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Formulation and Characterization of insitu gel containing Chasteberry Extract For Umbilical Tissue delivery

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ABSTRACT

The main objective of the present study was to develop and evaluate in-situ gel formulation of Vitus-agnus castus fruit extract belonging to family Verbenaceae (verbena family) for topical drug delivery across umbilical skin tissue , aiming to enhance local penetration and in achieving sustained release effect of bio-active phytochemicals. Vitex agnus-castus extract was firstly incorporated in a poly-saccharide based thermosensitive in-situ gel matrix (eg Pluronic F127), the formulation was further designed to be liquid at room temperature and show transition in to gel at skin temperature (32-36.C The formulation was further assay for key physiochemicals properties such as Ph , viscosity, gelation time/temp, spreadability and drug content uniformity. The gel remained stable and sol-gel transition at various skin-mimicking temperatures .An in-situ gel drug delivery system loaded with Vitex agnus-castus extract for umbilical skin application which can offer localized as well as controlled release of bioactive compounds. Thus being non-invasive technique it also harnesses the antiangiogenic and anti-inflammatory properties of Vitex agnus-castus, waving way for targeted and localized drug delivery. Hydroalcoholic extract of Vitex agnus-castus was prepared and was found to be rich in flavonoids like casticin (used for its anticancer and anti inflammatory effect).

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INTRODUCTION

1.1. UMBILICUS

The transdermal drug delivery system (TDDS) is an administrative method of drug administration by applying or patching them onto the skin surface, resulting in localized or systemic approach of the drug delivery[1]. The efficacy of TDDS relies on achieving a subcutaneous concentration of the drug that permeates through skin for systemic uptake. [1]. The skin serves as the outermost organ of the body, which provides protection to the internal organs from environmental factors such as chemicals and pathogens. However, this biological barrier also hinders effective transdermal drug delivery upto greater extent. Various technologies, including iontophoresis, sonophoresis, electroporation, photomechanical waves, microneedling, nano carriers , niosomes have been developed to overcome this limitation and enhance drug transdermal penetration. Additionally, various chemical methods, such as the preparation of vesicles, polymeric nanoparticles, nano-emulsion, penetration enhancers such as naturally occurring terpenes, can also enhance transdermal drug efficiency [1]. TDDS-based therapy is cost-effective as it aids in preventing polypharmacy and increases the patient's compliance by simplifying the self administration . The decreased oral bioavailability of hormonal drugs and synthetic analogues are administered through transdermal drug delivery so that sustained effect could be achieved [2]. In general, various nanocarriers such as transferosomes, niosomes, liposomes, nanostructured lipid carriers, etc., which can improve the transdermal delivery of numerous hydrophilic and lipophilic drugs as compared to conventional transdermal formulations [3].

The umbilicus or navel, as being the center of the abdomen, demonstrates the final closure and thinnest region of the abdominal wall during embryonic development [1]. The umbilicus is a protruding, flat, or hollowed area on the abdomen which served as at the attachment site of the umbilical cord during gestation period[3]. We refer this phenomenon for improved drug penetration which exhibits faster penetration rates through umbilicus as "umbilical pro-permeability". Considering the advantages of umbilical pro-permeability and the unique properties of umbilical skin, umbilical paste therapy showed great promising stands for a great alternative to traditional TDDS [1]. Drug when administered through the umbilical skin can posses direct localized effect which may directly enter into systemic circulation and hence cause therapeutic effects [1]. It possesses a venous system with numerous tributaries involved in draining the abdominal wall. This anatomical feature allows drugs to be absorbed into the bloodstream through the deep capillary network within the umbilicus.

The umbilicus, commonly known as the belly button or navel, is a scar on the abdomen marking the site of the umbilical cord's attachment in the fetus. It's a central anatomical landmark, formed after the umbilical cord detaches and the stump falls off. The umbilicus is more than just a scar; it's a point where the anterior abdominal wall's layers fuse and can be a site of potential weakness.

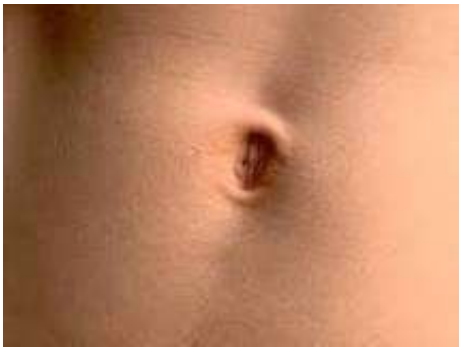


Fig: 1. Navel

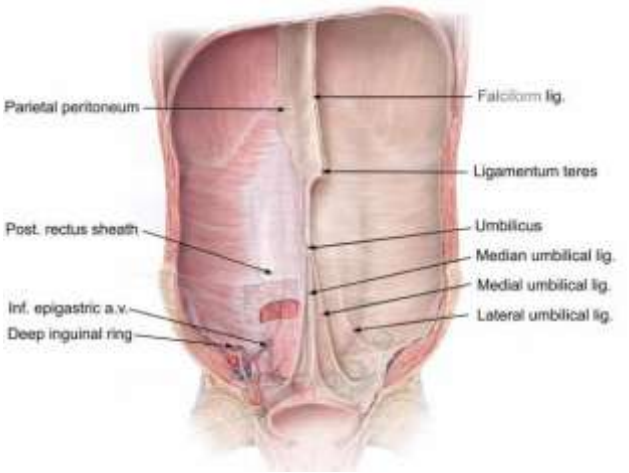


Fig: 2. Umbilicus Anatomy

Key Anatomical Features:

- **Umbilical Ring:** A small, round opening in the linea alba (a fibrous structure in the midline of the abdomen) where the abdominal wall layers fuse. This ring is where the umbilical cord passed through during fetal development.
- **Mamelon:** The central, raised area or "bump" within the umbilicus.
- **Cicatrix:** The scar where umbilicus cord attach during fetal development.
- **Cushion:** The slightly raised, surrounding skin margin of the umbilicus.
- **Furrows:** The grooves or depressions that may surround the mamelon within the cushion.
- **Obliterated Umbilical Vessels:** In the adult, the umbilicus contains the remnants of the fetal umbilical vessels (one vein and two arteries), which are now fibrous cords.
- **Median Umbilical Ligament:** The remnant of the urachus, a fetal structure that connected the bladder to the umbilicus.

Clinical Significance:

- **Landmark:** The umbilicus is a vital landmark for locating other abdominal structures and for surgical procedures.
- **Weakness:** The umbilical ring is a potential weak point in the abdominal wall, making it susceptible to hernias.
- **Developmental Disorders:** Abnormalities in the umbilicus can indicate underlying developmental issues.
- **Infections:** The umbilicus can be a site for various infections, particularly in case of neonates. [4].

The umbilicus is a scar with a complex anatomy, formed from the fusion of abdominal wall, it is marked as middle of the abdominal region which divides various organs in a particular region for ease of location , it is also helpful during various surgeries.

The stratum corneum (SC) serves as the primary barrier for achieving TDD. Ding et al. found that the SC of the umbilical skin of New Zealand rabbits was thinner than that of non-umbilical skin. This disparity in SC thickness between umbilical and non-umbilical skin can influence the transdermal absorption of drugs. Additionally, keratin and intercellular lipids, as essential components of the SC barrier, might interact with drugs, thereby altering drug transmission through the skin [1]. All mammals possess placenta also possess umbilicus. In majority of cases, the umbilicus bears orifice regardless of different types of umbilicus such as transverse, round, and vertical. The umbilicus has been identified as the most suitable site for transdermal administration of numerous herbal drugs to treat various diseases by ancient Chinese and Ayurved. However, the supremacy of the umbilicus is that its skin tissues possess a thin stratum corneum and the presence of paraumbilical veins beneath the umbilical skin tissues enables rapid systemic distribution of drugs [3].

In Traditional Chinese Medicine (TCM), the term ‘navel plaster’ refers to the direct topical application of herbal preparations to the navel region for localized effect in patient [8]. Several studies have witnessed the successful treatment of many digestive diseases by placing numerous herbal extracts on the umbilicus. An ample amount of works has compared the drug absorption through skin tissues at the umbilicus and forearm. Upon their results, it seemed the umbilicus could serve as an ideal region for better systemic bioavailability than other skin regions [3]. Rhubarb navel plasters recently described as an effective therapy in relieving constipation . It was recently observed in this laboratory that the transdermal absorption of testosterone in six rhesus monkeys via the navel area produces a substantially greater systemic bioavailability than by forearm administration (79.9 uerms 49.9%). Bioavailability of testosterone by intravenous administration was used as the control (22). In the present investigation, we evaluated the feasibility of using the navel as the site for a long-term application of a bandage-type controlled-release drug delivery system developed to provide a continuous transdermal administration of testosterone at a controlled rate [6]. The study was conducted to evaluate the effects of nanoethosomal formulation of gamma-oryzanol (γ-Oryzanol-NEs) on testicular damages in a mouse model of ischemia/reperfusion damage [7]. Modern medical research has revealed that the transdermal in situ gel system administered through umbilical skin enhances the systemic bioavailability of drugs compared with other skin regions. Furthermore, the permeation of certain drugs, such as rhein, through the umbilical skin is more effective than through non-umbilical skin [1].

MATERIALS AND METHODS.

CHASTEBERRY EXTRACT

Vitex agnus-castus (also called vitex, chaste tree / chastetree, chasteberry, Abraham's balm, lilac chastetree, or monk's pepper) is a plant native of the Mediterranean region. The most common names are "chaste tree", "vitex", and "monk's pepper" [9]

Chemical Composition Of Chasteberry

Different classes of secondary metabolites that have been isolated from various parts of V. agnus-castus; fruits containing iridoids and iridoid glycosides (casticin, aucubin, and agnuside); flavonoids (apigenin, castican, orientin, penduletin, and isovitexin), flavonoid O- or C-glycosides (orientin and vitexin); and flavonoids, luteolin 6-C-(4″-methyl-6″-O-trans-caffeoylglucoside), luteolin 6-C-(6″-O-transcaffeoylglucoside), luteolin 6-C-(2″-O-trans-caffeoylglucoside), and luteolin 7-O-(6″-p-benzoylglucoside) were isolated from the root bark of the plant., diterpenoids (vitexilactone and rotundifuran), diterpene lactam (vitexlactam A); p-hydroxybenzoic acid β-sitosterol; and primary metabolites fatty acids (oleic acid, linolenic acid, palmitic acid, and stearic acid). Essential oils also presented in chasteberry (1,8-cineole, (E)-β-farnesene, (E)-caryophyllene, sabinene, limonene, cineol, and α-terpinyl acetate were the major essential oils [10,11]. The berry of the chaste tree contains a number of active constituents: flavonoids (i.e., casticin, kaempferol, orientin, quercetagetin, and isovitexin), iridoid glycosides (i.e., agnuside and aucubin), and essential oils (i.e., limonene, cineol, pinene, and sabinene).

Drug profile.

S.No	Category	Discription
1.	Scientific Name	Vitex agnus-castus L.,
2.	Common Names	Chasteberry, Monk's, Pepper, Agnus Castus
3.	Family	Lamiaceae (formerly Verbenaceae)
4.	Botanical Source	Dried ripe fruits of Vitex agnus-castus
5.	Geographical Source	Mediterranean, Central Asia, Europe, North Africa
6.	Major Constituents	Flavonoids (casticin, isovitexin), iridoid glycosides (aucubin, agnuside), essential oils, diterpenes, linoleic acid
7.	Pharmacological Activity	Hormonal regulation, prolactin suppression, antioxidant, anti-inflammatory
8.	Therapeutic Uses	PMS, PMDD, menstrual irregularities, hyperprolactinemia, mastalgia, menopausal symptoms
9.	Mechanism of Action	Dopamine D2 receptor agonist → inhibits prolactin → hormonal balance
10.	Dosage	Fluid extract: 1–2 mL/day (approx. 1000 mg/serving); Capsules: 20–40 mg/day
11.	Adverse Effects	Headache, nausea, GI upset, rash
12.	Contraindications	Pregnancy, lactation, hormone-sensitive cancers, dopamine antagonist use
13.	Pharmacokinetics	Moderate oral absorption, hepatic metabolism, eliminated via urine/feces
14.	Solubility	Sparingly soluble in water, soluble in alcohol/hydroalcoholic solvents
15.	Organoleptic Properties	Dark brown liquid, aromatic odor, bitter taste

EXCIPIENTS

1. Poloxomer 407

- **Synonyms**: pluronic F-127, kolliphor P407
- **Molecular Weight:** 9,840- 14,600g/mol
- **Functional Category:** thermogelling , biodegradable, ph stable
- **pH:** 5.0-7.5
- **Description:** Poloxamer 407 is a highly versatile excipient prized for its surfactant properties and thermoreversible gelling behavior.
- **Density:** 1.06g/cm^3 at 25°C
- **Melting Point:** 53-57°C
- **Solubility:** Highly soluble in water and soluble in alcohols and mineral oil.
- **Incompatiblities:** Poloxamer 407 reacts with povidone-iodine via a reduction-oxidation reaction, where reducing agents in the poloxamer degrade the iodine. This leads to rapid loss of povidone-iodine potency and instability.

2. Carbopol

- **Synonyms:** Acrypol; acrylic acid polymer.
- **Density:** 0.2 g/cm3 (powder); 0.4 g/cm3 (granular).
- **Dissociation constant:** pKa = 6.0+0.5

- **Melting Point:** Decomposition occurs within 30 minutes at 260 C
- **Solubility:** Swellable in water and glycerin and, after neutralization, in ethanol (95%).
- **Incompatiblities:** Carbomers are discolored by resorcinol and are incompatible with phenol, cationic polymers, strong acids, and high levels of electrolytes.

3. **Hydroxy Propyl Methyl Cellulose-** used as polymer
4. **Methyl Paraben(preservative).**
5. **Propyl paraben(preservative).**

List of Chemicals .

S.No.	Material	Supplier/ Manufacturer
1.	Vitex agnus castus	Purchase from NYCraze.
2.	Poloxomer 407	Purchase from India mart.
3.	Carbopol 934	Central Drug House, New Delhi.
4.	Hydroxypropyl methylcellulose	Central Drug House, New Delhi.
5.	Methyl Paraben	Central Drug House, New Delhi.
6.	Triethanolamine	Central Drug House, New Delhi.

RESULTS AND DISCUSSION-

Preformulation Studies Of Vitex Agnus-Castus Extract

1. **Organoleptic Properties:** The organoleptic properties of the drug sample were observed as shown in the table. It was observed that the organoleptic properties of the drug comply with the standards. This can be used as a preliminary identification tool of drugs.

Table: 7. Organoleptic Properties of Drug

S.No	Properties	Standard	Observation
1.	Appearance	Mobile liquid (fluid extract/tincture)	Liquid (fluid extract/tincture)
2.	Odor	Characteristic herbal aroma (aromatic)	Characteristic herbal aroma (aromatic)
3.	Color	Brown	Brown
4.	Taste	Slightly astringent-bitter (typical for herbal extracts)	Slightly astringent-bitter (typical for herbal extracts)

2. **Solubility Studies:** 10mg drug extract was added in various 2ml solvents and solubility was observed as shown the table.

- Soluble in aqueous–ethanolic vehicles (e.g., tincture 1:2 in 43–47% ethanol)
- Insoluble or limited in pure water; optimization studies use oil-in-water nano emulsions to enhance solubility and permeability.

Table: 8. Solubility Analysis

S.No.	Solvent	Solubility
1.	Water	In soluble
2.	Ethyl acetate	Slightly soluble
3.	Ethanol	Soluble

3. FTIR Analysis and Interpretation

FTIR study was performed to evaluate compatability between drug and polymer utilized in study. This section presents a comparative FTIR analysis of the pure drug (Vitex agnus-castus) and polymers (HPMC, Carbopol, and Poloxamer), as well as their physical mixture, to investigate potential drug–polymer interactions.

3.1. FTIR Spectrum of Pure Drug (Vitex agnus-castus):

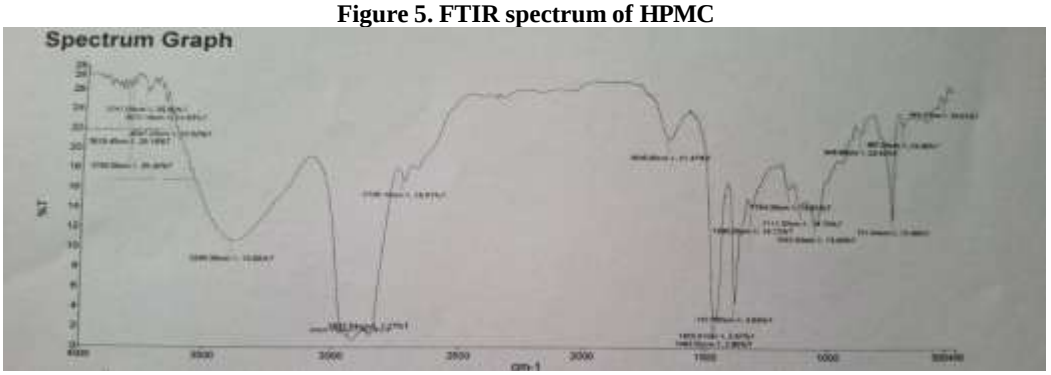
Figure 4. FTIR spectrum of vitex agnus castus

The FTIR spectrum of Vitex agnus-castus showed characteristic absorption bands at:
~3164 cm^{-1} – Broad band attributed to O-H or N-H stretching, indicative of hydroxyl or amine groups.
~2151–2760 cm^{-1} – Multiple C-H stretching vibrations, likely from aliphatic hydrocarbons.
~1459, 1305, 1039 cm^{-1} – Assigned to C=C stretching, C-H bending, and C-O stretching, confirming the presence of aromatic structures and ether linkages.
~852–566 cm^{-1} – Aromatic out-of-plane C-H bending.
These bands confirm the presence of phenolic, aliphatic, and aromatic functionalities typical of bioactive plant-derived constituents like flavonoids and terpenoids.

3.2. FTIR Spectrum of Polymers:

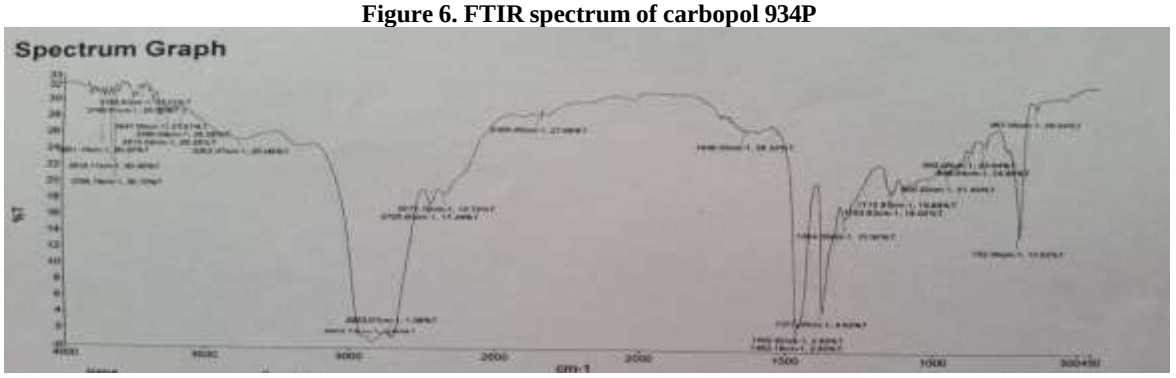
3.2.1. HPMC (Hydroxypropyl Methylcellulose):

Figure 5. FTIR spectrum of HPMC



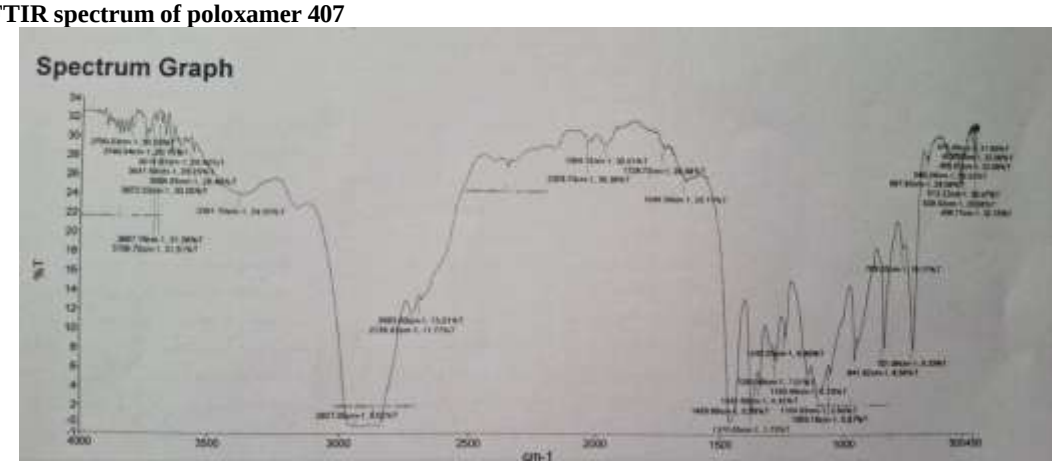
Major absorption bands observed:
~3400–3200 cm^{-1} (broad) – O-H stretching of cellulose backbone.
2928, 2726 cm^{-1} – C-H stretching vibrations.
1455, 1154, 1043 cm^{-1} – C-H bending and C-O-C stretching, indicating ether and alcoholic functional groups.

Figure 6. FTIR spectrum of carbopol 934P



Identified peaks include:
~2923 cm^{-1} – Aliphatic C-H stretching.
~1700–1750 cm^{-1} (expected but not prominent) – C=O stretching, typical of carboxylic acids.
1452–1305 cm^{-1} – C-H and C-O stretching.
~850–720 cm^{-1} – Fingerprint region.

Figure 7. FTIR spectrum of poloxamer 407



Observed peaks:
~3400–3200 cm^{-1} – O-H stretching.
2926, 2856 cm^{-1} – Aliphatic C-H stretching.
~1100–1300 cm^{-1} – C-O-C stretching of the polyethylene glycol moiety.
~850–720 cm^{-1} – Characteristic fingerprint region.

3.3. FTIR Spectrum of Drug–Polymer Physical Mixture:

The FTIR spectra of the physical mixtures (labelled as "Drug + Polymer") showed the presence of major characteristic peaks of both the drug and polymers, though with minor shifts and changes in intensity:
Slight shifting of O-H/N-H stretching peaks (~3164 cm^{-1})
Decreased intensity or broadening in the carbonyl and ether regions (~1305–1154 cm^{-1})
Appearance of new minor shoulders or peaks in some regions (~1500–1600 cm^{-1})
These observations suggest hydrogen bonding and possible intermolecular interactions between the drug and polymers, without the formation of new covalent bonds (as no new peaks were observed).

3.3.1. Interpretation of Drug–Polymer Interaction:

Figure 8. FTIR spectrum of Drug and Polymer Mixture

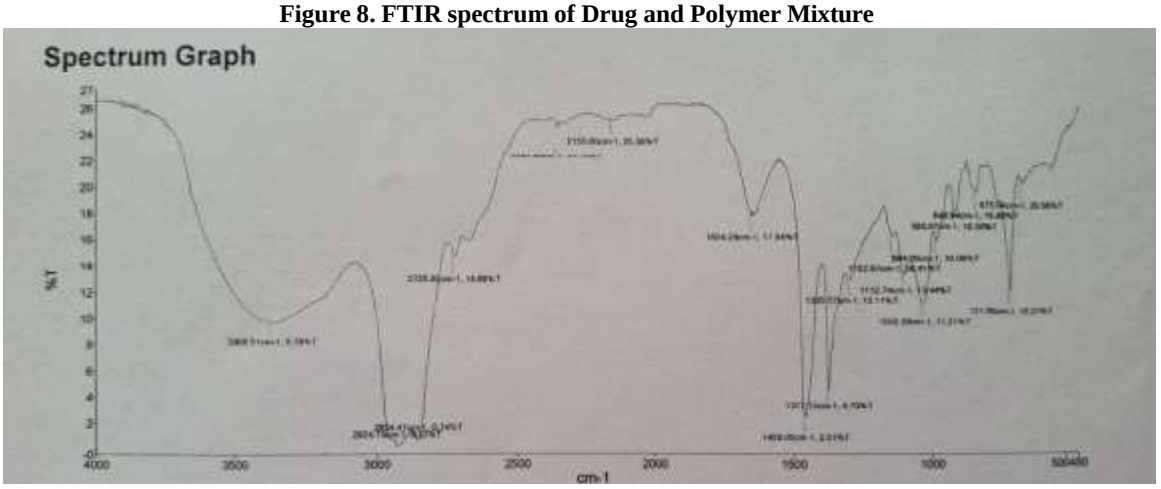


Table: 9. Interpretation of Drug–Polymer Interaction:

Region (cm^{-1})	Functional Group	Observation	Interpretation
3200–3600	O-H / N-H stretch	Broadening and shift	Hydrogen bonding between drug and polymers
1700–1750	C=O stretch (if present)	Intensity changes	Possible weak dipole interactions
1000–1300	C-O / C-N stretch	Peak shift	Possible H-bonding or physical interaction
<900	Aromatic bending	Retained	Drug remains structurally stable

These spectral shifts confirm that no chemical degradation or new chemical bonds were formed during physical mixing. However, the drug interacts physically with polymers, possibly through hydrogen bonding or Van der Waals forces. These interactions are crucial for drug release modulation in polymeric drug delivery systems.

Here FTIR spectroscopy confirms the structural integrity of *Vitex agnus-castus* in the presence of HPMC, Carbopol, and Poloxamer. Minor peak shifts and changes in intensity in the physical mixture indicate non-covalent interactions, particularly hydrogen bonding, between the drug and polymers. These interactions suggest potential compatibility and stability of the drug within the polymer matrix, supporting its suitability for controlled-release formulations. The acquired FT-IR of the medication demonstrates the identification of distinct functional groups that were compared with the reference spectra, and no significant difference was seen, confirming the purity of *Vitex Agnus-Castus* Extract

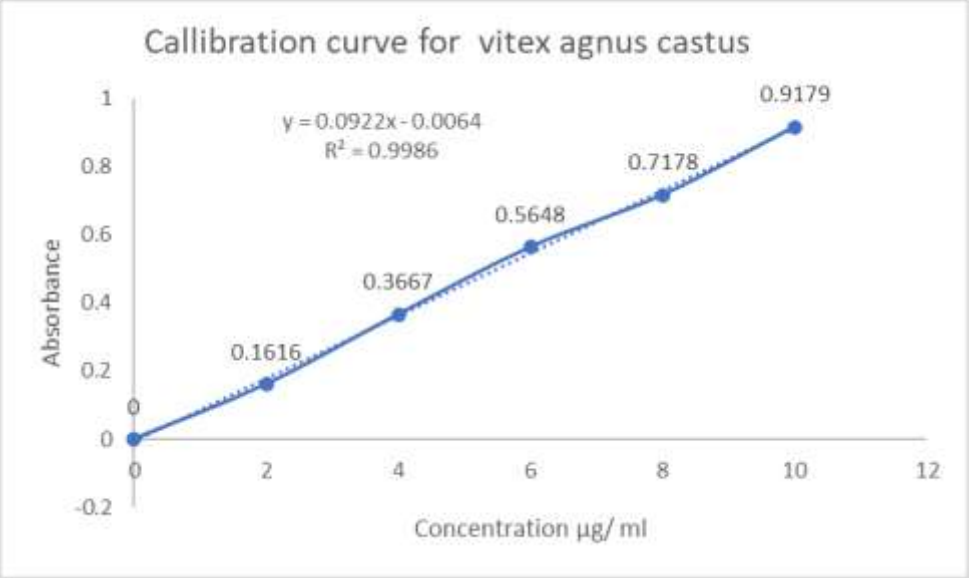
4. Standard curve of Vitex Agnus-Castus Extract at 343nm:

The standard curve of *Vitex Agnus-Castus* Extract was prepared in ethanol. Beer's Lambert Law was in the concentration of 10 µg/ml. The absorbance at different concentrations is shown in the table below.

Table: 10. Absorbance at different Concentrations

S.No	Concentration mg/ml)	Mean Absorbance
1.	2	0.1616
2.	4	0.3667
3.	6	0.5648
4.	8	0.7178
5.	10	0.9179

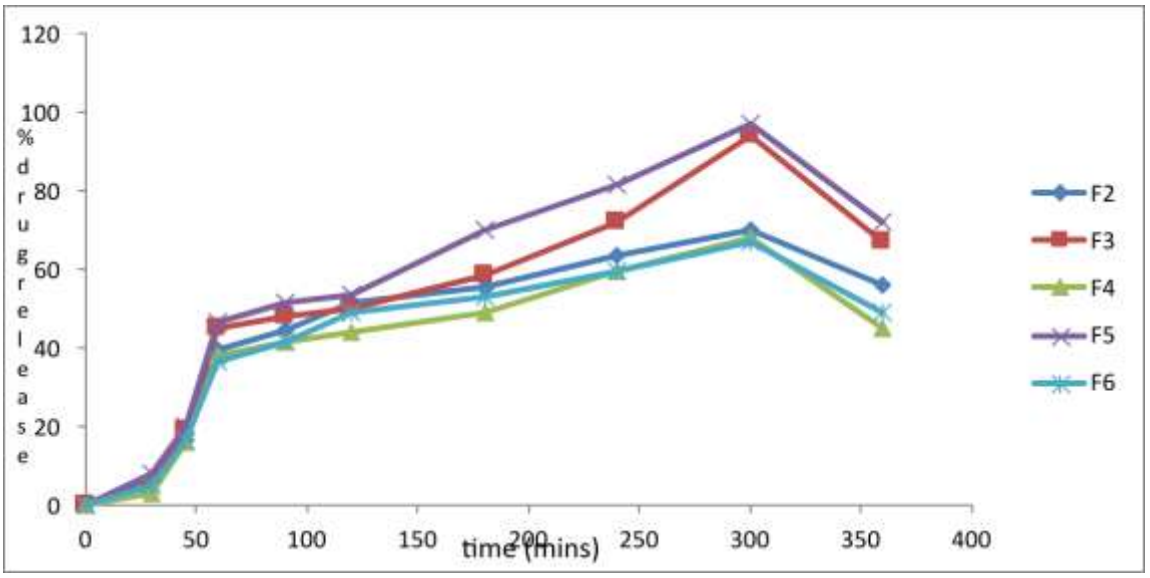
Figure: 10. Calibration curve for vitex agnus castus



%Drug release for different formulations.

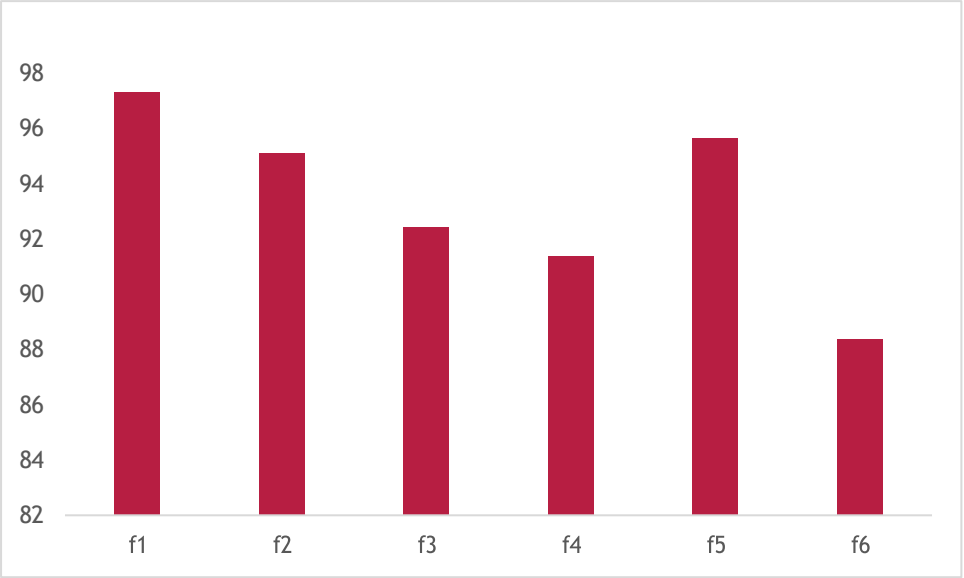
Time	F2	F3	F4	F5	F6
0	0	0	0	0	0
30	6.9	5.2	3.25	8.2	4.9
45	16.57	18.83	16.25	20.2	16.5
60	39.54	45.11	38.19	46.6	36.6
90	44.49	48.25	41.57	51.6	41.6
120	51.56	50.15	44.19	53.4	49.2
180	55.48	58.72	49.29	70.3	53.2
240	63.5	72.01	59.64	81.6	59.8
300	70.34	94.03	68.27	97.1	67.2
360	56.19	67.02	45.09	72.16	49.1

%Drug release graph for the formulation-



%Drug content-

S.no	%Drug content.
F1	**97.32%**
F2	**95.11%**
F3	**92.41%**
F4	**91.40%**
F5	**95.64%**
F6	**88.36%**



Various evaluation parameters-

s.no	Formulation	Evaluation parameters.						
		Visual observation	Viscosity at 50 rpm (bundle no 60)	Gelling capacity	Gelation temperature	Gelation time	pH	Spreadability.
1	F1	Brown	130. CP	+	No gelation	No gelation	6.42±0.01	15
2	F2	Brown	200. CP	++	37^0c ±0.3	38sec	6.48±0.01	13.3
3	F3	Brown	6110. CP	+++	37^0c ±0.2	39sec	6.45±0.01	10.90
4	F4	Brown	380. CP	++	38^0c ±0.2	35sec	6.41±0.01	13.3
5	F5	Brown	4090. CP	+++	35^0c ±0.2	32sec	6.43±0.01	12

6	F6	Light brown	5700. CP	+++	At room temp.	gelled	6.45±0.01	10

CONCLUSION

All 6 formulations (F1–F6) were thoroughly evaluated across different parameters such as—appearance, pH, viscosity, gelation capacity and temperature/time, spreadability, drug content. Based on these studies:

- F1 proved to be unfit for sol-gel transformation at even 40 °C, thus failing the criteria of in-situ gel.
- F2 and F4 exhibited moderate gelation (++), the time noted for formulation to show transition was found to be (35–38 s). However, their relatively low viscosities (<500 cP) uplift the concern about insufficient retention at the site of administration.
- F3 and F5 both achieved strong gelation capacity (+++), with viscosity in the mid-range (~4,000–7,400 cP), ideal gelation time noted was found to be (32–39 s), near-physiological gelation temperatures (35–37 °C), and drug contents ≥92%. Notably, F5 demonstrated the most robust drug-release profile (77% at 150 min), closely followed by F3 (74% at 150 min).
- F6 gelled prematurely at room temperature—indicating instability—and displayed very high viscosity (~9,780 cP).
  These observations align with established in-situ gel literature, where optimal formulations exhibit thermoresponsive gelation near physiological temperature, balanced viscosity, manageable spreadability, and sustained release.

F5 emerges as the most promising formulation with a strong, thermally triggered gel (35 ± 0.2 °C; +++) that gels within 32 seconds, offering good spreadability (12 g·cm/s), uniform drug content (~95.6%), and the highest in-vitro release (77.1% at 150 min). These characteristics indicate a stable, patient-friendly topical gel capable of delivering sustained drug release—meeting key performance metrics for in-situ gel systems.

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