KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI COLLEGE OF HEALTH SCIENCES

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMACEUTICS

STANDARDISATION OF ORAL LIQUID IBUPROFEN SUSPENSION FOR IMPROVED STABILITY AND EFFICACY

BY

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STANDARDISATION OF ORAL LIQUID IBUPROFEN FORMULATION FOR IMPROVED STABILITY AND EFFICACY

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BY

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KUMASI

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DECLARATION

I hereby declare that this dissertation is my own work for the Master of Philosophy degree in Pharmaceutics and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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DEDICATION

This work is dedicated to my mum, Madam Comfort Sefah, for her love, prayers and support which has brought me this far.

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To God be the Glory.

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ABSTRACT

Ibuprofen is a non-steroidal anti-inflammatory drug on the World Health Organization's (WHO) Model list of Essential Medicines. Due to its peripheral antiinflammatory action, ibuprofen is a useful drug in painful conditions associated with inflammation such as teething pain. This makes ibuprofen suspension one of the main analgesic, antipyretic and non-steroidal anti-inflammatory drugs available for paediatric care on the local market. Ernest Chemists Limited (ECL) a local pharmaceutical manufacturing company in Ghana ventured into the production of ibuprofen suspension. However the product was found to be unstable after 12months under real time stability studies.

The focus of this work was to find the cause(s) of the instability of the ECL ibuprofen suspension and to optimise and reformulate a standardised and stable oral ibuprofen suspension.

The procedures and properties of the ECL ibuprofen suspension were reviewed by examining the suspension from the raw material stage through processing, packaging, finished product specifications and storage conditions. The effect of environmental conditions such as storage temperature and light on the suspension was also investigated. Furthermore, the effects of product specific factors, like suspension pH on the stability of the product were also investigated.

It was deduced from the results of the investigations that the absence of a buffering agent and an antioxidant may have accounted for the instability of the suspension. It was proposed to include a buffering agent and antioxidant in the recommended recipe. Citric acid-sodium citrate system was selected as the buffering agent, while ascorbic acid was chosen as the antioxidant.

The one variable at a time (OVAT) principle was used to optimise the new formulation. The optimisation process led to the development of nine different formulations. One of the formulations was assessed as the best and selected as the preferred standard formulation for the ibuprofen suspension which was subjected to accelerated stability studies.

The standardised formulation passed the accelerated stability studies pointing to a potentially stable product.

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LIST OF ABBREVIATIONS

API: Active Pharmaceutical Ingredient

BP: British Pharmacopoeia

ECL: Ernest Chemists Limited

ICH: International Conference on Harmonisation

USP: United States Pharmacopeia

WHO: World Health Organisation

CHAPTER

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Pharmaceutical formulation is the process of combining active pharmaceutical ingredients (API) and other chemical substances (excipients) to produce a medicinal product (Hassan, 2012). In order to enhance product delivery to the patient, pharmaceutical products are formulated into specific dosage forms. Each dosage form requires specific pharmaceutical technology and the accompanying technical challenges for formulation development. Oral liquid formulations are usually solutions, emulsions or suspensions containing one or more active pharmaceutical ingredients in a suitable vehicle; the active pharmaceutical ingredient may be a solid or a liquid which might be used as such (International Pharmacopoeia, 2008). One of the major challenges with oral liquid products is poor aqueous solubility which impacts on bioavailability of the active ingredient (Kesisoglou *et al.*, 2007). The poor aqueous solubility may lead to the formulation of an oral liquid preparation as a suspension. The other reasons for suspension formulation include masking the bitter taste of the active ingredient, improving the stability of the active ingredient and to achieve controlled release of the product (Kulshreshtha *et al*., 2010).

Pharmaceutical formulations must be stable in order to ensure safety, quality and efficacy. The stability of a pharmaceutical dosage form is defined as the ability of the product in a specific container or container closure system to maintain its physical, chemical, microbial and safety specifications (Kommanaboyina and Rhodes, 1999). Physical, chemical and microbiological stability can be achieved by the addition of appropriate excipients such as buffering agents, antioxidants and preservatives (Nunn and Williams, 2005).

1.2 Rationale for the Research

Ibuprofen is one of the non-opioid analgesic and non-steroidal anti-inflammatory drugs (NSAID) on the World Health Organization's (WHO) Model list of Essential Medicines (WHO, 2015). On the local market ibuprofen suspension is one of the main analgesic, antipyretic and non-steroidal anti-inflammatory drugs available for paediatric use and is widely prescribed.

Ernest Chemists Limited (ECL) is a local pharmaceutical manufacturing company in Ghana. In view of the importance of ibuprofen in the health delivery system in Ghana, the company manufactured an ibuprofen suspension for the local market. The product failed real time stability studies under International Conference on Harmonization (ICH) storage conditions of 30 ± 2^0C and 75±5% relative humidity after 12 months of storage. There was a drop in the assay of the preparation. A market survey of locally manufactured brands also showed some of the products failing some of the test parameters.

1.3 Aim

The aim of the research is to investigate source(s) or cause(s) of the instability of the ECL ibuprofen suspension and to optimise, reformulate and standardise a stable oral ibuprofen preparation.

1.4 Objectives

i. Determine the causes of the instability of the ibuprofen suspension by ECL ii. Determine how to overcome the instability.

iii. Draw up an appropriate formula for the formulation. iv. Optimise and standardise the formulation.

v. Recommend appropriate manufacturing procedure if need be.

vi. Recommend appropriate packaging materials if need be.

vii. Recommend appropriate storage conditions if need be.

1.5 Benefits of Research

The following benefits will be realised at the end of the study;

- Contribute to the knowledge about the instability of ibuprofen suspensions.
- Contribute to the formulation development of oral liquid preparations.
- The study will help Ernest Chemists Ltd develop a stable and effective ibuprofen suspension for the market.

1.6 Ibuprofen

Ibuprofen is (±)-2-(p-isobutylphenyl) propionic acid, with molecular formula $C_{13}H_{18}O_2$, molar mass 206.28 and structure as illustrated in figure 1.1 (USP, 2015):

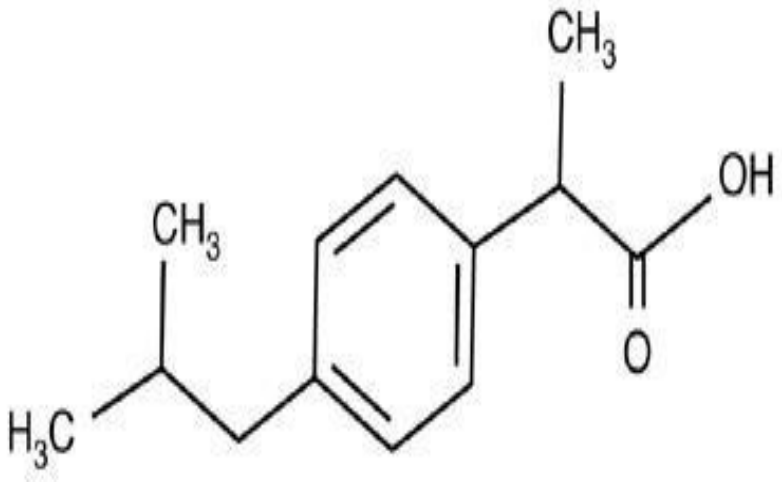

Figure 1.1 Structure of Ibuprofen

1.6.1 Properties of ibuprofen

Ibuprofen is a white or almost white crystalline powder or colourless crystals. It has a melting point of 75^0C to 78^0C. Ibuprofen is practically insoluble in water, freely soluble in acetone, methanol and methylene chloride (BP, 2014). The pKa is in the range of 4.5 to 4.6 (Potthast *et al*., 2005). It has a characteristic strong smell.

1.6.2 Stereochemistry of ibuprofen

Ibuprofen contains a chiral centre and therefore exists as a racemate (Romero and Rhodes, 1993). The two optical isomers of ibuprofen are identified by the prefixes R(−) and S(+). It is only the S(+) enantiomer which inhibits prostaglandin synthesis hence pharmacologically it is the S(+)- enantiomer which is active (Jamali *et al*.,

1988). However the R(−) enantiomer is readily converted to the active (S)- form by enzymes in the body (Lee *et al*., 1985). In view of this a racemic mixture of the two are used in production.

1.6.3 Uses of ibuprofen

Ibuprofen like other non-steroidal anti-inflammatory drugs (NSAIDs) is used worldwide in relieving the symptoms of pain, inflammation and fever (Steinmeyer, 2000; Rainsford, 2009). Ibuprofen is indicated for the treatment of mild to moderate

pain and as an adjunct to opioids in the management of moderate to severe pain (WHO, 1996). Ibuprofen is especially useful in situations where aspirin and paracetamol use do not result in adequate pain relief or where the use of opioid containing combinations will lead to central nervous system or gastro-intestinal adverse effects (Dionne, 2001). It is used in painful and inflammatory conditions such as dysmenorrhea, headache including migraine, post-operative pain, musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis (Martindale, 2002). Ibuprofen has also been used as an alternative to indomethacin in the treatment of patent ductus arteriosus (Bushra and Aslam, 2010).

1.6.4 Mode of Action of ibuprofen

The pharmacological effects of NSAIDs is exerted through the inhibition of enzymes that catalyse prostaglandin synthesis specifically prostaglandin H synthase (Vane, 1996). Prostaglandin H synthase consist of two isoenzymes normally referred to as cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) (Cashman, 1996). COX-1 naturally occurs in normal cells and whiles COX-2 is produced during inflammation (Xie, 1992). COX-1 produces prostaglandins that protect the stomach and kidneys whiles COX-2 produces prostaglandins responsible for pain and inflammation (Vane, 1998).

1.7 Pharmaceutical Dosage Forms

The active pharmaceutical ingredients are hardly used in their raw state and are most often administered in the form of pharmaceutical dosage forms through the addition of excipients such as preservatives, lubricants, binders, flavours, sweetening and bulking agents (Dutta, 2015).

The dosage forms are the means by which the pharmaceutical products are delivered to sites of action within the body. Among the reasons for pharmaceutical dosage form design is improving bioavailability, ensuring accurate dosing, masking unpleasant taste,

formulation of sustained release products, protecting from environmental conditions and gastric acids (Lesar, 2002). The pharmaceutical dosage form determines the physical form of the product.

1.7.1 Classification of Pharmaceutical Dosage Forms

Pharmaceutical dosage forms can be classified based on route of administration or physical form.

Using the physical form as a means of classification, dosage forms can be classified into solids, liquids and gases.

1.7.1.1 Solids

Solid products administered through the oral route are the most popular form of drug delivery (Bu *et al.,* 2011). Among these products are tablets, capsules, lozenges and powders. In addition there are other solid products like the pessaries and suppositories which are administered through the vaginal and anal regions.

1.7.1.2 Gases

Gaseous dosage forms consist of medical gases and aero-dispersions (Moynihan and Crean, 2009) and among these gases are the traditional ones, like oxygen and nitrous oxide, as well as recent discovered ones like nitric oxide, carbon monoxide and hydrogen sulphide (Nakao *et al.,* 2009).

1.7.1.3 Liquids

Liquid dosage forms are pharmaceutical formulations in which the active ingredient and excipients are dissolved or dispersed in a liquid medium. Oral liquid dosage forms offer unique opportunities to patients with swallowing difficulties and paediatrics who are unable to take solid dosage forms like tablets and capsules and also offer faster therapeutic response (Rubio-Bonilla *et al.,* 2010). Compared to tablets and capsules oral

liquids offer better dosage control. Liquids may be formulated as syrups, emulsions, aromatic water, elixirs, injections, liniments, tinctures and suspensions.

- **Syrups**

  Pharmaceutical syrups are concentrated sugar based aqueous liquid formulations which may contain active ingredients and other excipients such as preservatives, flavours, colour and other sweetening agents (Farukh and Khan, 2013).

- **Emulsions**

  Pharmaceutical emulsions are two phase preparations consisting of two immiscible liquids, with one (dispersed phase) uniformly dispersed in the other (continuous phase). The preparation is stabilized by a third agent, the emulsifying agent (Khan *et al.,* 2011).

- **Aromatic Water**

  Aromatic waters are clear saturated solutions of volatile oils or other aromatic materials in water. They are normally used for flavouring other than medical uses.

- **Elixirs**

  An elixir is a clear, water-alcohol based solution which may or may not contain an active pharmaceutical ingredient that is formulated for oral use.

- **Injections**

  Injections are sterile formulations administered parenterally. They are normally in the form of solutions, suspensions or emulsions of drug products, or of a solid that contains active ingredients to be dissolved or suspended before use (Korean Pharmacopoeia, 2012).

- **Liniments**

  Liniments are external formulations that are applied by rubbing into the skin.

- **Tinctures**

  Tincture is an alcoholic or alcohol-water solutions of plant extracts or pure chemical substances.

- **Suspensions**

  Not all active ingredients are sufficiently soluble to allow the formulation of such substances in the form of solutions. In such instances the substance can be formulated as a suspension. Pharmaceutical suspensions are coarse dispersion consisting of insoluble materials (internal phase) uniformly dispersed in a liquid (external phase). The external phase may be aqueous, organic or oily liquid for external applications (Raj and Angela, 2011). Suspensions are mostly unstable, in the absence of agitation the solid particles tend to settle at the bottom of the container over time. The ease of redispersing the sediments again will depend on the nature of the particles

  (Nutan and Reddy, 2010). The particle size of the dispersed phase ranges from 0.5 to 5.0microns (Lachman *et al.,* 1986). The particle size of the dispersed phase is very important in the formulation of the suspension.

1.8 Types of Suspensions

Pharmaceutical suspensions can be classified based upon their use: oral suspensions, ophthalmic suspensions, parenteral suspensions and suspensions for external use.

1.9 Properties of Good Suspensions

A good suspension should be smooth with good organoleptic properties, physically and chemically stable. In addition the suspension should have a low rate of sedimentation, uniform dispersion, easy to re-disperse upon agitation, easy to pour from the container and be able to resist microbial growth (Sushma *et al.*, 2013).

1.10 Advantages of Suspensions

The insoluble salts of some active substances may be more palatable and stable than the soluble salt. Suspensions allow the formulation of hydrophobic drugs in the liquid form. The chemical stability of certain active ingredients can be improved when formulated as suspensions e.g. procaine penicillin. Secondly the start and duration of action of suspensions can be controlled e.g. protamine zinc insulin suspension and also unpleasant and bitter taste can be masked e.g. metronidazole suspension. Lastly suspensions will be more rapidly absorbed than the corresponding solid dosage form (Marriott, 2010).

1.11 Disadvantages of Suspensions

Suspensions are difficult to formulate because of physical stability and sedimentation problems. They are bulky to handle (Jones, 2008). Compared to fixed dosage forms like tablets and capsules there is the possibility of dose variation.

1.12 Excipients for Suspension Formulation

Excipients are physiologically inactive materials used in formulations to enhance manufacturing, administration and to protect the formulation from issues concerning physical and chemical stability (Abraham and Mathew, 2014). Suspensions consist of the active pharmaceutical ingredient(s) and excipients. The functional excipients used in suspension formulation include sweeteners, preservatives, solvents, wetting agents, thickeners, colouring agents, flavours, suspending agents, antioxidants, pH adjusting and buffering agents.

1.12.1 Solvents and Co-solvents

Water is the most used solvent in pharmaceutical manufacturing because of its safety profile, physiological compatibility and good solubilising power. However its use is

sometimes limited by its promotion of microbial growth and the instability of some drugs in the presence of water.

Co-solvents are defined as water-miscible organic solvents that are used in liquid drug formulations to increase the solubility of poorly water-soluble substances or to enhance the chemical stability of a drug (Rubino and Yalkowsky, 1987) e.g. propylene glycol, glycerine, ethanol.

1.12.2 Preservatives

Preservatives are used to kill or inhibit the growth of microorganisms that cannot be prevented from the product through good manufacturing practices or those that get to the product during use (Bean, 1972). The inclusion of preservatives in multi-dose liquid and semi-solid products is mandatory and performance standards are defined in compendia monographs. The number of regulatory approved preservatives for multidose oral products is limited (Elder and Crowley, 2012a). A products formulation has a bearing on the efficacy of the added preservative. Some of the formulation factors affecting preservative action include pH, complexation with emulsifying agents and partitioning of the preservative between the components of an emulsion (Bean, 1972).

Table 1.1 Preservatives for Oral Pharmaceutical Products

Preservative	pH of Microbial Activity	Concentration
Methylparaben	4.0 – 8.0	0.015 – 0.20
Ethylparaben	4.0 – 8.0	Up to 0.25%
Propylparaben	4.0 – 8.0	0.01 – 0.02
Sodium benzoate	2.0 – 5.0	0.02 – 0.50
Benzoic acid	Below 4.5	Up to 0.15%
Potassium sorbate	Below 6.0	0.10 – 0.20
Sorbic acid	Below 6.0	0.025 – 0.10

1.12.3 Wetting Agents

Suspensions consist of insoluble materials dispersed in a liquid medium and some of the insoluble materials can easily get wet by the liquid facilitating easy dispersion. However many insoluble materials are too hydrophobic to easily get wet, hence form large porous clumps in the liquid or remain floated on the surface. Fine powders are prone to cause this problem because of the larger surface area (Nutan and Reddy, 2010). Wetting agents are materials that causes a liquid to spread more easily across or penetrate into the surface of a solid by reducing the surface tension of the liquid (Zontek and Kostka, 2012).

Surfactants with HLB value between 7 and 9 can be used as wetting agents and the commonly used wetting agents for oral use are polysorbates and sorbitan esters (Nutan and Reddy, 2010).

1.12.4 Sweetening Agents

The use of sweetening agents is the commonest way of masking taste and there are both natural and synthetic sweeteners available for this purpose.

Sucrose is widely used in oral pharmaceutical formulations and it may attack aluminium closures. Sucrose is also considered to be more cariogenic than other carbohydrates since it is more easily converted to dental plaque (Rowe *et al.*, 2009).

Table 1.2 Relative sweetness of commonly used sweeteners

Sweetening Agents	Relative Sweetness *	Comment
Aspartame	200	Not very stable in solution
Acesulfame potassium	137 - 200	Bitter after taste if used in higher concentration
Cyclamate	40	Banned
Glycerrhizin	50	Moderately expensive
Sorbitol	0.50 – 0.60	May be harmful if ingested in large amounts
Manitol	0.60	Negative heat of solution

Saccharin	450	Unpleasant after taste
Sucrose	1	Most commonly used
Sucralose	600	Synergestic sweetening effect
Glycerin	0.60	May explode if mixed with strong oxidising agents

*Sucrose is taken as a standard of 1 for comparison
Source: (Abraham and Mathew, 2014)

1.12.5 Suspending and Thickening Agents

Suspending agents stabilise suspensions by preventing the sedimentation of the solutes in the dispersion medium. They do increase the viscosity of the dispersion medium which helps prevent sedimentation and this makes most suspending agents also thickening agents. Suspending agents act by forming a film around the dispersed particle and decrease the inter particle attraction. A good suspension should exhibit thixotropic behaviour. At rest the suspension should be viscous enough to prevent sedimentation but have good flow characteristics when agitated. Suspension stability is dependent on the type of suspending agent rather than the physical properties of the API. The quantum of suspending agent is dependent on the presence or absence of other materials capable of modifying the viscosity of the medium. Examples of suspending agents are acacia gum, alginates, microcrystalline cellulose, carboxymethylcellulose and xanthan gum.

1.12.6 Buffering Agents

Buffers are used in pharmaceutical formulations to control the pH of the product and the control of pH is necessary to:

- Ensure physiological compatibility.
- Maintain/optimise chemical stability.
- Maintain/optimise preservative effectiveness.
- Optimise solubility/insolubility.

Examples of buffer salts used in pharmaceutical formulations include (Jones, 2015):

- acetates (acetic acid and sodium acetate): 1–2%
- citrates (citric acid and sodium citrate): 1–5%
- phosphates (sodium phosphate and disodium phosphate): 0.8–2%

1.12.7 Antioxidants

Antioxidants are molecules that are used to improve the stability of products by delaying the oxidation of the API and other excipients. Antioxidants can be grouped into three:

- Members of the first group are known as true antioxidants and they inhibit oxidation by reacting with free radicals and blocking chain reactions.
- The second group are made up of reducing agents which have lower redox potentials than the API or excipient. In solution they are oxidised in preference to the API or excipients. Reducing agents may also operate by reacting with free radicals.
- The third group consists of antioxidant synergists. They have little antioxidant effect but do enhance the antioxidants effect of the first group by reacting with heavy metal ions that catalyse oxidation (Trivedi and Patel, 2011).

1.12.8 Colouring Agent

Colours are added to pharmaceutical formulations to impart desired colours to the product and to improve elegance especially paediatric products (Allam and Kumar, 2011).

1.12.9 Flavouring Agent

Flavours do complement the sweetener in a formulation and helps to increase patient compliance (Basu and Sen, 2015).

1.13 Packaging and Storage Conditions for Suspensions

Suspensions should be packed in wide mouth closure with adequate air space above the product to enable agitation so as to enhance pouring of the product. It should be protected from freezing and the label should always bear the inscription, 'shake before use'.

1.14 Theory of Suspensions

Sedimentation means settling of particle or floccules in suspensions under gravitational force.

The sedimentation velocity is expressed by Stoke's equation.

$$v = \frac{d^2 (p_1 - p_2) g}{18 \eta}$$

Where:

v = velocity of sedimentation

d = diameter of the particle g

= acceleration of gravity

ρ_1 = density of the particle

ρ_0 = density of the vehicle

η = viscosity of the vehicle

Stoke's equation applies only to:

- Spherical particles in very dilute suspensions (0.5 to 2.0 g per 100 ml).
- Particles which freely settle without interference with one another (without collision).
- Particles with no physical or chemical attraction or affinity with the dispersion medium.

Pharmaceutical suspensions usually have concentrations of 5%, 10%, or higher percentage, so there are hindrances to particle settling.

1.14.1 Factors affecting Sedimentation

- **Particle Size**

  Sedimentation velocity (v) is directly proportional to the square of diameter of particle.

  $$V \alpha d^2$$

  It is apparent from the equation that the velocity of fall of a suspended particle is higher for bigger particles than it is for smaller particles. Reducing the particle size of the dispersed phase produces a slower rate of sedimentation (Nutan and Reddy, 2010).

- **Density of the Dispersed Phase**

  $$V \alpha (\rho_1 - \rho_2)$$

  The higher the density of the particles, the greater the rate of descent. If the density of the dispersed particles is lower than the density of the dispersion medium they will tend to float and floating particles are quite difficult to distribute uniformly in the vehicle (Mastropietro *et al*., 2013).

- **Viscosity of Dispersion Medium (η)**

  $$V \alpha 1/\eta_o$$

  Sedimentation velocity is inversely proportional to the viscosity of the dispersion medium. So the rate of sedimentation may be reduced by increasing the viscosity of the dispersion medium. Products having too high viscosities are not desirable, because they pour with difficulty and difficult to redisperse the suspended particles (Mastropietro *et al*., 2013).

1.15 Stability of Suspensions

Disperse systems are thermodynamically unstable and as a result many changes can occur during and after manufacture (Zatz, 1985). Physical stability can be defined as the ability of the disperse phase to remain uniformly distributed in the dispersion medium. Among the parameters that could change are colour, drug content, microbial attributes, uniformity, pH, viscosity, sedimentation and caking (redispersibility). The ability for the suspension to maintain its initial attributes is critical for pharmaceutical formulations since any change could adversely affect its safety, quality and efficacy. The periodic monitoring of the above mentioned parameters as per authentic procedures is important in the determination of the stability of suspensions. The stability of suspensions is the result of various physical and electrochemical forces

(Particle Sciences, 2009).

1.16 Interfacial Properties of Suspensions

In pharmaceutical suspensions, the disperse phase consists of finely divided particles in the dispersion medium. The increased surface area leads to surface free energy that is thermodynamically unstable. The dispersed particles will tend to regroup to decrease the total surface area and reduce the surface free energy, flocculation (agglomeration) and aggregation occurs because the system has a tendency towards a thermodynamically stable state.

Basically there are two types of interaction between particles – attraction and repulsion. The forces of attraction (called Van der Waals forces) are always present pulling particles together and when the attractive forces are dominant, the particles will adhere; if the repulsive forces are stronger then the particles will remain suspended separately (Particle Sciences, 2009).

1.16.1 Zeta Potential

Zeta potential is the charge that develops at the solid-liquid interface. It is another parameter that could be used in assessing the stability of pharmaceutical suspensions. It measures the electrostatic repulsive force between the particles (Weiner *et al.*, 1993). Zeta potential is an important physical property of particles in suspension. All materials will spontaneously acquire a surface electrical charge when brought into contact with a polar medium (Fairhurst, 2013).

The development of a net charge at the particle surface affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions (ions of opposite charge to that of the particle) close to the surface. Thus an electrical double layer exists around each particle. The liquid layer surrounding the particle exists as two parts; an inner region (Stern layer) where the ions are strongly bound and an outer (diffuse) region where they are less firmly associated. Within the diffuse layer there is a notional boundary inside which the ions and particles form a stable entity. When a particle moves (e.g. due to gravity), ions within the boundary move it. Those ions beyond the boundary stay with the bulk dispersant. The potential at this boundary (surface of hydrodynamic shear) is the zeta potential.

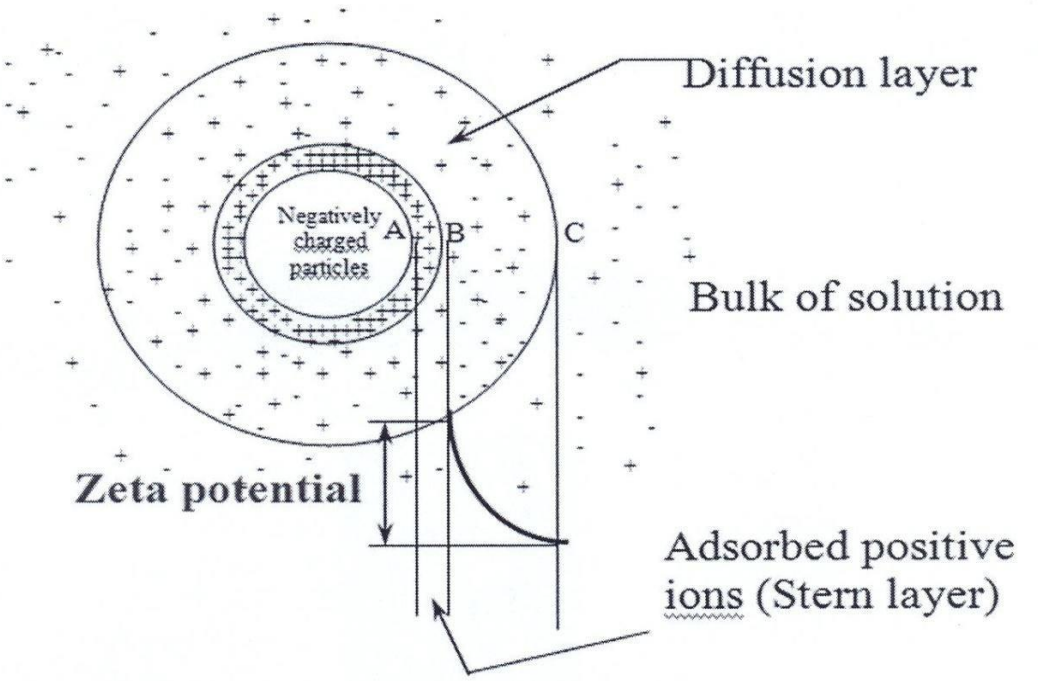

Figure 1.2 Double Layer

(Chen, 2013)

1.16.2 Flocculation

Flocculation is the process by which particles are caused to join together to form loosely connected aggregates (flocs) (Zatz, 1985).

1.16.3 Degree of Flocculation

A suspension partially flocculated and sufficiently viscous will give a product with desirable sedimentation properties. There is the need to control flocculation and viscosity to make redispersion easy. Flocculation control can be done through a combination of particle size control and the use of flocculating agents. The possible flocculating agents that could be used can be categorised into electrolytes, surfactants and polymers (Troy and Beringer, 2006).

1.16.4 Flocculating Agents

Flocculating agents are chemical additives that cause suspended solids to form aggregates called flocs (Heitner, 2004). There are three main types: electrolytes, surfactants and polymers.

- **Electrolytes**

  They act by decreasing the zeta potential, making it possible for the particles to come together to form loosely arranged structures (flocs). The flocculating ability increases with the valency of the ions. Hence calcium ions are stronger than sodium or potassium ions but trivalent ions are seldom used because of their toxicity (Patel, 2010).

- **Surfactants**

  Ionic as well as non-ionic surfactants can both be used as flocculating agents. Ionic surfactants act by neutralising the charge on the particles. Non-ionic surfactants are adsorbed onto more than one particle because of their long structure thereby, forming a loose flocculated structure (Patel, 2010).

- **Polymers**

  Polymers (both linear and branch chain) form gel-like networks that are adsorbed onto the surface of the dispersed particles, keeping them in a flocculated state. Hydrophilic polymers can also act as protective colloids. In this capacity, flocs are sterically prevented from adhering to one another and loose sediment is the result (Troy and Beringer, 2006).

1.16.5 Flocculated Suspension

In flocculated suspensions the particles tend to aggregate, leading to the formation of larger particles called flocs or floccules and because of their larger size they tend to settle faster. The floccules possess porous loose structure enabling the dispersion medium to flow through them during sedimentation. Secondly the floccules can entrap

a large amount of the liquid phase. Since the floccules are large the volume of the final sediment will also be large and can easily be redispersed. This makes flocculated particles less prone to compaction and cake formation than unflocculated particles.

1.16.6 Deflocculated Suspension

The dispersed particles in a deflocculated suspension remain as distinct separated units resulting in slow settling of the particles. The smallest particles take time to settle long after shaking giving rise to cloudy supernatant for an appreciable time. The slow rate of settling prevents the entrapment of liquid within the sediment. The sediments are therefore compact, cohesive and very difficult to redisperse upon agitation (Schott, 1976). This leads to caking.

1.17 Quality Control Tests for Suspensions

1.17.1 Sedimentation Volume

Sedimentation volume is a qualitative term which gives an indication of the quantum of settling that occurs in a suspension. The sedimentation volume, F, is the ratio of the equilibrium volume of the sediment, Vu, to the total volume of the suspension, Vo (Paul and Saha, 2012).

$$F=Vu/Vo$$

F has values ranging from less than one to greater than one.
Normally $F < 1$

When $F < 1$ then $Vu < Vo$

When $F = 1$ then $Vu = Vo$

The system is in flocculated equilibrium and shows no clear supernatant on standing.

When $F > 1$ then $Vu > Vo$

Sediment volume is greater than the original volume due to the network of flocs formed in the suspension, so loose and fluffy sediment.

The sedimentation volume gives only a qualitative account of flocculation.

1.17.2 Redispersibility

Suspensions must be homogeneously suspended prior to taking a dose in order to ensure uniformity of doses. Some pharmacopoeias require suspensions to be redispersed by shaking (Deicke, 1999). Redispersibility describes the ability of suspensions to uniformly disperse with little agitation after standing for some time (Patel, 2010). The ease of redispersibility is a major consideration in assessing the acceptability of a suspension.

1.17.3 Particle Size Distribution

Suspensions are thermodynamically unstable and separate on standing. The stability of the suspension is related to the particle size of the suspended particles (Murthy *et al*., 2015). Suspensions can be classified based on the particle size of the dispersed medium. Those with particle size greater than ~1μm are classified as coarse suspensions, whiles those below 1 mm are classified as colloidal suspensions (Manoharan *et al.*, 2010). The particle size of the dispersed phase has an effect on precipitation and aggregation. Normally finer particles produce more stable suspension. Particle size distribution has a direct influence on the texture and feel of the pharmaceutical formulation. An adequate particle size distribution is important in product processing as well as the safety, efficacy and quality (Silva *et al.*, 2013). Suspension stability also depends on the balance of the repulsive and attractive forces existing between the particles. The presence of weak or no repulsive force may lead to aggregation. In suspension formulation there is an optimum particle size distribution that gives a minimum viscosity whilst maintaining the volume fraction of particles.

1.18 Rheology

Rheology is the study of the deformation and flow of a material when it is subjected to an applied force (Barnes, 2000). A material will always respond to an applied force in a number of ways. The material may flow as a means of relieving the applied force and such materials are liquids. Materials tend to resist an applied force to some degree or else the material will not have an original form (Lubrizol, 2008).

1.18.1 Viscosity

Materials always tend to resist an applied force to some degree. It is this resistance to applied force that gives the material its original form. Viscosity is a measure of a flowing liquid to resist an applied force and the greater the resistance the higher the viscosity (Lubrizol, 2011).

1.18.2 Types of Flow Behaviour

Flow behaviour can be broadly classified into Newtonian and non-Newtonian. In Newtonian flow the viscosity is always the same regardless of the applied shear stress. Non- Newtonian systems are more complex and are characterized by large dissolved or solvated molecules, with a tendency to re-associate and a strong interaction with the solvent (Lubrizol, 2008).

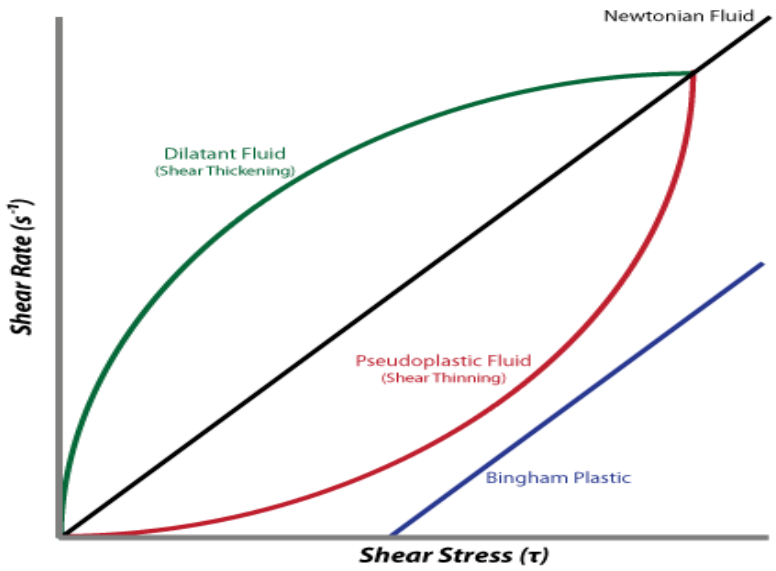

Figure 1.3 Types of Flow

(Physics for all, 2015)

Non-Newtonian flow can be divided into four types.

Dilatant — Occurs in suspensions that contain high concentrations of closely-packed solids. Examples: concrete, toothpaste.

Pseudoplastic — Viscosity decreases with increasing shear rates, flow characteristics are said to be pseudoplastic. This type of flow is encountered with large swollen or dissolved particles. Examples: solutions of guar gum, cellulosic thickeners, alginates.

Bingham Plastic — in this type of flow a minimum amount of force is needed for flow to start. Examples: catsup, PVC and styrene polymers (Lubrizol, 2008).

1.18.3 Thixotropy

Thixotropy is defined as, a decrease in viscosity under stress, followed by gradual recovery when the stress is removed. The effect is time dependent. Thixotropy can be observed when the structure of the disperse molecule (polymer) is broken upon the application of shear stress. The structure reforms with time when shearing force is removed.

1.18.4 Yield Value

Yield value (stress) is defined as the initial resistance to flow under applied stress. It is the stress that has to be applied to a material for flow to begin. Below the yield value (stress) the material will deform elastically (like stretching a spring), above the yield value (stress) the sample will flow like a liquid (Larson, 1999). In the case of

Bingham and Ellis plastic flow, a minimum shear stress is required to initiate flow. This minimum stress is known as the yield value or yield index. A practical application of yield value is the suspension of particles in a liquid. Unless the force of gravity operating on a suspended particle of a given mass exceeds the liquid's yield value, it will not descend (Lubrizol, 2002). Yield value is an important parameter in suspensions.

Particles will be continuously suspended in a liquid if the yield value of the liquid is enough to overcome the effect of gravity on the particles.

1.18.5 Yield Value and Viscosity

This is a simple experiment performed by Lubrizol Advanced Materials Inc. to demonstrate the importance of yield value as against viscosity in suspension formulation. Four suspending agents of varying concentrations were used in this experiment. The various concentrations were chosen because they produce gels of the same viscosity. Four different spheres of varying densities were suspended in the each of these gels. How the spheres were suspended after a month of storage is shown in figure 1.4. A ring on each indicates the initial positions of the spheres.

The experiment demonstrates that though the four gels have the same viscosities they do not possess the same suspending abilities.

A. Guar gum 2.1%
B. Carboxymethylcellulose 2.3%
C. Xanthan gum 6.0%
D. Carbopol® 940 NF polymer 0.4%

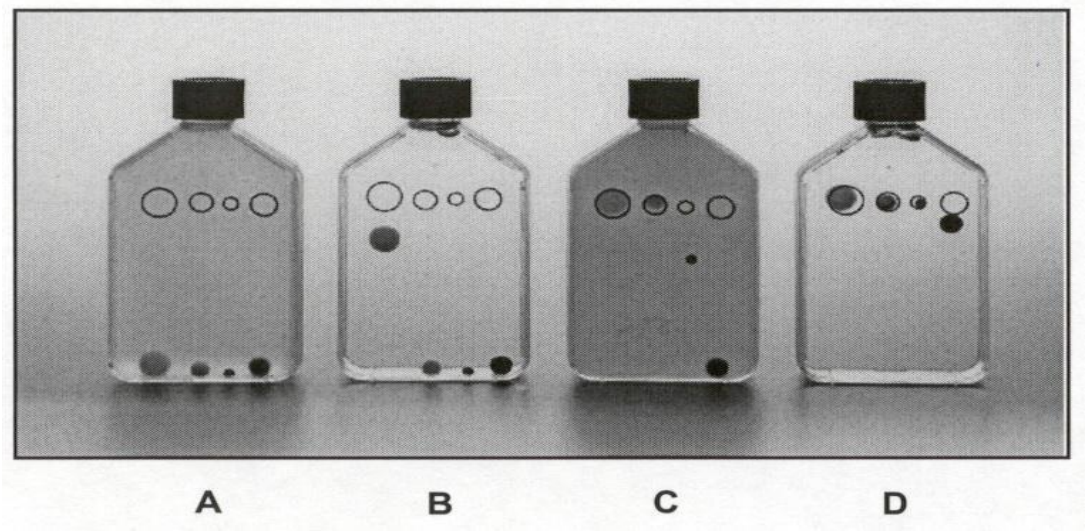

Figure 1.4 Viscosity vs. yield value of water-based gels

(Lubrizol, 2008)

1.19 Stability of Pharmaceutical Products

Pharmaceutical products degrade on storage and the extent of the degradation varies from product to product. It can be so acute as to render the product unfit for use within a short period. Pharmaceutical formulations are complex physico-chemical systems making them prone to degradation through physical, chemical and microbial reactions. There is a possibility of interaction between the active pharmaceutical ingredient and excipients or container-closure system (Monkhouse, 1984). Product instability can be further aggravated through poor formulation, packaging and storage (Shaikh and Sial, 1996). Therefore the active ingredient and excipients of a formulation must be compatible with one another to produce a stable product that is efficacious, safe and easy to administer (Patel *et al*., 2011).

Stability of a pharmaceutical product may be defined as the ability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications (Kommanaboyina and Rhodes, 1999).

It is the extent to which a product retains, within the specified limits, throughout its shelf life and use, the same properties and characteristics possessed at the time of its packaging. The instability may manifest as active ingredient or excipient degradation. The product degradation may be exhibited in the form of colour change, change in viscosity, phase separation, cracking or caking.

1.20 Factors Affecting the Stability of a Product

Challenges with formulation and stability are encountered more with liquid dosage forms than solid products. This is one of the reasons why a lot of products are first released on to the market as solid dosage forms. There are a number of factors affecting the stability of a drug and its dosage forms. These factors can be categorised into three (Tong and Zhang, 2006);

- Factors that are related to the active pharmaceutical ingredient and excipients.
- The product stability could be influenced by factors related to the formulation like active ingredient to excipient ratio, processing method and mixing or milling process (Collier *et al.,* 2010).
- Factors related to the product environment like storage temperature, relative humidity, packaging materials, light exposure and oxygen can affect the stability of the product (Singh and Singh, 2010).

1.21 Stability Testing

Stability testing provides evidence of how the quality of an active pharmaceutical ingredient or finish pharmaceutical product varies with time under the influence of various environmental factors as temperature, humidity and light (WHO, 2009: Velagaleti, 2010).

Stability studies are among the important processes done during pharmaceutical product development (Charde *et al*., 2014). It is a complex process because of the myriad of factors influencing the stability of pharmaceutical products. There are four main stability procedures; Accelerated stability testing.

- Real time stability testing.
- Retained stability testing.
- Cyclic temperature stress testing.

Accelerated stability testing is normally performed at the early stages of product development. The product or active ingredient is subjected to relatively high temperatures and or humidity as a worst case scenario. The data recorded from this provides some preliminary information on the future stability of the product.

Real-time stability testing is normally performed for longer period under more gentle conditions in order to determine how much degradation of the active ingredient has occurred during the entire shelf life of the product.

Retained sample stability testing is usually performed for all marketed products requiring stability data.

The stability sample is obtained from the retained samples. At least one batch is selected in a year. If the number of batches in a year exceeds 50, the samples should be taken from two or more batches.

Cyclic temperature stress testing is not a routine testing method for marketed products. The tests are designed on knowledge of the product so as to mimic likely conditions in market place storage. The cycle period is mostly 24 hours since the diurnal rhythm on earth is 24 hours.

Paul Schumacher in 1972 and Wolfgang Grimm in 1986 proposed dividing the world into four climatic zones in order to reduce the number of stability testing required of the target markets for pharmaceutical products. The climatic zones were I, II, III and IV. This concept was accepted and adopted in regulatory guidelines and pharmacopoeias. The WHO Expert Committee on Specifications for Pharmaceutical

Preparations in 2005 recommended the split of Climatic Zone IV into two zones: Climatic Zone IVa and Climatic Zone IVb. Ghana falls in Climatic Zone IVb (Markens, 2009).

The stability testing conditions for various zones could be found in WHO document on stability testing.

Table 1.3 ICH Stability Zones

ZONE	TYPE OF CLIMATE	MAJOR COUNTRIES	TEMPERATURE	RH
Zone I	Temperate zone	Canada, USA, Northern Europe	21^0C	45%
Zone II	Mediterranean / subtropical zone	Southern Europe, Japan, China	25^0C	60%
Zone III	Hot dry zone	India, Iraq	30^0C	35%
Zone IVa	Hot humid / tropical zone	Egypt, Kuwait, Iran	30^0C	70%

Zone IVb	Hot / higher humidity	Brazil, Ghana	30^0C	75%

Source: (Rios, 2015)

1.21.1 Importance of Stability Testing

One of the cardinal points for stability testing is ensuring safety and efficacy of the pharmaceutical product. A product could degrade into toxic decomposition products thereby rendering the product dangerous for administration to potential patients.

Secondly product degradation could also result in loss of activity resulting in treatment failures. For these reasons stability reports have become an important prerequisite for marketing authorisation of new pharmaceutical products. Stability testing helps in the following, arriving at the best formulation for the product, choosing the right excipients, the right container closure system, predicting the product shelf life as well as the storage conditions (Bajaj *et al.*, 2012).

1.22 Degradation Pathway

The major pathways of degradation for pharmaceuticals can be divided into thermolytic, oxidative and photolytic.

It includes oxidation, hydrolysis, photolysis and racemization (Charde *et al.*, 2013).

1.22.1 Thermolytic Degradation

In thermolytic degradation the process is driven by heat or significantly affected by temperature.

- **Hydrolysis**

  Hydrolysis is the commonest of the thermolytic degradation reactions accounting for over 50% of drug degradations. Hydrolysis is often the main degradation pathway for drug substances having esters, amides, anhydrides, imides, ethers, imines, oximes, hydrazones, semicarbazones, lactams, lactones,

thiol esters, sulfonates, sulfonamides, and acetals functional groups within their structure (Yoshioka and Stella, 2002).

Prevention of Hydrolysis

a) pH Adjustment

Most active substances are weakly acidic or weakly basic, they are more soluble when ionised however they degrade faster in the ionised state. Where the pH of a formulation has to be adjusted in order to improve the solubility of the active ingredient leading to the instability of the formulation then water miscible solvent can be introduced into the product to enhance stability by suppressing ionisation (Gokani and Desai, 2012).

b) Addition of Surfactant

Surfactant form micelles in solution and the particles of the drug substances are trapped in them. The hydroxyl groups such as OH are unable to penetrate the micelle cover to reach the drug particles thereby decreasing the hydrolysis rate (Saqib, 2008).

c) Formation of Salts and Esters

The formation of a less soluble salt or ester of a drug substance will reduce the rate of hydrolysis e.g. phosphate ester of Clindamycin (Saqib, 2008).

d) Desiccants

Storing with desiccants will reduce the rate of hydrolysis (Buckley and Newbold, 2005).

e) Complexing Agents

Complex formation reduces the rate of hydrolysis (Buyuktas, 2006).

1.22.2 Oxidative Degradation

Oxidation is a common chemical degradation pathway for pharmaceuticals. Oxygen the main mediator of this degradation pathway is abundant in the environment making the possibility of this reaction occurring very high.

Oxidation is second only to hydrolysis in terms of drug degradation pathways and it accounts for about 20 to 30% of drug degradations. It occurs through three primary mechanisms: electrophilic/nucleophilic, electron transfer and autoxidation.

Peroxide attack can lead to the oxidation of a drug molecule, which is typical of nucleophilic or electrophilic mechanism. The electron transfer process is similar to the nucleophilic or electrophilic process, the difference being that an electron is transferred from a low electron affinity donor (e.g., drug molecule) to an oxidising agent through the activity of transition metal (Zhou, 2009). Drug molecules with the following functional groups: alkenes, ethers, thioethers, amines and aromatic group (toluene, phenols, and anisole) are susceptible to oxidation.

Prevention of Oxidation

a) **Reducing Oxygen Content**

   Oxidation occurs in the presence of oxygen so by reducing the oxygen content of a product the rate of oxidation could be reduced e.g. filling under nitrogen (Brown and Leeson, 1969).

b) **Addition of Antioxidant.**

   Antioxidants act by being oxidised themselves; hence they are reducing agents such as thiols, polyphenols or ascorbic acid (Piechocki and Thoma, 2007).

c) **Addition of Chelating Agent**

   They form complexes with trace amounts of heavy metal ions and inactivate their catalysing ability e.g. disodium edetate, citric acid, tartaric acid (Piechocki and Thoma, 2007).

d) Adjustment of pH

The product could be formulated at the pH of optimum stability in order to reduce oxidation potential of the system (Gokani and Desai, 2012).

e) Changing the Solvent System

Some solvent system may have catalysing effect on oxidation reaction when used in the formulation e.g. Aldehydes, ethers, ketones may influence free radical reaction.

1.22.3 Photolytic Degradation

Light absorption by some drug substances can result in chemical reaction because light carries energy. Light sensitive products can be affected by sunlight (ultraviolet light) or by artificial light like fluorescent light (Welankiwar *et al*., 2013). The interaction between the drug and light radiation can lead to degradation of the active ingredient or excipients in the product (Tønnesen, 2008). Product degradation as a result of light absorption is directly initiated by the light energy hence temperature has very little effect on it (Zhou, 2009).

Prevention of Photo Degradation

a) Suitable Packaging

Using the appropriate packaging material can prevent photo degradation. Yellow-green glass protects products against light radiations in the U.V. range whiles amber protects products against U.V. radiation but not from I.R. radiation.

b) Use of Antioxidants

Photo degradation may be controlled through the use of antioxidants. The antioxidants act by undergoing preferential oxidation by donating an electron and/ or receiving the excess energy of the activated molecule (Piechocki and

Thoma, 2007). Ascorbic acid, α-tocopherol, and butylated hydroxytoluene act as free radical scavengers (Vinod *et al.,* 2015).

An example is sulphacetamide solution can be protected from photo degradation by antioxidants such as sodium thiosulfate or sodium metabisulphite.

1.22.4 Isomerisation

Another degradation pathway of drugs is through isomerisation. A lot of drugs are optically active hence there is the possibility of interconversion from one isomer to the other leading to changes in their pharmacological and toxicological properties (Fathima *et al*., 2011).

1.22.5 Polymerisation

Polymerisation is a continuous reaction between molecules which could lead to product degradation. More than one monomer reacts to form a polymer (Fathima et al., 2011). Polymerisation in glucose solution causes it to darken. This is attributed to the breakdown product [5- (hydroxyl methyl) furfural].

1.23 Available Dosage Forms of Ibuprofen

Ibuprofen is commonly available on the market in the form of tablets and suspensions. Parenteral formulation of ibuprofen was introduced in the United States in 2009. Topical formulation for the treatment of adult acne is available in Japan. In Ghana the main dosage forms available are tablets and suspensions.

1.24 Locally Manufactured Brands

Ibuprofen is manufactured locally as tablets and suspensions.

Table 1.4 Local manufacturers of ibuprofen products

Dosage Form	Product Name	Manufacturer
Tablets	Keuron	Amponsah-Efah Pharmaceuticals Ltd
	Emgiprofen	M & G Pharmaceuticals Ltd
	Enafen	Ernest Chemists Ltd

	Ibuprofen	Ayrton Drug Manufacturing Co. Ltd
	Anfen	Danadams Pharmaceutical Industry Ltd
Suspensions	Keuron	Amponsah-Efah Pharmaceuticals Ltd
	Pocupain	Pokupharma Ltd
	Ibuprofen	Geo Medicore Ltd
	Emgiprofen	M & G Pharmaceuticals Ltd
	Ibuprofen	New Global Pharmaceuticals Ltd
	Enafen	Ernest Chemists Ltd

CHAPTER 2 ASSESSMENT OF ERNEST CHEMISTS LIMITED IBUPROFEN

SUSPENSION AND OTHER LOCALLY MANUFACTURED BRANDS

2.1 Introduction and Background

Pharmaceutical suspensions are uniform dispersions of solid drug particles in a liquid medium usually an aqueous solution (Nielloud *et al*., 1998). APIs not stable in solution or soluble in aqueous or non-aqueous solvents are usually formulated in the suspension form (Sushma *et al*., 2013). Suspensions offer unique opportunities to part of the population who are unable to take solid dosage forms like children.

Ibuprofen an NSAID was introduced in 1969 as a better alternative to aspirin for the management of pain and inflammation. Ibuprofen is one of the most commonly prescribed NSAID (Bradbury, 2004) and also one of the recommended analgesics for painful conditions associated with inflammation such as dental pain by the Ministry of Health (MOH, 2010). Ibuprofen is practically insoluble in water (BP, 2014) and hence it is formulated in the liquid form as a suspension.

Ernest Chemists Limited (ECL) is an indigenous pharmaceutical company in Ghana. It is a key player in the pharmaceutical industry in Ghana. The company is into the importation and manufacture of pharmaceutical products in Ghana. The

manufacturing plant of ECL is situated in the heavy industrial area of the port city of Tema. The plant manufactures generic pharmaceuticals in the form of tablets, capsules, powders, oral and non-oral liquid preparations.

As part of its strategy to be a market leader in the manufacture of generics, the company veered into the manufacture of ibuprofen suspension. Samples of the ibuprofen suspension were put on real time stability studies. However it was found out there was a significant drop in the assay of the product after 12 months of storage.

The study is to identify possible cause(s) of the instability and to formulate a more stable ibuprofen suspension for the market.

The procedures and properties of the ECL ibuprofen suspension were as follows;

2.2 Materials and Equipment

The materials used by ECL for their ibuprofen suspension preparation consisted of ibuprofen from IOL Chemical and Pharmaceutical Ltd, India, propylhydroxybenzoate from Gujarat Organics Ltd, India, methylhydroxybenzoate from Sharon Labs Ltd, Israel, xanthan gum from Shandong Fufeng Fermentation Co. Ltd, China, saccharin sodium from Suzhou Fine Chemicals Co. Ltd, China, silicon dioxide (Aerosil 200) from Evonik Degussa AG, Germany, sucrose from Sugar Australia Pty Ltd, Australia, propylene glycol from Ineos NV, Belgium, simethicone emulsion 30% from Palmo Industrial Silicones Pvt. Ltd, India. amaranth from Irish Country Cold Group, UK, polysorbate 80 from Vasudha Chemicals Pvt Ltd, India and pineapple flavour from Irish Country Gold Group, UK. The rest are 60 ml amber coloured glass bottle from Rak Ghani, Pakistan, 28mm ROPP Aluminium Cap with expanded polyethylene liner from Archana Ampoules Pvt, India, self-adhesive label from SS Group, India, 28mm 10ml transparent plastic measuring cap from Archana Ampoules Pvt, India, 320gsm laminated aqua vanish jacket from SS Group, India and 3ply corrugated carton from Ghana carton, Ghana. HPLC grade methanol from Merck Life Science Pvt Ltd (India),

orthophosphoric acid from Merck Life Science Pvt Ltd (India), acetate buffer from Daejung Chemicals & Metals Co. Ltd (Korea), phthalate buffer from Daejung Chemicals & Metals Co. Ltd (Korea) and phosphate buffer – Daejung Chemicals & Metals Co. Ltd (Korea).

The equipments used by ECL in the suspension preparation and quality control analysis consisted of 1000 litre process vessel from GMP Machineries & Packaging (India), transfer pump from GMP Machineries & Packaging (India), digifil filling machine from Filquip Engineering (South Africa), ROPP Capping machine from Arol Closure Systems (Italy), labelling machine from Productive Systems (Pty) Ltd (South Africa) and shrink wrapper from Acepak (Pty) Ltd, South Africa, weighing scale (ME 204) from Metler Toledo, viscometer (Haake Viscotester 6L) from Haake, UK, pH meter(HI 2215 pH ORP Meter) from Hanna Instruments, HPLC (1200 Series) from Agilent Technologies and pycnometer from Merck, Germany.

2.3 Methods

2.3.1 Composition

The batch size for the preparation of ECL ibuprofen suspension was 1000 litres.

Table 2.1 Batch Composition of ECL Ibuprofen Suspension (1000 litres)

Raw Materials	Quantity (kg)
Sucrose	300.0
Saccharin Sodium	0.03
Ethanol 96%	0.01
Propylparaben	0.16
Methylparaben	1.84
Xanthan Gum	1.2
Colloidal silicon dioxide	8.0
Propylene Glycol	110.0
Dimethylpolysiloxane emulsion (30%)	1.0
Polysorbate 80	2.0
Ibuprofen	20.0
Amaranth	0.006

Pineapple Flavour	2.0
Purified water to	1000L

2.3.2 Mixing procedure

About 300 litres of purified water was added to the process vessel. The sucrose and saccharin sodium are added under stirring. The parabens are dissolved with the ethanol 96% and the xanthan gum suspended in the alcoholic solution. This was added to the bulk preparation whiles stirring. The colloidal silicon dioxide was moistened with water and milled once through the colloid mill. This was added to the bulk preparation. The propylene glycol was added. The ibuprofen powder, polysorbate 80, dimethypolysiloxane emulsion and about 60 litres of purified water are premixed together. The resultant mixture was milled in the colloid mill for about 20minutes and added to the bulk preparation. The amaranth was dissolved and added to the bulk suspension. The pineapple flavour was added and the volume adjusted to 1000 litres with purified water. The product was logged for quality control analysis.

2.3.3 Packaging process

The product was transferred from the storage tank using a transfer pump into the product holding port of the filling machine. The glass bottles were loaded unto the filling machine and four bottles were test filled. The filled volumes were checked and the necessary adjustments performed until the specifications were met. Once the filling specifications were achieved the whole batch production was filled, capped and labelled. The labelled products were packed into jackets, shrink wrapped and put into corrugated boxes.

2.4 ECL quality control test on ibuprofen suspension

The following parameters of the ibuprofen suspension were checked by the quality control section of ECL;

- **Description**

A bottle of the suspension was shaken to redisperse it. The contents were poured into a 100 ml beaker. The colour and other physical attributes like the appearance were recorded.

- **Redispersibility**

  A bottle of the suspension was allowed to stand undisturbed for not less than 3days. It was shaken to redistribute the sediments. The ease of redispersion was recorded.

- **Viscosity**

  A bottle of the suspension was shaken to redisperse it. The suspension was poured into a 100 ml beaker. The spindle L3 was rinsed with water and fitted onto the Haake viscotester.

  The spindle was immersed in the suspension and the viscometer started. The viscosity of the suspensions was read. The test was repeated twice.

- **pH**

  The pH meter was calibrated using the buffers acetate buffer (pH 4), phthalate buffer (pH 7) and phosphate buffer (pH 9.2). The bottle of suspension was shaken to redisperse it. The probe of the pH meter was rinsed with distilled water and insert into the suspension. The pH of the suspension was recorded when the reading was stable. The test was repeated twice.

- **Assay**

The relative density of the suspension was determined. Using the relative density an amount of suspension equivalent to 5 ml of the suspension (100 mg ibuprofen) was weighed. 50 ml diluent was added and shaken for 15minutes. The volume was adjusted to 100 ml with the diluent and filtered through a 0.45µ syringe membrane filter. A standard solution of 0.1% w/v ibuprofen in the diluent was prepared by weighing 100

mg of ibuprofen powder and adding 50 ml diluents. This was shaken for 15minutes. The volume was adjusted to 100ml with the diluent and filtered using

0.45µ syringe membrane filter. The two solutions were run on the HPLC using the chromatographic specifications below.

Diluent: (75% methanol: 24.7% distilled water: 0.3% orthophosphoric acid)

Chromatographic Specifications:

- Column: Zorbax Eclipse XDB-C18, 150x4.6 mm, 5 µ
- Mobile phase: 75% methanol: 24.7% water: 0.3% orthophosphoric acid
- Wavelength: 264 nm
- Flow rate: 1.5 ml/min
- Injection volume: 25 µl
- Temperature: 28^0C

The amount of ibuprofen in the sample was determined by comparing the areas of the sample and standard peaks.

2.5 Analysis of Locally Manufactured Ibuprofen Suspension

A market survey was conducted to find out about available locally manufactured brands of ibuprofen suspension. This was done visiting different pharmacy shops and purchasing locally manufactured ibuprofen suspension available.

2.5.1 Testing of Local Brands

The following tests were performed on the purchased locally manufactured brands; description, redispersibility, viscosity, pH and assay using the ECL quality control test methods (section 2.4).

2.6 Results and Discussion

2.6.1 Specifications of the ECL ibuprofen suspension

Composition – Each 5 ml of the suspension contained 100 mg Ibuprofen

Volume of suspension – 60 ml (57 – 63 ml)

Appearance – Pink coloured viscous suspension

Odour – Pineapple flavoured

Viscosity – 610 – 680mPas pH

– 3.5 – 5.0

Assay – 95.0 – 105.0%

Storage conditions – Store below 30°C.

The ECL ibuprofen suspension was a pink coloured viscous suspension with a pineapple flavour packed in an amber coloured glass bottle. The suspension contains 100 mg of ibuprofen per each 5ml of suspension. The viscosity of the suspension range from 610 to 680mPas with pH from 3.5 to 5.0. The content of ibuprofen in the suspension range from 95 to 105% and it should be stored below 30^0C.

2.6.2 Locally manufactured products

Four locally manufactured ibuprofen suspensions were found on the market.

Table 2.2 Locally Manufactured ibuprofen Suspensions

Product	Strength	Batch No.	Manufacturing Date	Expiry Date
1	100mg/5ml	A1M008	April, 2012	April, 2015
2	100mg/5ml	PP010	December, 2011	December, 2014
3	100mg/5ml	401	January, 2012	December, 2014
4	100mg/5ml	201	November, 2011	October, 2014

Viscosities of 4 Locally manufactured Products

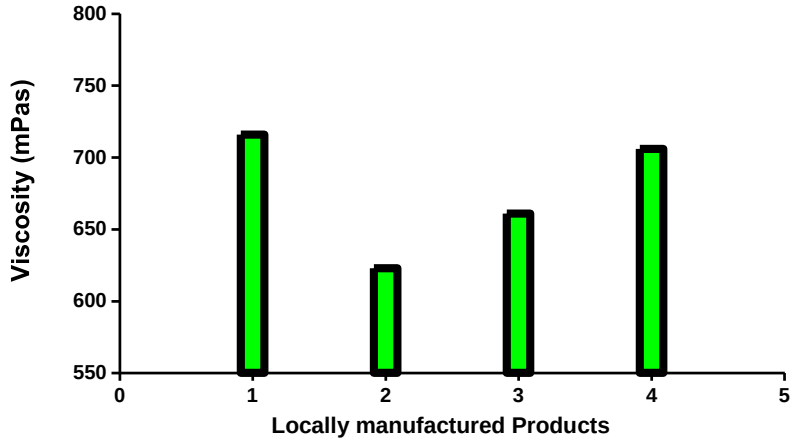

Figure 2.1 Viscosities of locally manufactured products

The results of the analysis of the locally manufactured products showed that the colour of the suspensions varied, two of the brands were yellow coloured whiles the other two were orange coloured. The mean viscosities ranged from 623 to 716mPas (figure 2.1) and appear to be more viscous than the ECL ibuprofen suspension.

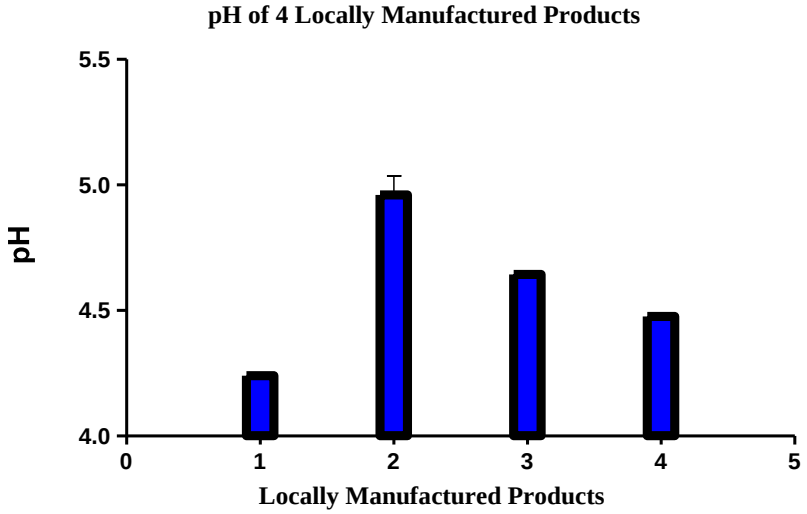

Figure 2.2 pH of Locally Manufactured Products

The pHs ranged from 4.24 to 4.96 (figure 2.2) and were all within the ECL acceptable limits of 3.5 to 5.0.

Content Assay of 4 Locally Manufactured Products

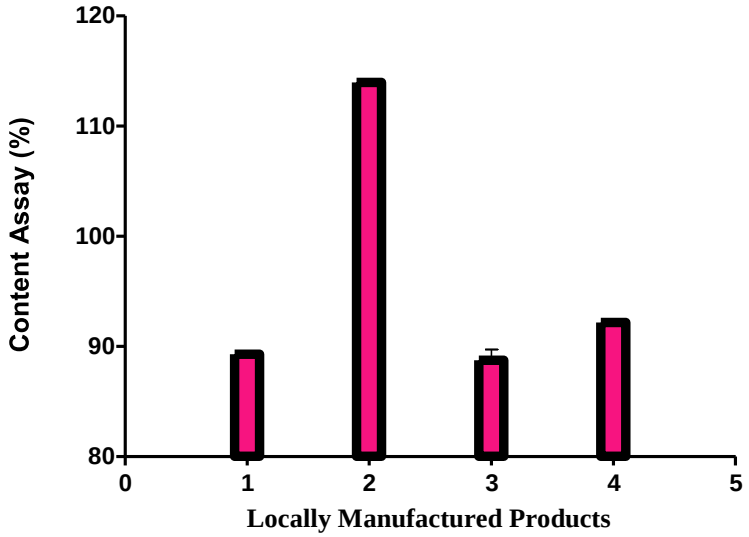

Figure 2.3 Content Assays of locally manufactured products

There were variations in the content assay results, it ranged from 88.8% to 114.0% (figure 2.3) and they were all outside the BP limits of 95 to 105%.

2.7 Conclusion

The results on the analysis of the locally manufactured ibuprofen suspension showed that all the four suspensions failed the content assay test and this might be an indication that local manufacturers might be having problems with the formulation of ibuprofen suspension.

CHAPTER 3 INVESTIGATIONS INTO THE PRODUCT FOR POSSIBLE INTERVENTIONS

3.1 Introduction

Pharmaceutical formulation is the process of converting an API into medicines in a suitable form for administration through a particular route. The process often involve the addition of excipients such as suspending agents, sweeteners, preservatives, antioxidants, binders and lubricants. Among the objectives of pharmaceutical formulation is to produce medicines that are efficacious, acceptable, convenient to use and stable.

Stability of a pharmaceutical product may be defined as the ability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications (Kommanaboyina and Rhodes, 1999). The stability of any pharmaceutical product is for a definite period and this makes stability studies critical in the product development process and also important in the marketing authorization. Stability studies are thus performed at all phases of the product development. The aim of stability testing is to provide evidence of how the quality of an API or FPP varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. The stability programme also includes the study of product-related factors that influence its quality, for example, API-excipients interaction, container closure systems and packaging materials. The product stability involves various changes that may occur in the product during processing and storage and the impact of those changes on its safety (Shaikh and Sial, 1996). The deterioration may be realised through changes in colour, odour, viscosity, precipitation and caking.

In order to determine the possible cause(s) of the problem the suspension was reexamined from the raw materials stage through to the processing, finished product and subsequent handling and storage. The investigations also focused on factors capable of affecting the stability of pharmaceutical suspensions such as composition of the suspension, preparation procedure, packaging materials, API-excipients interaction, microbial contamination and storage conditions like temperature and
light.

3.2 Materials and Equipments

The materials for the sample ibuprofen suspension preparation included ibuprofen from four different manufacturers namely IOL Chemical and Pharmaceutical Ltd,

India, Alka Laboratories PVT Ltd, India, Allied Chemicals and Pharmaceutical Ltd, India and Satwik Drugs Ltd, India. The other materials are **p**ropylhydroxybenzoate from Gujarat Organics Ltd, India, methylhydroxybenzoate from Sharon Labs Ltd, Israel, xanthan gum from Shandong Fufeng Fermentation Co. Ltd, China**, s**accharin sodium from Suzhou Fine Chemicals Co. Ltd, China, silicon dioxide (Aerosil 200) from Evonik Degussa AG, Germany, sucrose from Sugar Australia Pty Ltd, Australia, propylene glycol from Ineos NV, Belgium, simethicone emulsion 30% from Palmo Industrial Silicones Pvt. Ltd, India, amaranth from Irish Country Cold Group, UK, **p**olysorbate 80 from Vasudha Chemicals Pvt Ltd, India and pineapple flavour from Irish Country Gold Group, UK, HPLC grade methanol from Merck Life Science Pvt Ltd (India), orthophosphoric acid from Merck Life Science Pvt Ltd (India), acetate buffer from Daejung Chemicals & Metals Co. Ltd (Korea), phthalate buffer from Daejung Chemicals & Metals Co. Ltd (Korea) and phosphate buffer – Daejung Chemicals & Metals Co. Ltd (Korea), nutrient agar, potato dextrose agar, MacConkey agar, bismuth sulphite agar, petri dish and pipettes. The rest are mixing stirrer from Filquip Engineering, South Africa, magnetic stirrer (Stuart UC152) from Stuart, UK and weighing scale from Metler Toledo, Switzerland.

The equipments used were analytical weighing scale (ME 204) from Metler Toledo, viscometer (Haake Viscotester 6L) from Haake, UK, pH meter(HI 2215 pH ORP Meter) from Hanna Instruments, HPLC (1200 Series) from Agilent Technologies HPLC and pycnometer from Merck, Germany.

3.3 Methods

3.3.1 Composition of ECL ibuprofen Suspension

The composition of the ECL ibuprofen suspension (section 2.3.1) was reviewed by assigning the various excipients to their functional groups namely; preservatives,

sweetening agents, suspending agents, thickening agents, solvents and co-solvents, colouring agents and flavours. Secondly the appropriateness of the various excipients and the quantities employed in the formulation were assessed.

3.3.2 Preparation Procedure

The preparation procedure as used by ECL (section 2.3.2) was reviewed by analysing the various steps involved in the mixing operation and also the order of addition of the various materials.

3.3.3 Packaging Materials

The various materials used in packing the product (section 2.3.3) were assessed to find their appropriateness for the product. The materials were grouped into primary, secondary and tertiary packaging materials.

3.3.4 API - Excipient Interaction

The quantity of excipient required for the preparation of 500ml of the ibuprofen suspension was each weighed individually and mixed with 10g of ibuprofen powder. Separately each API-excipient blend was poured into an amber coloured glass bottle, capped and kept for 3 months in a real time stability room. The mixtures were retrieved from the point of storage monthly and visually inspected for any visible changes during the period.

3.3.5 Level of microbial Contamination

The pour plate method was used. Four media (nutrient agar, potato dextrose agar, MacConkey agar and bismuth sulphite agar) were used. The media were boiled to melt. The media were then stabilised at 45^0C in a water bath. Each medium was inoculated with 1 ml of ibuprofen suspension. The media – suspension mixture were poured into petri dishes and allowed to cool. The plates were incubated in an inverted position at

37^0C for 24 hours. However the plate containing the potato dextrose agar was incubated at 25^0C for 72 hours.

3.3.6 Effect of the API quality on the formulation of ibuprofen Suspension

The suspension preparation followed the same procedure used by ECL (section 2.3.2). The different suspensions were bottled in amber coloured glass bottles, labelled as suspension A, B and C. A fourth suspension was prepared using API similar to what was used by ECL (from the same manufacturer) in the preparation of their suspension. This was labelled as suspension D and used as the control. All the suspensions were kept under real time stability conditions (temperature 30 ± 2^0C and humidity 75 ± 5%). The parameters of the suspensions (description, redispersibility, viscosity, pH and assay) were assessed based on the ECL quality control test procedures (section 2.4) after 3, 6, 9, 12, 18 and 24 months of storage.

3.3.7 Effect of Storage Temperature on the Stability of ibuprofen Suspension

The materials needed for the preparation of 5 litres of ibuprofen suspension were weighed. The mixing procedure as used by ECL (section 2.3.2) was adopted for the suspension preparation. Some of the prepared suspension was filled into 48 amber coloured glass bottles. The products were divided into 4 groups of twelve bottles each. Each group was stored under different temperature conditions namely;

- 4 – 8^0C (stored in refrigerator)
- 20 – 25^0C (stored under air condition)
- 26 – 28^0C (stored under room conditions)
- 28 – 32^0C (stored in a real time stability room)

The parameters of the suspension (description, redispersibility, viscosity, pH and assay) were determined using the ECL quality control test (section 2.4) at the following time periods:

- before storage (initial)

- at the end of 3, 6, 9, 12, 18 and 24months

3.3.8 Effect of Light on the Stability of ibuprofen Suspension

A sample of the prepared suspension (section 3.3.6) were filled into two sets of glass bottles:

- First set comprised of 12 amber coloured glass bottles, same as that used by ECL (light resistant bottles).
- Second set consisted of 12 plain glass bottles.

The products were stored in the real time stability room (temperature 30 ± 2^0C and humidity 75 ± 5%). The suspensions were retrieved from their point of storage after 3, 6, 9, 12, 18 and 24 months and analysed for the following parameters: description, redispersibility, viscosity, pH and assay using the ECL quality control methods (section 2.4).

3.3.9 Effect of Suspension pH on the Stability of ibuprofen Suspension

Four (4) litres of ibuprofen suspension was prepared using the ECL preparation method (section 2.3.2). Some of the suspension was filled into 12 amber coloured glass bottles and the rest divided into 4 equal parts. Citric acid and sodium citrate were used to adjust the pHs of the various parts to:

- 3.0 – 4.0 (citric acid used)
- 5.0 to 6.0 (sodium citrate used)
- 6.0 to 7.0 (sodium citrate used)
- 7.0 to 8.0 (sodium citrate used)

Each part was bottled into 12 amber coloured glass bottles. The bottled products were stored in the real time stability room (temperature 30 ± 2^0C and humidity 75 ± 5%). The parameters (description, redispersibility, viscosity, pH and assay) of the various products were analysed after 3, 6, 9, 12, 18 and 24 months of storage using the ECL quality control methods (section 2.4).

3.4 Statistical Analysis of Results

The results were presented as Mean ± SD (standard deviation). GraphPad Prism version 5.01 for Windows (GraphPad Software Inc., San Diego - California) was used for the statistical analysis. Two-way analysis of variance (ANOVA) with Bonferroni posttests was performed to determine the differences between the test samples and the control. Possible correlations between the values were ascertained by Pearson's correlation tests. Tests with values of $P < 0.05$ were considered significant.

3.5 Results and Discussion

3.5.1 Composition of the ECL Ibuprofen Suspension

Table 3.1 Composition of 1 litre ECL Ibuprofen Suspension

Material	Quantity (g)	Functional Category
Sucrose	300.0	Sweetening agent
Saccharin Sodium	0.03	Sweetening agent
Ethanol 96%	0.01	Co-solvent
Propylparaben	0.16	Preservative
Methylparaben	1.84	Preservative
Xanthan Gum	1.2	Thickening agent
Colloidal silicon dioxide	8.0	Suspending agent
Propylene Glycol	110.0	Co-solvent
Dimethylpolysiloxane emulsion (30%)	1.0	Antifoaming agent
Polysorbate 80	2.0	Wetting agent
Ibuprofen	20.0	API
Amaranth	0.006	Colouring agent
Pineapple Flavour	2.0	Flavouring agent

The suspension was composed of the excipients found in most suspensions. In the case of the sweeteners, sugar was combined with saccharin sodium to enhance the sweetening ability leading to a lesser amount of the individual sweeteners being used. However since it was a paediatric formulation an alternative sweetener like sorbitol or

glycerine could have been used in place of the sugar because of the effect of sugar on dental caries in children (Edgar, 1998). Secondly liquid sorbitol or glycerine would enhance the viscosity of the suspension. The ethanol 96% served as a solvent for the dissolution of the parabens. However the parabens are soluble in the propylene glycol which could be used for that purpose. Furthermore since it was a paediatric formulation it was important to avoid the use of alcohol because of safety concerns with alcohol use in paediatric formulations (Svirskis *et al.,* 2013). The combination of the preservatives methylparaben and propylparaben leads to synergistic effect between the two resulting in a lesser quantity of each preservative being used. The cosolvent propylene glycol enhances the preservative action of the parabens (De Spiegeleer *et al*., 2006). Noticeably there was no buffering agent in the formulation to control the pH. Also there was no antioxidant in the formulation to prevent oxidation of the API since ibuprofen was sensitive to oxidation (Caviglioli *et al.,* 2002).

3.5.2 Preparation Procedure

In pharmaceutical production, processes such as granulation, drying, compaction, comminution and coating can result in partial or complete phase conversion, leading to poor product quality (Zhou, 2009). The preparation method used by ECL for the suspension preparation (section 2.3.2) consists mainly of two main processes:

- Normal mixing of the materials
- Colloid milling of the API

The normal mixing of materials employed in oral liquid preparation may not have any quality effect on the API. However the colloid milling of the API has the potential of affecting the integrity of the API (Zhou, 2009; Qiu *et al*., 2009). This process can be avoided with the use of micronised grade of the API.

3.5.3 Active Pharmaceutical Ingredient - Excipients Interaction

The API - excipient interaction is one of the major causes of product instability. Excipients are supposed to be pharmacologically inert but they can cause or participate in chemical or physical interactions with drug substances, which could affect the efficacy or safety of the pharmaceutical product. Although a lot of effort is put in the purification of excipients they may contain residues of the starting material, solvents and reagents. The multi-component nature of the excipients may lead to interaction between the excipients and the APIs (Fathima *et al.*, 2011) or excipient to excipient interactions. However the mixing of the API and the individual excipients did not result in any observable interaction indicating the possible compatibility between the API and the excipients.

3.5.4 Packaging materials

Table 3.2 Packaging materials used for ECL ibuprofen suspension

	Material	**Specification**
Primary	Container Cap	amber coloured glass bottle 28 mm ROPP Aluminium Cap with expanded polyethylene liner
Secondary	Label Measuring cap Jacket	Self-adhesive label paper 28mm 10ml transparent cap 320 gsm laminated aqua vanish paper board
Tertiary	Carton (shipper)	3 ply corrugated fibreboard

The packaging material forms part of the pharmaceutical product. The packaging material protects the product from environmental hazards such as dust, gases, moisture and microorganisms. Packaging materials (table 3.2) can be categorised into primary

(is in direct contact with the product), secondary (is the package which surrounds the primary package) and tertiary package which is used for transportation purposes (Nasa, 2014). The product container is amber coloured glass bottle. Glass is nonporous and impermeable it does not degrade and is chemically inert (Shivsharan *et al*., 2014). Amber glass is capable of protecting its contents from the harmful effects of sunlight by sifting out ultraviolet rays (Kumar, 2013). The protective ability of the container can be achieved only if there is a good seal between the container and closure. The closure is usually the most vulnerable and vital part of a container as far as stability and compatibility with the finished product are concerned (Keerthi *et al*., 2014). The closure used for the ibuprofen suspension is 28 mm ROPP aluminium screw cap. Aluminium and its alloys are resistant to corrosion (Oldring and Nehring,

2007) making aluminium ROPP caps ideal for the closure of ibuprofen suspension. However sucrose may attack aluminium closures (Rowe *et al*., 2009). If the aluminium cap is to be maintained as the closure then the sucrose in the formulation will have to be replaced with an alternative sweetener. The secondary packaging consists of label made from self-adhesive label paper and the outer jacket made from 320 gsm laminated aqua vanish paperboard. Paperboard is lightweight and strong making it ideal as a support for the primary packaging materials. The tertiary packaging material consists of shipper made from 3 ply corrugated fibreboard**.** Corrugated fibreboard has a higher resistance to bending than flat fibreboard, making it suitable to withstand the stress involve in handling, transportation and storage.

3.5.5 Microbial Analysis

Microbial contamination can result in product failures hence microbial test on the suspension was performed in order to determine if the instability was as a result of microbial contamination. The ibuprofen suspension is a non-sterile product and was not produced by aseptic processes and hence not expected to be totally free from microbial

contamination. The extent of contamination in non-sterile pharmaceuticals is regulated through acceptance criteria for microbiological quality established in official compendia.

Table 3.3 Level of microbial contamination test

Tests	Media	Results cfu/mL	Specifications
Total aerobic count	Nutrient agar	<10	Not more than 10^2cfu/mL
Fungi	Potato dextrose agar	1	Not more than 10^1cfu/mL
E. coli	MacConkey agar	Nil	Must be absent
Salmonella typhi	Bismuth sulphite agar	Nil	Must be absent

The microbial contamination test results (table 3.3) showed that the aerobic bacteria and fungi count were within the acceptable limits while E. coli and Salmonella typhi were also absent in the suspension. Microbial contamination of multi-dose pharmaceutical products by especially opportunistic organisms is prevented by the inclusion of preservatives (Elder and Crowley, 2012b). Ibuprofen suspension with acceptable microbial levels may be an indication of the efficacy of the added preservative.

3.5.6 Effect of the API quality on the formulation of ibuprofen suspension

The stability of finished pharmaceutical product is dependent on a number of factors. Among these factors are the API related ones like the chemical structure, impurity profile, the physical form and particle size of the API. The problem with the ECL ibuprofen suspension could have arisen as a result of quality issues with the API used for the formulation. In order to test this hypothesis, three samples of the ibuprofen suspension were prepared using ibuprofen (API) from different manufacturers. The results showed that all the suspensions appeared as viscous and pink coloured and readily redispersed and they maintained their appearance and redispersibility throughout the storage period. The appearance and redispersibility of the control

(suspension D) did not also change over the storage period. The similar behaviour of the four suspensions might probably be as a result of similarities in the quality of the various APIs.

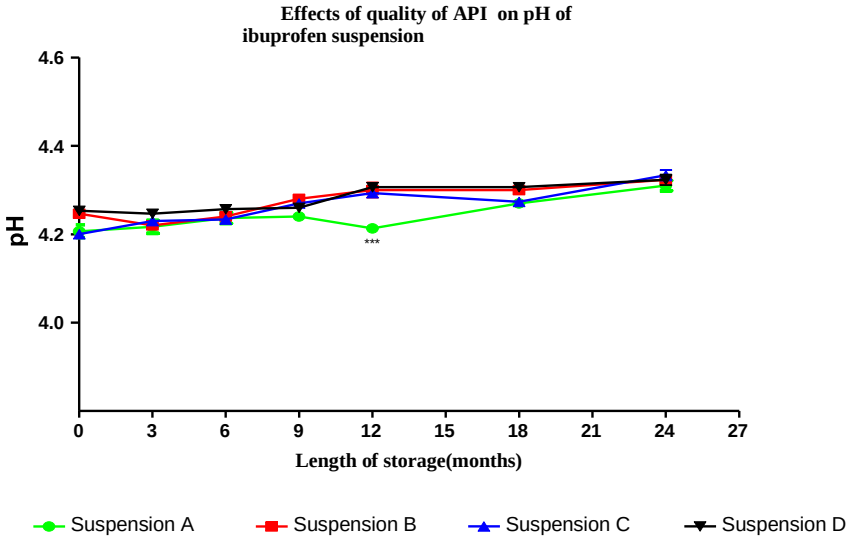

Figure 3.1 Relationship between Mean pH of ibuprofen suspension with different API and Storage Time

The initial pH of the suspensions ranged from 4.20 to 4.25 and at the end of the storage period the pH ranged from 4.31 to 4.32 (figure 3.1). The statistical analysis showed the variation in pH between the various suspensions and the control suspension was insignificant ($P > 0.05$). This may be happening because of the probable similarities between the different APIs. All the suspensions lack buffering agent and this makes them prone to variations in pH during the storage period.

Effects of quality of API

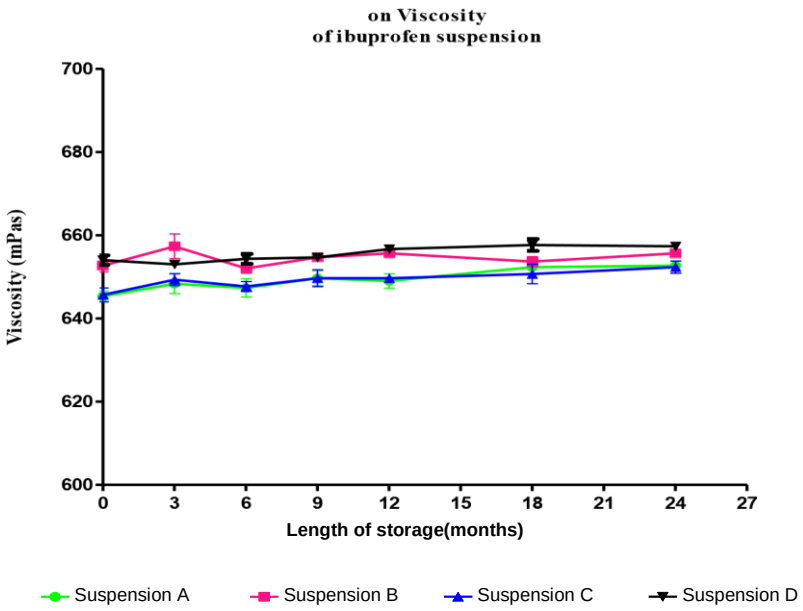

Figure 3.2 Relationship between Mean Viscosity of ibuprofen suspension with different API and Storage Time

The initial viscosities of the various suspensions ranged from 645 to 654 mPas. At the end of the storage period the suspensions recorded increases in their viscosities, ending at 652 to 657 mPas (figure 3.2). Statistical analysis showed that the differences between the viscosities of the various suspensions and that of the control were not significant ($P > 0.05$). All the suspensions contain the same and equal quantity of viscosity enhancing agent (xanthan gum). Xanthan gum is a very stable thickening agent and suspensions containing it are not expected to show significant variation in their viscosities. Secondly it could also be an indication of the similarities between the various APIs used in the preparation of the suspensions.

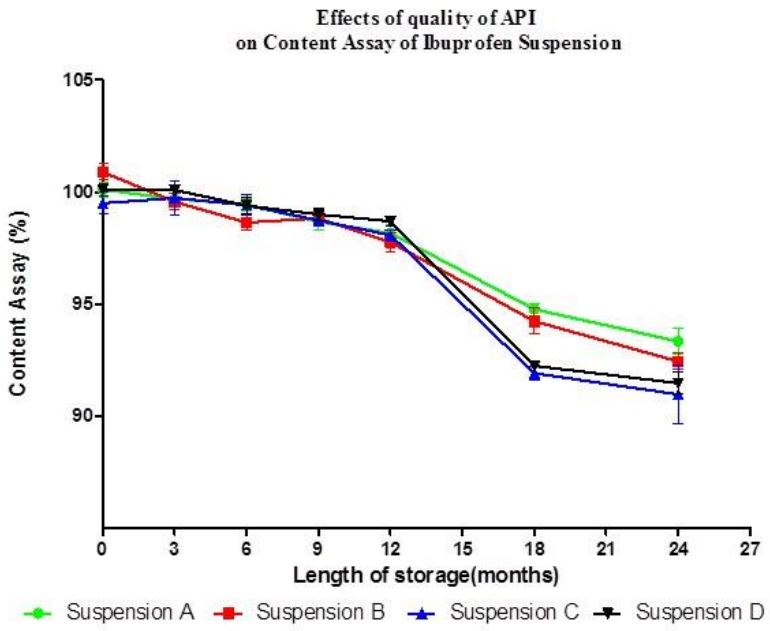

Figure 3.3 Relationship between mean assay of ibuprofen suspension with different API and storage Time

The results shows the initial content assays of the four suspensions ranged from 99.5 to 100.9%. At the end of the storage period the content assays of all the four suspensions ranged from 90.97 to 93.33% (figure 3.3) and were all below the BP lower limit of 95%. The suspensions all seem to exhibit the same behaviour of having their assays dropping below the 95% mark after 12 months. Statistically no significant differences ($P > 0.05$) were observed in the values of the content assays recorded for the four suspensions. This gives a probable indication of the similarities in the quality of the various materials used in the preparation of the suspensions.

3.5.7 Effect of Storage Temperature on the stability of ibuprofen suspension

Environmental conditions such as storage temperature can affect the stability of a formulation. The objective of this test was to find the possible effect(s) if any of storage temperature on the stability of the ibuprofen suspension. This was achieved by storing samples of the suspension at different temperature ranges and analysing specific parameters of the products at specified time intervals.

Initially all the suspensions appeared as pink viscous and readily redispersed suspensions. Over the 24 months period it was observed that the products stored at the various temperature ranges did not show any visible changes in their appearance and redispersibility. The results give probable indication that storage temperature may not have an effect on both the appearance and redispersibility of the ibuprofen suspension.

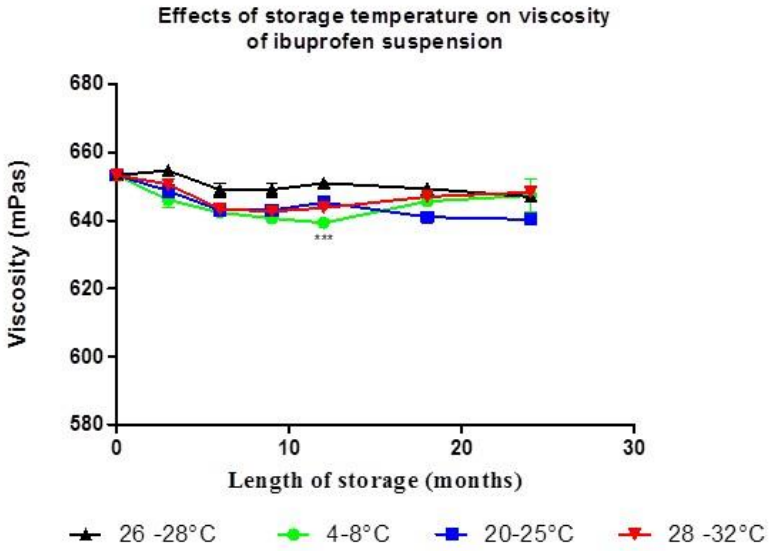

Figure 3.4 Relationship between Mean Viscosity of Suspension stored at different Storage Temperatures

The viscosities of the suspensions stored at the various temperature ranges were determined at specified time intervals. They all had an initial viscosity of 653 mPas but the viscosities reduced to 640 to 648 mPas (figure 3.4) after the storage period. Statistical analysis of the results showed that after 24 months of storage there were no significant differences ($P > 0.05$) between the viscosities of the suspensions stored at the various temperature ranges indicating the viscosities were probably not affected by the storage temperatures. Xanthan gum is the principal suspending agent in the formulation. Xanthan gum is a high molecular weight molecule with a rigid helical conformation. It forms complex molecular aggregates through hydrogen bonds and polymer entanglement. This rigid and ordered conformation makes xanthan gum relatively more stable as a thickener compared to other polysaccharides. Also xanthan

gum has the ability to maintain its viscosity until a definite melting temperature (Tm) is reached after which the viscosity decreases sharply due to a reversible molecular conformational change (Desplanques *et al*., 2012).

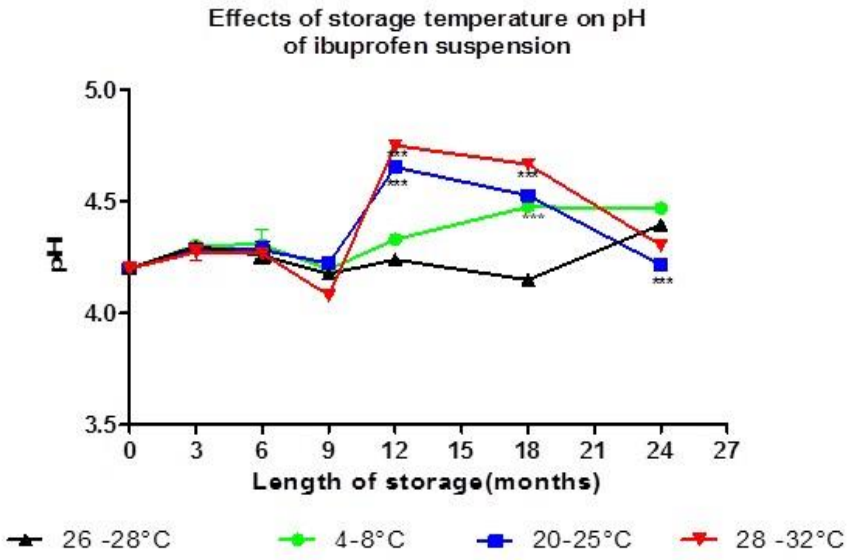

Figure 3.5 Relationship between Mean pH of Suspension stored at different Storage Temperatures

The suspensions had an initial pH of 4.20 but at the end of storage period the pHs were from 4.22 to 4.47 (figure 3.5). The pH readings recorded for the suspension stored at the various temperature ranges showed significant variations ($P < 0.001$) in pH over the 24 months period. The suspensions had no buffering agent and this made them vulnerable to variations in pH. This might explain the variations in the recorded pH of the suspensions.

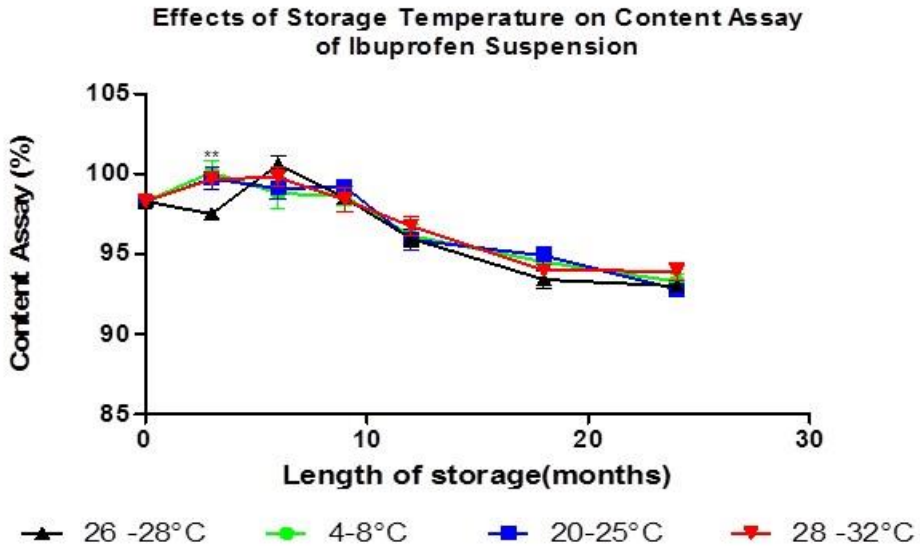

Figure 3.6 Relationship between Mean Assay of Suspension stored at different Storage Temperatures

The initial content of ibuprofen in the suspension was 98.30% however the results indicate a gradual reduction in the content of ibuprofen in the suspensions stored at all the temperature ranges after 6months (figure 3.6). After 24 months of storage each suspension had reduced level of ibuprofen, ranging from 92.80 to 93.90% giving a probable indication that the formulation cannot stay for 24 months. Statistically there were no significant ($P > 0.05$) differences in the assays of the various suspensions stored at the various temperature ranges after 24months of storage. This seems to suggest that storage temperature may not have an effect on the API content of the suspension.

3.5.8 Effect of light on the stability of ibuprofen suspension

Light is another environmental factor which can affect the stability of the suspension, The results show that for both suspensions stored in the plain glass and amber coloured glass there was no change in both the appearance and ease of redispersibility of the suspensions after 24 months of storage.

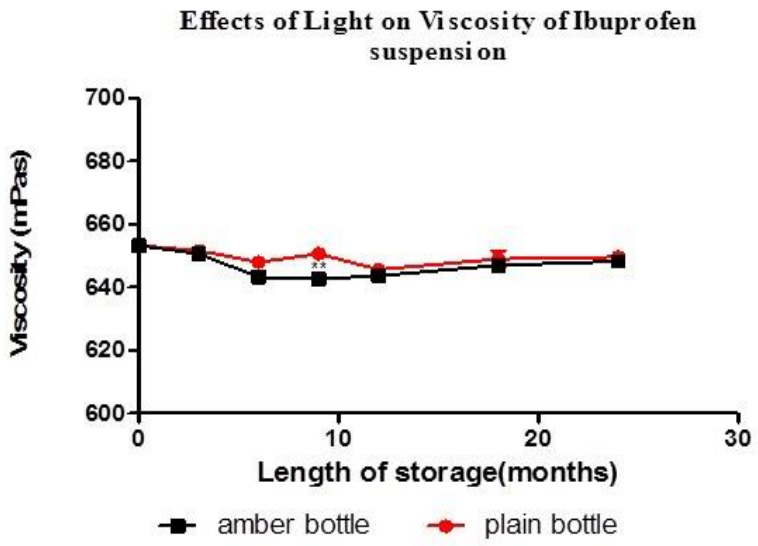

Figure 3.7 Relationship between Mean Viscosity of suspension stored under light and Storage Time

The initial viscosities of both suspensions were 653mPas and at the end of the storage period the viscosity of the suspension in the plain bottle was 650mPas while that of the suspension in the amber bottle was 648mPas (figure 3.7). The results showed no significant differences ($P > 0.05$) between the values recorded for both the suspensions in the plain bottles and the ones in the amber bottles. This might mean the appearance, ease of redispersibility and the viscosity may all not be affected by light. Viscosity of the suspension may not be affected by light because xanthan gum the main thickening agent in the suspension has good stability and viscosity properties.

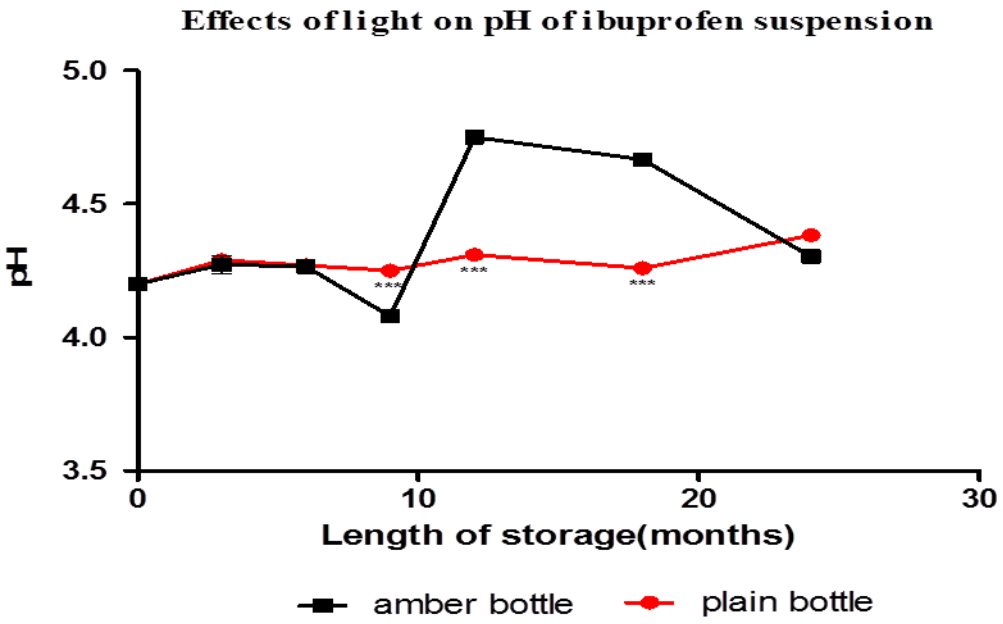

Figure 3.8 Relationship between Mean pH of suspension stored under light and Storage Time

The pH of the suspension increased from an initial value of 4.20 to 4.38 and 4.30 for the suspensions in the plain and amber bottles respectively (figure 3.8). There were significant differences ($P < 0.001$) in pH between the values recorded for the suspensions stored in the plain and amber bottles after 6 months of storage. The absence of a pH stabilising agent in the formulation may account for the wide variation in pH of the two samples. Also it might be an indication of the effect of light on the pH of the suspension.

On the content assay, both suspensions had their initial assays dropping from 98.30% to 92.51 and 93.90% respectively for the suspensions in the plain and amber bottles (figure 3.9).

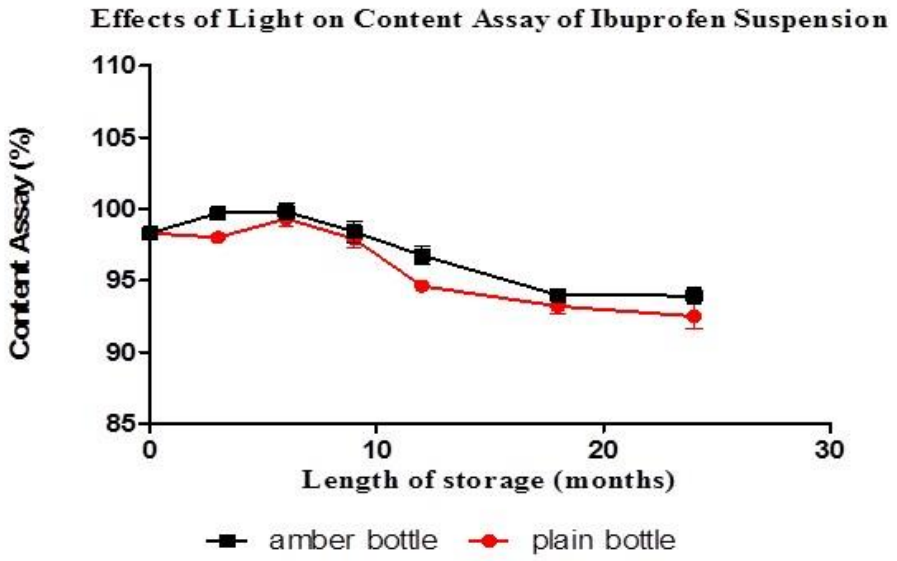

Figure 3.9 Relationship between Mean Assay of suspension stored under light and Storage Time

Statistically the differences in content assays at the end of the storage period were not significant ($P > 0.05$). This might be an indication that the content of ibuprofen in the suspension was unaffected by light.

3.5.9 Effect of Suspension pH on the stability of ibuprofen suspension

Hydrolysis is one of the important pathways of drug degradation (Charde *et al.*, 2013). Hydrolysis may be dependent on the pH of the medium. Secondly the pH of a medium affects the growth of microorganisms in the medium. The suspension pH has effect on the efficacy of preservatives used in preserving the suspension (Elder and Crowley, 2012b). Hence suspension pH is one of the important factors to be considered in product stability. In order to test the effect of suspension pH on the stability of the product, samples of the suspension with varying pHs were analysed over a time period.

The suspension appeared as a viscous pink suspension which was readily redispersed. At the end of 24 months of storage the appearance and ease of redispersibility of all the suspensions remained unchanged implying that the suspension pH probably does not have an effect on the appearance and dispersibility.

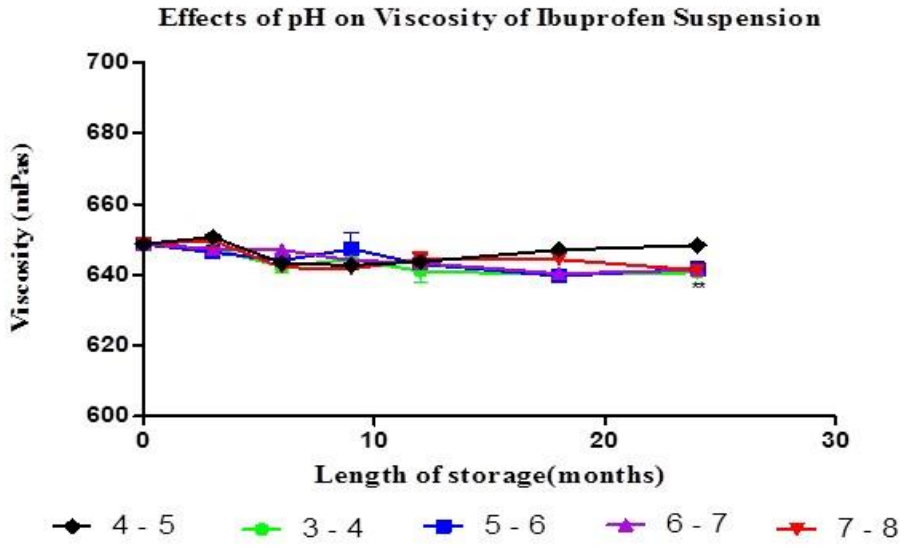

Figure 3.10 Relationship between Mean Viscosity of the suspension at different pH ranges and Storage Time

The suspensions had an initial viscosity of 649mPas and this reduced to 641 to 642mPas after 24 months (figure 3.10). Xanthan gum which is the main viscosity enhancing agent in the suspension is extremely stable over a wide pH range, 211(Mudoi *et al.*, 2013). In line with this, statistically the results also showed no significant differences ($P > 0.05$) between the viscosities recorded for the various suspensions.

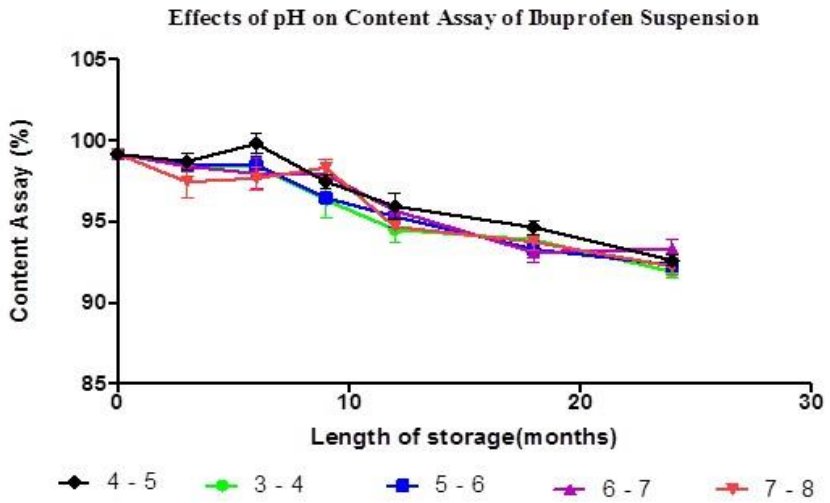

Figure 3.11 Relationship between Mean Content Assay of the suspension at different pH ranges and Storage Time

The initial content assay of the suspensions was 99.15% and there was a gradually reduction in the assays after 6 months of storage. The results showed that the content of ibuprofen in the suspensions after 24 months storage were 91.90 to 93.30% (figure 3.11) which was below the BP acceptable lower limit of 95.00%. However the results showed that the content assays for the various suspensions after 24 months of storage were not significant ($P > 0.05$). The results do suggest the pH of the suspension might not affect the stability of the formulation.

3.6 Conclusion

The results of the investigations point to variation in suspension pH and oxidation as the probable causes of the instability.

3.7 Proposed Interventions

Based on the results of the investigations the following changes were proposed to improve on the initial formulation made by ECL:

- Changing the sweetener (sucrose)
- Taking out the co-solvent ethanol 96%
- Adding a buffering agent
- Adding an antioxidant
- Changing the colouring agent

CHAPTER 4

PROPOSED FORMULA, OPTIMISATION AND STANDARDISATION

4.1 Introduction

Over the years pharmaceutical dosage forms were formulated based on the knowledge gained from the personal experience of formulation scientists. The processes of pharmaceutical product development are mostly dependent on intuitive and subjective reasoning rather than rational operations. Trial and error processes are inefficient, time consuming and costly.

Optimisation of a formulation is generally the process of making the product as perfect, effective and functional as possible under a given set of conditions. All the physical, chemical and biological properties must be factored in selecting the materials and manufacturing procedure. It could be achieved by a series of logical steps whiles carefully controlling the variables and changing one at a time until a satisfactory system is achieved. Optimisation is a useful tool to quantitate a formulation that has been qualitatively determined (Schwartz and Sharp, 1981). Standard formulation methods often involve running a grid search about a formulation or process. The initial point is either an educated guess or deduced from prior art however in this particular work the starting point was the proposed recipe.

The prime objective of standardization is that the chemical substances or formulated products must be clinically satisfactory. This means it should be safe, effective and stable and it should not have any unspecified variation in its amount and it is free from any toxicity or harmful effect.

The pharmaceutical manufacturing division of ECL developed and produced its brand of ibuprofen suspension. However the product failed real time stability studies after 12 months (section 2.7). In order to improve upon the original formulation a series of studies were performed on the initial suspension (chapter 3). Based on the results certain

interventions were proposed to the original formulation to generate a new recipe. This new recipe had to be optimised, standardised and lastly taken through accelerated stability studies as a means of checking on its stability profile.

4.1.1 Proposed Interventions

In order to improve on the original formulation by ECL the proposed interventions (section 3.6) were to be effected to generate a new formula. Among the interventions proposed are:

- **Changing the sweetener sucrose** - Sucrose has an effect on dental caries in children (Edgar, 1998). This makes it inappropriate in the current formulation because the product is for paediatrics. Furthermore sucrose may attack the aluminium ROPP closure for the product (section 1.12.4). This reemphasises the need for an alternative sweetener. Liquid sorbitol 70% and glycerine are possible replacements for sucrose. However sorbitol was chosen because it is relatively cheaper than glycerine.
- **Taking out the co-solvent ethanol 96%** – There were two co-solvents (ethanol 96% and propylene glycol) in the formulation. The role of ethanol 96% was the dissolution of the parabens. However there are safety concerns with the use of alcohol in paediatric formulations (Svirskis *et al*., 2013). Since the parabens are soluble in propylene glycol which could be used for that purpose, the ethanol could be taken out of the formulation.
- **Adding a buffering agent** – The results of the various investigative tests conducted on the original suspension by ECL showed fluctuations in the pH of the suspension. In order to minimise the fluctuations a buffering agent had to be included in the formulation. The proposed buffering agent for the formulation was the citrate buffer, citric acid – sodium citrate combination because the buffer pH is within the pH range of the ibuprofen suspension.

- **Adding an antioxidant** - Antioxidants are included in pharmaceutical formulations to minimise or retard oxidative processes that occur with some API or excipients when exposed to oxygen or in the presence of free radicals. Ibuprofen is sensitive to oxidation (Caviglioli et al., 2002) and the problem could have been as a result of oxidation. Antioxidants used for aqueous products include: ascorbic acid, sodium metabisulphite and sodium sulphite. Although very effective, the use of sulphites as antioxidants is controlled by restrictions set by World Health Organisation (WHO) due to health-related issues incurred by sulphite-sensitive individuals (Li et al., 2008). For this reason ascorbic acid was chosen as the antioxidant for the proposed formulation.
- **Changing the colouring agent** – The colouring agent amaranth is incompatible with the antioxidants sodium metabisulphite, sodium sulphite and ascorbic acid. If any of these antioxidants was added to the formulation then a suitable colouring agent was needed to replace the amaranth. Since it had been proposed to include ascorbic acid in the formulation then the colouring agent amaranth had to be replaced with one which was compatible with the antioxidant. Tartrazine was selected because it is compatible with ascorbic acid.

4.2 Materials and Equipments

The materials used for the sample ibuprofen suspension preparation consisted of ibuprofen from IOL Chemical Amb Pharmaceutical Ltd, India, propylhydroxybenzoate from Gujarat Organics Ltd, India, methylhydroxybenzoate from Sharon Labs Ltd, Israel, xanthan gum from Shandong Fufeng Fermentation Co. Ltd, China, saccharin sodium from Suzhou Fine Chemicals Co. Ltd, China), ascorbic acid from Hebei Welcome Pharmaceutical Co. Ltd, China, silicon dioxide (Aerosil 200) from Evonik Degussa AG, Germany, propylene glycol from Ineos NV, Belgium, simethicone

emulsion 30% from Palmo Industrial Silicones Pvt. Ltd, India, polysorbate 80 from Vasudha Chemicals Pvt Ltd, India, liquid sorbitol 70% from

Maize products, India, tartrazine and pineapple flavour from Irish Country Cold Group, UK.

The equipment for preparing and analysing the suspension consisted **of** magnetic stirrer (Stuart UC152) from Stuart, UK, weighing scale from Metler Toledo, Switzerland, viscometer (Haake Viscotester 6L) from Haake, UK, pH meter(HI 2215 pH ORP Meter) from Hanna Instruments, HPLC (1200 Series) from Agilent Technologies and stability chamber from Pharma Safe Sanyo Gallenkamp PLC.

4.3 Methods

4.3.1 Determining the quantities of the new excipients

Sorbitol liquid 70%

Quantity of sucrose in the original formulation was 300 g. Sorbitol has about 50 - 60% the sweetness of sucrose (table 1.2). To maintain the same level of sweetness the quantity of sorbitol needed was 500 - 600 g.

Concentration of the sorbitol liquid was 70%

500 – 600g sorbitol = 714 – 857g sorbitol liquid 70%

Density of sorbitol liquid 70% = 1.29

714 – 857g sorbitol liquid 70% = 553 – 664 ml sorbitol liquid 70%

The volume of the suspension was 1000 ml. The total volume of liquid excipients in the formulation was important as it determines the possible volume of purified water that could be used for the formulation. The co-solvent propylene glycol (110 g) was about 106 ml, this makes the volume of the sweetener important because if too much sweetener was added, there will be very little amount of water available to dissolve the other excipients. In view of the foregoing the minimum amount of sorbitol liquid 70% (553 ml) was proposed for the formulation.

Ascorbic Acid

Ascorbic acid was used as an antioxidant in aqueous pharmaceutical preparations at concentrations of 0.01–0.1% w/v (Rowe *et al.*, 2012).

Buffering agent – Citric acid – Sodium citrate

The proposed buffering agent for the formulation was citrate buffer, citric acid – sodium citrate combination.

Using the Henderson – Hasselbach equation :

$$pH = pK_a + \log \frac{[salt]}{[acid]}$$

pK_a of citric acid = 4.76 pH

of suspension = 3.5 – 5.0

Optimal buffering capacity was achieved when pH = pK_a and [salt] = [acid]

This implies optimal buffering capacity will be achieved at pH 4.76 which also falls within the pH limits of 3.5 to 5.0.

For optimal buffering activity: citric acid concentration

= sodium citrate concentration

Molar mass of citric acid = 192.12

Molar mass of sodium citrate = 258.07

Ratio of Citric acid to sodium citrate = 192.12: 258.07 (1: 1.3433)
Citric acid was used as a buffering agent in the concentration of 0.1 - 2.0% w/v (1.0 - 20 g) per 1000 ml (Rowe *et al.*, 2012).

Table 4.1 Proposed Formula for 1litre Ibuprofen Suspension

MATERIALS	QUANTITIES (g)
Sorbitol liquid 70%	500.00
Saccharin Sodium	0.03
Propylparaben	0.16
Methylparaben	1.84
Xanthan Gum	1.20

Colloidal Silicon dioxide	8.00
Propylene Glycol	110.00
Dimethylpolysiloxane emulsion 30%	1.00
Polysorbate 80	2.00
Ascorbic acid	0.10 - 1.00
Ibuprofen	20.00
Citric acid	1.00 – 20.00
Sodium citrate	q.s
Tartrazine (E102)	0.006
Pineapple Flavour	2.00

Experimental design

The experimental design was based on the traditional approach of changing one variable at a time (OVAT) whiles keeping the other factors constant. There are two independent variables citric acid and ascorbic acid. For each variable 3 levels were considered (low, mid and high levels).

The suggested quantity of citric acid was 1.00 – 20.00g. The 1.00g was considered as the low level, 10.50g as the mid-level and 20.00 g as the high level.

The suggested quantity of ascorbic acid was 0.10 – 1.00g. The 0.10g was considered as the low level, 0.55g as mid-level and the 1.00g as the high level.

Table 4.2 Level of formulation variables

Variables	**Levels**		
	Low	**Middle**	**High**
Citric acid	1.00	10.50	20.00
Ascorbic acid	0.10	0.55	1.00

Based on the different combination of the variables 9 different formulations of ibuprofen suspension were prepared.

Table 4.3 Different Formulations of Ibuprofen suspension

	VARIABLES (g)

DIFFERENT FORMULATIONS	Ascorbic acid	Citric acid	Sodium citrate*
Formulation 1	0.10	1.00	1.34
Formulation 2	0.10	10.50	14.07
Formulation 3	0.10	20.00	26.80
Formulation 4	0.55	1.00	1.34
Formulation 5	0.55	10.50	14.07
Formulation 6	0.55	20.00	26.80
Formulation 7	1.00	1.00	1.34
Formulation 8	1.00	10.50	14.07
Formulation 9	1.00	20.00	26.80

* Based on the ratio 1: 1.34 (section 4.3.1)

4.3.2 Optimising the formulation

4.3.2.1 Preparation of Ibuprofen Suspensions for Optimisation

Nine formulations of ibuprofen suspensions were prepared using the different amounts of ascorbic acid, citric acid and sodium citrate (table 4.3). About 150 ml of purified water was added to the beaker. The saccharin sodium and sorbitol liquid were added under stirring. The parabens were dissolved with about 20 g of propylene glycol and the xanthan gum suspended in the solution. This was added to the bulk preparation whiles stirring. The colloidal silicon dioxide was moistened with water and added to the bulk preparation. The propylene glycol was added. The citric acid, sodium citrate and ascorbic acid were added in that order. The ibuprofen powder, polysorbate 80, dimethypolysiloxane emulsion and about 60 ml of purified water were premixed together. The resultant mixture was added to the bulk preparation. The colour was dissolved and added to the bulk suspension. The flavour was added and the volume adjusted to 1000 ml with purified water.

4.3.2.2 Evaluation of 9 Formulations

The parameters (appearance, redispersibility, viscosity and pH) of the 9 suspensions were determined using the ECL quality control test (section 2.4).

4.3.3 Stability of standard formulation

The accelerated stability test procedure was employed to ascertain the stability of the standard formulation. Using the standard formula (table 4.5) 1000 ml standard ibuprofen suspension was prepared and the parameters of the suspension (appearance, redispersibility, viscosity, pH and assay) determined. The prepared standard formulation (section 4.8.1) was bottled using the same glass bottle and cap used by ECL. The products were placed in the accelerated stability chamber (temperature 40 ± 20C, relative humidity 75% ± 5%). Samples of the product were analysed after 3 and 6 months of storage for description, redispersibility, viscosity, pH and content assay.

4.4 Results and Discussion

4.4.1 Appearance and Redispersibility

All the 9 formulations appeared as yellow viscous suspensions. They all had the same appearance and behaviour towards redispersibility and in terms of appearance and redispersibility there was nothing to choose between the nine formulations.

4.4.2 Viscosity

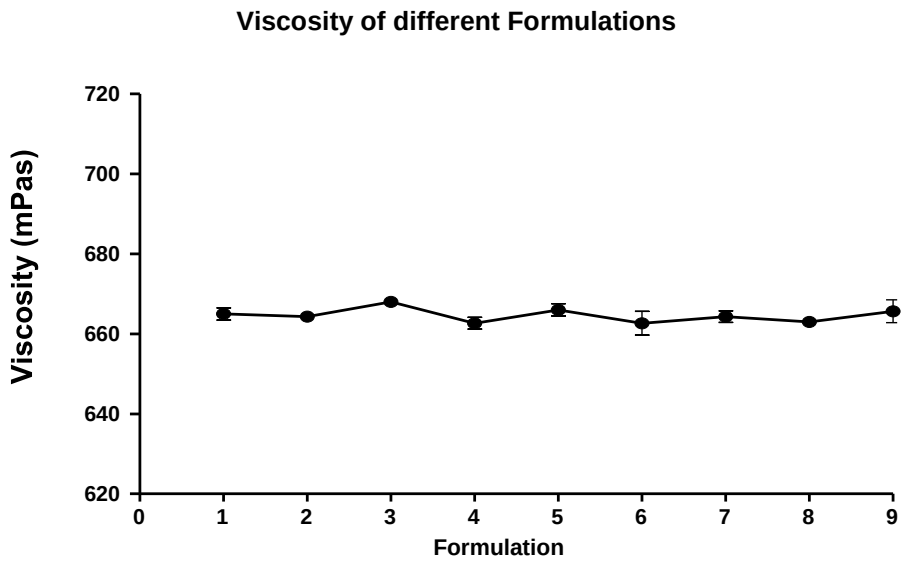

Figure 4.1 Viscosity of Different Formulation

The viscosities of the 9 formulations ranged from 663 to 668 mPas (figure 4.1) within the ECL specification limits of 610 to 680 mPas (section 2.6.1) for ibuprofen suspension. However according to Stokes law, sedimentation velocity is inversely proportional to the viscosity of the dispersion medium. As viscosity increases, there is decrease in the rate of settling giving rise to good dispersion of particles and enhanced physical stability. Based on this, formulation 3 may be better than the other formulations in terms of physical stability.

4.4.3 pH

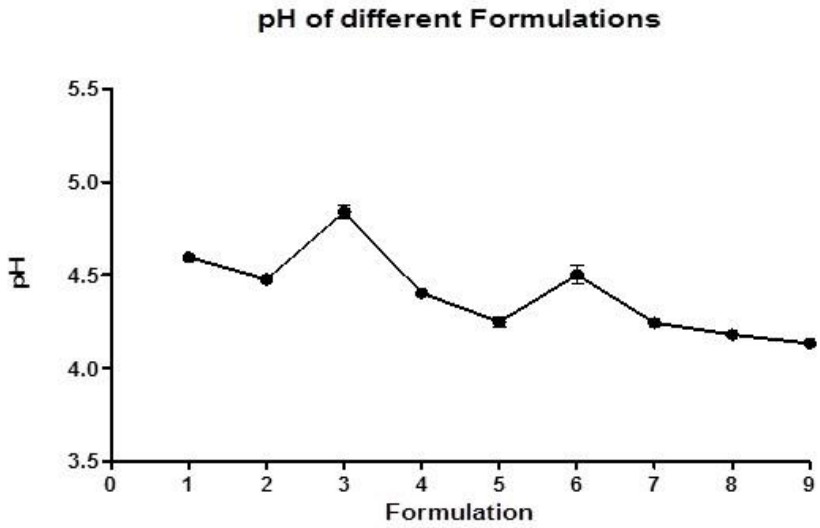

Figure 4.2 pH of Different Formulations

The 9 formulations had their pH ranging from 4.13 to 4.87 (figure 4.2) and all were within the acceptable limits of 3.5 to 5.0 (section 2.6.1) for ECL ibuprofen suspension. Buffering activity is optimum when the pH is the same as the pKa of the acid. The pKa of citric acid is 4.76. For optimum activity the pH of the suspension should be as close as possible to 4.76. The results show formulations 1 and 3 as those with pHs 4.60 and 4.87 respectively close to 4.76.

4.4.4 Optimised formulation

The results so far show formulation 3 might possess better qualities in terms of viscosity and pH and hence formulation 3 was therefore adopted as the standard formulation.

4.4.5 Standard Formulation

Based on the foregoing the proposed standard formulation for ibuprofen suspension was given as:

Table 4.4 Proposed standard formula for ibuprofen suspension

MATERIALS	PROPOSED QUANTITY (g)

Sorbitol liquid 70%	500
Saccharin Sodium	0.03
Propylparaben	0.16
Methylparaben	1.84
Xanthan Gum	1.2
Colloidal Silicon dioxide	8.0
Propylene Glycol	110.0
Dimethylpolysiloxane emulsion 30%	1.0
Polysorbate 80	2.0
Ascorbic acid	0.1
Ibuprofen	20.0
Citric acid	20.0
Sodium citrate	26.8
Tartrazine (E102)	0.0060
Pineapple Flavour	2.0

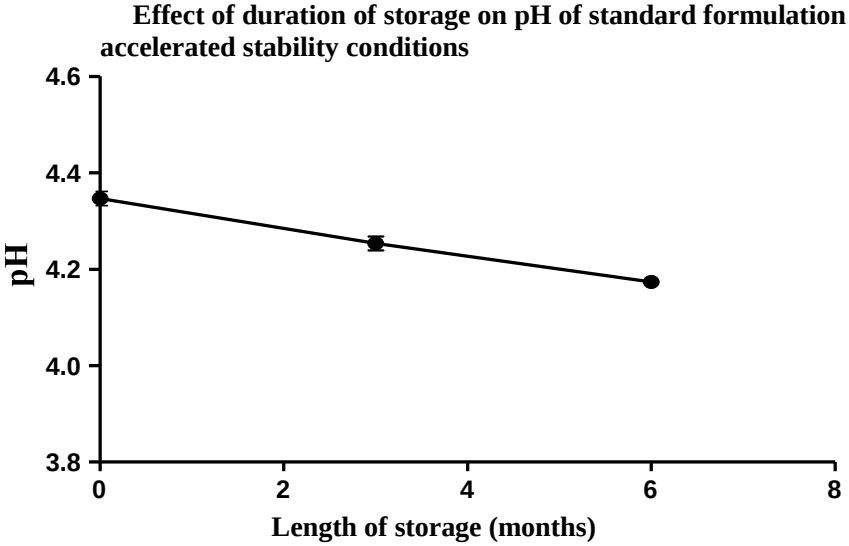

Figure 4.3 Relationship between pH and length of storage of standard formulation under accelerated storage conditions

Formulations were considered stable if the physical parameters did not change or the percentage change in a parameter is less than 5% of the original value. The pH of the standard ibuprofen suspension dropped from 4.35 to 4.17 at the end of the accelerated stability test (figure 4.3). This was still within the acceptable limit of 3.5 to 5.0. The

percentage drop in pH of 4.14% was not significant. This might be an indication of the effectiveness of the buffering agent.

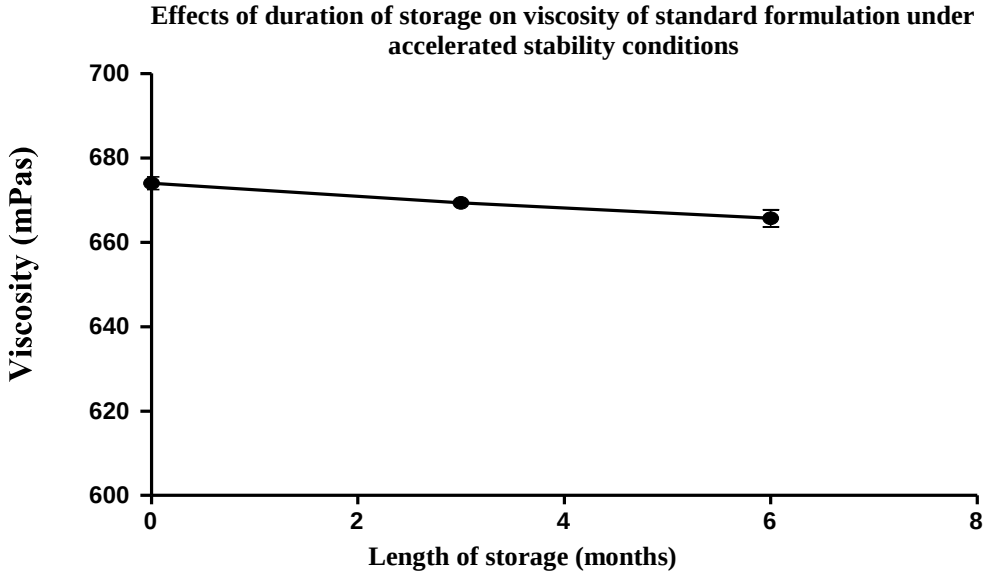

Figure 4.4 Relationship between viscosity and length of storage of standard formulation under accelerated storage conditions.

The initial viscosity of the suspension was 674 mPas and it dropped to 666 mPas at the end of the 6months period (figure 4.4). The percentage drop of 1.2% was not significant and the viscosity remained within the ECL acceptable criteria for viscosity of 610 to 680mPas.

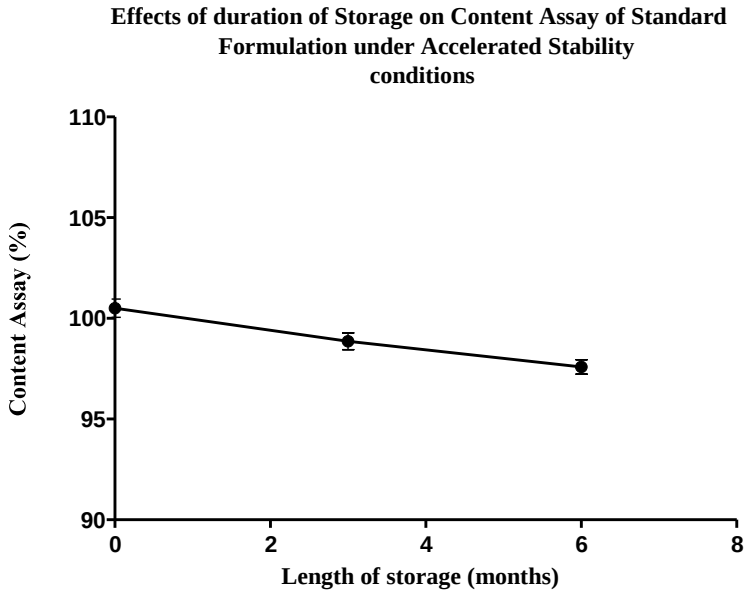

Figure 4.5 Relationship between content assay and length of storage of standard formulation under accelerated storage conditions

The assay of the suspension dropped from 100.5 to 97.6% (figure 4.5) a percentage drop of 2.9% which was not significant. After 6months test under accelerated stability studies the products satisfied all the test parameters indicating the possible stability of the formulation.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

5.1 Discussion

When an ibuprofen suspension produced by ECL was found to have failed real time stability studies due to low assay (content of ibuprofen) after 12 months of storage. It was imperative to find out the possible cause(s) of the problem and suggest an appropriate remedy. The ECL manufactured suspension was re-examined to find out the possible cause of the problem.

Composition of ECL Ibuprofen Suspension

Qualitatively the ECL ibuprofen suspension was composed of preservatives (methylparaben and propylparaben), sweetening agents (sucrose and saccharin sodium), solvents and co-solvents (ethanol 96% and propylene glycol), thickening and suspending agents (xanthan gum and colloidal silicon dioxide), wetting agent (polysorbate 80), antifoaming agent (dimethylpolysiloxane emulsion 30%), colour (amaranth) and flavour (pineapple).

These are the general components of suspensions however sucrose has an effect on dental caries in children (Edgar, 1998) and because the product is a paediatric formulation an alternative sweetener like sorbitol was recommended. Secondly there are safety concerns with the use of alcohol in paediatric formulations (Svirskis *et al*., 2013). The principal function of ethanol 96% in the formulation is the dissolution of the parabens. The parabens are however soluble in propylene glycol which could be used for that purpose, and leave out the ethanol.

API Quality

The quality of API used for the ECL ibuprofen suspension was examined by comparing the ECL ibuprofen suspension with other ibuprofen suspensions produced with alternative APIs from other API sources. The results showed that all the four suspensions maintained both their appearance and ease of redispersibility. The initial pH of the suspensions 4.21, 4.25, 4.20 and 4.25 increased to pH of 4.31, 4.32, 4.33 and 4.32 respectively after the storage period. The drop in pH over the storage period for the various suspensions was not significant ($P > 0.05$). Similarly the initial assay of 100.1, 100.9, 99.5 and 100.11% of the suspension dropped to 93.33, 92.44, 90.97 and 91.47% respectively after 24months of storage. In all the suspensions the ibuprofen content fell below the BP lower limit of 95% after 12months of storage. These similarities in the behaviour of the suspensions suggest the APIs might be of the same quality and therefore the API might not be responsible for the instability observed.

Preparation Procedure

Analysis of the processing method used by ECL for the suspension preparation showed that it did not consist of any extreme process which will affect the integrity of the materials.

Packaging Materials

The assessment of the packaging materials used for the product showed the primary packaging material consisted of amber coloured glass bottles and closures of ROPP aluminium screw caps. Glass is nonporous and impermeable to water vapour; it does not degrade and is chemically inert (Shivsharan *et al*., 2014). Amber glass has the potential of protecting its contents from the harmful effects of sunlight by sifting out ultraviolet rays (Kumar, 2013). Aluminium and its alloys are resistant to corrosion (Oldring and Nehring, 2007) making aluminium ROPP caps ideal for the closure of ibuprofen suspension. However sucrose may attack aluminium closures (Rowe *et al*., 2009). This further reemphasised the need to replace the sucrose in the formulation with an alternative sweetener like sorbitol. The secondary packaging consisted of selfadhesive label made from label paper and the outer jacket made from 320gsm laminated aqua vanish paperboard. The strong nature of paperboard enables it to hold the product safely. Furthermore the aqua varnish provides a smooth and attractive finish which enables the jacket to have high abrasion and rub resistance. The tertiary packaging material consisted of shipper made from 3ply corrugated fibreboard. The corrugated nature of the fibreboard gives it high resistance to bending and makes it able to withstand all the stress during transportation.

API - Excipient Interaction

When API was mixed with the individual excipients and observed over a period the results did not show any visible or noticeable interaction between the API and any of the excipients suggesting there might not be any visible interaction between the various excipients and the API.

Microbial Analysis

Microbial analysis of the ECL ibuprofen suspension showed that the product was free from both E. coli and Salmonella typhi. The aerobic bacteria count of <10 were within the acceptable limit of not more than 10^2 cfu/mL whiles the fungal count of 1 was within the acceptable limit of 10^1cfu/mL. Since the suspension is a non-sterile preparation it was not expected to be completely free of microbes. As the microbial levels are within the acceptable limits it might be an indication of the effectiveness of the preservatives in the formulation.

Effect of Storage Temperature on the Stability of the Suspension

On the effect of storage temperature on the ECL ibuprofen suspension it was observed after 24months of storage that the products stored at the various temperature ranges did not show any visible changes in their appearance and ease of redispersibility. At the end of the storage period the viscosity of the suspension stored at 4 – 8^0C dropped from an initial value of 653 to 647mPas. Likewise the viscosities of the suspensions stored at 20 – 25^0C, 26 – 28^0C and 28 – 32^0C dropped from their initial value of 653 to 640, 647 and 648mPas respectively. Statistical analysis of the test results showed that there were no significant differences ($P > 0.05$) between the viscosities of the suspensions stored at the various temperature ranges.

This might be due to the thickening agent (xanthan gum) in the suspension which has the ability to maintain its viscosity until the melting temperature (Tm) is reached after which the viscosity decreases sharply due to a reversible molecular conformational change (Desplanques *et al*., 2012).

On the effect of storage temperature on the pH of the suspension, the results showed fluctuations in the pH of the various suspensions. The pH of the suspension stored at 4 – 8^0C increased from 4.20 to 4.47 at the end of the storage period. The pH of the suspension stored at 20 – 25^0C increased from 4.20 to 4.65 after 12months of storage

and reduced to 4.22 after the storage period. Similarly the pH of the suspension stored at 28 – 32^{0}C increased from 4.20 to 4.75 after 12months of storage and came down to 4.30 after 24months. The fluctuations in the pH may be attributed to the absence of a buffering agent in the formulation.

There was significant drop in the API content of all the suspensions after 24 months of storage. The assay of the suspension stored at 4 – 8^{0}C dropped from 98.30 to 93.30%, whiles that stored at 20 – 25^{0}C dropped from 98.30 to 92.80%. Similarly the suspensions stored at 26 – 28^{0}C and 28 – 32^{0}C had their API contents dropping significantly to 93.01 and 93.90% respectively from an initial value of 98.3%. The significant drop in the assays demonstrates the instability of the ibuprofen formulation. However statistically there were no significant differences ($P > 0.05$) in the assays of the various suspensions stored at the different temperature ranges suggesting that storage temperature might not be responsible for the deterioration of the ibuprofen suspension.

Effect of Light on the Stability of Ibuprofen Suspension

The test results indicate that both the appearance and ease of redispersibility of the suspension were not affected by light after 24 months of storage. Similarly the results show no significant effect of light on the viscosity and assay of the suspension after the storage period. The viscosity of the suspension in the amber bottle dropped from 653 to 648mPas whiles that of the suspension in the plain bottle dropped from 653 to 650mPas. The assay of the suspension in the amber bottle fell from 98.3 to 93.9% whiles that of the suspension in the plain bottle dropped from 98.3 to 92.51%.

Effect of Suspension pH on the Stability of Ibuprofen Suspension

On the effect of suspension pH on the stability of the suspension the results showed that varying the pH of the suspension had no noticeable effect on the redispersibility and appearance of the product. The test on viscosity showed that the ibuprofen suspensions with pH within the ranges 3 to 4, 5 to 6, 6 to 7 and 7 to 8 had insignificant reduction in their viscosities from 649 to 648, 642, 641 and 641 respectively. At the end of the storage period, statistically there were no significant differences ($P > 0.05$) in the assays of the various suspensions. These seem to suggest the pH of ibuprofen suspension may not be a significant factor in its stability.

Proposed Interventions

In order to improve on the original formulation by ECL the interventions proposed included changing the sweetener from sucrose to sorbitol liquid. This was necessary because of the effect of sucrose on dental caries in children.

Also because of safety concerns with the use of ethanol in paediatric preparations it was recommended it should be taken out of the formulation and replaced with propylene glycol.

Variations in pH of the suspension were observed and to guard against this phenomenon it was proposed to include a buffering agent in the formulation. Citrate buffer was settled on because the pH of the buffer lies within the pH range of the ibuprofen suspension.

Ibuprofen is prone to oxidation and to protect the product from oxidation it was proposed to include an antioxidant in the formulation. The following antioxidants, sodium sulphite, sodium metabisulphite and ascorbic acid were considered. However ascorbic acid was chosen because of the safety concerns with the use of sulphites in sulphite-sensitive individuals.

Lastly because the colouring agent (amaranth) in the original formulation by ECL is incompatible with ascorbic acid it was replaced with tartrazine which is compatible with the antioxidant.

Proposed Recipe, Optimisation and Standardisation of the Ibuprofen Suspension

Based on the proposed interventions a provisional formula was developed which included the buffering agent (citric acid and sodium citrate), antioxidant (ascorbic acid), sweetener (sorbitol liquid) and the colour (tartrazine). Ethanol, sucrose and amaranth were no longer excipients in the proposed formula for the ibuprofen suspension. The one variable at a time (OVAT) principle was used to optimise the formulation. This resulted in the preparation of 9 formulations which were evaluated. The results of the evaluation showed all the formulations were redispersible and appeared as yellow viscous suspensions with formulation 3 comparatively having the highest viscosity of 668mPas. Since increase in viscosity enhances physical stability, formulation 3 with the highest viscosity was assessed to be superior to the other formulations in terms of physical stability. Optimal buffering capacity is obtained when pKa is equal to pH. The buffering capacity of the buffer in formulation 3 was therefore considered to be higher than the other formulations since the pH of formulation 3 (4.87) is closer to the pKa of citric acid (4.76). These factors led to formulation 3 being adopted as the standard formulation.

Stability of Standard Formulation

The Ghana Food and Drugs Authority (FDA, Ghana) defines significant change as a percentage change of 5% or more in the initial content of API(s) or failing to meet the acceptance criteria (FDA, 2013a). The standard formulation was a yellow viscous suspension which was readily redispersed. There were no visible changes in the colour

and appearance of the suspension when the product was subjected to accelerated stability test. Likewise the redispersibility remained unchanged during the six months of storage under accelerated test conditions of 38 to 42^0C and humidity of 70 to 80%.

Viscosity plays an important role in the physical stability of suspensions and although there was a drop of 1.2% in the viscosity of the suspension (674 to 666) under the accelerated stability conditions this was not significant.

The initial pH of the suspension was 4.35 and it came down to 4.17 after 6 months storage in the accelerated stability chamber but this was still within the acceptable limits of 3.5 to 5.0.

Lastly the percentage drop in assay (content of ibuprofen) of the suspension after the storage period in the accelerated stability chamber was 2.9% (100.5 to 97.6) which was insignificant.

The preliminary accelerated stability studies show a probable stable product. According to the FDA, Ghana guidelines on stability testing of finished pharmaceutical products (FPP) data from stability studies should be provided for real time stability studies (FDA, 2013b). Since the stability studies on the standard formulation focused on only accelerated stability studies it was inconclusive to declare the product as been stable, however the results do point to a probable stable formulation.

5.2 Conclusions

Among the many factors investigated, the most likely cause(s) of the instability could be oxidation and pH changes as such a new formula was proposed to include an antioxidant and buffering agent.

This new formula was used to develop formulations of ibuprofen suspension and the most stable and promising formulation selected and standardised.

The standardised formulation passed accelerated stability test indicating a potentially stable formulation.

5.3 Recommendation

The standard formulation should be put on real time (long term) stability studies to provide data for a definitive decision to be made on the stability of the proposed standard formulation.

REFERENCES

Abraham, J. and Mathew, F. (2014). Taste masking of paediatric formulation: a review on technologies, recent trends and regulatory aspects. *International Journal of Pharmacy and Pharmaceutical Sciences,* 6(1), pp.12-19.

Allam, K. V. and Kumar, G. P. (2011). Colorants - The cosmetics for the pharmaceutical dosage forms. *International Journal of Pharmacy and Pharmaceutical Sciences.* Vol 3 (3).

Bajaj, S., Singla, D. and Sakhuja, N. (2012). Stability testing of pharmaceutical products. *Journal of Applied Pharmaceutical Science,* 2(03), pp.129-138.

Barnes, H.A. (2000). *A Handbook of Elementary Rheology.* pp.5.

Basu, D. and Sen, D.J. (2015). Organoleptic agents: adaptability, acceptability and palatability in formulations to make it lucrative. *World Journal of Pharmacy and Pharmaceutical Sciences.* Volume 4, Issue 10.

Bean, H.S., (1972). Preservatives for Pharmaceuticals. *J. Soc. Cosmet. Chem.,* 23, pp. 703-720.

BP, (2014). The Stationery Office, Vol. I, pp.1164.

Bradbury, F., (2004). How important is the role of the physician in the correct use of a drug? An observational cohort study in general practice. *International Journal of Clinical Practice,* 58(144), pp.27-32.

Brown, M. and Leeson, L. J. (1969). Protection of oxygen-sensitive pharmaceuticals with nitrogen. *Journal of Pharmaceutical Sciences*. Vol. 58 (2), pp. 242-245.

Bu, X., Chandran, S., Spirig, J. and Wang, Q. (2011). Sample Preparation for Select Nonsolid Dosage Forms. *In Sample Preparation of Pharmaceutical Dosage Forms* (pp. 179-210). Springer US.

Buckley, I. and Newbold, A. (2005). Desiccants for Pharmaceutical Applications. *Pharmaceutical Technology Europe*.

Bushra, R. and Aslam, N. (2010). An Overview of Clinical Pharmacology of Ibuprofen. *Oman Medical Journal.* Volume 25, Issue 3.

Buyuktas, B.S. (2006). Investigation of the complexation and hydrolysis– condensation of titanium (IV) n-butoxide [Ti (OBun) 4] with some unsaturated mono and dicarboxylic acids. *Transition Metal Chemistry,* 31(6), pp.786-791.

Cashman, J.N. (1996). The mechanisms of action of NSAIDs in analgesia. *Drugs*, 52(5), pp.13-23.

Caviglioli, G., Valeria, P., Brunella, P., Sergio, C., Attilia, A. and Gaetano, B. (2002). Identification of degradation products of ibuprofen arising from oxidative and thermal treatments. *Journal of Pharmaceutical and Biomedical Analysis,* 30(3), pp.499-509.

Charde, M.S., Kumar, J., Welankiwar, A.S. and Chakole, R.D. (2013). Review: Development of forced degradation studies of drugs. *International Journal of Advances in Pharmaceutics*, 2(3), pp.34-39.

Charde, M.S., Shinde, M.M., Welankiwar, A.S. and Jitendra, K. (2014). Development of analytical and stability testing method for vitamin-A palmitate formulation. *International Journal of Pharmaceutical Chemistry*, 4(1), pp.39-51.

Chen, W. (2013). What is Zeta Potential? *American Filtration and Separation Society*, viewed 10 October 2016, http://www. afssociety.org/filtrationmedia/62-what-is-zeta-potential

Collier, J.W., Shah, R.B., Gupta, A., Sayeed, V., Habib, M.J. and Khan, M.A. (2010). Influence of formulation and processing factors on stability of levothyroxine sodium pentahydrate. *AAPS PharmSciTech*, 11(2), pp.818-825.

De Spiegeleer, B., Wattyn, E., Slegers, G., Van Der Meeren, P., Vlaminck, K. and Van Vooren, L. (2006). The importance of the cosolvent propylene glycol on the antimicrobial preservative efficacy of a pharmaceutical formulation by DOE-ruggedness testing. *Pharmaceutical Development and Technology*, 11(3), pp.275-284.

Deicke, A. and Süverkrüp, R. (1999). Dose uniformity and redispersibility of pharmaceutical suspensions I: quantification and mechanical modelling of human shaking behaviour. *European Journal of Pharmaceutics and Biopharmaceutics*, 48(3), pp.225-232.

Desplanques, S., Renou, F., Grisel, M. and Malhiac, C. (2012). Impact of chemical composition of xanthan and acacia gums on the emulsification and stability of oil-in-water emulsions. *Food Hydrocolloids*, 27(2), pp.401-410.

Dionne, R.A. and Berthold, C.W. (2001). Therapeutic uses of non-steroidal antiinflammatory drugs in dentistry. *Critical Reviews in Oral Biology & Medicine*, 12(4), pp.315-330.

Dutta, S. (2015). Natural polysaccharides as novel excipients for modified drug delivery systems: a review. *World Journal of Pharmaceutical and Life Sciences. Institutions*, 1(1), pp.50-84.

Edgar, W.M. (1998). Sugar substitutes, chewing gum and dental caries--a review. *British Dental Journal*, 184(1), pp.29-32.

Elder, D.P. and Crowley P.J. (2012a). Antimicrobial Preservatives Part One: Choosing a Preservative System. *American Pharmaceutical Review*.

Elder, D.P. and Crowley, P.J. (2012b). Antimicrobial Preservatives Part Two: choosing a preservative. *American Pharmaceutical Review*.

Fairhurst, D. (2013). An Overview of the Zeta Potential - Part 1: The Concept. *American Pharmaceutical Review.*

Farukh, S.F.B. and Khan, M.S. (2013). A New Approach of Syrup Manufacturing Using Fuzzy Time Control Discrete Event System. *International Journal of Computer and Electrical Engineering*, 5(2), p.256.

Fathima, N., Mamatha, T., Qureshi, H.K., Anitha, N. and Rao, J.V. (2011). Drugexcipient Interaction and its Importance in Dosage Form Development. *Journal of Applied Pharmaceutical Science*, 1(06), pp.66-71.

FDA (2013a). Guidelines for stability testing of active pharmaceutical ingredients and finished pharmaceutical products. Food and Drugs Authority, Ghana. 02, p. 15.

FDA (2013b). Guidelines for stability testing of active pharmaceutical ingredients and finished pharmaceutical products. Food and Drugs Authority, Ghana. 02, p. 12.

Gokani, R.H. and Desai, K.N. (2012). Stability Study: Regulatory Requirement.

International Journal of Advances in Pharmaceutical Analysis, 2(4), pp.73-78.

Hassan, B.A.R. (2012). Overview on Pharmaceutical Formulation and Drug Design. *Pharmaceut Anal Acta*, 3(10).

Heitner, H.I. (2004). Flocculating Agents. *Kirk-Othmer Encyclopedia of Chemical Technology*.

International Pharmacopoeia (2008). WHO Press, 1st Supplement, 4th edition, p.1547.

Jamali, F., Singh, N.N., Pasutto, F.M., Russell, A.S. and Coutts, R.T. (1988). Pharmacokinetics of Ibuprofen Enantiomers in Humans following Oral Administration of Tablets with Different Absorption Rates. *Pharmaceutical research*, 5(1), pp.40-43.

Jones, D. (2008). *Pharmaceutics-Dosage Form and Design*. Pharmaceutical Press, pp.26.

Jones, D.A. (2015) *FASTtrack: Pharmaceutics - Dosage Form and Design*. London: Pharmaceutical Press, United Kingdom, pp.9.

Keerthi, M., Prasanna, J.L., Sharuna, K.S. and Rao, N.R. (2014). A review on packaging for different formulations. *Asian Journal of Research in Pharmaceutical Science*, 4(3), pp.141-151.

Kesisoglou, F., Panmai, S. and Wu, Y. (2007). Nanosizing—Oral formulation development and biopharmaceutical evaluation. *Advanced Drug Delivery Reviews*, 59(7), pp.631-644.

Khan, B.A., Akhtar, N., Khan, H.M.S., Waseem, K., Mahmood, T., Rasul, A., Iqbal, M. and Khan, H. (2011). Basics of Pharmaceutical Emulsions: A Review. *African Journal of Pharmacy and Pharmacology*, 5(25), pp.2715-2725.

Kommanaboyina, B. and Rhodes, C.T. (1999). Trends in Stability Testing, with Emphasis on Stability during Distribution and Storage. *Drug Development and Industrial Pharmacy*, 25(7), pp.857-868.

Korean Pharmacopoeia, (2012). 10th edition, pp.17.

Kulshreshtha, A. K., Singh, O. N. and Wall, G. M. (2010). *Pharmaceutical Suspensions from Formulation Development to Manufacturing*. Springer, New York.

Kumar, S. (2013). Pharmaceutical Packaging Technology - A Review. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 4 (4).

Lachman, L., Lieberman, H.A. and Kanig, J.L. (1986). *The Theory and Practice of Industrial Pharmacy,* 3rd edition, Bombay: Varghese publishing house. pp. 479-494.

Larson, R.G. (1999). *The Structure and Rheology of Complex Fluids* (Vol. 150), New York: Oxford university press.

Lee, E.J., Williams, K.M., Day, R., Graham, G. and Champion, D. (1985). Stereoselective Disposition of Ibuprofen Enantiomers in Man. *British Journal of Clinical Pharmacology*, 19(5), pp.669-674.

Lesar, T.S. (2002). Prescribing Errors Involving Medication Dosage Forms. *Journal of General Internal Medicine*, 17(8), pp.579-587.

Li, H., Guo, A. and Wang, H. (2008). Mechanisms of oxidative browning of wine. *Food Chemistry*, 108: 1−13.

Lubrizol, (2002). Measurement and Understanding of Yield Value in Home Care Formulations. Lubrizol Technical Data Sheet, TDS-244, pp.1.

Lubrizol, (2008). Flow and Suspension Properties. Lubrizol Pharmaceutical Bulletin, 7th edition, pp.3.

Lubrizol, (2011). Oral Suspensions. Pharmaceutical Bulletin 22, pp. 3.

Manoharan, C., Basarkar, A. and Singh, J. (2010). Various Pharmaceutical Disperse Systems. In *Pharmaceutical Suspensions* (pp. 1). Springer New York.

Markens, U. (2009).Conducting Stability Studies: Recent Changes to Climate Zone IV. *Life Science*. Technical Bulletin, 13.

Marriott, J.F. (2010). *Pharmaceutical Compounding and Dispensing*. Pharmaceutical

Press, pp.116.

Martindale: The Extra Pharmacopoeia (2002). 33rd edition, Pharmaceutical Press, pp.44.

Mastropietro, D.J., Nimroozi, R. and Omidian, H. (2013). Rheology in pharmaceutical formulations-A perspective. *Journal of Developing Drugs*.

Ministry of Health (2010). *Standard Treatment Guidelines*. 6th Edition, pp. 407.

Monkhouse, D.C. (1984). Stability Aspects of Preformulation and Formulation of Solid Pharmaceuticals. *Drug Development and Industrial Pharmacy*, 10(8-9), pp.1373-1412.

Moynihan, H. and Crean, A. (2009). *Physicochemical Basis of Pharmaceuticals*. Oxford University Press, pp.17.

Mudoi, P., Bharali, P. and Konwar, B.K. (2013). Study on the Effect of pH, Temperature and Aeration on the Cellular Growth and Xanthan Production by Xanthomonas campestris Using Waste Residual Molasses. *Journal of Bioprocessing & Biotechniques*, 3:135.

Murthy, P.N., Devi, M.V., Sahoo, S.K., Mahapatra, A.K. and Khandai, M. (2015). Rapid Assessment of Sedimentation Stability in Paracetamol Suspension Formulations with Different Suspending Agents using near Infrared Transmission Measurements. *J. Chem. Pharm. Res.* 7(5), pp.186-195.

Nakao, A., Sugimoto, R., Billiar, T.R. and McCurry, K.R. (2009). Therapeutic antioxidant medical gas. *Journal of Clinical Biochemistry and Nutrition*, 44(1), pp.1-13.

Nasa, P. (2014). A review on pharmaceutical packaging material. *World Journal of Pharmaceutical Research*, 3(5), pp.344-368.

Nielloud, F., Marti-Mestres, G., Fortuné, R., Mestres, J. P. and Maillols, H. (1998). Formulation of insoluble drugs. Study of pharmaceutical suspensions by response surface methodology. *Analusis*, 26, 277-281

Nunn, T. and Williams, J. (2005). Formulation of medicines for children. *British Journal of Clinical Pharmacology*, 59(6), pp.674-676.

Nutan, M.T. and Reddy, I.K. (2010). General principles of suspensions. In *Pharmaceutical Suspensions* (pp. 39-65). Springer New York.

Oldring, P.K.T. and Nehring, U. (2007). *Packaging Materials: Metal Packaging for Foodstuffs.* Report prepared under the Responsibility of the International Life Sciences Institute (ILSI Europe) Packaging Materials Task Force, pp.7.

Particle Sciences (2009). Physical Stability of Disperse Systems. Particle Sciences - Technical brief, Volume 1.

Patel, R.M. (2010). Parenteral suspension: an overview. *International Journal of Current Pharmaceutical Research*, 2(3), pp.1-13.

Patel, V. P., Desai, T., Chavda, B., and Katira, R. (2011). Extemporaneous dosage form for oral liquids. *Pharmacophore*, 2(2), pp.86-103.

Paul, S. and Saha, D. (2012). Comparative studies on sedimentation parameter of aluminium hydroxide and sodium bicarbonate, magnesium trisilicate, magnesium carbonate suspension. *Asian Journal of Pharmacy and Technology*, 2(4), pp.133-134.

Physics for all (2015), Can you walk on water? (Non-Newtonian Fluid Pool) accessed 18th March 2016, http://www.physics4all.com/can-you-walk-on-water-nonnewtonian-fluid-pool/.

Piechocki, J. T. and Thoma, K. (eds.) (2007). *Pharmaceutical Photostability and Stabilisation Technology*. CRC Press, pp. 368.

Potthast, H., Dressman, J.B., Junginger, H.E., Midha, K.K., Oeser, H., Shah, V.P., and Barends, D.M. (2005). Biowaiver monographs for immediate release solid oral dosage forms: Ibuprofen. *Journal of Pharmaceutical Sciences*, 94(10), pp.2121-2131.

Qiu, Y., Chen, Y., Zhang, G. G. Z., Liu, L. and Porter, W. R. (eds) (2009). *Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice.* Academic Press, PP. 117.

Rainsford, K.D. (2009). Ibuprofen pharmacology, efficacy and safety. *Inflammopharmacology*, 17(6), pp.275-342.

Raj, S.P. and Angela, L.S. (2011). Stability of marketed albendazole suspensions and correlation between zeta potential and sedimentation. *Int. Res. J. Pharm*, 3, pp.154-156.

Rios, M. (2015). Special Report on Product Stability Testing: Developing Methods for New Biologics and Emerging Markets. *BioProcess International.* Vol. 13, No. 5.

Romero, A.J. and Rhodes, C.T., (1993). Stereochemical aspects of the molecular pharmaceutics of ibuprofen. *Journal of Pharmacy and Pharmacology*, 45(4), pp.258-262.

Rowe, R.C., Sheskey, P.J. and Quinn, M.E. (eds.) (2009). *Handbook of Pharmaceutical Excipients*. Pharmaceutical Press and American Pharmacists Association, pp. 43, 181, 706.

Rubino, J.T. and Yalkowsky, S.H. (1987). Cosolvency and cosolvent polarity. *Pharmaceutical Research*, 4(3), pp.220-230.

Rubio-Bonilla, M.V., Londono, R. and Rubio, A. (2010). Liquid dosage forms. *Pharmaceutical Sciences Encyclopedia*, 10, pp.1–32.

Saqib, S. H. (2008). Drug stability, Drug Stability blog, web log post, 31 March 2008, viewed 14 October 2016, http://drugstabilityucp.blogspot.com/.

Schott, H. (1976). Controlled flocculation of coarse suspensions by colloidally dispersed solids I: Interaction of bismuth subnitrate with bentonite. *Journal of Pharmaceutical Sciences,* 65(6), pp.855-861.

Schwartz, J.B. and Sharp, M. (1981). Optimisation techniques in product formulation. *J. Soc. Cosmet. Chem.*, 32, 287-30.

Shaikh, R. H. and Sial, A. A. (1996). Stability of pharmaceutical formulations. *Pakistan Journal of Pharmaceutical Sciences*, 9(2), pp.83-86.

Shivsharan, U.S., Raut E.S. and Shaikh, Z.M., (2014). Packaging of cosmetics: A review. *Journal of Pharmaceutical and Scientific Innovation*. 3 (4).

Silva, A.F., Burggraeve, A., Denon, Q., Van der Meeren, P., Sandler, N., Van Den Kerkhof, T., Hellings, M., Vervaet, C., Remon, J.P., Lopes, J.A. and De Beer, T. (2013). Particle sizing measurements in pharmaceutical applications: Comparison of in-process methods versus off-line methods. *European Journal of Pharmaceutics and Biopharmaceutics*, 85(3), pp.1006-1018.

Singh, S. and Singh, J. (2010). Development of accelerated stability protocol for sildenafil tablets–A European perspective review. *International Journal of Pharma and Bio Sciences.* 1(4).

Steinmeyer, J. (2000). Pharmacological basis for the therapy of pain and inflammation with nonsteroidal anti-inflammatory drugs. *Arthritis Research*, 2(5), pp.379385.

Sushma, G., Kumar, K. M., Ajay, B. and Ruchi, T. (2013). Advancements and patents in pharmaceutical suspension technologies. *Journal of Biological & Scientific Opinion*, 1(4).

Svirskis, D., Toh, M. and Ram, S. (2013). The use of ethanol in paediatric formulations in New Zealand. *European Journal of Paediatrics*, 172(7), pp.919-926.

Tong, W. and Zhang, G. G. Z. (2006). Stability and excipient compatibility studies. *Integrated Drug Product Development Process*. University of Utah.

Tønnesen, H. H. (2008). Photoreactivity of drugs. In: *Solar Radiation and Human Health.*

Trivedi, H.K. and Patel, M.C. (2011). A stability indicating method for the determination of the antioxidant sodium bisulfite in pharmaceutical formulation by RP-HPLC technique. *Scientia Pharmaceutica*, 79(4), p.909.

Troy, D. B. and Beringer, P. (eds) (2006). *Remington: The Science and Practice of Pharmacy.* Twenty-First Edition. Lippincott Williams & Wilkins, pp. 323.

USP (2015). United States Pharmacopeial Convention, Vol. 2, pp. 3827-3828.

Vane, J.R. (1996). Introduction: mechanism of action of NSAIDs. *Rheumatology* , 35(1), pp.1-3.

Vane, J.R. and Botting, R.M. (1998). Anti-inflammatory drugs and their mechanism of action. *Inflammation Research*, 47(2), pp.78-87.

Velagaleti, R. (2010). Stability and shelf life of pharmaceutical products. *Pharmaceutical Sciences Encyclopedia,* 17, pp.1–24.

Vinod, Budhwaar, V. and Nanda, A. (2015). Photochemical fate of pharmaceuticals: An updated review. *International Journal of Pharmaceutical Research and Bio-science,* 4(2), pp.54-70.

Weiner, B.B., Tscharnuter, W.W. and Fairhurst, D. (1993). Zeta Potential: A New Approach. *A Paper presented at the Canadian Mineral Analysts Meeting*.

Welankiwar, A., Saudagar, S., Kumar, J. and Barabde, A. (2013). Photostability testing of pharmaceutical products. *Int. Res. J. Pharm*, 4 (9).

WHO (1996). Cancer pain relief: with a guide to opioid availability. WHO, 2nd edition, p. 17.

WHO (2009). WHO Technical Report Series, No. 953.

WHO (2015). WHO Model list of Essential Medicines, 19th List.

Xie, W., Robertson, D.L. and Simmons, D.L. (1992). Mitogen_inducible

prostaglandin G/H synthase: A new target for nonsteroidal antiinflammatory drugs. *Drug Development Research,* 25(4), pp.249-265.

Yoshioka, S. and Stella, V.J. (2000). *Stability of Drugs and Dosage Forms* (pp. 139-150). New York: Kluwer Academic/Plenum Publishers.

Zatz, J.L. (1985). Physical stability of suspensions. *J. Soc. Cosmet. Chem*., 36, pp.393-411.

Zhou D. (2009). Understanding Physicochemical Properties for Pharmaceutical Product Development and Manufacturing II: Physical and Chemical Stability and Excipient Compatibility. *Journal of Validation Technology*, 15(3), pp.36-47.

Zontek, S.J. and Kostka, S.J. (2012). Understanding the different wetting agent chemistries. *USGA Green Sec. Rec*, 50(15), pp.1-6.

APPENDIX

Appendix A Analysis of Locally Manufactured Production

Parameter	**PRODUCT**			
	1	**2**	**3**	**4**
Description	Viscous yellow suspension	Viscous yellow suspension	Viscous orange suspension	Viscous orange suspension
Mean Viscosity (mPas) ± SD	716 ± 0.0006	623 ± 0.0026	661 ± 0.0016	706 ± 0.0039

Mean pH ± SD	4.24 ± 0.0200	4.96 ± 0.1308	4.64 ± 0.0058	4.48 ± 0.0058
Mean Assay (%) ± SD	89.30 ±0.2031	113.99 ± 0.0004	88.75 ± 0.0237	92.19 ± 0.0.3412

Appendix B .API – Excipients Interaction

Excipients	**Test Results**		
	1 month	**2 months**	**3 months**
Sucrose	No visible interaction observed	No visible interaction observed	No visible interaction observed
Saccharin sodium	No visible interaction observed	No visible interaction observed	No visible interaction observed
Ethanol 96%	No visible interaction observed	No visible interaction observed	No visible interaction observed
Propylparaben	No visible interaction observed	No visible interaction observed	No visible interaction observed
Methylparaben	No visible interaction observed	No visible interaction observed	No visible interaction observed
Xanthan gum	No visible interaction observed	No visible interaction observed	No visible interaction observed
Colloidal silicon dioxide	No visible interaction observed	No visible interaction observed	No visible interaction observed
Propylene glycol	No visible interaction observed	No visible interaction observed	No visible interaction observed
Dimethypolysiloxane emulsion 30%	No visible interaction observed	No visible interaction observed	No visible interaction observed
Polysorbate 80	No visible interaction observed	No visible interaction observed	No visible interaction observed

Appendix C Effect of API quality on the description of the ibuprofen suspension

Suspension	Description						
	Length of Storage(months)						
	0	3	6	9	12	18	24
A	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
B	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
C	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
D	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension

Appendix D Effect of API quality on the redispersibility of the ibuprofen suspension

Suspension	Redispersibility						
	Length of Storage(months)						
	0	3	6	9	12	18	24
A	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed
B	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed
C	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed
D	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed

Appendix E Effect of API quality on the Mean pH of the ibuprofen suspension

Suspension	Mean pH ± SD						
	Length of Storage(months)						
	0	**3**	**6**	**9**	**12**	**18**	**24**
A	4.21 ±0.0252	4.22 ±0.0252	4.24 ± 0.0208	4.24 ±0.0173	4.21 ±0.0153	4.27 ±0.0100	4.31 ±0.0200
B	4.25 ±0.0153	4.22 ±0.0100	4.24 ±0.0100	4.28 ±0.0100	4.30 ±0.0300	4.30 ±0.0100	4.32 ±0.0058
C	4.20 ±0.0207	4.23 ±0.0117	4.23 ±0.0232	4.27 ±0.0172	4.29 ±0.0175	4.27 ±0.0367	4.33 ±0.0208
D	4.25 ±0.0153	4.25 ±0.0115	4.26 ±0.0153	4.26 ±0.0100	4.31 ±0.0058	4.31 ±0.0153	4.32 ±0.0208

n=3, SD=Standard Deviation

Appendix F Effect of API quality on the Mean Viscosity of the ibuprofen suspension

Suspension	Mean Viscosity ± SD						
	Length of Storage(months)						
	0	3	6	9	12	18	24
A	645 ±2.0817	648 ±4.0415	647 ±3.7859	650 ±3.0551	649 ±3.0000	652 ±2.5166	653 ±0.5774
B	653 ±2.5166	657 ±5.1316	652 ±2.0000	655 ±1.5275	656 ±0.5774	654 ±2.0817	656 ±0.5774
C	646 ±2.8868	649 ±2.5166	648 ±2.0817	650 ±3.5119	650 ±0.5774	651 ±4.0415	652 ±2.5166
D	654 ±2.0000	653 ±1.7321	654 ±2.0817	655 ±1.5275	657 ±1.1547	658 ±2.5166	657 ±1.1547

n=3, SD=Standard Deviation

Appendix G Effect of API quality on the Mean Content Assay of the ibuprofen suspension

Suspension	Mean Content Assay ± SD						
	Length of Storage(months)						
	0	3	6	9	12	18	24
A	100.10 ±0.6245	99.70 ±0.8185	99.43 ±0.5024	98.67 ±0.6028	98.17 ±0.6028	94.77 ±0.4726	93.33 ±0.9853
B	100.90 ±0.6245	99.57 ±0.6506	98.64 ±0.6445	98.83 ±0.4034	97.77 ±0.7234	94.23 ±0.9866	92.44 ±0.6122
C	99.50 ±0.8544	99.73 ±1.2741	99.41 ±0.7695	98.73 ±0.3512	98.07 ±0.4034	91.91 ±0.5587	90.97 ±2.2745
D	100.11 ±0.4553	100.09 ±0.1153	99.40 ±0.6538	99.00 ±0.4822	98.70 ±0.4000	92.24 ±0.3508	91.47 ±0.8327

n=3, SD=Standard Deviation

Appendix H Effect of storage temperature on the description of the suspension

Storage Temperature	Description						
	Length of Storage (months)						
	0	3	6	9	12	18	24
$4 – 8^0$C	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
$20 –25^0$C	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension

26– 28^{0}C	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
28 - 32^{0}C	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension

Appendix I Effect of storage temperature on the redispersibility of the suspension

Storage Temperature	**Redispersibility**					
	Length of Storage(months)					
	0	**3**	**6**	**9**	**12**	
4 – 8^{0}C	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	re
20 – 25^{0}C	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	re
26 – 28^{0}C	Readily	Readily	Readily	Readily	Readily	
28 - 32^{0}C	redispersedReadily redispersed	redispersedReadily redispersed	redispersedReadily redispersed	redispersedReadily redispersed	redispersedReadily redispersed	redisp redisp

Appendix J Effect of storage temperature on the mean viscosity of the suspension

Storage Temperature	**Mean Viscosity (mPas) ± SD**						
	Length of Storage(months)						
	0	**3**	**6**	**9**	**12**	**18**	**24**
4 – 8^{0}C	653 ±3.0551	646 ±3.6056	642 ±2.0817	641 ±2.5166	639 ±2.0817	646 ±2.0817	647 ±8.0829
20 – 25^{0}C	653 ±3.0551	649	643	643	645	641	640
	3.055653	±3.2146	±2.6458	±1.0000	±2.0817	±1.7321	±0.5774
	653±	655	649	649	651	649	647
26 – 28^{0}C	± 3.0551	±2.0817	±3.6056	±3.6056	±2.000	±2.0817	±1.0000
28 - 32^{0}C	653 ± 3.0551	651 ±2.0817	643 ±2.0817	643 ±2.0817	644 ±2.0817	647 ±1.0000	648 ±2.0817

n=3, SD=Standard Deviation

Appendix K Effect of storage temperature on the mean pH of the suspension

Storage Temperature	**Mean pH ± SD**						
	Length of Storage (months)						
	0	**3**	**6**	**9**	**12**	**18**	**24**
4 – 8^{0}C	4.20 ±0.0173	4.30**2** ±0.0306	4.31 ±0.1222	4.20 ±0.0208	4.33 ±0.0100	4.48 ±0.0208	4.47 ±0.0436

20 – 25^{0}C	4.20	4.29	4.28	4.22	4.65	4.53	4.22
26 – 28^{0}C	±0.0173	±0.0100	±0.0643	±0.0208	±0.0115	±0.0208	±0.0208
	4.20	4.30	4.26	4.18	4.24	4.15	4.40
	±0.0173	±0.0361	±0.0493	±0.0058	±0.0173	±0.0100	±0.0153
28 - 32^{0}C	4.20	4.27	4.27	4.08	4.75	4.67	4.30
	±0.0173	±0.0586	±0.0379	±0.0200	±0.0100	±0.0115	±0.0058

n=3, SD=Standard Deviation

Appendix L Effect of storage temperature on the mean content assay of the suspension

Storage Temperature	**Mean Content Assay(%) ± SD**						
	Length of Storage (months)						
	0	**3**	**6**	**9**	**12**	**18**	**24**
4 – 8^{0}C	98.30 ±0.5292	100.10 ±1.3417	98.80 ±1.7059	98.60 ±1.0599	96.20 ±1.1676	94.50 ±0.8718	93.30 ±0.7211
20 – 25^{0}C	98.30 ±0.5292	99.70 ±1.1926	99.10 ±1.1533	99.20 ±0.7337	95.90 ±1.1533	94.90 ±1.0206	92.80 ±0.3000
26 – 28^{0}C	98.30 ±0.5292	97.50 ±0.6083	100.60 ±0.9165	98.50 ±0.6265	96.00 ±0.9385	93.40 ±0.9165	93.01 ±0.3606
28 - 32^{0}C	98.30 ±0.5292	99.70 ±0.4000	99.80 ±1.0536	98.40 ±1.3000	96.70 ±1.0778	94.00 ±0.5511	93.90 ±0.9455

n=3, SD=Standard Deviation

Appendix M Effect of light on suspension in plain bottle

Parameter	Length of Storage (months)						
	0	**3**	**6**	**9**	**12**	**18**	**24**
Description	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
Redispersibility	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed
Viscosity	653±3.0551	652±2.5166	648±2.6458	651±2.0817	646±3.2146	649±4.5826	650±2.0817
pH	4.20±0.0200	4.29±0.0200	4.27±0.0100	4.25±0.0100	4.31±0.0173	4.26±0.0200	4.38±0.0208
Assay	98.30±0.5292	98.00±0.2589	99.30±0.8228	97.90±1.0259	94.60±0.6245	93.20±0.9539	92.51±1.5077

Appendix N Effect of light on suspension in amber coloured bottle

Parameter	Length of Storage (months)						
	0	**3**	**6**	**9**	**12**	**18**	**24**
Description	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
Redispersibility	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed
Viscosity	653 ± 3.0551	651 ±2.0817	643 ±2.0817	643 ±2.0817	644 ±2.0817	647 ±1.0000	648 ±2.0817
pH	4.20 ±0.0173	4.27 ±0.0586	4.27 ±0.0379	4.08 ±0.0200	4.75 ±0.0100	4.67 ±0.0115	4.30 ±0.0058
Assay	98.30 ±0.5292	99.70 ±0.4000	99.80 ±1.0536	98.40 ±1.3000	96.70 ±1.0778	94.00 ±0.5511	93.90 ±0.9455

n=3, SD=Standard Deviation

Appendix O Effect of pH on the description of the suspension

pH	Description						
	Length of Storage (months)						
	0	3	6	9	12	18	24
3.0 –4.0	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
4.0 – 5.0	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
5.0– 6.0	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
6.0– 7.0	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension
7.0– 8.0	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension	Viscous pink suspension

Appendix P Effect of pH on the Redispersibility of the Suspension

pH	Redispersibility						
	Length of Storage(months)						
	0	3	6	9	12	18	24
3.0 – 4.0	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed
4.0 – 5.0	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed
5.0 – 6.0	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed
6.0 – 7.0	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed
7.0 – 8.0	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed	Readily redispersed

Appendix Q Effect of pH on the Mean Viscosity of the suspension

pH	Mean Viscosity(mPas) ± SD						
	Length of Storage (months)						
	0	3	6	9	12	18	24
3.0 – 4.0	649±1.5275	647±3.2146	642±3.2146	644±2.5166	641±5.0000	640±2.0000	641±2.0817
4.0 – 5.0	649±1.5275	651 ±2.0817	643 ±2.0817	643±2.0817	644±2.0817	647 ±1.0000	648±2.0817
5.0 – 6.0	649±1.5275	646±3.2146	644±1.0000	647±8.0829	643±1.0000	640±1.5275	642±3.5119
6.0 – 7.0	649±1.5275	647±2.5166	647±1.0000	644±1.0000	643±2.0817	640±2.0817	641±2.0817
7.0 – 8.0	649±1.5275	650±3.2146	642±2.0000	642±2.0817	644±3.5119	644±1.5275	641±1.5275

n=3, SD=Standard Deviation

Appendix R Effect of pH on the Mean Assay of the suspension

pH	Mean Assay(%)±SD						
	Length of Storage(months)						
	0	**3**	**6**	**9**	**12**	**18**	**24**
3.0 – 4.0	99.15 ±0.3122	98.40 ±0.4359	98.44 ±1.3427	96.33 ±1.9348	94.49 ±1.4126	93.87 ±1.0017	91.90 ±0.7000
4.0 – 5.0	99.15 ±0.3122	98.70 ±0.9165	99.80 ±1.0536	97.47 ±0.7095	95.94 ±1.3782	94.64 ±0.7727	92.60 ±0.6116
5.0 – 6.0	99.15 ±0.3122	98.45 ±0.5644	98.47 ±0.9018	96.47 ±0.7024	95.30 ±1.1136	93.29 ±0.8992	92.33 ±0.7095
6.0 – 7.0	99.15 ±0.3122	98.40 ±0.1000	97.95 ±1.6020	97.90 ±1.0440	95.67 ±0.5132	93.10 ±1.0440	93.30 ±1.0149
7.0 – 8.0	99.15 ±0.3122	97.45 ±1.7226	97.65 ±1.2379	98.31 ±0.8947	94.67 ±0.4619	93.73 ± 0.8021	92.23 ±0.9018

n=3, SD=Standard Deviation

Appendix S Results of evaluation of parameters

Parameter	FORMULATION								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Appearance	light yellow viscous suspension	light yellow viscous suspension	yellow viscous suspension	yellow viscous suspension	yellow viscous suspension	deep yellow viscous suspension	deep yellow viscous suspension	deep yellow viscous suspension	deep yellow viscous suspension
Redispersibility	readily redispersed	readily redispersed	readily redispersed	readily redispersed	readily redispersed	readily redispersed	readily redispersed	readily redispersed	readily redispersed
Viscosity (mPas)	665± 2.6458	664± 2.0817	668±2.0000	663± 2.5166	666± 2.6458	663± 5.1316	664 ± 2.5166	663± 2.0000	666±4.9329
pH	4.60±0.0351	4.45±0.0153	4.87±0.0656	4.40±0.0451	4.25±0.0500	4.49±0.0907	4.24±0.0416	4.18±0.0200	4.13±0.0115

n=3, SD=Standard Deviation

Appendix T Results of accelerated stability studies on standard formulation

Test Parameter	Results		
	Storage Period (months)		
	0	**3**	**6**
Appearance	yellow viscous suspension	yellow viscous suspension	yellow viscous suspension
Redispersibility	readily redispersed	readily redispersed	readily redispersed
pH	4.35 ± 0.0252	4.25 ± 0.0252	4.17 ± 0.0153
Viscosity (mPas)	674 ± 2.6458	669 ± 1.5275	666 ± 3.5119
Assay (%)	100.50 ± 0.7853	98.87 ± 0.7245	97.59 ± 1.0688

n=3, SD=Standard Deviation