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Formulation of HPV Dry Powder Wafers for Sublingual Vaccination

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Abstract

Vaccination has been used to prevent diseases since the early 1800s.Throughout the history of vaccination, attention has only been paid to the type of virus that can be administered instead of the method of vaccination. Traditional needle-dependent vaccination requires sanitation and refrigeration which is not available in developing countries such as India, where viruses such as the human papillomavirus (HPV) are very prevalent. The goal of this study was to develop a sublingual wafer formulation that is robust and heat-stable. The sublingual wafer delivery method is expected to lower the cost of vaccination because of the ease of transportation and storage. Dry powder vaccine formulations were prepared using the *Sievers et al* patented Carbon Dioxide Assisted Nebulization with a Bubble Dryer (CAN- BD) drying process. Excipients commonly used in the pharmaceutical industry for oral tablet formation were tested for wafer formation with the CAN-BD processed protein powder. Desired wafer properties were tested, i.e. binding capabilities, target dissociation time.

1. Introduction

1.1 History of vaccines

Perhaps the greatest success story in public health is the invention of vaccines. Millions of lives were saved and diseases were prevented from wreaking havoc in the body. Human beings have benefited from vaccines ever since Edward Jenner's creation of smallpox vaccine in 1790. Our road to discovering effective vaccination is neither neat nor direct. For example, during the 1830s, a vociferous anti-vaccination movement emerged. Mandatory vaccination was often misunderstood. When a Britain passed compulsory vaccination laws in 1821, the working-class Britons viewed the mandate as a direct government assault on their communities by the elite class.[14] Although scientist and doctors alike faced irrational protests, they still managed to develop many vaccines that would become essential for people around the world. Dr. Peebles was the first to isolate the measles virus and John Franklin Enders was responsible for the discovery of the polio virus. With the advent of vaccines, children of the developed nations were vaccinated, which contained outbreaks of illness such as smallpox. However, developing countries still lack the resources to effectively vaccinate most of the population. This has paved the way for research to develop more convenient and cost-effective vaccination method.

1.2 Sublingual Vaccination

Sublingual vaccination has been used many years to deliver drugs and small molecules to the bloodstream. The idea of sublingual vaccination is simple: a form of the vaccine (solution, suspension, wafer, film strip, etc.) is placed under the tongue. Due to the presence of high density blood vessels in the mucous membranes, the immune system cells capture the vaccine

and move the vaccine through the body without significant degradation. According to Dr. Cecil Czerkinsky, sublingual vaccination appears to disseminate immunity to a broader range of organs than the classical routes of injecting or ingesting vaccines.[7] Sublingual vaccination eliminated the need for the use of needle and the risks associated with the needle injection method. Overall, sublingual vaccination matches nasal vaccination in terms of the degree of immune response generation[7]; however, the sublingual route did not allow viruses to travel into the central nervous system, a rare but potentially harmful complication of intranasal vaccination. This hazard of nasal delivery is referred to as cribriform plate penetration.

1.3 HPV Vaccine

The human papillomavirus is a leading cause of cervical cancer which is the second most common cancer among women in the world. In 2008, there were 529,000 new cases of cervical cancer which resulted in 274,000 deaths (WHO 2008).[20] Cervical cancer is caused by persistent genital infection, and, according to the WHO, virtually all of the cervical infection is linked with the HPV virus, which is the most common viral infection of the reproductive tract. Although present VLP HPV vaccine has demonstrated remarkable efficacy, the high cost of the vaccine limit the availability to developing countries where resource are scarce. In 2008, the WHO estimated that more than 85% of the cervical cancer deaths are in developing countries. In addition to the high cost, the current HPV vaccines are needle-dependent and require refrigeration which hinders effective delivery in resource-poor areas[16]. As an alternative to the injectable HPV vaccine, the Sievers's group developed dry vaccine wafers which are thermally stable, and convenient for storage and delivery. Due to the wafer's thermal stability and ease of transportation, the cost of the vaccine will be greatly reduced which will enable the vaccine to

become available in underdeveloped parts of the world. Several excipients were tested for their wafer formation facilitation abilities.

1.4 Myo-Inositol

Myo-Inositol constitutes the majority of the placebo HPV powder. It was chosen due to its ability to increase viral stability and maintain relatively low hygroscopicity. Inositol is a naturally-

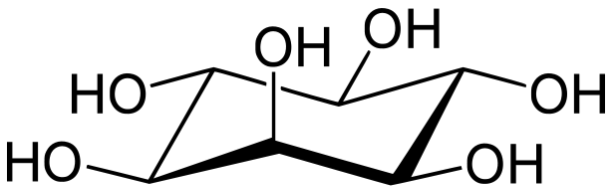

Figure 1. Structure of *myo*-Inositol from Sigma Aldrich

occurring nutrient found in various forms, the most common being *myo*-inositol. It is found in phospholipids which function as cellular mediators of signal transduction in metabolic regulation, and growth. Commercially, it is used in baby formula and derived from rice. Excipients that are plant derived from rice. Excipients that are plant-derived rather than animal-derived have the advantage of being free from hazardous contaminants.

1.5 Excipients Utilized

Four excipients were tested but only 2 were ideal for wafer formulation. Microcrystalline Cellulose (MCC) and (hydroxypropyl) methyl cellulose (HPMC) were able to aid wafer formation when they were present in relatively small amount. Both compounds are known pharmaceutical excipients. Microcrystalline cellulose is purified, partially depolymerized cellulose that is prepared by treating alpha-cellulose. MCC consists of crystalline aggregates

which are obtained through hydrolytic degradation of unpurified region of the cellulose polymer.

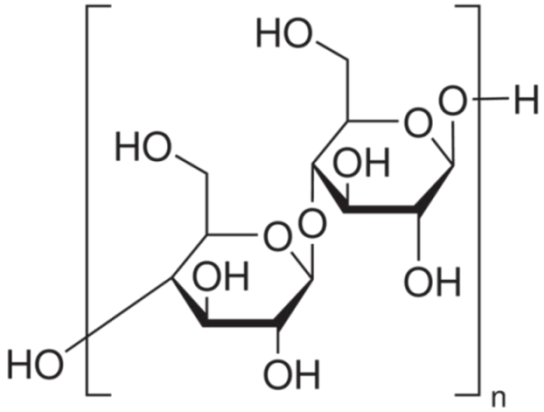

Figure 3. Microcrystalline Cellulose
provided by Sigma Aldrich

As an insoluble fiber, microcrystalline cellulose (MCC) does not get absorbed into the bloodstream; hence, it does not cause toxicity when taken orally[17]. The biggest disadvantage of the MCC is its low water-solubility[10]. Wafers that contain high amounts of MCC may demonstrate slow dissolution time. However, for this study, MCC content can be as little as 20% of the wafer mass, which is sufficient for robust wafer formation.

(Hydroxypropyl) methylcellulose (HPMC) is the other excipient that aided in wafer formation. HPMC is a non-ionic, water-soluble polymer that is derived from cellulose. In addition to pharmaceutical usage, HPMC is employed for making cement renders, ophthalmic

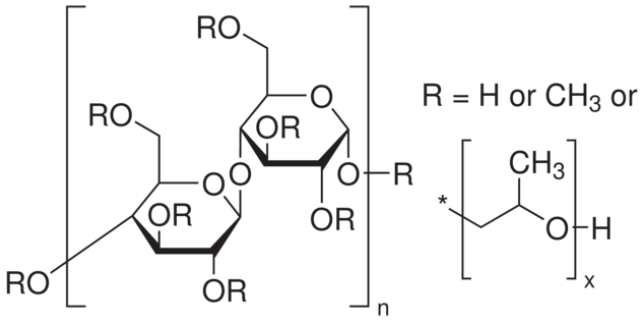

Figure 2 (Hydroxypropyl) methylcellulose
provided by Sigma Aldrich

lubricant, eye drops, etc. Benefits of using this excipient include its reversible gelation upon heating; HPMC is surface active and enzyme resistant. Hydrophilic polymer such as HPMC interacts with water via their hydroxyl groups. Water can plasticize the HPMC through hydrogen bonding which results in strong and compact formation which is very favorable for manufacturing sublingual wafer tablet by direct compression.[11, 13]

2-Experimental

2.1 Formulation tested

Four excipients were tested: Polyvinylpyrrolidone(PVP), HPMC, MCC, Corn Starch (high amyolose). Each formulation had different amount of excipients ranging from 20%-50% of the total wafer mass. The placebo HPV powder consisted of 98.5% Myo-Inositol and 1.5% Leucine, with a trace amount of ammonium acetate in order to improve respiratory fractions. Formulations that showed robust wafer formation were tested with lower excipient content. For example, the ability to produce robust wafers from 50% to 20% excipient content was continuously demonstrated by the MCC. However, the ability to produce robust wafers at 40% make up of total wafer mass was not successfully demonstrated by the PVP excipient; hence, no trials were conducted at lower excipient content.

2.2 Experimental apparatus.

Wafers were made with the KBr Port-A-Press Kit from the International Crystal Laboratories (Figure 4). A wrench was used to ensure that the same amount of pressure was applied to each pellet, thereby facilitating reproducibility of result. The wrench also ensured easy tweaking of the applied pressure. A strain gauge panel meter from Omega was attached to the pellet press to ensure accurate reading of results (Figure 5).

Placebo Powder was dispensed into a 4mm-sized die for compression (Figure 4). The 4mm wafer served to mimic the wafer size that will be tested during clinical trial.

Placebo powder was introduced into the die with a wafer dispensing device (Figure 6) that was developed by Dr. Stephen Cape. The device dispenses a consistent mass of powder to the die, thereby facilitating the manufacture of wafers with masses in a narrow range. During the production of wafers, the pellet press was placed in a Terra Universal glove box in which the relative humidity (%RH) of the environment can be adjusted. The glove box served as a humidifying chamber during the dry, winter season in Colorado. Water vapor and nitrogen gas were simultaneously pumped into the chamber to keep the relative humidity at a constant level which minimized the effect of humidity fluctuation in the lab. The glove box is made of static-dissipative PVC plating which reduces the effect of static forces between the micrometer-sized particles in the placebo powder. Static between particles negatively influenced wafer robustness.

Figure 4 Pellet press and die set for wafer making
(Provided by http://www.internationalcrystal.net/,
visited on March 19th, 2012

Figure 5 Strain gauge panel . (Image provided by gauhttp://www.omega.com/pressure/psc.html, visited March 18th, 2012

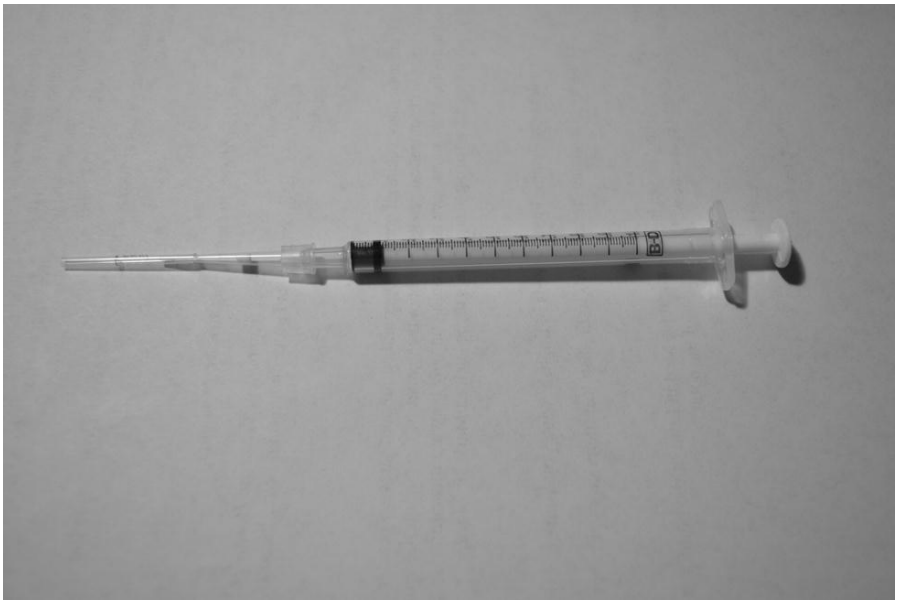

Figure 6. Powder dispensing device that was invented by Dr. Stephen Cape for the use of loading in the die a consistent amount of powder for wafer making.

2.3 Materials

The excipients tested for this study were: microcrystalline cellulose, (hydroxypropyl) methylcellulose with a viscosity of 100 centipoise (CP), polyvinylpyrrolidone of 10k molecular weight, and corn starch with trace amylose. The MCC used for this study was from Arcos Organics with an average particle size of 50µm. The rest of the excipients were obtained from Sigma Aldrich. The polyvinylpyrrolidone used for this study did not come as fine-sized particles therefore milling of the particle was required. The morphology of the milled and un-milled PVP was captured by using a scanning electron microscope (Figures 8, 9). The placebo powder for the HPV vaccine consisted of *myo*-inositol from Tsuno Rice Fine Chemicals (Katsuragi, Japan) (Figure 7) in addition to L-leucine. The placebo HPV powder was manufactured by Dave McAdams with the CAN-BD process (1200 psi CO_2, 65 ^{o}C, 0.3 mL/min H_2O flow rate).

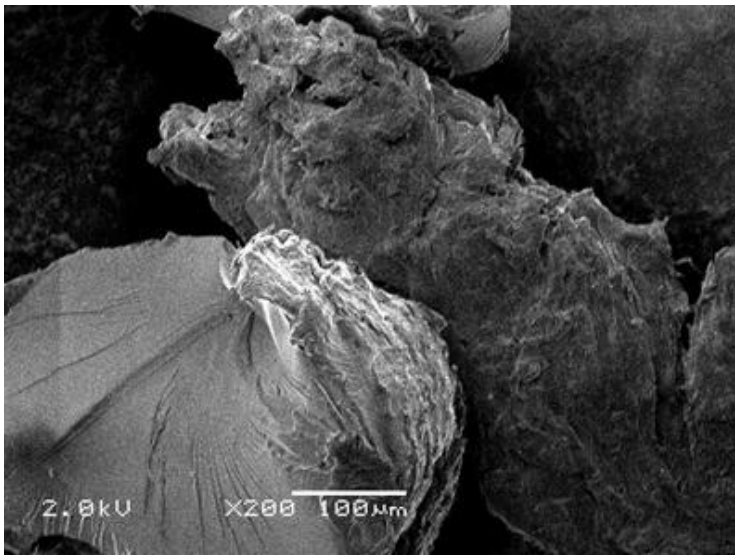

Figure 7 Scanning electron micrograph of unprocessed *myo*-inositol (Tsuno). Magnification-200x, Bar=100µM

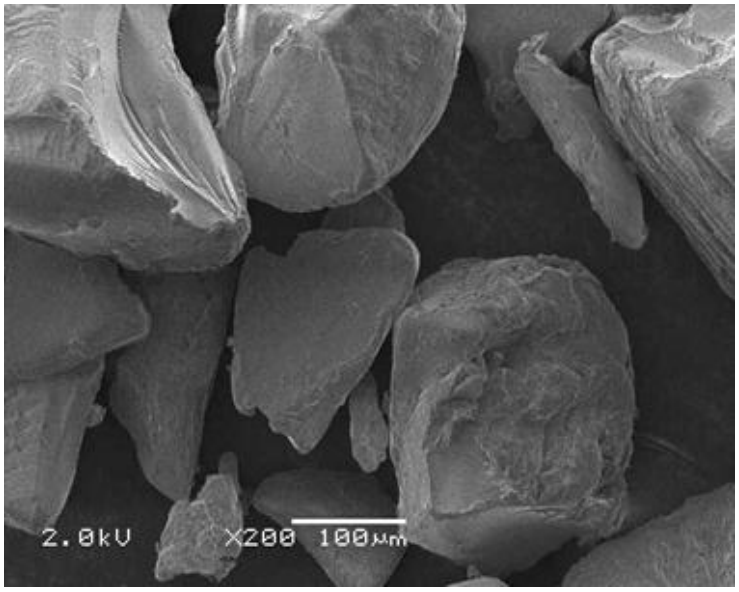

Figure 8. Scanning electron micrograph of unmilled polyvinylpyrrolidone. Magnification=200x, Bar=100µM

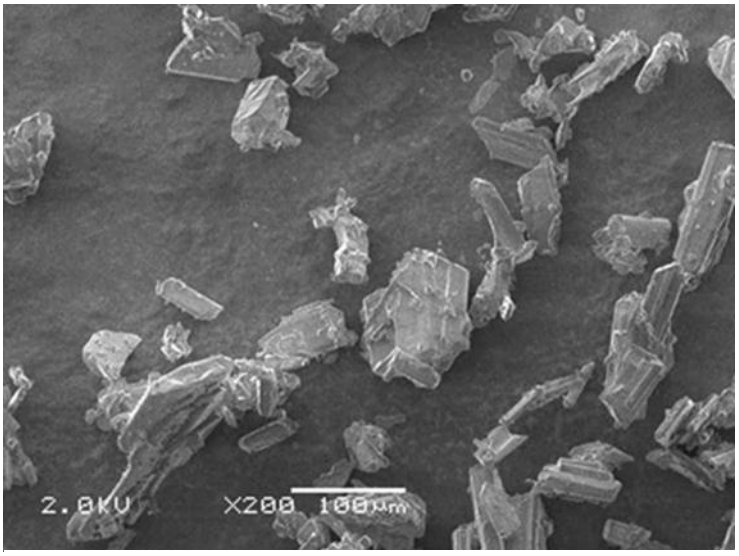

Figure 9 Scanning electron micrograph of milled polyvinylpyrrolidone. Magnification=200X. Bar=100µM

2.4 Dissolution Equipment and Method

The dissolution properties of wafers were tested. A scintillation vial filled with 3 mL of water at 37^{o} C (simulation of salivary temperature) was used to dissolve the vaccine wafers. No stirring or shaking of the dissolution vials was required. The water was kept in a warm water bath. Before every trial, the temperature was measured in order to confirm that it was at 37^{o} C.

2.5 Preparation of Wafer Formulation and Manufacturing of Wafers

When the formulations were produced, excipients and placebo powders were milled then they were thoroughly mixed with a vortex mixer. This was done in order to avoid uneven distribution of different excipients which would yield misleading results. As the wafers were made, the pellet press was held at the desired pressure for 30 seconds as a mean of improving wafer integrity.

Towards the beginning of the study, wafers were made at a humidity of 22±1 % relative humidity. However, wafers made under this condition only had a deceivingly robust appearance. When those wafers were transferred from vial to vial by using a pair forceps, crumbling of the wafers occurred. In order to solve this problem, more excipients were added into the formulation.

Dr. Stephen Cape suggested that making wafers at a higher humidity would improve wafer integrity without the need to add more excipient. Subsequently, wafers were manufactured at 40 %RH environment. Initially wafers would be made at 22 %RH and be left in a humidity chamber that was set at 40 %RH for 20-50 minutes. However, employing this method did not produce more robust wafers than wafers made from the original method.

Another method involved leaving formulated powder at 40 %RH for 20 minutes; then wafers were made at 40 %RH conditions. The powder was noticeably stickier, and the wafers made from these powders were very robust even when a relatively low pressure was applied (20lb). During subsequent trials, placebo powders were exposed to a 50%-60 %RH environment for 20 minutes before wafers were made.

3-Results

3.1 Characterization of wafers

All the wafers made were scored with a number to characterize their robustness, with 1 being the least robust, to 6 being the most robust (Figure 10).

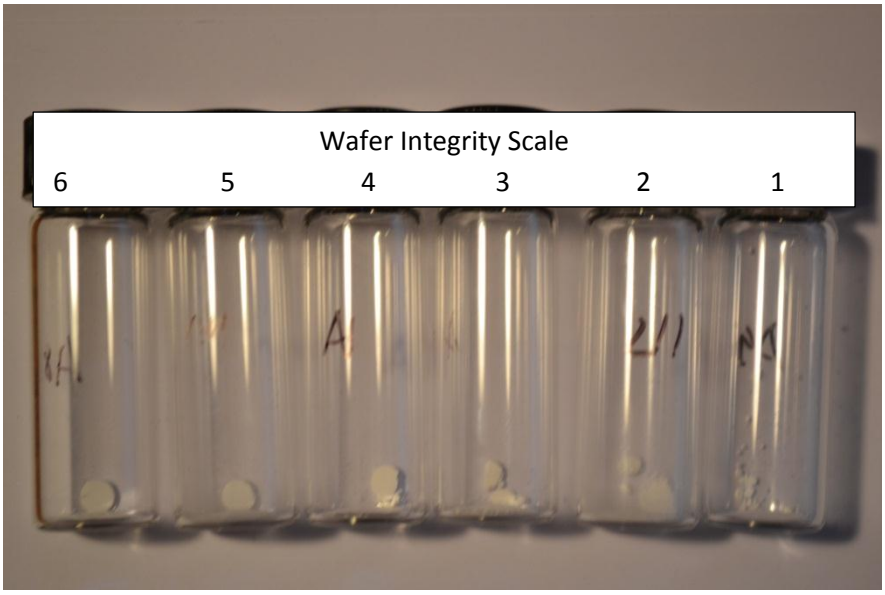

Figure 8. Image of wafers at different assigned integrity level

3.2 Factors Affecting Wafer Robustness

Humidity, applied pressure, and excipient content were all adjusted to test their relative effect on the wafer's robustness. The placebo powder's ability to form wafers without additional excipients was tested, and no robust wafers were formed. The placebo wafers with no excipients had an integrity ranging from 1-2 (Table 2). For each formulation, the higher the applied pressure, the more robust the wafer will be (Figure 14). In addition, added moisture content to the wafers compensated for low applied pressure. For example, at a pressure of 20 lb and 20%

RH humidity level, wafers made from different formulations can be described as having a deceivingly robust appearance. These wafers would generally crumble when being transferred with a pair of forceps. At 20 %RH, a pressure of 85 lb. of force was sufficient in producing a wafer with robust integrity. At 50 %RH, robust wafers can be made by applying as little as 20 lb. of force (Table 6). The amount of excipients that were present in a wafer also increases wafer robustness (Figure 12). Lastly, the mass of the wafers did not seem to have a significant effect on wafer integrity (Figure 11).

3.3 Factors affecting wafer disintegration

The content of excipients is one of the primary factors that affect wafer disintegration time. Although the original intent was to test wafer's dissolution properties, none of the wafers that were produced for this study completely dissolved, instead, the wafers disintegrated into small particles, therefore, disintegration properties were observed and recorded. Robust wafers needed to disintegrate in a reasonable amount of time (under 2 minutes) to be practically useful. Formulations that disintegrated too slowly (requiring >2 minutes) were not deemed as a viable option. PVP measured to have the slowest disintegration time whereas MCC and the mixture of HPMC and MCC were measured to have short(less than 30 seconds) disintegration time. The pressure applied had minimal effect on the dissolution time of the wafer (Figure 15), in addition, the wafer mass also did not significantly affect the dissolution time.

3.4 Viable Formulations

The microcrystalline cellulose and the 33% HPMC, 66% CMC mixture were demonstrated to have the best wafer vaccine characteristics. Both formulations disintegrated in less than 30 seconds and can be made into a robust wafer with an applied pressure of 30 lb. or less. Most

importantly, the two excipient formulations only required approximately 20-30% of the wafer mass to produce a robust wafer (Table 1), which allowed for maximal amount of active vaccine protein content to be present in the wafer.

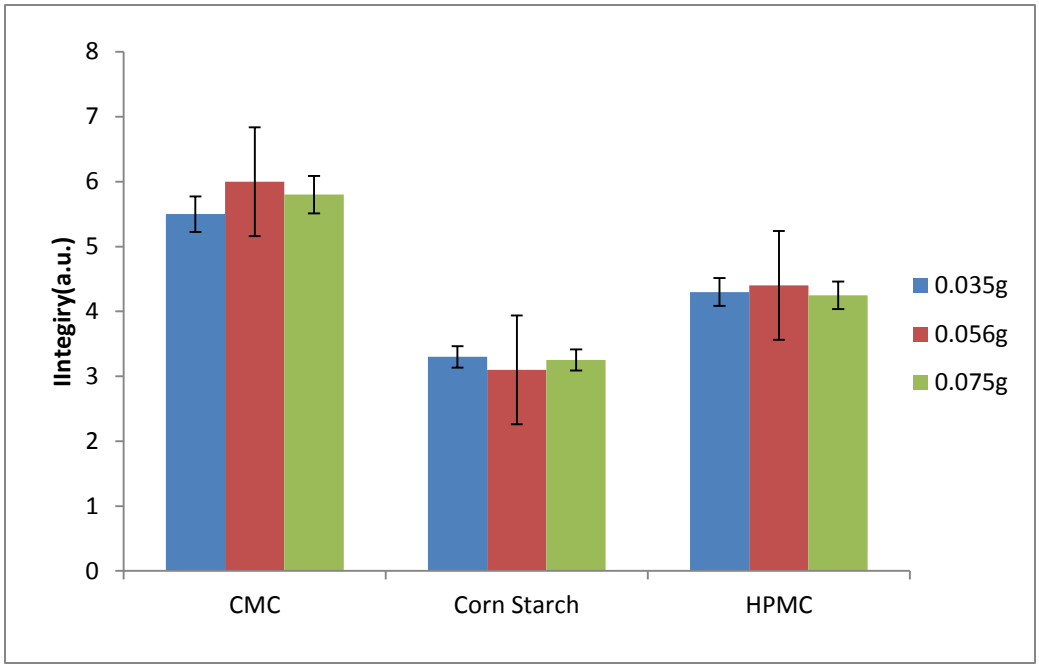

Figure 11. Average wafer integrity based on wafer mass. Errors bars indicate one standard deviation from the mean

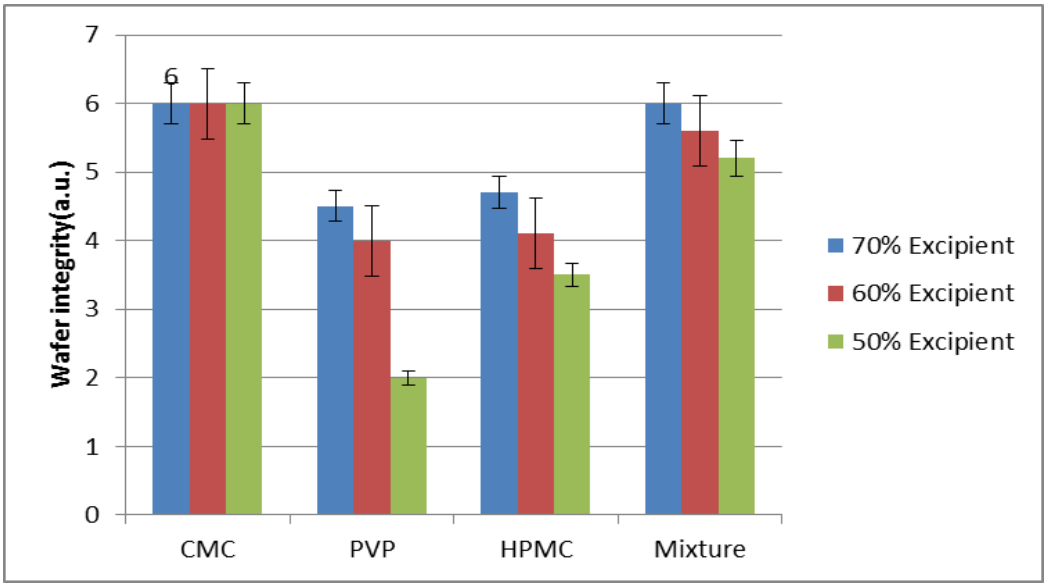

Figure 12. Average wafer integrity in relation to percent excipients in wafer mass. Errors bars indicate one standard deviation from the mean

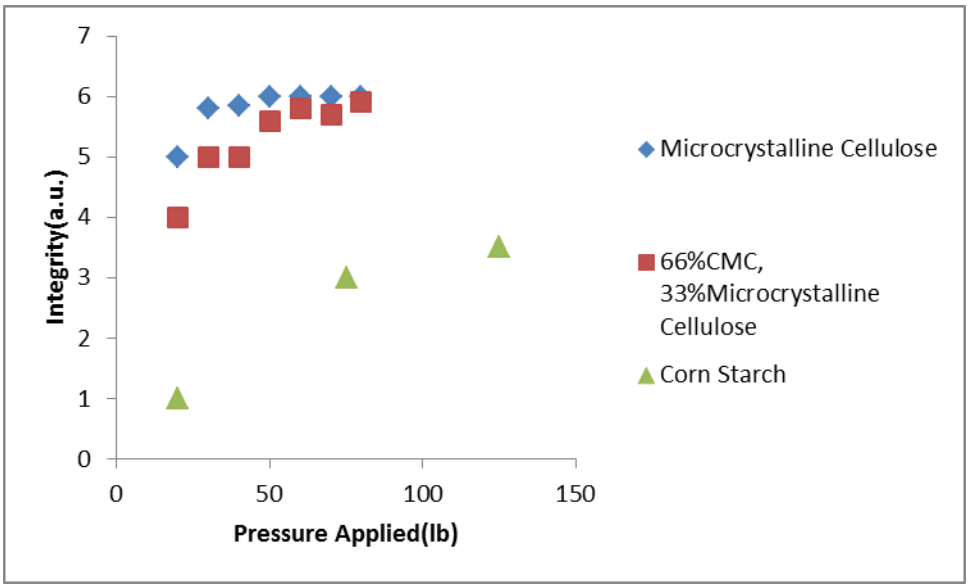

Figure 13. Wafer Integrity in relation amount of pressure applied

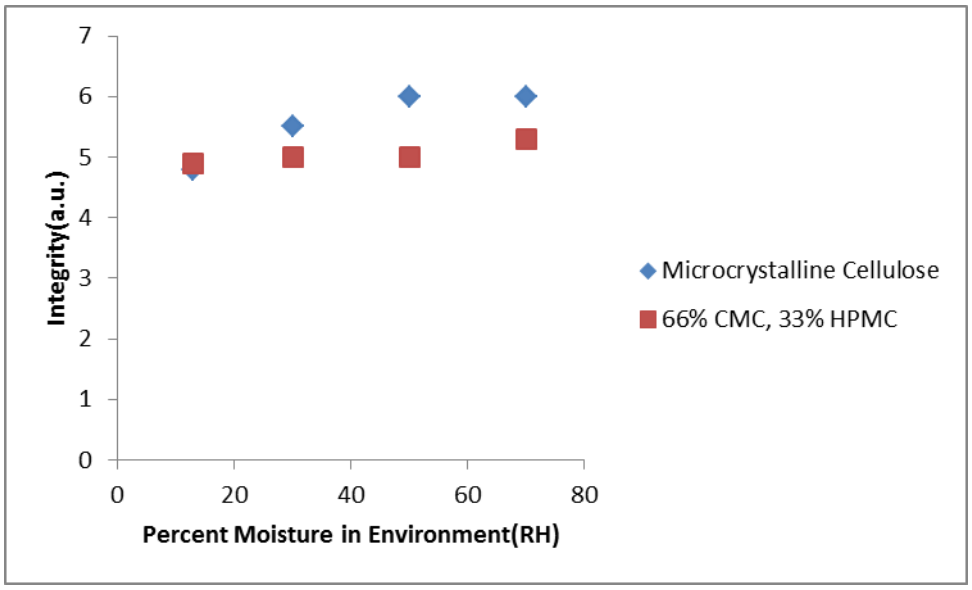

Figure 14. Wafer Integrity in relation to the percent of moisture in the environment where the wafers are made.

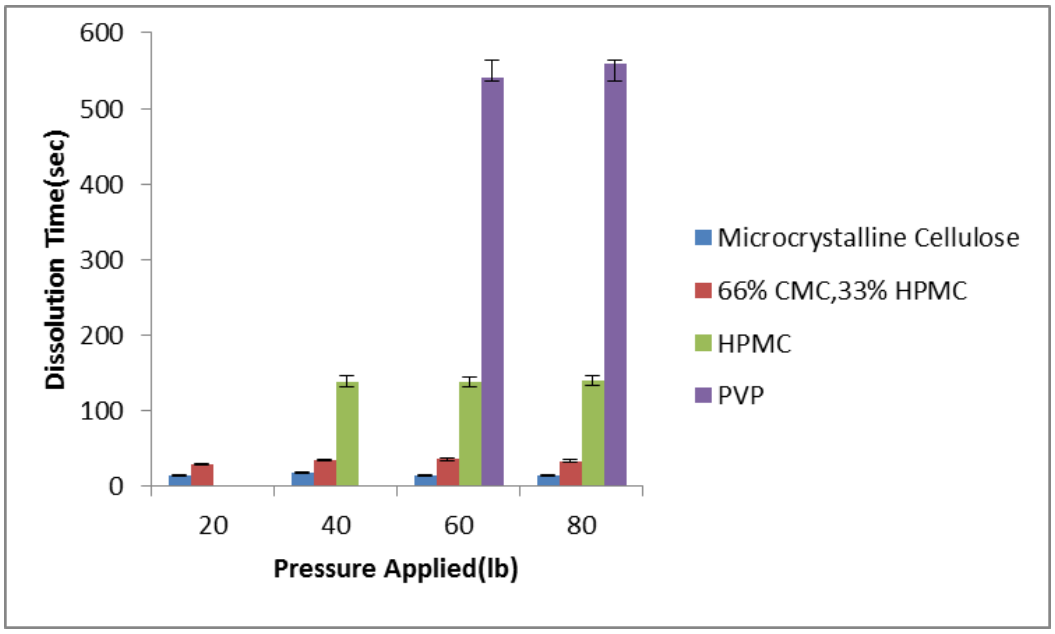

Figure 15. Disintegration time of wafers in relation to pressure applied in 3 mL of 37^{o} C water

Table 1 Dry Powder Optimization

Dry Powder Composition	Excipients for Wafer Formation	Processing Conditions	Wafer Robustness[2]	Average Dissolution Time[1]
98.5% *myo*-inositol, 1.5% leucine	Sorbitol (50% of total wafer mass)	70 atm compression pressure 6-8% RH 30 seconds compression time 4-mm die set used	Medium	80- 88 seconds
98.5% *myo*-inositol, 1.5% leucine	Polyvinylpyrrolidone (50% of total wafer mass)	175 atm compression pressure 10% RH 30 seconds compression time 4-mm die set used	Medium	420-460 seconds (7-8 minutes)
98.5% *myo*-inositol, 1.5% leucine	Starch (from corn with high amylose) (50% of total wafer mass)	350 to 440 atm compression pressure 20% RH 30 seconds compression time 4-mm die set used	Low	10-15 seconds
98.5% *myo*-inositol, 1.5% leucine	Microcrystalline cellulose (30% of total wafer mass)	35 to 70 atm compression pressure 50-70% RH 30 seconds compression time 4-mm die set used	High	16-20 seconds
98.5% *myo*-inositol, 1.5% leucine	Microcrystalline cellulose (33% of total wafer mass) with hydroxypropylmethyl cellulose (17% of total wafer mass)	70 to 140 atm compression pressure 50-70% RH 30 seconds compression time 4-mm die set used	High	60-90 seconds
97% *myo*-inositol, 1.5% leucine, 1.5% bovine serum albumin	Microcrystalline cellulose (33% of total wafer mass)	35 to 70 atm compression pressure 50-70% RH 30 seconds compression time 4-mm die set used	High	20-23 seconds
97% *myo*-inositol, 1.5% leucine, 1.5% bovine serum albumin	Microcrystalline cellulose (33% of total wafer mass) with hydroxypropylmethyl cellulose (17% of total wafer mass)	35 to 70 atm compression pressure 50-70% RH 30 seconds compression time 4-mm die set used	Medium	60-90 Seconds

[1]Dissolution tests were conducted with 3mL of 35.5-37.0^{o} C water.

[2]Wafer robustness was assessed as low, medium and high. Low signifies a poorly formed wafer where it is unable to stay intact. Medium robustness represents wafers that stayed intact but may have small chunks coming off during transferring. High robustness indicates a wafer that is intact with little to no loose powder coming off.

Table 2 Placebo Powder Wafer Characterization

Vial	EmptyVial Mass (g)	FullVial Mass(g)	Wafer Mass(g)	Pressure(psi)	Humidity	Wafer Integrity
1	5.70125	5.72996	0.02871	200	11	2
2	5.74651	5.79069	0.04418	200	9.1	1
3	5.68954	5.774453	0.084913	200	8	2
4	5.67013	5.7084	0.03827	300	6.6	1
5	5.72156	5.76933	0.04777	300	13	2
6	5.69906	5.74437	0.04531	300	9.5	2

4-Discussion

The primary objective of this study was to develop a wafer formulation that is robust and can disintegrate in water (surrogate for saliva) in a reasonable amount of time. The robustness of a wafer is usually proportional to the amount of excipient added. The placebo powder does not exhibit adhesive properties because the placebo powder was designed to have micro particles which do not agglomerate during dry powder inhalation. Ammonium acetate was also added in order to improve respiratory fractions. This eliminated the option of developing a placebo protein powder that contains adhesive characteristics.

During relatively hot (~80^{o}C) and humid conditions (<60 %RH), the placebo (DM110708) powder formed clumps which could have been due to the high relative humidity in the laboratory. The placebo powder is mainly composed of *myo*-inositol which contains 6 polar hydroxyl groups. The relative high moisture may have induced hydrogen bonding between the *myo*-inositol particles.[18]

Excipients that possessed more polymer-like properties were chosen to improve wafer robustness; however, there is the chance that the robust wafer might not disintegrate in an acceptable time-frame. The initial tests with polymers were conducted with polyvinylpyrrolidone, although it is a water soluble polymer, no complete dissolution occurred, however, complete disintegration in 3 mL of 37^{o} C water could take up to 4-6 minutes, which may be too long for efficient absorption under the tongue. A more hydrophilic polymer was desired in order to ensure timely dissolution. Dr. Stephen Cape has suggested microcrystalline cellulose as an option. MCC is known for its low solubility,[10] however, the MCC-placebo wafers all disintegrated within 30 seconds during dissolution testing. Dry mixing the hydroxyl propyl methyl cellulose also yielded robust wafers;

however, the disintegration time is around 2-3 minutes which may not be fast enough for wafer disintegration, although this is still being evaluated. The idea behind mixing the HPMC and MCC was to have the MCC help the wafer dissolve in time while the HPMC holds the wafer together under low pressures. However, extensive trials showed that wafers formed from the MCC excipients and the wafers formed from the HPMC -plus MCC mixture had similar robustness.

Pressure was applied to the powder mixtures in order to make them robust; however, the active protein or viral envelope in the vaccine may lose activity under high pressure. Studies were done to demonstrate the correlation between protein unfolding and high pressure[19]. Wafers were exposed to moisture, which can further enhance binding properties of the polymer excipients[12], in order to reduce the amount of pressure applied. However, moisture is also known to destabilize proteins as the hydrophilic exterior interacts with water, which also can result in protein unfolding[18]. This possible effect of moisture was minimized by drying out the wafer overnight with desiccants in a sealed vacuum pack. Karl Fischer titration was done on wafers in order to ensure that the wafers did not contain excessive moisture content. The moisture content of was determined to be around 0.08%-0.13%.

Upon disintegration, wafers that contain different excipients formed various kind of solution. The viscosity of solutions of HPMC was high because it is often used as a thickener. Sigma Aldrich product literature indicates that HPMC solubility is around 10mg/ml, but the water temperature needs to be 90 C^{o}, stirring and agitation is also required. MCC, on the other hand, dissociates into micro particles, however, it does not go into solution which maybe less conducive for absorption under the tongue.

In the section describing method used, it was mentioned that formulated powder which was left exposed to moisture formed better wafers than wafers that were exposed to moisture after they have already been made. This was due to the greater surface area for moisture exposure when exposing the powder before making the wafers[12]. In addition, moisture is spread evenly among the micro particles. The method where wafers were exposed to moisture after its formation only coats the outside of the wafer where the inside may remain dry and brittle. Karl Fischer was done on wafers made from the 2 methods where wafers were exposed to moisture and placebo powder was exposed to moisture before wafers were made. The difference in moisture content was only 4-6% where this difference in moisture content should not be significant enough to cause a difference in protein stability.

5-Conclusion

Through extensive trials during this study, two formulations was chosen: 70% (*myo*-Inositol, Leucine, ammonium acetate) and 30% MCC; 60% (*myo*-Inositol, Leucine, ammonium acetate) and 40% (2/3 HPMC, 1/3 MCC.) These two formulations performed satisfactorily both in wafer quality testing and dissolution tests. These two formulations can be made at 40% moisture conditions which, after Karl Fischer testing, do not add too much moisture to the wafer itself. This lessens the possibility of protein destabilization.

Although this study was primarily aimed toward application in HPV vaccine development, the sublingual wafer form of vaccines may be applied to other diseases such as the measles. If clinical trial results demonstrate that the efficacies of sublingual wafer vaccines are comparable to that of the needle-injected form, the sublingual form may be a solution for vaccination in underdeveloped regions of the world. The research on sublingual wafer formulation signals a new direction in vaccine research which may make vaccination more affordable, and efficient.

Acknowledgements

This research project would not have been possible without the support of many people. I wish to express my gratitude to professor Sievers who gave me the valuable opportunity to participate in such an amazing and meaningful project that could potentially vaccinate millions of underprivileged children. Deepest gratitude is also due to Dr. Stephen Cape for guiding me throughout research. I would also like to thank Dave McAdams, J'amie Manion who was always available to help me whenever I have questions regarding certain equipment in the lab. Finally I would like to extend my thanks to my supervising committee for taking the time to read this thesis. It has been an amazing two years in the Sievers's group, I have learned very much and this project would not have been possible without you. Thank you very much.

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Appendix

Table 3 Microcrystalline Cellulose Wafer Characterization with 40% Cellulose Microcrystalline as Wafer Mass

Powder Content: 60% DM110308 , 40% Cellulose Microcrystalline (50uM)						
Wafer Making Condition: Humidity-13% RH, 4mm die, kept at tested pressure for 30 seconds						
Dissolution Condition: 3mL wafer, water temp: 35.0 C- 37 C						
Sample	Empty(g)	Full(g)	Pressure(lb)	Wafer Integrity	Dissolution Time(sec)	
J7	6.16244	6.17031	85	6	35	
J8	6.22717	6.23123	85	6	30	
J9	6.18541	6.19301	85	5	29	
J10	6.18628	6.19528	100	4	N/A	
J11	6.17517	6.18318	100	5	20	
J12	6.18457	6.19301	100	5	21	
C1	6.32724	6.33352	100	6	513	
C2	6.35999	6.36761	100	6	475	
C3	6.31315	6.32279	100	6	490	
C4	6.29996	6.31063	80	5	460	
C5	6.31854	6.32756	80	5	455	
C6	6.36231	6.37109	80	5	480	

Table 4 .Microcrystalline Cellulose Wafer Characterization with 30% Cellulose Microcrystalline as Wafer Mass

Powder Content: 70% DM100308, 30% Cellulose Microcrystalline 50uM					
Wafer Making Condition: Humidity-13% RH, 4mm die, kept at tested pressure for 30 secon					
Dissolution Condition: 3mL wafer, water temp: 35.0 C- 37 C					
Sample	Empty(g)	Full(g)	Pressure(lb)	Wafer Integrity	Dissolution Time(sec)
F1	6.30428	6.31297	60	4	N/A
F2	6.33597	6.34509	60	4	N/A
F3	6.29178	6.30026	60	4	N/A
F4	6.34655	6.35538	80	4	N/A
F5	6.31245	6.32265	80	4	N/A
F6	6.35204	6.36422	80	4	N/A

Table 5 Microcrystalline Cellulose Wafer Characterization with 40% as Cellulose Microcrystalline as Wafer Mass at an Exposed Humidity of 40% RH

Powder Content: 60% DM110308 , 40% Cellulose Microcrystalline (50uM)						
Wafer Making Condition: Humidity-**40%** RH, 4mm die, kept at tested pressure for 30 seconds						
Dissolution Condition: 3mL wafer, water temp: 35.0 C- 37 C						
Sample	Empty(g)	Full(g)	Pressure(lb)	Wafer Integrity	Dissolution Time(sec)	
Z1	6.59829	6.60402	40	6	Instant	
Z2	6.55061	6.55685	40	6	Instant	
Z3	6.58216	6.59264	30	6	Instant	
Z4	6.49783	6.50405	40	6	Instant	
Z5	6.53815	N/A	30	N/A	Instant	
Z6	6.41846	6.42391	40	6	Instant	
Z7	6.60324	6.60972	30	5	Instant	
Z8	6.50961	6.50743	30	6	Instant	
Z9	6.61708	6.62291	30	6	Instant	
Z10	6.53721	6.54545	40	6	Instant	
Z11	6.48279	6.49156	40	6	Instant	
Z12	6.55291	N/A	30	N/A	Instant	

Table 6 Microcrystalline Cellulose Wafer Characterization with 50% as Cellulose Microcrystalline as Wafer Mass

Powder Content: 80% DM110308 , 20% Microcyrstalline Cellulose						
Wafer Making Condition: Humidity-**50% RH**, 4mm die, kept at tested pressure for 30 seconds						
Dissolution Condition: 3mL wafer, water temp: 35.0 C- 37 C						
Sample	Empty(g)	Full(g)	Pressure(lb)	Wafer Integrity	Dissolution Time(sec)	
X1	6.17301	6.18401	20	6	Instant	
X2	6.23274	6.24521	20	6	Instant	
X3	6.26265	6.27821	20	6	Instant	
X4	6.19648	6.20459	20	6	Instant	
X5	6.25059	6.26105	20	6	Instant	
X6	6.20125	6.21145	20	6	Instant	
X7	6.24616	6.25606	10	4	Instant	
X8	6.15557	6.15857	10	4	Instant	
X9	6.19921	6.20154	10	4	Instant	
X10	6.32228	6.32229	10	3	Instant	
X11	6.26526	6.27811	10	4	Instant	
X12	6.20767	6.21524	10	3	Instant	

Table 7 (Hydroxypropyl)Methylcellulose Wafer Characterization with 50% HPMC as Wafer Mass

Powder Content: 50% DM110308 , 50% HPMC 100CP						
Wafer Making Condition: Humidity-20% RH, 4mm die, kept at tested pressure for 30 seconds						
Dissolution Condition: 3mL wafer, water temp: 35.0 C- 37 C						
Sample	Empty(g)	Full(g)	Pressure(lb)	Wafer Integrity	Dissolution Time(sec)	
A1	6.33881	6.34653	100	6	163	
A2	6.32163	6.32768	100	6	175	
A3	6.35763	6.36541	100	6	150	
A4	6.32565	6.33398	125	6	170	
A5	6.32981	6.33623	125	6	180	
A6	6.31761	6.32616	125	6	163	

Table 8 Polyvinylpyrrolidone Wafer Characterization with 50% Polyvinylpyrrolidone as Wafer Mass

Powder Content: 50%{Myo(95%)-Leucine(5%)}, 50%PVP High Molecular Weight 20%RH							
Dissolution Condition: 3mL water, water temp-23C							
Vial	Empty(g)	Full(g)	Wafer(g)	Pressure(lb)	Die Size(mm)	Wafer Integrity	Dissolution Time
A	6.31977	6.36888	0.04911	100	7	4	8 min 39 sec
B	6.35781	6.39018	0.03237	100	7	2	8 min 22sec
C	6.33999	6.38843	0.04844	100	7	6	7 min 15 sec
D	6.40749	6.44594	0.03845	100	7	6	9 min 08 sec
E	6.38951	6.43391	0.04441	WGNM*	7	2	8 min 56 sec
F	6.29039	6.33272	0.04233	WGNM*	7	6	7 min 56 sec
G	6.41472	6.45008	0.03536	WGNM*	7	2	7 min 12 sec
H	6.36644	6.42125	0.05481	WGNM*	7	3	9 min 30 sec
I	6.28718	6.29645	0.00927	50	4	6	6 min 27 sec
J	6.36221	6.36961	0.00741	50	4	6	5 min 41 sec
K	6.31329	6.31952	0.00623	50	4	6	5 min 20 sec
L	6.37494	6.38618	0.01124	50	4	6	6 min 13 sec

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Table 9 Polyvinylpyrrolidone Wafer Characterization with 60% PVP as Wafer Mass

Powder Content: 40%[Myo(98.5%)-Leucine(1.5%)], 60% PVP High Molecular Weight						
Date: Oct 28th, 2010 Humidity: Under 10% RH Die size: 4mm						
Vial	Empty(g)	Full(g)	Wafer(g)	Pressure(lb)	Wafer Integrity	Dissolution Time
C1	6.29263	6.320271	0.02764	50	2	4 min 34 sec
C2	6.38587	6.40155	0.01568	50	6	3 min 42 sec
C3	6.33664	6.34963	0.01299	50	6	3min 59sec
C4	6.36432	6.37671	0.01239	WGNM	4	4 min 09 sec
C5	6.3445	6.35432	0.00982	WGNM	6	5 min
C6	6.33471	6.34481	0.01012	WGNM	6	4 min 3 sec

Table 10 Polyvinylpyrrolidone Wafer Characterization with 55% PVP as Wafer Mass

Powder Content 55% PVP Low Molecular Weight 45% Myo(CAN-BD) Die Size: 4mm						
Date: Oct 21st, 2010		**Humidity: Under 10%RH**				
Vial	Empty(g)	Full(g)	Wafer(g)	Pressure(lb)	Wafer Integrity	Dissolution Time
A1	6.40265	6.41009	0.00744	75	1	4 min 5 sec
A2	6.33966	6.34661	0.00695	75	1	4 min 15sec
A3	6.35792	6.36558	0.00766	75	1	4 min 6 sec
A4	6.40308	6.41007	0.00699	75	1	2 min 20 sec
A5	6.37863	6.38898	0.01035	150	3	4 min 36 sec
A6	6.36839	6.37124	0.00285	150	4	4 min 4 sec
A7	6.39497	6.34028	0.00345	150	3	4 min 10 sec
A8	6.34444	6.34944	0.00611	150	3	2 min 35 sec