Original Article

Preparation and in vitro evaluation of topical gel of 5-fluorouracil

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ABSTRACT

Topical drug administration through the skin can be defined as the application of drug contains formulation directly to the skin for prophylactic, protective, or therapeutic purposes. 5-Fluorouracil is an antineoplastic agent used for the treatment of skin diseases such as actinic keratosis. The aim of this work was development a gel formulation of 5-FU using NaCMC and PVA as a gelling agent. Six formulations (F1-F6) were prepared using different concentrations of NaCMC and PVA as a gelling agent, glycerin as a humectant, and methyl hydroxyl benzoate preservative. The effect of gelling agent type and concentration on the extrudability, spreadability, rheology, and in vitro drug release was investigated. All the prepared gel exhibited acceptable physical properties such as homogeneity, clarity and consistency, pH value, extrudability and, spreadability. The best formulation F1 prepared by using 4% NaCMC as a gelling agent,5% glycerin as a humectant, and 0.2% methyl hydroxyl benzoate as a preservative with pH value 6.38±0.04, drug content 97.2±0.2, extrusion time 12±0.5sec with highest drug release 98.12% through 3h.

Keywords: Anticancer, Sodium carboxymethylcellulose, Polyvinyl alcohol, Gelling agent, Spreadability, Rheology

Introduction

Topical drug delivery is a localized drug administration anywhere through rectal, vaginal, ophthalmic, and skin. Topical drug administration through the skin can be defined as the application of drug contains formulation directly to the skin for prophylactic, protective, or therapeutic purposes [1-4]. The topical drug delivery has many advantages: avoidance of first-pass metabolism, GIT irritation, and varied absorption conditions such as pH changes, gastric emptying, and presence of enzymes [5]. The gel is a semisolid network composed of a liquid phase constrained inside a three-dimensional matrix of natural or synthetic polymer. A high degree of chemical or physical cross-linking is established [6]. The main advantages of gel are easy to formulate, non-greasy, providing controlled release, and can be used for polar and non-polar drug administration [7]. 5-Fluorouracil (5FU) is an antineoplastic agent used to treat skin diseases such as actinic keratosis psoriasis, cutaneous premalignant, malignant lesions, and basal-cell carcinoma, 5-FU available in different dosage forms like cream: 0.5%, 1%,5% and injection:50 mg/mL. Chemically 5-FU is a white crystalline powder with a melting point of 282 °C. It is slightly soluble in ethanol, sparingly soluble in water, practically insoluble in chloroform and ether. It has a chemical formula C4H3FN2O2 with MW=130.1 [8]. The aim of this work was development a gel formulation of 5-FU using a Na carboxymethylcellulose (NaCMC) and polyvinyl alcohol (PVA) as a gelling agent. the influence of type and concentration of gelling agent was investigated, drug content, pH, spreadability, extrudability viscosity and In -vitro drug release of the prepared gel were evaluated.

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Materials and Methods

Materials

5-FU was purchased from Hyper-chem Ltd Co. China, NaCMC from Baerlocher, GMBH, Germany, PVA from H.I. media

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laboratories, India, glycerin and Methyl hydroxyl benzoate obtained from Gain Land chemical community, U.K.All other reagents and chemicals used were of analytical grade.

Construction calibration curve of 5-FU

A stock solution of 5-FU at a concentration (1mg/ml) in buffer pH5.5 was prepared and diluted to a series of standard solutions at a concentration range from (2.5,5,10,15, and 17.5 µg/ml). The absorbance of these standard solutions was measured at 5-FU λ max 266 nm by using a U.V spectrophotometer. After that, the absorbance of each prepared standard solution was plotted against the concentrations.

Preparation of 5-FU gel

Six formulations (F1-F6) were prepared, as shown in **Table 1**, using different NaCMC and PVA concentrations as a gelling agent. NaCMC formulations (F1-F3) were prepared by dispersed the desired amount of NaCMC insufficient quantity of D.W, which contain 0.2gm methyl hydroxyl benzoate as a preservative and 5ml glycerin as a humectant and increasing the gel spreadability. The dispersed solution was homogenized by the magnetics stirrer then left at room temperature for one day to complete the polymer swelling [9]. PVA formulations (F4-F6) were prepared by using the hot method in which 70ml D.W that contain 0.2gm methyl hydroxyl benzoate and 5ml Glycerin heated to 70 ºC in a beaker for 10 min followed by slow addition of PVA amount, then complete the volume to 100 ml by D.W. Finally the PVA dispersion was left to cool at room temperature for a viscous clear dispersion production [10].

Table 1. Composition of 5-FU 0.5% gel formulation (Expressed as % w/w)

Materials (g)	F1	F2	F3	F4	F5	F6
5-FU	0.5	0.5	0.5	0.5	0.5	0.5
NaCMC	4	5	6			
PVA				6	7	8
Glycerin	5ml	5ml	5ml	5ml	5ml	5ml
Methyl hydroxyl benzoate	0.2	0.2	0.2	0.2	0.2	0.2
D.W.	100	100	100	100	100	100

Evaluation of 5-FU

Visual examination

This test was considered for visual characteristics, which include homogeneity, clarity, and consistency [10].

pH determination

The pH of 5-FU gel was measured at room temperature using a digital pH meter, and the reading was taken in triplicate [11].

Drug content

A sample of 1gm of prepared gel, which is equivalent to 5mg 5-FU, was taken in a100ml buffer pH 5.5 of volumetric flask followed by flask sonication to get complete drug solubility and filtration through 0.45µm membrane filter. One ml of the filtered solution was diluted to 10 ml buffer pH 5.5 and analyzed at λ max 266 nm by a U.V spectrophotometer using the buffer pH 5.5 as blank [12].

Extrudability

A sample (15 gm) of the gel of each formulation was placed in a 60 ml feeding syringe ,followed by applied one gram pressure. The time required for the gel to be completely extruded from the syringe was recorded, an average of three readings was taken [13].

Spreadability

One of the important gel criteria to get the ideal quantities is to possess good spreadability. The term expressed the degree of an extended area in which the gel spreads quickly on the affected skin part. A sample weighted one gram of each formula was pressed between two slides with 500mg weight, then left for five minutes when no further spreading was expected; after that, the diameters of spread circles were measured in cm and used as spreadability comparative values; this test is done in triplicate and takes the average values [14].

Viscosity study

The viscosity is considered an important evaluation test of rheological study in semisolid dosage form, the determination of viscosity was done using a digital viscometer Myr VR3000 with spindle no. R7 at room temperature with an optimum speeds 2.5,4,6,10,20,30 and 50 rpm [15].

In vitro drug release study

Each formulation's sample (1 gm) was weighed using a digital balance and uniformly spread on the watch glass surface and, after that, gently lowered throughout 500ml buffer pH 5.5 as a dissolution media. The paddle's dissolution apparatus was positioned around 2.5 cm above the watch glass; the paddle rotates at 50 rpm and maintains at 37 ± 0.5 °C. At specified time intervals, an aliquot of 5ml dissolution media withdrawal is replaced immediately by the same dissolution media volume over three h. The samples were filtered through 0.45 mm filter paper and analyzed at appropriate λ max 266nm of 5-FU by using a UV-visible spectrophotometer to obtain the % drug release at each time interval [16].

Fourier-transformed infrared (FTIR) spectroscopic analysis

FTIR spectra of pure5-FU and physical mixture at ratio (1:1) of 5-FU and NaCMC were analyzed between 400 and 4000 cm–1

by KBr disc method, using FTIR lambda 7600-Australia spectroscopy [17].

Statistical analysis

Student t-test was used for statistical analysis when $p<0.05$. A significant statistical difference was considered, and the study was done in triplicate, where the data represent mean ± standard deviation.

Results and Discussion

Physical appearance

The six prepared gel formulations were vicious, homogenous, smooth, and glossy with no phase separations; as shown in **Table 2**, NaCMC formulations show translucent appearance. In contrast, the PVA formulation shows a white appearance.

Table 2. Physical properties of 5-FU gels

Formulation code	Color	pH	Drug content (%)
F1	Translucent	6.38±0.04	97.2±0.2
F2	Translucent	6.42±0.01	96.14±0.3
F3	Translucent	6.44±0.02	96.95±0.2
F4	White	6.27±0.01	90.06±0.6
F5	White	6.36±0.05	96.55±0.1
F6	White	6.45±0.04	93.3 ±0.4

Values represent mean ± S.D., n=3

pH determination

the pH of all formulations was between the range (6.27 - 6.45) as seen in **Table 2** so that it has the pH near the pH of the skin (4-5.5) and does not cause any skin irritation [18].

Drug content

The data of Drug content analysis from the six prepared formulations range between (90.06-97.2), as shown in **Table 2**, indicating the homogenous 5-FU distribution within the prepared gels.

Extrudability

The extrudability depends on the type and concentration of the polymer used, as depicted in **Figure 1**. As the concentration of polymer increases, the extrusion time will increase , and from the obtained results, the PVA gel was quickly extrudable than NaCMC gel.

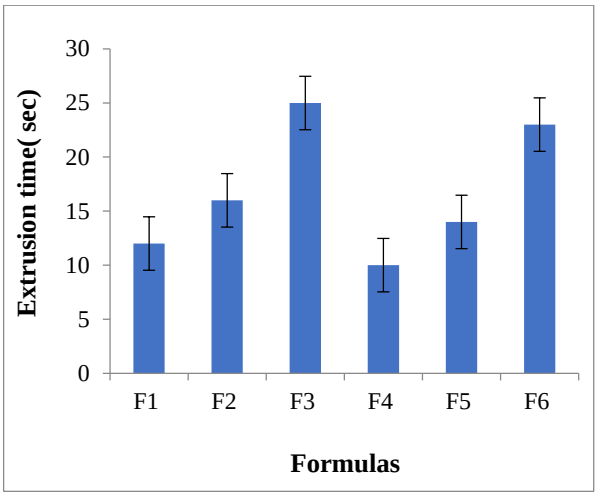

Figure 1. Extrudability of 5-FU gel (mean ± S.D.,n=3)

Spreadability

The spreadability values for 5-FU gel ranged from 4.5cm with gel prepared by NaCMC to 8 cm with gel prepared by PVA, indicating the ease of spreadability using a small shear amount. The result data indicate that the PVA gel is more spreadable than the NaCMC gel, also as the concentration of NaCMC polymer increase (F1-F3), the spreadability will decrease this is due to as polymer concentration increase, the chains repulsion and the cross-linking between the chains will increase so that the spreadability of the polymer will reduce [19], the same result seen in PVA based formulas.

Viscosity

From the obtained results for NaCMC based formulas, as the concentration of NaCMC increases, the viscosity of gel increases at the same velocity; this is due to the particles of the gel linked together by Vander Waals forces or H- bonding to produce an interlaced network , thus give the rigidity to the gel structure so that as the gelling agent concentration increase lead to increasing the gel viscosity [20]. Also from the result, it was seen, the viscosity decrease as the speed of spindle increase due to disarranged molecules caused to align their long axes in the flow direction so that this orientations reduce the internal resistance of the substances and thus reduce the viscosity [21], the same result was obtained with PVA based formulas, so that all formulas show non-Newtonian pseudoplastic flow behavior and the obtained viscosities for different 5-FUgels formulas, at low and high shearing rate indicate that NaCMC based formulas produced the high viscosity values than PVA based formulas.

It was found that F1 exhibit pseudoplastic with the time-dependent flow (thixotropic behavior) in which the down curve show displacement concerning the up curve and the down curve producing at any shear rate lower shear stress than on the up curve as result hysteresis loop formed between the up and down curve, this thixotropic behavior occur due to the gel needs time to rebuild to its original structure which destroys throughout the continuous measurement of the shear [19].

In vitro drug release study

The release profile of 5-FU gel from different prepared formulations in phosphate buffer pH 5.5 was study and the graphical representation of the drug release is shown in **Figure 2**; in the case of NaCMC based formulas, the 5-FU release can be ranked with following order F1> F2>F3 with (98.12, 93.5, 86.63) % drug release respectively, while for PVA based formulas the5-F.U. release can be ranked with following order F4> F5>F6 with (73.93,70.75,66.25) % drug release respectively. From the obtained data, it was found that the release of the drug significantly depended on the concentration and type of the polymer used; regarding the polymer concentration, it was seen as the polymer concentration increase, the drug release from the gel would decrease, this is due to as the polymer concentration increase leads to increasing the polymer cross-linking and reducing the migration of drug molecules [22]. Regarding the polymer types, NaCMC based formulas showed more drug release than PVA-based formulas; this is due to the three-dimension, and physical structural network of the PVA polymer since 5-FU entrapment within these structural networks revealed the high capability of PVA compared with NaCMC [20, 23].

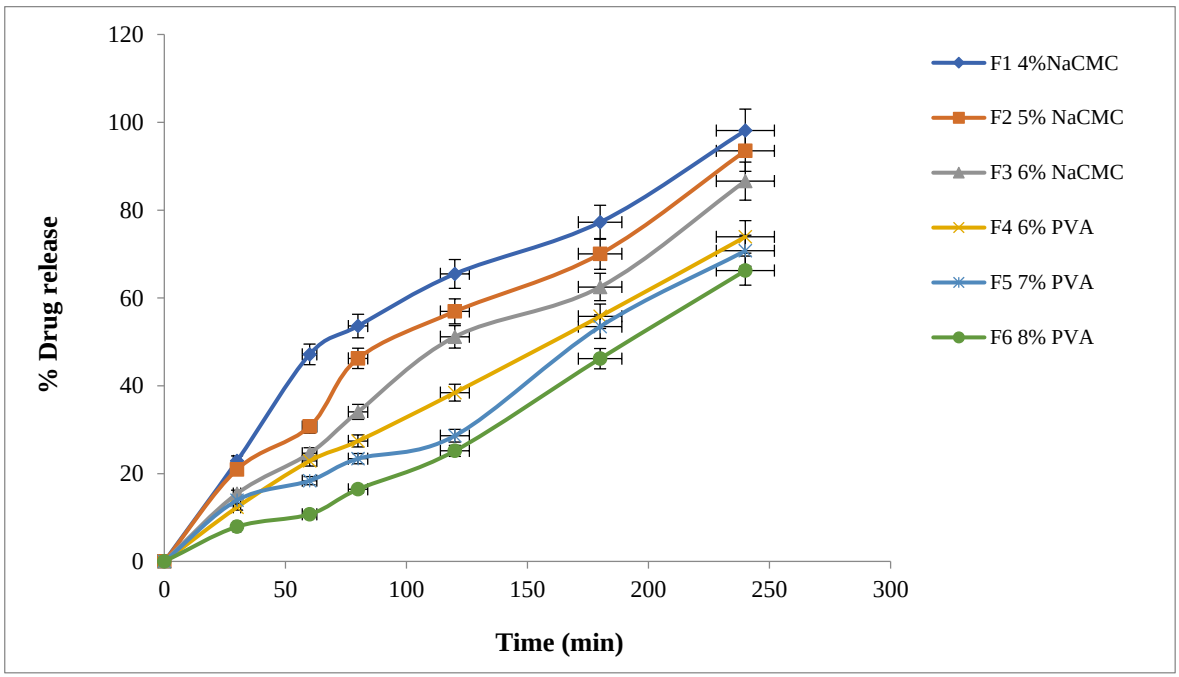

Figure 2. Drug release from the prepared gel formulation

FTIR spectroscopic analysis

The infrared spectra for pure 5-FU and Physical mixture of drug and NaCMC (1:1) were shown in **Figures 3 and 4**. The presence of characteristic peak of the drug in the physical mixture as seen in **Table 3**, indicates no interaction between the drug and the polymer used.

Table 3. FT-IR absorption bands of pure 5-FU and Physical mixture (1:1)

Characteristic groups	Pure drug	Physical mixture (1:1)
C=O of amide	1639	1633
C-F stretch	1242/1222/1180	1240/1222/1180
C-H bending of aromatic	991/933	991/930
N –H amide wag	867/760/ 640	867/760/ 640

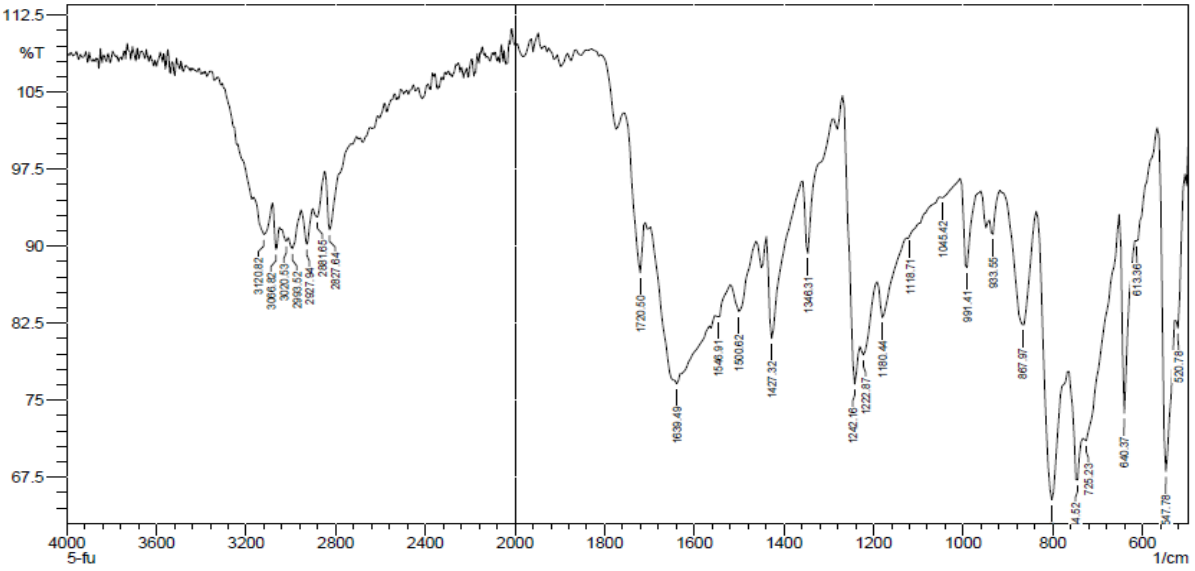

Figure 3. FTIR spectrum of pure 5-FU

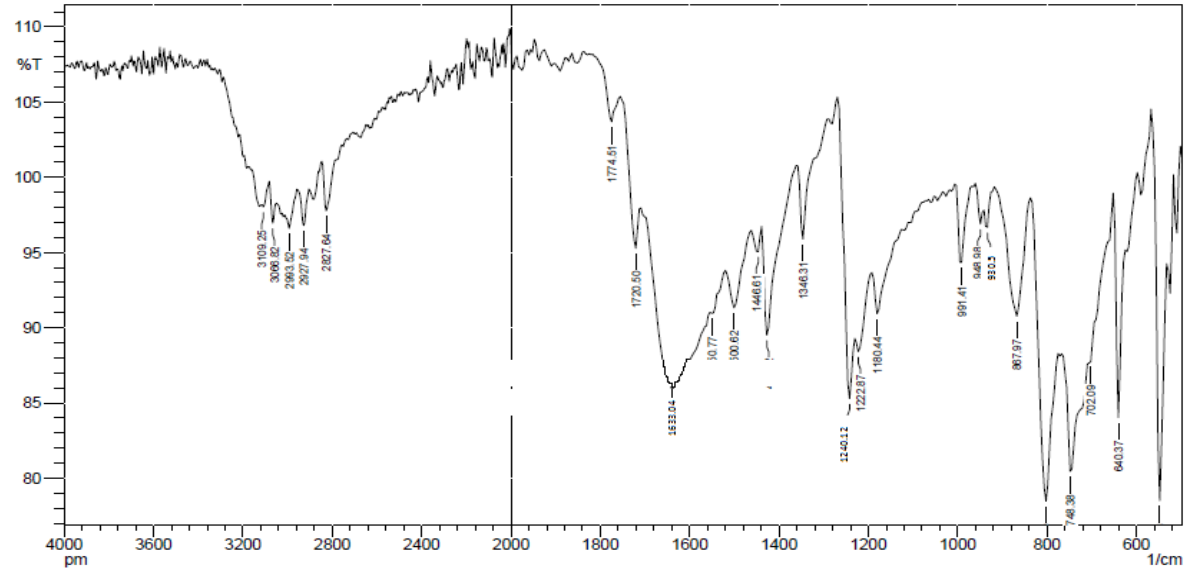

Figure 4. FTIR spectrum of physical mixture of 5- F U and NaCMC at ratio (1:1)

Conclusion

5-FU formulated as a topical gel using NaCMC and PVA as a gelling agent, the release of the drug from gels formulation affected by the type and concentration of the polymer used . The optimum 5-FU was obtained by using 4% NaCMC as a gelling agent,5% glycerin as a humectant and 0.2% methyl hydroxyl benzoate as a preservative with pH value 6.38±0.04, drug content 97.2±0.2, extrusion time 12±0.5sec with highest drug release 98.12 % through 3 h and this formula exhibit time depended on flow or thixotropic effect.

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Conflict of interest: None

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Ethics statement: The study was approved by the Local Ethics Committee of the college of pharmacy, University of Baghdad (Protocol C143-2020).

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