5-FLUOROURACIL MUCOADHESIVE LIQUID SUPPOSITORY
FORMULATION AND EVALUATION

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ABSTRACT
5-FU was formulated into a thermo-sensitive liquid suppository in combination with citrus pectin as Galectin-3 inhibitor, using poloxamer P407 as a thermo- gelling agent. Pectin addition retarded 5-FU solubility but the addition of cyclodextrin improved it, due to complex formation and decrease in crystallinity of 5-FU from 100 to 19.01% confirmed by XRD and FTIR. All the formulas showed sustained release due to P407 incorporation and non-Fickian release kinetics. The incorporation of carbopol C940 as a mucoadhesive polymer altered the gelation temperature of P407. The selected liquid suppository formula P4( P407:C940(19:1)) exhibited mucoadhesion strength of 2.476×103 dyne/cm², gelation temperature of 31.7°C and gel strength of 75 sec and showed pseudo-plastic flow with consistency of 3.7255 and 3.9934 mPa.s-n at 20° and 37° C respectively and permeation through sheep rectal tissue of 0.695cm/hr.

KEYWORDS: 5-FU, Pectin, Poloxamer 409, Carbopol 940, Rectal Cancer.

INTRODUCTION
Rectal cancers (RC) are usually adenocarcinomas, frequently with bad prognosis, which is thought to be due to Galectin 3, which is highly expressed in RC and is involved in cancer progression, angiogenesis and cancer cell adhesion. Galectin 3 is inhibited by citrus pectin in a dose dependent manner. [1] The chemotherapy of RC nearly universally involves the use of 5-fluorouracil (5-FU) given before curative surgery; improving sphincter preservation in
patients with low-lying tumors and improving quality of life. [2] 5-FU has erratic bioavailability and its’ antitumor activity is more dependent on the tumor exposure to 5-FU which depends on both the concentration and the time, than on the dose administered. [3] Synergy has been seen due to combination of 5-FU with Galectin 3 inhibitors. [4] Thermo-sensitive and mucoadhesive polymers have gained much attention recently to elevate patient discomfort, relief the alien feeling due to solid suppository insertion, and afford an elegant method of administration besides its’ ability to be retained at the site of administration. The main thermo responsive polymer used in rectal delivery of drugs are; Poloxamers which are series of closely related difunctional triblock copolymers surfactants of non-ionic nature, they comprise of a central block of relatively hydrophobic polypropylene oxide PPO surrounded on both sides by the blocks of relatively hydrophilic polyethylene oxide oxide PEO. When the poloxamer is placed in cold water, hydration layer surrounds the poloxamer molecule and the hydrophobic portion are separated due to hydrogen bonds, with increasing temperature de-solvation of the hydrophilic chains occurs as a result of breakage of hydrogen bonds which leads to increase chain friction and entanglement producing a hydrophobic association. This results in hydrophobic interaction amongst the PPO domain and gel formation. [5] While among the widely used mucoadhesive polymers, the polyacrylates (like Carbopol) are the most efficient due to the high molecular mass of polymer, that will ensure relatively high residence time on the mucosa, also the degree of cross-linking which diminishes the dissolution rate of hydrophilic polymer chains in aqueous environment providing comparatively greater cohesion of polymers to the mucosa. [6] Pectin is a polysaccharide with chains of linear regions (galactouronic acid) and their methyl esters, interrupted in places by rhamnose units causing a kink in the straight chain. The degree of esterification of the galactouronic acid and therefore the charge on the pectin molecule affects its gelling properties. [7] Pectin suffer from syneresis on aging and its’ mechanical properties are inferior to the synthetic polymers. Highly Methylated pectin show a strong tendency to interact with large intestinal mucosa, thus interrupting the biochemical processes mediating cell-cell adhesion in tumor growth. While Citrus pectin modified by heat (this reduces the chain length) is reported to lead to significant levels of tumor cell apoptosis comparable to fractionized pectin powder. While pH treatment; with mild base (which removes esterification) reduces cell- cell adhesion in tumor development by acting as ligands of Galectin 3. [8] The aim of this study is to formulate mucoadhesive liquid suppository of 5-FU in combination with Pectin.
MATERIALS

1. 5-Fluorouracil (5-FU) purchased from Ampla Pharmaceutics Inc., USA.
2. Unipectin® QC40 (Citrus pectin) Cargill, Redon, France.
3. Poloxamer 407 (Pluronic® F127) solid with general formula of PEO (101) PPO (56) PEO (110), melting point 52-57 °C and average molecular weight 9840–14 600 Sybronics, Actico, Jordan (a kind gift from Assistant Prof. Eman Alkhathery).
4. Carbopol 940 (C940) is Homo-polymer of acrylic acid cross-linked with Allyl ethers of pentaerythritol with average molecular weight of 40,000 - 60,000 HiMedia lab., Pvt Ltd, Mumdai, India.
5. Beta cyclodextrin CD HiMedia Lab., Pvt Ltd, Mumdai, India. All the reagents are of analytic grade, used without purification.

METHODS

Preparation of 5-FU Solid Mixture

Solid mixtures were prepared by trituration of the solid ingredients for a few minutes in a mortar and 10ml alcohol was added during trituration, the slurry was poured in a glass container and left aside overnight for evaporation of alcohol, as seen in table 1.

Table 1: Formulation guide for 5-FU, Pectin and Cyclodextrin solid mixture

<table>
<thead>
<tr>
<th>Code</th>
<th>5-FU (gram)</th>
<th>Pectin (gram)</th>
<th>Cyclodextrin (gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>M4</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>M5</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

C940 was dispersed in distilled water (1% w/v) by slowly sprinkling portions of the powder gradually in to the vortex of the mixture at high speed until all the powder is incorporated and mixing is continued for addition 10-15 min to allow complete hydration of C940. Propylene glycol is added to the dry 5-FU powder blend M5 (5-FU: Pectin: CD 1:2:2) with the other ingredients (methylparaben and EDTA), and triturated to obtain smooth slurry and then poured into the C940 dispersion, with continuous agitation until complete hydration. This mixture was placed in an ice bath and allowed to cool down to 4°C, for about 5-10 min, then P407 (according to the formulation guide in table 2) was added slowly small pieces at each time to allow full hydration, after all is in the dispersion, mixing was continued for at
least 5 more min, the mixture is stored in tightly closed glass container and stored at 4°C overnight for complete hydration.

Table 2: Formulation Guide of 5-Fu Liquid Suppository.

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Weight (gram)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P0</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU:Pectin:Cyclodextrin (1:2:2)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>equivalent to 5-FU</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Propylene glycol (ml)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Carbopol (940)</td>
<td></td>
<td>10</td>
<td>15</td>
<td>18</td>
<td>19</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Poloxamer (P407)</td>
<td></td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Distilled Water up to(ml)</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Characterization of 5-Fuand 5-FU Solid Mixture

1. Solubility Study

Pure drug was placed in a sealed glass container with excess water and placed in a shaking water bath set to 20°C and 37°C for 72 hours and then a sample of the supernatant was taken and diluted with water and measured spectrophotometry at 266nm, the same was done for drug solid mixtures M1 to M5

2. X-Ray Diffraction XRD

The crystalline nature of free 5-FU and 5-FU solid mixtures M1, M4, M5 were compared by placing the powder in aluminum plates, the XRD patterns was acquired using Cu radiation (wave 1.5406 Å, voltage 40 kV, current 30 mA) using Schimatzu LAD X XRD 600 in continuous mode at axis of 2-theta in the range 10-40 degrees with a step size of 0.05° 20 and step speed 5deg/min preset time of 0.6 seconds

3. Particle Size Analysis

The particle size for solid mixtures were measured by light scattering method using Schimatzu SALD 2101, in which 0.3g of the sample(M5,M4,M1) was suspended in 100ml water and to be introduced into the laser path, particle size and the particle distribution was obtained.

Evaluation of 5-FU Liquid Suppository

1. FTIR Study

The prepared liquid suppositories were spread slightly in petri dishes one week prior to the
test and allowed to air dry, each sample was scraped from the petri dish, and triturated in a marble mortar with and without KBr and analyzed by Schimatzu 8400S FTIR, the test was performed at room temperature in range from 400-4000 cm\(^{-1}\), and compared to the spectrum of the pure drug.

2. Viscosity

The viscosity of the liquid suppository was measured to ensure syringability of the formula, using NDJ 5S rotational (cup and bob) digital viscometer with spindle 4 attached, at different rotation speeds of 6, 12, 30 and 60 rpm, at 20°C (room temperature) and at 37°C (the formula was placed in a thermo-stated water bath for 30 min.) and the viscosity measured. The shear stress can be calculated \(^{(9)}\) as

\[
\sigma = \frac{T}{2\pi h R_b^2}
\]

And the shear rate \(\gamma' = \frac{4\pi R_b^2 \text{rpm}}{50(R_c^2 - R_b^2)}\)

Where: \(R_b\) and \(R_c\) are the radii of the bob (0.6cm) and cup (1.6cm) respectively and \(h\) (5cm) the effective height of the liquid in the cup, \(T\) is the torque.

Flow behavior was analyzed by regression analysis of the log Power Law (Ostwald de Waele so that log shear stress was plotted vs. log shear rate.

\[
\log \sigma = \log K + n \log \gamma' \quad (3)
\]
\[
\log \eta = \log K + (n - 1) \log \gamma \quad (3a)
\]

In which: \(\gamma'\) is shear rate (s\(^{-1}\)), \(\sigma\) is shear stress (dyne/cm\(^2\)), \(\eta\) is viscosity \(n\): is the flow index and \(K\) is the consistency index (Pa.s-n)

In this equation \(n\) is the index of the deviation from Newtonian flow behavior. The more the value of \(n\) differs from unity, the more non-Newtonian is the flow behavior. Newtonian behavior \(n=1\), for pseudo plastic flow (shear thinning) behavior, \(0 < n<1\), While for dilatant flow (shear thickening) \(n>1\)

3. Gel Strength

The gel strength was determined according to a previously adopted method reported by Choi et al. \(^{(10)}\) the liquid suppository (50 g) was placed in a 100-mL graduated cylinder and gelled in a thermostat at 36.5°C. The homemade apparatus (plunger) for measuring gel strength...
(weight: 35 g) was then placed onto the gelled suppository (immersed vertically into the cylinder, the diameter of the plunger should occupy about 68% of the inside diameter of the glass cylinder). The gel strength, which means the viscosity of gel at physiological temperature, was determined by the time in (sec) the apparatus took to sink 5 cm down through the gel (Figure 1).

4. Measurement of Gelation Temperature

The gelation temperature was measured using the method reported by Choi et al. and Miyazaki et al. (11) A 10-ml volume of the liquid suppository was transferred to a 20-ml transparent vial containing a magnetic stirring bar. The vial was heated at an increasing rate of 1°C/min with constant stirring rate at 50 rpm. The temperature at which the rotation of the bar stopped was taken as the gelation temperature.

5. Determination of Bio Adhesive Force

The bio adhesive force of the liquid suppository was determined by using the modified balance. [12] In brief, a section of tissue was cut from the sheep rectum and placed in the deep-freezer until use. The day of the experiment, the tissue was thawed by placing the tissue in Tyrodes solution for about 2 hrs. (NaCl 0.8%, KCl 0.02%, CaCl₂ 0.02%, MgCl₂ 0.01%, NaH₂PO₄ 0.005%, NaHCO₃ 0.1%, Glucose 0.1%, in D.W). As shown in figure 2; the tissue (E) was secured with mucosal side out onto glass vial by rubber band; the vial was attached to the balance instead of one of the pans by a height-adjustable hook. Next, glass plate (D) was placed under the mucosal tissue. The liquid suppository (F) was placed on the glass plate; then, the height of the vial was adjusted so that the liquid suppository could touch the mucosal tissue which was allowed to adhere for three minutes (preload time). On the other side of the balance, a plastic cup (A) was placed to collect water, was balanced with the other...
side. Water was added drop by drop to the plastic cup until the weight of water in the cup detached the glass slide from the mucosal tissue. Bio adhesive force, the detachment stress (dyne/cm²), was determined from the minimal weights that detached two surfaces from each other, was calculated using the following equation.

\[ F = \frac{980 \ m}{\pi r^2} \]  

(4)

Where \( F \) is the detachment force (dyne/cm²) of the liquid suppository per unit area of mucosa (cm²) \( (\pi r^2 \text{ in which } r \text{ is the radius of the vial}) \)

\( (r = 1.2 \text{ cm}) \) by the balance weight \( m \) (g) and 980 is acceleration due to gravity (cm/sec²). The rectal tissue was changed for each measurement.

Figure 2. Mucoadhesive force measuring device.

The Release of 5-FU From In Liquid Suppository

In Vitro Dissolution Test

Studies of 5-FU release from liquid suppository formulations were performed using the in vitro dialyzing method in which 5ml of the prepared liquid suppository formulation was syringed into a glass container closed at one end and the open end covered by a 1.8 cm cap in diameter of hydrophilic membrane of regenerated cellulose Millipore 0.45\( \mu \) (soaked for 1 hr. before the test in the phosphate buffer) this glass apparatus was fixed on the paddle with cable ties, on dissolution apparatus type 2 and 500ml phosphate buffer pH7 was used as a dissolution medium, within a period of 3hr and 50 rpm at 37°C. Aliquots (5ml) were taken at regular intervals and analyzed spectrophotometrically at 266nm, \(^{13} \) the experiments were repeated in triplicates.
Figure 3. Photograph of the ex vivo test, (left) the sheep rectal tissue covered the glass vial which is attached to the dissolution paddle by a cable tie. In vitro test is shown in right side of the picture.

**Ex Vivo Permeation Test**

Studies of drug permeation was performed for formula P4 for 24hrs at 37°C using the same apparatus as before but substituting the Millipore membrane by sheep rectum, which was thawed for about one hour in Tyrodes’ solution, 5ml of the prepared liquid suppository was placed in the glass cell (donor) and rotated at a rate of 50 rpm in dissolution apparatus type 2 and 500ml phosphate buffer pH 7 was used as the recipient. Aliquots were taken at regular intervals and analyzed spectrophotometrically at 266nm. (Figure 3)

**RESULTS AND DISCUSSION**

**Solubility Study**

The solubility of 5-FU in water at 20°C and 37°C is 12.4mg/ml and 13mg/ml respectively, the addition of Pectin significantly retarded dissolution of 5-FU at both temperatures (p <0.05) by 87.25% for 20°C and 72.88% for 37°C, when comparing M2 with the 5-FU solubility. There is significant difference (p<0.05) between M2 and M5 in solubility of 5-FU at both temperatures, and the solubility increased 86.7% at 20°C and 81.95% at 37°C when comparing M2 with M5 solubility, in which the solubility of 5-FU in M5 was recorded as 11.9mg/ml at 20°C and 19.53 mg/ml at 37°C. Pectin is acidic in nature, and 5-FU exhibits lower solubility in the acidic environment. [14] Therefore, the addition of β-cyclodextrin was necessary to compensate for the decrease in dissolution, and to increase the penetration of 5-FU in human cancer cell line, which can minimize the dosage and effect the cytotoxic potential of 5-FU [15].
Figure 4. The solubility of 5-FU and solid mixtures at 20° C and 37° C in the following order 5-FU-M1-M2-M3-M4-M5

Particle Size Analysis
There was insignificant difference in the particle size between the particle size of the pure drug and M5 (p=0.445). As 5-FU medium particle size was (344μ±0.151) M5 (276μ±0.229), M4 (247μ±0.232), M1 (304μ±0.238) Lowering the particle size into Nano range will aid absorption and bioavailability but it will also increase the rate of spreading of the particle higher into the colon, since the action of the drug is required for local action not to effect the normal colon cell located upstream the larger particle size will be retained at the place of administration. [16]

X ray Diffraction XRD
5-FU is crystalline in nature, and exhibits sharp signal (intensity) at the diffraction angles 20 of 28.5734° (d=3.1125 Å), 20.6166° (d=4.30468 Å) and 18.7883° (d=3.1125 Å) which is consistent with the standard 5-FU X ray diffraction chart with the intense peak at 20 of 28.680° and corresponding to the radial width of reflection (d=3.11259 Å) at the scattering angle. The intensities of the peak in the blends decrease proportionally as the content of 5-FU is reduced. The possible reduction in crystallinity may be due to complex formation. The increase in solubility of the complex is confirmed by X-ray diffractogram The % crystallinity from the XRD plots was calculated by using Fit Gaussian method with the help of origin software. [17] The Crystallinity percent was calculated by comparison with the area of the peak of the highest intensity at 20 values of 28.5734° using the following formula;

\[
\% \text{Crystallinity} = \frac{\text{Crystalline area}}{\text{Total area}} \times 100
\]  

(5)

5-FU showed 100% Crystallinity, \textbf{M1} 80.05%, \textbf{M4} 25.09%, \textbf{M5} 19.01%
Figure 5. X-ray diffraction of the combined charts pink 5-FU, red 5-FU: pectin 1:1, green 5-FU: pectin: CD 1:2:1, blue 5-FU: Pectin: CD 1:2:2

FT-IR Study

The characteristics peaks of 5-FU are present in the prepared liquid suppository of 20% Poloxamer formula P5 (figure 6) but with less intensity; meaning there is no chemical interaction between the formula ingredients and the drug. But the inclusion of the drug in CD decreased the intensities $^{[18]}$ of some bands; (1884 cm$^{-1}$, 2824 cm$^{-1}$) Also some regions of the spectra have become less intense around the 1728 cm$^{-1}$ and 1200 cm$^{-1}$ region, and 1000-600 cm$^{-1}$ region in the 20% Poloxamer film.

Figure 6. FTIR chart of 20% Poloxamer (P5) film.

Physiochemical Properties of the 5-FU Liquid Suppository

1. Gelation Temperature

The gelation temperature of the liquid suppository is an essential parameter, the sol–gel transition will ensure the rectal retention of the dosage form. The gelation temperature of the liquid suppository should be in the range of 30-36°C.
The formulas that apply to these criteria are P4 and P5, as seen in table 3 the gelation temperature increased as the concentration of poloxamer decreased. Choi et al. [19] reported that gelation temperature of P407 of 18-25% solution is 13-25°C (above critical micellar concentration) but the addition of carbopol (mucoadhesive polymer) increased the gelation temperature. [20] Poloxamers show sol to gel transition due to extensive hydrophobic interaction between the poly-oxypropylene domain and breakage of the hydrogen bonds between the hydrophilic domain, since the Carbopol chains are extensively hydrated with numerous hydrogen bonds, and cross linkers that extend into large network, that may separate the poly-oxypropylene domain therefore; the poloxamer will need a higher temperature for sol to gel transition. [21]

**Table 3: Physiochemical Properties of 5-Fu Liquid Suppository.**

<table>
<thead>
<tr>
<th>Formula code</th>
<th>pH</th>
<th>Gelation temperature (°C)</th>
<th>Gel strength (Sec)</th>
<th>Mucoadhesion (dyne/cm²)x10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>P5</td>
<td>4.5</td>
<td>30.6</td>
<td>100</td>
<td>2.619</td>
</tr>
<tr>
<td>P4</td>
<td>3.2</td>
<td>31.7</td>
<td>75</td>
<td>2.476</td>
</tr>
<tr>
<td>P3</td>
<td>3.6</td>
<td>36.5</td>
<td>40</td>
<td>1.3918</td>
</tr>
<tr>
<td>P2</td>
<td>3.6</td>
<td>37</td>
<td>25</td>
<td>1.269</td>
</tr>
<tr>
<td>P1</td>
<td>3.3</td>
<td>37.9</td>
<td>16.5</td>
<td>1.242</td>
</tr>
</tbody>
</table>

2. **Gel Strength**

The gel strength is related to the internal structure and the strength of the physical or chemical bonds forming the interplaying network of the long chains of the polymers involved in gel formation, it resembles consistency and is related to viscosity and depends on the type of polymer used. [22] The formula must have considerable gel strength to be applied by a syringe to the rectal area, weak gels will leak and strong ones are difficult for syringe manipulation, the criteria sets a range from 10-50 sec. But formula P4 and P5 gel strength was 75, and 100 sec. respectively and they were syringable, even in hot climate as Iraq. Gels with higher gel strength have been chosen outside the criteria. [23]

3. **Mucoadhesive Force**

The mucoadhesion is vital for the 5-FU in situ rectal suppository in order to ensure adhesion of the formula to the site of the tumor and remain there at least until mucin turn-over. The formulas P4 and P5 show the best mucoadhesive properties (table 3), both the poloxamer and Carbopol show bioadhesive properties, but since Carbopol is anionic polymer, the mucoadhesive properties are superior to nonionic poloxamer polymers, addition of drugs or additives may alter the mucoadhesive properties of the formula. The stronger effect of
Carbopol on the physiochemical properties, seem to be due the poloxamer long chains are inserted or are physically tangled between the Carbopol network, reinforcing the mucoadhesive properties of Carbopol by extensive hydrogen bonds with the oligo-polysaccharide chains of the rectal mucosa. \[^{[24]}\] In conclusion formula P4 fits all the above criteria.

**Rheological Behavior of Formula P4**

The flow behavior of formula P4 was shown to be shear thinning pseudo plastic at both 20°C and 37 °C (table 4)(figure 7), as the shear rate increases the viscosity decreases, this is essential for syringability. \[^{[25]}\] The temperature has a major effect on the viscosity of thermosensitive liquid suppository increasing the temperature near body temperature (54% increase in temperature) the viscosity increases significantly (p=0.0047). Therefore, as P405 concentration is raised from 10–15 to 20% (w/w), the poloxamer chains will be initially present as monomolecular micelles, in which the hydrophobic PPO segments are protected from the aqueous phase by the hydrophilic PEO segments. As the concentration of P405 is increased, inter-micelle aggregation increasingly occurs, which, in turn, results in an increase in the viscoelastic properties. Carbopol interferes with mono-micelle or multi-micelle formation creating rheological antagonism, \[^{[26]}\] as the concentration of P407 increased from 15 to 20% (w/w), rheological antagonism decreased. This may be explained by the increased molar ratio of Poloxamer to Carbopol and therefore, whilst the number of interactions between the two polymers has remained constant, the number of free poloxamer molecules that are free to self-associate has increased.

![Figure 7. Double Logarithmic representation of power law at 20 and 37°C of formula P4.](image-url)
Table 4: Flow Characters of formula P4 at 20 and 37°C.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Ostwald KmPa.s-n</th>
<th>n</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>3.7255</td>
<td>0.6483</td>
<td>0.9801</td>
</tr>
<tr>
<td>37°C</td>
<td>3.9934</td>
<td>0.581</td>
<td>0.9993</td>
</tr>
</tbody>
</table>

Rheological synergy was observed in formulas P4 and P5 in which increased temperature of the system facilitated hydrophobic bonding between the PPO segments of adjacent micelles (inter-micellar) of Poloxamer molecules and Carbopol interaction with the hydrophilic PEO regions of adjacent micelles through hydrogen and Van der Waals forces thus, increasing cross-link density and network formation this effect elevates the gelation temperature.

The Effect of P407 Concentration on the Release of 5-FU Form Liquid Suppository

The release of 5-FU from the formulas P0 to P5 in phosphate buffer pH 7 were plotted as seen in figure 8, and showed significant difference between P1-P5 (p<0.05) in terms of 5-FU release. The release kinetics of 5-FU in table 5 show diffusional exponent between 0.5 and 1. This type of transport is known as anomalous or non-Fickian transport drug release which occurs due to a combination of macromolecular relaxations and Fickian diffusion. Increasing the P407 concentration (above CMC) in formulas P3-P5 retarded the release of drug because of the higher formula viscosity, but formula P1 (below CMC) seemed to show enhanced release because poloxamers are amphiphilic surfactant that may enhance the release of drugs.

As the P407 consists of a large population of micelles in aqueous phase, the incorporated drug may be released by diffusion through gel matrix. Drug release can be affected by the viscosity of the gel, the size of the aqueous channels and the distribution of the drug between the micelles and the aqueous phase. [27]

Figure 8. The effect of Poloxamer concentration on the release of 5-FU from in-situ gel in phosphate buffer pH 7 and 37°C at 266nm.
Table 5: The Release Kinetics of 5-Fu Liquid Suppository.

<table>
<thead>
<tr>
<th></th>
<th>P0</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
</tr>
</thead>
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<tr>
<td><strong>Higuchi</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_h$</td>
<td>4.977</td>
<td>8.581</td>
<td>5.439</td>
<td>2.647</td>
<td>2.837</td>
<td>1.530</td>
</tr>
<tr>
<td>$R_2$</td>
<td>0.9331</td>
<td>0.9877</td>
<td>0.9510</td>
<td>0.9048</td>
<td>0.9659</td>
<td>0.9713</td>
</tr>
<tr>
<td>$T_{50}$ (hr.)</td>
<td>100.9</td>
<td>33.9</td>
<td>84.5</td>
<td>356</td>
<td>310.5</td>
<td>1067.3</td>
</tr>
<tr>
<td><strong>Korsmeyer</strong></td>
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<tr>
<td>$K_{KP}$</td>
<td>3.978</td>
<td>8.309</td>
<td>4.535</td>
<td>2.130</td>
<td>2.169</td>
<td>1.383</td>
</tr>
<tr>
<td>$R_2$</td>
<td>0.9932</td>
<td>0.9892</td>
<td>0.9947</td>
<td>0.9653</td>
<td>0.9841</td>
<td>0.9888</td>
</tr>
<tr>
<td>$n$</td>
<td>0.812</td>
<td>0.539</td>
<td>0.754</td>
<td>0.804</td>
<td>0.608</td>
<td>0.644</td>
</tr>
<tr>
<td>$T_{50}$ (hr.)</td>
<td>22.6</td>
<td>27.99</td>
<td>24</td>
<td>50.7</td>
<td>174.933</td>
<td>261.9</td>
</tr>
<tr>
<td><strong>Zero Order</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_0$</td>
<td>3.402</td>
<td>5.346</td>
<td>3.702</td>
<td>1.810</td>
<td>0.739</td>
<td>1.032</td>
</tr>
<tr>
<td>$R_2$</td>
<td>0.9774</td>
<td>0.8379</td>
<td>0.9656</td>
<td>0.9471</td>
<td>0.8306</td>
<td>0.9123</td>
</tr>
<tr>
<td>$T_{50}$ (hr.)</td>
<td>14.699</td>
<td>9.352</td>
<td>13.5</td>
<td>27.63</td>
<td>67.632</td>
<td>48.4</td>
</tr>
</tbody>
</table>

$K_h$ is the Higuchi constant (mg.ml-1hr.-1/2) while $K_{KP}$ is the Korsmeyer-Peppas constant (mg/ml.hr.-n) $T_{50}$ time for 50% release (hr.)

**Ex Vivo Release**

The similarity factor between the in vitro and the ex vivo was performed and was found to be similar ($f_2=91.97$), i.e. more than 50, validating the in vitro procedure for studying permeation and release. The permeation of 5-FU was obtained from the slope of the plot of cumulative amount permeated /cm2 of formula P4 studied after 8 hr. of release (figure 9), and the flux is obtained from linear regression and the permeability coefficient $K_p$ which is obtained from the relationship $K_p=J/C$ in which $C$ is the concentration of the drug in the gelled liquid suppository. The $K_p$ will be $0.0695$(mg/ hr.cm2) /0.1mg/cm3=0.695 cm/hr.

![Figure 9. The permeation of 5-FU from formula P4 in phosphate buffer pH 7 and 37°C measured at 266nm.](image-url)

Figure 9. The permeation of 5-FU from formula P4 in phosphate buffer pH 7 and 37°C measured at 266nm.
CONCLUSION
5-FU was formulated as mucoadhesive liquid suppository for rectal administration in combination with pectin, which retarded the solubility of the drug, therefore CD was added which decreased the crystallinity of the drug, inclusion of Carbopol greatly enhanced the mucoadhesion which is attributed to the anionic nature of the polymer and the high molecular weight.5-FU liquid suppository showed pseudo-plastic behavior which is essential for syringability, also the increase of P407 concentration sustained the release of 5FU.

ACKNOWLEDGEMENTS
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REFERENCES