A NOVEL *IN-VITRO* DISSOLUTION TEST FOR CHOLECALCIFEROL(D3) TABLETS WITH CUMULATIVE DRUG RELEASE PATTERNS AT ALTERED PH & AGITATION

Vothani Sarath Babu[1*], Amudala Sridhar Reddy [2], Vasanth Kumar PM[3], Chittoor Pallavi[4], Ch. Apparao[5],

[1]Faculty, Department of Pharmaceutics, S V U College of Pharmaceutical Sciences, S V University, Tirupati, 517002, AP, India

[2]Department of Pharmaceutics, S V U College of Pharmaceutical Sciences, S V University, Tirupati, 517002, AP, India

[3]Faculty, Department of Analysis, S V U College of Pharmaceutical Sciences, S V University, Tirupati, 517002, AP, India

[4]Department of Biochemistry, S V U College of Sciences, S V University, Tirupati, 517002, AP, India

[5]Professor & Principal of S V U College of Pharmaceutical Sciences, S V University, Tirupati, 517502, AP, India

Corresponding Author*:

Mr. VOTHANI SARATH BABU,

Faculty, Department of Pharmaceutics

S V University College of Pharmaceutical Sciences,

S V University, Tirupati

AP, India, Pin 517002

Abstract: Current work was carried out on dissolution apparatus (USP) that helps drug release of Vitamin cholecalciferol (D3) in dissolution process followed by dissolution monograph, that helps for the quantification of drug available in biological fluid at gastrointestinal tract and significant factors involved in change of pH of medium and agitation of dissolution media. The experiment results exhibited better drug release for Cholecalciferol Tablets (D3) drug in 0.1 N HCL and acidic buffer than pH 6.8 buffer with different agitation speeds.

Keywords: Vitamin cholecalciferol, buffers, Dissolution apparatus and Tablets

INTRODUCTION

Cholecalciferol is a steroid hormone produced in the skin when exposed to ultraviolet light or obtained from dietary sources. The active form of cholecalciferol, 1,25-dihydroxycholecalciferol (calcitriol) plays an important role in maintaining blood calcium and phosphorus levels and mineralization of bone. The activated form of cholecalciferol binds to vitamin D receptors and modulates gene expression. This leads to an increase in serum calcium concentrations by increasing intestinal absorption of phosphorus and calcium, promoting distal renal tubular reabsorption of calcium and increasing osteoclastic resorption. However, studies are also ongoing to determine whether or not cholecalciferol may also play certain roles in cancer, autoimmune disorders, cardiovascular disease, and other medical conditions that may be associated with vitamin D deficiency. [1]

When comes to the solubility of vitamin D3(cholecalciferol) it is highly soluble in fats and lipophilic substance than aqueous compounds. Due to low solubility of vitamin D3 it should come (BCS-II or IV) low solubility, high permeability or low solubility and low permeability.to increase the bioavailability of vitamin D3 it should be formulated in different novel dosage forms like nano-particles, microparticles, Nano emulation, self-suspended Nano emulation. When it comes to oral formulation of cholecalciferol formulation like tablets it must and show the good bioavailability after administration. But its low solubility/dissolving in aqueous solution it cannot be show enough bioavailability in administered patients. Hence dissolution is the rate limiting step in the oral formulation.

Dissolution is a physicochemical process by which a solid substance enters into solvent phase to form a solution. Release of drug from the formulated dosage form is the key parameter for the any orally-administered drugs to be systemically effective [2]. For in vitro dissolution of poorly soluble drugs, it is difficult to find adequate hydrodynamic conditions as agitation rate, medium composition and suitable volume, as well as a good discriminating power. In these conditions adequate dissolution cannot be achieved with aqueous solutions within physiologic pH ranges (1.2-6.8) Because of the different characteristics of early dosage form, the site absorption and dosing routes and applications, it is essential to give appropriate consideration to the following factors in the development of the test method include Apparatus selection, dissolution medium, agitation and temperature. [3,4]. Low solubility and permeability are problematic characteristics of class IV drugs; thus, the determination of in vitro release performance using different agitation rates, dissolution media, and dissolution apparatuses provides important information for improving the manufacture and evaluation of generic

formulations [5,6]. Despite the wide use of USP basket and paddle apparatuses (USP Apparatus 1 and 2, respectively) to monitor the physical quality of tablets and capsules, several investigations about the hydrodynamic environment that surrounds oral formulations have reported that these USP apparatuses do not adequately reproduce the natural environment of the gastrointestinal tract [7-9]. thus, it is necessary to document the in vitro release performance of poorly soluble drugs under different conditions to establish, in the best possible way, the environment in which the solid dosage forms will be within the gastrointestinal tract [10-12]
. Further, alternative apparatuses must be developed to achieve this goal. When the human pharmacokinetic (PK) data are available from a sufficient number of versions of a product with different dissolution profiles, a properly developed dissolution test may lead to the development of in vitro-in vivo correlation (IVIVC) for the drug products. A dissolution test can be used to guide product development and for quality control of products. When an effective IVIVC can be established, the in vitro dissolution test is a valid measure for the in vivo quality/performance of the product [13,14]. By contrast, a dissolution test is not a valid measure of in vivo performance when IVIVC has not been established. Furthermore, the same dissolution results for multisource products do not guarantee that the products will have the same in vivo performance [15] In the absence of IVIVC, the use of dissolution data is ambiguous for comparing different products [16,17].

Dissolution and drug absorption

After oral administration of any solid oral dosage forms (e.g., immediate release (IR) or modified release (MR) tablets or capsules), the APIs in the dosage forms must dissolve into the gastrointestinal (GI) fluid (solution) before the APIs can become therapeutically effective (e.g., locally in the GI tract or after being absorbed into the bloodstream to reach the site of action), The dissolution rate from the dosage form into the GI fluid may affect the drug absorption rate, hence the therapeutic effect of the dosage forms.[18,19]

· with publishing the first studies describing the relationship between rates of dissolution and absorption. Initially, dissolution testing was included in the USP with an intention of measuring the physiological availability of the product in the GI fluid. However, direct verification of the dissolution in the GI fluid is difficult. Notably, the GI fluid is not the final destination for drugs that are entering the systemic circulation. In general, a wide range of physiological conditions affect how quickly BCS class II medicines dissolve. Three key features of GI fluids that can influence the rate of medication release are pH, ionic strength, and buffer capacity [20,21]

After a drug dissolve into the physiological fluid in the GI tract, the drug will pass through the GI membrane and be transported into the bloodstream to reach the site of action and exert a

pharmacological effect. Assuming that the absorption mechanism is the same for different lots of the same product or the same product made by different manufacturers (generics), the dissolution in the GI tract can be inferred by measuring the bioavailability (BA) of the product after oral administration. Bioavailability is defined as the rate and extent to which the drug is absorbed from a drug product and becomes available at the site of action. [22]

The main aim of this study was to evaluate the in vitro release performance of Cholecalciferol tablets in the hydrodynamic environments generated by using USP apparatuses, agitations and dissolution media of physiological relevance to identify the rate and extent of release of the drug from formulated product [23,24].

EXPERIMENTAL

Materials and Methodology

Vitamin D3(Cholecalciferol) of Indian marketed tablet used as nutritional supplement, HCL (hydrochloric acid), disodium hydrogen phosphate, potassium dihydrogen phosphate, glacial acetic acid, sodium hydroxide (NaOH), potassium hydroxide (KOH), pH-meter, USP-dissolution apparatus USP-II(Paddle), UV-visible spectrometer.

Method:

***IN-VITRO*-DISSOLUTION GENERAL PROCEDURE**

The drug release pattern for Eight cholecalciferol tablets is carried out by using different buffer pH like 0.1N HCL,4.0,4.5,5.0,5.5,6.8 as a dissolution media(900ml) and maintain the bath temperature 37°C± 0.5. at different RPMs like 50,75,100 rpms by USP-II paddle as apparatus, 10ml of samples were collected after predetermined time interval i.e., 5, 10, 15, 30, 45, 60, 90 and 120 min and replaced by

freshly prepared medium at the same time in order to maintain sink condition. Further dilution of samples is made by taking 0.45ml sample and makeup its volume up to 10ml with respective medium. Absorbance of all the samples is taken at wavelength of 315nm by UV Spectrophotometer so that concentration in each time interval can be determined.

- For tablet 1- 0.1NHCL pH buffer used as a dissolution medium &50RPMs
- For tablet 2- 6.8 pH buffer used as a dissolution medium&50RPMs
- For tablet 3- 0.1NHCL pH buffer used as a dissolution medium &70RPMs
- For tablet 4- 6.8 pH buffer used as a dissolution medium&70 Rpm
- For tablet 5- 4.0 pH buffer used as a dissolution medium&100RPMs
- For tablet 6- 4.9 pH buffer used as a dissolution medium&100RPMs
- For tablet 7- 5.0 pH buffer used as a dissolution medium&100RPMs
- For tablet 8- 5.9 pH buffer used as a dissolution medium&100RPMs

Each tablet facing the different buffer solution and agitations every time

For tablet-1 we use the 0.1N HCL as a buffer and maintain the agitation speed at50rpm.hear the drug release from tablet is started immediately and the tablet gets disintegrated and release the drug into bulk of the solution or surrounding media. Were in case of Tablet-2 drug could not release until 5 to 10 minutes after that tablet get extrusion, after that the drug release slowly when compared to that tablet -1at pH-6.8buffer as dissolution media at 50rpms as stirring speed Maintain the bath temperature of 37°C± 0.5.and USP-II (paddle) as apparatus.

In Tablet -3 and Tablet-4 the buffer solution for dissolution media is similar to that of Tablet -1and Tablet-2and only difference in the agitations of apparatus. For Tablet-3&4 the rotation should be maintained at70 rpms. the only difference in the tablet1,2 and 3,4 is difference in their rotations (stirring speed/agitations), when focus on the release pattern of drug from medicament, the drug release pattern is increased in tablet-3 of dissolution medium by increasing the stirring speed and in tablet-4 slow release of drug from the medicament is observed but no significant change observed by increasing the stirring speed of the apparatus.

Regarding tablets 5, 6, 7, and 8, tablet 5&6's dissolution media is different. Tablet-5 uses a pH buffer of 4.0 while Tablet-6 uses one of pH 4.9; both mediums should be stirred at a speed of 100 rpm. Tablets 7 and 8 both have stirring speeds of 100 rpms; however, their buffer solutions differ. For tablet 7, we utilise 5.0 as a dissolving media, and for tablet 8 uses 5.5 pH buffer as a media. Hear the drug release should be decreased in tablet 5&6 when compared with tablet 1&3and slightly increased compare with tablet-2&4. when we observe the release pattern of Tablet-7&8 the release pattern should be decreased while comparing with Tablet5&6 and shows similar invitro release patterns of tablet 2&4.

By performing the all-dissolution studies for the 8-tablets from company manufactured strip at different pH buffers and stirring speed the percentage drug release should be following manner

Preparation of Calibration curve:

The standard solutions for the drug having concentration 2, 4, 6, 8, and 10µg/ml was prepared with phosphate buffer pH 6.8 from the stock solution. The absorbance of solutions of pure cholecalciferol drug were measured at 315 λ max and a calibration curve was plotted between absorbance v/s concentration to get the linearity and regression equation which has shown.

Results and discussion

The awareness of the analyte (the substance to be measured). A calibration curve, additionally referred to as a preferred curve, is a standard technique for figuring out the concentration of a substance in an unknown sample with the aid of the usage of evaluating the unknown to a hard and fast of popular samples of recognized attention. A calibration curve is one approach to the trouble of tool calibration; extraordinary preferred techniques may combo the standard into the unknown, giving an internal fashionable. The calibration curve is a plot of approaches the instrumental reaction, the so-referred to as analytical sign, changes

Figure 1: Calibration curve of cholecalciferol

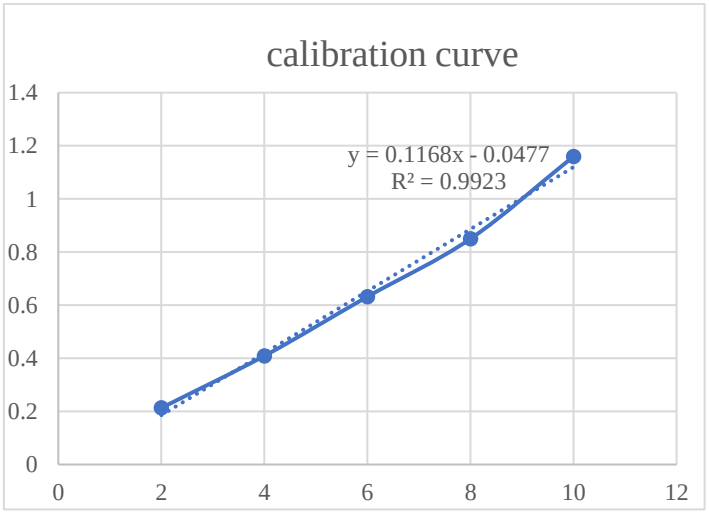


Table 1: Absorbance vs concentration

Concentration (µg/ml	Absorbance(nm)
2	0.213
4	0.409
6	0.632
8	0.85
10	1.16

Table 2 : Absorbance of Each tablet at regular intervels at different pH& agitations.

sample	TAB-1 0.1/50	TAB-2 6.8/50	TAB-3 0.1/70	TAB-4 6.8/70	TAB-5 4.0/100	TAB-6 4.9/100	TAB-7 5.0/100	TAB-8 5.5/100
10	2.412	0.144	1.64	0.37	0.148	0.254	0.982	0.184
20	2.532	0.179	1.832	0.461	0.214	0.363	1.312	0.217
30	2.62	0.192	1.978	0.48	0.241	0.383	1.4	0.317
40	2.73	0.224	2.187	0.546	0.316	0.475	1.651	0.357
50	2.802	0.289	2.221	0.78	0.377	0.532	1.73	0.416
60	2.962	0.296	2.45	0.82	0.502	0.605	1.844	0.56

Figure-2: Absorbance of tablet 1&2 at regular intervals at different buffers (0.1&6.8) and same agitations of media (50 rpm)

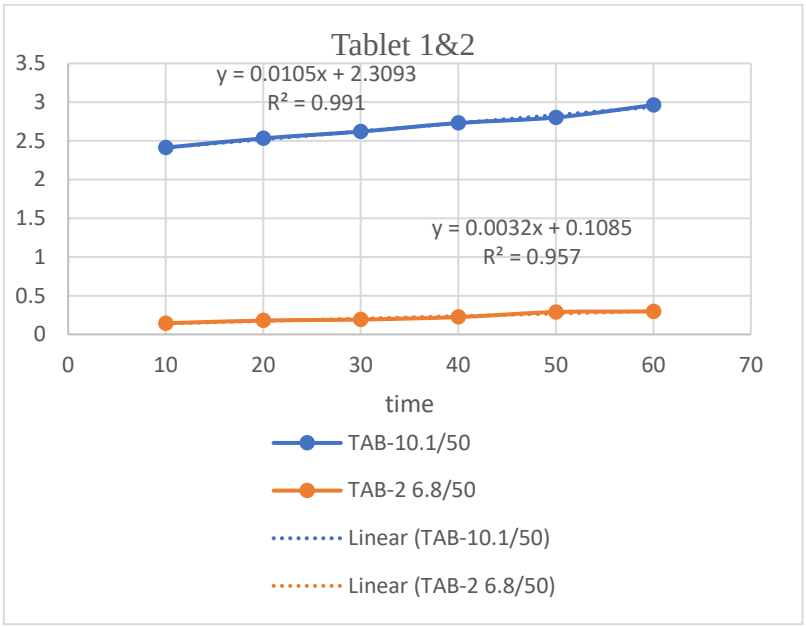


Figure-3: Absorbance of tablet 3&4 at regular intervals at different buffers (0.1&6.8) and same agitations of media but increased the agitations of media (70 rpm) compared to Tablet-1&2(50rpm)

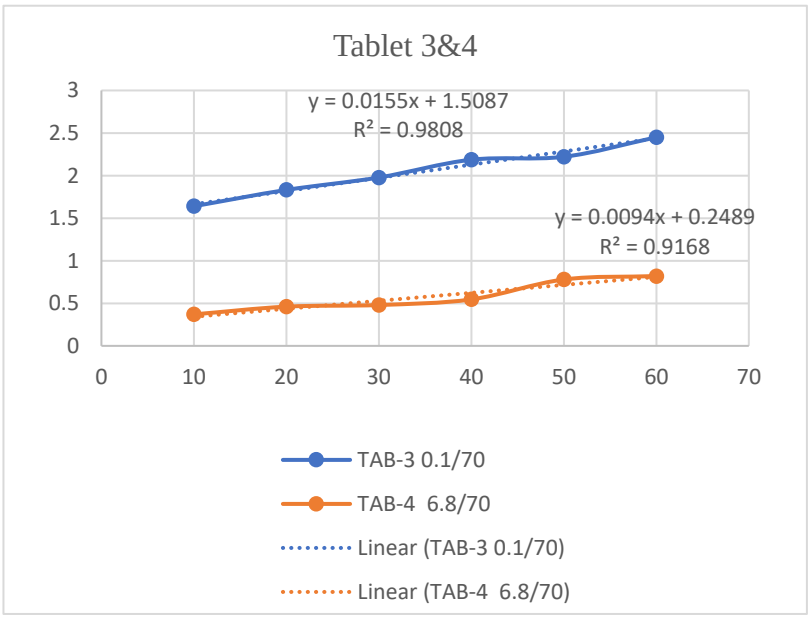
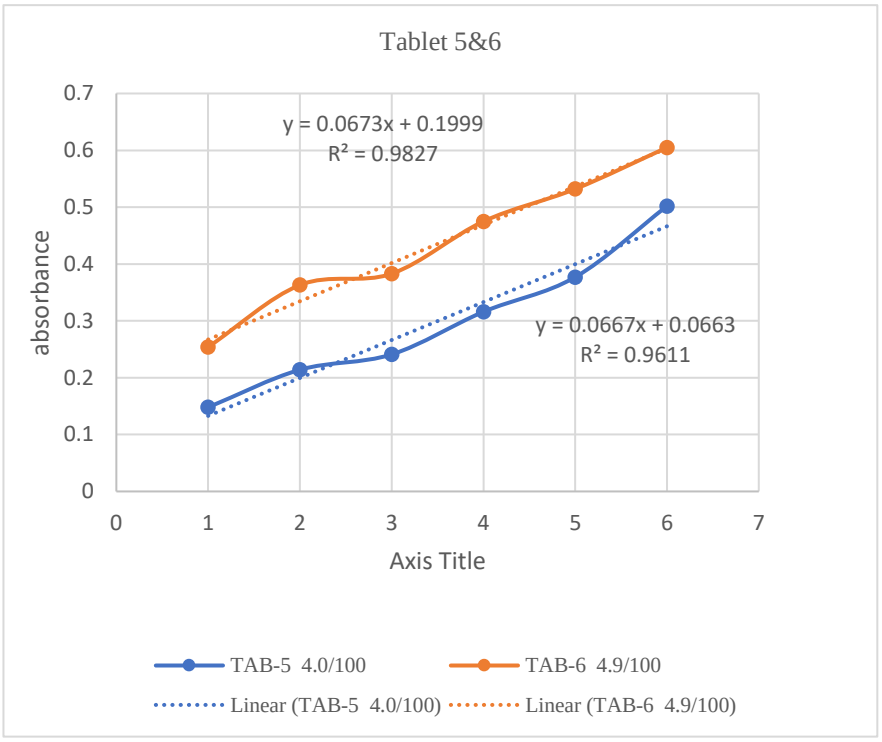
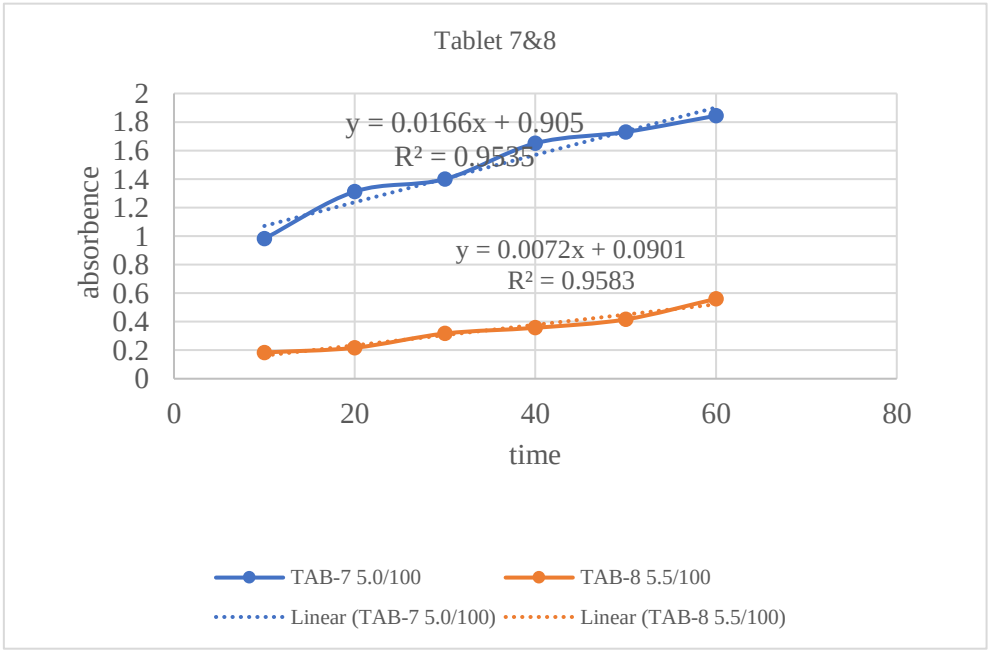


Figure 4: Absorbance of tablet 5&6 at regular intervals of different buffers (4.0&4.9) and same agitations of media but increased the agitations of media (100rpm) compared to Tablet-3&4(70rpm)



FigureNo-5: Absorbance of tablet 7&8 at regular intervals of different buffers (5.0&5.5) and same agitations of media but increased the agitations of media (100rpm) compared to Tablet-3&4(70rpm)



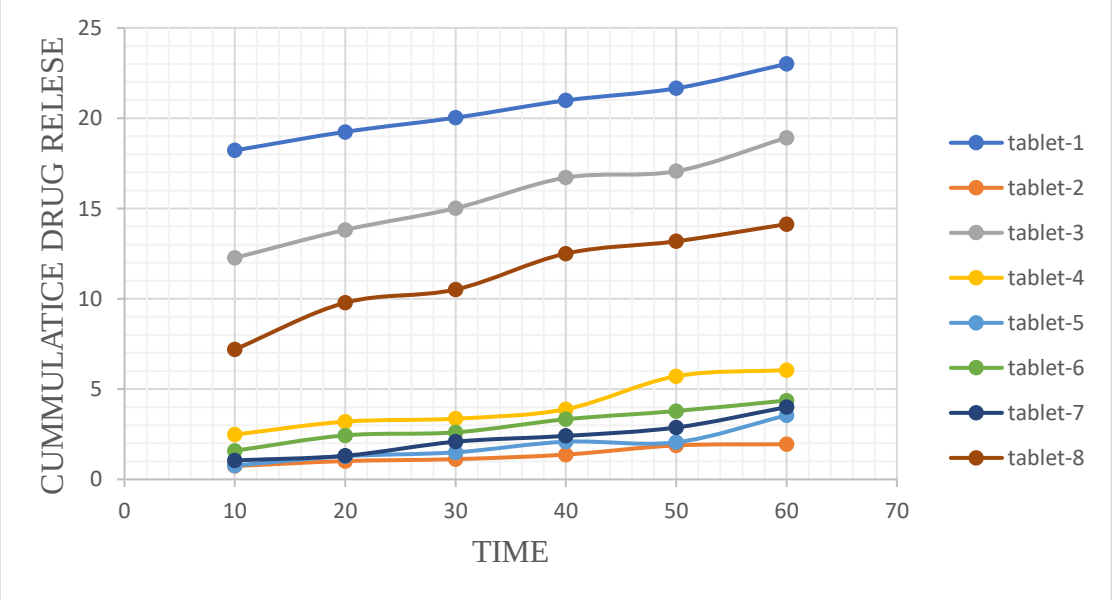
CUMULATIVE DRUG RELEASE OF CHOLECALCIFEROL AT DIFFERENT pH

Amount drug released at each regular interval is calculated by using slope and concentration of the standard calibrated curve and finally we calculate the drug release in 900 ml of media and cumulative drug release (the cumulative amount released at each sampling time is the sum of the amount in the receiver at that time Plus the amount in each sample that was removed and replaced with empty buffer.)

Table no-3: Represent the cumulative drug release of each tablet at regular interval of different buffers and agitations of medium

Time	tablet-1	tablet-2	tablet-3	tablet-4	tablet-5	tablet-6	tablet-7	tablet-8
10	18.21807	0.742038	12.26943	2.483476	0.77286	1.58964	7.199229	1.050257
20	19.24393	1.015852	13.81705	3.198472	1.285715	2.438369	9.782033	1.310372
30	20.02836	1.121644	15.01843	3.362568	1.500882	2.605976	10.51424	2.088168
40	20.98608	1.374396	16.71151	3.889636	2.087068	3.329234	12.5062	2.407915
50	21.6557	1.8828	17.0651	5.71405	2.05685	3.786	13.1836	2.8757
60	23.0065	1.94707	18.9227	6.05362	3.54587	4.36997	14.134	4.00113

FigureNo-6: cumulative drug release Vs Time



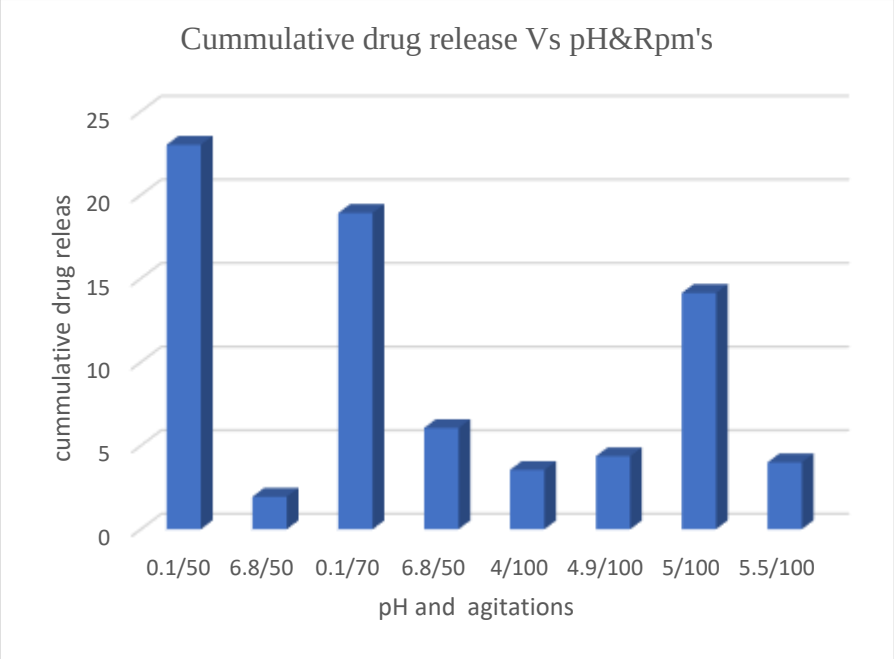
CUMULATIVE RELEASE OF DRUG AT DIFFERENT pH AND AGITATIONS

Cumulative drug release should be calculated and find out the final release of drug at last regular interval by this we can identify the better release pattern of drug at different pH and agitations

Table-4: cumulative drug release of each tablet at final intervals

S/NO	pH/Rpm's	Cumulative drug release
Tablet-1	0.1/50	23.0065
Tablet-2	6.8/50	1.94707
Tablet-3	0.1/70	18.9227
Tablet-4	6.8/50	6.05362
Tablet-5	4/100	3.54587
Tablet-6	4.9/100	4.36997
Tablet-7	5/100	14.134
Tablet-8	5.5/100	4.00113

Figure-7: cumulative drug release Vs pH& RPM's



DISCUSSION

1) Effect of pH of dissolution media on drug release:

The result of dissolution studies on cholecalciferol shows different release patterns at different dissolution buffer pH and stirring speed. When comes to the buffer pH of tablet 1&2 is 0.1N HCL and 6.8 pH phosphate buffer and rotations per minute is 50 maintained. Hear Rpm's should be same for two tablets but difference is their buffer pH. Release patterns should be altered due to the effect of pH on solubility release of drug into surrounding media.as shown in figure -2 and table-3 the amount of drug release in tablet-1 in 60 minutes interval is 23.0065 (0.1N HCL/50rpm's) and for tablet -2 is 1.94707(6.8 pH/50). there is huge difference in the release amount of drug.

When dissolution is performed for next two tablet (tablet-3&4) the dissolution medium is similar like tablet 1 and 2 but this time changed in agitation /rotations should be increased 50 to 70 Rpm's. hear the amount of drug release should be increased in both medium, at 0.1N pH (tablet-3) the amount of drug release is same as tablet-1, but in tablet-4 the invitro drug should be increased slightly (Cdr-6.05362) when compared with tablet -2, the main altered parameter is Rpm's/agitations.

When it comes to the tablet-5,6,7, &8 the pH of the buffer is different for four tablet follows 4.0.4.9 ,5.0 and 5.5 and stirring speed should be same for all four tablets of the medium is 100.Amount of drug release should be increase followed by increasing the pH of the medium.

2) Effect of Agitations on dissolution media on drug release.

The effect of agitation intensity on drug release was shown in figure ,2,3,4&5. The speed of rotation has much effect on drug release in terms of increasing agitation makes decrease in drug release. Hence, it can be expected that the release from the developed formulations may be dependent of the hydrodynamic conditions of the GIT. When we observe the amount drug release in figure 1&2 and 3&4 the cumulative drug release is differ in both due to the change in the agitation rate in medium. The dissolution rate increased with an increase in the agitation rate from 50 to 75 rpm. However, no significant increase in the dissolution rate was noted with an increase in the agitation rate from 75 to 100 rpm [25]. Exactly the amount drug release in tablet-2,4,5,6,7and 8 there is no effective release in further increase in the agitation speed in medium. So, the rate of stirring /agitation will also affect the amount of drug release it is clearly observed in figure-2 and figure-4 and 5. based on the solubility or BCS classification pH and agitations shows different effect on the bioavailability of drug

Conclusion:

The aim of current study to evaluate the development of marketed drug release by dissolution process to achieve drug release in biological system based on condition of media and agitations of media that helps to makes prepare the novel drug release patterns of the drug and also, alter to achieve better pharmacokinetic and Biopharmaceutical limitation of drugs.

Conflict of interest: There is no conflict of interest

Acknowledgment: I would like thank to Prof. Ch Apparao, Principal of S V U college of Pharmaceutical Sciences, for providing facility to carry out this research work.

References:

1. National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 5280795, Cholecalciferol. Retrieved July 26, 2022 from https://pubchem.ncbi.nlm.nih.gov/compound/Cholecalciferol#section=Solubility
2. Lachman, L., Lieberman, H. A., & Kanig, J. L. (1976). *The theory and practice of industrial pharmacy*. Philadelphia: Lea & Febiger
3. da Fonseca LB, Labastie M, de Sousa VP, Volpato NM. Development and validation of a discriminative dissolution test for nimesulide suspensions. AAPS PharmSciTech 2009; 10:1145-52.
4. Gao, Z., Ngo, C., Ye, W., Rodriguez, J. D., Keire, D., Sun, D., ... & Jiang, W. (2019). Effects of dissolution medium pH and simulated gastrointestinal contraction on drug release from Nifedipine extended-release tablets. *Journal of Pharmaceutical Sciences*, *108*(3), 1189-1194
5. Vangani S, Li X, Zhou P, et al. Dissolution of poorly water-soluble drugs in biphasic media using USP 4 and fiber optic system. Clin Res Regul Aff. 2009;26(1e2):8-19.
6. . Dokoumetzidis A, Macheras P. A century of dissolution research: from noyes and whitney to the biopharmaceutics classification system. Int J Pharm. 2006; 321:1-11..
7. M. Morihara, N. Aoyagi, N. Kaniwa, N. Katori, S. Kojim. Hydrodynamic flows around tablets in different pharmacopeial dissolution tests. Drug Development and Industrial Pharmacy 28 (2002) 655-662.
8. K. Greco, T.L. Bergman, R. Bogner. Design and characterization of laminar flow-through dissolution apparatus: comparison of hydrodynamic conditions to those of common dissolution techniques. Pharmaceutical Development and Technology 16 (2011) 75-87.
9. V. Todaro, T. Persoons, G. Grove, M.A. Healy, D.M. D'Arcy. Characterization and simulation of hydrodynamics in the paddle, basket and flow-through dissolution testing apparatuses - a review. Dissolution Technologies 24 (2017) 24-36.
10. Dressman JB, Amidon GL, Reppas C, Shah VP. Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms. *Pharm Res.* 1998;15:11–22
11. McConnell EL, Fadda HM, Basit AW. Gut Instincts: Explorations in Intestinal Physiology and Drug Delivery. *Int J Pharm.* 2008;364:213–226.
12. DeSesso JM, Jacobson CF. Anatomical and Physiological Parameters Affecting Gastrointestinal Absorption in Humans and Rats. *Food Chem Toxicol.* 2001;39:209–228.
13. Chakraborty, S., Pandya, K., & Aggarwal, D. (2014). Establishing prospective IVIVC for generic pharmaceuticals: methodologies assessment. *The Open Drug Delivery Journal*, *5*(1)..

14. Qureshi, S. A. (2010). In vitro-in vivo correlation (ivivc) and determining drug concentrations in blood from dissolution testing–a simple and practical approach. *The Open Drug Delivery Journal*, *4*(1).
15. Roudier, B., Davit, B. M., Beyssac, E., & Cardot, J. M. (2014). In vitro-in vivo correlation's dissolution limits setting. *Pharmaceutical research*, *31*(9), 2529-2538..
16. USP38 Chapter, 1088.
17. FDA Guidance for Industry. Extended-release oral solid dosage forms: development, evaluation, and application of in vitro/ in vivo correlations; September 1997.
18. Dokoumetzidis A, Macheras P. A century of dissolution research: from Noyes and Whitney to the biopharmaceutics classification system. Int J Pharm 2006; 321:1-11.
19. Edwards LJ. The dissolution and diffusion of aspirin in aqueous media. Trans Faraday Soc 1951; 47:1191-210
20. Galia, E., Nicolaides, E., Hörter, D., Löbenberg, R., Reppas, C., & Dressman, J. B. (1998). Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharmaceutical research*, *15*(5), 698-705.
21. Tsume, Y., Langguth, P., Garcia-Arieta, A., & Amidon, G. L. (2012). In silico prediction of drug dissolution and absorption with variation in intestinal pH for BCS class II weak acid drugs: ibuprofen and ketoprofen. *Biopharmaceutics & drug disposition*, *33*(7), 366-377.
22. Raúl Medina-López, Sergio Guillén-Moedano, Marcela Hurtado In vitro release studies of furosemide reference tablets: influence of agitation rate, USP apparatus and dissolution mediaADMET and DMPK, Vol. 8 No. 4 (2020), 411-423Published 29-06-2020;**DOI:** https://doi.org/10.5599/admet.801
23. Food and Drug Administration. Guidance for Industry. Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER), 2000. Available from: http://www.fda.gov/downloads/Drugs/.../ Guidances/ucm070246.pdf [Last accessed 2012 Mar 21].
24. Rivera-Leyva, J. C., García-Flores, M., Valladares-Méndez, A., Orozco-Castellanos, L. M., & Martínez-Alfaro, M. (2012). Comparative studies on the dissolution profiles of oral ibuprofen suspension and commercial tablets using biopharmaceutical classification system criteria. *Indian Journal of Pharmaceutical Sciences*, *74*(4), 312.

25. Shah VP, Gurbarg M, Noory A, Dighe S, Skelly JP. Influence of higher rates of agitation on release patterns of immediate-release drug products. J Pharm Sci. 1992 Jun;81(6):500-3. doi: 10.1002/jps.2600810604. PMID: 1522485.