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Preparation and Characterization of Polymorphs of Febuxostat and Study of their Influence on Dosage Form Designing

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ABSTRACT:

The aim of this research work was to prepare and characterize different crystal forms of Febuxostat which is Xanthine oxidase inhibitor, used as an Antigout agent, Febuxostat recrystallized by Ultrasonication from mixture of various batches of acetone-water, ethanol-water, methanol-water and the resultant mixture were stored for 7 days at room temperature. The crystals obtained from above method were characterized by FT-IR, Differential Scanning Calorimetry, Scanning Electron Microscopy and X-ray Powder Diffractometry to investigate changes in physical properties of drug.

X-ray Powder Diffractometry study reveals decrease in % Crystallinity which indicates amorphous nature. Scanning electron microscopy shows needle shaped morphology with methanol- water and acetone- water batches and irregular rod shaped morphology with ethanol-water batch, while Differential scanning colorimetry shows shift in Endothermic peak to quite lower temperature which indicates sharp decrease in melting point for batches E2, E5 and E10.FT-IR reveals arrangement of functional group which shows characteristic changes in frequencies for different batches of mixture of acetone-water, ethanol-water, and methanol-water.

KEYWORDS: Febuxostat; Recrystallization; Physicochemical characterization; % Crystallinity; Crystal habit

1. Introduction:

Modern pharmaceutical research and development indicates the great importance of the relationship between crystal structure and physical properties of drugs. Different crystalline forms of drugs, as well as amorphous forms, have different physical properties that mayaffect both stability and bioavailability. In this respect, the investigation for polymorphic phases is important requirement during very early stages of solid-state characterization on new drugs[1, 5].

Pharmaceuticals can exist in various solid form shaving different physical and chemical properties. These solid forms include ‘polymorphs’, solvates, de solvates and amorphous solids. This concept of variability broadly classified as polymorphism hereafter, was first introduced by Klaprothin1788[5], who identified the two different forms of calcium carbonate as calcite and

aragonite. Polymorphism has contributed significant variability in product performance in pharmaceutical, chemical and food industry and gives challenge to pharmaceutical scientists in producing drugs of consistent quality[5]. It also provides a unique opportunity, to engineer solids having 'tailor made' properties (challenges). Surprisingly, a very large number of Pharmaceuticals exhibit the phenomenon of polymorphism. 70% of barbiturates, 60% of sulfonamides and 23% of steroids exist in different polymorphic forms[15, 16].Those who study polymorphism are rapidly reaching the conclusion that all compounds, can crystallize in different solidforms[5,11].

Crystallization is a separation and purification technique commonly used in the chemical industry which involves diffusional process accompanied by formation of new heterogeneous phase. Many chemical substances can crystallize in different crystal forms a phenomenon known as polymorphism. Polymorphs are defined as crystalline phases that have different arrangements and/or conformations of molecules in the crystal lattice. From a pharmaceutical point of view, bioavailability, the ability to process and stability of the product are influenced by the existence of varied physical and chemical properties of these solid-state forms. Supersaturation, nucleation and crystal growth are the predominant physical phenomena associated with crystallization. Supersaturation is defined as the concentration of the solute in excess of saturated concentration under given conditions of temperature. It is composed of two zones. The metastable zone shows crystal growing without nucleating, whereas unstable region crystals appear after nucleation. Nucleation is a first step in crystal formation where molecule collides each other in solution leading to prenucleation cluster as population of cluster increases they begin to associate to form embryo[9].

Febuxostat is chemically 2-[3-Cyano-4-(2-methylpropoxy) phenyl]-4-methyl-1,3-thiazole-5-carboxylic acid, Freely soluble in dimethylformamide, soluble in dimethyl lsulfoxide, sparingly soluble in ethanol; slightly soluble in methanol and acetonitrile; and practically insoluble in water[2, 4, 6].

2. Materials and Methods

Preparation of Febuxostat polymorphs:

In this saturated solution of drug in solvents combinations like ethanol-water (100:0ml, 75:25ml, 50:50ml, and 25:75ml) and in same ratio with methanol-water and acetone-water solvent system were prepared and kept for 07 days at room temperature. These different ratios of ethanol-water, methanol-water and acetone-water solvent system were taken for the studying influences of solvent system on recrystallization of drug to get different solid forms for drug. Batch code of Febuxostat and their solid form were mentioned in Table No. 1.1

Preparation of calibration curve of Febuxostat in different solvent.

The calibration curve was prepared by dissolving 10 mg of accurately weighed Febuxostat in 10 ml ethanol and the final volume was made up to 100 ml using ethanol. This stock solution (100µg/ml) was diluted suitably with ethanol to get solutions containing 2, 4,6,8,10,12 and 14µg/ml of the drug. UV spectra were run to estimate λ_{max}. And finally standard calibration curve absorbance vs. concentration were plotted, similarly calibration curve in phosphate buffer (6.8) and methanol were determined as mentioned in Table No. 1.2

3. Characterization:

Differential Scanning Colorimetry (DSC):
The thermal behaviour of drug and their solid forms were carried out using Differential Scanning Calorimeter. For this 1 mg of drug sample was placed in a platinum crucible and the DSC thermograms were recorded at a heating rate of 20ºC/min in the range 20ºto 200ºC. Nitrogen gas was purged at the rate of 30 ml/min. to maintain inert atmosphere.

Scanning electron microscopy study:
The Scanning electron microscopic analysis of drug and their solid forms were carried out by JSM -6360A scanning electron microscope operated at acceleration of voltage 15 KV. Prior to estimation samples were coated with 20 nm thin platinum layer by auto fine coater to render them electrically conductive.

Fourier-transform infrared spectrometric characterization:
FT-IR spectra were obtained using a mode spectrum (Shimadzu Co. Japan) over the range of 500-3500/cm. Dry KBr was finely ground in a morter after which sample was added and mixed it properly.

Dissolution study of powder samples:
In-vitro dissolution study of powder sample of Febuxostat and its solid forms were performed using the USP dissolution apparatus type I (rotating basket type) in phosphate buffer pH 6.8 dissolution medium maintained at 37 ± 0.5^0C and speed is 75 rpm. The powder sample of amount 40mg were wrapped in muslin cloth and kept in basket. The drug release study carried out in 900ml of phosphate buffer pH 6.8dissolution medium. The sample was withdrawal at 5, 10,15,30,45,60,75,90 min and replaced with fresh dissolution medium filtered using Whatman filter paper (No 41). Samples were analyzed spectrophotometrically at 316.2 nm.

Aqueous solubility determination:
Saturation solubility is determined in distilled water in order to study the impact of solid forms on solubility of drug so; saturated solubility of Febuxostat and their solid forms were carried out in water. Febuxostat was added in water up to the saturation level and this was kept in vials up to 24 hr. at 37ºc. This vial was kept on magnetic stirrer. Resulting solution were filtered with the help of Whatman filter paper and appropriate dilution were made and take the UV absorbance at λ_{max}314.Similarly saturated solubility of solid forms were determined.

X-ray powder diffraction:
X-ray diffraction patterns for plain drug Febuxostat and their solid forms were obtained by measuring the 2θ in the range of 10 to 50^0 on a diffractometer. The XRPD patterns were recorded automatically using rate meter with time constant of 2 X 10^2 pulse/second and with the scanning speed of 20 (2θ)/min.

Preparation of Immediate Release Tablet:
From above study, the following batches as in Table No.1.3 were selected for further study in which these selected batches from different solid forms and the plain drug batch were formulated into directly compressed tablet and comparative account of influence of these solid forms on Dosage forms were studied in detail.

Dissolution study of Immediate release tablet:

In-vitro dissolution studies of Immediate Release Tablet tablets of Febuxostat and its various solid forms were performed using the USP dissolution apparatus (Apparatus II), in phosphate buffer of pH 6.8 dissolution medium maintained at 37 ±0.5°C and a speed of 50 rpm. Immediate Release Tablet from each batch were added into dissolution medium (n=3).The drug release studies were carried out in 900 ml of phosphate buffer of pH 6.8as dissolution media. Sample aliquots were withdrawn after 5, 10,15,30,45,60,75,90, minute interval and replaced with fresh dissolution medium, filtered using Whatman filter paper (No.41). Samples were analysed spectrophotometrically at 316.2 nm, with phosphate buffer of pH 6.8 as blank.

4. Results and discussion:

Differential Scanning Colorimetry (DSC):

The Batch E showed the melting endothermic at 212^0C followed by decomposition exothermic peak at 251^0C, as compare to melting point of plain drug (Batch E) there is significant decrease in melting point of solid forms (Batch E2, E5, E10) that's shows melting endothermic peak at 201^0C, 210^0C, 210^0C respectively and decomposition exothermic at 255^0C, 254^0C, 251^0C, respectively as in Fig No.1.2 and Table No. 1.4

In case E10 there is peak at 210^0C which is indicates that different morphology or crystalline nature as compared to plain drug E.

Scanning Electron Microscopy study:

The morphological and crystal habit of solid form batches (E2, E5, and E10) were studied by Scanning electron microscopy and compared with plain drug Febuxostat batch (E). Scanning electron microscopy images show that the batch E2, E5 and E10 from recrystallization showing the decrease in particle size with needle and irregular shape morphologies as in Fig. No. 1.3

Comparing Scanning electron microscopy of crystals obtained from methanol, ethanol and acetone solutions shows that the size and shape of crystals produced in these solvents are significantly different. The variations in face dimensions or the appearance or disappearance of some faces could be the cause of the change in morphology of Febuxostat crystals, obtained from different solvents. Under certain conditions of crystallization, the growth of one set of crystal faces may be retarded, or the other set of faces may be induced to grow faster. For instance, using different solvents as a crystallization medium with the same method changes the pattern of crystal growth from irregular to needle shape (with methanol and acetone). This can be explained by the interaction between the solvent molecules and different crystal faces which is believed to change the crystal morphology.

X-ray Powder Diffraction:

XRD spectra for all Febuxostat crystals are presented in above Fig. No. 1.4 Disappearance of sharp peaks in diffractograms of many samples indicates polymorphic modifications-Habit modification from crystalline to amorphous form. This is probably due to the higher crystal perfection or different preferred orientations of the crystals in the sample holder because of their different crystal habits. Therefore the abundance of the planes exposed to the X-ray source would have been altered, producing the radiation in the relative intensities of the peak.

Fourier-Transform Infrared Spectrometric Characterization:

Febuxostat and their solid forms show the IR-pattern in following Fig No. 1.5 FTIR spectroscopy has been successfully used for exploring the differences in molecular conformations, crystal packing and hydrogen bonding arrangements for different solid state forms of an organic compound. Spectral variations originate due to alteration in bonds that exhibit characteristic vibrational frequencies, leading to frequency shifts and splitting in absorption bands. The FTIR spectrum for solid form obtained from (Batches E, E2 E5, and E10) shows change in frequency and spectra as compare to pure drug (batch E) as shown in Table No.1.5

Dissolution study of powder samples:

The impact of solid forms on dissolution profiles were studied and comparative accounts of the same were done with plain drug as it shown in following Table No. 1.6 and Fig No. 1.6 Above data indicates that there in significant increase in % cumulative drug release of solid forms (E2, E5, E10) as compaired to plain drug (E), increase order of % cumulative drug release E<E5<E2<E10.

Dissolution study of Immediate release tablet:

From above dissolution data and dissolution profiles as in Table No. 1.7 and Fig. No.1.7 of various batches of immediate release tablet it is cleared that the different solid forms in immediate release tablet batches showing different released pattern as compared to batch of immediate release tablet containing plain drug F3>F1>F2>F.

5. Conclusion:

Crystallization medium has a major effect on Febuxostat crystal habit modification. Crystallization of Febuxostat results needle shape crystals from methanol and acetone and irregular rod shape with ethanol. From the data of solubility, IR study, *In-vitro* released study, DSC study, XRPD study and Scanning electron microscopy study, the solid forms (Batches E2, E5, and E10) showed significant variation form the plain Febuxostat (Batch E).

From above study it is clear that there is increase in solubility of Febuxostat and also its dissolution rate due to increase its Amorphous nature i.e. decrease in Crystallinity, and also shows its decrease in Melting point and the enhancement of Dissolution rate of immediate release tablet.

Acknowledgements:

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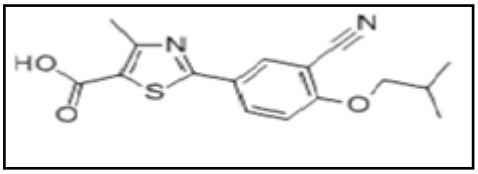

Fig. No. 1.1 Structure of Febuxostat

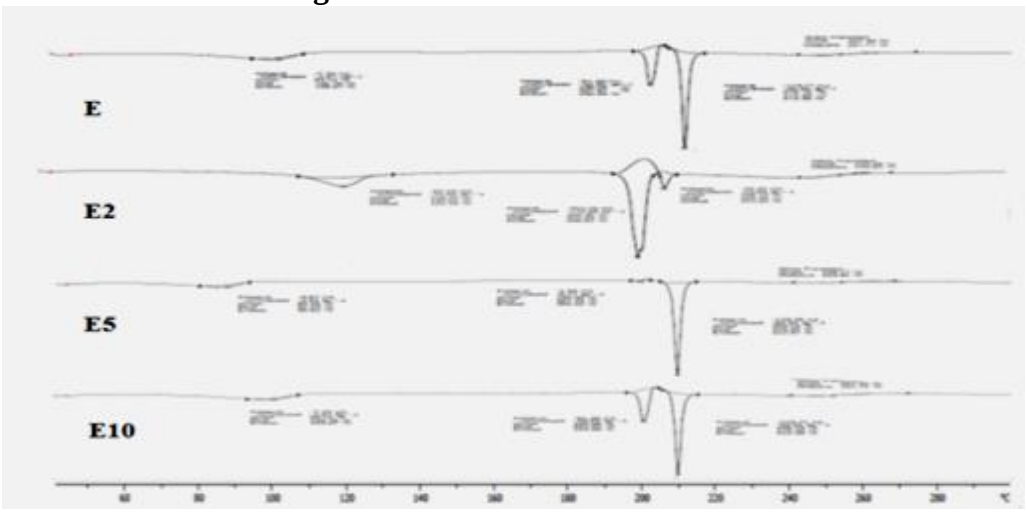

Fig. No. 1.2 DSC study for Febuxostat (Batch E) and Batches of Solid forms (Batches E2 E5 E10)

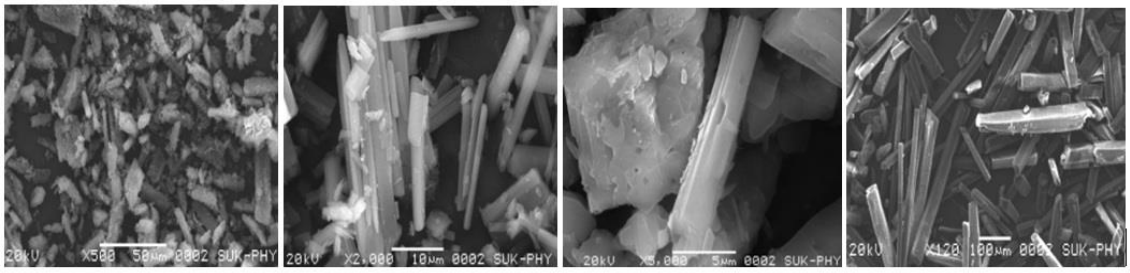

Fig. No.1.3 SEM images for the plain drug Febuxostat (Batch E) and its solid Forms (Batch E2, E5, E10)

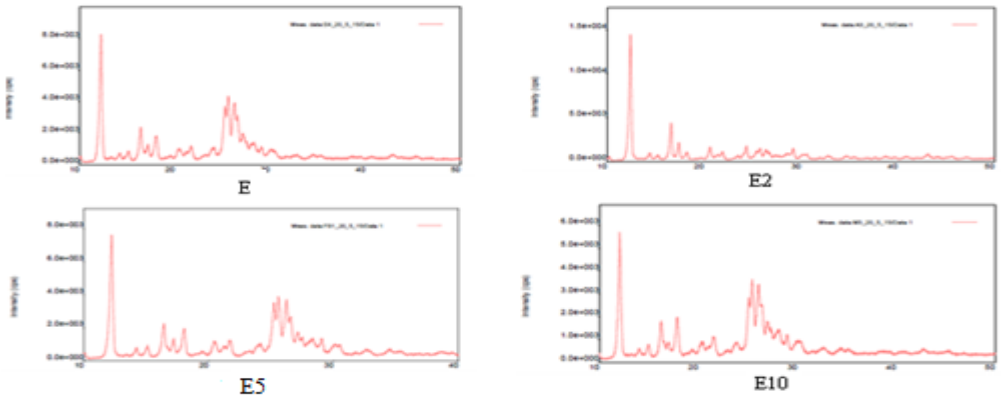

Fig. No. 1.4 Powder diffraction characterization (XRPD) Febuxostat (Batch E) and their Solid forms (Batch E2, E5, E10)

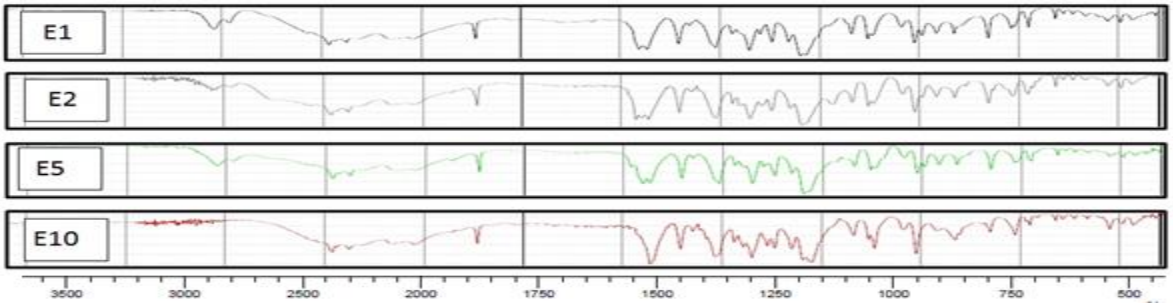

Fig. No. 1.5 Spectral region of 1060-1130, 1681-1590, 3060-2810 for Batch E and Batch E2, E5, E10.

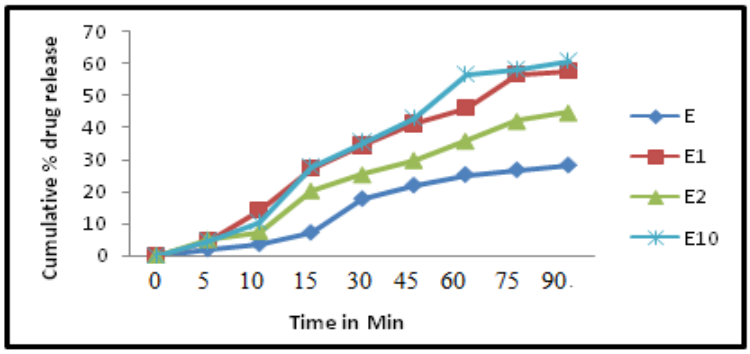

Fig. No. 1.6 Dissolution profiles of Febuxostat powder and its various solid form batches

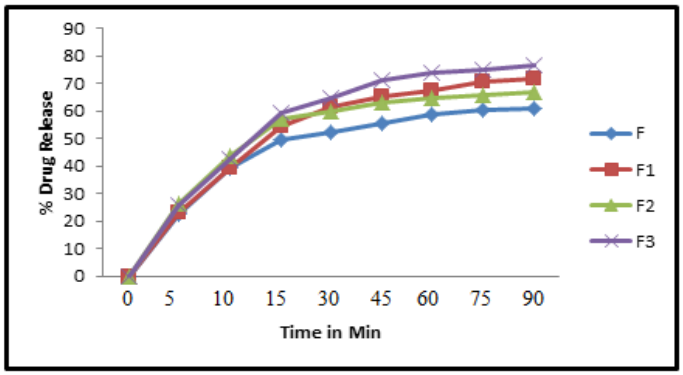

Fig. No. 1.7 Dissolution profiles for various batches of Immediate Release Tablet

List of Tables:

Table No. 1.1 Batch code of Febuxostat and their solid form.

Sr. No.	Batch Code	Sample (Solvent Qty in ml)
1	E	Febuxostat
2	E1	Recrystallize sample Acetone : Water (100:0)
3	E2	Recrystallize sample Acetone : Water (75:25)
4	E3	Recrystallize sample Acetone : Water (50:50)
5	E4	Recrystallize sample Acetone : Water (25:75)
6	E5	Recrystallize sample Ethanol : Water (100:0)
7	E6	Recrystallize sample Ethanol : Water (75:25)
8	E7	Recrystallize sample Ethanol : Water (50:50)
9	E8	Recrystallize sample Ethanol : Water (25:75)
10	E9	Recrystallize sample Methanol : Water (100:0)
11	E10	Recrystallize sample Methanol : Water (75:25)
12	E11	Recrystallize sample Methanol : Water (50:50)
13	E12	Recrystallize sample Methanol : Water (25:75)

Table No. 1.2 Particulars of the calibrations curves of Febuxostat in different solvent

Sr. No	Solvent	λ_{max} value	Equation of line (Y=mx+c)	Coefficient of regression ($r^{2)}$
1	Methanol	314.8	Y=0.0887x+0.0404	0.998
2	Water	314	Y=0.091x-0.0371	0.995

Table No. 1.3 Selected Batches from the solid forms were coded for the Dosage forms Immediate Release Tablet.

Codes for Batches of Batch of Directly Compressed Tablet	Selected batches from the different solid forms
F	Plain drug Febuxostat sample
F1	Recrystallized Samples (Acetone: water 75:25) (Batch E2)
F2	Recrystallized Samples (Ethanol: water 100:0) (Batch E5)
F3	Recrystallized Samples (Methanol: water 75:25) (Batch E10)

Table No. 1.4 Thermochemical parameters (DSC) and solubility of various solid forms

Sample	Onset of Temp. ^{0}C	Peak Temp. ^{0}C	Decomposition Temp. ^{0}C	Aq. Solubility
E	210.19	212.30	251.71	0.15
E2	199.49	201.99	255.81	0.40
E5	209.19	210.44	254.81	0.37
E10	209.11	210.13	251.71	0.48

Table No. 1.5 IR frequencies for the Batch E and Batch E2, E5, E10.

Batch E	Batch E2	E5	E10	Assignment
912-835	912-830	912-845	912-830	OH bend Carboxylic acid
1130-1060	1128-1080	1128-1060	1116-1060	C-O Stretch Carboxylic acid
1284-1250	1294-1258	1284-1258	1296-1250	C-O Stretch
1450-1420	1450-1410	1450-1427	1450-1415	C-C Stretch Ring
1681-1595	1604-1595	1684-1590	1604-1590	C=N Stretch
1704-1514	1714-1514	1701-1515	1704-1518	C=O Stretch Carboxylic acid
2227-2180	2229-2185	2227-2180	2230-2185	CN Stretching
3060-2810	3068-2810	3068-2810	3051-2815	O-H Stretch Carboxylic acid
3529-3400	3530-3410	3549-3402	3522-3410	O-H Stretch Alcohol

Table No. 1.6 Dissolution study of powder samples

Sr No.	Time (min)	Cumulative % drug release*			
		E	E2	E5	E10
1	0.0	0.0	0.0	0.0	0.0
2	5	1.95±0.18	4.62±0.26	5±0.25	4.45±0.19
3	10	3.61±0.20	14.02±0.19	7.52±0.21	10.29±0.14
4	15	7.20±0.92	27.12±0.18	20.26±0.11	27.78±0.23
5	30	17.81±0.21	34.45±0.11	25.26±0.11	35.27±0.25
6	45	22.07±0.14	41.27±0.17	29.85±0.28	43.01±0.19
7	60	25.40±0.21	46.32±0.19	35.89±0.27	56.60±0.6
8	75	26.89±0.13	56.7±0.16	42.12±0.16	58.07±0.20
9	90	28.39±0.22	57.75±0.27	44.78±0.25	60.63±0.15

***** All values are expressed as mean ± SD, n=3

Table No.1.7 Dissolution profiles for the various batches of directly compressed tablet.

Sr. No	Time (min)	Cumulative % drug release*			
		F	F1	F2	F3
1	0	0	0	0	0
2	5	22.52±0.15	23.2±0.11	26.12±0.12	25.57±0.21
3	10	39.21±0.21	39.22±0.17	43.74±0.16	42.61±0.19
4	15	49.56±0.11	54.34±0.22	56.92±0.12	59.16±0.15
5	30	52.08±0.16	61.39±0.23	60.04±0.24	64.83±0.17
6	45	55.74±0.17	65.38±0.25	63.18±0.22	71.09±0.21
7	60	58.86±0.22	67.71±0.19	64.65±0.11	74.01±0.22
8	75	60.30±0.14	70.61±0.13	65.85±0.14	75.26±0.11
9	90	61.19±0.22	71.84±0.23	67.05±0.21	76.80±0.15

***** All values are expressed as mean ± SD, n=3