



EVALUATION OF *IN VITRO* STABILITY STUDIES ON NUTRACEUTICALS IN ORAL SOLID DOSAGE FORMS WITH SPECIAL REFERENCE TO GLUCOSAMINE

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Article Received on: 10/08/13 Revised on: 21/08/13 Approved for publication: 22/08/13

DOI: 10.7897/2230-8407.04854

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

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ABSTRACT

Pure Glucosamine is very "hygroscopic" and degrades (breaks down) rapidly when exposed to moisture or air. To avoid this, Glucosamine needs to be bound to a stabilizer to be sold commercially. The sulfate and the HCL forms are two of the most common "agents" that Glucosamine is bound to ensure its stability. After Glucosamine is bound, it is stable and will not degrade before it can get to the store shelf. Hence it is very difficult to prepare Glucosamine base and instead find Glucosamine Sulfate or Glucosamine HCL. Thus an attempt was made to stabilize the Glucosamine formulation by using the combination of anti-oxidant, Desiccant to resolve the problems associated with the Glucosamine formulation. In the present formulation studies were carried for the stability studies as ICH guidelines with Real time and Accelerated stability studies and it was found to be stable during the study period of stability for three months. All the formulas with single desiccant and single ant-oxidant did not reduce the extent of the degradation. The tablets so prepared with conventional methods showed good results physical evaluation parameters and chemical parameters such as Assay and Dissolution values. The granules prepared by using these antioxidants and desiccant, were good in their flow properties. Glucosamine, an amino monosaccharides naturally occurring in the connective and cartilage tissues, contributes to maintaining the strength, flexibility and elasticity of these tissues. Glucosamine is a precursor to a molecule called a glycosaminoglycan, which is used in the formation and repair of cartilage. In recent years, glucosamine has been used widely to treat osteoarthritis in humans and animal models. *In vivo*, glucosamine is typically converted to N-acetyl-glucosamine. Non-steroidal anti-inflammatory drugs (NSAIDs) are effective in reducing inflammation but are not highly soluble and, in addition, may have undesirable side effects. Efforts have been made to improve the pharmaceutical properties of NSAIDs, such as permeability, solubility and stability, by creating NSAID prodrugs. Prodrugs are typically evaluated in relation to the drug's pharmacological and pharmacokinetic properties. These modifications may alter the physicochemical properties of the drug, which may in turn effect the administration options that optimize drug delivery.

Keywords: Nutraceuticals, *in-vitro*, stability study, glucosamine

INTRODUCTION

In present scenario, rapid industrialization and technological advancement has led to improved quality of life in terms of income, spending and lifestyle along with the economic growth. The first victim of this lifestyle change has been food habits. It has also imposed a major challenge in the form of 'lifestyle diseases'. Consumption of junk food has increased enormously, which has led to a number of diseases related to nutritional deficiencies. Nutraceuticals can play an important role in controlling them. The idea behind the mode of action of nutraceuticals is to provide functional benefits by increasing the supply of natural building blocks in the body. Replacement of these building blocks can work in two ways: to diminish disease signs or to improve performance. Nutraceuticals is a broad umbrella term used to describe any product derived from food sources that provides extra health benefits in addition to the basic nutritional value found in foods. Nowadays, Nutraceuticals are available in various forms including tablets. The formulations are a combination of natural ingredients, essential vitamins, amino acids and minerals, offer health benefits far beyond vitamin supplements alone. Oral route has been one of the most popular routes of drug delivery due to its ease of administration, patient compliance, least sterility constraints and flexible design of dosage forms. In the last few years controlled release dosage forms have made significant progress in terms of clinical efficacy and patient compliance. The objective of designing a controlled release system is to deliver drugs at a rate necessary to achieve and maintain a constant drug slowly over several hours, to protect the drug

the stomach from the irritating effects of the drug. In present scenario, rapid industrialization and technological advancement has led to improved quality of life in terms of income, spending and lifestyle along with the economic growth. The first victim of this lifestyle change has been food habits. It has also imposed a major challenge in the form of 'lifestyle diseases'. Consumption of junk food has increased enormously, which has led to a number of diseases related to nutritional deficiencies. Nutraceuticals can play an important role in controlling them. The idea behind the mode of action of nutraceuticals is to provide functional benefits by increasing the supply of natural building blocks in the body. Replacement of these building blocks can work in two ways: to diminish disease signs or to improve performance. Nutraceuticals is a broad umbrella term used to describe any product derived from food sources that provides extra health benefits in addition to the basic nutritional value found in foods'. Controlled drug delivery system is one which delivers the drug at a predetermined rate locally or systemically for a period of time i.e. it implies a predictability and reproducibility in the drug release kinetics which means that release of active ingredient from a controlled release drug delivery system proceeds at a rate profile that is not only predictable kinetically but also reproducible from one unit to other. Polymer structure features contributing to mucoadhesion² Leung S .S *et.al.* Studied, the expanded nature of mucin and polymer networks; which permits mutual interpenetration / Inter diffusion of mucin and adhesives results in an increase in contact area and establishment of physical entanglement of two different macromolecules. *In*

vivo and *In vitro* nasal mucoadhesion of some water-soluble polymer: A cationic polymer with higher charge density causes the stronger adhesion of aqueous solution on the mucosa. This fact suggests that electronic interaction between the polymer base and mucosa may be factor for adhesion³. Studies on the drug release kinetics from carbomer matrices: Durrani M.J *et.al* showed that, the drug solubility can influence the mechanism of drug release. Atenolol is a sparingly water-soluble drug demonstrated a square root time dependent drug release. While Furosemide, poorly water-soluble drug gave zero-order release. So the solubility is having the influence on the drug release in controlled release tablet⁴. It is totally independent on the various grade of the polymer. Gastro-Retentivity: Its drug delivery potential:⁵ Singh M *et.al* showed that, that the extent of drug absorption from GIT is determined by GI Physiology, irrespective of controlled release properties of the device. These include gastro retentive system, delayed release system and colon targeting. Various other approaches have been tried to retain the dosage form in the stomach as a way of increasing the overall rotation time and include floating system, high density pallets, bioadhesive system, swelling systems and shape system. Uses of Passage dealing precipitants have recently been highlighted. E.g. Salts of Myrestic acid. Improvement of drug release rate from carbopol 934P formulation: Nakanishi T, Kaiho F and Hayashi M having finding that, the carboxyl group of Carbopol is dissociate in the alkaline environment, electrostatic repulsion between negatively charged carboxyl groups causes uncoiling and expansion of molecules resulting in swelling of the polymer and gel formation. The gel is composed of closely packed swollen particles, whose swelling increases with increase in the pH; thus forming thicker and more rigid gel layer⁶. Aminophylline bio adhesive tablets attempted by wet granulation⁷ the wet granulation has limited bio adhesion, because wetting of Carbomer and there drying was impossible when their concentration exceed 10 %. Wet granulation did not allow obtaining of highly bio adhesive tablets. However wetting and drying steps did not alter the polymer structure or bio adhesive property. Factors affecting the bio adhesive properties of tablets consisting of Hydroxy propyl cellulose Carboxyvinyl polymer:⁸, Saoh K *et.al* having findings which shown that, the adhesion force was closely related to the moisture content on the mucus membrane. Namely the tablet did not stick to a very moist membrane, but stick tightly to one with little moisture. Hosseinali Tabandeh, *et al.* has designed the sustained release matrix tablet of Aspirin with Ethylcellulose, Eudragit RS100 and Eudragit S100 and studied the release profile, tablet hardness of the tablets⁹. Glucosamine HCl was found to be effective in controlling osteoarthritis (OA) symptoms in several clinical studies. In particular, two randomised, placebo-controlled, double-blind trials of 3-year duration in knee OA patients, showed that this symptom-modifying effect is sustained over long-term treatment courses. Moreover, both studies indicated that the drug also has a structure-modifying effect, as assessed by measurement of joint space narrowing on standardised plain radiographs by a valid technique and consistent within studies and patient populations.¹⁰

MATERIALS AND METHODS

Glucosamine Hydrochloride (Yantai Dongcheng Biochemicals Co. Ltd), Dicalcium Phosphate, Anhydrous (Rhodia), Carbomer (Lubrizon), Stearic acid (Taurus chemicals limited) Ammonio Methacrylate Copolymer Type

A and B (Eudragit Rohm Pharma Polymers / Degussa), Purified Talc (Luzenac), Ethanol (Ethyl alcohol-SD Fine Chemicals), IPA, Citric acid, Sodium starch glycollate, Tartaric acid, HPC, Colloidal silicon dioxide, PVPK 30, Ethyl cellulose, Crospovidone, Sodium metabisulphate, Mannitol, PEG 600, EDTA and Kieselgur. Analytical Method development, Drug Product specification, Description, Identification, Average mass, Uniformity of mass, Dimension, Hardness, Friability, Assay and dissolution.

Drug Release Profiles

1st hour -----between 20 % to 30 % of label claim
4th hour ----- between 40 % to 55 % of label claim
8th hour----- between 60 % to 75 % of label claim
12th hour-----Not Less Than 85 % of label claim

Dissolution conditions:

Apparatus: USP Type II (Paddle)
Medium : Distilled water.
Volume : 1000 mL.
RPM : 50.
Time intervals : 1st, 4th, 8th and 12th hour.
Temperature : 37, 0°C ± 0, 5°C.

Chromatographic Conditions

Column : BDS Hypersil C₈; 250 mm x 4, 6 mm; 5 µm or equivalent.
Flow rate : 0, 6 mL/minute
Detector : UV at 195 nm
Injection volume: 10 µL.
Temperature : Ambient.
Buffer: Mix 1, 0 mL of Phosphoric acid in 2000 mL of distilled water, adjust to pH 3, 0 with Potassium Hydroxide.
Mobile Phase: Mix 900 mL of Buffer and 100 mL of Acetonitrile. Sonicate for 15 minutes and filter through 0, 45 µ membrane filters and degas.

Manufacturing Procedure of B2, B3, B4, B5, B6, B7 and B8

Step 1: Sift item numbers 1, 2, 3 and 4 through #40 ASTM S.S. sieves and collected separately into polyethylene bags.
Step 2: Blended item 1, 2 and 3 of step 2 in a separate polybag and mixed for five minutes
Step 3: Load dry mix of step 2 into RMG and add Isopropyl alcohol at slow speed of impeller and Mix for 5 minutes (until to get a breakable dough mass)
Step 4: Load the wet mass into Rapid dryer, dry at 40°C for 15 minutes and to get LOD NMT 2 % w/w
Step 5: Dried granule pass through #20 and collected in poly bag.
Step 6: Blended step 5 and item no 4 for 2 minutes in polybag.
Step 7: Lubricated blend compressed by 23.5 X 11.50 mm punches at the average weight of 1875 mg.

Preparation of Coating Suspension

Step 8: Dissolve to disperse Ammonio Methacrylate Copolymer Type A and B in alcohol and Add purified talc, to getting homogenous suspension free from lumps.
Step 9: Loaded uncoated tablets into conventional coating pan.
Step 10: Applied coating suspension on uncoated tablets to desired weight gain achieved.
The physical and chemical parameters of the tablets were found to be satisfactory.

Stability Study

Stability study was carried out on the formulation B5. Tablet of batch B5 were packed in PVC blister and 180 cc HDPE container with silica gel bag and absorbent cotton. It was stored at $40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ relative humidity for 1, 2, 3 and 6 months. Tablets were evaluated for *in-vitro* drug release after six month. Result obtained was compared with data obtained for zero ambient time at ambient temperature. The results of *in-vitro* release study of Batch B5 after stability study.

In vitro Release Study

Glucosamine sulphate release was determined with six tablets per formulation using a USP Type II dissolution assembly

(Hansen SR-8 Plus, Chatsworth, CA, and Erweka DT-70, Milford, CT) in deionized water with a paddle speed of 50 rpm and bath temperature of $37.0 \pm 0.5^\circ\text{C}$. Samples were collected interval of 15 minutes, 30 minutes and 45 minutes. Each samples were collected were replaced with fresh equal volume of dissolution media. Six replicates were tested for each batch of the tablet formulations. Dissolution samples were filtered and this apparatus was equipped for filtered fraction collection diluted to appropriate concentrations for drug analysis. Each filtered sample was analyzed by HPLC method using Waters Alliance 2695 Separations module; 2996 PDA detector was used with BDS Hypersil C8 column (250 mm x 4.6 mm; 5 μm particle sizes)

Table 1: Shows the Data of Glucosamine Stability Study and *in-vitro* Release Study of Batch B5 for 6M

Time in hour	% Cumulative drug release profiles				
	Spec	Initial	1M	3M	6M
0	0	0	0	0	0
1	30	28	30	32	30
4	55	52	52	54	55
8	75	72	72	75	72
12	85	92	95	93	92

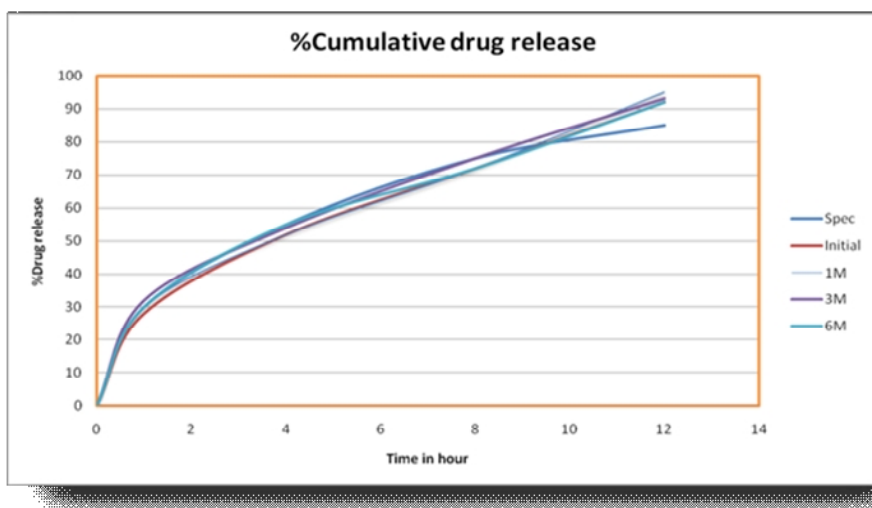


Figure 1: Dissolution Profile of Batch B5 for 6M Stability Data

The Batch B5 did not show any physical or chemical interaction between drug and polymer. It was concluded from the IR Study showed similar peak of drug in tablet formulation.

RESULTS AND DISCUSSION

In the present study the results shows various formulations of sustained release tablets of Glucosamine Hydrochloride were developed using various polymers viz, Carbomer and Ammonio Methacrylate Copolymer Type A and B different proportions and combinations by wet granulation technique. The tablets were evaluated for physical characterization, *in vitro* release study and stability studies (Figure 1 and Table 1). Results of *in vitro* release profile indicated that formulation (B5) was the most promising formulation as the extent of drug release from this formulation was high as compare to other formulations, which are suitable for once a day medication (Figure 1 and Table 1). Stability study was conducted on tablets of Batch B5 at $40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ RH for upto 6 months. Tablets were evaluated for *in vitro* release profile, after six month. No significant changes were observed in any of the studied parameters during the study

period, thus it could be concluded that formulation was stable (Figure 1 and Table 1). The synthesis and initial physicochemical evaluation of model mutual prodrugs as potential osteoarthritis therapeutics show some theoretical promise. Similar applications are of the pharmaceutical industry's interest. One objective was met with the synthesis of NSAID-glucosamine mutual prodrugs. These models may or may not improve the pharmaceutical properties of NSAIDs and glucosamine such as their permeability, solubility and stability in relationship to each drug's pharmacological and pharmacokinetic properties. The modifications have altered the physicochemical properties that will affect delivery methods by optimizing the drug's delivery, pharmacodynamic and pharmacokinetic properties. A drawback to the use of a concomitant (mutual prodrug) application of a product delivering an NSAID and glucosamine is that it currently would immediately raise

questions by FDA, since glucosamine is not considered a drug and its efficacy has not been proven. Hence, further *in vitro* and especially *in vivo* studies must be undertaken. Therefore, if these products or similar products show clinical significance a new drug application (NDA) would have to be filed due to the claims needed to protect the glucosamine entity. Predictive science inherently says that, like the “statin-drugs”, that were developed from nutraceuticals, glucosamine based products will be developed into “blockbuster” drugs with proven efficacies under FDA protocol. From the present investigation it is concluded that the tablets of batch B5 had considerable *in vitro* drug release. Tablets of Batch B5 was selected as an optimum batch and evaluated for further parameter like accelerated stability study and characterization using IR spectroscopy. The stability study revealed that there was not significant change in dissolution profile for a period of 6 months. Tablet of batch B5 did not show any physical or chemical interaction between drug and polymer, which was concluded from the IR Study showed similar peak of drug in tablet formulation. These modifications may alter the physicochemical properties of the drug, which may in turn effect the administration options that optimize drug delivery.

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Cite this article as:

Raju Merugu, M. Vijaya Bhaskara Reddy and Rajendra G. Lala. evaluation of in vitro stability studies on nutraceuticals in oral solid dosage forms with special reference to Glucosamine. Int. Res. J. Pharm. 2013; 4(8):265-268 <http://dx.doi.org/10.7897/2230-8407.04854>

Source of support: Nil, Conflict of interest: None Declared