ISSN 2348 –4012

VOLUME 6 | ISSUE 1 | JANUARY-JUNE 2019

Nirma University Journal of **Pharmaceutical Sciences**

An International Peer Reviewed Journal

NIRMA UNIVERSITY
INSTITUTE OF PHARMACY
NAAC ACCREDITED 'A' GRADE

Official Publication of
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For subscription related inquiries write to:

The Editor in Chief, Nirma University Journal of Pharmaceutical Sciences (ISSN 2348 –4012).

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Printed & Published by: Institute of Pharmacy, Nirma University

Printed at : Print Quick, Ahmedabad

B.PHARM-PROGRAMME VISION & MISSION

Vision

Striving to excel in pharmaceutical education, research & innovation to develop outstanding professionals catering to the health care needs of the humankind.

Mission

The institute aims to develop employable students, researchers and entrepreneurs by inculcating critical thinking, problem solving ability, ethical values and leadership skills. Institute provides vibrant environment for continuous learning by strengthening industrial collaboration for developing competent professionals.

B.PHARM-PROGRAMME EDUCATIONAL OBJECTIVES

PEO 1: To acquire effective knowledge of pharmaceutical sciences to be able to lead the industry and health care sector

PEO 2: To attain practical training and technical expertise in pharmaceutical sciences

PEO 3: To inculcate professional and ethical standards with effective interpersonal communication skills.

PEO 4: To develop interdisciplinary & holistic approach in Pharmaceuticals for societal cause, problem solving and lifelong learning.

PEO 5: To adapt and implement best practices in the profession by enrichment of knowledge and skills in research and critical thinking.

B.PHARM-PROGRAMME OUTCOMES

No.	Programme Outcomes
PO1	**Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
PO2	**Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines
PO3	**Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.

PO4 **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations

PO5 **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.

PO6 **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).

PO7 **Pharmaceutical Ethics:** Honor personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.

PO8 **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions

PO9 **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice

PO10 **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.

PO11 **Life-long learning:** Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis

PSO1 **Drugs and diseases:** Understand different classes of drugs, their mechanism of action, dynamics, kinetics, structure activity relationships, pathophysiology and pharmacotherapeutics of various diseases.

PSO2 **Drug development:** Ability to synthesize, develop and/or evaluate various pharmaceuticals and their formulations and cosmeceuticals products

PSO3 **Analytical skills:** Develop skills in qualitative and quantitative analysis of various pharmaceuticals.

PSO4 **Training:** Acquire technical knowledge and hands on training on equipments, instruments and software used in the field of pharmaceutical sciences.

NIRMA UNIVERSITY JOURNAL OF PHARMACEUTICAL SCIENCES
VOLUME 6| ISSUE 1 | JANUARY-JUNE 2019
ISSN 2348 –4012

Content

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EDITORIAL MESSAGE

Bioinformatics is the branch of biology that is concerned with the acquisition, storage, display and analysis of the information found in nucleic acid and protein sequence data. Genomes are complicated, and while a small number of your traits are mainly controlled by one gene, most traits are influenced by multiple genes. On top of that, lifestyle and environmental factors play a critical role in your development and health. The National Human Genome Research Institute (NHGRI) conducts a broad program of laboratory and clinical research to translate genomics into a greater understanding of human biology and develop better methods for the detection, prevention and treatment of heritable and genetic disorders.

Recent developments, including next-generation sequencing (NGS) and the growing role of hospital information technology (IT) systems and electronic health records, amass ever-increasing amounts of data before human genetics scientists and clinicians. However, they have ever-improving tools to analyze those data for research and clinical care. Correspondingly, the field of bioinformatics is turning to research questions in the field of human genetics, and the field of human genetics is making greater use of bioinformatics algorithms and tools.

There is a growing need for methods combining sequencing and computerized analysis and the scientists are trying hard to respond to these demands. With high-throughput procedures delivering more and more data, one needs to develop more efficient and more complex in-silico methods while making sure the associated analyses remain reproducible. Genomics, and -omics analysis as a whole, are now highly dependent on IT technologies, machine learning and statistics. On the other hand, the exact mechanism of how the drug molecule interacts with the biological targets and proteins together with its delivery inside the target etc. present substantial challenge in the scientific understanding of the subject. Computational methods go hand-in-hand with experimental techniques and thus minimize the investment in drug design and development. There is astounding development and progress in the computer simulations leading to better understanding of design and optimization of target, lead optimization, precise mechanism of drug delivery and finally safety and efficacy studies.

With every new issue our enthusiasm is growing. We are getting an annihilating response from the industrial experts, reviewers and the pharmacy community. In this issue we have invited research articles and review articles from industrial experts as well as postgraduate and undergraduate students spread across different facets of pharmaceutical field. We welcome contributions from all fields of research pertaining to health science. We hope the pharmacy fraternity will share our excitement and join us in this adventure.

Happy hours!!

Editorial Team, NUJPS

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Nirma Univ J Pharm Sci; 2019, 6(1) 1-14
© 2019, Nirma University, Ahmedabad, Gujarat, India
ISSN 2348 –4012

ARTICLE

COMPARATIVE *IN-VITRO* MULTIMEDIA DISSOLUTION STUDY OF CARBAMAZEPINE EXTENDED RELEASE TABLET 400MG AT VARIOUS PH RANGE.

Jignesh Shah, Vrunjan Shah, Deepika Koul, Aryan Gupta, Akash Goswami, Amit Patel, Dharmesh Shah, Kaushik Shah

Analytical Development Lab, Sushen Medicamentos Pvt. Ltd., Ahmedabad, Gujarat-382213, India.

Abstract:

Dissolution profiles of carbamazepine innovator formulation were evaluated and matched with the test formulation in multiple dissolution medium at various pH. Innovator formulation – Tegretol LP 400 was selected as a reference product. Different batches of innovator and test formulation of 400mg were selected for experiments. During development, the test formulation was formulated with respect to the dissolution profile of Tegretol LP 400 in defined medium – water. After achieving the matched dissolution profile in main medium - water, same test formulation was matched with innovator formulation in various pH medium. The similarity factor (f2) for test and reference product was obtained more than 50 for medium - water, acetate buffer pH 4.5 and phosphate buffer 6.8. During acidic medium at pH 1.2, the innovator formulation represented the typical behavior in dissolution and due to that high variability was observed in the results of intra-run. Same behavior of drug release was also observed for the repeated experiments as well as for other batch of innovator and even in the final test formulation. Hence, to prevent the high variability, surfactant was used for the dissolution in pH 1.2 medium. After various optimization experiments, the minimum optimum concentration of surfactant was achieved and the results of %RSD was found within acceptance criteria for all the formulations. The similarity factor (f2) of 12 units of test and reference product was found above 50. The results were also accepted for the other batch of innovator formulation and during the repetitive experiments.

Keyword: Keywords: Carbamazepine, Tegretol, Dissolution, Surfactant, SLS

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1. Introduction

Carbamazepine contains dibenzepine ring system which is structurally related to the tri-cyclic antidepressants [1–4] specifically used in therapy for seizures. Carbamazepine is highly insoluble in water at ambient temperature (25ºC) [5–8] with a solubility of 170mg/l. As the Carbamazepine is representing low water solubility, its bioavailability is also very low. Also polymorphism is observed for carbamazepine, which may affect its solubility. Upon multiple dosing of carbamazepine, it gets transformed to its metabolite and its half-life ($t_{1/2}$) is decreased [9]. The research says that central nervous system (CNS) side effects, related with immediate-release formulation of carbamazepine were reduced when patients were moved to an extended-release formulation [10]. So, sustained release formulation of carbamazepine is most effecting in maintaining the concentration in systemic circulation [9].

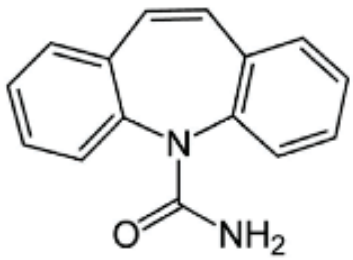

Figure-1 Carbamazepine Chemical Structure

Literature described the transformation of one polymorphic form to another dihydrate form, which is showing variable dissolution behavior of carbamazepine [11–13]. There have been several reports showing irregular dissolution [14–16], bioequivalence failures [17–19], and clinical failures of carbamazepine [20]. Certain literatures have been found which indicates the impact on polymers to make the drug release profile prolonged. But the optimization of polymer levels and combination of polymers plays a foremost role in achieving a successful extended release formulation which is correlating with the innovator formulation in each dissolution medium i.e. original medium as well as different biological pH.

In general, the enhanced dissolution pattern is depending upon either by dissolution medium pH change or by addition of the solubilizer, like surfactants and cyclodextrin derivatives in the preparation of dissolution medium [21–27]. SLS has been proven as the agent of choice because it is cost-effective and it holds good solubilizing capacity even at quite low concentrations. Already, several authors reported that SLS can be used to enhance dissolution of low water-soluble compounds [28]. Till date, many authors had published the articles on the addition and usage of SLS like sodium taurocholate or other surfactant for executing the dissolution of low soluble drug like carbamazepine using less dissolution media volume. Carbamazepine solubility was also distinctly increased in several nonionic surfactants [29]. But with the medium volume 1800mL according to USP method, very few articles were present which shows the surfactant assisted dissolution to get the higher release profile.

Even the data of innovator formulation in original medium as well as in the acidic medium has not been described at a large extent.

But here, the aim of carrying out the in-vitro drug dissolution at various biological pH to partially evaluate the effect of pH on drug release and absorption during in-vivo conditions. And specifically to evaluate the effect of surfactant on drug release and to set the minimum possible optimum concentration of surfactant to achieve desired drug release with a minimum variability in the results of both – innovator as well as test formulations.

2. Experimental

2.1 Chemicals and materials

Carbamazepine working standard (99.74%) was prepared from an API – Form-III, against a reference standard (99.9%) (B. No. 5) procured from EDQM. Methanol (HPLC grade, Merck, India), Hydrochloric acid (GR Grade, Merck, India), Sodium Chloride (GR Grade, Merck, India), Sodium Lauryl Sulphate (Emplura Grade, Merck, India), Sodium Hydroxide (GR Grade, Merck, India), Sodium Acetate (GR Grade, Merck, India), Acetic acid (GR Grade, Merck, India) and Potassium Dihydrogen Phosphate (GR Grade, Merck, India) were used for in-vitro dissolution experiments. Formulation of two different batches of innovator (RLD) product – Tegretol LP 400mg (Mfg. By: Novartis) were procured for comparing the release profile of test formulation.

2.2 Instrumentation

A dissolution apparatus system (708-DS, Agilent, Germany) with closed auto-sampler (850-DS, Agilent, Germany) with a 2 Liter bowl capacity, Double Beam UV Spectrophotometer (UV-1800, Shimadzu, Japan), Portable Dissolved Oxygen (DO) meter (HI9146, Hanna Instruments, Italy), Micro analytical balance (MSA6-6S-000-AM, Sartorius, Japan), pH meter (Thermo Orion Star II, Thermo, USA), Water purification system (Milli-Q, Millipore, USA), Dissolution Media Preparation Assembly and RO water system were used for dissolution experiment.

2.3 Instrumental method parameters

For carrying out dissolution experiment, USP dissolution apparatus – I (rotating basket with mesh size 10) with 2 liter capacity bowl were used. RPM of instrument was set to 100. The temperature of the medium was set to 37±0.5°C. 1800mL of dissolution medium was filled in each bowl [30]. Using a cannula with 70µ PTFE filter, 10mL sample from each bowl was withdrawn by auto-sampler connected with the dissolution apparatus and with PC. Same way, after withdrawal, 10mL of the respective medium was transferred to each bowl as media replacement to maintain the same volume throughout the experiment. The method was created in the computerized program to run the experiment. Standard and samples were analyzed through UV spectrophotometer using the wavelength 284nm.

2.4 Analytical Procedure

2.4.1 Standard and sample preparation

The carbamazepine working standard was weighed about 22.4mg and transferred to 100mL volumetric flask and dissolved in 25mL of methanol. Final volume was achieved with respective dissolution medium. After mixing, transferred 1mL of this solution to 25mL volumetric flask and diluted up to the mark with the respective medium.

The withdrawn samples were transferred to respective glass tubes kept inside the auto-sampler unit. 2mL of each sample was transferred to 25mL volumetric flask and diluted up to the mark with the respective medium.

UV Probe software was used from a computer which is connected with UV Spectrophotometer, and wavelength was set at 284nm. Initially, using respective dissolution medium, absorbance was corrected to zero. After correction, diluted standard solution was taken and analyzed. After that, diluted samples of each unit and each time points were analyzed. From the absorbance values, the % cumulative drug release of all units at each time point were calculated and evaluated for the acceptance of experiment (% drug release and %RSD).

2.5 Method development & optimization

For developing a generic formulation of Carbamazepine Extended Release Tablet with strength 400mg, from a reference of USP, it was found that dissolution should be performed with a media volume of 1800 mL to achieve the sink condition due to BCS Class-II drug. In its defined media – water, the dissolution profile of drug release from innovator formulation was observed for 24 hours and found reproducible within the acceptance level. In same manner, the test formulations were manufactured with different formulas according to QbD approach to match the drug release profile comparable to innovator formulation.

Initially analytical dissolution method was set according to USP using UV spectrophotometer. UV spectrum of Carbamazepine was scanned throughout the UV range. The λ_{max} 284nm was observed which was also correlated to the given value in USP (Refer figure-2).

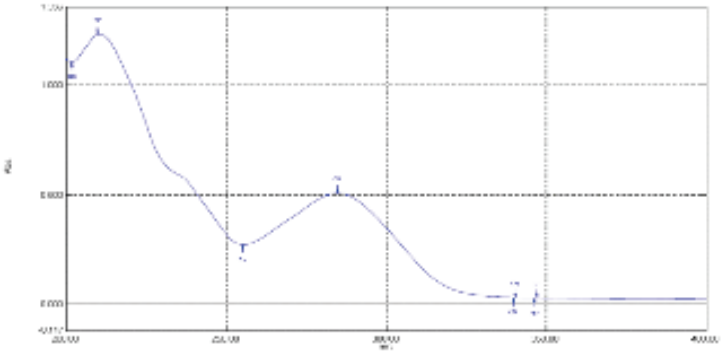

Figure-2 UV absorption spectrum of Carbamazepine

During the execution of experiment, behavior of innovator formulation was varying for each experiment and within the intra-units of respective individual experiment. To overcome this situation, the dissolution medium was degassed completely and dissolved oxygen was continuously monitored before the start of the experiment. Upon achieving the dissolved oxygen value below 5, medium was taken for the experiment. After that, the tablets of two different batches of innovator were evaluated with multiple experiments and results were found satisfactory with respect to drug release and %RSD.

Once the innovator profile has been evaluated, the formulation trials were started simultaneously and same were evaluated for drug release profile. After evaluation of release profile of all the formulations in water as a dissolution medium, the best formulation was selected and release profile was matched with innovator profiles. Table-1 represents the drug release profile of both batches of innovator formulation and the final test formulation in water.

Table-1 Drug release profile of both batches of innovator formulation and final test formulation in water

Sr. No.	B. No.		1	2	3	4	5	6	7	8	9	10	11
			1 Hr	2 Hr	3 Hr	5 Hr	6 Hr	8 Hr	10 Hr	12 Hr	16 Hr	20 Hr	24 Hr
RLD													
1	RLD (B-1)	% Release	13.5	21.7	29.5	43.0	48.9	58.9	66.4	72.5	80.5	84.8	88.7
		%RSD	2.1	5.0	3.6	3.7	3.9	4.1	3.0	3.1	2.9	2.4	2.9
2		% Release	13.4	22.2	30.0	43.5	49.8	59.3	66.9	72.9	81.7	86.4	89.7
		%RSD	7.3	6.5	6.7	5.2	4.4	4.4	4.8	3.4	3.6	2.4	1.7
3	RLD (B-2)	% Release	14.3	24.7	33.5	48.3	54.3	63.2	69.5	73.0	78.6	81.8	83.2
		%RSD	8.2	5.7	4.3	4.1	4.8	3.3	3.5	3.2	3.1	3.7	3.5
4		% Release	18.7	29.7	37.6	50.1	54.9	62.5	67.3	73.3	79.4	84.7	84.4
		%RSD	3.6	7.0	4.2	4.8	4.4	3.4	2.8	2.5	1.7	1.7	1.1
TEST													
1	Test (B-1)	% Release	15.6	24.9	32.5	44.9	49.7	59.2	65.7	71.2	79.3	84.5	88.4
		%RSD	3.5	2.6	2.2	1.4	1.0	1.5	1.5	1.8	2.0	2.5	2.9
2		% Release	15.5	24.6	32.1	44.3	49.7	58.1	64.8	71.0	79.3	83.7	87.0
		%RSD	4.5	4.1	3.0	3.3	3.5	3.1	2.8	3.3	3.3	3.4	2.6

Results represented that the profile of both formulation were found equivalent. Similarity factor (f2) for the 6 units as well as 12 units was found above 50. Table-2 represents the f2 value of experiments in water with multiple variables (Experiments, Batch, Formulation – Innovator or Test etc.).

Table-2 Similarity factor (f2 value) of experiments in water with multiple variables

F2 Value	RLD-1 - EXP-1	RLD-1 - EXP-2	RLD-2 - EXP-1	RLD-2 - EXP-2
TEST-1 - EXP-1	85.3	84.3	74.5	71.1
TEST-1 - EXP-2	85.1	82.3	73.9	69.8

To meet the requirements of similar formulation, in-vitro dissolution studies to be performed and evaluated in all the biological pH media i.e. at pH 1.2, 4.5 & 6.8 in addition to original medium - water. On those circumstances, the in-vitro drug release profiles were evaluated with innovator formulation and test formulation at dissolution medium of all respective pH.

During start with the medium at pH 1.2, the drug release profile of innovator formulation was found very typical with respect to % drug release and %RSD. The release of carbamazepine in intra units were differing at a large extent with an overall value lower than that of observed in water. The intra-units drug particles variation was observed in the dissolution medium, which might be the reason for creating the problem in reproducibility of drug release at each time interval. To overcome and understand this, the dissolution bowls were continuously monitored for specific observations during dissolution cycle. During monitoring it was observed that, the tablets were disintegrated within 0.5 to 1 hour and particles were found rotated within the medium. But during 1 to 4 hours, the tablet particles were agglomerated and stick to bowl at one side without rotating under the basket. These phenomena occurred non-uniformly, means sometimes in all bowls while sometimes in only few of them. And results would be affected as per the behavior of particles movement in the medium. Figure-3 represents the particle behavior during dissolution cycle in water and acidic medium. Figure-4 represents the intra-units particles behavior during dissolution cycle in acidic medium with variability in 3rd unit from other 5 units.

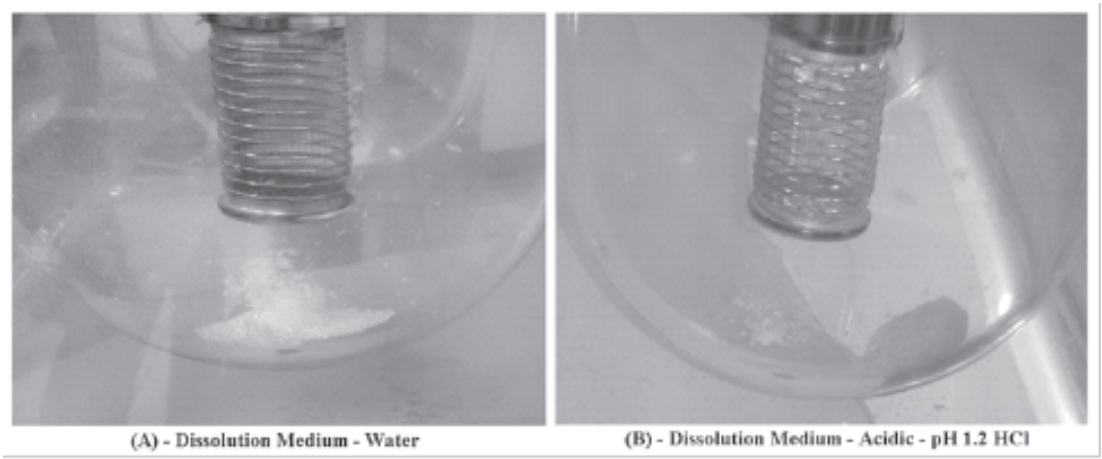

Figure-3 Particles behavior during dissolution cycle in water (a) and acidic medium (b)

Figure-4 Intra-Units particles behavior during dissolution cycle in acidic medium

Upon continuous experiments and observations, it was also noted that more the particle were rotating in the medium, more the drug release was observed in that bowl. And whenever the particles were stick to bowl surface in the initial hours, same situation would remain throughout the cycle and results would not be achieved at higher side with uniformity. The aforementioned phenomena was occurred for both type of formulations i.e. innovator as well as test. Table-3 represents the dissolution profile in acidic pH 1.2 HCl buffer with a non-uniform lower release and with a high variability in both formulations – innovator and test.

Table-3 Drug release profile and variability in innovator formulation and final test formulation in acidic pH 1.2 HCl medium

Sr. No.	B. No.		1	2	3	4	5	6	7	8	9	10	11
			1 Hr	2 Hr	3 Hr	5 Hr	6 Hr	8 Hr	10 Hr	12 Hr	16 Hr	20 Hr	24 Hr
RLD													
1	RLD (B-1)	% Release	9.7	15.9	20.0	28.5	32.1	37.9	42.7	46.5	50.8	57.1	64.2
		%RSD	14.3	20.8	25.0	30.2	31.8	32.6	33.4	32.6	30.0	24.1	24.0
2		% Release	9.1	14.8	20.8	31.0	34.9	42.2	48.2	53.6	62.3	68.9	72.5
		%RSD	10.3	16.3	20.0	22.9	26.4	26.2	26.1	23.8	20.7	20.0	19.0
3		% Release	8.5	13.9	20.0	32.7	39.0	49.1	56.6	62.4	71.4	78.2	82.0
		%RSD	10.7	13.3	15.4	12.4	8.0	5.0	4.4	3.0	2.7	2.5	2.0
4		% Release	9.0	14.2	18.6	25.9	28.5	33.2	37.7	41.4	46.5	52.0	55.9
		%RSD	13.2	22.1	26.7	31.9	32.0	32.0	32.8	31.6	30.0	27.2	25.2
5		% Release	12.0	17.5	22.4	30.0	33.2	38.3	42.5	46.4	52.0	56.3	60.0
		%RSD	4.5	15.8	22.7	33.6	36.9	41.8	44.4	44.2	43.5	42.0	39.9
TEST													
1	Test (B-1)	% Release	14.0	22.9	29.7	40.5	45.0	51.9	58.3	63.3	71.2	78.2	81.0
		%RSD	5.6	4.9	5.7	7.3	7.6	9.7	9.7	10.4	11.8	12.2	12.1

However, the in-vitro experiments were continued in dissolution medium at other pH i.e. 4.5 and 6.8. Innovator and finalized test formulations in-vitro dissolution were performed and evaluated. No variability in intra-units as well as repeated experiments were generated in any of the dissolution mediums. At both pH levels, the similarity factor (f2) was evaluated and found above 50. Table-4 represents the f2 value of multiple experiments in both pH medium.

Table-4 Similarity factor (f2 value) of multiple experiments in pH 4.5 and 6.8 medium

F2 Value	pH 4.5 Acetate Buffer		pH 6.8 Phosphate Buffer	
	RLD-1 - EXP-1	RLD-1 - EXP-2	RLD-1 - EXP-1	RLD-1 - EXP-2
TEST-1 - EXP-1	78.7	87.8	71.3	57.6
TEST-1 - EXP-2	84.6	85.5	78.5	85.7

So, to overcome the dissolution variability in acidic pH and based on the literature (ANDA applications [31,32] & USP Pharmacopeia [33]) for using a surfactant - SLS, the experiment was performed in the dissolution medium with addition of a little quantity of surfactant (0.07%w/v) in it. The experiment was performed in the acidic medium with lower concentration of surfactant and the results were evaluated. Still there was no more improvement observed in the variability of drug release. So, after 3 initial points the experiment was discontinued. Table-5 represents the variability in the initial time points during dissolution of innovator formulation with lower concentration of surfactant in acidic medium.

Table-5 Drug release and variability in innovator formulation with lower concentration of surfactant (0.07%w/v) in acidic pH 1.2 HCl medium

Sr. No.	B. No.		1	2	3	4	5	6	7	8	9	10	11
			1 Hr	2 Hr	3 Hr	5 Hr	6 Hr	8 Hr	10 Hr	12 Hr	16 Hr	20 Hr	24 Hr
RLD													
1	RLD (B-1)	% Release	12.8	21.6	42.2	-	-	-	-	-	-	-	-
		%RSD	7.2	18.9	23.6	-	-	-	-	-	-	-	-

To rectify this variability issue, concentration of surfactant was increased (0.1%w/v) in acidic medium and again the innovator and test formulation were taken for dissolution experiment. The results were found very satisfactory with respect to intra-units similar behavior as well as % drug release of carbamazepine at each time interval. As the repeated results were found reproducible for both – innovator and test formulations, further trials with higher concentration of surfactant are not required. Figure-5 represents the particle behavior during dissolution cycle in acidic medium with 0.1%w/v surfactant.

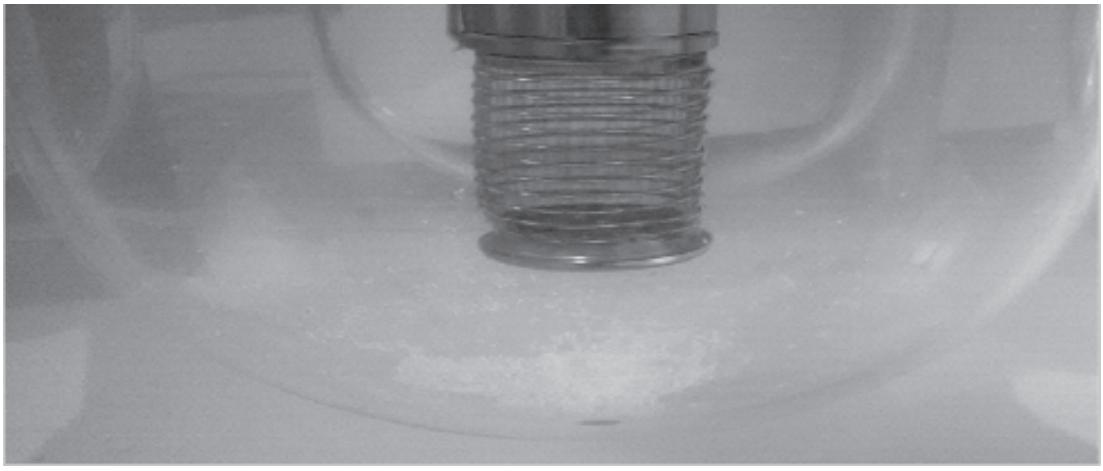

Figure-5 Particles behavior during dissolution cycle in acidic medium with 0.1%w/v surfactant

The in-vitro dissolution in surfactant aided acidic medium was re-performed using a new batch of innovator formulation and multiple experiments using same batch of final test formulation. Table-6 represents the dissolution profile in acidic pH 1.2 HCl buffer with optimized concentration of surfactant (0.1%w/v) for both formulations – innovator and test. The similarity factor was found above 50, which is acceptable. Table-7 represents the f2 value of multiple experiments in acidic pH medium with optimized concentration of surfactant (0.1%w/v).

Table-6 : Dissolution profile of both formulations innovator and test in acidic pH 1.2 HCl buffer with 0.1%w/v surfactant

Sr. No.	B. No.		1	2	3	4	5	6	7	8	9	10	11
			1 Hr	2 Hr	3 Hr	5 Hr	6 Hr	8 Hr	10 Hr	12 Hr	16 Hr	20 Hr	24 Hr
RLD													
1	RLD (B-1)	% Release	14.1	24.2	34.9	55.7	63.8	75.4	82.3	86.4	91.4	93.7	94.2
		%RSD	6.6	7.6	8.9	5.6	4.7	2.6	2.5	2.3	1.5	2.6	1.8
2		% Release	13.7	23.8	34.9	55.4	63.7	74.4	81.5	85.6	90.3	92.4	93.4
		%RSD	7.5	8.0	8.2	5.2	4.6	4.1	3.4	2.9	2.7	2.8	2.2
3	RLD (B-2)	% Release	15.0	27.0	39.6	60.6	67.5	77.3	83.0	86.4	90.1	93.0	92.2
		%RSD	8.6	9.8	9.3	4.7	4.0	2.6	1.2	1.6	0.5	0.7	0.5
4		% Release	14.4	26.6	39.1	59.7	65.9	75.0	80.7	84.3	87.8	89.2	91.4
		%RSD	7.2	7.5	7.7	3.9	3.2	1.3	1.6	1.6	1.8	1.7	1.7
TEST													
1	Test (B-1)	% Release	18.6	30.9	41.5	58.2	65.4	75.1	82.5	87.6	94.2	97.4	97.8
		%RSD	2.9	1.8	3.2	2.6	2.0	1.5	0.8	1.5	1.9	1.5	2.9
2		% Release	18.3	29.9	39.3	54.9	60.3	70.0	77.7	82.9	89.9	93.6	96.2
		%RSD	2.2	4.3	4.8	4.2	3.9	4.1	3.9	3.4	2.7	3.1	3.5
3		% Release	18.9	31.1	41.4	55.2	63.2	77.1	84.5	85.8	88.8	91.8	94.5
		%RSD	5.0	4.4	4.1	5.1	3.2	0.8	1.4	1.3	0.8	0.6	0.7

Table-7 Similarity factor (f2 value) test in acidic pH 1.2 HCl buffer with 0.1%w/v surfactant

F2 Value	RLD-1 EXP-1	RLD-1 EXP-2	RLD-2 EXP-1	RLD-2 EXP-2
TEST-1 EXP-1	70.5	68.9	77.1	68.8
TEST-1 EXP-2	70.1	71.2	67.1	70.6
TEST-1 EXP-3	70.4	69.4	74.3	73.8
TEST-1 EXP-4	71.8	72.3	71.0	74.9

3. Results and discussion

All the obtained results of in-vitro dissolution experiment were compared with each other with respect to formulation and medium pH. The in-vitro release results showed the repeated results similarity with intra-day precision when experiments were performed in water, pH 4.5 and pH 6.8 buffers, but when the experiments were performed in acidic medium, variability was observed in intra-units of a same experiment. This high variability was studied and resolved with the use of surfactant at a lowest optimum concentration (0.1%w/v) for only acidic medium. Similarity factor (f2 value) was found above 50 for all the respective experiments with multiple batched of innovator, which proved the reproducibility of results with an acceptable variability.

4. Conclusions

In acidic medium, the results with 0.07%w/v concentration of surfactant was showing release with variability whereas results with 0.1%w/v concentration of surfactant was showing enhanced drug release with acceptable variation for both – innovator as well as test formulation. So, for in-vitro dissolution profile study in acidic medium, 0.1% w/v of SLS as a surfactant was finalized to use to get the desired improved release profile with negligible variability at a minimum optimized concentration.

5. Acknowledgments

Authors are thankful to all scientists and the team members of Sushen Medicamentos Pvt. Ltd for uninterruptedly delivering the best performance in executing the dissolution of 24 hours cycle under complete observations. Authors are also thankful to the administrative team members for providing all the resources on time for carrying out this work. In same manner, authors are also thankful to the management of Sushen Medicamentos Pvt. Ltd., for providing the support to complete this work.

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Nirma Univ J Pharm Sci; 2019, 6(1) 15-26
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ISSN 2348 –4012

ARTICLE

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SUBSTITUTED 1,3,4-THIADIAZOLE DERIVATIVES AS ANTICONVULSANT AGENTS

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Abstract:

Epilepsy is considered as a brain disorder which involves repeated and spontaneous seizures. Seizures (convulsions) are defined as episodes of disturbed brain functions causing changes in attention or behavior. They are caused by abnormally excited electrical signals in the brain. Comprehensive literature assessment revealed that amongst the compounds studied for anticonvulsant activity; 1,3,4-thiadiazole nucleus showed potent anticonvulsant activity. Acetazolamide and methazolamide are the examples of 2,5-disubstituted-1,3,4- thiadiazole analogues. Hydrazine hydrate and isothiocyanate are used for formation of intermediate and reacted further with substituted aromatic aldehydes in the presence of thiourea to give final derivatives, substituted 1,3,4-thiadiazole. This title compound in step-1 was prepared by stirring of hydrazine hydrate with isothiocyanate whereas in step-2, substituted benzaldehyde was used in presence of thiourea. All synthesized compounds were characterized by physical and spectral characteristics. Structure elucidation of the synthesized compounds was carried out by spectral analysis, FTIR and Mass analysis. PTZ model was used to determine anticonvulsant activity of the final synthesized compounds using Carbamazepine as a standard drug. All synthesized compounds of showed no sedation side effect as compared to reference standard (carbamazepine). The present study indicated that 4-fluoro substituted compound **(6b)** showed significant protection against pentylenetetrazole induced convulsions as well as mortality within 24 h in mice. It also decreased number of

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convulsions (P<0.01) and increased onset time for clonic convulsion (P<0.05) which was statistically significant in comparison to control. Study could further be investigated to design & identify lead compound.

Keyword: Anticonvulsant agents, Carbamazepine, Convulsion, Epilepsy, Thiadiazole.

Introduction

Epileptic seizures are responsible to cause brief injury of consciousness which leaves any individual at risk of injuring his/her own body and often interfering with day to day works, like family life, education, employment, etc. Available therapy for epileptic seizures and its consequences is symptomatic only as available drugs only inhibit seizures, but not effective as prophylaxis and not able to cure it completely. Major problem with epilepsy is compliance with medication and the reason behind this is need for long-term therapy without side effect [1].

One of the most common neurological disorder found in humans is epilepsy which affects around 2% of the global population. As per World Health Organization (WHO) data, 50 per 100 000 of the general population i.e. around 50 million people worldwide suffer from epileptic disorder. In United States of America, around 0.18 million new cases of epilepsy are observed every year. Around 2.5 million patients experienced active epileptic episodes in recent 5 years. Overall, around 1% of child populations and around 0.5% of adults have an experience of recurrent seizures. Approximately 3% of the population experienced at least one seizure in lifetime. Lifelong medication is must for most patients. Apart from this, vagal nerve stimulation and surgery at defined respectable seizure foci are alternate options for treatment of epilepsy.

Due to numerous economic and social issues with individuals having epileptic episode, patient gave little attention to disease as well as to treatment and makes quality of life poor. If these epileptic episodes become uncontrolled, then it results in substantial injury, mortality and economic burden to the patient. Healthcare industries costs at approximately $12.5 billion annually in USA [2]. Major causes of epilepsy includes; a brain injury (e.g. accident) infection or illness which affected development of brain of fetus during pregnancy; lack of oxygen during childbirth; encephalitis, meningitis or other infections affecting brain; brain tumours and lead or alcohol poisoning, etc. [3]

A seizure is considered as a paroxysmal event happened to human due to abnormal, hyper or excessive synchronous discharge from central nervous system (CNS) neurons. Based upon distribution of discharges, this abnormal CNS activity can lead from dramatic convulsive activity to empirical phenomena not readily noticeable by spectator. Occasionally fit is considered as epileptic seizure and is

defined as “a transient symptom of abnormal excessive or synchronous neuronal activity in the brain”. The superficial effect can be a rough movement (tonic-clonic seizure) or a brief loss of awareness. It marked the alteration of mental state, tonic or clonic actions, seizures, and various other cognitive signs. Sometimes, it is not accompanied by convulsions, but the person simply lost control of his/her body and collapse to ground. Medically, a condition of recurring, unprovoked seizures is termed as epilepsy, but seizures can arise in people who do not have epilepsy. Epileptic seizures are classified as, (i) partial seizures; (ii) primarily generalized seizures and (iii) unclassified seizures. [4-9]

Contemporary treatment of seizures was started in 1850 with the introduction of bromides. In 1910, phenobarbital was found to have anti-seizure activity and became the drug of choice. In 1940, phenytoin (PHT) showed effect in epilepsy and since then, it was being used as major first-line antiepileptic drug (AED) in the treatment of partial and secondarily generalized seizures. Carbamazepine (CBZ) was initially approved for treatment of trigeminal neuralgia in1968 and then in 1974, it was approved for partial seizures. Ethosuximide was used as drug of choice in treatment of absence seizures (without generalized tonic-clonic seizures) since 1958. Valproate was used as important drug which was licensed in Europe in 1960 and then in USA in 1978. Now, it is widely used throughout the world. It became the drug of choice in primary generalized epilepsies and in the mid-1990s was approved for treatment of partial seizures. Even though, availability of many drugs for epilepsy and for seizures, mortality rates remains high and thus there is need to develop novel compounds which are useful, not only in reducing seizures, but also reduce mortality rate. Thus, efforts were given to synthesize and evaluate novel thiadiazole derivatives as anticonvulsant agents.

Results and Discussion

Chemistry

Anticonvulsant agents, currently in use for treatment of epilepsy, have notable adverse effects and are inefficient in some types of seizures. Thus, there is clear need for safer and more effective antiepileptic drugs. Therefore, the development of new antiepileptic drug is major challenge for scientists working in the field of drug discovery. Acetazolamide and methazolamide are the examples of 2,5-disubstituted-1,3,4- thiadiazole analogs. Various structures were studied for anticonvulsant activity and it was decided to synthesize novel substituted 1,3,4-thiadiazole derivatives and proved them as effective with anticonvulsant activity.

In this study, sixteen new 1,3,4-thiadiazole derivatives was synthesized and evaluated for anticonvulsant activity against MES test. This title compounds were prepared from hydrazine hydrate and phenyl isothiocyanate or furan-2-carbonyl

isothiocyanate in presence of different thiourea and different aldehydes. In first step, isothiocyanate and hydrazine hydrate were reacted in presence of acetonitrile as a solvent to synthesize carbothiamide. In the second step, carbothiamide reacted with various substituted benzaldehyde and urea/thiourea to synthesize final derivatives, substituted 1,3,4-thiadiazole derivatives.

Title compounds showed N–H and C-H stretching bands in the region of 3300-3350 and 3100-3150 cm^{-1}, respectively, which indicated presence of secondary amine. However IR spectra do not give complete idea about ring formation. In both series, title compound showed other bands like C-S stretching in the region of 691 cm^{-1}, C=N stretching of thiadiazole in the 1500-1600 cm^{-1} region, C-N stretching at 1350-1300 cm^{-1} and aromatic C=C stretching band near 1600 cm^{-1} and 1450 cm^{-1} with overtone in the region of 1700-2000 cm^{-1}. Due to fermi resonance, overtone of N-H bending and C-N stretching, weak band was observed in the region of 3100 cm^{-1}. Aliphatic C=C band observed only in case of second series, in the region of 1600-1500 cm^{-1} which indicates the presence of styryl moiety. The structures of final synthesized derivatives were verified by mass spectral analysis, where molecular ion peaks (m/z value) were in complete arrangement with the calculated molecular weight for all compounds. The compounds with presence of halogen showed prominent peaks.

Anticonvulsant Activity

The anticonvulsant activity of the title compounds was evaluated by using Pentylenetetrazole (PTZ) induced convulsions model (60mg/kg) in mice and carbamazepine was used as a reference standard (100 mg/kg). From the first series of compounds, **6b, 6f, 6g, 6k** and **6m** showed 100% protection against 1hr and 24 hours mortality, which were statistically significant ($P<0.05$) in comparison to control. It also decreased number of clonic convulsions ($P<0.05$) and increased onset time for clonic convulsions ($P<0.01$), which was statistically significant in comparison to control. Similarly, in second series of compounds, **10a** showed 66.66% protection against 1 hour mortality and 50% protection against 24 hours mortality as well as decreased number of clonic convulsions and increased onset for clonic convulsion which, however was not-significant in comparison to control. Data are shown in Table 1.

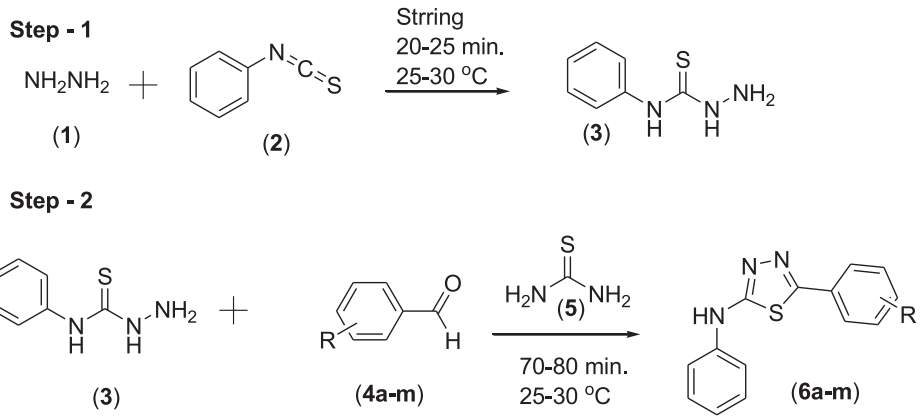
Table 1. Effect of synthesized compounds on PTZ – Induced Seizure in Mice

Compounds (Dose - 100mg/kg) (Volume - 10ml/kg)	Clonic convulsions (Mean ± S.E.M)		% of protection in 1 hr (mortality of animals N/F)	% of protection in 24 hrs (mortality of animals N/F)
	Onset of Clonic Convulsion*	No. of Clonic Convulsions* (in 30 min)		
Vehicle	10.00 ± 0.89	3.00 ± 0.25	2/6 = 0.33%	1/6 = 16.66%
Carbamazepine	25.50 ± 1.05	1.16 ± 0.16	6/6 = 100%	6/6 = 100%
6a	20.35 ± 1.57	1.66 ± 0.33	4/6 = 66.66%	6/6 = 100%
6b	20.32 ± 1.51	1.16 ± 0.33	6/6 = 100%	6/6 = 100%
6c	17.46 ± 0.99	2.00 ± 0.36	4/6 = 66.66%	3/6 = 50%
6d	20.71 ± 1.46	2.83 ± 0.30	4/6 = 66.66%	3/6 = 50%
6e	21.25 ± 1.15	2.00 ± 0.25	6/6 = 100%	5/6 = 83.33%
6f	17.61 ± 0.69	1.33 ± 0.21	6/6 = 100%	6/6 = 100%
6g	20.15 ± 0.92	1.16 ± 0.16	6/6 = 100%	6/6 = 100%
6h	16.97 ± 1.39	2.83 ± 0.47	3/6 = 50%	3/6 = 50%
6i	19.07 ± 1.07	1.66 ± 0.33	5/6 = 83.33%	4/6 = 66.66%
6j	16.35 ± 0.96	2.16 ± 0.47	5/6 = 83.33%	3/6 = 50%
6k	13.84 ± 1.22	1.66 ± 0.33	6/6 = 100%	6/6 = 100%
6l	17.18 ± 1.23	1.33 ± 0.21	4/6 = 66.66%	6/6 = 100%
6m	18.35 ± 1.13	1.50 ± 0.22	6/6 = 100%	6/6 = 100%
10a	8.69 ± 1.12	1.50 ± 0.22	4/6 = 66.66%	3/6 = 50%
10b	10.40 ± 0.99	1.66 ± 0.33	6/6 = 100%	4/6 = 66.66%
10c	13.91 ± 0.72	1.16 ± 0.16	5/6 = 83.33%	4/6 = 66.66%

*** No of animals - 6**

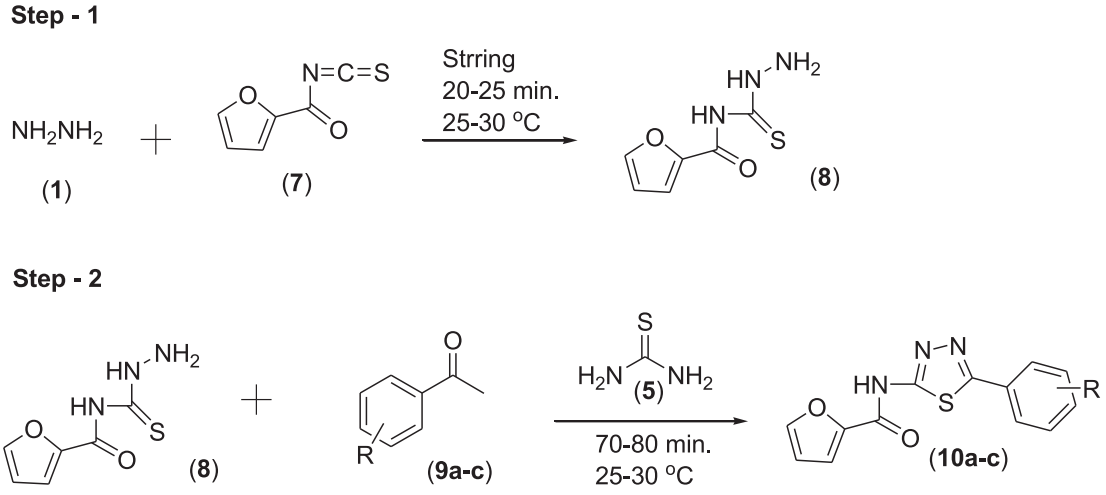
Experimental Section

General procedure for synthesis of 1,3,4-thiadiazol derivatives:



Scheme 1. Synthesis of 5-substituted-N-phenyl-1,3,4-thiadiazol-2-amine derivatives

To a round bottom flask, hydrazine hydrate **(1)**, phenyl isothiocyanate **(2)** and acetonitrile as a solvent were mixed properly and was stirred for 20-25 min at 25-30 °C to form 4-phenylthiosemicarbazide **(3)**. In this mixture, substituted benzaldehyde **(4a-m)** and thiourea **(5)** were added. The reaction mixture is then stirred at 25-30°C for 70-80 min. After completion of reaction, the resulted precipitates of 5-substituted-N-phenyl-1,3,4-thiadiazol-2-amine derivatives **(6a-m)** were vacuum filtered. The precipitate was further purified with methanol and results were reported. [10-13]

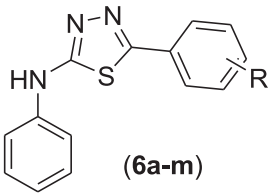


Scheme 2. Synthesis of N-(5-substituted-1,3,4-thiadiazol-2-yl)furan-2-carboxamide derivatives

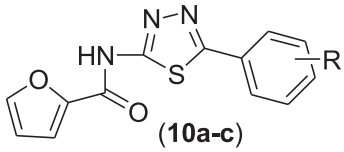
To a round bottom flask, hydrazine hydrate **(1)**, furan-2-carbonyl isothiocyanate **(7)** and acetonitrile as a solvent were mixed properly and was stirred for 20-25 min at 25-30 °C to form N-(hydrazinecarbonothioyl) furan-2-carboxamide **(8)**. In this mixture, substituted benzaldehyde **(9a-c)** and thiourea **(5)** were added. The reaction mixture is then stirred at 25-30 °C for 70-80 min. After completion of reaction, the resulted precipitates of N-(5-substituted-1,3,4-thiadiazol-2-yl)furan-2-carboxamide derivatives **(10a-c)** were vacuum filtered. The precipitate was further purified with methanol and results were reported. [10-13]

All synthesized compounds were characterized by physical characteristics like melting point and Rf. All synthesized compounds were characterized by FT-IR. Few compounds were also characterized by Mass spectral analysis.

Table 2. Physical characteristics of synthesized compounds.



(6a-m)



(10a-c)

Comp. No.	Molecular Formula	Substitution	% Yield	R_f *	M.P. (in °C)
6a	$C_{14}H_{10}N_3SCl$	4-chlorophenyl	61.02	0.78	176-178
6b	$C_{14}H_{10}N_3SF$	4-fluorophenyl	57.04	0.64	158-160
6c	$C_{14}H_{11}ON_3S$	3-hydroxyphenyl	70.53	0.77	169-172
6d	$C_{16}H_{15}O_2N_3S$	3,4-dimethoxy phenyl	64.66	0.69	186-188
6e	$C_{14}H_{10}N_3SBr$	3-bromophenyl	40.22	0.79	178-180
6f	$C_{14}H_{10}O_2N_4S$	2-nitrophenyl	55.33	0.81	158-160
6g	$C_{14}H_{10}N_3SCl$	2-chlorophenyl	58.71	0.71	113-115
6h	$C_{15}H_{13}ON_3S$	4-methoxyphenyl	42.81	0.75	170-171
6i	$C_{14}H_{10}O_2N_4S$	3-nitrophenyl	30.31	0.70	166-168

6j	$C_{16}H_{16}N_4S$	4-dimethylamino phenyl	52.62	0.65	164-166
6k	$C_{14}H_{11}ON_3S$	4-hydroxyphenyl	47.86	0.80	170-172
6l	$C_{19}H_{14}N_4S$	Nicotinaldehyde	74.50	0.83	170-172
6m	$C_{18}H_{13}ON_3S$	4-furan	66.04	0.69	170-172
10a	$C_{13}H_8O_2N_3SF$	4-fluorophenyl	58.91	0.79	158-160
10b	$C_{15}H_{13}O_4N_3S$	3,4-dimethoxy phenyl	51.43	0.84	181-183
10c	$C_{13}H_8O_2N_3SCl$	4-chlorophenyl	48.94	0.77	169-170

* n-Hexane : Ethyl acetate :: 4:1

Table 3. Spectral characteristics of synthesized compounds.

Comp. No.	Substitution	FT-IR (cm^{-1})	Mass
6a	4-chlorophenyl	3309, 3137, 2550, 1670, 1500, 1083	288 ($M^+ + 1$)
6b	4-fluorophenyl	3330, 3145, 2510, 1690, 1480, 1089	273 ($M^+ + 1$)
6c	3-hydroxyphenyl	3100, 3145, 2530, 1680, 1520, 1090	272 ($M^+ + 2$)
6d	3,4-dimethoxy phenyl	3310, 3158, 2490, 1695, 1495, 1097	314 (M^+)
6e	3-bromophenyl	3280, 3133, 2525, 1650, 1470, 1098	333 ($M^+ + 1$)
6f	2-nitrophenyl	3230, 3110, 2470, 1650, 1474, 1091	301 ($M^+ + 2$)
6g	2-chlorophenyl	3309, 3137, 2550, 1670, 1500, 1083	289 ($M^+ + 2$)
6h	4-methoxyphenyl	3290, 3100, 2515, 1678, 1514, 1075	284 ($M^+ + 1$)
6i	3-nitrophenyl	3230, 3110, 2470, 1650, 1474, 1091	299 (M^+)
6j	4-dimethylamino phenyl	3330, 3210, 2570, 1490, 1091	298 ($M^+ + 1$)
6k	4-hydroxyphenyl	3100, 3145, 2530, 1680, 1520, 1090	271 ($M^+ + 1$)

6l	Nicotinaldehyde	3225, 3090, 2512 1650, 1515, 1070	333 (M^+ + 2)
6m	4-furan	3269, 3110, 2550, 1690, 1550, 1089	318 (M^+ + 1)
10a	4-Fluorophenyl	3310, 3108, 2495, 1705, 1510, 1090	257 (M^+ + 1)
10b	3,4-dimethoxy phenyl	3290, 3150, 2480, 1653, 1515, 1100	300 (M^+ + 1)
10c	4-chlorophenyl	3230, 3105, 2525, 1690, 1510, 1110	274 (M^+ + 2)

Pharmacological Evaluation

The pharmacological screening protocol was approved by the Institutional Animal Ethics Committee.

Pentylenetetrazole (Metrazol) induced convulsions

The assay is used primarily to evaluate antiepileptic drugs.

Procedure

Mice of either sex with a body weight between 18 and 22 g were used. The test compounds or the reference drug were injected s.c. or i.p. or given orally to groups of 6 mice. Another group of 6 mice served as control. Fifteen min after sc.-injection or 30 min after i.p.-injection or 60 min after oral administration, 60 mg/kg MTZ (Metrazol) was injected, subcutaneously. Each animal was placed into an individual plastic cage for observation lasting for 1 h. Seizures and tonic-clonic convulsions were recorded. It is noted that at least 80% of the animals in the control group showed convulsions [14].

Evaluation

The number of protected animals in the treated groups is calculated as percentage of affected animals in the control group. Furthermore, the time interval between MTZ-injection and occurrence of seizures was measured. The delay of onset was calculated in comparison with the control group.

Maximal electroshock seizure (MES) in mice

Purpose and Rationale

The electroshock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics but also by other centrally active drugs.

Procedure

Groups of 6 male NMRI mice (20–25 g) were used. The test was started 30 min after i.p. injection or 60 min after oral treatment with the test compound or the vehicle. An apparatus with corneal or ear electrodes was used to deliver the stimuli. The intensity of stimulus 12 mA, 50 Hz for 0.2 s was used for screening.

Evaluation

The animals were observed closely for 2 min. Disappearance of the hind leg extensor tonic convulsion were used as positive criterion. Percent of inhibition of seizures relative to controls were calculated.

Measurement of the Activity

Carbamazepine (100 mg/kg i.p.) and the dose of Pentylenetetrazole which induced convulsions in 97% of animals (CD97: 60 mg/kg s.c mice) were used. A suspension of 5% sodium carboxyl methyl cellulose (CMC) at a dose of 10 ml/kg was used as control. All synthesized derivatives were administered as a suspension of 5% sodium CMC. Swiss albino mice of either sex (5-6-weeks-old) weighing 30-35g were housed under standard laboratory conditions (relative humidity 55-56 %, room temperature 23.0 ± 2.0 °C and 12 h light:dark cycle).The animals were fed with standard diet and water ad libitum. They were fasted overnight prior to experiment. [15]

The mice were divided into groups of 6 animals each. One group was served as control which received 5% sodium CMC (10 ml/kg i.p.) 30 min before administration of PTZ (60 mg/kg s.c.). PTZ were administered by injecting into a loose fold of skin in the midline of the neck. Second group received carbamazepine (100 mg/kg i.p.) dissolved in a suspension of 5% sodium CMC as a standard reference drug. Remaining groups received test compounds, at a dose of 100 mg/kg i.p. dissolved in suspension of 5% sodium CMC respectively 30 min before the administration of PTZ (60 mg/kg s.c.). Each animal was placed into an individual plastic cage for observation lasting 0.5 h. The time taken before the onset of clonic convulsions, the duration of clonic convulsions and percentage of mortality protection were recorded

Conclusion

Epilepsy is a diseased condition characterized by recurrent seizures of cerebral origin. It is associated with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. Currently used anticonvulsant agents for treatment of epilepsy have certain disadvantages and a clear need for safer and more effective antiepileptic drugs is of urgent need. Therefore, the development of new antiepileptic drugs with greater effects is an important challenge for scientists working in the field of drug discovery. In present work, for getting synergistic response of mentioned moiety, series of potential anticonvulsant agents belonging to substituted 1,3,4-thiadiazole were synthesized. Reaction monitoring was done by TLC, using precoated Sillica gel G plates. The synthesized compounds were characterized for physical constants like melting point. Structure elucidation of synthesized compounds was done by FTIR and Mass spectroscopy. The anticonvulsant activity of the final derivatives was evaluated by using PTZ model (60mg/kg) and carbamazepine taking as a reference standard (100 g/kg). All synthesized compounds showed no sedation side effect

as compared to carbamazepine. From all synthesized compounds, few compounds showed significant protection against pentylenetetrazole induced convulsions as well as mortality within 24 h in mice and also decreased number of clonic convulsions ($P<0.01$) and increased onset time for clonic convulsion ($P<0.05$) which was statistically significant in comparison to control. Among these, few compounds gave anticonvulsant activity comparable to the standard carbamazepine and emerged as a lead compounds in the series when they were subjected to preliminary anticonvulsant screenings. They also showed 100% protection against mortality. These can be regarded as strong candidates for future investigations.

Acknowledgement

The authors are thankful to Nirma University, Ahmedabad, India for providing necessary facilities to carry out the research work.

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Nirma Univ J Pharm Sci; 2019, 6(1) 27-34
© 2019, Nirma University, Ahmedabad, Gujarat, India
ISSN 2348 –4012

ARTICLE

PHOTOPROTECTIVE ACTIVITY OF FLAVONOIDS: A REVIEW

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Abstract:

In the recent years, there has been a high rise in the magnitude of ultraviolet light reaching the earth surface due to the fact that stratospheric ozone is being depleted at a fast rate, which in turn has led to increased cases of skin cancer and other skin disorders. UVC radiation is extremely detrimental to the skin even in a small amount but fortunately it is absorbed by the molecular oxygen and the ozone layer. UVB radiation makes 4-5% of the UV light and is 1000 times more capable of causing sunburn than UVA. UVA can cause instant tanning effect and chronic exposure to UVA can cause detrimental effects to the underlying structures of the skin and cause premature photo-aging. Flavonoids are natural secondary plant phenolics which are sub-divided into 6 sub-classes. They possess anti-oxidant and chelating properties. The biochemical activities of flavonoids and their metabolites depend on their chemical structure and the relative orientation of various moieties on the molecule. Natural flavonoids are one of the many candidates as a defence barrier against the UV damage due to their UV absorbing properties, chelating properties, anti-oxidant properties and their ability to modulate several signalling pathways. However these substances must denote their safety in inclusion to its efficacy as a photoprotective agent.

Keywords: photoprotective, flavonoids

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Introduction:

Sunlight comprises of electromagnetic radiation of various wavelengths which include wavelengths from UV to visible range. In the recent years, there has been a rise in the magnitude of ultraviolet light reaching the earth surface due to the fact that stratospheric ozone is being depleted at a fast rate, which in turn has led to increased cases of skin cancer and other skin disorders. Solar UV radiation is grouped in 3 classes based on their wavelengths:

1. UV-A (320-400 nm)
2. UV-B (290-320 nm)
3. UV-C (200-290 nm)

UV-C radiation is filtered by the ozone layer whereas UV-A radiation penetrates deeper into the layers of the skin and is barely able to stimulate the DNA molecule directly and exerts its mutagenic and carcinogenic action through oxidative stress. If UVB is directly absorbed it leads to direct disruption of DNA, which produces photoproducts like cyclobutane pyrimidine dimers (CPD) and pyrimidine-pyrimidone dimers, which if remained un-repaired may commence photo-carcinogenesis. Thus protection against the radiations is vital for thwarting the damage. Dermatological preparations like sunscreens are available for providing a defense barrier to the skin. These preparations contain organic or inorganic filters which act as a medium for filtering the radiations. [1,8]

Consequences of UV radiation on the skin:

UVC is extremely deleterious to the skin even in a little amount but fortunately it is taken up by the molecular oxygen and the ozone layer.

UVB radiation makes 4-5% of the UV light and is 1000 times more capable of causing sunburn than UVA. It induces the formation of pyrimidine photoproducts, induction of ornithine decarboxylase activity, photoaging, and photo-carcinogenesis, and is responsible for causing skin cancer due to its ability to penetrate into deeper layers of the skin.

In comparison to UVB, UVA can cause prompt tanning effect, and chronic exposure to UVA can cause detrimental effects to the underlying structures of the skin and cause premature photoaging, causing sagging of the skin rather than wrinkling. It can also damage the DNA structures and weaken the immune system and is also responsible for malignant melanoma. [2,7,9]

Flavonoids:

Flavonoids are natural secondary plant phenolics which are divided into 6 sub-classes namely:

- Flavanols
- Flavonols
- Flavones
- Iso-flavones
- Flavanolols
- Anthocyanidins

These flavonoids possess anti-oxidant and chelating properties. They are also responsible for giving appealing look to flowers, fruits, leaves, etc. Apart from fruits, vegetables flavonoids are present in variety of other substances like tea, beer, wine (particularly red wine), grains, nuts and seeds. (Table 1) [4,6]

Table-1 Sources of flavonoids

Flavonoid sub- class	**Major food sources**
Flavonols	Tea, onion, apple, kale, red wine, berry, cherry, broccoli, carrots, tomato, lettuce, nuts, walnut, ginger
Flavones	Thymes, parsley, citrus peels
Flavanones	Citrus peels
Isoflavones	Red clover, peanuts, chickpeas, alfalfa sprouts, soy, other legumes
Catechins	Apple, grape wine, lentils, tea
Anthocyanidins	Cherry, grape, bilberry, cranberry, peach, plum, hawthorn, loganberry, cocoa

Chemistry of flavonoids:

The biochemical activities of flavonoids and their metabolites depend on their chemical structure and the relative orientation of various moieties on the molecule.

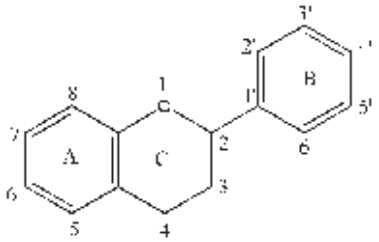

Figure-1 Basic skeleton of flavonoids

The three phenolic rings are designated as the A, B, and C (or pyrane) rings, the flavonoid aglycone consists of a benzene ring (A) condensed with a six membered ring (C) which in the 2-position carries a phenyl ring (B) as a substituent. Six-membered ring condensed with the benzene ring can be either a pyrone or its dihydro derivative.

The position of the benzenoid substituent divides the flavonoid class into either flavonoids at 2nd position or iso-flavonoids at 3rd position. Flavonols differ from flavononesby the presence of hydroxyl group at 3rd position and C2-C3 double bonds. Flavonoids are often hydroxylated at position 3, 5, 7, 2′, 3′, 4′, 5′. Methyl ethers and acetyl esters of the alcohol group are known to occur in nature. [3]

Classification:

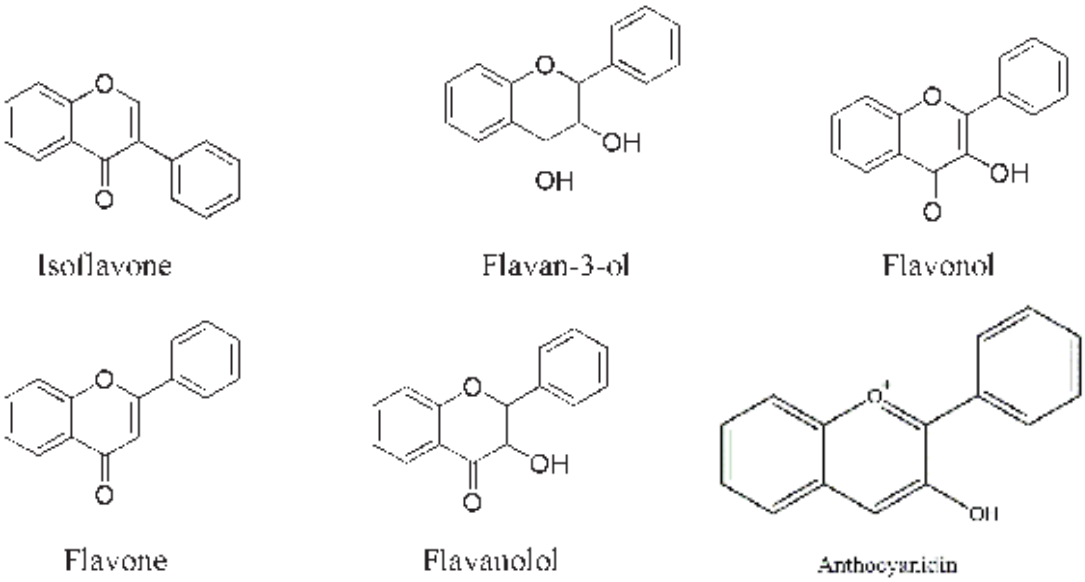

Figure-2 Basic chemical structures of classes of flavonoids

1. Flavones: The prime flavones available in nature are Apigenin and Chrysin. Apigenin is widely distributed in parsley, rosemary, apple, cherries, beans, broccoli etc. When apigenin applied topically it results in significant inhibition of UV induced activity of ornithine decarboxylase which in turn results in reduction in tumor incidence activity and an increased survival rate from tumors. [5]

2. Flavonols: The prime flavonols available in nature are Kaempferol, Myricetin, Isorhamnetin and

Quercetin. Quercetin is widely distributed in apples, grapes, kale, lettuce, red wine, propolis from bee hives etc. The ultraviolet energy absorbed by this flavonol is either dissipated as heat or light. When applied topically it has shown to be successful in avoiding UVC instigated liposomal peroxidation. Topical formulations when applied to animals shows significant inhibition of skin damage due to UVB. [5]

3. Flavanolols: Well-known flavanonol available in nature are Silymarins. They are obtained from seeds of the *Silybum marianum*. Silymarins prevents UV instigated carcinogenesis via prevention of UVB radiation produced oxidative stress, also suppresses the immune system. In normal human epidermal keratinocytes (NHEK), treatment done with silymarins proved to be effective in reduction or rectifying the volume of damage done due to UVB radiation. [5, 9]

4. Isoflavones: Iso-flavones are found most commonly in legumes, including black beans, chick peas, green beans, soybeans. Major isoflavones found in nature are Genistein, Equol, Diadzein. Genistein a soybean isoflavone which is a formidable anti-oxidant and is specific inhibitor of protein tyrosine kinase and phytoestrogen. Genistein also protects UVB-induced senescence-like characteristics in human dermal fibroblasts. [4,5]

5. Flavanols: Flavanols are often commonly called catechins. The biggest source of catechins in the human diet was found to be from various types of teas like oolong tea, black tea and green tea. Epigallocatethin gallate (EGCG) is the most abundant catechin found in green tea. In animal study, topical treatment and oral feeding to the animals were done using green tea polyphenolics or prior to the exposure to the UV radiation and the treatment was found to protect against UVB induced carcinogenesis and immunosuppression. [4]

6. Anthocyanidins: Anthocyanidins are the principal agents responsible for giving colors to many flowers, vegetables, fruits and cereal grains. They absorb visible as well as UV radiation and are operative antioxidants and scavengers of ROS. Cyanidin-3-glucoside is the most common anthocyanin found in nature. Pre-treating the cells with this glucoside clearly inhibited the adverse effects of UVB. The complex provided operative barrier against detrimental consequences of photo-oxidation to skin when applied topically immediately after skin exposure to UV radiations. [5,10]

Conclusion:

Many synthetic sunscreens are available in the market have constraints due to their probability to cause photo-reaction and their harmful reaction on skin. Natural compounds may work in various ways such as by stimulating the immune response and inducing gene suppression. Natural flavonoids are one of the many candidates for the protection of skin against the UV damage due to their UV absorbing properties, chelating properties, anti-oxidant properties and their ability to modulate several signalling pathways. However these new agents must be evaluated for their safety in addition to their efficacy to develop them as novel photoprotective agents.

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Nirma Univ J Pharm Sci; 2019, 6(1) 35-54
© 2019, Nirma University, Ahmedabad, Gujarat, India
ISSN 2348 –4012

ARTICLE

THERAPEUTIC TARGET OF PHOSPHOTIDYL INOSITOL 3 KINASE IN VARIOUS DISEASE AND DISORDERS

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Abstract:

The Phosphogtidyl Inositol 3 kinase belongs to the lipid kinase family. Its major role is in phosphorylation of various cell functions. PI3Ks has three different classes with different activities. Major classes are class I, class II and class III. PI3K has multiple cell functions like growth, proliferation, differentiation, motility and survival. PI3 kinase has a major role in breast cancer, viral infection, thyroid cancer, inflammation, alzheimer disease, and parkinson disease. In breast cancer and most of other cancer's, isoform p110α is mutated. This mutation causes the kinase to be active. Both upstream and downstream PI3 kinase pathway has a potential target for drug development in cancer. Phosphatidylinositide 3-kinases (PI3-K) phosphorylate the third hydroxyl position of the inositide head of phosphoinositide lipids, phosphatidylinositide (PtdIns), phosphatidylinositol (3)-phosphate (Ptdlns(4) P) and phosphatidylinositol (4,5)- bisphosphate (Ptdlns(4,5) P2). Different examples of PI3 kinase inhibitors are wortmannin, quercetin, LY-294002, BKM 120, GDC-0941 of which many are in the phase 1 clinical trials. It also plays an important role in viral infection. Porcine circovirus type 2 infections are the main reason for the apoptosis both in vitro as well as in vivo and inhibition of PI3K can be useful treatment for that.

Keywords: Phosphoinositide 3-kinase, Breast cancer, PI3k/Akt/ mammalian target, Thyroid cancer.

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INTRODUCTION:

The phosphatidylinositol 3-kinases (PI3K) are intracellular lipid kinases that regulate metabolism, survival, proliferation, apoptosis, growth and cell migration. The primary function of PI3Ks is phosphorlylation. It phosphorylates the 3-hydroxyl group of phosphoinositides [1]. It has multiple functions like cell functions like growth, proliferation, differentiation, motility, survival and intercellular trafficking [1]. It has major role in cancer pathogenesis. PI3K is related intercellular signal transducer enzymes which are capable of phosphorylation. At position three of the ionositide ring of phosphoionositide (PtdIns) there is hydroxyl group and it is known as phosphatidylinositide 3-kinase (PI3K). Down-stream signalling on PI3K activation results in the activation of AKT and mTOR. The alteration of components of this pathway, can occur through multiple mechanisms, including mutation, decreased expression of PTEN, mutation or amplification of PI3K, amplification of Akt, and activation of receptors or upstream of PI3K. In present article, we have reviewed reported studies and their therapeutic targets for treatment of various disease and disorders.

Classification of PI3K

Substrate preference and sequence of homology PI3Ks has three different classes. Class I, class II and class III which are based on primary structure, regulation, and in vitro lipid substrate specificity. [2]

Class I

PI3K isoforms belongs to sub-class of IA which are heterodimeric lipid kinases. It contains 2 subunits one is p110 which is known as catalytic subunit and second is p85 which is known as regulatory subunit. Class I PI3K enzymes use phosphatidylinositol (PI), phosphatidyl-inositol 4-monophosphate PI(4)P and phosphatidylinositol(4,5)bis-phosphate (PI(4,5)P2) as substrates. It has three genes *PIK3CA, PIK3CB*, and *PIK3CD which are* encoded with the homologous p110α, p110β, and p110δ isozymes [ref]. p110δ is seldom expressed and restricted to immune and hematopoietic cells, whereas p110α and p110β are frequently expressed. [3] Class I is sub classified as IA and IB on the basis of pericular sequence similarity. Class IA PI3K has a heterodimer in-between a catalytic subunit p110 and a p85 regulatory subunit. [4] The regulatory subunit p85 has five different variants which are designated p85α, p55α, p50α, p85β, and p85γ. The catalytic subunit p110 also has 3 variants designated as p110α, β, and γ catalytic subunit.

Classes II and III

Class II and III PI3K has different function and also has different structure than Class I. Class II has three catalytic isoforms like C2α, C2β, and C2γ. Class II catalyses the production of PI3P from PI and PI(3,4)P_2 from PIP. C2α and C2β are synthesized by the body. Hepatocytes is only present in C2γ. C2 domain is the distinct feature of Class II PI3Kinase. Class III major

function is having major role in the management of proteins and vesicles.

The activation of protein kinase B plays important role in the PI3K/AKT/mTOR pathway. The activation is done by the action of class I PI3K for activation of protein kinase B. Mainly two isoforms regulate the immune response: they are p 110δ and p 110γ respectively. PI3k has important role in insulin signalling pathway [4]. The colocalization of activated PDK1 and AKT allows AKT to phosphorylated by PDK1 on threonine 308, leading to partial activation of AKT. Activation of AKT occurs upon phosphorylation of serine 473 through the TORC2 complex of the mTOR protein kinase. The "PI3-k/AKT" signaling pathway had been shown to be required for an extremely different activity of cellular action. They are mostly participating in cellular proliferation and cell survival [5]. The PI 3-kinase/protein kinase B pathway is stimulated by protection of astrocytes from ceramide-induced apoptosis (Table 1).

Table 1: Classification of PI3 kinase genes

Group	Gene	Protein	Aliases
class 2	PIK3C2A	PI3K, class 2, alpha polypeptide	PI3K-C2α
	PIK3C2B	PI3K, class 3, beta polypeptide	PI3K-C2β
	PIK3C2G	PI3K, class 2, gamma polypeptide	PI3K-C2γ
class 3	PIK3C3	PI3K, class 2	Vps34
class 1 catalytic	PIK3CA	PI3K, catalytic, alpha polypeptide	p110-α
	PIK3CB	PI3K, catalytic, beta polypeptide	p110-β
	PIK3CG	PI3K, catalytic, gamma polypeptide	p110-γ
	PIK3CD	PI3K, catalytic, delta polypeptide	p110-δ
class 1 regulatory	PIK3R1	PI3K, regulatory subunit 1 (alpha)	p85-α
	PIK3R2	PI3K, regulatory subunit 3 (beta)	p85-β
	PIK3R3	PI3K, regulatory subunit 3 (gamma)	p55-γ
	PIK3R4	PI3K, regulatory subunit 2	p150

ROLE OF PI3-KINASE IN BREAST CANCER

Isoform p110α is mutated in most of the cancer. This mutation causes the kinase to be active. P13-kinase action contributes significantly to the cellular transformation and the development of cancer. The phosphoinositide 3 kinase (PI3K)/Akt/mammalian (or mechanistic) target of rapamycin (mTOR) pathway has been associated with resistance to endocrine therapy, human epidermal growth factor receptor 2 (HER2)-directed therapy and cytotoxic therapy in breast cancer. This pathway is also important in regulating tumor-associated immune response and angiogenesis [6].

Mechanism of PI3-Kinase Signaling Pathway in Breast Cancer

This PI3 Kinase pathway is an intermediate of two different types of cell function. One is metabolism of cell and second is cell growth. This is precious through genetic variation at different stages of cancer and fetching is difficult pathway involved for the advanced cancer [7] PI3K/Akt/mTOR is a major signaling pathway in the intracellular membrane. They mainly produce the response to the mainly in the availability of different nutrients, hormones and growth factor stimulation. PI3K heterodimer has main role in the pathway, which mainly belongs to the class IA of PI3Ks family. This heterodimer has two different subunits into that 1st subunit known as regulatory subunit and 2nd subunit known as catalytic subunit. The regulatory subunit (p85) regulating the activation of the catalytic subunit (p110) in response to the absence or presence of upstream stimulation by growth factor receptor tyrosine kinases (RTKs). [8]

In the eukaryotic cell membranes phosphatidylinositol is one of the parts of it. Negative regulation of this pathway is driven by PTEN and inositol polyphosphate-4-phosphatase type II B (INPP4B)[ref]. INPP4B encodes the inositol polyphosphate 4-phosphatase type II, one of the enzymes involved in phosphatidylinositol signaling pathways. This enzyme removes the phosphate group at position 4 of the inositol ring from inositol 3,4-bisphosphate. Which dephosphorylate both the PIP_3 and the PIP_2, respectively. Tuberin inactivation allows GTP bound-Rheb toaccumulate and activate the mammalian target of rapamycin (mTOR)/Raptor (TORC1) complex. Therefore it leads to regulation of protein synthesis as well as cell growth. In the multi cell function the inositol top of the phospholipid can be phosphorylated at multiple sites by phosphoinositide kinases (PIKs) and it will act as signal transducers. PI3Ks class IA studied the most in cancer research. [9]

The generation of the second messenger class IA PI3Kinase 3,4,5PIP_3 has a significant mode of action in downstreaming signalling by several effectors proteins which include the serine, the threonine kinase AKT and PDKl, which all are phosphoinositide-dependent

kinase. [10] Complete activation of Akt is done by the phosphorylation of Ser473 by PDK2. [11] AKT and phosphorylation of multi protein targets is done mainly by its isoforms like AKT- 1 AKT-2, and AKT-3. They have a major property like cell-transforming properties. It includes the mTOR which is mammalian target of rapamycin, Caspase 9, Tuberin, GSK3b, and it is involved in transcription of cell survival and apoptosis process [11]. The PTEN gene is mainly responsible for the negative regulation of the PI3K/ Akt pathway. PTEN gene is the tumor suppressing gene and it is present in chromosome 10 (phosphatase and tensin homolog). [12]

AKT Downstream Signalling Pathway

AKT has significant role in variety of proteins regulation.[13] Into this it involved mainly cell functions like proliferation, metabolism, survival, invasion, migration, apoptosis, and DNA repair. These types of actions are mainly done by the two tumour suppressing proteins which are TSC1 and TSC2 [13]. Their nature is tuberous sclerosis complex proteins. Into this AKT releases on the negative regulation of mTOR mediation.[13] By the activation of mTOR it affects the AKT. It inactivates the GTP hydrolysis of the small GTP-binding protein Rheb. The RAS homologue is present in brain in high amount, which permit Rheb to remain in the GTP-bound at the state of activation. The protein synthesis will contribute to the pathogenesis of multiple tumor types. It mainly dependson the availability of two things 1st is on the nutrients and 2nd is on energy sources of mTor. [14]

mTOR has two multiprotein complexes-mTORC'1 and mTORC2. mTORC 1 complex, it is made up of mTOR, raptor as well as mammalian LST8 (mLST-8/GβL) It is mainly having action in mTOR for phosphorylation and PRAS40 [15]. The activation of S6K and 4EBP1 both are responsible for the translation of initiation for the protein synthesis [15]. The list of main proteins due to which cell cycle are control, they are D-type cyclins, c-myc, and ornithine decarboxylase which regulated through this complex [16]. The nature of mTOR is to regulate protein synthesis. (Figure 1)

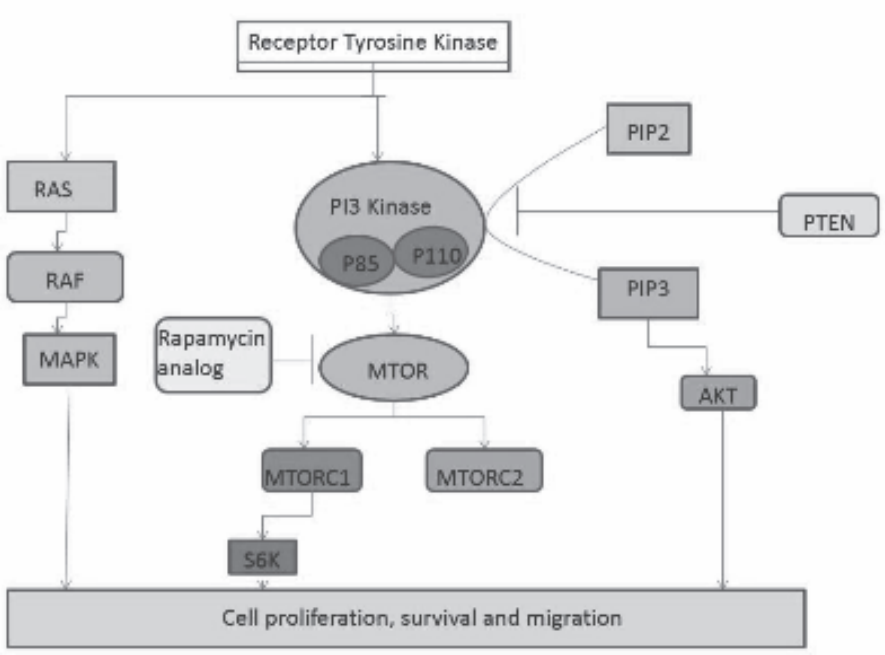

Figure 1: PI3K signaling through AKT in breast cancer, including multiple clinically relevant feedback loops.

PI3 kinase pathway as a target

Both upstream and downstream PI3 kinase pathway has a potential target for the drug development in breast cancer. From the preclinical and clinical studies it has been found that the complex of PI3k in different levels of cancer treatment is used alone or in combination with the chemotherapy or radiation or other targeted therapies for cancer. [17] The PI3K/AKT/mTOR pathway is complex and the most effective choice chain for the different types of cancer, into that the study of combination therapy and classification of analytical factors is very important.

PIK3CA Activation

PIK3CA mutations will induce a transformed phenotype *in vitro* and *in vivo*. It also include enhancement of cell proliferation and survival, growth factor independence, protection from apoptosis, and drug resistance. Mutation in PIK3CA oncogene is most common in breast cancer.Mutations in PIK3CA represent the most common genetic events in estrogen receptor–positive (ER+) breast cancer. Its occurenceis 30% to 50%. Less commonly observed are mutations in PTEN (2% to 4%), AKT1 (2% to 3%), and phosphatidylinositol-3-kinase regulatory subunit alpha (PIK3R1: 1% to 2%) [18,19]. The catalytic subunit p110α is encoded by the PIK3CA, which has a

significant action in the activation of AKT downstream signalling and mammary tumor sequence. In preclinical studies, cancer cells carrying PIK3CA mutation depend on the alpha catalytic subunit of PI3K for cell growth. [20] Although the presence of PIK3CA mutation in ER+ breast cancer has not been associated with de novo resistance to endocrine therapy, upregulation of PI3K pathway signaling has been observed in tumor cells grown under long-term estrogen deprivation in experimental models. [21]

Most widespread mutations in breast cancer are in exon 20 which is a catalytic domain. Numerous analysis have reported direct relationship between Pl3Kinase and Estrogen receptor (ER), progesterone receptor (PR) positivity, and over expression of HER2 [22]. The relation with pathologic sign and clinical outcomes is still not very well establishing [23]. PIK3CA-activating mutations and PTEN loss has an inverse bond. Current observation by many scientists has reported that, in tumors with PIK3CA mutation only 13% had PTEN loss, whereas 34% expressed PTEN normally [24].

PI3K Pathway and Breast Cancer Subtypes

Various gene expressions has shown somewhat significant effect in breast cancer. Examples of various genes are luminal A and B both, enriched HER 2, and basal-like tumors. [24] Classification is mainly based upon ER or PR position, HER2 expression, cytokeratin 5/6 (CK5/6), and EGFR nature. PI3Kinase pathway varies among the frequency and type of breast cancer. [23] Molecular transformations possibly havea different clinical force which is dependent on the breast tumor molecular backgrounds and the presence of treatment received.

Triple Negative Breast Cancer

PI3Kinase activation is mainly done by PTEN loss. This is mainly seen in triple negative for ER, PR, and HER2 and optimistic for CK 5/6 or EGFR. The PTEN pathway loss is connected with the basal-like phenotype. The nature and lack of direct therapies against these tumors hasd given chance to a talented growth in the search and discovery of possible targets in breast cancer which has significant clinical efficacy. The RAS/RAF/MEKinase pathway is the markers of the basal-like tumors sensitive to MEKinase inhibitors. From the pharmacogenomic observation for the breast cancer cell lines genes that are having the combination treatment with these two PI3K and MEK inhibitors generated a synergistic effect inhibiting basal-like cell lines. In particular design of clinical trials into which combination therapies including MEKinase and PI3Kinase inhibitors for the patient population. This might be a more effective or might be a solitary-pathway reserve therapy. [25]

ROLE OF PI-3KINASE IN THYROID CANCER

Genetic alterations have strong evidence for the oncogenes and pathology of thyroid cancer, mainly those that occurs due to encoding for main players of major signalling pathway. By the change in the genetic background with different types of thyroid tumors, and certain molecular signalling pathways are consequently changed into them. There is a typical example of the particular genetic modulation, which is the oncogenic BRAF mutation in the Ras MEK ERK/MAP kinase pathway (MAPK pathway). This ccurs commonly in PTC, particularly CPTC and TCPTC, and some ATC. [26] Other genetic modulations include the MAPKinase pathway. In perticular thyroid tumor genesis include RET/PTC and Ras mutations. [27] Recently, the importance of the phosphatidylinositol-3 kinase (PI3K)/Akt pathway in thyroid cancer has been discovered. [28] Recently studies haveparticularly spread the genetic backgrounds for the PI3K/Akt pathway. (Figure 2)

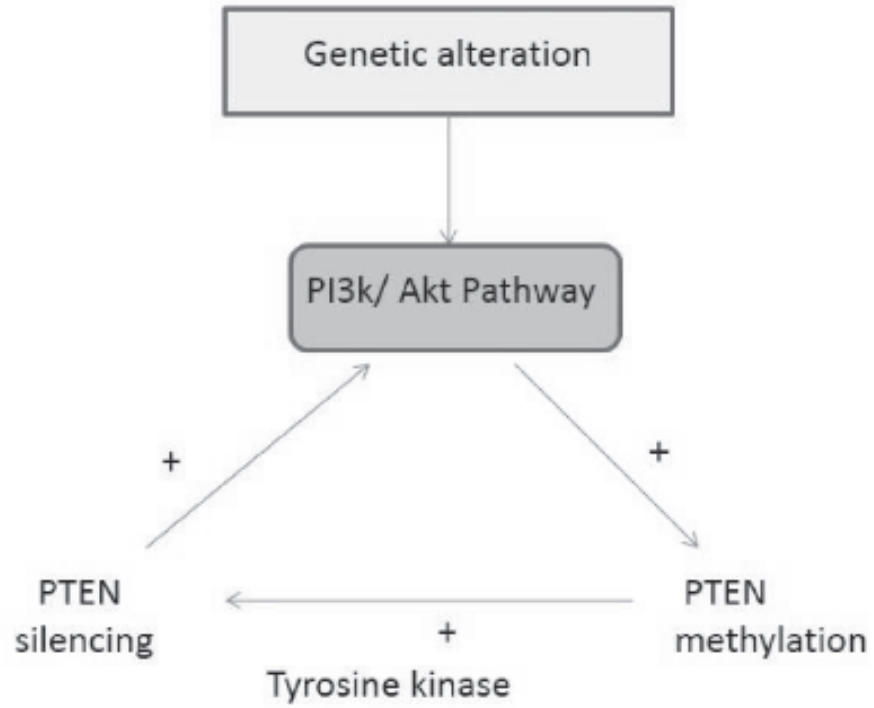

Figure 2: PI3k/ Akt signalling pathway in thyroid tumor.

PTEN, Phosphatase and tensin homolog; PI3K, Phosphoinositide 3-kinases; AKT, v-akt murine thymoma viral oncogenehomolog

PI3K class I is most important in human cancer. There are various genetic modulations that activate the PI3k/Akt pathway in thyroid cancer .In this mainly PPARγ/Pax8 rearrangement exists. PTEN expression is indirectly suppressed by PPARγ/Pax8 which is followed by the activation of the PI3K/Akt signalling. [29]

PI3K Inhibitors

The first synthetic PI3Kinase inhibitor was LY-294002 [30] SF-1126 is the prodrug of the conjugation of LY-294001 with Arg-Gly-Asp peptides which is in the phase I clinical trials[ref]. It has a multimodel Pap-PI3K inhibitor. One of the phase 1 clinical trial drug BKM 120 (a potent PI3K inhibitor) or BEZ235 (a PI3K/mTOR inhibitor) in combination with the endocrine therapy has main use in postmenopausal development in patients with hormone receptor—positive metastatic breast tumors. [31] One of the newly discovered PI3Kinase inhibitor also is GDC-0941 (Genentech Inc.) which is in a phase I clinical trial. This drug is also used in combination therapy with Paclitaxel and Bevacizumab for metastatic breast cancer.Another molecule XL-147 (Exelixis/Sanofi-Aventis) is in phase 1 clinical trial which is either used alone or combination with trastuzumab and paclitaxel. The XL147 agent is a selective PI3K inhibitor which is very potent inhibitor of the Class I PI3K family. (Table 2)

Table 2: PI3 kinase pathway inhibitors in phase 1 clinical trials

mTOR kinase inhibitors	Rapalogs	AKT inhibitors
MNLO128	Everolimus	Perifosine
AZD2014	Temsirolimus	MK2206
OSI027	Deforolimus	Ipatasertib
CC223		GSK2141795
		GSK2110183

ROLE OF PI3- KINASE IN INFLAMMATION

Phosphatidylinositide 3-kinases (PI3-K) phosphorylate the third hydroxyl position of the inositide head of phosphoinositide lipids, phosphatidylinositide (Ptdlns), phosphatidylinositol (3)-phosphate (Ptdlns(4) P) and phosphatidylinositol (4,5)- bisphosphate (Ptdlns(4,5) P2). [32] This results in formation of Ptdlns PIP, Ptdlns(3,4) PIP2, and Ptdlns(3,4,5) PIP3 formation respectively. This lipids bind to the pleckstrin domains of proteins, and

thus by controlling the activity and signal transduction in various molecules. PI3-kinases could be branched into 3 main classes based on their lipid substrate.

The Class I PI3-Kinase is the main isoform that is coupled to outside stimulus. [33] The enzymes of class 1 encode regulatory subunits encoded by all separate genes of PIK3 receptor. coding of p85, p50 and p55, encoded by PIK3receptor 2. Thus, regulatory subunits couple with class I - p110, p101,p108.and pl10 catalytic subunits. GPCR receptors stimulate PI3K by the GPCR interactions. [34] The p110 catalytic subunit has significant sequence homology to IA class catalytic subunits; even then, the regulatory subunits p85 are different from p101 and p87. Class IA and class IB phosphatidylinositide 3- kinases are stimulated through downstream in myeloid cells of toll/IL-1 receptors and selected specific inhibitors of isoform have been developed. [35] LPS/CD14 reaction regulates constant levels of PIP2 in the plasma membrane and the MAL adaptor protein expression. MAL produces the MyD88 adaptor by TIR-mediated recruitment. Tyrosine phosphorylation on the TIR domain of MAL/MyD88 or other TLR4 adaptor serves to recruit SH2 containing protein p85, the PI3-Kinase regulatory sub-unit by a src-related kinase. The catalytic subunits of P13-Kinase p110isoforms processed by the phosphorylation of PIP2 to PIP3. Downstreaming of the ras-dependent pathway and IL-1 receptor of class I B activation P13-Kinase y isoform, has been reportedto be linked with trafficking tumor growth and progression of myeloid cell. [32] The first selective P13-KS inhibitor is IC-87114. AS-604850 and AS-605240 are inhibitors of ATP-competitive PI3K Y isoform has been reported in murine colitis models of intestinal inflammation.

The regulatory subunits produce recruitment of plasma membrane receptor complex followed by receptor joining. The reaction between p85 and the receptor complex by a high-affinity interaction was mediated through the p85 Src homology 2 (S1-12) domain and the tyrosine-phosphorylated particular lines between the cytoplasmic tail of the receptor. The process in plasma membrane select the p110 catalytic domain to the where the phosphorylation of the main product Ptdlns(4,5) P2 to produce PtdIns(3,4,5) P3. It had been recently demonstrated that p85 through phosphorylation was regulated which determines its potential to associate with p110. [36] Selection p85 with signalling complexes in plasma membrane have association containing She, Orb2. and Gab2 which has also been reported in response to cytokines such as interletikin-1 t III). [37] The catalytic subunit, also binds to activated ras in the plasma membrane which might stabilize association after recruitment to the receptor complex. Phosphatidylinositide 3-kinases (PI3-K) phosphorylate the 3rd hydroxyl position of the inostide head of phosphoinositide lipids, phosphatidylinositide (PtdIns), phosphatidylinositol (3)-phosphate

(Ptdlns(4) P) and phosphatidylinositol (4,5)- bisphosphate (Ptdlns(4,5) P2). [38] This lipids bind to the pleckstrin same domains of proteins, and thus by controlling the activity and sintercellular localization of a signal transduction in various molecules. PI3-kinases could be branched into 3 main classes based on their in vitro lipid substrate specificity.

The Class l PI3-Kinase are main focus on study and its isoforms that are coupled to outside stimulus [39]. The enzymes of class 1 A encode regulatory subunits encoded by all separate genes PIK3receptorl coding of p85µ, and other transcriptase p50 and p55. Moreover, in the mucosa of people high levels of binding activity of NI kl3 with inflammatory bowel disease intestinal macrophages might express, and it was thought that these cells had not been downregulated are newly recruited monocytes that [40] . Studies on P13-K knockout mice give the idea regarding the P13-K negatively regulates through TLR2, 4, 5, and 9 was increased activation. In p85µ deficient mice and IPS-induced IL-12 secretion was increased in p110 deficient macrophages [41]. PI3-K appears to inhibit serine threonine kinase. PI3-K activation in response of GSK3 results in increased IL-10 production through CREB and its coactivator CRP binding to TLR stimulation leads to the inhibition. GSK3 also inhibits directly affect IL-10 expression of AP-1 DNA binding which is because of competition for the CBP coactivator was decreased due to Phosphoinositide-dependent kinase 1 (PDKI) was an main signaling component in the PI3-K pathway. In the primary macrophages developed from mice of myeloid tissue had increased TNFα and IL-6 mRNA expression.Their macrophages shows prolonged ubiquitination of TRAF-6 in response to LPS induction in PDK-1 dependent feedback mechanism on NFKB activation. [42]

Lamina propria T (LPT) cells are destitute of antigen-receptor with few T-cells proliferation in response to TCR/CD3-directed stimuli. [43] T-cell activation through CD58/CD2 or B7/CD28 contributes to the increase of T-helper cells, raised T-cell proliferation and reduced apoptosis, all are the characteristic of inflammatory bowel disease. [44] Much lesser T-cell proliferation was observed after TCR stimulation with monoclonal antibody (mAb) compared to dual stimulation with anti CD2 and anti-CD28 mAb and no proliferation was observed with anti-CD2 mAb alone. Hypo responsiveness was restricted to the mucosa and cannot be found in the mesenteric lymph nodes or Peyer's patches. Work through Kamanaka's group explains that the hypo-responsiveness of LPT cells. They showed that a/PTCR stimulation induces Foxp3+ regulatory T-cells (Treg) with high IL-10 production[44].

TLR Signalling

Recent development in the TLR Signalling describes the importance of PI3-K to

demonstratetheir role for targeting inflammatory disease. It has been reported that there is a cross talk between TLR activation and the PI3K/Akt pathway that leads to an anti-inflammatory action. Stimulation of P13-KJAKT inhibits GSK3 which attenuates excessive pro-inflammatory TLR9-mediated immune responses. It had been demonstrated that in CD4+ T-cells, GSK313 promoted the production of pro-inflammatory cytokines while lowering secretion of the anti-inflammatory IL-10 through differential regulation of NFKB and CREB activities [45]. It had also been demonstrated that in CD4+ and DNA stimulation is done by T-cell-dependent antigen by the PI3K dependent pathway directly increases proliferation and prevents energy and augments humoral responses.

In Intestinal Inflammation there occurs an increase in the over expression of PI3 kinase enzyme due to over expression of Wnt pathway leads to increase in beta catenin gene and thus increases its level and leads to development of inflammation and so one have to discover the drug to inhibit the PI3 kinase and thus prevents from inflammation.

ROLE OF P13-KINASE IN VIRAL INFECTION

Human immunodeficiency virus type 1 (HIV-1) can activate multiple signaling pathways within a target cell to facilitate viral entry and replication. A number of signal transduction pathways may be activated during engagement of the HIV-1 envelope with CD4 and/or the chemokine coreceptor. Binding to CD4 causes phosphorylation of receptor tyrosine kinase. [42]. Many viruses have evolved mechanisms to manipulate this signaling pathway to ensure successful virus replication. The human herpesviruses undergo both latent and lytic infection, but differ in cell tropism, growth kinetics, and disease manifestations. Herpesviruses express multiple proteins that target the PI3K/Akt cell signaling pathway during the course of their life cycle to facilitate viral infection, replication, latency, and reactivation.[46] Influenza A virus infection leads to activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and that this cellular reaction is dependent on the expression of the viral nonstructural protein 1 (NS1). [45]Phosphoinositide 3-kinases (PI3Ks) regulate an array of protein kinase signaling cascades that, in turn, control diverse cellular processes like cell survival, metabolism, proliferation, and inflammation/immunity. [46] The phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway has attracted much recent interestdue to its central role in modulating diverse downstream signalling pathways associated with cellsurvival, proliferation, differentiation, morphology and apoptosis.[47,48]

PI3K as a key in apoptosis

The PI3K pathway has the key role in the apoptosis pathway. PI3K modulates different cellular activities. Which mainly

include cell survival, growth, proliferation, migration and apoptosis. [49] Its main role is in the virus life cycle as well as from the virus entry through viral transcription and also in the synthesis of the protein. This will enhance the cell survival by the inhibition of apoptosis in infected cells as well as it also affects ncogenic transformation. [50] PCV2 infection is the main reason for the apoptosis both in vitro as well as in vivo. [51] Many reports shows that the PI3K/Akt pathway in virus which induces apoptotic responses of many other viruses. Therefore, it lead to the biggest question that the PI3K/Akt pathway is involved in PCV2 infection or it contributes to PCV2-induced cell survival and prevention of apoptosis, because PI3/Akt provides the favouring condition for the virus growth. One of the major possibilities is that the PI3K/Akt pathway which participates in the different functions of cell activities like preservation of host cell survival and it also involve in the blockage of apoptotic responses.

From the different studies it is observe that Akt must phosphorylate in the early stage of the PCV2 infection and its action is depend on PI3K. By the Inhibition of P13K activation it leads to activation of a PCV2 virus, which reduce viral DNA accumulation and also synthesis of protein. This leads to one of the major apoptotic responses in the PCV2-induced cells. Many evidences show positive result for these possibilities. Initially infection of the PCV2 is crucial process in to that the breakage of poly-ADP ribose polymerase and caspase-3 as well as DNA differentiation occures. This will lead to the increase in the apoptosis chances after inhibition of the P13K. From the different substantiation's it is recommended that PCV2 infection can be cure by the inhibiting PI3K/Akt pathway. It leads to premature apoptosis for virus growth after the infection [52]. PCV2 infection induces PI3K-dependent phosphorylation of Akt. Investigation from the different studies whether Akt activation in response to PCV2 infection occurred through the PI3K pathway with a specific PI3K inhibitor, LY294007. The concentrations of the inhibitor are maintained during the adsorption period and PCV2 infection [53]. One of the PI3K inhibitor, wortmannin is also use to treat the PCV2-infected cells and obtained positive response results. Thus, the PCV2-induced phosphorylation of Akt involves a PI3K-dependent mechanism. It is been hypothesized that in viral infection leads to activation of the PI3K/Akt pathway. The activation of Akt signaling pathway induced by early PCV2 infection occurs through a PI3K-dependent mechanism, and mediated through the virus infection with host cells. Thus it can be conclude that PI3K/Akt Signaling pathway is necessary for the growth of PCV2. and PI3K/Akt signaling pathway activation leads to the trigger of the circovirus type 2 infection which was required for efficient PCV2 replication as well as in the suppression of premature apoptosis for better virus growth after infection.[54]

ROLE OF PI-3KINASE IN SCHIZOPHERNIA

Schizophrenia is very serious disorder and mostly geriatric population is affected by this neurodegenerative disorder. In this disorder there is no specific biochemical test that conforms for the clinical diagnosis purpose. Impairment of signaling pathway of PI3K in a many of neurodegenerative brain disorders observed mainly in alzheimer disease. Protein-to-gene reverse approach leads to the evidence for the impairment of AKT GSK3 signalling pathway in schizophrenia. This also proves the association between schizophrenia and PI3K. The genetic association of PI3K with schizophrenia was confirmed in other populations [55].

PI-3KINASE IN NON-NEURONAL CELL

PI3K-Akt Pathwayis a signal transduction pathway that promotes survival and growth in response to extracellular signals.Akt has mainly three isoforms in mammalian cells they are AKT I, AKT2, and AKT3. This isoforms has different roles. It mainly includes different processes mainly development and metabolism of the neuronal cells. From all this different isoforms AKT1 is the most important isoforms as it is highly expressed isoform and it has strong evidence for its active role in schizophrenia. Another isoform of the Akt is Akt2 which is mainly involved in the regulationas well as in the metabolism of the insulin-regulated glucose homeostatis.

PI3K is expressed at a higher level and significant expression of AKT2 occurs in insulin-responsive tissues such as skeletal muscle, liver, heart, kidney, and adipose tissue.[56] During development of central nervous system (CNS), the expression of AKT1 and AKT2 level get increase during development, but was gradually reduced during postnatal development. In the adult brain expression of AKT1 and AKT2 is primarily weak. Unlike AKT I and AKT2, AKT3 was only expressed in some tissues, such as in the brain and testes, with reduce expression in skeletal muscle, pancreas, heart, and kidney. Moreover AKT3 was expressed in the brain and had a role in postnatal brain development [56].

PI3K-AKT PATHWAY *IN NEURONAL CELLS:*

For the normal function of the cell protein phosphorylations plays an important role in CNS. This mechanism is widely use for the regulation of the efficacy and specificity of the neurotransmitter which is release from the presynaptic terminal to the nerve impulse. [57] For the regulation of the neuronal size and survival PI3K-Akt plays an important role. Over express of Akt in cerebellar granule neurons prevents apoptosis during withdrawal of growth factors. Inhibition of the PI3K leads to cell survival in the neurons which is supported by growth factor.

PI3K-AKT *SIGNALLING IN SCHIZOPHRENIA*

From the various reports and many other different studies had support the impairment of the PI3K-Akt signaling plays major role in the pathology of schizophrenia. It is been observed that there is major reduction of the Akt protein level in the schizophrenia patients. AKT1 decreased in the hippocampus and frontal cortex in brain samples. This decrease in protein level in the brain was specific to AKT 1 isoform and other 2 isoforms AKT2 and AKT3 levels were unaffect. Many studies provided convergent evidence of reducing in AKT1 mRNA, protein, and activity levels in the prefrontal cortex and hippocampus, as well as in peripheral blood of schizophrenic patients [58].

ROLE OF PI3-KINASE IN PARKINSON DISORDER:

PI3K enhancer in the brain is a enhancer of PI3 kinase/Akt enhancer. This is the group which belongs to GTP binding proteins that comes to the α-subgroup of GTPase family. In the Parkinson disease there is increase in kinase level which is mainly responsible for the over expression of the neuronal gene therefore it might be hypothesised that decrease in the PI3 kinase level will help in the treatment of the disease [59]

The role of PIK enhancer GTPase in the balancing of the neuronal survival has strong evidence which is mainly based on the studies which has been done over the last 10yr. These studies lead to the strong conclusions that functional activities of PIK enhancer in neurone had not been explain very well. It is implicated in the regulating the activities of the transcription factors such as signal transducer and activator of transcription 5A after that prolactin stimulation. The neuro-philins mainly brain derived neuropathic factor (BDNF) are the main molecule that involve in the neuroprotective mechanism during the catastrophic damages. This BDNF protects against glutmate induced apoptotic cell death by the PI3K and extracellular signal regulated kinase pathway in vitro. [59] It is also been reported that increase in BDNF expression after the ischemia and seizer induction, this BDNF has a protective mechanism against the neuronal death. If insufficient BDNF is there then it will leads to the chronic neurodegenerative disease [60]

CONCLUSION:

It can be concluded that PI3K pathway inhibition has beneficial role in various disease and disorders. For the newer drug target and better management purpose it's inhibition can be useful. It can be use as a combination therapy for the various life threatening diseases like Cancer mainly breast cancer and thyroid cancer and inflammatory and neuronal. From the various preclinical data of newly discover PI3k inhibitory action better management therapy can be discover for the better human health services.

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Nirma Univ J Pharm Sci; 2019, 6(1) 55-68
© 2019, Nirma University, Ahmedabad, Gujarat, India
ISSN 2348 –4012

ARTICLE

INHALED FLUTICASONE PROPIONATE DRY POWDER FOR THE EFFECTIVE MANAGEMENT OF ASTHMA

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Abstract:

Dry powder inhaler has become an attractive platform for pulmonary drug delivery. Dry powder inhalers are commonly used to treat asthma and other pulmonary diseases. Dry powder inhalers chiefly consist of micronized drug admixed with the carrier particles which aid in the flow of the drug in the respiratory tract. The dry powder formulations of fluticasone propionate were prepared with different coarse and fine lactose grades such as Respitose® SV003, Respitose® SV010, Respitose® ML001, Respitose® ML006 and Lactohale® LH 230, Inhalac® 230 and Inhalac® 400 and evaluated for flow properties, emitted and fine particle dose, content and blend uniformity. The final coarse and fine lactose grades were Inhalac® 230 and Inhalac® 400 based on the flow properties. 32 factorial design was applied for the formulation optimization. The final device used was Breezhaler®. The optimized batch showed a fine particle fraction of 8.99 ± 3.71 with a mass median aerodynamic diameter of 3.40 ± 0.02 thus showing the efficiency of dry powder inhalers in the delivery of fluticasone propionate deep into the lungs.

Keywords: Dry powder inhaler, fluticasone, fine particle fraction, cascade impactor.

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1. Introduction

Asthma is a chronic disease which is generally caused due to factors such as dust mites, common cold, air pollutants and irritants etc. It is a condition in which the airways of a person becomes inflamed and swollen up leading to excess mucus production due to which breathing becomes difficult. (1-3) About 334 million people suffer from asthma worldwide. (4) The current treatment of asthma involves the use of pressurized inhalers.

Nowadays, dry powder inhalers are prioritized now over pressurized formulations for asthma due to their several advantages, prior one is the safety due to absence of any propellants in the formulation. (5) Dry powder inhalers are mainly composed of blend of drug in micronized form and carrier particles. These work on fluidization mechanism in which the drug and carrier blend is fluidized in the lungs. The carrier particles are retained in the upper airways while the drug particles with an aerodynamic diameter between 1-5 μm undergo deposition deep into the lower airways. The disaggregation between drug and carrier particles plays a key role in the efficient delivery of the drug deep into the lungs. The key steps in the delivery of drug from DPI formulations are detachment of drug particles from carrier, dispersion of drug in the air flow and finally deposition of drug in the lungs. (6-8)

Fluticasone propionate is a corticosteroid which is highly effective against asthma. (9, 10) It is an essential component of asthma treatment which binds to the glucocorticosteroid receptors with high affinity. This results in down regulation of pro-inflammatory mediators (Interleukins) and up regulation of anti-inflammatory mediators (IkappaB). Fluticasone propionate has been chosen as the model drug due to high lipophilicity which will result in high retention in the lung tissues. (11, 12) Though researchers are working on the development of nanoparticles, microparticles etc. for the delivery of fluticasone propionate in the lungs. But, considering developing nations, dry powder inhaler formulations will serve as an economical alternative to the above listed expensive formulations.

In this research work, we have developed dry powder inhaler formulation of fluticasone propionate with a view to improve the aerodynamic properties of the drug for the successful treatment of asthma. The designing of powder of such a potent drug is a challenge which should meet the desired aerodynamic properties for the proper deposition into the lungs. The deposition is a key factor influencing the efficiency of any inhaled drug delivery system. The purpose of the investigation is to evaluate the lung deposition of fluticasone propionate via cascade impactor study.

2. Materials and Methods

2.1 Materials

Fluticasone propionate was obtained as a gift sample from Zydus Cadila Helathcare

Ltd. Rotahaler® and Breezhaler® were purchased from a local vendor. Capsules of size '3' were provided as gift sample from Cipla Ltd. Mumbai. Respitose® SV003, Respitose® SV010, Respitose® ML001, Respitose® ML006 and Lactohale® LH 230 were obtained as a gift sample from DMV-Fonterra Excipients GmbH & Co. and Inhalac® 230 and Inhalac® 400 were obtained as a gift sample from Meggle, Germany. All the chemicals and reagents used were of analytical grade.

2.2 Preformulation studies

2.2.1 Drug excipient compatibility studies by Fourier transform infrared spectroscopy (FTIR)

In a dry powder inhaler formulation, the drug and carrier are in contact with each other. Hence, FTIR (Jasco FTIR 6100, Japan) studies were carried out in order to determine the compatibility of fluticasone propionate with the lactose. The powder blend was mixed with KBr in 1:1 ratio and spectrum was recorded in range of 4000-400 cm^{-1} after 24 hours using Spectra Manager II™ software.

2.2.2 Differential scanning calorimetry (DSC)

The drug excipient compatibility was determined using DSC. The DSC of pure drug and the blend of the micronized drug and the lactose were carried out using Mettler Toledo. The sample was sealed and was heated at 10°C at a temperature in the range of 25-250°C.

2.3 Preparation of formulation

2.3.1 Preliminary batches

Several coarse lactose grades such as Inhalac® 120, Inhalac® 230, Respitose® SV010, Respitose® SV 003, Respitose® ML 001 and fine lactose grades such as Inhalac® 250, Inhalac® 400, Respitose® ML006 and Lactohale® LH 230 were used for the preparation of preliminary batches. After preparation, these batches were further evaluated for flow properties, emitted and fine particle dose, content and blend uniformity. The functionality of devices, Rotahaler® and Breezhaler® were evaluated and the final selection was done based on the amount of drug left in the device. The selection criterion was based on the fact that less the amount of drug left in the device, the more efficient it is.

2.3.2 Preparation of fluticasone propionate dry powder formulation

The preblend of Inhalac® 230 and Inhalac® 400 was prepared using vortex mixer (Eie Instruments Pvt. Ltd., Ahmedabad). [13] After 24 hrs, the micronized drug was added into the lactose preblend and mixed for about 10 mins. The capsules were filled with blend equivalent to 50 mg of fluticasone propionate. The prepared batches were evaluated for flow properties, emitted and fine particle dose, blend and content uniformity.

2.3.3 Optimization of fluticasone propionate DPI

3^2 factorial design was used for optimizing process parameters of dry powder inhaler

formulation as well as develop the optimized formulation as given in **Table 1** and **Table 2**. Using this design, one can determine the effect of independent variables such as the amount of coarse lactose (X1) and fine lactose (X2) on dependent variables- flow properties, content uniformity, blend uniformity, emitted dose and fine particles dose. The independent variables were set at three levels. Nine batches were prepared as per the design. The experimental responses can be determined by the polynomial equation as given below:

Table 1 Independent and dependent variables of 3^2 full factorial design

Independent variables	Level 1 (-1)	Level 2 (0)	Level 3 (1)
Amount of coarse lactose	45.0	47.5	50.0
Amount of fine lactose	0.0	2.5	5.0
Y_1: Flow properties, **Y_2:** Content uniformity, **Y_3:** Blend uniformity, **Y_4:** Fine particle dose			

Table 2 Optimization of design batches using 3^2 full factorial design

Batch	X_1: Amount of coarse lactose	X_2: Amount of fine lactose
A1	-1	-1
A2	-1	0
A3	-1	1
A4	0	-1
A5	0	0
A6	0	1
A7	1	-1
A8	1	0
A9	1	1

$$Y = B_0 + B_1X_1 + B_2X_2 + B_{12}X_1X_2 + B_1X_1^2 + B_2X_2^2 \quad (1)$$

Where, Y represents the response (dependent variable) and X_1 and X_2 represents the factors (independent variables) and B_0, B_1 and B_2 represents the coefficients of respective factors.

2.4 Evaluation of the batches

2.4.1 Flow properties of drug and carrier preblend

The bulk and tapped volumes were determined as per United States Pharmacopoeia (USP) [14]. From these

volumes, tapped and bulk densities were calculated (n=3). Hausner's ratio was calculated to determine the type of flow using the formula:

$$Hausner's\ ratio = \frac{Tapped\ density}{Bulk\ density} \quad (2)$$

2.4.2 Content uniformity

Contents of ten capsules were individually dissolved in 100 mL of solvent (phosphate buffer pH 7.4: methanol (90:10)) via sonication for 5 minutes. The sample was analyzed using US-Vis spectrometer (Model 1800 UV-Visible spectrophotometer, Schimadzu, Japan) at a wavelength of 236 nm. The sample was considered homogenous if not more than one capsule was outside 85-115% of mean fluticasone propionate content and none was outside 75-125% of mean content. (n=3)

2.4.3 Blend uniformity

Weighed quantity of blend was dissolved in phosphate buffer pH 7.4: methanol (90:10) via sonication for about 5 minutes. The drug content was analyzed using UV-Vis spectrophotometer at 236 nm. The analysis was done in triplicates.

2.4.4 Emitted dose and Fine particle dose

The emitted and fine particle dose was determined using twin stage impinge fabricated as per European Pharmacopoeia[15]. It is a two stage device used for the assessment of aerosolized formulations. The coarse part of the formulation remains on the upper stage whereas fine fraction of the drug is collected in the lower stage. The upper stage was filled with 30 mL of solvent (phosphate buffer: methanol (90:10)) and the lower stage was filled with 7 mL of the solvent in order to resemble the respiratory tract of the lungs.

2.4.5 *In vitro* deposition studies of the optimized batch using eight stage non viable cascade impactor

2.4.5.1 Mass balance and *in vitro* deposition studies

The mass balance is necessary to be determined in order to ensure no drug loss in the system. The recovery of the total amount of active pharmaceutical ingredient from an impactor represents the mass balance.[16] After mass balance determination, *in vitro* deposition studies were carried out using eight stage nonviable cascade impactor (Tisch Environmental Inc TE-20-800). For mimicking the human throat, a throat piece was attached on the inlet cone which was placed on Stage '0' of the impactor. The mouthpiece adaptor was used according to Breezhaler® design. The adaptor was fitted on the throat piece and the impactor was connected to vacuum pump set at 28.3 L/min. Inspiration of single formulation

dose was done for about 8 sec. After the experimentation, the particles on each stage were washed with phosphate buffer: methanol (90:10) and analyzed using UV-Visible spectrophotometer. The cumulative percentage dose from stages 2 to 5 represents the fine particle fraction. (n=3)[17]

2.5 Scanning electron microscopy (SEM)

The surface morphology studies of coarse and fine lactose preblend was carried out using SEM (Oberkochen, Germany). The method used was plasma deposition in which gold coating was done under argon atmosphere onto the sample to make it conductive to scanning electron beam. The SEM was carried out at a working distance of 17.5 mm and at a voltage of 30 kV.

2.6 Statistical analysis

The batches were prepared three times each, and the results were expressed as mean ± standard deviation. A difference between means (Pd"0.05) was considered significant.

3.0 Results and Discussion

Dry powder inhalers comprise of formulations in which the micronized drug is admixed with the carrier particles. However, coarse carrier particles possess both high and low adhesion sites. The high adhesion sites do not allow easy detachment of drug which may lead to improper dosing of the formulation. Hence, the coarse lactose is firstly admixed with fine lactose so that most of the high adhesion sites are covered by the fines and major part of the drug adheres to the low adhesion sites which may facilitate the separation of the drug from the coarse lactose.[18]

3.1 Preformulation studies

3.1.1 Drug excipient compatibility studies

FTIR studies were carried out to determine the compatibility between the drug and the carrier. The FTIR spectra of pure futicasone propionate and its mixture with lactose has been shown in **Fig.1** which indicated the presence of identical peaks (3329 cm^{-1}, 2939.31 cm^{-1}, 1618 cm^{-1}, 1271 cm^{-1} and 736 cm^{-1}) in both pure drug and the mixture thus showing the compatibility between the drug and the carrier.

3.1.2 Differential Scanning Calorimetry

The DSC thermograms of drug and mixture are shown in **Fig.2.** The pure fluticasone propionate exhibited an endothermic peak at 290°C, representing the melting point of the drug. The representing peak was also seen in the mixture thus showing the compatibility of the drug with the carrier.

3.2 Preliminary batches

Preliminary batches were prepared using various coarse and fine lactose grades such as Respitose® SV003, Respitose® SV010, Respitose® ML001, Respitose® ML006, Lactohale® LH 230, Inhalac® 230 and Inhalac® 400. Out of these, Inhalac® 230 and Inhalac® 400 were the final coarse and fine lactose selected on the basis of flow properties.

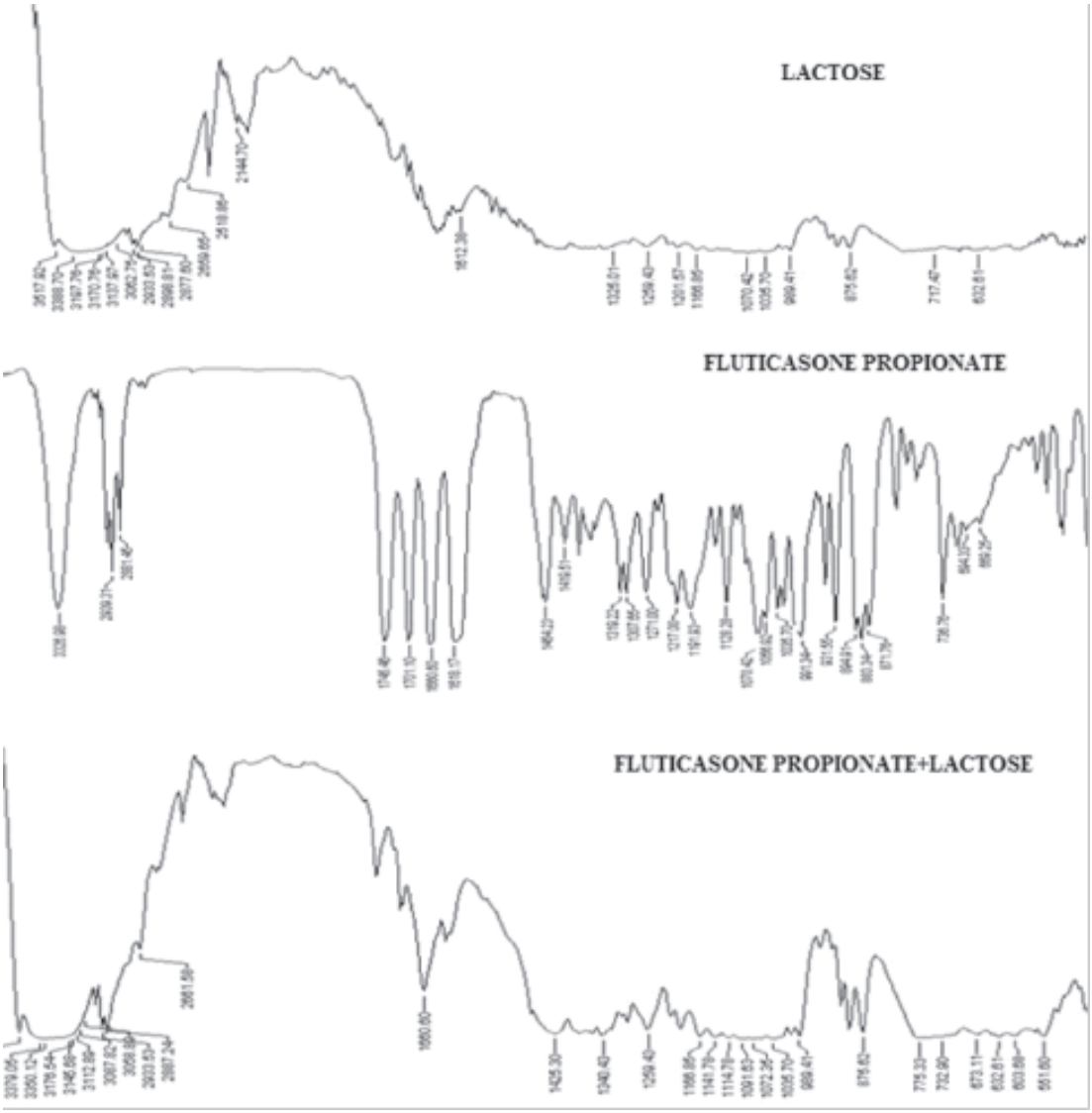

Figure 1. FTIR spectrum of lactose, Fluticasone propionate and combination of lactose and Fluticasone propionate

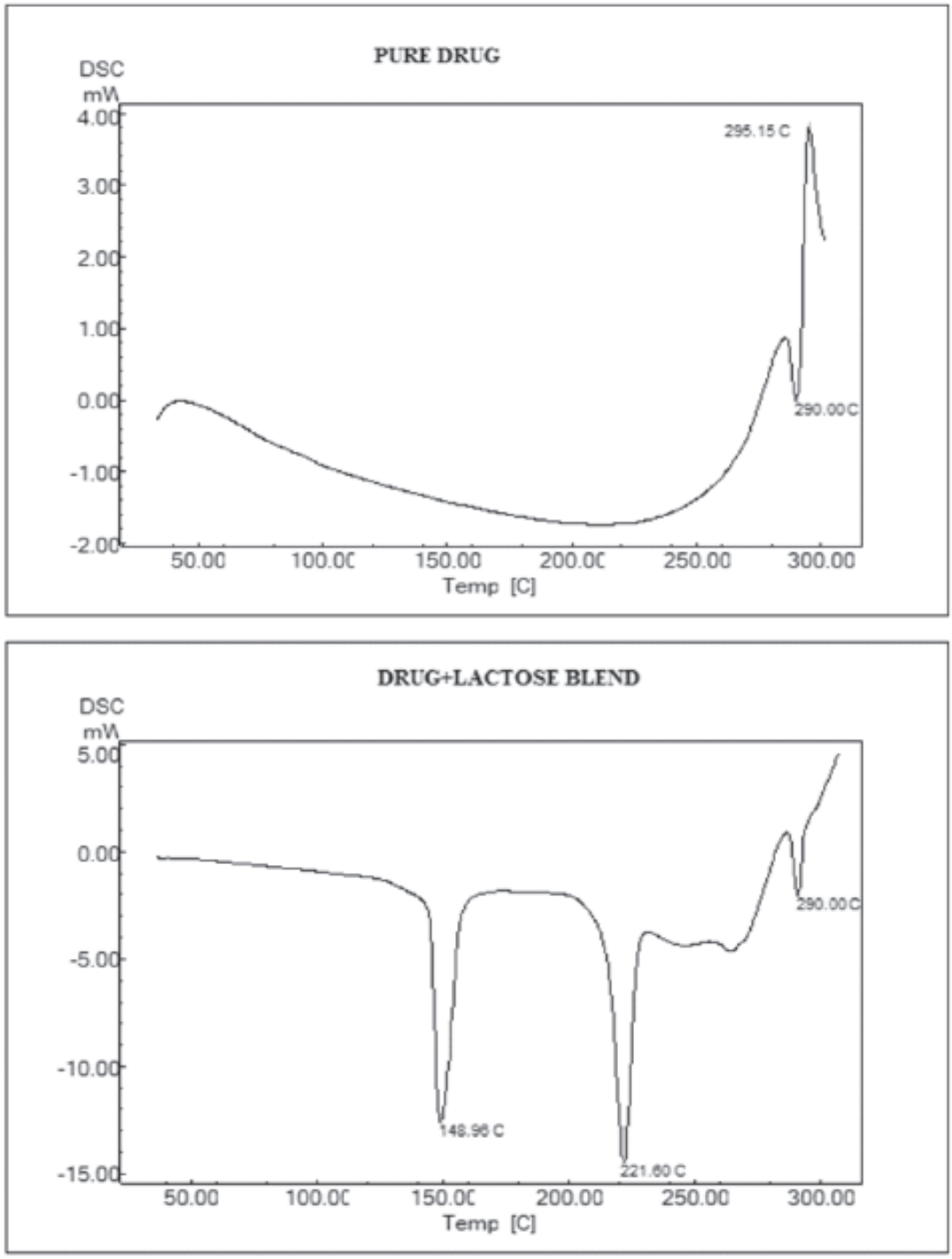

Figure 2. DSC of Fluticasone propionate alone and dry powder inhaler blend (optimized batch)

3.3 Optmization of the formulation

Design of Experiments (DOE) is used from a long time for optimizing the formulations. Numerous designs can be used for the optimization based on the data of the preliminary batches. Since few components are required in dry powder inhaler formulations and the amount of coarse and fine lactose were the important factors influencing the dry powder inhaler performance. Hence, 3^2 factorial design was used for the optimization considering amount of coarse lactose (X_1) and amount of fine lactose (X_2) as the independent variables. Nine batches were prepared and evaluated for following parameters:

3.3.1 Flow properties of the drug and carrier preblend

The micronized fluticasone propionate showed Hausner's ratio of 1.87, whereas design batches showed values between 1.18-1.21. The drug flow can be categorized as 'exceedingly poor' and 'fair flow' for the design batches as per USP [16] except batch A9 showed 'poor' flow. The poor flow might be due to the presence of high amount of fine lactose due to which large amount of drug remain unbound to low adhesion sites, thus leading to 'poor' flow. The results have been shown in **Table 3**.

Table 3 Evaluation of responses for design batches

Batch	X_1: CL	X_2: FL	Y_1: CU (%)	Y_2: BU (%)	Y_3: FPD (%)
A1	-1	-1	99.32 ± 1.26	98.30 ± 1.43	6.60 ± 0.41
A2	-1	0	100.79 ± 0.82	98.76 ± 4.59	7.10 ± 1.69
A3	-1	1	89.78 ± 2.37	88.89 ± 5.81	8.67 ± 2.31
A4	0	-1	111.02 ± 5.68	99.64 ± 4.28	6.50 ± 7.86
A5	0	0	109.98 ± 9.81	99.89 ± 0.67	7.40 ± 3.91
A6	0	1	85.01 ± 1.71	86.92 ± 5.83	8.50 ± 0.76
A7	1	-1	115.67 ± 4.82	101.87 ± 0.65	6.70 ± 3.62
A8	1	0	110.11 ± 4.36	101.2 ± 0.89	6.97 ± 4.21
A9	1	1	87.68 ± 2.21	91.98 ± 1.82	8.70 ± 5.31

* CL= coarse lactose, FL=fine lactose, CU= Content uniformity, BU=blend uniformity, FPD= fine particle dose

$$Y_{CU} = 99.72 + 1.52X_1 + 0.16\,X_2 - 0.12\,X_1X_2 + 0.35\,X_1^2 + 0.15\,X_2^2 \quad (3)$$

3.3.2 Content uniformity

The content uniformity of all the batches was within 85-115% of the label claim as per USP. The batches A3, A6 and A9 having the highest fine lactose content showed low content uniformity. This might be due to improper binding of the drug to the lactose due to high amount of fines which might have covered both high as well as low adhesion sites thus leading to low content uniformity. The results have been shown in **Table 3**.

The complete polynomial equation for content uniformity was described as follows:

From the above equation, it can be seen that the amount of coarse lactose and fine lactose both have a positive effect on the content uniformity. This might be due to the increase in number of high adhesion sites with the increase in the amount of coarse lactose. In order to cover these high adhesion sites, the fine lactose has to be increased which will lead to proper binding of drug to the low adhesion sites thus leading to proper content uniformity.

3.3.3 Blend uniformity

The blend uniformity of all the batches was within 85-115% of the label claim as per USP. However, again the batches A3, A6 and A9 with high amount of fines showed poor blend uniformity. This might be again due to insufficient binding of drug to the coarse lactose due to high amount of fines. The results have been shown in **Table 3**.

The polynomial equation for the blend uniformity was described as follows:

$$Y_{BU} = 108.83 + 6.69\,X_1 - 0.090\,X_2 - 0.12\,X_1X_2 - 3.35\,X_1^2 + 1.75\,X_2^2 \quad (4)$$

The above equation states that amount of coarse lactose has a positive effect on blend uniformity whereas the fine lactose amount has a negative impact on the blend uniformity. This might be due to improper binding of drug and coarse lactose due to increase in fines above certain extent, thus resulting in poor blend uniformity.

3.3.4 *In vitro* drug deposition studies by twin stage impinger

Twin stage impinger is a simple device which comprises of two stages and is used for the assessment of inhalation formulations. The powder is fractionated via upper impinger stage (simulated oro-pharynx) and then into lower impinger stage having defined aerodynamic particle size cut-off. The fraction of the drug which is accumulated in the lower impinger stage, is the fine particle fraction. This fraction undergoes deposition deep into the lower respiratory tract of the lungs. The results have been shown in **Table 3**.

The polynomial equation for fine particle fraction was described as follows:

$$Y = 7.16 + 0.10\,X_1 + 1.01\,X_2 - 0.018\,X_1X_2 - 0.010\,X_1^2 + 0.45\,X_2^2 \quad (5)$$

From the above equation, it can be seen that as the amount of coarse lactose and fine lactose are increased, they may lead to high fine particle fraction. This might be due to easy detachment of the drug from the coarse lactose due to its high adhesion onto the low adhesion sites of the coarse lactose. Thus lung deposition majorly depends on the optimum ratio of coarse and file lactose as well as mixing time or lactose with drug.

The contour plots and the overlay plot with the design space has been shown in **Fig. 3**.

3.3.5 Scanning electron microscopy

The SEM studies of preblend of coarse and fine lactose were done to see whether the fine lactose occupies the high adhesion sites of coarse lactose or not. The SEM images as shown in **Fig. 4** show that the fine lactose has covered majority of the high adhesion sites of the coarse lactose.

3.3.6 *In vitro* deposition studies of optimized batch using eight stage nonviable cascade impactor

3.3.6.1 Mass balance and *in vitro* deposition studies

The mass balance study of the optimized batch was done prior to *in vitro* deposition studies. The mass balance was 98.63% which was within the acceptable range of 85-115%, showing no drug loss in the system. Cascade impactor is the best tool for the *in vitro* evaluation of the inhalation products due to several merits. It helps in the determination of mass median aerodynamic diameter which plays a significant role in determining the

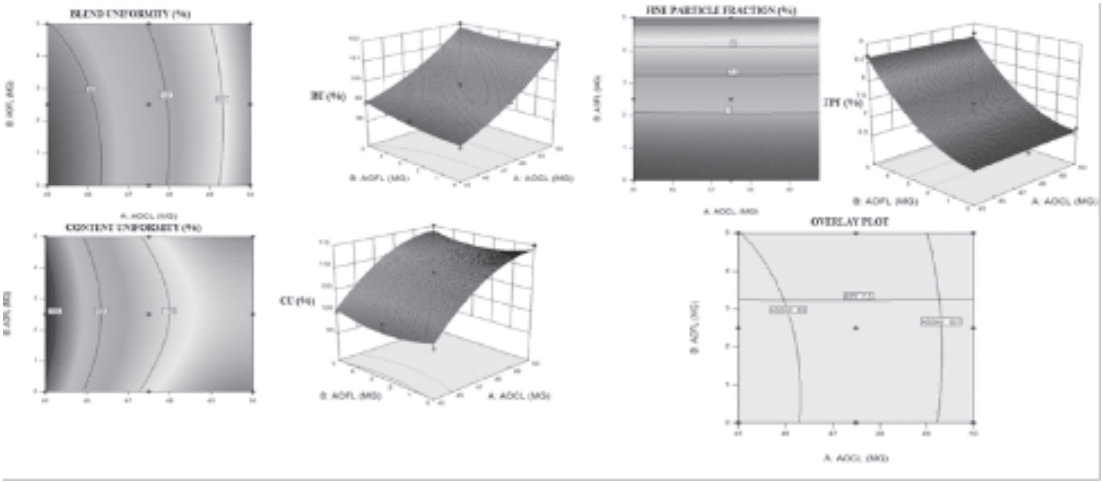

Figure 3. Response surface plots of design batches and overlay curve

Table 4 Mass balance and fine particle fraction of the optimized batch

Optimized batch	Mass Balance (%)	Fine particle fraction (%)	Mass median aerodynamic diameter (µm)
A_{10}	98.63 ± 3.41	8.99 ± 3.71	3.40 ± 0.02

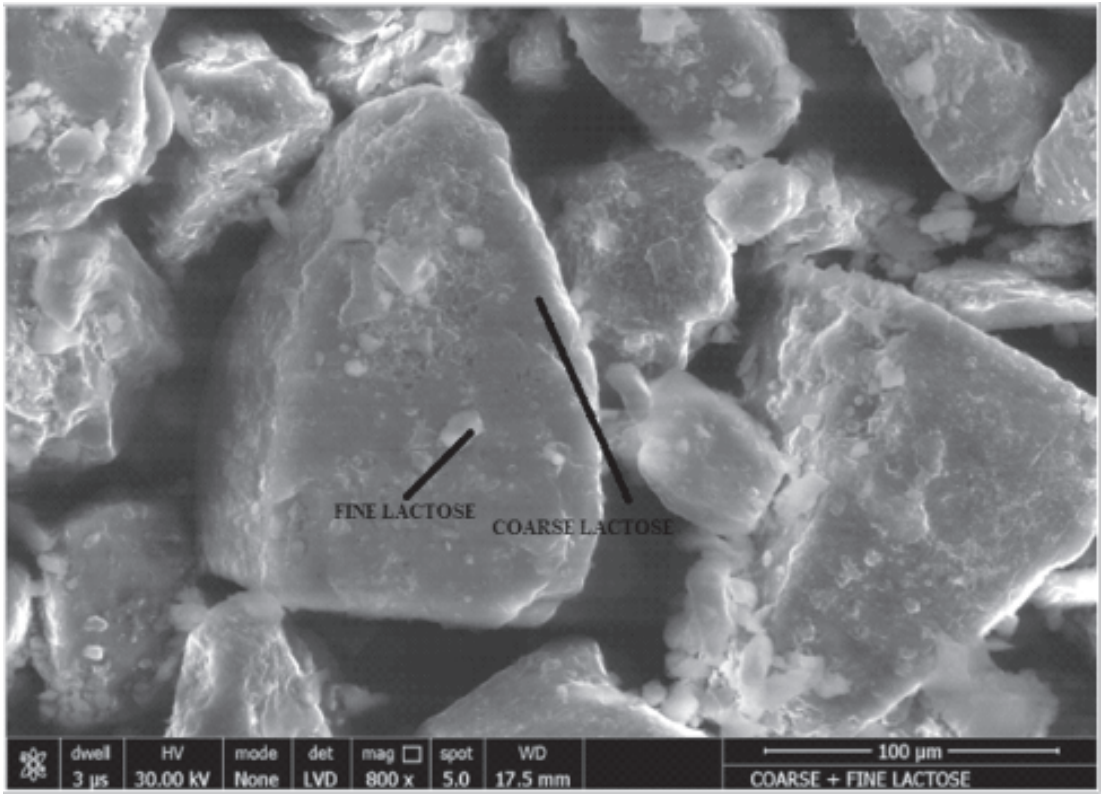

Figure 4. SEM image of dry powder inhaler blend (optimized batch)

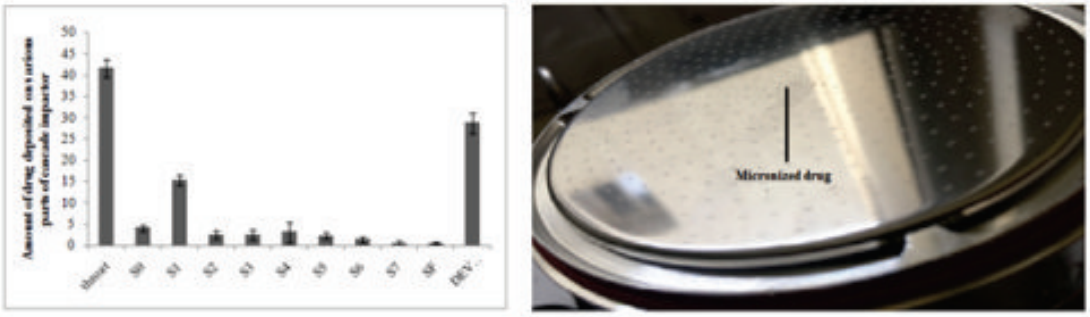

Figure 5. Amount of drug deposited on various stages of cascade impactor and image of S1 plate

deposition of the drug in several parts of the lungs. It also helps to determine the fine particle fraction of the drug. [19,20] It consists of eight stages with a cut-off diameter ranging between 0.7 and 10 µm. The mass median aerodynamic diameter as well as fine particle fraction of the optimized batch has been shown in **Table 4** and the amount of drug deposited at various stages has been shown in **Fig.5**.

Conclusion

The fluticasone propionate dry powder formulation was prepared and evaluated systematically. The optimized formulation showed mass median aerodynamic diameter between 1-5 µm and fine particle fraction of 8.99% using Breezhaler® device. This represents the effectiveness of the dry powder inhalation formulation in the efficient delivery of fluticasone propionate into the lungs which can be useful for the successful treatment of severe disease such as asthma.

Acknowledgement

The authors would thank Institute of Pharmacy, Nirma University for providing all the facilities for carrying out our research work. We also thank **Meggle Germany** and **DFE Pharma** for providing us inhalable lactose grades.

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