Sudan University of Science and Technology

College of Graduate Studies

Quality Assurance of Aspirin Collected From The Hawkers

توكيد جوده الاسبرين المتحصل عليه من الباعه المتجولين

A Thesis Submitted in Partial Fulfillment for the Requirements of the Degree of M.Sc in Chemistry

By
Mona Abbas Ibrahim Ahmed
(BS.C honor in chemistry)

Supervisor: Dr. Mohamed Sulieman Ali Eltoum

(Des 2020)

Dedication

To

My parent,

Brothers,

And Sisters

Acknowledgment

First of all my sincere thanks to Allah Almighty for helping and supporting me to complete this work.

It’s an opportunity to offer great thanks to my supervisor Dr. Mohamed Sulieman Ali Eltoum for his wonderful support.

The chance was extended to thanks Citypharma staff for opening their laboratory to do practical part of the research.
A lot of thanks for Mr. Mohammed the manager of Citypharma laboratory.

Finally to my colleague for their unlimited support.

Abstract

At the present research the effect of heat and storage condition was studied and investigated for Three group of commercial aspirin (acetyl salicylic acid) samples from different market in Khartoum state (Omdurman, Bahrie, Khartoum). The obtained samples were subjected to physical and chemical tests using different analytical techniques. The average weight of sample of Khartoum was 0.326g, Omdurman and ,Bahrie were 0.325g, 0.334g respectively. The average hardness were found of sample of Khartoum 3.005kg, Omdurman 3.065kg, Bahrie 3.395kg (Reference hardness not more than 10 and not less than 4kg). The average thickness of sample were also measured and it was found for Khartoum 3.1mm, Omdurman 3.15mm , Bahrie .15mm (Reference thickness not more than3.2mm). The result of average friability of sample of Khartoum 1.15%, Omdurman 1.35%, Bahrie 1.15% , (Reference friability not more than 1%). The obtained results showed that the dissolution were 63.86% for Khartoum sample and 63.70%, 66.20% for Omdurman and Bahrie respectively (Reference more than 75%). Finally the average assay of Khartoum was found 83.713% , Omdurman 78.56%, Bahrie 84.593% (Reference according to Britches pharmacopeia 98±102%) From the above obtained results in this research its clear that the aspirin marketed in the street in local market was less effective.

المستخلص

في هذا البحث تم دراسه تاثيردرجه الحراره وظروف التخزين علي تلاته مجموعات من عينات الاسبرين التجاري من اسواق ولايه الخرطوم (امدرمان وبحري والخرطوم علي التوالي). العينات التي تم الحصول عليها خضعت لي تحاليل فيزيائيه وكيميائيه باستخدام مختلف تقنيات التحليل .فنجد ان متوسط الوزن في عينات الخرطوم 0.326جرام, وامدرمان وبحري0.325 جرام 0.334 جرام علي التوالي ,(العينه المرجعيه للوزن 350+)جرام. وايضا متوسط الصلابه في عينات الخرطوم 3.005كيلوجرام وامدرمان 3.065كيلوجرام وبحري 3.395 كيلوجرام(العينه المرجعيه للصلابه لاتزيد من 10 واكثر من 4 كيلوجرام). ووجد ان متوسط السمك في العينات التي تم قياسها ووجد ان عينه الخرطوم 3.1ملي متر وامدرمان 3.15 ملي متر وبحري 3.15 ملي متر(العينه المرجعيه للسمك 3.2 ملي متر). ونتائج متوسط التفتيت في عينه الخرطوم هي 1.15هي % وامدرمان 1.35 %وبحري 1.15% (العينه المرجعيه للتفتيت اقل من 1%).ومن النتائج المتحصل عليها اظهرت ان 63.68%هي متوسط تذويب عينه الخرطوم وامدرمان وبحري 63.70% و 66.20% علي التوالي (العينه المرجعيه للتذويب اكتر من 75%). واخيرا متوسط تركيز عينه الخرطوم هي 83.713% وامدرمان 78.56 %وبحري 84.593 %(العينه المرجعيه للتركيز95 -105%).من النتائج المتحصله اعلاه في هذا البحث يتضح ان الاسبرين الذي يباع في الشارع والاسواق المحليه اقل كفاءه .

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Chapter one

1-Introduction and literature review

1-1 Introduction:

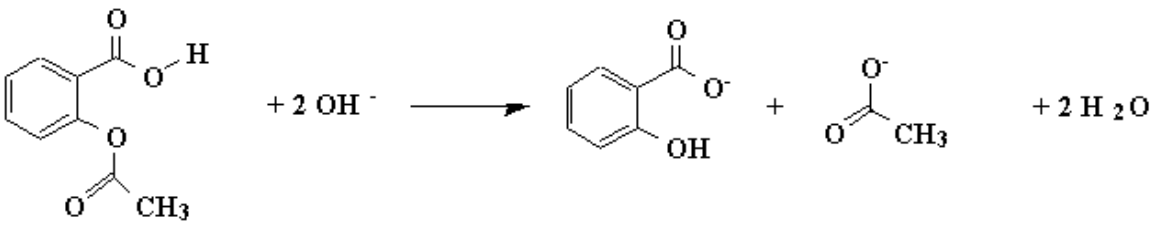
The term aspirin is used, in accordance with its widespread generic usage throughout the world, as the name for the chemical, acetylsalicylic acid.

Aspirin, acetylsalicylic acid, was first synthesized in 1893 by Felix Hofmann, a chemist for the German firm of Bayer. This compound had the medicinal properties of salicylic acid, an extract of willow bark, without the unpleasant taste or the high degree of irritation of the mucous membranes lining the mouth, gullet, and stomach [Rains, 2004].

Aspirin is both an organic ester and an organic acid. It is used extensively in medicine as a painkiller (analgesic) and as a fever-reducing drug (antipyretic). When ingested, acetylsalicylic acid remains intact in the acidic stomach, but in the basic medium of the upper intestinal tract, it hydrolyzes forming the salicylate and acetate ions. However, its additional physiological effects and biochemical reactions are still not thoroughly understood [Rains, 2004].

When ingested, acetylsalicylic acid remains intact in the acidic stomach, but in the basic medium of the upper intestinal tract, it hydrolyzes forming the salicylate and acetate ions:

Equation showed the hydrolysis of aspirin:



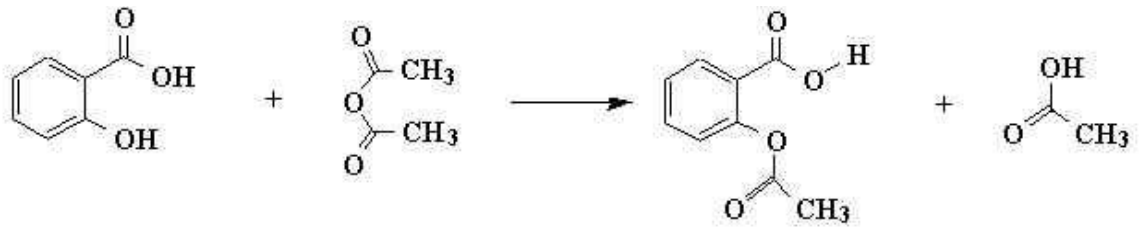
The analgesic effect of aspirin is probably due to the salicylate ion, however; its additional effects are still not well understood. Salicylic acid has the same

therapeutic effects as aspirin, but it causes more severe stomach upset [Rains, 2004].

Aspirin, acetylsalicylic acid, can be easily made from salicylic acid by an organic reaction know as esterification. Specifically in this lab, aspirin will be prepared by allowing salicylic acid to react with

acetic anhydride in the presence of sulfuric acid, which is a catalyst for the reaction. The reaction equation is shown below:

Equation showed the formation of aspirin:



Salicylic Acid Acetic anhydride Aspirin Acetic acid

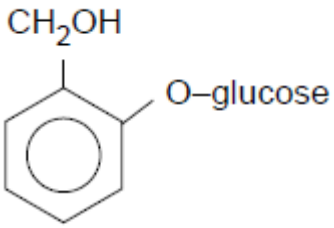
Aspirin is commonly used to reduce the risk of ischemic events in patients with cardiovascular disease. Aspirin inhibits platelets by irreversibly binding to cyclooxygenase (COX) and blocking the synthesis of thromboxane A2. However, patients treated with aspirin still suffer ischemic events, and laboratory assessment of platelet function reveals persistent platelet aggregation despite regular aspirin therapy in a significant proportion of patients at high risk of ischemic events. The concept of aspirin resistance and aspirin non-response has highlighted an area where current antiplatelet treatment may be suboptimal [Rains, 2004].

1-1-2 History of aspirin:

In the 5th century people used the bitter powder extracted from a willow bark to relieve pain.

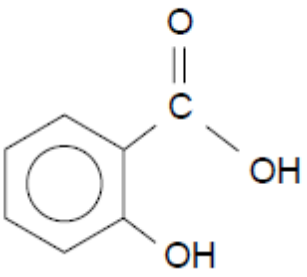
1830s A Scottish physician found that extracts of willow bark relieved symptoms of acute rheumatism.

1840s Organic chemists working with willow bark and flowers of the meadow sweet plant, spirea, isolated and identified the active ingredient as salicin (salix = Latin word for willow) .



Salicin

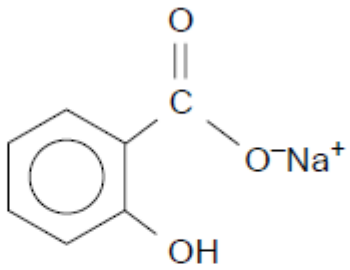
1870 Professor von Nencki of Basle demonstrated that salicin was converted into salicylic acid in the body [Rains, 2004].



Salicylic acid
(2-Hydroxybenzoic acid)

Salicylic acid was then given to patients with fevers and their symptoms were relieved. However, the compound caused severe irritation of the lining of the mouth, gullet and stomach [Rains, 2004].

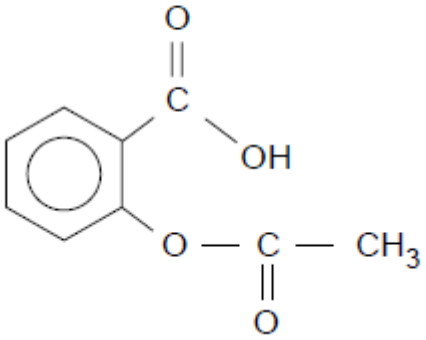
1875 Chemists made sodium salicylate and gave that to doctors to try on their patients. It still worked to help reduce pain and fever and did lessen the irritation, but tasted awful [Rains, 2004].



Sodium salicylate
(Sodium 2-hydroxybenzoate)

In the large doses used for treating rheumatism sodium salicylate frequently caused the patient to vomit.

1890s Felix Hofmann of the Bayer Company in Germany made aspirin which was found to have good medicinal properties, low membrane irritation and a reasonable taste [Rains ,2004].



Aspirin
(2-Ethanoyloxybenzenecarboxylic acid)

He called the new medicine aspirin (‘a’ for acetyl – the systematic name for the compound at the time was acetylsalicylic acid, ‘spir’ for spirea, the meadow sweet plant).

Nowadays chemists use the systematic name, ethanoyl, instead of acetyl; but the trivial name acetyl is still very common.

1898 Aspirin was sent for clinical trials, Bayer manufactured the medicine and patented the process [Rains, 2004].

1915 During World War I the British wanted aspirin but it was made by the Germans (Bayer & Co). So the British government offered a £20,000 reward to anyone who could develop a workable manufacturing process. This was achieved by George Nicholas, a Melbourne pharmacist, who subsequently gave his tablet the name ‘Aspro’.

1990s More than 10 million kilograms of aspirin are made in the US each year!

Nowadays aspirin is not only used as a painkiller but has also been proposed as effective in reducing the incidence of heart disease [Rains 2004].

Indications:

• Local analgetic effect.

• Antipyretic.

• Anti-inflammatory.

• Antiplatelet.

1-1-3 Common-side effects:

• Gastrointestinal complaints (stomach upset, dyspepsia, heartburn, small blood loss).

• Frequently, central effects (dizziness, tinnitus, hearing loss, vertigo, centrally mediated vision disturbances, and headaches.

• Prolonged and more severe bleeding after operations.

• Possibility to get gastric ulcer and kidney injury [Donald 2005].

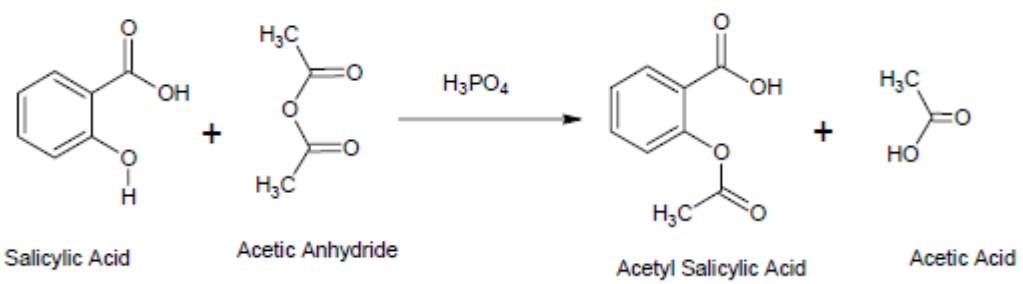
1-1-4 **Synthesis of Aspirin:**

Few synthetic organic compounds have enjoyed such widespread medicinal use as aspirin. Over 30 million pounds of it are consumed each year in the United States alone, as a first line of defense against the discomforts of colds, minor pains, headaches, and arthritis. Even though extracts of willow leaves and bark have been used for centuries for their pain-relieving (analgesic) and fever-reducing (antipyretic) properties, only in the late 1800s was the active ingredient of willow and poplar bark discovered to be salicylic acid [2]. This substance, it was found, could be produced cheaply and in large amounts, but its use had severe limitations because of its acidic properties. Membranes lining the stomach and passages leading to it are irritated by the acid. The side effects of salicylic acid use were often worse than the original discomfort. The breakthrough came in 1893 when a German chemist, Felix Hofmann, synthesized the acetyl derivative of salicylic acid; it proved to have the same kind of medicinal properties without the high degree of irritation to mucous membranes [Donald 2005].

Aspirin tablets also include a binder such as starch to hold the tablet in a stable shape. The usual 5-grain (an apothecary unit of mass) aspirin tablet contains 0.325 g of acetylsalicylic acid. Acetaminophen, sold as Tylenol, Datril, or Anacin 3, is another widely used analgesic. A newer analgesic, ibuprofen (brand names include Advil and Nuprin), became available over the counter in the United States during the 1980s, although it was available earlier as the prescription drug motrin [Donald 2005].

The contents of the stomach are acidic, and most of the ingested aspirin passes through the stomach unchanged. However, under the alkaline conditions in the intestines, aspirin forms sodium acetylsalicylate, which is absorbed through the intestinal wall. Few people suffer serious toxic effects from using aspirin, although some people are allergic to it. People suffering from ulcers may find their condition made worse by the use of aspirin. Aspirin has been cited as a possible contributing factor in Reye's syndrome, which can lead to death. In addition, aspirin seems to interfere with blood clotting. This anticoagulant property of aspirin limits its use for patients anticipating surgery but renders it effective in reducing heart attacks and strokes by preventing or hindering the formation of blood clots. Excess amounts, however, can cause pain, fever, or inflammation. Aspirin reduces the activity of the enzyme prostaglandin synthetase, thereby inhibiting prostaglandin synthesis. Acetaminophen and ibuprofen also work by reducing the level of prostaglandins in the body. Aspirin is prepared by acetylating salicylic acid in a process called esterification**.** Esterification is the reaction of a carboxyl (-COOH) group, and an -OH group to form a carboxylate ester group. In this case the source of the -OH group is the phenolic –OH attached to the ring of the salicylic acid. The acetyl group comes from the acetic anhydride and the reaction is catalyzed by phosphoric acid[Donald 2005].

Equation showed the formation of aspirin:



Most of the non-steroidal anti-inflammatory drugs (NSAIDs) are carboxylic acids.

Aspirin (acetylsalicylic acid, ASA) has been used since the turn of the lastcentury to reduce pain and fever, but the parent compound, salicylic acid, has been known and used since antiquity, owing to its common occurrence as a glycoside in willow bark. Acetylation merely decreases its irritating effect. Among the numerous other salicylates known and used, flufenisal has a longer duration of activity and fewer side effects than aspirin [Donald 2005].

1-1-5 Clinical trials of aspirin:

The first clinical trials of aspirin were performed by Witthauer in 1898 (Witthauer, 1899), and Wohlgemuth (1899) at the University of Leyden Medical Clinic in Berlin. Wohlgemuth studied a total of 10 patients given aspirin 1 to 3 g supplied by the Elberfelders (Bayer's) for the treatment of acute joint rheumatism, juvenile rheumatoid arthritis and a variety of other conditions. Some patients were given aspirin as an alcoholic solution (because of its insolubility in water). Remarkably, in the light of today's knowledge that alcohol markedly increases the irritancy of aspirin, no pain or other symptoms of gastric distress were reported in these patients [Donald 2005].

The early trials with aspirin were in patients with rheumatoid arthritis, yet as noted previously [Donald 2005].

1-1-6 Structure and reactions of aspirin:

The crystal morphology of aspirin has been shown to be a dimer with the hydrogen bonds formed across a centre of symmetry. The length of each hydrogen bond is 2.645 A. Quantum chemical calculations have shown that the dimer with two hydrogen bonds is more stable through two carboxyl groups. The

interaction energy of the hydrogen bond dimer is due to charge transfer and electrostatic interaction.

These features of the crystal structure of aspirin are obviously of importance for formulation of the drug, markedly affecting dissolution as well as absorption of oral forms of the drug.

Aspirin appears to have the characteristics of an acid anhydride [Sarkar ,Nahar 2019]. It has been suggested by Davidson and Auerbach (1953) that the acetylating capacity of aspirin may be accounted for by assuming equilibrium between aspirin and salicyloyl acetic anhydride [Sarkar, Nahar 2019].

1-1-7 Solubility of aspirin:

One gram dissolves in 300 ml water at 25º, in 100 ml water at 37º, in 5 ml alcohol or chloroform, in 10–15 ml diethyl ether.

Thermal decomposition of aspirin leads to the formation not only of salicylic and acetic acids but also acetyl salicylic acid, acetyl acetyl salicylic acid and cyclic polymers of salicylic acid. Thus, pyrolysis of aspirin with simultaneous distillation of products at 300 to 350°C (15m) produces these cyclic polymers termed salicylates [Sarkar, Nahar 2019].

1-1-8 Pharmaceutical incompatibilities and stability:

• Decomposed by boiling water or when dissolved in solution of alkali hydroxides and carbonates.

• Hydrolysis occurs in admixture with salts containing water of crystallisation.

• Aspirin forms a damp, pasty mass when titrated with acetanilide, phenacetin, antipyrine, aminopyrine, methenamine, phenol or phenyl salicylate[Sarkar, Nahar 2019].

• Powders containing aspirin with an alkali salt such as sodium bicarbonate become gummy on contact with atmospheric moisture.

• Solutions of the alkaline acetates and citrates, as well as alkalis themselves, dissolve aspirin, but the resulting solution hydrolyses rapidly to form salts of acetic and salicylic acids. Sugar and glycerol have been shown to hinder this decomposition [Sarkar, Nahar 2019 .].

1-1-9 Hydrolysis of aspirin:

Aspirin undergoes both spontaneous and enzymatic hydrolysis to salicylate, but the spontaneous hydrolysis is insignificant in the body. This is shown by the half-life of 15.5 hours in physiological buffered saline. The half-life should be little different or even longer at any point within the pH range of 1.2 to 8.0. This half-life of spontaneous hydrolysis is far longer than the half-life of elimination (about 10 minutes) and the half-life in plasma in vitro (about 30 minutes) [Sarkar, Nahar 2019].

Enzymatic hydrolysis of aspirin occurs in a variety of tissues, including the liver, gastrointestinal tract, hind limbs and blood. The major enzymes hydrolysing aspirin in human plasma are probably cholinesterases, since the hydrolysis of aspirin is inhibited by classical anticholinesterases [Sarkar, Nahar 2019].

1-1-10 Non-enzymic hydrolysis of aspirin:

The spontaneous hydrolysis of aspirin varies markedly with pH, and the presence of counter ions. Under the acidic conditions present in the stomach (i.e. pH 2 to 3), the rate of hydrolysis is much lower than at higher pH values (greater than 9 to 11), where the rate increases dramatically. The rate of hydrolysis of aspirin at pH 5 to 8 (such as it is in the upper intestinal tract) is about double that at pH 2, where it is at a minimum. The pH hydrolysis curve varies somewhat according to the buffer system employed [Sarkar, Nahar 2019].

The rate of hydrolysis of aspirin increases markedly in the presence of 10 to 90 per cent aqueous ethanol mixtures. This is of relevance in view of the frequent consumption of alcohol with aspirin. Interestingly, no appreciable hydrolysis occurs in absolute methanol or propanol, but does so in aqueous methanol mixtures where it occurs at a higher rate than that in water. Hydrolysis of aspirin in aqueous media is reduced by the addition of sorbitol. Ammonium ions and amines (e.g. histamine) and amino acids (at less than 20mmol per litre) also stimulate hydrolysis of aspirin, but porcine mucus (0.05 to 5.0 per cent) and pepsin (0.1 to 2.5 per cent) [Sarkar, Nahar 2019].

1-1-11 Aspirin anhydride:

This drug was first considered in 1908) as a possible precursor of aspirin in view of it being a dimeric product that would hydrolyse to aspirin, and so release aspirin in vivo[Sarkar, Nahar 2019].

Some physicochemical evidence suggested that this drug might have significant advantages over aspirin, but this later proved largely unfounded. An earlier report also claimed that this drug had fewer gastrointestinal side effects in man compared with aspirin, but later studies failed to substantiate these claims. This anhydride has low bioavailability in man, and is therapeutically less effective than aspirin in the treatment of rheumatoid arthritis; it also shows considerable gastric irritancy in animals [Sarkar, Nahar 2019].

1-1-12 Absorption of aspirin:

Aspirin is quite lipid-soluble in the un-ionised form with a log P value (logarithm of partition coefficient of the un-ionised form between octanol and water) of 1.19, while salicylic acid is much more lipid soluble with a log P value of 2.26. Given that the pKa values of aspirin and salicylic acid are 3.5 and 2.97, respectively, the un-ionised species are major forms only in the stomach and in the upper small intestine [Sarkar, Nahar 2019].

Once in solution, both aspirin and salicylate are totally absorbed from the gastrointestinal tract although several factors influence the rate of absorption of both aspirin and salicylate. The effective absorption of aspirin is, however, incomplete due to first-pass metabolism in the liver. Its effective absorption from solution is about 50 to 70 per cent in man, and is constant over a wide range of doses[Sarkar, Nahar 2019].

Various factors affect the rate of absorption of aspirin and salicylate. These include the physicochemical properties of the compounds, the pH of the gastrointestinal lumen, the surface area of the tract, the rate of gastric emptying, and intestinal transit times. The rate and extent of absorption is also greatly affected by the pharmaceutical formulation, which, together with the pH of the immediate environment, controls the dissolution of the salicylates within the gastrointestinal tract [Sarkar, Nahar 2019].

The gastric absorption of aspirin is limited, despite the high proportion of the un-ionised aspirin present, its absorption being restricted by the surface area of the mucosa. Thus, only about 12 per cent of the mass of aspirin is absorbed from an unbuffered solution after 10 minutes in the stomach, and the extent of absorption decreases to about 1 per cent if gastric pH is increased to above 6, because most of the aspirin is then in the less permeable ionised form. However, the slower gastric absorption obtained after increasing the pH of gastric contents is not reflected in slower overall absorption in vivo, since buffered solutions of aspirin are quickly emptied into the small intestine, where absorption is rapid[3].

On an empty stomach, solutions of aspirin salts are absorbed quite quickly in man with a half-life of absorption ranging from 5 to 16 minutes. With a short half-life of elimination, the rate of absorption of aspirin profoundly affects the pattern of plasma concentrations after its oral administration. In particular, the peak levels of aspirin are markedly reduced with slowing the rate of absorption, which occurs particularly if aspirin is administered with meals, or as sustained

release or enteric-coated preparations. For acute conditions, such as acute pain or as an acute antiplatelet agent after myocardial infarction, aspirin should be administered in solution or by chewing buffered tablets [Sarkar, Nahar 2019].

1-1-13 Accumulation of aspirin:

Because of its short half-life, aspirin does not accumulate significantly in plasma during its long-term administration, although since it irreversibly acetylates cyclooxygenases its effects may outlast the transient appearance of unchanged aspirin [Bettelheim, Landesberg 2000].

1-1-14 Presystolic metabolism of aspirin:

Aspirin is stable in gastric and duodenal fluids, and is therefore absorbed by the gastrointestinal tract as unchanged aspirin. While esterases are present in the gut wall and liver, the major site of presystemic metabolism of aspirin in man is in the liver [Bettelheim, Landesberg 2000].

1-1-15 Enteric-coated formulations of aspirin:

Many enteric formulations of aspirin have been prepared in order to decrease its upper gastrointestinal toxicity. Protection of the gastrointestinal tract is, however, only partial. Enteric coatings are applied to whole tablets or to granules that are presented in capsules. In the past the gastrointestinal absorption of such formulations was inconsistent and often incomplete, but modern coatings appear to provide more reliable release of aspirin. The rate of absorption of enteric-coated tablets of aspirin is still variable, largely due to the retention of intact tablets in the stomach, because absorption only occurs after passage of the enteric-coated tablets into the small intestine. Considerable numbers of intact enteric-coated tablets have been recovered from patients with pyloric obstruction [Bettelheim, Landesberg 2000].

Such patients should not be given enteric-coated tablets of aspirin or any other drug. Capsules containing enteric-coated granules of drug are safer in this condition [Bettelheim, Landesberg 2000].

1-1-16 Localisation of aspirin in the stomach:

Gastrointestinal discomfort and damage are clinically significant side effects of aspirin and other nonsteroidal anti-inflammatory drugs. As is well recognised, the therapeutic and side effects of drugs are dependent upon their uptake or binding at sites of action. With regard to the gastrointestinal toxicity of the salicylates and related drugs, it is of note that salicylate is concentrated in the parietal cells of the stomach and it has been suggested that the high concentrations of aspirin and salicylate in the parietal cell may initiate damage to the gastric mucosa. The localisation of aspirin and salicylate in the parietal cells is due to the high pH gradient at the parietal cell. Aspirin is also absorbed slowly from the skin, and it appears to be usefully applied to the skin in the treatment of herpes zoster and post-herpetic neuralgia, although further evaluation is required. The very low levels of aspirin in blood (about 2M) resulting from cutaneous application are still sufficient to reduce prostaglandin synthesis in the gastrointestinal tract with consequent gastric damage [Bettelheim, Landesberg 2000].

1-1-17 Distribution of aspirin:

Aspirin distribute widely throughout the body, although their volumes of distribution during the elimination phase are only about 10 litres after low doses in adults. Although aspirin is bound to plasma proteins to a lesser extent than other non-steroidal anti-inflammatory drugs, their low volumes of distribution indicate that binding to tissue constituents is still lower than to plasma proteins [Bettelheim, Landesberg 2000].

1-1-18 Binding of aspirin to plasma proteins:

Unchanged aspirin binds both irreversibly and reversibly to plasma proteins. Irreversible binding involves the transfer of the acetyl group to bind covalently to the plasma proteins. The precise extent of reversible binding of aspirin to plasma albumin is difficult to determine, because of both the covalent binding and hydrolysis to salicylate, but the reversible binding of aspirin is about 60 per cent, with the bulk of the binding being to albumin [Lednicer, Mitscher 1977].

1-1-19 Tissue uptake and effects of the aspirin:

Uptake of any drug at its site of action is clearly a prerequisite for its therapeutic or toxic effects, and with aspirin and similar drugs their anti-inflammatory effect is consistent with their more marked uptake in inflamed than in non-inflamed joints of the rat. Their toxic effects on the liver and kidney have also been related to their uptake in these tissues. Aspirin also permeate into the eye and persist in aqueous and vitreous humours for a longer time than in plasma. The presence of significant concentrations of aspirin may be responsible for its reported prevention of cataract formation through local acetylation of lens protein or other constituents of the eye [Lednicer, Mitscher 1977].

1-1-20 Acetylation of proteins by aspirin:

A minor, although pharmacologically important, mode of metabolism of aspirin is by acetylation of a variety of proteins. A large number of proteins are acetylated in vivo and in vitro, including plasma albumin, other plasma proteins, various enzymes, membranes of red blood cells, haemoglobin and renal proteins. The acetylation of albumin inhibits the reaction between glucose and albumin (glycosylation) and, to a lesser extent, the reaction between glucose and haemoglobin [Lednicer, Mitscher 1977].

The clinical significance of this latter reaction is not known at this stage, in contrast to the more detailed knowledge about the acetylation of prostaglandin endoperoxide synthase-1 synthase-2 (COX 1 and 2) by aspirin. Acetylation occurs at one serine of both these enzymes, and is at least partly responsible for the pharmacological properties of aspirin. In particular, the irreversible acetylation of cyclooxygenase of platelets causes the prolonged inhibition of platelet function by aspirin [Lednicer, Mitscher 1977].

1-1-21 Pharmacokinetics of aspirin:

Unchanged aspirin can be detected in plasma for about 1 hour after its intravenous or oral administration [Lednicer, Mitscher 1977].

Following its intravenous administration in man, it has a distribution half-life of about 3 minutes, an elimination half-life of 10 minutes and a clearance of about 800 ml blood/min. Aspirin is hydrolysed enzymatically in blood, but its clearance in blood accounts for only about 15 per cent of the total body clearance of the drug and the bulk of the clearance is considered to occur in the liver. By contrast, the clearance of aspirin in the rat is dose-dependent and at a low dose (40 mg/kg) is slightly greater than hepatic blood flow, indicating significant extra hepatic hydrolysis [Lednicer, Mitscher 1977].

1-1-22 Elimination of aspirin:

The major mode of elimination of aspirin is by hydrolysis to salicylate. Because of its rapid hydrolysis only small amounts of aspirin are excreted unchanged in urine, and essentially all the aspirin is eliminated in urine as salicylate and its further metabolites.

The salicylates are, in comparison with other NSAIDs and analgesics, of relatively low toxicity when taken at therapeutic dosages, and this is probably one of the main features accounting for the success of this group of drugs. Yet the

discovery (or rediscovery), of some minor side effects at various times leading to concern about the safety of these drugs, usually leads to improved understanding about the relative importance of these effects.

During the therapeutic use of these salicylates the following, in some cases potentially serious, adverse reactions can occur:

1. Upper gastrointestinal haemorrhage and/or ulceration with generalised pain, dyspepsia, diarrhoea, constipation and other signs of abdominal discomfort [gwermuth 2015].

2. Nephrotoxicity, principally from ingestion of aspirin in combination with other analgesic/ anti-inflammatory drugs; also, rarely, renal cell carcinoma [gwermuth 2015].

3. Hepatoxicity, more often manifest in certain disease states (e.g. systemic lupus erythematous and rheumatoid arthritis).

4. Hypersensitivity reactions in the form of rashes, angioedema, urticarial weals or asthma, and rarely Stevens–Johnson and Lyell's syndromes[gwermuth 2015].

5. Teratogenicity and reduced birth weight – the former especially from ingestion of large quantities of these drugs during the first trimester of pregnancy; this appears to be a general problem with many NSAIDs and analgesic drugs.

6. Central nervous system sensory reactions comprising tinnitus (known as 'salicylism' because of frequent occurrence at high doses of salicylates), loss of hearing, vertigo (with nausea) and myopia[gwermuth 2015].

7. Blood dyscrasias (rarely), e.g. agranulocytosis, pancytopenia, aplastic anaemia, thromocytopaenia.

8. Reye's syndrome in children.

Aspirin still has side effects. Haemorrhaging of the stomach walls can occur even with normal dosages. These side effects can be reduced through the addition of coatings or through the use of buffering agents. Magnesium hydroxide, magnesium carbonate, and aluminum glycinate, when mixed into the formulation of the aspirin (e.g., Bufferin), reduce the irritation[gwermuth 2015].

1-1-23**General standard test for drug detection definition as follow:**

i- Weight:

Is an official test performed for non-potent drugs in tablets, capsule, liquid vials, sachets, suppositories etc to ensure the uniformity of content active ingredient indirectly [British Pharmacopeia 2013].

ii- Hardness:

Hardness test or tablet break test is a physical test described by the major compendia to measure the hardness of the tablet and its ability to with stand tensile force during manufacturing steps ,it also reflect an idea about reproducibility[British Pharmacopeia 2013].

iii- Thickness:

Is performed as in house test to control the quality of the tablet and ensure the dimension of the tablet[British Pharmacopeia 2013]

VI -Disintegration:

This test is provided to determine whether tablet or capsules disintegrate within the prescribed time when placed in liquid medium under the experimental condition [British Pharmacopeia 2013]

V-Friability:

Reduction in the mass of the granules or spheroid or in the formation of fragment of granules or spheroids, occurring when the granules or spheroids are subjected to mechanical strain during handling [British Pharmacopeia 2013].

vi- Dissolution**:**

A suitable test may be carried out to demonstrate the appropriate release of the active substances [British Pharmacopeia 2013].

7-Assay:

Is a quantitative chemical test described by the compendia to measure the content of active ingredient per unit dose of finished product or to measure the quantity of substance in a bulk of raw material [British Pharmacopeia 2013].

1-2 **Previous Study On analysis** :

In HPLC method there are several factors such as, the interaction between the solute components and the stationary phase that affected the chromatography resolution. HPLC is a good method for analysis of drugs because it has good selectivity and sensitivity values with small levels and in a complex matrix.

Therefore, the HPLC systems can be easily used to separate a wide range of Chemical components. ASP is a no steroidal anti-inflammatory [Muchtaridi, yuliani, Sopyan 2015], ant rheumatic, antithrombotic; chemically it is 2-acetoxy benzoic Acid an impurity in a drug substance as defined by the International Conference on Harmonisation (ICH) Guidelines, is any component of the drug substance that is not the chemical entity defined as the drug Substance and affects

the purity of active ingredient or drug Substances [Muchtaridi ,Yuliani ,Sopyan 2015] .

The impurity profile of pharmaceuticals is of increasing importance as drug safety receiving more and more attention from the public and from the media. Therefore, identification, quantification, and control of impurities in the drug substance and drug product, are an important part of drug development and regulatory assessment.

There are many methods to investigate the assay analysis of aspirin [Khan,Bandewar ,Zameeruddin, Bharkad 2017]. Literature show that UV method was developed and validated the determination of concentration of aspirin (ASP), impurities from such as salicylic acid (SAL) and heavy metal ions (HMI) in ASP tablets (AAS)[Saeed,Hamzah,Ahmed 2018].

The proposed method can help research studies, quality control and routine analysis with lesser resources available. The results of the assay of pharmaceutical formulation of the developed method are highly reliable and reproducible and is in good agreement with the label claim of the medicines [Saeed,Hamzah,Ahmed 2018].

Another Method developed to aspirin in pharmaceutical dosage by Uv method showed that the aspirin is an antipletelet agent while omeprazole is proton pump inhibitor used in combination for treatment of stroke and other cardiovascular disease. On extensive literature survey it was found that very few methods are reported for Simultaneous estimation of aspirin and Omeprazole in combined dosage form by any analytical technique. These methods were developed on single Aspirin only or combination with other drugs by using UV spectroscopy in tablet dosage form [Yosprala,2016].

And Aspirin [2-(acetyloxy) benzoic acid], acts as an inhibitor of cyclooxygenase. Which results in the inhibition of the biosynthesis of

prostaglandins. It also inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis [Yosprala,2016].

Analytical method for Simultaneous estimation of Aspirin and Omeprazole using Methanol as a solvent on the basis of solubility. The maximum Absorption (λ max) of Aspirin and Omeprazole were found at 276 and 301 nm. respectively [13].

Linearity range for aspirin was given at 10-50 μg/ml with %RSD value 0.997 and Omeprazole was 2-10 μg/ml with %RSD value 0.997. The method was validated for precision and % RSD was found less than 2.0 for both aspirin and omeprazole. The proposed method was statistically validated for standard deviation, relative standard deviation, Coefficient of variance and the results were within the range. Hence the above method was simple, cheap, cost Effective. [Chaudhari,Phalak 2020]

Another Method developed and validated for simultaneous determination of aspirin in pharmaceutical dosage by a sensitive, specific, precise and cost effective High Performance Liquid Chromatographic method of analysis for aspirin in presence of its degradation products [Kumar.Jamadar Krishnamurthy Bhat ,Musmade ,2010].

The method employed Hypersil BDS C18 (100 x 4.6 mm 5μ) column as stationary phase.The mobile phase consisted of sodium perchlorate buffer (pH2.5): acetonitrile: isopropyl alcohol (85:14:1 % v/v). It is pumped through the chromatographic system at a flow rate of 1.5 ml min−1.The UV detector is operated at 275 nm. This system was found to give good resolution between aspirin and its degradation products. Method was validated as per ICH guidelines [Suresh Kumar ,Latif Jamadar,Krishnamurthy Bhat2010 ,Indian pharmacopoeia 2004 ,Mark index2001,Grudeep ,chatwal ,anand2001,Bec;ett,Stnlale 2001].there

is another Method developed and validated of simultaneous determination of Aspirin in pharmaceutical dosage by using Hplc method.

A simple, accurate and reproducible RP-HPLC has been developed for simultaneous determination of Aspirin and Ticlopidine Hydrochloride in Bulk and Tablet dosage form [Shinde Sampat,Kachave ,Chaudhari 2013]. The chromatography was carried out on the Inertsil ODS C18, 250mm x 4.6mm, 5µm column using a mobile phase composition of 0.02M. Potassium dihydrogen o-phosphate (pH 3.7) and Methanol at a flow rate of 1.0 ml/min that provides an optimal resolution of components in an acceptable elution time. The detection was made at 254 nm. The retention times of Aspirin and Ticlopidine Hydrochloride are 3.3min and 7.3min respectively. The calibration curves were linear over the range 16µg/mL to 24µg/mL for Aspirin and 40µg/mL-60µg/mL for Ticlopidine HCl. The interday and intraday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for the Simultaneous determination of Aspirin and Ticlopidine Hydrochloride in bulk and its tablet dosage forms. Accuracy (recoveries: 99.44 and 99.60%) and reproducibility were found to satisfactory. The proposed method was validated as per ICH and USP guidelines and it was found suitable for the routine quality control analysis of the drugs in tablet dosage forms [Shinde Sampat ,Kachave ,Chaudhari 2013].

Research Objective:

The aims of this research are to:

Study the effect of heat and storage on the concentration of active ingredient in aspirin by subjected three group of sample obtained from local market (from hawkers) in Khartoum, Omdurman, and bahrie to physiochemical test which include: weight, hardness, thickness, friability, dissolution, and assay.

From the result obtained one could conclude about the efficiency of the active ingredient in aspirin from local market and street seller.

Chapter two

2-MATERIALS AND METHODS

Chapter two

2-Material and Method

2-1 Samples:

Three group of samples of aspirin were obtained from different market of Khartoum state (Khartoum, Omdurman, bahrie) and storage at room temperature.

2-2 Chemicals:

All chemical used in this research were analytical grade type and includes:

-sodium hydroxide (scharlau Indian).

-hydrochloric acid (scharlau Indian).

-sodium acetate (scharlau Indian).

-glacial acetic acid (scharlau Indian)..

- iron (III) chloride solution (sdf).

-sulphuric acid (scharlau Indian).

-phenol red (scharlau Indian).

-Ethanol (scharlau Indian). .

- Distilled Water.

2-3: Instruments:

-Balance (sensitive balance 4 digits model AG204 serial number 1118432646 Switzerland).

- Hardness instrument (Bulk Scientific model AN350 India).

- Thickness instrument (fernier).

- Disintegration instrument (Bulk Scientific model LT -74 Serial number AE 20B-03-500A India).

- Friability instrument (Bulk Scientific Serial number 400063 India).

- Dissolution instrument (Bulk Scientific model DA -6D serial number 040340 India).

- UV Spectrophotometer (model T500 Serial Number AE1608032 India)

-Burette (GA).

- Flask.

-heater.

-beaker.

- Cylinder (GA)

2-4 Methods:

2-4-1 Method of weight:

The sample (20 tablets of aspirin) was weighed by Balance QC. The results obtained by Balance QC were recorded [British Pharmacopeia 2013].

2-4-2Method of Hardness:

The sample (10 tablet of aspirin) was comprised by hardness QC instrument even brake. The results obtained by hardness QC instrument were recorded [British Pharmacopeia 2013].

2-4-3 Method of Thickness:

The sample (10 tablets of aspirin) was putted by fernier QC. The results obtained by fernier QC were recorded [British Pharmacopeia 2013].

2-4-4 Method of Disintegration:

The sample (aspirin 6 tablets) was putted in disintegration device even all aspirin dissolved. The time required to dissolve all aspirin was recorded [British Pharmacopeia 2013].

2-4-5 Method of Friability:

The sample (20 tablets of aspirin) was weighed by Balance QC and putted in friability device to four minutes and then was weighed. The results obtained by Balance QC were recorded [British Pharmacopeia 2013].

Calculation:

Friability = $(A - A^{-} / A)$ %

Where:

- A = weight of tablets before friability device.
- A^{-}= weight of tablets after friability device.

2-4-6 Identification:

0.5 g of the powdered tablets was boiled for 2 to 3 minutes with 10ml of 5M sodium hydroxide, and was cooled. An excess of 1M sulphuric acid was added to solution, the crystalline precipitated was produced. The crystalline precipitated was dissolved in water, and then the iron (III) chloride solution was

added to aqueous solution, a deep violet colour was produced [British Pharmacopeia 2013].

2-4-7 Salicylic acid:

A quantity of the powdered tablets containing 0.10 of aspirin was shacked with 4 ml of ethanol (96%) and was diluted to 100ml with water at a temperature not exceeding 10°. The solution was filtered, 50 ml of filtrate was transferred to a Nasser cylinder, and the 1 ml of freshly prepared ammonium iron (III) sulphate solution was added to the filtrate in Nasser cylinder. A violet colour was obtained [British Pharmacopeia 2013].

2-4-8 Method of Dissolution test:

The medium of a pH 4.5 buffer was prepared by mixing 29.9g of sodium acetate and 16.6ml of glacial acetic acid with sufficient water to produce 10 litters, and then the aspirin tablet (sample) was taken into buffer solution. The basket was rotated at 50 revolutions per minutes. 20ml of the media was taken and filtered. The absorbance of filtrate was determined at 250nm using spectrophotometer. The same procedure was repeated for standard [British Pharmacopeia 2013].

Calculation:

Dissolution % = absorbance of sample / absorbance of standard ×100.

2-4-9- Method of Assay:

Powder of 20 tablets was weighed, and then was added to 30ml of 0.5M sodium hydroxide. The solution was titrated with 0.5M hydrochloric acid using phenol red solution as indicator. The volume of acid required was recorded.[British Pharmacopeia 2013]

Calculation:

The different between titration represent the amount of sodium hydroxide required to titrate the aspirin.

Each ml of 0.5M sodium hydroxide is equivalent 0.04504

Volume of acid × 0.4504 × A.wt × 1000/ equivalent wt ×molarity of sodium Hydroxide

Where:

Volume of acid = volume of acid titrate the solution of aspirin and sodium hydroxide -volume of acid titrate the sodium hydroxide =2.3

Method of blank :

0.25g of starch and 0.25g cellulose was weighted and then was added to 30ml of 0.5M sodium hydroxide. The solution was titrated with 0.5M hydrochloric acid using phenol red solution as indicator. The volume of acid required was recorded.

Chapter three

3-RESULT AND DISCUSSION

Chapter Three

3-Result and Discussion

3-1 Results:

3-1 Result of weight:

Table (3-1) Result of weight:

Sample	Average weight of Khartoum g	Average weight of Omdurman g	Average weight of Bahrie g	
1	0.321	0.324	0.337	
2	0.331	0.327	0.331	
Average of weight g	0.326	0.325	0.323	

The average of weight less than standard.

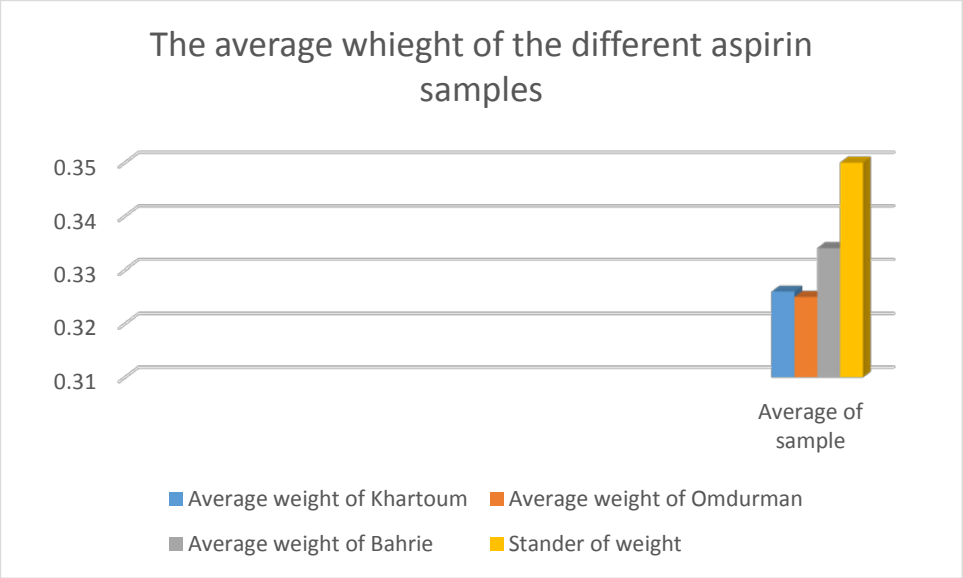


Fig (3-1) the average weight of the different aspirin samples

3-2Result of Hardness:

Table (3-2) Result of hardness:

Sample	**Average hardness of Khartoum kg**	**Average hardness of Omdurman kg**	**Average hardness of Bahrie kg**	Standered of hardness **kg**
1	3.01	3.04	3.39	10-4
2	3.0	3.09	3.4	
Average of hardness	3.005	3.65	3.395	

The average of hardness effect by heat and become less than standard.

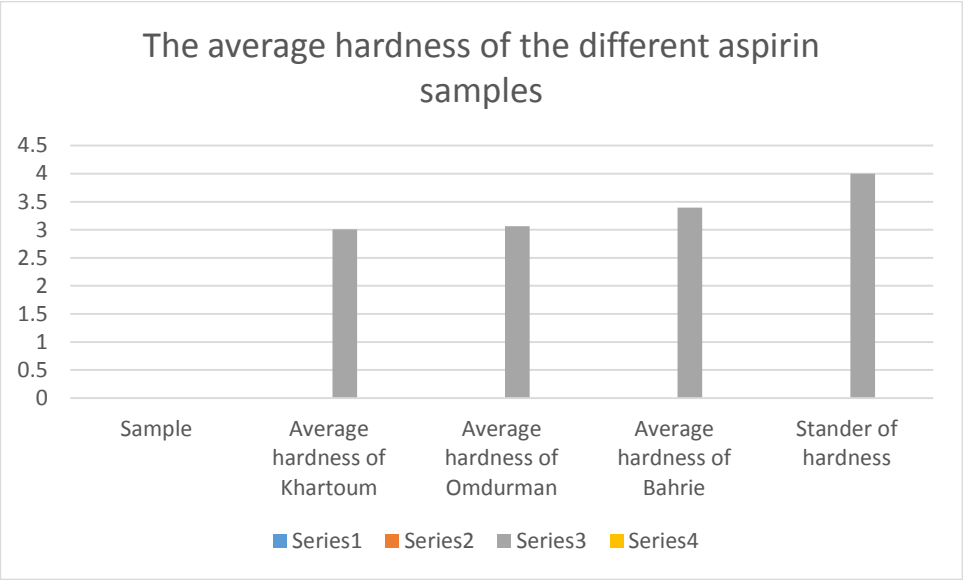


Fig (3-2) the average hardness of the different aspirin samples.

3-3Result of Thickness:

Table (3-3) Result of thickness:

Sample	**Average thickness of Khart** mm **oum**	**Average thickness of Omdurman**	**Average thickness of Bahrie** mm	Stander of thickness mm
1(mm)	3.1	3.1	3.1	3.2
2(mm)	3.1	3.2	3.2	
Average of thickness	3.1	3.15	3.15	

The thickness less than stander.

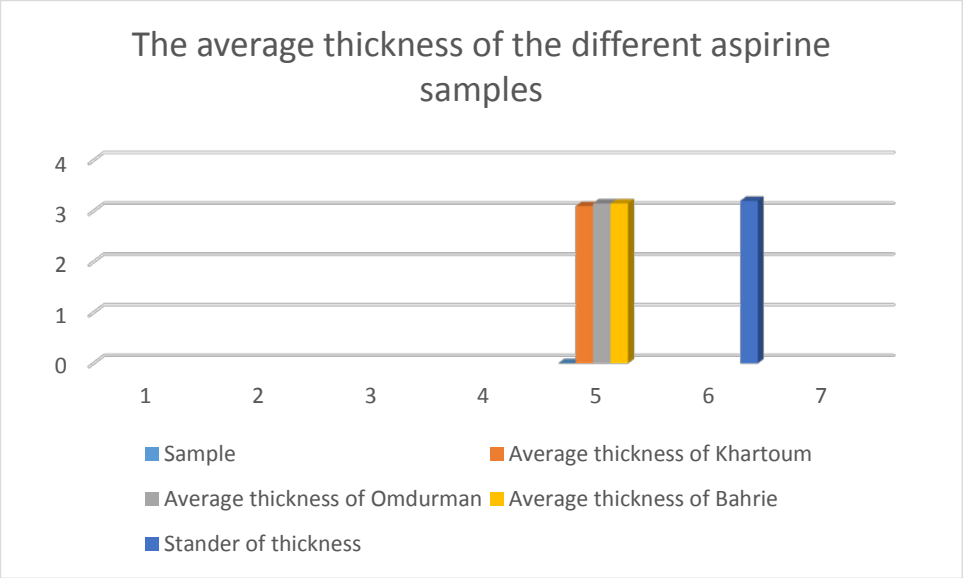


Fig **(3-3) the average thickness of the different aspirin samples.**

3-4 Result of Disintegration:

Table (3-4) Result of disintegration:

Sample	Khartoum (sec)	Omdurman (sec)	Bahrie (sec)	Stander of disintegration
1.disintegration	14	16	17	
2.Disintegration	15	14	16	Less than minute
Average of disintegration	14.5	15	16.5	

The result of disintegration less than standard.

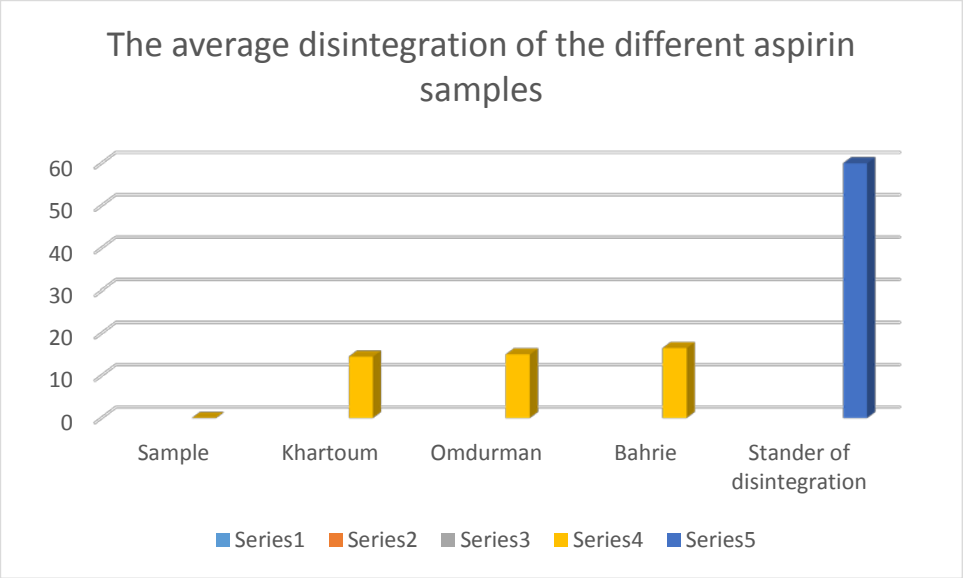


Fig **(3-4) the average disintegration of the different aspirin samples.**

3-5 Result of Friability:

Table (3-5) Result of friability:

Sample	Loss of friability in Khartoum (%)	Loss of friability in Omdurman (%)	Loss of friability in Bahrie(%)	Stander of Friability
Friability	1.2	1.4	1.1	Not more than 1%
Friability	1.1	1.3	1.2	
Average of friability	1.15	1.3	1.15	

The result of friability effected by heat and become the tablet more broken and these lead the result differ than stander.

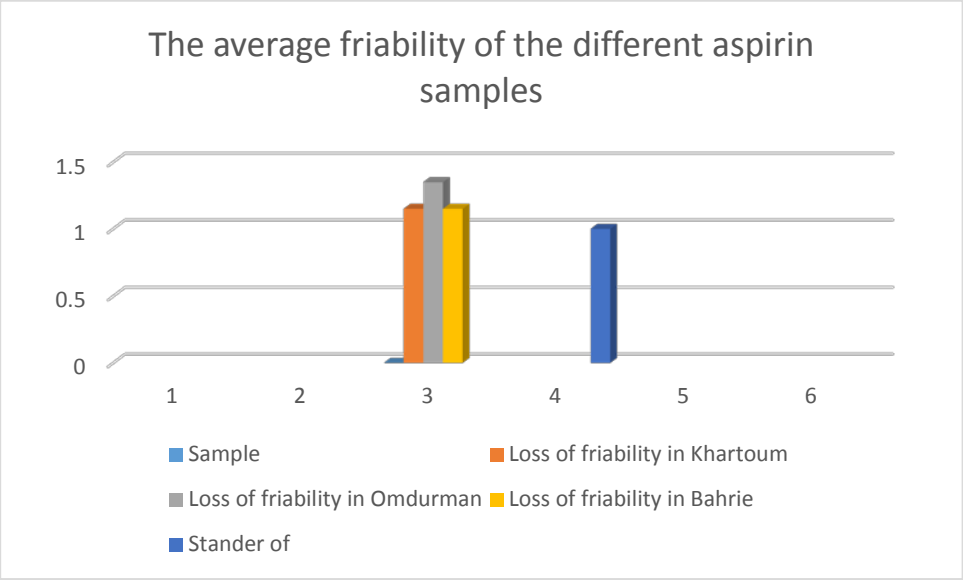


Fig **(3-5) the average friability of the different aspirin samples.**

3-6 Chemical properties:

3-6 Identification:

i- Result:

The aspirin tablet contains ASA.

ii- Salicylic acid:

Non free salicylic acid

3-7 Result of Dissolution test:

Table (3-6) result dissolution of Khartoum:

i-Sample one:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	Stander of dissolution
Absorbance of sample	0.230	0.232	0.227	0.225	0.232	0.229	
Absorbance of standard	0.350	0.350	0.350	0.350	0.350	0.350	More than 75%
Dissolution%	65.70	66.30	64.90	46.00	66.30	65.40	

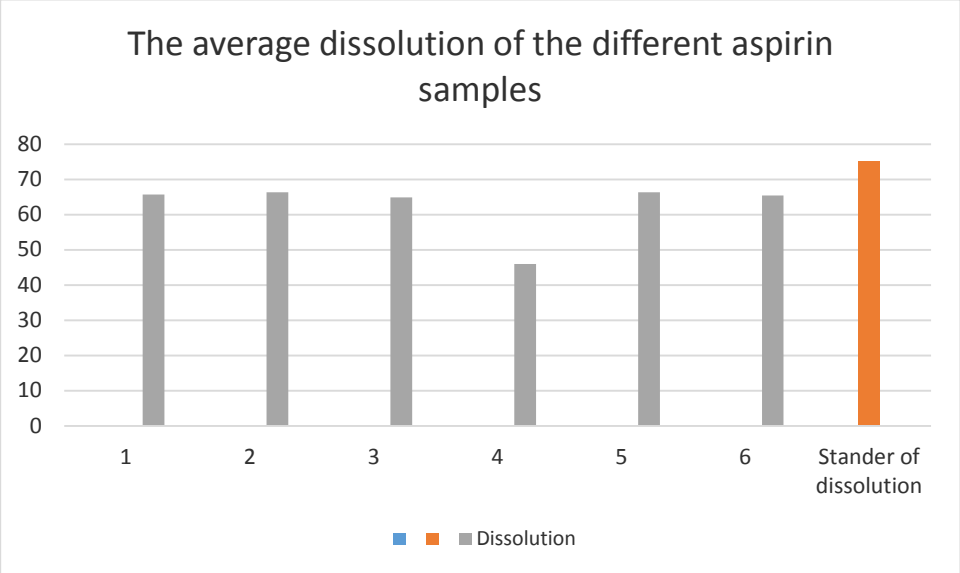


Fig **(3-6) the average dissolution of the different aspirin samples.**

Table (3-7) result dissolution of Khartoum:

ii- Sample two:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	Stander of dissolution
Absorbance of sample	0.225	0.230	0.235	0.227	0.223	0.222	
Absorbance of standard	0.351	0.351	0.351	0.351	0.351	0.351	More than 75%
Dissolution%	64.10	65.50	67.00	64.70	63.50	63.20	

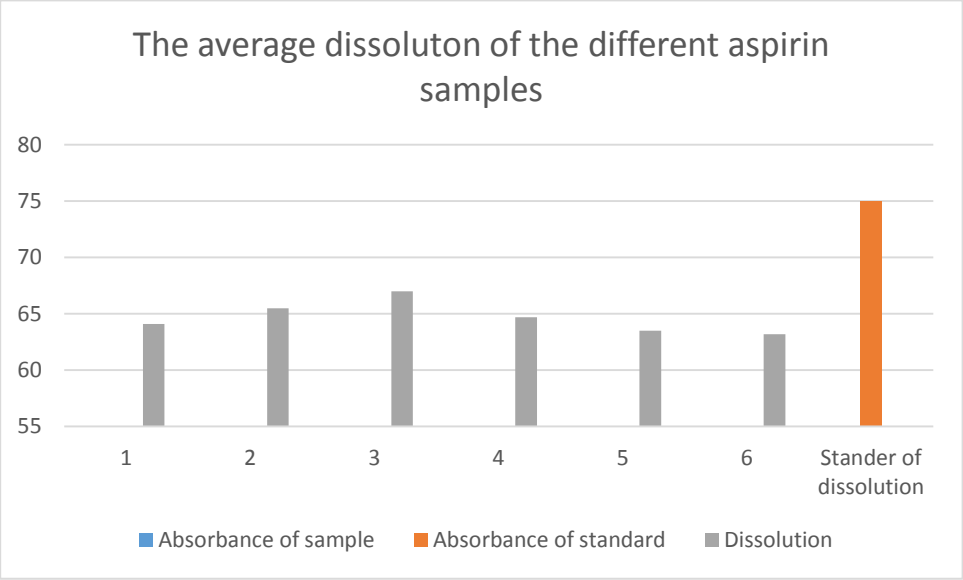


Fig **(3-7) the average dissolution of the different aspirin samples.**

Table (3-8) result dissolution of Omdurman:

i- Sample one:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	Stander of dissolution
Absorbance of sample	0.219	0.218	0.213	0.228	0.227	0.230	
Absorbance of standard	**0**.352	0.352	0.352	0.352	0.352	0.352	More than 75%
Dissolution%	62.20	61.90	60.50	65.00	64.50	65.30	

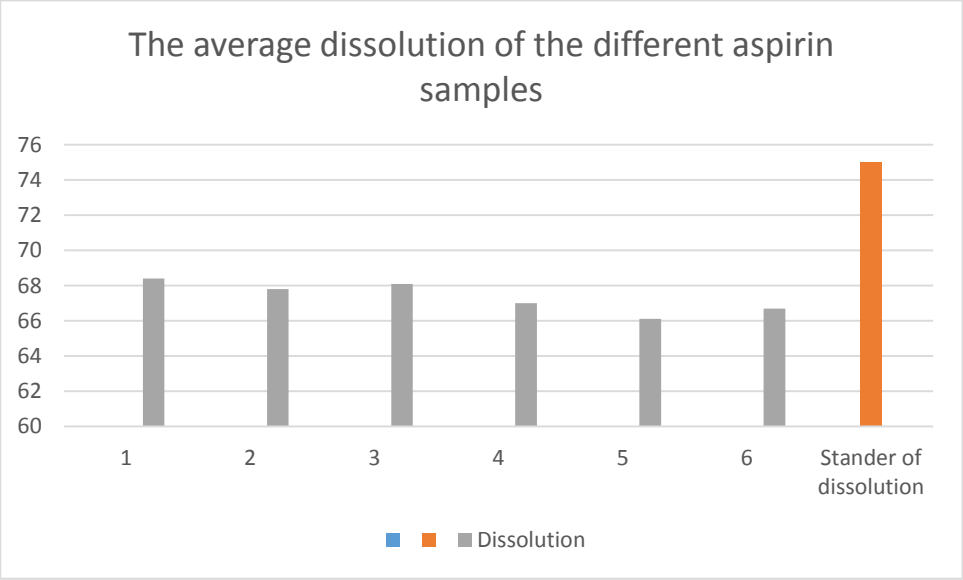


Fig **(3-8) the average dissolution of the different aspirin samples.**

Table (3-9) result dissolution of Omdurman:

ii- Sample two:

Sample	1	2	3	4	5	6	Stander of dissolution
Absorbance of sample	0.235	0.232	0.230	0.228	0.225	0.229	
Absorbance of standard	0.350	0.350	0.350	0.350	0.350	0.350	More than 75%
Dissolution%	67.10	66.30	65.70	65.70	64.30	65.40	

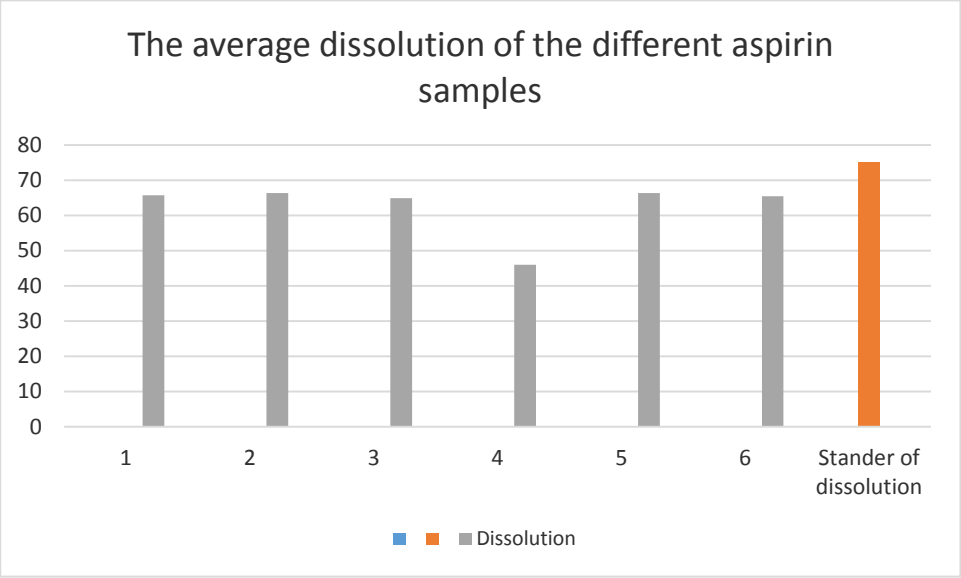


Fig **(3-9) the average dissolution of the different aspirin samples.**

Table (3-10) result dissolution of Bahrie :

i- Sample one:

Sample	1	2	3	4	5	6	Stander of dissolution
Absorbance of sample	0.240	0.238	0.239	0.235	0.232	0.234	
Absorbance of standard	0.351	0.351	0.35	0.351	0.351	0.351	More than 75%
Dissolution%	68.40	67.81	68.10	67.00	66.12	66.70	

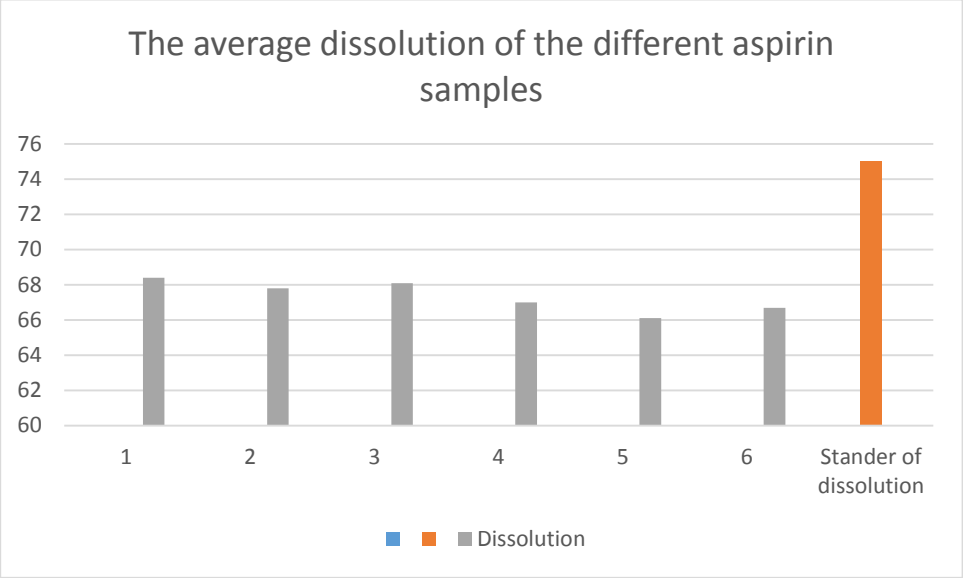


Fig **(3-10) the average dissolution of the different aspirin samples.**

Table (3-11) result dissolution of Bahrie :

ii- Sample two:

Sample	1	2	3	4	5	6	Stander of dissolution
Absorbance of sample	0.233	0.230	0.228	0.225	0.231	0.227	
Absorbance of standard	0.352	0.352	0.352	0.352	0.352	0.352	More than 75%
Dissolution%	66.20	65.31	64.80	63.91	65.60	64.52	

The result of dissolution test effected by heat and become less than stander.

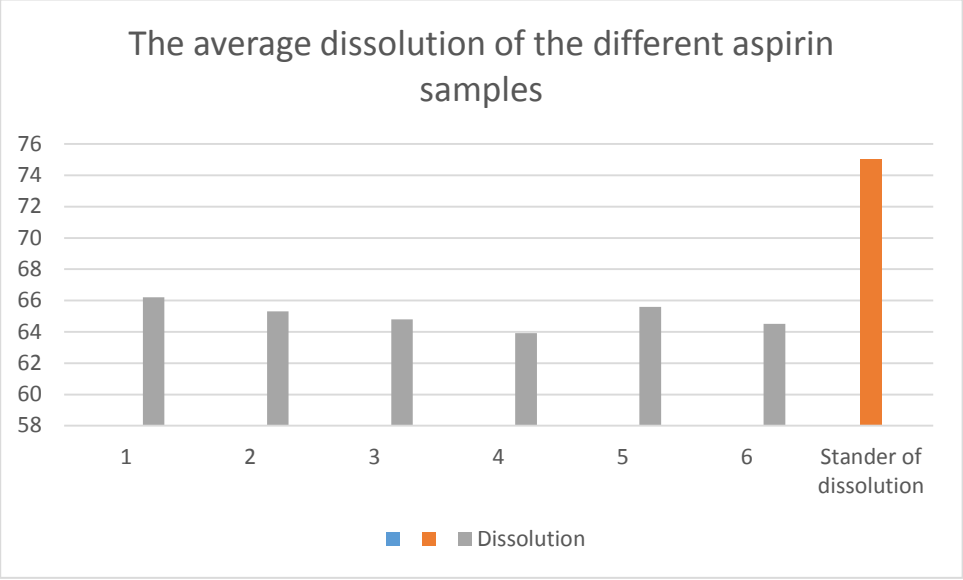


Fig **(3-11) the average dissolution of the different aspirin samples.**

3-8Assay:

The volume of titration blank 45.

The result of titration in Khartoum:

i- Sample one:

Assay = 9.1× 0.49× 0.04504 ×0.321 × 1000 / 1.610× 0.5 = 80.08%

ii- **Sample two:**

Assay=9.5×0.04504×0.49×0.331×1000/0.5×1.660=83.612%

The result of titration in bahrie:

i-Sample one:

Assay=9.5×0.04504×0.49×1000×0.337/0.5×1.685= 83.814%

ii-Sample two:

Assay =9.7×0.49×0.04504×1000×0.331/0.5×1.660=85.372%

The result of titration in Omdurman:

Assay=9.0×0.49×0.04504×0.327×1000/0.5×1.635=79.45%

ii- Sample two:

Assay=8.8×0.04504×0.49×1000×0.324/0.5×1.620=77.685

Table (3-12) result assay of aspirin samples:

Sample	Average assay of Khartoum%	Average assay of Omdurman%	Average assay of Bahrie%	Stander of assay
1	80.08	79.45	83.81	100±2
2	83.61	77.68	85.37	
Average of assay%	81.84	78.56	84.59	

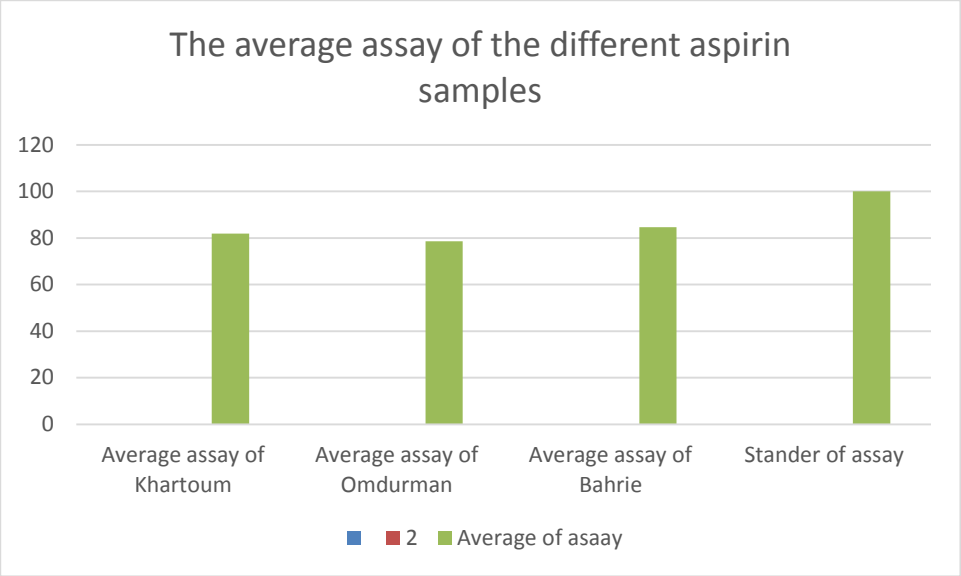


Fig **(3-12) the average assay of the different aspirin samples.**

- The result of assay test effected by heat and become less than stander and these mean the concentration of acetyls salicylic acid effect become less.

Frome the results in compare with B.p there is variation in standard result, and these variation come from bad store in bad condition.

For example:

The result of friability in stander pharmacopeia less than% 1 but in this research the result more than% 1.

The result of hardness between 4kg and less than 10kg but in the result of research less than 4kg.

The result of dissolution in stander pharmacopeia more than %75 but in these research less than %75.

The stander result of assay 100% which is far greater than the results obtained in this research (Khartoum, Omdurman, Bahrie)

All these variation in result come from bad store and heat and became the less effected.

Conclusion:

The following points may be considered as a conclusion to the forgoing article:

1- The result of physical tests were carried out should be achieve the allowed value.

- The hardness of aspirin tablet should be less 10kg more than 4 kg.
- The thickness of aspirin tablet should be less 6mm more than 3.2 mm.
- The disintegration of aspirin tablet should be less than minute.
- The friability of aspirin tablet should be less 1%.

2- The final product (aspirin tablet) should be not containing salicylic acid because the salicylic is very toxic.

3- The dissolution of aspirin tablet must be more than 75%.

4- The assay of any drug must be 100%±2.

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