Characterization and Evaluation of Warfarin (Anticoagulant Drug) in Immediate Release Tablet Dosage Form

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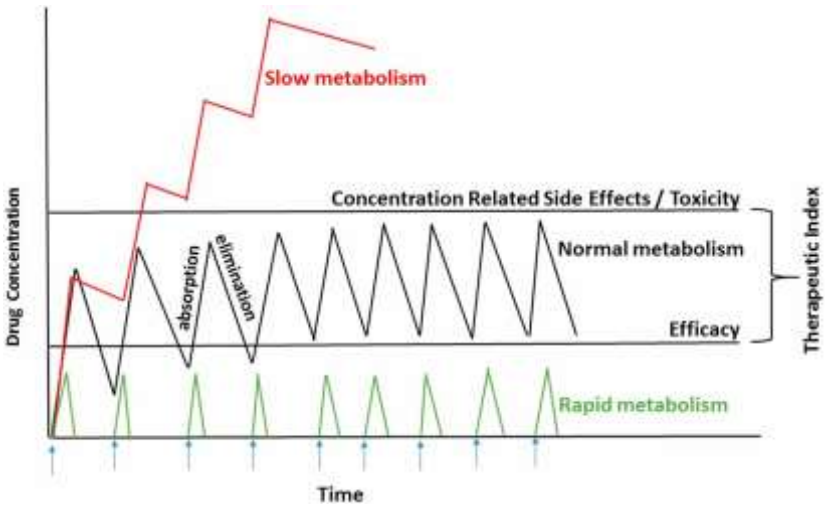
Abstract:- Anticoagulants and antiplatelet drugs eliminate or reduce the risk of blood clots. They are often called blood thinners, but these medications don't really thin your blood. Instead, they help prevent or break up dangerous blood clots that form in your blood vessels or heart. Most conventional (immediate release) oral drug products, such as tablets and capsules, are formulated to release the active drug immediately after oral administration. In the formulation of conventional drug products, no deliberate effort is made to modify the drug release rate. In this paper we characterize and evaluate the anticoagulant drug in immediate release tablet dosage form.

Keywords: Anticoagulant, Immediate Release, Density, Drug Delivery System,Warfarin

I. INTRODUCTION

Medications are only one part of a successful treatment plan. They are appropriate when they provide benefit, improve function and have either no or mild, manageable side effects. The use of medications or drugs for any purpose requires patient consent. Pharmacotherapy can be defined as the treatment and prevention of illness and disease by means of drugs of chemical or biological origin. Drug Delivery System (DDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time and place of release of drugs in the body. This process includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action. The drug concentration at the appropriate site should be above the minimal effective concentration (MEC) and below the minimal toxic concentration (MTC). This concentration interval is known as the therapeutic range.



Dosage forms can control the rate of release of a drug and/or the location of release, and they can be classified into immediate-release and modified-release dosage forms.

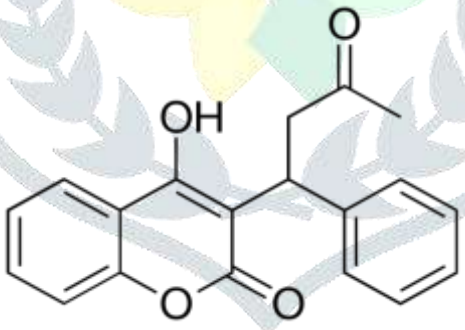
- Immediate release – Drug release immediately after administration
- Modified release – Drug release only occurs some time after the administration or for a prolonged period of time or to a specific target in the body .

Immediate Release Formulations:

Many dosage forms are designed to release the drug immediately or at least as quickly as possible after administration. This is useful if a fast onset of action is required for therapeutic reasons. Immediate release allows the drug to dissolve in the GI contents, with no intention of delaying or prolonging the dissolution or absorption of the drug .

The term immediate release pharmaceutical formulation includes any formulation in which the rate of release of drug from the formulation and/or the absorption of drug, is neither appreciably, nor intentionally, retarded by galenic manipulations. Immediate release delivery systems give a fast onset of action and for a therapeutic action the drug should be in solution, therefore disintegration of the dosage form and dissolution of the drug may have to occur first depending on the dosage form. Immediate release systems usually release the drug in a single action following a first order kinetics profile.

Tablets are defined as 'solid preparations each containing a single dose of one or more active ingredients and obtained by compressing uniform volumes of particles.
They are intended for oral administration. Some are swallowed whole, some after being chewed, some are dissolved or dispersed in water before being administered and some are retained in the mouth, where the active ingredient is 'liberated'. Thus, a variety of tablets exists and the type of excipients and also the way in which they are incorporated in the tablet vary between the different types .



Structure of Warfarin

II. CHARACTERIZATION

Characterization of Warfarin

Characterization of Warfarin was performed. Warfarin characterized for different micromeritics properties such as bulk density, tapped density, compressibility index, Hausner's ratio and particle size. Warfarin was characterized for LOD, API calculations (Moisture and assay compensations) done, pH solubility profile checked at different pH media. Analytical wavelength was determined for the Drug. Warfarin characteristics were identified by FT-IR.

Selection of Excipients and Drug Excipients Compatibility Studies

Drug excipient compatibility study of the "candidate drug" with different excipients was performed to assess their interactions with the drug to select suitable excipients which will ensure the development of a stable as well as the therapeutically effective and safe dosage form. Selection of excipients was carried out based on the compatibility study.

Evaluation of Physical and Process Parameters

Evaluation of process parameters such as weight variation, thickness, hardness, assay, impurities and residual solvent level. Drug release data, drug release characteristics of developed formulations for *in-vitro*drug release using dissolution apparatus, in order to identify and optimize the critical factors and parameters influencing the process.

- **Pharmacokinetics**

Warfarin drug displays prolonged absorption. Thus, despite a short clearance half-life of about 6 hours, the apparent half-life during repeat dosing is about 12 hours, which allows twice-daily dosing to provide effective anticoagulation, but it also means that when the drug is stopped for surgery, anticoagulation persists for at least a day.

- **Absorption**

The absolute bioavailability of warfarin drug is approximately 50 % for doses up to 10 mg of anticoagulant drug. Food does not affect the bioavailability of anticoagulant drug. Maximum concentrations (Cmax) of anticoagulant drug appear 3 to 4 hours after oral administration of anticoagulant drug. Anticoagulant drug is absorbed throughout the GI tract with the distal small bowel and ascending colon contributing about 55 % of anticoagulant drug absorption. Anticoagulant drug demonstrates linear pharmacokinetics with dose-proportional increases in exposure for oral doses up to 10 mg. At doses ≥25 mg, anticoagulant drug displays dissolution-limited absorption with decreased bioavailability.

- **Distribution**

Plasma protein binding in humans is approximately 87 %. The volume of distribution (Vss) is approximately 21 liters.

- **Metabolism**

Approximately 25% of an orally administered anticoagulant drug dose is recovered in urine and feces as metabolites. Anticoagulant drug is metabolized mainly via CYP3A4 with minor contributions from CYP1A2, 2C8, 2C9, 2C19, and 2J2. O-demethylation and hydroxylation at the 3-oxopiperidinyl moiety are the major sites of biotransformation. Unchanged anticoagulant drug is the major drug-related component in human plasma; there are no active circulating metabolites.

- **Elimination**

Anticoagulant drug is eliminated in both urine and feces. Renal excretion accounts for about 27 % of total clearance. Biliary and direct intestinal excretion contributes to elimination of anticoagulant drug in the feces. Following intravenous administration, anticoagulant drug is eliminated with a dominant half-life of ~ 6 hours. Following oral administration, the apparent half-life is ~12 hours because of prolonged absorption. Anticoagulant drug is a substrate of transport proteins: P-gp and breast cancer resistance protein.

- **Mechanism of Action**

Anticoagulant drug is an oral, reversible, and selective active site inhibitor of FXa. It does not require antithrombin III for antithrombotic activity. Anticoagulant drug inhibits free and clot-bound FXa, and prothrombinase activity. Anticoagulant drug has no direct effect on platelet aggregation, but indirectly inhibits platelet aggregation induced by thrombin. By inhibiting FXa, anticoagulant drug decreases thrombin generation and thrombus development.

III. EXPERIMENTAL WORK

Characterization of Anticoagulant drug:

Drug substance characterization is the first step to formulation development process.
The drug substance was characterized for following parameters.

1. Bulk Density:-

Bulk density or apparent density is defined as the ratio of mass of a powder to the bulk volume. The bulk density of a powder depends primarily on particle shape, particle size distribution and the tendency of the particles to adhere to one another. It is determined by measuring the volume of a known mass of powder sample by putting into a graduated cylinder.

Method: Accurately weighed 25 g of test sample (Candidate drug) was taken and sifted through #40 and transferred it into 100 ml graduated measuring cylinder. The volume of the mass without compacting was observed. Then unsettled apparent volume level was noted, V_o
The bulk density was calculated by following formula

$$\textbf{Bulk Density} = (M) / (V_o)$$

Where, M = Weight of the test sample
V_o = Unsettled apparent volume

2. Tapped Density:-

The tapped density is a limited density attained after "tapping down" usually in a device that lifts and drops a volumetric measuring cylinder containing the powder from a fixed distance. Tapped density was determined by using Electrolab USP Apparatus.
Method: Accurately weighed 25 gm of candidate drug was screened through #40 ASTM and transferred into a 100 mL graduated measuring cylinder without compacting. Cylinder containing the sample was tapped mechanically by raising the cylinder and allowing it to drop under its own weight using a suitable mechanical tapped density tester that provides a fixed drop of 14 ± 2 mm at a nominal rate of 300 drops per minute. Cylinder was tapped 500 times initially and tapped volume, V_a was measured to the nearest graduated unit. Tapping was repeated an additional 750 times and tapped volume, V_b was measured to the nearest graduated unit. When the two volumes is less than 2%, V_b was declared as final tapped volume, V_f 1250 taps was incremented till the difference between succeeding measurements was observed more than 2 % .
It was calculated by the following formula

$$\textbf{Tapped Density} = (M) / (V_f)$$

Where, M= Weight of the test sample
V_f = Final tapped volume

3. Compressibility Index:-

The compressibility index is the measure of property of powder to be compressed. The packing ability of drug was evaluated from change in volume, which is due to rearrangement of packing occurring during tapping.
It is indicated as Carr's index / compressibility index and can be calculated as follows:

Carr's Index (%) = (Tapped density– Bulk density) X 100 / Tapped density

Table: Scale of flowability by Carr's Index and Hausner's Ratio

Sr.No.	Carr's Index (%)	Hausner's Ratio	Flow Characteristic
1.	< 10	1.00–1.11	Excellent
2.	11–15	1.12–1.18	Good
3.	16–20	1.19–1.25	Fair
4.	21–25	1.26–1.34	Passable
5.	26–31	1.35–1.45	Poor
6.	32–37	1.46–1.59	Very poor
7.	>38	>1.60	Very Very poor

4. Hausner's Ratio:-

This is an indirect index of ease of powder flow. Hausner's Ratio is an indication of flowability of a powder. It is the ratio of tapped density to the apparent density

Hausner's ratio was calculated as**:**

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density}}{\text{Bulk density}}$$

5. UV Spectral Analysis:-

Preparation of Standard Solution (Stock I)

An accurately weighed amount of Warafarin Drug (15 mg) was dissolved in Sodium phosphate buffer pH 6.8 in 150 ml volumetric flask and then volume was made up to the mark which gives 100 µg/mL stock solution. Sodium phosphate buffer pH 6.8 using as blank.

Preparation of different Concentration Solutions

The aliquots 0.5, 1, 1.5, 2, 2.5,3,3.5,4 ml of stock solution pipette out into 10 mL volumetric flask. These dilutions produced 5, 10, 15, 20, 25 µg/ml concentrations of Warafarin.

Determination of Analytical Wavelength (λ max)

From the standard stock solution, 1 ml was taken out in 10 ml volumetric flask and volume was made up to 10 ml with Sodium phosphate buffer pH 6.8. The resulting solution containing 10µg/ml was scanned over complete UV range (i.e. 200–400 nm) using Shimadzu UV–Visible spectrophotometer for determination of λ maxof the drug

6. Loss on Drying (LOD):-

LOD test is designed to measure the amount of water and volatile matters in the sample, when sample is dried under specified conditions. The quantity of moisture present in the Warafarin was determined by using moisture analyzer and can be calculated as follows.

Moisture content of the drug was determined with the help of moisture analyzer at 105˚C on auto mode. About 1 gram of drug was taken and placed evenly as a layer on the balance. Then, the reading was taken as LOD (% w/w moisture content)

$$\text{LOD (\%)} = \frac{\text{(Initial Weight – Final Weight)}}{\text{Initial Weight}} \times 100$$

7. API Calculation (Moisture and Assay Compensation):-

The quantity of Warafarin to be dispensed is based on assay on dried basis and moisture content by using following formula:

$$\text{Potency} = \frac{\text{Theorotical Qty. of Drug in batch x 100 x 100}}{\text{Assay X (100– Loss on Drying)}}$$

8. Determination of Aqueous pH Solubility:-

The solubility of drug was determined as per BCS classification system. The solubility was checked in 250 ml of different media and water. The highest amount of dose was accurately weighed and transferred in individual volumetric flask containing different buffer solutions and sonicated for 30 minutes. The extracted drug samples were suitably diluted and analyzed with UV- Visible spectrophotometer

Table: Solubility Chart

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble,Insoluble	10,000 and over

IV. RESULT AND DISCUSSION

Characterization and Identification of Warfarin Drug

1. Physicochemical Characterization of Warfarin Drug:

Physical parameters such as bulk density, tapped density,Carr's index andHausner's ratio of Anticoagulant Drug were determined as per method described in previous section.

Table: Physical Properties of Anticoagulant Drug

Parameters	Observed Value
Bulk Density (g/mL)	0.224
Tapped Density (g/mL)	0.425
Carr's Index (%)	47.79
Hausner's Ratio	1.925

From above data it was interpreted that drug has poor flow properties which may affect content uniformity.

2. Solubility Profile:

The solubility of Anticoagulant Drug has been tested in different pH buffer solutions. Data indicates that solubility of drug increases with increase in pH.

Table: Solubility Profile of Anticoagulant Drug

Medium	Solubility (mg/250mL)
0.1N HCL	0.30
Phosphate buffer pH 7.4	10.00
Phosphate buffer pH 6.8	9.50
Acetate buffer pH 4.5	0.50

3. Ultraviolet (UV) Spectral Analysis:

UV spectrum of Warfarin Drug was recorded on UV-Visible spectrophotometer at 200-400 nm. The sample exhibits maxima at 222 nm which is similar to reported value. The UV spectrum of drug is shown below.

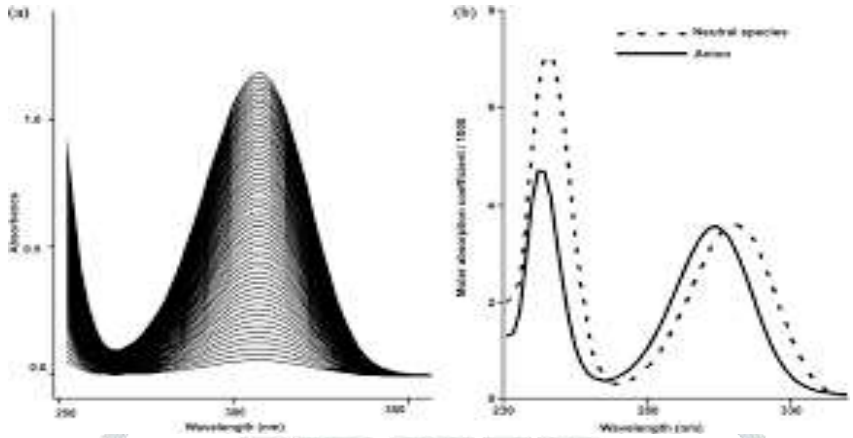


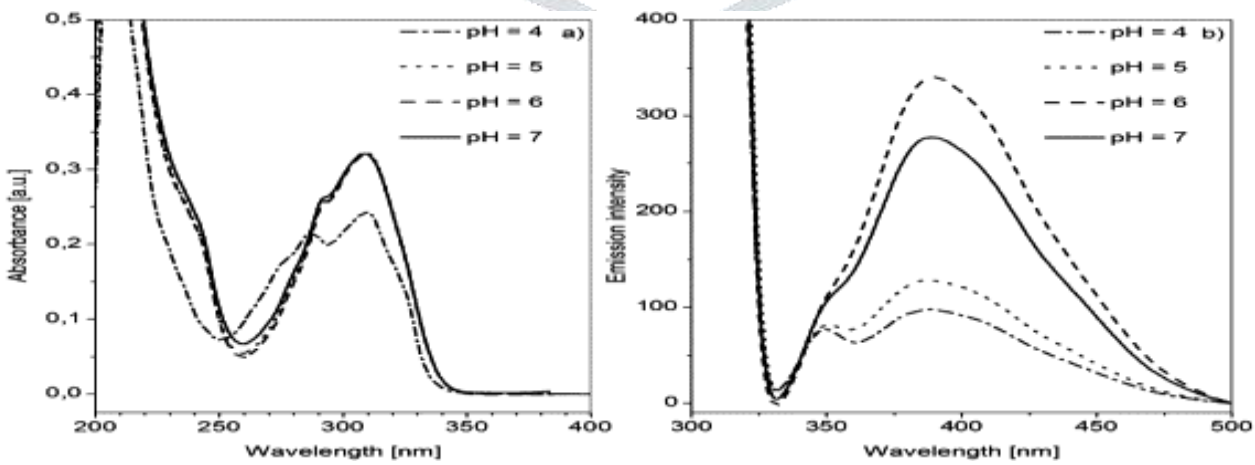
Figure: UV Spectrum of Warfarin Drug

4. Standard Calibration Curve of Warfarin in Sodium phosphate buffer pH 6.8:

The standard solution in the range of 5 to 25 µg/ mL was prepared and analyzed by using UV-Visible Spectrometer. The absorbance of these solutions was measured at 222.3 nm (λmax) Sodium phosphate buffer pH 6.8 using as blank. The linearity equation obtained is y = 0.043x - 0.027 with good correlation coefficient (R^2 =0.999)

Table: Standard Calibration curve of Warfarin Drug

Sr.No	**Concentration (µg/mL**	**Absorbance**
1.	5	0.178
2.	10	0.422
3.	15	0.615
4.	20	0.739
5.	25	1.062



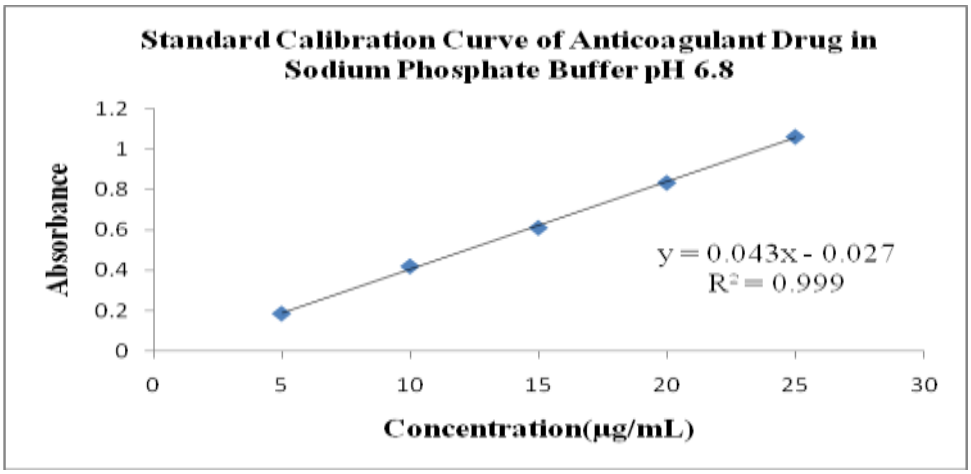


Figure: Standard Calibration Curve of Warfarin Drug

5. Determination of LOD:

LOD of the Anticoagulant Drug was determined by the infrared moisture balance. One gram of sample was placed and heated at 108 ^{o}C auto mode. LOD was directly noted from the value displayed by instrument. LOD of drug sample was found to be less than 0.5 % w/w.

6. Infrared Spectrum:

The infrared (IR) analysis of Anticoagulant Drug was carried out using potassium bromide disk method. The spectrum provided below in figure. The wave number and assignment of the most important bands are described in the following table

Table: Symmetric and Asymmetric Vibrations

IR Values (cm^{-1})	Functional Groups
3325.0	N-H str. of amine
3250.6	N-H str. of amide
2942.7	CH str. of CH2
2754.2	CH str. of CH2
1677.9	C=O of amide

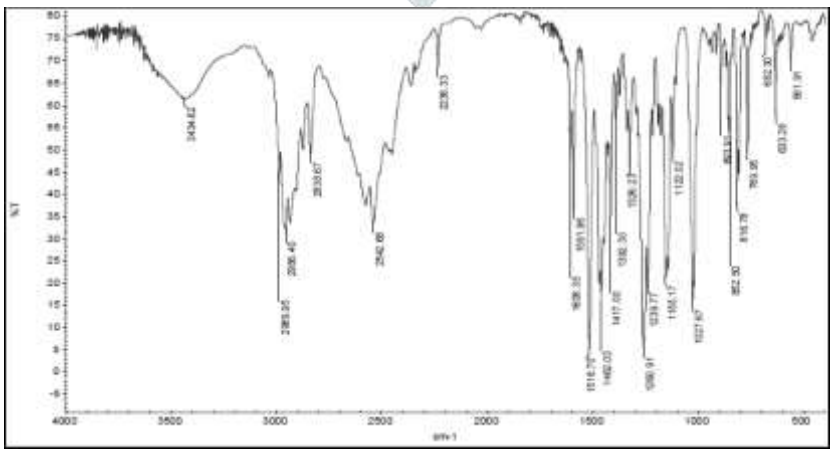


Figure: IR spectrum of Warfarin Drug

Interpretation:-Table gives the interpretation of peak obtained in the IR spectra along with their corresponding functional groups. IR results show the presence of above groups in the IR spectra of drug which confirmed that the drug is Warfarin

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