50.28±6.26	78.94±9.39	0.1524

* Each batch was prepared four times and tested for release.

Table XIII : Significance of the Molecular Weight, Polydispersity and Viscosity of the Polymer on the Release Characteristics of the Microcapsules

	POLYMER CHARACTERISTICS		
	Molecular Weight (Dalton)	**Solution Viscosity (cp)**	**Polydispersity**
RELEASE CHARACTERISTICS OF MICROCAPSULES			
Burst Release	NS	0.000 (1^{st})	0.008 (2^{nd})
Steady State Release	NS	NS	NS
Total Release in 1 hr	NS	0.000 (1^{st})	0.004 (2^{nd})
Total Release in 8 hrs	0.000 (1^{st})	NS	0.002 (2^{nd})

The numbers shown in the table were p-values which were only given when the variables were significant.

* The ranking given in the parenthesis were the order of the influence of the polymer or microcapsule properties on the response which were calculated by Minitab Stepwise Regression Program

the polydispersity of the polymer by using Stepwise Regression Analysis, Table XIII. Burst release is lower with higher viscosity of the polymer solution and smaller polydispersity.

The Least Significant Difference(LSD) Test is a statistical method to determine whether the difference between the arguments is significant by comparing the difference to the calculated LSD value, the arguments are significantly different if the difference is larger than the LSD value. From the LSD Test, there is no significant difference of the burst release between the polymers except for the one with molecular weight 216,380 Da which shows the lowest burst release. The detailed statistical data were given in Appendix I, Table X.

The data of steady state release given in Table XII show no significant differences between the six polymers. The observation was demonstrated by LSD Test, Appendix I, Table XI. Therefore, the steady state release of the six polymers was not different from one another and was not affected by the physical-chemical properties of the polymer.

The total release in one hour was affected by first the solution viscosity and then the polydispersity of the polymer, Table XIII. The total release in one hour increases with decreasing solution viscosity and increasing polydispersity. The detailed statistical data were given in Appendix I, Table XII.

The total release in eight hours was only influenced by the viscosity of the polymer

solution, Table XIII. The total release in eight hours increases with decreasing solution viscosity, Appendix I, Table XIII.

Overall, cellulose acetate butyrate with varying molecular weights affected ascorbyl palmitate microcapsules in more than one way. There were all acceptable fine flowing microcapsules and depending on the primary purpose of the microcapsules, a suitable batch of cellulose acetate butyrate can be selected in order to satisfy formulation requirements.

V. CONCLUSION

The ascorbyl palmitate microcapsules produced by six different cellulose acetate butyrate fractions with mean molecular weight of 53,000 to 265,000 were acceptable spherical particles with particle sizes changing from 24.10 to 57.14um. Emulsion non-solvent microencapsulation method was used for microcapsule production.

The viscosity of the cellulose acetate butyrate solution (6% in acetone) that was used for microencapsulation was affected by the molecular weight, the butyryl and the hydroxyl content of the polymer in the given order. The solution viscosity increased with increasing molecular weight and hydroxyl content, but decreased with an increase in the butyryl content.

The Stepwise Regression Analysis carried out demonstrated that the polydispersity of the polymer was also influenced by the polymer molecular weight and hydroxyl group content. The polydispersity increased with increasing polymer molecular weight and hydroxyl content.

The microcapsule yield prepared with six fractions of cellulose acetate butyrate was influenced by the polymer molecular weight. Microcapsule yield decreased as the polymer molecular weight increased.

Molecular weight, solution viscosity and the polydispersity of the polymer had significant effects on the mean particle size. The mean molecular weight was the most

important factor which was followed by the solution viscosity and polydispersity. The size of microcapsules increased with increasing molecular weight, solution viscosity and decreased with increasing polymer polydispersity.

The only significant factor that affected particle size distribution of the microcapsules was the molecular weight of the polymer. Higher molecular weight cellulose acetate butyrate produced microcapsules with wider particle size distribution.

Degree of aggregation was only affected by the molecular weight of the polymer. The degree of aggregation decreased as the molecular weight of the polymer increased.

Drug loading was significantly influenced by the solution viscosity and the polydispersity of the polymer in the given order. Higher viscosity of the polymer solution and narrower polydispersity of the polymer produced microcapsules with low drug loading.

Statistical analysis of the release profiles demonstrated two different release rates of ascorbyl palmitate from the microcapsules which can be the result of the burst effect and steady state release.

The burst release was affected by the solution viscosity of the polymer and its polydispersity. Basically, the burst release was lower with higher solution viscosity and smaller polydispersity of the polymer.

The steady state releases of the polymers were not significantly different from one

another.

The total release in one hour was significantly influenced by solution viscosity and polydispersity. The amount of total release in one hour increased with decreasing solution viscosity and increasing polydispersity.

The viscosity of the polymer solution was the only factor which affected the total release in eight hours. The total release in eight hours increased with decreasing viscosity of the polymer solution.

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APPENDIX I

Table I : Solubility Data of Ascorbyl Palmitate in Deionized Water Containing 1.5% Sodium Thiosulfate

Repetition	**1**	**2**	**3**	**4**
UV Absorbance	0.2022	0.2196	0.2095	0.1974
Corresponding Solubility (g/L)	0.9118	0.9900	0.9448	0.8902
Solubility : 0.9342 ± 0.0434 g/L				

Table II : Properties of Cellulose Acetate Butyrate Coated Microcapsules.

Polymer Brand No.	Mw (Daltons)	Acetyl Content (%)	Butyryl Content (%)	Hydroxyl Content (%)	Viscosity (cP)	Polydispersity	Batch	Yield (%)	Mean Particle Size (um)	Size Distribution (um)	Degree of Aggregation (%)	Drug Loading (%)
381-0.1	53937	13.5	38.0	1.3	1.97	3.111	2.1	98.33	25.57	7.25	20.70	11.43
							2.2	93.89	21.96	6.76	18.99	13.32
							2.3	98.33	23.88	7.69	19.22	11.40
							2.4	96.11	26.21	8.16	17.74	11.40
553-0.4	67840	2.0	46.0	4.8	3.16	3.248	1.1	100.00	19.24	10.20	12.61	12.99
							1.2	97.22	25.44	10.73	11.76	14.40
							1.3	100.00	26.91	8.90	13.39	12.68
							1.4	98.89	24.81	9.37	12.22	9.12
381-2	155437	13.5	38.0	1.3	11.31	3.208	4.1	93.33	49.00	14.90	12.32	13.77
							4.2	96.67	46.99	16.06	14.86	12.17
							4.3	95.56	50.73	20.48	12.78	11.70
							4.4	99.44	52.28	17.81	13.40	10.34
531-1	171439	3.0	50.0	1.7	11.19	3.404	3.1	98.89	42.53	12.94	10.36	12.40
							3.2	97.78	38.24	11.04	13.26	9.72
							3.3	95.00	34.12	12.39	11.20	11.19
							3.4	94.44	37.87	9.69	9.24	11.62
171-15S	216380	29.5	17.0	1.1	49.94	3.844	5.1	94.44	62.29	20.88	8.82	9.90
							5.2	98.33	52.00	18.12	11.11	6.99
							5.3	93.33	54.63	17.89	7.80	7.80
							5.4	94.44	59.63	17.66	9.64	7.96
381-20	264362	13.5	37.0	1.8	51.45	4.402	6.1	93.89	47.95	23.59	8.72	10.94
							6.2	92.78	50.17	22.80	8.98	12.36
							6.3	95.00	44.95	20.11	10.10	8.46
							6.4	96.67	44.87	16.68	10.63	11.69

Table III : Statistical Regression Analysis Data of the Polymer Characteristics on the Viscosity of Cellulose Acetate Butyrate Solution.

Stepwise regression of Viscosity on 4 predictors, with N = 24

STEP	1	2	3
CONSTANT	-17.07	19.22	15.29
Mw	0.00025	0.00021	0.00022
T-RATIO	9.27	11.05	14.12
butyryl		-0.78	-0.93
T-RATIO		-5.84	-8.03
hydroxyl			3.39
T-RATIO			3.52
S	9.88	6.24	5.03
R-SQ	79.61	92.23	95.20

The regression equation is
Viscosity = 15.3 +0.000222 Mw - 0.930 butyryl + 3.39 hydroxyl

Predictor	Coef	Stdev	t-ratio	p
Constant	15.292	5.641	2.71	0.013
Mw	0.00022244	0.00001575	14.12	0.000
butyryl	-0.9297	0.1157	-8.03	0.000
hydroxyl	3.3876	0.9637	3.52	0.002

s = 5.028 R-sq = 95.2% R-sq(adj) = 94.5%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	3	10029.3	3343.1	132.22	0.000
Error	20	505.7	25.3		
Total	23	10535.0			

SOURCE	DF	SEQ SS
Mw	1	8386.8
butyryl	1	1330.2
hydroxyl	1	312.4

Table IV : Statistical Regression Analysis Data of the Polymer Characteristics on Polymer Polydispersity.

Stepwise regression of Polydispersity on 4 predictors, N = 24

STEP	1	2
CONSTANT	2.714	2.445
Mw	0.00001	0.00001
T-RATIO	8.62	9.46
hydroxyl		0.085
T-RATIO		2.30
S	0.226	0.207
R-SQ	77.14	81.74

The regression equation is
Polydispersity = 2.44 +0.000006 Mw + 0.0851 hydroxyl

Predictor	Coef	Stdev	t-ratio	p
Constant	2.4445	0.1523	16.06	0.000
Mw	0.00000595	0.00000063	9.46	0.000
hydroxyl	0.08511	0.03702	2.30	0.032

s = 0.2071 R-sq = 81.7% R-sq(adj) = 80.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	2	4.0326	2.0163	47.00	0.000
Error	21	0.9009	0.0429		
Total	23	4.9336			

SOURCE	DF	SEQ SS
Mw	1	3.8059
hydroxyl	1	0.2268

Table V : Statistical Regression Analysis Data of the Polymer Characteristics on Yield

Stepwise regression of yield on 3 predictors, with N = 24.

STEP	1
CONSTANT	99.78
Mw	-0.00002
T-RATIO	-3.79
S	1.93
R-SQ	39.56

The regression equation is
Yield = 99.8 -0.000020 Mw

Predictor	Coef	Stdev	t-ratio	p
Constant	99.7822	0.9032	110.48	0.000
Mw	-0.00001991	0.00000525	-3.79	0.001

s = 1.930 R-sq = 39.6% R-sq(adj) = 36.8%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	53.603	53.603	14.40	0.001
Error	22	81.909	3.723		
Total	23	135.512			

Table VI : Statistical Regression Analysis Data of the Polymer Characteristics on Mean Particle Size

Stepwise regression of size on 3 predictors, with N = 24

STEP	1	2	3
CONSTANT	18.32	72.58	137.47
Mw	0.00014	0.00025	0.00018
T-RATIO	6.84	7.00	7.77
Polydispersity		-20.0	-39.7
T-RATIO		-3.43	-8.23
Viscosity			0.67
T-RATIO			6.05
S	7.56	6.19	3.77
R-SQ	68.02	79.50	92.75

The regression equation is
Mean particle size = 137 +0.000185 Mw - 39.7 Polydispersity + 0.668 Viscosity

Predictor	Coef	Stdev	t-ratio	p
Constant	137.47	14.54	9.46	0.000
Mw	0.00018479	0.00002377	7.77	0.000
Polydispersity	-39.694	4.821	-8.23	0.000
Viscosity	0.6680	0.1105	6.05	0.000

s = 3.773 R-sq = 92.8% R-sq(adj) = 91.7%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	3	3643.2	1214.4	85.29	0.000
Error	20	284.8	14.2		
Total	23	3928.0			

SOURCE	DF	SEQ SS
Mw	1	2672.0
Polydispersity	1	450.6
Viscosity	1	520.6

Table VII : Statistical Regression Analysis Data of the Polymer Characteristics on Particle Size Distribution

Stepwise regression of size distribution on 3 predictors, with N = 24

STEP	1
CONSTANT	5.022
Mw	0.00006
T-RATIO	8.08
S	2.71
R-SQ	74.79

The regression equation is
Distribution = 5.02 +0.000060 Mw

Predictor	Coef	Stdev	t-ratio	p
Constant	5.022	1.270	3.95	0.001
Mw	0.00005960	0.00000738	8.08	0.000

s = 2.713 R-sq = 74.8% R-sq(adj) = 73.6%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	480.39	480.39	65.28	0.000
Error	22	161.91	7.36		
Total	23	642.30			

Table VIII : Statistical Regression Analysis Data of the Polymer Characteristics on Degree of Aggregation

Stepwise regression of aggregation on 3 predictors, with N = 24

STEP	1
CONSTANT	18.02
Mw	-0.00004
T-RATIO	-5.70
S	2.30
R-SQ	59.64

The regression equation is
aggregation = 18.0 -0.000036 Mw

Predictor	Coef	Stdev	t-ratio	p
Constant	18.024	1.078	16.72	0.000
Mw	-0.00003570	0.00000626	-5.70	0.000

s = 2.303 R-sq = 59.6% R-sq(adj) = 57.8%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	172.39	172.39	32.51	0.000
Error	22	116.66	5.30		
Total	23	289.05			

Table IX : Statistical Regression Analysis Data of the Polymer Characteristics on Drug Loading

Stepwise regression of drug loading on 3 predictors, with N = 24

STEP	1	2
CONSTANT	12.2036	-0.4775
Viscosity	-0.053	-0.135
T-RATIO	-3.32	-3.41
Polydispersity		4.1
T-RATIO		2.24
S	1.62	1.49
R-SQ	33.41	46.25

The regression equation is
drug loading = - 0.48 - 0.135 Viscosity + 4.09 Polydispersity

Predictor	Coef	Stdev	t-ratio	p
Constant	-0.478	5.679	-0.08	0.934
Viscosity	-0.13477	0.03948	-3.41	0.003
Polydispersity	4.086	1.824	2.24	0.036

s = 1.494 R-sq = 46.2% R-sq(adj) = 41.1%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	2	40.318	20.159	9.03	0.001
Error	21	46.858	2.231		
Total	23	87.175			

Table X : Statistical Analysis Data of the Polymer Characteristics on the Burst Release.

Stepwise regression of burst release on 3 predictors, with N = 24

STEP	1	2
CONSTANT	0.4662	-0.5349
Viscosity	-0.0036	-0.0101
T-RATIO	-3.59	-4.28
Polydispersity		0.32
T-RATIO		2.95
S	0.104	0.0896
R-SQ	36.96	55.41

The regression equation is
burst = - 0.535 - 0.0101 Viscosity + 0.323 Polydispersity

Predictor	Coef	Stdev	t-ratio	p
Constant	-0.5349	0.3407	-1.57	0.131
Viscosity	-0.010131	0.002368	-4.28	0.000
Polydispersity	0.3226	0.1094	2.95	0.008

s = 0.08960 R-sq = 55.4% R-sq(adj) = 51.2%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	2	0.20947	0.10474	13.05	0.000
Error	21	0.16860	0.00803		
Total	23	0.37807			

Table XI : Statistical Analysis Data of the Polymer Characteristics on the Steady State Release

Stepwise regression of steady state release on 3 predictors, with N = 24

No variables entered or removed

One-Way ANOVA Analysis

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F	p
FACTOR	5	0.00726	0.00145	0.74	0.601
ERROR	18	0.03514	**0.00195**		
TOTAL	23	0.04240			

LEVEL	N	MEAN	STDEV
381-20	4	0.15245	0.05514
171-15S	4	0.20257	0.06048
381-2	4	0.18790	0.02641
531-1	4	0.16748	0.04074
381-0.1	4	0.16660	0.03882
553-0.4	4	0.15795	0.03391

$LSD = t_{0.025,18} (2MS_E/4)^{1/2} = 0.0656$ while $MS_E = 0.00195$

None of the differences between any two of the polymers is larger than LSD. Therefore, there is no significant difference on the steady state release between the six polymers.

Table XII : Statistical Analysis Data of the Polymer Characteristics on the Total Release in One Hour

Stepwise regression of total release in 1 hour on 3 predictors, with N = 24

STEP	1	2
CONSTANT	0.6419	-0.1267
Viscosity	-0.00388	-0.00887
T-RATIO	-5.35	-5.40
Polydispersity		0.248
T-RATIO		3.26
S	0.0745	0.0622
R-SQ	56.53	71.15

The regression equation is
1 hour = - 0.127 - 0.00887 Viscosity + 0.248 Polydispersity

Predictor	Coef	Stdev	t-ratio	p
Constant	-0.1267	0.2363	-0.54	0.598
Viscosity	-0.008865	0.001643	-5.40	0.000
Polydispersity	0.24763	0.07591	3.26	0.004

s = 0.06215 R-sq = 71.1% R-sq(adj) = 68.4%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	2	0.20003	0.10002	25.89	0.000
Error	21	0.08112	0.00386		
Total	23	0.28115			

Table XIII : Statistical Analysis Data of the Polymer Characteristics on the Total Release in Eight Hours

Stepwise regression of total release in 8 hours on 3 predictors, with N = 24

STEP	1
CONSTANT	0.9551
Viscosity	-0.00341
T-RATIO	-7.50
S	0.0467
R-SQ	71.89

The regression equation is
8 hour = 0.955 - 0.00341 Viscosity

Predictor	Coef	Stdev	t-ratio	p
Constant	0.95515	0.01365	69.97	0.000
Viscosity	-0.0034102	0.0004547	-7.50	0.000

s = 0.04667 R-sq = 71.9% R-sq(adj) = 70.6%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.12252	0.12252	56.26	0.000
Error	22	0.04791	0.00218		
Total	23	0.17043			

APPENDIX II

SELECTION OF SPAN TYPE AND CONCENTRATION

Between Span 60 and 80, in order to select the suitable Span type and concentration that produced the widest differences between the microcapsules that were prepared with the highest molecular weight and the lowest molecular weight polymers, a factorial design was used[109-111]. The variables selected were the highest molecular weight (Mw = 264,362 Daltons) and the lower molecular weight polymer (Mw = 67,840 Daltons), Span 60 and Span 80 and the concentration levels of 0.2 and 1%w/w. The data obtained (yield, particle size, size distribution, drug loading and degree of aggregation) were analyzed by an ANOVA General Linear Model to examine not only the contribution of single factors, but also the effects of interactions between the three preselected variables, namely, polymer molecular weight, surfactant type and concentration. Tabulation of the data were presented in Table I and Table II.

Investigation of both tables demonstrated that polymer molecular weight is a significant factor in the overall characteristics investigated. Particle size was significantly affected by polymer molecular weight, surfactant type and the interaction of the three factors (polymer molecular weight, surfactant type and surfactant concentration). Low molecular weight polymer produced smaller particles and narrower size distribution. Span 60 produced smaller particle size microcapsules than Span 80. However surfactant type is not a significant factor in size distribution. The concentration effect was not significant. However the interaction of polymer molecular weight, surfactant type and

surfactant concentration was significant to particle size as well as particle size distribution.

This preliminary study indicated that the study was worth investigating polymer molecular weight effect further, since polymer molecular weight affected all the pre-selected responses. Span 80 was selected as a suitable surfactant because it gave a trend of lesser degree of aggregation and produced microcapsules with higher drug loading. Therefore 1% Span 80 was used in the final formula.

Table I : Selection of The Surfactant Type and Concentration; The Effects of Polymer Molecular Weight, Span Type and Concentration on the Yield, Physical Properties and Drug Loading.

VARIABLES			RESPONSES				
Polymer Mw (Daltons)	Surfactant Type	Surfactant Concentration(%)	Yield (%)	Particle Size(um)	Size Distribution	Degree of Aggregation(%)	Drug Loading(%)
			98.89	31.91	13.84	13.07	11.55
67,840	Span 80	0.2	96.67	27.68	9.13	11.58	12.57
			100.00	26.53	9.30	13.19	10.30
			100.00	19.24	10.20	12.61	12.99
67,840	Span 80	1.0	97.22	25.44	10.73	11.76	14.40
			100.00	26.91	8.90	13.39	12.68
			94.95	21.52	9.73	14.19	8.96
67,840	Span 60	0.2	96.46	26.11	9.22	13.00	8.80
			100.00	27.37	9.20	10.03	8.93
			95.56	25.38	7.20	13.45	7.77
67,840	Span 60	1.0	97.41	22.81	10.68	13.51	7.77
			100.00	24.91	10.64	11.67	6.16
			93.33	33.32	19.69	9.16	10.87
264,362	Span 80	0.2	94.44	40.06	16.01	10.64	9.30
			94.44	40.17	15.59	7.94	8.10
			93.89	43.55	22.51	8.72	10.94
264,362	Span 80	1.0	92.78	44.95	20.11	8.98	12.36
			95.00	44.87	16.68	10.63	8.46
			91.92	34.53	22.85	11.33	8.78
264,362	Span 60	0.2	92.93	37.13	19.28	13.26	8.30
			100.00	41.73	19.27	10.8	6.13
			86.67	30.35	19.91	8.45	7.01
264,362	Span 60	1.0	91.48	31.58	11.82	13.52	7.27
			100.00	34.75	14.10	11.37	5.76

Table II : p-Value of the Effect of the Factors Using ANOVA Analysis According to 95% Confidence Level.

	FACTORS						
RESPONSES	**Molecular Weight (Mw)**	**Surfactant Type (ST)**	**Surfactant Concentration (SC)**	**Interaction of MW & ST**	**Interaction of MW & SC**	**Interaction of ST & SC**	**Interaction of MW & ST & SC**
Mean Particle Size	**0.000**	**0.006**	0.373	0.079	0.198	0.120	**0.004**
Size Distribution	**0.000**	0.472	0.431	0.864	0.652	0.101	**0.042**
Degree of Aggregation	**0.023**	**0.013**	0.410	0.692	0.640	0.328	0.113
Drug Loading	**0.004**	**0.000**	0.866	0.133	0.956	**0.009**	0.510

The values reported in each cell are the "p-Value". The ones printed in bold are significant at $\alpha = 0.05$.

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