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CHEMICAL KINETICS AND STABILITY STUDIES OF AMLODIPINE BESYLATE

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ABSTRACT

The drug Amlodipine besylate was selected to study its chemical kinetics and degradation kinetics under variable conditions such as different temperatures, pH, light, buffers, solvent system and surfactants by applying the principles of chemical kinetics. The solubility of drug was checked in water and different solvents like Dimethyl formamide, methanol, ethanol, propylene glycol, PEG 400, PEG 600 and glycerol. The dug was found to be fully soluble in methanol, DMF, PEG 600, PEG 400, and Propylene glycol. The stock solution was then scanned between 200-400nm and the λmax was found to be 239 nm where the absorbance for 40 μg/ml was 1.1726 (Fig –1). The standard graph of the drug was plotted between concentration and absorbance whish gave a linear plot and the Lambert Beers law was obeyed in the concentration range of 0 to 30μg/ml.

Keywords *- Amlodipine besylate, chemical kinetics, drug concentration, solubility.*

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INTRODUCTION

Chemical Kinetics[1] is the discipline that is concerned with the mechanism by which a chemical process gets to its final state from its initial state and the rate in which this reaction proceeds. Therefore, chemical kinetics involves the study of rate of chemical change and the way in which this rate is influenced by the condition of the concentration of reactants, products, and other chemical species that may be present, and by factors such as solvent, pressure, and temperature, applied to pharmaceutics, such information permits a rational approach to the stabilization of drug products, and prediction of shelf life and optimum storage conditions. Kinetics principle has found applications in pharmacy with regard to drug stability, dissolution, pharmacokinetics, drug action. In the rational design and evaluation of dosage forms for drugs, the stability of the active components must be a major criterion in determining their suitability. Several forms of instability can lead to the rejection of a drug product. First, there may be chemical degradation of the active drug, leading to a substantial lowering of the quantity of the therapeutic agent in the dosage form. Many drugs (e.g., Digoxin and Theophylline) have narrow therapeutic indices, and they need to be carefully titrated in individual patients so that serum levels are neither to high that they are potentially toxic, nor to low that they are ineffective, for these drugs, it is of paramount interest that the dosage form reproducibly deliver the same amount of drug.[2] Second, although chemical degradation of the active drug may not be extensive, a toxic, product may be formed in the decomposition process. Dearbon described several examples in which the products of degradation are significantly more toxic than the original therapeutic agent. Thus the conversions of Tetracycline to Epianhydrotetracycline, Arsphenamine to Oxophenamine

and p-Aminosalisylic acid to m-Aminophenol in dosage forms give rise to potentially toxic agents that, when ingested, can cause undesirable effects.[3] Recently, Nord et. al. reported that the anti-malarial Chloroquine can produce toxic reactions that are attributable to the photochemical degradation of the substance.[4] Phototoxicity has also been reported to occur following administration of Chlordiazepoxide and Nitrazepam[5]. Another example of an adverse reaction caused by a degradation product was provided by Neftel et. al who showed that infusion of degraded penicillin G led to sensitization of lymphocytes and formation of antipenicilloyl antibodies.[3-6]Stability of a pharmaceutical product may be defined as the capability of a particular formulation, in a specific container, to remain within its physical, chemical, microbiological, therapeutic, and toxicological specifications assurances that the packaged product will be stable for its anticipated half life

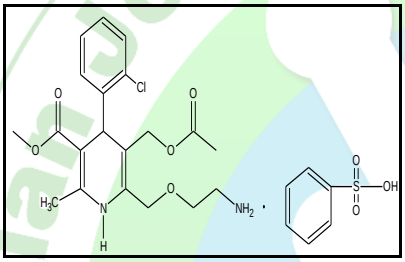

Figure 1- Structure of Amlodipine Besylate

Amlodipine Besylate is a calcium ion influx inhibitor (slow channel blocker or Ca^{++} ion antagonist) and inhibits he transmembrane influx of Ca^{++} ions into cardiac and smooth muscle.The mechanism of the antihypertensive action of Amlodipine is due to a direct relaxant action on vascular smooth muscle. Amlodipine is well absorbed orally with peak blood levels occurring 6-12 hrs. post dose. Oral administration of a single therapeutic dose gave a mean absolute bioavailability of 64% (range 52-88%). The volume of distribution is approximately 20 L/Kg. The absorption of Amlodipine is unaffected by food. The terminal plasma elimination half-life is about 35-50 hrs. and is consistent with once daily dosing. Steady state plasma levels are reached after 7-8 days of consecutive dosing. Amlodipine is extensively metabolized by the liver to inactive metabolite with 10% of the parent compound and 60% of metabolites excreted in the urine. ***In vitro*** studies have shown that approximately 97.5% of circulating Amlodipine is bound to plasma proteins.

Amlodipine Besylate is chemically 3-ethyl 5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl-1, 4 dihydro-6-methyl pyridine-3, 5-dicarboxylate monobenzene sulfonate. Presently the drug is available only in its solid form. Liquid forms may be beneficial for elderly patients or as parenterals may be useful for quicker or also for sustained release. In the light of this fact it was thought worthwhile to undertake kinetic study of the drug in liquid forms in order to obtain the overall stability profile of the drug under varied conditions such as temperature, light, pH, and different solvents.[10-12]

MATERIALS & METHODS [13-17]

CHEMICAL NAME	OBTAINED FROM
Amlodipine Besylate	Torrent Research Centre, Ahmedabad.
Acetic Acid	Suvidhinath Laboratories, Baroda.
Boric acid	E. Merck (India) Limited, Mumbai.
Disodium hydrogen phosphate	Central Drug House (P) Limited, Bombay.
Hydrochloric acid	Reachem Laboratory Chemical Pvt. Ltd., Mumbai.
PEG600	Chemical Solvent Center, Bombay.
PEG400	Chemical Solvent Center, Bombay.
Propylene Glycol	Chemical Solvent Center, Bombay.
Potassium chloride	Central Drug House (P) Limited, Bombay.
Potassium dihydrogen phosphate	Central Drug House (P) Limited, Bombay.
Sodium hydroxide	Central Drug House(P) Limited, Bombay.
Sodium lauryl sulphate	E. Merck (India) Limited, Mumbai.
Sodium acetate	E. Merck (India) Limited, Mumbai.
Tween 80	Genuine Chemical Co., Mumbai

INSTRUMENTS	NAME OF THE BRAND
Cyclo Mixer	CM 101, Remi Equipments
Refrigerator	Kelvinator, India.
Weighing balance	Adventurer OHAUS, NJ, USA.
U.V/VIS Spectrophotometer	Jasco, V-350, Japan Servo Co. Ltd., Japan.
pH meter	Metzer Instruments, Mathura.
Oven	KEMI, KUHS-2.

METHOD

Degradation kinetics of Amlodipine Besylate was studied by observing its degradation under factors like temperature, pH, buffers, solvent systems and light. Kinetic analysis was done by using parameters like rate constant and half-life of reaction. A plot was drawn between time (min) and log % undecomposed to find out the rate constant with the help of which half-life was calculated.[18-19]

Preparation of Calibration Curve [20-22]

Standard Stock Solution

25 mg. of Amlodipine Besylate was accurately weighed and transferred into a 250-ml. volumetric flask and dissolved in 1 ml Methanol and made up the volume to 250 ml. with water. This was the standard stock solution containing 100 µg/ml. of Amlodipine Besylate.

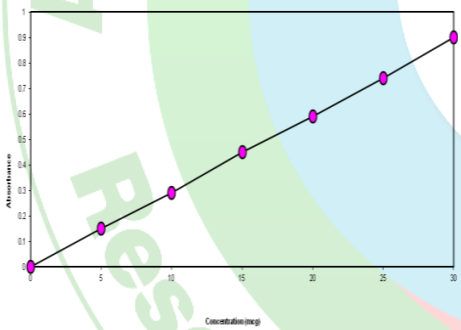

Figure 2 – Calibration curve of amlodipine besylate

Proof of spectrophotometric method as the Stability indicating method of estimation [23-25]

In order to prove U.V. method as stability indicating method of estimation, two sets of drug solution of equal conc. were prepared. One set of undegraded drug was prepared in water and absorbance was noted for a fixed conc. of drug from the set. In another set, drug was taken and its solution was prepared in 1 N HCl and was kept at high temperature for a fixed time to cause intentional degradation of the drug and absorbance was noted for a fixed conc. of the drug. To this solution a definite amount of the drug was added and absorbance was measured. The observed value was checked for any difference with that for pure drug in the absence of degradation product. The procedure followed is below: -

Spectrophotometric Scanning of Amlodipine Besylate

From the standard stock solution prepared in water, UV scan was taken between wavelengths 200-400 nm. against the water as blank and the wavelength of maximum absorbance was noted from the scan (Fig.-1).

Standard Plot

The stock solution of Amlodipine Besylate was prepared by taking 25 mg of Amlodipine Besylate in a 250 ml volumetric flask and dissolving in 1 ml of Methanol and making up volume with water. From this prepared stock solution, aliquots of 0.5 to 3.0 ml. were transferred into a series of 10 ml volumetric flasks and the final volume was made with water to give the concentration ranging between 5-30 µg/ml. The absorbance was measured at 239 nm against water as blank and the amount of Amlodipine Besylate present in the sample solution was computed from its calibration curve (Fig.- 2 & Table- 1).

Table 1 - Calibration curve data for Amlodipine besylate

S. No.	Conc.	Absorbance
0	00.00	00.000
1	0.500	0.1502
2	10.00	0.2922
4	15.00	0.4501
5	20.00	0.5964
6	25.00	0.7421
7	30.00	0.9012

Stock solution of undegraded drug- 25 mg of Amlodipine Besylate was accurately weighed and transferred to a 250 ml volumetric flask. To this 1 ml of Methanol was added and shaken well to dissolve the drug. To this water was added up to the volume to give a stock solution having conc. of 100 µg/ml. From this, 4 ml was withdrawn and transferred to a 10 ml volumetric flask, made up volume with water and shaken well to give a conc. of 40 µg/ml and then scanned between wavelength region of 200-400 nm against water as blank and the absorbance was noted at 239 nm (Fig. –1).

Stock solution of degraded drug in 1 N HCl- 25 mg of Amlodipine Besylate was accurately weighed and transferred to a 250 ml volumetric flask. To this 1 ml of Methanol was added and shaken well to dissolve the drug. To this 1 N HCl was added up to the volume to give a stock solution having conc. of 100µg/ml. This stock was

subjected to 90^0 C for 15 minutes in order to degrade the drug. From this 2 ml of sample was withdrawn and transferred to a 10 ml volumetric flask and made up to volume with water to give a conc. that should be equal to 20µg/ml. This was then subjected for scanning between range of 200-400 nm and the absorbance was noted at 239 nm (Fig. – 3)

Solution of undegraded drug along with degraded drug in 1 N HCl- 4 ml was withdrawn from stock solution of undegraded drug and transferred to a 10 ml volumetric flask. To the same flask 2 ml was added taken from stock solution of the degraded drug in 1 N HCl. The volume of this flask was made up with water to give a conc. of 60 µg/ml and shaken well. This was then kept for scanning between range of 200-400 nm and the absorbance was noted at 239 nm (Fig. – 4).

Effect of temperature: -

Method: [26-27]

25 mg of Amlodipine Besylate was accurately weighed and transferred to a 250 ml volumetric flask. To this 1 ml of Methanol was added and shaken well to dissolve the drug and the volume was made up with 1 N HCl maintained initially the temperature at which the stability studies required to be carried out. This gave a stock solution having conc. 100µg/ml.

Table 2 - Parameters for degradation at various temperatures:

S. No.	slope	K (min^{-1})	Half life (min)	log K	log K + 3
1	-0.07978	0.18374	3.77160	-0.73579	2.26420
2	-0.21249	0.48937	1.41609	-0.31036	2.68964
3	-0.59980	1.38133	0.50168	0.14029	3.14029
4	-0.99581	2.29334	0.30217	0.36047	3.36047
5	-1.20761	2.78112	0.24918	0.44422	3.44422

Effect of pH at room temperature: - [28-30]

To study the effect of hydrogen ion and hydroxyl ion conc. on the rate of hydrolysis of the drug, different solutions of HCl and NaOH with different normalities were prepared which corresponds to different pH as indicated in the table:

Table3: Preparation of drug solution of different pH

pH	HCl	pH	NaOH
0.18	1.0 N	13.10	0.2 N
0.24	0.8 N	13.15	0.4 N
0.30	0.6 N	13.25	0.6 N
0.40	0.4 N	13.30	0.8 N
0.48	0.2 N	13.35	1.0 N

Method:

25 mg of Amlodipine Besylate was accurately weighed and transferred to a 250 ml volumetric flask. To this 1 ml of Methanol was added and shaken well to dissolve the drug and the solution of desired pH initially maintained at 65^0 C and in which stability study required to be carried out (already prepared) was added to made up the volume to give a stock solution having conc. of 100 µg/ml. It was kept at room temperature throughout the study. From the stock, 2 ml samples were withdrawn at 0, 5, 10, 15, 20, 25, 30, 40, 50, and 60 minutes time intervals and transferred to a series of 10 ml volumetric flasks. The volume of flasks was made with water and the absorbance was measured at 239 nm against blank.

Table 4: Parameters for degradation of drug at Various pH at room temperature

S. No.	pH	Slope	K (min^{-1})	Half life (min)	log K	log K+3
1	0.18	-0.07978	0.18374	3.77160	-0.73579	2.26420
2	0.24	-0.05733	0.13202	5.24916	-0.87936	2.12064
3	0.28	-0.0488	0.11238	6.16658	-0.94931	2.05068
4	0.40	-0.04645	0.10696	6.47859	-0.97075	2.02925
5	0.48	-0.02035	0.04686	14.7878	-1.32917	1.67082
6	11.0	-0.04380	0.10087	6.86999	-0.99622	2.00377
7	12.0	-0.07767	0.17888	3.87402	-0.74743	2.25257
8	13.0	-0.09633	0.22184	3.12375	-0.65394	2.34605
9	13.10	-0.19934	0.45909	1.50950	-0.33810	2.66189
10	13.15	-0.3304	0.76090	0.91076	-0.11856	2.88132
11	13.25	-0.47044	1.08342	0.63963	0.03480	3.03480
12	13.30	-0.05041	1.16107	0.59686	0.06486	3.06486
13	13.35	-0.70909	1.63302	0.42436	0.21299	3.21299

Table 5: Parameters for degradation of drug at various pH at 65 ^{0} C

S. No.	pH	Slope	K	Half life	log K	log K+3
1	0.11	-0.78295	1.80314	0.38432	0.25603	3.25603
2	1.22	-0.15838	0.36476	1.89987	-0.43799	2.56200
3	2.19	-0.07153	0.16473	4.20682	-0.78322	2.21677
4	4.3	-0.06893	0.15873	4.36568	-0.79932	2.20068
5	6.28	-0.01071	0.02465	28.1088	-1.60811	1.39189
6	8.09	-0.02345	0.05400	12.8388	-1.26761	1.73239
7	10.13	-0.09015	0.20761	3.33783	- 0.68273	2.31726
8	11.82	-0.60916	1.40289	0.49397	0.14702	3.14702
9	13.70	--1.21858	2.80639	0.24693	0.44814	3.44814

Effect of Buffers:[31-35]

To study the effect of buffer species, which can also catalyze hydrolysis of the drug other than H^+ ion and OH^+ ions, stability studies were carried out in:

Acetate Buffer pH 3.4

Citro Phosphate Buffer pH 5

Phosphate Buffer pH 7.4

Alkaline Borate Buffer pH 9

Table 6: Parameters for degradation of drug in Boric Buffer

S. No.	Medium	Slope	K	Half life	log K	log K+3
1	0.4 M Boric acid	-0.02472	0.05693	12.17102	-1.24459	1.75540
2	0.6 M Boric acid	-0.03669	0.08449	8.20133	-1.07315	1.926848

Table 7: Decomposition of the drug with surfactants during autoclaving

S.No.	Surfactant	% used	Absorbance	conc. of drug decomposed (µg/ml)
1	SLS	0.5	0.4833	3.71
2	SLS	1.5	0.5012	3.19
3	Tween 20	5	0.4974	3.32
4	Tween 20	10	0.4985	3.28

CONCLUSION

Chemical kinetics and stability are the terms that are used to describe the rate of chemical change and ability of a drug to maintain its integrity throughout the time period from manufacturing till its use by the consumer, which is the most important requirement for a drug to show its therapeutic effect. The drug chosen in the study was Amlodipine besylate and as the stability of any chemical drug substance is influenced by various factors like temperature, pH, solvents, buffers, light etc. therefore all these factors were covered during the stability study of Amlodipine besylate.

As per literature survey no work was done on stability study of Amlodipine besylate except two extemporaneously prepared dosage forms and the drug is having ester groups, so it was expected to degrade by hydrolysis when conditions for hydrolysis are provided like hydrogen and hydroxyl ions, high temperature, and solvent composition. Principles of chemical kinetics were applied and the parameters like rate constant, half life were calculated which gave an idea regarding the stability profile of the drug in various conditions of temperature, light, pH, and solvent system. When the stability study was carried out at different temperatures, the K value was found to be highest for **90 ^{0} C i.e. 2.781/min**

When the study was carried out to observe the effect of hydrogen and hydroxyl ions on hydrolysis, it was concluded that degradation was influenced by specific acid base catalysis. The K values were found to be high for low and high pH and maximum stability of the drug was around neutral pH i.e. pH 7. The drug was less stable at other pH values with the least stability at very high and very low pH values (pH 0.11, pH 13.7). The drug was found to be less stable in sunlight showing more degradation with 1 N NaOH compared to 1 N HCl.

When an attempt was made to sterilize the liquid solution of the drug by autoclaving in order to get an idea about formulation of liquid/parenteral preparation of the drug, the drug was degraded and thus was not able to withstand the process of sterilization by autoclaving.

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