

Stability Assessment Approaches for Amoxicillin Nano-suspension as Promising Tool for Drug Delivery System

Abstract

While nano-suspension amoxicillin is one of the approaches for improving dissolution rate of amoxicillin that is widely used as antibiotic. Stabilizing these nano. dosage forms is always a challenge. The present study utilizes Nanoprecipitation solvent evaporation technique for preparation of amoxicillin nanoparticles and proposed stability study approach of the same. The methodology based on investigating stability of amoxicillin Nano suspension based on the in vitro dissolution and particle size, the optimized drug: Polymer ratio re-formulated into nano-suspensions and compared with marketed suspensions. Samples initially characterized and then subjected to stability testing at ambient temperature and relative humidity up to 6 months assayed using a validated HPLC method. During initial characterization, increase in saturation solubility and dissolution rate observed in all samples. During stability testing, there was gradual decrease in saturation solubility and dissolution rate of the samples, over the period of 3 months. This study considers long-term isothermal measurements, consistent with short-term non-isothermal (accelerating) measurements, providing predictive model to calculate the isothermal degradation periods. While nonisothermal method ignores the effect of the storage conditions. While isothermal and nonisothermal treatments would allow accurate and fast prediction of the drug-loaded nanoparticle shelf life correlating parameters obtained using a single mathematical model. The isoconversional method does not consider crystalline state of the sample, the accuracy of the prediction would be assessed by comparison of estimated shelf life versus data coming from traditional stability studies.

Keywords: Amoxicillin Nano-Suspension; Stability Assessment; Isothermal-none isothermal; Isoconversion

Introduction and literature review

Nano-suspensions frequently used as an approach for solubility enhancement, one of the overlooked aspects in the development of this dosage forms are the stability concerns and the degradation of the carried drugs. Amoxicillin is subject, in common with all penicillin's, to hydrolytic degradation of the B-Lactam ring to produce under alkaline conditions. Penicilloic acid which subsequently decomposes to penilloic acid by decarboxylation. The reaction is first order (B.P.C,94). Under acidic conditions, amoxicillin hydrolyses to penicillenic acid. At the same time, base-catalysed self-amino lysis. dimerization occurs, by nucleophilic attack of P-lactam carbonyl moiety of one molecule by the free side chain amino group of a neighbouring molecule. Concentrations of amoxicillin and the pH of the solution determine the corresponding input of the hydrolysis and amino lysis to the overall degradation reaction. ⁽¹⁾

The degradation of amoxicillin trihydrate over pH 3-10.5 at 35°C. follows first order kinetic. The activation energy was 90.75 KJ/mol. ⁽²⁾

T90 for amoxicillin trihydrate is 3.2 years at 20°C ⁽³⁾ recommended that suspension stored in original container at room temperature will have expiration date of 7 days after reconstitution. Polymerisation occurs during storage of concentrated solutions. The reactive 13-lactam bond of one molecule is opened by reaction with side-chain of another molecule and a (timer is formed. The process can continue to form higher polymers such polymers shown to be highly antigenic in animal and considered to play part in eliciting pencilloyl Specific reaction in man. The hydrolysis rate constant of amoxicillin at PH 8.1 is 1.1×10^{-6} at 35 °C ⁽⁴⁾. The electron withdrawing group NI12 played the significant role in the initial rupture of the 13-lactam ring which was responsible for the overall degradation ⁽⁵⁾. Amoxicillin. [3-lactam ring, is more susceptible to hydrolytic degradation when the pH deviated from the isoelectric point (pH 4.8) ⁽⁶⁾ and the zwitter ionic form of the drug was the most stable species (Mendez et al., 1989). In strong acid medium the main degradation products are amoxicillin penamaldic and amoxicillin penicilloic acid. As the increases, the formation of penicamaldic acid decreases and penicilolic acid increases. In the neutral and basic region, the degradation products are amoxicillin penicilloic acid and 2-(6'-phenylpiperazine-2,5-dione-3"-y1)5,5%-dimethylthiazolidone-4-carboxylic acid ⁽⁷⁾.

Stability Testing of New Drug Substances

Stability protocols for new dosage forms should follow the guidance in the parent stability guideline in principle. However, a reduced stability database at submission time (e.g., 6 months accelerated and 6 months long term data from ongoing studies) may be acceptable in certain justified cases.

Kinetics in Heterogeneous Drug Delivery Systems

Most pharmaceutical nano-particulated systems are highly complex and not homogeneous at molecular or atomic levels, so approaches based on gas kinetic vacuum probably are not the best. The transformations of substances such systems comprise changes in physical state, crystallization, melting, solubility, chemical reactions, etc. that can be thermally stimulated by heating or cooling processes. The speed in theory which assumes that each reactant molecule is surrounded mostly by and form to which they are transformed are determined by the kinetic characteristic of the system.⁽²⁾

The classical method of assessing pharmaceutical stability has three main problems that must be considered: First, a mathematical model that attempts to match the kinetics of solid substances must be created. The second element to be considered is the effect of relative humidity on the reaction kinetics, and the third lies essentially in the time required to perform the determination of stability.⁽⁶⁾

In the evaluation of the reaction kinetics in heterogeneous systems, the only concentration of importance is at the reaction interface, which cannot be quantified in a practical way; for this reason, the way of monitoring the development of the reaction is measuring the degree of conversion of the reagent or the product.⁽⁷⁾ For the purposes of the present study, stability is considered to be where corresponds to the extension of change in a specific parameter of interest, the amount of degraded substance for example; in this particular case, is the initial concentration of substance and is the amount of substance measured at time.⁽⁸⁾

In acceleration models, the degree of conversion increases with time; on the contrary, in the deceleration models, the conversion rate decreases over time; sigmoidal ones share the characteristics of both and represent autocatalytic process. The three types of kinetics can spread on dozens of heterogeneous reaction models. Frequently, experimental data do not fit any model, or if it does, it will fit one of them at the beginning of the process and other at the

end. The challenges increase when model fitting is performed on experimental data obtained in a nonisothermal run at a single heating rate. ^(9,10)

To overcome this complexity, the isoconversion paradigm was developed: it allows the calculation of the Arrhenius equation constants, without the need to determine the order of the reactions.

Nano-particles Stability assessment:

The isoconversion is a methodology created by Kujirai and Akahira in 1925, who studied the decomposition of materials under isothermal conditions, allowing them the determination of the activation energy as a function of mass loss; in 1948, Dakin proposed some kinetic models in the decomposition of complex materials; and, in the 1950s, instrumentation was generated that allowed the study of materials under nonisothermal conditions. The isoconversion stability test uses some samples as maintained at different temperature and humidity conditions, in order to determine the values of the kinetic constants at different temperatures. ^(6,11,12)

Adapting well-known isothermal and non-isothermal methods to nanoparticles would allow correlating kinetic parameters obtained in a single mathematical model and predicting the shelf life faster than traditional methods. Unlike the traditional approaches, the iso-conversional method:

- a. considers nano-suspension as heterogeneous systems, with no specific kinetic order
- b. set degradation to the maximum
- c. kinetics will be considered same for all processes regardless of the conditions
- d. Includes the effect of humidity by a modification of Arrhenius equation.

Processing nanoparticles for isothermal and non-isothermal treatments would allow accurate and fast prediction of the drug-loaded nanoparticle shelf life correlating parameters obtained using a single mathematical model. The accuracy of the prediction would be assessed by comparison of estimated shelf life versus data coming from traditional stability studies. There are several medicines with bioavailability or stability problems that are integrated into new drug delivery system such as silymarin, colchicine, genistein, curcumin, etc. and showed improved bio-pharmaceutic properties of the drugs. ^(11,12,13,14)

MATERIALS AND METHOD CHEMICALS:

Nanoprecipitation technique was employed to produce nanoparticles of amoxicillin, a poorly water soluble drug, to improve the solubility. We used micro-nanosize amoxicillin particles, polymer concentration, and organic to aqueous solvent ratios were adjusted. The nanosuspension showing the lowest particle size of 280 nm were obtained by 1:1 ratio of drug to polymer using a solvent evaporation technique at laboratory scale with acceptable zeta potential. Two commercial brands of amoxicillin (125 mg/5 ml) were used for comparative study. It was available as dry powder containing 125 mg amoxicillin (as amoxicillin trihydrate) per 5 ml for reconstitution in water for oral use. The samples were purchased from a national manufacturing facility.

Amoxicillin Nano-suspension preparation:

Chemicals: Amoxicillin (Active ingredient), polyvinyl pyrrolidone K30, surface active agent Tween80, Benzoalkonium chloride, Ethanol, Domestic amoxicillin trihydrate powder for 125mg/5ml oral suspension. The rest of the chemicals were of analytical grade.

The sample of amoxicillin procured for study was identified and estimated for its purity. The sample of amoxicillin was identified by melting point, FTIR, Differential Scanning Colorimetry.

METHODS:

1. Calibration Curve – for amoxicillin particle: Maximum wavelength was determined by scanning on UV –Visible spectrometer These results are surmise in tables.
2. Melting Point – was measured with use of Thieles tube apparatus and burner. The range of melting temperature was noted.
3. FTIR Spectroscopy Analysis Fourier –transform infrared (FT –IR) spectra of moisture free powdered sample of amoxicillin ,PVP, Tween 80 and physical mixture were obtained using a spectrophotometer (FTIR –Shimadzu ,India)
4. Differential Scanning Calorimetry (DSC) Analysis DSC scans of pure drug sample and polymer were recorded

Preparation:

Isothermal Stage

Nanoparticles were weighted in 18 open aluminum capsules for DSC; all the capsules of the same type of nanoparticle (AMOX: PVP 30 T80 (1:1), AMOX: PVP 30 T80 (1:2), or AMOX: PVP 30 T80 (1:3)) were placed in specially designed supports, subjecting the nanoparticles to the established humidity and temperature conditions (3 different conditions).the nanoparticles to the selected conditions during the selected time (2 storage times). Using HPLC the amount of API remaining in specific intervals were recorded.

Nonisothermal Stage

For determination, the remaining 9 capsules of isothermal stage were submitted at three different heating rates (3 capsules for each speed). the kinetic parameters were calculated for the isothermal and nonisothermal methods.

Accelerated Stability Study Stage

Another set of 12 samples were subjected to 40°C and 75% RH in accelerated traditional stability study. Samples will be taken at 1, 2, 3, and 6 months and analyzed for API content.

Chemical analyses:

A. Principle: Isocratic HPLC with UV detector. B.P -2007.(15) USP 2007. (16)

B. Reagents: HPLC grade materials to prepare the following solutions:

1. Mobile phase A: Mixture of 50 ml acetonitrile. 50ml of 0.2M potassium dihydrogen orthophosphate, adjust with 0.5ml dilute acetic acid to PH 5 – Dilute to 1000ml with purified water.

2. Mobile phase B: Mixture of 400 ml acetonitrile with 50ml of 0.2M potassium dihydrogen orthophosphate, adjust with 0.5ml dilute acetic acid to PH 5 – Dilute to 1000ml with purified water.

3. Mobile phase ratio: Mixture of 850 ml mobile phase A and 150ml of mobile phase B. Mix for 15 minutes and degas.

C. Chromatographic conditions:

1. Columns: Stainless steel column 125 mm 4mm Nucleosil C 18 packed with octodecylsilane 5µm , Merck. Flow rate: flow rate of 1 ml/min. Detection: At wave length of 254nm. Injection volume: 20µl volume

D. Assay preparation:

Preparation of the reference standard:

143.6 mg of Amoxicillin trihydrate internal reference standard was used which is equivalent to 125mg amoxicillin in anhydrous state to 100ml volumetric flask, completed to 100ml with mobile phase. Stirred in a magnetic stirrer for 15 min. Filtered and the first 10ml filtrate was discarded.

Preparation of sample:

1. Reconstitute the domestic sample by adding water to 75ml and shake well,
2. Take 5ml of the suspension to 100ml volumetric flask.
3. Complete to volume with the mobile phase - stir for 15 min and then filter
4. the first 10ml of filtrate was discarded

Procedure:

1. 20µl of the standard preparation samples were injected into the chromatographic system.
2. chromatograph, record the retention time and the area under the main peak were recorded
3. column was washed by running the mobile phase for 15min and reference standard and the tested sample reinjected each for three times.
4. standard deviations were obtained for the replicates of not more than 2%. Tailing factor not more than 2.5

Calculation:

Weight content per 5ml were determined as follow:

$(L/D) \times (C/P) \times (R_u/R_s)$ where: L: labelled conc. Per ml- D: conc. Of the test preparation- C: reference standard conc. - P: declared potency of reference standard - R_u : peak area of tested sample - R_s : peak area of reference standard

Multiplied by 5 to obtain the content per 5ml then the percentage of the content to the labelled quantity.

*The same repeated for the all samples and after 7 days for samples after constitution and storage at room temperature for 7 days.

Physical tests method:

The following physical tests carried out for the sample twice during the study, initially and at the end of the study after storage of 52 weeks.

1. Appearance: all the products were described after visual examination for (colour, odour and taste)
 2. PH: (B.P 2007 –pH Eur method 2.2.3)
 3. Deliverable volume: USP test for multi-dose containers.
 4. Reconstitution time and sedimentation volume: To evaluate the stability of the suspendability function of the resulting suspension. The method was described by (B.P.C-1994)
 5. Particulate contamination: (B.P 2007, Particulate contamination subdivides particles. PH Eur. Method 2.9.19) Microscopic particle count test method.
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RESULT AND DISCUSSION:

Composition of Various Nano-suspensions Formulation Evaluation Parameter 1. Particle Size Analysis Particle size and particle size distribution was determined by photon correlation spectroscopy (PCS) using a zeta sizer average, measuring range (20-1000nm) Viscosity were determined by Brookfield viscometer. For better bioavailability, we control Amoxicillin extent of dispersion by straightforwardly encapsulating the particles in the presence of polymers, and surfactants. Because of the high surface energy of these particles triggers agglomeration of the drug crystals. These phenomena controlled by the addition of various additives to ensure adequate stabilization. The main function of the stabilizer is to coat the drug particles to prevent Ostwald maturation and agglomeration of the nano-suspension and to achieve a physically stable formulation by providing a steric or ionic barrier. Suitable wetting of drug particles as well as their electrostatic and steric stabilization by excipients is necessary to produce stable nano-suspensions^(3,4)

1. Construction of Calibration Curve using UV spectrometer

The UV spectrometer method selected for the estimation of amoxicillin, showing absorbance at λ max 343.43nm

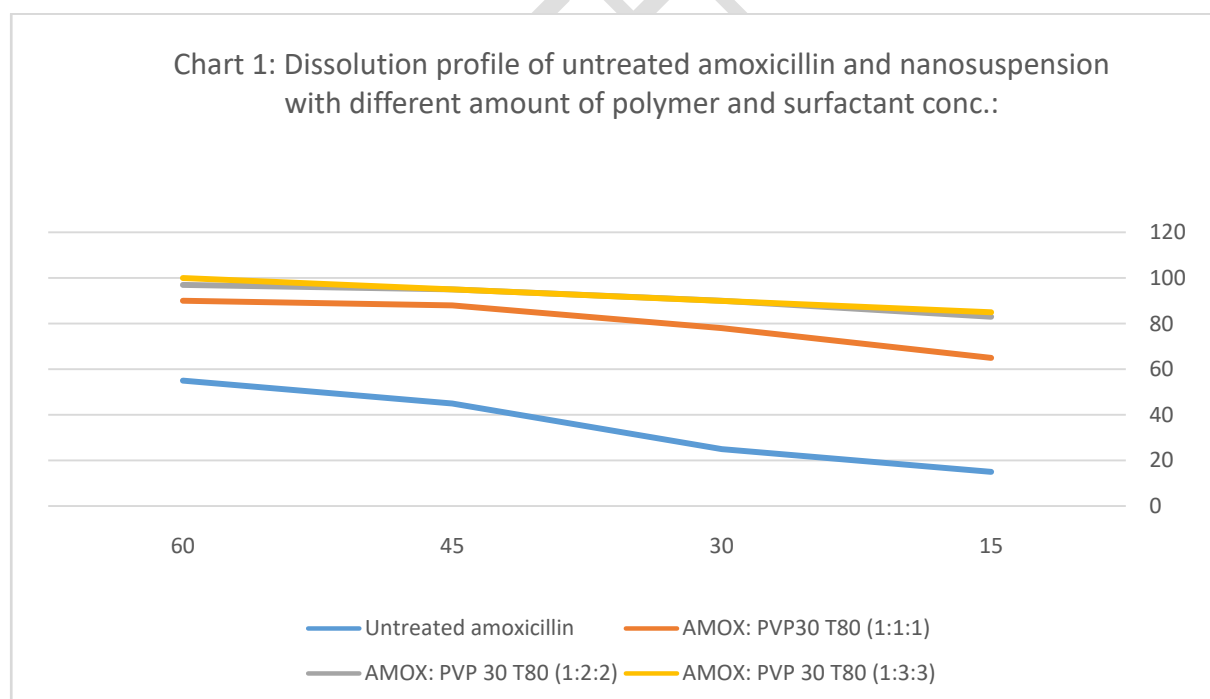
2. Melting Point –

The melting point were determined by Thieles tube apparatus by using paraffin oil, thermometer. The melting point found to be 197-204°C.

3. FTIR

FTIR used to assess the interaction between excipients and the drug molecule in the solid state. The FTIR spectra taken by pure drug, PVP, Tween80, reconstituted nano-suspension. The FTIR spectra of all sample show in figure--Row amoxicillin and precipitated nanoparticle exhibited same FTIR spectra the amoxicillin show peak at 3468 for N-H and the range is from 3300-3500, and the nano-suspension show the peck at 3543 which demonstrate that the chemical structure of the drug is not changed before and after the precipitation process.

Nano-suspension were prepared using Nanoprecipitation solvent evaporation technique (aqueous phase and organic phase) the aqueous phase containing different amount of polymer and surface active agent at 25c° as ratio 1:1, 1:2, 1:3, 1:4, 1:5 (Chart 1). The organic phase containing active ingredient dissolved in an ethanol at 25c°. the organic phase were poured into aqueous phase subsequently stirred on mechanical stirrer. Then allow the volatile solvent to evaporate. Addition of organic solvent by mean of a syringe positioned with the needle directly into surfactant containing water. Organic solvent were left to evaporate off under a slow mechanical stirrer of the nano-suspension at room temperature for 8 h. (Table1) Composition of Amoxicillin Suspension. (Chart 1).



4. DSC

The physical state of raw amoxicillin and reconstituted nanoparticle of nano-suspension examined by DSC. Raw amoxicillin exhibited a melting point with fusion enthalpy whereas DSC scan of PVP, a broad endotherm ranging from 207-234 observed due to presence of residual solvent.

The kinetic parameters of a decomposition reaction can be estimated using differential scanning calorimetry (DSC). The sample subjected to different heating rates, in DSC, the maximum decomposition increase as temperature increases. According to Kissinger's method, if is plotted as a function of the inverse of, the activation energy of the reaction, can be determined from the slope of the line. The values of the decomposition rate constant can be calculated for a reference temperature. Low values would imply a lower energy barrier to overcome for degradation to occur and, therefore, less stability; low values of would indicate longer times until the substance comes out of the specification by decomposition.

The shelf life of a drug delivery system is the period during it remains under the acceptable limits, for example, an API content $\geq 95\%$ (or a value of); it can be calculated by where the reference temperature, , is 298.15 K (25°C) ⁽¹⁶⁾

The kinetic parameters and the shelf life time used to compare API from different batch, manufacturer, time, or storage conditions and make inferences about their stability and decomposition progress. In the case of drug delivery systems, decisions can be made about which formulation has the greatest stability or whether a formulation in the process of development is similar or not to a market one, of known stability.

The validity of the predictions of shelf life must be verified because the calculations are made by assuming that (i) the reactions are of first order; (ii) the values of the kinetic parameters are similar for the temperatures in the study range; and (iii) the effect of moisture is not significant. Studies that propose new methods of analysis or that provide specific information on an active principle such as kinetic parameters and degradation pathways are very useful in the pharmaceutical industry because they can save resources, as well as guarantee the safety, quality, and effectiveness of medicines.

Thermal analysis techniques such as differential scanning calorimetry (DSC) allow the characterization and identification of physicochemical changes in the solid state of different compounds, thus streamlining the process of preformulation and development of drugs and

substances of pharmacological interest. Its use is not limited solely to the determination of purity, polymorphs, solvates and hydrates, melting point, and component quantification but also allows the characterization of the surface properties of powders, such as humectants, adsorbents, and surface energy, thus constituting a useful tool in decision making in the case of problems related to the production process.

Another advantage of using a non-isothermal method is that it is not based on any assumptions about the reaction mechanism and thus does not require any prior knowledge of the initial concentration of the API, which eliminates the prolonged reaction times and storage requirements required by conventional techniques.

Isoconversional Methods

Modern methods maintain the principle of isoconversion through flexible integration procedures for the determination of activation energy. Moreover, it was observed that the activation energy obtained from the free models is independent of the heating rate, but certain studies indicate that there would be dependence of the heating range.

The kinetic isoconversion methods were based on the elimination of the reaction model from kinetic calculations. The principle states that the rate of conversion, under certain environmental conditions, depends solely on temperature. For the experimental determination, it is necessary to establish a specification, an acceptance limit to which the samples must not reach, in a determined time, in order to remain safe and effective. HPLC (USP method) were used to assay the amoxicillin content initially, after one month, after 6 month

Table (1) Amoxicillin content for 6 groups of the same sample batch (sample 1) during storage:

Period of time	1	2	3	4	5	6
0	101.33	101.33	101.33	101.33	101.33	101.33
0	101.52	101.52	101.52	101.52	101.52	101.52
Mean	101.43	101.43	101.43	101.43	101.43	101.43
After 6 months	99.52	94.4	98.534	93.58	97.876	95.943
Final	92.46	93.45	91.9	92.45	90.9	93.12
Final	91.87	93	90.05	91	90.5	91

Final	90.94	92.8	90.12	90.8	89.82	92.7
Final	89.7	90.1	89.23	90.1	89.25	90.6
Mean	91.24	92.34	90.33	91.09	90.12	91.86

High temperature accelerate deterioration of a pharmaceutical product⁽¹⁶⁾. Suspensions are most likely to show loss of content uniformity as a function of time due to changes in sedimentation volume and sedimentation time. If the attraction between the particles due to relatively weak London-Van der Waals forces is greater than the repulsion forces which are due to the electrical double layer that surround each particle, the particles come together to form loose floccules that settle rapidly to produce a readily dispersed sediment. If the repulsion is greater than attraction, the suspension deflocculated, that each article settles slowly and individually to form sediment and the particles may aggregate to form in-dispersible sediment crystals.

There were no significant changes in appearance, odour or colour. Samples showed no significant deviation from 75 ml with standard deviation of 0.52 The deliverable volume was 76 ml and the sedimentation volume was 70/77 ml, PH ranging from 5.9- 5.96 and the reconstitution time was 2-3 shakes and there was no significant variation in the powder weight. Four bottles contain between 95-100% of the required weight out of 10 bottle, six bottles contain between 100%-105%. Thus, the product defined as physically stable.

The product was greatly affected by temperature that the contents declined below 90% after the storage, the degradation of the powder followed pseudo-zero order reaction of kinetics with a K_0 1.3mg.month.

The regression equation for samples was:

$$C = 124.0216 - 0.9832 \times t, \text{ the shelf life from the true [SRI, was 12 months]}$$

The reconstituted suspension degradation reaction followed first order reaction with a rate constant $K_1 = 1.1 \times 10^{-2}$ month. The I.SR1, equation was:

$\ln C = 4.8372743 - 0.0114333454 \times t$ and the concentration reached 80% alter 15 days at ambient conditions (29.7 °C /31.4% RH) and 90% after 7 days.

In plotting degradation versus time, the shape of the curve would be the same at different temperatures, being more pronounced at elevated temperatures and with a tendency to look like a straight line as the temperature decreases⁽¹⁰⁾. it can be said that the lines and k start from zero as the origin and can be described by $K_i = \alpha_i/t_i$

If the degradation value is the same for the lines k_1 and k_2 , one has to $k_1t_1 = k_2t_2$

Combining the Effect of Temperature and Relative Humidity

The combination of Arrhenius equations and moisture sensitivity ⁽⁶⁾ gives an equation that considers the effects of temperature and humidity on API stability.

This equation states that the rate of degradation, or kinetic constant, depends on temperature and relative humidity.

Because the equation has 3 constants, and two independent variables, the minimum number of experiments required to solve the equation is three, being necessary to increase the number of points to improve the accuracy of the measurements ⁽¹³⁾ In most studies, two to three weeks may be suitable to determine the shelf life of a product under environmental conditions. Some authors suggest considering the variation of 5°C and 5% of RH for 14-day assay.

These measurements can be used to estimate the isoconversion time in each condition. (1) First, draw a line through the data to find the intercept with the limit specification. (2) The two points closest to the specification limit can be used to interpolate or extrapolate the time to reach the specification. (3) A single point closest to the specification can be used to determine.

To determine the relationship between the variables with the degradation, two methodologies can be used: (1) transform the Arrhenius equation to the linear form and determine the constants by least squares regression and (2) make a least squares fit in the exponential form.

Schematic representation of methodology combining isothermal isoconversional and nonisothermal approaches for the assessment of shelf life in nanoparticles.

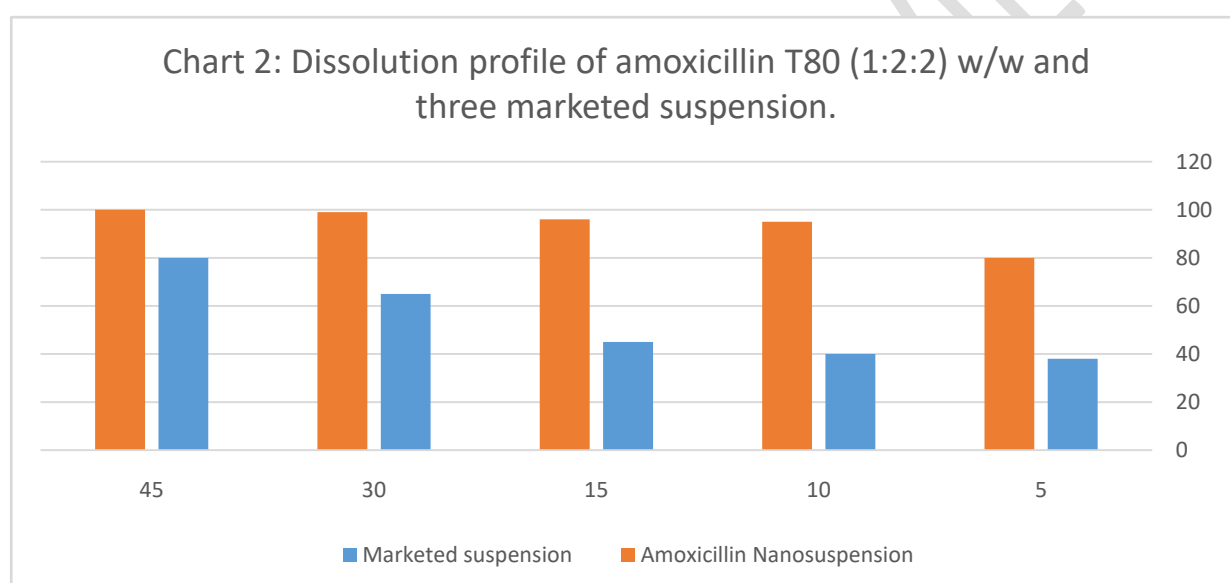
Finally, by combining isothermal isoconversional and nonisothermal method, their individual limitations could be overwhelmed, allowing a faster and accurate shelf life estimation that could be contrasted against data obtained from accelerated or long-term stability studies.

Particle Size Analysis –

Amoxicillin was sieved before the preparation process. To avoid blocking during the process, it was considered important to perform pre-milling using a high-speed over-head homogenizer. As amoxicillin is poorly water soluble and hydrophobic in nature, it was necessary to disperse

it in the aqueous polymeric solution. The mean particle size and particle size distribution affect the saturation solubility, dissolution rate, physical stability, even In vivo behavior of nano-suspension. The polydispersive index in range of 0.1-0.22 indicate a fairly narrow size distribution can be determined by photon correlation spectroscopy.

Amoxicillin Nano-suspension compared with commercial amoxicillin suspensions. The rate of drug dissolution from the formulations under study compared at 5 min intervals in the first 15 min, followed by 15 min interval for a period of 1 h. It is evident from Chart 2 that the nano-suspension showed almost two-fold faster release compared to the marketed products in first 5 min and 96% release in 15 min, which would induce faster rate of absorption, rapid onset of action and improved patient safety by decreasing gastric irritation after oral administration.



CONCLUSION

It should be emphasized that despite the promising data on amoxicillin nano-suspension, none of the formulations have made it to the clinical stage. Due to the difficulties in calculating shelf life. However, isothermal and nonisothermal strategies permits to calculate its dynamic parameters and to set up a scientific model to expect its shelf life. The accuracy of the proposed model assessed by comparison of predicted shelf life versus actual coming from long-term stability studies. Isothermal isoconversional methods and nonisothermal treatments could allow the establishment of mathematical predictors of stability with less uncertainty than traditional approach for nanoparticles. According to our findings, we agree with the possibility to calculate the value of the kinetic constants based on a fixed degradation limit, regardless of

the shape of the curve. Thereby, a sample submitted to intense conditions of temperature and humidity will reach a predetermined limit in a specific time. The isoconversion method is beneficial for the calculation of degradation kinetics because it only considers the time to reach the specification.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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