Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.537

Physicochemical and Spectrometric Evaluation of Co-Processed Maize Starch B.P. and Alpha-Lactose Monohydrate

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ABSTRACT

Co-processing is based on the concept of two or more excipients interacting at molecular level, the objective of which is to provide a synergy of improved functionality as well as masking the undesirable properties of individual materials. The aim of this research was to evaluate the physicochemical and spectrometric properties of co - processed Maize Starch B.P. and α - lactose monohydrate. Various proportions of Maize Starch B.P. and α - lactose monohydrate (90:10, 92.5:7.5, 95:5, 97.5:2.5), based on principles of co-processing were prepared using the solvent evaporation method (co – drying), and characterized using Angle of repose, Carr's index, Hausner's ratio and swelling capacity. The proportion of excipients (92.5:7.5) with the best flowability and compressibility was selected and further investigated for compatibility studies to check for excipient – excipient interaction, using Fourier Transform Infrared (FTIR). All the co-processed excipients showed good flow properties (as reflected in the Angle of repose of 27.0^{o} and Hausner's ratio of 1.21 for Batch II that was selected for further studies) and no new peaks were formed from the Fourier Transform Infrared (FTIR) study conducted on the co – processed excipient. The result obtained revealed that, co – processing Maize Starch B.P. with α - lactose monohydrate will produce an excipient with desirable physicochemical properties suitable for pharmaceutical tablet formulations.

Keywords: Co – processing, maize starch, α- lactose monohydrate, FTIR

INTRODUCTION

Pharmaceutical excipients are substances other than the active pharmaceutical ingredients which are included in the manufacturing process of finished pharmaceutical products. These inactive, non-medicinal substances are intentionally included in a pharmaceutical product to serve different purposes such as stabilization and bulking up solid formulations or to confer therapeutic improvement on the active ingredients, by enhancing drug absorption (Borbas *et al*., 2016), reducing viscosity or enhancing solubility (Lesney and Mark, 2001). Excipients are also useful in manufacturing processes, in order to facilitate powder flowability or non-stick properties, likewise aiding stability of medications *in vitro* by prevention of denaturation or aggregation over the expected shelf-life of the finished products.

When formulating direct compression tablets, choice of pharmaceutical excipient is of immense importance. The process of compression comprises of transitional repacking, deformation at point of contact, fragmentation and/or bonding, deformation of the solid body, decompression and ejection (Parott, 1990). Out of these, the tableting of a powder is predominantly affected by compressibility (deformation) and compactibility (bonding) attributes of powder

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under pressure (Jain, 1999). Ideal diluents should consist of a mixture of a plastic material and a brittle material, thus incorporating merits of both mechanisms (Armstrong *et al.,* 1996). The presentation of most coprocessed excipients is such that small amount of plastic material is fixed between or on the particles of the larger amount of the brittle material. Such combinations help improve functionalities such as flow properties, compaction performance, strain-rate sensitivity, lubricant sensitivity or moisture sensitivity, or hornification (Nachaegari and Bansal, 2004). Cellactose, a coprocessed excipient with larger amount of brittle material (75% lactose) and a smaller amount of plastic material (25% cellulose) which prevent the storage of too much elastic energy during compression, leads to a small amount of stress relaxation and a reduced tendency of capping and lamination (Casahoursat *et al.,* 1988).

Over the years, scientists have found out that excipients made up of single components do not always provide requisite performance to allow certain active pharmaceutical ingredients to be formulated or manufactured (Patilatul *et al.,* 2013). The excipients industry to date has been an extension of the food industry (Steinberg *et al.,* 2001). Moreover, excipients are evolutions of the food industry, which has helped to maintain a good safety profile. Increasing regulatory pressure on purity, safety and standardization of the excipients have resulted in the formation of an international body, the International Pharmaceutical Excipients Council (IPEC).

A co-processed excipient is a physical combination of two or more excipients designed to modify their properties in a manner not achievable by simple mixing, and without significant chemical change. They have improved functionalities as compared to individual excipients like compressibility, better flow property, reduced lubricant sensitivity (Harsha and Kishori, 2014).

Several experimental works have been carried out on the use of maize starch and lactose monohydrate as co-processed excipients. Some of them include: a study carried out to describe the differences in compaction properties between microcrystalline cellulose (MCC) and α- lactose monohydrate physical mixture and co-processed MCC with α-lactose monohydrate (Cellactose) (Adi and Moawia, 2008), use of coprocessed particles of maize starch(MS) and acacia gum(Ac) (StarAc) as a multifunctional excipient in the formulation of metronidazole tablets using direct compression method (Olowosulu *et al.,* 2015), a work done by Muzikova and Zvolankova (2007) to evaluate the differences in properties of tablets from two co-processed dry binders based on α-lactose monohydrate and cellulose (MicroceLac 100 and Cellactose 80).

This research work was aimed at investigating the physicochemical and spectrometric properties of co-processed maize starch and lactose monohydrate as a standard excipient.

MATERIALS AND METHODS

Experimental Maize

The experimental Maize Starch B. P. and α – lactose monohydrate were sourced from the Laboratory of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe, Nigeria.

Determination of Organoleptic Properties

These include:

Taste*:* the taste of the powder on the tongue was determined.

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***Colour**:*visual test using the naked eyes was used to determine the colour.

***Odour**:* The powder smell using the nose was determined.

***Texture**:* The feel of the powder was determined using touch.

Determination of pH

Each powder sample weighing one gram (1g) was dispersed in 100 ml of distilled water. This was shaken for 5 min. A pH meter was used to determine the pH of the supernatant.

Determination of Solubility

Each powder sample weighing one gram (1g) was dispersed in 10 ml each of hot distilled water, cold distilled water and 95 % ethanol, shaken for 5 min and allowed to stand for 24 hours. 5 ml of each of the supernatant was withdrawn and heated to dryness on water bath, the weight of the dry residue was expressed as a percentage (%) with reference to the volume of the solution, and solubility of the material in solvent determined as percentage (%) w/v.

Angle of Repose

This was calculated using the funnel and stand method. A plugged glass funnel was fixed on laboratory stand at a height of 10 cm from the flat surface. Thirty gram (30 g) of the sample was placed in the funnel. The plug was then detached and the sample allowed to flow. The height and diameter of the heap formed was noted. The angle of repose (θ) was obtained as:

$$Tan\ \theta = \frac{H}{r}$$

Where

θ is the angle of repose; H is the height of the conical powder heap; r is the radius of circular base

Bulk Density

A quantity of each powder weighing thirty gram (30 g) was poured into 100 ml glass measuring cylinder at an angle of 45 ° using a glass funnel. The cylinder was then dropped on the flat, wooden platform from a height of 2.5 cm three times at the interval of 2 s. The volume occupied by the powder was taken as bulk volume.

$$Bulk\ density = \frac{weight\ of\ powder\ in\ grams}{volume\ of\ powder\ in\ millilitre}$$

Tapped Density

The cylinder containing the powder from bulk density was then tapped on the flat, wooden platform 300 times until the volume occupied by the powder became constant. This was recorded as volume. The bulk and tapped densities were computed as the ratio of weight to volume. The results obtained were used in calculating the Carr's index and Hausner's ratio. The equations are as follows:

$$Tapped\ \ density = \frac{weight\ of\ powder\ in\ gram}{volume\ of\ powder\ in\ millilitre}$$

$$Carr's\ \ index = \frac{tapped\ density - bulk\ density}{tapped\ density} \times 100$$

$$Hausner's\ ratio = \frac{tapped\ density}{bulk\ density}$$

Swelling Capacity

A ten gram (10 g) powder sample was poured into a 100 ml graduated cylinder and the tapped volume recorded after 100 taps. The powder was then mixed and shaken with 80 ml of purified water until all the particles were well dispersed. The mucilage was made up to 100 ml and the sediment volume of the swollen powder was observed after 24 h. The

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swelling capacity was calculated in percentage (%), as expressed below:

$$S=\frac{Vv}{Vx} \times 100$$

Where

S is the swelling capacity; Vv is the swollen volume of the sediment; Vx is the tapped volume

True Density

The method as described by Odeku *et al.,* 2005 was adopted. The particle density was calculated using a pycnometer with xylene as the displacement fluid. An empty 50 ml pycnometer bottle was weighed (W). The bottle was filled with xylene and the excess wiped off. The bottle was weighed a second time (W1). The differences between W1 and W were obtained (W2). Two gram (2 g) of the sample was weighed (W3) and transferred into a pycnometer bottle. The excess solvent was then wiped off and the bottle was weighed again (W4). The true density, ρ_t (g/cm³) was determin from the equation below:

$$\rho_t = \frac{W2 \times W3}{50[W3 - W4 + W2 + W]}$$

Where

ρ_t is the particle density; W is the weight of empty bottle; W2 is the weight of xylene

W3 is the weight of powder sample; W4 is the weight of bottle, sample and xylene

Powder Porosity

This was derived from the values of the true density using the formula below:

$$\varepsilon = (1 - \frac{BD}{\rho t}) \text{ x } 100$$

Where

BD is bulk density; ρ_t is the true density; ε is the porosity

Packing Fraction

This was derived from the values of the true density and bulk density using the equation below:

$$P_f = \frac{BD}{\rho t}$$

Where

BD is the bulk density; ρ_t is the true density and P_f is the packing fraction.

Formulation Studies

Preparation of co-processed excipient consisting of α- lactose monohydrate and Zea mays

Solvent evaporation was used and the solvent was water. These procedure was carried out using co-drying method (Olowosulu *et al*., 2011).

Four batches of suspensions were prepared from mixtures of α-lactose monohydrate and *Zea mays* at different ratios of 90:10, 92.5:7.5, 95:5 and 97.5:2.5. The quantities of powders at the above ratios were weighed individually and mixed uniformly using the doubling up method. Suitable amount of water was added to the respective ratios and stirred for 5 min to form a cohesive mass. The mixture was later poured on a tray and dried using a hot air oven at 60 ℃ for 1 h. The dried product was weighed to determine percentage (%) recovery, passed through 1.7 mm mesh size and stored for further processing.

Analysis of co - processed granules

The co - processed granules were subjected to the following physicochemical tests: Angle of repose, bulk and tapped densities, Carr's compressibility index, Hausner's ratio, powder porosity, packing fraction, true density and swelling capacity. The procedures used are the same as described above for maize starch and lactose powders.

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Compatibility studies

Spectra of lactose monohydrate, *Zea mays* and co – processed excipients were studied using Fourier transform infra – red (FTIR) spectrophotometer. Initially, five milligrams (5 mg) of pure sample (lactose monohydrate, *Zea mays*) and co – processed excipient were individually crushed with potassium bromide (KBr) to 200 mg resulting in a transparent pellet of about 1 mm thickness. The pellets prepared were analyzed with FTIR spectrophotometer (Perkin Elmer L1600401 Spectrum Two DT GS, UK) using KBr as the beam splitter. The instrument was operated under dry air and scanned at spectra region of 4000 – 400 cm^{-1}.

RESULTS AND DISCUSSION

The results of organoleptic properties of *Zea mays,* α-lactose monohydrate and co-processed excipient are shown in Table 1. All are white, tasteless and odourless, with fine texture except coprocessed excipient with coarse texture. These complied with BP (2002) specifications.

Table 1: Organoleptic properties of *Zea mays* (ZM), α-lactose monohydrate (α-LM) and co – processed *Zea mays* and α-lactose monohydrate (COP)

Properties	ZM	α-LM	COP
Colour	White	White	White
Taste	Tasteless	Tasteless	Tasteless
Odour	Odourless	Odourless	Odourless
Texture	Fine	Fine	Coarse

The results of physicochemical tests are shown in Table 4. The Angle of repose of powder is a measure of the degree of cohesiveness of the powder (Aulton, 2007). An Angle of repose value above 50° is an indication of poor flow characteristic of a powder while an Angle of repose close to 25° shows a very good flow. Due to the cohesiveness of the particles which could retard the powder flow, the Angle of repose for *Zea mays* was 43.6°, which indicates poor flow. α – Lactose monohydrate showed fair flow in its Angle of repose of 39.7°, which could be due to the cohesiveness of the powder and the moisture present in the powder (Alderborn, 2007).

Hausner's ratio and Carr's compressibility are parameters used to express powder and granules flowability. The smaller the degree of compression, the better the flow of the powder. When the compression ratio is 5 – 15 %, the powder flow is excellent while the flowability of 18 – 21% gives a good flow. When the ratio is from 23 – 28 %, the powder flow is average, and is poor when the compression ratio is 21 – 35% (Aulton, 2007). When the Hausner's ratio is 1.2, the powder has a good flowability and filling ability while a ratio of 1.3 indicates poor flow. A ratio of 1.4 has fair flow properties (Aulton, 2007). In Table 4 above, it could be observed that both *Zea mays* and α – lactose monohydrate has poor flow based on the values obtained for Carr's compressibility index and Hausner's ratio.

Density is the ratio of mass of material to the volume occupied by the same material. The bulk, tapped and true densities of the materials are shown in Table 2. The values of bulk, true and tapped densities in ascending order are as follows: ZM < α - LM. The tapped density is usually higher than the bulk density because of diminished void spaces while the bulk density is always less than the true density because the bulk powder contains more inter - particulate pores (Staniforth and Aulton, 2007). Particle packing and consolidation have great impact on

DOI: 10.56892/bima.v7i4.537

flowability of powders, and sometimes used as an indirect method of measuring or quantifying powder flow. There is a direct relationship between particle packing and bulk and tapped densities. Thus, the results of bulk and tapped densities in Table 2 show that α – lactose monohydrate consolidates more easily than *Zea mays.* Hence the flowability is in the order: α - LM > ZM. Also, from the results of the packing fraction and porosity in Table 4, α – lactose monohydrate exhibited a larger volume reduction than *Zea mays.* Thus, it would appear under the applied tapping pressure that the polygonal shape and larger particles size promote closer packing of particles than the void shape and smaller particle size of *Zea mays*.

There is a direct relationship between the swelling power and amylopectin content, suggesting that the higher the amylopectin content, the greater the swelling power (Tester *et al.,* 2004). *Zea mays*, due to the high level of amylopectin content showed a higher swelling power than α – lactose monohydrate which is as a result of absence of long chain of amylopectin.

The results of the effects of various disintegrant concentrations on the physical properties of the co – processed excipients are presented in Table 4. An Angle of repose values above 50 ° is an indication of poor flow characteristics of a powder, while Angle of repose close to 25° shows a very good flow (Velasco *et al.,* 1995). The Angle of repose of the various concentrations of the co – processed granules exhibited good flow properties ranging from 25.9° to 27.2°. This could be due to the fact that the granules had larger particles than the single excipients.

The true, bulk and tapped densities increased with increase in concentrations from 36:4 % w/w to 39:1 % w/w. Lower bulk density has been associated with better dilution potential, due to its ability to accommodate more of the poorly compressible powders in its pore spaces (Thoorens *et al.,* 2014). Particle packing and consolidation have great impact on powder flowability, and sometimes used as an indirect method of measuring or quantifying powder flow. There is a direct relationship between particle packing and bulk and tapped densities. Thus, the results of bulk and tapped densities in Table 4 show that (IV) consolidates more easily, followed by (III) whereas, (II) and (I) consolidate poorly. Carr's compressibility index of 5 – 15% shows excellent flow properties, while 18 – 21 %, fair flow and 23 – 28 %, poor flow. Hausner's ratio of 1.2 indicates a good flow property (Aulton, 2007). It could be observed that the values for Hausner's ratio were between 1.17 and 1.21(Table 4), which means good flow properties, while that of Carr's index were between 17.7 % and 21.6 %, which also indicates fair flow properties.

Also, from the results of packing fraction and porosity in Table 4, (I) exhibited largest maximum volume reduction, followed by (IV) while (II) was the lowest. Ideally, since (I) has the highest concentration of α – lactose monohydrate, it should have the highest maximum volume of reduction while (IV) should have the least.

There is a direct relationship between the swelling power and amylopectin content, suggesting that the higher the amylopectin content, the greater the swelling power. High swelling capacity is observed in batches (III) and (IV) while batch (I) showed no swelling. This may be due to high concentration of lactose monohydrate (I), which has negative swelling property and might have affected the swelling power of *Zea mays.*

Hence, batch II (92.5: 7.5) was selected for compatibility evaluation because it has more desirable properties (Angle of repose of 27^{o};

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Hausner's ratio of 1.21; porosity of 84.3 %) in order to draw a conclusion on its suitability as an excipient in pharmaceutical tablet formulations.

Table 2: Results of physicochemical tests on ZM, α-LM and COP

Physicochemical test	ZM	α-LM	COP (Batch 2)
Angle of repose (°)	43.6	39.7	27
True density (g/mL)	1.5	1.7	3.0
Bulk density (g/mL)	0.54	0.64	0.47
Tapped density (g/mL)	0.79	0.86	0.57
Hausner's ratio	1.46	1.26	1.21
Carr's index (%)	31.64	25.58	20.7
Swelling capacity (%)	28.66	-	32.25
Packing fraction	0.36	0.38	0.16
Solubility			
• Hot dist. water	Insoluble	Insoluble	Insoluble
• Cold dist. water	Insoluble	Soluble	Insoluble
• 95% ethanol	Insoluble	Soluble	Insoluble
pH	.46	7.66	7.01
Porosity (%)	64	62	84.3

Keys: ZM = *Zea mays* α – LM = α – lactose monohydrate COP = co – processed

Table 3: Preparation of co-processed excipients

Batches	ZM: α-LM (g)	Weight recovered (%)
I	36.0: 4.0	96.3
II	37.0: 3.0	97.8
III	38.0: 2.0	94.6
IV	39.0: 1.0	96.0

Table 4: Results for physicochemical properties of various batches of co-processed excipients

Properties	Batches of ZM : α-LM			
	I	II	III	IV
Angle of repose(°)	25.9	27.0	26.1	27.2
True density (g/ml)	2.5	3.0	3.5	3.3
Bulk density (g/ml)	0.46	0.47	0.56	0.57
Tapped density(g/ml)	0.55	0.57	0.66	0.69
Hausner's ratio	1.19	1.21	1.17	1.21
Carr's index (%)	18.7	20.7	17.7	21.6
Swelling capacity (% w/w)	0.00	32.25	36.5	37.0
Packing fraction	0.184	0.157	0.160	0.172
Solubility:				
• Hot dist. water	Insoluble	Insoluble	Insoluble	Insoluble
• Cold dist. water	Insoluble	Insoluble	Insoluble	Insoluble
• 95% ethanol	Insoluble	Insoluble	Insoluble	Insoluble
pH	6.95	7.01	7.10	7.08
Powder porosity (%)	81.6	84.3	84	82.8

FT – IR is used to determine if there is molecular interaction between materials used or intended to be used in a formulation. If the interaction is not chemical, no new moieties are formed to warrant incompatibility. The functional groups present or absent in a molecule are detected by FT – IR spectroscopy. These functional groups absorb infrared photons of characteristic energies which are reflected as a plot of photon energy

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versus intensity of absorption called the infrared spectrum. The characteristic bands are reflected due to physical blends or chemical reactions when two or more substances are mixed (Silverstein and Webster, 1998). The FT – IR spectrum of COP when compared with the individual spectra of ZM and α – LM did not show any chemical reaction occurring during co – processing because their characteristic bands were maintained in the spectrum of the co – processed mixture (Figures 1 – 3). The improved functionality observed in COP could be as a result of physical modification that occurred in its particle structure.

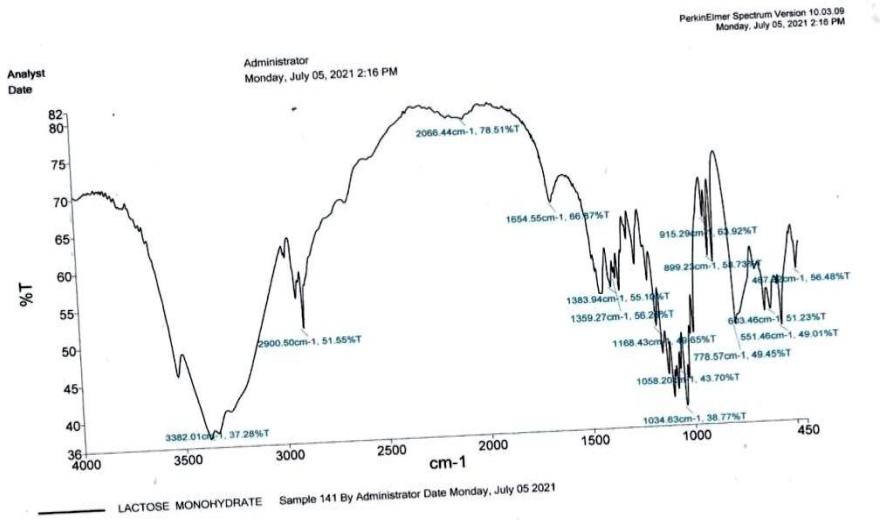

Figure 1: FTIR spectrum of lactose monohydrate

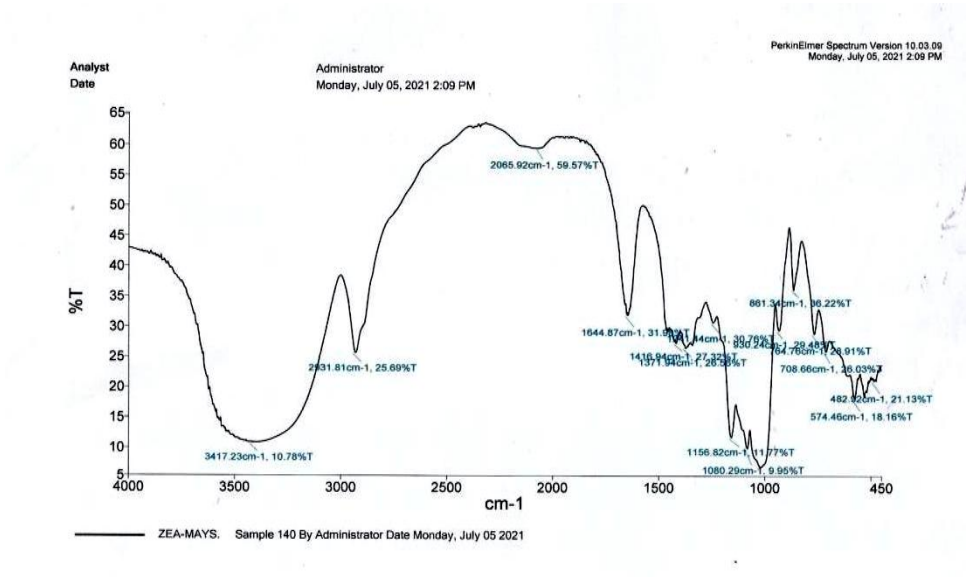

Figure 2: FTIR spectrum of *Zea-mays*

DOI: 10.56892/bima.v7i4.537

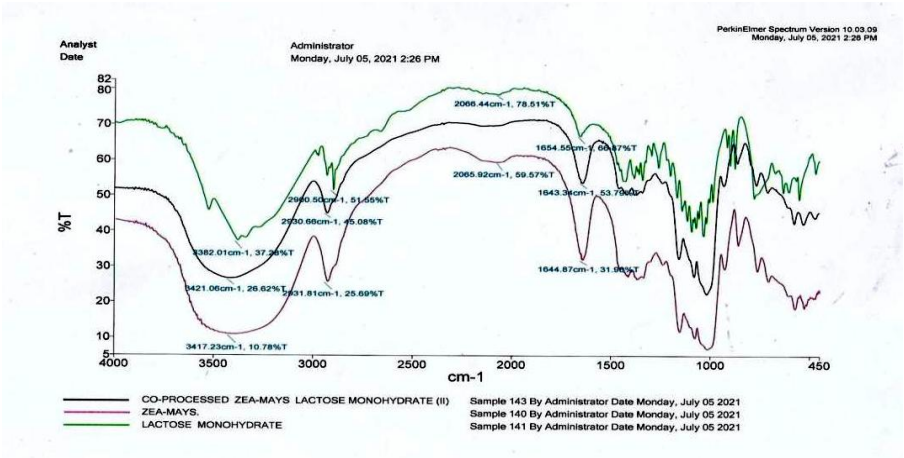

Figure 3: FTIR overlay of co-processed *Zea-mays* and lactose monohydrate, *Zea-mays and* lactose monohydrate

CONCLUSION

The research carried out on co – processed excipient (COP), made up of *Zea mays* and α – lactose monohydrate has shown that, modification of the individual excipients by way of co – processing produced granules with better physicochemical properties, for formulation of tablets with desirable characteristics.

REFERENCES

Adi, I., Arida, Moawia, M. and Al- Tabakha. (2008). Cellactose, a Co-Processed Excipient: A comparison study. *Pharmaceutical Development and Technology*, 13(2): 165-175.

Alderborn, G. (2007). Tablets and compaction. In: Aulton, M. E. (Ed), *Aulton's Pharmaceutics: The design and manufacture of medicines*. Churchill Livingstone Elsevier, London, 3rd Edition, 31:441- 482.

Armstrong, N. A., Roschiesen, G., Al-Aghbar, M. R. (1996). Cellactose as a tablet diluent. *Manuf. Chem.*, 42: 325-330.

Aulton, M. E. (2007). "The Design and Manufacture of Medicines". China, Churchill Livingstone. *Pharmaceutics Elsevier*; pp. 442-457.

Borbas, E., Sinko, B., Tsinman, O., Tsinman, K., Kiserdei, E. (2016). Investigation and Mathematical Description of the Real Driving Force of Passive Transport of Drug Molecules from Supersaturated Solutions. *Molecular Pharmaceutics,* 13(11): 3816 – 3826.

Casahoursat, L., Lemagen, G., Larrouture, D. (1988). The use of stress relaxation trials to characterize tablet capping. *Drug Dev Ind Pharm,* 14(15-17): 2179-99.

Harsha, K., and Kishori, J. (2014) Co – processed excipients – a review. *World Journal of Pharmaceutical Research,* 3(3).

Jain, S. (1999). Mechanical properties of powders for compaction and tableting: an overview. *Pharmaceutical Science Technology Today*, 2(1): 20-31.

Lesney, M. S. (2001). "More than just sugar in the pill". *Today's Chemist at Work*, 10(1): 30 - 6. Retrieved August 13, 2013.

Muzikova, J. and Zvolankova, J. (2007). A study of the properties of tablets from co-processed dry binders composed of α-lactose monohydrate and different types of cellulose. *Ceska Slov* Farm. 56(6): 269- 75.

DOI: 10.56892/bima.v7i4.537

Nachaegari, S. K. and Bansal, A. K. (2004). Coprocessed excipients for solid dosage form. *Pharm Technol*, 28: 52-65.

Olowosulu, A. K., Oyi, A. R., Isah, A. B., and Ibrahim, M. A. (2015). The use of multifunctional starch based co-processed excipients (StarAc) in the formulation of metronidazole tablets by direct compression. *African Journal of Pharmaceutical Research and development,* 7(2): 101-108.

Parrott, E. L. (1990). Compression. In: Lieberman HA, Lachman L, Schwartz JB, editors, *Pharmaceutical Dosage Forms:* Tablets. 2: 153-82.

Patilatul, Kundusubrata and Srinivasan. (2013). A Review on co-processed excipients: A novel approach in formulation development. *International Journal of Pharmaceutical Innovations*, 3: 25-41.

Silverstein, R., M., and Webster, F., X. (1998). "Spectrometric Identification of Organic compounds", 6th edition, John Wiley & Sons., New York.

Staniforth, J., N., and Aulton, M., E. (2007). Powder Flow. In: *Aulton, M., E. (Ed.) Aulton's Pharmaceutics: The Design and Manufacture of Medicines,* Churchill Livingstone, Elsevier, London, 3rd Edition, 29: 410 426.

Steinberg, M., Blecher, L., and Mercill, A. (2001). From inactive ingredients to pharmaceutical excipients. *Pharmaceutical Technology,* 25 (7): 62-64.

Tester, R., F., Karkalas, J., J., and Qi (2004). Starch composition, fine structure and architecture (Review). *Journal of Cereal Science,* 39: 151- 165.

Thoorens, G., Krier, F., Leclerq, B., Carlin, B., and Evrard, B. (2011). Microcrystalline cellulose, a direct compression binder in a quality by design environment – A review. *Int. J. Pharm,* 473(1- 2): 64-72.

Velasco, M., V. and MunRuiJimenz – Castellanus M. R. (1995). Studies on maltodextrins as Direct Vehicles. *Drug Dev.,* 1: 2-5.