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Research Article

Pre-formulation Study of Tamoxifen and Excipients in the Formulation of Nanoparticle Drug Delivery System

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Abstract

The inactive ingredients (excipients) are an integral part of the pharmaceutical drug delivery system. If not selected wisely and added without a proper scientific approach can lead to the instability of the API, low therapeutic outcome, or untoward effects. I correct study pre-formulation studies were carried out to confirm that the excipients used in the preparation of the nanoparticles were compatible with tamoxifen. The HPLC-UV assay, FTIR spectra, XRD study, and thermogravimetric analysis of the stored preparation showed that the nanoparticles were stable, indicating that the ingredients were not reactive and compatible with each other. The tamoxifen drug content was above 98%. Also, the FTIR spectrum of the optimized and physical mixture showed that the API retained its major (1357.89 cm^{-1}, 1589.34 cm^{-1}, 1739.79 cm^{-}, 12870.08 cm^{-1}, and 3402.43 cm^{-1}) characteristic peaks. The XRD confirmed that the drug is very well dispersed in amorphous form with no extraneous peaks. The TGA isotherm indicated that the melting point of the optimized formulation was 400°C, which is significantly higher than the pure tamoxifen melting point (150°C). The TGA results indicated that the formulation is heat stable.

Keywords: Tamoxifen, incompatibility, PLGA nanoparticles, stability, HPLC-UV analysis.

1. Introduction

Drug delivery are technologies, formulations, approaches and system for delivering a pharmaceutical entity, to achieve its desired therapeutic effect in the body safely based on nanoparticles (Mustafa et al. 2016). The materials which are in the size range of 1 and 100nm are termed nanomaterials which are applied in tissue engineering, biosensors, microfluidics, and other drug delivery systems (Orive et al. 2004). Nanomedicines are developed by using curative agents at the nanoscale. Nanostructures are exploited as delivery mediators by drug encapsulation or linking therapeutic drugs and hence with more controlled release and precise targeting to deliver them at a particular site (Lam et al. 2017). The substances that range from 10nm to 1000nm are called nanoparticles, these are solid and colloidal(Singh and Lillard Jr 2009). The two different types of nanoparticles include nanocapsules and nanospheres. The confinement of a drug to a cavity surrounded by a polymeric membrane gives rise to the formation of nanocapsules. In comparison, a matrix system comprised of physically and uniformly dispersed drugs is called a nanosphere (Singh and Lillard Jr 2009).

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are employed to study the compatibility examination between active pharmaceutical ingredients (APIs) and excipients (Chadha and Bhandari 2014).

The active pharmaceutical ingredient (API) is formulated into a suitable dosage form and delivery system along with suitable inactive ingredients (excipients) to make the API palatable, mask the bitter taste, impart stability, and control the drug delivery and release (Fathima et al. 2011). On the one hand, the ideal excipient is inert with no pharmacological and/or toxic or untoward effects. On the other hand, it must be compatible and may not interact with the API or other added substances (Ahuja and Scypinski 2001, Khan et al. 2022). If the added substance interacts or is incompatible with the formulation ingredients, it will physically or chemically destabilize the formulation resulting in a change in appearance, color, odor, and flavor, or even result in degradation of the API resulting in subtherapeutic response or even toxic degradative product (McDaid et al. 2003, Canbay and Doğantürk 2018). Silicone dioxide interacts with nitrazepam resulting in retarding the dissolution rate of the nitrazepam tablets (Fathima et al. 2011), resulting in decreased bioavailability. The magnesium stearate interacts with captopril forming hydrogen bonds and degrading the API (Li and Wu 2014). Similarly, the diclofenac sodium interacts with methyl methacrylate polymer (enteric film former)(Sabnis, Rege, and Block 1997). An extensive study has been recently carried out to analyze the compatibility of acetazolamide with commonly used excipients like mannitol, β cyclodextrin, methylcellulose, chitosan lactose, starch 1500, PVP k-30, etc. the result showed that acetazolamide was compatible only with the methylcellulose and Chitosan, while incompatible with all the other commonly used excipients (Rojek and Wesolowski 2022). Therefore, suitable and safe excipients must be added to the formulation to avoid instability and have the required therapeutic outcome (Ullah et al. 2020). Tamoxifen, a selective estrogen receptor modulator, belongs to the non-steroidal triphenylethylene derivatives (Bullock and Blackwell 2008), used widely for the treatment and prophylaxis of estrogen receptor-positive breast cancer (O'Regan and Jordan 2002).

2. Materials and Methods

2.1. Chemicals

Tamoxifen citrate (purity 99.9%), purchased from Huzhou Zhanwang Pharma Co., Ltd., China, PLGA (75:25, MW 66000-107000), Sigma Aldrich; Acetonitrile (purity >99.9%) was purchased from Fisher Scientific PVT. U.K. Ltd. (Loughborough, U.K), Methanol (purity >99.9% sigma), Ethanol (Scharlau, reagent grade 99.8%), Dimethyl sulphoxide, $DMSO_4$ (Honey well, Germany), Dichloromethane, DCM (Sigma Aldrich), potassium dihydrogen phosphate were purchased from Sigma Aldrich (St. Louis, MO, USA), Ortho-phosphoric acid), Disodium Hydrogen Phosphate, Di-potassium hydrogen phosphate, (Scharlau chemie, Spain), Pluronic E127 (Sigma Aldrich), distilled water [Millipore ultrapure water sys. (Milford, USA)].

2.2. Instruments

Analytical balance (BEL engineering, Italy), Centrifuge (DLAB, California U.S.), Multiple channel magnetic stirrers (DLAB, MS-H-S10, California U.S.), Vortex mixer (Fisher Scientific®, USA), Probe Sonicator (Bandelin, Sonopuls, Germany), PH-2601 BenchTop P.H. Meter (METER8, China), Freez Dryer (Human Lab), Bath sonicator (NEY, Ultrsonik). The Series '200 HPLC system' (Perkin-Elmer, Norwalk-CT, USA) included a pump, degasser vacuum (online), autosampler, column oven, and UV-Vis-detector. Perkin Elmer Total Chrom® *Workstation (*version 6.3.1) connected to the HPLC system through network chromatography interface (NCI 900) was used to acquire and evaluate data. A250 × 4.6mm id ACE Generix 5 C_{18} column (Weber Consulting, Hungary) with a Perkin-Elmer (30 × 4.6 mm), 10 µm, C_{18}pre-guard column cartridge was used to accomplish analyte separation.

UV-Vis spectrophotometer (Perkin (Elmer Series 200, Lambda 25), FTIR spectrophotometer

(Shimadzu Japan), X-Ray diffractometer (JDX-3532, JEOL, Japan).

2.3. Compatibility Studies (Drug, Excipients)

Drug excipients compatibilities studies are a prerequisite for nano-formulations to ensure that the excipients used in the preparation have no interaction with the API. To perform these studies, binary mixtures (1:1) and nanoparticles were prepared of drug with polymer (PLGA) and surfactant (Pluronic). The physical mixture and the nanoparticles were evaluated for Drug content, Physical consistency, FTIR studies, XRD, TGA.

2.4. Drug Content Determination

The optimized nanoparticle formulation was assessed for the drug content by the HPLC-UV method (Khattak et al. 2022) in triplicate after storing for three months. Briefly, 50 µl sample was injected via autosampler into the HPLC system coupled with U.V. detector at a mobile flow rate of 0.8 ml/min at 236nm. The mobile phase comprises ACN: Buffer (60:40v/v). The pH of the phosphate buffer was 6.0 with a column oven temperature of 35°C.

2.5. Physical Consistency

After storing at stress conditions, the samples were visually observed for any change in color and physical consistency.

2.6. FTIR Studies for Incompatibilities

FTIR studies for incompatibilities were performed for the drug and its physical mixtures with excipients and optimized nano-formulations to investigate any possible interactions. The % transmittance (%T) was recorded in the spectral range of 4000-500 cm^{-1}.

2.7. X-RAY Diffraction (XRD)

The XRD pattern of the pure drug, polymer, stabilizer and their physical mixture in 1:1 and the optimized formulation was studied at an angular range (2θ) of 10°-40° using JDX-3532, JEOL, Japan, X-ray diffractometer.

Thermo-Gravimetric (TGA)Analysis

TGA analysis was performed to assess the phase transition, thermal decomposition, and solid-gas reactions (oxidation, reduction) at controlled temperatures in a controlled environment. The prepared sample (≈ 200mg) was placed in a pan that was buoyed by precision balance. The pan lies in a furnace and is heated/cooled during the experiment. The experiment mass was monitored throughout the experiment. A sample purge gas (inert, reactive) controlled the sample environment. The sample was heated at a rate of 5 °C min^{-1} up to 600 °C. Alumina was used as the reference sample.

3. Results and Discussions

3.1. Drug Content Determination

Due to heat and moisture between the drug and excipients, drug degradation may occur by physical and chemical interaction, resulting in decreased drug content. Samples containing the drugs were run in triplicate to determine the drug content by the HPLC=UV method. The results are presented as mean ± S.D. in table 1. The results obtained showed non-significant differences amid the initial and final concentration of the drug content in the physical mixture under stress conditions confirming no interaction between the drug and excipients.

Table 1: Drug Content Determination.

	% Drug Content ± SD
Analysis time	**Sample1(drug, PLGA, excipients)**
Day 1	99.28±0.13
1 week	98.54±0.60
2 weeks	98.22±0.11
1month	98.32±0.32

3.2. Physical Consistency Assessment

After storing under stress conditions, the samples were observed for any kind of change in color and physical consistency. Upon visual examination, no change in color and physical consistency were observed.

3.3. FTIR Studies for Incompatibilities

FTIR studies for incompatibilities were performed for the drug and its physical mixtures with excipients and optimized nano-formulations to investigate any possible interactions. The % transmittance (%T) was recorded in the spectral range of 4000-500 cm^{-1}.

The FTIR spectra are shown in Fig 1. Tamoxifen citrate showed characteristics bands at 1357.89 cm^{-1} owing to N=O stretching, C=C stretching at 1589.34 cm^{-1}, at 1739.79 cm^{-1} corresponds to ketone group, C-H stretching at 2870.08 cm^{-1}, and O.H. group at 3402.43 cm^{-1}. PLGA displayed characteristics peaks at 960.55 cm^{-c}orresponding to aromatic compound, C-O bending at 1045 cm^{-1}, ester carbonyl group at 1242cm^{-1}, C=C stretching at 2276 cm^{-1}, at 3410.15 cm^{-1} owing to C-H stretching and O.H. group at 4436.28 cm^{-1}. Pluronic showed peaks at 840 cm^{-1} that corresponds to aromatic compound, C-O stretching at 1060.85 cm^{-1}, C-H bending at 1465.9 cm^{-1}, C=O stretching at 1608.63 cm^{-1}, C-N stretching at 2164.13 cm^{-1}, C-H stretching at 2966.52 cm^{-1}, and O.H. group at 4008.08 cm^{-1}. The physical mixture showed all characteristic peaks of the drug and excipients that confirm no incompatibility.

3.4. X-Ray Diffraction

The X-ray diffraction was performed for the visualization of a crystal internal atomic arrangement to confirm the crystalline nature of the analyzing substance. The X-ray diffractogram of the drug (tamoxifen citrate), formulation excipients, and drug-loaded polymeric nanoparticles (PLGA) were studied over an angular range (2θ) of 3°to 70°; (JDX-3532, JEOL, Japan, X-ray diffractometer).

Tamoxifen citrate diffractogram showed intense peaks between 5° to 30° at 2θ, verifying the crystalline nature of the drug. No intense or crystalline peak was observed for PLGA, thus verifying the amorphous nature of the polymer. Pluronic F-127 shows a characteristic peak at 20° and 23° at 2θ, showing its crystalline nature.

The XRD pattern of tamoxifen citrate loaded PLGA nano-particles showed no intense peaks corresponding to the drug, confirming that the drug is encapsulated as an amorphous form in the nanoparticles associated to the dilution effect of polymer (Mudgil and Pawar 2013, Pignatello et al. 2002, Gupta et al. 2010), the amorphous dispersion of drug in the polymer will results consistent and predictable drug release. The two peaks in the diffractogram correspond to the stabilizer (pluronic F-127). The XRD spectra of pure drug, PLGA, Pluronic F127, physical mixture, and optimized formulation are shown in Figure 2.

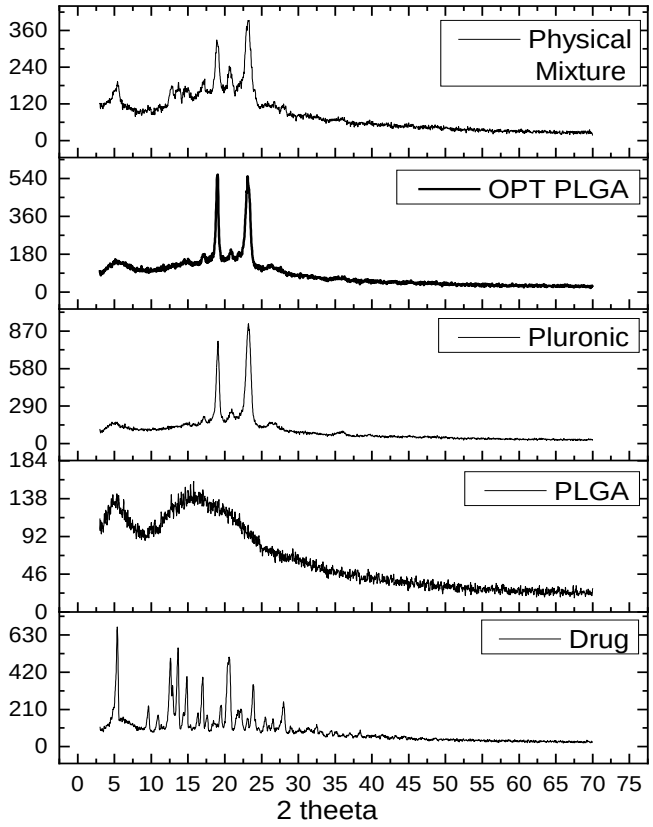

Figure 2: XRD Spectra of Pure Drug, PLGA, Pluronic, Physical mixture, and Optimized drug-loaded PLGA Nanoparticles

3.5. Thermo-gravimetric Analysis (TGA)

TGA measures the mass of the sample as it is heated, cooled, or held at constant temperatures in a defined atmosphere. It is used to characterize materials by measuring their change in mass as a function of temperature. TGA is frequently used

along with DSC because the techniques provide complemental information, facilitating the interpretation of the results.

These methods have been used to rule out drug and polymer interactions. The physical interaction and the thermal characteristics between the tamoxifen and PLGA constituting nano-formulation were investigated by TGA.

The TGA curve of pure tamoxifen citrate showed that the drug starts to degrade at 150°C, having a degradation peak at 280 °C with a sharp shoulder at 190 °C. The PLGA TGA curve showed degradation at 250 °C followed by thermal stability up to 300 °C. The thermal decomposition of PLGA appears with a peak centered at 360 °C with a shoulder at 320 °C. The surfactant (Pluronic) starts to degrade at 250 °C with a large central thermal decomposition peak at 330 °C. The physical mixture of drug, PLGA, and surfactant starts to degrade at 190 °C with a shoulder at 200 °C. The mixture showed a large thermal decomposition peak centered up to 400 °C. The thermal decomposition of optimized PLGA nano-formulation starts at 275 °C and has a major peak of thermal decomposition at 330 °C, ending at 400 °C. The TGA results confirmed that the thermal stability of tamoxifen encapsulated in the polymeric nanoparticles was enhanced, which is attributed to the capability and behavior of polymers in enhancing the thermal stability of drugs. The TGA spectra are shown in Figure 3.

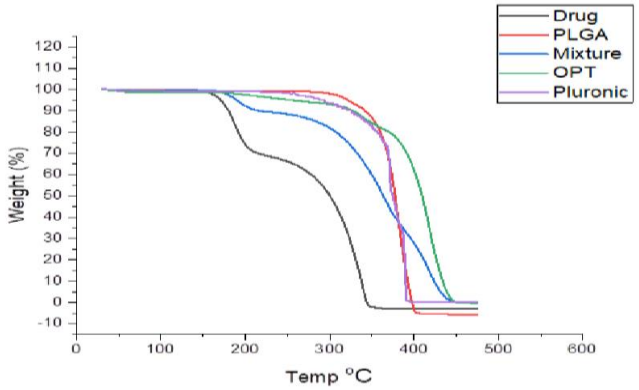

Figure 3: TGA Spectra; Drug, PLGA, Pluronic F127, Physical mixture, and Optimized PLGA nanoparticles formulation.

4. Conclusion

The current showed that there was no interaction or incompatibility among the API (Tamoxifen) and the excipients (PLGA, Pluronic) as the drug content was above 98%. Furthermore, the FTIR spectrum of the developed nanoparticles showed no interaction between the drug and added ingredients. The XRD further confirmed the results, and the TGA studies depicted that the PLGA used as a polymer imparts stability to the drug.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Study Approval

Yes. The study was approved by the Advanced studies and Research board, University of Peshawar.

Consent Forms

NA.

Authors Contribution

MAK, and ZI; conceptualized the study, MAK; wrote the final manuscript, SG, SP; helped in the formal analysis, TH, and AT; did the experimental analysis, and ZI; supervised the whole project.

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