The Effects of Low Molecular Weight Polar Molecules on the Physical and Chemical Properties of Hard Gelatin Capsule Shells

By

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Submitted to the graduate degree program in Pharmaceutical Chemistry and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science.

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Date Defended: 7 December 2017

The Thesis Committee for Evelyn Yanez certifies that this is the approved version of the following thesis:

The Effects of Low Molecular Weight Polar Molecules on the Physical and Chemical Properties of Hard Gelatin Capsule Shells

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Prof. Forrest

Date Approved: 22 December 2017

Abstract

Liquid filled capsule formulations offer opportunities to enhance both solubility and the oral bioavailability of new chemical entities [1,5,7]. Excipients used in liquid based formulations can range from lipophilic vehicles, solubilizing agents, surfactants, emulsifying agents and adsorption enhancers [2,3,4]. Commonly used solubilizing agents in commercially available oral formulations are ethanol, propylene glycol (PG) and glycerin [11]. These low molecular weight polar and hygroscopic molecules can penetrate and plasticize the capsule shell and affect the moisture content, compromising the capsule physical integrity and are therefore used in lower quantities [1,39]. This research aims to investigate the effects of these commonly used low molecular weight polar solubilizing agents in lipophilic excipients (cremophors and miglyols) on the compatibility with hard gelatin capsules monitored over stress conditions for a 3 month period at 25°C/60% RH, 30°C/65% RH and 40°C/75% RH storage conditions. The capsule physical properties such as brittleness and elasticity were determined by measuring the glass transition temperatures and the texture analysis of the gelatin films upon exposure to the solubilizing agents. Scanning Electron Microscopy (SEM) was used to document physical changes to the capsule shell. Additionally, the extent of gelatin cross-linking upon exposure to the solubilizing agents was evaluated using dissolution testing and measuring the aldehydes content, a by-product of cross-linking, using p-amino benzoic acid (PABA) derivatization high-pressure liquid chromatography (HPLC) method.

Results from this research suggest that propylene glycol and glycerin with two and three hydroxyl groups respectively are more incompatible with hard gelatin capsule shells than ethanol with one hydroxyl group.

Acknowledgements

I want to start by thanking Dr. Fernando Alvarez-Nuñez for all his helpful advice, guidance, and amazing patience throughout this project as my adviser in the Pharmaceutics Department at Amgen Inc. I also like to acknowledge the teaching, support, and counsel of Dr. Laird Forrest and the education and assistance I received from the rest of University of Kansas Pharmaceutical Chemistry Department faculty and staff.

The support from my colleagues both at Amgen and Genentech is deeply appreciated. I would like to give special thanks to Dr. Rick Chiu, for his dissolution training, Julie Calahan for her thermal analysis monitoring training, and Dr. Mark Ragains and Dr. Karthik Nagapudi for their encouragement and scientific discussions. And Dr. Joseph Lubach from Genentech for constant encouragement.

Lastly I would like to thank my mother Marta, brothers and friends, specifically my spouse Sajeevi, for their loving support and kindness and for giving me the encouragement to complete what I started.

Mama Noi, Mama Tere, Abuelito Rafa, Papa Chepe, Papi y Mami en el nombre de Dios todo poderoso ya terminé mis angelitos. Gracias Mahte por estudiar conmigo.

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Introduction

Theoretical Background and Significance

One of the many challenges in the pharmaceutical industry is the increasing number of poorly soluble (<10μg/mL, lipophilic) new chemical entities (NCEs) [5]. Lipid-based liquid formulations offer opportunities to enhance both solubility and the oral bioavailability of NCEs. In addition to enhancing the solubility, liquid formulations can be used to address challenges with low dose/high potency drugs, and low melting point drugs [38]. Since the active pharmaceutical ingredient (API) is in solution, liquid formulations have the advantage of overcoming any polymorphic conversions of the API. Of course, this is the case of finding an adequate solubilization system. Liquid formulations can also address formulation development challenges such as content uniformity at low doses, poor flow properties, particle size issues, cross contamination in processing and exposure [33] as well as reducing food effects providing faster timelines to first in human (FIH) studies [32].

Hard Gelatin Capsules

Liquid formulations use excipients which are either liquids or semi-solid in nature that are typically filled into capsules. There are two types of liquid-filled capsules (LFC): hard and soft capsules. The “hard” capsule consists of two separate parts, each a semi-closed cylinder in shape and has a self-locking and tapered rim feature to lock after filling. The “soft” capsule is a one-piece container, which has a variable shape and can be either seamed, along its axis, or seamless.

The majority of the hard capsules are made either with gelatin or hydroxypropyl methylcellulose (HPMC). Gelatin capsules are more susceptible to humidity changes, incompatible with

hygroscopic substances and can cross-link at high humidity and high temperatures (40°C/75%RH) (Appendix 1).

Gelatin

Gelatin is the commercial protein derived from native protein collagen by hydrolysis [6] which is present in animal skin, white connective tissue and bones [44]. The protein fractions consist almost entirely of amino acids joined together by amide linkages to form linear polymers, varying in molecular weight from 15,000 – 250,000Da [7]; see Table 1 for amino acids percentages in gelatin. There are two types of gelatin, Type A (pH3.8-6.0) is derived from acidic hydrolysis of pork skin. Type B gelatin (pH 5.0-7.4) is derived from basic hydrolysis of bones and animal skin and contributes. Gelatin used in the pharmaceutical industries is a blend of these two types [8].

Table 1- Amino Acids and their Content in Gelatin [7, 40].

Amino Acid	Percentage (%)	Abbreviation
Glycine	25.5	Gly or G
Proline	18.0	Pro or P
Hydroxyproline	14.1	Hyp
Glutamic acid	11.4	Glu or E
Alanine	8.5	Ala or A
Arginine	8.5	Arg or R
Aspartic acid	6.6	Asp or D
Lysine	4.1	Lys or K
Leucine	3.2	Leu or L
Valine	2.5	Val or V
Phenylalamine	2.2	Phe or F
Threonine	1.9	Thr or T
Isoleucine	1.4	Ile or I
Methionine	1.0	Met or M
Histidine	0.8	His or H
Tyrosine	0.5	Tyr or Y
Serine	0.4	Ser or S
Cysteine	0.1	Cys or C

Gelatin has the properties required to meet the technical needs of the pharmaceutical capsule industry. These include solubility, solution viscosity, and thermally reversible gelation properties in aqueous solution. It produces strong, clear, flexible, high-gloss films, which dissolve readily under the conditions existing in the stomach.

Liquid-Filled Capsule Formulation Excipients

Excipients used in lipid-based formulations can range from lipophilic vehicles, solubilizing agents, surfactants, emulsifying agents and adsorption enhancers. Commonly used excipients for liquid and semi-solid formulations in hard gelatin capsules are listed in Appendix 2.

Commonly used solubilizing agents in commercially available oral formulations are ethanol, propylene glycol (PG) and glycerin[11]. These low molecular weight polar and hygroscopic molecules can penetrate and plasticize the capsule shell and affect the moisture content, compromising the capsule physical integrity. Cole, et al. [1,39], shows that ethanol, propylene glycol and glycerin, at a 100% level are incompatible with hard gelatin capsules and should be avoided. Chen et al., investigated the compatibility of lipophilic excipients (capmul CMC and cremophor EL) on hard gelatin capsules as a function of glass transition and texture changes [26]. Stress studies with amoxicillin and formaldehyde contaminated lactose in hard gelatin capsules and acetomionophen in soft gelatin capsules showed significant decrease in dissolution [41,42].

Aims of the Study

This study investigates the physical and chemical integrity of gelatin capsule shell as a function of the interactions between low molecular weight co-solvent molecules and gelatin, a biopolymer. The investigation focused on the impact of co-solvents with increasing hydrophilicity on gelatin capsule shell physicochemical integrity. The co-solvents used were low molecular weight solubilizing agents; ethanol, propylene glycol (PG) and glycerin which are commonly used in oral formulations. The hydrophilicity increases from ethanol, PG to glycerin. In determining the co-solvent impact on physical and chemical integrity of gelatin, the solubility parameters, hydrogen bonding, polarity, and dispersion index of the co-solvents were considered. These interactions ultimately could impact the performance of the dosage form. The physical and chemical integrity of the gelatin capsules were monitored over a 3-month period at 25°C/60% RH, 30°C/65% RH and 40°C/75% RH storage conditions. The capsule physical properties such as brittleness and elasticity were determined by measuring the glass transition temperatures and the texture analysis of the gelatin films upon exposure to the solubilizing agents. Scanning Electron Microscopy (SEM) was used to document physical changes to the capsule shell. The well-known effects of gelatin cross-linking upon exposure to the solubilizing agents was evaluated using dissolution testing and measuring the aldehydes content, a by-product of cross-linking, using p-amino benzoic acid (PABA) derivatization HPLC method[5].

Materials and Methods

Materials

Excipients

Ethanol absolute, Ph Eur/BP/JP/USP grade, (synonym: Alcohol, ethyl alcohol) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless liquid.

Propylene Glycol, meets USP testing specifications grade, (synonym: 1,2-Propanediol) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless oily liquid.

Glycerin, meets USP testing specifications grade, (synonym: Glycerol, 1,2,3-Propanetriol) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless oily liquid.

Active Pharmaceutical Ingredient (API)

Ibuprofen (synonym: α-Methyl-4-(isobutyl)phenylacetic acid, (±)-2-(4-Isobutylphenyl)propanoic acid)) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a white powder.

Gelatin Capsules

Two- piece hard capsules were gelatin Licaps (Capsugel Greenwood, SC, USA). The capsule type was natural transparent and of size 0. Size 4 was used for preliminary studies.

Fillers

Caprylic/Capric Triglyceride, medium-chain triglycerides, (Miglyol 812N) was obtained from Briechle-Fernandez Marketing Services (Eatontown, NJ, USA) in the form of a clear oily liquid.

Propylene Glycol Dicaprylate/Dicaprate (Miglyol 840) was obtained from Cremer Oleo (Cincinnati, OH, USA) in the form of a clear oily liquid.

Polyoxyl 35 hydrogenated castor oil (synonym: Macrogolglycerol ricinoleate, PEG-35 castor oil, Cremophor EL, Kolliphor EL) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a yellow oily liquid.

Polyoxyl 40 hydrogenated castor oil (synonym: Macrogolglycerol hydroxystearate, PEG-40 castor oil, Cremophor RH40, Kolliphor RH40) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a yellow oily liquid.

Reagents

For HPLC analysis of concentration of Ibuprofen used high-pressure liquid chromatography (HPLC-Dissolution) and the following reagents:

Acetonitrile, HPLC grade, (synonym: ACN, methyl cyanide) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless liquid.

Water, HPLC grade, was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless liquid.

Phosphoric acid, NF reagent, (synonym: orthophosphoric acid) was obtained from J.T. Baker Chemicals (Center Valley, PA, USA) in the form of a clear colorless liquid.

The Dissolution media of pH 7.2 phosphate buffer was made with the following reagents in 0.2M solutions and mixed according to USP 711 monograph.

Potassium phosphate monobasic, NF grade, (synonym: Potassium dihydrogen phosphate) was obtained from Sigma-Aldrich (St. Louis MO USA) in the form of a white powder.

Sodium hydroxide, ACS grade, (synonym: soda caustic) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of white pellets.

For HPLC analysis of concentration of acetaldehyde and formaldehyde used high-pressure liquid chromatography (HPLC-Aldehyde) and the following reagents:

Acetaldehyde, >99.9%, (synonym: ethanal) was obtained from Supelco and distributed by Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless liquid.

Formaldehyde solution 37%, HPLC grade, (synonym: Formalin) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless liquid.

4-Aminobenzoic acid, ACS reagent grade, (synonym: PABA, 4-aminobenzoic acid) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless liquid.

Isopropyl alcohol, HPLC reagent, (synonym: IPA, isopropanol, 2-propanol) was obtained from Sigma-Aldrich (St. Louis MO USA) in the form of a clear colorless liquid.

Sodium cyanoborohydride solution, reagent grade, (synonym: sodium cyanotrihydridoborate) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless liquid.

Trifluoroacetic acid, HPLC reagent, (synonym: TFA) was obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of a clear colorless liquid in 1mL glass ampules.

Methods

Scanning Electron Microscopy (SEM)

To measure surface morphology changes in the gelatin capsule shell SEM images were taken. SEM data were obtained using a SEM NeoScope (JOEL, Peabody, MA, USA) Benchtop instrument to evaluate the brown specs been in the microscope at variable magnifications. A small amount of sample was mounted on double-sided carbon tape on a 35mm aluminum SEM stub and the sample was not gold-coated using a sputter-coater. The SEM analysis was conducted at an accelerating voltage of 10kV using secondary electron backscattering signal detection.

Differential Scanning Calorimetry (DSC)

DSC was used to evaluate the Tg value variations of the gelatin capsule shells. DSC analysis was conducted on a TA Instruments Q100 instrument (TA Instruments, New Castle, USA). A sample size of approximately 5-10mg,was obtained by cutting out a circle with a standard hole puncher, the sample was then weighed out into a standard aluminum DSC pan. The pan with a pin-hole was crimped. The sample was heated at 10°C/min from ambient temperature to 300°C under dry nitrogen at 50mL/min.

Thermogravimetric analysis (TGA)

To measure the moisture content differences in the gelatin capsule shells, TGA analysis was performed. TGA was conducted on a TA Instruments Q500 instrument (TA Instruments, New Castle, USA). A sample size of approximately 5-20mg was obtained by cutting out a circle, with a standard hole puncher, the sample was then weighed out into a platinum pan. The sample was heated at 10°C/min from ambient temperature to 400°C under dry nitrogen at 25mL/min.

Dissolution Analysis

The dissolution rate was measured using a USP type II dissolution apparatus (paddle method, Varian VK 7010; VanKel Agilent (Vankel/ Agilent Technologies, Woburn, MA, USA) with a heater and autosampler collection systems (VK 750D, VK 810 and VK 8000). A total of 900mL of pH 7.2 phosphate buffer was used as the medium as per USP 711 monograph. The temperature was maintained at 37˚C ± 0.5˚C, and the paddle speed of 50rpm. The Ibuprofen capsules were dropped manually with thin stainless steel wire spiral sinkers (Distek, Township, NJ, USA). At the predetermined time points, analiquots of 1.5mL were collected and filtered through a 0.45um membrane, and the concentration was determined using UV/Vis as described in HPLC method.

High Pressure Liquid Chromatography (HPLC)

Two separate HPLC methods were used. One was used to quantify the dissolution of the model drug (Ibuprofen) in gelatin capsules to infer the potential chemical changes to the gelatin capsule shell. The second one was used to quantify the residual aldehyde content in the gelatin capsule shell.

HPLC-Dissolution

HPLC-UV was performed on an Agilent 1100 series (Agilent Technologies, Foster City, CA, USA) HPLC equipped with a quaternary pump, DAD detector, auto sampler, and a Symmetry C18 150×4.6mm 5µm particle size column. The mobile phase made (65% water, 35% acetonitrile, 0.025% phosphoric acid) at a flow rate of 2.0mL/min. Column temperature was maintained at 30°C throughout and data was collected at 224 and 254nm with a reference wavelength of 460nm (20nm bandwidth, 0.05 steps, averaged). Concentration of standards and samples were approximately 0.01mg/mL. Used USP Ibuprofen

HPLC- Aldehyde

High pressure liquid chromatography-ultraviolet spectroscopy (HPLC-UV) was performed on an Agilent 1100 series HPLC (Agilent Technologies, Foster City, CA, USA) equipped with a quaternary pump, DAD detector, auto sampler, and a Zorbax EclipseXBD-C18 150×4.6mm 5µm particle size column. The mobile phase made (0.1%TFA in acetonitrile, 0.1%TFA in water) at a flow rate of 1.0mL/min, run time 25 minutes. Column temperature was maintained at 38°C throughout and data was collected at 305 and 254nm with a reference wavelength of 460nm (20nm bandwidth, 0.05 steps, averaged). Concentration of standards was about 0.004mg/mL (2µg/mL for formaldehyde and 6ug/mL for acetaldehyde) and samples were approximately 0.01mg/mL.

Moisture Sorption Analysis

Moisture sorption analysis was used to measure the moisture adsorption/desorption of the gelatin capsule shell. Moisture sorption data was collected at 25°C using a VTI (TA Instruments, New Castle, USA), vapor sorption analyzer. A sample size of approximately 4-10mg was used in a standard platinum pan. Hygroscopicity was evaluated from 5 to 95%RH in increments of 5%RH. Data for adsorption and desorption cycles were collected. Equilibrium criteria were set at 0.001% weight change in 10 minute with a maximum equilibration time of 180 minutes.

Texture Analysis Data

The gelatin film breaking force was measured to evaluate the brittleness and elasticity of the gelatin films. The film breaking force was measured with a texture analyzer, TA-XT2i (Texture Technologies Corp., Scarsdale, NY, USA) with small film extensibility rig for 5 samples and

puncture probe (TA-XT Plus, TA-108s5 and TA-8). The small film extensibility rig was changed to hold three samples and the samples were held in place by round base magnetic rings and kept in place with a metal washer (Grainger, Houston, TX, USA), see Figure 1. These modifications were made in-house to accommodate the small film size of the gelatin capsule shell.

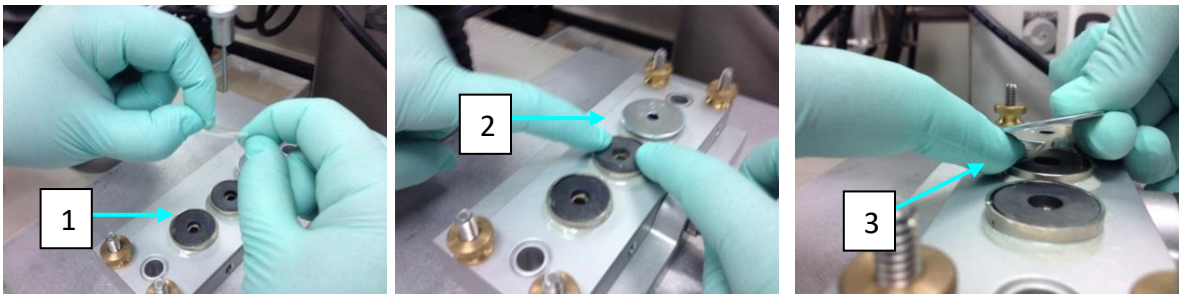


Figure 1. Texture Analysis Small Film Rig Changed with Magnetic Rings (1) and Metal Washer (2) to Fix Gelatin Film in Place as Shown (3).

Thermal Activity Monitor (TAM)

Microcalorimetry (TAM) was used to determine the extent of chemical and/or physical interactions between the co-solvents and the gelatin capsules. TAM data was collected for 5-9 days (TAM model 2277; Thermometric AB, Sweden EU). A feasibility experiment with a hard gelatin capsule empty and exposed to ethanol, propylene glycol, and glycerol loaded one at a time into a stainless steel calorimeter cell, sealed and lowered into the measuring position of the calorimeter.

Hansen Solubility Parameters (HSPs)

Calculated HSPs using Molecular Modeling Pro software version 6.3.5. Norgwyn Montgomery software Inc. Website: www.chemsw.com.

Wolfram Mathematica

Calculated observed versus predicted puncture force plots using the linear algebra approach from Wolfram Mathematica software version 11(Wolfram Research, Champaign, IL, USA). Website: www.wolfram.com/mathematica/.

Statistical Analysis Software (JMP)

Data collected for from thermal and texture analysis modeled using JMP Statistical Discovery software version 11.1.1. SAS Institute Inc. Website: www.jmp.com/en_us/.

Results and Discussion

Baseline Physical Characterization of Gelatin Capsule

The baseline physical characterization data for empty gelatin capsule shell films was generated using DSC, TGA, DVS, SEM and the texture analyzer. The DSC data generated, as shown in Figure 2 shows a Tg of 60.5˚C which is in the range of values reported in the literature.

The TGA data shown in Figure 3 also falls within the manufacturer's listed range with a 15% weight loss. The equilibrium moisture adsorption/desorption data generated using the dynamic sorption system (DVS) shows minimum hysteresis which could be attributed to the internally locked water content. As shown in Figure 4, the gelatin shell moisture adsorption desorption isotherm shows minimal hysteresis indicating that of all moisture gained with increasing relative humidity (RH) and is lost when lowering the %RH. The SEM images (Figure 5 and Figure 6) show gelatin capsule shells to have very smooth surface from the manufacturing process. The measured thickness of the gelatin film was about 40um. The brittleness and elasticity of the capsule shell were measured with the texture analyzer by measuring the force required to break the film. As shown in Figure 7 and Figure 8, a repeatable force of 3508g at 1.33mm distance for 2.7 seconds is required to puncture the film.

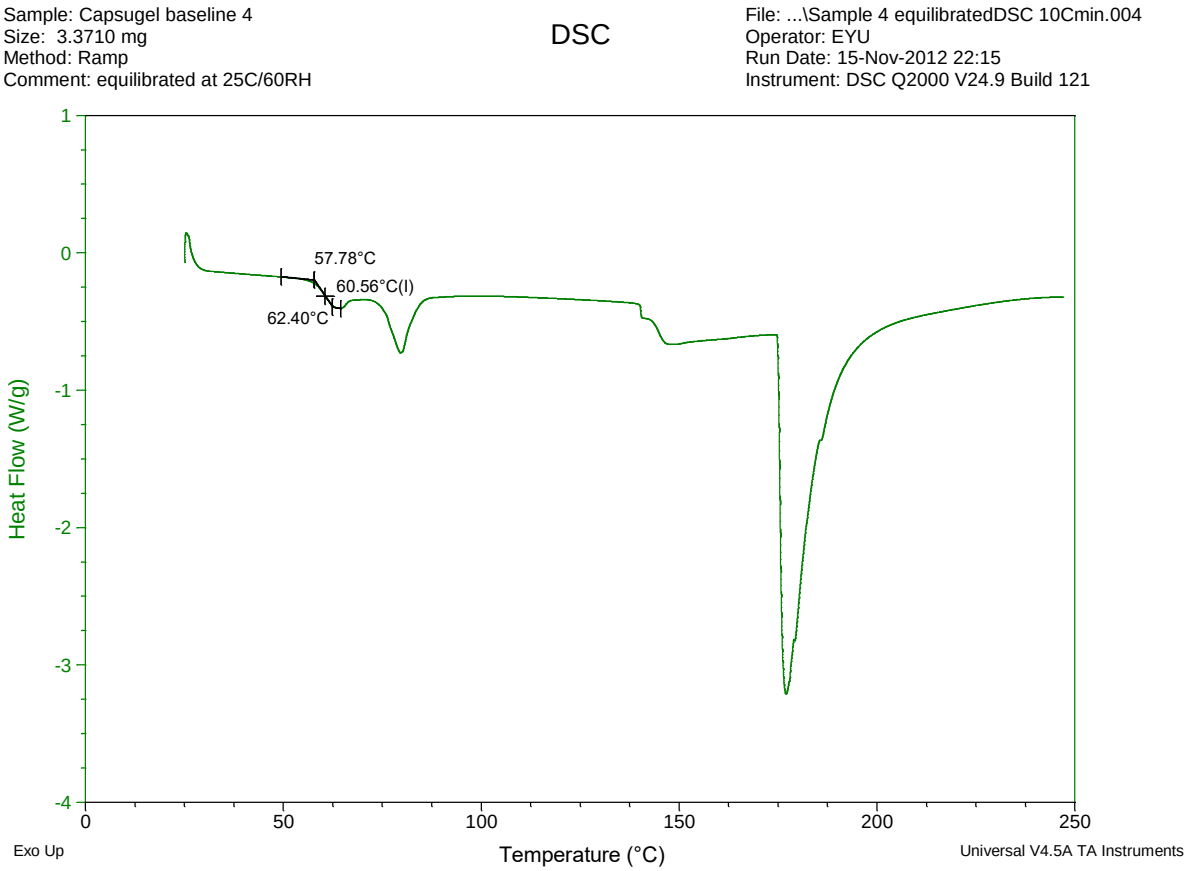


Figure 2. Differential Scanning Calorimetry (DSC) of Capsugel LiCaps™ Size 0 Gelatin Capsules Shows Tg at 60.6˚C.

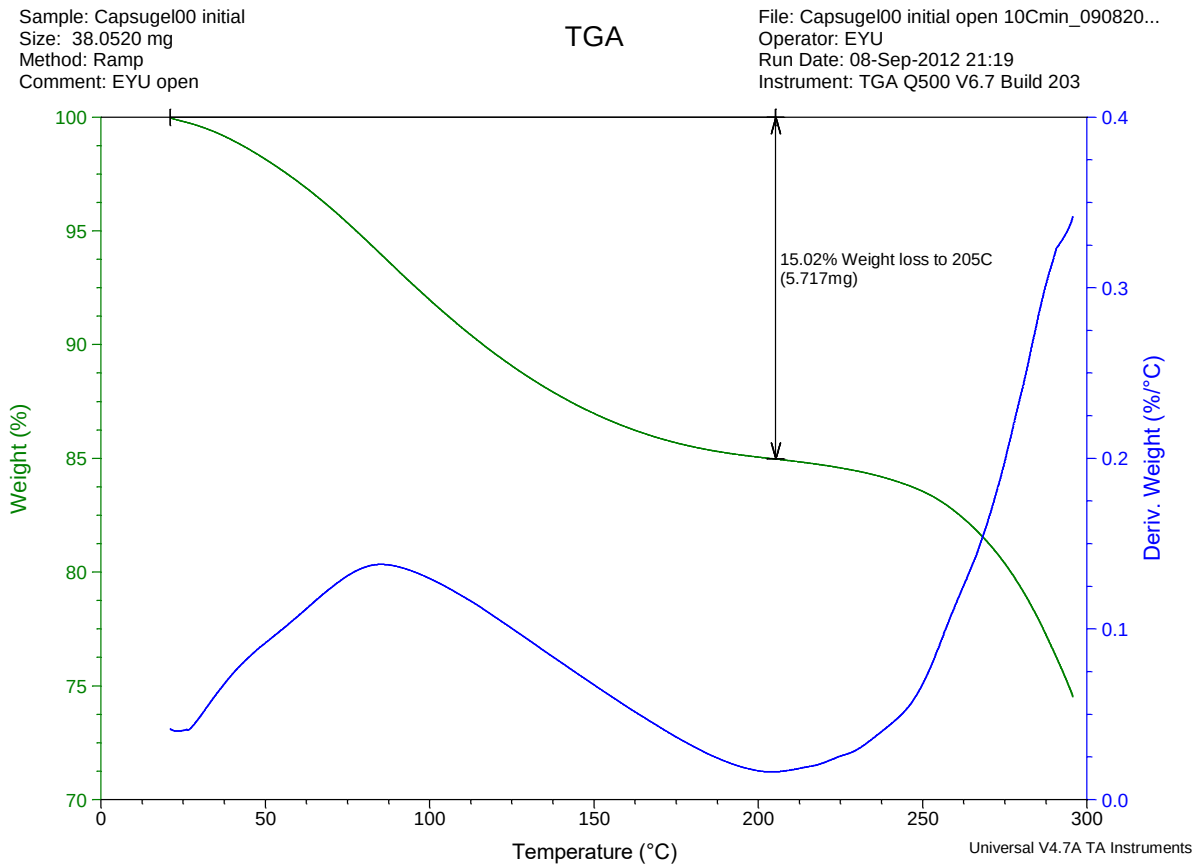


Figure 3. Thermogravimatric Analysis (TGA) of Capsugel LiCaps™ Size 0 Gelatin Capsules Shows Weight Loss of 15.0% at 205˚C after Conditioning.

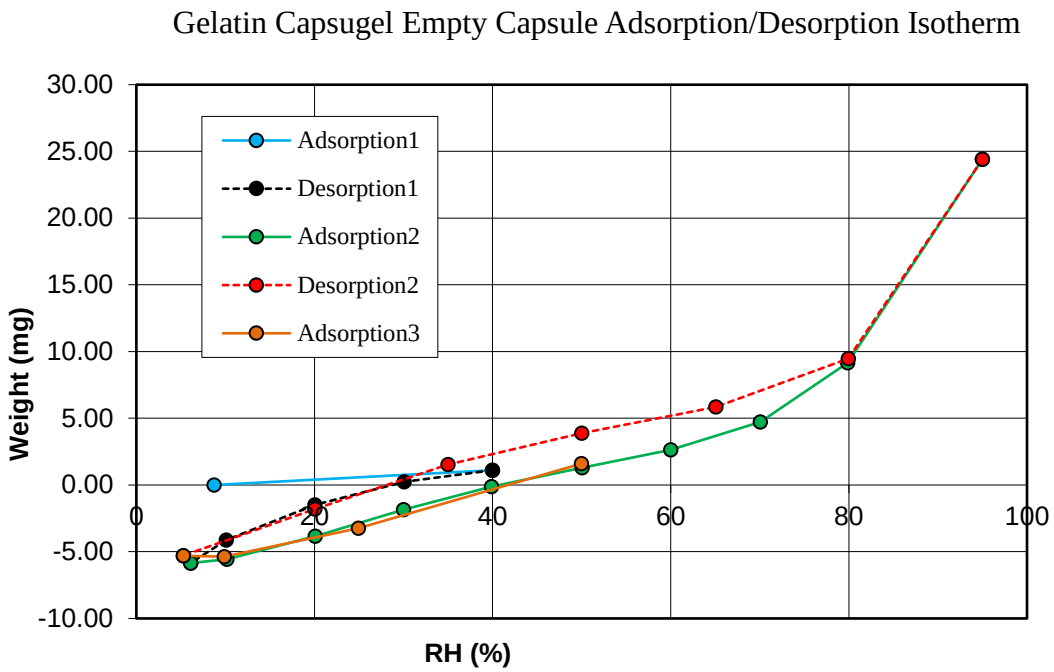
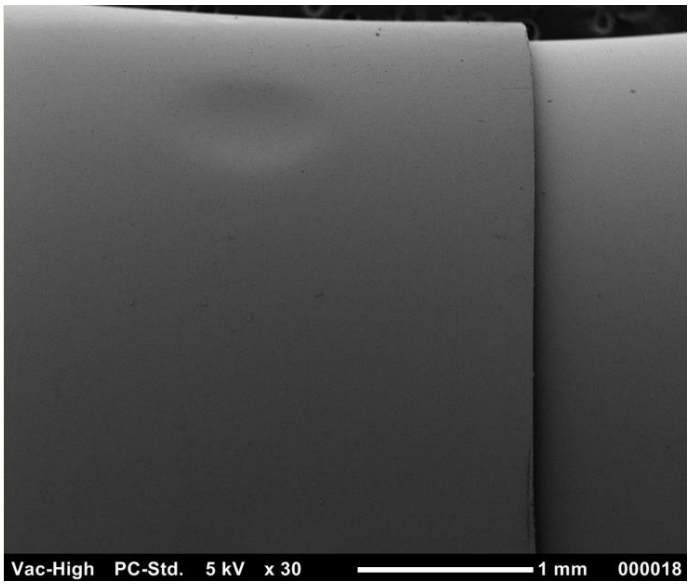


Figure 4. Moisture Sorption (DVS) of Capsugel LiCaps™ Size 0 Gelatin Capsules Shows Adsorption and Desorption Isotherms.



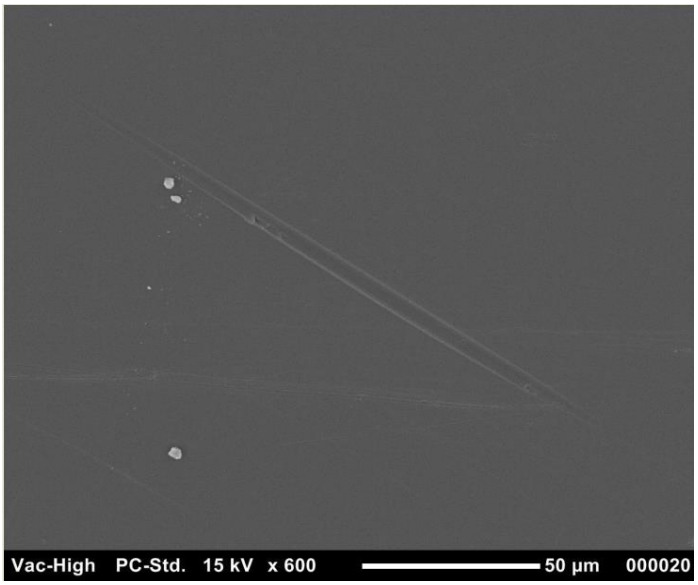


Figure 5. Scanning Electron Microscope (SEM) of Capsugel LiCaps™ Size 4 gelatin capsules shows images of gelatin capsule shell. SEM shows smooth gelatin capsule shell and the photo on the bottom shows ejection mark from the manufacturing process of the capsule.

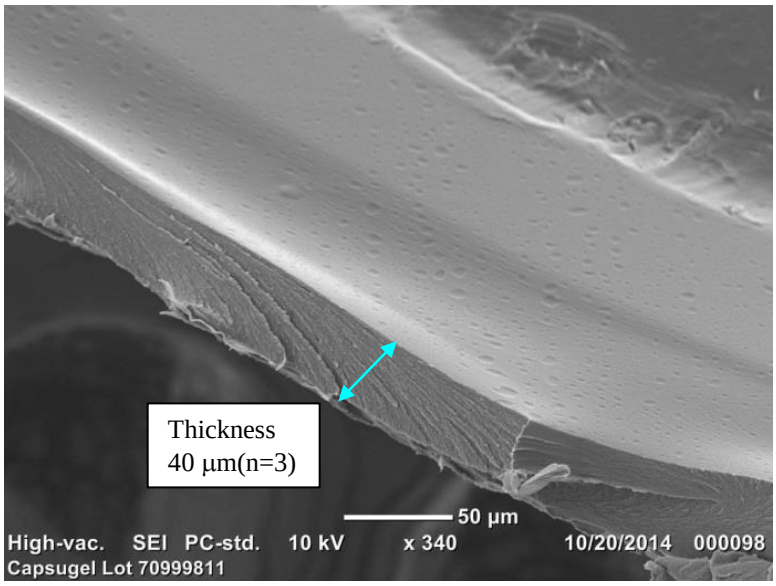


Figure 6. Scanning Electron Microscope (SEM) of Capsugel LiCaps™ Size 0 Gelatin Capsules Shows Images of Gelatin Capsule Shell Film. Gelatin Film Thickness of about 40µm.

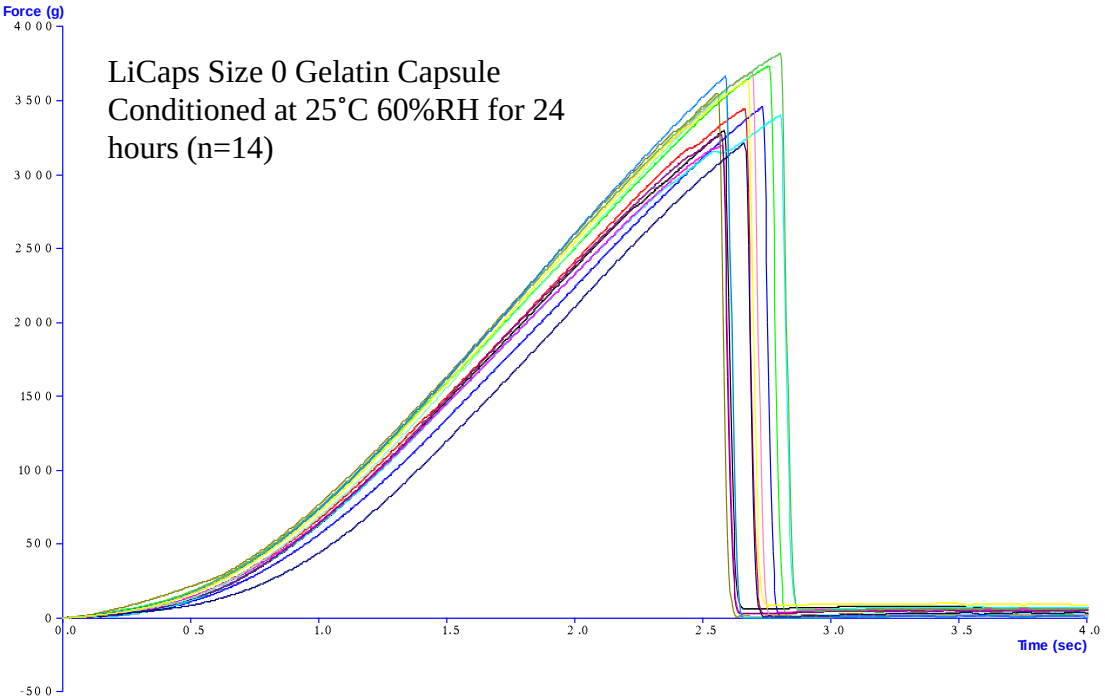


Figure 7. Texture Analysis (TA) of Capsugel LiCaps™ Size 0 Gelatin Capsules Shows Texture Analysis Profile of Gelatin Capsule. The gelatin capsule film was placed on the holder and secured in place by magnets as shown in Figure 8. The graph shows the load placed on the film as a function of time or distance in the x-axis and force needed for sample to fail/ break in y-axis. The texture analysis instrument records the load or force the probe applies to the film and the maximum load before breaking is shown as a peak. A repeatable force of 3508g, at 1.33mm distance for 2.7 seconds is required to puncture the film.

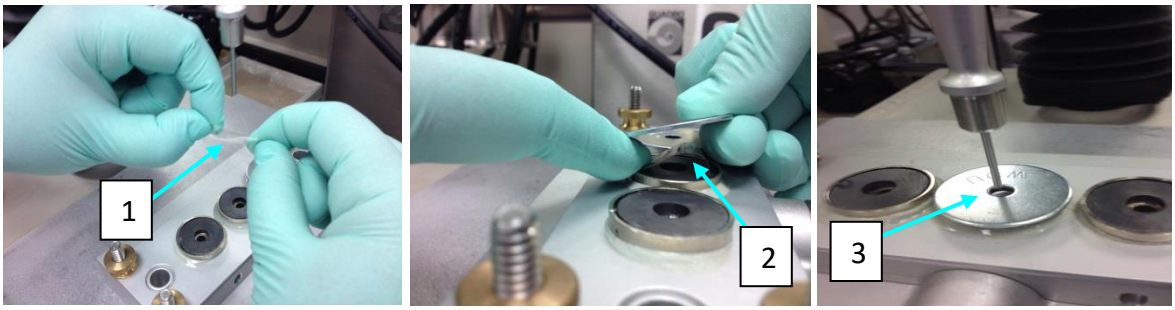


Figure 8. Texture analysis small film rig changed with magnetic rings and metal washer to fix gelatin film in place. Removed the rounded end of the bottom half of the gelatin capsule shell and made one cut to have a flat film (1), placed between magnetic ring and metal washer (2) and performed test in a film firmly in place (3).

Making Formulations in Gelatin Capsule

Once the empty gelatin capsule shell's baseline physical characteristics were determined, the capsules were filled with the formulations of interest and placed on stability, as shown in Table2. Capsules were filled using a metal semi-manual capsule filler machine (Figure 9) and a manual multichannel pipette and stored vertically on stability at 25˚C/60%RH, 30˚C/65%RH and 40˚C/75%RH humidity chambers. As shown in Table 2, all the capsule formulations had ethanol, propylene glycol and glycerin at 5% and 10% (v/v) levels. The remaining composition in the formulations was filled with non-ionic surfactants (Cremophor RL and Cremophor RH40) or medium chain triglycerides (Miglyols 812N and Miglyol 840). The formulation composition and the stability storage conditions are summarized in Table 2. The compositions used in this study are within the ranges used in commercially available formulations (Appendix 2).

Table 2 Formulation Composition and Storage Conditions

Number	Co-Solvent	Co-Solvent (%)	Filler	Stability Storage
1	Ethanol	5%	Cremophor EL	25˚C/60%RH 30˚C/65%RH 40˚C/75%RH
2	Ethanol	10%	Cremophor EL	
3	Propylene Glycol	5%	Cremophor EL	
4	Propylene Glycol	10%	Cremophor EL	
5	Glycerin	5%	Cremophor EL	
6	Glycerin	10%	Cremophor EL	
7	Ethanol	5%	Cremophor RH40	
8	Ethanol	10%	Cremophor RH40	
9	Propylene Glycol	5%	Cremophor RH40	
10	Propylene Glycol	10%	Cremophor RH40	
11	Glycerin	5%	Cremophor RH40	
12	Glycerin	10%	Cremophor RH40	
13	Ethanol	5%	Miglyol 812N	
14	Ethanol	10%	Miglyol 812N	
15	Propylene Glycol	5%	Miglyol 812N	
16	Propylene Glycol	10%	Miglyol 812N	
17	Glycerin	5%	Miglyol 812N	
18	Glycerin	10%	Miglyol 812N	
19	Ethanol	5%	Miglyol 840	
20	Ethanol	10%	Miglyol 840	
21	Propylene Glycol	5%	Miglyol 840	
22	Propylene Glycol	10%	Miglyol 840	
23	Glycerin	5%	Miglyol 840	
24	Glycerin	10%	Miglyol 840	

This study will only focus on the effects of ethanol, propylene glycol and glycerin on gelatin capsule shell. However significant differences between cremophor and miglyols formulations will be discussed, if observed.

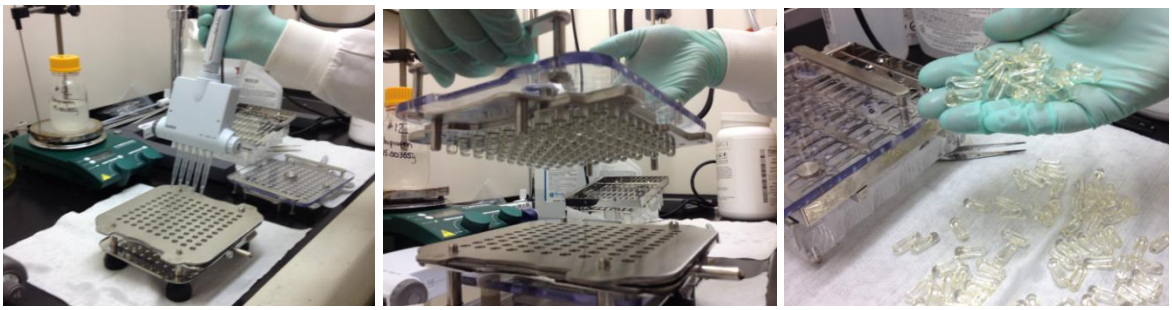


Figure 9. Semi-Manual Capsule Filler Machine Used to Fill Capsugel LiCaps™ Size 0 Gelatin Capsules.

Thermal Analysis Data to Determine Physical Stability

As shown in Figure 10, the DSC data shows that ethanol, propylene glycol and glycerin act as plasticizers lowering the Tg at all storage conditions. The lower Tg in comparison to starting material shows ethanol, propylene glycol and glycerin are all acting like plasticizers. Among the three co-solvents, ethanol, with the least amount of hydroxyl groups, shows the least change in elasticity compared to the baseline Tg.

Figure 10. Differential Scanning Calorimetry of Stress Gelatin Capsules Shows Glass Transition Data of Stress Samples at a Lower Tg than the Starting Gelatin Capsule Shell (Tg Baseline in Purple at 60.5˚C).

Texture Analysis Data to Determine Physical Stability

Texture analysis was used to compare changes to gelatin capsule shells as it was exposed to ethanol, propylene glycol and glycerin at stress conditions for 3 months. The principal of a texture measurement system is to physically deform a test sample in a control manner and measure it's response in the form of break force, distance and time. Lower break force, shorter distances and shorter times are consistent with brittle materials in comparison to higher break force, longer distance and longer times for elastic materials. The distance measured is the distance traveled before breaking or puncturing the film. A repeatable force of 3508g, at 1.33mm distance for 2.7 seconds is required to puncture the film for an initial condition gelatin capsule film.

The baseline distance and force data for empty gelatin capsule shell films were 1.3mm and 3508g, respectively. This same approach (i.e. addition of glycerin as a plasticizer for gelatin) is used in soft gelatin capsule manufacturing to enhance elasticity in gelatin capsule.

Figure 11 summarizes the distance measurements as a function of storage conditions for all tested formulations. Out of the 3 co-solvents, glycerin/cremophor formulations show higher distances compared to the baseline independent of the storage conditions. This indicates that the glycerin/cremophor formulations have more elastic gelatin capsules in comparison to the rest of the formulations. Furthermore, the 10% glycerin/cremophor formulations had higher distances in comparison to the 5% glycerin/cremophor formulations, showing them to be more elastic than the 5% formulations.

As discussed above, glycerin/cremophor formulations show more gelatin capsule shell elasticity than glycerin/miglyols formulations. This could be due to the compatibility differences of the co-solvent filler. It can be explained as glycerin being more compatible with miglyol than with cremophor. If this is the case, then in the glycerin/ cremophor formulation glycerin is available to interact with gelatin. The direct interactions of glycerin with gelatin can reduce the affinity of gelatin for moisture which could lead to gelatin becoming more elastic [51]. This same approach (i.e. addition of glycerin as a plasticizer for gelatin) is used in soft gelatin capsule manufacturing to enhance elasticity in gelatin capsule.

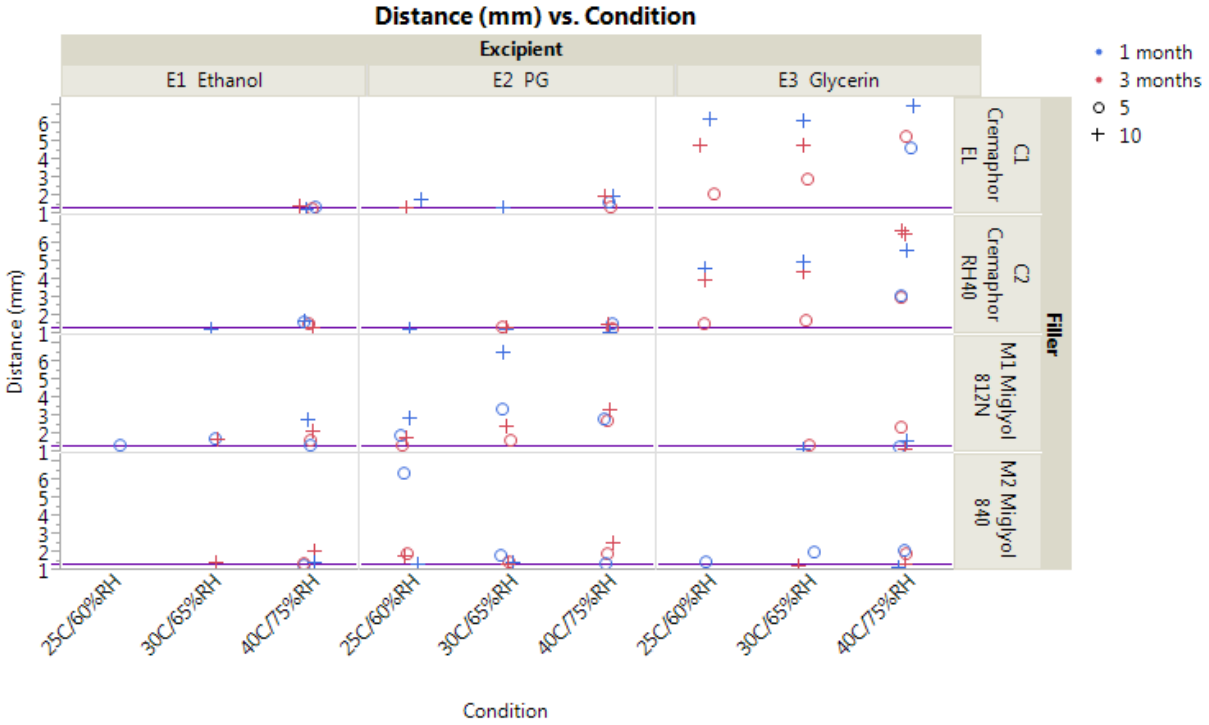


Figure 11. Texture Analysis of Stress Gelatin Capsules Shows Distance Texture Analysis Data of Stress Samples, Starting Gelatin Capsule Shell (Distance Baseline in Purple at 1.33mm as Shown in Figure 7).

The break force data as shown in Figure 13 co-relates with the distance data shown in Figure 12 with glycerin/cremophor formulations having the most impact to the gelatin film elasticity. Furthermore, Figure 12 shows PG to increase gelatin elasticity to some extent but not to the same level as glycerin. In both cases, the 10% glycerin has a more pronounced effect compared to 5%.

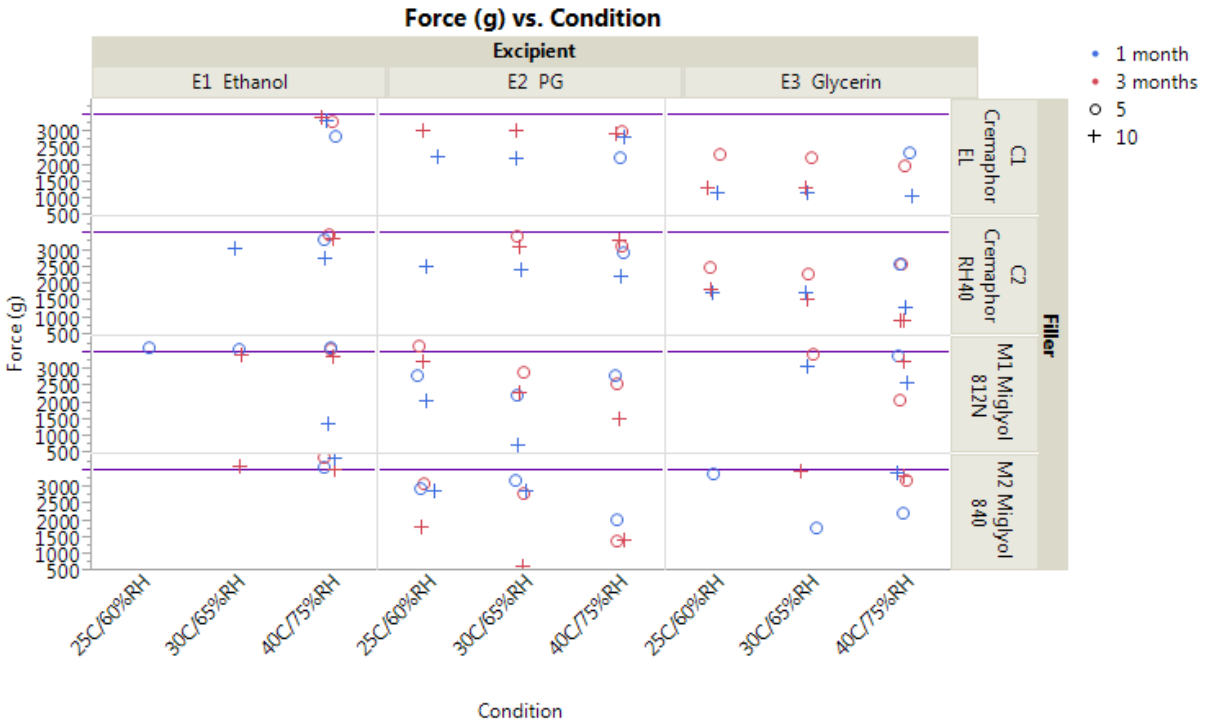


Figure 12. Texture Analysis of Stress Gelatin Capsules Shows Break Force Texture Analysis Data of Stress Samples, Starting Gelatin Capsule Shell (Break Force Baseline in Purple at 3508g as Shown in Figure 7)

To determine the correlation between elasticity and Tg, the distance data from the texture analyzer were plotted as a function of Tg, see Figure 13. The observations were similar This same approach (i.e. addition of glycerin as a plasticizer for gelatin) is used in soft gelatin capsule manufacturing to enhance elasticity in gelatin capsule.

The observations were similar to Figure 11 where a direct co-relation with Tg to elasticity (i.e. distance) could not be deduced. The overall Tgs for all formulations are lower than that of the baseline.

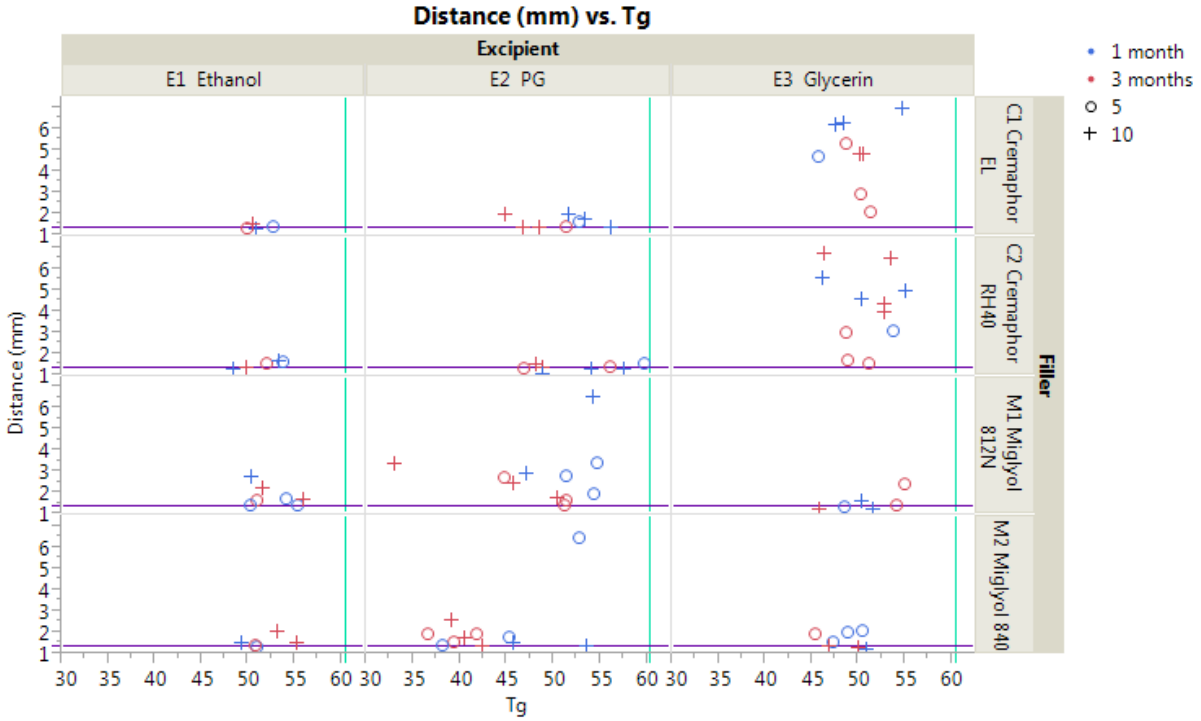


Figure 13. Texture Analysis of Stress Gelatin Capsules Shows Elasticity Texture Analysis Data versus Tg of Stress Samples. Empty Gelatin Capsule Shell (Distance Baseline in Purple at 1.3mm and Tg Baseline at 60.5˚C)

When elasticity was determined as a function of break force, observations are similar to the ones observed for distance; where a direct co-relation with Tg to elasticity (i.e. break force) could not be deduced. The overall Tgs for all formulations are lower than that of the baseline.

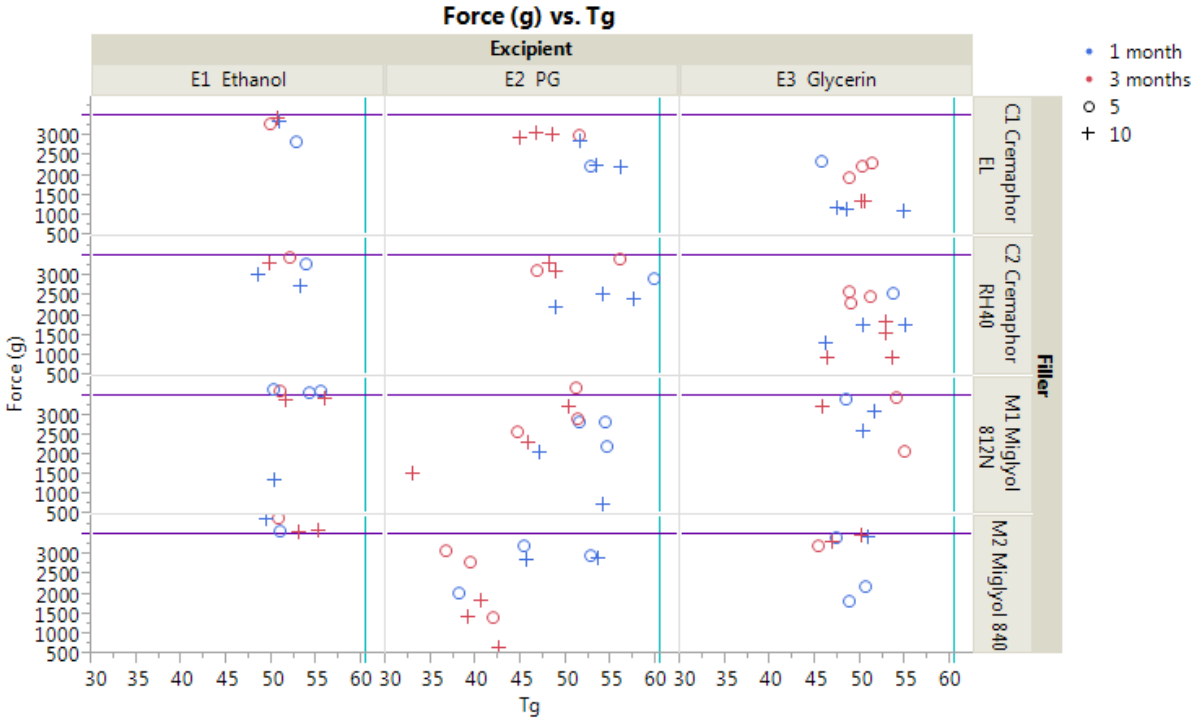


Figure 14. Texture Analysis of Stress Gelatin Capsules Shows Force Texture Analysis Data versus Tg of Stress Samples. Empty Gelatin Capsule Shell (Force Baseline in Purple at 3508g and Tg Baseline at 60.5˚C).

In summary, the texture analysis data with respect to both distance and force showed that glycerin/cremophor formulations to have the greatest impact on increasing the gelatin films' elasticity, even though the measured Tg values did not indicate this pheomenon. Among the three co-solvents evaluated, ethanol has the least impact on gelatin elasticity with both cremophor and miglyol with PG/miglyol 812 having a moderate effect and glycerin/cremophor with the most increase elasticity compared to baseline.

This trend shows that the increasing number of hydroxyl groups (i.e. increasing hydrophilicity) results in softening the gelatin film. The interactions of glycerin with gelatin at the concentrations tested in this study could influence the elasticity of the gelatin film. Glycerin with the highest number of hydroxyl groups can disrupt the hydrogen bonding between gelatin molecules (i.e. C=O…H-N), facilitating the mobility of the polarized groups in gelatin thus making the capsule shell more elastic.

The elasticity differences (longer distances) seen between the two filler types, cremophor and miglyol can be attributed to the potential gelatin cross-linking as discussed in section where the residual aldehyde content is discussed. The cross-linking resulted in the gelatin film becoming more elastic.

Dissolution Data to Determine Chemical Stability

In order to assess the impact of gelatin cross-linking on dissolution performance, formulations with Ibuprofen as a model active pharmaceutical ingredient were evaluated. The dissolution data of Ibuprofen under stress conditions of ethanol and glycerin performed better than propylene glycol regardless of filler in the formulation (Figure 15).

The unstressed (initial) capsule exhibits a classic immediate release profile. Rupture occurred by 5 minutes and complete release by 30 minutes. A decline in dissolution performance was observed for all formulations when stressed 40C/75% RH for 3 months. All stressed formulations had lower % dissolved at all time points compared to initial. The most impact to performance was seen in propylene glycol-based formulation. In this case capsule rupture time for this formulation was delayed and % dissolved at final was < 50%.

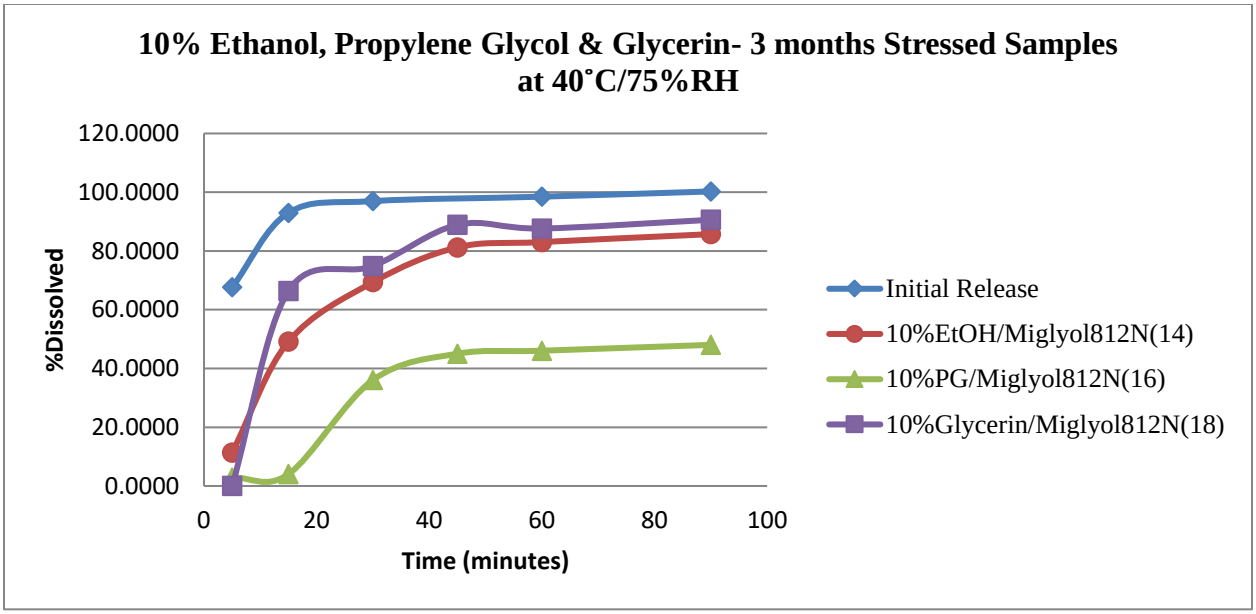


Figure 15. Dissolution of Stress Gelatin Capsules Shows 10% Ethanol, Propylene Glycol and Glycerin at 40˚C/75%RH for 3 Months in Comparison to a Non-Exposed or Stress Sample.

Glycerin exposed capsules at 10%, formulation 18, was stressed at 40˚C/75% RH for 1 and 3 months. As expected, the dissolution performance declined with increasing time points, and most significantly at 3 months (Figure 16). This trend was observed across all formulations.

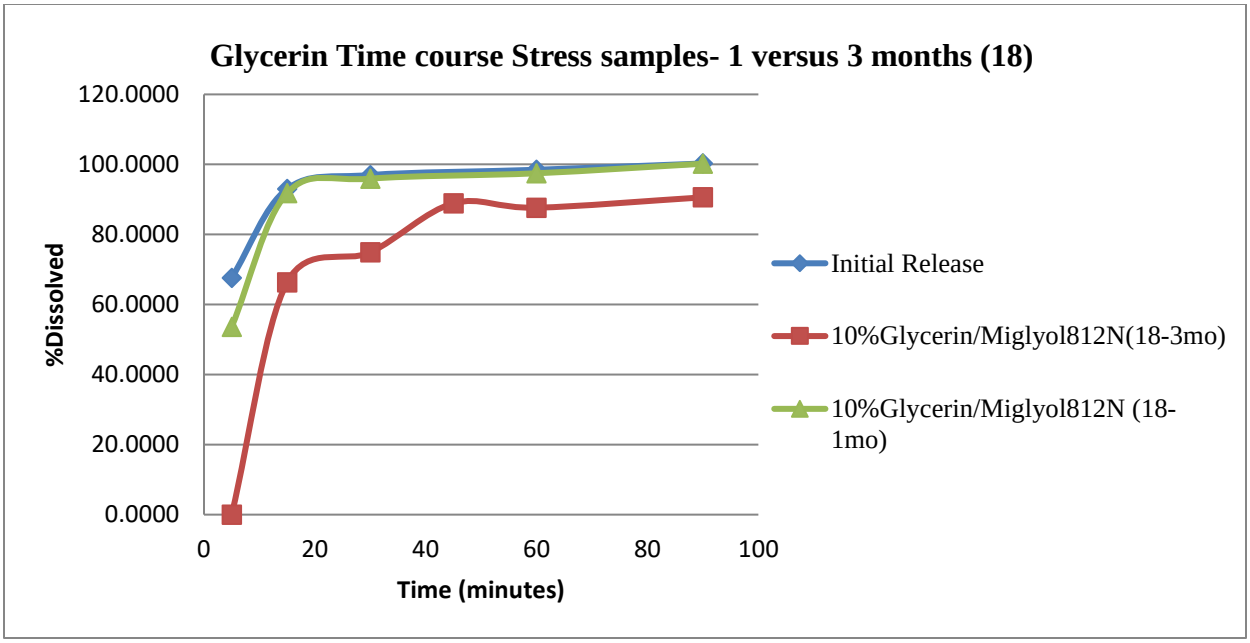


Figure 16. Dissolution of 10% Glycerin Stress Gelatin Capsules Stored at 40˚C/75%RH Shows a Slower Release for 3 months in Comparison to 1 Month and a Non-Exposed or Stress Sample.

Focusing in on the propylene glycol-based formulation from Figure 17, capsule performance as a function of stress conditions was assessed. Per expectations, the dissolution performance declined with increasing stress conditions. This trend was observed across all formulations with the most significant impact from stress at 40˚C/75% RH. At 40˚C/75% RH, it is apparent there is a delay in dissolution, with a final dissolution % of 48% at the final time point.

In summary, the dissolution data shows that propylene glycol is more incompatible to the gelatin capsule shell than glycerin and ethanol. The change in gelatin elasticity may contribute to the slower release profiles of PG. The DSC and texture analysis data narrows the reactivity to the glycerin and PG co-solvents.

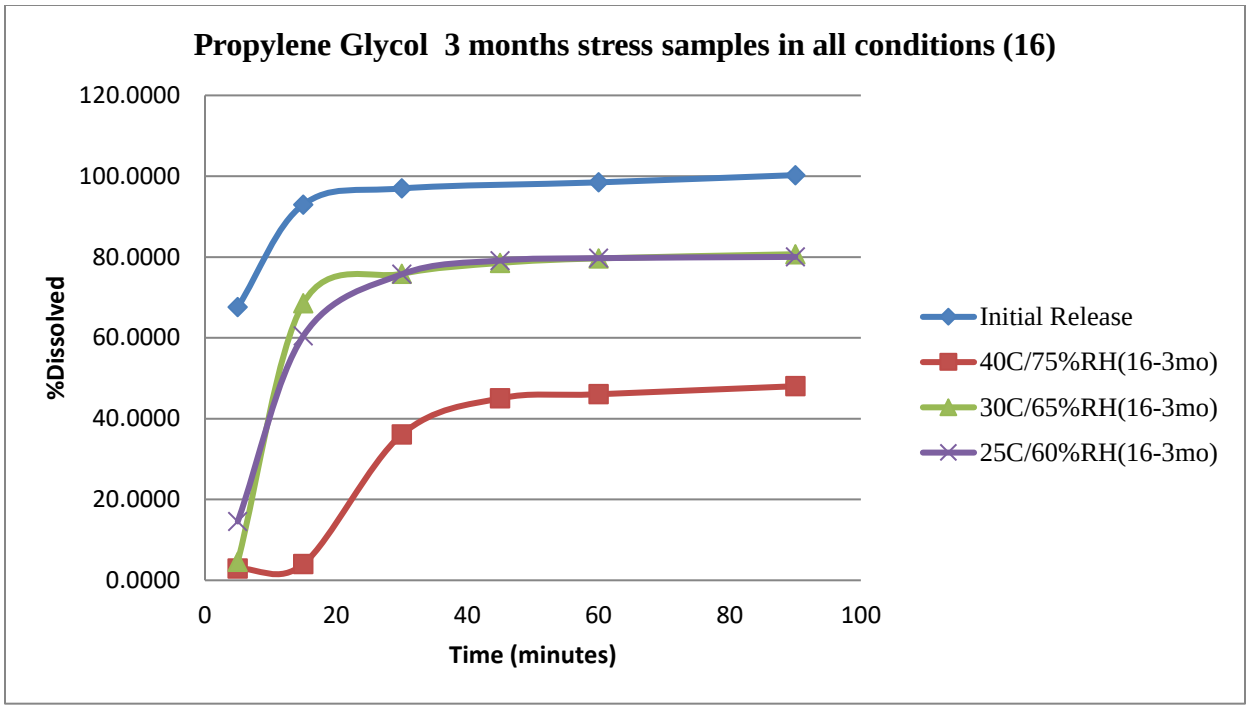


Figure 17. Dissolution of 10% Propylene Glycol 3-Month Stress Gelatin Capsules at 25˚C/60%RH, 30˚C/65%RH and 40˚C/75%RH. Propylene Glycol at 40˚C/75%RH Declines in Dissolution as Expected.

In the interest of filler type impact on dissolution performance, cremophor and miglyol-based formulations were measured at 40°C/75% RH at 3 months (Figure 18). Overall, the cremophor-based formulations enhance the reactivity of the excipients in the capsules, thereby negatively impacting the dissolution performance. Despite the differences between cremophor and miglyol, the time, stress condition and excipient related trends, discussed above, are the same between the two fillers (Figure 17, Figure 18 and Figure 19).

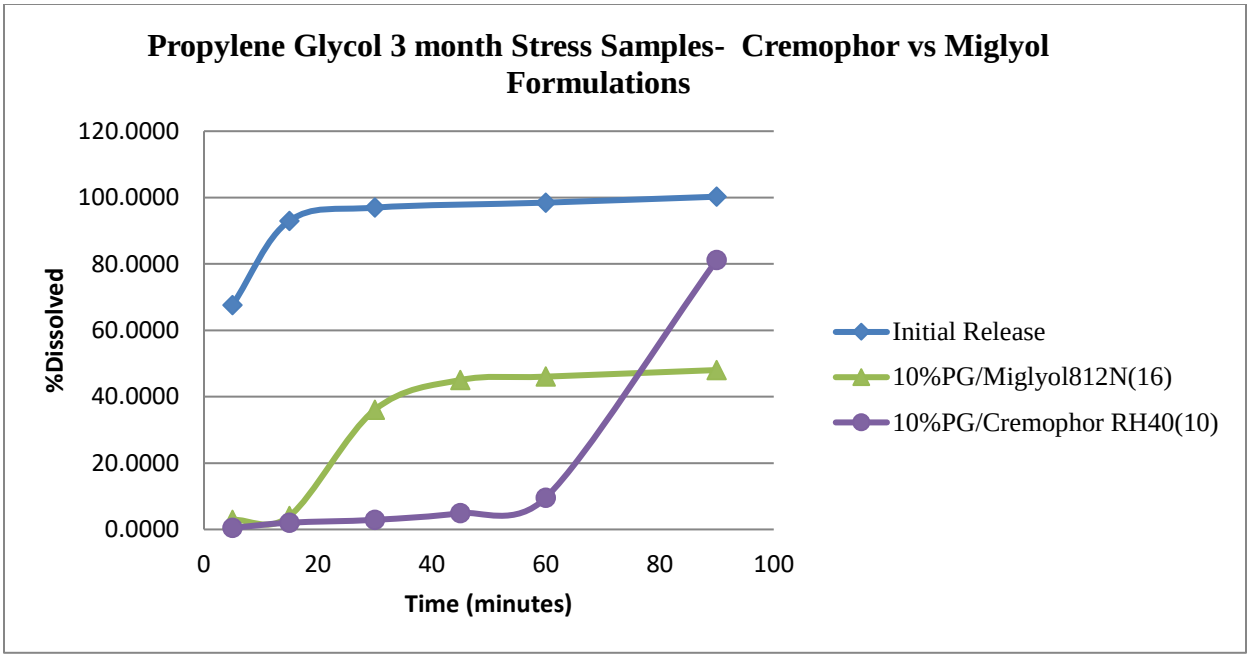


Figure 18. Dissolution of 10% Propylene Glycol 3-Month Stress Gelatin Capsules for Cremophor and Miglyol-Based Formulations at 40°C/75%RH. Cremophor-Based Formulations are More Succeptible to Dissolution Changes that Miglyols.

Visual and SEM Data to Determine Physical Stability

Appearance is useful to further explain data and in this study visual observations as well as electron scanning microscopy (SEM) observations were noted. Visually, the majority of the formulations after 3 months at 40˚C/75%RH appeared slightly more yellow. The impact of each solvent on appearance is discussed below.

As shown in Figure 19, capsules exposed to propylene glycol show the biggest visual change compared to initial appearance followed by glycerin and ethanol. As expected, the appearance also changes with the storage condition with 40˚C /75% RH having capsules that looked to be partially deformed. These visual observations were further confirmed by the SEM images and the corresponding capsule shell thickness measurements. As shown in Figure 20 and

Figure 21, Propylene glycol exposed capsule shell is significantly thicker compared to the shells exposed to glycerin. This increase in wall thickness seems to force the capsule to shrink thus deforming the capsule (Figure 19). The measured shell thickness values are shown in Table 3.

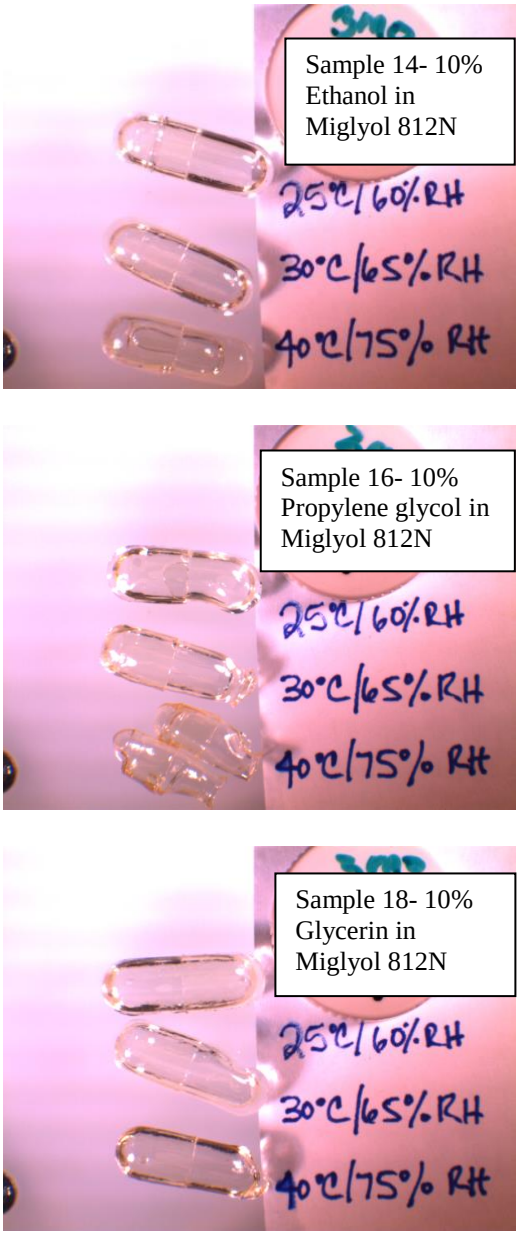
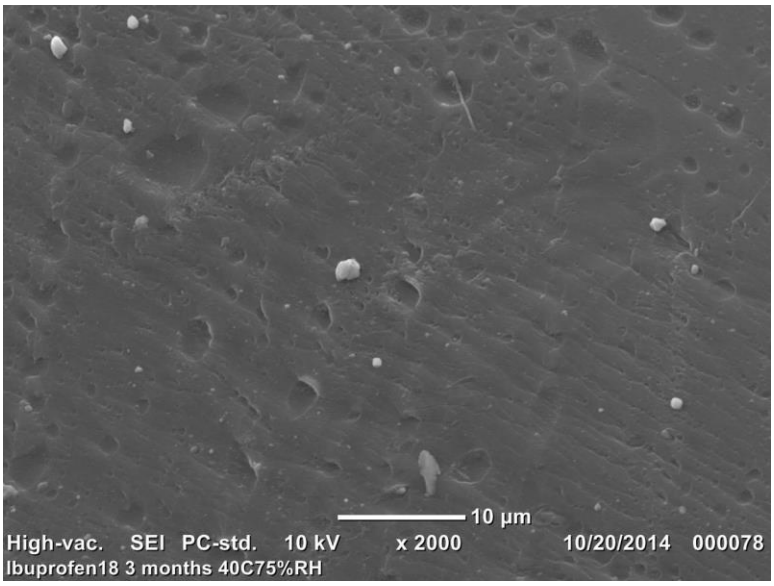


Figure 19. Visual Appearance of 3-Month Stress Gelatin Capsules Exposed to Ethanol, Propylene Glycol and Glycerin (Samples 14, 16 and 18) for All Storage Conditions.



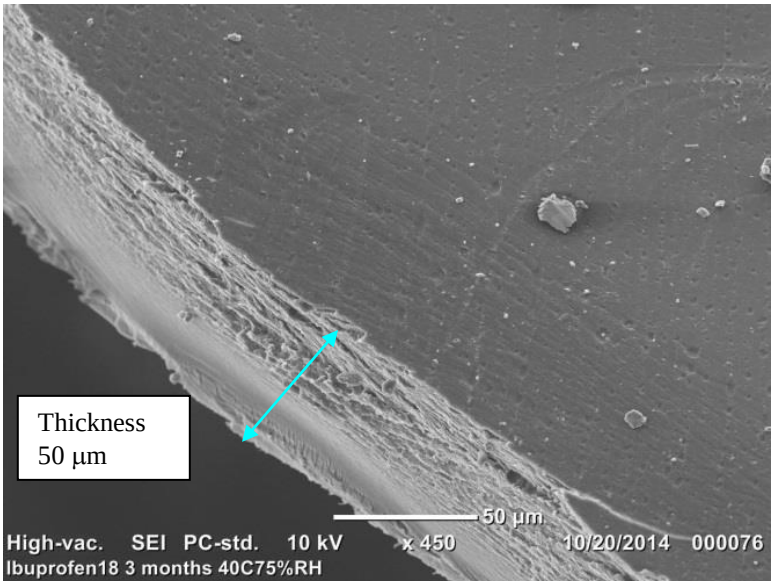
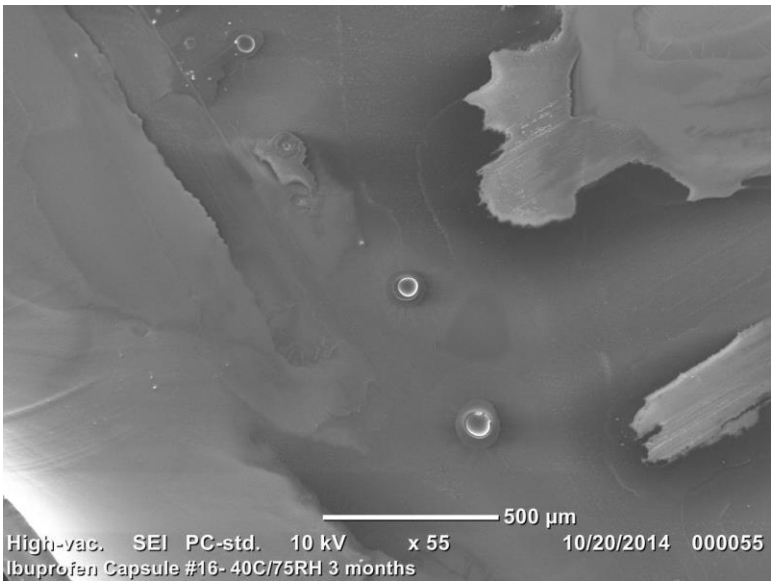


Figure 20. SEM of Sample 18- 10% Glycerin in Miglyol 812N, 3-Month 40˚C/75%RH. SEM Photos of the Inner Side of the Gelatin Capsule Film and a Size View to Measure the Thickness of the Film. The SEM Photos of Glycerin-Exposed Capsules, Sample 18, Inner Side of the Film Show a Damaged/Deformed Film in Comparison to Non-Exposed Capsule.



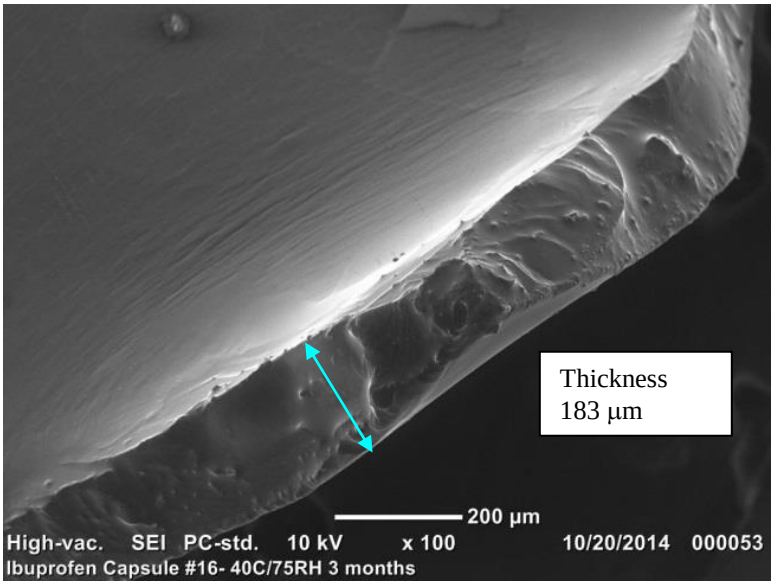


Figure 21. SEM of Sample 16- 10% Propylene Glycol in Miglyol 812N, 3-Month 40˚C/75%RH. SEM Photos of the Inner Side of the Gelatin Capsule Film and a Size View to Measure the Thickness of the Film. The SEM Photos of Propylene Glycol-Exposed Capsules, Sample 16, Inner Side of the Film Show a Smooth Slightly Swollen Top Layer Film in Comparison to Non-Exposed Capsule.

Table 3. SEM Measured Thickness of Glycerin and Propylene Glycol-Exposed Capsules, Samples 18 and 16, for 3 Months at 40˚C/75%RH.

Sample	Thickness measured by SEM (n=3)	
	Initial	40˚C/75%RH
Non-exposed gelatin capsule shell film	40µm (Figure 6)	n/a
Glycerin/Miglyol 812N (18)	n/a	50µm (Figure 20)
Propylene glycol/ Miglyol 812N (16)	n/a	183µm (Figure 21)

Thermal Activity Monitoring Data to Determine Chemical Stability

Since the data discussed up to this point shows propylene glycol to have the greatest impact on capsule performance, the compatibility of the capsule shell with each of the three excipients (propylene glycol, glycerin, ethanol) was measured using microcalorimtery. Samples for this study were prepared by filling each of the excipients at 100% directly into the gelatin capsule. The samples were then loaded into a thermal activity monitor (TAM) microcalorimeter to monitor the rate of reaction between the individual excipients and the gelatin film (Figure 22). The compatibility screening studies show the thermal heat flow adsorbed and released compared to an empty gelatin capsule shell (Figure 22 in green). All the mixtures of the co-solvents in the gelatin shell showed higher heat flow changes than the empty shell, with the highest heat production rate (i.e. reaction rate) for propylene glycol followed by glycerol and ethanol. This microcalorimetry data indicates that out of the three co-solvents tested, ethanol is the most compatible and propylene glycol is the least compatible with the gelatin capsule shell. The TAM data eliminates the impact of the filler as the measurements can be done with gelatin capsule filled with only the co-solvents. The TAM results further confirm the ranking order of excipient reactivity with gelatin capsules observed with DSC (Tg), texture analysis (elasticity), dissolution and appearance (visual and SEM).

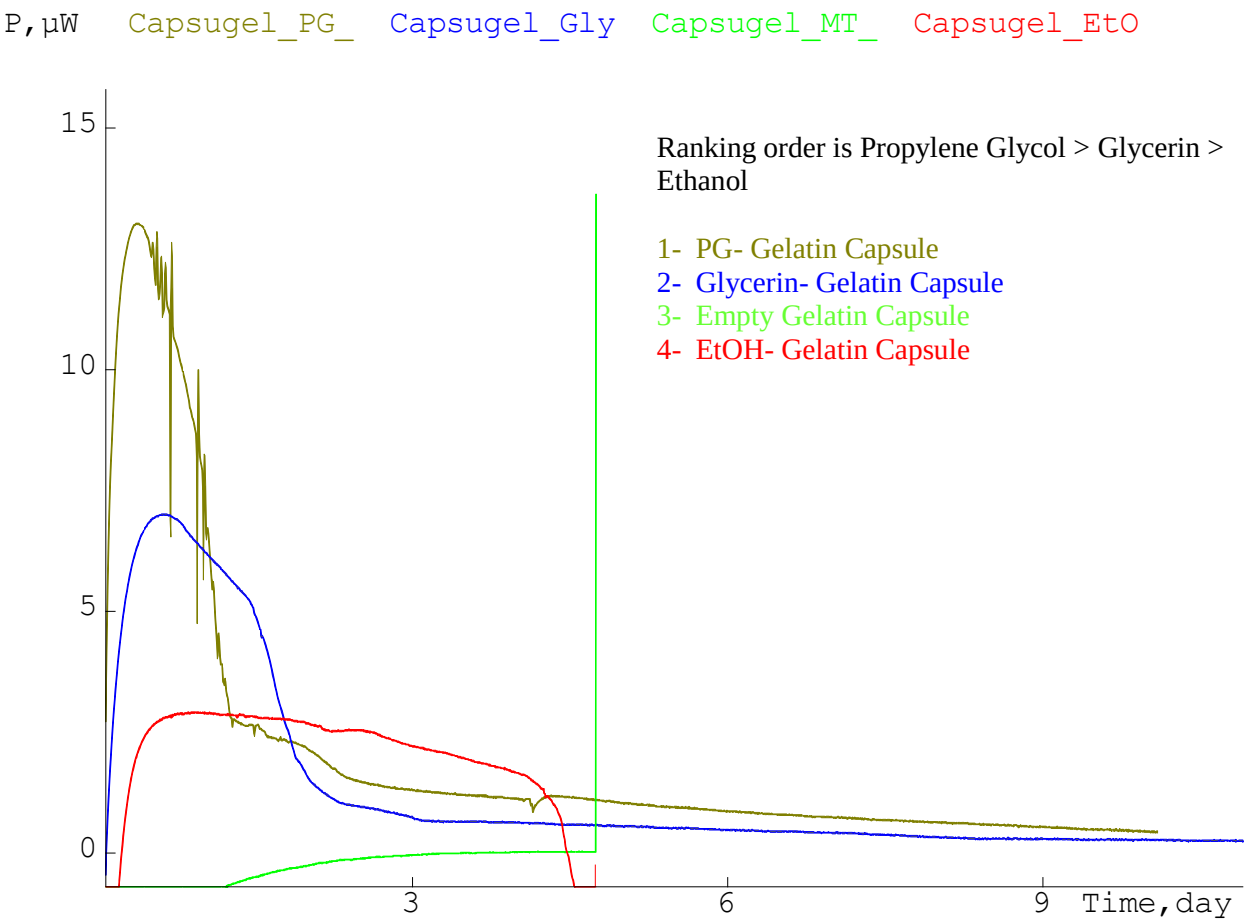


Figure 22. Thermal Analysis Monitoring (TAM) Microcalorimetry of Ethanol, Propylene Glycol and Glycerin.

Cremophor and Miglyol (Filler) Effect to Determine Chemical Stability Variations

The dissolution behavior observed between cremophors and miglyol- based formulations (Figure 18) was independent of the excipients (ethanol, propylene glycol or glycerin) used as can be seen in Figure 23.

In order to better understand the dissolution behavior differences observed between the cremophors and miglyols formulations, the DSC, texture analysis, appearance of these formulations were looked at.

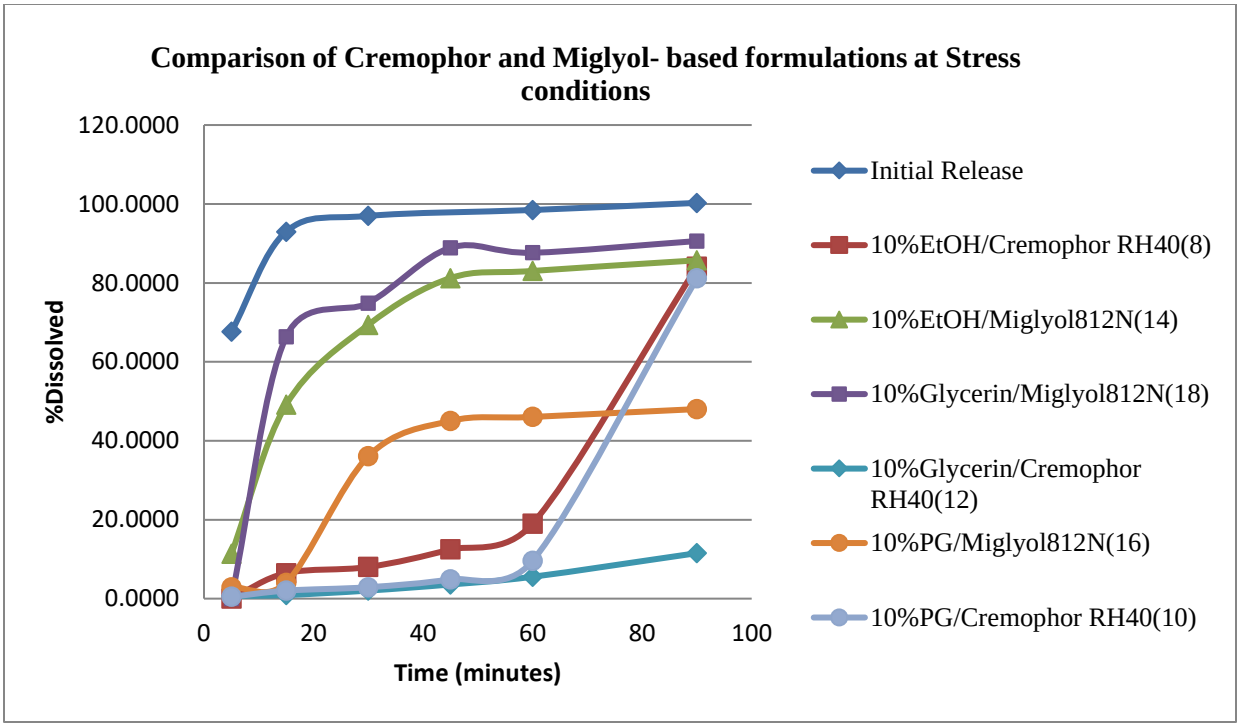


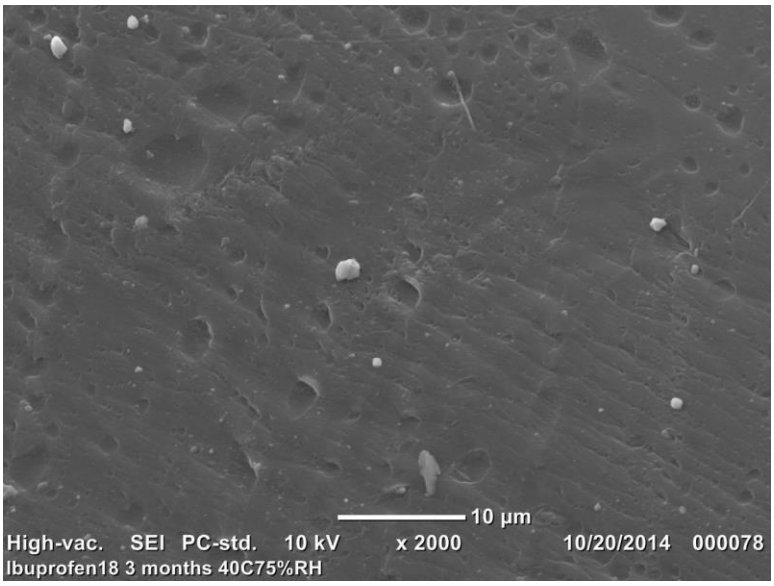
Figure 23. Dissolution of 3-Month Stress Gelatin Capsules for Cremaphor and Miglyol-Based Formulations at 40˚C/75%RH. Cremaphor-Based Formulations are More Susceptible to Dissolution Changes Than Miglyols.

DSC and texture analysis did not show any significant trends between cremophors and miglyols. However, both the visual and SEM images showed differences: visually the capsule shell walls were intact for cremophor formulations while for miglyols, the shell appears to be deformed and shrunken. The SEM images show that the cremophor formulations capsule shells are significantly thicker in comparison to the miglyols, see Figure 24, Figure 25,Figure 26 and Table 4.

Figure 24. Visual appearance of 10% propylene glycol for 3-Months Appearance of 10% Propylene Glycol for 3 Months at 40˚C/75%RH, showing Cremaphor and Miglyol-Based Formulations.

Table 4. SEM Measured Thickness of Glycerin-Exposed Capsules in Miglyol and Cremaphor-Based Formulations, Samples 18 and 12, for 3-Month at 40˚C/75%RH.

Sample	**Thickness measured by SEM (n=3)**	
	Initial	**40˚C/75%RH**
Non-exposed gelatin capsule shell film	40µm (Figure 6)	n/a
Glycerin/Miglyol 812N (18)	n/a	50µm (Figure 25)
Glycerin/Cremophor RH40 (12)	n/a	110µm (Figure 26)



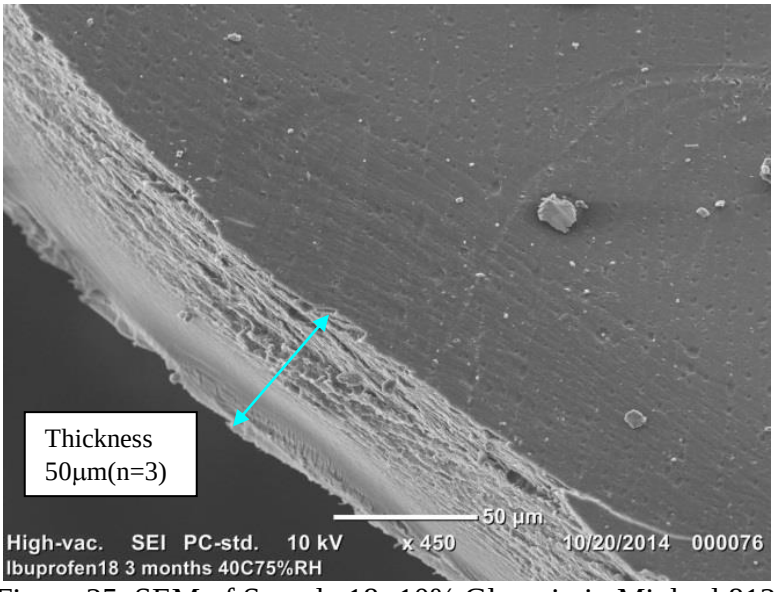
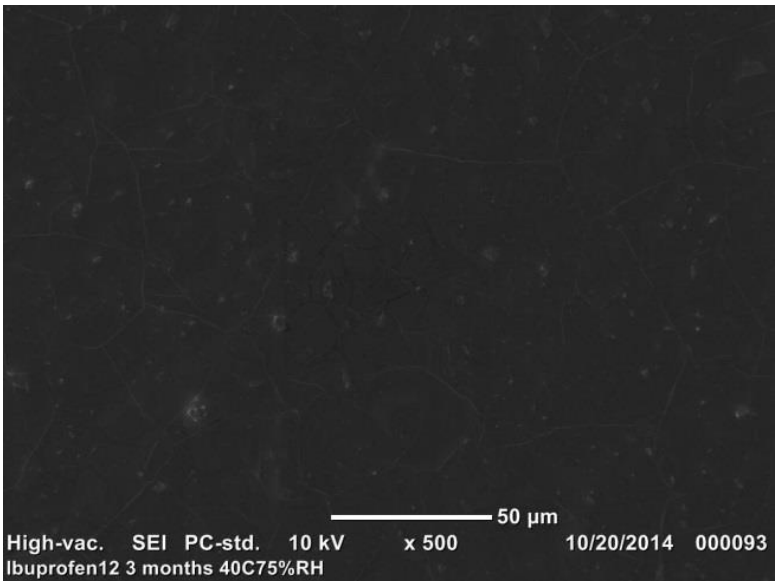


Figure 25. SEM of Sample 18- 10% Glycerin in Miglyol 812N, 3-Month Sample at 40˚C/75%RH.



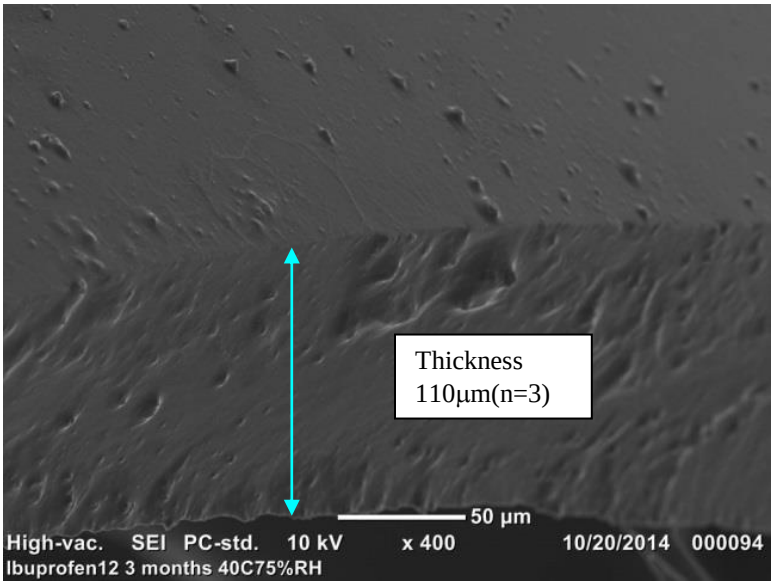


Figure 26. SEM of Sample 12- 10% Glycerin in Cremophor RH40, 3-Month Sample at 40˚C/75%RH.

Aldehyde Testing to Determine Chemical Stability Variations

The hardening of gelatin has been shown to affect the in vitro dissolution of capsules and it has been shown that this is due to cross-linking between protein chains in gelatin [52]. Several studies have been carried out to show that the gelatin cross-linking event involves the reaction between lysine-lysine, lysine-arginine and arginine-arginine in the presence of aldehydes. The cross-linking process results in a formation of a swollen rubbery water insoluble membrane during dissolution testing, this water insoluble gelatin film act as a barrier, restricting drug release [52]. In order to test if this is the cause behind the observed dissolution behavior (Figure 23), the amount of aldehyde present in the cremophor and miglyol formulations were measured (Table 5). The data confirms that cremophor formulations contain higher amounts aldehydes compared to miglyol formulations. This is in agreement with Li et. al., measurements of presence of aldehydes in cremophor [53].

Table 5. Total Aldehyde in Miglyol and Cremaphor-Based Formulations, Before and After Storage for 3 Months at 40˚C/75%RH.

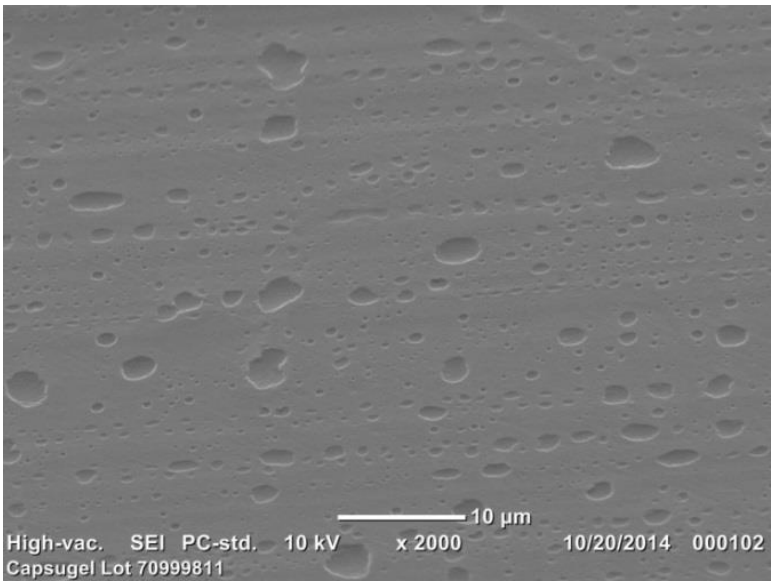
Sample	**Total Aldehydes (μg/mL)**	
	Initial	40˚C/75%RH
PG/Miglyol 812N (16)	0	0
PG/Cremophor RH40 (10)	56	10
Ethanol/Miglyol 812N (14)	0	0
Ethanol/Cremophor RH40 (8)	79	21
Glycerin/Miglyol 812N (18)	0	0
Glycerin/Cremophor RH40 (12)	57	47

Aldehyde-Induced Pellicle Formation in Dissolution Data- Chemical Stability Variations

A formation of a swollen rubbery water insoluble gelatin cross linked membrane was observed during the dissolution testing for the cremophor formulation as shown in Figure 27. This water-insoluble gelatin film acts as a barrier (pellicle), restricting drug release which explains the dissolution behavior of cremophor-based formations seen in Figure 23.

SEM images of the cremophor-based formulation capsules before and after dissolution testing shows gelatin film surface changes indicating potential gelatin cross-linking (Figure 28).

Figure 27. Pellicle formation during dissolution for Sample 6 and 12 -10% glycerin in cremophor-based formulations, after 3-month storage at 40˚C/75%RH. Image represents samples taken out of dissolution bath after completion of testing.



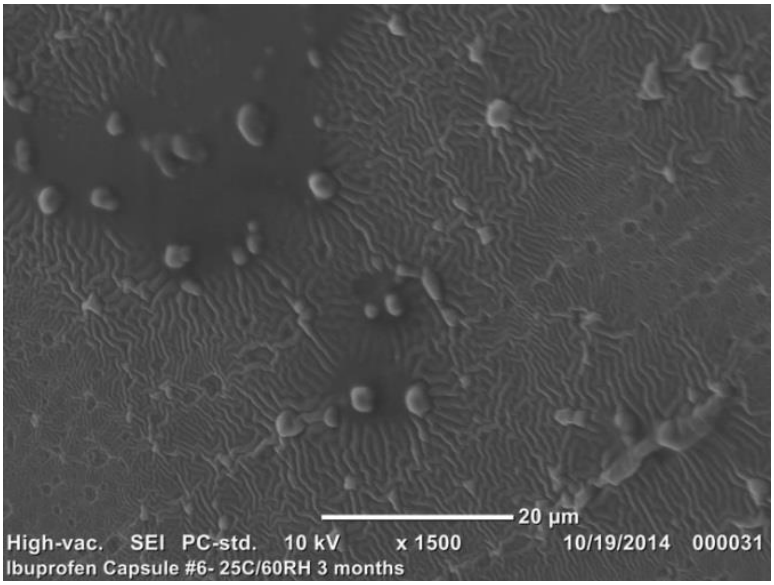


Figure 28. SEM Photos of Gelatin film initial from (left) of Sample 6 -10% Glycerin/cremophor EL, after 3-month storage at 25˚C/60%RH (right). These photos are show inside less smooth view of the gelatin capsule film.

Discussion

Among the 3 excipients studied, propylene glycol-based formulations had the biggest impact in the gelatin capsule shell. Table 6, shows the summary of all performed test on the ethanol, propylene glycol, glycerin exposed samples for 3 months at the three storage conditions.

Table 6. Summary of Results from All Tests Performed.

Test	Conclusion	Notes
Texture Analysis	Glycerin and PG > Ethanol	Most elastic is glycerin, then propylene glycol followed by ethanol
DSC, Tg	PG and Glycerin > Ethanol	All act as plasticizers to gelatin capsule film. PG showed most change to Tg.
SEM & Visual	PG > Glycerin > Ethanol	PG shows the most change to the capsule shell
Dissolution	PG > Glycerin and Ethanol	PG shows most delays in dissolution
TAM	PG > Glycerin > Ethanol	PG is most incompatible alone in gelatin capsule, followed by glycerin and ethanol
Formulation Filler	Cremophor > Miglyol	Cremophor-based formulations had aldehydes which lead to cross-linking in the gelatin capsule shell

In order to understand the interactions between the excipients and the gelatin capsule shell, Hansen solubility parameters (HSP) were calculated. HSPs are based on the cohesive energy which is the energy required to separate the constituents atoms of the material. Cohesive energy is the net effect of all the inter atomic/ molecular interactions including Van de Waals interactions, covalent bonds, ionic bonds, hydrogen bonds, electrostatic interactions, induced dipole and permanent dipole interactions. An understanding of the cohesive energies is important to explain and predict how substances will behave when they are mixed and predict physico-chemical properties (e.g. solubility, glass transition) of drugs and excipients. Solubility parameters can be estimated from molecular structure using molecular modeling and molecular dynamics calculations [59].

Hansen parameters can be used to predict the interactions and incompatibilities between molecules in a multi component mixtures. Hansen has been used specifically to predict solubility of a polymer in a solvent or the compatibility of a polymer and a plasticizer. The calculated Hansen solubility parameters focus on 3 major types of interactions in common organic materials, the London dispersion interactions, the polarity interactions and the hydrogen boding interactions between molecules. These are derived from atomic forces and have also been called dispersion interactions (E_D). Finding the dispersion cohesive energy is the starting point for calculating the 3 Hansen parameters for a given liquid.

The permanent dipole-dipole interactions cause a second type of cohesion energy, the polar cohesive energy (E_P). These are inherently molecular interactions and are found in most molecules to one extent or another. The dipole moment is the primary parameter used to calculate these interactions.

The third major cohesive energy source is hydrogen bonding (E_H). Hydrogen bonding is a molecular interaction and resembles the polar interactions in this respect. Alcohols, glycols and other hydrophilic materials have high hydrogen bonding parameters.

The basic equation governing the assignment of HSPs is the total cohesion energy (E), must be the sum of the individual energies that make it up.

$$\mathbf{E = E_H + E_P + E_D} \qquad \text{Equation 1.}$$

Dividing this by the molar volume gives the square of the total solubility parameter as the sum of the squares of the Hansen components.

$$\mathbf{\delta^2 = \delta_H^2 + \delta_P^2 + \delta_D^2} \qquad \text{Equation 2.}$$

The Hansen solubility parameter δ_d is the component of the cohesive interaction between molecules due to the London dispersion forces, the Hansen solubility parameter δ_p is the

component of the cohesive interaction between molecules due to dipole polar interactions, and the Hansen solubility parameter δ_h is the component of the cohesive interaction between molecules due to the formation of hydrogen bonds [60].

The calculated Hansen solubility parameters for any drug are determined by adding each structural group contribution and dividing the sum by the molar volume, which is directly calculated from the chemical structure [60, 61]. Using these calculated parameters the equation for the solubility parameters (Hansen Distance), Ra, between to materials based on their respective partial solubility parameter components was derived as shown below.

$$(Ra)^2 = 4(\delta D_2-\delta D_1)^2 + (\delta P_2-\delta P_1)^2 + (\delta H_2-\delta H_1)^2 \qquad \text{Equation 3.}$$

Table 7. Summary Table of "Neat" Calculated Hansen Solubility Parameters (in $MPa^{1/2}$) and Distance Measurements to Gelatin (Ra).

Solvent	Molar Volume	Hydrogen Bonding, δH	Polarity, δP	Dispersion, δD	Solubility Parameter	Ra
Ethanol	59.87	19.39	8.8	15.76	26.47	15.0
PG	74.87	23.33	9.33	16.75	30.2	14.6
Glycerin	70.74	29.55	12.25	17.52	36.47	18.1
Cremophor EL	1464.65	16.41	2.23	16.69	23.52	13.2
Cremophor RH40	1616.09	15.84	2.02	16.79	23.17	13.1
Miglyol 812	480.9	18.53	6.72	17.35	26.29	11.5
Miglyol 840	348.31	12.7	4.59	14.82	19.78	16.9
Gelatin	1609.42	16.57	6.08	22.99	28.99	-

Calculated the gelatin Hansen parameter from the average of the three chains shown for collagen in the literature using Molecular Modeling Pro software (Figure 29, [48]).

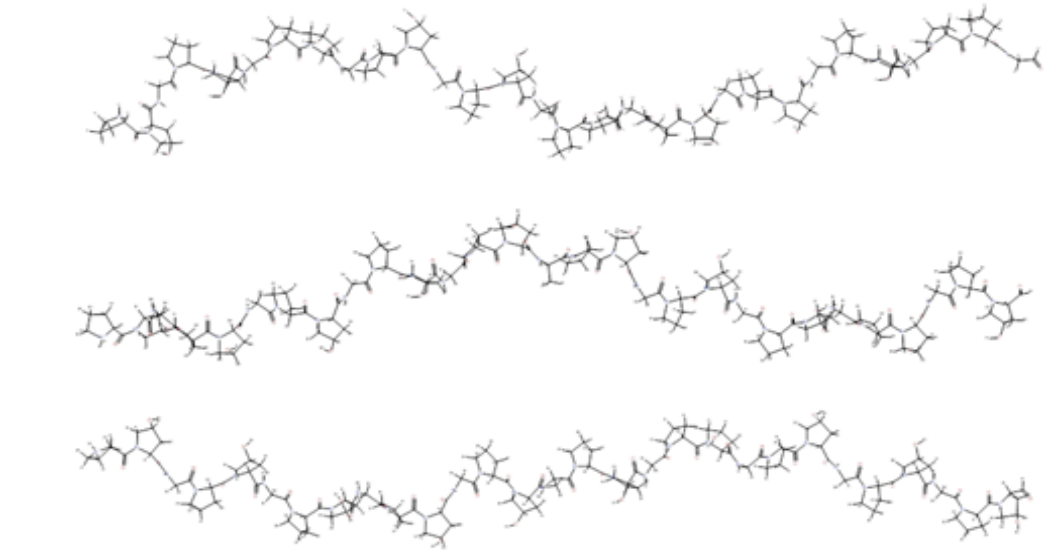


Figure 29. Collagen 3 Helix Structure.

Based on the Hansen solubility parameters distance, Ra, propylene glycol has the smallest Ra thus making it the most reactive (soluble) with gelatin among the 3 excipients studied.

Data obtained for the puncture force needed to break the gelatin capsule shell was used to look at the HSP predictability of the formulations' impact to the gelatin capsule shell elasticity (i.e. plasticizing effect). The calculated HSP values in Table 7 were used to determine the predicted

puncture force for each of the formulations tested. The predicted puncture force determination was performed to find the best fit of the data by using linear algebra [55] (Equation 4).

$$\mathbf{A\,x = b}$$ Equation 4

Where the dimensions of A is a matrix of HSP, b is the matrix of the observed puncture forces from texture analysis, and x is a matrix containing the best fitting weights.

The Hansen Parameter *differences* for all compositions excipients and fillers were entered into a matrix A.

The best fitting weights that allow one to predict the resulting Puncture Forces (matrix b) from the corresponding Hansen Parameter differences can be calculated by the matrix expression (Equation 5).

$$\mathbf{(A^T A)^{-1} A^T b = x}$$ Equation 5.

Where x will contain the best possible coefficients for predict the values in b given the values in A.

The observed versus predicted puncture force plots generated using the linear algebra approach for the 5% co-solvents and 95% fillers at 40˚C/75%RH are discussed below. This discussion is applicable to also to 10% co-solvent and other storage conditions.

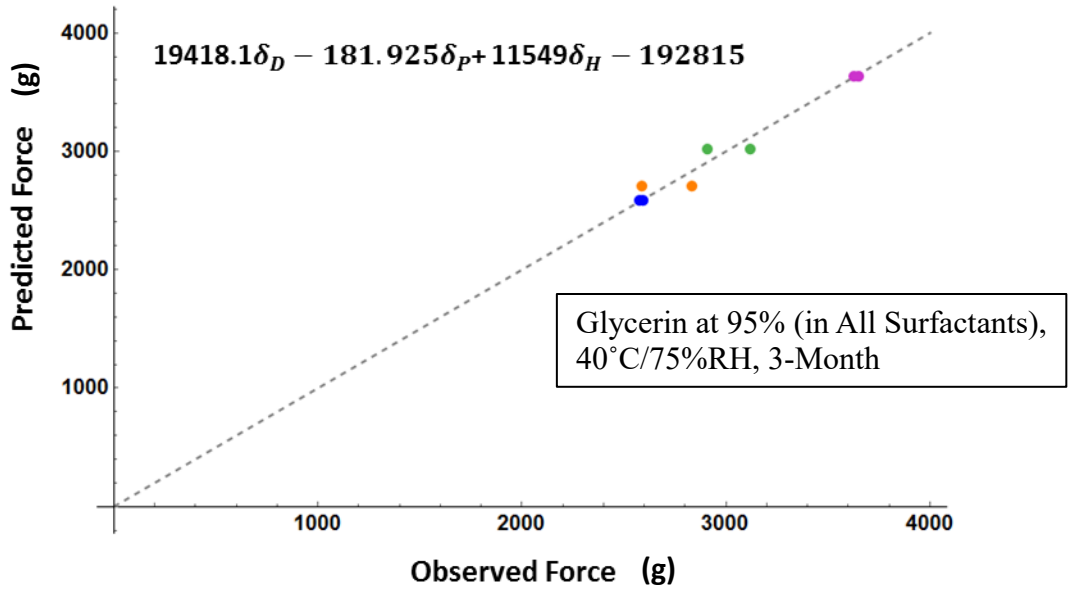


Figure 30. Observed versus predicted puncture force plot for 5% glycerin in all fillers. Cremophor EL, cremophor RH40, miglyol 812, miglyol 840 data are shown in green, blue, magenta and orange colors repectively.

Plasticization weakens the gelatin and compromises the mechanical properties of the gelatin (reflected in a reduced puncture force). The larger the value of Puncture Force, the smaller the influence of the Solvent/Surfactant composition. One can simply read from the plot which Solvent/Surfact composition is best suited as the "filler" for a liquid filled capsule.

This suggests that the larger the Hansen Parameters difference between gelatin and the Solvent/Surfactant composition, the smaller the plasticization will be. Intuitively this is correct. If the differences were actually smaller, we would expect the gelatin to be in danger of dissolving. The closer one is to this latter situation, the greater will be the plasticization.

In the case of 95% glycerin (Figure 30), the values obtained from "x" do a good job of predicting what force will be required to puncutre the gelatin.

The relative values of the weights are approximately consistent when comparing surfactants across the three solvents (Figure 31and Figure 32).

For situations where the fit is not as good, it is possible that the composition of the solvent/surfactant is not ideal, i.e. mixing may be imperfect resulting in heterogeneous mixtures.

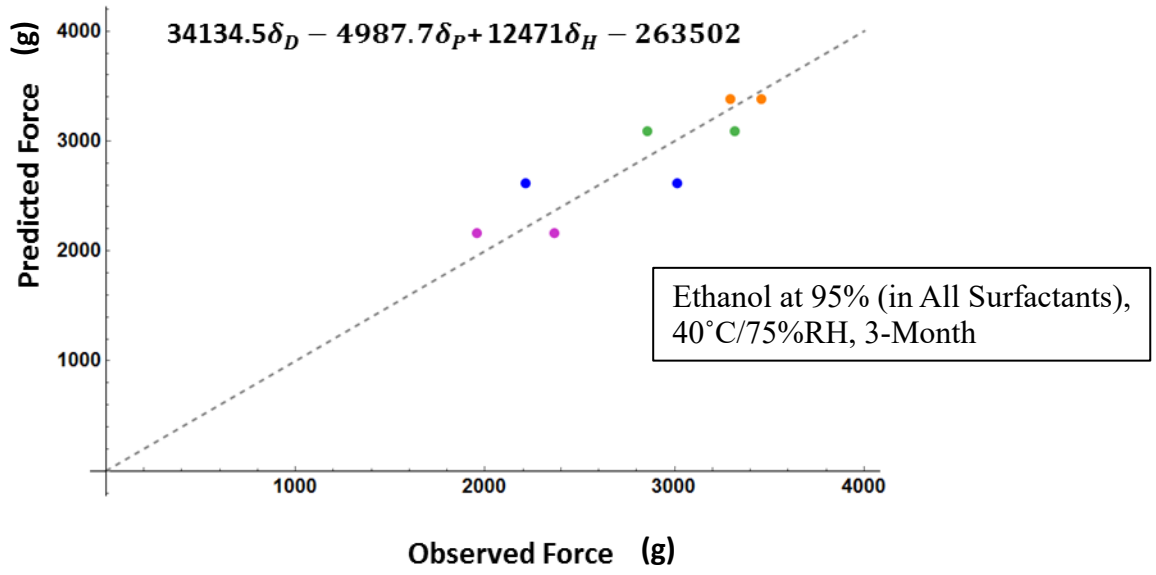


Figure 31. Observed versus Predicted puncture force plot for 5% ethanol in all fillers. Cremophor EL, cremophor RH40, miglyol 812, miglyol 840 data are shown in green, blue, magenta and orange colors repectively.

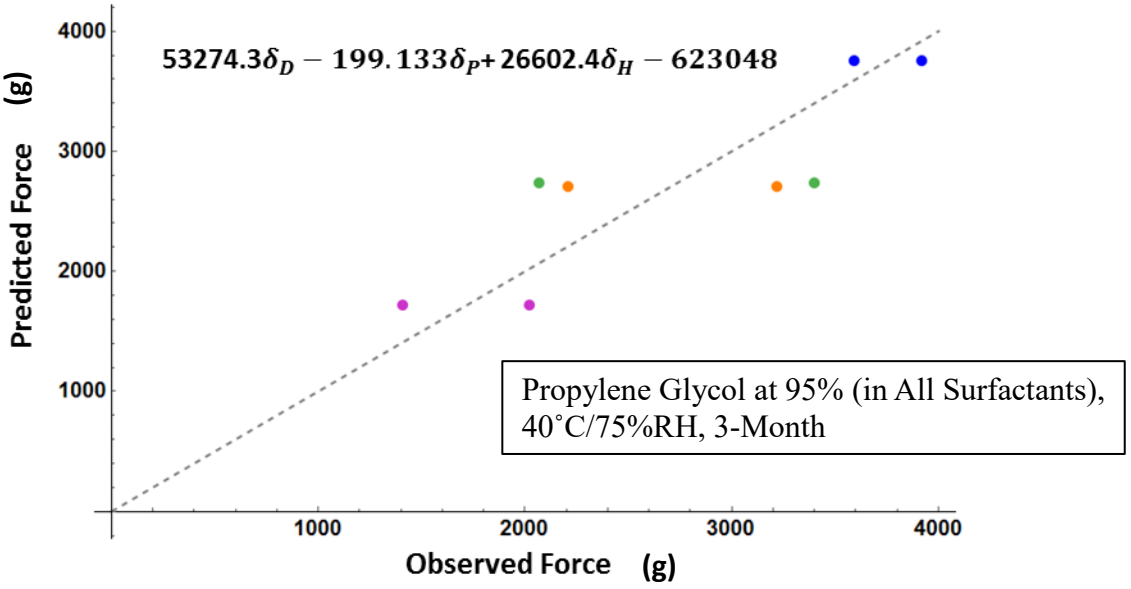


Figure 32. Observed versus Predicted puncture force plot for 5% propylene glycol in all fillers. Cremophor EL, cremophor RH40, miglyol 812, miglyol 840 data are shown in green, blue, magenta and orange colors repectively.

The figures above (Figure 30,Figure 31 and Figure 32) show that when using glycerin as a co-solvent, the filler miglyol 812 will have a less plasticizing effect on gelatin than the other 3 fillers tested. Similarly, with ethanol it is miglyol 840 and with propylene glycol it is cremophor RH40. Hansen solubility parameters data shows propylene glycol has the shortest distance in the HSP sphere space. From physical properties of the solvent perspective as well as Hansen polarity measurement, we conclude that glycerin and propylene glycol are more polar solvents than ethanol. But the viscosity, surface tension and density, shown in Table 8Table 8 below, would not allow glycerin to diffuse as quickly as propylene glycol to react with the film.

Table 8. Properties of Ethanol, Propylene Glycol and Glycerin at 25˚C.

Solvent	**Molecular Formula**	**Dielectric constant (ε)**	**Specific Density (g/mL)**	**Surface Tension (dynes/cm)**	**Viscosity (Pa.s)**
Ethanol	C_2H_6O	24.5	0.789	22.3	0.00102
Propylene glycol	$C_3H_8O_2$	32.1	1.036	40.1	0.042
Glycerin	$C_3H_8O_3$	42.5	1.261	64.0	1.412

Ethanol in the other hand can diffuse quickly in the gelatin capsule film and is therefore minimized in concentration in soft gelatin capsule formulations [(50)]. The water-soluble organic solvents in commercial available solubilized oral formulations are polyethylene glycol 400 (PEG 400), ethanol, propylene glycol, and glycerin along with many water-soluble non-ionic surfactants. The most common water-soluble organic solvent in PEG 400 for soft gelatin capsules [(49)].

Gelatin capsules with propylene glycol are more damaged (i.e. break and leak) under stability compared to glycerin because it is more lipophilic and volatile than glycerin [(50)].

Conclusion

The co-solvent compatibility with gelatin capsule shell study showed that glycerin and propylene glycol with three and two hydroxyl groups respectively to be more incompatible than ethanol with one hydroxyl group. This reactivity was based on changes in the glass transition, elasticity from texture analysis, dissolution release and observations from visual and SEM techniques.

Hansen solubility parameters, viscosity and surface tension were used to explain the results. The calculated Hansen solubility parameters predict propylene glycol to be the least compatible among the three co-solvents because of it's proximity to the gelatin capsule shell.

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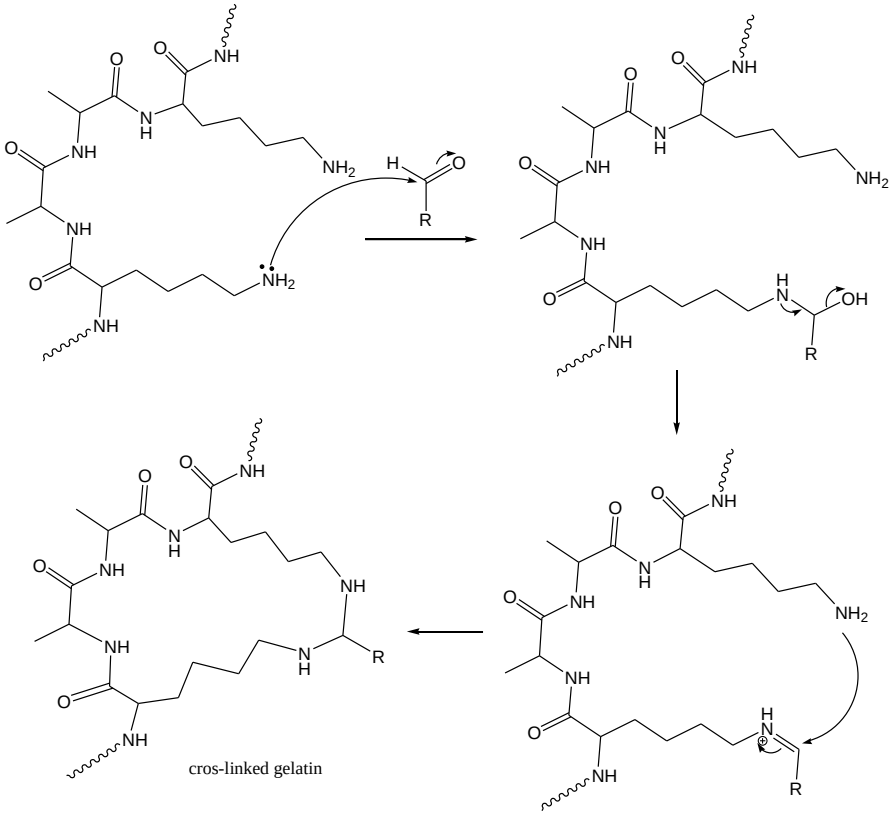
Appendices

Appendix 1: Cross-linking of Gelatin

One of the common modifications observed in gelatin capsules is the cross-linking of gelatin which results in slower shell disintegration leading to slower formulation release. The cross-linking can either be internal or external. Internal cross-linking occurs when capsules experience high humidity, high heat, light or UV- vis radiation exposures [(7)]. External cross-linking is due to exposure to external agents such as aldehydes, peroxides, metal ions and sulfonic acids which chemically alter the gelatin structure (Figure A). Small amounts of aldehydes and peroxides are present in some commonly used excipients and are well characterized in the literature. Not only aldehydes but the presence of chemicals such as saccharides and dyes [(8)]; imines and ketones [(12)]; calcium carbonate [(13)], hydrogen peroxide, sulfuric acid and p-toluene sulfonic acid [(14)]; and benzene and carbodiimides [(F10)] are also known to induce cross-linking.

Figure A. Cross-linking in gelatin a reaction of amine in Lysine amino acids with Aldehyde (8).



The cross- linking causes the formation of swollen, very thin, tough, rubbery, water insoluble membrane, also known as pellicle. The pellicle acts as a barrier and restricts the release of drug. It is not disrupted easily by gentle agitation, and the dissolution values drop often to the point of rejection [8, 12]. For gelatin capsule products, cross-linking renders the gelatin capsule shell insoluble in aqueous media and decreases the in vitro rate of dissolution [25]. Digenis et al., describes the mechanistic rationalizations that explain gelatin cross-linking in stress conditions relevant to pharmaceutical situations, see Figure A[8].

Digenis et al., described the following chemical events to be involved in the cross-linking process [8,7]:

1. The reactivity of the gelatin to arise from the trifunctional amino acid lysine. The lysine residues, which are proximal to each other, are oxidatively deaminated to yield terminal

aldehyde groups. One of the aldehyde groups is attached by a free ε-amino group of a neighboring lysine to yield an imine, which subsequently undergoes a series of adol-type condensation reactions to produce a cross-linked product containing pyridinium ring(s).

2. The lysyl ε-amino group reaction with aldehyde yielding a hydroxymethylamino derivative, which latter reacts with another hydroxymethylamino lysine residue to form dimethylene ether, which in turn rearranges and results in the development of cross link.
3. A formation of an aminal (amine form of acetal) from the reaction of cationic imidine intermediate with a free amino group. The pH of the environment plays and important role in this type of reaction.

In addition to lysine-lysine cross-linking, lysine-arginine and arginine-arginine cross-linking was also reported [7].

Appendix 2: Commonly used liquid filled capsule formulation excipients

Excipients used in lipid based formulations can range from lipophilic vehicles, solubilizing agents, surfactants, emulsifying agents and adsorption enhancers. Commonly used excipients for liquid and semi-solid formulations in hard gelatin capsules are listed in Table A and B.

The chemical structure, hydrophilic lipophilic balance and aldehyde impurity information for the cremophors and miglyols is listed in Table C.

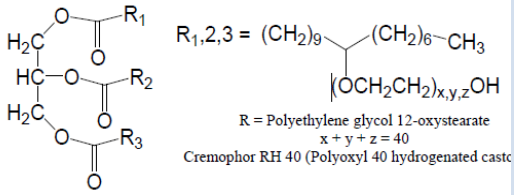
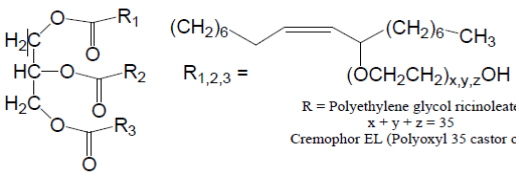
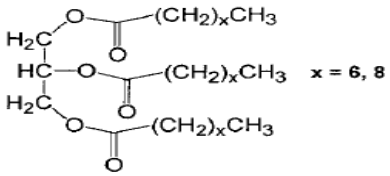
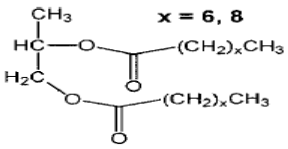
Table A. Excipients used in formulations of liquid and semi-solid hard gelatin capsules [39].

Refined Specialty Oils	**Medium chain triglycerides (MCTs) and related esters**	**Semi-solid lipophilic vehicles / Viscosity modifiers for lipophilic liquid vehicles**	**Solubilizing agents, surfactants, emulsifying agents adsorption enhancers**
Arachis oil	Akomed E	Hydrogenated Specialty oils	Capryol 90
Castor oil	Akomed R	Arachis oil: Groundnut 36 Castor oil: Cutina HR	Gelucire 44/14, 50/13
Cottonseed oil	Captex 355	Cottonseed oil: Sterotex Palm oil: Softisan 154	Cremophor RH 40
Maize (corn) oil	Labrafac CC	Soybean oil: Akosol 407	Imwitor 191, 308(glycerin content <5%), 380, 742, 780 K, 928, 988
Olive oil	Labrafac PG	Aerosil	Labrafil M 1944 CS, M 2125 CS
Sesame oil	Lauroglycol FCC	Cetoseryl alcohol	Lauroglycol 90
Soybean oil	Miglyol 810	Cetyl alcohol	PEG MW > 4000
Sunflower oil	Miglyol 812	Gelucires 33/01, 39/01, 43/01	Plurol Oleique CC 497
-	Miglyol 829	Glyceryl behenate (Compritol 888 ATO)	Poloxamer 124 and 188
-	Miglyol 840	Glyceryl palmitostearate (precirol ATO5)	Softigen 701, 767
-	Softisan 645	Softisans 100, 142, 378, 649	Tagat TO
-	-	Steryl Alcohol	Tween 80

Table B. Maximum amounts of solubilizing excipients used in commercially available oral formulations [34,36].

Excipient	**Estimated maximum amount administered orally**	**Product, Drug**
Ethanol	3.1 mL, b.i.d. 4.2 mL, q.d.	Norvir, oral solution (Ritonavir) Kaletra, oral solution (Lopinavir/Ritonavir)
Propylene Glycol	51 grams, b.i.d.	Agenerase, oral solution (Amprenavir)
Glycerin	3.1 grams	Reference 36
Cremophor EL (Polyoxyl 35 Castor oil, Surfactant)	620 mg, b.i.d.	Norvir, capsule (Ritonavir)
Cremophor RH 40 (Polyoxyl 40 hydrogenated Castor oil)	≥400 mg	Reference 36
Medium chain triglyceride (Miglyols)	20 mL, q.d.	Sustiva ,30 mg/mL oral solution (Efavirenz)

Table C. Hydrophilic lipophilic balance (HLB) values for Cremophor and Miglyols used in study.

Product (Composition)	Structure	HLB	Aldehyde Content
Cremophor RH40 Hydrogenated Castor Oil Ethoxylated with 40 EO		14-16	Contains traces of Formaldehyde and Acetaldehyde *
Cremophor EL Castor Oil Ethoxylated with 35EO		12-14	Contains 0.8ug/g Formaldehyde and <0.2ug/g Acetaldehyde *
Miglyol 812 Caprylic/ Capric Triglyceride Glycerin w/ 60%C8 & 40%C10		15.36	No aldehyde content found
Miglyol 840 Propylene Glycol Dicaprylate/ Dicaprate Propylene Glycol w/ 70%C8 & 30%C10		Not found	No aldehyde content found

* Li et al. (2006) Detection and quantification of low molecular weight aldehydes in pharmaceutical excipients by head space gas chromatogrphy J Chromatogr A 1104:1-10.