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A Study of Drug-Excipient Interaction and Drug Product Stability Using Dry (Powder) Film Coating in Comparison with Conventional (Aqueous) Film Coating

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ABSTRACT

The objective of this work was to study drug-excipient interactions in solid dosage forms when coated with dry powder film-coat compared with conventional aqueous film. Free films of Eudragit RL, (ERL) with or without drugs (Metoprolol Succinate or Diclofenac sodium) were prepared by casting method and characterized by FTIR, NMR, or DSC. Tablets of either drugs were prepared by wet granulation and coated in a fluidized-bed by ERL aqueous dispersion or micronized powder. Dissolution behavior and color change study were carried out for tablet batches on zero time and after 3 months of storage in stability chambers. The results of free films showed a greater possibility of drug polymer interaction in the aqueous dispersion than dry powder films. The results of dissolution rate revealed a greater rate change in aqueous- than in dry-coated tablets. This was confirmed by the Color change study which showed more intense yellowing in aqueous-coated tablets.

Keywords: Drug-excipient interaction, eudragit RL, diclofenac sodium, dry powder, coating.

INTRODUCTION

Solid pharmaceutical dosage forms like tablets, capsules, granules, pellets etc. are coated for many purposes such as protection from moisture, light or oxygen; masking of odor or taste; acid resistance in gastric fluids; and to modify drug release from these dosage forms as controlled or delayed action. Traditionally, coating is carried out using either organic solution or aqueous solution or dispersion of certain polymers sprayed, after mixing with other substances

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such as plasticizers, onto dosage forms[1-4]. However, solvent-based coatings suffer from many potential disadvantages such as toxicity, air pollution or residual remnants of organic solvents. Also, the removal of solvents is highly energy consuming and takes longer processing time[5-6]. These all reflected as an increase in the cost of the process as well as the drug product per se.

Among serious efforts to overcome the above-mentioned problems of solvent-based coating, many recent works have been published regarding new coating technologies which are independent of solvent referred to as "solventless" coating. These may include, but not limited to, compression coating, hot melt coating, supercritical fluid spray coating, photocurable coating and dry powder coating [7]. However, the later, dry powder coating, was the most widely investigated. In this process, powdered (micronized) coating materials are directly applied, with or without wetting, onto solid dosage forms, and then heat-cured to form a coat [8]. Several dry coating technologies, including plasticizer-dry-coating, electrostatic-dry-coating, heat-dry-coating and plasticizer-electrostatic-heat-dry-coating have been developed and extensively reported [8-10].

Drug-excipient interaction, among other factors, may affect drug product quality and performance [11-13]. One example of such interactions is the action of the drug as a plasticizer for the polymeric film [14-15]. Plasticizers are generally added to polymers to increase their flexibility and hence durability, increase the permeability for the drug and promote film formation [3,11]. Therefore, the action of drug as a plasticizer is an extra- or over-plasticization and should be seriously taken in consideration and extensively investigated [11,15]. Sieppmann et al (2006) studied the plasticization effect of 3 different drugs, namely, chlorpheniramine, metoprolol tartrate and ibuprofen and found that they acted as good plasticizers for Eudragit RS polymers, and this effect was directly proportional to drug load [16]. The presence of solvent (ex. water) may complicate physical interaction between drug and polymer [17]. This type of interaction may simply occur during coating process due to the presence of solvent or even after coating (i.e. within the final dosage form) by solvent migration from core to coat [11]. In critical cases such as enteric coating of gastric antiulcer drugs, ex. proton pump inhibitors (PPIs), which are sensitive to acidic materials (ex. Eudragit L-100-55), a subcoat of different polymer have been applied [18-20].

Eudragit RL (ERL), generically referred to as Polymethacrylate, is widely used as film former for controlled release dosage forms [21-22]. Polymethacrylate polymers include ERL and Eudragit RS (ERS). However, ERL possesses higher permeability and hydrophilicity than ERS, since the content of quaternary ammonium moieties is greater in ERL [23]. These polymers are weekly cationic in nature and therefore are prone to interaction with anionic drug moieties [2, 16, 24,25].

It has recently been shown that metoprolol free base and metoprolol tartrate act as plasticizers for Eudragit RL based networks in the dry state [17, 18, 26]. Omari (1995), studied the interaction of diclofenac sodium (DS) with Eudragit RL and RS films prepared either from organic solutions or aqueous dispersions of these polymers [24]. Except for the study by Adeyeye et al (2004), who reported that solid state mixtures of DS and Eudragit polymers (RS or L100-55) showed lower extent of interaction than in liquid state [27], drug excipient interaction was not investigated particularly during powder dry coating.

In this work, both metoprolol succinate (MS) and diclofenac sodium (DS) were selected as model drugs. The objective was to study drug-excipient interaction as well as the effect of certain processing parameters on the performance and stability of dosage forms when coated by dry ERL powder in comparison with conventional aqueous coating technique. In first part, free films of ERL alone or with drugs were prepared by casting method using aqueous dispersion or dry powder of the polymer. Free films were characterized using Fourier-transform infrared spectroscopy (FTIR), Nuclear magnetic resonance (NMR) and Differential scanning calorimetry (DSC). In the second part, tablets of either MS or DS, were prepared by wet granulation method and coated with ERL polymers using dry powder coating or conventional aqueous coating techniques. The tablets were tested for physical properties and drug release behavior. A stability (aging) study also was conducted for up to 3 months under different storage conditions during which tablets were retested for their dissolution behavior at predetermined periods. A color change study was also performed using adobe photoshop.

METHODOLOGY

Metoprolol Succinate (MS) and Diclofenac sodium (DS) were donated by Hikma Pharma PLC, Amman, Jordan; Eudragit RL 100 was donated by Evonik industries AG, Germany; Triethyl citrate (TEC) was purchased from Parchem, NY, USA; Lactose anhydrous was donated by Al-Taqaddom Pharm. Co. Amman, Jordan; microcrystalline cellulose (Avicel) was donated by FMC, PA, USA; Polyvinylpyrrolidone (PVP) was purchased from BASF Corp. (Mt. Olive, NJ, USA), Magnesium stearate and talc were purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA). Other solvents and reagents are of pharmaceutical grades.

Free films preparation

Free films of ERL aqueous dispersion, were prepared by casting technique reported by Lehman (1997) [28]. A 5g ERL plus 1g (20% of dry polymer) triethyl

citrate (TC) as a plasticizer were added to 20g of distilled water (DW) (termed later as Aqueous Dispersion Film, ADF) and stirred, using high sheer propeller (IKA, Germany) for at least 3hr in a hot water bath (>80°C). The weight then was corrected by DW under stirring until cooling to room temperature (RT). The mixture was dried on a Teflon tape (Taixing Chuanda Plastic Co., Ltd., China) at RT for 24 hr. The casting area was 15cm x 15cm and the casting volume was 25-30ml. ADF was then cured in an oven at 60°C for 2 hr which then peeled off, labeled and stored in double plastic cases at RT until further use. Free films containing drugs were prepared in the same way except that a 0.5g of either MS or DS was added to ADF and equilibrated using magnetic stirrer for 2 hr prior to casting.

Free films of polymer powder (termed later as dry powder film, DPF) was prepared by micronization of ERL100 pellets using an electric chopper (Moulinex Co. France). The particle size under 60µ-sieve (5g) was taken, mixed well with TC (1g=20% of polymer), using mortar and pestle, distributed evenly by a ruler on Teflon tape and cured in an oven at 75-80°C for 6 hr. The film was then peeled off and stored in double plastic cases until further use. For DPF with drugs, 0.5 g of either MS or DS was added to the polymer-plasticized mixture and further mixed homogeneously and continue with the same procedure.

Free Film Characterization

NMR

Proton Nuclear magnetic resonance (H^1-NMR) spectra for the free films prepared were determined using NMR spectrometer (Bruker 400MHz Avance III, USA). Samples were dissolved in DMSO or $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard.

DSC

The glass transition temperature (Tg) of the polymeric systems was determined by differential scanning calorimetry (DSC 821; Mettler Toledo AG, Giessen, Germany). Film

samples of approximately 6 mg were accurately weighed into aluminum pans, which were sealed and perforated. The samples were heated (at 5 °C/min) under a nitrogen atmosphere from 0 to 100 °C. The Tg and heat flow energy (mJ) were determined.

FTIR

FTIR spectra were determined Using FTIR spectrometer (Bruker, Billerica,

MA, USA) and KBr pellets. The scanning range was 4000-400 cm^{-1}. Spectra for drugs, ERL polymer, free films of polymer with or without drugs were obtained.

Tablets Preparation

Tablets of either MS or DS were prepared by wet granulation method using PVP solution (10%) as a binder. Formulae are shown in Table 1. Granules were mixed with appropriate amount of magnesium stearate and compressed into tablets using single punch tableting machine (Korsch, GMPH, Germany) tooled with 12mm shallow concave punch. Tablets were characterized for their content and weight uniformity, hardness, friability and disintegration time. Table 2 shows tablet properties.

Table 1. Tablet formula of either Diclofenac sodium or metoprolol succinate.

Ingredients	Wt (mg/tab)
MS or DS	50
Lactose anhydrous	200
Microcrystaline cellulose	200
PVP (as 10% solution)	~2
Mg stearate	~1%
Total	~450

Table 2. Physical properties of tablets prepared from DS or MS.

Tablet properties	Value
Hardness (N)	20-35
Friability (%)	< 1
Disintegration time (min)	5-10
Weight uniformity (mg) ± 5%	450
Content Uniformity (mg) ± 5%	50

Tablets Coating

Tablets prepared in the previous section were coated with ERL aqueous dispersion using fluid bed (Wurster) system (Aeromatic STREA1, AG, Switzerland). Coating formulation and conditions were as reported in a previous work [29]. In brief, a certain weight of tablets was loaded in the coating chamber and after preheating the weight was taken again as initial weight (Wi). The process then started at a low spray rate which increased gradually under a suitable atomizing and fluidizing air rates. The coated tablets were then cured in a static tray-

oven at 60°C for 24 hours. Th final weight (Wf) was taken and the amount of coat was calculated as percentage coat to core ratio (%CCR=[Wf-Wi/Wi]x100).

Dry powder coating was conducted in the same coating apparatus, with a simple modification. A side hole (6mm in diameter) was drilled in the lower side of the Wurster chamber to facilitate powder delivery (see fig1). ERL micronized powder (<60 micron) was fed using powder feeder (AccuRate® Tuf-Flex™ feeders, Schenck Co. USA) connected via silicon tubing, 15mm in diameter from feeder side and 5mm from the other side to fit the drilled hole. Powder delivery was performed with the aid of compressed air via a separate airway hose (2mm in diameter) inserted directly in the silicon tubing. The process was carried out by spraying TEC -as a plasticizer- by the Wurester's bottom spray nozzle, using a peristaltic pump (VELP Scientifica, SRL, Italy), onto tablets to wet their surfaces, followed immediately by direct powder application. These 2 steps were repeated in a reciprocal intermittent way till the end of predetermined quantity of coat. Feeding rate and other coating conditions are shown in Table 3. At the end of the process, the tablets were cured in a static oven on Teflon-lined trays at 60°C for 24 hours. To prevent sticking during the curing step (and later in stability test), the cured tablets were dusted with 1-2% talc based on the weight of the coated tablets. Percentage CCR was calculated as in aqueous process.

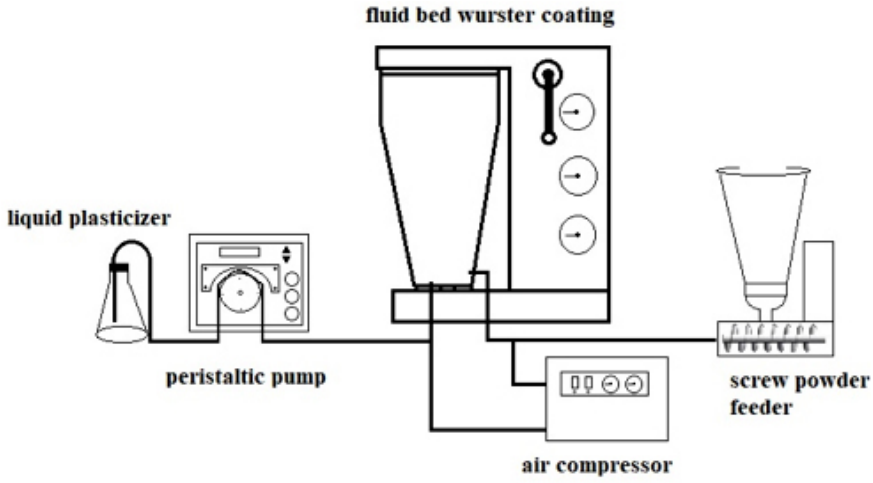


Figure 1. Fluid bed coating system with modifications for dry powder coating.

Table 3. Conditions employed during dry coating process

Condition	Values
Tablets batch weight (g)	250
Plasticizer Spray rate (ml/min)	1-2
Atomizing air pressure (bar)	1.5
Fluidizing air rate (m^3/hr)	50
Inlet air temperature (ºC)	55
Outlet air temperature (ºC)	45
Powder feeding rate (g/min)	2-5

A total of eight tablet batches were prepared of both DS (4 batches) and MS (4 batches) coated with ERL-ADF or ERL-DPF. Table 4 shows these batches and their CCR percentages. It is noteworthy that the objective of this work is to study interaction of drugs with excipient (coating polymer or other additives) using either solvent-dependent (aqueous dispersion) or solventless (dry powder) techniques irrespective of coat ratio. Therefore, the CCR value will not be considered as an investigating parameter in this research.

Table 4. Tablet batches of DS or MS prepared in this work and coated with either ERL-ADF or ERL-DPF of different coat-to-core ratios (%CCR).

No	DS tablets		MS tablets	
	Coated with	%CCR	Coated with	%CCR
1	ERL-ADF	2.7	ERL-ADF	1.0
2	ERL-ADF	4.0	ERL-ADF	4.0
3	ERL-DPF	2.4	ERL-DPF	1.6
4	ERL-DPF	3.0	ERL-DPF	4.4

Dissolution of coated tablets

Dissolution of coted tablets was conducted in 1000ml of gradient pH profile dissolution media corresponding to pH 1.2 (0.1N HCl) (for 2 hr) and 6.8 (phosphate buffer) using USP II (paddle) method apparatus (Esico International, India) operated at 75 rpm (±3rpm) and 37ºC (±1 ºC). The change in pH was done *in situ* by addition a precalculated amount of concentrated solution of tribasic sodium phosphate directly to the medium [30]. Five milliliter samples were withdrawn at predetermined intervals up to 20-24hr, replaced immediately with fresh medium, filtered through Millipore filter (Merck, Germany) and analyzed spectrophotometrically (SCO-TECH, GmbH, Germany) at λ_{max} 276 nm and 222 nm for DS and MS, respectively. Average of at least 3 replicates was calculated.

Dissolution Kinetics

The percentage of drug released was first calculated, then the average of three independent replicates along with standard deviation were measured. Data was then fitted to 2 kinetic models: zero order and first order equations. For zero order, the linear regression and linear equation was calculated for the first 6 time points. In case of first order, the percentage of drug released transformed to natural Logarithm then the linear regression was conducted. linear equation was used is , where a is the slope and b is the intercept with the y axis. Both a and b were calculated for all data points using Microsoft Excel.

Stability study of coated tablets

All tablet batches prepared in this work (see Table 4) were included in stability study. Adequate quantities of tablets from each batch were filled in plastic (HDPE) bottles, closed and stored in a closed cabinet at room temperature (RT, 20±3°C), or in stability chambers (Binder GmbH, Germany) at 40°C (±3°C) and 50°C (±3°C) for 3 months. After 1, 2 and 3 months, samples of tablets were withdrawn and inspected visually for any change in surface appearance, analyzed for color changes (see next section) and retested for their dissolution behavior and compared with the initial data (at zero time).

Color change study

Two batches of each of MS or DS tablets coated with either ADF or DPF were selected; namely: MS tablets of CCR 1% and 4.0% ADF and 1.6% and 4.4% DPF and DS tablets of CCR 2.7% and 4% ADF and 2.4% and 3% DPF. As a row of three tablets, a photo using camera was taken (Fig 2). Two different types of analysis were then conducted: qualitative and quantitative.

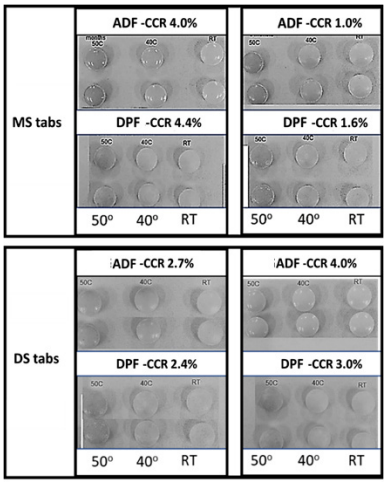
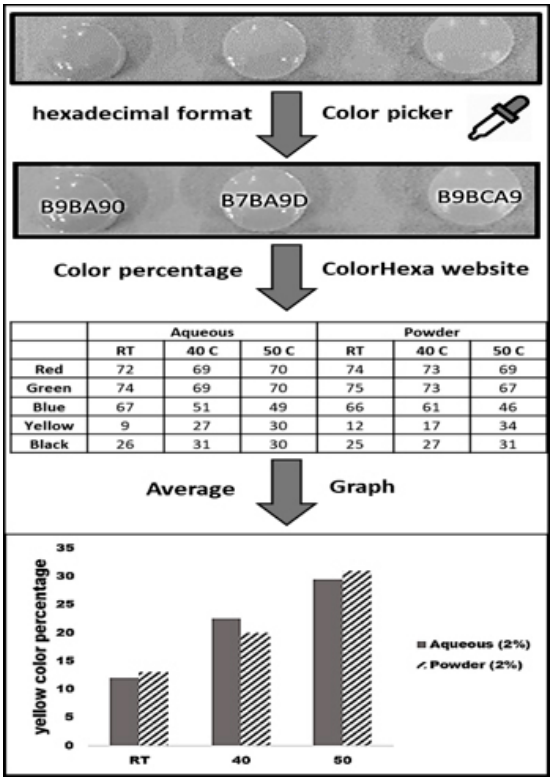


Figure 2. Images (in black and white) for selective tablets of different CCR and at different storage conditions to study color variation.

Qualitative analysis: an image processing was conducted using Adobe Photoshop to show the density of yellow color in black and white images. The images were split into the original channels (red, blue and green).

Quantitative analysis: To endorse the variation of yellow color between the tablets, the intensity of yellow color was measured. The procedure is summarized in Fig 3. Using unprocessed colored images, the hexadecimal code of the tablet color was identified using color picker tool from Adobe photoshop. The regions with extreme shadow and light have been excluded. The hexadecimal code was then applied on ColorHexa website (www.colorhexa.com) to get the intensity of red, blue, green, yellow and black color. The average yellow color was then calculated for multiple images of the same formulation. Later, the averages of three independent images were graphed using Microsoft excel.



	Aqueous			Powder		
	RT	40 C	50 C	RT	40 C	50 C
Red	72	69	70	74	73	69
Green	74	69	70	75	73	67
Blue	67	51	49	66	61	46
Yellow	9	27	30	12	17	34
Black	26	31	30	25	27	31

Figure 3. The image analysis steps. the hexadecimal format of each tablet color was identified using color picker from Adobe illustrator, then the percentages of colors (Red, Green, Blue, Yellow, Black) for each hexadecimal name were identified using Colorhexa website (www.colorhexa.com).

RESULTS AND DISCUSSION

Free films characterization

The functional groups of Eudragit polymers (and in some cases, the charges associated) make them readily reactive with drug substances[31]. Interaction of

the Ammoniomathacrylate copolymers (ex. ERL) (Fig 4) with other molecules is, most probably, attributed to their quaternary ammonium groups (QAGs) content[22,24,31]. In coating technology, studying the effect of such interactions on different properties of the final polymer films and later on the drug dosage form, free film (vs applied film) technique is usually adopted. Such technique has been established as a successful tool in the development of a film coating systems [32-33]. In this work, free films of ERL either from aqueous dispersion or dry powder with or without drugs were prepared using casting method and characterized for drug- polymer interactions using methods such as FTIR, NMR and DSC.

In a previous work[24], a stoichiometric ionic interaction was detected between DS solution and ERL powder and ERL aqueous dispersion and was supported by evidences from FTIR spectroscopy and X-Ray diffraction. However, the interaction was slower in case of powder than aqueous dispersion due to particle surface area difference[24].

In this work, ADF and DPF of DS were prepared in 1:10 ratio and characterized using FTIR, NMR and DSC. Fig 5 shows the FTIR spectrum of ERL-DS ADF and ERL-DS DPF (the complete spectra of the 2 drugs as well as pure polymer were shown in the associated supplement file). In aqueous films two peaks were appeared at 1557 and 1574 cm^{-1} which, most probably, correspond to secondary amine in DS molecule (see structure in Fig 4). In dry films these peaks were not shown or insignificant. This could be explained as follows: the ionic interaction between carboxylate anions of DS and quaternary ammonium cations of ERL in case of ADF, introduce some modification (such as deshielding) to the secondary amine of DS made it detectable by the FTIR spectrometer. This did not happen in DPF. In FTIR of ERL with MS (see supplement file), some small peaks appeared, however, not significant.

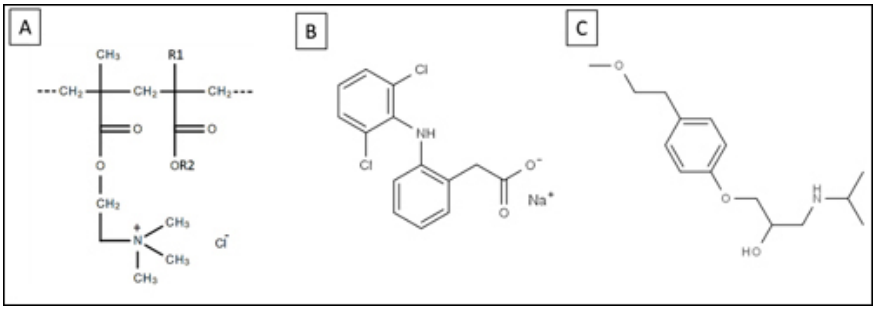


Figure 4. Chemical structure of (A) ERL polymer, (B) Diclofenac Sodium (C) Metoprolol Succinate

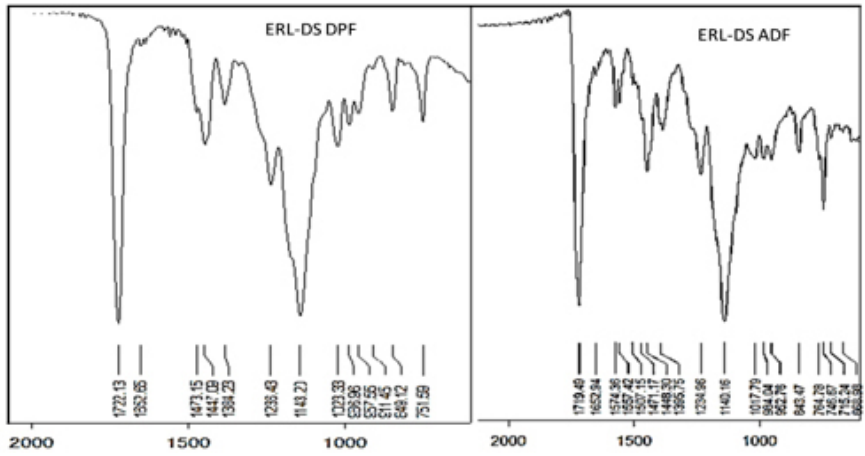


Figure 5. FTIR spectra of ERL free films: A) ERL-DS DPF B) ERL-DS ADF

Metoprolol interaction with polymethacrylate polymers was extensively investigated by Siepman et al (2006) and Glaessel et al (2009, 2010)[16-17, 26]. They found that metoprolol tartrate (and chlorpheniramine maleate and ibuprofen) act as efficient plasticizers for Eudragit RS as indicated by the significantly decreased Tg with increasing drug loading, irrespective of the type of drug[26]. In this work, MS were added in 1:5 ratio to ERL aqueous dispersion (without TEC) and cast on Teflon tape for 24hr. A clear transparent film was obtained (see Fig 1 A in supplement file) which is in agreement with the results reported in literature[16-17]. DS in a similar experiment, failed to form a film (see Fig 1 B in supplement file). Attempts to prepare a dry powder film containing drug and polymer only were unsuccessful even at higher temperatures (up to 80°C).

A strong evidence on MS interaction with the polymer was obtained using proton NMR spectroscopy of the free films (Fig 6). In spectrum of ERL-MS ADF (Fig 6B) in the region around 3.5 ppm where the protons of QAGs are expected to resonate, 2 peaks were depicted at 3.55 and 3.59 ppm. In the spectrum of the DPF (Fig 6A) only one peak is obtained at 3.59 ppm. In aqueous films, MS as a water-soluble drug, will dissolve and an electrostatic or hydrophilic interaction with the polymer is expected. This interaction led up to formation of a new peak in that region. In the DPF no new peak observed. The same can be said for interaction of DS with ERL NMR spectrum, however not in the same degree of clarity (Fig 6 C and D).

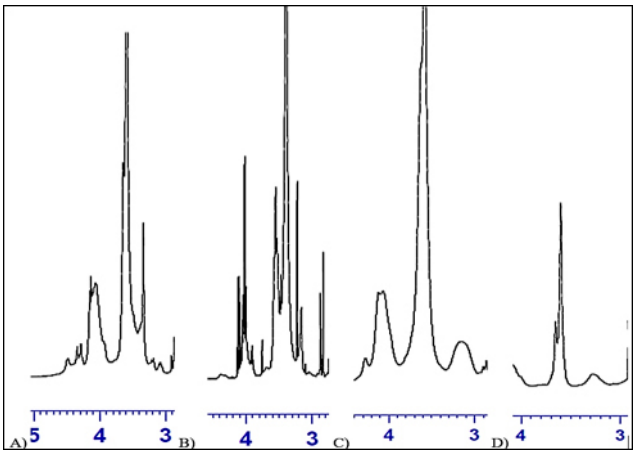


Figure 6. Proton NMR spectra of free films: A) ERL-MS DPF, B) ERL-MS ADF, C) ERL-DS DPF and D) ERL-DS ADF.

Thermal study using DSC for free films of ERL alone or containing drugs showed that the transition behavior of these films was different in aqueous from that in dry films (Fig 7 and table 5). The glass transition temperature (Tg) of ERL100 was reported to be 58-68°C[34-35]. In this work, DSC thermograms showed that in aqueous films Tg was reduced by TEC to 54°C and further reduced to 48 and 53 after interaction with DS and MS, respectively (Table 5). In dry films TEC reduced Tg to below 50°C, however addition of either drugs (with TEC) increased it to 53°C and 54°C for DS and MS, respectively. The explanation to this behavior is that, in the aqueous systems, drugs may synergize plasticization effect of the TEC while in dry films antiplasticization effect occurs[36]. This may also explain the difference in heat flow magnitudes during transition which was significantly higher in aqueous in comparison to dry films as shown in Table 5.

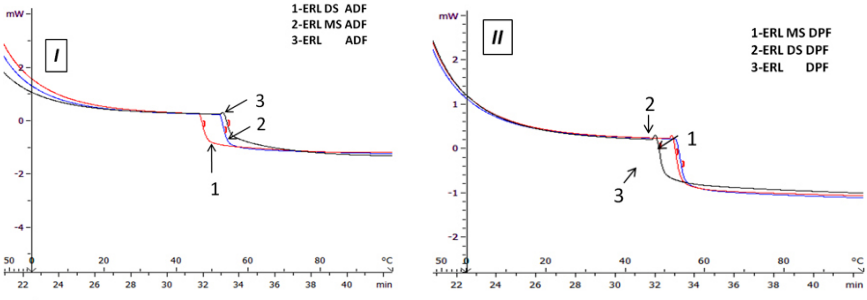


Figure 7. DSC thermograms of free films of ERL with or without drugs. I) aqueous dispersion films (ADF). II) dry powder films (DPF).

Table 5. Glass transition temperature (Tg) and Heat flow of ERL free films with or without drugs according to method of preparation (aqueous or dry)

Film Preparation	Tg Midpoint (°C)	Heat Flow (mJ)
ERL ADF	54	- 46
ERL-DS ADF	48	- 24
ERL-MS ADF	53	- 21
ERL DPF	49	- 15
ERL-DS DPF	53	- 18
ERL-MS DPF	54	- 25

Coating Process of Tablets with ERL ADF or DPF

The coating process was performed using fluid bed (Wurster) system for both aqueous conventional coating and powder dry coating. However, to suit the technique for dry coating, a modification in the apparatus (see experimental section) was done in order to facilitate dry powder feeding to coating chamber. In literature, several trial investigations have been published. Some of these include fluid bed system and centrifugal granulator[9], modified Wurester apparatus[37], rotary fluid bed[38] and modified lab scale spheronizer[39-41]. In most of these studies, modifications were made to the original apparatus without technique standardizations. In our work, since (up to the authors' knowledge) no standard apparatus can be obtained, the introduced modifications to fluid bed system helped to reach good success. However, standardization and validation of such technique still required.

All tablet batches prepared in this work (see Table 4 in experimental section) were investigated for coat uniformity, color changes and stickiness during coating. In general, all batches were of good coat and color uniformity, however, dry coated tablets showed somewhat more stickiness than those coated with aqueous dispersion. This might be attributed to high percentage (40%) of the highly hydrophilic plasticizer (TEC) used in case of dry process. Therefore, use of dusting talc has become an essential requirement in order to minimize this phenomenon. All batches also were tested for their dissolution behavior (i.e. at zero time) for the purpose of initial drug release performance and as a reference for comparison in later studies (see results in the next section).

Stability Study

Stability is the extent to which a product retains, within the specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its manufacturing. Stability testing thus evaluates the effect of environmental factors on the quality of the drug substance or a

formulated product[42-43]. In this study, different tablet batches prepared (Table 4) were stored at different conditions as explained in experimental section. Tablets were tested for color changes and dissolution behavior.

Color Change Study

Many recent articles were published using color change as stability indicator [44-46]. In this study the discoloration of tablets via the formation of yellow color was studied qualitatively and quantitively.

In the qualitative color analysis, as shown in Fig 2, black and white picture doesn't represent the real case, as the change in tablets color is undistinguished specially between 40°C and room temperature (RT), as well as between aqueous and dry (see Fig 2). Therefore, image processing was conducted by splitting images into the original channels. As seen in Fig 8 there is a distinct change in color using blue filter between the stored tablets at different temperatures, while no differences were observed using red and green filter. Yellow color appears as black color under blue filter. Therefore, it's clear that the intensity of yellow color is proportional to the heat; as the storage temperature increases, the yellow color intensity increases (Fig 8 blue filter).

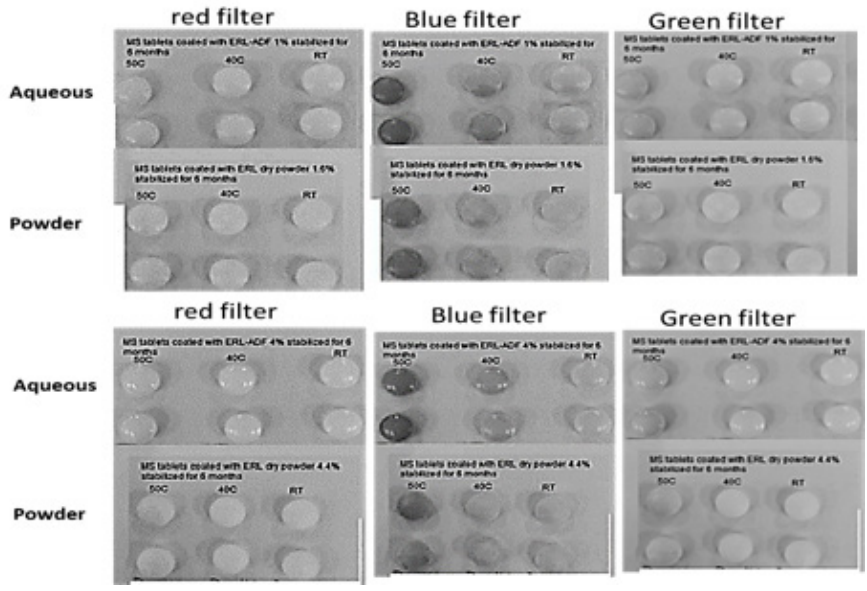


Figure 8. Tablet images after split into red blue and green channel. The upper image is MS tablets coated with 1% ERL-ADF (Aqueous) and 1.6 % ERL-DPF (Powder), the lower image is MS tablets coated with 4.0% ERL-ADF (Aqueous) and 4.4% ERL-DPF (Powder).

The quantitative analysis of yellow color is needed to differentiate the effect of coating method on the discoloration of tablets. As has been illustrated in Fig 9 (A and B), in case of MS, the yellow color was more intense in aqueous coating than in dry coating across the range of temperatures. The analysis suggests potential benefits of using dry over aqueous coating. Meanwhile, it was not the case with DS (Fig 9 C and D), the intensity of yellow color was somewhat similar between aqueous and dry coating.

The source of yellow-brown color is highly suggested due to a Maillard reaction (browning reaction), which has been observed in several drug formulations[18,47-49] including metoprolol[47]. Browning reaction is initiated by the heat, and that explains the increasing in the intensity of yellow color was associated with the elevating of the storage temperature. All formulations in this study contained lactose and polyvinylpyrrolidone which play parts of Maillard reaction under the stress of temperatures[50]. Also, this reaction peaks in the presence of water and occurs better in alkaline than in acid conditions. This explains the higher color intensity in aqueous coating systems than dry powder coating systems. Meanwhile explains why the MS tablets with aqueous coat (Metoprolol pKa=9.5), has more intense yellow color than DS tablets (Diclofenac pKa=4.5)[50].

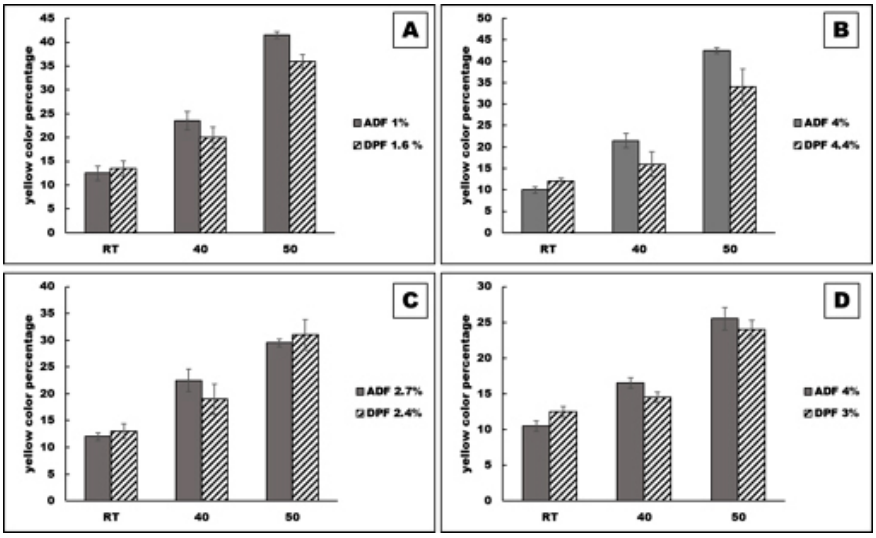


Figure 9. Comparison of the density of tablet's yellow color at different storage conditions; room temperature (RT), 40°C and 50°C, for both MS and DS. A) MS tablets: 1.0% ADF vs 1.6% DPF, B) MS tablets: 4.0% ADF vs 4.4% DPF, C) DS tablets: 2.7% ADF vs 2.4% DPF, D) DS tablets: 4% ADF vs 3.0% DPF. The error bar represents the standard deviation of the mean.

Dissolution: Dissolution of different tablet batches stored at various storage conditions were conducted in pH profile media (acidic pH 1-2 for 2 hrs then basic pH 6.8 up to 22 hrs) and compared with the results at zero time. A total of 88 graphs were plotted. A representative example are shown in Fig 10.

The drug release kinetics was studied by fitting the data of cumulative amount drug dissolved vs time to two kinetic equations: zero order and first order models[51]. Due to the fact that a large quantity of data were obtained, a representative results of 1 batch were shown in Table 6 (other data are shown in Table 2 in supplement file). From these data it is clear that zero order model is the one of best fit.

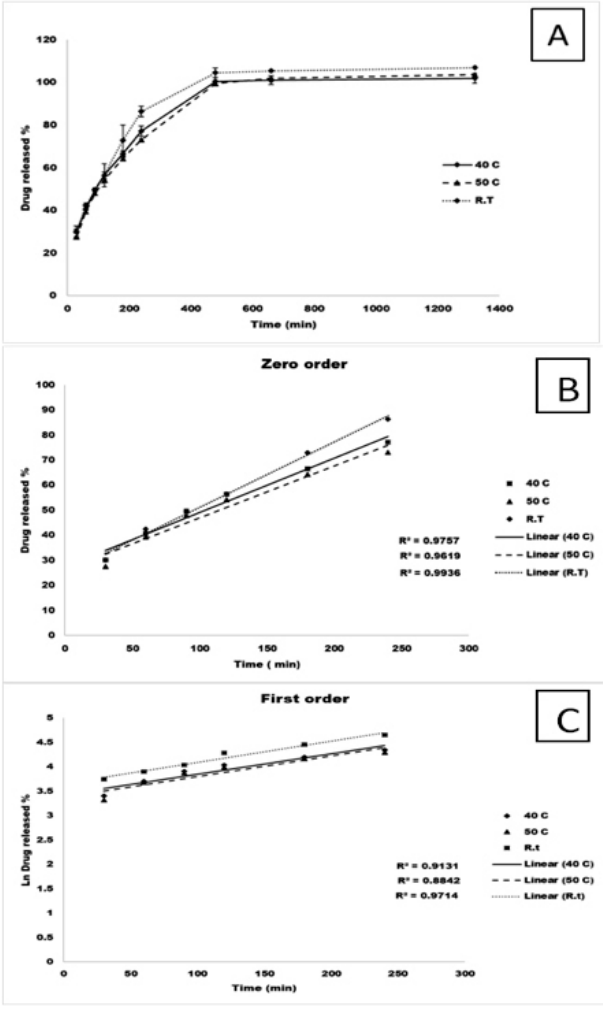


Figure 10. A model for the kinetics of drug release, the example is MS tablets coated with ERL-DPF 1.6 % CCR after 3 months of storage at different temperature. A) cumulative drug release data. B) the data fitted into zero order kinetics. C) the data transformed into natural logarithm and then fitted in to first order kinetics. Each experiment was conducted in triplicate, the error bar in graph (A) represents ±SD. The linear regression (R^2) is shown on the graph for each storage temperature.

Table 6. An example of kinetics data. MS tablets coated with ERL-DPF 1.6 % CCR different storage time in different storage temperatures. *a*= slope of the linear equation, *b*= is the intercept with the y axis, EXP*(b)* = inverse natural logarithm *(b)*, R^2= the coefficient of determination

		Zero order			First order		
Storage time (months)	**Temperature (C)**	**R^2**	**a**	**b**	**R^2**	**A**	**EXP (b)**
0		0.986	0.3125	24.12	0.9555	0.0052	30.35618
1	R.T	0.9272	0.2182	26.423	0.8128	0.0044	34.62237
	40	0.9764	0.2076	26.706	0.9253	0.0035	32.23974
	50	0.9706	0.2091	27.701	0.9072	0.0036	30.93536
2	R.T	0.9634	0.2035	29.557	0.9118	0.0035	40.97655
	40	0.9751	0.2114	26.841	0.9164	0.0039	29.89825
	50	0.9699	0.2079	26.527	0.9047	0.004	29.27693
3	R.T	0.9936	0.2612	24.97	0.9712	0.0049	37.12535
	40	0.9757	0.2168	27.429	0.9131	0.0042	30.90444
	50	0.9619	0.2075	26.176	0.8842	0.0042	29.22721

To compare the effect of two coating processes (aqueous vs dry powder), data of zero order kinetic model of 2 batches of each drug (see Table 1 in supplement file) were considered. Dissolution rate constants (a) from this table (Table 1 in supplement file) were taken as a comparison parameter and included in Table 7.

As the kinetics are more fitted into zero order, the drug release is independent on the initial concentration, and totally depends on the rate constant (a), which can mathematically be driven from the slope of linear equation. According to Table 7, the rate of release is smaller in aqueous coating than in dry coating. However, (a) values in aqueous coating were doubled with storage time while no significant changes in (a) were observed in dry coating method. One-way ANOVA were conducted to test if the change in slope (a) is statistically different between the two methods. The percentage ratios of (a) values at storage temperatures to its value at zero time were used. According to the results in Table 8, the F-value is greater than the F-critical value for the alpha level ($p<0.001$). Therefore, the change in dissolution rates are significantly different between dry and aqueous coating and, hence, dry powder coating can improve stability of drug product with respect to dissolution rate in comparison with aqueous coating.

Table 7. Zero order dissolution rate constants (a) for the different batches from table 7.

	Storage time/Temp		RT	40C	50C
DS tablets coated ERL-ADF 2.7%					
	0	0.0243			
	1		0.0571	0.0461	0.0539
	2		0.0506	0.0415	0.0655
	3		0.0374	0.035	0.0294
DS tablets coated ERL-DPF 3.0%					
	0	0.3072			
	1		0.2916	0.2194	0.332
	2		0.3101	0.3081	0.3405
	3		0.2896	0.2872	0.2033
MS tablets coated ERL-ADF 4.0%					
	0	0.1271			
	1		0.2218	0.2244	0.2293
	2		0.2338	0.2344	0.2424
	3		0.2167	0.251	0.2301
MS tablets coated ERL-DPF 4.4%					
	0	0.2258			
	1		0.2228	0.232	0.2264
	2		0.2368	0.2567	0.2589
	3		0.2407	0.1997	0.2242

Table 8. One-way ANOVA test of the percentage ratios of (a) values at storage temperatures to its value at zero time

Drug	Source of Variation	Square sum	Medium square	F[a]
MS	Dry and aqueous coating	2.883333	2.883333	605.0239
DS	Dry and aqueous coating	4.239504	4.239504	33.76046

F critical= 4.493998

[a] Significant for $P < 0.001$.

Drug interaction with coating materials was studied under solvent-dependent (aqueous) coating process in comparison with solventless (dry powder) coating technique. Studies on free films of ERL alone or containing drugs (MS or DS) showed a significant decrease in interaction extent in dry powder coating relative to aqueous coating. These results were confirmed using FTIR, NMR and DSC characterization methods. Tablets of either MS or DS were prepared and coated successfully in a modified Wurester fluid bed using ERL micronized

powder (solventless) as a film former and TEC as wetting agent and plasticizer. These tablets in different %CCR were tested initially for physical properties and dissolution behavior and compared to those coated with ERL aqueous dispersion. A stability study was conducted for dry coated as well as aqueous coated tablets at different storage conditions (RT, 40°C and 50°C) for up to 3 months. Dissolution testing for the stability batches showed that release rate constant calculated from zero order kinetics possessed greater change extent in aqueous than dry powder coating as indicated by ANOVA test. The results of color change study supported the above results of free films and stability batches and showed that yellowing (due to Millard reaction) in aqueous coated tablets was significantly higher than in dry coated ones. However, the need for validation process of dry powder coating method is considered a major limitation of this study, which is hopefully, a future work.

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Conflict of Interest

No conflict of interest

REFERENCES

1. Porter, S. C. Coating of Pharmaceutical Dosage Forms, in Gennaro, A.R. (edr), *Remington: The Science and Practice of Pharmacy*, Mack Pub. Co. **1995**, 1650-1659.

2. Omari, D. Study of Interactions of Film coating Materials with Drug Formulation Components in Solid Dosage Forms, PhD Thesis, Cairo University, Egypt, **2003**.

3. Hogan, J. Film Coating Materials and Their Properties, in Cole, G, *Pharmaceutical Coating Technology*, Tayler & Francis. **1995**, 6-52.

4. Haris, M.; Ghebre-Sellassie, I. Aqueous polymeric coating for Modified Release Oral Dosage forms in McGinity, W and Felton, L. *Aqueous Polymeric Coating of Pharmaceutical Dosage Forms*, 3rdEdn., Informa healthcare. **2008**, 47-66.

5. Aulton, M.; Twichell, A. Film coat Quality, in Cole, G. *Pharmaceutical Coating Technology*, Tayler& Francis. **1995**, 363-408.

6. Jones, D. Pharmaceutics-Dosage Form and Design, Pharmaceutical Press. **2008** ,244-251.

7. Bose, S.; Bogner, R. H. Solventless Pharmaceutical Coating Processes: A Review. *Pharm Dev Technol*. **2007**, *12*, 115-131.

8. Luo, Y.; Zhu, J.; Ma, Y.; Zhang, H. Dry coating, a novel coating technology for solid pharmaceutical dosage forms. *Int J Pharm*. **2008**, *358*, 16-22.

9. Obara, S.; Maruyama, N.; Nishiyama, Y.; Kokubo, H. Dry coating: An innovative enteric coating method using a cellulose derivative. *Eur. J. Pharm. Biopharm*. **1999**, *47*, 51-9.

10. Pearnchob, N.; Bodmeier, R. Dry Powder Coating of Pellets with Micronized Eudragit® RS for Extended Drug Release. *Pharm. Res*. **2003**, *20*, 1970-1976.

11. Diane Bruce, L.; McGinity, J. Polymer Interactions with Drugs and Excipients, in McGinity, W and Felton, L. *Aqueous Polymeric Coating of Pharmaceutical Dosage Forms*, 3rdEdn., Informa healthcare, **2008**, 369-407.

12. Jenquin, M. R.; Liebowitz, S. M.; Sarabia, R. E.; McGinity, J.W. Physical and chemical factors influencing the release of drugs from acrylic resin films. *J. Pharm. Sci*. **1990**, *79*, 811-816.

13. Crowley, M. M.; Fredersdorf, A.; Schroeder, B.; Kucera, S.; Prodduturi, S.; Repka, M. A.; McGinity, J. W. The influence of guaifenesin and ketoprofen on the properties of hot-melt extruded polyethylene oxide films. *Eur. J. Pharm. Sci*. **2004**, *22*, 409-418.

14. Wu, C.; McGinity, J. W. Influence of ibuprofen as a solid-state plasticizer in Eudragit® RS 30 D on the physicochemical properties of coated beads. *AAPS PharmSciTech*. **2001**, *2*, 35–43.

15. Wu, C.; McGinity, J. W. Non-traditional plasticization of polymeric films. *Int. J. Pharm*. **1999**, *177*, 15-27.

16. Siepmann, F.; Le Brun, V.; Siepmann, J. Drugs acting as plasticizers in polymeric systems: A quantitative treatment. *J. Control. Release*. **2006**, *115*, 298-306.

17. Glaessl, B.; Siepmann, F.; Tucker, I.; Rades, T.; Siepmann, J. Deeper insight into the drug release mechanisms in Eudragit RL-based delivery systems. *Int. J. Pharm*. **2010**, *389*, 139-46.

18. Bharate, S. S.; Bharate, S. B.; Bajaj, A. N. Interactions and incompatibilities of pharmaceu-

tical excipients with active pharmaceutical ingredients: a comprehensive review. *J. Excipients Food Chem.* **2010**, *81*, 117-122.

19. Das, A.; Sathyamoorthy, N.; Garikapati, D. Development of enteric coated pantoprazole tablets with an aqueous based polymer. *Int. J. ChemTech Res.* **2013**, *5*, 2394-2404.

20. Omari, D. M. Formulation and In vitro/In vivo evaluation of esomeprazole enteric coated minitablets. *J. Drug Deliv. Sci. Technol.* **2017**, *39*, 156-165.

21. the United States Pharmacopoeia (USP) and the National Formulary (NF). USP 24- NF19. Rockville, Md. **1999**, 2000- 2209.

22. Rohm Pharm Info Sheets, GMBH, Darmstadt. **2002**, Info: O/E 2-4, 7.7E 1-4. 7.9/E 1-4.

23. R.C. Rowe, P.J. Sheskey, M.E. Fenton. Handbook of Pharmaceutical Excipients: Pharmaceutical Excipients. **2012,** 544-557.

24. Omari, D. M. The use of Drug-Excipient Interactions in The Design of Controlled Release Dosage Forms. MSc. Thesis, Jordan Univ. Sci. Tech. Amman. **1995**, 137-140.

25. Mehta, A. M. Processing and Equipment Consideration for Aqueous Coatings, in McGinity, W and Felton, L. *Aqueous Polymeric Coating of Pharmaceutical Dosage Forms*, 3rdEdn. Informa healthcare. **2008**, 67-103.

26. Glaessl, B.; Siepmann, F.; Tucker, I.; Siepmann, J.; Rades, T. Characterization of quaternary polymethacrylate films containing tartaric acid, metoprolol free base or metoprolol tartrate. *Eur. J. Pharm. Biopharm.* **2009**, *73* ,366-72.

27. Adeyeye, M. C.; Mwangi, E.; Katondo, B.; Jain, A.; Ichikawa, H.; Fukumori, Y. Dissolution stability studies of suspensions of prolonged-release diclofenac microcapsules prepared by the Wurster process: I. Eudragit-based formulation and possible drug-excipient interaction. *J. Microencapsul.* **2005**, *22*, 333-342.

28. Lehman, K, in McGinity, J.W.*.Aqueous polymeric coating for pharmaceutical dosage forms*, 2nd ed. Marcel Dekker, New York. **1997**, 101-176.

29. Omari, D. M.; Sallam, A.; Abd-Elbary, A.; El-Samaligy, M. Lactic acid-induced modifications in films of Eudragit RL and RS aqueous dispersions. *Int. J. Pharm.* **2004**, *274*, 85-96.

30. Lehman, K. Practical course in film coating of pharmaceutical dosage forms with Eudragit. Darmstadt: Pharma polymer. **2001**, 144-147.

31. Diane Bruce, L.; McGinity, J. W. Polymer Interactions with Drugs and Excipients, in McGinity, W and Felton, L. *Aqueous Polymeric Coating of Pharmaceutical Dosage Forms*, 3rdEdn., Informa healthcare. **2008**, 369.

32. Allen, D. J.; Demarco, J. D.; Kwana, K. C. Free films I: Apparatus and preliminary evaluation. *J. Pharm. Sci.* **1972**, *61*, 106-110.

33. Parikh, N. H.; Porter, S. C.; Rohera, B. D. Tensile Properties of Free Films Cast from Aqueous Ethylcellulose Dispersions. *Pharm. Res.* **1993**, *10*, 810-815.

34. MatWeb: Material Property Data; www.matweb.com/search/datasheet.aspx

35. Kumar, M.; Sachan, N. An Overview on Polymethacrylate Polymers in Gastroretentive Dosage Forms Pravin Gupta1. *Open Pharm. Sci. J.* **2015**, *2*, 31-42

36. Mishra, A.; Pathak, A. K. Plasticizers: A Vital Excipient in Novel Pharmaceutical Formula-

tions. *Current Res. Pharm. Sci.* **2017**, *7*, 1-10.

37. Ludwig, W.; Szafran, R. G.; Kmiec, A.; Dziak, J. Dry powder coating in a modified wurster apparatus. *Procedia Eng*. **2012**, *42*, 437-446.

38. Kablitz, C. D.; Harder, K.; Urbanetz, N. A. Dry coating in a rotary fluid bed. *Eur. J. Pharm. Sci.* **2006**, *27*, 212-219.

39. Zheng, W.; Cerea, M.; Sauer, D.; McGinity, J. W. Properties of theophylline tablets powder-coated with methacrylate ester copolymers. *J. Drug Deliv. Sci. Technol*. **2004**, *14*, 319-325.

40. Sauer, D.; McGinity, J. Properties of theophylline tablets dry powder coated with Eudragit ® e PO and Eudragit® L 100-55. *Pharm. Dev. Technol.* **2009**, *14*, 632-641.

41. Sauer, D. An Investigation of Formulation Factors and Processing Parameters for the Powder-Coating of Tablets, PhD Dissertation, The University of Texas at Austin, **2008**.

42. Cao, Z.; Zhang, X.; Liu, L.; Zhao, L.; Li, H.; Wang, J. Effect of pH on the aggregation of α-syn12 dimer in explicit water by replica-exchange molecular dynamics simulation. *Int. J. Mol. Sci.* **2015**, *16*, 14291-304.

43. ICH Expert Working Group, ICH Guideline Q1A(R2) Stability Testing of New Drug Substances and Products, International Conference of Harmonization, **2006**, 873.

44. Li, Y.; Polozova, A.; Gruia, F.; Feng, J. Characterization of the Degradation Products of a Color-Changed Monoclonal Antibody: Tryptophan-Derived Chromophores, *Anal. Chem.* **2014**, *86*, 6850–6857

45. Mochizuki, K.; Takayama, K. Prediction of color changes in acetaminophen solution using the time-temperature superposition principle. *Drug Dev Ind Pharm.* **2016**, *42*, 1050-1057.

46. Katsura, S.; Yamada, N.; Nakashima, A.; Shiraishi, S.; Gunji, M.; Furuishi, T.; Endo, T; Ueda, H.; Yonemochi, E. Investigation of Discoloration of Furosemide Tablets in a Light-Shielded Environment. *Chem. Pharm. Bull.* **2017**, *65*, 373-380,

47. George, R. C.; Barbuch, R. J.; Huber, E. W.; Regg, B.T. Investigation into the yellowing on aging of sabril® tablet cores. *Drug Dev. Ind. Pharm*. **1994**, *20*, 3023-3032.

48. Reddy, R. B.; More, K. R.; Gupta, L.; Jha, M. S.; Magar, L. Identification, synthesis, isolation and characterization of new impurity in metoprolol tartrate tablets. *J. Pharm. Biomed. Anal.* **2016**, *117*, 104-108.

49. Fathima, N.; Mamatha, T.; Qureshi, H. K.; Anitha, N.; Venkateswara Rao, J. Drug-excipient interaction and its importance in dosage form development, *J. Appl. Pharm. Sci.* **2011**, *1*, 66-71.

50. Labuza, T. P.; Reineccius, G. A.; Monnier, V. M.; O'Brien, J.; Baynes, J. W. Maillard Reactions in chemistry, food and health. Woodhead. **1998**, 175–198.

51. Siepmann, J.; Siepmann, F. Mathematical Models of Drug Dissolution: A Review. *Int J Pharm.* **2013**, *453*, 12-24.

SUPPLEMENT DATA

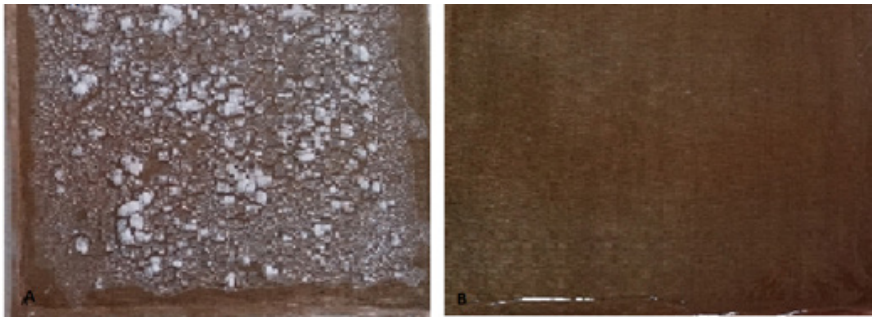

Figure 1. Interaction of model drugs with ERL aqueous dispersion without TEC a) 1 g DS unable to form a film. b) 1g MS formed a clear transparent film.

Table 1. Zero order kinetic model data of representative batches (2 of MS and 2 of DS) to compare aqueous coating with dry coating.

Drug	Coating	%CCR	months	St Temp	R2	a	b	Drug	Coating	%CCR	months	St Temp	R2	a	b
diclofinac	aqueous	2.70%	0		0.9859	0.0243	-0.301	diclofinac	dry	3.00%	0		0.9977	0.3072	-0.7767
			1	R.T	0.9865	0.0571	-1.363				1	R.T	0.9748	0.2916	9.2784
				40	0.995	0.0461	0.1604					40	0.9956	0.2194	4.6484
				50	0.9167	0.0539	-2.0673					50	0.9914	0.332	8.0902
			2	R.T	0.9822	0.0506	-1.0695				2	R.T	0.9976	0.3101	-0.149
				40	0.9697	0.0415	-1.8966					40	0.9992	0.3081	4.8497
				50	0.9787	0.0655	0.0655					50	0.9951	0.3405	11.259
			3	R.T	0.9798	0.0374	0.2378				3	R.T	0.9993	0.2896	3.6549
				40	0.9681	0.035	-1.6313					40	0.9627	0.2872	13.013
				50	0.9391	0.0294	0.6438					50	0.9919	0.2033	1.9912
metoprolol	aqueous	3.60%	0		0.9397	0.1271	8.7767	metoprolc	dry	4.40%	0		0.9515	0.2258	28.467
			1	R.T	0.9193	0.2218	23.611				1	R.T	0.9868	0.2228	31.596
				40	0.9412	0.2244	20.62					40	0.9834	0.232	25.795
				50	0.9333	0.2293	16.976					50	0.9858	0.2264	21.703
			2	R.T	0.9517	0.2338	18.343				2	R.T	0.9735	0.2368	24.595
				40	0.9439	0.2344	15.464					40	0.9788	0.2567	19.337
				50	0.9435	0.2424	14.088					50	0.9665	0.2589	18.711
			3	R.T	0.9661	0.2167	20.951				3	R.T	0.9657	0.2407	24.305
				40	0.9809	0.251	14.282					40	0.9737	0.1997	28.4
				50	0.9543	0.2301	17.9					50	0.9848	0.2242	22.014

FTIR Data

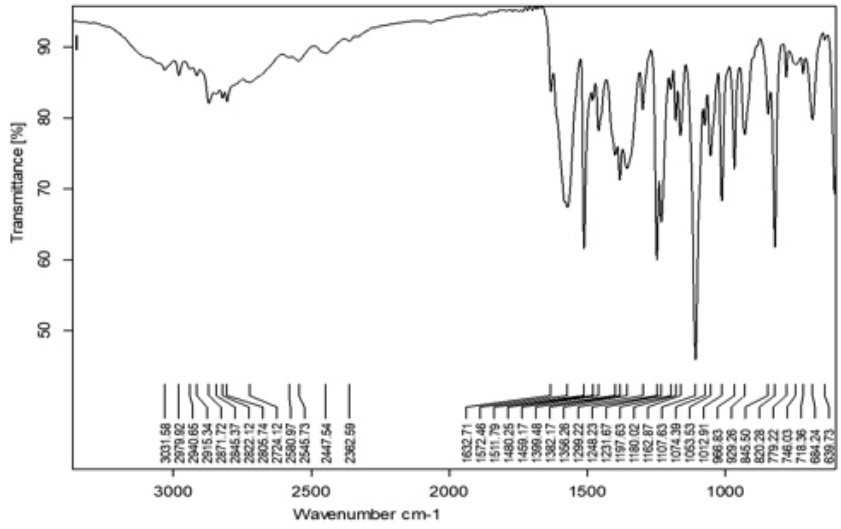

Figure 2. FTIR of metoprolol succinate

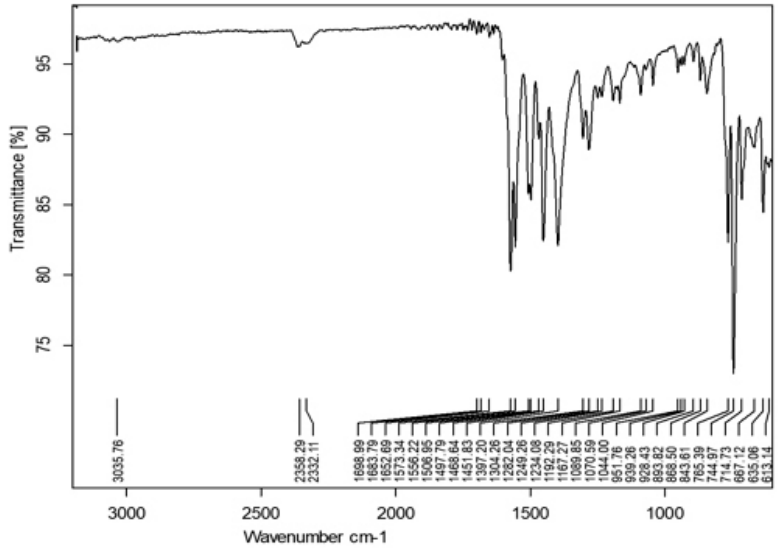

Figure 3. FTIR of Diclofenac sodium

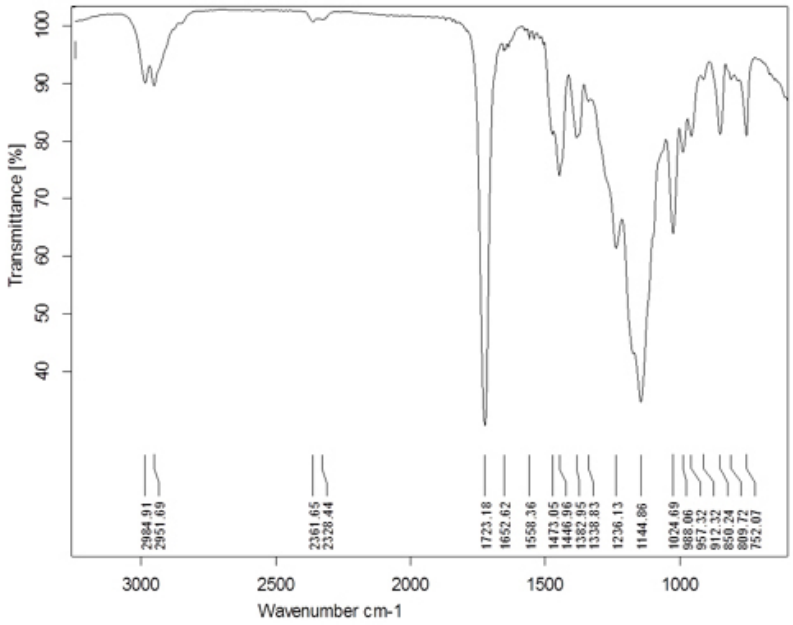

Figure 4. FTIR of ERL-100

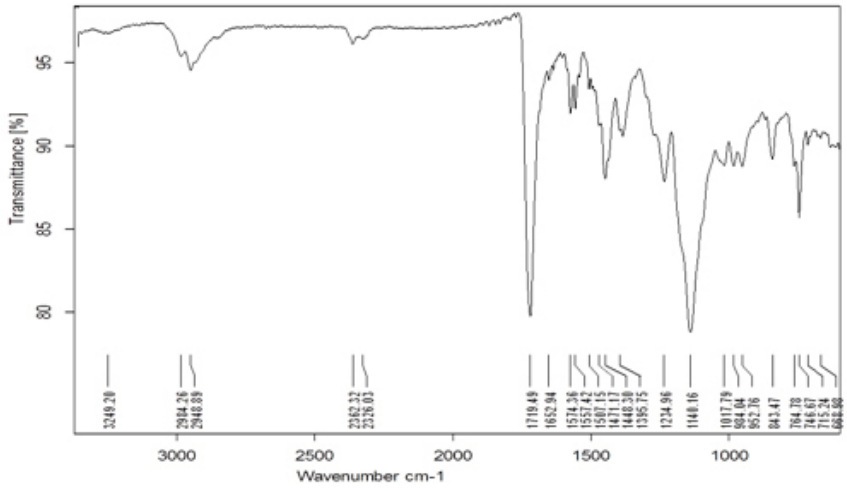

Figure 5. FTIR of ERL-DS ADF

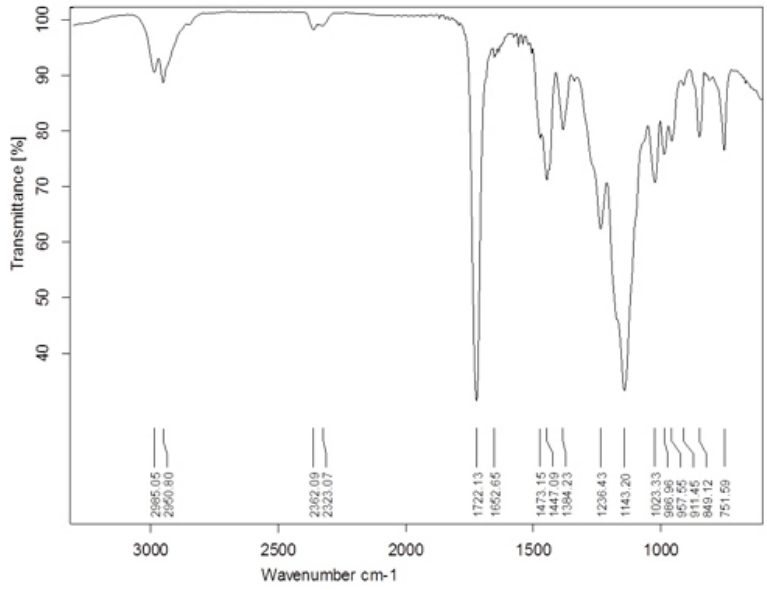

Figure 6. FTIR of ERL-DS DPF

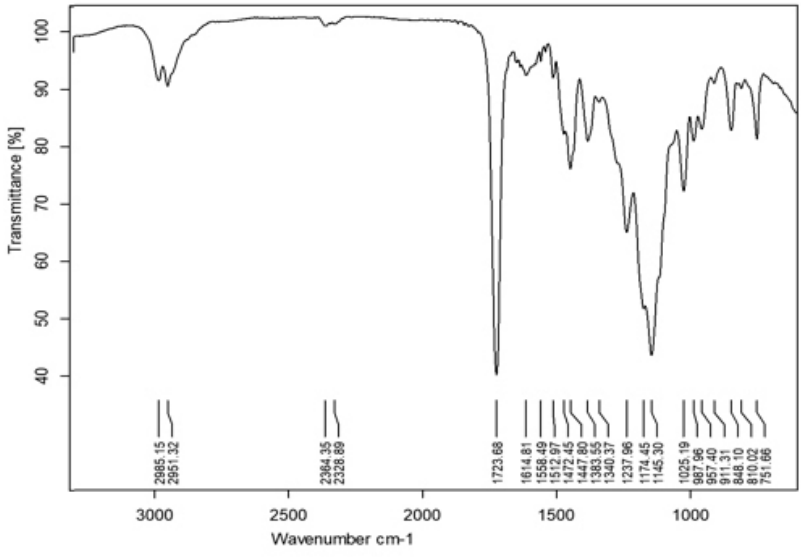

Figure 7. FTIR of ERL-MS ADF

Table 2. Kinetic data of different tablet batches in this work. For more details see Table 6 in the article, where Met: metoprolol, Dic: diclofenac sodium, Type: type of coating, To: storage temperature in Celsius, Stg: storage duration in months, Per% : percentage of coating, R.T: room temperature.

					Zero order			first order			
drug	Type	Per %	Stg	T°	R^2	a	b	R2	a	EXP (b)	b
Met	dry	1.60	0		0.986	0.212	24.12	0.955	0.005	30.35	3.413
			1	R. T	0.927	0.218	26.42	0.812	0.004	34.62	3.5445
				40	0.976	0.207	26.70	0.925	0.003	32.23	3.4732
				50	0.970	0.209	27.70	0.907	0.003	30.93	3.4319
			2	R. T	0.963	0.203	29.55	0.911	0.003	40.976	3.713
				40	0.975	0.211	26.84	0.916	0.003	29.898	3.3978
				50	0.969	0.207	26.52	0.904	0.004	29.276	3.3768
			3	R. T	0.993	0.261	24.97	0.971	0.004	37.125	3.6143
				40	0.975	0.216	27.42	0.913	0.004	30.904	3.4309
				50	0.961	0.207	26.17	0.884	0.004	29.227	3.3751
Met	dry	4.40	0		0.951	0.225	28.46	0.871	0.003	31.000	3.434
			1	R. T	0.986	0.222	31.59	0.941	0.003	41.268	3.7201
				40	0.983	0.232	25.79	0.921	0.004	29.341	3.379
				50	0.985	0.226	21.70	0.922	0.004	25.459	3.2371
			2	R. T	0.9735	0.2368	24.595	0.9664	0.0045	36.64584	3.6013
				40	0.9788	0.2567	19.337	0.8881	0.0054	24.24475	3.1882
				50	0.9665	0.2589	18.711	0.8523	0.0056	23.24291	3.146
			3	R.T	0.9657	0.2407	24.305	0.8558	0.0044	28.76905	3.3593
				40	0.9737	0.1997	28.4	0.8933	0.0036	31.98925	3.4654
				50	0.9848	0.2242	22.014	0.9095	0.0043	26.94237	3.2937
Met	aqueous	1.00	0		0.9408	0.2378	20.792	0.8357	0.0053	24.29814	3.1904
			1	R.T	0.9555	0.2034	20.66	0.8386	0.0045	23.91008	3.1743
				40	0.9077	0.2134	21.673	0.7827	0.0047	23.97712	3.1771
				50	0.9075	0.1918	23.018	0.7705	0.0043	24.7667	3.2095
			2	R.T	0.9417	0.2064	24.592	0.8225	0.0042	27.36323	3.3092
				40	0.9298	0.2044	23.28	0.7966	0.0044	25.66941	3.2453
				50	0.9813	0.2155	17.573	0.885	0.0047	22.22683	3.1013

			3	R.T	0.9675	0.2078	23.448	0.8723	0.0041	27.27853	3.3061
				40	0.9562	0.2064	18.559	0.8251	0.0047	22.1536	3.098
				50	0.9744	0.2167	17.693	0.8579	0.0048	22.19795	3.1
Met	**aqueous**	**4.00**	**0**		0.9397	0.1271	8.7767	0.8158	0.0061	10.41555	2.3433
			1	R.T	0.9193	0.2218	23.611	0.7836	0.0046	25.98709	3.2576
				40	0.9412	0.2244	20.62	0.7977	0.0048	23.63432	3.1627
				50	0.9333	0.2293	16.976	0.7627	0.0055	19.66224	2.9787
			2	R.T	0.9517	0.2338	18.343	0.8107	0.0051	21.99687	3.0909
				40	0.9439	0.2344	15.464	0.8567	0.0052	20.54875	3.0228
				50	0.9435	0.2424	14.088	0.8488	0.0055	19.21133	2.9555
			3	R.T	0.9661	0.2167	20.951	0.8756	0.0044	25.21654	3.2275
				40	0.9809	0.251	14.282	0.8688	0.0054	20.39317	3.0152
				50	0.9543	0.2301	17.9	0.8297	0.005	22.11818	3.0964
Dic	**dry**	**2.40**	**0**		0.9816	0.3892	-9.2499	0.858	0.0085	9.625348	2.2644
			1	R.T	0.9817	0.3273	-7.3125	0.9258	0.0096	6.920583	1.9345
				40	0.9817	0.3489	-0.7333	0.8773	0.0084	11.13619	2.4102
				50	0.9747	0.361	-5.8693	0.9012	0.0094	9.189854	2.2181
			2	R.T	0.9865	0.405	-9.3294	0.9245	0.012	6.579113	1.8839
				40	0.9952	0.2749	-3.66	0.9443	0.0102	6.732187	1.9069
				50	0.9795	0.382	-6.902	0.9486	0.0108	7.96375	2.0749
			3	R.T	0.9846	0.3496	-9.4822	0.9292	0.0101	6.456582	1.8651
				40	0.9888	0.3168	-4.8514	0.9247	0.0089	8.19808	2.1039
				50	0.9837	0.339	-7.0188	0.9287	0.0094	7.597354	2.0278
Dic	**dry**	**3**	**0**		0.9977	0.3072	-0.7767	0.9391	0.0095	8.369549	2.1246
			1	R.T	0.9748	0.2916	9.2784	0.8491	0.0075	15.65828	2.751
				40	0.9956	0.2194	4.6484	0.9182	0.0076	10.73919	2.3739
				50	0.9914	0.332	8.0902	0.978	0.0069	18.45989	2.9156
			2	R.T	0.9976	0.3101	-0.149	0.9382	0.009	10.33463	2.3355
				40	0.9992	0.3081	4.8497	0.9482	0.0077	14.26773	2.658
				50	0.9951	0.3405	11.259	0.9368	0.0068	20.35649	3.0134

			3	R.T	0.9993	0.2896	3.6549	0.9246	0.0069	13.7646	2.6221
				40	0.9627	0.2872	13.013	0.8924	0.0057	20.64762	3.0276
				50	0.9919	0.2033	1.9912	0.8866	0.0074	8.641706	2.1566
Dic	aqueous	2.7	0		0.9859	0.0243	-0.301	0.959	0.0098	0.630274	-0.4616
			1	R.T	0.9865	0.0571	-1.363	0.9484	0.0093	1.291753	0.256
				40	0.995	0.0461	0.1604	0.9554	0.0072	1.960697	0.6733
				50	0.9167	0.0539	-2.0673	0.9817	0.0099	0.924964	-0.078
			2	R.T	0.9822	0.0506	-1.0695	0.919	0.0091	1.209371	0.1901
				40	0.9697	0.0415	-1.8966	0.787	0.0157	0.186766	-1.6779
				50	0.9787	0.0655	0.0655	0.9348	0.0071	1.637548	0.4932
			3	R.T	0.9798	0.0374	0.2378	0.9851	0.0065	1.851692	0.6161
				40	0.9681	0.035	-1.6313	0.9462	0.012	0.271702	-1.30305
				50	0.9391	0.0294	0.6438	0.9911	0.0077	0.935195	-0.067
Dic	aqueous	4.00	0		0.9994	0.0245	-0.6078	0.829	0.0139	0.285932	-1.252
			1	R.T	0.9012	0.0299	0.1988	0.912	0.0075	1.097133	0.0927
				40	0.994	0.0484	0.4963	0.9171	0.0071	2.169724	0.7746
				50	0.9439	0.0134	0.0881	0.961	0.0063	0.6827	-0.3817
			2	R.T	0.9818	0.0336	-0.5651	0.9657	0.0082	1.014504	0.0144
				40	0.9474	0.0373	-0.643	0.9951	0.0076	1.266428	0.2362
				50	0.9276	0.007	0.6418	0.982	0.0038	0.891544	-0.1148
			3	R.T	0.965	0.0331	-0.4349	0.8834	0.0087	0.902578	-0.1025
				40	0.9584	0.0203	-0.8317	0.8985	0.0109	0.267536	-1.3185
				50	0.8857	0.0189	-0.7867	0.9968	0.0091	0.370834	-0.992