Original Research Article

Formulation and characterization of hydrochlorothiazide solid lipid microparticles based on lipid matrices of *Irvingia* fat

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Abstract

Introduction: The purpose of this study was to improve the solubilization, bioavailability, and permeability of hydrochlorothiazide (HCTZ) by the formulation and characterization of HCTZ solid lipid microparticles (SLMs) based on fat derived from *Irvingia gabonensis* var. *excelsa* (*Irvingia wombolu*) and Phospholipon®90G (P90G). **Materials and Methods:** *Irvingia* fat was extracted from the nut of *I. gabonensis* var. *excelsa* using petroleum ether (40-60°C). HCTZ loaded SLM were formulated using hot homogenization method with 5% w/w *Irvingia* fat/P90G at each of 1:0, 9:1, 4:1, and 3:1 ratios, 1% w/w HCTZ, 1.5% w/w Labrasol® surfactant and distilled water. Subsequently, particle size analysis, pH, syringeability, drug encapsulation efficiency (EE%), yield, freeze-thaw cycle test, drug release, diffusion, and kinetics were evaluated. **Results:** The SLM dispersions showed a particle size range of 10.15 ± 2.36 to 13.50 ± 6.88 μm and pH of 5.6-6.4 while dispersions containing 3:1 *Irvingia* fat/P90G passed through most of the needles (18G, 21G, and 22G) after syringeability studies. A single freeze-thaw cycle caused loss of physical integrity. The EE% of the SLMs were ≥80%, with high yield. The highest drug release and diffusion was observed with SLMs prepared with 3:1 *Irvingia* fat-P90G mixture (HDP3) and Higuchi model best described the kinetics of the HCTZ release by Fickian diffusion. **Conclusion:** The release and permeability of HCTZ was improved by its incorporation into *Irvingia* fat and P90G (3:1) as SLMs.

Key words: Encapsulation efficiency, Fickian diffusion, homogenization

INTRODUCTION

Currently, approximately 40% of the marketed immediate release oral drugs are categorized as practically insoluble (<100 μg/ml).[1] Lipid-based formulations are typically reputed to improve the solubility and bioavailability of per orally administered poorly soluble drugs.[2] Usually, the solubility of an amorphous drug is higher than that of the corresponding crystalline drug. The difference in the solubility between amorphous form and crystalline form has been reported to be between 1.1- and 1000-fold.[3] Improved bioavailability, protection of sensitive drug molecules from the outer environment (water, light) and even controlled release characteristics were claimed by incorporation of poorly water soluble drugs in the solid lipid matrix.[4] Solid lipid microparticles (SLMs) are usually presented as emulsion systems, differing from conventional emulsions by their particulate nature and can be lyophilized into discrete microparticles.[5] Moreover, lipid matrices (LMs) can be structured with phospholipids for improved functionality. The composition of the lipid matrix and their physicochemical properties such as crystallization of the components are important for the drug loading capacity of nanoparticles and microparticles.[6]

Irvingia gabonensis var. *excelsa* (*Irvingia wombolu*) is a tropical African tree, whose seed or nut contains fat.[7,8] Fat extracted from the nut of this plant can be used for food, pharmaceutical and cosmetic applications.[8] Fat derived from the nut of the plant is generally regarded as safe and has been consumed locally in Africa for centuries. An investigation of its applicability in lipid-based drug delivery systems is, therefore, desirable.

Hydrochlorothiazide (HCTZ) is a thiazide diuretic and belongs to Biopharmaceutics Classification System class IV with its low aqueous solubility and low membrane permeability. Consequently, HCTZ is, therefore, a good candidate for advanced lipid formulation in order to achieve improved bioavailability which could lead to dose reduction and decreased side-effects. In addition, reduced side effect of HCTZ may promote patient compliance and eventually therapeutic performance of the formulation.

The aim of this study was to improve the efficacy and safety of HCTZ by formulation and characterization HCTZ SLMs based on fat derived from *I. gabonensis* var. *excelsa* (*I. wombolu*) and Phospholipon®90G (P90G).

MATERIALS AND METHODS

Materials
Phospholipon®90G (Phospholipid GmbH, Koln, Germany), HCTZ (Juhel Pharmaceuticals, Nigeria), Labrasol® (Gattefosse, France), *Irvingia* fat was prepared in Department of Pharmaceutical Technology and Industrial Pharmacy Laboratory, University of Nigeria, Nsukka, Nigeria.

Extraction of fat from *Irvingia gabonensis* var. *excelsa* (*Irvingia wombolu*)
Fat was extracted from the nut of *I. gabonensis* var. *excelsa* with petroleum ether (40-60°C) using column extraction and concentrated with a rotary evaporator. The nuts were milled to coarse form and dried prior to extraction. Further purification was carried out by heating a 2% w/w suspension of a 1:9 ratio blend of activated charcoal and bentonite in the lipid at 50°C for 1 h. Thereafter, the suspension was vacuum-filtered using Buchner funnel. This type of fat is commonly called "dika fat." The yield of extracted *Irvingia* fat was calculated using Eq. 1:

$$\text{Yield \%} = \left(\frac{\text{weight of extracted Irvingia fat}}{\text{weight of dried Irvingia nuts}} \right) \times 100 \qquad (1)$$

Formulation and optimization of unloaded solid lipid microparticles
Solid lipid microparticles were formulated using hot homogenization method. *Irvingia* fat (5% w/w) was melted at 60°C while aqueous Labrasol® surfactant solution (1.5% w/w) was maintained at the same temperature in an electronic thermostat water bath for 5 min. Appropriate quantity of HCTZ was dissolved in the molten *Irvingia* fat to formulate SLM dispersions containing 1% w/w HCTZ. The surfactant solution was then added to the HCTZ loaded molten *Irvingia* fat with gentle stirring. The mixture was homogenized at 20,000 rpm for 5 min with an Ultra-turrax® mixer while submerged in the water bath. Afterwards, the dispersion (HD SLM dispersion) was allowed to cool and subsequently stored in a refrigerator (4°C). The procedure was repeated using *Irvingia* fat/P90G LMs at 3:1, 4:1, and 9:1 mass ratios to form PDP3, PDP4, and PDP9 SLM

dispersions respectively. The P90G was pre-heated at 80°C prior to lipid fusion.

Characterization of the solid lipid microparticles
Particle size analysis, morphology, and polydispersity
The particle size and morphology were studied by viewing thin slide samples of dispersions with a Motic® digital light microscope and images captured with a Motic® camera (Motic, Xiamen, China) and analyzed using Motic® images plus 2.0 software (Motic, Xiamen, China). The polydispersity index was calculated as the ratio of standard deviation to the mean particle size of the SLM.

pH of SLM dispersions
The pH of the dispersions was evaluated using a validated pH meter (HANNA Instruments, Padova, Italy). The electrode part of the pH meter was immersed into 50 ml quantities of each dispersion, and the reading recorded.

Drug encapsulation efficiency, drug loading capacity, and yield (%)
The content of HCTZ in the microparticles was determined using UV spectrophotometric method. The SLM dispersion was centrifuged at 3000 rpm for 20 min and the supernatant assayed with a UV-VIS spectrophotometer (Spectrumlab 752s, UK) at 273 nm respectively after appropriate dilutions. A laboratory desktop centrifuge (Model SM 800B, Uniscope Surgifriend Medicals, England) was used for centrifugation. The drug encapsulation efficiency (EE%) of the loaded microparticles was calculated using Eqs. 2 and 3.

$$\text{EE \%} = \left(\frac{\text{Real drug loading}}{\text{theoretical drug loading}} \right) \times 100 \qquad (2)$$

$$\text{EE \%} = \left(\frac{w_{\text{total}} - w_{\text{free}}}{w_{\text{total}}} \right) \times 100 \qquad (3)$$

where w_{total} = weight of the drug added to the system and w_{free} = weight of free drug dissolved in medium/supernatant.

The drug loading capacity (DLC %) was calculated using Eq. 4:

$$\text{DLC \%} = \frac{w_{\text{total}} - w_{\text{free}}}{w_{\text{total}} - w_{\text{free}} + w_{\text{lipid}}} \times 100 \qquad (4)$$

where w_{lipid} is the weight of lipid added to the system.

The percentage yield of the SLMs after the hot homogenization preparation process, was calculated using Eq. 5:

$$\text{Yield \%} = \left(\frac{\text{actual weight of SLM}}{\text{theoretical weight of SLM}} \right) \times 100 \qquad (5)$$

Syringeability
The syringeability of the formulations was assessed by passing each dispersion through different needle gauges of varying size

(18G, 21G, 22G, and 23G). The smallest needle gauge that an entire sample passes through is taken as the syringeability of that sample formulation.

Freeze-thaw cycle test

The temperature of storage of the SLM dispersions was varied between 4°C, 25°C, and 40°C for 1 cycle. The physical stability and drug content of the different formulations were evaluated after 1 week and 1 month. A single freeze-thaw cycle was performed.

Differential scanning calorimetry of lyophilized solid lipid microparticle dispersions

An appropriate volume of each dispersion was lyophilized using a lyophilizer/freeze-drier (York Scientific Industries Pvt. Ltd., Model YSI 280, India). This process converted the dispersions to powder. Differential scanning calorimetry (DSC) was then performed on samples of HCTZ SLM dispersion using a DSC instrument (NETZSCH DSC 204 F1, Germany) at a temperature range of 30-400°C and heating rate of 10 K/min on an aluminum pan with a pierced lid. The DSC of the SLM samples was used to assess their thermal property, crystallinity and to observe any chemical interaction between components of the SLM.

Drug release and diffusion studies of solid lipid microparticle dispersions

Appropriate volumes of SLM dispersions equivalent to 25 mg of HCTZ were each separately enclosed in dialysis membrane tubing (molecular weight cutoff 5000-8000) with hermetically sealed ends. The diffusion surface area was maintained by using membrane with the same length (3 cm) and width (2.5 cm) for all the tests. The enclosed dispersions were submerged in 900 ml of simulated gastric fluid in a beaker mounted on a magnetic stirrer assembly, and the medium was maintained at 37 ± 1°C and stirred at 50 rpm. A series of 5 ml volumes of the test solution were withdrawn at 30 min interval for 6 h and assayed at 273 nm using an Spectrumlab 752s UV-VIS spectrophotometer.

Kinetics and mechanism of drug release and diffusion

The cumulative amount of HCTZ released from the formulated dispersions at different time intervals were fitted to the following plots; zero order kinetic model using cumulative percentage drug release versus time or "Q versus t";[9] first order kinetic model using log cumulative of percentage drug remaining versus time or "Log $(100 - Q)$ versus t";[10] Higuchi model using cumulative

percentage drug release versus square root of time or "Q versus $t^{1/2}$".[11,12] Zero order kinetics describes drug release that is not dependent on the concentration, while first order kinetics is used to explain the concentration dependent kinetics. Higuchi model is usually used to describe drug release that follows Fickian diffusion and could be observed in some porous matrices. The linearity of these plots was determined by their R^2 values and the plot (model) with the highest linearity was taken as that which described the kinetics and mechanism of drug release.

Statistical analysis

The results obtained were analyzed using One-way analysis of variance. Descriptive and inferential statistics were employed in data analysis. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

An *Irvingia* fat yield of 30.5% was obtained. The particle size of the SLMs were within the range of 10.15 ± 2.36 to 13.50 ± 6.88 µm [Table 1, Figure 1]. The particles were micro-sized and mostly spherical or ellipse shaped. The particle sizes of the dispersions did not follow any definite P90G concentration related pattern. Moreover, some of the formulations were highly polydispersed, so no significant differences ($P < 0.05$) could be deduced. The polydispersity indices showed that some of the SLMs were not quite uniformly distributed in terms of particle size. This may be because of the nature of materials used (*Irvingia* fat, P90G, and Labrasol®). The relatively low melting point of *Irvingia* fat, the self-emulsifying nature of Labrasol® and the stickiness of P90G might have promoted particle size fluctuations during the SLM production.

The particle size of the formulations would have affected other properties such as syringeability and drug release. The pH of the SLM dispersions were slightly acidic [Table 1], which could be because of the presence of fatty acids in *Irvingia* fat. The pH were between 5.6 and 6.4. Table 1 also shows that the drug EE% and yield of the SLMs were relatively high, and effective loading capacity was observed. This suggested that the hot homogenization production method employed was effective. Studies have shown that the composition of the lipid matrix (fat and P90G) can influence the degree of drug encapsulation.[13] In the evaluation of the syringeability of the dispersions, HD and HDP9 passed through needle of 18G gauge size only, as HDP4 passed through 18G and 21G only, while HDP3 passed through

Table 1: Some physicochemical properties of the HCTZ SLM dispersions

Formulation	pH	Particle size (µm)	Polydispersity index	Encapsulation efficiency (%)	Loading capacity (%)	Yield (%)
HD	6.4	13.05±1.62	0.12	80.23±0.1	13.83	91±0.5
HDP9	6.4	13.00±6.62	0.51	81.36±0.2	13.99	91±0.5
HDP4	5.6	10.15±2.36	0.23	80.51±0.1	13.87	93±0.5
HDP3	5.9	13.50±6.88	0.51	83.05±0.1	14.24	89±0.5

HCTZ: Hydrochlorothiazide, SLM: Solid lipid microparticle

18G, 21G, and 22G only. None of the SLM dispersions were "syringeable" through needle gauge 23G. The ease of passage through the needles of different gauge sizes, improved with increasing P90G content since only batch HDP3 passed through needles with a smaller orifice size (22G). The "deformability" of P90G might have facilitated the passage of formulations containing P90G.

Freeze-thaw cycle

After a single freeze-thaw cycle (4°C to 25°C to 40°C), the SLMs lost its physical integrity and stability. The SLMs melted at the highest temperature, coalescing at the top of the dispersion with subsequent solidification. However, there was no significant difference ($P > 0.05$) in drug content before and after a single freeze-thaw cycle and also when evaluated after 1 week and 1 month. Refrigeration at 4°C increased the visualized viscosity of the dispersion.

The result showed that storage condition for this product should be optimized and maintained especially in the tropics where environmental temperature could rise high causing physical instability and loss of microparticle integrity. The risk of instability under such conditions is further heightened considering the melting temperature of *Irvingia* based formulations (39-43°C). Therefore, selection of packaging materials would be vital, since poor conductors of heat would be preferred. Labels of these products should also clearly indicate storage under cool conditions.
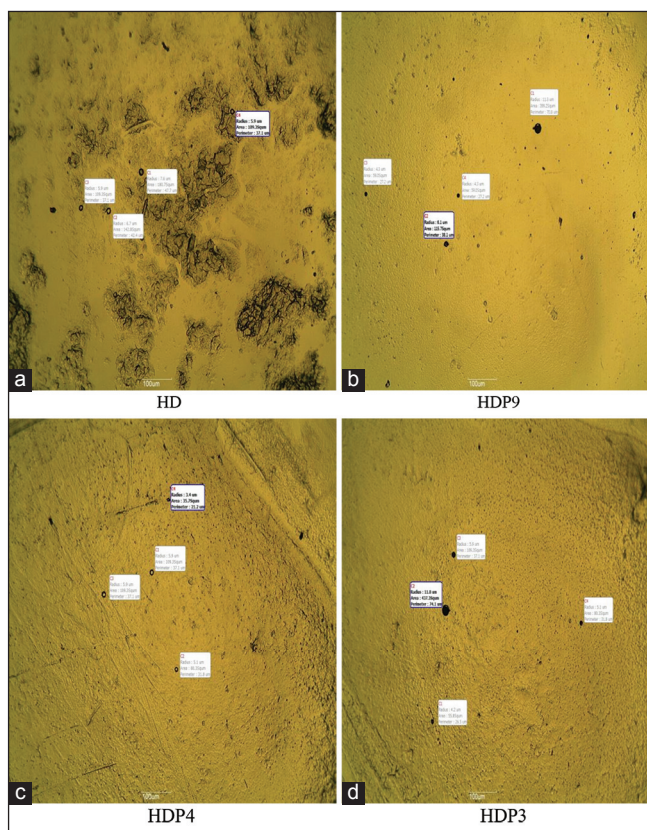
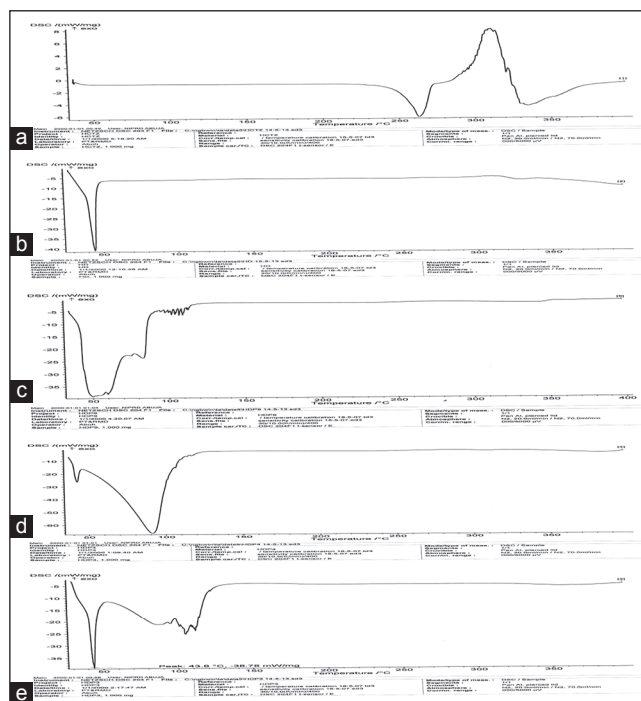### Differential scanning calorimetry thermogram of freeze-dried solid lipid microparticle powder

The DSC thermogram of freeze-dried SLM samples was used to evaluate their thermal properties and possible drug-excipients interaction. The thermograms [Figure 2] showed that there was no definite chemical reaction between the ingredients. The melting peaks of the SLM powders were close to the melting point of *Irvingia* fat (42°C). This showed that the thermal property of the samples was predominantly that of the *Irvingia* fat carrier. This was very clear in HD microparticles where *Irvingia* fat was the only lipid base. Here the drug was in an amorphous state with no visible peak of its own within the matrix. The peak signified thermally induced phase transition of the SLM and heat capacity was at its maximum at this point. However, as the P90G content increased, the HCTZ was increasingly partially recrystallized with the emergence of shoulders on the dominant peak. This observation may be because of high drug loading of the lipid matrix with concomitant liberation and partial crystallization of the drug molecules. The composition of the LM and their physicochemical properties such as crystallization of the components are important for the drug loading capacity of nanoparticles.[6]

Drug release and diffusion

The drug release and diffusion profile of HCTZ loaded SLM dispersions are presented in Figure 3. SLM dispersions prepared with 3:1 *Irvingia* fat/P90G LMs had the highest

Figure 1: Photomicrographs for HD (a), HDP9 (b), HDP4 (c), and HDP3 (d) solid lipid microparticle dispersions
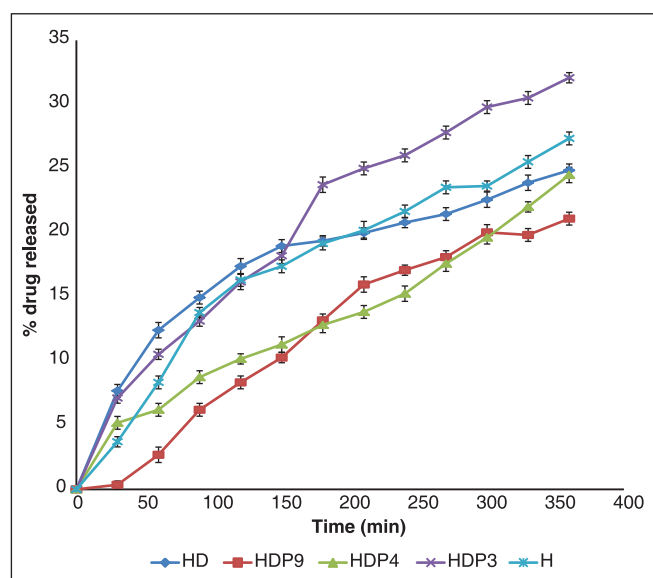

Figure 2: Differential scanning calorimetry thermograms of hydrochlorothiazide (a) and solid lipid microparticles HD (b), HDP9 (c), HDP4 (d), and HDP3 (e)

drug release through the dialysis membrane (significantly different at $P < 0.05$). After 6 h of study, a maximum of 30% drug release was observed in HDP3. The higher P90G content in this preparation might have improved penetration through the membrane. Phospholipids have been shown to disrupt membranes.[14] In addition, the stickiness of P90G might have increased the adhesion of the drug loaded SLM to the wall of the membrane sac, thereby facilitating more permeation. The low solubility of HCTZ in *Irvingia* fat implies that the HCTZ loaded SLM existed as solid dispersion and solid solution since the drug would have dispersed or dissolved in the lipid matrix. The aqueous solubility and other physicochemical characteristics of drugs are important in drug release. The release profile showed a steady climb with a gradual, but continuous increase in drug release. This behavior could be attributed to uniform distribution of HCTZ within the lipid matrix. The slightly higher EE% observed with SLMs containing a 3:1 lipid matrix may have contributed to the higher drug release and diffusion observed with the batch. However, different factors can influence the drug release and diffusion profile. These includes the following:

1. Drug release from LMs of SLMs,
2. Aqueous solubility of the drug,
3. Presence of free drug,
4. Diffusion rate toward and across the membrane wall,
5. Permeability of drug across dialysis membrane,
6. Partition coefficient between liquefied LMs and water, and
7. Volume of medium in the receiver compartment.

The application of SLMs as a delivery device has been shown to improve solubilization and dissolution of drugs. The lipid particles form a crude emulsion, and this promotes the solubilization of the co-administered lipophilic drugs.[15,16] This process is facilitated by the presence of surface active agents.

Moreover, controlled delivery and enhanced permeability of the drug was achieved.
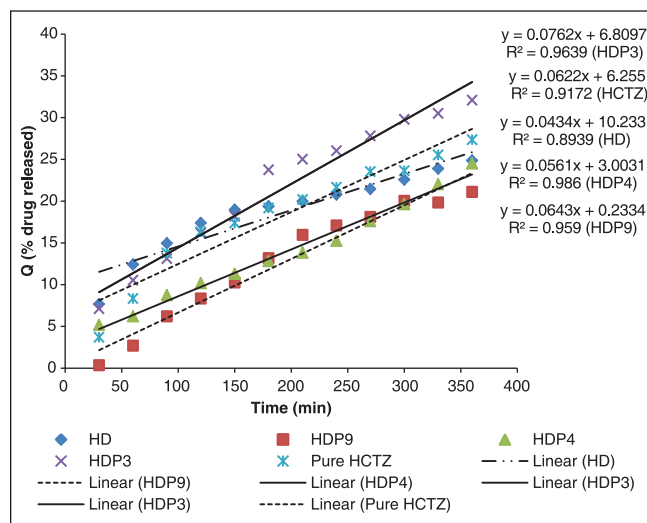
Kinetic and mechanism of drug release

Figures 4-6 and Table 2 show that the kinetics of HCTZ release from the SLM dispersions through dialysis membrane, predominantly obeyed Higuchi model since the plot of cumulative percentage drug released against the square root of time had the highest R^2 values.

Formulations HD, HDP9, HDP3, and HCTZ fitted more with Higuchi model. This implied that the mechanism of drug release might be Fickian diffusion. The time dependent drug release process, therefore, followed sink conditions. The Fickian diffusion release occurred by molecular diffusion of the drug due to the chemical gradient. Only batch HDP4 fitted more with zero order which suggested that it might have been independent of concentration.

In general, the release process of the formulations seems to be most suitably described by Higuchi kinetic model because HCTZ intrinsically has low membrane permeability. This might have favored attainment of sink conditions, where further release was facilitated by excessive volume of dissolution medium (900 ml). This effectively increased the concentration gradient by decreasing the concentration of dissolved drug in the external dissolution medium (receiver compartment).

CONCLUSION

The incorporation of HCTZ into SLMs based on *Irvingia* fat and P90G modified the release of HCTZ drug and improved its permeability. This observation would possibly improve the bioavailability of the drug, allow for dose reduction and reduce associated side effects.

Figure 3: Percentage drug release profile of membrane enclosed hydrochlorothiazide loaded solid lipid microparticles

Figure 4: Zero order kinetic model for hydrochlorothiazide loaded solid lipid microparticles
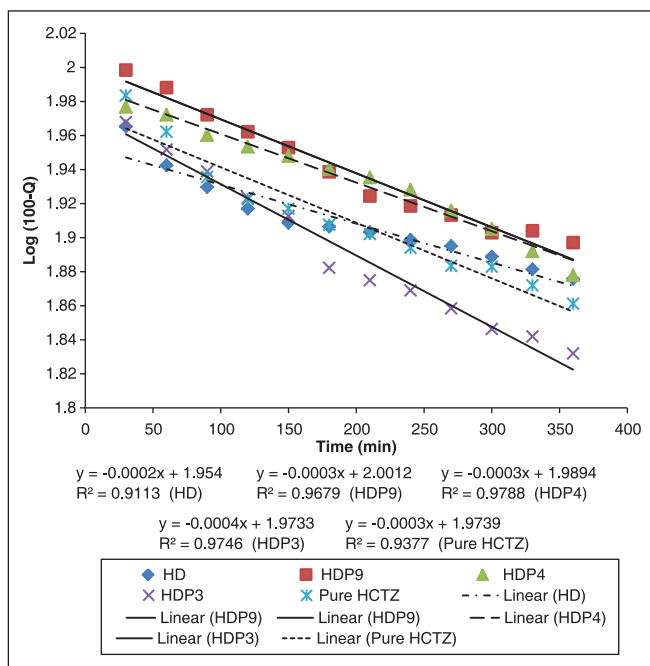
Figure 5: First order kinetic model of hydrochlorothiazide loaded solid lipid microparticles
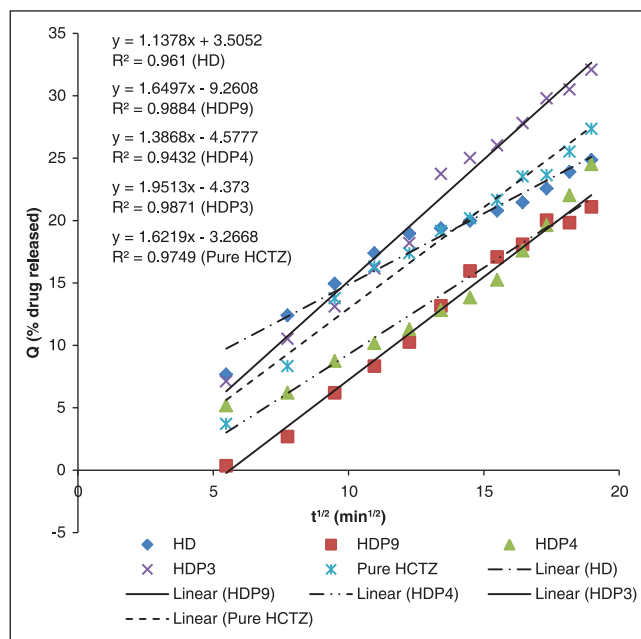

Figure 6: Higuchi model for hydrochlorothiazide loaded solid lipid microparticles

Table 2: R^2 values for different kinetic models

Formulation	R²		
	Zero order	First order	Higuchi
HD	0.8939	0.9113	0.9610
HDP9	0.9590	0.9679	0.9884
HDP4	0.9860	0.9788	0.9432
HDP3	0.9639	0.9746	0.9871
Pure HCTZ	0.9172	0.9377	0.9749

HCTZ: Hydrochlorothiazide

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