APPLICATION OF STARCH AND ITS DERIVATIVE FROM CASSAVA (*Manihot esculenta* Crantz.) AS DISINTEGRANTS

IN PHARMACEUTICAL TABLETS FORMULATION

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Abstract

The main aim of the research work focused on the application of the starch and its derivative prepared from cassava (*Manihot esculenta* Crantz.) and their application in pharmaceutical tablets formulation. The cytotoxicity of aqueous solution of local cassava starch (LCS) and its derivative carboxymethyl cassava starch (CMCS) were evaluated by brine shrimp cytotoxicity assay. According to the brine shrimp cytotoxicity assay, LCS and CMCS were observed to be free from cytotoxicity (LD_{50} > 1000 ppm), up to 1000 μg/mL concentration. The morphological characteristics of LCS and CMCS were recorded by using standard plate count method and Gram staining method indicating, the absence of *Escherichia coli* in both samples. LCS and CMCS were applied as disintegrants with different weights in chlorpheniramine maleate tablets formulation. 2.3 % (w/w) of LCS and 1.2 % (w/w) of CMCS were found to be most suitable for chlorpheniramine maleate tablets formulation determined by their physical properties such as moisture content, thickness, hardness, friability and disintegration times in distilled water as well as 0.1 M HCl solution.

Keywords: cytotoxicity, microbiological characteristics, tablets formulation, physical properties

Introduction

Carboxymethylation of polysaccharides is a widely studied conversion since it is simple and leads to products with a variety of promising properties. The polysaccharides (10 g) is activated with 80 mL of 40 % sodium monochloroacetic acid in the presence of 4 mL of 2 M NaOH solution.This

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first step is one mole of polysaccharides transformed into an alkoxide form. In the second step etherification occur to give synthesis yielding the carboxymethyl (CM) polysaccharide derivative. Not only cellulose and starch but also various polysaccharides from different sources are applied as starting materials (Ashok and Jitendra, 2012). Toxicology is the study of the adverse effects of chemicals, physical or biological agent on living organisms and the ecosystem. Brine shrimp lethality assay was applied for toxicity test since it is considered as a useful for preliminary assessment of toxicity. Its has been established as a safe, practical and economic method for determination of bioactivities of synthetic compound as well as plant products. Brine shrimp lethality bioassay is a rapid and comprehensive bioassay for the bioactive compounds of natural and synthetic origin. This method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in the microwell scale. The assay is widely used in the evaluation of toxicity of fungal toxics, heavy metals, pesticides, and medicines etc. (Lee *et al.,* 1999). Reasons for microbial analysis are to meet certain set standards, to estimate the shelf-life of the product, to determine quality of the products and for public health purpose (Kiiyukia, 2003). Local cassava starch were used as binder and disintegrant in solid dosage form, but due to poor flow ability their utilization is restricted. So, starch can be modified by using chemical modification. Modified starches have been developed known as disintegrant, which are used to improve the efficiency of solid dosage forms. This is achieved by decreasing the disintegration time which in turn enhances drug dissolution rate. Disintegrants are an essential component in tablet formulation. A disintegrant is added to facilitate the rupture of bonds and subsequent disintegration of the tablets. This increases the surface area of the drug exposed to the gastrointestinal fluid. The oldest and still the most popular disintegrants are corn, potato starch that has been well dried and powdered. Nowadays cassava starches are the most widely used as disintegrant in pharmaceutical industry. Local starch has certain limitation and the concentration of starch in a conventional tablet formation is normally up to 10 % w/w. So, modified starches are now commonly used and it has specialized characteristics. Modified starch are used as disintegrants, it was found that the higher dissolution rates, find dispersion of particle form and disintegrant within two minutes. There are two methods of disintegrating agents into the tablet, (1)

Internal addition and (2) External addition. In external addition method, the disintegrant is added to the sized granulation with mixing prior to compression. In internal addition method the disintegrant is mixed with other powders before wetting the powder mixtures with the granulation fluid (Desia *et al.,* 2016). In this research, local cassava starch and carboxymethyl cassava starch were investigated the cytotoxicity of aqueous solution by brine shrimp cytotoxicity assay. It was indicated the microbiological analysis and to be used as potential pharmaceutical disintegrating agents for tablet formulation.

Materials And Methods

In the experiments, the chemicals used were procured from the British Drug House (BDH) Chemical Ltd., England. All specific chemicals used were cited in detail in each experimental section. In all investigation, the recommended methods and standard procedures involving both conventional and modern techniques were employed. Instruments employed in this work consists of conventional laboratory wares, glassware and other supporting facilities. Some of the instruments used in this study were balance (310 ± 0.1 mg) (LA 310 satorius AG, Gottingen, Germany), Granulating & Drying Machine (Model No. Strea-1 Fuji Sangyo. Co., Ltd., Japan), Mixer, Labo-Mill (Model No-OT 21, Yamato Co., Ltd., Japan), Stainless Steel Sieve (16 mesh, 32 mesh, 24 mesh, 100 mesh), Tableting Machine (Model No. Clean Press 19, Kyoto, Japan), Y-cone Blender (Model No-H 25, Yamato Co., Ltd., Japan). Infrared Moisture Meter (Kett) (Model No. F.1 A, Kyoto, Japan), Electronic Digital Caliper (Model No. E-23112, Peacock Co., Ltd), Hardness tester (Model No. D-63512 (Hainburg) Germany), Friabilator (Model No. D-63512 (Hainburg) Germany), Disintegration tester (Model No. D-63512 (Hainburg) Germany).

Preparation of Local Cassava Starch and Carboxymethyl Cassava Starch

The local cassava starch (LCS) was prepared by using conventional method. The local cassava starch (10.00 g) was dispersed in 200 mL of isopropanol/water (4:1, v/v) aqueous solution and 4 mL of 2 M NaOH solution was added with stirring. The mixture was stirred at room temperature for 10 min. 40 % sodium monochloroacetate (80 mL) was added and the mixture was

stirred for further 30 min. The pH of the mixture was then adjusted to about 5.0 by addition of 50 % glacial acetic acid. The carboxymethyl starch was filtered by Buchner funnel and washed with ethanol (95 %) until neutral condition was reached. The modified starch was dried at 50 °C for 6 h.

Study of Cytotoxicity and Microbiological Screening of the Local Cassava Starch and Carboxymethyl Cassava Starch

(a) Investigation of cytotoxicity by brine shrimp bioassay

The sample solution was prepared by dissolving 5 mg of respective sample in 5 mL of distilled water. The stock solution was tenfold diluted serially with distilled water to get the sample solution with the concentrations of 1000, 100, 10 and 1 μg/mL. Test solution (1 mL) was mixed with 9 mL of artificial sea water and placed in the chamber of ice cup. Alive brine shrimp (10 napulli) was taken with pasteur pipette and placed into each chamber which was kept at room temperature for about 24 h. After 24 h incubation, the number of survival brine shrimp was counted and 50 % lethality dose (LD_{50}) was calculated (Dockery and Tomkins, 2000). The control solution was prepared as the above procedure by using distilled water instead of sample solution. The cytotoxicity of different doses of tested samples are described (Table 1).

(b) Investigation of microorganisms by Agar Plates Count method

The agar plates count method was used to detect the microbiological action on local cassava starch and carboxymethyl cassava starch. Sample (0.1 mL) was spread into nutrient agar plates and potato dextrose agar plates, and then incubated in an incubator at 32 °C for 24 h. Single colony was transferred into EMB medium and incubated at 32 °C for 48 h.

Application of Local Cassava Starch and Carboxymethyl Cassava Starch in Tablet Manufacturing Process

Chlorpheniramine maleate tablets manufacturing

In this research work, chlorpheniramine maleate tablets were prepared by wet granulation method. Different weights of the disintegrants were used to

prepare tablets. Firstly, chlorpheniramine maleate (4 mg) was mixed with rate controlling polymers, calcium phosphate, calcium sulfate, colour in mixer granulator and then the filling agent lactose monohydrate powder added as compression aid. The mixture was wetted by addition of water and then granulated. Granules were dried in fluidizing, granulating and drying machine at 50 °C for 12 h and then passed through the 16 mesh sieve. The dried granules were mixed with local cassava starch (1.8 % and 2.3 %) or carboxymethyl cassava starch (1.2 %, 1.8 % and 2.3 %) powder in a motor mixer. The complete mixture was compressed into plain tablet by using 5 mm diameter, deep punches and dies in the rotary tablet press.

Quality Control and Measurement of Tablet Properties for Chlorpheniramine Maleate Tablets

(a) Determination of moisture contents of tablets

Sample (5 g) was placed in the pan of the Infrared Moisture Meter (Kett), which was measured under infrared lamp at 80 °C for 10 min. By direct reading from the infrared moisture meter, it indicated the loss of moisture in percentage from the samples. The test was repeated for three times and the average moisture content in percentage was calculated.

(b) Determination of thickness of tablets

The thickness of five tablets of chlorpheniramine maleate prepared with different weights of the LCS and CMCS was measured by Electronic digital caliper (Model No. E 23112, Peacock Co., Ltd). The means and standard deviation were also calculated for each sample.

(c) Determination of tablets hardness

Twenty tablets were randomly selected from each different weights of the tablet. Digital tablet hardness tester was employed to determine the mechanical strength of the tablets. A tablet is placed between two anvils, force is applied to the anvils and the crushing strength that just causes the tablet to

break is recorded, the average force required to crush the tablets sample was calculated.

(d) Determination of disintegration time of tablets

Six tablets from each different weights of sample were utilized for disintegration studies in distilled water and 0.1M hydrochloric acid at 37 °C ± 0.5 °C using a pharma test disintegration apparatus. Tablets were placed in each of the six tubes of the basket. The basket was mechanically raised and lowered in the immersion medium. The distintegration time of tablet was recorded when all particles from the tablet passed freely through the mesh of apparatus.

(e) Determination of tablets friability

To evaluate the degree of friability of the tablets from each tablet sample, ten tablets were randomly selected, dusted and weighed. These tablets were placed in a roche friabilator which rotated 100 times in 4 min. Afterwards the tablets were once again dusted and reweighed to determine the percentage loss of weight.

$$\% \text{ Friability} = \frac{W_1 - W_2}{W_1} \times 100$$

where,

W_1 = weight of tablets before operation

W_2 = weight of tablets after operation

Results and Discussion

Cytotoxicity of Local Cassava Starch and Carboxymethyl Cassava Starch

The cytotoxicity of different doses (1000 ppm, 100 ppm, 10 ppm, 1 ppm) of LCS and CMCS against *Artemia salina* (brine shrimp) is shown in Table 1. The cytotoxic effects were expressed in terms of mean ± SEM (standard error mean) and LD_{50} (50 % Lethality Dose). In this experiment,

potassium dichromate and caffeine were used as cytotoxic standard. As shown in (Table 1), LCS and CMCS were not cytotoxic to brine shrimp up to maximum dose of 1000 μg/mL. The LD_{50} standard $K_2Cr_2O_7$ and caffeine are 43.74 μg/mL and 1000 μg/mL respectively. According to the results, LCS and CMCS were found to be free from cytotoxicity.

Table 1. Cytotoxicity of Different Doses of Local Cassava Starch and Carboxymethyl Cassava Starch against *Artemia salina* (Brine Shrimp)

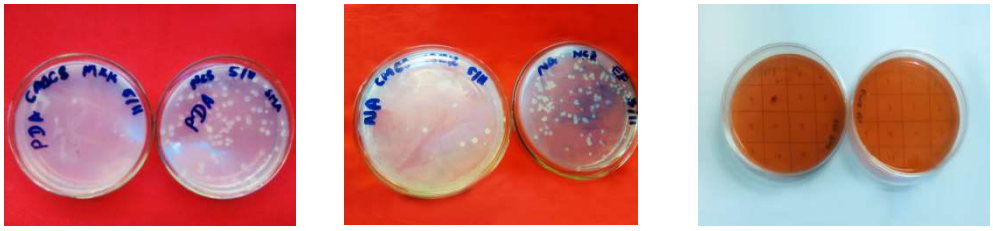
Sample	Brine Shrimp Survivals (Mean ± SEM) in Various Concentrations (μg/mL)				LD_{50} (μg/mL)
	1000	**100**	**10**	**1**	
LCS	10.00	10.00	10.00	10.00	>1000
CMCS	10.00	10.00	10.00	10.00	>1000
$K_2Cr_2O_7$*	0	0	8 ± 2.00	9.33 ± 0.67	44.19
Caffeine*	5 ± 1.15	7 ± 0.57	7.66 ± 1.20	10	1000

* Used as Cytotoxic Standards

SEM = Standard Error Means

The Morphological Characteristics of Local Cassava Starch and Carboxymethyl Cassava Starch

The morphological characteristics of LCS and CMCS were recorded by using standard plates count method and Gram staining method. The type of colonies on Nutrient Agar medium and Potato Dextrose Agar medium were observed. The stains have the morphology of coccus shape and Gram (-)ve in both samples and they may be in the same genus. These colonies are transferred to Eosin Methylene Blue (EMB) medium, on the EMB medium, the colour of colony is not red, therefore it was found to be confirmed that *Escherichia coli* are absent in the LCS and CMCS. For each stain, the type of colonies on Nutrient Agar medium, Potato Dextrose Agar medium and EMB medium are shown in Figure 1 and its morphological results are described (Table 2).



(a) **(b)** **(c)**

Figure 1. Types of colonies on (a) nutrient agar medium (b) potato dextrose agar medium (c) EMB medium

Table 2. The Morphological Characteristics of Microorganisms Observed in Local Cassava Starch and Carboxymethyl Cassava Starch

Stain	Morphology	Colony on N/A	Colony on PDA	Colony on EMB	Gram stain
LCS	Coccus with spore in long chain	Round and white colony grow on agar thickly	Small white colony grow on agar	Colony is not red	(-)ve
CMCS	Coccus with spore in long chain	Round and white colony grow on agar thickly	Small white colony grow on agar	Not grows	(-)ve

Application of Local Cassava Starch and Carboxymethyl Cassava Starch as Disintegrants in Tablet Manufacturing Process

Manufacture of tablet formulation

Chlorpheniramine maleate tablets were prepared in Credit Pharmaceutical Industry Co.Ltd. Different weights of local cassava starch (1.8 %, 2.3 %) and carboxymethyl cassava starch (1.2 %, 1.8 %, 2.3 %) were used as disintegrants in preparation of tablets. Prepared chlorpheniramine maleate tablets and procedure for tablet manufacturing process are shown in (Figure 2).

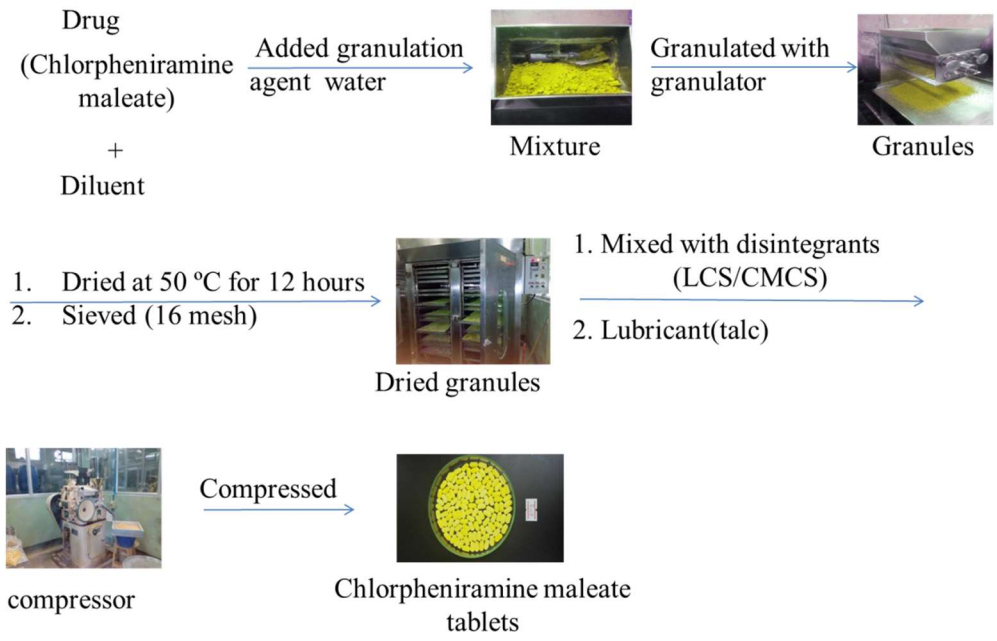


Figure 2. Determination of the Starch and its Derivative in Procedure for Tablet Manufacturing Process
LCS (1.8 %), (2.3 %)
CMCS (1.2 %), (1.8 %), (2.3 %)

Quality Control and Measurement of Tablet Properties for the Prepared Chlorpheniramine Maleate Tablets

(a) Moisture contents of the prepared chlorpheniramine maleate tablets

The moisture contents of prepared chlorpheniramine maleate tablets were measured by using the Infrared Moisture Meter (Kett) (Model No. F. 1 A, Kyoto, Japan) (Figure 3). Table 3 shows the moisture contents of chlorpheniramine maleate tablets prepared with different weights of LCS and that of CMCS used as disintegrants in tablets formulation. LCS used as disintegrant in different weight are in the range of 2.33 ~ 2.27 and CMCS used as disintegrant in different weights are in the range of 2.38 to 2.53, which are within the reported data range. Otherwise, it is important that the moisture contents be kept as low as possible during storage to prevent microbial

spoilage, hydrolysis and enzymatic decomposition. The resulting data are within the official limited (British Pharmaceutical Codex, 1994).

(b) Thickness of the prepared chlorpheniramine maleate tablets

The thickness of the prepared chlorpheniramine maleate tablets were measured by using the Electronic Digital Caliper (Model No. E-23112, Peacock Co., Ltd) (Figure 3). The thickness of chlorpheniramine maleate tablets data are described in Table 3. Chlorpheniramine maleate tablets were prepared by wet granulation method and by using 5 mm diameter, deep punches and dyed in the rotary tablet press. The thickness is generally less than half of the diameter. The thickness of tablets prepared with different weights (1.8 % and 2.3 %) of LCS used as disintegrants in tablet formulation was observed to be in the range of 2.74 ~ 2.89 mm and (1.2 %, 1.8 % and 2.3 %) CMCS used as disintegrant in tablets formulation was observed to be in the range of 2.75 ~ 3.02 mm, increased with increasing the weights of starches.

(c) Hardness (Crushing strength) of the prepared chlorpheniramine maleate tablets

The hardness of the prepared chlorpheniramine maleate tablets were measured by using the Hardness tester (Model No. D-63512 (Hainburg) Germany). The hardness of tablet with different weights (1.8 % and 2.3 %) of LCS and that of CMCS (1.2 %, 1.8 % and 2.3 %) used as disintegrants in tablets formulation are shown in Figure 3.

It was observed that, as the disintegrants weights increased, the hardness (crushing strength) increased. Therefore, hardness (crushing strength) is directly proportional to the weight of the disintegrant. The production of tablet with LCS (1.8 %) is complicated due to difficulties in tablet ejection, resulting in picking, sticking, capping and cracking of tablet high friability, lower hardness and disintegration time. The ejection of tablet has been found easier while using CMCS (1.2 %) as disintegrant, because this composition could minimize picking, sticking, capping, cracking etc., and subsequent of the tablet hardness and increased disintegration time and decreased the friability. Chlorpheniramine maleate tablets formulated with CMCS disintegrants exhibited higher values of hardness (crushing strength) then those formulated with LCS disintegrants. The hardness of the prepared tablets formulated with

both LCS (49 ~ 53 N) and CMCS (59 ~ 76 N) occurred in the range of the official limit of 50 ~ 300 N (British Pharmaceutical Codex, 1994).

(d) Friability of the chlorpheniramine maleate tablets prepared with local cassava starch and carboxymethyl cassava starch in different weights

The friability of the chlorpheniramine maleate tablets prepared with local cassava starch and carboxymethyl cassava starch in different weights were measured by using the friabiliator (Model No. D-63512 (Hainburg) Germany) (Figure 3).

The friability of the tablet with different weights of LCS and CMCS that used as disintegrants in tablets formulation are described in (Table 3). It was found that when the disintegrants weights increased, the values of friability of the tablet decreased. In tablet formulation, the higher values of hardness of tablets were observed with the lower values of friability of the tablets. The friability of the tablet is inversely proportional to the hardness of tablet.

The friability of the tablets was found to be higher in 1.8 % LCS than 2.3 % LCS used as disintegrants, and that of tablets formulated with CMCS was found in the order of 2.3 % < 1.8 % < 1.2 % CMCS used as disintegrants in the tablet formulation. The tablets that less than 1 % of their weights, friability of these tablets is good (Mohammad Saleem, 2014). But LCS has to be used in concentration above 2.3 %. Below 2.3 % there is insufficient, friability of the tablets ability is decreased, therefore transportation and packing is difficult. Above 2.3 %, the incompressibility of starch makes it difficult to compress tablets of sufficient hardness. CMCS has to be used in concentration is 1.2 %, there is sufficient, friability of the tablet ability is good therefore CMCS is more suitable used as disintegrant in tablets formulation.

(e) Disintegration time of the chlorpheniramine maleate tablets prepared with local cassava starch and carboxymethyl cassava starch in different weights

The disintegration time of the prepared chlorpheniramine maleate tablets formulated with local cassava starch and carboxymethyl cassava starch in different weights was measured by using Disintegration tester (Model No. D-63512 (Hainburg) Germany) (Figure 3).

Table 3 shows the resulted disintegration time of the tablets in distilled water and 0.1 M HCl solution. It was found that the disintegration time decreased with increasing the disintegrant weights of starches in the tablet formulation in distilled water as well as in 0.1 M HCl solution. When LCS was used as disintegrant in tablet formulation, the disintegration time were observed 2 to 3 min in distilled water and within 8 min in 0.1 M HCl solution. On the other hand, while CMCS was used as disintegrant in tablet formulation, the disintegration time was observed 4 to 5 min in distilled water and within 9 min in 0.1 M HCl solution.

Tablets containing disintegrants generally passed the official disintegration test for uncoated tablets within 5 to 15 min (Baker and Jaiyeoba, 2009). This may imply a faster onset of action and as such may be useful in immediate release formulations. Tablets containing disintegrants showed good olution profile.

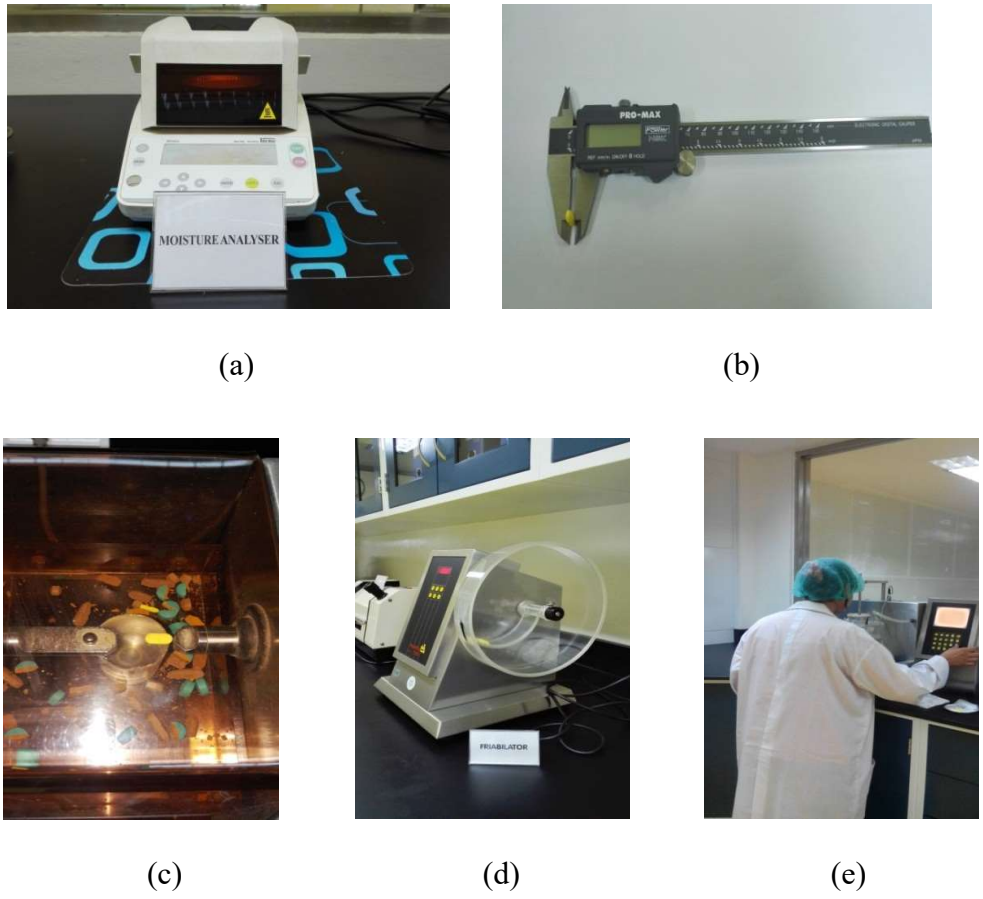
The results show that CMCS is potentially useful as a disintegrant and may be suitable to use in various tablets formulation.

Table 3. Physical Properties of the Chlorpheniramine Maleate Tablets Prepared with Different Weights of Local Cassava Starch and Carboxymethyl Cassava Starch

Parameters	Properties of Tablets in Different weights of starch (%)						
	1.2		1.8		2.3		Official *
	I	II	III	IV	V	VI	Limited
Moisture	-	2.38	2.33	2.42	2.27	2.53	1-3 %
Thickness	-	2.75±0.04	2.74±0.03	2.83±0.02	2.89±0.05	3.02±0.06	-
Hardness (N)	-	59	49	69	53	76	50- 300
Friability (%)	-	0.1959	0.3611	0.1936	0.2229	0.1619	< 1**
Disintegration Time in water (s)	-	313	162	305	129	254	> 240
Disintegration Time in HCl (0.1 M) (s)	-	565	505	557	503	549	540-900

I, III, V = LCS
II, IV, VI = CMCS

*British Pharmaceutical Codex(1994)
**Mohammad *et al*.,(2014)



(a) (b)

(c) (d) (e)

Figure 3. Photographs of

(a) Infrared moisture meter (Kett) (Model No. F. 1 A, Kyoto, Japan)

(b) Electronic digital caliper (Model No. E-23112, Peacock Co., Ltd)

(c) Hardness tester (Model No. D-63512 (Hainburg) Germany)

(d) Friabilator (Model No. D-63512 (Hainburg) Germany)

(e) Disintegration tester (Model No. D-63512 (Hainburg) Germany)

Conclusion

From the overall assessment of the present work concerning with the starch and its derivative from cassava (*Manihot esculenta* Crantz.) and their application in pharmaceutical tablets formulation, the following inferences could be deduced. Both LCS and CMCS were observed to be free from cytotoxic effect and harmful bacteria *E. coli*. Consequently, LCS and CMCS were applied as disintegrants with different weights in chlorpheniramine maleate tablets formulation. LCS (2.3 % w/w) and CMCS (1.2 % w/w) were observed to be the best conditions as the disintegrants in the chlorpheniramin maleate tablets formulation according to their quality assessment of tablets such as moisture content, thickness, friability, hardness, disintegration time in distilled water as well as 0.1 M HCl solution. CMCS was found to be better than LCS in the tablet formulation as an alternative disintegrant. Therefore CMCS is so biodegradable and non-toxic product that the finding increases the number of application including the food and pharmaceutical area. According to these inferences, carboxymethylated polysaccharides in particular CMCS based on renewable resources are useful in many applications.

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