



Stability Testing of Amoxicillin Nano-suspension as Promising Tool for Drug Delivery System

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Author's contribution

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

While nano-suspension amoxicillin is one of the approaches for improving dissolution rate of amoxicillin antibiotic used widely. Stabilizing these nanodosage 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 was based on investigating stability of amoxicillin nano suspension *in vitro*, the optimized drug (polymer ratio reformulated into nano-suspensions) and we compared our results with marketed suspensions. Samples were 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. As per our results, we agree with the possibility to calculate the value of kinetic constants based on a fixed degradation limit, regardless of the shape of the curve. 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.*

1. INTRODUCTION

Nano-suspensions are frequently used as an approach for solubility enhancement. One of the overlooked aspects in the development of these dosage forms are the stability concerns and the degradation of the carried drugs. Amoxicillin is subject, in common with all penicillins, to hydrolytic degradation of the β -Lactamring under alkaline conditions. Decomposition of penicilloic acid to penilic acid follows first order kinetic reaction [1]. Under acidic conditions, amoxicillin hydrolyses to penicillenic acid. At the same time, base-catalysed self-amino lysis, and dimerization occurs by nucleophilic attack of β -Lactamcarbonyl 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]. Nano-suspensions are also advantageous in achieving quick onset of action for drugs that are completely but slowly absorbed, i.e. those having high t_{max} values. This is illustrated by the study carried out for naproxen, a nonsteroidal anti-inflammatory drug [3]. Oral Nanosuspensions have several advantages for drug delivery. Among many administration routes of drug delivery, due to its advantages such as ease of ingestion, versatility to accommodate various types of drug candidates, low production cost, high safety, good patient compliance, and pain avoidance [4,5].

1.1 Stability Testing of New Drug Substances

Newly developed pharmaceuticals stability protocol should consider the original guidelines in principle. Considering that six months accelerated stability study plus six months long term in special cases can be accepted. Storage conditions particularly temperature and humidity, are significantly affecting drug stability. The advice by the manufacturers for storing reconstituted antibiotic suspensions that they have to be kept refrigerated in order preserve the stability of these drugs. It has been reported that many patients and various community pharmacies do not follow this recommendation unintentionally. Storing suspensions under optimal storage conditions and fluctuated circumstances can be justified by different

reasons such as lack of refrigerators in the properties, irregular power supply, or lack of the information in case of the patients. Consequently, these suspensions might be exposed to instability factors which may result in some degrees of degradation of the products, which may lead to loss of effectiveness depending on how severe this exposure. However, these nano products might be more sensitive if preserved under same condition which makes it highly complicated to propose a stability protocol. The objective of this study was to investigate the possibility of proposing stability protocol for these sensitive product considering isothermal and none isothermal methods for faster and more reliable shelf life calculation.

1.2 Kinetics in Heterogeneous Drug Delivery Systems

Heterogeneity of nano-particle molecules affects its physiochemical properties. Moreover, it can be stimulated by temperature assuming 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 main factors that makes proposing stability protocol for pharmaceutical product more complicated is the time required for the process; relative humidity effect; and the mathematical model that goes in consistency with the products particle kinetics [6]. To bypass this complexity, the isoconversion is proposed as it ease the Arrhenius equation constants calculation, without determining the order of the reactions.

In heterogeneous systems it's difficult to quantify the reaction interface so monitoring the reaction development can be done by measuring the degree of product conversion [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].

The degree of conversion increases with time in under accelerated conditions; On the other hand, in long term studies the conversion rate decreases over time; sigmoidal ones share the characteristics of both and represent autocatalytic process. These types of kinetics can spread on many of heterogeneous reaction

models. Frequently, experimental data do not fit any model, or if it does, The difficulties increases when model fitting is performed on experimental data obtained in a none isothermal run at a single heating rate [9,10].

1.3 Nano-particles Stability Assessment

Kujirai and Akahira in 1925, invented the isoconversion method by studying materials decomposition under isothermal conditions, to determination activation energy as a function of mass loss. In1948, 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 none isothermal conditions. The isoconversion stability test uses some samples to calculate kinetic constants at different temperatures [6,11,12].

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 the procedures
- D. Arrhenius equation is to be modified to consider the relative humidity effect.

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].

2. MATERIALS AND METHODS

To improve the solubility Nanoprecipitation technique was used for the preparation amoxicillin nanoparticles. We used micro-nanosize amoxicillin particles, polymer concentration, and organic solvent 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 125 mg/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.

2.1 Methods

1. Calibration Curve – for amoxicillin particle were reported using scanning spectrophotometer.
2. Melting Point – was measured using Thieles tube apparatus
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

2.2 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 separate chambers under three different heat and relative humidity values. Using HPLC the amount of API remaining in specific intervals were recorded.

None isothermal Stage: Nine capsules remained from isothermal stage were divided in to three groups each contains three capsules and kept under three different temperatures rates for the purpose of calculating its kinetic parameters were calculated for the isothermal and none isothermal methods.

Accelerated Stability Study Stage: Twelve product samples were kept in a stability chamber in 40°C and 75% RH samples were examined four times during six 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 solutions
- C. Chromatographic conditions considered
- D. Assay was prepared

Samples and reference standard where prepared.

2.3 Procedure

1. 20 μ l of the standard preparation samples were injected into the chromatographic system.
2. retention time and the area under the main peak were recorded.
3. Column was washed by running the mobile phase for 15min
4. Standard deviations were obtained.

2.4 Calculation

Weight content per 5ml was determined as follow:

(L/D) CP/100) (ru/rs) where: L: labelled conc. Per ml- D: conc. Of the test preparation- C: reference standard conc. - P: declared potency of reference standard - Ru: peak area of tested sample - Rs: peak area of reference standard

*The same was repeated for the all samples.

Physical analyses method:

1. PH
2. Organoleptic properties tested visually for changes in colour odour
3. Deliverable volume: USP test for multi-dose containers.
4. Reconstitution time and sedimentation volume: The method was described by (B.P.C-1994).

3. RESULT AND DISCUSSION

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 using polymers and surface active agents. These particles tend to agglomerate due to high energy in the surface. However, the agglomeration

process can be managed using stabilizers which will cover the particles and stop nano-suspension Ostwald maturation and agglomeration and form ionic barrier to facilitate the particles physical stability. Moreover, better electrostatic and steric stability using additives is important to formulate 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 343.43 nm

2. Melting Point – melting point reading was 197-204°C using Thieles tube.

3. FTIR

Identical FTIR spectra was reported by amoxicillin and nano-particle precipitate, amoxicillin peak range is from 3300-3500, and the nano-suspension peak at 3543 which indicates similarity in chemical structure even after 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 25°C 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 25°C. After eight hours organic solvent were evaporated (Chart 1).

4. DSC

Differential scanning calorimetry (DSC) can be used firstly for the physical state of raw amoxicillin and reconstituted nano-particle of nano-suspension. However, Due to presence of solvent residues the raw amoxicillin the broad endotherm was 207-234. Secondly, following Kissinger's method, if plotted as a function of the inverse of the activation energy of the reaction kinetic parameters would be easily determined from the slope of the line. Decomposition rate constant would be estimated using reference temperature. Decreased amount reflects less energy barrier against degradation, Accordingly, higher rate has decreased stability and lower decomposition rate indicates more time for the substance to be out of specification. The shelf life of a DDS is when it contain more than or equal to ninety five percent of the active pharmaceutical ingredients which can be calculated at a room temperature as a reference [16].

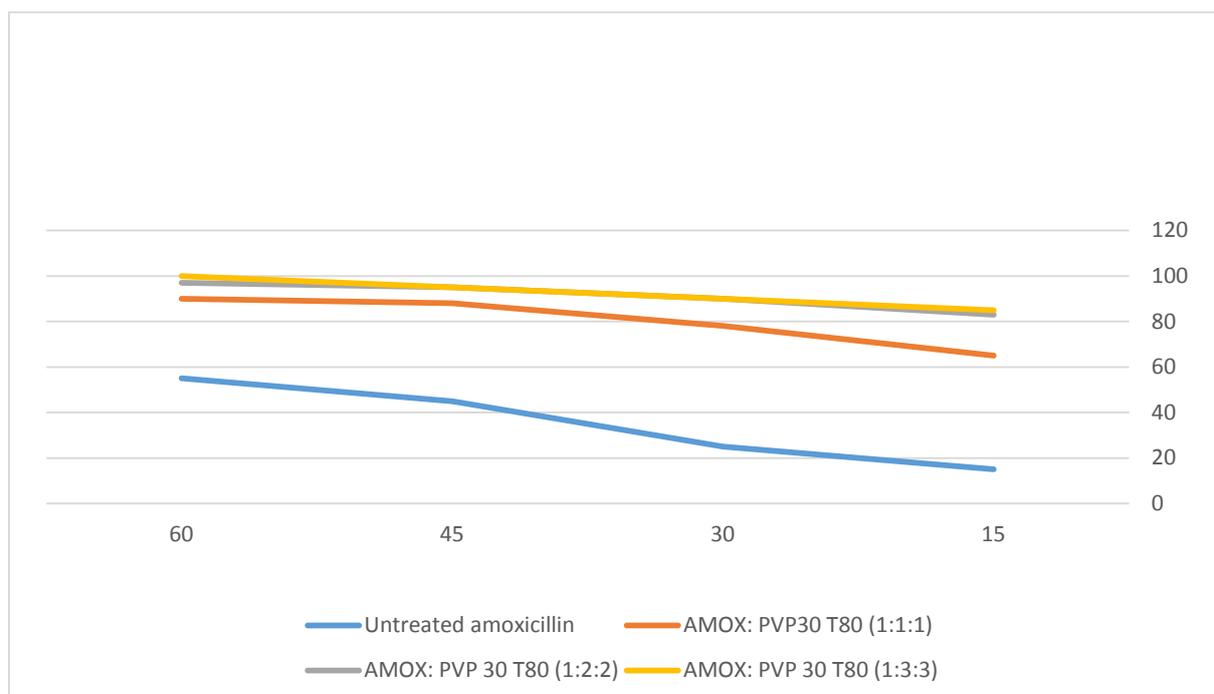


Chart 1. Dissolution profile of untreated amoxicillin and nanosuspension with different amount of polymer and surfactant conc

Since the calculation is performed assuming that the reaction is first-order, it is necessary to verify the validity of the shelf life prediction. This will help pharmaceutical manufacturer deciding whether the newly developed formula has the same stability as the commercially available product.

Information about active pharmaceutical ingredient concentration and reaction mechanism is not necessary if we are conducting none isothermal technique, which will reduce the needed time compared to any other conventional method.

3. ISOCONVERSIONAL METHODS

The latest method maintains the principle of isoconversion by determining the activation energy using a flexible integration method. Furthermore, free model activation energy did not depend on the heating rate. The isoconversion method was based on the idea that temperature is the only factor affecting conversion rate with exclusion of reaction order from calculations. For experimental decisions, it is necessary to establish specifications and tolerance limits that the sample must not reach within a given time in order to maintain a safe

and effective condition. First, after 1 month and 6 months, the amoxicillin content was measured using HPLC (USP method).

High temperature accelerate deterioration of a pharmaceutical product [16]. Difference in sedimentation rate and volume may affect its content uniformity because particles attraction (London-Van der waals forces) is more than repulsion forces as a result of electrical double layer. In addition, floccules formation may affect the rate of sedimentation but if the repulsion is greater than London-Van der waals forces deflocculating may occur which might lead to sediment crystals [17].

However, minor difference in organoleptic properties were noticed. Samples showed minor 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 rang was from 5.85- 5.9 (Table 2) and the reconstitution time was two to three shakes and there was minimum variation in the sample weight. 4 samples consist of 95 to 100% of the actual weight out of 10 samples; six samples contain around 101%. Thus, the sample result for physical test indicates acceptable stability range.

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

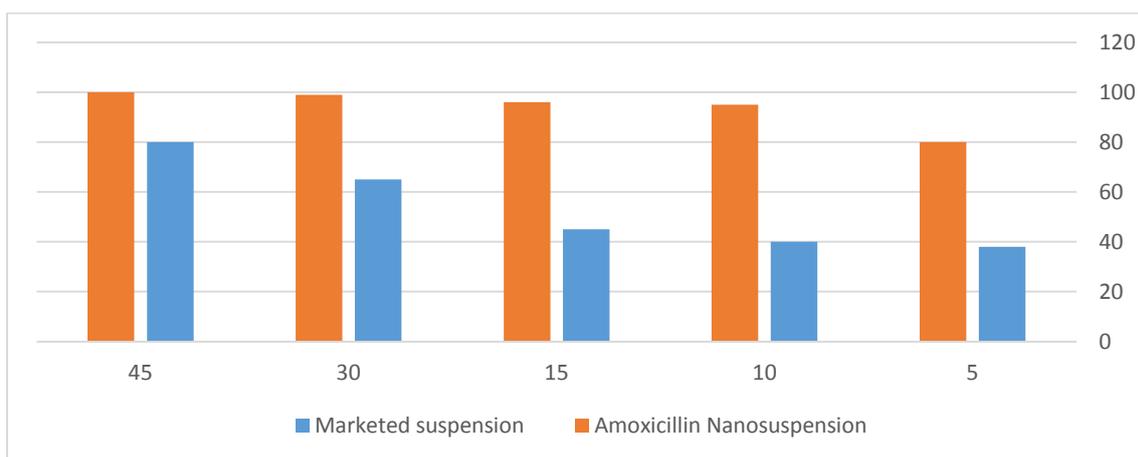


Chart 2. Dissolution profile of amoxicillin T80 (1:2:2) w/w and commercial amoxicillin suspensions

The product was significantly affected by heat that the constituent decreased to 90% after the storing, the degradation followed pseudo-zero order for the powder with a K_0 1.3 mg. month.

The regression equation for samples was:

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

After reconstitution sample degradation reaction followed first order reaction with arate constant $K_i = 1.1 \times 10^{-2}$ month. The I.SR1, equation was:

$\ln C = 4.8372743 - 0.0114333454 \times t$ – concentration after two weeks reached 80% at ambient conditions (29.7°C /31.4% RH) and after one week it was around 90%.

When considering the degradation rate during specific time its noticeable that the rate is much higher in elevated temperature and linear with temperature decrease [10]. Accordingly lines and k start from zero as the origin and can be described by $K_i = c_i/t_i$

If the degradation value is the same for the lines k_1 and k_2 , one has to $k_1 t_1 = k_2 t_2$

Amoxicillin Nano-suspension compared with commercial amoxicillin suspensions {Samples were purchased from a national manufacturing facility and asked to be transported to our laboratory under standard storage conditions.} The products were tested initially and data loggers were supplied to be used in measuring and recording temperature and humidity every 90 minutes during transportation. Physical changes were examined (Table 2) and no changes were detected. Dissolution rate were reported three times at the first fifteen min. for the next hour it was reported each fifteen min. As shown in (Chart 2) the nano-suspension dissolution rate were 100% faster than the commercial amoxicillin samples for the first 5 min and 96% release in 15 min, which may indicate higher rate of drug absorption, rapid onset of action better bioavailability and less gastric discomfort which may increase patient compliance.

Physical changes: The product retained its physical characters no changes were detected.

Table 2. The product PH and Volume

Test	1	2	3	4	5	6
PH	5.76	5.79	5.81	5.79	5.89	5.81
Volume	75.9	75.5	76.1	75.2	75.4	75.62ml

4. 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 non-isothermal strategies allow to calculate its dynamic parameters and to set up a scientific model to expect its shelf life. Compared to accelerated long-term studies, the use of non-isothermal/isothermal models together may allow fast and reliable shelf life calculation in case of a Nano product with less uncertainty. As per our results, we agree with the possibility to calculate the value of kinetic constants based on a fixed degradation limit, regardless of the shape of the curve.

DISCLAIMER

The products used for this research are commonly and predominantly used 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.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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