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SCREENING AND EVALUATION OF SOME PHARMACUETICAL FORMULATION ADDITIVES

Manisha Dudhal, Nilesh Dorage, Abhay Shirode

ABSTRACT

In any type of pharmaceutical dosage form, formulation additives play an important role for maintaining stability of drug in formulation and for desired drug release from the dosage form. A formulation additive varies from type of dosage form such as for tablet, capsule, cream, injectables etc. Formulation additives must be compatible with active drugs substance of a particular formulation. Hence screening of different formulation additives is an important aspect of preformulation studies . Our mini research project was aiming at screening of different formulation additives for Nanolipid carrier system of ziprasidone hydrochloride.

INTRODUCTION

Novel Drug Delivery System opportunity for formulation scientists to overcome the many challenges. Drug discovery and delivery research attained a stable and unique position among research based industries during 1980-2005. But today, drug discovery research is considered as an expensive, uncertain and risky business.

The method by which a drug is delivered can have a significant effect on its efficacy. Many new ideas for controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology. Drug delivered can have significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived and concentrations above or below the range can be toxic or produce no therapeutic effect. The main goal for developing novel drug delivery systems is to minimize drug degradation and loss, to prevent harmful side effects and to increase bioavailability. Targeting is the ability to direct the drug loaded systems to the site of interest.

Since ligand–receptor interactions can be highly selective, this could allow a more precise targeting of the site of interest.

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Several drug delivery systems have been formulated and are being investigated for nasal and pulmonary delivery which includes

liposomes, pro-liposomes, microspheres, gels, prodrugs, cyclodextrins, among others. Nanoparticles composed of biodegradable polymers show assurance in fulfilling the stringent requirements placed on these delivery systems, such as ability to be transferred into an aerosol, stability against forces generated during aerosolization, biocompatibility, targeting of specific sites or cell populations in the lung, release of the drug in a predetermined manner, and degradation within an acceptable period of time.

Novel Drug Delivery System can broadly classified as:

- Oral drug delivery system.
- Mucosal drug delivery system.
- Nasal drug delivery system.
- Ocular drug delivery system.
- Transdermal drug delivery syste□□Parenteral drug delivery system.
- Targeted drug delivery system.
- Inhalation / Pulmonary drug delivery system.

Advantages of NDDS:

1. Improve therapy by increasing the duration of action and reducing the side effects.

2. Improve patient compliance through decreased dosing frequency and convenient route of administration.

3. Achieve targeting of drugs to a specific site to reduce unwanted side effects and obtain maximum efficacy.

4. Optimization of duration of action of drug.

5. Improved bioavailability of some drugs.

Disadvantages of NDDS:

1. Stability problems.

2. Increased cost per unit dose

AIM:

To screen various lipids,oil and surfactants to select most suitable excipients for the NLC dispersion of ziprasidone HCL.

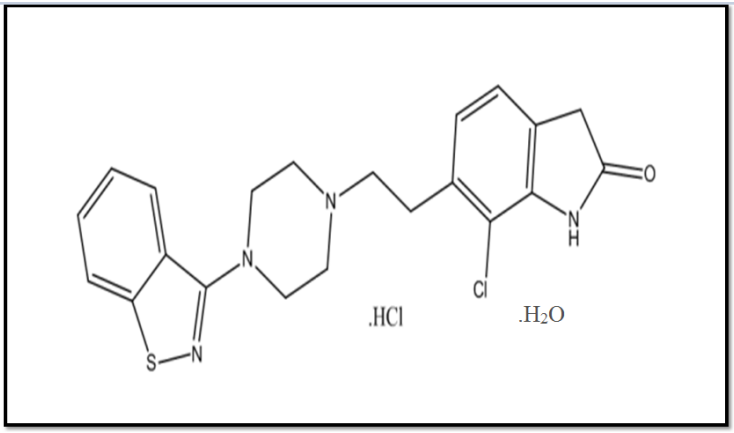
MATERIAL AND METHOD

Drug Profile
Ziprasidone Hydrochloride Monohydrate (ZHM)

Chemical IUPAC Name: 5-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one

Chemical Formula: C21H21ClN4OS

Chemical Structure



Molecular Weight: 467.41

Melting Point: 300°C-304°C

Appearance

Solubility: It is insoluble in water but it is soluble in-

- Dimethyl sulfoxide (DMSO): >10 mg/mL,
- Dimethyl formamide(DMF): ~0.16 mg/ml and
- 1: 2 solution of DMSO: phosphate buffer solution (pH 7.2): ~0.33 mg/ml.

Therapeutic Category: Antipsychotic

Storage: Store at room temperature and away from excess heat and moisture

Table 1: Selected Excipients

Nanostructured lipid carriers (NLCs) component	Excipients
Solid lipids	PrecirolATO5
Liquid liquid	Labrafil M1944
stabilizer	Gelucire50/13

Methods:

Drug excipients compatibility studies were carried out using FTIR and DSC.

RESULT AND DISCUSSION:

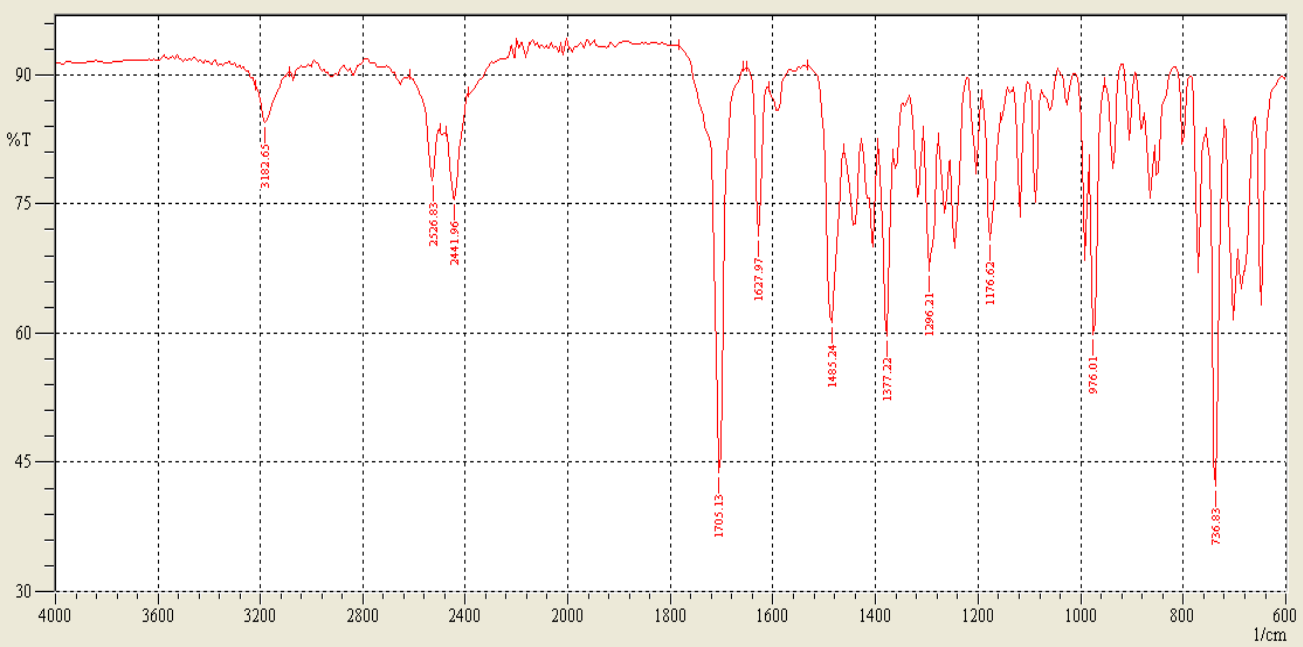


Figure 1: FTIR spectra of ZHM

Table 2: Observed absorption peak with the predicted functional group of ZHM

SR.NO	PEAK/BOND	ABSORPTION WAVELENGTH(CM-1)
1	N-H stretching band	3182.65
2	C-H stretching band	2526.83
3	C=o stretching band	1705.13
4	C=N stretching band	1627.97
5	C-Cl stretching band	736.83
6	C-N stretching band	1377.22

Differential Scanning Calorimetry (DSC)

DSC thermogram of ZHM obtained a sharp endothermic peak at 300.09°C which directly indicated its melting point. The reported value of melting point for ZHM is in the range of 300-304^{O}C[103-105]. Hence, the experimental value is in close *agreement* with reported one.

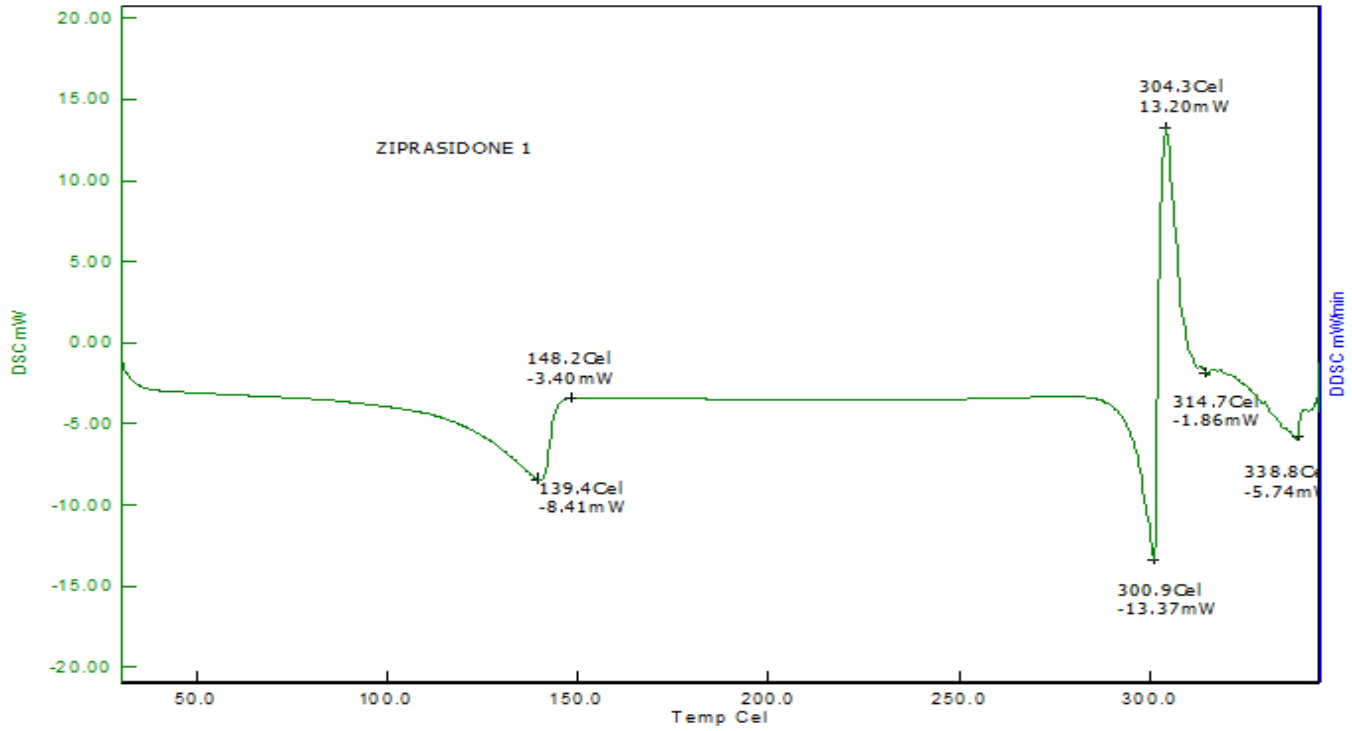


Figure 2: FTIR spectra of ZHM HCL monohydrate with excipient

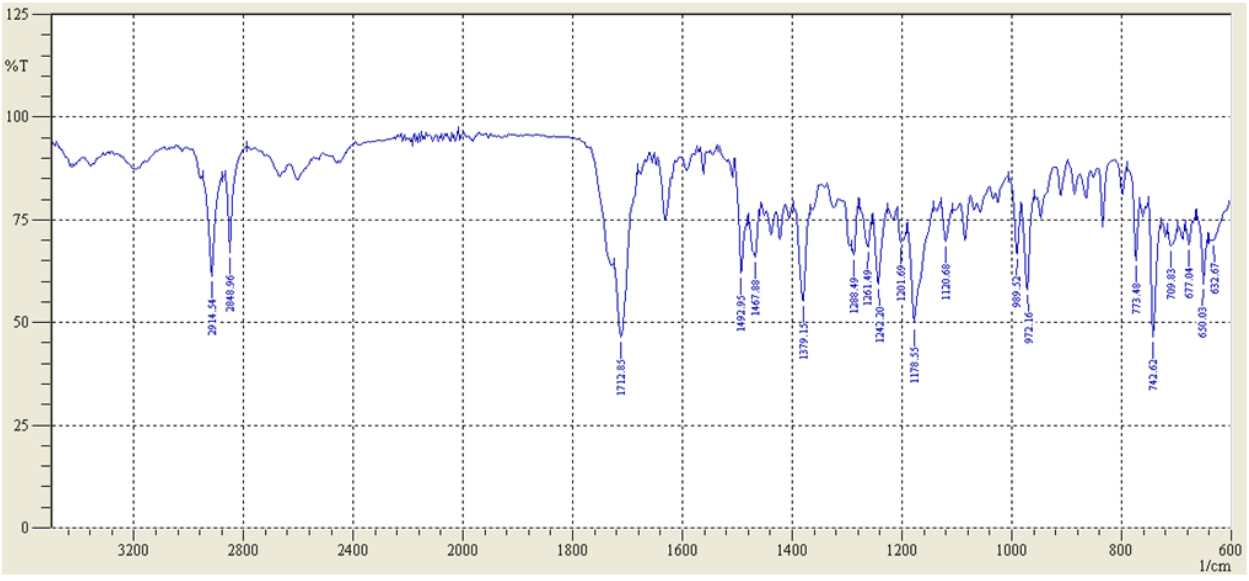


Figure 3: FTIR spectrum of ZHM With Precirol ATO 5

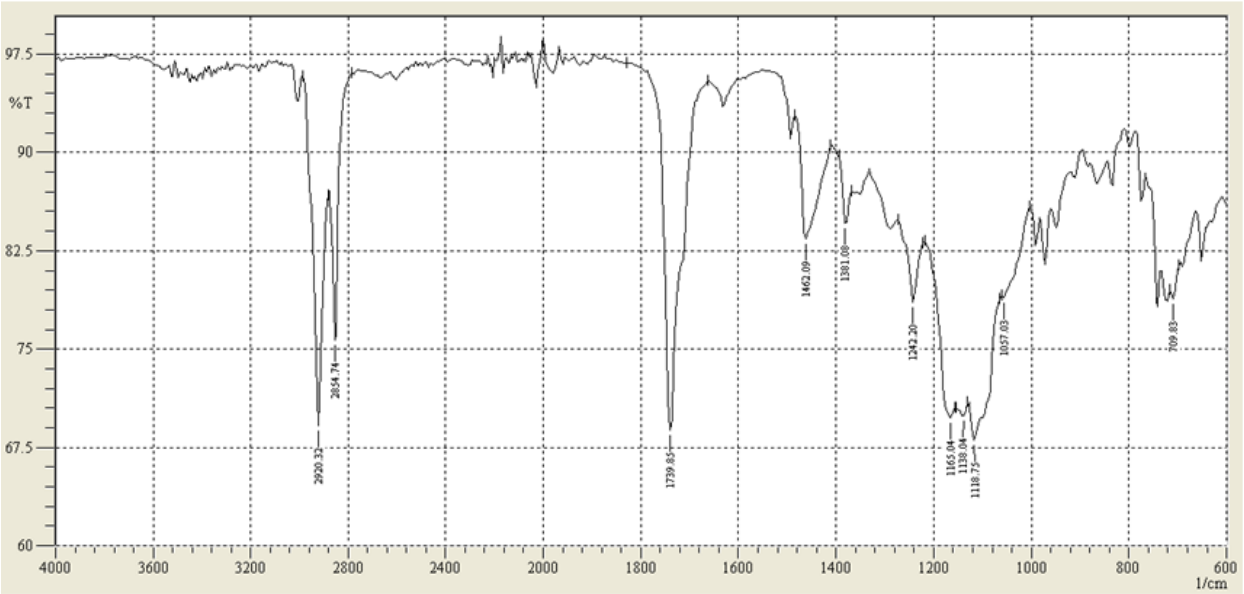


Figure 4: FTIR spectrum of ZHM with LABRAFIL M1944

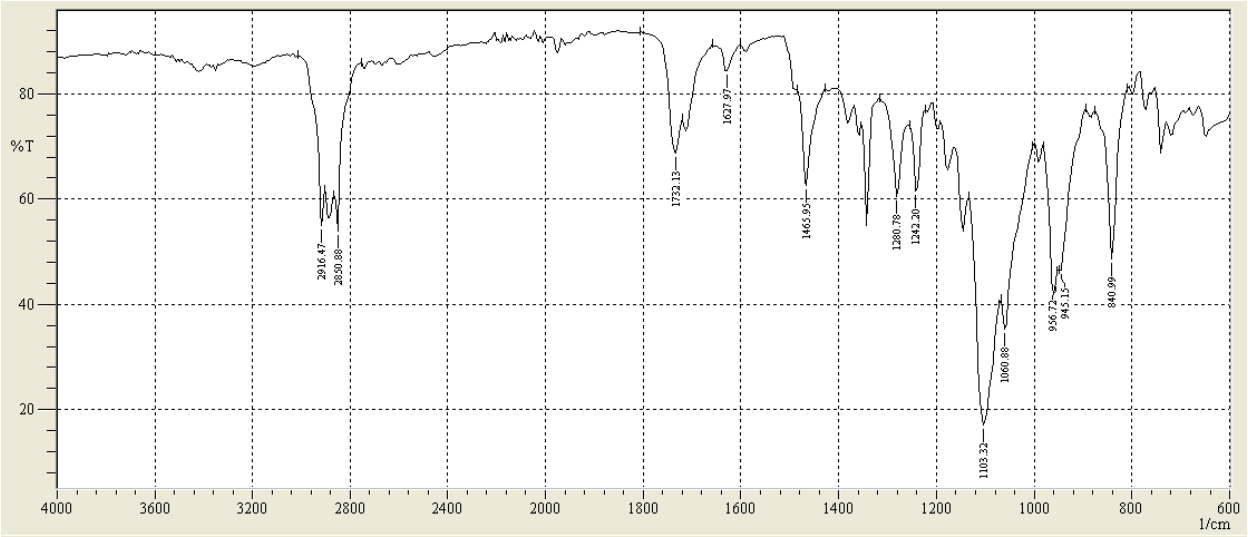


Figure 5: FTIR spectrum of ZHM with Gelucire 50/13

The FTIR spectra obtained for pur drug and with selected excipients i.e precirol ATO5, LabrafilM1944 , Gelucire50/13 indicated that there were no major changes observed in the peaks in the FTIR spectrum of pure drug and mixture. These observations indicated that both drug iscompatible with all formulatiom additives.

DSC STUDIES:

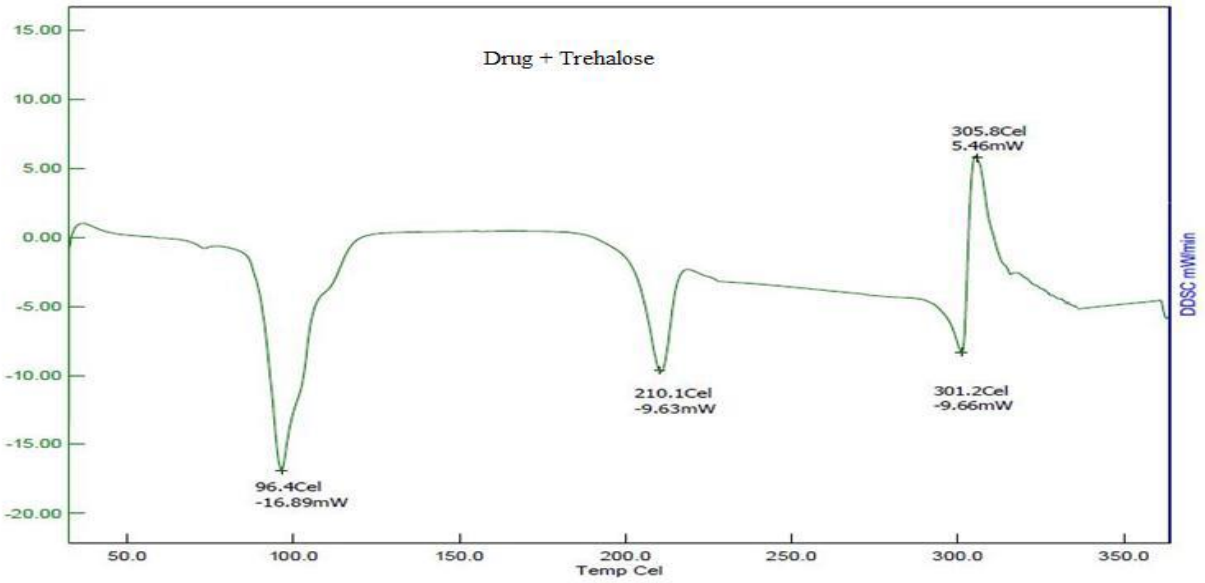


Figure 6: DSC thermogram of ZHM and labrafilM1944 mixture (1:1)

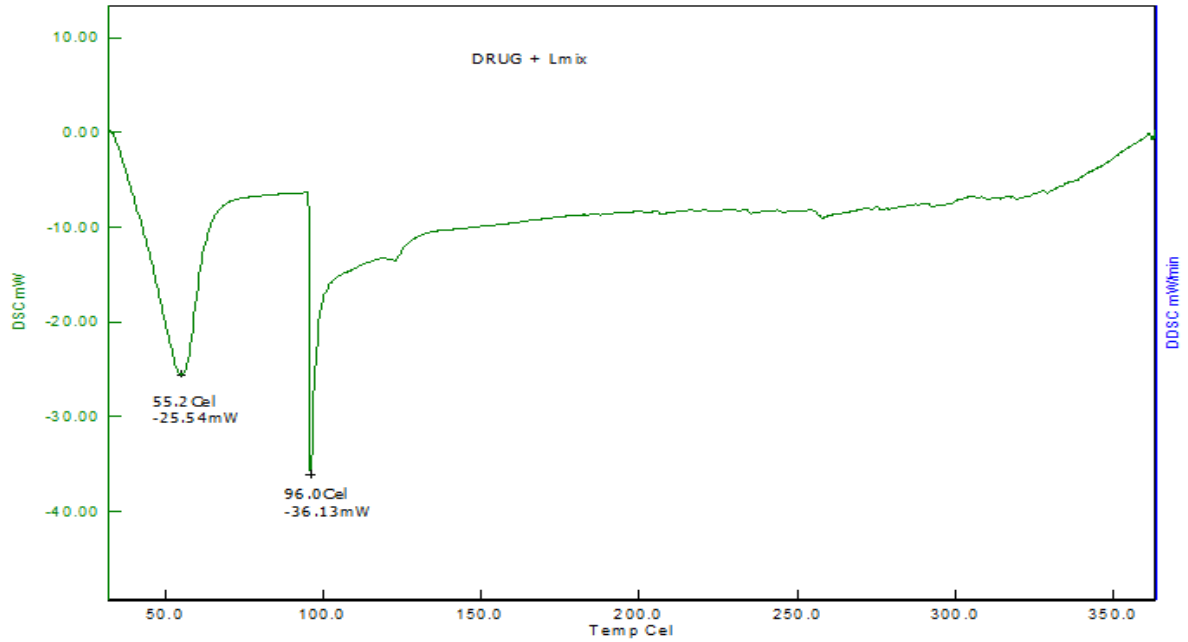


Figure7: DSC thermogram of ZHM and precirol ATO 5 mixture (1:1)

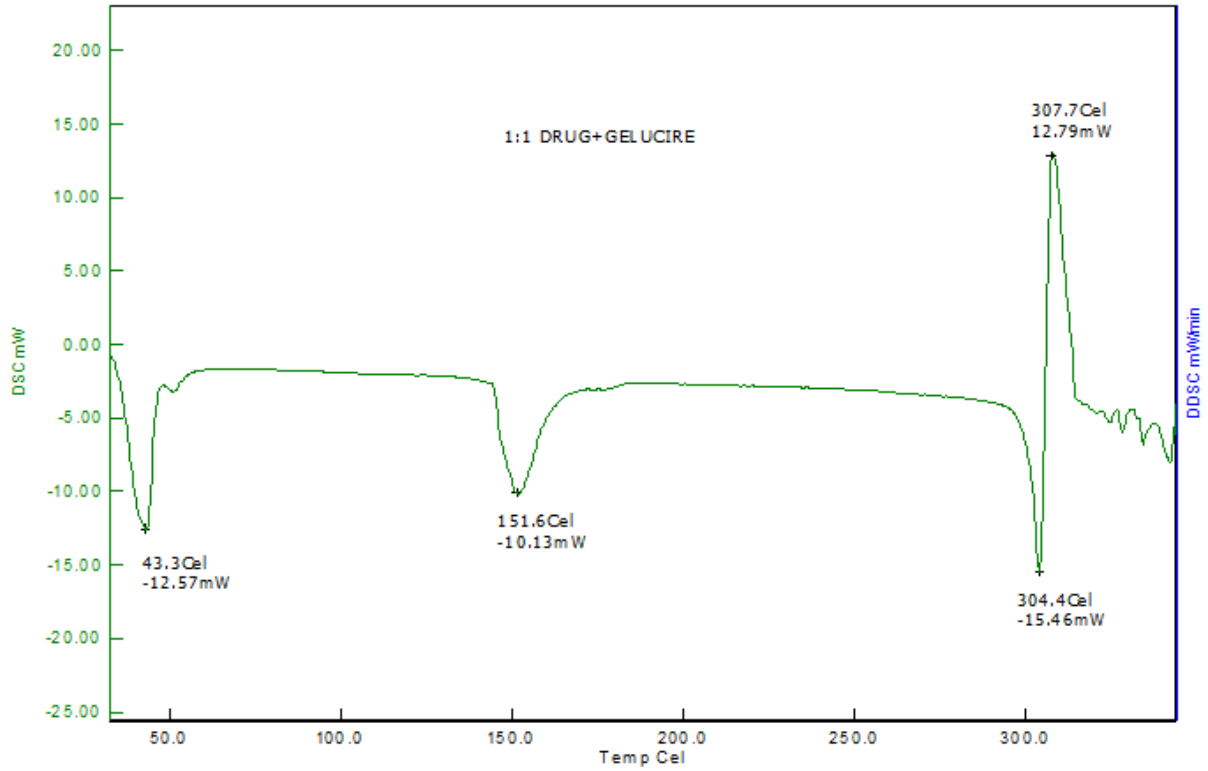


Figure8: DSC thermogram ZHM and gelucire 50/13 mixture (1:1)

CONCLUSION

ZiprasidoneHCL Monohydrate and with selected excipients i.e precirolATO 5 , Labrafil M1944, Gelucire 50/13 indicates that there were no major changes observed in the characteristic of pure drug and in the mixture. These observation indicates that drug is compatible with all formulation additives

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SYNTHESIS OF CHALCONES AND ITS ANTI-CANCER ACTIVITY

Sanjana Durve, Gauri Ghag and Mrs. Deepali M. Jagdale

Considering the various side effects of anti-cancer drugs, various targeted therapies are evolving. Considering this, two novel chalcone derivatives were synthesized and evaluated for their *in-vivo* anticancer activity using zebra fish model. Both the drugs showed prominent anti-angiogenic activity. It concludes that both the drugs target VEGFR and hence can be used as targeted anticancer agents in various cancers such as breast cancer, pancreatic cancer etc.

INTRODUCTION

1.1. Cancer

Cancer, in its most basic definition, is the abnormal uncontrolled growth of cells. Normally, human cells grow and divide to form new cells as the body needs them .When cancer develops, however, this orderly process breaks down.

1.2.Vascular Endothelial Growth Factor Receptor (VEGFR): A prospective target

VEGFs signal through three tyrosine kinase receptors, known as Flt-1 (VEGFR-1), Flk-1/KDR (VEGFR-2) and VEGFR-3 predominantly expressed by endothelial cells. A degree of specificity has been shown for growth factor-receptor binding. Recognition of the VEGF pathway as a key regulator of angiogenesis has led to the development of several VEGF-targeted agents, including agents that prevent VEGF-A binding to its receptors. In addition, anti-VEGF therapy will cause blockade of incorporation of haematopoietic and endothelial progenitor cells.

1.3.Chalcones

Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids.

RATIONALE

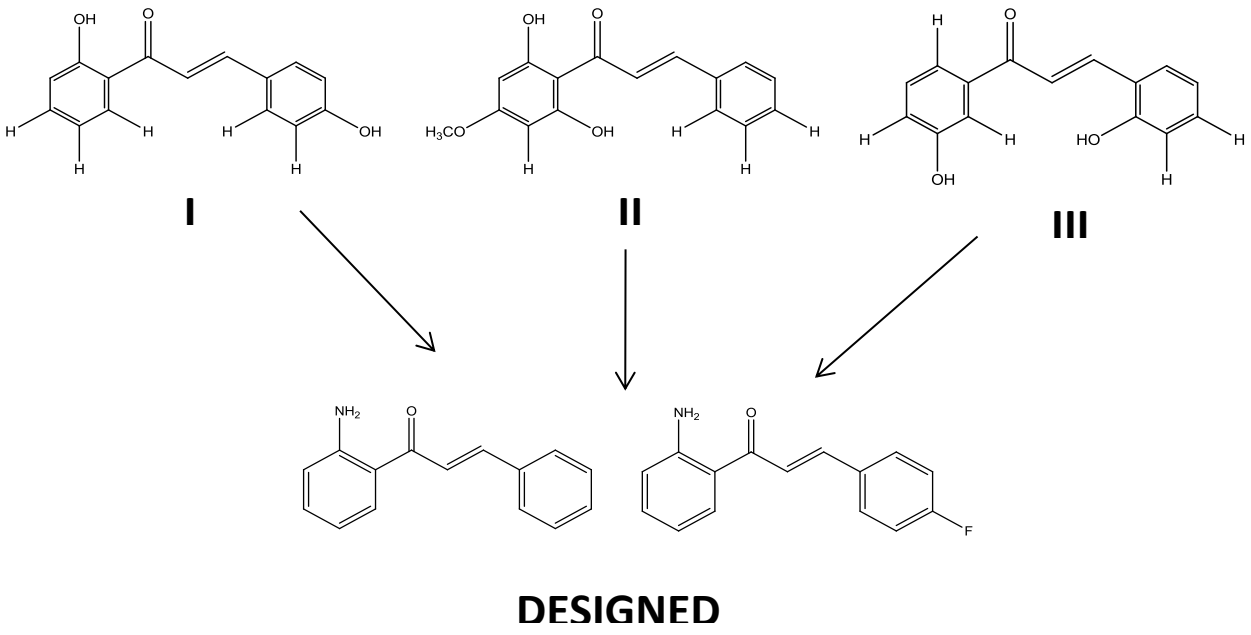

Fig. 1: Designing of the compounds.

VEGF and its tyrosine kinase receptors (VEGFRs) are the key regulators in angiogenesis. VEGF-A, the most important member of VEGF, binds and activates the VEGFR2 (KDR), subsequently activate the main signalling pathway. Therefore, we speculated that the VEGF pathway might play a role in the pro-angiogenic effect of chalcone-based derivatives. This prompted us to synthesize above chalcone derivatives and evaluate them for in-vivo anti-VEGF activity.

METHODOLOGY

3.1. <u>Procedure of synthesis</u>:

1. A suspension of aromatic ketone (40 mmol) and aromatic aldehyde (40 mmol) in ethanolic NaOH was stirred for 4 h at 15-25^{o}C.
2. The reaction mixture was poured into cold water to precipitate solid compound which was recrystallized with ethanol or acetone in order to obtain pure chalcone.

3.2. Procedure for biological evaluation.

Toxicity studies were done to calculate maximum tolerable dose of the test compounds (1mg/150ml, 2mg/150ml, 3mg/150ml). From that the administration dose was decided and administered. Administered dose was half of minimum tolerable dose. The wild type of zebra fishes were kept for acclimatization for 3 days. Fishes were divided in 3 groups containing 3 fishes each that were kept in 250 ml glass beakers containing 150 ml of fish water. On 1st day fishes were anesthetized by ice and their tail was cut up to 50%. Drug was administered on alternate days till day 5. Images were observed.

RESULT

4.1. Chemical Evaluation

Compound 2a:

Molecular structure:-

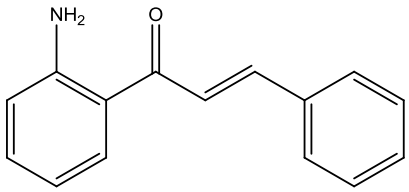

Figure: 1

1) IUPAC name: (E)-1-(2-aminophenyl)-3-phenylprop-2-en-1-one.
2) Molecular weight: 223
3) Molecular formula: $C_{15}H_{13}ON$
4) Nature: yellowish orange crystals
5) Melting point: 74 ^{o}C
6) Yield (%w/w): 84.03 %.

FTIR spectra

IR spectra was recorded on a Quest ATR Diamond Accessory (Black) P31482 & Shimadzu 8100 infrared spectrophotometer.

Compound 2b:

Molecular structure:-

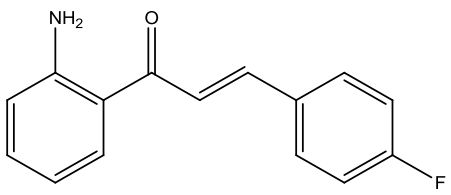

Figure: 2

1) IUPAC name: (E)-1-(2-aminophenyl)-3-(4-fluorophenyl)prop-2-en-1-one.
2) Molecular weight: 241
3) Molecular formula: $C_{15}H_{12}ONF$
4) Nature: Dark yellow crystals
5) Melting point: 94 ^{o}C
6) Yield (%w/w): 66.27%

FTIR spectra

IR spectra was recorded on a Quest ATR Diamond Accessory (Black) P31482 & Shimadzu 8100 infrared spectrophotometer.

4.2.Biological Evaluation

4.2.1. Control group

The fishes in control group were allowed to grow in water without the addition of any drug. Their tail developed normally in the water.

4.2.2 Test compounds

Test dilutions of both the compounds 2a and 2b exhibited *in-vivo* anti-angeogenic activity. Inhibition of tail growth of zebra fish is given in table 1

Table 1: Inhibition of tail growth of zebra fish

Sr. No	Area of Original Tail	Area of New Tail Growth	Average % Regeneration
Vehicle Control			
1	3246	1220	40.39
2	3648	1340	
3	3925	1840	
Group 2A			
1	3169	525	23.50
2	2950	829	
3	2879	773	
Group 2B			
1	2957	531	21.63
2	3222	824	
3	3159	681	

The doses were shown to be toxic at higher drug concentration of 60µg/ml and 90µg/ml and most of the fishes died at this concentration. On the rest of the concentrations deformities on the tail of the fishes were observed.

DISCUSSION

5.1. Synthesis of chalcones

The IR spectra of these chalcones show the presence of C=O, C=C and two sharp bands of NH_2 at 1643-1647 cm^{-1}, 1570-1573 cm^{-1} and 3279-3425 cm^{-1} respectively. C=O and C=C peaks are characteristic peaks of chalcones, as these peaks denote the presence of α,β-unsaturated compounds.

5.2. Biological Evaluation

The growth of tail was observed lesser in fishes administration with 2a and 2b than that of control. Hence it can be concluded that the drugs have antiangiogenesis effect. The data helps in the preliminary idea of toxic and efficacious range of drugs. Since the synthesized compounds are seen to inhibit formation of vasculature in the tail region they could have a potential role as a VEGF inhibitor. The *in-vivo* studies further support the claim for a better evaluation of the compounds. Testing can be done on higher animals.

CONCLUSION

Both the compounds showed good anti-angiogenic effect. As angiogenesis is one of the important factors required for cancer cell growth, inhibition of angiogenesis will led to death of cancer cells. Hence we can effectively conclude that the present derivatives will be effectively used as anti-cancer agents.

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6. Pro-Angiogenic Effects of Chalcone Derivatives in Zebrafish Embryos *in Vivo* Yau-Hung Chen 1,*, Chao-Yuan Chang 1, Chiung-Fang Chang 2, Po-Chih Chen 3, Ya-Ting Lee 1, Ching-Yuh Chern 3,* and Jen-Ning Tsai.,*Molecules* **2015**, *20*, 12512-12524.

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SIMPLE, EFFICIENT AND GREEN METHOD FOR THE SYNTHESIS OF PHENYLHYDRAZONE DERIVATIVES.

Minakshi Sonone, Leena Tandel, Dhiraj Nikam

1. ABSTRACT

A simple, efficient and ecofriendly method at ambient temperature was used to synthesize few phenylhydrazone derivatives by using phenylhydrazide hydrochloride and mixture of aldehyde in water and ethanol ratio (1:1). All derivatives were purified by recrystallization using suitable solvents. The structures of synthesized compounds were confirmed by IR spectral data and melting point.

Keywords: phenylhydrazone, aldehyde, melting point

2. INTRODUCTION

The Fischer indole synthesis is a chemical reaction that produces the aromatic heterocycle indole from a (substituted) phenylhydrazine and an aldehyde or Ketone under acidic conditions. The reaction was discovered in 1883 by Hermann Emil Fischer . Today anti migraine drugs of the triptan class are often synthesized by this method.[1]

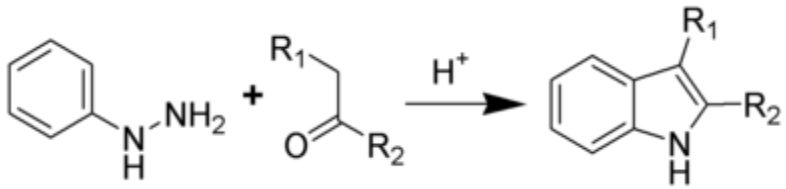


Fig.1.General structure of phenyhydrazone analogues of aldehydes

Phenylhydrazine is prepared by oxidizing ani line with sodium nitrite in the presence of hydrogen chloride to form the diazonium salt , which is subsequently reduced using sodium sulfite in the presence of sodium hydroxide to form the final product [2]

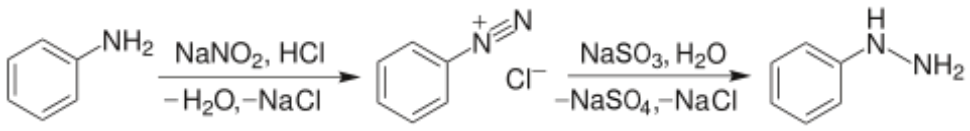


Fig.2. Synthesis of Phenylhydrazine

Traditional method of synthesis involves the reaction of a phenylhydrazide with a carbonyl compound under reflux conditions in diluted media. An industrial drawback of this method is the amount of solvents used (ethanol or toluene usually) inducing a lot of waste and thermal energy needed for an overall time consuming process. In addition, water formation during the reaction may induce incomplete conversions.[3]

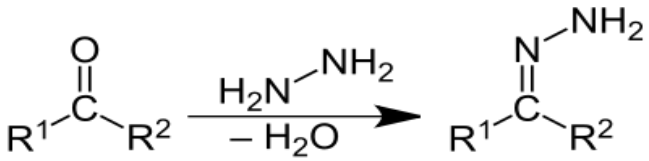


Fig.3. General reaction mechanism for synthesis of phenyhydrazone

Nowadays there is an urgent need for research and industry to invent new, clear and less expensive ways to produce chemicals. All areas of chemistry play important roles in this context. Among them, catalysis and solvent-free systems are the most explored, using different types of energy to perform reactions, including microwave, ultrasound, photochemical and mechanical procedures.[4]

2.1 Solvent free approach

Solvent free approach in recent times has gained lot of interest because of the huge number of advantages associated with it. From the literature survey, we learned that A.R. Hajipour *et al.* put forward one step synthesis of hydrazone and semicarbazone derivatives from the various aldehydes and ketones under solid-phase conditions.[5] Further Allen C.F.H *et al*. showed solvent free reaction conditions are convenient because the reaction media is easy to handle without the need for extensive purification. Yields are high and reactions proceed to completion rapidly.[6]

In this article, we report a simple, efficient and rapid method of synthesis of derivatives of phenyhydrazone aldehydes which are of prime importance in organic chemistry.

2.2 Bilogical activities of phenylhydrazones

Antitubercular,antimicrobial, anticonvulsant, antiepileptic, anti-inflammatory, antioxidant

and antihyperalgesic ,Anti HIV activities.[7]

3. AIM AND OBJECTIVE

- To synthesize phenylhydrazone derivatives in simple, effective and green method .
- To characterize phenylhydrazone derivatives by I.R. spectral analysis and their melting point.

4. RATIONALE

Literature survey revealed that most of the reported procedures used for this purpose require the use of solid supports or other auxiliaries, solvents for the extraction from the solid supports, high temperatures, long reaction time, expensive and not readily available reagents and tedious work-up procedures. Thus, there is need for the protocol for the development of catalytic-free process with readily available reagents which operate under milder conditions.

Some of the methods like mild, convenient solvent free procedure for synthesis of phenylhydrazone put forward by A.R. Hajipour *et al.* in their research showed the importance of green chemistry as the approach was environmentally friendly with no use of harmful solvent. [3]

Encouraging from this method, we have made an attempt to prepare phenylhydrazone derivatives by just simple mixing of phenylhydrazide hydrochloride and mixture of aldehyde with ethanol water mixture with further purification done by recrystallisation.

5. MATERIAL AND METHODS

5.1 Experimental section:

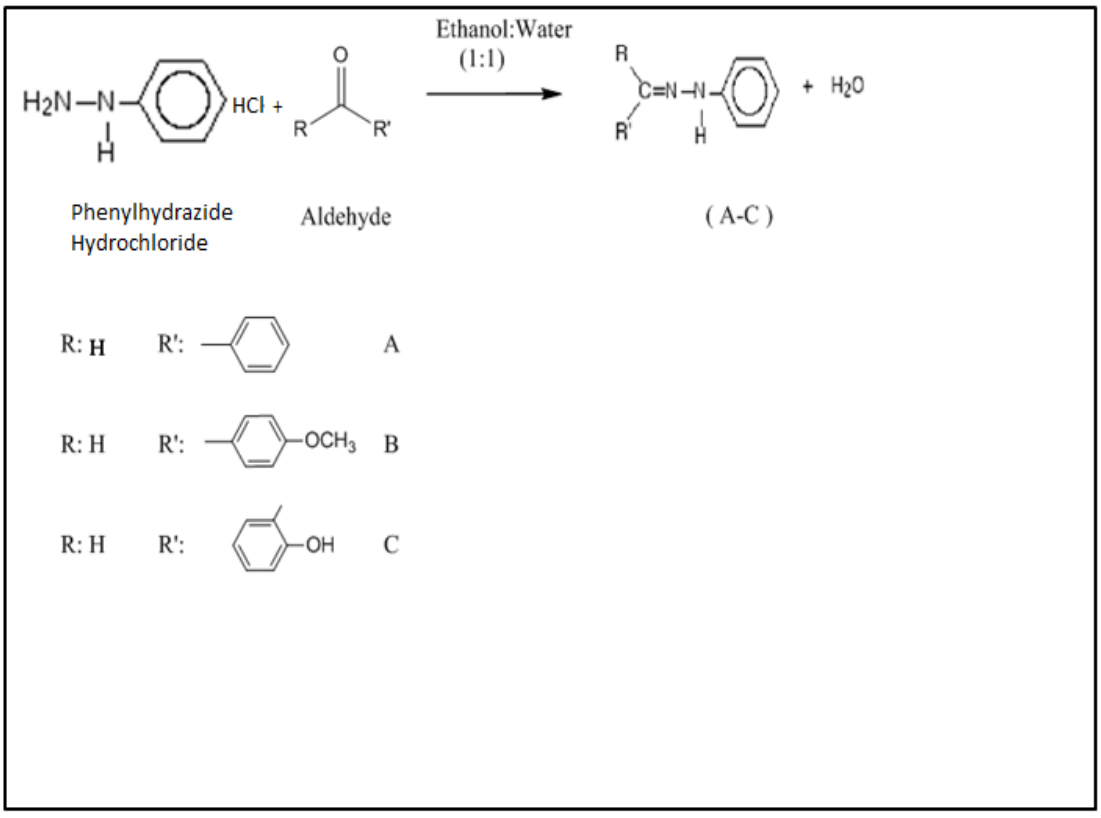
All chemical used were obtained from commercial sources. Melting points were measured in open capillary tube on veego (VMP-D) melting point apparatus. TLC was done using silica gel 60 F_{254} TLC plates. Infrared spectra were recorded on Schimadzu FTIR spectrometer.

5.2 General procedure for preparation of phenylhydrazone:

- In a clean and dry 250 ml conical flask, a solution of phenylhydrazide hydrochloride in water was prepared having 25 mmol of phenylhydrazide hydrochloride (conical flask 1).

- In another clean and dry 250 ml conical flask, a solution of respective aldehyde in water and ethanol mixture (1:1) was prepared having 25 mmol of aldehyde (conical flask 2).
- Phenylhydrazone were obtained by simple mixing of the solution from conical flask 1 into solution of conical flask 2.
- Derivative of the aldehydes obtained were purified by recrystallisation using 95% ethanol. The entire reaction was monitored by Thin Layer Chromatography.
- Characterization of the derivatives were done by

1. Obtaining IR spectrum using FTIR spectrometer.
2. Determining melting point.

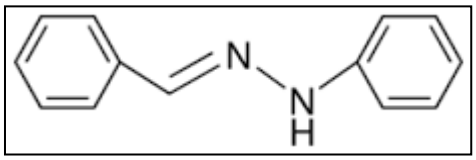


General Scheme for synthesis of phenylhydrazone derivatives

6. RESULT AND DISCUSSION

Benzaldehyde Phenylhydrazone (A)

- Structure:



N-(benzylideneamino)aniline

- Molecular weight: 196.247g/mol
- M.P: 155-157°C
- Percentage yield: 90%
- R_f value: 0.5
- TLC solvent: chloroform(2ml):methanol(2 drops)
- IR spectra:

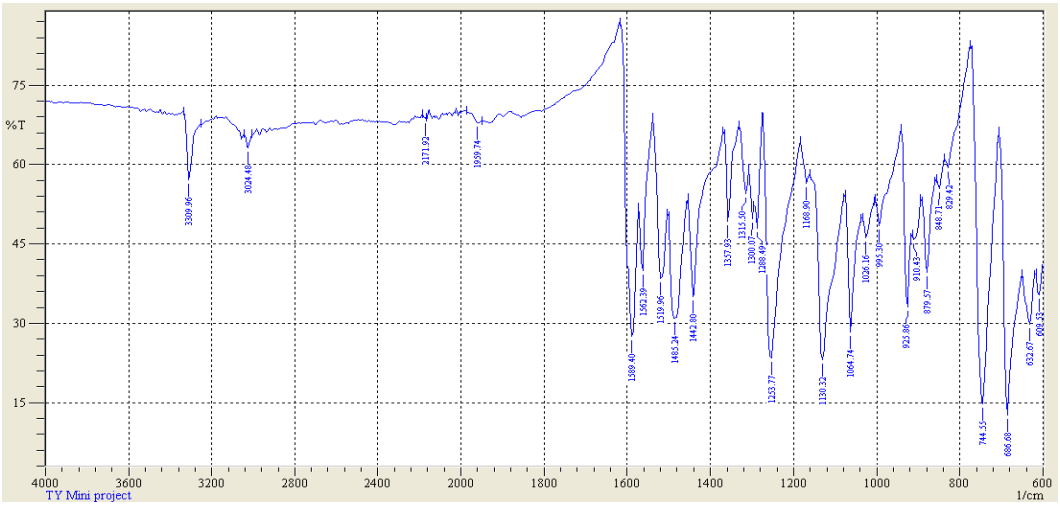


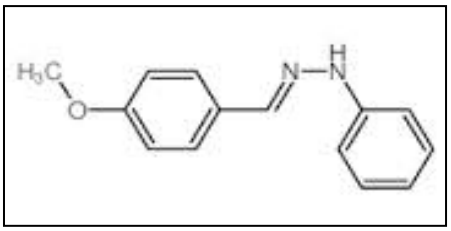
Fig.4. IR spectra N-(benzylideneamino)aniline

Group	Wave number (cm^{-1})
O-H Stretching (H- BONDED)	3309.96
SP2 C-H STRETCH	3024.48
CHARACTERISTIC TO BEXZALDEHDE DERIVATIVE	2171.92
C=O STRECTCHCONJUGATED	1589.40
C=C STRETCH CONJUGATED	1485.24
C-C BENDING	1253.77
C-O BENDING	1130.32
AROMATIC C-H BENDING	744.55
N=N STRETCHING	1562.39
N-H BENDING	1537.93
Ph-N	1130.32

Table1.IR Interpretation of N-(benzylideneamino)aniline

Anisaldehyde Phenylhydrazone (B)

- Structure:



(1E)-1-(4-Methoxybenzylidene)-2-phenylhydrazine

- Molecular weight: 226.273g/mol
- M.P: 108-110˚C
- Percentage yield: 90%
- R_f value: 0.4
- TLC solvent: chloroform(2ml):methanol(2 drops)

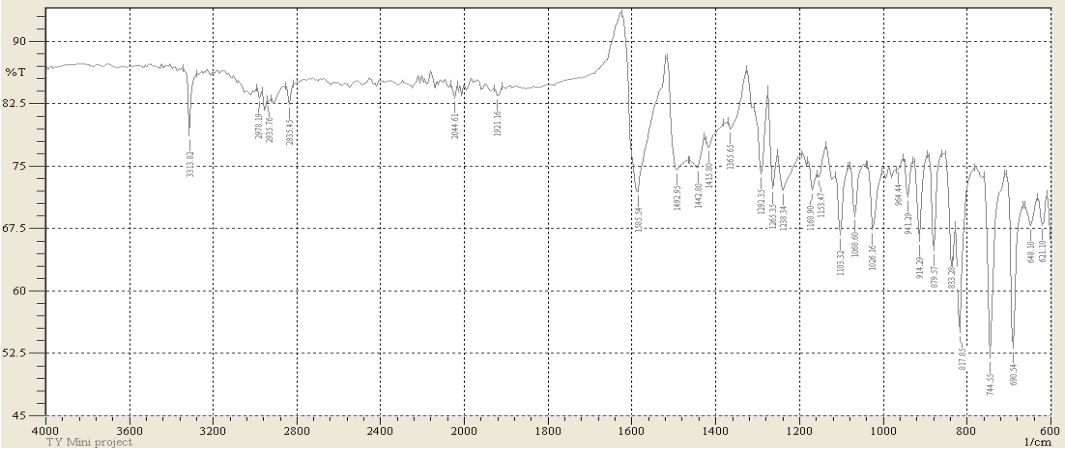


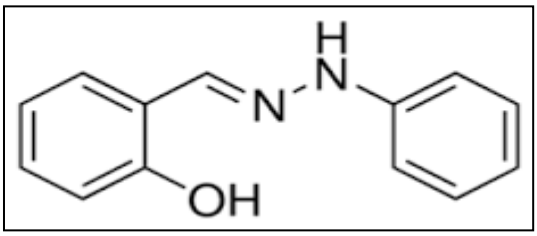
Fig.5.IR spectra (1E)-1-(4-Methoxybenzylidene)-2-phenylhydrazine

Group	Wave number (cm^{-1})
O-H Stretching (H- BONDED)	3313.82
SP2 C-H STRETCH	2978.19
C-H STRETCH ALDEHYDE	2835.45
C=O STRECTCHCONJUGATED	1585.54
C=C STRETCH CONJUGATED	1492.95
C-C BENDING	1265.35
C-O BENDING	1103.32
AROMATIC C-H BENDING	744.55
N=N STRETCHING	1442.80
Ph-N	1168.90
CHARACTERISTIC TO ANISALDEHYDE DERIVATIVE	2044.61

Table 2. IR Interpretation of (1E)-1-(4-Methoxybenzylidene)-2-phenylhydrazine

Salicylaldehye phenylhydrazone (C)

- Structure:



2-[(E)-(Phenylhydrazono)methyl]phenol

- Molecular weight: 212.25 g/mol
- M.P: 143-145 ˚C
- Percentage yield: 92%
- R_f value: 0.5
- TLC solvent: chloroform(2ml):methanol(2 drops)

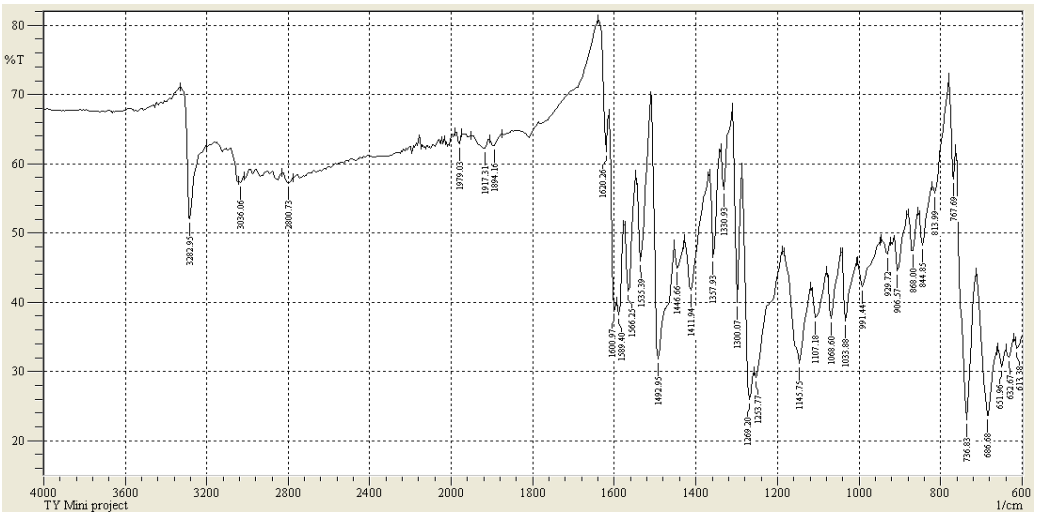


Fig.6.IR Spectra of 2-[(E)-(Phenylhydrazono)methyl]phenol

Group	Wave number (cm^{-1})
O-H Stretching 9(H- BOND)	3282.95
SP2 C-H STRETCH	3036.66
C-H STRETCH ALDEHYDE	2800.73
C=O STRECTCHCONJUGATED	1600.97
C=C STRETCH CONJUGATED	1492.95
C-C BENDING	1269.20
C-O BENDING	1107.18
AROMATIC C-H BENDING	736.83
N=N STRETCHING	1566.25
N-H BENDING	1535.39

Table 3. IR Interpretation of 2-[(E)-(Phenylhydrazono)methyl]phenol

Phenylhydrazone derivatives were synthesized by condensation of phenylhydrazine with benzaldehyde/anisaldehyde/salicylaldehyde at ambient temperature under catalytic free conditions in shorter reaction times with excellent yields (85-95%).

All these compounds were confirmed by their analytical and spectral data. Melting points were confirmed by comparing with reference standard from literature. The presence of peaks in the range of 1600-1685 cm-1 (C=O stretching) and 1450-1580 cm-1 (N=N stretching) from IR spectra confirmed the formation the product.

The method is superior to other introduced methods as it produces in most cases quantitative yields of the desired products with no waste, or any need of tedious purification procedures.

No difference was found when the final product of our synthesis was compared to the currently reported methods. The yields of the product are comparable to any of the current reported methods which can be illustrated by comparing the yield of product A with that of different methods in the table below.

Product code	Reaction conditions	Yield (%)
A	Ball-milling, 25°C, 30 min[3]	100
A	1:1 ratio of water and methanol stirring at 25°C (current method)	90
A	Water and ethanol solvent system, stirring for 15 min at 1000 rpm, 25°C[8]	97
A	Silica gel, NaOH[4]	92

7. CONCLUSION

A simple, efficient and eco-friendly protocol for the synthesis of phenylhydrazone derivatives under catalytic-free conditions. The method developed is comparable to any of the currently reported methods for the synthesis of highly useful intermediate phenylhydrazone

8. REFERENCES:

1 Naimi-Jamal, Mohammad Reza, Mokhtari, Javad, Dekamin, Mohammad Gorban, Javanshir, Shahrzad Iran, J. Chem. Chem. Eng. Vol. 31, No. 2, 2012.

2. Mohammed Zameer Ahmed, N. T. Patel1, K. A. Shaikh2, M. M. Kendre1, Shaikh Shahid1, And M. A. Baseer1

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SIMPLE, EFFICIENT AND GREEN METHOD FOR THE SYNTHESIS OF SEMICARBAZONE DERIVATIVES.

Jignesh Panchal, Prashant Nimbalkar, Dhiraj Nikam

1. ABSTRACT

A simple, efficient and green method was used to synthesize few semicarbazone derivatives by using semicarbazide hydrochloride and mixture of aldehydes or ketones in water and ethanol ratio (1:1). All derivatives were purified by recrystallization using suitable solvents. The structures of synthesized compounds were confirmed by IR spectral data and melting point.

Keywords: Semicarbazones, aldehydes and ketones.

2. INTRODUCTION

- In organic chemistry, a semicarbazone is a derivative of imines formed by a condensation reaction between a ketone or aldehyde and semicarbazide. The terminal amine group of semicarbazide, which behaves very similarly to primary amines.[1]

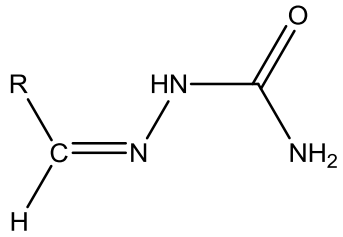
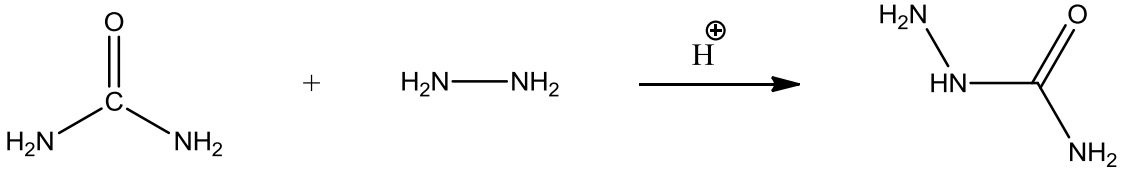


Fig.1 General Structure of semicarbazone analogues of aldehydes

- Semicarbazide is prepared by reacting urea with hydrazine in presence of acid catalyst which is shown below in Fig.2.



Urea Hydrazine Semicarbazide

Fig.2. Synthesis of semicarbazide

2.1 Biological activities of semicarbazones:

- They are found to possess various pharmacological properties such as antimicrobial, anticonvulsant, antiepileptic, anti-inflammatory, antioxidant and antiproliferative activities.
- Ozair*et al*., synthesized a series of *N*-(4,6-substituted diphenylpyrimidin-2-yl) semicarbazones and tested for their anticonvulsant activity against the two seizure models, maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ).
- Afrasiai*et al* synthesizedNi (II) complexes of ortho-napthaquinonethiosemicarbazone and semicarbazone weresynthesized and spectroscopically. The X-ray crystal structure ofboth the complexes describe a distorted octahedral coordination with two tridentate monodeprotonatedligands. *In vitro* anticancer studies on MCF-7 human breast cancer cells reveal thatthe semicarbazone derivative along with its nickel complex is more active in the inhibition ofcell proliferation than the thiosemicarbazone analogue.
- They also act as intermediate for the synthesis of biologically important heterocyclic moieties such as 1,3,4-oxadiazoles, 1,2,3-triazoles and metal complexes.[3]
- Traditional method of synthesis involves the reaction of a semicarbazide with a carbonyl compound under reflux conditions in diluted media. An industrial drawback of this method is the amount of solvents used (ethanol or toluene usually) inducing a lot of waste and thermal energy needed for an overall time consuming process. In addition, water formation during the reaction may induce incomplete conversions.[4]

2.2 General Mechanism for synthesis of semicarbazones:

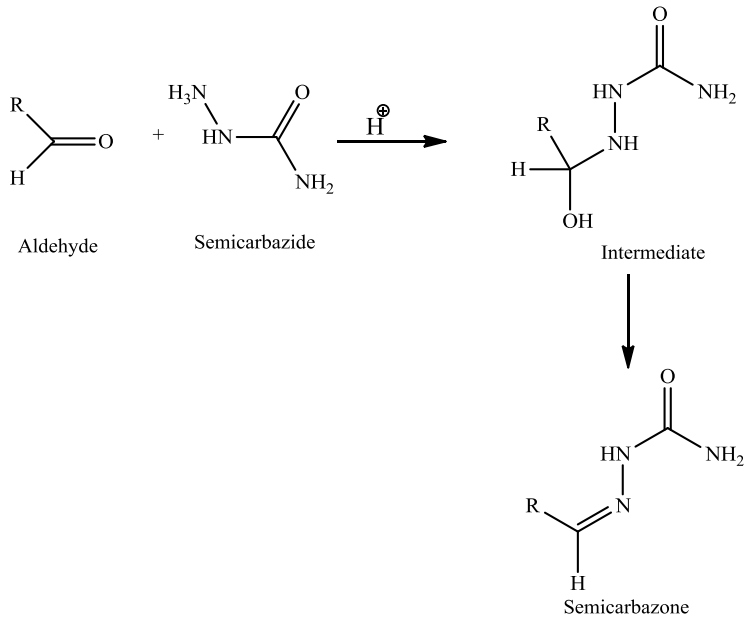


Fig.3. General Reaction mechanism for synthesis of semicarbazones

Nowadays there is an urgent need for research and industry to invent new, clear and less expensive ways to produce chemicals. All areas of chemistry play important roles in this context. Among them, catalysis and solvent-free systems are the most explored, using different types of energy to perform reactions, including microwave, ultrasound, photochemical and mechanical procedures.

Solvent free approach in recent times has gained lot of interest because of the huge number of advantages associated with it. From the literature survey, we learned that A.R. Hajipour*et al.*put forward one step synthesis of hydrazone and semicarbazone derivatives from the various aldehydes and ketones under solid-phase conditions.[4] Further R. Kamakshi*et al*. showed solvent free reaction conditions are convenient because the reaction media is easy to handle without the need for extensive purification. Yields are high and reactions proceed to completion rapidly.[5]

3. AIMS AND OBJECTIVES

- To develop a simple, efficient and green method for synthesis of semicarbazones derivatives.
- To characterize semicarbazone derivatives by I.R. spectral analysis and their melting point.

4. MATERIAL AND METHODS

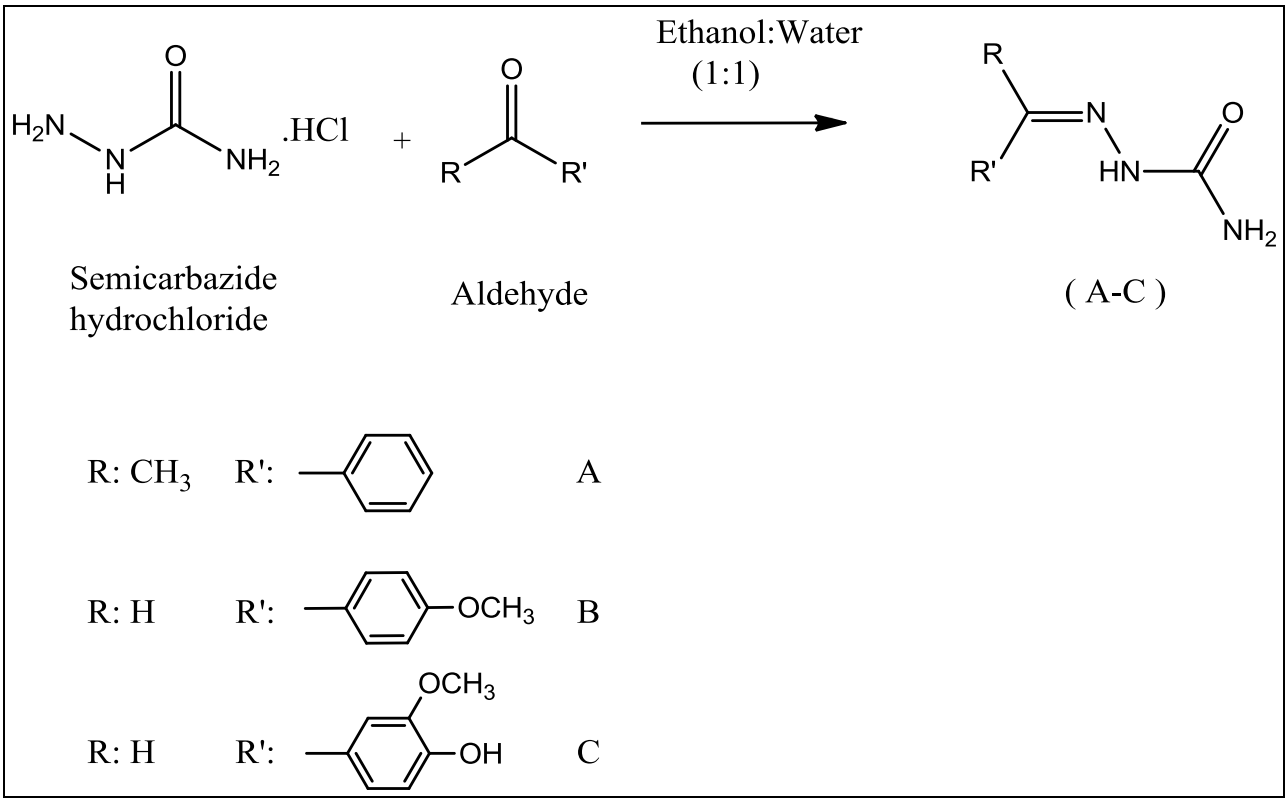
4.1 Experimental section:

All chemical used were obtained from commercial sources. Melting points were measured in open capillary tube on veego (VMP-D) melting point apparatus. TLC was done using silica gel 60 F_{254} TLC plates. Infrared spectra were recordedonSchimadzu FTIR spectrometer.

4.2 General procedure for preparation of semicarbazones:

- In a clean and dry 250 ml conical flask, a solution of semicarbazide in water was prepared having 25 mmol of semicarbazide hydrochloride (conical flask 1).

- In another clean and dry 250 ml conical flask, a solution of respective aldehyde in water and ethanol mixture (1:1) was prepared having 25 mmol of aldehyde (conical flask 2).
- Semicarbzones were obtained by simple mixing of the contents of above conical flask.
- Derivative of the semicarbazones obtained were purified by recrystallisation using 95% ethanol. The entire reaction was monitored by Thin Layer Chromatography.
- Characterization of the derivatives were done by
  1. Obtaining IR spectrum using FTIR spectrophotometer.
  2. Determining melting point using veego.

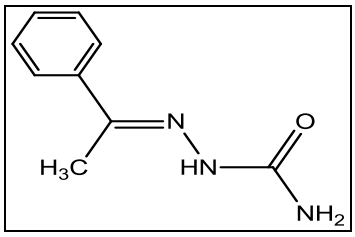


General Scheme for synthesis of semicarbazone derivatives

5.RESULT AND DISCUSSION

5.1 Acetophenone Semicarbazone (A)

- Structure:



- IUPAC: (E)-2-(1-phenylethylidene) hydrazinecarboxamide.
- Molecular weight:177.20314 g/mol

Group	Wave number (cm^{-1})

- M.P: 202-204 [Standard MP: 198°C]
- Percentage yield: 90%
- Rf value: 0.5
- IR spectra:

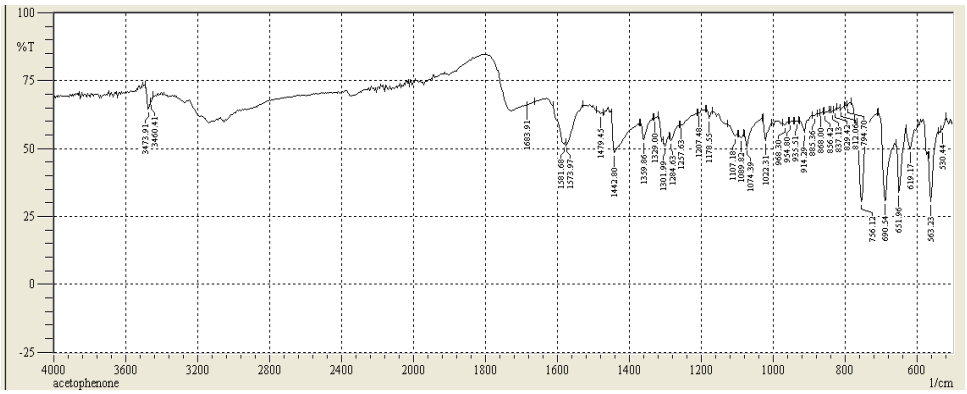


Fig.4. IR Spectra of (E)-2-(1-phenylethylidene) hydrazinecarboxamide

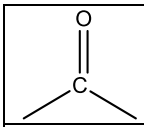
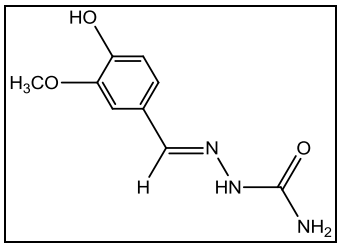
	1683
C═N	1573.97
C—N	1178.55
N—H	3473
C—C	914.29

Table1.IR Interpretation of (E)-2-(1-phenylethylidene)hydrazinecarboxamide.

5.2 Vanillin Semicarbazone (C)

- Structure:



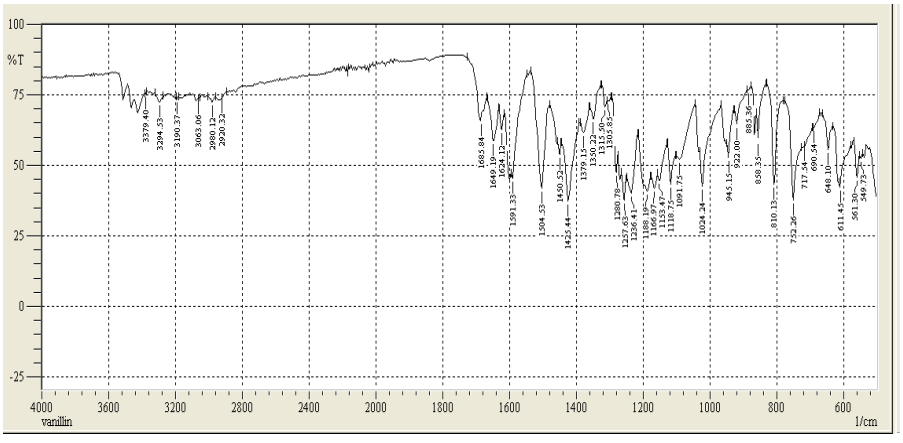
- IUPAC: (E)-2-(4-hydroxy-3-methoxybenzylidene)hydrazinecarboxamide
- Molecular weight:209 g/mol
- M.P: 237-239˚C [Standard MP: 230˚C]
- Percentage yield: 92%
- Rf value: 0.5
- IR spectra:
- 

Fig.5.IR Spectra of (E)-2-(4-hydroxy-3-ethoxybenzylidene)hydrazinecarboxamide

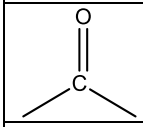
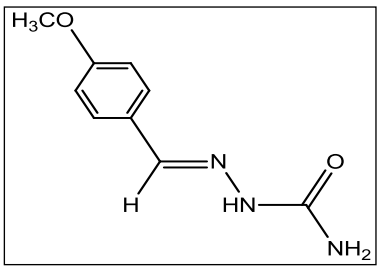
Group	Wave number (cm^{-1})
	1685
C═N	1649.19
C—N	1118.75
N—H	3379
C—C	945.15

Table 2.IR Interpretation of (E)-2-(4-hydroxy-3-thoxybenzylidene)hydrazinecarboxamide

5.3 Anisaldehyde Semicarbazone (B)

- Structure:



- IUPAC : (E)-2-(4-methoxybenzylidene)hydrazinecarboxamide
- Molecular weight: 189.21384 g/mol
- M.P: 211-213 ˚C [Standard MP: 210˚C]
- Percentage yield: 88%
- Rf value: 0.6
- IR spectra:

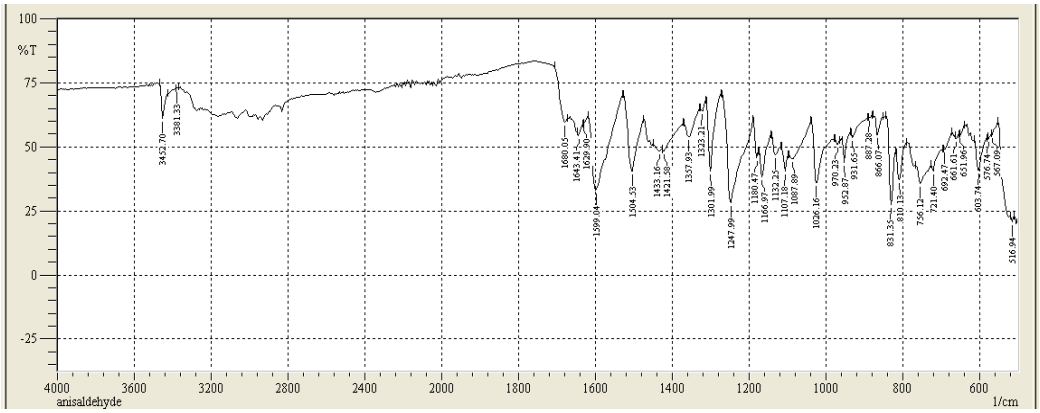


Fig.6.IR Spectra of (E)-2-(4-methoxybenzylidene)hydrazinecarboxamide

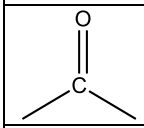
Group	Wave number (cm^{-1})
	1680.05
C═N	1599.04
C—N	1180.47
N—H	3452
C—C	952.87

Table 3.IR Interpretation of (E)-2-(4-methoxybenzylidene)hydrazinecarboxamide

- Semicarbazone derivatives were synthesized by condensation of semicarbazide hydrochloride with acetophenone/anisaldehyde/vanillin at ambient temperature under catalytic free conditions in shorter reaction times with excellent yields (85-95%).

- All these compounds were confirmed by their analytical and spectral data. Melting points were confirmed by comparing with reference standard from literature. The presence of peaks in the range of 1650-1685 cm-1 (C=O stretching) and 1555-1610 cm-1 (C=N stretching) from IR spectra confirmed the formation the product.

- The method is superior to other introduced methodsas it produces in most cases quantitative yields of the desired products with no waste, or any need of tedious purification procedures.

- No difference was found when the final product of our synthesis was compared to the currently reported methods. The yields of the product are comparable to any of

the current reported methods which can be illustrated by comparing the yield of product A with that of different methods in the table below.

Product code	Reaction conditions	Yield (%)
A	ball-milling, 25 °C, 30 min	100
A	2 : 1 ratio of water and methanol stirring at 25°C	97
A	Water and ethanol solvent system, stirring for 15 min at 1000 rpm, 25°C	90
A	Silica gel, NaOH	92

6. CONCLUSION

Thus, we have developed a simple, efficient and green method for the synthesis of semicarbazone derivatives under catalytic-free conditions. The method developed is comparable to any of the currently reported methods for the synthesis of highly useful intermediate semicarbazones.

7. REFERENCES:

1. Naimi-Jamal, Mohammad Reza, Mokhtari, Javad, Dekamin, Mohammad Gorban, Javanshir, Shahrzad Iran, *J. Chem. Chem. Eng*. Vol. 31, No. 2, 2012.

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FORMULATION AND EVALUATION OF SODIUM ALGINATE BEADS LOADED WITH CHELATING AGENT.

Aniket Londhe, Rupali Madapura, Neha Dand

ABSTRACT

Microparticles from nontoxic biopolymer were prepared as a solution for the adsorption process of heavy metals in polymeric porous beads. The aim of the study is to propose a new method to remove bivalent ions (Pb^{2+}; Cd^{2+}) from synthetic wastewater using calcium alginate microparticles. The alginate (a natural polymer obtained from marine algae) was tested as adsorbent material, due to its good affinity for the bivalent metal ions. Additionally a chelating agent was loaded into a polymeric carrier prepared in an aqueous environment. Calcium alginate beads using sodium alginate employing ionotropic gelation technique with calcium chloride as cross linking agent. The chelating agent was simultaneously incorporated during gelation stage. The beads were evaluated for % drug content.

Key words: Sodium alginate, calcium chloride, ionotropic gelation, chelating agents

INTRODUCTION

Environmental pollution by toxic metals arises from industrial, agricultural effluents and waste disposal from various sources. Many industries such as metal plating facilities, mining operations and tanneries discharge waste containing heavy metal ions. These toxic metals can cause accumulative poisoning, cancer and brain damage when found above the tolerance levels. The agencies for the environmental monitoring have set permissible limits for heavy metals levels in drinking water because of their harmful effects. The removal and rapid decontamination of heavy metals (Cd, Pb, Cu, Hg) become very important for the environmental remediation. Many processes have been used for the removal of heavy metals from waste waters, such as chemical precipitation, coagulation, solvent extraction, membrane separation, ion exchange and adsorption. For dilute metal concentrations, ion exchange, reverse osmosis and adsorption can be applied. However, the common use of ion exchange and reverse osmosis is restricted by the high operating cost. As an alternative to chemical precipitation, membrane filtration, or ion exchange, adsorption processes with wide variety adsorbents have been tested [1].

The adsorbents used should have some specific properties, such as a high ability to reduce the concentration of heavy metals below the acceptable limits, high adsorption capacity and long lifetime. Thus, it is a continuing need to identify and develop low-cost and efficient adsorbents for facile and efficient removal process. Heavy metal adsorption was studied on various adsorbents such as activated carbon [2], fly ash [3], and bioadsorbents (adsorbents

from plant- and animal-origin materials, for example bark/tannin-rich materials, humus, peat moss, modified cotton and wool, chitin, chitosan, seaweed, and biopolymers [4-6].

Alginate is a biopolymer with many applications in drug delivery systems, cell encapsulation, food industry, cosmetics. In wastewater treatment could play an important role in removal heavy metal ions due its advantages, such as facile obtaining procedure, biodegradability, biocompatibility, economic and environmental friendliness.

Alginic acid is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks) or alternating M and G-residues (MG-blocks).The chemical compound sodium alginate is the sodium salt of Alginic acid. Its empirical formula is $NaC_6H_7O_6$. Sodium alginate is a gum, extracted from the cell walls of brown algae. It is soluble in cold and hot water with strong agitation and can thicken and bind. In addition to its use as a thickening, gel forming and colloidal stabilizing agent in the food and beverage industries, it is also used as a binder in tablet formulation. Its unique property of forming water insoluble calcium alginate gel through ionotropic gelation with Ca^{+2} ions under simple and mild conditions has made it possible to encapsulate both macromolecular agents and low molecular weight therapeutic agents [7].

RATIONALE

Alginate has an innate strong affinity for divalent metal ions. As majority of metal impurities found in water are divalent in nature, alginate can prove to be an attractive and economic alternative to the existing water treatment options. Furthermore. the chelating ability of these microparticles can be improved by incorporating a chelating agent in them. The basis behind this particular process was to load a natural polymeric carrier with a chelating agent. Disodium edetate was chosen because of its versatile nature. It is freely soluble in an aqueous medium and shows no evidence of toxicity (when taken in a dose less than 3gms). In this study, EDTA was incorporated into calcium alginate beads by simultaneous method due to the unavailability of other more efficient chelating agents like Dimercaptosuccinic acid (DMSA), 2,3-Dimercapto-1-propanesulfonic acid (DMPS)

AIM

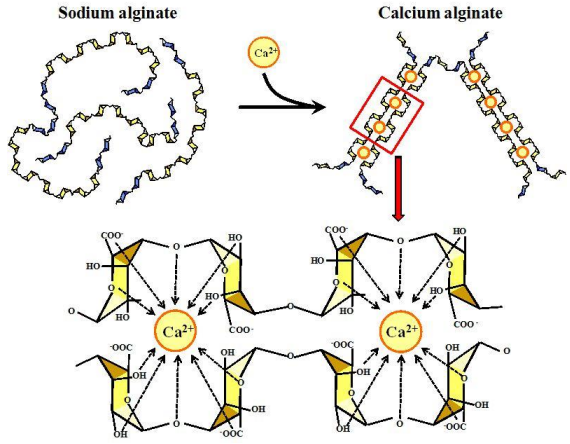
1. To Formulate Calcium alginate beads by ionotropic gelation method
2. To load chelating agent in the beads
3. To analyse these beads for % drug content

MATERIALS

- Sodium Alginate
- Calcium Chloride 95% LR (Research lab- Fine Chem Industries)
- Ethylenediamine Tetra Acetic Acid Disodium Salt (Research lab- Fine Chem Industries)
- Overhead Stirrer – (Remy Motors Ltd)
- Ultrasonicator Bath

METHODOLOGY

Alginate, a linear polysaccharide consisting of mannuronic (M) and guluronic acid (G)residues, is considered as a stimuli-responsive polymer which undergoes sol-geltransition in the presence of di-valent or multi-valent cations such as Ca++ and changespolymer hydrophilicity in response to external pH environment. Due to spatialarrangement of the ring and hydroxyl oxygen atoms of the monomers as shown in Fig 1, poly-guluronate (GG block) can form a stronger type of cooperative bonding withcalcium ions (or other multi-valent cations), resulting in ionotropic gelation. Basedupon the steric arrangements of the junction zones, this characteristic property has beendescribed as an 'egg-box' model or zipper mechanism [13].



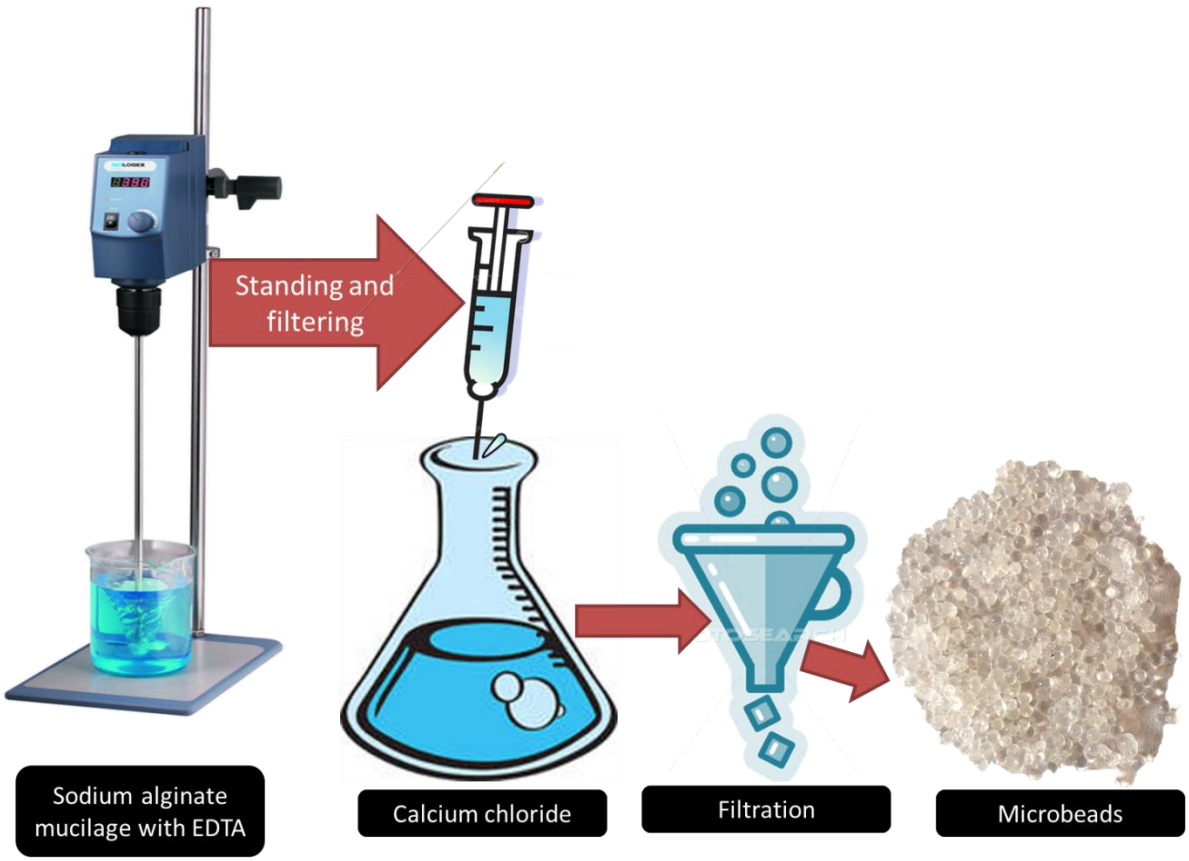
Schematic illustration of the molecular structure of alginate hydrogel

Experiment

Batch	Sodium Alginate	Calcium Chloride	Chelating agent
A1	2%	1%	200mg
A2	2.5%	1%	200mg
A3	5%	1%	200mg
A4	7.5%	1%	200mg
A5	10%	1%	200mg

The batches were prepared using the simultaneous method. The calculated amount of Sodium alginate was weighed and added to distilled water. An overhead stirrer was used (100rpm) to form mucilage. A weighed amount of the chelating agent was added into the dispersion and again stirredat 100rpm till it formed uniform mucilage. It was the isolated for around 60 mins to let the bubbles, so formed during the stirring operation to disappear. The time of isolation was altered with respect to the concentration of sodium alginate used. Since, higher concentration had greater viscosity, it required more time to settle. It was then passed through a muslin cloth to ensure its freedom from particulate matter and leftover bubbles.

A 1% w/v Calcium Chloride solution was prepared with water in a beaker having a wider base. The wider base helps to give more surface area for formation of the beads.Using a syringe the sodium alginate solution was dropped into the calcium chloride solution. It was left isolated for 90 mins to allow the chelating agent to get loaded into the beads. The formed beads were then filtered using a filter paper. They were dried in the hot air oven for three hours at 60ºC for curing. Care should be taken to not let the oven overheat and turn the beads brown [14].



Schematic representation of manufacturing of alginate beads

METHOD FOR % DRUG CONTENT

The dried microbeads were crushed in mortar. 25 ml of distilled water was added to dissolve EDTA. Ultrasonicator was used to aid the solubilisation of EDTA. The solution was filtered in filled in the burette.20 ml of 0.05 M $ZnCl_2$ solution was nearly neutralised with dropwise addition of 2 M NaOH solution. To this the 25 ml of water was added. Furthermore 10 ml of NH_3/ NH_4Cl buffer was added to dissolve the formed precipitate. Then 5 ml of excess buffer is added. 40 mg of Mordant black II is introduced and the prepared solution is titrated against the solution of crushed microbeads until end point is reached i.e. blue colour is discharged [15]. [Factor: 1 ml of 0.05 M EDTA ~ 0.003269 g of Zn^{+2}]

RESULT AND DISCUSSION

In this experiment we created a gel out of sodium alginate. A gel is a soft substance that has the properties of both liquids and solids. In this experiment, long chains of repeating molecules in the alginate – called polymers – became tangled into a net or mesh. When calcium chloride was added, the calcium ions in the solution crosslinked the polymers in the alginate, attaching them to each other at many points. This cross-linking created a flexible, soft solid – a gel bead. During the longer soak, more calcium ions were able to move further into the mesh of the gel bead, resulting in more cross-linking and a firmer texture.

Batch A1 – The beads prepared were very soft and could not be hardened. Hence slightly higher concentration of sodium alginate was used to prepare microbeads of batch A2 but those too proved to be too soft to handle.

Thus a 3rd batch having 5% sodium alginate was prepared. The beads prepared had acceptable morphology. The beads were assayed by the above mentioned method. The beads did not show any entrapment of EDTA.

Thus to entrap more EDTA the concentration of sodium alginate was increased from 5% to 7.5%. But the results of titration showed no improvement. Thus a further increase in the concentration of the polymer was attempted. The 10% solution of sodium alginate was too thick to handle. Hence this batch could not be formed.

The inability to detect EDTA by the complexometric titration method can be attributed to the following reasons:

1. EDTA is highly water soluble hence it could not be entrapped in the microbeads and remained in the filtrate.
2. The EDTA already chelated with calcium chloride used to cause gelation thus no free EDTA was available to be detected
3. The method is not sensitive enough to detect such low levels of EDTA

CONCLUSION

In this study, EDTA was incorporated into calcium alginate beads by simultaneous method due to the unavailability of other more efficient chelating agents like Dimercaptosuccinic acid (DMSA), 2,3-Dimercapto-1-propanesulfonic acid (DMPS) . Thus the above study can be continued further by incorporating other chelating agents. Further evaluation such as surface morphology, porosity, adsorption behaviour etc needs to be done.

The microbeads of sodium alginate prepared by ionotropic gelation can prove to be an effective alternative to currently available method to soften water, remove common heavy metal effluents found in industrial waste water, heavy metal poisoning etc. These unloaded beads have a whole spectrum of uses ranging from immobilisation of enzymes, delivery of drugs, controlled release of drugs, wound healing, food industry etc.

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SYNTHESIS OF CHALCONES BY GREEN METHOD

Nikita Mohite, Sheetal Mahadik, Rhea Mohan

ABSTRACT: The beginning of green chemistry is frequently considered as a response to the need to reduce the damage of the environment by hazardous chemicals and the processes used to produce them. This project presents an example of the implementation of green chemistry principles in synthesis of drug through chalcones synthesis. Chalcones constitute an important class of compound possessing diverse type of biological properties including antibacterial, antiplasmodial, trypanocidal etc properties. Grindstone technique was used for synthesis of chalcones. The chalcones synthesized were 1,3-diphenyl-2-propen-1-one,3-(4-hydroxy-3-methoxy-phenyl)-1-phenylprop-2-ene-1-one,3-(3-hydroxyphenyl3-1-phenylprop-2-en-1-one,3-(3-methoxyphenyl)-1-phenylprop-2-en-1-one.TLC was done to check the purity of products.

Keywords: chalcones, green chemistry, grindstone technique, TLC.

INTRODUCTION:

It is widely acknowledged that there is a growing need for more environmentally acceptable processes in the chemical industry. Green chemistry has grown from a small idea into a new approach to the scientifically based environmental protection[1]. **Green chemistry**, it is area of chemistry and chemical engineering focused on the designing of products and processes that minimize the use and generation of hazardous substance[2] . Twelve principles that form the basis of green chemistry

12 principles of green chemistry

Green chemistry metrics are basically measure aspects of a chemical process relating to the principles of green chemistry. These metrics serve to quantify the efficiency or environmental performance of chemical processes, and allow changes in performance to be measured. The motivation for using metrics is the expectation that quantifying technical and environmental improvements can make the benefits of new technologies more tangible, perceptible, or understandable. Two factors that measures the efficiency of process are[3]:

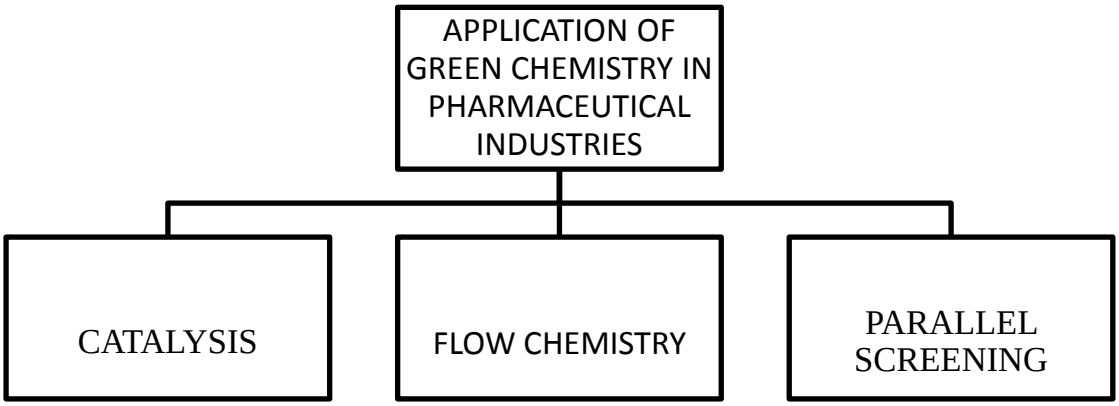
a. Atom economy b. E-factor

INDUSTRY SEGMENT	PRODUCT TONNAGE	E-FACTOR(KG WASTE/KG PRODUCT)
OIL REFINING	**10^6 -10^7**	**<0.1**
BULK CHEMICALS	**10^4-10^6**	**<1-5**
FINE CHEMICALS	**10^4-10^2**	**5-50**
PHARMACEUTICALS	**10-10^2**	**25-100**

Fig. A .R SHELDON "green chemistry and catalysis"

The above table clearly shows that maximum waste is generated from pharmaceutical industries, for a industry that mainly focuses on reducing the disease burden and

improving standards of patients it is ironical that it generates more ratio of waste per kg. Green chemistry is a tool, which when implemented right, can help the industry achieve its environmental goals[1].



CATALYSIS: Traditional organic synthesis features stoichiometric quantities of reagents, leading to large quantities of by products, which add to the burden of wastage. The right catalyst technology enhances product value, while minimizing waste streams, and improving cycle times. It is estimated that, this new biocatalytic method for pregabalin will cut down Pfizer's organic chemical waste production by 200,000 MT in the period from 2007-2020.

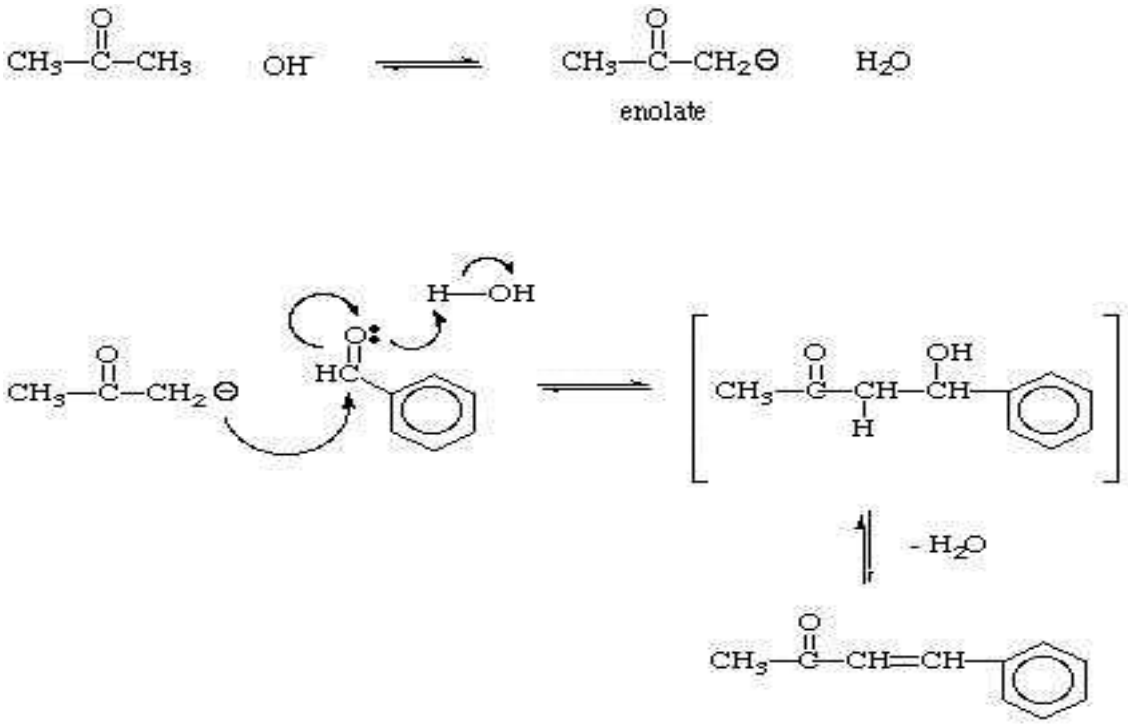
FLOW CHEMISTRY: A sustainable production method, that is yet to demonstrate its full potential in the scale-up of APIs, is the incorporation of continuous manufacturing processes. Flow chemistry allows for superior reaction mechanics and better rates of heat and mass transfer over conventional batch reactors, leading to safe and economic processes.

PARALLEL SCREENING: Molecules in the API business are typically NCEs or novel intermediates and reported procedures do not always work as expected. P a r a l l e l r e a c t i o n screening saves a substantial amount of time and enables timely activities during process research, provided it is planned and utilized well.

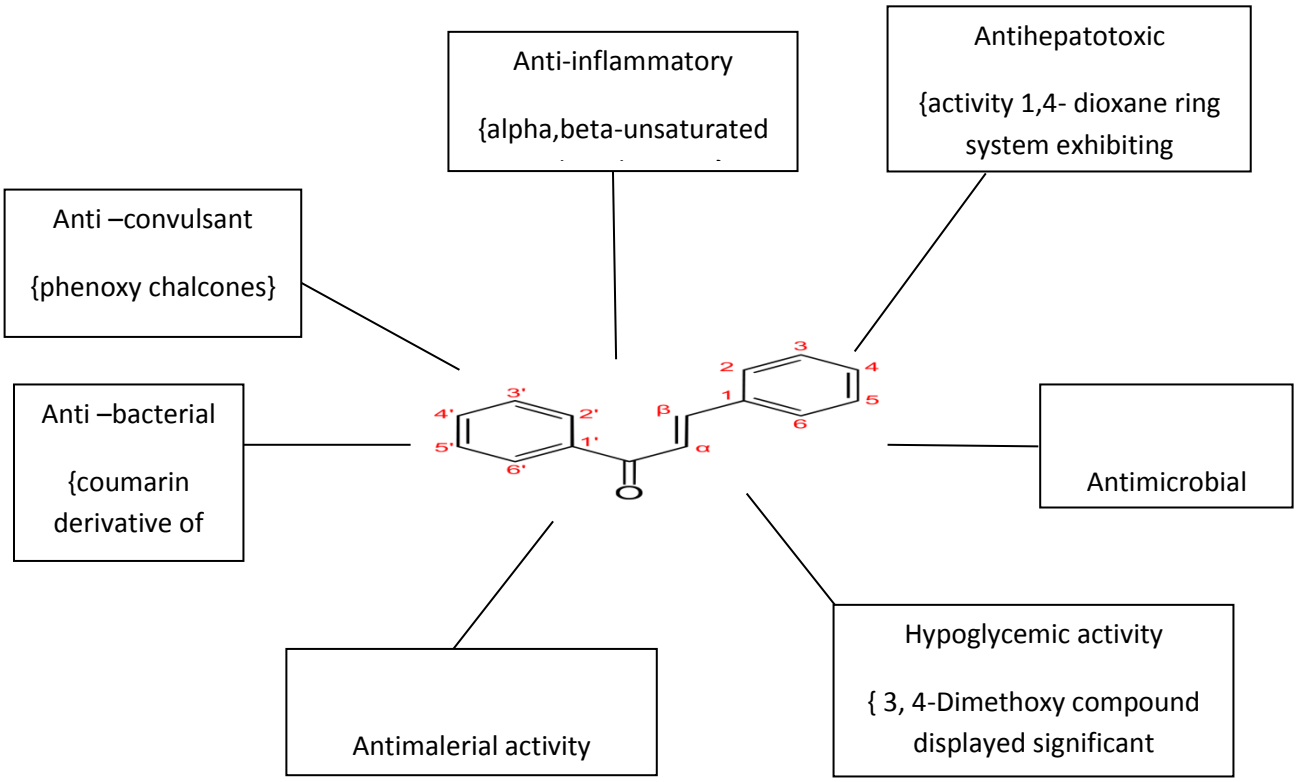
Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids. Benzylideneacetophenone is the parent member of the Chalcone series.

AIM AND RATIONALE:

The chemistry of chalcones has generated intensive scientific studies throughout the world. Especially interest has been focused on the synthesis and biodynamic activities of chalcones. Chalcones are 1, 3-diphenyl-2-propene- 1-one, in which two aromatic rings are linked by a three carbon α, β- unsaturated carbonyl system. Different methods are available for the preparation of chalcones2.The most convenient method is the Claisen-Schmidt condensation of Equimolar quantities of arylmethylketone with aryl aldehyde in the presence of alcoholic alkali[4].



There are wide uses of chalcones:



Considering all above point mentioned we tried to devise a green method for synthesis of chalcones, which is more efficient, convenient , environmental friendly and a solvent less technique.

MATERIALS AND METHOD:

CHEMICALS: vanillin, benzaldehyde ,acetophenone ,solid sodium hydroxide

Recrystalisation solvents: absolute alcohol, alcohol(60%,40%), Diluted hydrochloric acid.

APPARATUS: 100ml, 500ml beaker, glass rod ,mortar pestle, recrystalisation flasks etc.

METHODOLOGY:

A mixture of 4-Hydroxy-3-methoxybenzaldehyde (0.05mmol)/benzaldehyde respective substituted acetophenones (0.05mmol)and sodiumhydroxide was ground together in mortar with pestle for 5 min and left to harden at room temperature for 30 min. The solid was dissolved in cold distilled water and acidified with dilute HCl and kept aside overnight. The solid that separated was filtered, dried and purified[5,6].

PURIFICATION METHOD:

Two methods were tried: a. ether extraction

b. recrystallisation

To check the presence of impurities TLC was carried out using chloroform as a mobile phase and silicon plates as a stationary phase.

RESULTS AND DISCUSSIONS:

We tried to synthesize chalcones by green technique and good amount of product was obtained . to check the purity of product TLC was carried out .

Compound	Rf value
Compound 1{vanillin + acetophenone}	0.3
Compound 2{benzalydehyde + acetophenone}	0.87

We had some problem in purification of the product if in future any convenient and eco-friendly recrstallisation solvents can discovered it can open up windows , as chalcones has a widespread activity it will bring about a prominent change in field of chemistry.

CONCLUSION:

we have redeveloped a simple , efficient and more eco-friendly method for synthesis of chalcones by grindstone .The notable advantages of present method are no organic solvent required ,waste minimization, simple operation, clean reaction profile, easy work up, shorter reaction time(5-10 min), and eco-friendly as compared to conventional method.

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STUDY OF ANTI-MICROBIAL ACTIVITY OF *Psidium guajava* and *Manilkara zapota* LEAF EXTRACT.

Ashish Salunkhe, Ashwini Salunkhe, Sandeep Nikam

ABSTRACT

Natural drugs are boom to mankind. They have few side effects as compared to allopathic medicines. This project is related to herbal extract, having potent anti-microbial activity. *Psidium guajava*, is an important food crop and medicinal plant in tropical and subtropical countries is widely used like food and in folk medicine around of the world. *Psidium guajava* linked with the antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, antiinflamatory and antinociceptive activities and also used as hypoglycemic agent. *Manilkara zapota* contains Linoleidic acid, Oleic acid, unsaturated fatty acids, Linoleic acid, Tannins, Flavonoids. Crude extract of *Manilkara zapota and Psidium zuajava* showed anti-microbial activity against gram positive Bacillus subtilis, Staphylococcus aureus and gram negative Escherichia coli, Salmonella typhii. The concentrations of leaf extract are made and evaluated for antimicrobial activity. DMSO is used as standard for antimicrobial assay. This study provides significant insights into the therapeutic effect of *Manilkara zapota* and *Psidium guajava* on gram positive and negative microbes and opens the way for further studies on identification of novel anti-microbial targets of *Manilkara zapota* and *Psidium guajava.*

INTRODUCTION

Ayurvedic preparations are getting popularity worldwide over past few years. This is mainly because allopathic medicines are proven to be producing more side effects as compared to the ayurvedic medicines. Ayurvedic medicines are made up of medicinal herbs, minerals and metals or any combination thereof.

The boiled water extract of Psidium guajava plant leaves and bark are used in medicinal preparations which are utilized as remedies for dysentery, diarrhoea and upper respiratory tract infections while guava fruit paste and cheese are popular dishes in Florida, the West Indies and parts of South America. In Malaysia, Psidium guajava is used for stomach ache and gastroenteritis; Leaf, root, and bark extracts are used for treatment of diarrhoea, leukorrhea, cholera, external ulcers, and skin diseases. Psidium guajava leaf extract contains guajava polyphenol that has an anti-oxidation action. The flower and leaf of the plant have been reported to have antibiotic activity. In the present study

antimicrobial potentiality of the P. guajava leaves was investigated against a few clinically isolated as well as standard microbial cultures.

Manilkara zapota belonging to family Sapotaceae and genus Manilkara. The common names of Manilkara zapota are Sapote, Sapodilla, Manilkara zapotilla, Manilkara achras, Mimusopusmanilkara, Achraszapota, and Achrassapota.

The leaves were found to contain:-

- Unsaturated fatty acids representing 32.32 % of the total fatty acids
- Oleic acid (13.95%)
- Linoleidic acid (10.18 %)
- Linoleic acid (5.96 %) were the major ones
- The seeds were found to contain
- Flavonoids
- Tannins (mainly from unripe fruits)
- Triterpenes
- Saponins (mainly from the seeds)

RATIONALE

In most cases antimicrobial combinations are employed to broaden the spectrum of coverage. This clinical application as likely to be successful as long as the combinations are not antagonistic. Most examples of antibiotic antagonist are those in which a bacteriostatic agent renders a bactericidal agent "static". Synergistic combinations should allow the use of lower concentrations of drug in combination and thus diminish the incidence of dose-related antibiotic toxicity, but the concept has met with only limited success so far. The reason of taking the herbal drugs is they were widely available, cheap and quite safe. It also has mild side effects of nausea and vomiting. Combination of two extracts is made and synergistic activity gives the antimicrobial activity. Synergism is a positive interaction created when two agents combined and exert an inhibitory effect that is greater than the sum of their individual effects. Combination therapy can be used to expand the antimicrobial spectrum, to prevent the emergence of resistant mutants, to minimize toxicity and to obtain synergistic antimicrobial activity, it could be an alternative to monotherapy for patients with invasive infectious that are difficult to treat, such as those due to multi-resistant species and for those who failed to respond to respond to standard treatment. Antimicrobial compounds used in combination might promote the effectiveness of each agent, with efficacy being achieved using a lower dose of each drug. Pharmacological benefits would accrue, with one drug clearing infection from one body

system while the other clears it from different site. In addition, synergism in antimicrobials could be utilized in an attempt to prevent or delay the emergence in vivo of resistant populations of the pathogenic organisms.

MATERIALS

1) Agar Slants:- Agar slant were prepared form Nutrient agar powder. Suspend 28gm of nutrient agar powder in 1000ml of distilled water, heat till boil to dissolved completely. Then poured into 9 test tubes approximately 10ml each, plugged with cotton and tied with thread and sterilized in autoclave for 15-20 minutes at 121.60 C at 15 lbs pressure. After sterilization the test tubes were placed on slanted position to prepare slants of nutrient agar in test tubes. Then cultures were inoculated in zig-zag manner and incubated at 370C for 24-48 hours.

2) Nutrient broth:- Nutrient broth was prepared using following ingredients:-

- Peptone – 0.5gm
- Meat extract – 0.3gm
- Sodium chloride – 0.5gm
- Distilled water – 100ml

3) Nutrient agar (for petri plates):- Nutrient agar for the 25 petri plates was prepared using 16.8gm of nutrient agar in 600ml of distilled water. Bring to boil to dissolve completely and plugged with cotton, tied with thread and sterilized using autoclave at 121.60C at 15 lbs pressure for 15-20 minutes.

4) Soft agar:- Soft agar was prepared by using 1-1.5gm of agar-agar powder in 100 ml of distilled water. Bring to boil to dissolved completely. Then in hot conditions transferred to 25 test tubes each containing 2-3ml of soft agar then each test tube were plugged with cotton and tied with thread and sterilized using autoclave at 121.6 °C at 15 lbs pressure for 15-20 minutes. After sterilization cooled till room temperature.

5) Saline :- Saline was prepared using 0.85gm of sodium chloride in 100ml of distilled water. Dissolved completely and plugged with cotton and sterilized using autoclave 121.60C at 15 lbs pressure for 15-20 minutes.

Apparatus used:-

1) 9 Test tubes

2) 8 250ml volumetric flasks

3) 30 petri plates

4) Hot air oven

5) Incubator (370)

6) Autoclave

7) Burners

METHODYLOGY

Plant material:-

Psidium guajava and Manilkara zapota leaves were collected.

Extraction :-

Leaves of P. guajava and Manilkara zapota were collected air dried and then powdered. 10 grams of each leaf powder were used for methanolic extraction. The extraction method is used is Soxhlet extraction method. In solvent extraction, the sample was extracted in solvent kept on a rotary shaker overnight and then the filtrate was collected. The solvent was then evaporated to get the concentrated solution of extract then the solvent were used for anti-microbial assay.

Microorganisms Studied :-

The Gram-positive and Gram-negative bacteria were procured from National Chemical Laboratory, Pune – 411 008 and Department of Microbiology, KarmaveerBhaurao Patil College, Vashi, Navi Mumbai-400 703. Four micro-organisms were used those are as following:-

- Gram Positive:- 1) *Bacillus subtilis* 2) *Staphylococcus aureus*
- Gram Negative:- 1) *Escherichia coli* 2) *Salmonella typhii*

Standard solution used:-

Ciprofloxacin drug is taken as standard for the anti-microbial assay.

Anti-microbial study :-

Antimicrobial activity was performed by well method. Nutrient Agar slants was prepared. After autoclave flask was removed from autoclave and in hot conditions approximately 10ml of agar was taken in 9 test tubes and kept tilted for solidification. A loopfull of old culture taken in aseptic area and spread in zig-zag manner on the slant. Applied same procedure for other slants.Prepared 2 slants of same organism. After inoculation kept it for incubation for 24-48 hours at 37^0C. After incubation Nutrient broth was prepared by taking peptone, meat extract, sodium chloride and distilled water. All slants are removed from incubator and loopfull of growth is taken in aseptic area and transferred to the broth. Applied same procedure for all flasks of broth. 2 flasks were prepared for each micro-organism. After transfer of micro-organism to the broth, the flasks were place in incubator for 24-48 hours at 37^0 C. After the 24 hours growth of flask were tested against the 0.5 McFarland reagent. In between the procedure 25 patri plates are sterilized in hot air oven. After sterilization patri plates were cooled. Mean while nutrient agar was prepared using 16.8gm agar powder in 600ml of water heated the solution till boiling and sterilized in autoclave at 121^0 C at 15 lbs pressure for 15-20 minutes. After autoclave flask were removed from autoclave and in hot conditions agar were poured in plates (approximately 10-15ml of agar per plate) in aseptic conditions. The plates were cooled. Soft agar were prepared using 1.5gm agar agar power in 100ml of distilled water and cooled. After cooling soft agar were poured into petri plates along with the micro-organism and spreaded using glass spreader whiche were previously sterilized using alcohol. After spreading wells are boured per plate 3 using metal borer in aseptic area. Therdiftrent concentrations of of two leaf extract were prepared (Psidium Guajava : Manilkara Zapota) and 1ml of extraction mixture were place in well using 1ml micro pipette which was previously sterilized using alcohol in aseptic area. After placing the mixture into well labeled . Similarly standard drug were pored into well. After that all plates were placed in incubator for 24-48 hours at 37^0 C.

RESULTS

In the present study P. guajava leaf and Manilkara zapota leaf extracts extracted in methanol were investigated at five different concentrations for their antimicrobial potentiality against 2 gram negative micro-organism and 2 gram positive micro-organism clinically important microbial strains. The extract concentration was more active against gram-positive bacterial strains studied. The zone of inhibition was measured in the millimeter (mm).Details of the result are shown in Table :-

1) *Bacillus subtilis*

M.Zapota : P.Guajava	D1	D2	D3	D-Avg
Standard	24.5	15	12	17.1
2:0	11.5	12	12.5	12
1.6:0.4	12.5	11.5	13.5	12.5
1:1	20	15.5	15.5	17
0.6:1.4	20.5	19	19	19.5
0:2	19.5	21	19.5	20

2) *Staphylococcus aureus*

M.Zapota : P.Guajava	D1	D2	D3	D-Avg
Standard	15.5	17	12.5	15
2:0	18	19	12.5	16.5
1.6:0.4	12.5	11.5	13.5	12.5
1:1	15	17	13.5	15.1
0.6:1.4	20.5	16.5	16.5	17.8
0:2	20	17.5	16.5	18

3) *Escherichia coli*

M.Zapota : P.Guajava	D1	D2	D3	D-Avg
Standard	14.5	12	16.5	14.3
2:0	12.5	12.5	10.5	11.8
1.6:0.4	11.5	12.5	13	12.3
1:1	15.5	15	16	15.5
0.6:1.4	15	13	15	14.3
0:2	14	15	12.5	13.8

4) *Salmonella typhii*

M.Zapota : P.Guajava	D1	D2	D3	D-Avg
Standard	18	18.5	19	18.5
2:0	14	12.5	11	12.5
1.6:0.4	14.5	13.5	14.5	14.1
1:1	12.5	13	12.5	12.6
0.6:1.4	14	14.5	15.5	14.6
0:2	14	15	12.5	13.8

CONCLUSIONS

The concentration of extracts of both leafs shows anti-microbial activity. Alcoholic extract of both plant leaves was highly active against gram positive while the extracts were equally active against gram-negative strains. The acetone extract of P.guajava and Manilkara zapota should further be studied for its phytochemical constituents in order to elucidate the active principle within the extract which can turn out to be a novel antimicrobial agent of the future.

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STANDARDIZATION OF SENNA TABLETS

Anagha More, Namrata Nikam, V. N. Garge

ABSTRACT

Cassia leaves and pods extracts has been used in traditional or herbal medicine since ancient times. The pods and leaves contain anthraquinone glycosides that have a significant laxative effect. These tablets can be used as an alternative source of laxative medicine in Sudan due to the abundance of Cassia acutifolia as a wild plant.

Standardization is the process of implementing and developing technical standards. Standardization can help to maximize compatibility, interoperability, safety, repeatability, or quality. It can also facilitate commoditization of formerly custom processes.

Key Words: Standardization, Herbal Medicines

INTRODUCTION

In today's competitive world where everyone is exposed to a fierce and continuous struggle for existence, due to changes in the lifestyle, often related to today's hectic pace of life, our food habits, overexertion, insufficient rest, intake of unhealthy diet especially one without enough fibres leads to the fairly common and uncomfortable complaint, called "Constipation". It usually involves difficult or irregular bowel movements, accompanied by hard, dry motion, which can be painful to pass. Senna works by gently stimulating the bowel to encourage bowel sennosides is relatively well understood; they first demonstrate their activity through an interaction with the intestinal bacteria, by which they are hydrolyzed and then reduced to the anthrone stage as the actual active form. The sennosides and other anthraquinone derivatives possess a laxative effect. movement for relief from occasional or nonpersistent constipation. However, the sennoside content of the senna used in the manufacture of senna tablet is standardized to ensure the constant desired amount of active ingredient and predictable result from every dose. Also most of the times the API used for preparation of senna tablets is the calcium salt of sennosides known as calcium sennosides. At the same time if excipients.

Objectives:

- To understand the methods of quantitative analysis of glycosides (e.g. Digitalis & senna)
- To understand the spectrophotometer and relationship between absorption of light and color
- To know some physical and chemical properties of digitalis and senna constituents.
- To learn some analytic laboratory skills.

Principle:

The Senna contains mainly combined anthraquinones (sennosides) with only small amount of free anthraquinones. Since senna is assayed for the content of sennosides, removal of free anthraquinones should be done first by extraction with an organic solvent.

On Hydrolysis of anthraquinone glycoside into free anthraquinone (**rhein**).

On Addition of standard alkali e.g. 1N KOH (Borntrager's test) gives a rose red color which is measured colorimetrically at 500 nm.

Aim: Evaluation of a marketed ayurvedic dosage form (tablets). Tablets have been evaluated on various parameters such as friability, weight variation, and assay.

RATIONALE

Even as the government plans to introduce tax sops for ayurvedic medicines, the state lacks proper checking facilities for those products.

Of the 2,000 to 3,000 ayurvedic products in the market, only 300 to 400 samples undergo testing at the Drugs Testing Laboratory in Thiruvananthapuram, the lone testing centre in the state.

The ayurvedic industry in the state is growing by leaps and bounds registering a turnover of `600 crore, but the kashayam, choornam and lehyam that customers buy could be laden with high quantity of heavy metals, synthetic steroids, allopathic medicines and alcohol.

There are only three ayurveda drug inspectors in the state, who just collect about 30 to 40 samples each month, which means not all products are being analysed. “The samples that are tested at the laboratory are less when compared to the bulk of products,” said Krishnakumar, ayurveda drug analyst at the Drugs Testing Laboratory. Drugs Controller of Kerala C S Satheesh Kumar said the whole system had to be streamlined for making it foolproof. “There are proposals for setting up drug testing laboratories at Pathanamthitta, Thrissur and Kozhikode,” he said and added that it could strengthen the whole system.

When the Excise Department registered 1,000 cases of spurious ayurvedic products in the past two years, the Drugs Testing Laboratory took action only against two or three products, sources said.

They alleged that there is a nexus between the manufacturers of ayurvedic products and higher officials. And of the 300 to 400 samples tested every year, only a few cases of sub-standard medicines are detected.

"Between 2007 and 2010, a total of 14 samples with high quantity of alcohol and steroids were detected. While 27 samples were found to have a higher content of heavy metals, 36 samples had allopathic presence," Krishnakumar said.

Noting that there was no particular standard for checking ayurvedic products, Krishnakumar said the Centre should take steps to fix standards for ayurvedic products.

He said there was a lack of proper facility at the drugs testing laboratory and that it was not able to handle all the products manufactured in the state.

"The standardisation methods of ayurvedic drugs should be relevant and evaluative," he said. It is also said that there is no system to establish the toxic levels in ayurvedic drug formulations where metals are a major component.

With an increase in the popularity of ayurveda, drug manufacturing units have mushroomed and commercialisation has taken place on a large scale. This has resulted in compromising on quality.

Materials

1. Senna tablets (Any ayurvedic dosage form)
2. Colorimetry
3. Beaker, separating funnel, stirrer, mortar, separating funnel, filter paper.

Chemicals: Hydrochloric acid, Chloroform, Sodium carbonate, Sodium sulphate, Potassium hydroxide.

Method:

1. Triturate 2 tablet, in a mortar, with 20 ml of warm dist. Water. Transfer the mixture quantitatively into a conical flask.

2.Digest the mixture on a boiling water bath for 15 minutes and filter into a 250 ml separatory funnel.

3. Cool the mixture, add 2 drops HCI, and shake gently with 2 quantities each of 15 ml CHCI3; discard the CHCl3 layer.

4. Filter the aqueous layer through a piece of cotton into a 100 ml volumetric flask.

5. Pipette 10 ml of the filtrate into a 250ml round bottom flask.

6. Adjust PH of the solution to pH7-8 with few drop of 5% w/v solution of Na_2CO_3

7. Add mixture of (8mlof15%$Fecl_3$+12mlH_2O) and mix.

8. Heat on a boiling water bath under reflux for 20mins.

9. Add 1.3ml conc.HC1 to render acidic and continue reflux for 20minutes.

10. Cool, transfer the mixture quantitatively to a separator and extract with 3 quantities each of 25ml of ether, receiving the ether extract into a 100ml volumetric flask; complete to volume with ether.

11.Pipette 10ml of ethereal extract into a beaker, evaporate just to dryness on a water bath and dissolve the residue in 10ml INKOH accurately measured; filtering in case of any turbidity.

12.Without of the resulting solution at 500nm, using 1 cm cuvette.

Observation: absorbance - 0.12 at 500nm using 1 cm cuvette

Calculation:

Formula = A × 1.8 × 100/ 4

= 0.12 × 1.8 × 100/4

= 540 mg..... For 2 tablets

= 270 mg.....for 1 tablet.

5% +- deviation is allowed

Label claim is 273 mg

Limit: 259 - 286 mg.

Inference: content of senna in tablet is lies within the limit of 5%+-

Result : Given tablet passes the test.

DISCUSSION

Tests performed

Friability: Friability test is conducted using Roche friabilate or. For this, usually 10 tablets are selected randomly from each batch and their initial weight (W0) is noted. These tablets are then transferred to the drum of friabilate or and rotated at appropriate rpm for definite time period. After which the tablets are collected and weighed again (W). The percentage friability is then

Calculated by the following formula:

F= (1- W0/W) *100

Test is performed on 10 tablets.

W1 (weight of tablets before test): 8220 mg

W2 (weight of tablet after test): 8212 mg

Formula: W1 - W2 / W1 × 100

= 8220 - 8212/ 8220 × 100

= 0.09 %

Observation: Tablets are cracked, break

Inference: 4 tablets out of 10 are break

Result : Given tablets fails Friability test.

weight variation:

* Weight variation test is performed to check that the manufactured tablets have a uniform weight.

* As per USP twenty tablets are weighed individually and an combined weight is taken, the average weight is obtained by dividing the combined weight by 20, now the average weight is compared to the individual weight of the tablet,

* For a tablet to pass the test not more than 2 tablets should lie out of the specified percentage and if no tablet differs by more than two times the percentage limit.

W 1 = 820 mg	W 11 = 810 mg
W 2 = 810 mg	W 12 = 840 mg
W 3 = 860 mg	W 13 = 790 mg
W 4 = 840 mg	W 14 = 820 mg
W 5 = 820 mg	W 15 = 830 mg
W 6 = 810 mg	W 16 = 810 mg
W 7 = 830 mg	W 17 = 820 mg
W 8 = 790 mg	W 18 = 810 mg
W 9 = 830 mg	W 19 = 810 mg
W 10 = 810 mg	W 20 = 820 mg

Average Wgt = 1680/20

= 819mg

5% deviation is allowed

5/100 × 819 = 40.95

Lower limit = 778.05 Upper limit = 859.95

Inference: one tablet out of 20 lies outside the 5% deviation.

Result: Given tablets pass the wet variation test.

RESULT :

test	Limit	Observation	Inference
Assay	259 - 286 mg	270 mg	Passes the test
Weight variation test	778.05-859.95	One tablet lies outside the limit	passes the test
Friability	NMT 1 %	Tablet crack, break	Fails the test

CONCLUSION:

The given senna tablets (SAMAY TABLETS) passes weight variation test & % content test (assay) and fails Friability test.

Ayurvedic preparation doesn't have regulations for standards so spurious, substandard drugs are also in the market. Hence regulations on Ayurvedic drugs are required.

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ANALYTICAL STUDY OF PARACETAMOL BY HPTLC

Kirtika Chatri, Sonali Bhagat, V. V. Khanvilkar

ABSTRACT :

Study involves quantitative analysis of four brands (samples) of paracetamol tablets; using HPTLC method. The method is accurate, precise, stability-indicating and takes less than 30 minutes for most samples.% content and content uniformity for each sample was calculated using peak areas of the samples and that of standard. The result Obtained was compared with that of standard sample to see it is within specified limit with official book (NLT 95.0% and NMT 105.0% according to IP, 2007).

KEYWORDS:

Paracetamol, Tablet content uniformity, HPTLC method.

INTRODUCTION:

Paracetamol (acetaminophen) is one of the most popular over-the-counter analgesic and antipyretic drug.

It is easily available as a generic medication with different trade names.

Content uniformity is a pharmaceutical analysis parameter for the quality control of capsules or tablets. Content uniformity tests are performed to check uniformity of drug content in various formulation units.

In this multiple capsules or tablets are selected at random and a suitable analytical method is applied to assay the individual content of the active ingredient in each capsule or tablet.

High performance thin layer chromatography (HPTLC) is a chromatographic technique that utilizes the capillary action of a solvent and a stationary phase to separate compounds in a sample mixture.

Due to ease of operation, less cost and time involved it has gained popularity amongst analysts.

RATIONALE:

Paracetamol is easily available which is commonly used by patients. Approximately 100 number of paracetamol tablets brands are available in market.The rationale of the study was to find out paracetamol content of four brands to check content uniformity amongst them HPTLC is a simple technique and method of HPTLC analysis was available hence the content uniformity was decided to be checked by HPTLC analysis.

MATERIAL :

REQUIREMENTS:

REAGENTS:-Methanol, Toluene, Chloroform.

TABLET POWDER**:-**Dolo 650, Crocin, Paracip, Alkem.

APPARATUS:-10 × 10 C18 silica 60 F HPTLC plate, twin trough chamber,

Linomat 5 applicator, UV chamber, CAMAG TLC scanner 5.

METHOD :**HPTLC METHOD**

I) FOR CALIBRATION CURVE:-

1. Preparation of stock solution :-

   Dissolve 10 mg of Paracetamol in 10 ml of methanol to make stock solution of 1000 ppm.

   Preparation of mobile phase:-

   Toluene: methanol: chloroform = 5: 2: 3

2. Application of stock solution

   Apply stock solution in concentrations of 5, 10, 15, 20, 25 ppm [Volume to be applied (in µl): 5, 10, 15, 20 and 25]

3. Dimension of plate :

   7cm × 10 cm (For 6 spots i.e. 5 Stock+1 Sample)

II) FOR SAMPLE SOLUTION

1. Preparation of sample solution :

   Weigh & powder 20 tablets. Weigh quantity of tablet powder equivalent to 10 mg of Paracetamol& dilute it to 10 ml with methanol.

   Sonicate for 10 min for complete dissolution of Paracetamol.

Filter the solution.

2. Application of Sample solution :

Apply the sample solution in concentration of 15 ppm [volume to be applied (in µl):15]

III) DEVELOPMENT OF PLATE :

a) Saturation of chromatographic chamber-

Pour the mobile phase in Twin Trough chamber. Place the filter paper vertically in the chamber. Cover the chamber with a lid & allow the mobile phase to run across the filter paper until the filter paper completely gets saturated with the mobile phase.

b) Open the lid of the saturated chamber and insert the plate on which spots were applied, into the front trough so that the back of the plate rests against the front wall of the chamber and the silica layer faces the inside of the chamber. Close the lid. Let the mobile phase ascend until it reaches the mark. Open the lid and remove the plate. Dry the plate and scan it under CAMAG TLC scanner 5.

CALCULATIONS:

1) For preparation of sample solution :

For Dolo650 :

Average weight of tablet = 827 mg

827 mg of tablet powder = 650 mg of Paracetamol

X mg of tablet powder = 10 mg of Paracetamol

$$X = \frac{10 \times 827}{650} = 12.27 \text{ mg}$$

For Crocin:

Average weight of tablet = 673 mg

673 mg of tablet powder = 500 mg of Paracetamol

X mg of tablet powder = 10 mg of Paracetamol

$$X = \frac{10 \times 673}{500} = 13.46 \text{ mg}$$

For Paracip:

Average weight of tablet = 620 mg

620 mg of tablet powder = 500 mg of Paracetamol

X mg of tablet powder = 10 mg of Paracetamol

$$X = \frac{10 \times 620}{500} = 12.4 \text{ mg}$$

For Alkem:

Average weight of tablet = 601 mg

601 mg of tablet powder = 500 mg of Paracetamol

X mg of tablet powder = 10 mg of Paracetamol

$$X = \frac{10 \times 601}{500} = 12.02 \text{ mg}$$

Table 1:

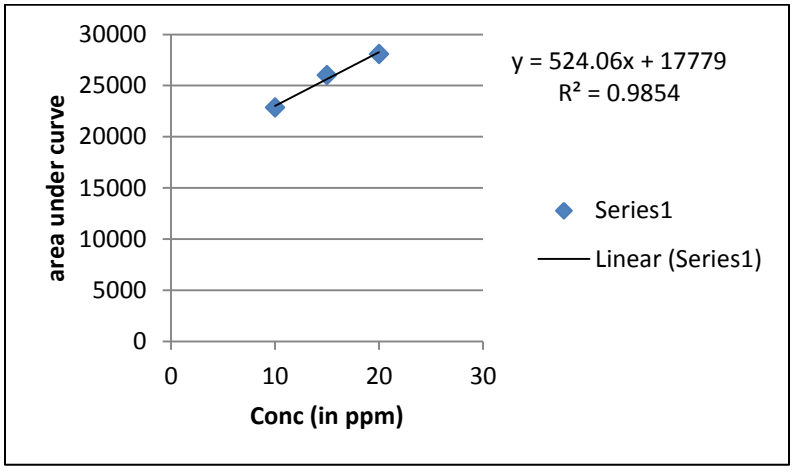
Sr. no.	Tablet Brands	Weight of Tablet powder to be taken
1.	Dolo 650	12.72 mg
2.	Crocin	13.46 mg
3.	Paracip	12.4 mg
4.	Alkem	12.02 mg

OBSERVATION TABLE:

Table 2:

Sr no	Concentration (in ppm)	Volume spotted on HPTLC plate from stock solution(µl)	Plate 1	Plate 2	Average
1	5	1	19291.9	16243.9	17767.9
2	10	1	24868.9	20802.2	22835.55
3	15	1	27625.3	24392.6	26008.95
4	20	1	29810.6	26341.7	28076.15
5	25	1	31263.9	27808.7	29536.3
6	Dolo	1	26743.8	24449.6	25596.7
7	Crocin	1	25262.2	24541.2	24901.7
8	Paracip	1	25004.5	25494.2	25249.35
9	Alkem	1	25347.4	23731.8	24539.6

Calibration curve obtained with absorbance recorded at λ_{max} 243 nm



Concentration of paracetamol Y = 524 x + 17779

Table 3:

Sr no	Tablet brand	Concentration (µg)
1	Dolo 650	14.91
2	Crocin	13.59
3	Paracip	14.24
4	Alkem	12.9

CALCULATIONS:

Sample applied = 15 µl

1) Dolo 650 :

14.91 µg in applied 15 µl (0.015ml)

y µg present in 10 ml of sample solution

$y=\frac{10\times14.91}{0.015}$ = 9940 µg= 9.94 mg

2) Crocin:

13.59 µg in applied 15 µl (0.015ml)

y µg present in 10 ml of sample solution

$y = \frac{10\times13.59}{0.015}$= 9060 µg=9.06 mg

3) Paracip:

14.24 µg in applied 15 µl (0.015ml)

y µg present in 10 ml of sample solution

$y=\frac{10\times14.24}{0.015}$= 9493.3 µg = 9.49 mg

4) Alkem:

12.9 µg in applied 15 µl (0.015ml)

y µg present in 10 ml of sample solution

$y =\frac{10\times12.9}{0.015}$= 8600 µg= 8.6 mg

I) CALCULATION OF PARACETAMOL IN ACTUAL TABLET POWDER:

1) Dolo 650 :
   12.57 mg powder contains 9.94 mg of Paracetamol
   827 mg powder contains Z mg of Paracetamol
   $Z = \frac{827 \times 9.94}{12.57} = 646.25$ mg

2) Crocin:
   13.46 mg powder contains 9.06 mg of Paracetamol
   673 mg powder contains Z mg of Paracetamol
   $Z = \frac{673 \times 9.06}{13.46} = 453$ mg

3) Paracip:
   12.4 mg powder contains 9.49 mg of Paracetamol
   620 mg powder contains Z mg of Paracetamol
   $Z = \frac{620 \times 9.49}{12.4} = 474.66$ mg

4) Alkem:
   12.02 mg powder contains 8.6 mg of Paracetamol
   601 mg powder contains Z mg of Paracetamol
   $Z = \frac{601 \times 8.6}{12.02} = 430$ mg

II) % content uniformity of tablets :

1) Dolo 650 :

$$\% \text{ content uniformity} = \frac{\text{calculated weight}}{\text{dose}} \times 100$$

$$= \frac{646.25}{650} \times 100$$

$$= 99.42\% \text{ w/w}$$

2) Crocin:

$$\% \text{ content uniformity} = \frac{\text{calculated weight}}{\text{dose}} \times 100$$

$$= \frac{453}{500} \times 100$$

$$= 90.6 \% \text{ w/w}$$

3) Paracip:

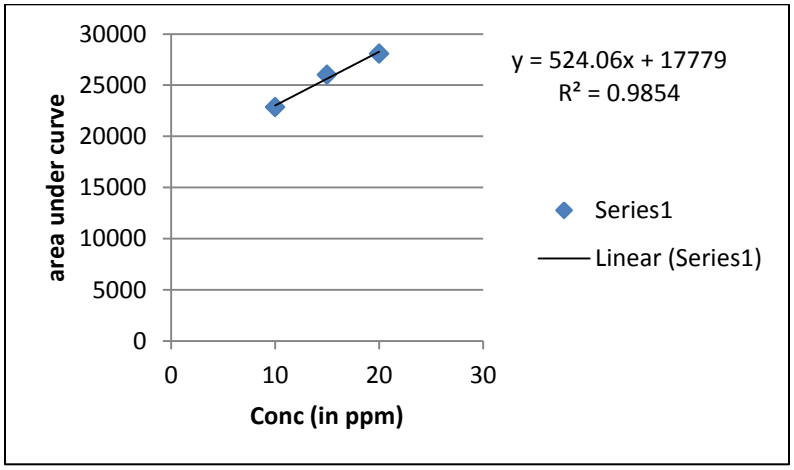
$$\% \text{ content uniformity} = \frac{\text{calculated weight}}{\text{dose}} \times 100$$
$$= \frac{474.66}{500} \times 100$$
$$= 94.93 \text{ \% w/w}$$

4) Alkem:

$$\% \text{ content uniformity} = \frac{\text{calculated weight}}{\text{dose}} \times 100$$
$$= \frac{430}{500} \times 100$$
$$= 86 \text{ \% w/w}$$

RESULT:

Calibration curve obtained with absorbance recorded at λ_{max} 243 nm



Brand Name	Average area obtained for 15µl sample	Concentration present in 15µl based on equation of line Y=mx+c	Actual tablet content	% Tablet content
Dolo 650	25596.7	14.91	646.25 mg	99.42% w/w
Crocin	24901.7	13.59	453 mg	90.6 % w/w
Paracip	25249.35	14.24	474.66 mg	94.93 % w/w
Alkem	24539.6	12.9	430 mg	86 % w/w

CONCLUSION:

% content and content uniformity in mg for each sample was calculated using HPTLC. Peak areas of the sample and that of standard was compared to see if it is within specified limit with official book and all were found to be in specified limit (NLT 95.0% and NMT 105.0% according to IP,2007).

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