International Journal of Chemical Sciences and Applications ISSN 0976-2590.
Vol 2, Issue 1, 2011, pp 100-107 http://www.bipublication.com

ENHANCEMENT OF BIOAVAILABILITY USING NATURAL INGREDIENTS AND ESTIMATION OF DRUG CONTENT BY ORTHOGONAL POLYNOMIALS

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ABSTRACT:

The study is based upon enhancing the bioavailability of analgesic tablets by introducing the seeds of *Lallemantia royleana Benth*, (Commonly known as Balanga) and also to estimate the amount of methionine (hepato protective) present in the formulation by applying orthogonal polynomials. The novel formulation is evaluated in terms of its bioavailability by comparing the parameters like disintegration time and dissolution time, with that of the standard formulations.

There are many methods to enhance bioavailability; one of the methods is the reduction of disintegration time. Balanga seeds are used for the above purpose as the seeds are known to contain plenty of mucilage within its chemical constituents. The mucilage absorbs water by imbibition and helps in tablet disintegration.

Keywords: Balanga seeds, bioavailability, disintegration time, orthogonal polynomials, hepato protective.

INTRODUCTION

Disintegration: It can be defined as the process by which the tablet breaks down or loses cohesion. It is the time required for the tablet to break down into aggregates.

Several mechanisms of tablet disintegration have been proposed. Even though these concepts are listed separately, inter-relationships probably occur in almost all tablet formulations [1]

(i) Effect of water absorption

The water absorbed by the tablet initiate disintegration, but this depends on the solubility of the drug and other ingredients present.

(ii) Swelling

The grains of the disintegrant, particularly of starches, swell in the presence of water and exert pressure on the granules to force them apart. Shangraw et al reported that tablets of water insoluble drugs disintegrated faster with starches than those of water soluble drugs due to the diminished water absorption capacity of the starches in the latter case.

Imbibition is defined as the displacement of one fluid by another immiscible fluid. This process is controlled and affected by a variety of factors. The capillary number (*Ca*) and the mobility ratio (*M*) have the greatest importance. It is also defined as the phenomenon by which the living or dead plant cells absorb water by surface attraction.

Porosity of tablets

It has been shown that penetration of water into a tablet is proportional to its mean pore diameter or porosity. The porosity and permeability of tablets decrease as the tabletting pressure is increased, and as the

porosity decreases, the disintegration time increases. Though no quantitative relationships have been reported between disintegration and penetration times, generally short disintegration times are associated with rapid fluid penetration and drug absorption. Thus the above parameter is related directly to the absolute bioavailability of drugs.[2, 3]

Balanga:

Chemical Constituents:

10.8% of fixed oil is present. Verbenone (16.4%) and trans-carveol (9.8%) were the major components of the oil [4]. The exact chemical constituents responsible for the specific therapeutic activity are not known. When moistened with water the seeds become voluminous and form translucent mucilage.

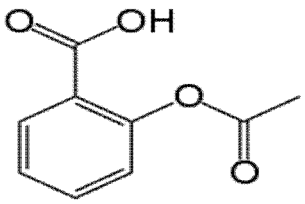
Atherosclerosis:

Atherosclerosis (also known as **arteriosclerotic vascular disease** or **ASVD**) is the condition in which an artery wall thickens as the result of a build-up of fatty materials such as cholesterol[(5)] It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, in large part due to the accumulation of macrophage white blood cells and promoted by low-density lipoproteins (plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL)[6,7]. It is commonly referred to as a hardening or furring of the arteries. It is caused by the formation of multiple plaques within the arteries.

INTRODUCTION TO ASPIRIN:

DRUG PROFILE OF ASPIRIN

Chemical Structure:



Aspirin was the first discovered member of the class of drugs known as Non-Steroidal. [8]Anti-Inflammatory Drugs (NSAIDs), not all of which are salicylates; although they all have similar effects and most have inhibition of the enzyme cyclooxygenase as their mechanism of action.

Mechanism of thrombolytic action of Aspirin:

Aspirin also has an antiplatelet effect by inhibiting the production of thromboxane, [9] which under normal circumstances binds platelet molecules together to create a patch over damage of the walls within blood vessels.[10-12] Because the platelet patch can become too large and also block blood flow, locally and downstream, aspirin is also used long-term, at low doses, to help prevent heart attacks, strokes, and blood clot formation in people at high risk for developing blood clots.[13,14] It has also been established that low doses of aspirin may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue.

Effect on Prostaglandins and Thromboxanes:

Aspirin's ability to suppress the production of prostaglandins and thromboxanes is due to its irreversible inactivation of the cyclooxygenase (COX) enzyme. Cyclooxygenase is required for prostaglandin and thromboxane synthesis.[15] Aspirin acts as an acetylating agent where an acetyl group is covalently attached to a serine residue in the active site of the COX enzyme. This makes

aspirin different from other NSAIDs (such as diclofenac and ibuprofen), which are reversible inhibitors.

Low-dose, long-term aspirin use irreversibly blocks the formation of thromboxane A_2 in platelets, producing an inhibitory effect on platelet aggregation. This anticoagulant property makes aspirin useful for reducing the incidence of heart attacks. 40 mg of aspirin[16-18] a day is able to inhibit a large proportion of maximum thromboxane A_2 release provoked acutely, with the prostaglandin I2 synthesis being little affected; however, higher doses of aspirin are required to attain further inhibition.

Methodology:

Extraction of Balanga Mucilage:

Crushed seeds were extracted by hot benzene in a large extraction flask, and after removal of the solvent by distillation; the crude fixed oil was left behind as a bottle green and somewhat opalescent liquid.

It was treated with animal charcoal and Fuller's earth and was ultimately obtained as transparent light green oil. [19]

Three different formulations were prepared as follows. Formulation-I was prepared according to the standard procedure without the incorporation of balanga mucilage. Formulation-II was prepared by adding 2 gms of balanga mucilage. Formulation-III was prepared by adding 4 gms of balanga mucilage. These three formulations were compared by conducting the evaluation tests for tablet dosage forms such as weight variation, hardness, friability, disintegration and dissolution tests.

Standard Formulation:

S.No.	Ingredients	Qty (for 200 tablets)
1.	Aspirin	60 gms
2.	ibuprofen	40 gms
3.	methionine	10 gms
4.	Starch	q.s
5.	Starch Paste	q.s
6.	Lactose	q.s
7.	Mannitol	q.s
8.	Dicalcium Phosphate	q.s
9.	Talcum	q.s
10	Dried Starch	q.s
11	Aerosil	q.s

Weight of each individual tablet was found to be – 625 mg (containing 300 mg of aspirin)

Experimental Formulations:

S.No.	Ingredients	Formulation-I for 200 tablets (Qty in gms)	Formulation-II (a) for 100 tablets (Qty in gms)	Formulation-II (b) for 100 tablets (Qty in gms)
1.	Aspirin	60 gms	30 gms	30 gms
2.	ibuprofen	40 gms	20 gms	20 gms
3.	methionine	10 gms	05 gms	05 gms
4.	Starch	3.16 gms	1.58 gms	1.58 gms
5.	Starch paste	q.s	q.s	q.s
6.	Lactose	q.s	q.s	q.s
7.	Mannitol	q.s	q.s	q.s
8.	Dicalcium Phosphate	q.s	q.s	q.s
9.	Talcum	q.s	q.s	q.s
10.	Dried Starch	q.s	q.s	q.s
11.	Aerosil	q.s	q.s	q.s
12.	Balanga mucilage	Nil	**2 gms**	**4 gms**

Weight of each individual tablet was found to increase with the incorporation of balanga mucilage as follows:

Formulation-II (a) – 644 mg (containing 300 mg of aspirin+200mg of ibuprofen+50mg methionine)

Formulation-II (b) – 664 mg (containing 300 mg of aspirin +200mg of ibuprofen+50mg methionine)

Estimation of Methionine in the pharmaceutical dosage forms by applying orthogonal polynomials.

Methods: A novel method for the estimation of DL-Methionine in various pharmaceutical dosage forms like tablets and syrups was developed. The absorbance of the Solution was measured at 570 nm. Beer-Lambert's law was observed to obey in the range of 10-100 mcg. In the estimation of methionine in pharmaceutical dosage forms, the quadratic polynomial coefficient was computed by measuring the fluorescence of the drug in 0.01N hydrochloric acid and six points equally spaced at 5nm levels on the emission spectrum from 550 to 575 nm were plotted. The quadratic polynomial coefficient was found to be linear (directly proportional) to the concentration in the range 0.5 to 2.0mcg.

METHOD-A: For quantitative analysis, asprin +ibuprofen+ Methionine suspension (20 mg) was transferred to 100 ml. calibrated flask and made up to the mark with water. In the case of tablets accurately weighed powder equivalent to 20 mg of Methionine was transferred to 100 ml of calibrated flask and made up to the mark with water. Weigh accurately 50 mg of the reference standards and transferred into a 250 ml calibrated flask and make up to the mark with water. Filter the test reference standard solution, and pipette 5 ml of the test and reference standard into 25 ml calibrated flasks. The flasks are kept for 20 mts in the boiling water along with the blank. The violet blue colored chromogen having a maximum absorbance was recorded at 570 nm. The flasks were allowed to cool to room temperature before being made upto mark with water and the absorbance of the test and reference standard solutions was read against blank at 570 nm.

RESULTS:

Both isomers of Methionine D and L forms gave Rf values 0.32 and 0.40 values respectively. The test sample showed the presence of both the isomers. The violet blue chromogen gave linear responses on the concentration range 10- 100 mcg. The recovery experiments gave 98 to 99% recovery with 99 to 100% reproducibility by method A, as shown in table **1.**

METHOD-B:

The fluorescence readings of the standard Methionine solution was scanned from 550 nm to 575 nm at 5 nm intervals using 0.01N hydrochloric acid as blank. The excitation wavelength to measure the fluorescence reading was set at 570 nm as the excitation maxima occurred at this wavelength. The quadratic polynomial coefficient was calculated for six points of 5 nm intervals for every segment from 550 nm to 575 nm

$P2f=(5)\ F_{550} + (4)\ F_{555} - (2)\ F_{560} - (2)\ F_{565} - (5)\ F_{570} + (4)\ F_{575}$…Eq 1

Where F is the value of fluorescence and the subscript denotes denotes the wavelength at which it is measured and the figures in the brackets taken from standard texts on numerical analysis. The quadratic polynomial coefficient (P2f) was more for the segment 550 to 575 nm and hence was chosen for the method. [20, 21] The amount of drug content in the dosage form was calculated from equation 2.

Vt / Vs x Ws/ Wt x A.W/D x 100 = % of the label claim. …Eq. 2

Where Ws and Wt are the weights of the standard drug and the sample preparation.
A.W is the average weight of the tablet and D the labeled drug content, all the weight being expressed in the mg. Vt and Vs are the values of quadratic polynomial coefficient calculated for the test and standard respectively using Eq….
Active ingredients like asprin, Ibuprofen and excipients like mannitol, Parabens, Sugar, Essence and coloring agents did not interfere with proposed method as indicated by the quantitative recovery. The proposed method can be used for quantitative analysis of methionine in market formulations.

Table.1. Assay of methionine by the proposed method

% Amount Found

Formulation	Proposed method	Recovery studies	reproducibility
Standard	98.9	98.8	99.1
Formulation-1	99.2	99.1	98.9

Table-2 Assay of methionine applying orthogonal polynomials coefficient

% Amount Found

Formulation	Proposed method	Recovery studies	reproducibility
Standard	98.1	97.5	99.0
Formulation-1	99.2	98.8	98.6

RESULTS OF ESTIMATION: Recoveries were quantitative with good reproducibility. Excepients like mannitol, dicalcium phosphate and talc. Essences, sugars and coloring agents did not interfere with proposed methods.

Evaluation Tests as per I.P.

Weight Variation Test:

20 tablets are weighed individually. The average weight is calculated and the individual weights are compared with the average weight.

Standard:

Average weight of tablets	Maximum Difference Allowed
130 mg or less	10%
130-324 mg	7.5%
More than 324 mg	5%

Observation:

S.No.	Formulation-I (without balanga)	Formulation-II (a) (2gms of balanga mucilage)	Formulation-II (b) (4gms of balanga mucilage)
1.	624 mg	645 mg	664 mg
2.	622 mg	646 mg	665 mg
3.	625 mg	648 mg	666mg
4.	622 mg	642 mg	666 mg
5.	624 mg	641 mg	662 mg
6.	623 mg	645 mg	665 mg
7.	626 mg	645 mg	668 mg
8.	628 mg	644 mg	664 mg
9.	622 mg	645 mg	663 mg
10.	623 mg	644mg	665 mg
11	624 mg	644 mg	664 mg
12.	622 mg	646 mg	666 mg
13.	622 mg	648 mg	641 mg
14.	626 mg	645 mg	664 mg
15.	623 mg	644 mg	664 mg
16.	623 mg	644 mg	664 mg
17.	625 mg	642 mg	664 mg
18.	623 mg	644 mg	665 mg
19.	624mg	644 mg	666 mg
20.	626 mg	646 mg	661 mg

Total weight – 12477 mg Total weight – 12892 mg Total weight – 13267 mg
Average weight – 623.0 mg Avg weight – 644.6 mg Avg weight – 663.3 mg

Hardness Test:

S. No.	Formulation-I (without balanga) (in kg/cm^2)	Formulation-II (a) (2gms of balanga mucilage) (in kg/cm^2)	Formulation-II (b) (4 gms of balanga mucilage) (in kg/cm^2)
1.	6	5.5	3.0
2.	5.4	5.0	4.0
3.	5.0	5.5	3.5
4.	6.2	5.0	3.0
5.	5.5	4.5	3.0

Monsanto hardness tester is used. The hardness of uncoated tablets should be between 3-7 kg/cm^2.

Observations: (Next table)

Friability Test:

Roche friabilator is used. 20 tablets are selected at random and weighed. They are placed in a friabilator and operated for 100 revolutions at 25 rpm. The tablets are dropped at a distance of 6 inches with each revolution. The tablets are then dusted and reweighed. The acceptable limit is 0.5 to 1%.

Observations:

DISINTEGRATION TEST:

The disintegration apparatus is used. It consists of 6 glass tubes that are 3 inches long, open at the top and bottom is held against a 10 no. screen. Tablet is placed in each tube and the basket rack is positioned in a 1 liter beaker of water at 37+/- 2^{0}C such that the tablet remains 2-5 cm below the surface of liquid on their upward movement and descend not closer than 2-5 cm from the bottom of the beaker. A standard motor device is used to move the basket assembly containing the basket up and down through a distance of 5-6 cm at a frequency of 28-32 cycles/min. limit specified is 5-30 min.

S.No.	Initial weight (W1)gm	Final Weight (W2) gm	Friability = (W1-W2)/W1 X 100
Formulation-I	12.49	12.38	0.88%
	12.40	12.32	0.64%
	12.52	12.42	0.79%
	12.41	12.34	0.56%
	12.44	12.38	0.48%
Formulation-II	12.88	12.79	0.95%
	12.92	12.81	0.85%
	12.84	12.78	0.46%
	12.88	12. 75	01.00%
	12.91	12.81	0.77%
Formulation-III	13.28	13.11	1.2%
	13.22	13.05	1.2%
	13.32	13.19	0.97%
	13.29	13.15	1.05%
	13.34	13.18	1.1%

Observations:

S. No.	Formulation-I (time in min)	Formulation-II (a) (time in min)	Formulation-II (b) (time in min)
1.	7 min	4 min 10 sec	2 min 5 sec
2.	6 min	4 min 30 sec	1 min 50 sec
3.	6 min 20 sec	4 min 50 sec	1 min 40 sec
4.	7 min 10 sec	4 min 10 sec	2 min 3 sec
5.	5 min 50 sec	3 min 50 sec	1 min 10 sec
6.	6 min 2 sec	3 min 55 sec	2 min 18 sec

DISCUSSION:

The above study has been conducted by the incorporation of natural disintegrant in increasing quantities followed by the comparison of their disintegration times. The pharmaceutical parameters had few notable variations like the friabilitiy increased as the quantity of disintegrant increased. Also the hardness of the formulation with more

amount of disintegrant reduced but was within the pharmacoepial acceptable limits. The disintegrating times however showed considerable decrease and the formulation -2 (having 4gms of mucilage) disintegrated much faster than the other two formulations.

In the other part of the study the content of methionine was estimated by using orthogonal polynomials. The above data was compiled and was compared with the other method of **BEER LAMBERTS** law.

A single point determination of the fluorescence for the estimation of methionine in the dosage forms showed a coefficient of variation of around 2% while by sampling of the fluorescence at 6 points the variation was reduced to 1%. This observation was consistent with the fact that larger observations improve the precision of the experiment. On the other hand the estimation of methionine by method 1 (Beer-Lamberts Law) both the isomers of methionine D and L forms gave Rf values of 0.32 and 0.40 respectively. The linear responses were in the range of 10-100mcg. The experimental recovery was 98-99% with 99-100% reproducibility.Active ingredients like asprin, ibuprofen and other excepients did not interfere with the experimental readings.The above study concludes that the estimation of compounds applying orthogonal polynomials coefficient is a convenient tool.

CONCLUSION:

It is hereby ascertained that the bioavailability of tablets could be enhanced by the use of disintegrants which are natural in origin. Natural disintegrants are far more superior to the synthetic ones. The disintegrant (balanga mucilage) is found to be absorbing water instantly followed by its imbibition, this results in the easy break down of the tablets in turn resulting in the deaggregation of all the granules. Further more, mucilage of balanga seeds also helps in the dissolution of the drug. The high water absorption tendency of balanga mucilage which encircles each drug particle retains water within the vicinity and promotes the drug solvation.

Also, the easy availability of balanga encourages its incorporation into tablets and other drug formulations. Balanga mucilage is found to be superior than other suitable disintegrants like aerosil (silica) in terms of acting, availability etc.

Aerosil has been found to be unsuitable as a disintegrating agent because it has carcinogenic properties, it is highly expensive and further more it has very low density which creates difficulty in granulating tablets. Balanga mucilage has been found to be much more effective in all these aspects.

The mucilage of balanga is further more known to have various other properties like healing, laxative, etc. Balanga mucilage is easily available and easily obtained at any place and is compatible with all other ingredients of the formulation. The mucilage is found to be stable and does not interact with other drugs or excipients. The mucilage of balanga is thus found to have a lot of properties which could be made use of in making the formulation much more superior and feasible. The above study needs to be conducted in much more elaborate way and has a lot of scope and potential in upgrading the formulations in drug industry.

Also the method of estimating drug content by using orthogonal polynomials is much more feasible and reliable and is also precise.

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