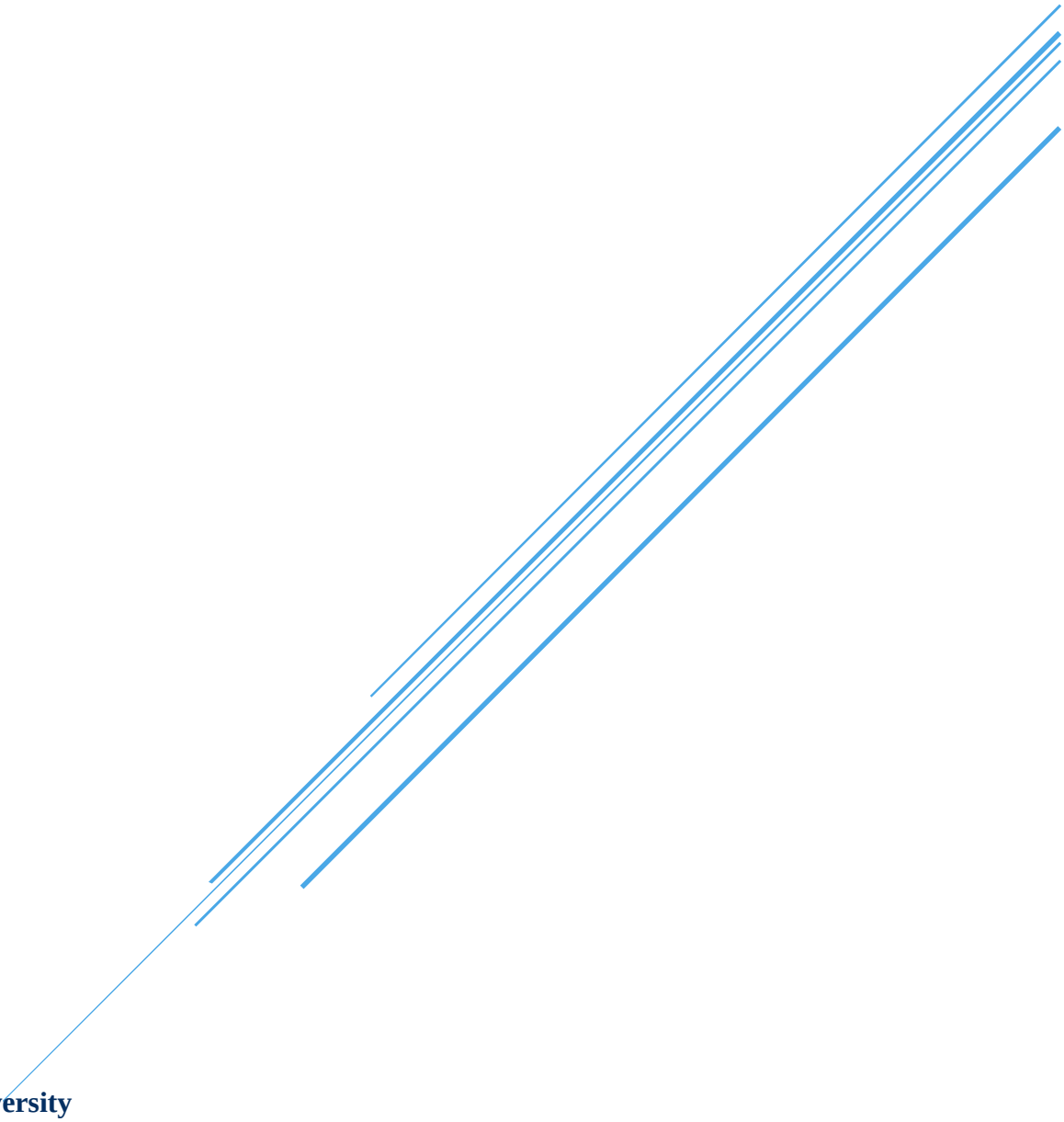
Development of Gabapentin Expandable Gastroretentive Controlled Drug Delivery System

MSc Degree Thesis



April - 2019
Birzeit University

Faculty of Pharmacy, Nursing and Health Professions

Master Program in Industrial Pharmaceutical Technology

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تطوير نظام دوائي ثابت الافراز قابل للتوسع متواجد في المعدة لفترة طويلة يحتوي على مادة الجابابنتين

This Thesis was submitted in partial fulfillment of the requirements for the Master's degree in Industrial Pharmaceutical Technology from the Faculty of Graduate Studies at Birzeit University, Palestine

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Contents

List of Abbreviations

API: Active Pharmaceutical Ingredient

BCS: Biopharmaceutical Classification System

FTIR: Fourier-Transform Infrared Spectroscopy

G-GR: Gastroretentive Gabapentin

G-IR: Immediate Release Gabapentin

HCL: Hydrochloric acid

HPLC: High Performance Liquid Chromatography

HPMC: Hydroxypropyl Methylcellulose

KOH: Potassium Hydroxide

LAT: L-amino Acid Transporters

MMC: Migrating Myoelectric Complex

N/A: Not Applicable

PEG 400: Polyethylene Glycol 400

PG: Propylene Glycol

PP 407: Poloxamer P407

PVP K30: Polyvinyl Pyrrolidone K30

RPM: Revolutions Per Minute

UV: Ultra Violet

XRD: X Ray Diffraction

D10%: Time required to release 10% of the active ingredient during dissolution test

D50%: Time required to release 50% of the active ingredient during dissolution test

D90%: Time required to release 90% of the active ingredient during dissolution test

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Abstract

Expandable drug delivery systems are one of many gastroretentive delivery systems which have emerged during the last few years. Expandable systems are usually folded in a capsule and expand to dimensions greater than the pyloric sphincter upon contact with gastric fluid. This prevents them from being evacuated by gastric emptying. The main objective of developing such systems is to increase the residence time of a specific drug in stomach; controlling its release, increasing its bioavailability and patient's compliance, decreasing its side effects and dosing frequency. An expandable gastroretentive drug delivery system containing Gabapentin was developed using experimental design. Compared to the multi layered Accordion Pill™ system, the developed system was able to expand to the required dimensions and obtain controlled release of the drug using one layer instead of three or more layers. This system was able to unfold at stomach pH in less than 15 minutes and obtain a controlled release of 78.1 ± 4.7 % in 6 hours following first order release kinetic model. It is rigid in stomach and its rigidity decreases at intestinal pH. FTIR analysis indicated the occurrence of hydrogen bonding in Gabapentin when present in the developed system. XRD test results indicated that Gabapentin physical properties changed from crystalline in the typical state to amorphous in the developed system.

أنظمة توصيل الدواء القابلة للتوسع هي واحدة من العديد من أنظمة التوصيل المتواجدة في المعدة لفترات طويلة التي ظهرت خلال السنوات القليلة الماضية. عادة ما يتم طي الأنظمة القابلة للتوسع في كبسولة وتوسعها يحصل

لأبعاد أكبر من أبعاد العضلة العاصرة البوابية عند ملامسة سائل المعدة. هذا يمنع إخلاءها أثناء حصول عملية إفراغ المعدة. الهدف الرئيسي لتطوير مثل هذه الأنظمة هو زيادة وقت إقامة دواء معين في المعدة و بالتالي التحكم في افرازه، زيادة التوافر البيولوجي له، و التقليل من آثاره الجانبية وعدد الجرعات اللازم اخذها يوميا. تم تطوير نظام توصيل دوائي قابل للتوسع يحتوي على مادة الجابابنتين. بالمقارنة مع نظام التوصيل الدوائي متعدد الطبقات، كان بإمكان النظام المطور التوسع الى الحجم المطلوب و التحكم بافراز الدواء باستخدام طبقة واحدة بدلا من ثلاثة او اكثر. هذا النظام بإمكانه التوسع في وسط المعدة الحمضي في أقل من 15 دقيقة و افراز 78.1 ± 4.7 ٪ من المادة الفعالة خلال 6 ساعات باتباع الدرجة الأولى من النموذج الحركي للإفراز. هذا النظام صلب في المعدة و تقل صلابته في الوسط المعوي. أشارت نتائج اختبار جهاز "فوريه" لتحويل طيف الأشعة تحت الحمراء الى حدوث ترابط هيدروجيني في مادة الجابابنتين عند وجودها في النظام المطور. أشارت نتائج اختبار حيود الأشعة السينية الى ان الخصائص الفيزيائية للجابابنتين تغيرت من البلورية في الحالة النموذجية إلى غير متبلورة في النظام المطور.

Part One: Introduction

1.1. Gastroretentive drug delivery systems

Many gastroretentive drug delivery systems have emerged during the last few years; these systems include floatable, bioadhesive, high density, magnetic and expandable gastroretentive drug delivery systems (Lopes, et al. 2016). These systems are intended to increase the residence time of a specific drug in stomach. This prolonged retention has many advantages including controlling the drug release, enhancing the bioavailability specifically for drugs with narrow absorption window and decreasing the side effects and dosing frequency as less doses are needed to obtain the same effect (Lopes, et al. 2016). In order to be suitable for a gastroretentive delivery system the drug should have a wide therapeutic window, a biological half-life ranging from 2-8 hours (Jantzen and Robinson 2002), and optimally taken in multiple daily doses. The main factors affecting gastric retention of drug dosage forms are the fed or fasted state, density and size of the drug delivery system.

1.1.1. Floatable systems

Floatable systems exhibit lower density than the gastric medium (i.e. lower than 1.004 g/cm^3) upon contact with gastric fluid, thus remain buoyant and resident in the stomach for extended period of time without affecting the rate of gastric emptying (Whitehead, et al. 1998). These systems can be either effervescent (i.e. gas producing) or non-effervescent. Both gastric contents and floating force affect the residence time of floating systems in the stomach (Mayavanshi and Gajjar 2008).

1.1.2. Bioadhesive systems

Adhesion to a biological membrane (ex. mucus layer) is the main mechanism by which bioadhesive systems prolong their residence time in stomach. These systems are most beneficial in localized drug delivery. However, they are usually sensitive to gastric environmental changes such as mucus layer regeneration (Bardonnet, et al. 2006).

1.1.3. High density systems

High density systems have the opposite principle of floating systems. They display higher density than the gastric medium and sink to the bottom of the stomach at which they remain for prolonged periods, resisting gastric emptying (Bardonnet, et al. 2006).

1.1.4. Magnetic systems

Magnetic attraction is main principle used in developing magnetic systems. These systems contain a small magnet and extend their residence time in stomach with the help of an external magnet which is usually placed below the abdomen (Murphy, et al. 2009). The major drawback of these systems is the need of an external device, which is not the case in other drug delivery systems.

1.1.5. Expandable systems

Pyloric sphincter is a rounded band of smooth muscle which represents a valve between the stomach and the intestines (Bonewit-West, Hunt and Applegate 2016). It usually relaxes (i.e. opens) during gastric emptying process, allowing gastric contents to exit

to the intestines (Bonewit-West, Hunt and Applegate 2016). Expandable systems expand to dimensions greater than the pyloric sphincter in it relaxed state, which provides prolonged gastric residence time. This technique can be called mechanical resistance. In addition to expansion to the suitable size some factors must be considered when developing these systems. The developed system should be easily swallowed and should not block the pyloric sphincter, thus gastric emptying process after expansion. In addition, its residual should be easily evacuated from the stomach after complete drug release (Klausner, Lavy, et al. 2003) (Nadav, et al. 2014). These systems are usually folded into capsules and expand upon contact with the gastric fluid. They are usually made from biodegradable polymers (Klausner, Eyal, et al. 2003). Many shapes have been developed which can folded into a capsule including ring, tetrahedron, and disc shapes (figure 1).

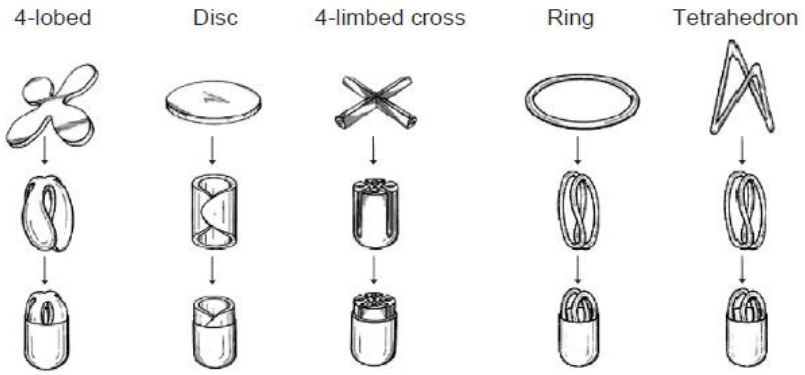

Figure 1. Different expandable system shapes (Caldwell, Gardner and Cargill 1988).

1.2. Physiological properties of the stomach

The stomach's gastric medium represents an acidic medium which mainly ranges from pH 1.1 to 4 (Dressman, et al. 1990) (Mojaverian and Chan 1988). Its gastric epithelial cells are separated from the gastric medium by a mucus membrane which represents a protective barrier for these cells (Bonewit-West, Hunt and Applegate 2016). The stomach possesses an evacuating mechanism called gastric emptying, during which a series of contractions result in evacuating the stomach contents to the intestine through the pyloric sphincter. The pyloric sphincter relaxes to 12.8 ± 7mm during gastric emptying (Paul and Charles 1981) (Salessiotis 1972).

1.2.1. Factors affecting the pH of the stomach

The pH of the stomach is affected by more than one factor including diet, disease, age, drugs and to a lesser extent gender. Fed state increases the pH up to 4 (Mojaverian and Chan 1988), while in fasted state the pH ranges from 1.1 to 1.25 (Dressman, et al. 1990) (Russell, et al. 1993) (Lui, et al. 1986). Both AIDS and pernicious anemia can decrease gastric acid secretion resulting in an elevation in gastric pH (Holt, Rosenberg and Russell 1989) (Lake-Bakaar, et al. 1988). About one fifth of the elderly have a gastric pH above 5 as a result of a decrease or an absence of gastric acid secretion (Varis, et al. 1979). The use of drugs like proton pump inhibiters and H_2 receptor blockers increases the pH. Men were reported to have more acidic gastric pH than women (Feldman and Barnett 1991) (Prewett, et al. 1991).

1.2.2. Factors affecting gastric emptying

Contractions in fasted state are known as migrating myoelectric complex (MMC). MMC cycle controls the gastrointestinal motility patterns and is divided into 4 phases which either describes a contraction or a non-contraction period. Digesting food causes a disruption and prolongation in this cycle, which leads to a delay in gastric emptying. Studies have demonstrated that drugs taken on an empty stomach are usually evacuated within one hour from ingestion (Talukder and Fassihi 2004). Body posture also has an effect on gastric emptying (Ollerenshaw, et al. 1987). Regarding floatable systems, upright position is the preferred posture which ensures the system is distant from the pyloric sphincter and decreases the possibility of premature evacuation. However, the supine position is preferred in non-floatable systems (Garg and Gupta 2008). The emotional state was also related to gastric emptying, since lower gastric emptying rates were observed in depressed patients and higher emptying rates were observed in anxious patients (Talukder and Fassihi 2004). Patients with diabetes mellitus type I and type II show 30-50% decrease in gastric emptying (Triantafyllou, et al. 2007), while patients with parkinsonism usually have delayed gastric emptying along with constipation (Krygowska-Wajs, et al. 2009). Caloric intake displayed an effect on gastric emptying rates as well. Gastric emptying of fatty meals was longer compared to regular meals. Fatty meals form an oily layer above the gastric contents which makes them harder to evacuate (Kutchai 1996). Women were found to have slower gastric emptying than men and elderly were found to have slower gastric emptying than younger people (Haus and Fell 1984) (Reddy, Sinha and Reddy 1999).

1.3. Developed expandable system

Originally, the expandable system was intended to be developed as a triple layered system composed of an internal layer enveloped in two outer layers. This is the main technique used in Accordion Pill™ system, in addition to one or two immediate release layers covering the outer layers (Nadav, et al. 2014) (Kagan, et al. 2006) (Nadav, et al. 2017). The internal layer represents the matrix from which the drug will be released. The outer layers will control the system's degradability and unfolding in stomach and to a lesser extent will assist in extending the drug's release. After several attempts to develop the triple layered system experimentally; tests results have demonstrated that this system was not applicable. As a result, one layered system was developed which represents a matrix system comprised of the active ingredient (Gabapentin), a plasticizer to increase the flexibility of the layer (Poloxamer P407), a mixture of hydrophobic polymers stable at stomach pH to sustain the release of the drug (Eudragit® polymers), and a swellable hydrophilic polymer which will undergo expansion as a result of fluid absorption (Gelatin). This system was developed using experimental design with the assistance of Design Expert® software which used D-optimal reduced quadratic design to obtain the optimal formula. Assay, dissolution, unfolding, degradability and elasticity tests were performed on the developed formulations. XRD and FTIR analyses were performed to study the developed systems physical and chemical properties, respectively.

1.4. Excipients used in developed formulations

Different excipients were used in the development of the expandable drug delivery system obtained. They include hydroxypropyl methylcellulose (HPMC), Eudragit® polymers, polyethylene glycol 400 (PEG 400), propylene glycol, povidone K30 (PVP K30), gelatin, poloxamer P407, potassium hydroxide (KOH).

1.4.1. Hydroxypropyl methylcellulose (HPMC)

Hypromellose or HPMC is a partly O-methylated, O-(2-hydroxypropylated) cellulose. It is an odorless tasteless white powder. It is hygroscopic and has a glass transition temperature of 170-180 ^{o}C. HPMC can be divided to numerous grades according to its viscosity. It has many uses in pharmaceutical industry including the use as a controlled release agent especially in high viscosity grades (Rowe, Sheskey and Quinn 2009). HPMC 100000 (high viscosity grade) was used in this research.

1.4.2. Eudragit® polymers

Eudragit® polymers or polymethacrylates are synthetic anionic and cationic polymers of methacrylic acid, methacrylic acid esters and dimethylaminoethyl methacrylates in variable ratios. Eudragit® L-100 [Poly(methacrylic acid, methyl methacrylate) 1:1], Eudragit® L100-55 [Poly(methacrylic acid, ethyl acrylate) 1:1] and Eudragit® S-100 [Poly(methacrylic acid, methyl methacrylate) 1:2] were used in this research for the as sustained release agents (Rowe, Sheskey and Quinn 2009). Eudragit® L100, L100-55 and S100 are supplied as a powder and are ionizable thus soluble at pH 6, 5.5 and 7, respectively (Dias, et al. 2007).

1.4.3. Polyethylene glycol 400 (PEG 400)

Polyethylene glycol is divided to different grades according to its molecular weight. Mainly, grades above 1000 are solids and below are liquids at ambient temperatures. PEG 400 was used as a plasticizer in the developed formulations (Rowe, Sheskey and Quinn 2009).

1.4.4. Propylene glycol

Propylene glycol or 1,2- dihydroxypropane is an odorless, colorless, viscous liquid. Its boiling point is 188 ^{o}C. It was used as a plasticizer in the developed formulations. Different other uses are available in pharmaceutical industry including the use as a preservative, extractant or solvent (Rowe, Sheskey and Quinn 2009).

1.4.5. Povidone K30 (PVP K30)

Povidone or polyvinylpyrrolidone (PVP) is a fine white odorless powder. Povidone is divided to different grades according to its K value (i.e. its viscosity in aqueous solution, relative to that of water) which represents its molecular weight. PVP K30 grade (i.e. 50000, molecular weight) was used in the developed formulations as a sustained release agent (Rowe, Sheskey and Quinn 2009).

1.4.6. Gelatin

Gelatin is an odorless, tasteless, yellow, brittle solid. It is hygroscopic and absorbs 5 to 10 times its weight of water thus swells. It was used in the developed formulations to

help expand the layer to the required dimensions. It can also be used as a gelling agent, suspending agent, film forming agent and as viscosity increasing agent (Rowe, Sheskey and Quinn 2009).

1.4.7. Poloxamer P407

Poloxamer is a nonionic polyoxyethylene-polyoxypropylene copolymer. It has different grades which differ in their molecular weights and physical state. Poloxamer P407 occurs as an odorless, tasteless, white, prilled granules. It was used as a plasticizer in the developed formulations (Rowe, Sheskey and Quinn 2009).

1.4.8. Potassium hydroxide (KOH)

Potassium hydroxide occurs as a white fused mass (Rowe, Sheskey and Quinn 2009). It is an alkalizing agent and was used in the developed formulations to control the degree of degradability of the developed layers and to assist in the dissolution and solvation of Eudragit® polymers during formulations preparation.

Part Two: Gabapentin

2.1. Overview of Gabapentin

Gabapentin is mainly used as an anticonvulsant agent and for neuropathic pain (Sean 2009). It has a molecular weight of 171.24g/mole, molecular formula $C_9H_{17}NO_2$, and melting point of 162-166 ^oC. pKa_1=3.68 at 25 ^oC (carboxylic acid), pKa_2=10.70 (primary amine). It is a white, crystalline solid; freely soluble in water, alkaline and acidic solutions (O'Neil 2013). Fed or fasting states do not change the solubility of Gabapentin because its solubility is independent of the pH (Cuiping, et al. 2013). Gabapentin is not considered a hazardous substance (Sigma-Aldrich 2017). According to the biopharmaceutical classification system (BCS) it is considered a class III drug which means it has high solubility and low permeability.

2.2. Stability

Gabapentin is chemically stable under recommended storage conditions (tightly closed container, in a dry and well-ventilated cool place) (Sigma-Aldrich 2017). 100 mg/ml oral suspension of Gabapentin prepared using Oral Mix and Oral Mix SF suspending agents did not show any degradation through 90 days of storage at 25 ^oC (Friciu, Roullin and Leclair 2017). Studies have indicated that Gabapentin aqueous solution is stable for 2 years at pH 6 and room temperature (Zour, et al. 1992).

2.3. Suitability

Gabapentin has a broad therapeutic index, is considered safe in overdose (Alan and Charles 2000), and has an approximate half-life of 6 hours (Cuiping, et al. 2013); this makes it a proper candidate for gastroretentive systems.

2.4. Pharmacokinetics

2.4.1. Absorption

Gabapentin absorption mainly occurs in the duodenum and the jejunum (Bockbrader 1995) (Stewart, et al. 1993) (Shell, Louie-Helm and Markey 2002). It has low absorption window. L-amino acid transporters (LAT) are the main transporters responsible for the uptake of Gabapentin in the small intestine. Expression of LAT is decreased along the small intestine and it is absent in the colon (Dave, et al. 2004). Saturation of these transporters prevents proportional increase in bioavailability with dose and usually occurs in immediate release dosage forms (figure2) (Stewart, et al. 1993) (Dave, et al. 2004) (Uchino, et al. 2002).

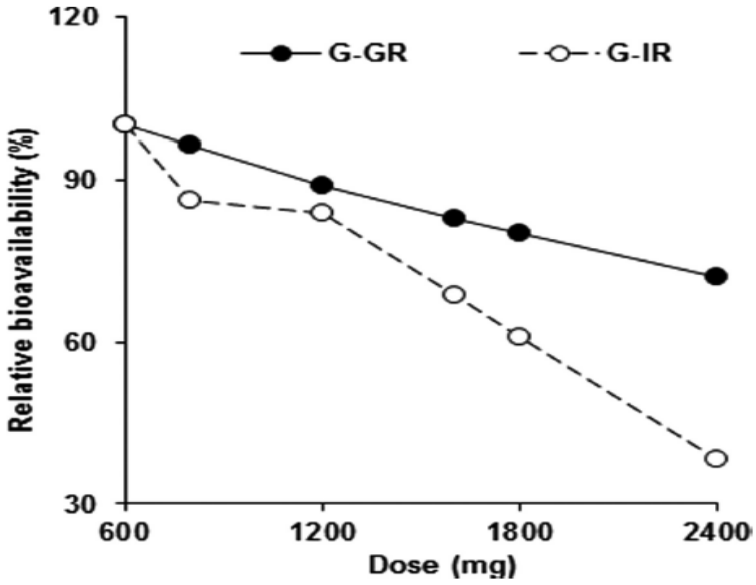

Figure 2. Relative bioavailability of swellable floatable Gabapentin (G-GR) vs immediate release Gabapentin (G-IR) (Cuiping, et al. 2013).

So, by developing a gastroretentive system which controls the release of Gabapentin over prolonged periods; the saturation of these transporters will be avoided. In addition, an extended release system of Gabapentin which releases the drug over 8 hours and is not gastroretentive; would be an inappropriate system, since it would reach the intestine in about 4 hours leading to half of the drug concentration or less being absorbed (Cuiping, et al. 2013).

2.4.2. Distribution

Binding of Gabapentin to plasma proteins is about 3% and it has an apparent volume of distribution of 58L (Davies and Morris 1993). Both indicate that Gabapentin is well distributed in body tissues, which correlates to its efficacy.

2.4.3. Metabolism and Excretion

Gabapentin metabolism is negligible. It has a systemic clearance of 1.6 ml/min/kg. Elimination half-life is about 6 hours independent of dose (Cuiping, et al. 2013). Renal clearance of intact Gabapentin represents about 70% of total clearance and is believed to be through glomerular filtration (Bockbrader 1995) (Pfizer-Incorporation 2011).

2.5. Administration

2.5.1. Epilepsy

The initial dose of Gabapentin for epilepsy is usually 300 mg once daily for the first day, twice daily for the second day, and three times daily for the third day. A 300 mg increase in dose can be then done each two to three days until epilepsy is controlled. Usually the administered dose could range from 0.9-3.6 g per day. 4.8 g daily dose was reported to be well tolerated (Sean 2009).

2.5.2. Neuropathic pain

For neuropathic pain the usual administered dose can reach up to 1.8 g daily in three separate doses (Sean 2009).

Part Three: Experimental Design

3.1. Materials

Materials used and their suppliers are summarized in the following table (table 1).

Table 1. Materials used and their suppliers

Material	**Supplier**
Eudragit® L100, Eudragit® L100-55, Eudragit® S100	Evonik Industries (Marl, Germany)
Sodium acetate, potassium hydroxide, polyethylene glycol 400, starch, magnesium stearate	Acros Organics (New Jersey, USA)
Poloxamer P407, gelatin, citric acid, sodium bicarbonate, propylene glycol, hydroxypropyl methylcellulose, povidone K30, microcrystalline cellulose	Sigma Aldrich (Missouri, USA)
Hydrochloric acid, potassium phosphate monobasic, acetonitrile	Carlo Erba Reagents (Barcelona, Spain)
Absolute Ethanol, talc	Fisher Scientific (Leicestershire, United Kingdom)

3.2. Instruments

Instruments used and their properties are summarized in the following table (table 2).

Table 2. Instruments used and their properties

Instrument	**Properties**
High performance liquid chromatography (HPLC) apparatus	Agilent HPLC 1200 Series consisting of a degasser (Model G1379B), a binary pump (Model G1312A), auto sampler ALS (Model G1329A), auto sampler thermostat FC/ALS Therm (Model G1330B), thermostat column compartment (Model G1316A) and a variable wavelength detector (Model G1314B). Separation was performed on Thermo Scientific Hypersil C18 column (150 * 4.6 mm, 5 µm BDS). The mobile phase consisted of buffer (1.2 g KH2PO4/1 liter water, adjusted to pH 6.9 with KOH)/Acetonitrile (90:10, v/v). The HPLC system was operated at a flow rate of 1.0 ml/min at 40 ^{o}C. The

	UV detector was set at 200 nm. Retention and stop times were 3.09 and 6 minutes, respectively. Injection volume was 40 µl. Chromatograms of the blank and Gabapentin 0.6mg/ml dissolution standard (HCL) analyses performed on the HPLC are shown in figures 3 and 4, respectively.
Fourier-transform infrared spectroscopy (FTIR)	FTIR spectra were obtained test using Tensor II FTIR Spectrometer, Bruker. The spectra of raw materials were collected by compression of about 1% wt. in KBr tablets, while for developed layers the spectra were collected using small pieces of the layer without being first compressed in KBr tablets. The IR spectra were obtained at spectral region of 400-4000 cm^{-1} using 4 cm^{-1} resolution.
X-Ray powder diffraction (XRD)	XRD analyses were performed on Miniflex 600 x-ray diffraction unit, Rigaku according to the following

	conditions. 40 kV F.F tube, 15 mA beam, scintillation counter (Kβ filter) detector, slit conditions DS/SS = 1.25°, RS = 0.3 mm, incident side and receiving side Soller slit = 5°, incident height limiting slit = 10 mm, scan speed = 2°/min.
Dissolution apparatus (Pharma Test DT70)	USP Apparatus II: paddle, speed: 50 RPM, temperature: 37 ^{o}C, medium: 0.085N HCL, pH 1.2 and acetate buffer, pH 4.1, volume: 500 ml

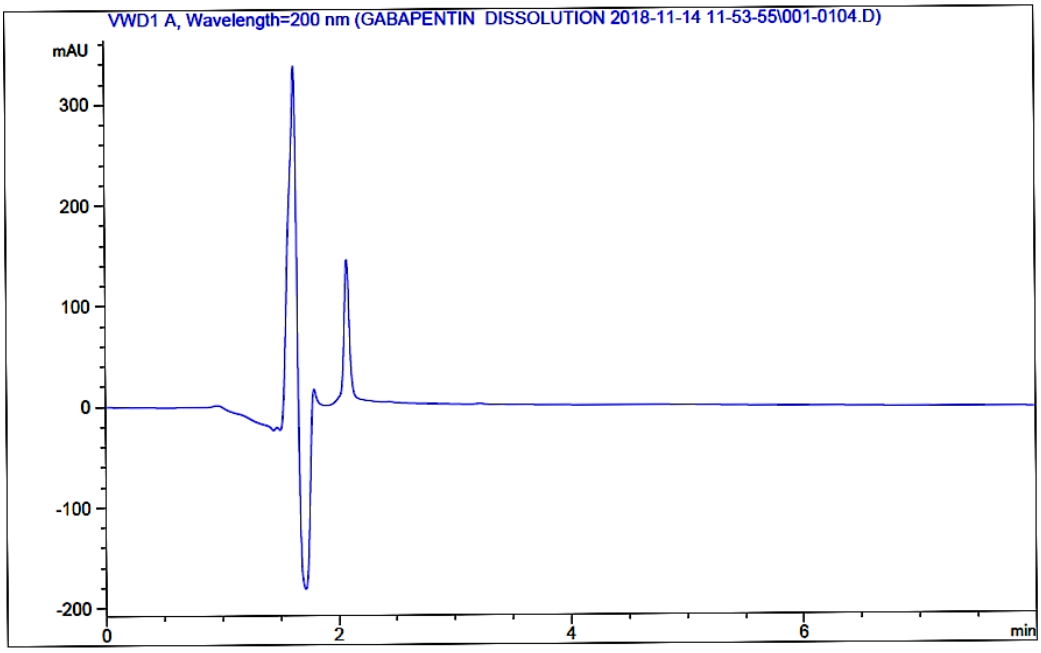

Figure 3. Chromatogram of the blank analyzed using HPLC

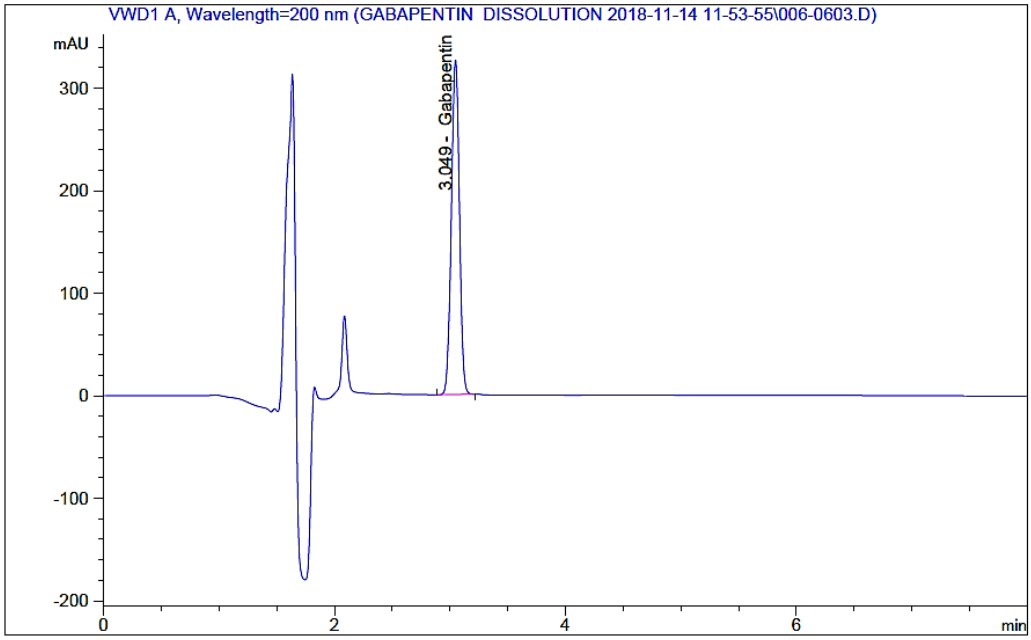

Figure 4. Chromatogram of Gabapentin 0.6mg/ml dissolution standard (HCL) analyzed using HPLC

3.3. Methodology

3.3.1. Triple layered system development

Triple layered system, composed of an internal matrix layer enveloped in two expandable outer layers, was intended to be developed. Outer layers will ideally have greater dimensions than the internal matrix layer in order to cover its surroundings (Figure 5). Outer layers and internal layer dimensions were 24*45 mm^2 and 21*40 mm^2, respectively (Nadav, et al. 2014). The layers dimensions are governed by the size of the capsule to be used and the opening size of the pyloric sphincter in its relaxed state (i.e. the system should unfold to at least 20 mm in stomach to avoid premature evacuation). These dimensions were later modified during experimental design based

on unfolding test results. Several techniques were attempted to obtain a method which leads to a miscible mixture of the ingredients. Initially, hot melt method was used. Proposed procedure was to melt the first outer layer and place it in a mold of the required dimensions. The internal layer having smaller dimensions will be melted, poured in another mold and then cut to the required dimensions. After it solidifies, it should be placed on the first outer layer. Finally, the second outer layer should be added as a melt on the previously solidified two layers while in original mold.

Figure 5. Developed triple layered system with the outer layers covering the internal layer.

3.3.1.1. Hot melt method (outer layers)

Outer layers were mainly intended to help expand the internal matrix layer upon contact with gastric fluid. Ingredients and concentrations in the following formula F1 (table 3) were selected based on previous literature review (Nadav, et al. 2014) (Nadav, et al. 2017). The purpose was to first develop the outer layers; then the ingredients can be selected, and their amounts can be modified based on tests results obtained (mainly unfolding test**).**

Table 3. Outer layer F1 formula.

Ingredient	Amount (mg)
Eudragit® S100	471
Eudragit® L100	235
Eudragit® L100-55	235
Gelatin	942
Propylene Glycol	942
Potassium Hydroxide	60

Ingredients of formula F1 were added to a beaker and heated using water bath for about 30 minutes, but no change occurred (i.e. ingredients were still in solid state). Propylene glycol was heated using water bath prior to other ingredients addition, but no change was observed. As a result, different melting techniques were used. Ingredients were added to a beaker and heated on hot plate which resulted in aggregate formation. Gradual addition of ingredients to prewarmed propylene glycol was tried, but an aggregate was formed (figure 6).

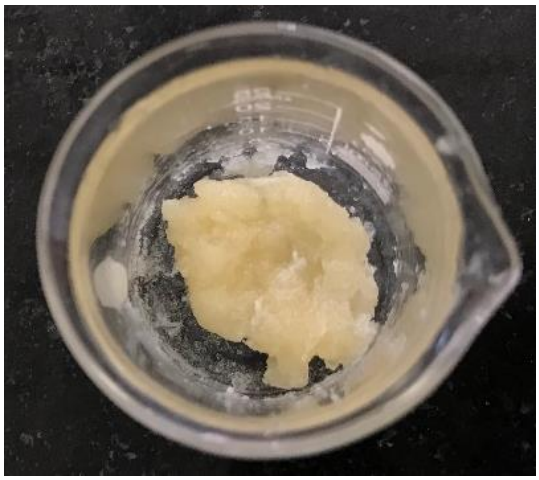

Figure 6. Aggregate formation during outer layers development using hot melt method.

Gelatin was grinded to ease its melting and mixed with the rest of the ingredients (except PG and KOH). The mixture was placed in water bath for 30 minutes with no change observed. It was then transferred to the hot plate and the rest of the ingredients were added but a solid aggregate was formed.

3.3.1.2. Solvent evaporation method (outer layers)

After failing to obtain a miscible mixture using hot melt method, solvent evaporation method was used. The purpose of using this method was to dissolve the ingredients in a common solvent which can be easily removed. Water, absolute ethanol and a mixture of both solvents were used to dissolve the ingredients. 2 ml of water were warmed in a beaker using the hot plate. Ingredients of formula F1 were added to the beaker while on plate. Ingredients addition started with gelatin, followed by Eudragit® S100, L100 and L100-55, respectively. KOH and PG were added subsequently. The resultant mixture formed a gel. The amount of water was doubled, and the ingredients were

added by the same order. This led to a non-homogeneous mixture in which some ingredients were not fully dissolved (figure 7).

Figure 7. Undissolved particles observed during outer layers development using solvent evaporation method.

Eudragit® polymers used have higher solubility at alkaline pH (Rowe, Sheskey and Quinn 2009) (Dias, et al. 2007). Ingredients addition, to 4 ml prewarmed water, was started with KOH in order to increase the pH of the solvent; facilitating Eudragit® polymers dissolution. Eudragit® S100, L100 and L100-55 were added, followed by gelatin. A highly viscous solution was obtained. KOH addition helped dissolve a portion of the added Eudragit® polymers. 1:1 mixture of absolute ethanol and water was used as solvent. A gel was formed after complete addition of Eudragit® polymers (figure 8).

Figure 8. Gel formation during outer layers development using solvent evaporation method.

Since Eudragit® L100-55 was the least soluble in water, its addition was performed immediately after KOH. This aided in dissolving a large portion of the polymer. The remaining portion of Eudragit® L100-55 was dissolved after the gradual addition of Eudragit® S100 and L100, respectively. Gelatin and PG were subsequently added. The resultant mixture was homogeneous. Ingredients addition were performed by the same order to absolute ethanol instead of water; which led to a homogeneous mixture. Several experiments were performed using water and absolute ethanol. In ethanol, ingredients were found to be more soluble and the resultant mixture was less viscous. Compared to water and water-ethanol solvents; absolute ethanol was more easily evaporated after being involved in the formulation. As a result, it was selected as the ideal solvent.

3.3.1.3. Heating technique

Four homogeneous samples of formula F1 were prepared using solvent evaporation method. Samples 1 and 2 were heated for 4 hours at 40 ºC using the oven. Samples 3 and 4 were heated using the microwave for 10 minutes at low and high micro power, respectively. Layers 1 and 2 were less sticky and more easily handled than layers 3 and 4. In addition, microwave produced layers contained numerous small air bubbles which could cause differences in drug release rate with each sample (i.e. prevent repeatability of the results) (figure 9).

Figure 9. Sticky outer layer after being heated using microwave.

3.3.1.4. Hot melt method (internal layer)

The nature and the concentration of excipients used in the following formula IA (table 4) were selected based on previous literature review (Nadav, et al. 2017) (Nadav, et al. 2014). The purpose was to first develop the internal layer; then according to the test results obtained (mainly dissolution test) modifications can be applied on the formula to optimize the drug release.

Table 4. Internal layer IA formula.

Ingredient	Amount (mg)
Gabapentin	600
Eudragit® L100	128
Poloxamer P407	64
Polyethylene glycol 400	64

Ingredients (Except API) were added to a beaker and heated using water bath, but no change was observed. The mixture was then transferred to the hot plate which resulted in a viscous gel that was difficult to handle. Using the hot plate, poloxamer P407 was melted at first, followed by PEG 400 and Eudragit® L100 addition. The resultant was a solid aggregate.

3.3.1.5. Solvent evaporation method (internal layer)

Poloxamer P407, PEG 400, Eudragit® L100 and Gabapentin were added to a beaker containing prewarmed water, respectively. The beaker was placed on a hot plate. Poloxamer P407 was not completely soluble in water and formed a cloudy solution. PEG 400, Gabapentin and Eudragit® L100 were added to prewarmed water on hot plate, respectively. The addition of Eudragit® L100 caused a latex to form. The mixture could not be handled. Absolute ethanol was used as an alternative to water. Ingredients were soluble in ethanol and resulted in a slightly viscous solution (figure 10).

Figure 10. Slightly viscous solution obtained during internal layer development using solvent evaporation method.

3.3.1.6. Cooking bags

Formulations F1 and IA were prepared using solvent evaporation method. Absolute ethanol represented the solvent. Both formulations were heated for an hour using the oven at 50 ^{o}C. Although formulations prepared using the oven were less sticky than microwave prepared formulations; they were still sticky to the mold used. Different molds were used including aluminum, glass and porcelain molds with no change observed. Oven cooking bags were used to decrease the stickiness of the layers to the molds. Both formulations were spread on a piece of glass covered with a cooking bag (figure 11).

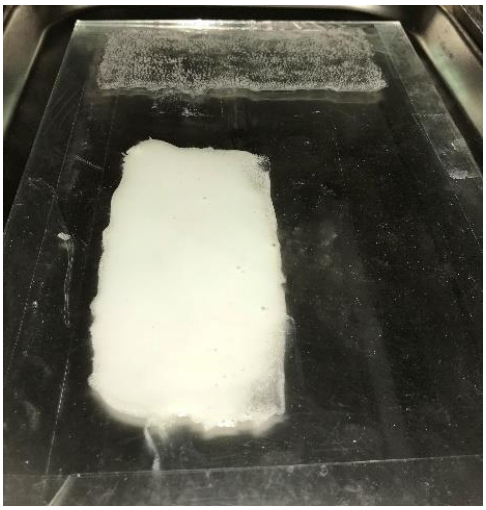

Figure 11. Internal and outer layers spread on a cooking bag.

The glass piece was transferred to the oven and heated for about 5 hours at 37 ^{o}C. The resultant layers were easily removed (figure 12, 13). Oven temperature and formulations heating time were modified continuously according to the visual inspection of layers solidification.

Figure 12. Internal layer easily removed after using cooking bag.

Figure 13. Outer layer easily removed after using cooking bag.

3.3.1.7. Internal and outer layers initial development

The outer layer intended to be developed was supposed to be slightly sticky in order to enhance its adherence to the internal layer. The Outer layer prepared from formula F1 lacked stickiness and elasticity (i.e. cannot be folded). The prepared internal layer from formula IA lacked elasticity. Numerous formulations were prepared based on the previous formulations F1 and IA in an attempt to enhance prepared layers physical characteristics and handling. Compared to outer formula F1; formula O1 ingredients quantities were decreased and it was prepared using the same amount of solvent (i.e. 5ml) in an attempt to enhance its elasticity (table 5).

Table 5. Outer layer O1 formula.

Ingredient	Amount (mg)
Eudragit® S100	209
Eudragit® L100	104
Eudragit® L100-55	104
Gelatin	418
Propylene Glycol	418
Potassium Hydroxide	26

Formula IA ingredients were decreased to 50% in formula I1 which was prepared using the same solvent volume (i.e. 5 ml) in an attempt to enhance its elasticity. Increasing excipients quantities and decreasing Gabapentin amount was attempted in formulations IB and I2, respectively (table 6). Developed formulations properties are shown in table 7.

Table 6. Formulations I1, I2 and IB.

	Amount (mg)			
Formula	**Gabapentin**	**Eudragit® L100**	**Poloxamer P407**	**PEG 400**
I1	300	64	32	32
IB	300	150	75	75
I2	258	64	32	32

Table 7. Developed formulations O1, I1, I2 and IB properties.

Formula	Stickiness	Comment
O1	Low Stickiness	Thin layer, good elasticity
I1	Sticky	Difficult to handle
IB	Sticky (figure 14)	Difficult to handle
I2	Non-sticky	Good elasticity

Figure 14. Developed formula IB.

Comparing developed layers obtained; outer layer O1 and internal layer I2 had the best characteristics.

3.3.1.8. Triple layered system development

Outer layer O1 and internal layer I2 were cut to the required dimensions (24*45 mm^2, 21*40 mm^2, respectively). First triple layer T1 was prepared by placing I2 in between two O1 layers. In order to increase outer layers stickiness and enhance their stickiness to the internal layer, T1 was heated in the oven at 50 ^oC for 30 minutes. Triple layer T2 was prepared by placing I2 in between two O1 layers after spreading some ethanol on the internal layer in order to enhance the stickiness. The layers were folded manually to accordion shape. "00" sized hard gelatin capsules were used (23.3*8.18 mm^2). The layers dimensions were decreased accordingly. Outer layers: 22*40 mm^2, Internal layer: 19*35 mm^2. T1 and T2 did not unfold properly during dissolution test. Outer layers detached during the test. Significant increase in excipients concentration were attempted in formula I5 to study their effects on the developed systems (table 8).

Table 8. Formula I5.

Ingredient	**Amount (mg)**
Gabapentin	923
Eudragit® L100	492
Poloxamer P407 (P-P407)	246
Polyethylene glycol 400	246

The resultant layer was non sticky and had good elasticity.

Further decrease in ingredients quantities and preparation using same solvent amount was attempted in formula O4 without changing the concentration percentages of each ingredient in the formula (table 9).

Table 9. Formula O4.

Ingredient	**Amount (mg)**
Eudragit® S100	175
Eudragit® L100	87
Eudragit® L100-55	87
Gelatin	351
Propylene Glycol	351
Potassium Hydroxide	22

Prepared outer layer O4 was very thin. Amount of the active ingredient in each internal layer was calculated based on the weight of the developed layer after deduction of the moisture content value. Internal layer I5 was cut into two biases of required dimensions and weighed. Outer layer O4 was cut into four outer biases of required dimensions and weighed. Two triple layers T5-1 and T5-2 were prepared using I5 and O4 prepared biases (i.e. each triple layer is composed of two outer O4 layers and one internal I5 layer of the required dimensions). T5-1 and T5-2 were stored in the desiccator for 2 days and both released about 90% of the drug during the first hour in dissolution test. This means that immediate release of the drug was obtained. Polyvinyl pyrrolidone

(PVP K30) and hydroxypropyl methylcellulose (HPMC) were added to the formulations, in an attempt to control the drug release. In the following internal layer formulations (I6- I12), outer layers were not used to cover developed internal layers (i.e. the purpose was to first examine the drug release then the outer layers can be used once a suitable release is obtained). The amount of Gabapentin was not changed in the following formulations (table 10). Compared to formula I5, amount of poloxamer P407 was decreased to half and was replaced by the addition of HPMC and PVP in formulations I6 and I7, respectively. 1:1 ratio of API to Eudragit® L100 was used in formula I8. 1:1:1 ratio of API, Eudragit® L100 and HPMC was attempted in formula I9. 1:1:1 ratio of API, Eudragit® L100 and PVP was attempted in formula I10. Eudragit® L100 was removed and its amount was replaced by HPMC and PVP in formulations I11 and I12, respectively. Developed formulations properties are shown in table 11.

Table 10. Internal layer formulations I6 - I12.

	Ingredients (mg)					
Formula	**Gabapentin**	**Eudragit® L100**	**Poloxamer P407**	**PEG 400**	**PVP K30**	**HPMC 100000**
I6	923	492	123	246		123
I7	923	492	123	246	123	
I8	923	923	246	246		
I9	923	923	246	246		923
I10	923	923	246	246	923	
I11	923		246	246		923
I12	923		246	246	923	

Table 11. Developed formulations I6 - I12 properties.

Formula	Elasticity	Dug release	Comment
I6	Elastic	≈ 90% in 30 minutes	Immediate release
I7	Elastic	≈ 90% in 30 minutes	Immediate release
I8	Elastic	≈ 90% in 2 hours	Immediate release
I9	Low elasticity*[b]	N/A*[c]	Difficult to fold
I10	N/A	N/A*[c]	Formed a gel during preparation
I11	Not elastic*[a]	N/A*[c]	Cannot be folded
I12	Not elastic*[a]	N/A*[c]	Cannot be folded

*[a] Not elastic indicating breakage of the developed layer upon folding. *[b] Low elasticity indicating difficulty in folding the developed layer. *[c] N/A: Dissolution test not applicable due to low elasticity

Previous data obtained during outer layers development were entered to Design Expert® software which provided the following outer layers formulations (table 12). Their properties are shown in table 13.

Table 12. Formulations O5 - O9.

	Ingredients (mg)					
Formula	**Eudragit® S100**	**Eudragit® L100**	**Eudragit® L100-55**	**Gelatin**	**Propylene glycol**	**KOH**
O5	188	94	94	376	376	2
O6	288	288	288	126	145	15
O7	288	288	288	141	145	
O8	288	288		155	460	
O9		288	192	211	453	7

Table 13. Developed formulations O5 - O9 properties.

Formula	**Elasticity**	**Comment**
O5	Elastic	Not sticky
O6	Not elastic	Cannot be folded
O7	N/A	Formed lumps during preparation
O8	Low elasticity	Difficult to fold
O9	Elastic	Highly sticky

The outer layer with the best characteristics O5 (i.e. elastic and easy to handle) was chosen to cover upcoming internal layers (table 14). 2:1:1 ratio of API, Eudragit® L100

and PVP was used in formula I13, respectively. 2:1:1 ratio of API, Eudragit® L100 and HPMC was used in formula I14, respectively. Eudragit® L100 was removed in formula I16 and 2:1:1 ratio of API, HPMC and PVP was used, respectively. Maximum soluble amount of Eudragit® L100 and HPMC was used in formulations I18 and I19, respectively. Compared to formula I19, a decrease in HPMC amount was applied in formula I20 as a result of difficulties (i.e. long mixing time, requirement of accurate gradual addition) faced during mixture preparation of formula I19. Further decrease in HPMC was applied in formulations I21 and I22, respectively. Developed formulations properties are shown in table 15.

Table 14. Formulations I13, I14, I16, I18 - I22.

	Ingredients (mg)					
Formula	**Gabapentin**	**Eudragit® L100**	**HPMC 100000**	**Poloxamer P407**	**PEG 400**	**PVP K30**
I13	1591	795		423	423	795
I14	1591	795	795	423	423	
I16	1591		795	423	423	795
I18	1591	1267	795	423	423	
I19	1591	795	1388	423	423	
I20	1591	795	1062	423	423	
I21	1591	795	900	423	423	
I22	1591	900	795	423	423	

Table 15. Developed formulations I13, I14, I16, I18 - I22 properties.

Formula	**Elasticity**	**Drug release after 1.5 hours**	**Comment**
I13	N/A	N/A	PVP and Eudragit® L100 did not mix
I14C*[a]	Elastic	≈ 90%	Immediate release
I16	Not elastic*[b]	≈ 90%	Immediate release
I18C*[a]	Elastic	≈ 90%	Immediate release
I19C*[a]	Not elastic*[b]	≈ 90%	Immediate release, difficult to prepare.
I20C*[a]	Low elasticity	≈ 90%	Immediate release
I21C*[a]	Elastic	≈ 90%	Immediate release
I22C*[a]	Elastic	≈ 90%	Immediate release

*[a] Internal layers covered with outer layer O5. *[b] Dissolution test was performed on the layer without previously folding it, due to lack of elasticity.

None of the developed formulations was able to control the drug release. As a result, one layered system with different formulations was intended to be developed.

3.3.2. One layered system development

After failing to control the drug release in previous attempts, new formulations were developed on the basis of one layered system. Compared to the previous triple layered system; this system should be able to control the drug release and unfold in stomach to

the required dimensions using one layer instead of three. This also includes less laboratory work required to develop the system.

3.3.2.1. Formulations development

Internal I14 and outer O5 layer ingredients were mixed together (formula X1). The layer was heated in the oven at 70 ^{o}C for two hours. Dissolution test was performed on the layer. It released about 90% of the active ingredient during the first hour. Proposed reason of the immediate release is the large amount of the solvent used, as an increase in the solvent will increase the release. Several new formulations were developed based on previous experience (table 16). Eudragit® L100, S100 and L100-55 were all used in the following formulations in an attempt to maximize the controlled release effect on the drug. Developed formulations properties are shown in table 17.

Table 16. Formulations I25 - I27.

	Ingredients (mg)					
Formula	**Gabapentin**	**Eudragit® L100**	**Eudragit® S100**	**Eudragit® L100-55**	**Gelatin**	**HPMC100000**
I25	1591	889	444	444	376	
I26	1591	889	444	444		795
I27	1591	795	397	397		

Table 17. Developed formulations I25 - I27 properties.

Formula	Elasticity	Drug release after 3hours
I25	Not elastic	≈ 60%
I26	Low elasticity	≈ 90%
I27	Low elasticity	≈ 60%

New formulations based on formula I25 were prepared. No new formulations were developed based on I27 because of the need of an ingredient which will absorb water and help expand the layer in stomach. HPMC was found to increase the release of drug, so it was excluded from following formulations. The addition of a plasticizer was attempted in following formulations in order to enhance prepared layers elasticity (table 18). Developed formulations properties are shown in table 19.

Table 18. Formulations I25/2, 6, 7, 8.

	Ingredients (mg)						
Formula	**Gabapentin**	**Eudragit® L100**	**Eudragit® S100**	**Eudragit® L100-55**	**Gelatin**	**PP 407**	**PEG 400**
I25/2	1591	889	444	444	376	423	423
I25/6	1591	889	444	444	376	50	
I25/7	1591	889	444	444	376	150	
I25/8	1591	889	444	444	376	100	

Table 19. Developed formulations I25/2, 6, 7, 8 properties.

Formula	Elasticity	Drug release	Comment
I25/2	Elastic	≈ 90% in 3 hours	Immediate release
I25/6	Elastic	≈ 66% in 7 hours	Formed a sticky layer
I25/7	Elastic	≈ 70% in 7 hours	Formed bubbles
I25/8	Elastic	≈ 60% in 5 hours	Non-sticky

PEG 400 and propylene glycol were found to increase the release of the drug, so they were excluded from following formulations. Increased amount of poloxamer P407 was found to induce bubble formation. Based on previous experience; ingredients effects on the developed layers characteristics were summarized in the following table (table 20).

Table 20. Ingredients effects on developed layers characteristics.

Ingredient	Effect
Eudragit® L100	Decreases drug release and increases prepared mixture viscosity as its concentration increase.
Eudragit® S100	Decreases drug release and increases prepared mixture viscosity as its concentration increase.
Eudragit® L100-55	Decreases drug release and increases prepared mixture viscosity as its concentration increase.
PVP K30	Decreases layers elasticity as its concentration increase, forms a gel during preparation if mixed with Eudragit® L100.
HPMC	Increases drug release and decreases prepared system elasticity as its concentration increase.
Gelatin	Enhances system expansion upon contact with liquids as its concentration increase.

KOH	Increases drug release as its concentration increase, helps dissolve Eudragit® polymers during system preparation.
Propylene glycol	Increases drug release and enhances prepared system elasticity as its concentration increase.
PEG 400	Increases drug release and enhances prepared system elasticity as its concentration increase.
Poloxamer P407	Enhances prepared system elasticity and decreases its stickiness as its concentration increase. Above a certain limit it enhances bubble formation in the prepared system.

Data obtained from previous experiments were entered to Design Expert® software which provided the following formulations (table 21). D-optimal reduced quadratic design was used.

Table 21. Formulations I25/8 A – I25/8 S.

	Ingredients (mg)					
Formula	**Gabapentin**	**Eudragit® L100**	**Eudragit® S100**	**Eudragit® L100-55**	**Gelatin**	**Poloxamer P407**
I25/8 A	1591	703	500	350	500	200
I25/8 B	1591	1000	500	326	226	200
I25/8 C	1591	1000	500	500	253	-
I25/8 D	1591	1000	500	253	500	-
I25/8 E	1591	853	200	500	500	200
I25/8 F	1591	901	370	370	500	110
I25/8 G	1591	853	500	200	500	200
I25/8 H	1591	1000	353	200	500	200
I25/8 I	1591	553	500	500	500	200
I25/8 J	1591	1000	500	200	426	126
I25/8 K	1591	703	350	500	500	200
I25/8 L	1591	1000	200	353	500	200
I25/8 M	1591	753	500	500	300	200
I25/8 N	1591	1000	253	500	500	-
I25/8 O	1591	753	500	500	500	-
I25/8 P	1591	753	500	500	300	200
I25/8 Q	1591	1000	500	500	100	153

I25/8 R	1591	1000	326	500	226	200
I25/8 S	1591	1000	200	500	353	200

Formulations provided by Design Expert® software were prepared and each was tested for unfolding, elasticity and drug release. FTIR analysis was performed to study these formulation's chemical characteristics.

Part Four: Performed Tests

4.1. Dissolution test

Medium used in performed dissolution tests was hydrochloric acid dissolution medium (pH 1.2), which represents the lower pH of the stomach. An additional test using acetate buffer dissolution medium (pH 4.1) which represents the higher pH of the stomach was performed on the final optimized formula. Paperclips were used to hold the layers to the bottom of the bucket during layers development for the purpose of dissolution rate studies (figure 15). High performance liquid chromatography (HPLC) was used to analyze dissolution test samples. Method used in HPLC analysis was according to the USP with some modifications.

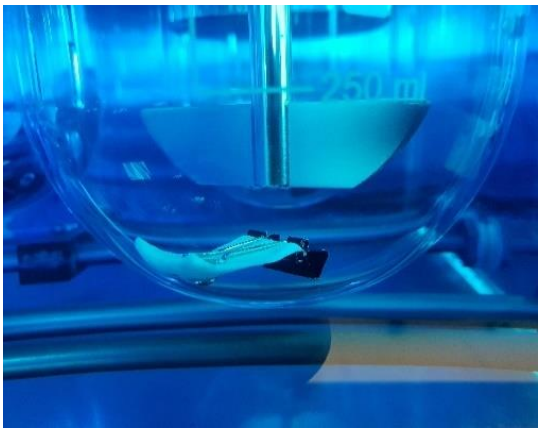

Figure 15. Dissolution test performed on a sample held by paperclips.

4.2. Assay test

Numerous techniques were used to dissolve the developed layers and to obtain the highest assay test result. Different solvents were used (ex. DMSO, water, absolute ethanol, the mobile phase) to dissolve the tested layers. Sonication, shaking, and

heating were tried to help extract the active ingredient from the tested layers. A piece taken from each developed layer was cut into small parts, weighed and placed in a volumetric flask containing absolute ethanol. The flask was then sonicated for 30 minutes and shaken for 1 hour at room temperature. 5ml was withdrawn from the resultant solution and diluted to 50ml with the mobile phase. About 2ml was withdrawn from the diluted solution and placed in a vial to be tested using HPLC. Solution stability test was performed on Gabapentin and was found to be stable for one month at room temperature. HPLC was used to analyze assay test samples.

4.3. Unfolding test

Unfolding test was performed using USP Apparatus II method. Hydrochloric acid dissolution medium (pH 1.2) was used to test all samples. An additional test using acetate buffer dissolution medium (pH 4.1) was performed on the final optimized formula. Capsules were disintegrated within 3-5 minutes. Developed layers are folded in a capsule (figure 16) and should unfold within 15 minutes of ingestion to prevent premature evacuation and avoid the release of the drug in the intestines instead of the stomach. The unfolded layer dimensions should exceed the pyloric sphincter dimensions in its relaxed state (i.e. 20 mm). The layers displayed increased stickiness upon contact with the dissolution medium. Different antiadhesive excipients were used to prevent layer sticking.

Figure 16. Prepared layer folded in a “00” sized hard gelatin capsule.

4.4. Young’s modulus test

Young’s modulus test is a measure of a material resistance to elongation upon stress and is equal to stress over strain. Higher young’s modulus values indicate higher layer rigidity (i.e. relatively larger force is needed to cause deformation). The most rigid layer will represent the optimal layer, as it will resist unplanned deformation (i.e. after unfolding) caused by gastric emptying forces which could lead to premature evacuation. The test was performed manually by attaching the layer to a retort stand from one side and to a weight from the other (figure 17). Same weight was used to test all samples. Time under stress was 2 minutes. Dimensions were measured using a digital caliper.

Figure 17. Young's modulus test performed manually.

4.5. Degradability test

This test was performed to inspect the degradability of the optimized formula at intestinal pH (i.e. 6.5) using USP apparatus II method. The layer was placed directly in the medium without being first loaded in a capsule. Its rigidity should be decreased at alkaline pH. Rigidity was inspected manually using Young's modulus test. Results will help us predict the layer's behavior in the intestines in case of premature evacuation.

4.6. Fourier-transform infrared spectroscopy (FTIR) analysis

FTIR analysis is a technique used to study and identify the interactions between reactive groups in different compounds. This analysis helps us understand the nature

of the bonds which have formed during different formulations development and relate test results with the release obtained. FTIR analysis was performed on different developed formulations, the physical mixture of the ingredients used in these formulations and on each ingredient alone. FTIR compatibility test was also performed to study the compatibility of Gabapentin with the excipients used in developed formulations.

4.7. X-Ray powder diffraction (XRD) analysis

XRD analysis is used to determine the physical properties of tested samples. It differentiates between crystalline and amorphous materials. Results will demonstrate the physical state of Gabapentin before and after being involved in the developed system.

Part Five: Results and discussion

5.1. Antiadhesive excipients selection for developed layers unfolding test

The following antiadhesive excipients were intended to prevent developed layers stickiness and assist in the unfolding process (table 22). A successful unfolding test is obtained when the tested layers unfold to at least 20mm in 15 minutes. This test was performed using USP dissolution Apparatus II method and HCL dissolution medium (pH 1.2). Tested layers were removed from the dissolution apparatus after 10 and 15 minutes to measure their lengths.

Table 22. Different antiadhesive excipients effects on the total time required for the unfolding process (pH 1.2)

	Length of the tested layer (mm)	
Antiadhesive excipient	**After 10 minutes**	**After 15 minutes**
Talc	12	12
Microcrystalline cellulose	-*	-
Starch	10	12
Magnesium stearate	-	-
Magnesium stearate and talc	-	-
Magnesium stearate, citric acid and sodium bicarbonate	-	-
Talc, citric acid and sodium bicarbonate	18	20
Citric acid and sodium bicarbonate	21	24

-*: No unfolding occurred

Citric acid and sodium bicarbonate were tested on three additional samples to assure repeatability of the results (table 23).

Table 23. Citric acid and sodium bicarbonate mixture effect on the total time required for the unfolding process (pH 1.2)

	Length of the tested layer (mm)	
Antiadhesive excipient	**10 minutes**	**15 minutes**
Citric acid and sodium bicarbonate (sample 1)	22 mm	25 mm
Citric acid and sodium bicarbonate (sample 2)	26 mm	26 mm
Citric acid and sodium bicarbonate (sample 3)	25 mm	25 mm

Both ingredients were grinded using mortar and pestle and spread as fine powder on the previously prepared layers. This combination produces CO_2 gas which pushes the layer folded parts away from each other. It also decreases time required for capsule shell disintegration. The ideal ratio of citric acid to sodium bicarbonate was 1:10, respectively. An increase in citric acid ratio would lead to aggregates formation during grinding it with sodium bicarbonate which decreases the resultant powder stickiness to the prepared layers (i.e. sodium bicarbonate-citric acid grinded powder is more effective when it is trapped in between the developed layers folded parts as it helps push the folded parts away from each other).

5.2. Dissolution, capsule shell disintegration, unfolding (pH 1.2) and Young's modulus tests

The following table (table 24) displays the time at which each developed system released 10, 50 and 90% of the active ingredient (D10%, D50% and D90%, respectively) during dissolution test.

Table 24. D10%, D50% and D90% of the developed formulations I25/8 A-S.

	Time (hours)		
Formula	**D10%**	**D50%**	**D90%**
I25/8 A	<0.25	≈ 1.5	-*
I25/8 B	<0.25	≈ 3	-
I25/8 C	<0.25	6	-
I25/8 D	<0.25	4	-
I25/8 E	<0.25	1	6
I25/8 F	<0.25	≈ 1.5	-
I25/8 G	<0.25	2	-
I25/8 H	<0.25	1	-
I25/8 I	<0.25	2	-
I25/8 J	<0.25	≈ 1.5	6
I25/8 K	<0.25	2	-
I25/8 L	<0.25	0.5	-
I25/8 M	<0.25	2	-
I25/8 N	<0.25	2	-
I25/8 O	<0.25	1	-
I25/8 P	<0.25	2	-
I25/8 Q	<0.25	1	4
I25/8 R	<0.25	1	4

I25/8 S	<0.25	≈ 0.75	4

-*: 90% release of the active ingredient required more than 6 hours

The following table (table 25) represents the drug release percentage at 6 hours, Capsule shell disintegration time, unfolding (pH 1.2) and Young's modulus tests results.

Table 25. Dissolution, capsule shell disintegration, unfolding (pH 1.2) and Young's modulus tests results

Formula	**Drug release % (6 hours)**	**Capsule shell disintegration time (minutes)**	**Unfolding test**	**Young's modulus (N/mm^2)**
I25/8 A	81.8	2.1	Fail	0.0117
I25/8 B	73.1	2.5	Fail	0.0079
I25/8 C	49.8	3.6	N/A[*a]	N/A[*a]
I25/8 D	60.0	2.4	N/A[*a]	N/A[*a]
I25/8 E	91.2	3	Pass[*b]	0.0142
I25/8 F	78.2	3.4	Pass	0.0067
I25/8 G	77.5	2.8	Pass	Fail[*c]
I25/8 H	84.9	3.7	Fail	0.0174
I25/8 I	78.7	3.6	Fail	0.0086
I25/8 J	89.5	4	Pass	0.0258
I25/8 K	79.4	2.7	Fail	0.0115
I25/8 L	82.8	3.6	N/A[*a]	N/A[*a]

I25/8 M	71.3	2.4	Pass	0.0133
I25/8 N	72.0	3.8	N/A*a	N/A*a
I25/8 O	81.4	3.4	N/A*a	N/A*a
I25/8 P	70.3	3	Fail	0.0164
I25/8 Q	100	3.7	Pass	0.0258
I25/8 R	94.6	2.8	N/A*a	N/A*a
I25/8 S	98.6	2	N/A*a	N/A*a

*a: test is not applicable due to low layer elasticity, *b: a layer passes the unfolding test if it unfolds within 15 minutes of being in contact with the dissolution medium, *c: failing in Young's modulus test when the layer is cut upon stress.

Tests results data were entered to Design Expert® software which provided ternary graphs that explain the relationship between excipients quantities with Young's modulus (figure 18), dissolution (figure 19) and unfolding (figure 20) tests. The three axis variables are Eudragit® polymers. Gelatin and poloxamer P407 factor values were set to the centroid.

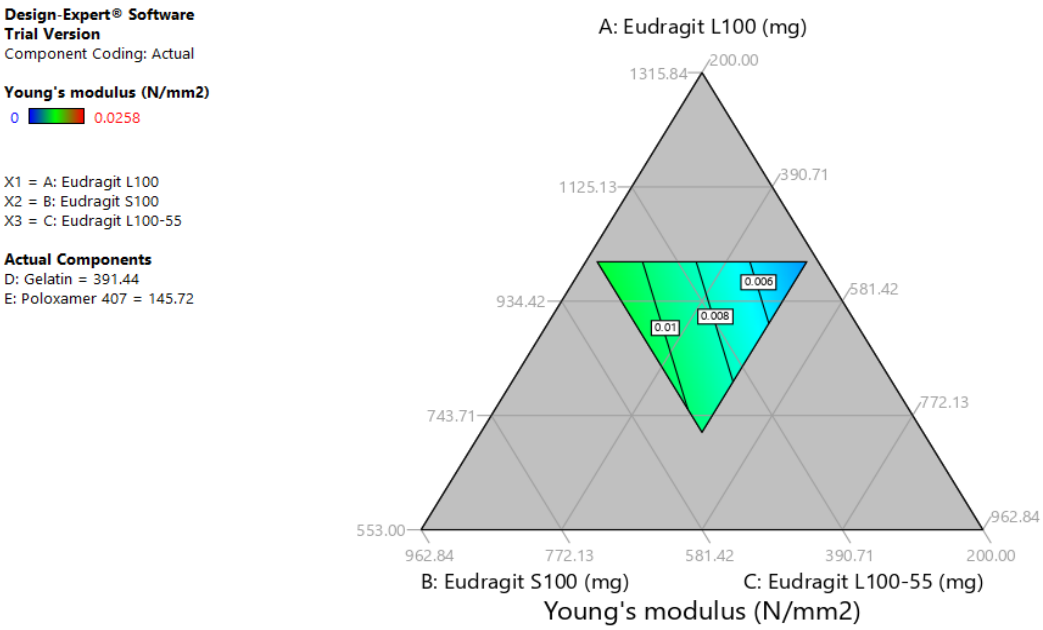

Figure 18. Excipients quantities relationship with Young's modulus test.

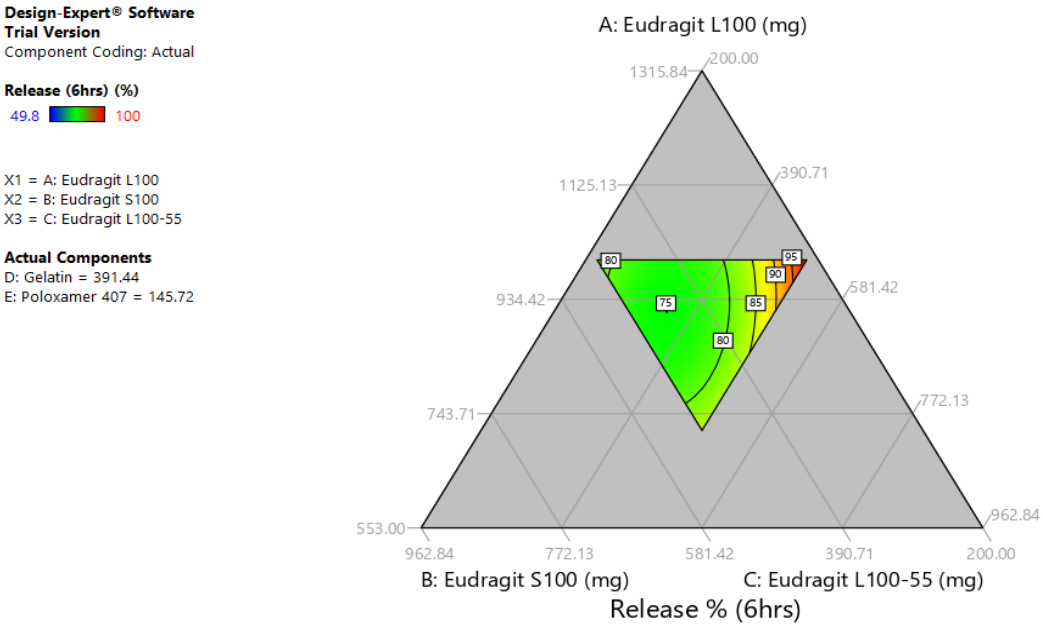

Figure 19. Excipients quantities relationship with drug release at 6 hours.

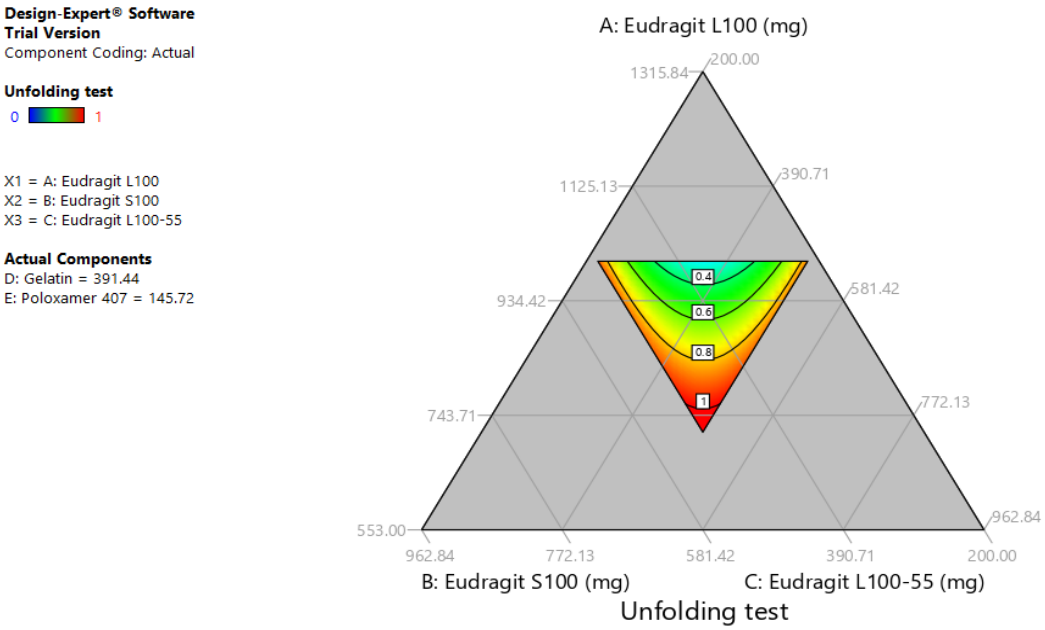

Figure 20. Excipients quantities relationship with the unfolding test.

Criteria of the optimized formula were set as a release of not less than 70% at 6 hours, a successful unfolding test and a Young's modulus of not less than 0.015 N/mm^2. The software provided the optimized formula I25/8 T (table 26) with a desirability factor of 1 (i.e. highest prediction factor). Dissolution, capsule shell disintegration, unfolding (pH 1.2) and Young's modulus tests results of optimized formula I25/8 T are shown in table 27.

Table 26. Ingredients quantities in optimized formula I25/8 T.

Ingredients (mg)					
Gabapentin	**Eudragit® L100**	**Eudragit® S100**	**Eudragit® L100-55**	**Gelatin**	**Poloxamer P407**
1591	1000	500	467	170	115

Table 27. Dissolution, capsule shell disintegration, unfolding (pH 1.2) and Young's modulus tests results of optimized formula I25/8 T

Formula	Drug release % (6 hours)	Capsule shell disintegration time (minutes)	Unfolding test	Young's modulus (N/mm^2)
I25/8 T	78.1 ± 4.8*	2.4	Pass	0.017 ± 0.003

*n=3 (formula I25/8 T was manufactured 3 times)

First order release kinetics was obtained (figure 21), which was not the case in previously developed formulations (figure 22) (Table 28). Based on the release kinetic equation obtained (Y = 9.0442x + 23.761), 8.4 ± 0.62 hours are required to release 100% of the active ingredient.

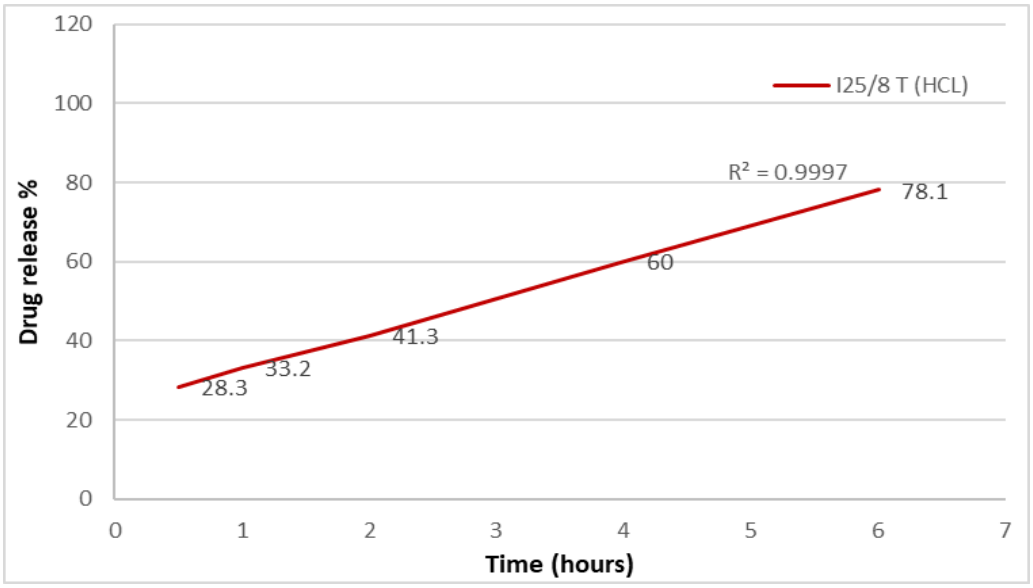

Figure 21. Mean dissolution of 3 formulations of optimal formula I25/8 T in HCL dissolution medium. (R.S.D < 5%)

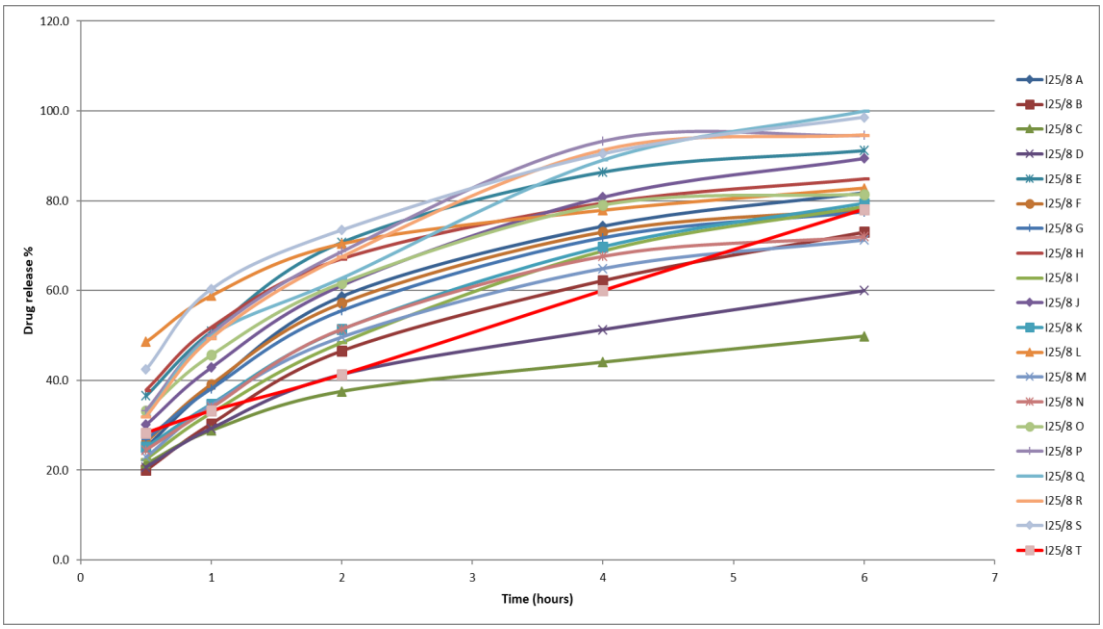

Figure 22. Drug release comparison between optimized formula I25/8 T and different developed formulations. (n=3, R.S.D < 5.6%)

Table 28. Release kinetic equation for optimal formula I25/8 T and different developed formulations

Release kinetic equation	Formula
$Y = 9.83x + 29.214$, $R^2 = 0.8883$	I25/8 A
$Y = 9.3138x + 21.323$, $R^2 = 0.9421$	I25/8 B
$Y = 4.7632x + 23.493$, $R^2 = 0.9085$	I25/8 C
$Y = 6.7519x + 22.278$, $R^2 = 0.9335$	I25/8 D
$Y = 9.4522x + 41.649$, $R^2 = 0.8611$	I25/8 E
$Y = 9.0642x + 30.296$, $R^2 = 0.879$	I25/8 F
$Y = 8.9487x + 29.785$, $R^2 = 0.8977$	I25/8 G
$Y = 7.9473x + 42.802$, $R^2 = 0.8635$	I25/8 H
$Y = 10.11x + 22.957$, $R^2 = 0.95$	I25/8 I
$Y = 10.487x + 32.599$, $R^2 = 0.9219$	I25/8 J
$Y = 9.7285x + 25.824$, $R^2 = 0.9443$	I25/8 K
$Y = 5.6783x + 52.389$, $R^2 = 0.8587$	I25/8 L
$Y = 8.4474x + 25.851$, $R^2 = 0.9056$	I25/8 M
$Y = 8.5623x + 26.809$, $R^2 = 0.8927$	I25/8 N
$Y = 8.5293x + 37.166$, $R^2 = 0.8676$	I25/8 O
$Y = 10.876x + 38.671$, $R^2 = 0.861$	I25/8 P
$Y = 11.849x + 34.783$, $R^2 = 0.9402$	I25/8 Q
$Y = 11.024x + 37.19$, $R^2 = 0.8754$	I25/8 R

$Y = 9.4032x + 47.682$, $R^2 = 0.8949$	I25/8 S
$Y = 9.0442x + 23.761$, $R^2 = 0.9997$	I25/8 T

5.3. Assay test

The resultant assay test value following the previously mentioned dissolving method was 98 ± 1.2%. Gabapentin was dissolved in the dissolution medium (HCL) and was stable after one month of storage at room temperature (i.e. assay test result was 98.2 ± 0.9%).

5.4. Dissolution and unfolding tests in acetate buffer (pH 4.1)

Dissolution and unfolding tests were performed on the optimal formula I25/8 T (n=3). 83.4 ± 5.3% release at 6 hours in acetate buffer (Figure 23) and successful unfolding test results were obtained. These results assure that the optimal formula will have the same behavior at both higher and lower pH of the stomach.

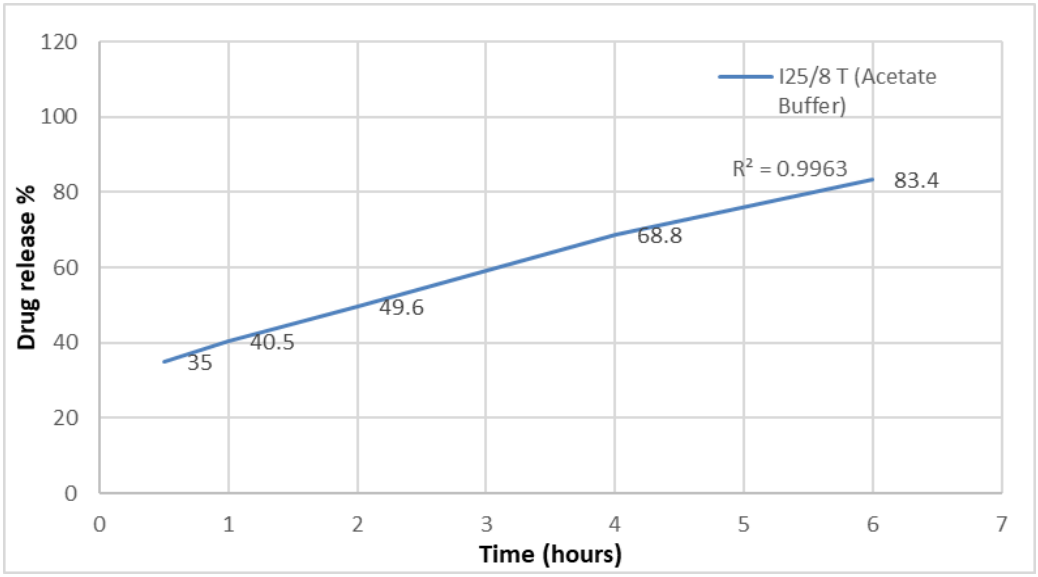

Figure 23. Mean dissolution of 3 formulations of optimal formula I25/8 T in acetate buffer dissolution medium. (R.S.D < 5%)

5.5. Degradability test (pH 6.5)

Developed layers demonstrate a significant increase in rigidity and thickness in wet state compared to dry state. Samples from optimized formula I25/8 T were tested at pH 1.2 and 6.5 which represent the pH of the stomach and the intestines, respectively. Young's modulus for the wet samples tested at pH 6.5 was 0.110 N/mm^2 after 5 hours, while for samples tested at pH 1.2 was 0.302 N/mm^2 after 6 hours. Layers tested at pH 6.5 were 1.24mm thick, while layers tested at pH 1.2 were 2.02mm thick. Both rigidity and thickness decreased significantly at intestinal pH. This should be the case in such systems in order to prevent possible side effects if premature evacuation occur.

5.6. Fourier-transform infrared spectroscopy (FTIR)

5.6.1. FTIR analysis

FTIR spectra for formulations I25/8 T, I25/8 E, pure Gabapentin and the physical mixture of Gabapentin with all excipients were obtained (Figure 24). Regarding Gabapentin, it normally shows no peak in the -NH stretching regions (3300-3500 cm^{-1}), since it is a zwitterion in the solid state (Reece and Levendis 2008) (Ibers 2001). The peaks at 3443cm^{-1} in both Gabapentin and physical mixture are most probably due to hydroxyl groups stretching vibration of water molecules absorbed from moisture. The two peaks at 2930cm^{-1} and 2860cm^{-1} are stretching vibrations of $-NH_3^+$ (Chimatadara, et al. 2007). The peak at 2151cm^{-1} represents stretching vibration of the side chain and/or CN group (Hsu, Ke and Lin 2010). peaks at 1545cm^{-1} and at 1614 cm^{-1} are due to vibrations of NH_3^+ deformation and the ionized asymmetric carboxylate group, respectively (Hsu, Ke and Lin 2010). Carboxylic acid hydroxyl group and CO stretching vibrations can be seen at 2925cm^{-1} and 1724cm^{-1}, respectively. The shift in the hydroxyl group peak from 3443 cm^{-1} in Gabapentin and physical mixture to 3269cm^{-1} in formula I25/8 T indicates the occurrence of hydrogen bonding. This bonding is most probably related to the sustained release of the drug. Almost no peak can be observed in formula I25/8 E in the hydroxyl region which indicates weaker hydrogen bonding. This weak bonding is most probably related to the less extended release of the drug. Formulations I25/8 S and I25/8 P also had relatively less controlled lease and exhibited similar characteristics to formula I25/8 E in FTIR (Figure 25). The split in the carbonyl peak at 1694cm^{-1} observed only in developed formulations is most

probably an overtone of the 847cm^{-1} original peak (Fermi resonance). FTIR spectra of pure Gabapentin and each excipient alone were also obtained (Figure 26).

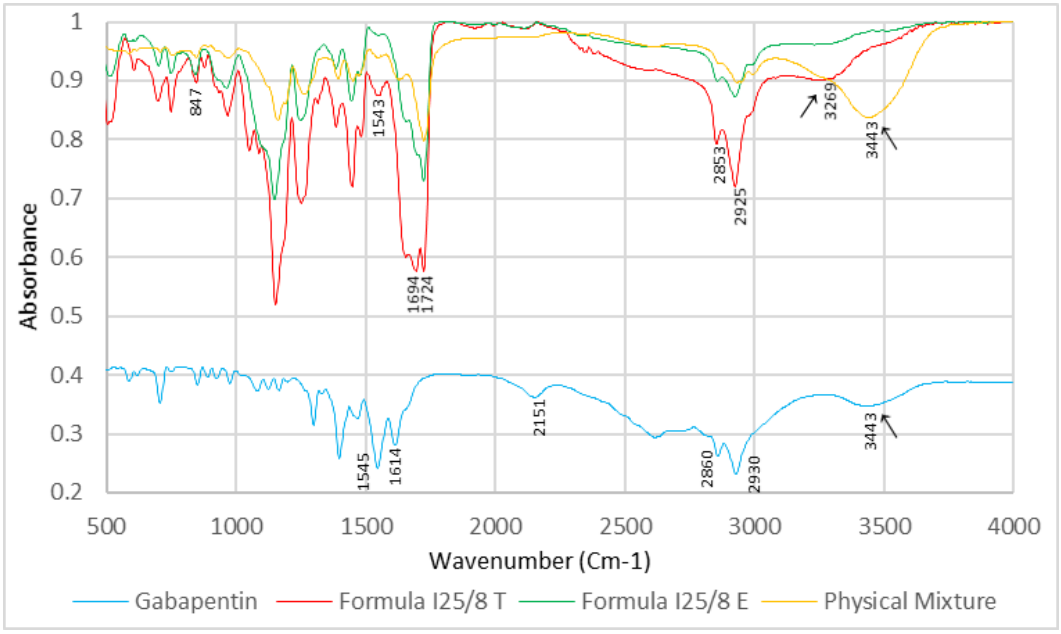

Figure 24. FTIR spectra for formulations I25/8 T, I25/8 E, pure Gabapentin and the physical mixture of Gabapentin with all excipients.

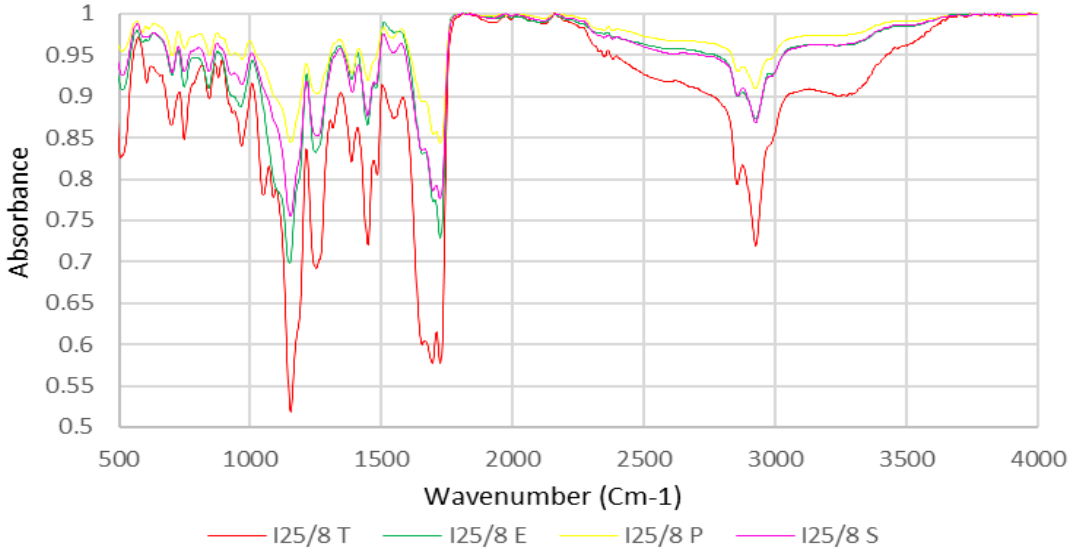

Figure 25. FTIR spectra for formulations I25/8 E, I25/8 P, I25/8 S and I25/8 T.

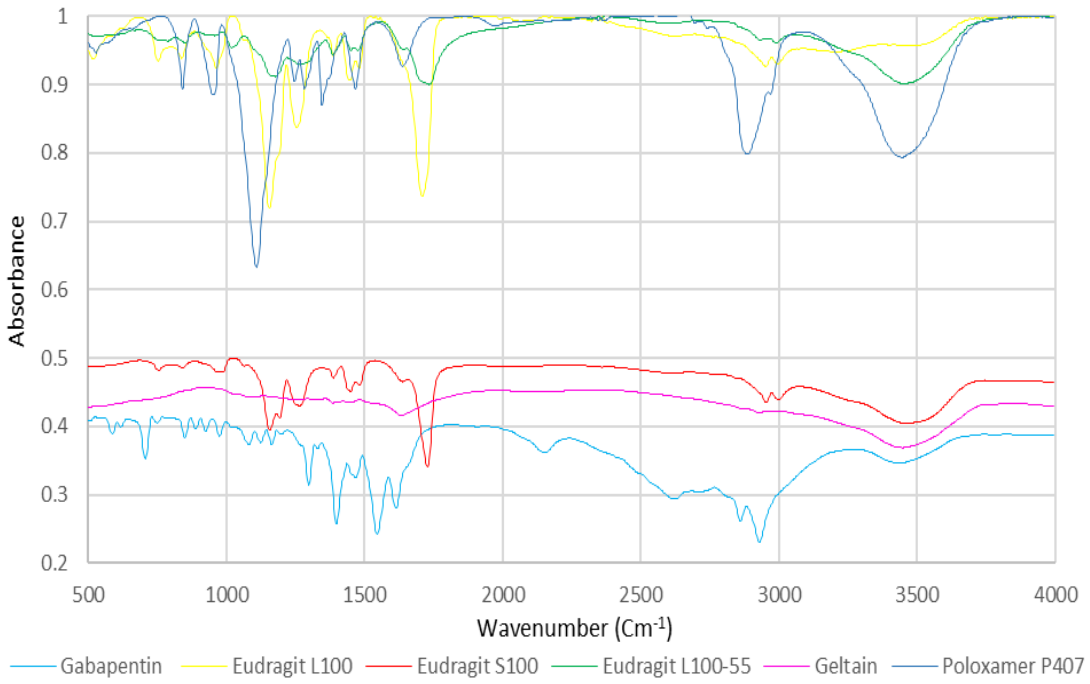

Figure 26. FTIR spectra for Gabapentin, gelatin, Eudragit® L100, Eudragit® S100, Eudragit® L100-55 and poloxamer P407.

5.6.2. FTIR compatibility study

Variations on the major chemical groups of Gabapentin before and after being involved in the developed formulations were studied. Main characteristic bands of Gabapentin were observed at 2930cm^{-1}, 2860cm^{-1} (stretching vibrations of -NH_3^+) and at 1545 cm^{-1}. The characteristic stretching bands observed in the optimal formula I25/8 T at 2925cm^{-1}, 2853cm^{-1} and at 1543cm^{-1} indicate that no change has occurred on the major chemical groups of Gabapentin after being involved in the developed formulations and proves that it is compatible with the excipients used in theses formulations.

5.7. X ray diffraction (XRD)

XRD patterns of pure Gabapentin, developed formula I25/8 T and the physical mixture of all ingredients were obtained (Figure 27). The most characteristics peaks of Gabapentin can be observed at 2θ = 15.7, 19.2, 22.9 and 31. XRD patterns of pure Gabapentin revealed that it was in crystalline state before being involved in the developed formula. On the other hand, XRD diffraction patterns of the developed formula showed a broad peak which represents a typical profile of an amorphous material. These patterns assure that Gabapentin physical state was changed from crystalline to amorphous after being involved in the developed formula. XRD patterns of pure Gabapentin and each excipient alone were also obtained (Figure 28).

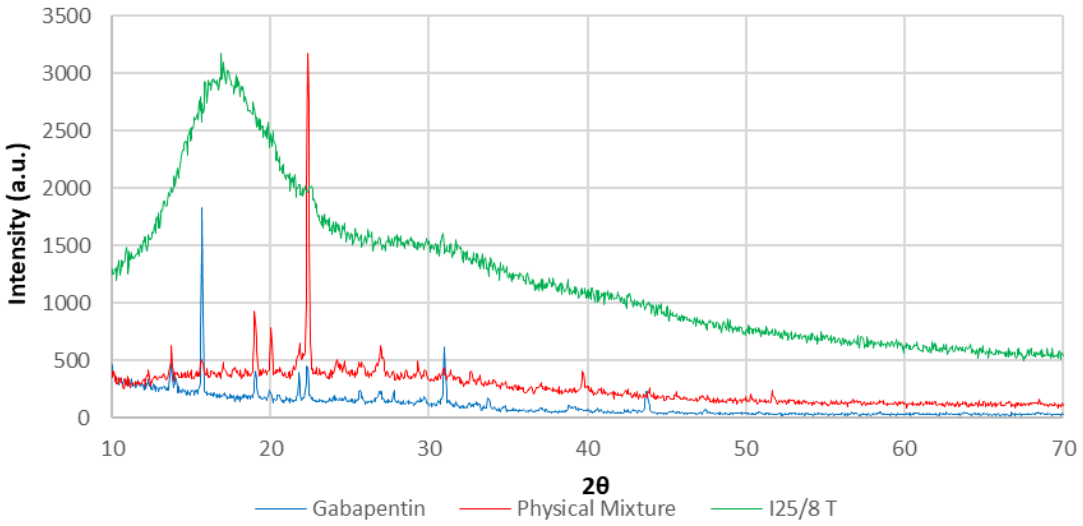

Figure 27. XRD patterns of pure Gabapentin, developed formula I25/8 T and the physical mixture of all ingredients.

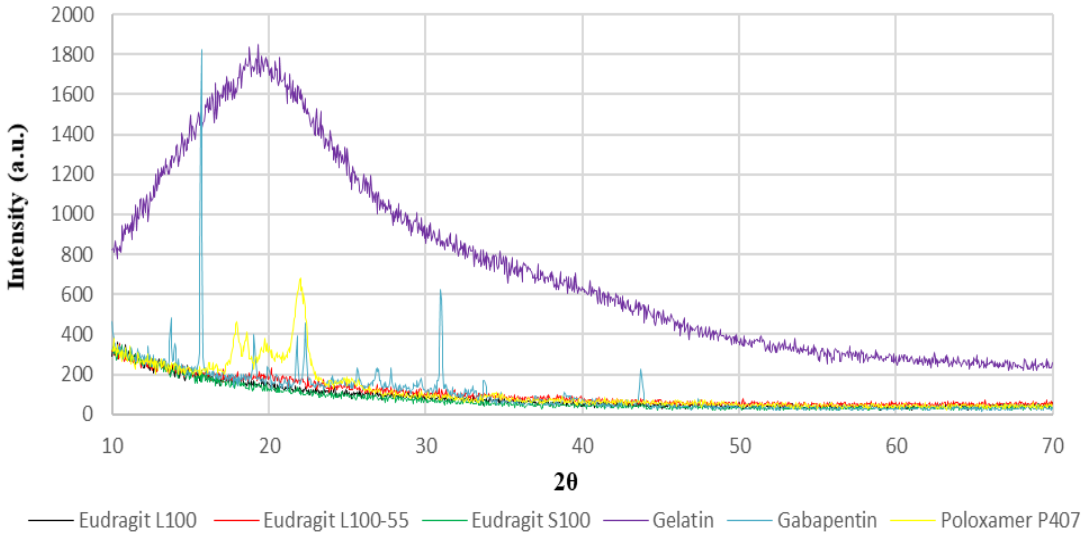

Figure 28. XRD patterns of pure Gabapentin, Eudragit® L100, Eudragit® S100, Eudragit® L100-55, gelatin and poloxamer P407.

Conclusion

The purpose of this study was to develop an expandable drug delivery system which extends Gabapentin release for at least 6 hours and retains in stomach for prolonged periods irrelevant to fed/fasted state. This extension usually involves enhanced bioavailability, deceased side effects and dosing frequency. During this study, one layered gastroretentive expandable delivery system containing Gabapentin was developed using design of experiments. This system was able to unfold in less than 15 minutes, which ensured the avoidance of premature evacuation. Drug release followed first order release kinetic model and was successfully extended to at least 6 hours. About 8.4 hours are required to release all of the Gabapentin present in the system. Youngs's modulus test result was above the set limit (0.015 N/mm^2) and indicated high rigidity in stomach. Degradability test results demonstrated significant decrease in the system's rigidity and thickness at intestinal pH. FTIR analysis proved that Gabapentin is compatible with the excipients used in the developed formulations and indicated the occurrence of hydrogen bonding in Gabapentin after being involved in the developed system. This bonding might be responsible for the drug's controlled release. The shift in the physical state of Gabapentin from crystalline in typical state to amorphous in the developed system was confirmed by XRD analysis. Further studies should be conducted considering the stability of the developed formulations; which, as a result of lack of resources and enough time, were not performed.

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